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Characterization of Fruit, Seed Traits and Seedling Growth Performance of Different Seed Sources of *Calophyllum inophyllum*

Karthik H N [a++*], Chetan Bhanu Rathod [b#] and Aravind B Rathod [c#]

[a] *Department of Forest Products and Utilization, College of Forestry, Sirsi, UAS Dharwad, India.*
[b] *Department of Silviculture and Agroforestry, College of Forestry, Sirsi, UAS Dharwad, India.*
[c] *Indian Institute of Forest Management, Bhopal, Madya Pradesh, India.*

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

Calophyllum inophyllum is an oil-bearing tree species with seeds containing 50-70 per cent oil, mainly used for non-edible purposes. Belonging to the Clusiaceae family, it is commonly known as Undi in Kannada. This littoral tropical tree grows above the high-tide mark along coastlines and is frequently found on sandy beaches. Its prominence has increased due to high demand as biofuel

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[++] *Assistant Professor;*
[#] *PhD Scholar;*
**Corresponding author: E-mail: karthikhnforestry@gmail.com;*

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and commercialization. The study aimed to examine the fruit and seed traits from various sources and the impact of seed source variation on germination and seedling growth of *Calophyllum inophyllum*. Experiments were conducted at the College of Forestry, Sirsi, with fruits collected from seven different regions from coastal taluks of Uttara Kannada district, Karnataka. Data on fruit, seed, and plant parameters were recorded. Results revealed that there was no such statistically significant difference was observed in fruit parameters, seed parameters, seedling parameters and germination percentage of *Callophyllum inophyllum* among the selected seed sources inferring no greater variations. However, the seeds from the Ramangindi (48.66%, 17% higher than the next best region, Ankola) region in Kumta taluk performed better in germination of seeds, fruit and seed traits, as well as growth compared to other sources.

Keywords: Seed source; Calophyllum inophyllum; germination percentage; seed length; seed width.

1. INTRODUCTION

Calophyllum inophyllum, commonly known as Alexandrian laurel or “Undi” in Kannada, belongs to the Clusiaceae family and thrives in tropical coastal regions above the high tide mark. It is predominantly found on sandy beaches across East Africa, Southern India, Malaysia and Australia (Chinthu et al., 2023). The tree is slow-growing, often reaching heights of 8-20 meters, with a broad, irregular crown. Its flowers are small and white, typically blooming twice a year in late spring and autumn. The seeds of *C. inophyllum* are notable for their high oil content, ranging from 50-73 (%), making the species significant for biofuel production (Ramanadane et al., 2007). Oil is commonly called Tamanu oil, is primarily used for medicinal purposes and cosmetics, believed to aid in tissue regeneration. The oil also serves industrial uses, including varnish production and soap manufacturing (Novod, 2008).

The wood of *C. inophyllum* is highly valued for its durability and versatility. Historically, it was used by Pacific Islanders to construct canoes and more recently for furniture, railway sleepers, and plywood. It is also sought after for its ability to endure harsh environments. Additionally, the wood is utilized in boat building due to its fine texture and strength (Chinthu et al., 2023). The timber is slightly heavier, stronger and more durable than that of other Calophyllum species. The wood is often fine textured and the grain is more interlocked. Sapwood is yellow-brown with a pink tinge and is well defined from the heartwood, which is red-brown, pink-brown or orange-brown. The density is 560-800 kg/cubic m at 15% moisture content (MC), with an energy value of about 19,100 kJ/kg.

Tannins are commonly present, especially in the bark (11.9%) but often also in the leaves. A decoction of the bark is sometimes used to toughen and dye fishing nets. The seed oil and the latex have occasionally been used in dyeing batik cloth in Java. In traditional medicine, various parts of the tree serve numerous purposes. The bark, rich in tannins, can be used as an astringent, while the leaves are utilized in treatments for eye inflammations and skin conditions. In some regions, the oil extracted from the seeds is applied to treat rheumatism and ulcers. Moreover, the latex and sap of the tree are poisonous and have been historically used to create poison arrows (Pawar & Patil, 2020).

