

Effect of growing media and arbuscular mycorrhiza fungi on seedling growth of *Leucaena leucocephala* (Lam.) de Wit.

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ABSTRACT : Present study was carried out to assess the effect of growing media and arbuscular mycorrhiza fungi (AMF) inoculation on growth of *Leucaena leucocephala* under nursery conditions. The study consisted of six different growing media viz., T1- soil (1), T2- soil + sand (2:1), T3- soil + vermicompost (2:1), T4- soil + perlite (2:1), T5- soil + sand + vermicompost (1:1:1) and T6- soil + perlite + vermicompost (1:1:1), and four mycorrhizal treatments viz., *Acaulospora scrobiculata* (individual); *Glomus intraradices* (individual); *A. scrobiculata* + *G. intraradices* (combined) and control (un-inoculated). Among growing media, all studied parameters (shoot and root length, fresh shoot and root weight, dry shoot and root weight) were significantly higher in soil + vermicompost media (T3). On the other hand, AMF inoculations significantly enhanced all the observed parameters, barring few exceptions. The combined inoculation of *A. scrobiculata* + *G. intraradices* gave higher values of growth parameters than individual inoculations. Plant showed maximum dependency (%) for dry matter production on combined inoculation of *A. scrobiculata* and *G. intraradices*. Thus, it can be concluded that potting media consisting of soil and vermicompost intermixed with *A. scrobiculata* + *G. intraradices* together should be used for raising *L. leucocephala* seedlings under nursery conditions.

Key words: *Acaulospora scrobiculata*, *Glomus intraradices*, mycorrhizal dependency, seedling quality index and vermicompost.

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1. INTRODUCTION

The production of quality seedlings of woody perennials is pre-requisite for any plantation programme (Kumar *et al.*, 2017). To survive in the harsh environment of a field, a plant needs a well-developed root system. The development of a healthy root system depends not only on the genetic properties of the plant but also on physical and chemical properties of the substrate used (Wilson *et al.*, 2001). Thus, selection of proper growing medium is very important for getting good germination and quality seedlings in nursery. A good growth medium provides sufficient anchorage to the plant, serves as a reservoir for nutrients and water, allows oxygen diffusion to the roots and permits gaseous exchange between roots and the atmosphere outside the root substrate (Unal, 2013). A favourable growing medium may consist of two or more ingredients e.g. soil + sand, soil + FYM/vermicompost, soil + perlite, soil + sand + vermicompost, soil + perlite + vermicompost etc. Addition of organic matter in potting media is important because it supplies essential nutrients required by the seedlings (Khan *et al.*, 2006).

According to Agbo and Omaliko (2006), the performance of seedlings in real field conditions is determined by their performance under nursery

conditions. When the nursery raised seedlings are transplanted, these may face transplanting shocks in the fields and consequently plants become weak and poorly established (Hartmann and Kester, 1986). Navarro-Garcia *et al.* (2011) postulated that preconditioning of young seedlings with efficient arbuscular mycorrhiza fungi (AMF) not only makes plant stronger but also helps in their establishment in the fields. The positive effects of AMF inoculation on seedling's growth under nursery conditions are well documented (Kumar *et al.*, 2005; Smith *et al.*, 2009; Jha *et al.*, 2017). According to Huang *et al.* (1985), mycorrhizal plants generally have greater shoot and root dry weight, leaf area and root length than non-mycorrhizal plants. Thus, early inoculation of seedlings with AMF under nursery conditions can be beneficial in two ways i.e. superior and stronger growth of the seedlings and better performance in the fields. Caravaca *et al.* (2003) suggested that native AMF species can produce more vigorous seedlings than non-native species.

