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Response of *L. leucocephala* L. (Ipillpil) to Different Soil Media and Phosphorus Fertilization

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Authors' contributions

All the work of the paper was carried out between the authors. All the authors made corrections, read and approved for final publication mutually.

Research Article

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ABSTRACT

The research was conducted in shade house to investigate the effect of different soil media and phosphorus (P) fertilization in tubes on early growth of the *Leucaenaleucocephala*. Three growth media sandy medium (only sand), clayey medium (sand and clayey soil ratio; 1:1) and farm yard manure (FYM) medium (sand, clayey soil and FYM ratio; 1:1:1) were prepared. Each medium was fertilized with three rates of phosphorus, i.e., 0, 20, 40 mg P kg⁻¹ medium. Nitrogen and potassium were also applied as basal dose @ 50 N and 100 mg K₂O kg⁻¹ media, respectively. The results indicated that shoot length (20 cm), root length (16 cm), shoot dry weight (947 mg plant⁻¹) and root dry weight (134 mg plant⁻¹) were the highest in FYM medium with 20 mg P kg⁻¹. The P concentration in shoot and root, total P uptake and P recovery were also significantly improved when plants were grown in FYM medium supplied with 20 mg P kg⁻¹. It is concluded from this study that *L. leucocephala*seedling growth was improved with P application, particularly in FYM medium compared to clayey and sandy media. Further, the P level i.e. 20 mg P kg⁻¹ was found best among all P rates used. This shows the significance of P and FYM medium in improving growth for the best survival of *L*.

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leucocephala seedlings and its raising for plantation on large scale.

Keywords: Phosphorus; sand; clay; farm yard manure; seedlings; root length; growth.

1. INTRODUCTION

Pakistan is a semi-arid and forest deficit country with less than 4.8 % of its area under forest cover [1]. As a general rule 20 to 25% of a country should carry forests to cater for its socio economic and environmental needs in an effective manner. To achieve this objective, fast growing multipurpose trees should be planted extensively on farmlands and along roadsides, canal sides and railway sides.

L. leucocephala (Local name Ipillpil) has been one of the most productive fast growing, semi ever green and versatile multipurpose tropical legume tree [2] and have high biomass productivity [3,4] and beneficial effects in the recovery of degraded soils [5,6]. Feria et al. [7] also investigated that the crop produces very high production of biomass and re-sprout capacity (more than 50 tons/ha/year, especially in annual crops,).

It has diversified uses; high quality forage for ruminants, fuel wood, charcoal, pulp and timber [8]. It helps to enrich soil and aids neighboring plants because its foliage is rich in nitrogen content and natural leaf drop returns this to the soil beneath the shrubs [5]. It has been known for production of bio ethanol, conversion from the biomass crops to ethanol [9] and described for the expanding range of xylooligosaccharides applications includes products for the food industry as novel sweeteners and prebiotics [10] and for the pharmaceutical industry [11]. According to recent study of Huijgen et al. [12] the biomass of the crop can be converted into spectrum of marketable products (food, feed, materials and chemicals) and energy (fuels, power and heat). Pulping and papermaking of variety *Leucaenadiversifolia* by soda-anthraquinone–ethanol was studied, in order to investigate the effects of cooking variables on properties of pulp and paper [13,14]. [15] reported that *L. leucocephala* pulp, wood chips, water, soda and anthraquinone were mixed in the desired proportions for making high quality paper sheets.

Physical properties of soil especially texture has great influence on plant growth and productivity. Sandy soils are generally less fertile and have little water holding capacity and hence cannot stand drought. Clay soils are generally more fertile and have more water holding capacity which results water logging during rains. A loam soil is a mixture of all three components of texture i.e. sand, silt and clay in suitable proportion (50-70% sand, 20% clay, and 30-40% silt) [16] and is preferred texture for nursery management. Because of its workability and ability to either hold water during a drought or drain water in rains [17,18].

