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Assessment of Seed Viability and Vigour in Neem (*Azadirachta indica* A. Juss.)

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Abstract

Rapid loss in viability of neem (*Azadirachta indica* A. Juss.) seed is a major problem. Present effort was undertaken for developing a set pattern for assessing of viability and vigour in seed of various mother tree age of neem (Age I-06 years, Age II-15 years, Age III-25 years and Age IV->30 years old). Various viability test viz. triphenyle tetrazolium chloride test, electrical conductivity, excised embryo test, and germination test have been performed on seeds obtained from mother tree age classes. Inconsistency was observed with the TTC and EC test in germination of seed in laboratory as well as nursery. While various vigour tests viz. cold test, chemical stress test (methanol stress test), and accelerated ageing test alongwith ageing index, germination test (G%, MGT and GV) and various seedling growth parameters like seedling length (cm), number of leaves, collar diameter (cm), total biomass (g) alongwith mathematical indices i.e. vigour index, sturdiness quotient, volume index, quality index, root shoot ratio in nursery as well have been taken for study and showed better consistency. On the basis present study results of various viability and vigour test indicated that mother tree age class II performed better in comparison to others and it can be recommended for seed collection. Further it is also recommended that viability of neem seed may be assessed using various laboratory tests like excise embryo test and germination test (G%, MGT and GV) and vigour test may be taken preferably by cold germination test, chemical (methanol) stress test, accelerated ageing test in laboratory and germination alongwith various seedling growth parameters seedling length (cm), number of leaves, collar diameter (cm), total biomass (g) alongwith mathematical indices like Vigour Index, Sturdiness quotient, Volume Index, Quality index, root shoot ratio in nursery as discussed in this study.

Key Words: germination percentage, mean germination time (MGT), germination value (GV), *Azadirachta indica*, vigour

Introduction

Seed vigour is the sum total of those properties of a seed, which determine the potential level of performance and activity of a non dormant seed or seed lot during germination and seedling emergence. Seed vigour is an important factor determining seed quality in forest trees (Savcenko and Podzarova 1970; Vlase and Voinescu 1972). The seed vigour characteristic may be specific to species or provenance within a species or within the same seed lot. Lack of proper meth-ods to evaluate viability and vigour can make it very difficult to judge the quality of seed at the time of procuring seeds for nursery sowing. All these factors greatly influence the seed viability and vigour especially from the commercial point of view.

Several studies have been performed on various forestry tree species seed (viz. Heydecker 1972; Sharma and Purohit 1980; Paliwal and Thaper 1982). It was suggested

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that seeds with low vigour fail to produce normal seedlings when oriented in an opposite manner. High vigour seeds, on the other hand, produce almost normal seedlings no matter how oriented in the seedbed. The phenomenon was subsequently reported in *Shorea robusta* (Sharma and Purohit 1980) and in Pine seeds in general (Paliwal and Thapar 1982) in a few more species. The concept of seed vigour (Moore 1968; Heydecker 1972) has been developed to distinguish those seeds in the population which, though, germinate under favourable conditions in a germina¬tor but are unlikely to be capable of producing seedlings in the field, where weak or delayed germination is often fatal.

The Neem (*Azadirachta indica* A. Juss.) belongs to Meliaceae family. It is an attractive, unique and evergreen multipurpose tree of tropical arid and semi arid region. It is generally propagated by seeds in nurseries, however, the seed have a short storage life and rapid loss in seed viability is a major problem in propagating neem for tree planting programmes. Its seed of Asian origin have been shown more or less recalcitrant (Gamene et al. 1996), while those of African provenances as orthodox (Bellefontaine and Audinet 1993). Seed is short lived and starts deteriorating faster (Ezumah 1986; Maithani et al. 1989). Any kind of seed start deterioration just after ripens. During collection, transportation, drying and storage of seeds must inevitably lead to some poor quality if they are not handled properly. Additionally, excessive dryness can render seeds more liable to cracking, brushing or abrasion on threshing. The effects of storage temperature and relative humidity have received more attention in the context of seed deterioration than have those of any other factors. The effects of moisture and storage temperatures have been studied further, where these parameters impinge on the intrinsic factors affecting seed vigour and viability. The standard germination tests by which tree seed quality can be determined, although the procedure is very time consuming requiring several weeks. In case of seed showing complicated dormancy, the test may take even longer to accomplish. The object of indirect tests of viability is to make a quick estimate of the ability of seeds to germinate, particularly, those exhibiting dormancy. Loss in vigour can be thought of as an intermediate stage in the life of the seed, occurring between the onset and termination of decline (death). Decline in vigour is extremely difficult to measure. Gill and Delouche (1973) found the rate of germination and seedling growth of corn to be the most consistent and sensitive measures of the progress of deterioration. Indirect tests can be performed within a few hours and are thus a great help in cases where the results of the tests are required as soon as possible.

