Chapter: I

Seed Germination
Vigour and Viability
1.1. Introduction

The high germinability and storability which are indicators of seed qualities is of key importance in respect to ecology, agriculture and the economy [300, 2, 301]. Seeds during storage are constantly exposed to adverse condition hence undergo gradual deterioration that is reflected in reduced percent of germination, storability and tolerance to abiotic stress [302]. Ageing is an irreversible degenerative process that occurs during storage. Damage of seed during storage is inevitable and decrease of seed quality is proportionately depends upon storage conditions i.e. temperature and relative air humidity. Seed moisture content, duration of storage, type of seed and initial quality of seed are other factors that contribute to seed ageing [303, 304, 305]. Additionally, storage conditions like high temperature and relative humidity (RH), a protocol commonly categorized as “Accelerated Ageing” is widely used for promoting rapid loss of seed germination and storage ability [306, 204]. Further, the chemical composition of oil seeds triggers specific metabolic reactions that contribute to rapid loss of germinability during storage. In fact, the seeds rich in lipids have limited longevity [131]. The neem seeds are rich in oil content (51%) [22], hence it would be interesting to unravel the longevity and storability in these seeds during differential drying.

Briefly, two categories of seed storage behavior i.e. orthodox and recalcitrant have been proposed [5]. Seeds that are desiccation tolerant and hence can be dried to sufficiently low water content without influencing their germinability and storability are characterized as orthodox such as Citrus spp. [307, 308, 38], Quercus spp. [309, 310, 48, 46], Cattleya aurantiaca [311, 312] and Manihot esculenta Crantz [313]. In contrast, the seeds that are shed from the mother plant with high water content and damaged by dehydration and are generally short-lived are classified as recalcitrant such as Shorea robusta [17], Theobroma cacao [314], Araucaria bidwillii [315] and Acer pseudoplatanus [316], Avicennia marina [317], Syzygium cuminii [297], Dipterocarpus sp. [318], Gmelina arborea, Hopea ponga, Jatropha curcas [64, 319, 320]. Recent studies identified a third category of seeds which are categorized as “intermediate seeds” because their storage behaviour permits slight dehydration but not like orthodox seeds [10, 321]. Large number of seeds such as Oil palm (Elaeis guinensis), coffee (Coffea arabica), papaya (Carica papaya) and mahogany (Swietenia macrophylla) [306], kauri spp (Agathis australis and Agathis
macrophylla) [322] and multipurpose neem tree *Azadirachta indica* [323, 324, 121], *Coffea arabica* [10], *Carica papaya* [12], *Elaeis guineensis* [325], *Fagus sylvatica* [326], Citrus [327], *Elaeis guinensis* [328] which were previously classified as recalcitrant are now categorized as intermediate seeds.

During storage, the seed water content, one of the prime factors that regulate seed viability and vigour in non-orthodox seeds, depends absolutely on rate of dehydration. Two types of drying rate such as slow (natural) and or rapid (using silica gel) are widely used in determining the seed longevity and the magnitude of desiccation tolerance in non-orthodox seeds [329]. The rapid drying compared to slow drying permits drying of non-orthodox seeds to significantly low water contents without affecting the seed viability and vigour. The differential rates of drying are commonly employed for determining the desiccation tolerance in non-orthodox seeds; recalcitrant or intermediate. The rapid drying alleviated significantly the desiccation tolerance in large number of seeds such as *Hevea brasiliensis* [61], *Araucaria hunsteinii* [60], *Avicennia marina* [64], *Scadoxus membranaceus* [63], *Castanospermum australe* [62], *Landolphia kirkii* Dyer [59], *Camellia sinensis* [47], *Ekebergia capensis* [54], *Castanospermum australe, Trichilia dregeana* [65], *Clausena lansium* [66], embryos of Cocoa and Ginkgo [330] and *Shorea robusta* [18]. Response of seeds or excised axes of non-orthodox seeds to drying depends absolutely on the rate at which water is lost [55, 54, 50, 51, 18]. To overcome the hindrance due to seed coverings, embryonic axis is subjected to flash drying especially for cryostorage [62]. This drying technique assisted dehydration of axes to sufficiently low water contents far more rapidly with minimal loss of viability in *Shorea robusta* [18], *Quercus robur* [57], *Landolphia kirkii* [55], and *Quercus rubra* [48]. In contrast, the slow-drying conditions facilitate aqueous-based deleterious processes as seed tissues have to spend a longer time period at intermediate water contents [55, 56, 331, 57]. Higher water contents (the water contents of undried seeds) allow aqueous-based degradative processes resulting from the disturbance of ongoing metabolism [332, 54, 53, 333]. Dehydration of seeds or axes below the critical water content (intolerance level) result in severe damage that occurs because of the removal of bound water from intracellular surfaces or biomolecules [53]. Thus, it is difficult to specify a cut-off-moisture content in case of different drying rates that is commonly referred as “critical moisture contents’
[CMC] at which viability is lost without specifying parameters relating both to the seeds or axes [54, 70]. The knowledge of CMC of the seeds help in the process of drying as well as the proper methodology suggested for storage. Berjak group [70, 329] had reviewed drying rate effect on recalcitrant seeds and suggested that different deleterious pathways are probably involved in desiccation induced cellular damage under different storage conditions. Studies on the effect of differential drying on seeds and axes by Pammenter et al. [55], Come and Corbineau [56], Pritchard and Manger [57] and Varghese et al. [18, 58] concluded that the rapid rate of drying of seed does not allow adequate time for sub-optimal water content (lower than CMC) mediated cellular damage to occur.

