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Physiological and biochemical changes in the seeds of *karanj* (*Pongamia pinnata* L) under different storage conditions

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ABSTRACT

An experiment was conducted to elucidate the possible physiological and biochemical changes associated with seed deterioration during storage of *Karanj* (*Pongamia pinnata* L.) seed. The seeds of variety 'Bak 49' ('IC430529') were extracted, processed and stored at three different levels of relative humidity (RH) (75%, 33% and 5.5%) and temperatures (4°C, 20°C and ambient). The seed viability pattern, physiological and biochemical parameters under different conditions were monitored at regular intervals to assess the effect of storage. Among the different RH treatments, 33% RH showed significantly higher values for viability and vigour over 5.5% and 75% RH at all temperatures. The biochemical parameters like electrical conductivity of seed leachates and lipid peroxidation under different treatments showed significantly increased values with seed deterioration. The level of total soluble sugars increased gradually whereas total soluble proteins and enzyme activity (dehydrogenase and acid phosphatase) decreased with storage period in all the treatments. The optimal conditions for extending seed storability in *karanj* without having any adverse effect on physiological and biochemical parameters were 4°C and 20°C and 33% RH. This study could possibly be helpful for conventional storage of *pongamia* seeds on large scale and can be further exploited in other orthodox tree species.

Key words: *Pongamia pinnata*, Physiological and Biochemical changes, Seed storage, Seed deterioration

Great importance is being given to the cultivation of biodiesel plants with an aim to reduce the dependence on imported petroleum products. Though the use of biodiesel derived from non edible oils is quite widespread in many countries, in the Indian context the most important viable option is the use of non-edible oils mostly of tree origin. Among tree species which can yield non-edible oil as a source of energy in the form of biodiesel, *Pongamia pinnata* L. has been a suitable species due to its hardy nature, high oil recovery (32–44%, Naresh *et al*. 2007), oil quality and easy harvesting. *Karanj* is an important minor non-edible oilseed tree, which is found throughout India up to an altitude of 1200 m (Anonymous 1965). Conservation of genetic diversity through storage of seed is the most common and economical method. but, the seed storage problems are most common in tropical countries like India, because of predominance of hot and humid tropical and subtropical climate with great variation in temperature, rainfall and relative humidity (RH) across the year.

P. pinnata is propagated by seeds; therefore, conservation of genetic resources of this species through storage of seed would be most economical. However, use of seed for conservation of genetic resources would need better understanding regarding the seed storage behaviour and the various physiological and biochemical changes occurring in the seed during deterioration. The present investigation was undertaken to study the effect of temperature and relative humidity on seed deterioration of *pongamia* seeds to standardize the suitable ideal conditions for their conventional storage.

MATERIALS AND METHODS

The present investigation was conducted at National Bureau of Plant Genetic Resources (NBPGR), New Delhi. The experimental materials comprised of the freshly harvested pods of *Pongamia pinnata* variety 'Bak 49' ('IC 430529') collected from Adilabad district of Andhra Pradesh. The seeds were carefully extracted without any damage, cleaned and dried. Saturated solutions of various salts were used to equilibrate the seeds to specific moisture levels, viz 75%, 33% and 5.5% RH with sodium chloride, magnesium chloride and RH zinc chloride respectively. (Rockland 1969a). Temperatures and relative humidity were monitored regularly with thermo-hygrometer. The seeds desiccated to different levels of moisture were pre-humidified in closed air tight desiccators over water (100% RH) for 24 hr before

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plating to avoid any imbibitional injury. The storage conditions were kept constant. The seed viability was assessed by the 'between paper method' of germination. The seedling vigour index (SVI) was calculated using the method of Abdul-baki and Anderson (1973). The biochemical parameters, viz electrical conductivity of seed leachates was estimated using Conductivity meter, total soluble proteins (Lowry *et al*. 1951), total soluble sugars (Mc Cready *et al*. 1950), lipid peroxidation (Heath and Parker 1968) and enzymes – dehydrogenase (Kittock and Law 1968) and acid phosphatase activity (Leigh and Walker 1980) were monitored at regular intervals. Seed moisture content was determined gravimetrically (ISTA 1999). Emergence of both radical and plumule were recorded after the 15th day of plating the seeds. Germination percentages were recorded every alternate day. The data was analysed statistically by adopting factorial CRD (Panse and Sukatme 1985). The data recorded as percentage were transformed to the respective angular (arc sine) value before subjecting them to statistical scrutiny. Differences among means were tested for significance using least significant difference tests (LSD).