Leucaena leucocephala (Lam.) de Wit, a member of family Fabaceae, is a fast growing tropical legume tree. In India, it is mostly cultivated for its fodder and other important uses like hedge plant, green manure, timber and as a bio energy crop (Devi *et al.*, 2013). It

has high nitrogen-fixing potential and also grown as a shade tree for cocoa, coffee and tea plantations (Hogberg and Kvarnstrom, 1982). It has succeeded as a preferred species for agroforestry and farm forestry interventions (Brewbaker, 1987). It generally acts as a shelterbelt, provides shade and protects variety of crops from wind during early stage of the growth. Keeping in view the multifarious uses of *L. leucocephala* and influence of the growing media and AMF inoculation on quality seedling production, the present study was carried out at ICAR-Central Agroforestry Research Institute (CAFRI), Jhansi.

2. MATERIALS AND METHODS

Study consisted of two factors viz., six growing media [T1- soil (1), T2- soil + sand (2:1), T3- soil + vermicompost (2:1), T4- soil + perlite (2:1), T5- soil + sand + vermicompost (1:1:1) and T6- soil + perlite + vermicompost (1:1:1)] and four mycorrhizal treatments [*Acaulospora scrobiculata* (individual), *Glomus intraradices* (individual), *A. scrobiculata* + *G. intraradices* (combined) and control (un-inoculated)]. Thus, study consisted of 24 treatment combinations (6×4) and each treatment was replicated ten times. Both the AM species were included in the study due to their better adaptability in this region. Prior to use these, the mycorrhization of *A. scrobiculata* (spores: 56/100 g soil and root colonization index: 23.525%) and *G. intraradices* (spores: 38/100 g soil and root colonization index: 39.475%) were assessed. Before sowing, seeds of *L. leucocephala* were treated with hot water. The study was carried out in root trainers (volume: 300 cc; individual type, fitted in iron stands). The treatments were imposed in the substrate by mixing mycorrhizal inocula with substrate @ 10 kg inoculum in 1000 kg substrate. For individual inoculations (either *A. scrobiculata* or *G. intraradices*), 10 kg inoculum were mixed in 1000 kg substrate but for combined inoculation (*A. scrobiculata* + *G. intraradices*), 5 kg of each inoculum were mixed in 1000 kg substrate. This mycorrhiza-substrate mixture was filled in respective root trainers. The root trainers were transferred to net-house and watered as per need. After 75 days of sowing, plants were harvested and analyzed for collar diameter (mm), shoot length (cm), root length (cm), fresh shoot weight (g), fresh root weight (g), dry shoot weight (g), dry root weight (g) and total dry weight (g). Root: shoot ratio (%) was determined by dividing dry weight of root with dry weight of shoot. Mycorrhizal dependency (MD) and Dickson quality index were calculated by using the formulae given by Plenchette *et al.* (1983) and Dickson *et al.* (1960), respectively.

$$\text{Mycorrhizal dependency (\%)} = \frac{M - NM}{M} \times 100$$

Where M is the dry weight of individual mycorrhizal plants and NM is the mean dry weight of corresponding non-mycorrhizal plants.

$$\text{Seedling quality index} = \frac{\text{Total dry weight of plant (g)}}{\frac{\text{Plant height (cm)}}{\text{Collar diameter (mm)}} + \frac{\text{Shoot dry weight (g)}}{\text{Root dry weight (g)}}}$$

Statistical analysis

All the data on seedling growth parameters were subjected to two-way analysis of variance. For each factor analyzed, the means of the different treatments were compared and ranked using Fischer F test ($P < 0.05$). The mean of the experiment was analyzed statistically using a general linear model for analysis of variance in CRD. Least Significant Difference (LSD) was used to compare treatment differences. The statistical analysis was performed using the statistical package SYSTAT version 12 and graph was prepared using MS Excel.