C. inophyllum is also known for its role in environmental conservation. The tree is planted for shade and acts as an efficient coastal protector, stabilizing sandy soils. Its ornamental value makes it popular for roadside planting in India (Chinthu et al., 2023).

In recent years, energy conservation and the pursuit of alternative energy sources have gained significant importance due to the global energy crisis. The increasing demand for carbon-neutral energy alternatives has highlighted the potential of *Calophyllum inophyllum*, an important tree species used for biodiesel production. This study aimed to investigate variations in fruit and seed traits among different seed sources and their impact on germination and seedling growth attributes of *C. inophyllum*, which is abundant in the study area and no such recent research was done before from the selected study area. This research may facilitate the early evaluation of selection criteria for key traits in both laboratory and nursery settings, potentially leading to improved field performance.

2. MATERIALS AND METHODS

The present study was carried out at the College of Forestry, Sirsi, located at 14°26'N latitude, 75°50'E longitude and 619 meters above mean sea level. The area experiences an average annual rainfall of 1069 mm, predominantly between June and September, with temperatures ranging from 15°C to 35°C. Seeds were collected from various coastal locations in the Uttara Kannada district (Table 1). Hundred Ripened fruits from each source were measured for pod traits, including pod length, breadth, thickness and weight. The seeds were then extracted from the pods and evaluated for seed traits such as length, breadth, thickness and weight. Fifty Seeds from each sources were sown in nursery beds and germination and seedling parameters were measured at 30 and 60 Days After Sowing (DAS).

Fruits from each locality were treated as separate treatments, labelled T_1 to T_7. Observations on pod and seed parameters were made before sowing the seeds in nursery beds. The experiment was designed using a Factorial Completely Randomized Design (CRD) with three replications and seven treatments. As a pre-sowing treatment, the fruits were soaked in water overnight.

2.1 Parameters Recorded

Germination Percent:

$$\text{Germination (\%)} = \frac{\text{Number of seeds germinated}}{\text{Number of seeds sown}} * 100$$

Seedling traits: After completion of germination, observation was taken on seedling parameters. The seedlings (10) from each replication were selected in different sources. The following observations were recorded from thirty randomly selected sample plants in each source.

Shoot length (cm): The height of the shoot was measured from ground level to growing tip and expressed in centimetres.

Root length (cm): The root length was measured from collar region to the tip of main root and expressed in centimetres.

Number of leaves per seedling: Total number of leaves was counted from each replication and average number of leaves per seedling was recorded.

Shoot dry weight per seedling: The dry weight was assured by destructive sampling three seedlings from each source were selected for every observation. The plant was uprooted from nursery bed and shoot were separated, the fresh weight was taken and average fresh weight per seedling was recorded. The samples were oven dried at 60-80ºC for 48 hours and shoot dry weight per seedlings were recorded in grams.

Root dry weight per seedling: Root dry weight was assessed by destructive sampling of three seedlings from each replication. The plant was uprooted from nursery bed and roots were separated and washed thoroughly with tap water. The average fresh weight was recorded per seedling. The samples were oven dried at 60-80ºC for 48 hours and root dry weights per seedlings were recorded in grams.

Collar diameter: Collar diameter was measured slightly above ground level and was expressed in mm.

Seedling height: Seedling height was measured from ground level to the tip of leaves and was expressed in cm.

Shoot to root Ratio:

$$\text{Shoot to root Ratio} = \text{Shoot length} / \text{Root length}$$

Seedling Biomass: Seedling Biomass was recorded replicate wise and average fresh weight per seedling was reported. The samples were oven dried at 60 ℃- 80 ℃ for 48 hours and dry weight was recorded per seedling in grams.