Water content (WC) of seed plays a critical role in the determination of seed longevity after harvest and during storage [5, 334, 11, 321, 335, 17]. Generally, the seeds are dried to low WC as high WC (the WC of un-dried seeds) is commonly responsible for susceptibility of the seeds to fungal or other pest attack [53, 54]. In addition to WC, the water content in the cell also be expressed as absolute and relative WC [336, 337, 59], water potential, water activity [338, 339, 52], cell volume [340, 341, 342], intracellular viscosity [343, 344], intermolecular proximity [345, 346] and structural water [347]. Additionally, WC is frequently expressed on a fresh or dry mass basis. Whilst WC on a fresh mass (FM) basis gives an accurate picture of the proportion of a seed constituted by water, water content on a dry mass (DM) is usually preferred when dealing with studies on the effects of water loss or uptake. This situation arises because WC on a DM basis is a linear expression of the amount of water in tissues [348]. Data should be preferably expressed on dry mass basis as the calculated values that are being normalized does not change as the amount of water changes and the proportional change in ‘water content’ reflects the proportional change in the amount of water in the tissue.

Sensitivity of seeds to high temperatures is strongly dependent on their water content as viability loss is faster with increasing water content (or moisture content) [302, 172, 338]. Oxidative reactions are largely responsible for ageing of dry seeds [126], but they are dependent on the seed water content. At water contents higher than 0.25 g H₂O g dry matter (DM), mitochondrial
respiration is estimated to occur [332], and to be one of the major sources for the production of active oxygen species (AOS). High seed moisture and high temperature further accelerate the seed deterioration in Zea mays L. [349, 350], Gossypium hirsutum L. [203] and Capsicum annuum Linn. [351].

The non-orthodox seeds can be dried to limited water content without the loss of germinability and the water content at which the germinability is reduced from the initial percent of germination is called Critical Water Content [CWC] [327, 54, 90]. If drying continues further below CWC, viability is eventually reduced to zero. For planning and execution of seed drying for storage of desiccation sensitive seeds, it is a pre-requisite to know the CWC of a species [352, 181]. Several tropical and temperate seeds like Araucaria hunsteinii [60]; Shorea robusta [17, 119, 120], Clausena lansium (Lour) and Litchi chinensis (Sonn.) [353] that are desiccation-sensitive are characterized by high CWC (> 30%). There are certain limitations of drying such as drying below CWC will not improve longevity and may even have deleterious effects on seed survival during storage [10, 354]. Seeds of several tropical tree species that are desiccation sensitive are characterized by high CWC or CMC (>30-40%) [355, 356, 353, 357, 358]. The values of CWC for recalcitrant species vary depending on the species [55, 359]. CWC for recalcitrant seeds such as Hopea odorata [360], Acer saccharinum [361], Madhuca indica [362], Dipterocarpus obtusifolius [319], Dipterocarpus turbinatus [319], Symphonia globulifera [363], Dipterocarpus costatus [356], Parashorea malaanonan [356], Araucaria araucana [298], Cotylelobium burckii [298], Trichilia tessmannii [298], Shorea robusta [364], Artocarpus heterophyllus [297], Euterpe edulis [365], Garcinia kola [181] and Syzygium cuminii [297] ranges from 30-50%. Dehydration of non-orthodox seeds below CWC resulted in rapid loss of germinability in seeds of Fagus sylvatica [366], Azadirachta indica [22], sun flower [339], Pongamia pinnata [367], Syzygium cuminii [16], Shorea robusta [368, 17]. In the present study, slow and rapid drying method will be examined to determine the longevity of neem seeds below CWC (10.9 Moisture content) [21].
All seeds are not alike in respect to longevity as they show large variation depending upon their initial viability, seeds water content, storage temperature and above all their storage behaviour. The most widely accepted single criterion for estimating seed deterioration is to measure percent germination [369, 370, 179, 371]. The seeds with 85 or above percent germination, especially in crops, produce healthy seedlings whereas seeds with lower germination percent tend to produce weak seedlings [372]. Seeds producing weak seedling are unable to survive once exposed to field conditions [373, 374, 375, 376] and finally result into abnormal or poor seedlings that are unable to establish [377]. During ageing, seeds lose their vigor, viability and germinibility [378]. The ageing or loss of vigor is evident by delayed germination and emergence, slower growth, increased susceptibility to stress, and ultimately decline in germinability [379, 204]. Germination ability and subsequent seedling establishment are the most important and proven parameters to assess the quality of seed [380, 381, 382].

