Seed Morphology and Tetrazolium Quick Seed Viability Testof Malabar Neem (*Melia dubia* Cav.) under Laboratory Condition

ABSTRACT

Melia dubia Cav. commonly known as Malabar Neem, is an economically important fast-growing indigenous multipurpose tree species of India.It has a wide range of applications from medicinal utilities such as treating pain, fever, and infections, to agricultural and industrial uses*viz.*, fodder, seed oil, resin, fuel wood, timber and its wood being utilized in the paper, matchbox, and plywood industries.Malabar Neem generally is distributed in tropical and sub-tropical regions of India and introduced in many countries of South Africa, the Middle East, North and South America, Brazil, Bermuda, Argentina, Southern Europe, Southeast Australia, and Asia Pacific regions.Nevertheless,it is a most promising tree species which is highly suitable for agroforestry or farm forestry with a life cycle of 8 to 12 years that gaining economic importance both in domestic and global markets and plantations in degraded lands. Consequently, the demand of Melia seedlings among Indian farmers has been increased, however the poor seed viability and very low seed germination hampered the good quality nursery production and large-scale plantations. Before seed sowing in nursery, there is need to determine the actual seed viability and to know the required seed quantity of Melia to be sown in forest nursery. Therefore, the present research study was conducted at Dr. Y. S. Parmar University of Horticulture and Forestry, Nauni, Solan, Himachal Pradesh of India to study the seed morphology and seed viability testing protocol using tetrazolium (TZ) test.The seeds of Melia species stained with 0.5, 1.0, 1.5 and 2.0 % solution of TZ and incubated at 30°C temperaturefor 24hours. The tetrazolium test performed with 100 seeds in 4 replications in CRD design and analyzed with OPSTAT statistical software. The length, width and weight of fruit, drupe and seed were recorded in the range of 9.40-12.97 mm, 6.55-9.70 mm&2.84-2.99 g; 5.84-14.32 mm, 2.76-9.72 mm&1.672-1.798 g; and 1.2-1.98 mm, 1.02-2.98 mm & 3.12-3.74 g, respectively.The colour of fruit was yellow with oval shape having smooth surface.The result of TZ test showed that the seeds of Malabar Neemsoaked in 0.5 %tetrazolium solution at 30 °C temperature for 24 hours found to be the highestseed viability percentage (40.25 %) which is recommended as a quick seed viability protocol in *M. dubia*. Overall, the results of the present research study fulfilled all the gaps to test quick seed viabilityof a seed lot before seed sowing in nursery for accurate knowledge of the seed requirement in quantity for the purpose of quality seedling production in nursery.

Keywords:*Melia dubia, Agroforestry tree species, Seed Morphology,Seed Viability, Tetrazolium Test, Nursery Seedlings*

INTRODUCTION

Melia dubia Cav. commonly known as Malabar Neem, is a member of the Meliaceae family. It is an industrially and economically important fast growing indigenous multipurpose tree species of India, which can be harvested on a short rotation period [1]. It is a deciduous tree which can grow upto 30 m height. The leaves are 2-3 pinnate, imparipinnate, estipulate, attenuate, rachis 10-30 cm long, slender, swollen at base, terete, scurfy tomentose when young; pinnae 3-7 pairs, 10-20 cm long; leaflets 2-11 on each pinna, opposite, estipulate; petiolule 3-10 mm long, slender; leaf lamina 4.5-9 x 2-4 cm in size, ovate-lanceolate, acute, base oblique, obtuse, round or attenuate, apex acuminate, margin crenate, glabrous at maturity. Flowers bisexual, 5-6 mm long in size, greenish-white. Fruit is a drupe, 2 x 1 cm in size, dorsally compressed with longitudinal ridges, yellow in ripen colour, fleshy; seeds 1 - 3 or 4, 1 cm in size [2]. Malabar Neem generally is distributed in tropical moist deciduous forests in the statesof India such as Tamil Nadu, Kerala, Karnataka, Himalayas, Sikkim, North Bengal, Upper Assam, Khasi Hills, Gujarat, Hills of Orissa, Deccan plateau, Nallamalai Hills and Western Ghats at an altitude ranging between 400 and 1800m above mean sea level [2, 3]. This species is native to Southern Asia, and has been introduced in many countries in South Africa, the Middle East, North and South America, Brazil, Bermuda, Argentina, Southern Europe, Southeast Australia, and Asia Pacific regions [4].