Table 1. Locations of seed collection

Treatments	Name of locality	Taluk	NL	EL
T_1	Ramangindi	Kumta	14 º 21 ‘ 40 “ 13	74 º 24 ‘ 24 “ 33
T_2	Apsarakonda	Honnavar	14 º 23 ‘ 50 “ 65	74 º 44 ‘ 59 “ 36
T_3	Dhareshwar	Honnavar	14 º 38 ‘ 28 “ 22	74 º 40 ‘ 27 “ 27
T_4	Kasarkod	Honnavar	14 º 26 ‘ 30 “ 87	74 º 43 ‘ 15 “ 22
T_5	Ankola	Ankola	14 º 63 ‘ 58 “ 15	74 º 28 ‘ 52 “ 23
T_6	Eco-beach	Honnavar	14 º 26 ‘ 06 “ 57	74 º 43 ‘ 28 “ 75
T_7	Karki	Honnavar	14 º 30 ‘ 70 “ 11	74 º 43 ‘ 51 “ 26

Statistical analysis: Collected data was subjected to statistical analysis using OPSTAT (Sheoran et al., 1998) and ANOVA table was constructed.

3. RESULTS AND DISCUSSION

The natural regeneration of plant species through seeds relies on seed production, germination capacity and the successful establishment of seedlings. Seeds from healthy, well-formed trees offer greater assurance that the resulting plants will exhibit good form, higher survival rates and better resistance to stress. A tree species may cover a vast geographical range and thrive in diverse environment with varying climate and topography. These populations, adapted to different local conditions, are referred to as provenances. Understanding the adaptability of a species' populations is crucial for forest restoration, especially as global warming and environmental changes disrupt natural forests (Ledig and Kitzmiller, 1992).

Provenance trials compare seeds from various climatic conditions to assess seed quality. These trials are a key step in tree species improvement, providing essential knowledge of genetic and environmental variations. Provenance trials allow for direct genetic comparisons among seed sources grown in multiple "common gardens" and help to quantify the impact of changing climates on wood production (Kitzmiller, 2005). A significant decision in forest management is selecting the appropriate seed sources for reforestation to ensure successful outcomes (Shu et al., 2012). This can be supported by seed zone and seed transfer guidelines, which reduce the risk of planting poorly adapted trees (Hamann et al., 2000). Morphological variation in seed characteristics across natural populations is valuable for tree improvement programs, as seed quality influences seedling performance, growth, and biomass allocation. Generally, germination variation among species is linked to the altitude, longitude and latitude of seed origin (Singh et al., 2004; Saikia et al., 2009).

3.1 Seed Source Variation on Fruit and Seed Traits

The data depicted in the Table 2 represents the effect of seed sources on fruit parameters of *Callophyllum inophyllum.* The results indicate that there are no statistically significant differences in fruit and seed parameters among the seven different seed sources.

Though, Ankola (T5) exhibited the highest fruit length, followed by Apsarakonda (T2), with Dhareshwar (T3) and Karki (T7) showing similar lengths. The shortest fruit length was observed in the Kasarkod region (T4). Apsarakonda (T2) had the highest fruit width, followed by Karki (T7) and Ramangindi (T1), while the lowest width was recorded at Eco-Beach (T6). Ramangindi (T1) had the highest fruit weight, followed by Dhareshwar (T3) and Apsarakonda (T2). The lowest fruit weight was observed in Eco-Beach (T6). And, Kasarkod (T4) exhibited the greatest seed length, followed by Ramangindi (T1), Apsarakonda (T2), and Ankola (T5). Kasarkod (T4) also showed the greatest seed width, followed by Ramangindi (T1) and Karki (T7), with Ankola (T5) exhibiting the lowest width. Ramangindi (T1) had the highest seed weight, followed by Apsarakonda (T2) and Kasarkod (T4), with Dhareshwar (T3) showing the lowest seed weight (Table 3).