Both sandy soils and clay soils can be enormously improved by the generous addition of organic matter. Soil organic matter comes from the remains of plants and animals. Original source of soil organic matter is raw plant residues such as tops roots of trees, grasses and other plant parts and the secondary source of organic matter is organic manures such as, compost, green manure and farmyard manures etc. Farm yard manure (FYM) refers to all the materials that can be collected from living places of farm animals, including dung, urine and straw etc. FYM is rich in compounds of N (1.72-4.04 %) and K (0.83-3.47 %) and in microorganisms. It improves the soil structure (aggregation), which holds more nutrients and water and becomes more fertile. FYM also encourages soil microbial activity which promotes

the soil's trace mineral supply and improving plant nutrition. Use of FYM alone cannot satisfy the plant requirement due to low nutrient status which slow decomposition rate of the manure. Enrichment of FYM with chemical fertilizer in proper ratio is required to attain the best possible advantages of the inputs [19].

Phosphorus is a vital component ATP the "energy unit" of plants which play an important role in capturing and converting the sun's energy into useful plant compounds, forms during photosynthesis and processes from the beginning of seedling growth through to the formation of grain and maturity [16]. Thus phosphorus is essential for plant health and vigor [20,21]. Rashid et al. [22] showed severe to moderate P deficiency in most fields of Attock and Chakwal districts of Pothohar Plateau, Punjab Province, Pakistan in soil nutrient indexing study. As *L. leucocephala* is native to alkaline soils of Central America [23], so it can be well adopted in alkaline soils of Pakistan as well, especially in Pothohar plateau including Islamabad, Rawalpindi, Chakwal, Jhelum and Attock districts. Hill [24] stated that fertilizer application plays an important role for the establishment and early growth of *L. leucocephala*.

2. MATERIALS AND METHODS

The research was conducted at the shade house of Rangeland Research Program, National Agricultural Research Center, Islamabad to investigate the effect of P fertilization in different soil media on seedling growth of *L. leucocephala* so that it can be planted intensively and extensively in the soils of Pakistan. Seeds of *L. leucocephala* were purchased from local market. Bulk samples of sand, clayey soil and farmyard manure (FYM) were collected from different areas of Islamabad. Three growth media sandy medium (only sand), clayey medium {(sand and clayey soil (1:1)} and FYM medium {(sand, clayey soil and FYM (1:1:1)}, were prepared. Three growth media were air dried ground to pass through 2 mm sieve and filled in plastic tubes having capacity of one kg.

All the media used for this trial were examined for their physio-chemical properties using methods of hand book 60 [25] before addition of fertilizer and sowing of L. *leucocephala*. The physico-chemical data of soil media used are presented in Table 1. Nitrogen and potassium were applied as basal dose to all the tubes @ 50 and 100 mg kg⁻¹ media, respectively. Analytical grade Ammonium sulphate and Potassium sulphate were used as N and K source respectively. The rates of phosphorus used were 0, 20, 40 mg kg⁻¹ soil media. Analytical grade phosphoric acid was used as P source. Sandy medium + P₀, Sandy medium + P₂₀, Sandy medium + P₄₀, Clayey medium + P₀, Clayey medium + P₄₀, FYM medium + P₂₀ and FYM medium + P₄₀ treatments were applied in three repeats with completely randomized design (CRD), as under:

Each treatment consisted of 30 pots containing one seed in each pot. Germination count was recorded after 30 days of sowing. After 6 months, the crop was harvested and 10 plants were randomly selected for data collection regarding the parameters such as, shoot and root length, P concentration in shoot and root. Roots and shoots were oven dried at 70°C for shoot and root dry matter. Composite samples of the upper, middle and lower branches were taken from 10 randomly selected seedlings. Similarly composite root samples were also taken. Plant samples were brought to soil laboratory in labeled paper bags. The samples were then oven dried at 70°C for 24 hours and ground in a crushing grinding mill. Phosphorus concentration was measured using standard method [26]. Total P uptake and P recovery by the seedlings were also calculated.

Parameter	Unit	Sandy *	Clayey**	FYM ***	
pH (1:1)		8.03	7.83	7.34	
EC (1:1)	dS/cm	0.21	0.50	0.68	
Sand	%	90.00	44.00	59.00	
Silt	%	8.00	25.00	15.00	
Clay	%	2.00	31.00	26.00	
CaCO₃	%	8.50	6.40	5.00	
Organic matter	%	0.32	0.75	1.85	
NO ₃ –N	mg kg⁻¹	0.14	0.25	4.25	
Р	mg kg⁻¹	1.86	2.76	3.36	
К	mg kg⁻¹	25.00	50.00	59.00	
Cu	mg kg ⁻¹	0.29	0.76	0.92	
Fe	mg kg⁻¹	0.63	0.40	5.35	
Mn	mg kg⁻¹	2.38	3.19	4.25	
Zn	mg kg⁻¹	0.47	0.55	0.98	

