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The Effect of Pre-sowing Treatments with *Glomus mosseae* and GA_3 on the Leaves Physiology of *Melia azedarach* Seedling

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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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

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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 (%).

1. 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 [1]. These fungi offer several benefits to their host, including enhanced phosphorus uptake [2], increased nitrogen absorption [3], production of plant growth hormones [4], defense against soil-borne diseases [5], and improved plant growth and productivity [6].

The colonization of plant roots by AM fungi has been recognized for its potential as a bio-protectant and biofertilizer [7], providing protection against parasitic fungi and nematodes, while also promoting plant growth and yield [8]. Around 80% of vascular plants have their roots colonized by AM fungi, making them an essential component of a healthy soil-plant system (Budi et al., 2012) [9]. They contribute to soil quality and improve plant fitness [10]. Neem, a tree species, particularly relies on mycorrhizal fungi, as they colonize its roots extensively [11].

Most plants form symbiotic partnerships with mycorrhizal fungi within their roots, providing an ideal ecological niche for fungal development and completing their sexual cycle [12]. This common symbiotic relationship between AM fungi and plant roots enhances the survival and growth of the majority of plants in natural ecosystems [13]. 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 [14].

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 [15]. 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 [16]. It serves multiple purposes and is classified as an agro-forestry/social forestry species, making it highly valuable [17].

However, the main challenge in establishing forest plantations of *Melia azedarach* lies in its poor seed germination [15]. Wulandini and Widyani [18] 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.

2. MATERIALS AND METHODS

2.1 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.

2.2 Experimental Design and Treatment Combinations

A Complete Randomized Design (CRD) was utilized in the study with twenty-six treatments, including a control group, and five replications per treatment. Each replication involved sowing 250 *Melia azedarach* seeds to assess the effects of pre-sowing treatments. The seeds were subjected to various treatments, such as soaking in tap water for different durations (24, 48 and 72 hours), concentrated sulfuric acid (H_2SO_4) for varying times (4, 6 and 8 minutes), gibberellic acid solutions of different concentrations (200, 300 and 400 ppm) for 24 hours, and cow dung slurry for different durations (2, 4 and 6 days). The treated seeds were sown in two types of soil: normal soil and soil inoculated with *Glomus mosseae*. Additionally, a control treatment involved sowing untreated seeds in non-inoculated soil. The seeds were 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. Physiological parameters of the seedlings were determined at 90 and 180 DAS (Days After Sowing). Chlorophyll and carotenoid content were determined using a chemical method. Mature and fully expanded leaves from the middle section of the plant were selected for the measurements. To extract the pigments, 50 mg of fragmented leaf tissue was placed in a vial with 50 ml of di-methyl sulphoxide (DMSO) and heated at 60°C until the tissue lost its chlorophyll (2-4 hours). The resulting liquid extract was then transferred to a graduated tube and brought up to a volume of 10 ml with DMSO. Absorbance readings were taken at 480, 645 and 665 nm using a spectrophotometer with a DMSO blank. Concentrations were calculated using [19] equations, handheld photosynthesis system, LCi-SD Bioscientific Ltd. The procedure involves preparing and calibrating the instrument, attach a leaf, set parameters, activate the system for recording, and collected data is then documented. 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 [20], and sporocarps were determined according to the method given by Gerdemann and Nicolson [21]. Plant survival percentage was calculated using the following formula.

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

2.3 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.

3. 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 seeds without treatment sown in *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 mmol m^{-2} s^{-1}) followed by Gibberellic acid 300 ppm for 24 hrs with *Glomus mosseae* (0.188 mmol m^{-2} s^{-1}). Whereas, stomatal conductance in *Glomus mosseae* was 0.170 mmol m^{-2} s^{-1} with lowest stomatal conductance in control *i.e.* 0.107 mmol 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 mmol m^{-2} s^{-1}) followed by Gibberellic acid 300 ppm for 24 hrs with *Glomus mosseae* (0.289 mmol m^{-2} s^{-1}) and Gibberellic acid 400 ppm for 24 hrs with *Glomus mosseae* (0.281 mmol m^{-2} s^{-1}) whereas, minimum was recorded in control *i.e.* 0.217 mmol 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 mmol H_2O m^{-2} s^{-1}) followed by Cow dung slurry for 6 days with *Glomus mosseae* (3.19 mmol H_2O m^{-2} s^{-1}) and Conc. H_2SO_4 for 8 min with *Glomus mosseae* (3.19 mmol H_2O m^{-2} s^{-1}) with lowest transpiration rate in control *i.e.* 1.81 mmol 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 mmol H_2O m^{-2} s^{-1}) followed by Cow dung slurry for 6 days with *Glomus mosseae* (5.18 mmol H_2O m^{-2} s^{-1}) and Conc. H_2SO_4 for 8 min with *Glomus mosseae* (5.12 mmol H_2O m^{-2} s^{-1}) whereas, minimum was recorded in control *i.e.* 3.45 mmol H_2O m^{-2} s^{-1}.

