

Physio-Biochemical Responses During Seed Desiccation in *Elaeocarpus tuberculatus* Roxb.

Research Article

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Abstract

Understanding the physio-biochemical responses in *Elaeocarpus tuberculatus* Roxb. (often called the Wild Rudraksha) involves looking at its behaviour as a recalcitrant (desiccation-sensitive) seed. Unlike common garden seeds that can be dried and stored for years, these seeds are native to moist, tropical environments (like the Myristica swamps of the Western Ghats) and die when they lose much water. The most critical change is the drop in moisture content during desiccation in *E. tuberculatus*, the seed loses its ability to germinate. As water leaves the cells, the phospholipid bilayer of the cell membranes becomes unstable. This leads to electrolyte leakage, where essential minerals and salts seep out of the seed resulting in the loss of viability. The lack of water triggers the formation of Reactive Oxygen Species (ROS). These “free radicals” attack the fats (lipids) in the cell membranes, creating malondialdehyde (MDA). High MDA levels are a primary marker of seed death. Free radical scavenging antioxidant enzymes like peroxidase (PO) and polyphenol oxidase (POD) show a sharp decrease in activity, leaving the seeds vulnerable to oxidative stress. The results of the present study suggests that the lack of raffinose-mediated vitrification and uncontrolled oxidative stress are the primary drivers of recalcitrant nature of seeds in *E. tuberculatus*. These findings provide a vital physiological framework for the *ex-situ* conservation and cryopreservation of this endemic species.

Keywords: *Elaeocarpus Tuberculatus* Roxb; Seed Viability; Desiccation; Physiology; Biochemistry

Introduction

The Western Ghats of India, one of the World's hotspots of biodiversity is characterised by altitudinal, as well as latitudinal gradients with tropical climatic factors. The environmental heterogeneity makes this area an extremely heterogeneous biogeographic zone, with a large amount of plant and animal diversity and support a high degree of endemism (30%) having 4500 higher plant species [1]. Many of the trees of this biome are evolutionarily primitive as far as their morphological, anatomical or physiological parameters are considered. The main propagule of these trees are large sized seeds that generally prefer riparian climate for the successful sustenance of genetic diversity. Large sized seeds, high

moisture content, carbohydrate as predominant reserve food and vascularisation of the integuments are considered as primitive seed characters [2].

Elaeocarpus tuberculatus Roxb. (Elaeocarpaceae) commonly known as the Wild Rudraksha, is a prominent canopy tree species restricted to the evergreen and Shola forests of the Western Ghats, India reaching a height of 20-30 meters [3]. The species serves as a vital keystone component in riparian and Myristica swamp ecosystems. Additionally, the plant is noted for its medicinal properties and highly ornamented seeds. Flowers are white in colour. The fruit is drupe and contain a single seed with two halves. Despite its ecological and economic importance, the natural regeneration

of this species is severely hindered by the recalcitrant nature of its seeds. Another important characteristic of this species is the seed years, i.e. the species exhibits masting habit of 2-5 years. The edaphic factors of their habitat also have a marked effect on the production of seeds. Unlike orthodox seeds that undergo maturation drying to achieve a quiescent state, seeds of *E. tuberculatus* are shed at high moisture contents and remain metabolically active. Any reduction in their critical moisture threshold triggers a sequence of deteriorative physio-biochemical changes, including membrane disruption, electrolyte leakage, and the accumulation of reactive oxygen species (ROS). High vulnerability to viability loss, predation of the seeds and the poor seed years will definitely pose threat to most of the endemic taxa of the mega diversity spot of Western Ghats. This study aims to investigate these changes to identify the specific metabolic 'tipping points' that lead to viability loss giving emphasis to the changes in sugars and anti-oxidant enzymes that will provide vital data for the ex-situ conservation and long-term storage of this ecologically sensitive species.

Materials and Methods

Fruit/seeds of *Elaeocarpus tuberculatus* were harvested from five healthy selected matured trees from Ponnudi hills, Trivandrum, Kerala (8°45' and 8°47'N latitude and 77°1' and 77°4' E longitude) at an altitude of 700m. The location experiences a mean temperature of 16-30°C, relative humidity in the range of 60-90%, light intensity of 2000-20000 lux/m² with an average rainfall of 2500-3000mm/year. To avoid damage and soil borne pathogens, mature fruits were collected by gently shaking of fruit bearing branches. Collected fruits were brought to the laboratory in polyethylene bags, washed and spread on absorbent paper to check fungal and other microbial attack before de-husking.