Table 1.Physico-chemical analysis of soil media (Sandy, Clayey and FYM)

Sand only, **Sand+Clayey soil (1:1), *** Sand+Clayeysoil+FYM (1:1:1)

Statistical analysis of the data was performed to calculate summary statistics for the different growth parameters, P concentration, P uptake and P recovery % of the plant using M. STAT-C package, LSD test at 5 % level of significance was used for any significant difference among treatment means [27].

Phosphorus uptake by shoots, root, total uptake and recovery was calculated by using the formulas.

P uptake by shoots = P Conc. (%) in shoots x Dry wt. of shoots P uptake by roots = P Conc. (%) in roots x Dry wt. of roots Total P uptake = P uptake by shoot + P uptake by root F = fertilizer C = Control $P \operatorname{Re} \operatorname{cov} ery(\%) = \frac{(Total \ P \ Uptake)_F - (Total \ P \ Uptake)_C}{P \ Applied}$

3. RESULTS

The results of the study revealed that soil media and its interaction with P fertilizer significantly improved different growth parameters and P uptake.

3.1 Shoot and Root Length

Effect of different P fertilizer rates on shoot and root length of L. using different media is given in Table 2. The results showed that maximum shoot length was evaluated by P_{20} withFYM medium followed by clayey medium which were 49 % and 38 % more than sandy medium, respectively. Similarly, maximum root length was found by P_{20} with FYM medium followed by clayey medium which were 18 % and 37 % more than sandy medium. The P_{40} treatmentSSS significantly produced lower shoot and root length than P_{20} in all media. Shoot

and root length obtained by P₄₀ was in the order: FYM medium>Clayey medium>Sandy medium. Sandy medium was found least effective in all media.

 Table 2. Effect of soil media and phosphorus fertilization on shoot and root length of *L. leucocephala* (n=10)

Treatments	Shoot ler	Shoot length (cm)			Root length (cm)		
	Sandy*	Clayey**	FYM***	Sandy	Clayey	FYM	
Po	11.63 h ^ψ	14.67 e	15.47 d	10.43 e	11.8 cd	10.2 e	
P ₂₀	13.47 f	18.53 b	20.10 a	11.47 d	13.5 b	15.66 a	
P ₄₀	12.67 g	16.57 c	16.97 c	10.23 e	12.3 c	13.23 b	
LSD Shoot length (0.05 = 0.45	SD Root leng	th(0.05) = 0	71*Sand on	lv **Sand+C	lavev soil	

LSD Shoot length (0.05) = 0.45 LSD Root length (0.05) = 0.71*Sand only, **Sand+Clayey soil (1:1), *** Sand+Clayeysoil+FYM (1:1:1) LSD = Least significant difference Ψ = Figure sharing same letter(s) do not differ significantly

3.2 Shoot and Root Dry Weight

There were significant main and interactive effects of application of P fertilizer and soil media (Table 3) on shoot dry matter (SDM) and root dry matter (RDM). Maximum SDM (947 mg) was investigated by P_{20} with FYM medium followed by clayey medium (887 mg) which were 43 % and 34 % more than sandy medium, respectively. Similarly, maximum RDM (134 mg) was determined by P_{20} with FYM medium followed by clayey medium (112 mg) which were 43 % and 19 % more than sandy medium (94 mg), respectively.

Table 3. Effect of soil media and phosphorus fertilization on shoot and root dry weight of *L. leucocephala* L. (n=10)

Treatments	Shoot dry weight (mg plant-1)			Root dry weight (mg plant-1)			
	Sandy*	Clayey**	FYM***	Sandy	Clayey	FYM	
P ₀	432 i ^ψ	537 h	634 g	60 h	71 g	86 f	
P ₂₀	662 e	887 c	947 a	94 e	112 c	134 a	
P ₄₀	652 f	874 d	934 b	88 f	104 d	129 b	

LSD shoot dry weight (0.05) = 6 LSD Root dry weight (0.05) = 3 *Sand Only, **Sand and clayey soil (1:1), *** Sand, clayey soil and FYM (1:1:1)

LSD = Least significant difference

^{*w*} = Figure sharing same letter(s) do not differ significantly

3.3 Phosphorus Concentration (PC %)

Main and interactive effect of P fertilizer rates and media on phosphorus concentration (PC) of shoot and root of Leucaena is given in Table 4. Maximum PC (0.26 %) in shoot was measured by P_{20} with FYM medium followed by clayey medium which were 38 % and 26 % more than sandy medium, respectively. Similarly, maximum PC % in root was viewed by P_{20} with FYM medium followed by clayey medium which were 51 % and 39 % more than sandy medium (0.17 %), respectively.

