



# RESEARCH ARTICLE

# Effect of source and seed storage conditions on biochemical attributes of *Simarouba glauca* DC

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#### **Abstract**

Oilseeds are vital in India's agriculture and nutrition, providing essential oils and proteins. They are a cornerstone of the edible oil industry, with India being one of the largest consumers globally and a major importer of edible oils from Indonesia and Malaysia. Investigations were carried out within the fifteen seed sources of *Simarouba glauca*, to elucidate information on the best seed source in terms of storage potential and seed source. The results of the study on physical and physiological observations illustrated that Akola seed source performed better for hundred seed weight (98.64 g), germination (75.75 %), root length (12.42 cm), shoot length (9.33 cm), dry matter production (0.972 g) and vigour index (75). The above results showed that among the seed variation, Akola seeds were resistant to adapt seed storage variability within 4, 8 and 12 months. This study thus provides valuable information for selecting and preserving *Simarouba glauca* seeds for future planting and conservation efforts.

**Keywords:** biochemical properties; oilseeds; physiological parameters

# Introduction

Oilseeds make up to 10 % of global production and are the world's fourth biggest source of edible oils (1). After cereals, oilseeds (groundnut, sunflower, soybean, seasmum, niger seed, mustard, safflower) are India's main source of protein and fat. 13-15 % of global oilseed area and 10-12 % of edible oil consumption are attributed to India (2). 72 % of oilseeds are grown in rainfed areas, leading to low productivity (3). Hence, a further increase in area for edible oil crops can lead to a decrease in productivity of food crops. This needs exploration of alternatives that can be useful in meeting the increasing demand. Among these, tree-bearing oilseeds (TBOs) are gaining significant importance.

Simarouba glauca, the "Lakshmi taru" or "Paradise tree," is a versatile tree that thrives in degraded soils and it can tolerate a wide range of temperatures (30-45°C) and altitudes (up to 1000 m above sea level) (4). This tree has the potential to yield 2000-2500 kg of oil per hectare per year. The chemicals present in fruit, pulp, seeds and bark are of high medicinal value, mainly due to the presence of amoebicide, anthelmintic, antiviral and help to cure gastritis, ulcers and malaria (5). The oil contains about 63 % unsaturated fatty acids and it is fit for human consumption (6). The oil is also used in the manufacture of soaps, lubricants, paints,

cosmetics, etc (7). It was first introduced by the National Bureau of Plant Genetic Resources (NBPGR) in the research station at Amravati in Maharashtra in 1966. In India, it is mainly grown in Odisha and Maharashtra and is also cultivated in Gujarat, West Bengal, Tamil Nadu and Rajasthan (8).

The factors that significantly affect how long seeds can be stored include the genetic makeup of the plant, the seed's previous history, its moisture content, the type of container used for storage and the storage temperature (9). Large scale production of good quality seed requires storage for more than one season. If not properly guarded, seed material will rapidly detoriate and completely lose its viability in due course. Understanding the storage potential of seeds is essential for effective seed management. Furthermore, the seeds are influenced by their origin due to climatic and edaphic factors (10). The seed source variations are prominent in many oilseeds with respect to their storage potential and the same is depicted in the current study (11). Delineation of the best seed source for individual species is an important milestone in establishing successful population of trees. Few studies focus on storage conditions for different seed sources. Thus, to address this gap, the present study is focused on seed source variation and effect of storage in terms of Simarouba glauca biochemical attributes. Through laboratory analysis, we tried to investigate the durability of

Simarouba glauca seeds based on different source of their collection.

#### Materials and methods

The experimental materials for the present study consisted of fifteen seed sources of Simarouba glauca DC selected from six agroclimatic zones of Tamil Nadu, three from Karnataka and one each from Maharashtra, Orissa and Gujarat. Seeds from individual trees from a source were mixed and used as a seed source. The seed sources and their geographic location are listed in Table 1 with a visual representation in Fig. 1 (ordered from Left to right). The experiment was carried out at Forest College and Research Institute, Tamil Nadu Agricultural University, Mettupalayam (11°9'N; 76°56'E; 300 m MSL; 830 mm rainfall) during 2021-2024. The seeds collected from different sources were cleaned with the help of a seive thoroughly to remove ill-filled, immature and insectdamaged seeds. The seeds were first dried under shade to bring the moisture content to  $8 \pm 0.5$  %. The initial physical and physiological parameters were recorded from the selected samples. After initial evaluation, the seeds were wrapped source-wise in plastic containers and stored under ambient conditions (32  $\pm$  2°C and 60  $\pm$  5 % relative humidity). Samples were drawn at 4, 8 and 12 months after storage and the following observation were recorded *viz*: germination (%), quick viability test, oil content, seed protein content, free fatty acid content, total phenol content, polyphenol oxidase activity, dehydrogenase activity, peroxidase activity, catalase and a amylase activity. The detail of the various seed sources along with geographical location is mentioned in Table 1.