The first sign of ageing in seed is lengthening of germination time [376]. Seeds after prolonged storage under natural conditions [ambient temperature and RH] that causes ageing leads to increased period of lag time required for germination as well as duration between imbibition and radicle emergence [383]. Compared to aged seeds high vigour seeds take shorter time to germinate with lesser duration between imbibition and radicle emergence [384]. In addition to percent germination estimated for quantifying the seed quality the germination index [GI], method for measuring seed vigour, is another method used frequently [383]. Czabator [385] proposed "germination index (GI)" to quantify the variation in the lag period for germination and establish its close relationship with the seed performance. It was found that GI is an expression of speed and totality of germination, and their interaction. A significant drop in the GI value was reported for desiccating and/or ageing seeds of Ammopiptanthus mongolica [124], Pongamia pinnata [367].

Viability tests can be used as significant indicators of seed germinability during ageing [386, 387]. The viability test is the measure of seed ability to produce enough of H⁺, released from the dehydrogenases activity [TCA cycle] during mitochondrial respiration. The tetrazolium
chloride (TZ) test is one of the most valuable and rapid techniques for analyzing seed quality [388]. The test evaluates the viability of seeds even when the seeds are in a state of dormancy [389]. The efficiency of the TTC test depends on the development of seed preconditioning, post conditioning preparation and staining methods adopted and standardized for each species [390]. Methodologies for carrying out the TTC test are constantly being refined, with specific manuals being available for the seeds of Gossypium hirsutum [391], Zea mays [392], Phaseolus vulgaris [393], Arachis hypogea [394] and Glycine max [395]. A very good correlation between TTC staining and seed viability was investigated in Allium cepa [305], Pinus wallichiana [396], Kielmeyera coriacea [397] and Shorea robusta [368].

The semi-permeable cellular membranes regulate the transport of materials into and out of the cell. Therefore, their role in maintaining seed viability and vigor is of key importance. Solute leakage is a common phenomenon observed immediately following the imbibition of water by dry seeds. During maturation drying of seed development the membranes are disorganized thus its repair and re-orientation to become semi-permeable essentially requires some time after imbibition. The plasma membrane in the dried seeds is in gel phase, which is disrupted during imbibition under the pressure of penetrating water, i.e. rapid rehydration [398]. The lag period for the repair of the membrane in an imbibed seed is a primary cause of solute leakage in the healthy seeds. The rate of leakage corresponds to the degree of cell membrane damage and or repair in response to ageing [187, 188]. Damage to the organization of cell membranes during seed ageing may constitute an important factor in explaining seed deterioration [399, 400, 175]. Membrane has been shown to be a key site of injury during seed ageing primarily due to oxidation and damage of its components especially lipids and protein [223]. The aged seeds are generally marked by increased solute leakage [401, 402, 403]. Electrical conductivity is used as useful indicator of seed viability and vigor. Electrolyte leakage of high vigor seeds is less than that of low vigor seeds and correlated with decline in germination in the seeds of Glycine max [404], Phaseolus vulgaris [405] and Lens culinaris [406], Shorea robusta [17], Euterpe edulis [365], Hopea ponga [407], Knema attenuate [408], Pongamia pinnata [367], Syzygium cuminii [16], Fagus sylvatica [316] and in peas [409]. Enhanced electrolyte leakage loss estimated in the damaged seeds of Lotus corniculatus [410], Artocarpus heterophyllus [411], Euterpe edulis [365], Fagus sylvatica [316] and Hopea ponga [407] was an indicator of loss in membrane integrity as a result of dehydration and is accountable for reduced germination and vigour of
seeds. Oil seeds such as soybean, mustard, sal, neem and mahua are more prone to desiccation induced deterioration due to high oil contents as the unsaturated lipid of the membrane undergoes high rate of peroxidation in presence of atmospheric oxygen thus damaging the membranes. Specific conductivity of leachates from imbibing seeds is widely used for testing seed vigour in *Shorea robusta* [412], *Azadirachta indica* [192], *Madhuca indica* [413] and in several crop species [177, 414]. The rate of leakage is directly correlated with the cell damage and repair in response to ageing [187, 188, 175]. Desiccation induced loss of viability in many recalcitrant seeds exhibits good negative correlation with electrolyte leakage [22, 415, 69, 17, 416].

In the present study, the effect of slow and rapid rate of drying (monitored by recording loss of water content) was investigated on neem seed longevity by estimating the percent germination, vigour (GI and specific conductivity) and viability.

### 1.2 Materials and Methods

#### 1.2.1 Site of Sample Collection

The neem fruits were collected from plus trees; a phenotype judged by appearance – Morphological characters such as; Tall with proportionate girth, good canopy, heavy flowering, rigorous fruiting, resistance to disease and insect attack or to other adverse environmental factors [417] in both Pt. Ravishankar Shukla University campus and Government Science College campus, Raipur (21°14'14"N latitude and 81°38'55"E longitude; elevation: 298 meters above sea level). Nearly 100 plus trees were marked in the area for collection of mature fruits.