The wood of Malabar Neem tree is used majorly in plywood industry and screened out as an alternate species for pulpwood [5]. The wood of Melia is also used for packing cases, pencils, match box sticks, photo frames, mini furniture like stools, benches, wooden tables, interior decoration, window, doors, wooden racks, musical instruments, tea powder boxes, cigar boxes, wooden building, ceiling planks, agricultural implements etc. [6]. Malabar Neem is one of the fastest growing trees in the world and considered as a money-spinning tree of short rotation due to its high demand in pulpwood, plywood and timber industries [3]. It is a good fuel and fodder yielding tree species. Its uses range from medicinal applications, such as treating pain, fever, and infections, to agricultural and industrial uses, with its seeds as a source of neem oil and its wood being utilized in the paper, matchbox, and plywood industries [7].Malabar neem is one such alternative species suitable for timber, plywood, pulpwood and fuel wood [8]. Thus, the species has a ready and assured market due to its multifarious utilities. The species is also highly adaptable in different environment conditions.

Nevertheless,Malabar Neem is a most promising tree species which is highly suitable for agroforestry or farm forestry with a life cycle of 8 to 12 years that gaining economic importance both in domestic and global markets [1].Consequently, there is good demand of Melia seedlings among farmers in Indian subcontinent. But the poor seed viability and very low seed germination hampered thegood quality nursery production and large-scale plantations of Melia in India [9]. The low seed viability and prolonged germination period resulted uneven germination and poor-quality seedlings. Therefore, there is a need of precise seed viability determination before to seed sowing in nursery which has a great importance for the reproduction of the species. The accuracy of required quantity of seeds of Melia to be sown in forest nursery is mainly dependent upon the viability of the seeds.One of the most accepted and reliable method of tree seed viability is the tetrazolium test[10]. Historically, a topographical method or tetrazolium test (TZ) of seed viability evaluation was developed first for agricultural cereal crops [10], later on conifer tree species [11] and many more crops [12].Seed dormancy is very common in most of the forest tree species [13,14]. However, TZ testprovides a quick informationonseed viability of any crop species even it isin a state of seed dormancy [15]. In addition, there is very scanty information available on seed viability testing in *Melia dubia* using tetrazolium test.Seed morphology is also important to identify the good quality seeds by measuring seed dimensions, seed weight, colour, shape and surface of fruit and seeds. A comparative research study of seed morphology in response to desiccation tolerance and physiological parameters in 71 Eastern Australian rainforest species was conducted by Hamilton et al. (2013) for the purpose of long-term biodiversity conservation [27].Keeping all the points in mind, the present experiment was conducted in Tree Seed Technology Laboratory with two primary objectives *viz.*, first to study the drupe and seed morphological characteristics of Malabar Neem, and second to evaluate the seed viability of Malabar Neem using quick tetrazolium test.

EXPERIMENTAL DETAILS

Seed collection and extraction

Fruits of Malabar Neem tree was collected from the experimental farm of forestry, College of Horticulture and Forestry, Neri, Hamirpur, Himachal Pradesh, India during the year of 2024at latitude (31.695790 N), longitude(76.4677291 E)and altitude (620 m from MSL)as geolocation presented in Figure 1. The recorded data of mother tree were recorded and presented in Table 1. After the collection of fruits, they were sun dried for 2-3 daysso that the fruit pulp gets dried and removed. This process is known as depulping.The pulp removed and then seeds (kernels) were extracted from the drupes (Figure 2). Then, seeds exposed for surface sterilization and dried, this is known as seed drying.Further, kernels were mixed thoroughly to maintain homogeneousness in the seed lot as per ISTA rules [28].