# **Biochemical parameters**

The seeds were soaked in water for eight hours and sown in sand medium. The experiment was set up in a completely randomized design with four replications of one hundred seeds each. The following biochemical parameter studies are:

#### **Germination (%)**

The method for germination per cent was in accordance with (12). Four replicates of one hundred seeds were kept in sand medium. After thirty days, the seedlings were evaluated and the mean number of normal seedlings (based on morphology and seed viability testing) was counted and expressed in per cent.

Table 1. Details of the seed sources

# Oil Content (%)

The seeds were decoated and the kernels were dried at 50 °C for 16 hr and allowed to cool in a desiccator. The seeds were then pulverized in a porcelain mortar. The thimble was then placed in a Soxhlet apparatus. The oil content was estimated using a Soxhlet extractor according to the procedure outlined previously (13). The percentage of oil was calculated using the formula.

Oil Content = 
$$\frac{\text{Oil weight (g)}}{\text{Sample weight (g)}} \times 100$$
 (Eqn. 1)

#### **Seed Protein Content**

The protein content of the seeds was determined using the calorimetric method (14). To estimate the protein content, the oil-free meal, obtained after extracting the oil from the ground seed material, was analyzed. The protein percentage was then calculated using a specific formula.

Protein (%) = 
$$3.78 + (6.16 \times OD \text{ value})$$

# Free fatty acid content

The total free fatty acid content of the oil was measured using a volumetric method. This value was calculated as a percentage of oleic acid (15).

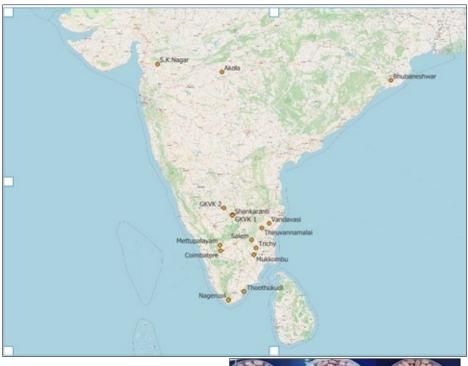
#### **Total Phenol content**

The total phenol content was estimated using a previously described method (16).

# Polyphenol oxidase activity

Two sets of 250 mg seed samples were homogenized in 5 mL of 0.1 M sodium phosphate buffer (pH 6.5) and centrifuged at 10,000 rpm for 10 min at 4 °C. The reaction mixture was prepared by combining 0.2 mL of enzyme extract, 0.2 mL of 0.01 M catechol (substrate) and 1.5 mL of 0.1 M sodium phosphate buffer (pH 6.5). A blank sample was also prepared for each set. After incubation, the absorbance of each sample was measured using a UV spectrophotometer. The phenol oxidase activity was calculated as the change in absorbance per min.

Sl. No.	Name of the seed sources	District/ Location	State	Latitude	Longitude
1.	Thiruvannamalai	Thiruvannamalai	Tamil Nadu	12°15'N	79°07'E
2.	Vandavasi	Thiruvannamalai	Tamil Nadu	12°30'N	79°30'E
3.	Salem	Salem / Danishpet	Tamil Nadu	11°36'N	78°35'E
4.	Coimbatore	Coimbatore	Tamil Nadu	11°1'N	76°58'E
5.	Mettupalayam	Coimbatore	Tamil Nadu	11°19'N	76°56'E
6.	Trichy	Trichy / Kumulur	Tamil Nadu	11°10'N	78°49'E
7.	Mukkombu	Trichy	Tamil Nadu	10°48'N	78°42'E
8.	Thoothukudi	Thoothukudi	Tamil Nadu	8°48'N	78°11'E
9.	Nagercoil	Kanyakumari /Keeriparai	Tamil Nadu	8°21'N	77°22'E
10.	GKVK 1	Bangalore/GKVK campus	Karnataka	12°58'N	77°35'E
11.	GKVK 2	Tumkur / Tiptur	Karnataka	13°20'N	77°08'E
12.	Shankaranti	Bangalore / Hebbal	Karnataka	12°56'N	77°35'E
13.	Akola	Akola	Maharashtra	20°42'N	77°02'E
14.	Bhubaneshwar	Bhubaneshwar	Orissa	20°15'N	85°52'E
15.	S.K.Nagar	Banaskantha	Gujarat	21°07'N	73°40'E



