



Scientific validation of traditional knowledge on techniques for sorting *Myristica* spp. seeds practiced by the farming community of Palakkad district, Kerala, India

K A Suresh Kumar^{1*}, C Anilkumar², K G Ajithkumar³ and T M Prajith¹

¹Department of Botany, Government College Chittur, Palakkad – 678 104, Kerala, India

²Conservation Biology Division, Jawaharlal Nehru Tropical Botanic Garden and Research Institute, Pacha, Palode, Thiruvananthapuram – 695 562, Kerala, India

³Department of Botany, Government College for Women, Thiruvananthapuram – 695 014, Kerala, India

*sureshtvmala74@gmail.com

Received: 30 June 2021

Accepted: 10 October 2021

Abstract

Palakkad is well known for agro biodiversity and agriculture based traditional knowledge. This paper deals with scientific analysis of traditional knowledge related to sorting viable seeds by various rural farming communities in Palakkad district of Kerala state. The present study was undertaken with the objective of documenting traditional knowledge of farmers related to sorting of viable seeds, *viz.* floating and shaky seeds never sprout. Another important objective was to pave the way for value addition of such traditional knowledge by giving logical and scientific interpretation. The seeds of various *Myristica* species (*Myristicaceae*) were considered for the scientific validation of the documented traditional knowledge because the distribution of these endemic and endangered tropical forest trees are restricted to a unique but highly fragile and fragmented fresh water ecosystem called Myristica swamps. The value added and validated traditional knowledge is documented and made available as knowledge repository to sustainable farming as well as community conservancy.

Keywords: Desiccation, Myristica swamps, Traditional knowledge, Viability

1. Introduction

Palakkad is known as “the granary of Kerala state”. Agriculture is the dominant economic activity and supports nearly 76% of the rural population (Premakumar *et al.*, 2015). Palakkad is well known for agro biodiversity and agriculture based traditional knowledge. Traditional knowledge or indigenous knowledge refers to the knowledge, innovations and practices of indigenous and local communities around the world. It is the accumulated knowledge, skills and technology of the local people, derived from the direct interaction of human beings with their environment over centuries and adapted to their culture and tradition. This knowledge should be identified, documented and transferred to future generation. Otherwise such precious knowledge treasures will be vanished from our society because most of the

traditional knowledge is non documented and carry over from one generation to next only through oral traditions like legends, proverbs, customs, folklore songs, tales, etc. (Moller, 2009). In much ethnic and rural community, traditional knowledge has been orally passed for generations from person to person. Traditional knowledge has many advantages over modern sophisticated technology. First of all, it is affordable to the economically weaker section. Secondly, unskilled farmers can also practice without much training. All the resources for the development of traditional knowledge-based technologies will be locally and readily available. Traditional knowledge prevailed among the farmer community, related to agriculture especially regarding collection and preservation of seeds is yet to be

explored for the conservation of forest trees which produce recalcitrant seeds. Roberts (1973) classified the seeds into two groups *viz.*, orthodox and recalcitrant based on their storage behavior. Recalcitrant seeds lose viability once they are dried to moisture content below critical moisture content (CMC). These seeds bypass maturation drying and maintain higher moisture content in the seeds but are extremely sensitive to desiccation and are metabolically active at shedding. On the contrary, orthodox seeds are characterized by maturation drying and metabolic quiescence. The orthodox seeds are remarkable in maintaining the cellular architecture under very low moisture content and they can be stored for long period of time without damaging the embryo (Pammenter and Berjak, 1999). The limited storage potential of recalcitrant seeds is a big problem in the maintenance of seed banks for long term conservation. Sub-zero, and in some cases higher than zero temperature significantly damage the recalcitrant seeds, and therefore temperature cannot be reduced greatly. This situation may limit the scope of modification in seed storage environment and even difficult to improve the storage life of recalcitrant seeds (King and Roberts, 1979; Roberts *et al.*, 1984). Traditional knowledge related to seed sorting techniques prevail among the farmer community can be explored to overcome the hurdles in propagation of recalcitrant seed producing trees like *Myristica malabarica* Lam. and *Myristica magnifica* Bedd. Among these *Myristica malabarica* was categorized as vulnerable species as per the red data book of IUCN (IUCN Threat Status: Vulnerable B1+2c) (Anonymous, 2020). The population of *M. magnifica* is also drastically declining due to the fragility of *Myristica* swamp ecosystem where the distribution of these species are confined and this species is categorized as endangered species by IUCN (IUCN Status: Endangered B1+2c) (Anonymous, 2020).