There are several viability test methods which are used in agricultural crops for rapid assessment of seed viability through direct or indirect means viz. size, colour, cutting test, excised embryo test, conductivity of seed leachate, triphenyle tetrazolium test and X-ray. Some of these are used in forest seeds and have been recommended by ISTA (1993). However, it is indispensable to follow standard pattern for testing seeds for germination in order to ensure uniformity and reproducibility of results. Testing under field conditions is normally unreliable and therefore, laboratory methods have been evolved (ISTA 1976). However, ample literature is not still available on assessment of viability and vigour in neem.

Accordingly the present study was undertaken to contribute in connection with developing a set pattern/method for assessing of viability and vigour in neem which may provide reliable practical guidelines for seed testing.

Materials and Methods

Four different mother ages of neem seed trees were selected around the Jodhpur city (latitude 24°40'N and longitude 71°15'E), Rajasthan, India as per plantation year mentioned in Table 1. Twenty trees of each age class were selected and in each age class, ten trees were selected randomly among the twenty trees. Seeds from all ten trees were collected and mixed lots were prepared for experiments. The temperature was mean min 20-22°C and mean max 33-34°C and humidity between 22-59% during the experiment entire period.

Collection, extraction and processing of seed

The green-yellow colour (Kumar and Mishra 2007) fruits were collected directly from tree branches (Kumar and Mishra 2007) from above mentioned seed stand. Seeds were extracted (Kumar et al. 2007), and dried as done by Kumar et al. 2008.

Table 1. Description of neem plantations used in the study

Age classes	No. of trees	Year of plantation	Average height (m)	Clear bole (m)	Average girth (cm)	Crown diameter (m)	Location
I (6 yrs)	10	1995	7.55 (1.19)	1.89 (0.72)	77.25 (9.52)	5.10 (0.84)	AFRI Campus & Nursery
II (15 yrs)	10	1986	10.20 (2.07)	1.45 (0.47)	103.75 (29.94)	10.00 (1.73)	DMRC, Jodhpur
III (25 yrs)	10	1976	12.85 (1.35)	1.92 (0.57)	118.95 (23.45)	10.88 (1.53)	JNVU, Jodhpur
IV (>30 yrs)	10	1965-1968	14.10 (1.79)	2.19 (0.68)	177.50 (21.70)	13.15 (2.04)	Pal Road, Jodhpur

Values in parenthesis are standard deviation.
Abbreviations: AFRI-Arid Forest Research Institute, DMRC-Desert Medicine Research Centre and JNVU- Jai Narayan Vyas University.

Viability and vigour test

Viability test

Triphenyle tetrazolium chloride test (TTC): 1% TTC (2,3,5-Triphenyle tetrazolium chloride) solution was prepared following Moore (1973). Topography of each seed was examined on the basis of stained pattern, the area of seed stain including plumule radicle axes. Seeds were categorized into five categories viz. completely stained, partially unstained with stained embryo, half stained with stained embryo, half stained without embryo and unstained embryo and the last one with complete unstained embryo.

Electrical conductivity test (EC): Electrical conductivity of seed leachate was measured following Agrawal and Dadlani (1992).

Excised embryo test (EET): Intact and decoated seeds were cut into two halves with embryonic axes (plumule and radicle) were incubated at $30\pm1^{o}C$ on top of paper in petridishes. Two replications of 50 seeds obtained from each mother tree age were used. The emergence of radicle to about half cm was considered as germination of seed. Germination data was recorded and germination percentage, mean germination time (MGT) and germination value (GV) was calculated.