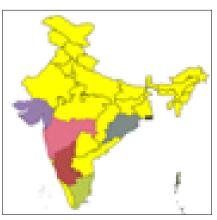




Fig. 1. Geographical location of various seed sources and variation.

# **Dehydrogenase activity**

The dehydrogenase activity differed significantly among the seed sources and periods of storage. Among the seed sources, Trichy recorded the highest dehydrogenase activity of 1.032, followed by Mettupalayam (0.942) and both were on par with each other. The minimum value was recorded in  $S_2$  (0.720). For the period of storage, while increase in the storage period, there was a progressive reduction in dehydrogenase activity (Fig. 6). It ranged from 0.897 to 0.766 during the initial and final period of analysis. While viewing the interaction effects, Trichy at the initial period had maximum activity of 1.241, followed by Mettupalayam at the fourth month of storage (0.987). The reduced dehydrogenase activity of 0.616 was observed in  $S_2$  at 12 months.

# **Catalase activity**

The catalase activity was calculated using phosphate buffer (pH 6.8), 4 mL of 0.3 N hydrogen peroxidase (substrate) and 0.2 mL of enzyme extract. The substrate was then titrated against  $0.1 \, N \, KMnO_4$ .

# $\boldsymbol{\alpha}$ - amylase activity

The samples were ground in 1.8 mL of cold 0.02 M sodium phosphate buffer (pH 6.0) and spun at 2000 RPM for 10 min. The resulting mixture contained 1 mL of a 0.067 % starch solution and 0.1 mL of enzyme extract. The reaction was halted after a 10 min incubation at 25°C by adding 1 mL of iodine solution, which comprised 60 mg of potassium iodide and 6 mg of iodine in 100 mL of 0.05N hydrochloric acid.

#### **Results**

The Simarouba glauca seeds collected from fifteen sources were evaluated for variation in storage potential. The following are the results of the experiment.

# **Germination (%)**

The result on germination per cent showed significant variation due to seed sources and periods of storage (Table 2). Among the sources, highest germination of 75.75 % was recorded in Akola ( $S_{13}$ ) followed by Mettupalayam ( $S_{5}$ ) (75.00) and Trichy ( $S_{6}$ ) (74.00), which were on par with each other statistically. The minimum percent of 56.25 was recorded in Coimbatore ( $S_{4}$ ).

#### **Seed oil content (%)**

The seed oil content was significantly ( $p_{0.05}$ = 0.003) influenced by the seed sources and periods of storage. Among the sources, Trichy (53.63 %) seed source performed well and showed maximum oil content, followed by Mettupalayam (51.43 %). Whereas, during the storage period, maximum oil content was observed in the initial month of storage (48.57 %) and decreased over time (43.33 %) of storage. As the storage period advanced, the loss in oil content was significant. Also, the total loss from the initial to the final period of storage was 5.24 %. Fig. 2 shows the variability pattern in seed oil content due to the period of storage and the source of collection.

# Seed protein content (%)

The variation in protein content among the seed sources and periods of storage was recorded. Among seed sources, Akola  $S_{13}$  recorded maximum protein content of 24.38 % followed by Mettupalayam (22.27 %). The least was recorded in Shankaranti  $S_{12}$  (14.35 %). Irrespective of sources, the seeds in the initial period showed maximum protein (19.05 %), which was reduced to 17.25 % at twelve months after storage. While reviewing deeply, a significant loss in protein content was noticed as the storage period advanced, with a gross loss of 1.8 % from the initial value. Fig. 3 shows the impact of seed source and duration of storage on seed protein content. The results showed that no significant variations were noticed for the interactions of seed sources and periods of storage.