2. Materials and methods

2.1. Study area

Palakkad, the largest district in Kerala, selected as study area, located in the central Kerala, lies in between 10° 21'N to 11° 14'N and 76° 02'E to 76° 54'E (Fig. 1). Palakkad district covers an area of 4475.8 sq. kms and has a total population of 2809934 as per census 2011.

2.2. Field survey

Field survey was conducted to collect the information from farming community through informal interview with selected farmers during October to December, 2020. A total 32 farmers within the age group of 50-75 years were participated in the survey. The information collected, regarding customary techniques for sorting of viable seeds are listed below.



Fig. 1. Location map of study area

2.2.1 Floating seeds fail to sprout (In Malayalam: *Parum vithu mulakkilla*)

Farmers used the table salt (Sodium Chloride) solution to sort the viable seeds. Salt solution will be prepared by dissolving table salt in pure water and seeds will be poured to the vessel containing the salt solution. The floated seeds will be removed and discarded. The seeds sank in the salt solution will be taken out for rising the seedlings. Before using the seeds for rising seedlings, it will be washed thoroughly in fresh water to remove the salt content on the surface of the seeds because they had the knowledge that the presence of excess salt adversely affects the seed germination. The farmers used a crude technique to determine the quantity of salt to be added. They keep hen's egg in a vessel containing fresh water and salt will be added little by little and stir it well (Plate 1.a & b) without breaking the egg until the egg floats.

The concentration of salt required to float the hen's egg was estimated in laboratory. A known volume of distilled water was taken in a beaker containing a fresh hen's egg and weighed. After taring up, sodium chloride was added in pinches and stirred it well. Adding of salt was continued till the egg get floated and the weight of the added salt was recorded. From these values the concentration of salt solution was estimated as 10 %.

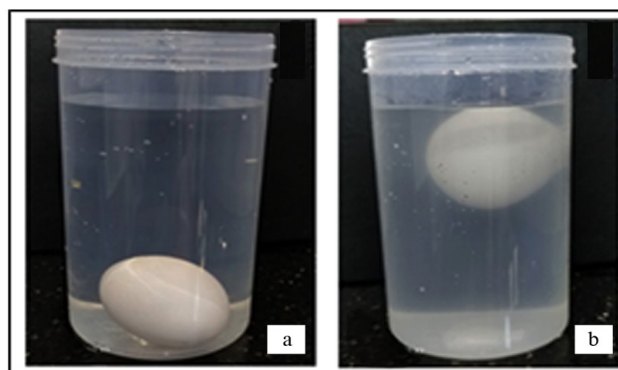


Plate 1. a. Egg in distilled water; b. Egg in 10% salt solution

2.2.2. Shaky seeds fail to sprout (In Malayalam: *Kulungum vithu mulakkill*)

Farmers shake the seeds by keeping seeds near the ear and listen to its sound. Those seeds which produce sound while shaking will be considered as non-viable to be discarded. On the other hand, quiet seeds are taken as viable seeds for rising seedlings.

Collected data were documented. The physiological parameters *viz.* moisture content, electrolyte leakage and total dissolved solids (TDS) of the seed, 1% 2, 3, 5 - Triphenyl Tetrazolium Chloride (TTZ) stainability of the embryonic tissue *etc.* were analyzed to assess the seed viability. Correlation analysis has been done to interpret the scientific basis of the traditional knowledge mentioned above.