Germination test (Germination percentage, MGT and GV): Germination test was determined for germination percentage, mean germination time (MGT) and germination value (GV) in laboratory using four replications of 100 seeds following Kumar et al. (2007). Germination data were recorded daily up to 21 days. Speed of germination in terms of Mean Germination Time (MGT) in days was calculated as per method used by Rawat and Thapliyal (2003) and germination value was calculated following Djavanshir and Pourbeik (1976).

Vigour test

Laboratory

Cold germination test: Seeds obtained from all mother tree age were taken for test in low temperature i.e. $10\pm1^{o}C$ to assess the vigour of seeds. The soil was placed uniformly in thick layer (appro. 2 cm) on bottom of the tray and water ($10\pm1^{o}C$) was added to bring the soil to 70% of its water holding capacity. The trays were then covered and put in a refrigerator at $10\pm1^{o}C$ for 7 days and then they were transferred to $25\pm1^{o}C$ for a further 4 days. Trays were incubated in a seed germinator for further germination studies. The results of a cold test were usually expressed as percentage of normal seedlings produced.

Chemical stress test (Methanol stress test): Chemical stress test was studied and standardized for neem seed as per the method of Musgrave et al. (1980). Seeds were dipped into 100 ml pure methanol in a 250 ml of conical flask for 1, 2, 5, 10, 15 and 20 minutes. Four replications with 100 seeds were taken for each case. Seeds were removed timely from methanol and washed in distilled water and tested for various germination parameters. 10 minutes incubation time (G50) was standardized for all the treatments.

Accelerated ageing test (AAT) and ageing index (AI): Seeds of different mother trees were aged as per method used by Vanangamudi et al. (2000). Ageing index (A.I.) was developed as per Wang et al. (1992).

Nursery

Nursery beds (size $5.0\times1.0\times0.45$ m) were prepared and filled with sand following Willan (1985). Four replications with 100 seeds of each lot were used. Germination data was recorded up to 21 days. A seed was considered germinated when hypocotyls emerged 1 cm above the ground. Seedlings were uprooted after 21 days of sowing and were washed thoroughly and studied for following parameters to assess the vigour of seeds:

Seedling length: Ten uprooted seedlings randomly selected in each replication were individually measured in cm with the measuring scale. According to Ninenstaedt (1981) and Compwell and Sorenson (1984), maximum seedling length was considered for good quality seedling.

Collar diameter: Ten uprooted seedlings randomly selected in each replication were individually measured in cm with the Vernier calliper upto two decimal places. Seedling diameter is related to field performance in the same manner as seedling height (Mullin and Svaton 1972). A quality seedling should possess the largest diameter.

Number of leaves: Ten uprooted seedlings randomly selected in each replication were individually measured manually for number of leaves. Maximum number of leaves was considered for good quality seedling.

Total biomass: 4×10 seedlings were randomly selected and roots and shoots were separated. They were dried at $80\pm1^{o}C$ in a forced air oven till constant weight (72 h). Biomass was expressed in grams (g). A quality seedling should be as heavy as possible to produce good growth.

Sturdiness quotient (S.Q.): The sturdiness quotient is the height in centimeter divided by stem diameter in millimeter (Ritchie 1985). It reflects the stocky or spindly nature of seedlings.

Volume index (Vol. I.): Maximum volume index was considered for good quality seedling. It was calculated by using following formula -

$$V.I. = Diameter\ (mm)^2 * Height\ (cm)$$

Vigour index (V.I.): The more vigour, the better the seed or seedling is able to cope with early stresses and survive. Vigour index was calculated following Abdul Baki and Anderson (1973).

Quality index (Q.I.): The quality index was calculated following Dickson et al. (1960).

Root shoot ratio: The root shoot ratio was work out following Racey et al. (1983).

All the data were collected and analyzed through ANOVA using SPSS computer package. The p values <0.05 were taken as significant.