Table 1 Equilibrium seed moisture content at various levels of relative humidities and temperature on wet weight basis in *P. pinnata*

Salt (%RH)	4°C	20°C	Ambient
NaCl (75%)	14.1(7)	13.8(8)	13.2(11)
$MgCl_2$ (33%)	7.8(8)	6.9(10)	6.0(12)
$ZnCl_2$ (5.5%)	3.5(12)	3.0(12)	2.94(14)

() indicated the days to equilibrate at respective temperature and relative humidity

RESULTS AND DISCUSSION

High temperature and moisture content during storage enhance seed deterioration (Mc Donald 1999). Relative effects of seed moisture content and temperature on seed longevity differ with species as well as the structural and biochemical composition of the seed. Under the present investigation temperatures and relative humidity had significant effect on seed deterioration in *P. pinnata*, where the seeds were equilibrated to different moisture levels over various saturated salts and stored at three different temperatures for a period of 120 days. The seeds were equilibrated to different RHs for 7–14 days where they showed 2.9 to 14% moisture content at different temperatures (Table1).

At high relative humidity of 75% there was a significant decline in the seed viability at all the temperatures. The viability decreased to 20 and 50% respectively at 4°C and ambient temperature within 20 days of storage while at 20°C the viability was maintained at 70% subsequently declined after 40 days of storage. The seeds showed poor germinability after 60 days of storage (10–20%) where the seedlings were abnormal with stunted growth. However at 33% RH the seed viability remained significantly higher at all the temperatures. The viability started declining at ambient temperature (50% after 60 days of storage) while at 4°C and 20°C the viability was maintained well (85%) even after 120 d of storage. The seeds, equilibrated at 5.5% RH showed 2.9 to 3.5% moisture content at various temperatures, an ultra dry storage condition which is not congenial for P*ongamia* seeds. The viability was maintained up to 68 and 60% at 4°C and 20°C till 80th day of storage but at ambient temperature the seeds started deteriorating after 40th day of storage (30% viability). Among

Table 2 Effect of storage conditions on the seed viability in *P. pinnata*

Salt	RH	Temperature	Storage period(days)						
			0	20	40	60	80	100	120
NaCl	75%	4°C	100	20(26.92)	15(23.18)	15(23.18)	15(23.18)	10(18.91)	0(4.5)
		20°C	100	70(57.10)	40(33.52)	20(26.92)	20(26.92)	20(26.92)	0(4.5)
		Ambient	100	50(45.29)	20(26.92)	10(18.91)	10(18.91)	10(18.91)	0(4.5)
$MgCl_2$	33%	4°C	100	100	98(82.97)	95(77.75)	92(74.11)	90(72.05)	85(67.62)
		20°C	100	100	98(82.97)	98(82.97)	90(72.05)	85(67.62	85(67.62)
		Ambient	100	95(77.75)	85(67.62	85(67.62	50(45.29)	40(33.52)	30(23.52)
$ZnCl_2$	5.5%	4°C	100	85(67.62)	78(62.38)	70(57.10)	68(55.86)	50(45.29)	40(39.52)
		20°C	100	85(67.62	70(57.10)	60(39.52)	60(39.52)	40(33.52)	30(23.52)
		Ambient	100	65(54.03)	30(23.52)	20(26.92)	20(26.92)	10(18.91)	10(18.91)

Source	C.D at 5%
Storage Period (A)	3.041
Salt (B)	1.755
Temperature (C)	1.755
A × B	5.267
A × C	5.267
B × C	3.041
A × B × C	9.124

all the RH treatments, 33% RH showed significantly higher germination compared to 5.5 and 75% RH at all the temperatures (Table 2). Similar studies leading to loss of germination at higher humidity and temperature has been reported in sunflower and sesame seeds (Bass *et al*. 1963, McDonald 1999). Under present investigation, at higher RH (75%), the survival rates are significantly lower at all the temperatures. The seeds could retain the viability only for a short period of one month, indicating that storage of *Pongamia* seeds even for a short period is difficult in the tropical regions with high temperature and high humidity. However, at low RH (5.5%) which is an ultra-dry condition the viability of seeds was not as good as at 33% RH, though the seeds maintained the viability up to a period of 2 months at 20°C. Increased rates of seed deterioration has been reported in many orthodox species stored above or below the optimum moisture content at which maximum longevity of viability was observed by Vertucci *et al.* (1994). The increased deterioration of very dry seeds observed in several studies may be a result of membrane damage due to the removal of structural water. Thus, over drying seeds may expose macromolecular surfaces to free radical attack and reduce the longevity of seed viability (McDonald 1999).