2.3. Seed moisture content

Moisture content of mature seeds of different species of *Myristica* at fresh and desiccated stages were calculated by measuring the difference between fresh and dry weight through constant hot air oven method (Anonymous, 2008). For calculating the dry weight of the seeds, the sample materials were taken in a pre-weighed bottle and weighed in an electronic balance, then dried it in a hot air oven at 130°C for 1 hr until the weight become constant. For each test 5 replicates of samples were used. Dry weight of each sample of each test was recorded after cooling to room temperature in a desiccator. Moisture content was expressed in percentage (%) (Anonymous, 1999).

2.4. Seed germination test

Mature seeds were kept in standardized conditions for germination test to assess the viability potential of the seeds. Viability of the seeds was determined on the basis of the percentage of seeds germinated. The seeds were considered as germinated while the radicle came out to the seed coat at length of 5mm (Anonymous, 1993). Germination tests were carried out in five replicates of fifty seeds each. Mature seeds were wrapped with sterilized tissue paper and kept it in a seed germinator (KEMI SEED GERMINATOR KSG-2) without light ($30 \pm 2^\circ\text{C}$, 80 % RH).

2.5. Viability test

Viability of the seeds of different species of *Myristica* at fresh and desiccated stages was assessed through Dehydrogenase activity (DHA) test (Kittock and Law, 1968) and each test was conducted with five replicates of one seed (embryo) each preconditioned by 4 hr water soaking and subsequent 24 hr incubation of individual seeds in 3 ml of 1% TTZ. The intensity of the stain on the embryo was observed by naked eye and the colour variations were recorded. Stained embryonic tissues were then individually soaked in 5ml of methyl

cellosolve solution for 4 hrs. The extract was decanted and intensity of colour was measured in a spectrophotometer (UV-VIS spectrophotometer, Shimadzu UV - 1800) at 480 nm.

2.6. Electrolyte conductivity

Electrolyte conductivity of mature seeds at fresh and desiccated stages was measured according to the method followed by Bonner (1996). One seed from each in five replicates with equal weight from all seed lots were incubated in 40ml of deionized distilled water and kept in a closed container for 24 hr at 28°C in the laboratory. Care should be taken to immerse the seeds completely in the deionized distilled water. The conductivity of the solution was measured with conductivity meter (Systronics, DDR, type 306). Conductivities were expressed as micro Siemens (μs).

2.7. Total Dissolved Solid (TDS)

Total Dissolved Solid (TDS) of mature seeds at fresh and desiccated stages were measured by following the method used to measure electrolyte conductivity. One seed from each in five replicates from all seed lots were incubated in 40 ml of deionized distilled water which was sufficient to sink the seeds completely and kept in a closed container for 24 hr at 28°C in the laboratory. Care should be taken to select the seeds with equal weight. The Total Dissolved Solid in the solution was measured with a conductivity meter (Systronics, Conductivity TDS meter 308). The measurement of Total Dissolved Solid (TDS) was expressed as parts per million (ppm).

2.8. Statistical analysis

All experiments were repeated 5 times and the data were statistically analyzed by one way ANOVA and the values are expressed as mean \pm standard error. Significance of differences between means values were tested by Least Significant Difference (LSD) ($P < 0.01$). Statistical analysis was carried out using SPSS 11.0 package.

3. Results and discussion

From the fresh seeds of *M. malabarica* Lam. and *M. magnifica* Bedd., viable seeds (Plate 2. a-f) were sorted by exploring the traditional indigenous technique followed by the farming community of Palakkad District of Kerala State. It was found that compared to distilled water, salt (Sodium Chloride) solution was better medium to sort the viable seeds more effectively (Table 1 & 2). The concentration of the salt solution used for the sorting of the viable seeds by the farmers at which the hen's egg floated was estimated as 10% by using modern laboratory equipment. It was noted that while 16 to 18 % non-viable seeds were floated in 10% salt solution (T_2), it

was 6 to 8 % in distilled water (T₁) (Table 1). The seeds sorted by salt solution registered 94 to 95 % germination under standard conditions (T₄) but it was 81 to 82 % when seeds were sorted by distilled water

(T₃) (Plate 3. a & b) (Table 1 & 2). The higher degree effectiveness of the 10 % salt solution in sorting the viable seeds can be attributed to increased density of the solution by the added solute.