Desiccation and viability assessment studies

Seeds were spread in the laboratory conditions to determine the change in moisture content and germination during desiccation on air drying. The whole seeds were sampled at regular intervals (48 hr) from 0 to 18 days after harvest for the analysis of moisture content. Another set of seeds in triplicates was used for germination test. Before sowing for germination, the seeds were transferred to humidity chamber in order to reduce the imbibitional damage while transferring directly into the water. Moisture content (MC) of the seeds at each interval was determined according to ISTA rules [4], High Constant Air Owen method, i.e., by drying at 130±1°C for 1 hour and was calculated on fresh weight basis and expressed as percentage of moisture (MC).

For estimation of Seed vigour index, ten seeds each in triplicates were taken from the seed lots during different desiccation periods at respective intervals (48 hr from 0 to 18 days after harvest) and sown in garden pots filled with clean sand and kept in the shady area for seed germination. The number of seeds germinated on each day was noted and the seed vigour index (SVI) was calculated according to the formula given by [5].

Tetrazolium reduction activity assay

Dehydrating seeds collected at specific intervals mentioned

elsewhere after cutting into pieces were incubated in 1% 2, 3, 5-triphenyl tetrazolium chloride (in 10 mM sodium phosphate buffer pH 7.2) in darkness for two hours. After three washes in distilled water, 5 ml acetone was added and incubated at room temperature in darkness for 16-18 hours to elute the red colour formazan formed. Optical density of the elute was measured at 480 nm in Systronics model 106 spectrophotometer and expressed as A 480 nm/g.fwt.

Effect of desiccation in seeds were evaluated by measuring electrolyte leakage and lipid peroxidation. Five seeds in triplicate from different desiccation period were soaked in 25 ml distilled water after chopping into pieces and covered with a lid to reduce evaporation during incubation and also to check dust contamination. After 24 hours the electrical conductivity of the leachate was measured using a dip cell conductivity meter (Systronics DDR 306). The specific conductance was expressed as $\mu\text{scm}^{-1}\text{g}$.

Lipid peroxidation was measured by measuring the concentration of thio barbituric acid reactive substances (TBRS), equated with malondialdehyde (MDA) [6]. One-gram fresh tissue was homogenized in 1 ml distilled water with sterilized silica in a mortar and pestle. 2 ml of 5% thiobarbituric acid (TBA) prepared in 20 % trichloroacetic acid was added to the homogenate. The reaction mixture was incubated in a boiling water bath for 30 minutes. A Pink colour was developed by the reaction of various lipid peroxidation products (viz. MDA) with TBA. The test tubes were immediately transferred to 0°C in a freezer to stop the reaction. The colour of the supernatant after centrifugation (at 3000 rpm for 15 minutes) was measured at 540 nm and 600nm respectively. After subtracting the absorbance of the non-specific (at 540 nm) the net absorbance was expressed at 540 nm $\text{mg}^{-1}\text{dr.wt.}$ of the seeds.

Biochemical analysis

Metabolites including total soluble sugars (TSS), starch, phenol, protein, lipids and individual sugars (glucose, galactose, arabinose, raffinose, and sucrose) and the activity of enzymes like peroxidase, polyphenol oxidase were also analysed for studying the stress induced changes occurring during the desiccation of the seeds. All operations except enzyme assay were carried out immediately after moisture analysis. For enzyme assay the de-coated seeds were soaked in water before assay.

Extraction and estimation of metabolites

One gm tissue of sample was ground in known volume of 80% ethanol (v/v) in distilled water and centrifuged at 4000 rpm for 10 minutes. The residue was again washed thrice with ethanol and after centrifugation the supernatant was collected and made up to 10ml with 80% ethanol. The supernatant served as source for the quantification of total sugars, phenolics and amino acids. Five ml of the extract was kept in a china dish and kept in hot air oven at 60°C and was dissolved in 5 ml distilled water and was centrifuged at 3000 rpm for 10 minutes and the supernatant served as the source for soluble sugar estimation. The left-over residue was ground with 4 ml 30% Perchloric acid (PCA), centrifuged at 3000 rpm for 10 minutes each and the residue was re-extracted with 15% PCA. The combined supernatant was used for the starch estimation.

Total soluble sugar (TSS) content was estimated by using the method proposed by Montgomery [7]. Total soluble sugar was calculated using glucose standard graph and expressed as mg g⁻¹ dr. wt. Content of starch in tissues was estimated according to Mc Cready et al. [8]. The starch content was calculated by multiplying the value with a constant value of 0.9 and expressed as mg g⁻¹ dw using D-Glucose as the standard. Phenolic content of the tissues was estimated by following the method of Swain and Hillis [9] and was calculated from standard graph drawn using catechol as the standard and expressed as mg g⁻¹ dr. wt. Total protein content in *E. tuberculatus* seeds were determined using the method of Lowry et al. [10]. Bovine serum albumin (BSA) was used as standard and the total protein quantity expressed as mg g⁻¹ dr. wt. Total extractable lipids were estimated using the method of Bligh and Dyer [11]. Total weight of lipids in the tissues and expressed as mg g⁻¹ dr. wt.