3. RESULTS AND DISCUSSION

Among different media, significantly higher collar diameter and shoot length was recorded in plants grown in T3 (Table 1). Root length was also recorded maximum in T3 but it was comparable with T1. On the other hand, among mycorrhizal treatments, collar diameter and shoot length were recorded maximum in combined inoculation of *A. scrobiculata* and *G. intraradices*, while root length was in *A. scrobiculata*-inoculated plants. Two-way interactions were also found statistically significant for studied parameters. Values of all growth parameters were recorded maximum in plants inoculated with *A. scrobiculata* + *G. intraradices* grown in T3, except root length.

Significantly higher fresh and dry weight of shoot and root were recorded in plants grown in T3, irrespective of AMF inoculations (Table 2). On the other hand, all mycorrhizal treatments increased the biomass of *L. leucocephala*. Maximum fresh shoot weight, dry shoot weight, dry root weight and total dry weight were recorded in plants inoculated with *A. scrobiculata* + *G. intraradices*. Dry root weight was significantly higher in *A. scrobiculata*-inoculated plants. In each tested growing media, AMF inoculations significantly enhanced all biomass related parameters, barring few exceptions. Values of biomass related parameters were recorded maximum in *A. scrobiculata* + *G.*

Table 1. Effect of AM inoculation on growth of *L. leucocephala* grown in different growing media.

| Growing media | Treatment | | | | Mean |
|-----------------------------|-----------------|--------------|-------------|---------|------|
| | Control | As* | Gi | As + Gi | |
| Collar diameter (mm) | | | | | |
| T1** | 3.01 | 3.14 | 2.94 | 3.39 | 3.12 |
| T2 | 2.89 | 2.87 | 3.09 | 3.02 | 2.97 |
| T3 | 3.16 | 3.48 | 2.93 | 3.60 | 3.29 |
| T4 | 3.41 | 3.25 | 3.17 | 3.15 | 3.25 |
| T5 | 2.99 | 2.93 | 2.93 | 3.54 | 3.10 |
| T6 | 2.52 | 2.91 | 2.59 | 2.43 | 2.61 |
| Mean | 3.00 | 3.10 | 2.94 | 3.19 | |
| Shoot length (cm) | | | | | |
| T1 | 18.3 | 20.5 | 17.5 | 16.6 | 18.2 |
| T2 | 19.0 | 24.2 | 22.1 | 29.4 | 23.7 |
| T3 | 23.0 | 27.7 | 26.4 | 31.2 | 27.1 |
| T4 | 25.8 | 24.8 | 24.0 | 25.3 | 25.0 |
| T5 | 23.4 | 22.8 | 23.6 | 28.1 | 24.5 |
| T6 | 18.4 | 21.3 | 18.1 | 15.6 | 18.4 |
| Mean | 21.3 | 23.6 | 22.0 | 24.4 | |
| Root length (cm) | | | | | |
| T1 | 19.5 | 19.0 | 20.6 | 19.4 | 19.6 |
| T2 | 17.1 | 19.9 | 19.8 | 19.6 | 19.1 |
| T3 | 18.7 | 21.3 | 20.5 | 18.3 | 19.7 |
| T4 | 19.0 | 19.9 | 19.3 | 19.6 | 19.4 |
| T5 | 19.2 | 18.5 | 18.8 | 18.3 | 18.7 |
| T6 | 18.3 | 18.3 | 16.8 | 16.2 | 17.0 |
| Mean | 18.6 | 19.5 | 19.3 | 18.6 | |
| LSD _{0.05} | | | | | |
| | Collar diameter | Shoot length | Root length | | |
| Growing media | 0.02 | 0.2 | 0.1 | | |
| AM inoculation | 0.01 | 0.1 | 0.1 | | |
| Interaction | 0.03 | 0.3 | 0.2 | | |

*As- *Acaulospora scrobiculata*, Gi- *Glomus intraradices*

**T1- soil (1), T2- soil + sand (2:1), T3- soil + vermicompost (2:1), T4- soil + perlite (2:1), T5- soil + sand + vermicompost (1:1:1) and T6- soil + perlite + vermicompost (1:1:1)

intraradices inoculated plants grown in T3, except fresh root weight which was recorded higher in *A. scrobiculata*-inoculated plants grown in T5 and it was found comparable with the value recorded from *A. scrobiculata* + *G. intraradices* inoculated plants grown in T3.