#### 1.2.2 Neem seed: Collection & Extraction

The seed is ovoid or spherical, pointed above and has a thin testa. It is exarillate with a small adaxial sacrotesta [418]. The mature fruit occasionally comprises of two to three seeds also. The mature, yellow fruits were harvested (84 days after anthesis) from trees either by hand plucking or collecting the freshly dropped fruits over a cloth spread on the ground after shaking the
branches. These fruits were brought to the laboratory within an hour of harvesting. In order to minimize the variation in seed quality/difference, the fruits of almost identical size, shape, colour and health were sorted out. A few seeds from the lot were depulped manually to determine the initial seed moisture content (IMC) [419] at the time of harvest. The seeds were extracted from the fruits by soaking them in 20% H₂SO₄ (Merck, India) for 30 min. followed by macerating them manually. Acid treatment [420] to the fruits rendered easy depulping of the fruit tissue and extraction of the seeds. The seeds with hard off-white or cream endocarp were rubbed gently over a wire mesh and then thoroughly washed under running tap water to remove the traces of pulp adhering to the endocarp [121]. The seeds, with endocarp, were dried to their IMC under shade for 12 h [421] and stored in perforated trays at ambient conditions [27-32°C and 35-45% relative humidity (RH)]. Extraction of seed with endocarp from the fruit was completed within 18 h (time required for depulping, extraction and drying to IMC) of harvest. The neem seeds were harvested consecutively for 3 years (from 2008-2010) and all experiments were repeated three times with five replications.

1.2.3 Seed drying and storage

The freshly extracted mature neem seeds were divided equally into three lots for conducting following experiments:

(i) The seeds were subjected to slow drying by spreading them in one layer in perforated baskets at ambient conditions (27-30°C, 35-45% RH).

(ii) The rapid drying was achieved by arranging seeds in net bags in one layer between silica gel in air-tight plastic boxes. Separate plastic boxes were used for seeds with different target moisture contents (TMC). The self-indicating silica gels were replaced in every 4 h. The corresponding target seed weight for each TMC was calculated as follows described by DFSC/IPGRI [422]:

\[
\text{Weight of seeds (g) at TMC} = \frac{100-\text{IMC}}{100-\text{TMC}} \times \text{initial seed weight}
\]
The water loss from the desiccating seeds was monitored by weighing the seeds and simultaneously recording the duration of drying.

Seeds were harvested from each lot separately at desired intervals for various analyses described as follows:

1.2.4 Moisture Content

The moisture content (MC) of the seeds was determined on fresh weight basis following the method given by International Seed Testing Association [423]. Five replicates of ten seeds each were taken and the fresh weight was determined using Sartorius (Germany) balance with 0.1 mg accuracy. Following fresh weight determination then seeds were kept for drying at 96°C in the hot air oven for 48 h and then there dry weight was determined. The seed MC was determined as follows:

\[
\text{Seed Moisture Content (\%) = \frac{\text{Seed Fresh Weight} - \text{Seed Dry Weight}}{\text{Seed Fresh Weight}} \times 100}
\]

1.2.5 Water content

Water content (WC) was determined gravimetrically for five replicates of 10 seeds, after drying for 48 h at 96°C [423], and expressed as g H₂O g⁻¹ DM (dry mass) using the formula:

\[
\text{WC (g H₂O g⁻¹ DM) = \frac{\text{Seed Fresh Weight} - \text{Seed Dry Weight}}{\text{Seed Dry Weight}}}
\]

Neem seeds were rich in oil hence the WC was recalculated for zero amount of oil [424].
1.2.6 Germination

The hard, creame coloured endocarp of the seeds were removed by slight hammering and breaking of the seed coat, slight enough to forestall any damage to the seed kernel. Excised seeds obtained after breaking hard endocarp (now referred as “Seed”) were surface sterilized with 1% sodium hypochlorite solution (BDH, India) for 15 min [425], thoroughly washed 4-5 times with distilled water (DW), and allowed it to germinate in the dark at 26-28° C on two layers of moistened filter paper towels in Petri dishes [21]. Germination was recorded at every 24 h as the radicle emerges (1 mm radicle) to complete germination (5 mm radicle), until 100 percent germination was achieved. Distilled water was supplied to the germinating seeds as and when necessary. The experiment was terminated when the seeds showed partial or complete decay with fungal infestation and/or no germination, even after a week since the last seed germinated. Germination tests were performed with fifteen seeds in five replicates.

1.2.7 Germination Index:

The germination index (GI) of the seed was estimated to evaluate the speed and efficiency of germination, as described by Varghese [192]. The data obtained during the germination test was used for estimating GI in the following manner:

\[ GI = MDG \times PV \]

Where, \( MDG = \) Mean Daily Germination

\( PV = \) Peak Value

MDG value is a measure of the totality of germination and is calculated as the final germination value achieved during germination test divided by the number of days required for germination percent recorded. PV was calculated as maximum value of germination percentage on any day divided by the number of days taken to achieve that germination percentage.
1.2.8 Viability test

The tetrazolium (TZ) test that is recommended for testing seed viability was performed according to [419]. The test is based on the ability of dehydrogenase enzymes (activated by soaking and incubation in DW) in the actively respiring areas of the living tissues in embryo and endosperm [388] to produce hydrogen ions, which in turn react with the colourless solution of tetrazolium salt to form a stable red-coloured triphenyl formazan which is insoluble in water [426]. The dead tissues, on the other hand, did not stain. The test was performed by imbibing the seeds in dark for 18 h in water at 27-32°C as per details described in the germination test. After soaking, the papery brown seed cover was removed carefully using a forceps and scalpel [427]. De-coated seeds were immersed in 1% 2, 3, 5 Tri-phenyl Tetrazolium Chloride (Sigma, USA) [428] solution prepared in ethanol (Merck, India) and incubated in dark at 27-32°C for 12 h. The red coloured formazan, formed in different parts of seed tissues, was extracted in ethanol and the absorbance of the ethanolic solution was read at 520 nm using a spectrophotometer (ATI-Unicam, UK) [121]. Formation of formazan was expressed as A520 g⁻¹ DM (dry mass) of seed tissue (cotyledon or embryonic axis).

