Original Research Article

Harnessing the Power of Symbiosis: Maximizing Physiological Growth in *Melia azedarach* through AM Fungi and Pre-sowing Seed Treatments

Abstract

Melia azedarach, a versatile tree belonging to the *Meliaceae* family, presents a significant challenge in achieving successful seed germination for forest plantations. The robust nature of *Melia azedarach* seeds necessitates pre-treatments to overcome physical barriers and enhance water absorption. Natural ecosystems often benefit from the symbiotic relationship between Arbuscular mycorrhizal (AM) fungi and plant roots, which promotes survival and growth. This study examined the impact of *Glomus mosseae*-inoculated soil on *Melia azedarach* seeds treated with pre-sowing techniques at the Nursery of the Forestry Department, CCSHAU, Hisar in 2019. *Glomus mosseae* was sown at a rate of 400–500 sporocarps per kg of soil, and its influence was evaluated in terms of physiological parameters, survival rate, root colonization percentage, and sporocarp count. Each replication of the experiment involved 250 seedlings and was repeated five times. Results demonstrated that soils inoculated with *Glomus mosseae* and treated with gibberellic acid at 200 ppm for 24 hours prior to sowing exhibited significantly higher physiological parameters (chlorophyll and carotenoid content, photosynthesis rate, transpiration rate, and stomatal conductance), survival percentage, root colonization percentage, and sporocarp count (per 100 g of soil). Therefore, the combined use of *Glomus mosseae* and gibberellic acid at 200 ppm for 24 hours is recommended to enhance physiological growth and plant survival in *Melia azedarach*.

Keywords: *Glomus mosseae, Melia azedarach,* photosynthesis rate, root colonization (%) and plant survival (%).

Introduction

Arbuscular mycorrhizal fungus (AMF) is a soil microorganism that plays a vital role in establishing a healthy relationship between soil and plants. By forming a symbiotic partnership, mycorrhizal fungi contribute to plant growth and survival by reducing stress factors (Sylvia & Williams, 1992). These fungi offer several benefits to their host, including enhanced phosphorus uptake (Goussous and Mohammad, 2009), increased nitrogen absorption (Rotor and Delima, 2010), production of plant growth hormones (Herrera-Medina et al., 2007),

defense against soil-borne diseases (Bakhtiar et al., 2010), and improved plant growth and productivity (Duponnois et al., 2005).

The colonization of plant roots by AM fungi has been recognized for its potential as a bio-protectant and biofertilizer (Berruti et al., 2016), providing protection against parasitic fungi and nematodes, while also promoting plant growth and yield (Wei et al., 2016). Around 80% of vascular plants have their roots colonized by AM fungi, making them an essential component of a healthy soil-plant system (Vierheirlig, 2004; Budi et al., 2012). They contribute to soil quality and improve plant fitness (Barea et al., 2002). Neem, a tree species, particularly relies on mycorrhizal fungi, as they colonize its roots extensively (Habte et al., 1993).

Most plants form symbiotic partnerships with mycorrhizal fungi within their roots, providing an ideal ecological niche for fungal development and completing their sexual cycle (Sandeep et al., 2015). This common symbiotic relationship between AM fungi and plant roots enhances the survival and growth of the majority of plants in natural ecosystems (Lipnicki, 2015). Within the root cells, arbuscles, which are branched hyphae, serve as sites for nutrient exchange between the fungi and the plant (Afzal et al., 2011). The primary advantage of mycorrhiza for forest plants is their efficient accumulation of nutrient ions and water in the rhizosphere. By enhancing nutrition, growth, dry matter production, and drought resistance, mycorrhizal fungi facilitate the availability of nutrients and water to both the host plant and the fungus (Luyindula and Haque, 1992).

Melia azedarach, a significant tree species in social forestry projects, is well-known for its therapeutic benefits. Researchers are particularly interested in finding optimal seed germination methods for this species (Azad et al., 2010). It is a fast-growing tree commonly planted for its ornamental value along roadways. The wood of *Melia azedarach* is utilized in various applications, including toys, boxes, athletic equipment, musical instruments, furniture, and fuelwood due to its calorific value of 5043-5176 kcal/kg (Shukla and Bhatanagar, 1988). It serves multiple purposes and is classified as an agro-forestry/social forestry species, making it highly valuable (Gupta, 1993).

However, the main challenge in establishing forest plantations of *Melia azedarach* lies in its poor seed germination (Azad et al., 2010). Wulandini and Widyani (2007) suggest that the seeds of *Melia azedarach* have a hard coating, and pre-treatments are used to remove this physical barrier and improve water absorption. This plant exhibits characteristics typical of those highly susceptible to arbuscular mycorrhizal symbiosis, including a coarse root structure and a lack of root hairs. The objective of this study is to explore efficient approaches for

maximizing the physiological growth of Melia azedarach by utilizing AM fungi and pre-sowing seed treatments.