Thus, the results of the present study showed that among growing media, all growth parameters (shoot length, root length, fresh shoot weight, fresh root weight, dry shoot weight and dry root weight) were

significantly higher in growing media containing soil and vermicompost (T3) when compared with other media. This could be due to the presence of vermicompost. Vermicompost contains high organic matter which increases water and nutrient holding capacity of the medium for supply to the plants. According to Bachman and Metzger (2008), vermicompost is reported to have bioactive compounds considered to be beneficial for root growth, root initiation, germination and growth of the

Table 2. Effect of AM inoculation on biomass of *L. leucocephala* grown in different growing media.

| Growing media | Treatment | | | | Mean |
|------------------------------------------|--------------------|-------------------|------------------|-----------------|------------------|
| | Control | As* | Gi | As + Gi | |
| Fresh shoot weight (g) | | | | | |
| T1** | 1.623 | 2.511 | 2.164 | 1.889 | 2.047 |
| T2 | 1.904 | 2.397 | 2.766 | 3.185 | 2.563 |
| T3 | 2.451 | 3.648 | 2.944 | 4.451 | 3.373 |
| T4 | 3.089 | 2.986 | 2.577 | 2.739 | 2.848 |
| T5 | 2.461 | 2.576 | 2.509 | 3.352 | 2.725 |
| T6 | 1.720 | 2.095 | 1.546 | 1.538 | 1.725 |
| Mean | 2.208 | 2.702 | 2.418 | 2.859 | |
| Fresh root weight (g) | | | | | |
| T1 | 0.522 | 1.254 | 1.104 | 0.808 | 0.922 |
| T2 | 0.554 | 1.224 | 1.253 | 1.057 | 1.022 |
| T3 | 0.704 | 1.391 | 1.077 | 1.528 | 1.175 |
| T4 | 1.026 | 1.287 | 1.008 | 0.924 | 1.061 |
| T5 | 0.766 | 1.542 | 0.973 | 1.054 | 1.084 |
| T6 | 0.395 | 0.984 | 0.667 | 0.508 | 0.638 |
| Mean | 0.661 | 1.280 | 1.014 | 0.980 | |
| Dry shoot weight (g) | | | | | |
| T1 | 0.580 | 0.727 | 0.608 | 0.619 | 0.633 |
| T2 | 0.579 | 0.694 | 0.655 | 0.829 | 0.692 |
| T3 | 0.800 | 1.038 | 0.829 | 1.233 | 0.975 |
| T4 | 0.997 | 0.840 | 0.749 | 0.836 | 0.855 |
| T5 | 0.736 | 0.618 | 0.683 | 0.970 | 0.752 |
| T6 | 0.601 | 0.553 | 0.410 | 0.443 | 0.502 |
| Mean | 0.716 | 0.745 | 0.657 | 0.822 | |
| Dry root weight (g) | | | | | |
| T1 | 0.269 | 0.361 | 0.302 | 0.375 | 0.327 |
| T2 | 0.252 | 0.324 | 0.374 | 0.377 | 0.332 |
| T3 | 0.290 | 0.365 | 0.303 | 0.445 | 0.351 |
| T4 | 0.365 | 0.345 | 0.327 | 0.328 | 0.341 |
| T5 | 0.265 | 0.282 | 0.243 | 0.316 | 0.277 |
| T6 | 0.160 | 0.218 | 0.168 | 0.157 | 0.176 |
| Mean | 0.267 | 0.316 | 0.286 | 0.333 | |
| Total dry weight (g⁻¹) | | | | | |
| T1 | 0.847 | 1.089 | 0.909 | 0.991 | 0.959 |
| T2 | 0.832 | 1.021 | 1.038 | 1.208 | 1.025 |
| T3 | 1.092 | 1.404 | 1.128 | 1.677 | 1.325 |
| T4 | 1.360 | 1.185 | 1.074 | 1.165 | 1.196 |
| T5 | 1.000 | 0.901 | 0.924 | 1.285 | 1.027 |
| T6 | 0.760 | 0.770 | 0.578 | 0.601 | 0.677 |
| Mean | 0.982 | 1.062 | 0.942 | 1.155 | |
| LSD _{0.05} | | | | | |
| | Fresh shoot weight | Fresh root weight | Dry shoot weight | Dry root weight | Total dry weight |
| Growing media | 0.024 | 0.012 | 0.008 | 0.003 | 0.011 |
| AM inoculation | 0.020 | 0.010 | 0.007 | 0.003 | 0.009 |
| Interaction | 0.049 | 0.025 | 0.017 | 0.007 | 0.021 |