Figure 1. Geolocation of fruit collection of *Melia dubia*for the present research study

Table 1: Recorded data of Malabar Neem mother tree

Tree Height (m)	DBH (cm)	Girth (cm)	CBH (m)	Crown Length (m)	Crown Width (m)
9.5	20.70	65	5.5	4	3.5

Figure 2. Extracted drupes from fruits after removing pulp and extracted seeds or kernels from the dried drupes of*Melia dubia*

Recording of Fruit, Drupe and Seed (Kernel) data

The length and width of fruits, drupes and kernels were measured with the help of Vernier Calliper (Mitutoyo) in millimetre. The Weight of fruits, drupes and kernels were taken with the help of Analytical Balance (Fisher Scientific). The colour, shape and surface of fruits, drupes and kernels were recorded by visual observation with the help of standard Munsell Colour Chart.

TetrazoliumSeed Viability Testing

The tetrazolium test (TZ) was employedwith four replications of 100 seeds from the seed lotof *M. dubia*.Seeds of theMalabar Neem were stained in 0.5, 1.0, 1.5 and 2.0% solution of tetrazolium test prepared in the double distilled water. The seeds of Melia were placed in the Petridishesin four replications to incubate with tetrazolium solution at 30°C temperature in the darkness for24hours (Figure 3).

The chemical 2,3,5-Triphenyl Tetrazolium Chloride (TTC) enters into the living embryo of the seed which produce a reddish or dark purple colour due to water insoluble compound called formazan by the activity of dehydrogenase enzyme [16]. After completion of incubation time, the Tetrazolium solution was drained out and seeds of Melia were washed in distilled water.Then, red- or purple-coloured seeds were counted as viableseeds and non-coloured seeds as non-viableseeds (Figure 4).

Figure 3. Dipping of excited seeds of *Melia dubia*in tetrazolium solution

Figure 4: Data recording of purple-coloured seeds asviable and non-coloured seeds as non-viable seeds of *Melia dubia*

Statistical Analysis

The collected experimental data in the laboratory condition were analysed in CRD with four replications by using OPSTAT statistical software package [17] for appropriate interpretation of data and ANOVA was constructed. Then, treatment means were compared at 5 % level of significance to find out the best tetrazolium concentration for seed viability in Melia as per suggested per Gomez and Gomez [18].

RESULTS AND DISCUSSION

Fruit, Drupe and Seed Morphology

Fruit length of Malabar Neem was found in the range of 9.40 to 12.97 mm with the mean of 11.24 mm. Fruit width was recorded in the range (6.55-9.70 mm) with the mean of 8.35 mm. Fruit weight was recorded in the range between 2.84 to 2.99 g with the mean of 2.964 g. The colour of fruit was yellow with oval shape having smooth surface (Table 2).

Table 2: Fruit characters of *Melia dubia*

Characters	Range	Mean
Fruit Length (cm)	9.40-12.97 mm	11.24 mm
Fruit Width (cm)	6.55-9.70 mm	8.35 mm
Fruit Weight (g)	2.84-2.99 g	2.964 g
Fruit Colour	Yellow	-
Fruit Shape	Oval	-
Fruit Surface	Smooth	-

Drupe length of Malabar Neem was found in the range of 5.84-14.32 mm with the mean of 9.53 mm. Drupe width was recorded in the range of 2.76-9.72 mm with the mean 5.16 mm. Drupe weight was recorded in the range between 1.672 to 1.798 g with the mean of 1.734 g. The colour of drupe was whitish yellow with ovoid-ellipsoid shape having asperoussurface. The number of seeds per drupe was observed in the range of 1 to 4 having a mean value 2.2 (Table 3).

Table 3: Drupe characters of*Melia dubia*

Characters	Range	Mean
Drupe Length (cm)	5.84-14.32 mm	9.53 mm
Drupe Width (cm)	2.76-9.72 mm	5.16 mm
Drupe Weight (g)	1.672-1.798 g	1.734 g
Drupe Colour	Whitish Yellow	-
Drupe Shape	Ovoid-ellipsoid	-
Drupe Surface	Asperous	-
No. of seeds/ Drupe	1-4	2.2

Seed (Kernal) length of Malabar Neem was found in the range of 1.2-1.98 mm with the mean 1.42 mm. Seed width was recorded in the range (1.02-2.98 mm) with the mean of 1.84 mm. Seed weight of 100 seeds was recorded in the range between 3.12 to 3.74 g with a mean value of 3.45 g. The colour of seed was black with narrow fusiform shape having smooth surface (Table 4).