Plate 2. a. Fruit of *Myristica malabarica* Lam.; b. Seed with aril of *M. malabarica* Lam.; c. Mature seeds of *M. malabarica* Lam.; d. Fruit of *Myristica magnifica* Bedd.; e. Seed with aril of *M. magnifica* Bedd.; f. Mature seeds *M. magnifica* Bedd.

Table 1. Sorting of viable seeds by distilled water and salt solution

Sl. No.	Scientific Name	Control - Seeds germinated (%)	T ₁		T ₂		T ₃		T ₄	
			Seeds floated (%)	Seeds germinated (%)	Seeds floated (%)	Seeds germinated (%)	Seeds sank (%)	Seeds germinated (%)	Seeds sank (%)	Seeds germinated (%)
1	<i>Myristica malabarica</i> Lam.	67.9±0.84	10.4±0.75	0	18±0.89	0	90.4±0.75	72±0.63	81.6±0.75	92±0.89
2	<i>Myristica magnifica</i> Bedd.	68.4±0.75	06±1.41	0	16.4±0.75	0	93.6±0.75	73.6±1.16	84±0.89	93.6±0.75

± Standard Error, %: Percentage. T₁: Seed floated in distilled water, T₂: seed floated in salt solution, T₃: Seeds sank in distilled water, T₄: Seeds sank in salt solution

Table 2. Variation of seed germinability in T₃ and T₄

Sl. No.	Scientific Name	Percentage of seed germination (%)			F-value (P-value)
		C	T ₃	T ₄	
1	<i>Myristica malabarica</i> Lam.	67.9±0.84 ^c	72.00±0.63 ^b	92.00±0.89 ^a	261.16** (<0.001)
2	<i>Myristica magnifica</i> Bedd.	68.40±0.75 ^c	73.60±1.16 ^b	93.60±0.75 ^a	214.13** (<0.001)

** Significant at 0.01 level, Means having different letter as superscript differ significantly within a row. C: Control, T₃: Seeds sank in distilled water, T₄: Seeds sank in salt solution