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 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 contentof *Melia azedarach*

Treatments	With *Glomus mosseae*				Without *Glomus mosseae*			
	Photosynthesis (μ mol CO_2 m^{-2} s^{-1})		Stomatal conductance (mmol m^{-2} s^{-1})		Photosynthesis (μ mol CO_2 m^{-2} s^{-1})		Stomatal conductance (mmol 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

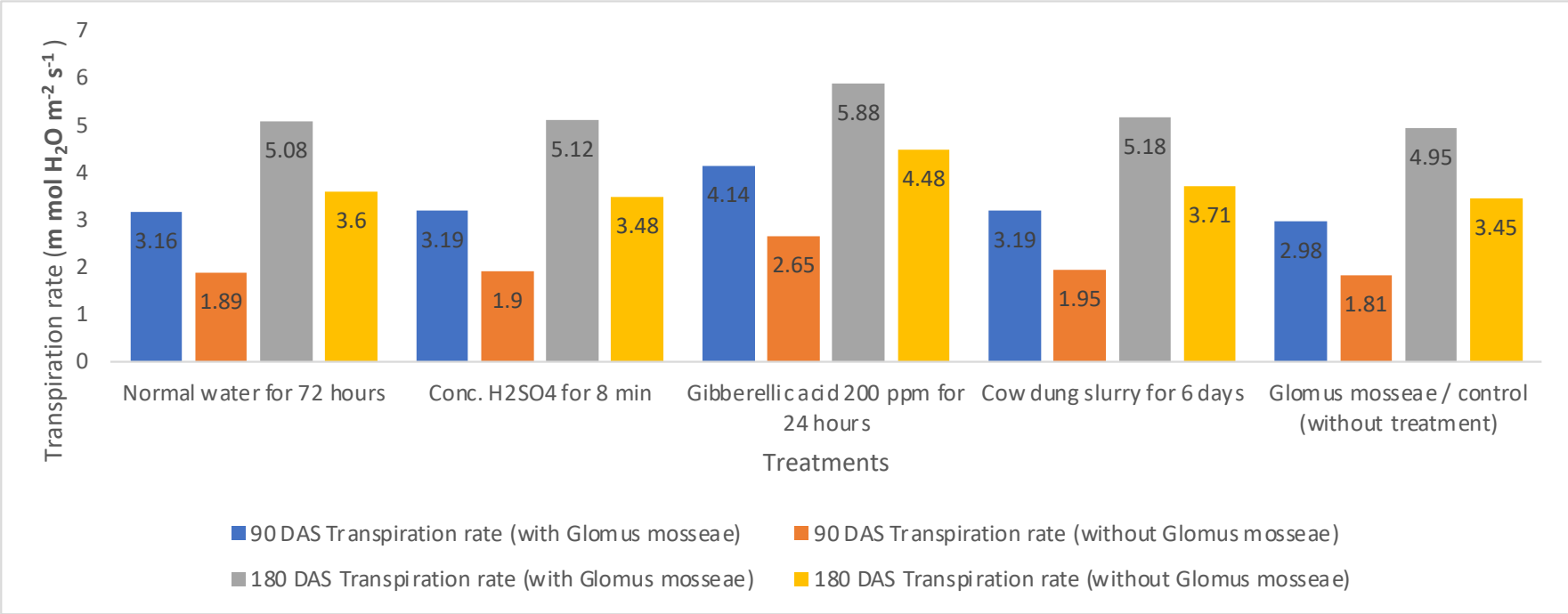

Fig. 1. Effect of pre sowing treatments of seeds in combination with and without *Glomus mosseae* on transpiration rate at 90 and 180 DAS

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 days	88.11	88.45	86.47	76.21	68.95	55.21
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