RESULTS

The viability and vigour tests on neem seeds obatined from various mother tree Age classes were carried out to assess the seed viability and vigour in lab and nursery. The results are given as follows:

The test of Triphenyle tetrazolium chloride test (TTC) was repeated many times as per method described. Unfortunately it was found unfit for neem seeds. Stained seeds were not matched with actually obtained germination in lab and nursery. Besides this, it used to develop patches or leaves light stained on cotyledons. While germination was also observed when seeds showed in dead (unstained) embryo in TTC test. Therefore data was not presented.

The conductivity of the solution reflected the general level of viability of the seed sample. In fresh seeds, electrical conductivity of leachate obtained from seeds of all mother tree age classes was measured. Seed of all mother tree age showed EC ranging from 144.02-295.01 μS/g (Table 2). However, no significant difference was observed among them and significant reduction in EC was observed in all aged seed. It was higher in fresh seeds ranging from

Table 2. Electrical conductivity and germination of seeds obtained from various mother tree age classes neem seed

Age classes	Electrical conductivity (μS/g)			Germination (%)		
	Fresh/ 0 days	35 days	65 days	Fresh/ 0 days	35 days	65 days
I	267.56^a	153.37^b	147.82^b	$92.25\ (73.9)^b$	$31.50\ (34.2)^e$	0
II	279.26^a	188.82^b	144.02^b	$96.25\ (79.0)^a$	$37.75\ (37.9)^d$	0
III	268.91^a	171.38^b	149.75^b	$93.50\ (75.3)^b$	$15.25\ (23.0)^f$	0
IV	295.01^a	195.17^b	161.56^b	$74.75\ (59.9)^c$	$5.00\ (12.9)^g$	0

Values are means of four replications.
In each column values not followed by same letter are significantly different ($p>0.05$).

267.56-295.01 µS/g of all mother tree age and decreased (upto 144.02 µS/g) with the storage period increased (after 65 days). In case of germination of fresh seeds, it was recorded >90% in first three mother tree age classes, while it was observed 74% in age IV only. However, there was absolute zero germination after 65 days stored seeds of all mother tree age classes (Table 2).

Excised embryo test (EET) was performed as described in materials and method in seed obtained from all mother tree age classes. The age class IV exhibited significantly lower germination percentage (76.50%), higher MGT (7.91 days) and low GV (106.49) as compared to age class I, II and III. However, mother tree age class I, II and III exhibited no significant difference in terms of germination percentage, MGT and GV. Mother tree age class II exhibited maximum germination percentage (97.5%), lower MGT (5.81 days) and highest GV (183) (Table 3).

Table 4 depicted the effect of cold test on vigour of seeds obtained from different mother tree age classes. The age class I exhibited not only higher germination (42%), GV (12.66) and lower MGT (16.16 days) but also higher number of normal seedling percentage (28%) as compared to rest of the age classes. However, age class I, II and III exhibited non-significant difference in respect to germination percentage, MGT, GV and ability to produce normal seedlings percentage which is ranging from 22 to 28%.

First of all chemical stress test (Methanol) was standardized for neem seed. Table 5 indicated the effect of methanol stress on germination percentage and mean germination time (MGT) of freshly collected neem seed. It was observed that 10 minutes incubation in methanol was found sufficient to get G_{50} (<50% seed viability) and mean germination time. It was compared with seed germination percentage (97%) and MGT (8.08 days) observed in control (Table 5). This incubation period was used for chemical stress test on seeds obtained from all mother tree age classes.

Freshly collected seeds from different mother age classes exhibited germination percentage ranging from 68 to 94.5% and MGT ranging from 8.64 to 12.12 days in control. When seeds were incubated in methanol for 10 minutes and were then assessed for germination percentage and MGT. It was observed that age class II showed higher

Table 3. Effect of excise embryo test (EET) on seed germination of different mother tree age classes under controlled environment

Age classes	Germination (%)	Mean germination time (MGT) (days)	Germination value (GV)
I	96.25 (79.29)^a	5.86^b	175.50^a
II	98.50 (83.94)^a	5.66^b	183.51^a
III	97.25 (80.71)^a	5.81^b	177.67^a
IV	76.50 (61.10)^b	7.91^a	106.49^b

Values are means of four replications and each replication contains 100 embryos. Values in parenthesis are arc sin transformed.
In each column values not followed by same letter are significantly different ($p > 0.05$).