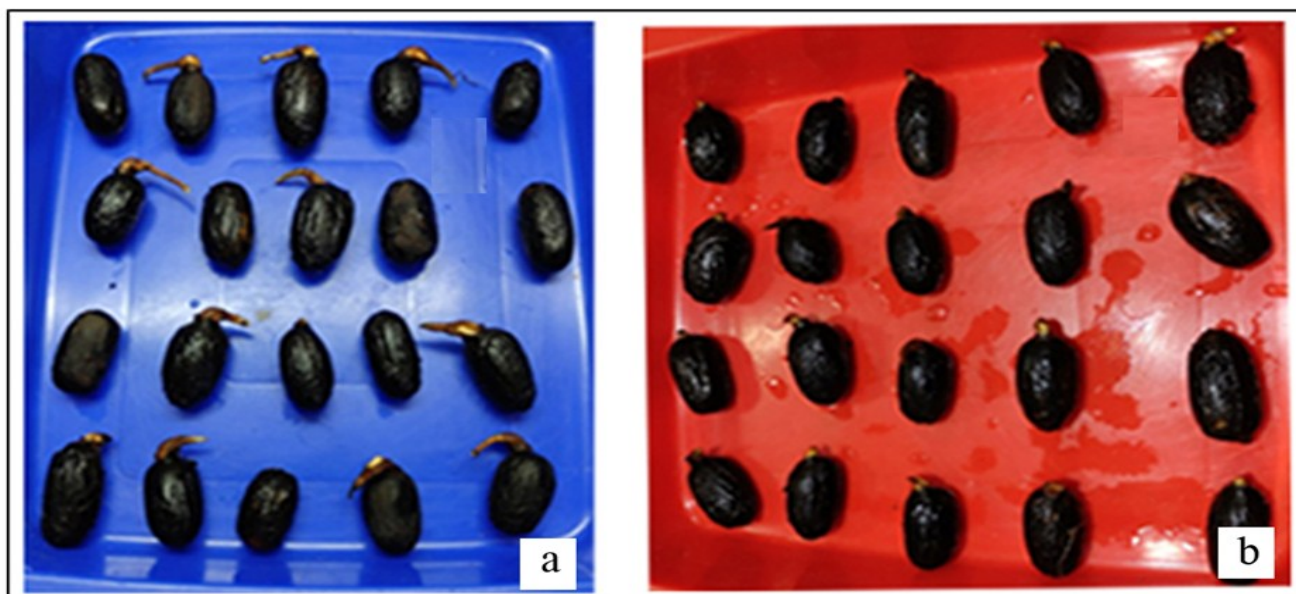


Plate 3. a. Germinated seeds of *Myristica malabarica* Lam.; b. Germinated seeds of *Myristica magnifica* Bedd.

Similar to the germinability, other physiological parameters to estimate the seed viability viz. moisture content of seeds, TTZ stainability, electrolyte conductivity, and total dissolved solids (TDS) also proved the potentiality of this traditional knowledge to sort out viable seeds from non-viable seeds. When the submerged seeds in distilled water (T₃) and salt solution (T₄) were incubated in tetrazolium chloride (TTZ) solution, the embryonal tissue was stained deeply in deep red colour. The absorbance of formazan as an indicator of the intensity of the TTZ absorbed by embryonic tissue, was very high in seeds which sank in distilled water and salt solution (Table 3 & 4). But the embryonal tissue was unstained and formazan absorbance was very low with the samples taken from the seeds floated in distilled water (T₁) and 10 % salt solution (T₂) (Table 3 & 4). Tetrazolium test was most

widely used rapid biochemical assay for measure the viability of seeds (Khajjak *et al.*, 2016). Kamarudeenkunju (2003) also reported similar pattern detection of desiccation induced viability loss through TTZ staining method in many trees of Western Ghats like *Hopea parviflora* Bedd. and *Vateria indica* L.

The electrolyte leakage represented as conductivity measurement was fivefold higher in floated seeds than the seeds sank in distilled water and 10 % salt solution (Table 3 & 4). The measurement of total dissolved solid (TDS) were also in the same direction. It was two to three folds higher than that of floated seeds (Table 4). Total dissolved solid (TDS) is a measurement of the total of organic and inorganic substances dissolved in a liquid. This includes anything present in water other than the pure water molecules. Being an evaluation index of cell membrane permeability, high electrolyte

Table 3. Physiological features of seeds sorted by distilled water

Scientific name	Variable	T1	T3	t-value (P-value)
<i>Myristica malabarica</i> Lam.	Average Moisture content (%)	8.07 ± 0.19	28.60 ± 0.93	21.71**
	Leachate conductivity (µS)	572.77 ± 1.15	122.07 ± 0.88	311.61**
	TDS (µS)	251.43 ± 1.69	112.62 ± 0.70	75.78**
	Formazane Concentration -OD TTZ	0.0023 ± 0.0007 US	0.339 ± 0.030 R	11.24**
	Average Moisture content (%)	8.15 ± 0.11	30.3 ± 0.98	22.55**
<i>Myristica magnifica</i> Bedd.	Leachate conductivity(µS)	579.03 ± 0.85	139.06 ± 0.81	375.91**
	TDS (µS)	263.85 ± 0.84	111.06 ± 0.74	137.16**
	Formazane Concentration -OD TTZ	0.0022 ± 0.0005 US	0.347 ± 0.017 R	20.10**

** Significant at 0.01 level, ± Standard Error, %: Percentage, TTZ: 2, 3, 5 Triphenyl Tetrazolium Chloride, OD- Optical Density, TDS: Total Dissolved Solids µS: Micro Siemen, US: Unstained. . C: Control, T₁: Seed floated in distilled water, T₃: Seeds sank in distilled water.