## **Total phenol content**

Differences in phenol content varied significantly among

seed sources and storage periods, as shown in Fig. 4. Among the sources evaluated phenol content was high in Trichy (3.213 mg g $^{-1}$  of seed), which was significantly different from all other sources. The low phenolic content was recorded in S $_{12}$  (2.389 mg g $^{-1}$  of seed), followed by S $_{4}$  (2.465 mg g $^{-1}$  of seed), which were on par with S $_{15}$ , S $_{14}$  and S $_{3}$  (2.494, 2.538 and 2.557 mg g $^{-1}$  of seed), respectively. While advancing storage period, it was found that decreased activity of phenols decreased from 3.301 mg g $^{-1}$  of seed to 2.260 mg g $^{-1}$  of seed during initial 12 months of storage, respectively. While considering the interaction effect, Trichy at the initial period registered maximum phenol content of 3.683 mg g $^{-1}$  of seed, followed by Nagercoli (3.629 mg g $^{-1}$  of seed).

# Polyphenol oxidase activity (OD value)

Among the seed sources and periods of storage, the results were positively related with each other and polyphenol oxidase activity. While comparing different seed sources, Trichy recorded the highest polyphenol oxidase activity (0.184), followed by Mettupalyam (0.174), which were on par with each other in (Fig. 5). The lowest polyphenol oxidase activity was recorded by  $S_{12}$  0.096. As the storage period increased, a decrease in polyphenol oxidase activity was observed from 0.153 to 0.127. No significant effect was observed for interaction.

# **Dehydrogenase activity (OD value)**

The dehydrogenase activity differed significantly among the seed sources and periods of storage. Among the seed sources, Trichy recorded the highest dehydrogenase activity of 1.032, followed by Mettupalayam (0.942) and both were on par with each other. The minimum value was recorded in  $S_2$  (0.720). For the period of storage, while increase in the storage period, there was a progressive reduction in dehydrogenase activity (Fig. 6). It ranged from 0.897 to 0.766 during the initial and final period of analysis. While viewing the interaction effects, Trichy at initial period had maximum activity of 1.241 followed by Mettupalayam at fourth month of storage (0.987). The reduced dehydrogenase activity of 0.616 was observed in  $S_2$  at 12 months.

# **Catalyse activity**

Statistically significant differences were observed among seed sources and periods of storage (Fig. 7). Maximum catalase activity was registered in Trichy (57.47  $\mu g \, H_2 O_2 m g^1$  of seed min<sup>-1</sup>),

Table 2. Effect of seed sources and period of storage on seed germination (%)

Trantmont and	Name of the seed sources (S)	Period of storage				Maan
Treatment code		Initial (P₀)	4 months (P <sub>1</sub> )	8 months (P <sub>2</sub> )	12 months (P <sub>3</sub> )	Mean
S <sub>1</sub>	Thiruvannamalai	9.14	9.03	8.56	6.31	8.26
$S_2$	Vandavasi	9.48	9.21	8.74	6.42	8.46
$S_3$	Salem	10.07	9.98	9.73	6.94	9.18
$S_4$	Coimbatore	9.13	9.03	8.91	6.43	8.37
<b>S</b> <sub>5</sub>	Mettupalayam	13.14	13.08	12.56	9.94	12.18
$S_6$	Trichy	11.00	12.97	11.37	8.61	10.98
$S_7$	Mukkombu	9.46	9.21	8.24	6.23	8.28
S <sub>8</sub>	Thoothukudi	10.45	10.24	9.54	7.72	9.48
$S_9$	Nagercoil	9.63	9.32	8.45	6.41	8.45
S <sub>10</sub>	GKVK – 1	10.30	10.18	9.55	7.15	9.29
S <sub>11</sub>	GKVK – 2	9.52	9.24	8.91	6.14	8.45
S <sub>12</sub>	Shankaranti	11.20	11.00	10.78	8.07	10.26
S <sub>13</sub>	Akola	13.76	13.27	12.46	10.20	12.42
S <sub>14</sub>	Bhubaneshwar	10.46	10.18	9.43	8.17	9.54
S <sub>15</sub>	S.K. Nagar	10.39	10.22	9.72	7.49	9.45
	Mean	10.47	10.41	9.79	7.47	
		S	М	SxM		
	SEd	0.496	0.256	0.782		
	CD (P=0.05)	0.993	0.512	1.563		