Since *C. inophyllum* is an important TBO's, it's seed traits are necessary measure which links to its yield and also helps in further selection and improvement of species for commercial traits to obtain higher yield. Shreekumar and Gunaga (2017) did the similar work using *C. Inophyllum* seeds from some regions of Maharashtra and Karnataka, and reported wide range of variations in fruit and seed traits unlike our results and Maharashtra source found to be the superior. In another study by Yuniastuti et. al., (2021) reported lower morphological characters' variance of 26% in *C. inophyllum* among costal sources of Indonesia, which somewhat agrees with our study. The seed germination and seedling vigour are also affected by seed size and other factors like dormancy, moisture, *etc.* the bigger sized seeds of *C. inophyllum* produces quick, uniform and maximum germination as well as vigorous seedling and higher dry biomass as compared to those of medium and small sized seeds. Genetic quality seeds are essential for production of quality seedlings in large quantities (Shreekumar and Gunaga, 2017).

Warringa et al. (1998) attributed the factors contributing to early development of inflorescence and partitioning of assimilates at anthesis to the variations in seed size. Schmidt (2001) had opined that the seeds exhibit variation in size but this is neither due to the less accumulation of reserve food material nor earlier abscission of seeds. Environmental influences during the development of the seeds combined with genetic variability are reported to cause variations in seed dimensions. Jijeesh and Sudhakara (2013) reported that the larger seed size gives a better germination.

3.2 Seed Source Variation on Germination and Seedling Attributes

The data depicted in Table 4 represent the effect of seed sources on germination percentage of *Callophyllum inophyllum.* However, none of the germination and seedling parameters showed statistically significant difference between the seed sources.

Calophyllum inophyllum, typically requiring 40-50 days to germinate, showed minimal germination at 30 DAS. Among the treatments, Ramangindi (T1) exhibited the highest germination rate at 5.33%, followed by T3 and T7 at 2%, T2 and T6 at 1.33%, and the lowest rate in T4 and T5 at 0.66%. At 60 DAS, Ramangindi (T1) again demonstrated superiority with a germination rate of 48.66%, while Eco-Beach (T6) showed the lowest rate at 28%.

Effect of seed sources on seedling parameters of *Callophyllum inophyllum* is represented in Table 5. Seedling parameters like root length, shoot length, shoot to root ratio, number of leaves and collar diameter were measured at 60 days after sowing (DAS) due to insufficient growth at 30 DAS, as germination had only just begun and no significant shoot or root growth was measurable. Root length was recorded from the base of the stem to the root tip, with Ramangindi (T1) exhibited the greatest root length at 9.38 cm, followed by Ankola (T5), Apsarakonda (T2), and Eco-Beach (T6) with the shortest root length. Ankola (T5) showed the highest shoot length at 12.29 cm, followed by Karki (T7), Ramangindi (T1), and Eco-Beach (T6) with the lowest shoot length. Karki (T7) exhibited the highest shoot-to-root ratio at 1.63, followed by Dhareshwar (T3), Ankola (T5), and Apsarakonda (T2) with the lowest ratio. Ramangindi (T1) had the greatest number of leaves at an average of 5 leaves per plant, while Eco-Beach (T6) had the least at 3.33 leaves.

Collar diameter was measured 3 cm above the ground level using Vernier caliper, results showed that T_7 (Karki) had the largest collar diameter of 3.16 mm and smallest diameter of 2.43 mm was found in Ankola (T_5.).

The superior performance of seeds from the Ramangindi region, in terms of both germination percentage and seedling growth, likely reflects the genetic adaptations of these seeds to the specific environmental conditions of that area. Ajeesh et. al., (2014) reported higher germination with bigger and medium seeds of *C. inophyllum* which holds good with our results where Ramangindi seeds have higher seed weight among the seven selections. Similarly, Zerbib (2007) reported that Provenance trials often show that seed populations adapted to local conditions perform better than those from other regions, particularly in response to climatic factors such as temperature, rainfall and soil type. The higher germination of the seeds might be due to the variation in seed biochemical components which increases with seed weight.