1.2.9 Leachate conductivity

Solute leakage loss was measured in terms of electrolytic conductivity by following Varghese and Naithani [121]. The seed steep water from 15 imbibing seeds in five replicates kept for germination was collected and used for the estimation of electrolyte conductivity. The seed leachate was collected after 24 h of seed imbibitions. The 3 mL of seed leachate was made up to volume of 30 mL using DW. The electrical conductivity was estimated using digital conductivity meter (Elico, India). Results were expressed as mS seed⁻¹.

1.3 Results

1.3.1 Water content

The fresh neem seeds shed from the plant with 1.454 g H₂O g⁻¹ DM water content [WC] [calculated at zero oil]. Rapid decline in water content was discernible within 7 days of storage.
period (SP) (0.388 g H$_2$O g$^{-1}$ DM) (Figure 1.1a). Nearly 73% loss in water content was discernible within 7 days of storage. Thereafter, a steady decline in seed water content was recorded. The lowest safe water content \{LSWC\}, below which the seed germination dropped form 100% was 0.205 g H$_2$O g$^{-1}$ DM. The seeds of 20, 60, 90 days of storage showed gradual loss of water content respectively to 0.167, 0.123, 0.116 g H$_2$O g$^{-1}$ DM. The seeds of 130 days storage which were nonviable recorded 0.102 g H$_2$O g$^{-1}$ DM. Approximately 93% decrement in WC was discernible as the fresh seeds were constantly kept in storage for 130 days. The decline in seed water content exhibited a negative correlation \(r = -0.61\) with seed storage during natural drying.

The freshly harvested neem seeds when subjected to rapid drying using silica gel [as described in the Materials and Methods] dried to 0.18 g H$_2$O g$^{-1}$ DM within 8 h (Figure 1.1b). Rapid drying declined the seed water content from 1.454 g H$_2$O g$^{-1}$ DM to 0.906 g H$_2$O g$^{-1}$ DM within 3 h. A strong negative correlation \(r = -0.99\) was noted between loss of WC and hours of drying.

**Figure 1.1a**

**Figure 1.1b**

**Figure 1.1.** The loss of water content at zero oil of mature neem seeds during natural drying (a) and rapid drying (b). Each value is mean ± SD of five replicates of ten seeds each. Data are significantly different \(p \leq 0.05\).
1.3.2 Germination

The freshly harvested seeds of neem showed 100% germination. The seeds exhibit 100% germination up to 7 days of SP but further increase in the period of storage under natural drying conditions observed decreasing trend in germinability. Steady decline in germinability was discernible to 90, 50 and 30% respectively at 20, 60 and 90 days of SP. The seeds became non-viable by 130 days of SP (Figure 1.2a). Percent germination showed a strong negative correlation \( r = -0.99 \) with period of storage. On the other hand, a positive correlation \( r = 0.58 \) was expressed between seed WC and %G of naturally dried seeds.

In case of rapidly dried seeds, 100% germination maintained in seeds dried to WC 0.906 g H\(_2\)O g\(^{-1}\) DM at 3 h (Figure 1.3b). Eighty percent germination was retained in seeds having WC 0.483 g H\(_2\)O g\(^{-1}\) DM. Seeds of WC 0.18 g H\(_2\)O g\(^{-1}\) DM showed 60% germination. Percent germination of rapidly dried seeds exhibited a negative correlation \( r = -0.89 \) and a positive correlation \( r = 0.90 \) with drying and seed WC respectively.

1.3.3 Germination Index

Unlike percent germination, germination index (GI) was maintained maximum only in freshly harvested seeds. An abrupt loss of GI from 10000 to 4250 was observed in 7 days of SP (Figure 1.2a). Although the seeds of 7 days of storage showed 100% germination the GI decreased markedly by 57.5%. Further storage of seeds resulted in steep decline in GI values to 928.13, 187.5 and 68.75 on 20, 60 and 90 days stored seeds. Compared to fresh seeds [undried seeds] nearly 145 fold decrement in GI was recorded in seeds of 90 days of SP. A negative correlation \( r = -0.7 \) and a strong positive correlation \( r = 0.98 \) existed with SP and WC respectively in naturally dried seeds.

GI values decreased significantly from 10000 to 4000 as the neem seeds rapid dried from 1.454 to 0.906 g H\(_2\)O g\(^{-1}\) DM (0-3 h of drying) (Figure 1.2b). There was gradual decline in GI value to 1333.3 and 1200 in seeds rapid dried respectively to WC 0.483 and 0.18 g H\(_2\)O g\(^{-1}\) DM. Compared to fresh neem seeds [1.454 g H\(_2\)O g\(^{-1}\) DM] nearly 8.3 fold decline in GI was
discernible in seeds dried to WC 0.18 g H₂O g⁻¹ DM. A negative correlation (r = -0.95) and a strong positive correlation (r = 0.96) were recorded with period of rapid drying and WC respectively.