Table 4: Seed (Kernal) characters of *Melia dubia*

Characters	Range	Mean
Seed Length (mm)	1.2-1.98 mm	1.42 mm
Seed Width (mm)	1.02-2.98 mm	1.84 mm
100 Seed Weight (g)	3.12-3.74 g	3.45 g
Seed Colour	Black	-
Seed Shape	Narrow Fusiform	-
Seed Surface	Smooth	-

Suresh and Devakumar (2017) categorized fruits of Malabar Neem into three categories *viz.*, small, medium and big size for the study of seed morphology [19]. They reported fruit length (small-27.50 mm, medium-31.75 mm and big-36.84 mm), fruit width (small-19.85 mm, medium-24.76 mm and big-27.89 mm), and fruit weight (small-9.68 g, medium-12.66 g and big-17.45 g) which was higher than the result of present study due to climatic conditions.They also reported seed length (small-14.25 mm, medium-14.36 mm and big-14.48 mm), seed width (small-3.64 mm, medium-3.74 mm and big-3.86 mm) and seed weight (small-0.16 g, medium-0.20 g and big-0.23 g) which was also higher than the result of present study due to climatic conditions.Malabar Neem was introduced in College of Horticulture and Forestry, Neri, Hamirpur to know the growth performance in the subtropical region of Himachal Pradesh at 680 m elevation height. It is also noticed that Malabar Neem tree is producing twice flowering and fruiting in a single year which divide the energy to produce flowerings due to climatic conditions. Therefore, the size of fruit is lower in the Northen Indiathan the reported size by Suresh and Devakumar (2017) in Southern India.

Seed Viability

Analysis of variance showed significant differences ($p<0.05$) among treatments of tetrazolium concentrations (Table 4). For imbibed seeds of Malabar Neem in tetrazolium solution, the lowest concentration (0.50 %) of tetrazolium solution was observed the highest (40.25 %) of viable and vigourous seeds, however the lowest seed viability percent (26.50 %) was recorded in 2.00 %

tetrazolium solution (Table 5& Figure 5). In case of 1.00 % TZ solution, the seed viability percent was recorded 30.25 % and 28.25 % seed viability in 1.50 % TZ solution. When the concentration of tetrazolium solution was increased then the seed viability percentage was decreased (Table 6).

Table 5: Analysis of variance (ANOVA) in CRD for seed viability of *Melia dubia*

Source of Variation	DF	Sum of Squares	Mean Squares	F-Calculated	Significance
Treatments	3	454.19	151.40	14.28	0.001
Error	12	127.25	10.60		
Total	15	581.44			

Table 6: Seed viability of*Melia dubia*

Treatments	**Mean**	**S.E.**
0.50 % TZ Solution	40.25	2.29
1.00 % TZ Solution	30.25	1.11
1.50 % TZ Solution	28.25	1.65
2.00 % TZ Solution	26.50	1.19
Mean	**31.31**	
SEm (±)	1.63	
C.D.	5.07	
C.V.	10.40	