Table 4. Effect of cold test on seed germination and production of normal seedlings of different mother tree age classesunder controlled environment

Age classes	Germination (%)	MGT (days)	GV	Normal seedling (%)
I	42 (40.40)^a	16.16^c	12.66^a	28.00 (31.96)^a
II	36 (36.85)^a	17.32^b	9.29^b	26.25 (30.83)^a
III	33 (35.07)^b	17.61^b	7.21^b	22.00 (27.95)^b
IV	23 (28.62)^c	19.11^a	3.18^c	10.25 (18.64)^c

Values are means of four replications and each replication contains 100 seeds. Values in parenthesis are arc sin transformed.
In each column values not followed by same letter are significantly different ($p > 0.05$).

Table 5. Standardization of chemical stress on seed germination percentage and mean germination time (MGT) of seeds under controlled environment

Chemical stressed period (minutes)	Germination (%)	MGT (days)
1	95.50 (78.13)^a	7.62^e
2	93.00 (75.10)^a	7.29^e
5	84.50 (66.94)^b	9.31^d
10	46.50 (43.01)^c	16.24^c
15	34.50 (35.98)^d	17.61^b
20	21.00 (27.21)^e	19.37^a
Control	97.00 (80.21)^a	8.08^e

Values are means of four replications and each replication contains 100 seeds. Values in parenthesis are arc sin transformed.
In each column values not followed by same letter are significantly different ($p > 0.05$).

germination percentage (45%) and lower MGT (16.81 days) as compared to rest of the mother tree age classes. Seeds of age class IV showed lower germination percentage (14%) and higher MGT (21.29 days) (Table 6).

Accelerated ageing test (AAT) as per method used by Vanangamudi et al. (2000), it was performed on seeds obtained from all mother tree age classes and Index of Ageing was prepared as per ageing index (A.I.) proposed by Wang et al. (1992). The initial germination percentage was significantly lower in age class IV (68%) and it was higher in age class II (97%) and III (97.50%) followed by age class I (94.5%) (Table 7). However, there was no significant difference between age class II and III. After ageing of seeds a significant rapid reduction in germination was observed in age class IV (19%) followed by Age class I (39%) and III (34%). Age class II showed significantly lower reduction in germination (46.50%). Similarly the ageing index was also higher in age class IV (0.73) followed by age class I (0.59) and II (0.52). However, there was no significant difference between age classes I and III. The data indicate that the age class II showed lower ageing index (0.52). The lower ageing index is proved to be the best vigour (Table 7).

Fresh seeds of mother tree age classes I, II and III showed significantly higher mean germination percentage ranging from 93.5 to 94% followed by age class IV (72.5%) under controlled conditions. However, age II and III showed significantly lower MGT 7.62 days and 7.88 days respectively followed by age I (9.27 days). Similar trend was observed with GV (Table 8). However, no significant difference was recorded between age class II and III with respect to MGT and GV. Age IV showed higher MGT (11.23 days) and lower GV (37.89) (Table 8).

In nursery also age class II showed maximum germination percentage (96.25%) in fresh seeds followed by age class III (93.5%) and age class I (92.25%). However, there was no significant difference between age classes I and III in terms of germination and MGT (Table 9). Age class II

Table 6. Effect of methanol stress on seed germination percentage and mean germination time (MGT) of different mother tree age classes under controlled environment

Age classes	Germination (%)		Mean germination time (MGT) (days)	
	Control	Stressed	Control	Stressed
I	94.50^b (76.91)	37.00^b (37.48)	9.32^b	18.76^c
II	97.00^a (80.21)	45.00^a (42.15)	8.64^c	16.81^d
III	97.50^a (81.06)	30.00^c (33.19)	8.65^c	20.28^b
IV	68.00^c (55.60)	14.00^d (21.96)	12.12^a	21.29^a

Values are means of four replications and each replication contains 100 seeds. Values in parenthesis are arc sin transformed.
In each column values not followed by same letter are significantly different ($p > 0.05$).