![Figure 1.2a](image1.png) ![Figure 1.2b](image2.png)

**Figure 1.2a**

**Figure 1.2b**

**Figure 1.2.** Percentage germination and GI during natural drying (a) and rapid drying (b) of neem seeds. Each value is means of five replicates ± SD. Data are significantly different (p ≤ 0.05).

1.3.4 Viability (TTC test)

The red colour compound of reduced triphenyl terazolium chloride, widely used for testing seed viability, decreased in both axis and cotyledon of neem seeds undergoing ageing and or loss of WC during natural drying (Figure 1.3a). A gradual decline was recorded from 63.48 to 57.41 A₅₂₀ g⁻¹ DM and 38.89 to 33.39 A₅₂₀ g⁻¹ DM respectively in axis and cotyledons of fresh seeds natural dried for 7 days.

Similar rate of decline recorded from 57.41 to 35.07 A₅₂₀ g⁻¹ DM and 33.39 to 17.42 A₅₂₀ g⁻¹ DM respectively in the axes and cotyledons of seeds dried from 7 to 90 days of storage. Compared to
fresh seeds the intensity of red colour of reduced TTC declined steeply by 2.25 and 3.41 fold respectively in axis cotyledon of 130 days old seeds. A strong negative correlation in both axis (with SP, \( r = -0.93 \); with WC \( r = 0.8 \)) and cotyledon (with SP, \( r = -0.95 \), with WC, \( r = 0.79 \)) was expressed.

Like natural drying, the seeds rapidly dried exhibited sharp decline in TZ colouration as desiccated to 0.906 g H\(_2\)O g\(^{-1}\) DM (Figure 1.3b) i.e. from 63.48 A\(_{520}\) g\(^{-1}\) DM to 51.15 A\(_{520}\) g\(^{-1}\) DM (axis) and 38.89 A\(_{520}\) g\(^{-1}\) DM to 29.47 A\(_{520}\) g\(^{-1}\) DM (cotyledons). Further, a steady decline in viability estimated by recording the intensity of red colour of TTC evident from 42.09 A\(_{520}\) g\(^{-1}\) DM at WC 0.483 g H\(_2\)O g\(^{-1}\) DM, 39.47 A\(_{520}\) g\(^{-1}\) DM at WC 0.18 g H\(_2\)O g\(^{-1}\) DM in axis and 25.33 A\(_{520}\) g\(^{-1}\) DM at WC 0.483 g H\(_2\)O g\(^{-1}\) DM, 23.5 A\(_{520}\) g\(^{-1}\) DM at WC 0.18 g H\(_2\)O g\(^{-1}\) DM in cotyledon. About 1.6 times decrement in viability was noticeable in both axes and cotyledons of seeds of WC 0.18 g H\(_2\)O g\(^{-1}\) DM then the fresh seeds. A positive correlation was exhibited in both axes (\( r = 0.98 \)) and cotyledons (\( r = 0.98 \)) with WC, with drying period in axis (\( r = -0.99 \)) and cotyledon (\( r = -0.97 \)) and with % G (\( r = 0.84 \) and \( r = 0.79 \)) in axis and cotyledons respectively.

**Figure 1.3a**

**Figure 1.3b**

**Figure 1.3.** Changes in viability of neem seeds during natural drying (a) and rapid drying (b).

Each point is mean of five replicates ± SD. Data are significantly different (\( p \leq 0.05 \)).
1.3.5 Leachate conductivity

Steady increments in the specific conductance were registered in naturally dried neem seeds (Figure 1.4a). The least value (2.15 mS seed$^{-1}$) of specific conductivity was measured in the leachates of 0 day old seeds whereas highest conductivity (9.72 mS seed$^{-1}$) was estimated in the leachates of non-viable seeds of 130 days of SP. Storage results in the desiccation of seeds leads to 50.1% escalation in the leachate conductivity i.e. from 2.78 mS seed$^{-1}$ on 7 days stored seed to 5.58 on 20 days of SP. A negative correlation ($r = -0.75$) with seed WC, ($r = -0.95$) with %G and also a positive correlation ($r = 0.95$) with SP were established.

Compared to natural drying the loss of electrolyte leakage was substantially low [3.2 mS seed$^{-1}$] in seeds rapid dried WC 0.18 g H$_2$O g$^{-1}$ DM (Figure 1.4b). A strong correlations were observed; negative $r = -0.96$ and $r = -0.89$ respectively with WC and %G, and a strong positive correlation $r = 0.99$ with drying period.

