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Original Article

Effects of Arbuscular Mycorrhizal Fungi on the Germination of *Terminalia arjuna* Plants Grown in Fly Ash Under Nursery Conditions

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ABSTRACT

Terminalia arjuna (Roxb) Wight & Arn is a tropical evergreen woody tree endemic to India that grows along river banks and canals. It has a significant medicinal value, particularly for heart disorders, where tree bark is used to treat circulatory ailments. The stiff seed coat is a key impediment to obtaining good and uniform germination, particularly in inert and low-moisture-holding capacity growing media such as coal-burned fly ash. The current study was designed to look into the effect of arbuscular mycorrhizal fungi on seed germination and seedling growth in *Terminalia arjuna* using fly ash as a growing medium. The 360 seeds were sown in two sets, for each treatment used in mycorrhizae and control without mycorrhiza inoculation. The results showed that seeds germinated with mycorrhiza inoculation had higher germination (77.77%), germination capacity (83.88%), germination energy (19.44%), germination value (24.16%), mean daily germination (9.33 seeds), peak value (2.59), cumulative germination (77.77%), and germination speed (23.40%) than seeds germinated without mycorrhiza inoculation. The use of mycorrhizal fungi in fly ash showed promising results, which could serve as a foundation for fly ash restoration and stability programs utilizing *Terminalia arjuna* as one of the powerful trees.

Keywords: Fly ash, forest, mycorrhizal fungi, restoration, seed germination

Introduction

Terminalia arjuna belongs to the Combretaceae family. The arjuna can be found growing all throughout the Indian Subcontinent, but it is most common in Uttar Pradesh, Bihar, Maharashtra, Madhya Pradesh, West Bengal, Odisha, south and central India, Sri Lanka, and Bangladesh (Soni & Singh, 2019). Seed germination is the most important stage of a plant's life cycle. Water, air, temperature, and light are all required for the seed germination process, which begins with imbibition, activation, and subsequent manifestation (Biswas et al., 2011; Chandra et al., 2021b; Kumar et al., 2023).

Fly ash is one of the most significant industrial solid wastes of the 21st century. Fly ash contains several hazardous elements, including mercury (Hg), arsenic (As), cadmium (Cd), barium (Ba), selenium (Se), nickel (Ni), chromium (Cr), vanadium (V), zinc (Zn), and lead (Pb) (Dwivedi & Jain, 2014). In addition to other poisons, these toxic metals can hinder plant seed germination, growth, and development (Ahmad et al., 2014). Fly ash is primarily used in the production of cement, bricks, concrete, wood replacement goods, wasteland reclamation, road construction, and the filling of underground mining wastes (Kaur & Goyal, 2015). One of its most promising applications is in the forestry industry, where it can be used in nurseries for tree planting. This will help to keep harmful heavy metals in wood biomass for a longer period of time (Chandra et al., 2021a).

Mycorrhiza symbiosis serves as a bridge between plant and fungal tissues, allowing materials to exchange. Mycorrhizal fungi produce hyphae with a large surface area for resource exchange. In addition, several mycorrhizal fungal genera develop intraradical vesicles, which act as fungus storage organs (Bhardwaj et al., 2023; Van der Heijden et al., 1998). In their roots, the majority of terrestrial plants form symbiotic partnerships with fungi. These symbiotic associations, known as mycorrhizae, act as conduits for the exchange of nutrients and energy between plants and soils (Cardon & Whitbeck, 2007). Plants and mycorrhizae have a symbiotic relationship that benefits both sides. Plants acquire minerals such as nitrogen, phosphorus, and potassium from mycorrhizae, and mycorrhizae receive carbon from plants (photosynthesis products) (Garcia & Zimmermann,

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2014; Hijri & Ba, 2018). The increased availability of mineral resources will hasten expansion. Fungi, in general, help plants become more resistant to abiotic pressures such as temperature, salinity, and flooding, as well as biotic challenges such as infections and insects (Yeh et al., 2019). The goal of this experiment is to evaluate how arbuscular mycorrhizal fungi affect seed germination in *T. arjuna* grown on fly ash growing media.

Material and Methods

Experiment Site

Seed germination studies were conducted at the departmental nursery of the Forestry, Wildlife, and Environmental Science department of Guru Ghasidas Vishwavidhyalaya, Bilaspur, Chhattisgarh, India (3.112550′N, 35.599170′E). Bilaspur has a tropical climate with moderate temperatures and moderate rainfall throughout the year. The city has an average annual temperature of 28–29°C and an annual rainfall of roughly 580 mm, having a very bright climate all year.