Table 7. Effect of accelerated ageing (AA) on seed germination and ageing index (AI) of different mother tree age classes under controlled environment

Age classes	Germination (%)		Ageing index (AI)
	Control	Aged	
I	94.50 $(76.91)^b$	39.00 $(38.65)^b$	0.59^b
II	97.00 $(80.21)^a$	46.50 $(43.01)^a$	0.52^c
III	97.50 $(81.06)^a$	34.00 $(35.69)^b$	0.65^b
IV	68.00 $(55.60)^c$	19.00 $(25.82)^c$	0.73^a

Values are means of four replications and each replication contains 100 seeds. Values in parenthesis are arc sin transformed.
In each column values not followed by same letter are significantly different ($p > 0.05$).

Table 8. Effect of different mother tree age classes on germination of fresh seeds under controlled environment

Parameters	Age classes			
	I	II	III	IV
Germination (%)	93.5 $(75.39)^a$	95.5 $(78.13)^a$	94.0 $(76.12)^a$	72.5 $(58.42)^b$
MGT (days)	9.27^b	7.62^c	7.88^c	11.23^a
Germination value	59.61^b	67.30^a	63.68^a	37.89^c

Values are means of four replications and each replication contains 100 seeds. Values in parenthesis are arc sin transformed.
In each column values not followed by same letter are significantly different ($p > 0.05$).

Table 9. Effect of different mother tree age classes on germination and seedling performance of fresh seeds in nursery

Parameters	Age classes			
	I	II	III	IV
Germination (%)	92.25 (73.9)^b	96.25 (79)^a	93.5 (75.3)^b	74.75 (59.9)^c
MGT (days)	11.63^b	11.47^b	11.68^b	13.96^a
Germination value	37.79^b	39.88^a	38.18^b	23.50^c
Seedling length (cm)	13.08^b	13.87^a	13.17^b	13.19^b
Number of leaves	2.60^a	2.70^a	2.45^b	2.35^b
Collar diameter (cm)	0.186^a	0.196^a	0.189^a	0.185^a
Total biomass (g)	0.069^a	0.073^a	0.070^a	0.060^b
Vigour index	6.37^b	6.99^a	6.55^b	4.51^c
Sturdiness quotient	3.81^b	3.57^b	3.68^b	4.59^a
Volume index	28.12^b	30.04^a	28.19^b	23.42^c
Quality index	0.84^a	0.91^a	0.82^a	0.66^b
Root shoot ratio	0.22^a	0.24^a	0.20^a	0.19^b

Values of germination percentage, mean germination time (MGT) and germination value (GV) are means of four replications and each replication contains 100 seeds. Seedling traits were means of 10 randomly selected seedlings in each replication. Values in parenthesis are arc sin transformed.
In each column values not followed by same letter are significantly different ($p > 0.05$).

showed higher GV (39.88) as compared to age classes I (37.79) and III (38.18). Age class IV showed higher MGT (13.96 days) and lower GV (23.50) (Table 9).

Age class II showed maximum seedling length (13.87 cm) followed by age class IV (13.19 cm), III (13.17 cm) and I (13.08 cm). However, no significant difference was observed among age classes I, III and IV with respect to seedling length. Age classes II (2.7) and I (2.60) showed significantly more number of leaves than age classes III and IV with 2.45 and 2.35 respectively. No significant difference was observed among all age classes with respect to collar diameter. Age classes II (0.073 g), III (0.070 g) and I (0.069 g) showed significantly higher total biomass as compared to age IV (0.060 g). Age class II showed maximum vigour index (6.99) followed by age class III (6.55) and age class I (6.37) as compared to IV (4.51). However, there was non-significant difference among age class I and age III with respect to vigour index. Age classes I, II and III showed significantly lower (good) sturdiness quotient with 3.81, 3.57 and 3.68 respectively than age class IV (4.59). Age class II showed maximum volume index (30.04) followed by age classes III and I with 28.12 and 28.19 respectively as compared age class IV (23.42). Age classes I, II and III showed significantly higher quality index and root shoot ratio as compared to age class IV. However, no significant difference was observed among age class I, II and III with respects to both parameters (Table 9).