*As- *Acaulospora scrobiculata*, Gi- *Glomus intraradices*

**T1- soil (1), T2- soil + sand (2:1), T3- soil + vermicompost (2:1), T4- soil + perlite (2:1), T5- soil + sand + vermicompost (1:1:1) and T6- soil + perlite + vermicompost (1:1:1)

plant. It also has balanced composition of nutrients (Zaller, 2007). Vermicompost mixed with the soil (T3) might have affected the physical, chemical and biological properties of the media, as the organic matter acts as glue for soil aggregation and is a source of soil nutrients. Organic matter may also improve nutrient availability and phosphorus absorption.

The root: shoot ratio was also computed for *L. leucocephala*. High root to shoot ratio indicates high absorption and storage capacity of water, which may be of advantage especially in the condition of limited moisture in the soil (Jaenicke, 1999). In present study, maximum values of root: shoot ratio and Dickson quality index were recorded in T1 when compared with other media which could be due to the better nutrition available in the soil (Table 3). Two-way interaction between different growing media and AM inoculation was also found statistically significant, showing that mycorrhizal treatments (*A. scrobiculata*,

G. intraradices and *A. scrobiculata* + *G. intraradices*) significantly enhanced root: shoot ratio and Dickson quality index of plants grown in T1 (soil) when compared with un-inoculated plant (control). According to Tsakalimi *et al.* (2009), seedling quality index can be a good indicator for the out planting performance of nursery raised seedlings. The probability of nursery raised plants performance under field conditions increases with the increase in the index value (Bayala *et al.*, 2009).

Results also showed that AMF significantly enhanced all the observed parameters, barring few exceptions. This could be due to more volume of soil explored for available nutrients and water by mycorrhizal plants than non-mycorrhizal plants. According to Shukla *et al.* (2012), improvement in plant growth upon AM inoculation is mainly due to the enhancement of mineral nutrient status of the plants. The plants with thriving mycorrhizal root systems are able to perform

Table 3. Effect of AM inoculation on root: shoot ratio and seedling quality index of *L. leucocephala* grown in different growing media.