Table 2. Effect of seed sources on Fruit parameters of *Callophyllum inophyllum*

Seed source	Fruit length (cm)	Fruit width (cm)	Fruit weight (gm)
Ramangindi (T_1)	2.94 ± 0.23^a	2.6 ± 0.23^a	7.57 ± 0.67^a
Apsarkonda (T_2)	3.08 ± 0.65^a	2.69 ± 0.65^a	6.45 ± 1.81^a
Dhareshwar (T_3)	2.53 ± 0.23^a	2.57 ± 0.23^a	6.49 ± 4.32^a
Kasarkod (T_4)	2.53 ± 0.45^a	2.51 ± 0.45^a	5.54 ± 0.18^a
Ankola (T_5)	3.13 ± 0.25^a	2.46 ± 0.25^a	5.99 ± 1.06^a
Eco-Beach (T_6)	2.97 ± 1.00^a	2.32 ± 1.00^a	4.58 ± 1.00^a
Karki (T_7)	3.02 ± 0.39^a	2.62 ± 0.39^a	5.79 ± 0.97^a
C.D (0.01%)	N/A	N/A	N/A
SEm (±)	0.23	0.18	1.15

Note**

1. Groups sharing the same letter (e.g., 'a') are not significantly different from each other
2. Different letters (e.g., 'a' vs. 'b') indicate significant differences between the means of those respective groups
3. The larger the differences in letters, the greater the significance of the contrast between the groups.

Table 3. Effect of seed sources on seed parameters of *Callophyllum inophyllum*

Seed source	Seed length (cm)	Seed width (cm)	Seed weight (gm)
Ramangindi (T_1)	2.07 ± 0.12 [a]	1.87 ± 0.05 [a]	4.28 ± 0.47 [a]
Apsarkonda (T_2)	1.99 ± 0.11 [a]	1.79 ± 0.04 [a]	4.13 ± 1.14 [a]
Dhareshwar (T_3)	1.98 ± 0.17 [a]	1.78 ± 0.12 [a]	2.68 ± 0.87 [a]
Kasarkod (T_4)	2.12 ± 0.12 [a]	1.91 ± 0.12 [a]	3.89 ± 1.49 [a]
Ankola (T_5)	1.90 ± 0.08 [a]	1.66 ± 0.04 [a]	3.75 ± 0.61 [a]
Eco-Beach (T_6)	1.96 ± 0.14 [a]	1.78 ± 0.09 [a]	3.54 ± 1.05 [a]
Karki (T_7)	1.98 ± 0.16 [a]	1.79 ± 0.13 [a]	3.44 ± 0.85 [a]
C.D (0.01%)	N/A	N/A	N/A
SEm (±)	0.12	0.08	0.46

Note**

1. Groups sharing the same letter (e.g., 'a') are not significantly different from each other
2. Different letters (e.g., 'a' vs. 'b') indicate significant differences between the means of those respective groups
3. The larger the differences in letters, the greater the significance of the contrast between the groups.

Table 4. Effect of seed sources on germination percentage of *Callophyllum inophyllum*

Seed source	30 DAS (%)	60 DAS (%)
Ramangindi (T_1)	5.33 ± 1.15 [a]	48.66 ± 1.03 [a]
Apsarkonda (T_2)	1.33 ± 2.31 [a]	33.33 ± 9.43 [a]
Dhareshwar (T_3)	2.00 ± 2.08 [a]	37.33 ± 19.05 [a]
Kasarkod (T_4)	0.66 ± 0.94 [a]	28.66 ± 7.50 [a]
Ankola (T_5)	0.66 ± 0.94 [a]	41.33 ± 4.04 [a]
Eco-Beach (T_6)	1.33 ± 0.94 [a]	28.00 ± 2.31 [a]
Karki (T_7)	2.00 ±1.15 [a]	30.00 ± 4.00 [a]
C.D (0.05%)	N/A	N/A
SEm (±)	1.37	5.23

Note**

1. Groups sharing the same letter (e.g., 'a') are not significantly different from each other
2. Different letters (e.g., 'a' vs. 'b') indicate significant differences between the means of those respective groups
3. The larger the differences in letters, the greater the significance of the contrast between the groups.