DISCUSSION

Various vigour and viability test (Tetrazolium chloride test, electrical conductivity test, excised embryo test, cold test, chemical (methanol) stress test, accelerated ageing test and germination along with seedling performance) had been carried out to assess the vigour of mother tree age classes. Inconsistency was observed with the tetrazolium chloride test (TTC) as germination in laboratory as well as nursery was shown in unstained and erratically stained cotyledons of seed obtained different mother tree age classes.

In case of electrical conductivity in present studies of leachate of seeds obtained from all the mother tree Age classes were observed higher as fresh and declined as seeds were completely lost their viability deteriorated. In contrast of this experiment Varghese and Naithani (1997) reported that EC of leachate exhibited remarkable gradual increase with deterioration in ageing of neem seeds. Since then the relationship has not been confirmed in these experiments and further study is required. Loss of membrane integrity as consequence of decline in vigour could also be attributed to perturbation to the normal membrane, in severely deteriorated seeds such as those subjected to accelerated aging. It is expected that repair of membrane damaged, i.e. the synthetic mode of turnover, will be severely impaired as an indirect consequence of loss of metabolic integrity. Repair of membrane can occur if seeds are hydrated slowly. Unaged seeds, which are hydrated in atmospheres of high relative humidity before being placed in water, do not leak appreciable quantities of solute (Simon and Raja Harun 1972). The initial slow hydration of the seeds probably allows for ordered rearrangement of the membrane before the inrush of water associated with imbibitions from the dry state, which might otherwise be too sudden and disruptive (Powell and Matthews 1977). Stored seeds in an imbibed state in which they remain metabolically active (Powell et al. 1983, 1984) and helps to maintain their viability (Villiers 1974) to repair the membrane.

Mother tree Age I, II and III showed insignificant difference in terms of germination percentage, MGT and GV in excised embryo test (EET). Only older one Age class IV showed significantly lower germination, higher MGT and lower GV as compared to other. Similarly in cold test younger mother tree Age classes (I & II) showed higher germination, lower MGT and higher GV followed by Age class III and IV. They also produced higher no normal seedlings. While, only mother tree Age class II showed higher germination percentage and lower MGT in chemical stress test as compared to other. In the same way mother tree Age class II showed higher germination percentage and lower Ageing index in accelerated ageing test as compared to others. There was significantly lower reduction as compared to control.

Mother tree Age I, II and III showed insignificant difference in terms of germination percentage, MGT and GV under controlled conditions. However, Age class II and III exhibited insignificant difference in terms of MGT and GV. In nursery experiment also of present studies, Age class II showed maximum germination percentage in fresh seeds followed by Age class III and Age class I. However, there was no significant difference between age classes I and III in terms of MGT. Age class II showed higher GV as compared to all. Age class IV showed lower germination percentage alongwith higher MGT (13.96 days) and lower GV.

In case of seedling vigour performance, Age class II showed maximum seedling length, number of leaves, total biomass, vigour index, sturdiness quotient, volume index, quality index and root shoot ratio as compared to others. According to Ninenstaedt (1981), Compwell and Sorenson (1984), maximum seedling length was considered for good quality seedling and seedling diameter is related to field performance in the same manner as seedling height (Mullin and Svaton 1972). A quality seedling should possess the largest diameter and should have as high as root shoot ratio as possible to insure the best survival in field (Racey et al. 1983).

The present results are in accordance with Masilamani et al. (2000) who reported higher germination, seedling emergence, root length, shoot length, vigour index and dry matter production in 20 year old tree and poorest in 30 year old trees of neem. Jindal et al. (1994) also reported similar results in Neem. Maideen et al. (1997) also recommended collecting the seeds from young tree age above 5 year in *Casuarina equisetifolia*. Ramprasad and Kandya (1992) also recommended that the seeds should be collected from mature or nearly mature trees, over mature or immature tree should be avoided, since seeds from them may be of low viability. Over all vigour tests showed better consistency and mother tree age class II performed better in comparison of age classes III, I and IV. Present studies are in conformity with Masilamani et al. (2000) who suggested that over matured tree should be avoided for seed collection.

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