Extraction and Estimation of different sugars through HPLC

Sample preparation

One gram seed sample was soaked in 95% ethanol and crushed in a mortar to get a fine paste. The mixture was then refluxed for 2 hours in a water bath. The clear filtrate from the reflux was concentrated in a rotary evaporator to remove the alcohol. Distilled water was then added to the mixture to make the volume to 5 ml. Insoluble materials if any, was filtered off. Lipids were removed by adding petroleum ether and were separated in a separating funnel. The aqueous extract thus obtained was filtered through a Whatman No. 1 paper and the filtrate was used for HPLC analysis [12].

HPLC analysis

The aqueous solutions obtained in the sample preparation were filtered through a 0.45µm Nylon filters and then passed through a guard column in HPLC (Shimadzu LC 2010A HT) system. Acetonitrile-water (70:30) mixture was used as the mobile phase in the column of LC-NH₂ at a flow rate of 1ml/min. 20µl of the sample was injected into the column for detecting the sugars. Refractive index detector was used for the detection of sugars. Standard solution of the sugars like glucose, galactose, arabinose, raffinose, sucrose, glycerol and ethylene glycol at concentration of 1mg/5ml were injected and the peak areas were calculated by the automated computer integrator. Concentrations of the sugars were calculated in comparison with those of the standard solutions. Retention times for glucose, galactose, arabinose, raffinose, and sucrose were also noted.

Extraction of enzymes

One-gram fresh seed tissue of each sample was homogenised in a pre-chilled mortar and pestle with a pinch of purified sand and 0.1 M cold sodium phosphate buffer (pH 7.0). The homogenate was centrifuged at 5000 rpm for 20 minutes using refrigerated centrifuge and the supernatant was collected. Proteins from the supernatant were precipitated in cold acetone twice and re-suspended in the extraction buffer after the evaporation of the acetone. The extract was centrifuged and the supernatant served as the enzyme source for both peroxidase and poly phenol oxidase assay. All the extraction procedures were carried out at 4°C.

Peroxidase enzyme assay

Peroxidase enzyme activity was measured according to the method of Chance and Maehly [13] by recording the change in absorbance at 470 nm due to oxidation of guaiacol in presence of hydrogen peroxide. The change in absorbance was recorded in spectrophotometer (Systronics model 106) at 470 nm. The enzyme activity was measured as change in absorbance per minutes using guaiacol as substrate.

Polyphenol oxidase

The polyphenol oxidase activity was measured by following the method of Yamaguchi et al. [14]. The assay mixture consisted of 1.0 ml 0.01M catechol and 1.0 ml 0.1M proline in 2.0 ml 0.1M Sodium phosphate buffer (pH 6.8). 1.0 ml enzyme extract was added quickly and the kinetics of the enzyme was recorded at 525 nm in spectrophotometer (Systronics 106) and the enzyme activity was expressed as change in absorbance per minute.

Statistical analysis

The data obtained from the experiments were analysed following Analysis of Variance (ANOVA) and the ratio obtained were checked for significance at 5% level. The means of each treatment were separated following the Least Significance Difference (LSD) by Duncan's Multiple Range Test (DMRT) at 5% level.

Results

Elaeocarpus tuberculatus belongs to typical evergreen canopy species and are restricted to cool, humid riparian environment. The fruit of *E. tuberculatus* is a drupe with a fleshy pericarp and matures approximately 150 days after anthesis (DAA). The fruit/seed dispersal is through the agency of frugivores, especially monkeys, bats and giant squirrels. Natural regeneration is hindered by several biotic and abiotic factors. A large number of beetles are found as seed predators in the field and also under storage of the seeds (Plate 2). Some of them make holes in the seed coat and find their way in to the seed locule. Large number of larvae of insects is found inside the fruit during different stages of fruit development. Most of them lay eggs when the fruits are young. A large number of young fruits with holes are found lying beneath the mother trees (Figure 8) (A, B, C and D).

Effect of desiccation on Moisture content and germination

Freshly harvested seeds of *E. tuberculatus* possessed 49.21% MC with 91.67 % germination during the span of 23 days (Figure1). When the seeds are exposed to open laboratory conditions (28±2°C & 65 % RH), no significant change in moisture and germination percentage was observed up to the 4th day. Subsequently a gradual decrease in MC and germination percentage was recorded. On the 12th day MC reduced to 30.77% with 58% reduction in germination percentage compared to the control seeds. As the desiccation reached the 16th day, seeds have only 19.61% MC with only 20% germination and complete viability loss in *E. tuberculatus* seeds was recorded at 17.79 % MC.