Seedling vigour gradually reduced with storage period. However, the loss of vigour was significantly higher at high RH of 75% where maximum abnormal seedlings were formed after storage. The vigour index at 20 and 4°C and 33% RH were at par with the control. Storage under high RH of 75% resulted in a significant decline in the vigour index after 20th day of storage. At lower RH of 5.5% although the decline was significant but it occurred after 80 days of storage i.e. at a much slower rate compared to the 75% RH. At lower RH of 5.5% the vigour index was highest at 4°C where the seedlings performed the best (Table 3). Loss of vigour is associated with seed deterioration and is influenced by composition of seed, particularly the food reserves or the efficiency of mobilization of nutrients. In the present study, the highest vigour was observed in the control seeds, which decreased with seed storage under different conditions of temperature and humidity as has been observed in sunflower (Ellis *et al.* 1996). The present study showed a decrease in shoot length which was found to be directly proportional to the seed viability and it also confirms the earlier reports in a number of crops (Halder and Gupta 1983, Harrington 1972, Dey and Basu 1982, Yadav *et al.* 1987, Dharamlingam and Basu 1990).

Degradative changes in cellular membranes are (is one of) the early events of seed ageing (Heydecker 1972). Enhanced solute leakage from imbibed seed is associated with the loss in seed vigour and viability (Dadlani and Agarwal 1985, Makkawi *et al*. 1999, Maristela and Vieira 2007). In the present study also, increase in electrolyte leakage was noted before the reduction in germination. The minimum conductivity of leachates was observed in the seeds equilibrated at 33% RH on the 20th day of storage at 4°C and 20°C respectively where the viability was maximum (Table 4). Increase in the amount of electrolytes is found to be proportional to the seed deterioration, attaining maximum values when seeds lost viability completely during storage at high humidity (Tables 2,4). At these levels the seeds showed very low germination. Therefore seed deterioration primarily resulted in increased leakage of solutes leading to an increase in the electrical conductivity which could be interpreted in terms of irreversible changes in membrane

Table 3 Effect of storage conditions on the vigour index in *P. pinnata*

Salt	RH	Temperature	Storage period (days)			
			0	40	80	120
NaCl	75%	4°C	4419	387	185	0
		20°C	4419	1120	357	0
		Ambient	4419	433	177	0
$MgCl_2$	33%	4°C	4419	3871	2382	1494
		20°C	4419	3662	2395	1413
		Ambient	4419	2542	2052	477
$ZnCl_2$	5.5%	4°C	4419	2691	1816	742
		20°C	4419	2093	1278	441
		Ambient	4419	714	343	87

Source	C.D at 5%
Storage Period (A)	59.84
Salt (B)	42.31
Temperature (C)	42.31
A × B	103.65
B × C	73.29
A × C	103.65
A × B × C	179.54

Table 4 Effect of storage conditions on the electrical conductivity (mmhos/cm/gfwt) of *P. pinnata*

Treatment	Storage Temp.	Storage period (Days) 0	40	80	120
NaCl	4°C	0.286	0.861	0.813	1.37
	20°C	0.286	0.598	0.637	0.481
	Ambient	0.286	0.73	0.789	1.52
$MgCl_2$	4°C	0.286	0.366	0.372	0.619
	20°C	0.286	0.33	0.366	0.533
	Ambient	0.286	0.625	0.665	0.876
$ZnCl_2$	4°C	0.286	0.375	0.381	0.47
	20°C	0.286	0.417	0.497	0.76
	Ambient	0.286	0.736	0.84	1.08

Source	C.D at 5%
Storage Period (A)	0.021
Salt (B)	0.015
Temperature (C)	0.015
A × B	0.037
A × C	0.037
B × C	0.032
A × B × C	0.065

structure and loss of unique chemical structure of membranes essential for maintaining seed viability.