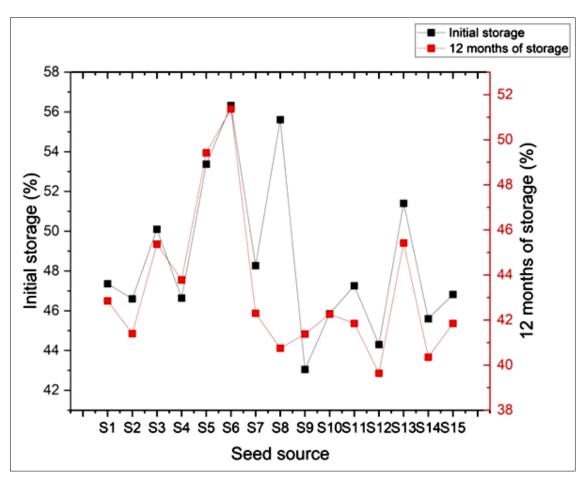
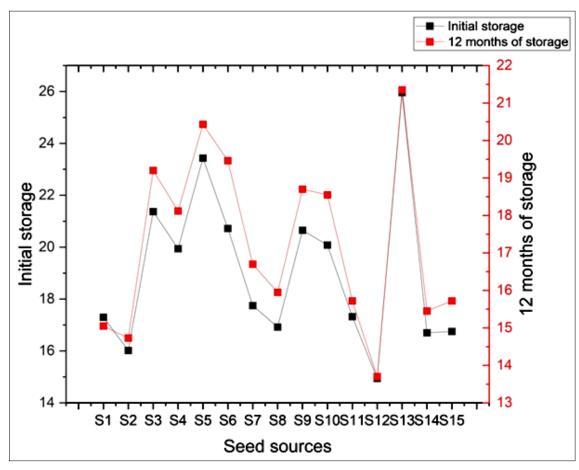


Fig. 2. Effect of seed sources and period of storage on seed oil content.



**Fig. 3.** Effect of seed sources and period of storage on seed protein content.

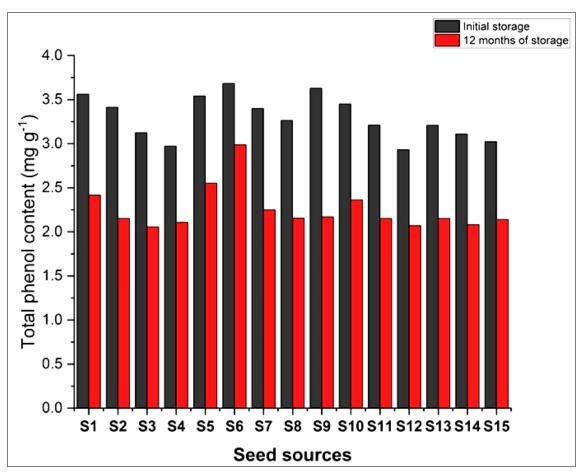


Fig. 4. Effect of seed source and period of storage on total phenolic content (mg g<sup>-1</sup>).

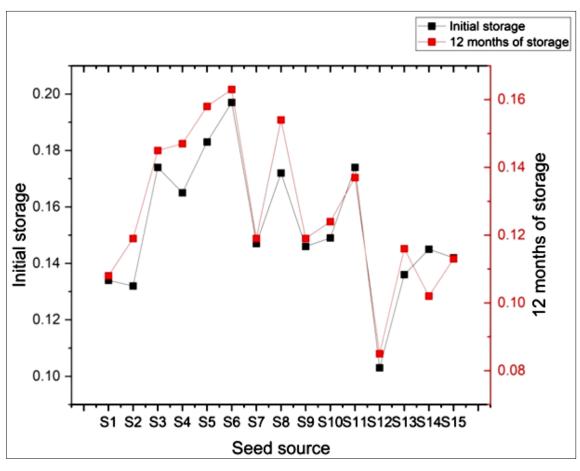


Fig. 5. Effect of seed source and period of storage on poly oxidase activity.

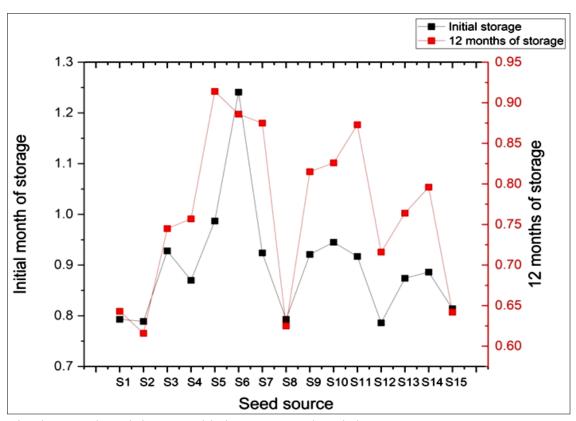


Fig. 6. Effect of seed source and period of storage on dehydrogenase activity (OD value).

which was independently superior to other sources. There is not much difference in catalase activity among the seed sources.