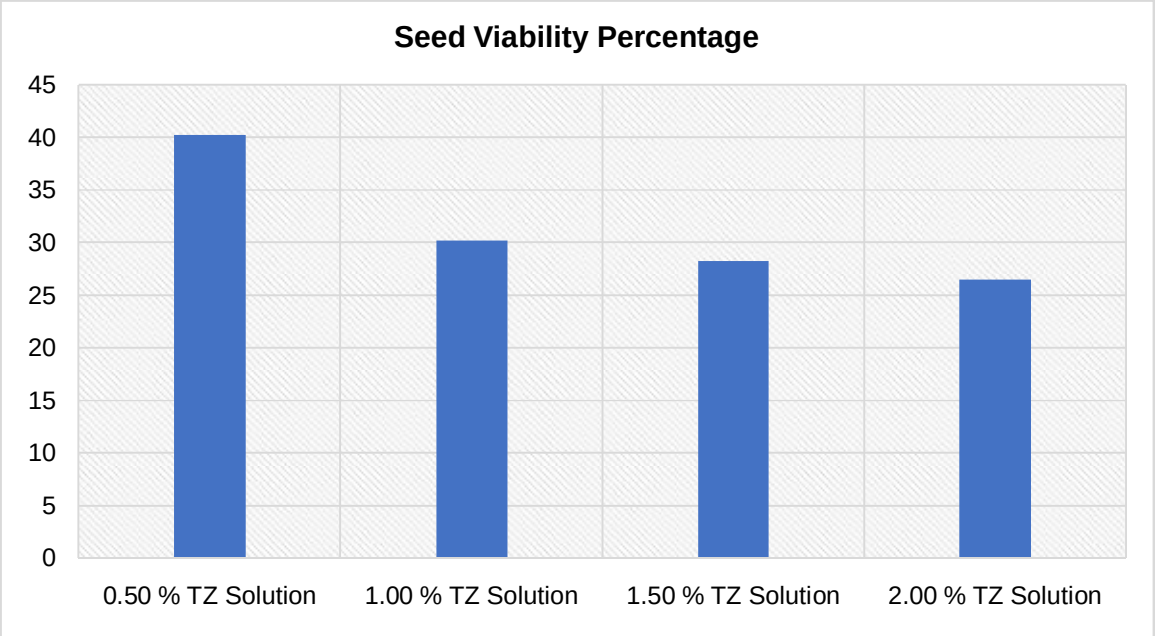

Figure 5: Seed viability of *Melia dubia*

Ajay *et al.* (2022) reported that GA_3 @ 100 ppm-soaked kernels of Malabar Neem for 24 hours significantly found to be the maximum seed viability percentage (41.68) with 0.1 % tetrazolium test with compare to control (20.87 %) [20]. This confirmed from the result of the present study that the seed viability raged between 26.50 % to 40.25 % with a mean value of 31.31 % seed viability. Phartyal *et al.* (2003) reported that the seeds of *Ulmus wallichiana* took stain very lightly when put in low concentration of TZ (0.1%) for a short duration (4-8 hours) and the colour intensity increased with an increase in temperature, incubation time and concentration of TZ solution. They found that the seed soaked at 40℃ for 24 hours in 1.0 and 0.5% solution of TZ showed 100% seed viability in*Ulmus wallichiana* [21]. Similarly, Aslam *et al.* (2010) studied the efficacy of tetrazolium test in determining the seed viability of *Pinus wallichiana* against the germination test. The percentage seed viability and laboratory germination recorded 79.82% and 82.69%, respectively which has further proved that tetrazolium test is a rapid, very effective and valuable research technique to know the seed viability of any crop [22]. The seeds of *Pinus roxburghii*soaked in 0.5 % tetrazolium solution at 25°C temperature for 24 hours was observed the highest seed viability percentage (64.75 %) which is recommended and standardized quick seed viability protocol in the chir pine species [23]. In various tree species, the tetrazolium test standardizedby different scientists for quick seed viability testing such as 0.01 % TZ solution in *Swietenia macrophylla* [24]; 0.5 % TZ solution in *Moringa oleifera* [25]; 0.5 % TZ solution in *Platymiscium floribundum*;0.1 % TZ solution in *Lonchocarpus muehlbergianus*; 0.5 % TZ solution in *Acacia polyphylla*[26]. A quick seed viability test was performed on four tropical forest tree species *viz.*, *Albizia lebbek, Acacia nilotica, Azadirachta indica* and *Casuarina equisetifolia* of India and a positive correlation was found between seed viability percentage estimated Tetrazolium Test by with germination test [29].Various seed viability tests *viz.*, triphenyle tetrazolium chloride test, excised embryo test, electrical conductivity, and germination test were performed on seeds of Neem belongs to Meliacease family obtained from mother tree of different age classes and the consistency was observed with the TTC (triphenyle tetrazolium chloride) and EC test in germination of seed in laboratory as well as nursery condition [30].Thus, the results from the present research study validate to the previous research studies conducted by various scientists across the globe for different forest tree speciesand confirmed its suitability for prediction of seed viability in Malabar Neem.

CONCLUSION

From the present research, it can be concluded that the seeds of *Melia dubia* should be soaked in 0.5 % tetrazolium solution at 30°C temperature for 24 hours to test quick seed viabilityof a seed lot before seed sowing in nursery for accurate knowledge of the seed requirement in quantity for the purpose of quality seedling production.

Disclaimer (Artificial intelligence)

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

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