Treatments	Shoot P c	oncentratio	n (%)	Root P concentration (%)			
	Sandy* Clayey** FYM***		Sandy	Clayey	FYM		
P ₀	0.160 eψ	0.172 d	0.187 d	0.125e	0.134 e	0.154 d	
P ₂₀	0.191 d	0.240 bc	0.263 a	0.174 c	0.242 b	0.263 a	
P ₄₀	0.184 d	0.225 c	0.252 a	0.161 cd	0.236 b	0260 a	

Table 4. Effect of soil media and phosphorus fertilization on shoot and root P concentration of L. leucocephala (n=10)

LSD Shoot P concentration (0.05) = 0.017 LSD Root P concentration (0.05) = 0.017 *Sand only, **Sand+Clayey soil (1:1), *** Sand+Clayeysoil+FYM (1:1:1) LSD = Least significant difference

 ψ = Figure sharing same letter(s) do not differ significantly

3.4 Phosphorus Uptake by Shoot and Root

Effect of different P fertilizer rates and media on P uptake by shoot and root of L. is given in Table 5. There were significant main and interactive effects of application of P fertilizer and soil media on P uptake. Maximum P uptake (2495 μ g) in shoot was investigated by P₂₀ with FYM medium followed by clayey medium (2129 μ g) which were 98% and 69% more than sandy medium (2129 μ g), respectively. Similarly, maximum P uptake (354 μ g) in root was observed by P₂₀ with FYM medium followed by clayey medium (270 μ g) which were 117% and 62% more than sandy medium (163 μ g), respectively.

Table 5. Effect of soil media and phosphorus fertilization on total P uptake of shoot and root of L. leucocephala (n=10)

Treatment	Shoot P uptake (µg plant ⁻¹)			Root P uptake (µg plant ⁻¹)		
	Sandy*	Clayey**	FYM***	Sandy	Clayey	FYM
P ₀	0692 h ^ψ	0926 g	1189 f	076 i	096 h	134 g
P ₂₀	1262 e	2129 c	2495 a	163 e	270 e	354 a
P ₄₀	1200 f	1967 d	2354 b	143 f	247 d	335 b

LSD Shoot P uptake (0.05) =37 LSD Root P uptake (0.05) =5

*Sand only, **Sand+Clayey soil (1:1), *** Sand+Clayeysoil+FYM (1:1:1)

LSD = Least significant difference

 $^{\psi}$ = Figure sharing same letter(s) do not differ significantly

3.5 Total Phosphorus Uptake

Effect on total P uptake is given in Table 6. There were significant main and interactive effects of application of P fertilizer and soil media on total P uptake of the plant. Maximum total P uptake (2848µg) was investigated by P_{20} with FYM medium followed by clayey medium (2400µg) which were 97% and 67% more than sandy medium (1442µg), respectively. Total P uptake by the plant with P_{20} was found 31% more in FYM medium than clayey medium.

Treatment	Total P uptake (µg plant ⁻¹)			Phosphorus Recovery (%)			
	Sandy*	Clayey**	FYM***	Sandy	Clayey	FYM	
Po	0767 h ^Ψ	1022g	1317 f	0.00	0.00	0.00	
P ₂₀	1442 e	2400 c	2848 a	4.11 c	8.62 b	9.57 a	
P ₄₀	1343 f	2213 d	2888 b	1.80 f	3.72 e	3.27 c	
					(

Table 6. Effect of soil media and phosphorus fertilization on total P uptake and percent recovery of L. leucocephala (n=10)

LSD P uptake (0.05) =39 *Sand only, **Sand+Clayey soil (1:1), *** Sand+Clayeysoil+FYM (1:1:1) LSD = Least significant difference