Effect of desiccation on Seed vigour index

Increase in desiccation period affected the time taken for

germination of the seeds thereby the seed vigour index (SVI) also decreased accordingly. SVI value of *E. tuberculatus* was always less than 1 (Figure 2), since the seeds took more than 20 days for germination. Fresh seeds took 23 days to register 90±2.5 % germination. Their seed vigour index value was 0.55. Index value decreased gradually from an initial value of 0.55 to 0.07 on the 12th day. Even though mean germination days increased gradually from the 12th day onwards there was no change in vigour index and attained a constant value of 0.07.

Effect of desiccation on tetrazolium staining pattern and formazan concentration

Studies on the tetrazolium staining pattern and the colour development on the embryo showed marked variation during different desiccation periods. Embryos of fresh seeds showed uniform intense red colour with TZ. During the successive days of desiccation, staining pattern and the intensity of staining reduced. On the 6th day onwards margins of the cotyledons become unstained and the colour became rose red. By the 12th day the cotyledonary margins become white in colour and the embryonal axis was rose red. On the 16th day the axis was feebly stained and no staining on the cotyledons (Table 1).

The measurement of formazan concentration was in agreement with the moisture loss and tetrazolium staining pattern. The decreasing trend in the absorbance of the formed formazan was found in *E. tuberculatus*. On the 12th day onwards the formazan concentration decreased rapidly (Figure. 3).

Effect of desiccation on leachate conductivity

Colour of the seed leachate was yellow in all the samples studied. More than two-fold increase in specific conductance has been recorded during the desiccation. Specific conductance of the leachate

Table 1: Tetrazolium staining pattern of the embryo during desiccation of *Elaeocarpus tuberculatus*

DAH	Moisture content (%)	Germination (%)	Tetrazolium staining pattern
0	49.21±0.11 ⁱ	91.67±1.33 ^b	The axis and cotyledons-stained deep red.
2	47.80±0.18 ^b	90.65±1.58 ^b	Uniformly stained. The vasculature on the cotyledons clearly visible
4	44.66±0.21 ^c	77.33±0.61 ^c	Uniform staining. Lighter in colour than in the previous stage
6	38.08±0.44 ^f	74.08±0.31 ^f	Axis and cotyledons uniformly stained with unstained margins.
8	37.26±0.45 ^f	67.28±0.63 ^e	Distal portions of the cotyledons stained red. White patches appeared on the axis.
10	34.88±0.25 ^e	53.17±0.48 ^d	Unstained area on the margins of the cotyledons increased. The axis not deeply stained
12	30.77±0.47 ^d	33.89±0.38 ^c	The cotyledonary margins more white in colour. Axis stained rose red
14	22.48±0.76 ^c	32.41±0.61 ^c	Only light staining on the axis and the cotyledons.
16	19.61±0.44 ^b	20.38±0.81 ^b	Only axis feebly stained
18	17.79±0.56 ^a		No Staining

*Values represent the means followed by ± standard error. The figures super scribed by the same letters in the same column are not statistically significant at p=0.05 level based on DMRT.

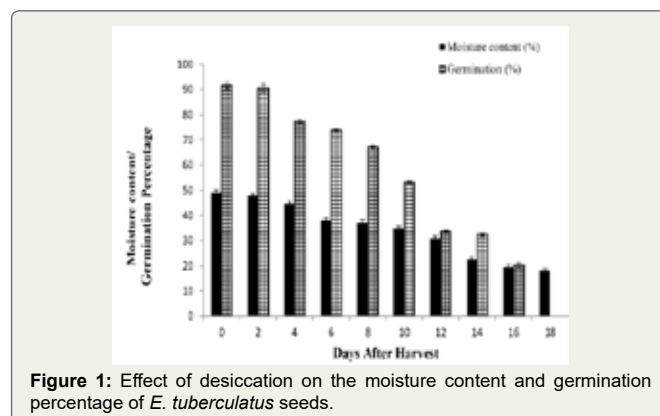


Figure 1: Effect of desiccation on the moisture content and germination percentage of *E. tuberculatus* seeds.