Seed Collection and Treatment

The experiment was conducted on May 20, 2022 (summer season), and started by collecting seeds from the local provenance of Bilaspur. Before sowing the seed, it was subjected to normal water treatment by soaking it for 48 hours in cold water. The experiment was conducted in fly ash media collected from the ash ponds of the National Thermal Power Company, Bilaspur, and used as growing media. Fly ash weighing 1.5 kg was filled in polythene bags, and watering was done for fly ash settlement. Then, after the seeds had been sown for a short while, each poly bag contained one seed along with 25 g of a mixture of inoculums, which included infected or colonized roots, spores (*Claroideoglomus etunicatum*, *Funneliformis mosseae*, and *Acaulospora scrobiculata*), substrate, and holes made from fly ash. Two treatments with and without mycorrhizal fungi were laid out as per a randomized block design. The polythene bags were laid out row by row in two sections of 720 polyethylene bags and watered daily.

Data Collection

The counting of the number of germinating seeds started on the first day and continued until the experiment was finished (30 days). During the trial, the germination percentage was recorded daily. The germination capacity, germination energy, peak value, cumulative germination, and germination speed of the seed that germinated on the day of sowing were all recorded (Tiwari et al., 2022). On the basis of seed germinating at the end and sowing time, the mean daily germination was calculated:

$$\text{Germination}(\%) = \frac{\text{Total number of seed germination}}{\text{Total number of seed sown}} \times 100 \quad (1)$$

$$\text{Germination capacity}(\%) = \frac{\textit{Total seed germinated + seed found viable after cutting}}{\text{The total number od seeds shown}} \times 100 \quad (2)$$

$$\text{Germination energy}(\%) = \frac{\textit{No.of seed germinated till the highest germination}}{\text{Total number of seed shown}} \times 100 \quad (3)$$

$$\text{Germination value} = \text{Mean daily germination} \times \text{Peak value} \quad (4)$$

$$\text{Mean daily germination}(\text{MDG}) = \frac{\textit{Cumulative}(\%)\textit{ of seed germination at the end of the test}}{\text{Day since sowing to end of the test}} \quad (5)$$

$$\text{Peak value}(\text{PV}) = \frac{\text{Cumulative germination}(\%)}{\text{Day since sowing}} \quad (6)$$

$$\text{Cumulative germination}(\%) = \frac{\text{Cumulative total of germination seeds}}{\text{Total number of seed shown}} \times 100 \quad (7)$$

$$\text{Germination speed} = \frac{\text{Number of newly germination see at time}}{\text{Number of days since sowing}} \quad (8)$$

Results

Seeds without mycorrhizae started germinating on the eighth day and terminated on the 30th day (total of 22 days of germination). The minimum seed germination was 10 on the 8th, 10th, 16th, 18th, 21st, and 30th days of sowing. while a maximum of 40 seeds germinated on the ninth day (Table 1). Total 200 seeds germinated against total 360 seeds, which means 55.55% cumulative seed germination without mycorrhiza inoculation (Figure 1). The germination capacity was 59.44%, with 13.88% germination energy (Table 2). The germination value scored was 12.32, whereas the mean daily germination was found to be 6.66. The peak value of seed germination was observed at a minimum of 0.34 on the eighth day and a maximum of 2.99 on the 13th day (Figure 2). The germination speed was highest at 4.44 on the ninth day of the experiment, while the minimum speed was 0.33 on the 30th day (Figure 3).

The mycorrhiza-treated seed germinated from the 8th day to the 28th day (Table 1). The maximum seed germination found was 50 on the 12 days of the experiment, while a minimum of 10 seeds germinated on the 13th, 16th, 17th, 18th, and 26th days. Mycorrhizae's impact on seed germination on *T. arjuna* recorded 280 out of 360 seeds (cumulative seed germination: 77.77%), demonstrating 22.22% better seed germination than control seeds without any inoculants (Figure 1). Also, higher germination capacity and germination energy (83.88% and 19.44%, respectively) were observed with mycorrhizae fungi (Table 2). Similarly, germination value and mean daily germination were 24.16 and 9.33 as compared to seeds without mycorrhizal fungi (12.32 Germination valuve [GV] and 6.66 Mean Daily Germination [MDG]). Seed germination was at its peak with a value of 4.16 on the 14th day and a minimum of 1.38 on the eighth day of the experiment (Figure 2). The minimum seed germination speed was observed at 0.38 on the 26th day, and the maximum data were observed at 5.00 on the 8th day of seed germination (Figure 3).