# a - amylase activity

The results were recorded for  $\sigma$  - amylase activity. The results showed significant variation between seed sources and periods of storage. The highest activity of  $\sigma$ -amylase was noticed in Trichy (0.157 mg maltose  $g^1$  of seed min<sup>-1</sup>), which was on par with Mettupalayam (0.1533 mg maltose  $g^1$  of seed min<sup>-1</sup>). The minimum activity was recorded by  $S_{11}$  (0.123 mg maltose  $g^1$  of seed min<sup>-1</sup>) and  $S_2$ ,  $S_{12}$  (each 0.124 mg maltose  $g^1$  of seed min<sup>-1</sup>). Among the periods of storage, initial amylase activity was 0.178 mg maltose  $g^1$  of seed min<sup>-1</sup>, which was decreased to 0.88 during twelfth months of storage (Fig. 8). While considering the interaction effect, the magnitude of decrease was higher, that is from 0.198 ( $S_6$  at  $P_0$ ) to 0.073 mg maltose  $g^1$  of seed min<sup>-1</sup>. ( $S_2$  at  $P_3$ )

**Table 3.** Effect of seed source and period of storage on free fatty acid

# Free fatty acid

The results for sources and periods of storage showed statistically positive differences for free fatty acid content below (Table 3). The minimum free fatty acid content of 1.13 % was noticed in Trichy, which was on par with Mettupalayam (1.14 %) and Akola (1.15 %), as against the maximum content of 1.72 % was recorded by  $S_{12}$ . A steady increase in free fatty acid content was noticed as the storage period advanced. Despite the storage period, a high value of 2.76 % was observed over twelve months of storage when compared to the initial period (0.55 %).

# **Discussions**

The study found that longer storage periods negatively impacted the oil content of seeds. However, the rate of

Treatment code	Name of the seed sources (S)	Period of storage				Moon
		Initial (P₀)	4 months (P <sub>1</sub> )	8 months (P <sub>2</sub> )	12 months (P <sub>3</sub> )	Mean
S <sub>1</sub>	Thiruvannamalai	0.54	0.96	1.53	2.25	1.32
$S_2$	Vandavasi	0.61	1.04	1.65	2.35	1.41
S₃	Salem	0.54	0.93	1.88	2.54	1.47
$S_4$	Coimbatore	0.57	0.95	1.76	2.95	1.55
<b>S</b> <sub>5</sub>	Mettupalayam	0.48	0.76	1.25	2.06	1.14
$S_6$	Trichy	0.43	0.74	1.31	2.10	1.13
$S_7$	Mukkombu	0.56	1.05	1.95	3.06	1.65
S <sub>8</sub>	Thoothukudi	0.50	0.85	1.76	3.18	1.57
$S_9$	Nagercoil	0.68	0.92	1.89	3.07	1.64
S <sub>10</sub>	GKVK – 1	0.62	0.86	1.90	2.96	1.58
S <sub>11</sub>	GKVK – 2	0.47	0.85	1.88	2.84	1.51
S <sub>12</sub>	Shankaranti	0.69	0.97	1.79	3.45	1.72
S <sub>13</sub>	Akola	0.48	0.76	1.35	2.01	1.15
S <sub>14</sub>	Bhubaneshwar	0.52	0.87	1.62	2.73	1.43
S <sub>15</sub>	S.K. Nagar	0.59	0.92	1.85	2.79	1.53
	Mean	0.55	0.90	1.71	2.76	
		S	М	SxM		
	SEd	0.067	0.034	0.135		
	CD (P=0.05)	0.133	0.069	0.026		