Materials And Methods

A. Planting materials and study site:

In 2019, an experiment was conducted at the nursery of the Department of Forestry, CCS Haryana Agricultural University in Hisar. During particularly hot summer days, the average monthly maximum and minimum temperatures could reach up to 48°C. The region experiences relative humidity ranging from 5% to 100%, and winter temperatures often drop below freezing, accompanied by frost. The growth and germination assessment utilized drupes of uniform size. Each treatment involved the random selection of 750 *Melia azedarach* drupes.

B. Experimental design and treatment combinations:

A Complete Randomized Design (CRD) was employed for the study, consisting of twenty-six treatments, including a control group, with five replications for each treatment. In each replication, 250 seeds were sown to investigate the impact of pre-sowing treatments. After germination, the seedlings were allowed to grow to assess their initial growth performance. The details of the pre-sowing seed treatments used in the experiment are provided below:

- **Seed Treatment:**

Each treatment involved the use of 750 *Melia azedarach* drupes that were chosen at random.

- **Normal Water Treatment:**

Seeds were soaked in ordinary tap water for 24, 48 and 72 hours after which they were removed and sown directly.

- **Conc. H_2SO_4 Treatment:**

Seeds were soaked in Conc. H_2SO_4 for 4, 6 and 8 minutes after which the seeds were washed with tap water.

- **Gibberellic acid treatment:**

Seeds were soaked in 200, 300 and 400 ppm gibberellic acid solutions for 24 hours.

- **Cow dung slurry treatment:**

1. Seeds were soaked Cow dung slurry for 2, 4 and 6 days.

- ***Glomus mosseae* treatment:**

Soil application of *Glomus mosseae* were applied at the rate of 400-500 sporocarp/kg of soil at the time of sowing of drupes of *Melia azedarach*

- **Control seeds:** Seeds were not given any treatment.

These treated seeds were then sown 2-3 cm deep in sterile sandy soil, individually inoculated with *G. mosseae* at a rate of 450-500 sporocarps/kg of soil, in polythene bags. The sowing of untreated seeds in non-inoculated soil served as the control. This experiment followed a completely randomized design, with each treatment replicated five times, and 250 seedlings per replication were raised and maintained. Physiological parameters of the seedlings were determined at 90 and 180 DAS (Days After Sowing). Chlorophyll and carotenoid content were calculated using Wellburn's (1994) equations, while photosynthesis, transpiration rate, and stomatal conductance were measured using a handheld photosynthesis system, LCi-SD Bioscientific Ltd. Additionally, mycorrhizal colonization in roots and sporocarp numbers in the soil were assessed at 60, 120, and 180 DAS. Mycorrhizal colonization was calculated using the method described by Phillips and Hayman (1970), and sporocarps were determined according to the method given by Gerdemann and Nicolson (1963). Plant survival percentage was calculated using the following formula.

$$\text{Plant Survival (\%)} = \frac{\text{Total number of seedlings survived}}{\text{Total number of seedlings}} \times 100$$

C. Analysis of Data

Analysis of Variance (ANOVA) was performed to examine the effects of seed treatments, and the Critical Difference at the 5% level of significance was used to determine whether there were significant differences between the means. The statistical analysis was conducted using OPSTAT.

Results

3.1 Effect of pre sowing treatments of seeds in combination with *Glomus mosseae* on physiological growth of *Melia azedarach*

As a result of various pre-sowing treatments, the data in Table 1 indicated a considerable increase in total chlorophyll of Melia azedarach at 90 and 180 days after sowing. At 90 DAS, Gibberellic acid 200 ppm for 24 hrs with *Glomus mosseae** treatment had considerably

greater total chlorophyll (23.12 µg/ml), followed by Gibberellic acid 300 ppm for 24 hrs with *Glomus mosseae** (21.70 µg/ml), and the lowest total chlorophyll in the control, 12.30 µg/ml. At 180 DAS, the total chlorophyll in the Gibberellic acid 200 ppm for 24 hrs with *Glomus mosseae** treatment was significantly higher (30.32 µg/ml), followed by the Gibberellic acid 300 ppm for 24 hrs with *Glomus mosseae** (28.71 µg/ml), while the lowest level was found in the control, which was 17.01 µg/ml.