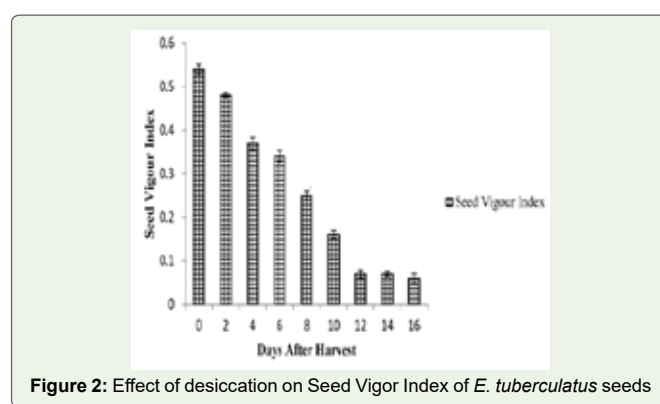


Figure 2: Effect of desiccation on Seed Vigour Index of *E. tuberculatus* seeds

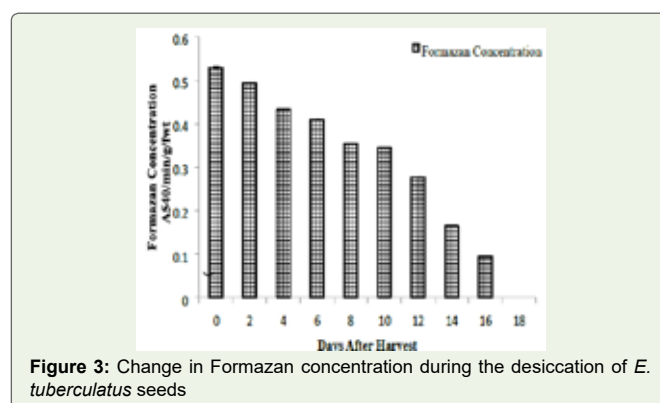


Figure 3: Change in Formazan concentration during the desiccation of *E. tuberculatus* seeds

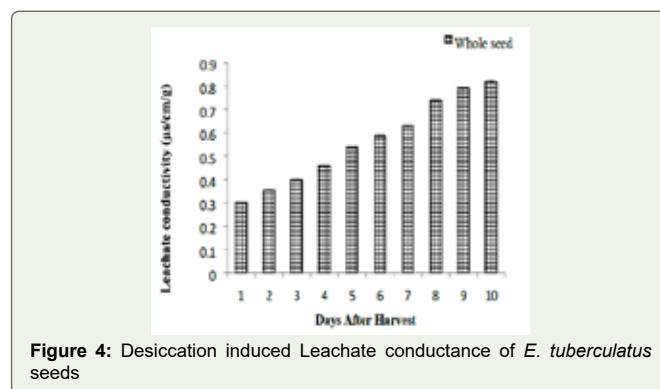


Figure 4: Desiccation induced Leachate conductance of *E. tuberculatus* seeds

Table 2: Change in Metabolite content during desiccation of *Elaeocarpus tuberculatus* seeds

DAH	Moisture content (%)	Total Soluble Sugar (mg g ⁻¹ d.wt.)		Starch (mg g ⁻¹ d.wt.)		Phenolics (mg g ⁻¹ d.wt.)		Protein (mg g ⁻¹ d.wt.)		Lipids (mg g ⁻¹ d.wt.)
		Emb	End	Emb	End	Emb	End	Emb	End	Whole seed
0	49.21± 0.11 ⁱ	52.8±0.21 ^a	58.6±0.38 ^a	123.0±1.35 ^h	157.2±1.47 ^h	97.8±0.952 ^a	160.9±1.36 ^{bc}	92.2±0.72 ^d	103.3±0.98 ^h	64.3±0.32 ^h
2	47.80±0.18 ^h	51.9±0.07 ^a	59.4±1.13 ^a	120.9±1.1 ^h	161.6±0.85 ⁱ	100±1.19 ^a	161.5±1.09 ^{bcd}	91.6±0.96 ^d	101.6±0.87 ^g	61.2±0.74 ^g
4	44.66±0.21 ^g	51.4±0.52 ^a	64.9±1.72 ^b	113.8±1.25 ^g	157.5±0.56 ^h	106.5±0.46 ^b	163.6±0.72 ^{cd}	91.6±0.73 ^d	98.5±1.73 ^f	54.3±1.2 ^f
6	38.08±0.44 ^f	58.1±0.44 ^{cd}	66.5±1.45 ^{bc}	96.8±0.7 ^e	138.9±0.55 ^g	106±1.76 ^b	143.3±1.15 ^a	86.4±0.55 ^c	99.3±0.48 ^f	53.3± 1.2 ^{ef}
8	37.26±0.45 ^f	59.4±0.54 ^d	68.5±0.46 ^c	100.1±0.5 ^f	133.4±1.15 ^f	114.2±1.21 ^c	159.2±1.14 ^b	85.3±1.28 ^c	86.6±0.43 ^e	51.0±0.42 ^e
10	34.88±0.25 ^e	59.7±1.24 ^d	68.5±0.24 ^c	84.6±1.1 ^d	103.8±1.38 ^e	128.3±0.48 ^d	165.1±1.33 ^{de}	82.1±0.72 ^b	82.1±1.08 ^d	36.9±0.98 ^d
12	30.77±0.47 ^d	56.8±1.12 ^{bc}	69.2±0.23 ^c	71.8±0.95 ^c	98.4±0.4 ^c	135.0±0.98 ^e	168.4±1.65 ^{ef}	79.4±1.27 ^b	76.8±0.78 ^c	34.4±0.89 ^c
14	22.48±0.76 ^c	57.5±0.67 ^{bcd}	58.1±0.52 ^a	67.6±1.14 ^b	77.4±1.1 ^b	139.5±1.24 ^f	170.3±1.26 ^f	79.3±1.10 ^b	68.9±1.97 ^b	31.3±0.63 ^b
16	19.61±0.44 ^b	55.9±0.61 ^b	59.4±0.91 ^a	57.1±1.2 ^a	53.7±0.88 ^a	131.3±1.41 ^d	173.5±1.27 ^g	76.5±0.59 ^a	64.3±0.92 ^a	28.2±0.44 ^a