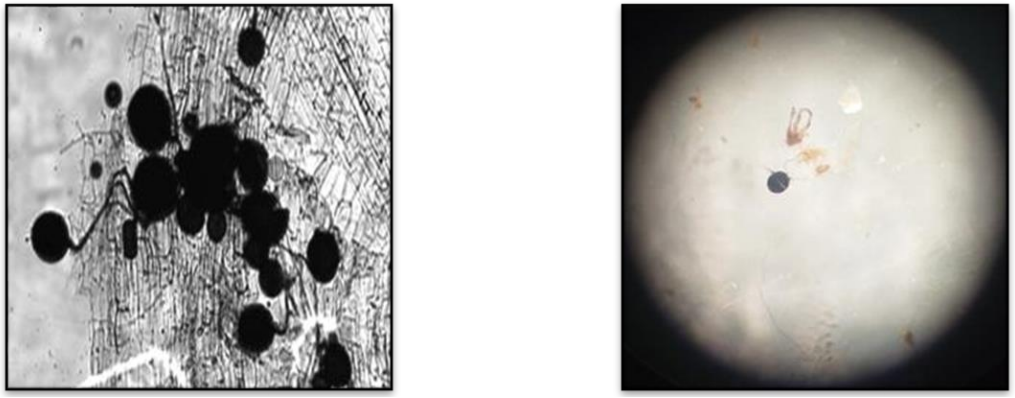

Fig. 2. (a) Glomus mosseae spores in roots of Melia azedarach by using phase *contrast microscope (b) Glomus mosseae sporocarp isolated from soil*

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.*

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.

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. [22] 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. Kaya et al. [23] in maize reported that application of GA_3 improve the water stress tolerance by maintaining membrane permeability, enhancing chlorophyll concentration in leaves. Romanowska et al. [24] showed that the effect of Gibberellic acid on photosynthesis of pea seedling and reported higher photosynthesis on treating with Gibberellic acid which is similar to our findings. Also, [25] 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. [26] and reported the effect of GA_3 on photosynthesis and chlorophyll content of *Camellia oleifera* leaves. This indicate that addition of high GA_3 inhibits chlorophyll synthesis in the plants.

Aslanpour et al. [27] 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. This rise a question on the maturity of leaf from which the chlorophyll was measured. For this case, it is very important to show the position of the leaf, from which chlorophyll was measured, to clarify whether the *Glomus fasiculatum* affected the chlorophyll content, etc. Wang et al. [28] reported that arbuscular mycorrhizal fungi inoculation was a promising strategy in enhancing photosynthesis content and stomatal

conductance of *Zelkova serrata* leaves. Also, Ruiz-Lozano and Aroca [29] 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*. For this case, it is very important to understand the effect of Gibberellic acid on root colonization (%), number of sporocarp (per 100 g of soil) and plant survival percentage. Several, biotic and abiotic factors also effects plant survival percentage over time. Hartman and Kester [30] 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. [31] showed that Gibberellic acids were required for arbuscular mycorrhiza development in the legume Lotus japonicas. Khalloufi et al. [32] 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*. Rodríguez et al. [33] 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. [34] found highest root colonization of spota plant treated with *Glomus mosseae* than control. Jasper et al. [35] observed maximum root colonization in Glomus sp. inoculated plants whereas no inoculation was found in uninoculated plants of Acacia sp. Jha et al. [36] found more colonization in AM inoculated plants than non-inoculated *Jatropha curcas L.* plants. Mridha et al. [37] reported root colonization % and spore population in different agroforestry trees. Budi et al. [9] 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. Based on the findings, it can be inferred that the *Glomus spp.* leads to a significant increase in nearly all the observed parameters, suggesting that the presence of *Glomus mosseae* greatly enhances the physiological mechanism.

5. CONCLUSION

The combined *Glomus mosseae* inoculation and pre-sowing treatment with Gibberellic acid (200 ppm for 24 hours) significantly enhanced physiological growth (chlorophyll content, carotenoid content, photosynthesis rate, transpiration rate, and stomatal conductance) and plant survival in *Melia azedarach* seedlings. Furthermore, this combination led to higher sporocarp numbers and increased root colonization compared to the control and seedlings without any treatment inoculated with *Glomus mosseae*. These results emphasize the successful establishment of *Melia azedarach* seedlings in nursery conditions by employing *Glomus mosseae* soil treatment combined with Gibberellic acid pre-sowing treatment.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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