| Growing media | Treatment | | | | Mean |
|------------------------------|-------------------|-------|------------------------|---------|-------|
| | Control | As* | Gi | As + Gi | |
| Root: shoot ratio (%) | | | | | |
| T1** | 0.480 | 0.503 | 0.495 | 0.610 | 0.522 |
| T2 | 0.443 | 0.471 | 0.667 | 0.462 | 0.511 |
| T3 | 0.368 | 0.349 | 0.375 | 0.367 | 0.365 |
| T4 | 0.365 | 0.412 | 0.437 | 0.396 | 0.402 |
| T5 | 0.364 | 0.452 | 0.359 | 0.323 | 0.375 |
| T6 | 0.291 | 0.397 | 0.424 | 0.368 | 0.370 |
| Mean | 0.385 | 0.431 | 0.459 | 0.421 | |
| Dickson quality index | | | | | |
| T1 | 0.103 | 0.115 | 0.158 | 0.150 | 0.124 |
| T2 | 0.093 | 0.096 | 0.116 | 0.103 | 0.102 |
| T3 | 0.109 | 0.130 | 0.096 | 0.147 | 0.120 |
| T4 | 0.132 | 0.120 | 0.109 | 0.111 | 0.118 |
| T5 | 0.097 | 0.094 | 0.085 | 0.117 | 0.098 |
| T6 | 0.068 | 0.078 | 0.061 | 0.064 | 0.068 |
| Mean | 0.100 | 0.108 | 0.097 | 0.115 | |
| LSD _{0.05} | | | | | |
| | Root: shoot ratio | | Seedling quality index | | |
| Growing media | 0.006 | | 0.001 | | |
| AM inoculation | 0.005 | | 0.001 | | |
| Interaction | 0.011 | | 0.003 | | |

*As- *Acaulospora scrobiculata*, Gi- *Glomus intraradices*

**T1- soil (1), T2- soil + sand (2:1), T3- soil + vermicompost (2:1), T4- soil + perlite (2:1), T5- soil + sand + vermicompost (1:1:1) and T6- soil + perlite + vermicompost (1:1:1)

better in comparison to their un-inoculated counterparts (Smith and Smith, 2012). Increase in growth parameters in AM inoculated plants has been reported by several workers in *L. leucocephala* (Lins *et al.*, 2006; Habte and Antal, 2010). Further, results showed that combined inoculation of *A. scrobiculata* and *G. intraradices* gave higher values of growth parameters than individual inoculations. In present study, dependency of *L. leucocephala* on AM inoculation for dry matter production was also found maximum on combined inoculation of *A. scrobiculata* and *G. intraradices*, irrespective of potting substrate (Figure 1) which showed that both the AMF species used under the study worked synergistically with each other. Synergism in between these two species has been reported earlier in various plant species under Bundelkhand region (Shukla *et al.*, 2018). Wehner *et al.* (2010) suggested that increase in richness of AM species is responsible for better growth of the plants as compared to individual AM inoculant. Different AM species (even different strains of AMF) have different strategy for colonization of plant roots, which may be related to taxonomic differences at the family level. In this study, two different AM species, having different mode of actions were included. *A. scrobiculata* forms vesicles inside root, spores outside the roots, does not produce sporocarps, etc. while *G. intraradices* produces vesicles, forms spores inside the roots and sporocarps in the rhizosphere (Jha *et al.*, 2014 and 2017). This could explain the reason why combined inoculation performed better than individual inoculation in present study. On the other hand, plants grown in media containing more than two substrates (T4, T5 and T6) exhibited MD values in negative ranges for most of the inoculation treatments.

Thus, based on the results obtained from the present study, it can be concluded that potting substrate consisting of soil and vermicompost intermixed with *A. scrobiculata* + *G. intraradices* together should be used

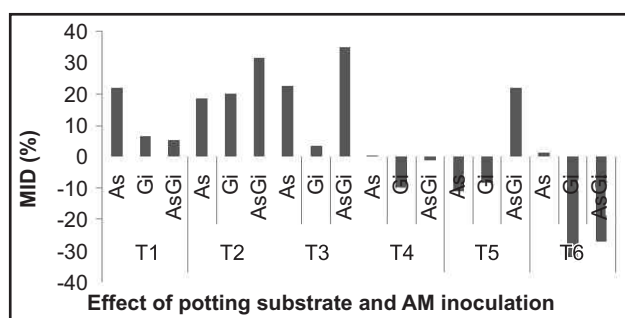


Fig. 1. Effect of growing media and AM inoculation on mycorrhizal dependency (MD; %) of *L. leucocephala*.

for raising the quality seedlings of *L. leucocephala* under nursery conditions and such seedlings are likely to perform well in the real field conditions.

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