Table 4. Physiological features of seeds sorted by salt water

Scientific name	Variable	T2	T4	t-value (P-value)
<i>Myristica malabarica</i> Lam.	Average Moisture content (%)	8.86 ± 0.09	31.20 ± 0.93	23.97**
	Leachate conductivity (µS)	560.66 ± 1.05	104.01 ± 0.75	352.27**
	TDS (µS)	180.52 ± 0.83	91.19 ± 0.74	80.58**
	Formazane Concentration - OD	0.0031 ± 0.0007	0.357 ± 0.016	21.62**
	TTZ	US	R	
<i>Myristica magnifica</i> Bedd.	Average Moisture content (%)	9.16 ± 0.48	31.98 ± 0.6	29.96**
	Leachate conductivity (µS)	568.28 ± 0.93	111.14 ± 0.89	355.87**
	TDS (µS)	206.76 ± 1.04	90.44 ± 1.01	80.49**
	Formazane Concentration - OD	0.0032 ± 0.0006	0.376 ± 0.032	11.75**
	TTZ	US	R	

** Significant at 0.01 level, ± Standard Error, %: Percentage, TTZ: 2, 3, 5 Triphenyl Tetrazolium Chloride, OD- Optical Density, TDS: Total Dissolved Solids µS: Micro Siemen, US: Unstained. C: Control, T₂: seed floated in salt solution, T₄: Seeds sank in salt solution

leakage and TDS during desiccation in seeds of *M. malabarica* Lam. and *M. magnifica* Bedd., indirectly infer the damage on structural integrity of cellular membranes in the tissues of the seeds (Cheng Hong - Yan *et al.*, 2005). The seeds with high viability usually possess well organized cellular membrane structure, with low of electrical conductivity (Goel *et al.*, 2002). Effective application of electrolyte conductivity and TDS methods for the estimation of seed viability has been reported in other species *viz.* *Glycine max* L. (Vieira *et al.*, 2004), *Medicago sativa* L. (Wang *et al.*, 1996), *Sorghum sudanense* Stapf. (Hsu *et al.*, 2000) and *Zea mays* L. (Cordova - Tellez and Burris, 2002). High vigor of seeds usually possess well organized cellular membrane structure, with low electrical conductivity values and TDS (Goel *et al.*, 2002). The desiccation induced damage occurred in cell membranes of seeds and discharge of more electrolytes leachate due this membrane damage has been reported in seeds of

Saraca asoca (Sorensen *et al.*, 1996), *Machilus thunbergii* Sieb. and Zucc. (Lin and Chen, 1995) and *Theobroma cacao* L. (Li and Sun, 1999). The desiccation of seeds adversely affect the structural integrity of the cellular membrane system which was reflected by the increased electrolyte leakage. According to Simon (1978) and Bewley (1985) progressive increasing leakage of electrolyte from the seeds as a consequence of seed desiccation can be taken as a reliable index of seed viability. The viability loss in desiccation sensitive recalcitrant seeds related to irreversible solute leakage has been reported by several authors (Berjack *et al.*, 1989; Fu *et al.*, 1990).

Non shaky seeds showed 93 to 94 % germination while shaky seeds not at all germinated (Table 5). While analyzing the physiological feature like germinability, moisture content of seeds, TTZ staining, electrolyte conductivity and total dissolved solids (TDS) of shaky and non shaky seeds, it was understood that the shaky

Table 5. Physiological features of seeds sorted by shaking of seeds

Scientific name	Variable	T5	T6	t-value (P-value)
<i>Myristica malabarica</i> Lam..	Moisture content (%)	6.1 ± 0.14	31.32 ± 0.66	37.50**
	Leachate conductivity (µS)	587.32 ± 0.83	107.29 ± 0.83	408.93**
	TDS (µS)	275.98 ± 0.8	92.85 ± 0.60	182.78**
	Formazan Concentration -OD	0.0032 ± 0.0015	0.338 ± 0.039	8.56**
	Germination (%)	0	90.80 ± 1.02	
<i>Myristica magnifica</i> Bedd.	TTZ	US	R	
	Moisture content (%)	6.34 ± 0.12	31.82 ± 0.79	32.06**
	Leachate conductivity (µS)	592.46 ± 1.09	114.46 ± 1.10	309.61**
	TDS (µS)	293.04 ± 1.25	95.54 ± 1.21	113.44**
	Formazane Concentration -OD	0.0014 ± 0.0004	0.354 ± 0.042	8.48**
	Seed Germinated (%)	0	89.60 ± 1.17	
	TTZ	US	R	