The data on carotenoid content presented in Table 1 revealed that there was no significant difference found among all the pre-sowing treatments. At 90 DAS, Gibberellic acid 200 ppm for 24 hrs with *Glomus mosseae** treatment had a considerably greater carotenoid content (5.22 µg/ml), followed by Gibberellic acid 300 ppm for 24 hrs with *Glomus mosseae** (5.12 µg/ml), with the control having the lowest carotenoid content (4.85 µg/ml). At 180 DAS, the carotenoid content was substantially greater in the Gibberellic acid 200 ppm for 24 hours with *Glomus mosseae** treatment (5.70 µg/ml), followed by Gibberellic acid 300 ppm for 24 hours with *Glomus mosseae** (5.59 µg/ml), while the lowest level was found in the control, or 5.22 µg/ml.

The analysis of data presented in Table 2 revealed significant influence of different pre-sowing treatments on photosynthesis of *Melia azedarach* at 90 and 180 days after sowing.

At 90 and 180 DAS, the photosynthesis was significantly higher in Gibberellic acid 200 ppm for 24 hrs with *Glomus mosseae** treatment (6.59 and 9.99 µ mol CO_2 m^{-2} s^{-1}, respectively) followed by Gibberellic acid 300 ppm for 24 hrs with *Glomus mosseae** (6.11and 9.35 µ mol CO_2 m^{-2} s^{-1}, respectively) and Gibberellic acid 400 ppm for 24 hrs with *Glomus mosseae** (6.05 and 9.28 µ mol CO_2 m^{-2} s^{-1}, respectively) with lowest photosynthesis in control *i.e.* 3.85 and 6.58 µ mol CO_2 m^{-2} s^{-1}, respectively.

It is quite evident from the data presented in Table 2 that the stomatal conductance of *Melia azedarach* was significiantly increased by different pre-sowing treatments inoculated with *Glomus mosseae* as compared to *Glomus mosseae* and control . The data was recorded at 90 and 180 days after sowing (DAS). At 90 DAS, the stomatal conductance was significantly higher in Gibberellic acid 200 ppm for 24 hrs with *Glomus mosseae** treatment (0.199 m mol m^{-2} s^{-1}) followed by Gibberellic acid 300 ppm for 24 hrs with *Glomus mosseae** (0.188 m mol m^{-2} s^{-1}). Whereas, stomatal conductance in *Glomus mosseae** was 0.170 m mol m^{-2} s^{-1} with lowest stomatal conductance in control *i.e.* 0.107 m mol m^{-2} s^{-1}. At 180 DAS, the stomatal conductance was significantly higher in Gibberellic acid 200 ppm for 24 hrs with

*Glomus mosseae** treatment (0.312 m mol m^{-2} s^{-1}) followed by Gibberellic acid 300 ppm for 24 hrs with *Glomus mosseae** (0.289 m mol m^{-2} s^{-1}) and Gibberellic acid 400 ppm for 24 hrs with *Glomus mosseae** (0.281 m mol m^{-2} s^{-1}) whereas, minimum was recorded in control *i.e.* 0.217 m mol m^{-2} s^{-1}.

The effect of pre-sowing treatments on transpiration rate of *Melia azedarach* has been presented in Fig. 1. Transpiration rate of *Melia azedarach* due to different pre-sowing treatments was found statistically significant at 90 and 180 days after sowing (DAS). At 90 DAS, the transpiration rate was significantly higher in Gibberellic acid 200 ppm for 24 hrs with *Glomus mosseae** treatment (4.14 m mol H_2O m^{-2} s^{-1}) followed by Cow dung slurry for 6 days with *Glomus mosseae** (3.19 m mol H_2O m^{-2} s^{-1}) and Conc. H_2SO_4 for 8 min with *Glomus mosseae** (3.19 m mol H_2O m^{-2} s^{-1}) with lowest transpiration rate in control *i.e.* 1.81 m mol H_2O m^{-2} s^{-1}.

At 180 DAS, the transpiration rate was significantly higher in Gibberellic acid 200 ppm for 24 hrs with *Glomus mosseae** treatment (5.88 m mol H_2O m^{-2} s^{-1}) followed by Cow dung slurry for 6 days with *Glomus mosseae** (5.18 m mol H_2O m^{-2} s^{-1}) and Conc. H_2SO_4 for 8 min with *Glomus mosseae** (5.12 m mol H_2O m^{-2} s^{-1}) whereas, minimum was recorded in control *i.e.* 3.45 m mol H_2O m^{-2} s^{-1}.