Table 5. Effect of seed sources on seedling parameters (60 DAS) of *Callophyllum inophyllum*

Seed source	Root length (cm)	Shoot length (cm)	Shoot to root ratio	No. of leaves	Collar diameter (mm)
Ramangindi (T_1)	9.38 ± 0.42 [a]	11.75 ± 0.84 [a]	1.26 ± 0.35 [a]	5.00 ± 1.73 [a]	2.88 ± 0.16 [a]
Apsarkonda (T_2)	8.60 ± 1.30 [a]	10.6 ± 1.75 [a]	1.24 ± 0.18 [a]	4.00 ± 0.00 [a]	2.60 ± 0.14 [a]
Dhareshwar (T_3)	7.20 ± 1.71 [a]	10.1 ± 2.82 [a]	1.41 ± 0.45 [a]	4.33 ± 1.53 [a]	2.72 ± 0.34 [a]
Kasarkod (T_4)	8.19 ± 2.11 [a]	9.74 ± 1.58 [a]	1.26 ± 0.24 [a]	3.67 ± 0.58 [a]	2.57 ± 0.10 [a]
Ankola (T_5)	9.03 ± 1.03 [a]	12.29 ± 0.12 [a]	1.37 ± 0.11 [a]	4.33 ± 0.58 [a]	2.43 ± 0.06 [a]
Eco-Beach (T_6)	1.09 ± 0.25 [a]	8.81 ± 1.09 [a]	1.29 ± 0.03 [a]	3.33 ± 0.58 [a]	2.60 ± 0.17 [a]
Karki (T_7)	7.69 ± 1.44 [a]	12.13 ± 0.17 [a]	1.63 ± 0.32 [a]	4.00 ± 1.00 [a]	3.16 ± 0.42 [a]
C.D (0.01%)	N/A	N/A	N/A	N/A	N/A
SEm (±)	0.91	1.18	0.19	0.47	0.16

Note**

1. Groups sharing the same letter (e.g., 'a') are not significantly different from each other
2. Different letters (e.g., 'a' vs. 'b') indicate significant differences between the means of those respective groups
3. The larger the differences in letters, the greater the significance of the contrast between the groups.

Similar results were also reported by Dwivedi (1993) in *Azadirachta indica* and Devagiri et al. (1998) in *Dalbergia sissoo.* They found that the variation observed in the seed characters may be attributed to adverse environment and differences in their distribution range this in turn affect the germination of seeds. Similarly, Palanikumaran et al. (2015) reported that he sizes and shape of seeds is variable depending on the structure and form of the ovary and environmental conditions under which plant is growing. Jijeesh an Sudhakar (2013) reported that the higher germination of the seeds might be due to the variation in seed biochemical components which increases with seed weight.

4. CONCLUSION

Seed source variation is a critical factor influencing the growth, vigor, and ultimately, the quality and quantity of *Calophyllum inophyllum*. While this study did not identify significant statistical differences at 99% confidence level with respect to fruit, seed, seedling, and germination parameters among the seven selected seed sources. Though, seeds from the Ramangindi region of Kumta taluk consistently exhibited superior performance, particularly in germination (48.66%, 17% higher than the next best region, Ankola), fruit characteristics, and some seedling growth parameters. This suggests that genetic factors specific to this region may contribute to enhanced plant performance. To further explore the potential of *C. inophyllum* for bioenergy applications, future research should delve deeper into the oil yield and fatty acid profiles of seeds from different regions. By identifying seed sources with optimal oil content and favourable fatty acid compositions, we can optimize biodiesel production and maximize the economic and environmental benefits of this valuable tree species.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc) and text-to-image generators have been used during writing or editing of this manuscript.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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