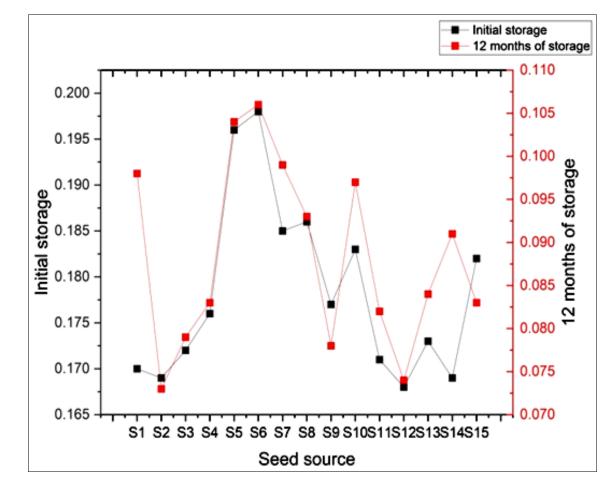
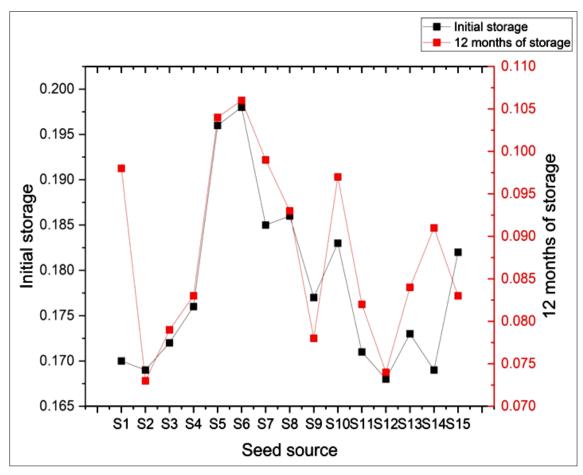


Fig. 7. Effect of seed source and period of storage on catalase activity (OD value).



**Fig. 8.** Effect of seed source and period of storage on  $\alpha$  - amylase activity (OD value).

decline in seed quality varied significantly among the different crops examined (17). As Simarouba glauca is greatly accepted for its oil and medicinal value, the storage of seeds and their prolonged viability is necessary for its vigorous growth and care. As seed ageing is one of the intriguing and challenging scientific problems of universal concern, it directly affects the seeds' biochemical processes (18). Therefore, proper attention is needed to understand the effects of seed storage and source. Based on the mentioned seed sources and storage pattern, the study was taken up to investigate the storage potential of Simarouba glauca from six agroclimatic zones of Tamil Nadu, along with Karnataka, Maharashtra, Orissa and Gujarat. The result on the biochemical attributes showed that the storage shows low oil content and high fatty acid, this may be due to the production of free fatty acid, since these fatty acids produce free radicals which readily combine with enzyme and nucleic acids causing their inactivation and thus reducing the germination (19). The above results are by the nutritional and storage effects on oil content and composition of sesame seeds (20). Similarly, variation in oil and free fatty acid content across different seed sources was reported in Madhuca latifolia (21). Also, the highest protein content was obtained in Akola (24.38 %), followed by Mettupalayam and the protein content also decreased with age due to prolonged ageing, but the decrease was slow as compared with other biochemical attributes. This can be one of the reasons for the maintenance of the viability of the seed for 12 months. The decreased level of the seeds was in agreement with the findings of (22) in Acaia nilotica; in Sesame (23). The observation on total phenol content and polyphenol oxidase activity is interlinked. The phenolic compounds are oxidized by polyphenol oxidase. So, the activity of polyphenol oxidase is determined either by presence or absence of phenolics. Total phenol content was decreased with ageing, which may explain the reduced activity of polyphenol oxidase. The results conformed to the previous findings (24). Measuring enzyme activity was one of the first biochemical methods used to assess seed deterioration and predict germination potential. Certain enzymes lose their activity as seed viability declines. However, the enzyme activity of individual seeds can vary based on the availability of precursors needed for enzyme synthesis. Polyphenol oxidase is a copper protein and widely occurring in nature and catalyzes the aerobic oxidation of phenolic compounds into quinones and also responsible for higher seed colour change from light to dark colour. Also all the enzyme activities were found to be higher in the Trichy source compared to other seed sources. The activity of catalyzed the oxidation of IAA. The presence of polyphenol oxidase inhibits the IAA oxidation, which leads to the induced activity of peroxidase to counteract with inhibitory effect of polyphenol oxidase. A similar observation was previously recorded (25). Also, a decrease in catalase and amylase with storage periods demands better utilization of seeds of simarouba of use. Generally, catalase and peroxidase activity decreased due to the aggregation of hydrogen peroxide as the seed neutralized free radicals. As the trend was similar during the initial and twelve months after storage this also led to a steep decline in viability from 87 to 52 % throughout storage. From a holistic view, three