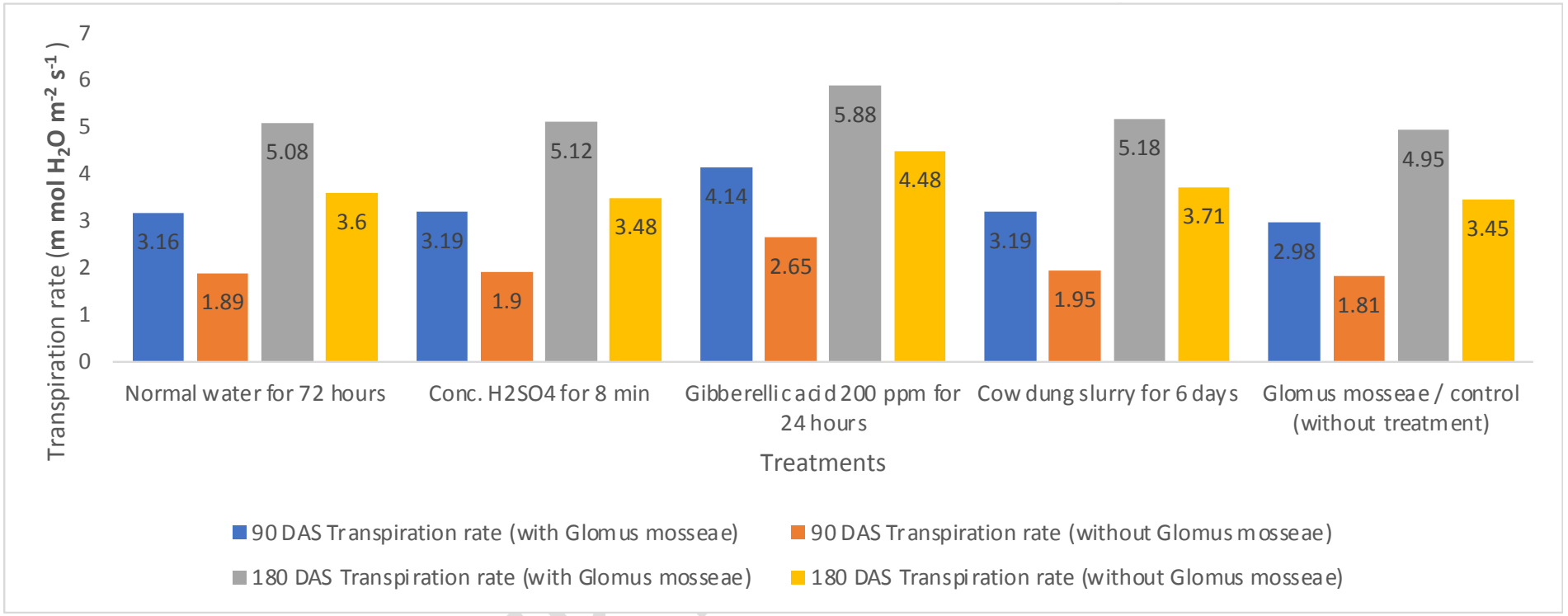
Table 1: Effect of pre sowing treatments of seeds in combination with *Glomus mosseae* on chlorophyll and carotenoid content of *Melia azedarach*

Treatments	With *Glomus mosseae*				Without *Glomus mosseae*			
	Total chlorophyll (µg/ml)		Carotenoid content (µg/ml)		Total chlorophyll (µg/ml)		Carotenoid content (µg/ml)	
	90 DAS	180 DAS	90 DAS	180 DAS	90 DAS	180 DAS	90 DAS	180 DAS
Normal water for 24 hours	18.91	23.66	4.98	5.31	12.56	17.15	4.85	5.23
Normal water for 48 hours	19.23	23.91	4.99	5.29	13.25	17.98	4.88	5.24
Normal water for 72 hours	19.55	24.17	4.99	5.30	13.32	18.17	4.86	5.22
Conc. H_2SO_4 for 4 min	19.09	23.91	5.00	5.32	13.06	17.40	4.86	5.22
Conc. H_2SO_4 for 6 min	19.53	24.17	5.02	5.30	13.13	17.73	4.86	5.23
Conc. H_2SO_4 for 8 min	19.42	24.49	5.02	5.31	12.81	17.64	4.87	5.22
Gibberellic acid 200 ppm for 24 hours	23.12	30.32	5.22	5.70	17.57	22.25	4.93	5.28
Gibberellic acid 300 ppm for 24 hours	21.70	28.71	5.12	5.59	16.57	20.91	4.92	5.26
Gibberellic acid 400 ppm for 24 hours	21.05	27.37	5.09	5.56	15.14	20.01	4.91	5.25
Cow dung slurry for 2 days	19.23	24.07	4.98	5.32	12.62	17.64	4.87	5.24
Cow dung slurry for 4 days	19.48	24.40	4.97	5.34	13.06	17.89	4.85	5.23
Cow dung slurry for 6 days	19.30	24.72	4.98	5.32	13.32	18.15	4.89	5.23
Glomus mosseae/ control	18.84	23.52	4.85	5.30	12.30	17.01	4.96	5.22
C.D. at 5% level of significance	1.29	1.67	N/S	N/S	1.29	1.67	N/S	N/S