![Figure 1.4a](image1.png)  ![Figure 1.4b](image2.png)

**Figure 1.4a**  **Figure 1.4b**

**Figure 1.4.** Loss of electrolytes from neem seeds during natural drying (a) and rapid drying (b). Each value is mean ± SD of five replicates of five seeds each. Data are significantly different at p≤ 0.05.
1.4 Discussion

In last two decades, plethora of literature that has been accumulated to describe the seed storage behaviour revealed large number of tropical tree seeds as desiccation sensitive or non-orthodox. Initially, all non-orthodox seeds were described as recalcitrant but later based on the critical water content that was intermediate between recalcitrant and orthodox several seeds such as oil palm, coffee, papaya, mahogany [306], kauri spp [322], neem [323, 324, 121], Coffe [10], Fagus [326] and Citrus [327] were classified as Intermediate. Unlike the recalcitrant seeds that are highly sensitive to desiccation the seeds with intermediate storage behaviour permit slight drying. The rapid drying technique was successfully used to improve the desiccation tolerance and survival in the intermediate seeds like *Hevea brasiliensis* [61], *Clausena lansium* [66], *Scadoxus membranaceus* [63], *Araucaria hunsteinii* [60], *Castanospermum australi*, *Trichilia dregeana* [65], *Castanospermum australe* [62], *Camellia sinensis* [47], *Landolphia kirkii* Dyer [59], *Ekebergia capensis* [54], and embryos of Cocoa and Ginkgo [330]. Therefore, this chapter provides an insight to the effect of slow and rapid drying on seed water content, germination, viability and vigour of intermediate neem seeds. The yellow and ripe neem seeds showing 100% germination were shed with relatively high Initial Water Content [IWC: 1.454 g H$_2$O g$^{-1}$ DM WC]. During storage, the slow drying [natural drying] of neem seeds resulted in steep decline in WC to 0.388 g H$_2$O g$^{-1}$ DM within first 7 days i.e. net loss of 73% but without loss in germinability. Later, the loss of water content that was significantly slow was accompanied by loss of germinability (Figs. 1.1a, 1.2a) as also revealed by obtaining a strong negative correlation ($r = -0.99$) between percent germination the storage period. For example, the slow drying of neem seeds for 60 days of storage resulted in 0.123 g H$_2$O g$^{-1}$ DM WC along with 50% loss of germinability whereas further storage of seeds for 130 days resulted in absolutely non-viable seeds with WC 0.102 g H$_2$O g$^{-1}$ DM was also substantiated by recording a positive correlation ($r = 0.58$) between seed WC and %G of naturally dried seeds. In contrast, the rapid drying [using silica gel] of fresh mature neem seeds [IWC: 1.454 g H$_2$O g$^{-1}$ DM] for 3hr reduced the water content to 0.906 g H$_2$O g$^{-1}$ DM but no reduction in germination i.e. maintained 100% (Figs. 1.1b, 1.2b). Even the reduction of WC to 0.18 g H$_2$O g$^{-1}$ DM by 8hr of rapid drying resulted in only 40% loss of germination i.e. seeds survived with 60% germination. Our results clearly revealed that higher desiccation tolerance with germinability was achieved by rapid drying compared to
slow drying in neem seeds was also indicated by recording a negative correlation ($r = -0.89$) and a positive correlation ($r = 0.90$) of rapid drying and seed WC, respectively, with percent germination. Relatively reduced percent of germination in slow dried neem seeds could be due to the exposure of seeds tissues for longer duration to intermediate water content that permits aqueous-based damaging reactions [discussed in the 2-4 chapters] [57, 331, 56, 55]. Drying of desiccation sensitive seeds below critical water content that is still very high allow aqueous-based deleterious pathways causing disturbance in the ongoing metabolism [53, 333, 54, 332]. Our results corroborate findings on *Avicennia marina* [429, 49], *Landolphia kirkii* Dyer [59], *Landolphia kirkii* [63], *Araucaria hunsteinii* [60], *Quercus robur* [48], *Scadoxus membranaceus* [63], *Hevea brasiliensis* [61], *Castanospermum australe* [62] and *Camellia sinensis* [47], where desiccation- sensitive seeds have been shown to retain viability to a lower moisture content if dried rapidly as compared to natural drying.

GI is an indicator of the maximum percentage germination at a given time and is a widely used index [430, 431] for seed quality. The germination index monitored both in the slow and rapid drying exhibited continuous loss even when the percent germination of neem seeds was maintained 100% up to 7 days of storage in slow dried seeds and for 3hrs during rapid drying. Our results clearly revealed that the loss of GI precedes the decrease in percent germination as also reported in other seeds, *viz.*, *Shorea robusta* [412], *Madhuca indica* [413], *Azadirachta indica* [192]. During slow drying, GI was rapidly declined by more than 50% i.e. from 10000 to 4250 within 7 days of storage (Fig. 1.2a). Loss of GI was about 145 fold in seeds subjected to slow drying for 90 days. Compare to slow drying the magnitude of loss of GI was relatively slow in rapid dried seeds. For example, the GI reduced from 10000 to 4000 in the neem seeds rapid dried from 1.454 to 0.906 g H$_2$O g$^{-1}$ DM for 3 hr (Fig. 1.2b). Further rapid drying of neem seeds to 8 hr resulted in net loss of 8800 of GI in seeds with WC 0.18 g H$_2$O g$^{-1}$ DM. Distinct variation was discernible in GI values in seeds exposed to slow and rapid drying. The rate of drying has been shown to have a marked effect on the survival of desiccation-sensitive seeds during storage [47, 46, 45]. The percent germination and GI tests were widely used both in the field and laboratory conditions for the assessment of priming, salt stress, and drought stress [432, 433, 434] effect. GI also gives information on the uniform or synchronized germination of seed.
population, and an indicator of individual germination rates that are close to the mean rate of germination for the population as a whole.