Understanding of various biochemical activities and compounds would help in assessing reasons for ephemeral behavior of *Pongamia* seeds as the data indicated a good correlation of increased electrolyte leakage with increased lipid peroxidation content (Fig 1), which indicated the destruction of membranes caused by peroxidation of lipids during deterioration. In other way the lipid peroxidation contents increased with loss of seed viability and vigour

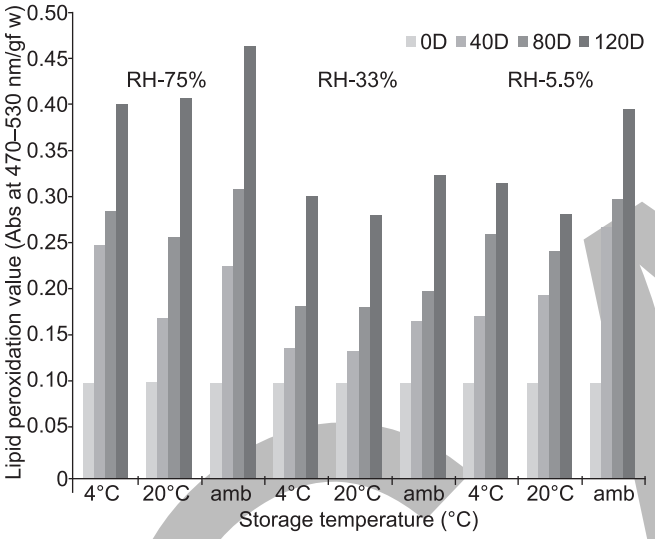

Fig 1 Effect of storage conditions on the lipid peroxidation values of *P. pinnata*

(Shahzad *et al*. 2000). Moisture content has been observed to be inversely proportional to oil content. Neergaard (1977) reported that seeds with high oil content have lower moisture than those high in protein and starch. The amount of soluble protein expressed as mg/g fresh wt. was found to be 82.0 in fresh seeds which significantly decreased with decline in seed viability (Table 5). At 33% RH and 4°C and 20°C there was no significant change in the level of proteins up to 40th day,whereas, a drastic reduction in the level of proteins was observed after 40th day at ambient temperature (Table 5). Moreover, seeds at lowest humidity (5.5% RH) maintained stable protein levels up to 80th day of storage. The total protein content of deteriorated seeds decreased progressively with storage period. Cherry and Skedsen (1986) hypothesized that the irreversible loss of some essential proteins in the aged seeds leads to loss of seed viability. The decline in the total protein content due to impaired protein biosynthetic activity with the gradual loss of seed viability have been reported in seeds of rye (Hallam *et al.* 1973), pea (Bray and Chow 1976, Powell and Mathews 1977), sal (Nautiyal *et al*. 1985) and pigeon pea (Kalpana and Madhav Rao 1994, 1997).

The total soluble sugar content was found to be 22.68 mg/g fwt. in fresh seeds which increased with seed

Table 5. Effect of storage conditions on the total soluble protein content (mg/gfwt.) in *P. pinnata*

Treatment	Storage Temp.	Storage period (Days)			
		0	40	80	120
NaCl	4°C	82.28	38.70	36.43	24.84
	20°C	82.28	55.20	48.40	35.06
	Ambient	82.28	45.61	47.53	19.19
$MgCl_2$	4°C	82.28	78.59	62.47	45.43
	20°C	82.28	79.75	63.41	46.38
	Ambient	82.28	62.00	55.13	26.45
$ZnCl_2$	4°C	82.28	68.91	50.27	18.20
	20°C	82.28	64.18	53.85	29.26
	Ambient	82.28	48.07	39.27	20.85

Source	C.D at 5%
Storage Period (A)	2.069
Salt (B)	1.463
Temperature (C)	1.463
A×B	3.583
A×C	3.583
B×C	2.534
A×B×C	6.207

deterioration and storage time (Table 6). In general, the value of sugars significantly increased with storage period in all the treatments. The seeds at lowest humidity (5.5%) performed well up to 40th day of storage without much decline in the sugar values.

The activities of dehydrogenase and acid phosphatase declined with seed deterioration. In the fresh seed, activity of dehydrogenase (D OD/g fresh wt.) was found to be 0.49. Similar activity (0.47 and 0.45) was maintained at 4°C and 20°C of 33% RH on the 40th day of storage which significantly declined to 0.35 and 0.36 within 120 days of storage (Fig 2). At higher RH of 75%, the activity reduced