Table 2: Effect of pre sowing treatments of seeds in combination with *Glomus mosseae* on photosynthesis and stomatal conductance and carotenoid content of *Melia azedarach*

Treatments	With *Glomus mosseae*				Without *Glomus mosseae*			
	Photosynthesis (μ mol CO_2 m^{-2} s^{-1})		Stomatal conductance (m mol m^{-2} s^{-1})		Photosynthesis (μ mol CO_2 m^{-2} s^{-1})		Stomatal conductance (m mol m^{-2} s^{-1})	
	90 DAS	180 DAS	90 DAS	180 DAS	90 DAS	180 DAS	90 DAS	180 DAS
Normal water for 24 hours	5.41	8.95	0.175	0.249	3.88	6.60	0.107	0.219
Normal water for 48 hours	5.42	8.90	0.172	0.250	3.90	6.65	0.110	0.219
Normal water for 72 hours	5.44	8.99	0.171	0.252	3.96	6.85	0.111	0.220
Conc. H_2SO_4 for 4 min	5.39	8.92	0.173	0.253	3.98	6.75	0.109	0.218
Conc. H_2SO_4 for 6 min	5.49	8.93	0.172	0.256	3.89	6.65	0.106	0.218
Conc. H_2SO_4 for 8 min	5.55	8.99	0.171	0.258	3.95	6.64	0.108	0.217
Gibberellic acid 200 ppm for 24 hours	6.59	9.99	0.199	0.312	5.12	8.40	0.162	0.237
Gibberellic acid 300 ppm for 24 hours	6.11	9.35	0.188	0.289	4.88	7.95	0.155	0.236
Gibberellic acid 400 ppm for 24 hours	6.05	9.28	0.181	0.281	4.75	7.85	0.148	0.234
Cow dung slurry for 2 days	5.61	8.91	0.174	0.249	4.01	6.90	0.108	0.220
Cow dung slurry for 4 days	5.52	8.95	0.175	0.250	3.99	6.90	0.109	0.224
Cow dung slurry for 6 days	5.60	8.98	0.175	0.250	4.00	6.84	0.110	0.221
Glomus mosseae/ control	5.34	8.89	0.170	0.248	3.87	6.58	0.107	0.217
C.D. at 5% level of significance	0.38	0.61	0.011	0.018	0.38	0.61	0.011	0.018

Fig. 1: Effect of pre sowing treatments of seeds in combination with and without *Glomus mosseae* on transpiration rate at 90 and 180 DAS



3.2 Effect of pre sowing treatments of seeds inoculated with *Glomus mosseae* on plant survival (%) of *Melia azedarach*

The plant survival (%) as influenced by different pre-sowing treatment are presented in Table 3. Plant survival percentage was recorded at 60, 120 and 180 days after sowing. At 60 DAS, the plant survival percentage was significantly higher in treatment with Gibberellic acid 200 ppm for 24 hrs with *Glomus mosseae** (95.21%) followed by Gibberellic acid 300 ppm for 24 hrs with *Glomus mosseae** (90.51%). Whereas, minimum was recorded in control *i.e.* 75.45%. At 120 DAS, the plant survival percentage was significantly higher in treatment with Gibberellic acid 200 ppm for 24 hrs with *Glomus mosseae** (94.32%) followed by Gibberellic acid 300 ppm for 24 hrs with *Glomus mosseae** (88.64%) and Gibberellic acid 400 ppm for 24 hrs with *Glomus mosseae** (87.65%). Whereas, minimum was recorded in control *i.e.* 65.49%. At 180 DAS, the plant survival percentage was significantly higher in treatment with Gibberellic acid 200 ppm for 24 hrs with *Glomus mosseae** (93.52%) followed by Gibberellic acid 300 ppm for 24 hrs with *Glomus mosseae** (87.94%) and Gibberellic acid 400 ppm for 24 hrs with *Glomus mosseae** (86.49%). Whereas, minimum was recorded in control *i.e.* 55.56%.