seed sources of Simarouba glauca viz., Akola, Trichy and Mettuaplayam possess better storability and are worthy for future afforestation. The present study examined variations in seed sources, with seeds collected from Akola (Maharashtra) exhibiting the highest hundred-seed weight, followed by Mettupalayam and Trichy, while the lowest was recorded in Mukkombu, Tamil Nadu. Similar variations in physical parameters due to seed sources have been reported in teak (26) and sandalwood (27). Germination percentage, a key indicator of seed growth potential and survival, showed that the heavier seeds from Akola had the highest germination rate (89 %), with minimal reduction up to the eighth month, followed by a sharp decline to 48 % between the ninth and twelfth months of storage. A similar trend was observed in seeds from Mettupalayam. These results align with the previous study (11) in Acacia nilotica.

Beyond initial germination potential, seed storage capacity is also influenced by its source. In this study, seeds from Trichy recorded the highest oil content (53.63 %), followed by Mettupalayam (51.43 %) and showed the lowest free fatty acid content (1.13 %) at the beginning of storage. However, as storage duration increased, free fatty acid content rose from 0.55 % to 2.76 % from initial to twelve months, also the study showed a progressive reduction of oil content and an increase of fatty acid. During storage, oil degradation into free fatty acids is catalyzed by the enzyme lipase. The increase in free fatty acids negatively affects germination, as they generate free radicals that interact with enzymes and nucleic acids, causing their inactivation and reducing seed viability (26). Additionally, long-chain unsaturated fatty acids cause mitochondrial swelling, impairing their function. The most significant decline in oil content occurred between four and eight months of storage, with oil loss and free fatty acid increase intersecting at 12 months, leading to reduced germination and vigor due to free radical formation, which disrupts biochemical processes.

Akola-sourced seeds had the highest protein content (24.38 %), followed by Mettupalayam (22.47 %). Protein content decreased with prolonged storage, though the rate of loss was lower compared to oil content, likely contributing to the viability of high-oil-content seeds for up to twelve months. Protein degradation occurs due to the conversion of free amino acids during aging, consistent with findings in Withania somnifera (28). Total phenol and polyphenol oxidase activity declined over time, with Akola, Trichy and Mettupalayam seeds showing better performance, possibly due to moderate phenol content. Poor storability in other seed sources may result from free radical formation through epidermal phenolics, leading to membrane integrity loss, as observed in sunflower (29).

Enzyme activity measurement is an early biochemical technique used to assess seed deterioration and predict germination potential. Enzyme activity varies among seeds depending on precursor availability for specific enzyme synthesis. Polyphenol oxidase, a copper-containing protein widely found in nature, catalyzes the aerobic oxidation of phenolic compounds into quinones. In this study, enzyme activity was highest in Trichy-sourced seeds, followed by Mettupalayam. Peroxidase and polyphenol oxidase activities

declined over storage, suggesting that polyphenol oxidase may have influenced peroxidase activity. Trichy seeds also exhibited the highest dehydrogenase activity, followed by those from Mettupalayam.

#### Conclusion

This study aimed to investigate the mechanism behind the loss of oil content during prolonged seed storage. As enzymes convert oil into free fatty acids, it indirectly affects the plant's physiology and germination capacity. Therefore, the study recorded the essential biochemical characteristics of Simarouba glauca, a medicinal plant. The studies on biochemical attributes showed that Trichy registered higher oil and total phenol content and the lowest fatty acid content. However, Akola -S<sub>13</sub> (24.38) recorded higher seed protein content. The studies on enzyme activities showed that all the measured enzyme activities were highest in the Trichy-S6 seed source. These included polyphenol oxidase (0.184), dehydrogenase (1.032), peroxidase (0.0314), catalase (57.47) and α-amylase (0.157). Overall, three seed sources, Akola, Trichy and Mettupalayam, possess better storability potential and are worthy of future afforestation.

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#### **Authors' contributions**

DS carried out the collection of plant materials and seed source collection, collected the data, biochemical attributes and drafted the manuscript. IS helped in the analysis of biochemical attributes and facilities required and soil sample collection. KTP and SUK assisted in the design of the study and helped in the statistical analysis and MVJ provided an interpretation and appraisal of the study.

# **Compliance with ethical standards**

**Conflict of interest:** Authors do not have any conflict of interests to declare.

# **Ethical issues:** None

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