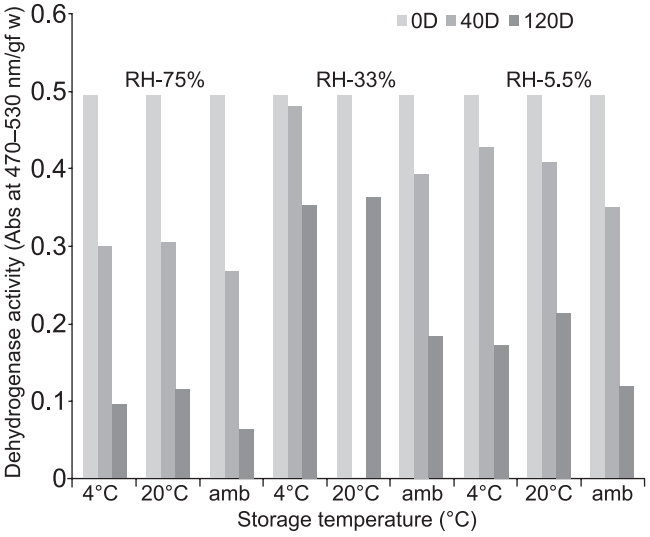

Fig 2 Effect of storage conditions on the Dehydrogenase activity in *P. pinnata*

Table 6. Effect of storage conditions on the total soluble sugars (mg/gfwt) in *P. pinnata*

Treatment	Storage Temp.	Storage period (Days)			
		0	40	80	120
NaCl	4°C	22.68	45.96	76.65	120.51
	20°C	22.68	40.83	49.21	71.44
	Ambient	22.68	44.55	58.66	88.61
$MgCl_2$	4°C	22.68	32.42	42.06	66.93
	20°C	22.68	34.68	43.08	68.24
	Ambient	22.68	35.45	46.39	80.71
$ZnCl_2$	4°C	22.68	35.35	54.35	73.71
	20°C	22.68	34.14	48.12	74.24
	Ambient	22.68	40.24	55.30	82.54

Source	C.D at 5%
Storage Period (A)	2.76
Salt (B)	1.95
Temperature (C)	1.95
A × B	4.78
A × C	4.78
B × C	3.38
A × B × C	8.29

significantly with values of 0.06–0.11 from 0.493 (control) at all the temperatures. In the fresh seed, activity of enzyme acid phosphatase was found to be 0.406 which significantly declined within 120 days of storage (Fig. 3). At ambient temperature the activity declined significantly (from 0.296 to 0.185). At higher RH of 75%, the activity reduced significantly at all the temperatures. The stored *Pongamia* seeds with time exhibited a declining trend in the quantities of proteins, enzyme dehydrogenase and acid phosphatase activity which are similar with the earlier reports of Nautiyal and Purohit (1985) and Chandel *et.al.* (1995).

It can be inferred from the data that storage at ambient

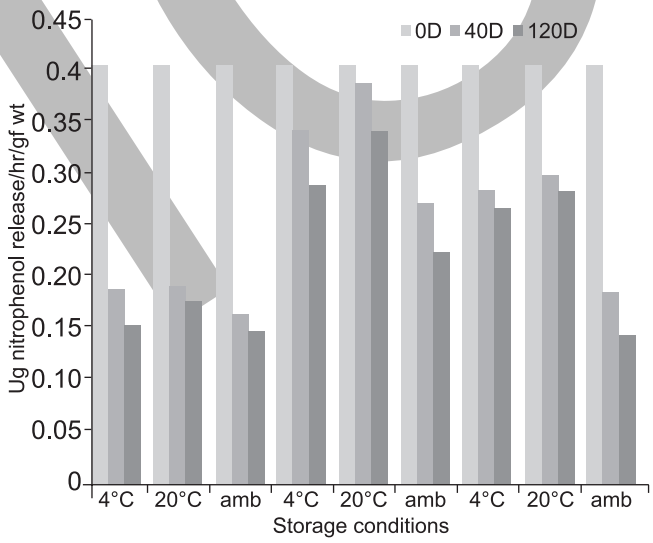

Fig 3 Effect of storage conditions on the Phosphatase activity in *P. pinnata*

temperatures and high humidity is associated with substantial decline in the activities of enzymes such as dehydrogenase and acid phosphatase. The stresses during storage under high humidity and fluctuating ambient temperatures are known to induce the formation of reactive oxygen species such as superoxide anion, hydroxyl radicals and hydrogen peroxide (Scandalios, 1993). Hence, formation of reactive oxygen species in deteriorating seeds and simultaneous inhibition of enzymes such as dehydrogenase and acid phosphatase and resultant deterioration in the membrane integrity in seeds could be the potential reasons of seed deterioration under high humidity and fluctuating ambient temperature in *Pongamia* seeds as observed in peanut (Sung and Jeng, 1994). During seed deterioration a cascade of events might be occurring in the seed leading to the loss in seed viability and ultimately leading to the seed death.

In Conclusion, for *Pongamia pinnata* seeds the optimum storage conditions are 4°C and 20°C and 33% RH without having any adverse effect on physiological and biochemical parameters which can be further exploited in other forestry species for extending seed storability.

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