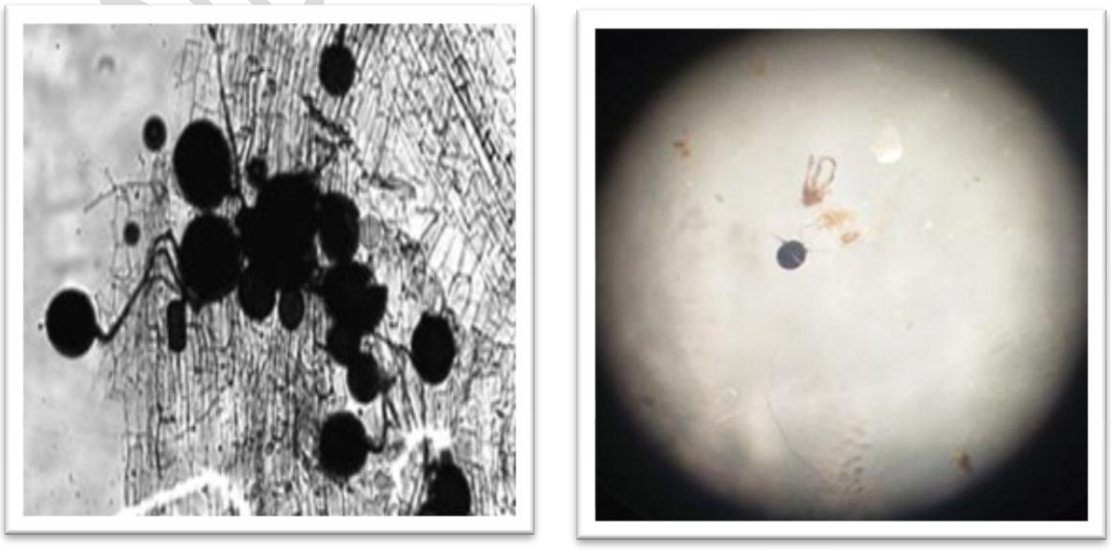
Table 3: Effect of pre sowing treatments of seeds inoculated with *Glomus mosseae* on plant survival (%) of *Melia azedarach* at 60, 120 and 180 DAS

Treatments	**Plant survival (%) (with *Glomus mosseae*)**			**Plant survival (%) (without *Glomus mosseae*)**		
	60 DAS	120 DAS	180 DAS	60 DAS	120 DAS	180 DAS
Normal water for 24 hours	87.94	86.70	85.79	76.52	66.87	56.96
Normal water for 48 hours	88.58	88.01	86.91	76.63	67.95	60.52
Normal water for 72 hours	86.94	85.70	83.79	76.90	67.98	60.14
Conc. H_2SO_4 for 4 min	85.94	84.70	83.72	75.90	66.97	60.98
Conc. H_2SO_4 for 6 min	88.52	88.52	86.71	76.21	64.56	54.95
Conc. H_2SO_4 for 8 min	88.20	88.10	86.11	76.95	66.62	57.96
Gibberellic acid 200 ppm for 24 hours	95.21	94.32	93.52	84.69	83.64	81.57
Gibberellic acid 300 ppm for 24 hours	90.51	88.64	87.94	82.59	80.56	79.82
Gibberellic acid 400 ppm for 24 hours	88.99	87.65	86.49	78.95	74.59	72.65
Cow dung slurry for 2	88.11	88.45	86.47	76.21	68.95	55.21

days						
Cow dung slurry for 4 days	89.34	88.14	85.48	77.69	66.97	59.21
Cow dung slurry for 6 days	88.10	88.13	86.46	77.35	66.94	58.61
Glomus mosseae/ control	88.94	88.70	86.79	75.45	65.49	55.56
C.D. at 5% level of significance	6.29	6.00	5.71	6.29	6.00	5.71

3.3 Effect of pre sowing treatments of seeds inoculated with *Glomus mosseae* on root colonization (%) and number of sporocarp (per 100 g of soil) of *Melia azedarach*

The result related to root colonization (%) at different observation period are shown in Table 4. Root colonization percentage was recorded at 60, 120 and 180 days after sowing. Similar trend of root colonization (%) was observed at 60, 120 and 180 DAS and the root colonization percentage was significantly higher in treatment with Gibberellic acid 200 ppm for 24 hrs with *Glomus mosseae** (33.89, 60.23 and 79.34%, respectively) followed by Gibberellic acid 300 ppm for 24 hrs with *Glomus mosseae** (30.25, 55.64 and 75.23%, respectively) and Gibberellic acid 400 ppm for 24 hrs with *Glomus mosseae** (28.69, 50.69 and 70.25%, respectively). Whereas, minimum was recorded in Normal water for 24 hrs with *Glomus mosseae** treatment at 60 and 180 DAS *i.e.* 21.35 and 60.55%, respectively and at 120 DAS minimum was recorded in *Glomus mosseae** treatment *i.e.* 40.56%. whereas no root colonization (%) or zero root colonization (%) was recorded in treatments without *Glomus mosseae.*



a **b**

***Fig 2. (a)Glomus mosseae* spores in roots of *Melia azedarach* by using phase contrast microscope (b) *Glomus mosseae* sporocarp isolated from soil**