Discussion

The positive response of mycorrhizal fungi on seed germination was recorded in *T. arjuna* (Ballina et al., 2017; Dalling et al., 2011; Huante et al., 2012). Williams et al. (1995); Tiwari et al. (2022) observed that the seeds start germination, but numerous infective microorganism attacks on the seed result in poor seed germination, whereas mycorrhizal fungi act as protection agents, building a shield around the seed, resulting in higher seed germination (Tiwari et al., 2022; Williams et al., 1995). The results of this study suggest that the use of mycorrhizae might be effective in accelerating seed germination in *T. arjuna* under nursery conditions. Additionally, by accelerating germination and plant

Table 1.
Seed Germination Attributes Without and With Mycorrhiza Treatments in Terminalia arjuna *Using Fly Ash Growthing Medium*

	Seed Germination (Number)		Cumulative Total Germination (Number)		Cumulative Germination (%)		Peak Value		Germination Speed	
Day	M−	M+	M−	M+	M−	M+	M−	M+	M−	M+
8	10	40	10	40	2.77	11.11	0.34	1.38	1.25	5.00
9	40	30	50	70	13.88	19.44	1.54	2.16	4.44	3.33
10	10	30	60	100	16.66	27.77	1.66	2.77	1.00	3.00
11	30	20	90	120	25.00	33.33	2.27	3.03	2.72	1.81
12	20	50	110	170	30.55	47.22	2.54	3.93	1.66	4.16
13	30	10	140	180	38.88	50.00	2.99	3.84	2.30	0.76
14	00	30	140	210	38.88	58.33	2.77	4.16	00	2.14
15	00	00	140	210	38.88	58.33	2.59	3.88	0.00	0.00
16	10	10	150	220	41.66	61.11	2.60	3.81	0.62	0.62
17	00	10	150	230	41.66	63.88	2.45	3.75	0.00	0.58
18	10	10	160	240	44.44	66.66	2.46	3.70	0.55	0.55
19	20	00	180	240	50.00	66.66	2.63	3.50	1.05	00
20	00	00	180	240	50.00	66.66	2.50	3.33	00	00
21	10	00	190	240	52.77	66.66	2.51	3.17	0.47	00
22	00	00	190	240	52.77	66.66	2.39	3.03	00	00
23	00	00	190	240	52.77	66.66	2.29	2.89	00	00
24	00	00	190	240	52.77	66.66	2.19	2.77	00	00
25	00	00	190	240	52.77	66.66	2.11	2.66	00	00
26	00	10	190	250	52.77	69.44	2.02	2.67	00	0.38
27	00	00	190	250	52.77	69.44	1.95	2.57	00	00
28	00	30	190	280	52.77	77.77	1.88	2.77	00	1.07
29	00	00	190	280	52.77	77.77	1.81	2.68	00	00
30	10	00	200	280	55.55	77.77	1.85	2.59	0.33	00

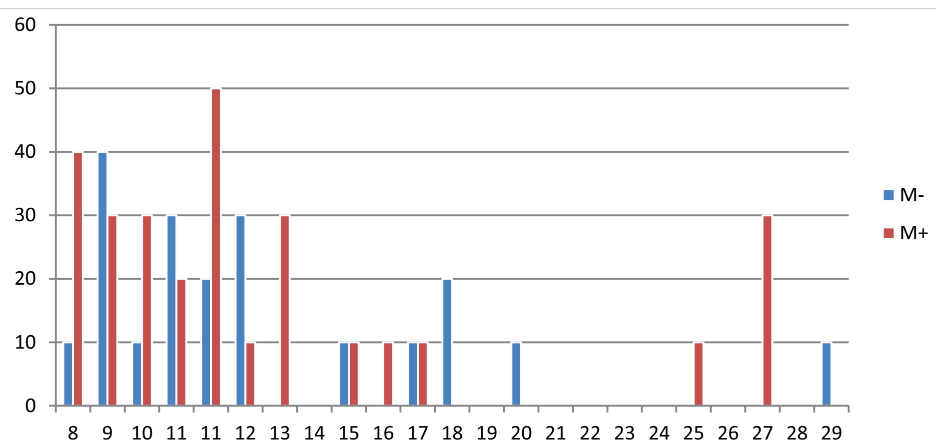

Figure 1.
Number of Seeds Germinated (Numbers/Day) Without and With Mycorrhizal Inoculations in Terminalia arjuna.

development, mycorrhizae could have a significant impact on the rehabilitation efforts of fly ash. Numerous studies, such as Smith and Read (2008), Yuan et al. (2016), Huang et al. (2018), Alghamdi (2019), Shao et al. (2020), and Figura et al. (2021), also reported accelerated seed germination due to the positive influence of mycorrhiza. However, the suppressive impact of mycorrhiza has also been reported by several researchers (Ballina et al., 2017; Louarn et al., 2012; Maighal et al., 2016; Varga, 2015; Wu et al., 2014).