*Values represent the means followed by ± standard error. The figures super scribed by the same letters in the same column are not statistically significant at p=0.05 level based on DMRT.

Table 3: Change in different sugar content during desiccation of *Elaeocarpus tuberculatus* endosperm- HPLC analysis

Moisture content (%)	Glycerol (mg g ⁻¹ d.wt.)	Glucose (mg g ⁻¹ d.wt.)	Sucrose (mg g ⁻¹ d.wt.)	Arabinose (mg g ⁻¹ d.wt.)	Raffinose (mg g ⁻¹ d.wt.)
49.38±0.6	0.259	0.546	0.322	5.491	Nil
45.81±1.3	0.227	0.175	0.334	5.936	Nil
39.25±1.6	0.239	8.231	2.314	8.427	Nil
34.23±1.4	0.258	13.375	8.835	12.720	Nil
30.25±1.9	0.270	13.751	10.889	19.686	Nil
20.20±1.5	1.543	6.808	19.133	18.461	Nil

Table 4: Change in different sugar content during desiccation of *Elaeocarpus tuberculatus* embryo- HPLC analysis

Moisture content (%)	Glycerol (mg g ⁻¹ d.wt.)	Glucose (mg g ⁻¹ d.wt.)	Sucrose (mg g ⁻¹ d.wt.)	Arabinose (mg g ⁻¹ d.wt.)	Raffinose (mg g ⁻¹ d.wt.)
49.38±0.6	0.144	0.724	0.150	Nil	Nil
45.81±0.9	0.178	2.633	0.784	Nil	Nil
39.25±0.05	0.226	4.238	0.815	Nil	Nil
34.23±1.0	0.442	6.653	0.893	Nil	Nil
30.25±0.5	1.249	10.171	4.722	Nil	Nil
20.20±0.04	0.461	8.209	4.073	Nil	Nil

from the desiccating seeds showed an increasing trend. Fresh seeds registered a value 0.301µs specific conductance which rose to 0.791µs on the 16th day. About 30% increase in specific conductance occurred between the 2nd and 4th day (Figure. 4).

The TSS content in the embryos and endosperm of the fresh seeds was 52.8 ±0.21mg g⁻¹dr.wt. and 58.6±0.38 mg g⁻¹dr.wt. respectively (Table 2). In embryo the increase in total soluble sugar content was negligible during desiccation without much significant difference (Table. 2). While in the endosperm the TSS content showed an increase up to the 12th day, with a maximum level and later decrease on the 14th and the 16th day of desiccation.

In embryos, starch content was high in fresh seeds (123.0 mg g⁻¹dr. wt. at 49.21% MC), thereafter, starch content decreased gradually as the desiccation continued (Table 2). Approximately 39% decrease in starch content has been observed during the desiccation. Similar pattern of decreasing trend has been observed in the endosperm also. Highest concentration of starch (161.6 mg g⁻¹dr.wt.) was registered at the 47.80% MC in the endosperm on the 2nd day of which slowly decreased with increase in desiccation.

In general, higher level of phenolic content has been recorded in

endosperm tissues with an initial content of 160.9 mg g⁻¹dr. wt. (Table. 2). As desiccation increased not much change was noticed. On the other hand, in embryos the phenolic content was low (156.2±1.2 mg) with increase in desiccation, the phenolic content also increased with a maximum of 199.5±0.08 mg g⁻¹dwt⁻¹ on the 14th day.

Analysis of total protein content in the seed of *E. tuberculatus* revealed that there was a gradual decreasing trend during desiccation in both embryo and endosperm (Table 2). Negligible difference in protein content has been observed during successive days of desiccation (Table.2).

Total lipid content of the whole seeds of *E. tuberculatus* on harvest was 64.0 ±0.32 mg g⁻¹dwt, which decreased significantly as the desiccation progressed (Table 2).