In the present study, number of sporocarp in soil was recorded at 60, 120 and 180 days after sowing and presented in Table 4. Number of sporocarp (per 100 g of soil) followed the similar, trend as that of root colonization (%) at 60, 120 and 180 days after sowing. At 60 DAS, the number of sporocarp was significantly higher in treatment with Gibberellic acid 200 ppm for 24 hrs with *Glomus mosseae** (188.20) followed by Gibberellic acid 300 ppm for 24 hrs with *Glomus mosseae** (176.97) and Gibberellic acid 400 ppm for 24 hrs with *Glomus mosseae** (170.26). Whereas, minimum was recorded in Normal water for 24 hrs with *Glomus mosseae** treatment *i.e.* 148.36. Similarly, 120 and 180 DAS, the number of sporocarp was significantly higher in treatment with Gibberellic acid 200 ppm for 24 hrs with *Glomus mosseae** (289.36 and 388.73, respectively) followed by Gibberellic acid 300 ppm for 24 hrs with *Glomus mosseae** (278.54 and 369.78, respectively). Whereas, minimum was recorded in *Glomus mosseae** treatment *i.e.* 250.12 at 120 DAS and at 180 DAS in Normal water for 24 hrs with *Glomus mosseae** treatment *i.e.* 338.24.

Table 4: Effect of pre sowing treatments of seeds inoculated with *Glomus mosseae* on root colonization (%) and number of sporocarp (per 100 g of soil) of *Melia azedarach*

Treatments	With *Glomus mosseae*						Without *Glomus mosseae*					
	Root colonization (%)			Number of spores (Per 100 g of soil)			Root colonization (%)			Number of spores (Per 100 g of soil)		
	60 DAS	120 DAS	180 DAS	60 DAS	120 DAS	180 DAS	60 DAS	120 DAS	180 DAS	60 DAS	120 DAS	180 DAS
Normal water for 24 hours	21.35	41.54	60.55	148.36	252.24	338.24	0.00	0.00	0.00	0.00	0.00	0.00
Normal water for 48 hours	21.50	40.88	60.65	151.23	253.64	339.80	0.00	0.00	0.00	0.00	0.00	0.00
Normal water for 72 hours	21.58	40.87	60.71	151.50	251.35	339.87	0.00	0.00	0.00	0.00	0.00	0.00
Conc. H_2SO_4 for 4 min	22.54	39.87	62.65	149.87	255.69	337.92	0.00	0.00	0.00	0.00	0.00	0.00
Conc. H_2SO_4 for 6 min	22.40	41.59	61.42	152.95	252.97	339.81	0.00	0.00	0.00	0.00	0.00	0.00
Conc. H_2SO_4 for 8 min	21.59	42.69	62.69	153.91	253.94	335.64	0.00	0.00	0.00	0.00	0.00	0.00
Gibberellic acid 200 ppm for 24 hours	33.89	60.23	79.34	188.20	289.36	388.73	0.00	0.00	0.00	0.00	0.00	0.00
Gibberellic acid 300 ppm for 24 hours	30.25	55.64	75.23	176.97	278.54	369.78	0.00	0.00	0.00	0.00	0.00	0.00
Gibberellic acid 400 ppm for 24 hours	28.69	50.69	70.25	170.26	270.63	360.87	0.00	0.00	0.00	0.00	0.00	0.00
Cow dung slurry for 2 days	22.64	41.32	63.88	153.99	254.81	337.61	0.00	0.00	0.00	0.00	0.00	0.00
Cow dung slurry for 4 days	22.98	42.31	64.21	152.54	253.67	338.64	0.00	0.00	0.00	0.00	0.00	0.00
Cow dung slurry for 6 days	22.96	43.54	64.69	153.21	252.47	339.87	0.00	0.00	0.00	0.00	0.00	0.00
Glomus mosseae/ control	21.40	40.56	61.64	150.24	250.12	340.36	0.00	0.00	0.00	0.00	0.00	0.00
C.D. at 5% level of significance	1.30	2.41	3.49	8.43	13.81	18.48	0.00	0.00	0.00	0.00	0.00	0.00