Table 2.
Comparative Seed Germination Data in Without Mycorrhizae and With Mycorrhizae

Parameter	Without Mycorrhizae	With Mycorrhizae
Seed germination (%)	55.55	77.77
Germination capacity (%)	59.44	83.88
Germination energy (%)	13.88	19.44
Germination value	12.32	24.16
Mean daily germination	6.66	9.33
Peak value	1.85	2.59
Cumulative germination (%)	55.55	77.77
Germination speed (numbers/day)	16.39	23.40

No doubt, the mycorrhizae in this study showed a positive influence on the seed germination of *T. arjuna* when fly ash was used as growing media. This may be attributed to the hyphal penetration of the seed, which softens the seed coat (Pankaj et al., 2021). There are studies showing a higher response of mycorrhizal fungi for improving the growth and survival of seedlings (Dovana et al., 2015; Hagh-Doust et al., 2022; Khalediyan et al., 2021), root growth (Falcón et al., 2021), and plant growth (Khalediyan et al., 2021; Weisany et al., 2015, 2016) due to higher nutrient and water supply and physiological adjustment (Bhardwaj et al., 2023; Huang et al., 2018; Khalediyan et al., 2021; Naranjo et al., 2011).

Conclusion

At the moment, utilizing fly ash and stabilizing fly ash dumping sites are huge challenges. Fine particles of fly ash settled on the soil surface in the fly ash-producing area, interfering with seed germination and plant and crop growth. This investigation aimed to mitigate the challenges of seed germination in fly ash by employing mycorrhiza-based solutions. The findings of this study imply that mycorrhizae are beneficial for *T. arjuna* seed germination. These results demonstrate the effects of fungal assemblages on seed germination in fly ash. Thus, mycorrhiza inoculation could be a green solution for improving seed germination and may be used for the restoration of fly ash areas. However, more work is needed to better understand the role of fungi in determining the seed germination of forest species.

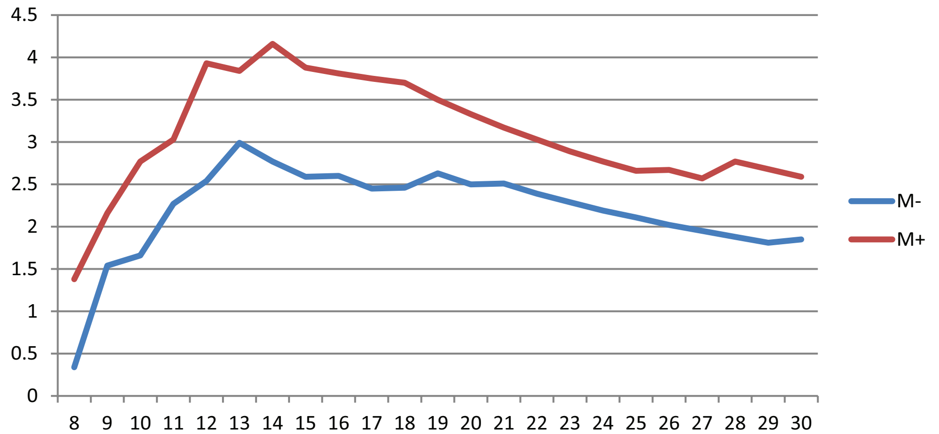

Figure 2.
Seed Germination Peak Value Without and With Mycorrhizal Inoculations in Terminalia arjuna.

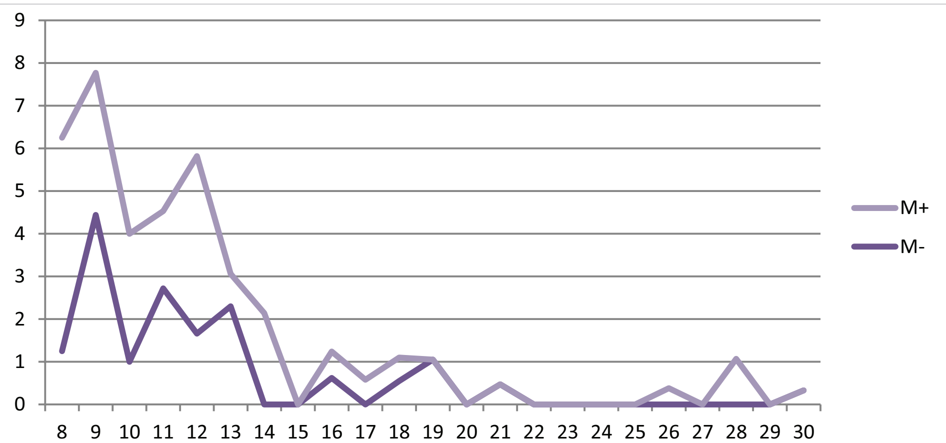

Figure 3.
Seed Germination Speed (nos/day) Without and With Mycorrhizal Inoculations in Terminalia arjuna.

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