HPLC analysis of simple sugars

The HPLC analysis revealed the presence as well as increase of Glycerol, Glucose and sucrose content in the embryos of *E. tuberculatus* during desiccation. But arabinose and raffinose were not detected in any of the samples studied (Table 4). Of the sugars observed, glucose was in much higher concentration compared to glycerol and sucrose. As the moisture content of the seed decreased, the content of these

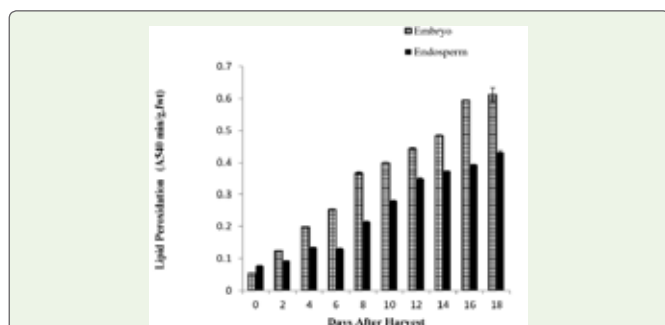


Figure 5: Effect of desiccation on lipid peroxidation on the embryo and endosperm of *E. tuberculatus* seeds

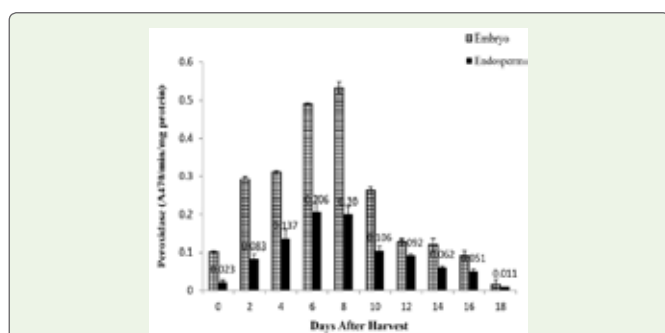


Figure 6: Change in Peroxidase activity in the embryo and endosperm of *E. tuberculatus* seeds during desiccation

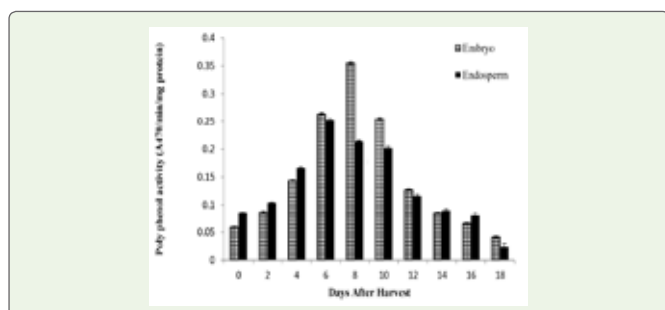


Figure 7: Change in poly phenol oxidase activity in the embryo and endosperm of *E. tuberculatus* seeds during desiccation

sugars increased. But during the later stages when the moisture content was 20.20%, the content of these sugars decreased (Table 4). There was a sharp increase in the levels of these sugars at 30.25% MC. At this MC, glucose content was 40% higher than the seeds with 34.25% MC. Similarly, the sucrose content increased abruptly at this stage. Threefold increase in the concentration of glycerol was observed when the MC was reduced to 30.25%.

In the endosperm glycerol content did not show much difference up to the 5th day of desiccation (MC 20.20%), which was very high (Table.3), whereas glucose, sucrose, and arabinose recorded a gradual increase from the initial moisture content (49.38%). Interestingly both raffinose and arabinose were not found in the embryos but arabinose was found in the endosperm at a higher concentration. The presence of raffinose was not detected in the seeds of *E. tuberculatus*.

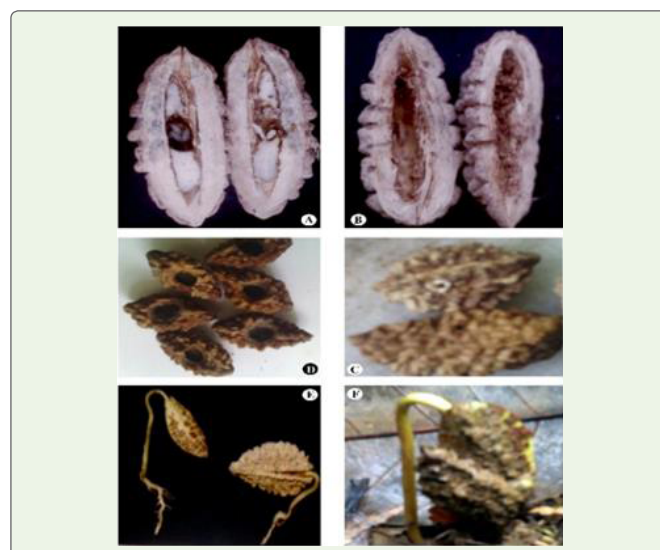


Figure 8: A, B and C infected seeds, D different sized seeds, E and F seed germination