4. Discussion

Physiological parameters of *Melia azedarach* like chlorophyll, carotenoid content, photosynthesis, transpiration rate and stomatal conducatance were found significantly higher in Gibberellic acid 200 ppm for 24 hrs with *Glomus mosseae* followed by Gibberellic acid 300 ppm for 24 hrs with *Glomus mosseae* and Gibberellic acid 400 ppm for 24 hrs with *Glomus mosseae* except for carotenoid content which had no significant difference among treatments. Khandaker *et al.* (2015) conducted similar experiment to investigate the effects of Gibberellic acid on chlorophyll content in *Syzygium samarangense* and results revealed increase in chlorophyll content on treating with Gibberellic acid. Experiment conducted by Al-rawi *et al.* (2016) reported that Gibberellic acid significantly gave higher chlorophyll content and leaf area in peach trees. Kaya *et al.* (2006) in maize reported that application of GA_3 improve the water stress tolerance by maintaining membrane permeability, enhancing chlorophyll concentration in leaves. Romanowska and Poskuta (1984) showed that the effect of Gibberellic acid on photosynthesis of pea seedling and reported higher photosynthesis on treating with Gibberellic acid. Also, Iftikhar *et al.* (2019) investigated on wheat plant and reported the effect of Gibberellic acid on growth, photosynthesis and chlorophyll content. Similar, work was done by Wen *et al.* (2018) and reported the effect of GA_3 on photosynthesis and chlorophyll content of *Camellia oleifera* leaves.

Aslanopour *et al.* (2019) showed similar work on grape and results indicated that inoculation with mycorrhiza fungi had a positive effect on chlorophyll index in a leaf of *Glomus mosseae* fungi. *Glomus intraradices* and *Glomus fasiculatum* had positive effect on transpiration rate and stomatal conductance. Whereas, *Glomus fasiculatum* fungi had highest positive effect on the photosynthesis. There were significant effect on chlorophyll content, photosynthesis, transpiration rate and stomatal conductance due to the effect of different treatments on plant growth parameters. Wang *et al.* (2019) reported that arbuscular mycorrhizal fungi inoculation was a promising strategy in enhancing photosynthesis content and stomatal conductance of *Zelkova serrata* leaves. Also, Ruiz and Aroca (2010) showed the effect of arbuscular mycorrhiza (AM) plants on stomatal behavior and water use efficiency and reported that the rate of stomatal conductance was higher in AM than in non AM plants.

The Significantly higher values of root colonization (%), number of sporocarp (per 100 g of soil) and plant survival percentage was reported in Gibberellic acid 200 ppm for 24 hrs with Glomus mosseae (79.34%, 388.73 and 93.52% respectively) followed by Gibberellic acid 300 ppm for 24 hrs with Glomus mosseae and Gibberellic acid 400 ppm for 24 hrs + Glomus mosseae. Several, biotic and abiotic factors also effects plant survival

percentage over time. Hartman and Kester (1983) stated that there are three conditions that must be fulfilled before germination begins, viz., seed must be viable, adequate inner conditions (eg. living embryo, physiological and biochemical factors etc.) and appropriate environmental condition. Takeda et al. (2015) showed that Gibberellic acids were required for arbuscular mycorrhiza development in the legume Lotus japonicas. Khalloufi et al. (2017) investigated that there was an positive interactive effect between Gibberellic acid and arbuscular mycorrhiza fungi which alleviates growth by modifying the hormonal balance of the Solanum lycopersicum . Rodriguez et al. (2016) showed that Gibberellic acid and abscisic acid perform essential functions and antagonize each other by oppositely regulating arbuscular mycorrhiza formation in tomato roots.

Saritha et al. (2014) found highest root colonization of spota plant treated with Glomus mosseae than control. Jasper et al. (1989) observed maximum root colonization in Glomus sp. inoculated plants whereas no inoculation was found in uninoculated plants of Acacia sp. Jha et al. (2014) found more colonization in AM inoculated plants than non inoculated Jatropha curcas L. plants. Mridha and Dhar (2007) reported root colonization % and spore population in different agroforestry trees. Budi et al. (2012) reported on inoculation Melia azedarach which showed enhanced root colonization (%), increased height, diameter,shoot biomass and root biomass in comparison to the uninoculated control plant.

5. Conclusion

Glomus mosseae were beneficial in promoting physiological growth (chlorophyll content, carotenoid content, photosynthesis rate, transpiration rate, and stomatal conductance) and plant survival of *Melia azedarach* seedlings when inoculated individually in soil with the combination of seeds treated with Gibberellic acid 200 ppm for 24 hours prior to sowing of seed. The number of sporocarp per 100 g of soil and the root colonization of seedlings were both higher in the Gibberellic acid 200 ppm for 24 hours with *Glomus mosseae* condition than in the control (un-inoculated) and the seedlings that were inoculated with *Glomus mosseae* without any pre-sowing treatment. The usage of *Glomus mosseae* as a soil treatment prior to the sowing of seeds treated with 200 ppm of Gibberellic acid for 24 hours of *Melia azedarach* is therefore considered to be highly successful in the establishment of seedlings under nursery conditions.

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