** Significant at 0.01 level, ± Standard Error, %: Percentage, TTZ: 2, 3, 5 Triphenyl Tetrazolium Chloride, OD- Optical Density, TDS: Total Dissolved Solids µS: Micro Siemen, US: Unstained. T₅: Shaky seeds, T₆: Non shaky seeds

seeds showed physiological resemblance with seeds floated in fresh water and 10 % salt solution and non-shaky with seeds sank in fresh water and 10 % salt solution. The moisture content of the fresh mature seeds of *M. malabarica* Lam. and *M. magnifica* Bedd. were 29 %, 30 % respectively and at this stage the seeds showed high germination percentage (Table 5). They were not shaky and do not produced sound while shaking. The tissues of the embryonic axis at this stage shown deep red colour while incubated in TTZ solution and absorbance of formazan was very high. Electrolyte leakage and TDS were recorded least value. The moisture content of fresh, mature seeds on exposure to open laboratory condition ($28 \pm 2^\circ\text{C}/60\% \text{ RH}$) for 8 days was declined drastically from 29 - 31 % to 5 - 6 % and became shaky. Such seeds started to produce sound while shaking and their tissues in the embryonal axis remained unstained with TTZ solution. The electrolyte leakage and TDS recorded very high values (Table 5).

In general, the loss of germination potential, reduced TTZ stainability and formazan absorbance, enhanced electrolyte leakage and TDS are the consequences of desiccation induced structural degradation of membrane system and macromolecules of the cell. Direct correlation between moisture content and germination percentage of *M. malabarica* Lam. was reported by Babu *et al.* (2010).

Based on the observations and analysis of results, it was understood that the most basic and critical factor which determine the viability of recalcitrant seeds was seed moisture content. The longevity of seeds was mainly affected by the reduction in moisture content below a critical value that may vary considerably among different species (Sanhewe and Ellis, 1996; Tweddle *et al.*, 2003) reported on the desiccation - sensitive seeds which are most common in tropical rainforests. Protein denaturation (Simon *et al.*, 1976) membrane instability (Wolfe, 1978) and lack of considerable proportion of unsaturated fatty acids (Mello *et al.*, 2010) are the possible reason behind deleterious effect of non-ambient temperature. Water serves as a protectant of macromolecular structure and controls the level of metabolic activity in plants. Thus the loss of water may strongly affect the nature of physical and biochemical reactions (Vertucci and Farrant, 1995). The logical and scientific analysis of the results obtained signify that the seeds of *Myristica* spp. with proper moisture content only sink in salt solution and these seeds register highest rate of germination. More number of seeds sunk in fresh water may be attributed to the lower density of distilled water compared to that of 10 % salt solution. The scientific inference of the observation regarding this traditional

knowledge is that the salt solution can be used for sorting viable seeds. The recalcitrant seeds with moisture content below the critical moisture level were shaky and less dense than water and that is why they made sound while shaking and floated while kept in distilled water and 10 % salt solution. These crude, but effective technique followed by the farmers to sort the viable seeds can be practiced with unskilled labour by utilizing locally available resources with affordable cost.

4. Conclusion

Traditional knowledge related to agriculture is a great treasure prevailed among the farmer community. Traditional knowledge has two powerful advantages over scientific knowledge like it has little or no cost, and is readily available. This type of knowledge should be identified, documented, validated scientifically and transferred to future generation before being vanished from our society. The present study was intended to provide protection and preservation to the traditional knowledge of local farmer's rural techniques related to sorting of viable seeds especially recalcitrant seeds like *Myristica malabarica* Lam. and *Myristica magnifica* Bedd. which are ecologically and economically unique.

Acknowledgements

The authors are thankful to the farmers and various farming communities of the Palakkad District for their help in field work and sharing their valuable knowledge on seeds. First author also thanks the Kerala University for the sanctioned PhD Programme.

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