Strong relationship of TZ test with the percent germination in slow and rapid dehydrating neem seeds was discernible. Viability tests can be used as significant indicators of seed survival subjected to various stress conditions [386, 387]. Gradual reduction in the development of pink coloured formazan, as a result of TZ reduction both in the axis and cotyledon of neem seed was distinguishable with increasing period of slow or rapid drying. The TZ activity was relatively higher in the axes than in the cotyledon (Fig.1.3a). Comparatively, the axis and cotyledon of rapid dried seeds maintained higher levels of reduced-TZ at all dehydrated stages than the natural drying. The rate of decline of viability was similar in the axes and cotyledons. Similarly, the viability in the rapid drying neem seeds estimated separately in the axes and cotyledon showed steady decline as a function of loss of water content. The magnitude of viability loss both in axes and cotyledon was proportionately comparable with germination percent in the rapid and slow dried seeds as also evident by estimation the statistical correlation where loss of percent germination exhibited relatively high positive correlation with loss of viability in slow [axis: 0.92 A$_{520}$ g$^{-1}$ DM and cotyledons: 0.94 A$_{520}$ g$^{-1}$ DM] than the rapid [axis: 0.84 A$_{520}$ g$^{-1}$ DM and cotyledons: 0.79 A$_{520}$ g$^{-1}$ DM] drying (Figs. 1.3a, 1.3b). Positive correlation between germination and viability in neem seeds corroborates the findings in other seeds such as *Shorea robusta* [368], *Kielmeyera coriacea* Mart [397], *Pinus wallichiana* [396], and *Allium cepa* [305]. Seed viability determined by TTC test is directly influenced by desiccation or dehydration of seed during prolonged storage [367, 435, 436, 180, 124].

In desiccation sensitive seeds, the drying induced structural disorganization process, as a result the dried seeds temporarily lose their membrane integrity [437]. The amount of solute leakage is the marker of the capacity of a seed to reconstitute the plasma membrane system and repair all other biological damage that occurs in seeds during drying [437, 438]. Thus, seeds with less physiological potential or poor quality as a result of the deteriorative process have a reduced capacity for membrane reorganization resulting in greater loss of electrolytes in the imbibition medium, ultimately leading to decreased seed reserves and reduced seed viability and vigour. In
our study, minimum levels [2.15 mS seed^{-1}] of electrolyte leakage that was estimated in the fresh seeds exhibited increasing pattern with the loss of seed water content. Almost 4 fold increment was recorded in non-viable seeds when slow dried to water content 0.102 H\textsubscript{2}O g^{-1} DM. Seeds during imbibition liberates amino acids, fatty acids, sugars, ions and enzymes in quantities varying according to the organizational state of the cellular membrane systems [439, 440, 437, 441]. The loss of electrolyte leakage was comparatively higher in the seeds subject to slow drying than the rapid drying. For example, the solute leakage was 8.28 mS seed^{-1} in seeds slow dried to 0.116 H\textsubscript{2}O g^{-1} DM whereas, it was more than 2 fold less i.e. 3.16 mS seed^{-1} in seeds rapid dried to relatively low water content i.e. 0.483 H\textsubscript{2}O g^{-1} DM (Fig. 1.4b). The slow dried seeds exhibited strong positive correlation (r = 0.95) with storage period, a negative correlation (r = -0.75) with seed WC and also a negative correlation (r = -0.95) with %G clearly suggesting the key association of solute leakage with storage period and germination during loss of water content. The rapid dried seeds with 60% germinability showed very low solute leakage [3.2 mS seed^{-1}] compared to 90% germinability seeds [5.58 mS seed^{-1}] that were slow dried to 0.167 H\textsubscript{2}O g^{-1} DM (Fig. 1.4a). Strong correlation of withdrawal of water was further substantiated by recording a strong correlations negative correlation with %G (r = -0.89) and positive correlation r = 0.99 with rapid drying period. Our study is in agreement to the findings of others [439, 440, 437, 441] and postulates that the magnitude of electrolyte leakage is directly proportional to membrane damage that leads to proportionate loss of seed germinability, vigour and viability. Decreased seed germination and vigour are directly related to increased quantities of solute leakage in the imbibitional medium due to loss of membrane integrity [442, 443, 444]. The low vigour seeds produced weak seedlings that are unable to survive or establish once reintroduced into a habitat [445, 446, 375]. This method has been successfully used to predict germination and seedling growth for wide range of species [447, 448, 449], as well as viability of tissues of land plant [450, 451] and of diverse species of algae [452, 453].

We conclude that the storage behaviour of the neem seeds may be characterized as intermediate as they are shed form the plant with relatively high water content and sensitive to drying when desiccated below critical water content 0.205 g H\textsubscript{2}O g^{-1} DM, an intermediate water content compared to orthodox and recalcitrant seeds. Our data on the comparison of pattern of %G, GI,
viability (TZ test) and vigour (specific conductivity) in the slow and rapid drying conditions clearly pinpoint the effective lowering of CWC thus improving the desiccation tolerance by rapid drying. Thus arguably it would be interesting to investigate the underlying biochemical pathway that contributes in improving the desiccation tolerance in neem seeds exposed to rapid drying by effectively lowering the CWC and comparing it with the effect of slow drying.