 ψ = Figure sharing same letter(s) do not differ significantly

3.6 Phosphorus Recovery Percentage

Main and interactive effects of application of P fertilizer and soil media on P recovery percentage of the seedling were significant (Table 6). Maximum P recovery (9.6%) was investigated by P_{20} with FYM medium followed by clayey medium (8.6%) and sandy medium (4.1%) which were 133% and 110% more than sandy medium, respectively. Similarly maximum P recovery (3.7%) was investigated by P_{40} with clayey medium followed by FYM medium (3.3%) and Sandy medium (1.8%) which were 107% and 82.0% more than sandy medium, respectively. Interactive effect of P fertilizer and FYM medium resulted in increased P recovery % of applied P to the plant. P recovery % by P_{20} was obtained 193% more than P_{40} in FYM medium. The treatment P_{20} produced significantly higher P recovery % than P_{40} in all media. P recovery % recorded by P_{20} was in the order: FYM medium>Clayey medium>Sandy medium.

4. DISCUSSION

The results of the study indicated that soil media significantly affected the plant growth parameters (such as shoot and root length and shoot and root dry matter). Plant growth parameters were the highest in FYM medium followed by clayey and sandy media The FYM medium responded likely better than clayey and sandy medium. Better growth can be attributed to better nutrients availability, greater water holding capacity and improvement of other soil physical characteristics such as bulk density, mechanical resistance, aeration, aggregate stability due to presence of FYM (organic matter) in the FYM medium. The results are similar to Atul and Sharma [28] who studied that sand + clay + FYM (1:1:1) was found best medium for survival and establishment of seedlings of Albizial ebbeck and Dalbergia sissoo. Chaplot and Mahnot [29] and Verma et al. [30] gave similar views that growth of L. Leucocephala was found maximum when FYM was mixed with soil. The results are also fairly in agreement with the findings of Gupta [43] who reported that the best growth of Azadira chtaindica were found in the mixture of sand, clay and FYM in the ratio of 1:1:1 in nursery experiments of Jodhpur, India. It was also revealed in a study by Memon and Tunio [31] that mineral fertilizer with farmyard manure on silty clay loam soil significantly increased wheat grain yield kg/ha. El-Sallami [32,33] also studied the response of L. leucocephala seedlings to soil (clay and sand) mixed with farmyard manure (FYM)) during 2000 and 2001 seasons at an experimental farm in Assiut, Egypt and observed that the soil mixture significantly increased all growth measurement i.e. number of branches, offsets and pods per plant, fresh weights of aerial parts and offsets, and fresh and dry weights of roots, shoot: root ratios and seed yield.

As regards the effect of P fertilization on *L. leucocephala* growth it significantly enhanced the plant growth parameters {(such as shoot and root length and shoot and root dry matter) (Table 2,3)}. The growth parameters were the highest at level of 20 mg P kg⁻¹ medium followed by 40 mg P kg⁻¹ medium and control treatment with no added P fertilizer. Similar results were put forth by Brauwers et al. [34] who studied the effect of P fertilizer application on the development of *Tabebuia caraiba* seedlings under nursery conditions and found that phosphorus fertilization contributed towards better growth in terms of plant height, number of leaves and stem base diameter. Similarly the response of potted seedlings of L. leucocephala to phosphorus (P) was observed by Ezenwa [35] that applying P at 7.5 mg/kg soil significantly increased shoot height by 14% over the control. Dixit et al. [36] also expressed similar views regarding the effect of P application on the seedlings of 3 species of *Acacia* and observed that the growth parameters were improved with the phosphorus fertilization. The results of the study corroborate with the findings of Gupta et al. [37,38] in glasshouse experiments on L. leucocephala who investigated that application of P gave best height and diameter growth and biomass parameters seedlings.

Similarly reports have come from Singh [39] who observed in an experiment that the spray of P_2O_5 increased the shoot weight and number of leaves of *L. leucocephala* seedlings. Similar claims were made by Garza et al. [40] i.e. the application of super phosphate increased the root dry weight, top dry weight and total biomass of *L. leucocephala*. Aslam et al. [41] also investigated that the grain and straw yields of different cultivars of wheat were improved with the phosphorus fertilization. Recently Salee et al. [42] concluded similar types of results that higher dose of phosphorus was best for achieving more cotton yields than its lower rates.