Discussion

Desiccation tolerance/sensitivity of seeds is a physiological phenomenon regulated by a balance between endogenous metabolic activity as well as exogenous environmental stress. In *E. tuberculatus*, the results revealed that there was a positive correlation between moisture loss and germination percentage (Figure 1). Fresh seeds possessed high initial moisture content (49.38%) and registered 90% germination, and took 23 days to complete germination. When the seeds exposed to open laboratory conditions (28±2°C& 65% RH), showed a significant decrease in moisture content on the 4th day and complete loss of viability was recorded on the 18th day. Following the classification of seeds [15,16,17] *E. tuberculatus* exhibits typical recalcitrant behaviour. The critical moisture content (CMC) defined by different authors at which 50% viability loss [18] was identified at an MC of 30.25% in *E. tuberculatus*. The rapid loss of moisture content and simultaneous loss of viability as reported in *Theobroma cacao* [19], *Aporosa lindleyana* [20] and *Vateria indica* [21-23]. The moisture content in the seeds at the time of harvest in tropical and temperate trees lies between 40% to 60% and for avoiding desiccation damage maintaining high moisture content is essential [24-26]. Such type of decrease in moisture content and viability was shown by some other recalcitrant seeds like *Shorea robusta* [26], *Avicennia marina* [27], *Myristica malabarica* [28], *Mesua ferrea* [22] etc.

The Seed Vigour Index (SVI) proved to be a sensitive marker for desiccation-induced deterioration on germination. The low SVI (0.55) and the prolonged germination period (23 days) in fresh seeds suggest inherent constraints on rapid emergence. This may be because of: a. Physical Obstruction due to the thick, highly lignified seed coat that likely imposes mechanical resistance to radicle protrusion and b. Chemical Inhibition due to high phenolic concentrations, as observed, may act as metabolic inhibitors, a phenomenon documented in other recalcitrant taxa [29]. As desiccation progresses, the drastic decline in SVI to 0.07 reflects an irreversible collapse of metabolic vigour long before total embryo death occurs.

At high hydration levels, cell membranes maintain a liquid-crystalline state, ensuring selective permeability. Dehydration in *E. tuberculatus* triggers a transition to a gel phase, leading to physical “broaching” of the plasma membrane [30]. The observed two-fold increase in leachate conductivity within 16 days confirms that viability loss is driven by the irreversible efflux of essential solutes as reported [31]. This negative correlation between electrolyte leakage and moisture content is a hallmark of desiccation-sensitive species, where the cellular machinery fails to maintain structural integrity under water stress [32,33].

The pivotal point of viability loss is closely linked to lipid peroxidation. The exponential increase in malondialdehyde (MDA) levels, particularly in the embryo, suggests that reactive oxygen species (ROS) leads to an uncontrolled “free radical attack” on membrane lipids. The embryo’s higher MDA concentration relative to the endosperm (40% higher) reflects its greater physiological activity and higher susceptibility to oxidative failure as reported in Cocoa and jack seed [34]. In response to this stress, antioxidant enzymes like peroxidase (POD) and polyphenol oxidase (PPO) show altered activity. While POD levels initially increased—likely as a compensatory mechanism - the subsequent decline at sub-critical moisture levels suggests protein denaturation and metabolic collapse [35]. The inverse correlation between MDA accumulation and antioxidant enzyme efficiency is a primary driver of recalcitrant nature in this species. Similar observations were also made in *Azadirachta indica* [36] and in *Limonia aureum* seeds [37].

The biochemical shift from starch to total soluble sugars (Table 2) during desiccation suggests active starch hydrolysis or gluconeogenesis from lipid reserves. According to the water Replacement Theory [38], sugars like sucrose protect membranes by hydrogen-bonding to lipid head groups, substituting for water molecules. However, *E. tuberculatus* lacks the full “sugar suite” required for true desiccation tolerance. While sucrose was abundant, the total absence of raffinose in the embryo is a critical finding. Raffinose-family oligosaccharides (RFOs) are essential for vitrification (glass formation), which prevents sucrose from crystallizing and maintains the cytoplasmic matrix in a stable state [39]. Though the endosperm contained arabinose, its absence in the vital tissues of the embryo further explains the species’ inability to survive drying.

Conclusion

Elaeocarpus tuberculatus produces recalcitrant seeds whose survival is strictly bound to high hydration levels. The lethal transition occurring below 30% MC is driven by lipid peroxidation of the embryo and a failure in membrane integrity. This will definitely lead to the failure of all types of anti-oxidant defence system in these seeds. The lack of raffinose-mediated vitrification and the mechanical barriers of the seed coat further complicate its natural regeneration. For conservation purposes, ex-situ strategies must focus on moist storage or cryopreservation of excised embryos to bypass the limitations of whole-seed desiccation.

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