The results of the study indicated that phosphorus uptake and utilization were significantly affected by soil media and phosphorus fertilization. In general phosphorus uptake was the highest in FYM medium followed by clayey and sandy media (Table 6). The similar trends of results were found in shoot and root P concentration as mentioned in Table 4. Phosphorus uptake was greater due to the facts that shoot and root biomass was greater in FYM medium as compared to clayey and sandy media (Table 3). The results are in close conformity with those found by Atul and Sharma; Chaplot and Mahnot; Aslam et al.; Gupta [28,29,41,43] who concluded that the application of FYM in the medium significantly increased the biomass of the plant.

As regards the effect of P fertilization on total P uptake, it was found that the P fertilization significantly increased the total P uptake by shoot and root (Table 6). The total P uptake was the highest at level of 20 mg P kg⁻¹ medium followed by 40 mg P kg⁻¹ medium and control treatment with no added P fertilizer. The highest total P uptake due to the fact that the biomass of shoot and root was the highest at 20 mg P kg⁻¹ medium. Similar results were also reported by Besfert [44] who concluded that P supply tomato plant increased the total P uptake. The results are also in close conformity with the investigation of Mehmood et al. [45] who reported that phosphorus uptake by wheat was increased with increasing levels of phosphorus. Aslam et al.; Mian et al.; Ilyas et al. [41,46,47] also gave same similar results that phosphorus uptake was increased with increase the level of P. Navale and Gaikwad [19] also reported that application of FYM combined with inorganic fertilizer, increased phosphorus uptake of Soybean. Aulakh et al. [48] studied the effect of phosphorus on soybean-wheat production and found that 60 kg P₂O₅ ha⁻¹ enhanced P uptake and, total biomass of both crops. Dubey et al. [49] investigated that total phosphorus uptake by *Linum usitatissimum* increased with increasing level of phosphorus.

Results of the study revealed that P recovery was significantly affected by media and P fertilization. The P recovery was the highest in FYM medium followed by clayey and sandy media. Higher P recovery in this medium is attributed to increase of biomass production and P Concentration of the plant. Better recovery can be described to better nutrients availability of the medium (Table 1) and better physical condition due to presence of FYM in the medium with which physical properties such as bulk density, mechanical resistance, aggregate stability, pore size distribution might have been in desirable balance. The results are quite similar to the findings of Gupta [43] who determined that application of FYM in the mixture increased the biomass and P recovery of the plant. Hussain and Adhi Kari [50] also investigated that application of FYM to soil increased the biomass and recovery of the nutrients in the wheat crop.

The results of the study revealed that P recovery was significantly affected by the P fertilization. In general P recovery was the highest at level of 20 mg P kg⁻¹ medium followed by 40 mg P kg⁻¹ medium. The highest phosphorus recovery with 20 mg P kg⁻¹ medium is due to with greater P uptake and biomass production. The results are in good agreement with the findings of Mohsin et al. [51] who investigated that phosphorus fertilizer enhanced p recovery of Chickpea. Xu et al. [52] also claimed that P application significantly increased tree growth biomass production and P use efficiency and P recovery of *Eucalyptus grandis*. Dubey et al. [49] reported that phosphorus application significantly improved phosphorus use efficiency and P recovery by in soybean wheat production system.

The results of study revealed that Leucaenagrowth parameters and phosphorus uptake and utilization at higher level of P (40 mg kg⁻¹) were affected adversely. In this treatment greater proportion of P was not utilized by plant and remained unutilized. The results are similar to the findings [53] who found that phosphorus recovery was reduced with increasing level of P. Dubey et al. [49] also claimed that phosphorus recovery was decreased at higher rates of phosphorus in *Eucalyptus grandistree*.

5. CONCLUSIONS

It is concluded from this study that biomass and root length of *L. leucocephala*seedlings were significantly improved with P fertilizer, the medium level i.e. 20 mg P Kg⁻¹ in FYM medium (sand, clayey soil and FYM ratio; 1:1:1) as compared to clayey (sand and clayey soil ratio; 1:1) and sandy media. This shows the significance of P and FYM medium for the seedling growth. Therefore the FYM medium and P fertilization at medium level i.e. 20 mg P Kg⁻¹ can be recommended for best survival *L. leucocephala*seedling and its raising for plantation on large scale.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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