Conclusions
Like most of the recalcitrant and intermediate seeds the freshly mature neem (Azadirachta indica A. Juss) seeds are shed from the tree at high moisture content (40-42%, fresh weight basis). These seeds are desiccation-sensitive and exhibit absolute loss of viability within 140-160 days after harvest, during storage at ambient condition (27-32°C, 35-45% RH). The desiccation-sensitivity of neem seeds make its storage behaviour enigmatic as it was variously described as intermediate as well as recalcitrant. Our results suggest that neem seeds are intermediate in storage behaviour. Inspite of more than 70% reduction in moisture content initially, the neem seeds showed 100% germination when dried up to intermediate moisture contents (10.9%), a relatively desiccated state compared to recalcitrant seeds (LSMC 28-40% moisture content). However, they showed rapid loss of viability when dried below intermediate moisture content (Gamene et al., 1996). Thus it is concluded that neem seeds, although desiccation-sensitive can be dehydrated safely to comparatively lower critical moisture contents than the recalcitrant seeds but sufficiently higher than the orthodox seeds. As suggested by Berjak and coworkers (1984 - 1997), the increasing desiccation-sensitivity in seeds in general may be analogous to sensitivity of dehydrating recalcitrant seed and/or germinating orthodox seeds. This desiccation-sensitivity may be due to loss of "structured water" which resulted in disorganization of metabolism, loss of stability of sub-cellular structures and membranes; the cumulative effect of which is the loss of viability. Different levels of initial moisture content and desiccation-sensitivity in the embryonic axes and cotyledon was ascribed to the difference in their lipid composition. Comparatively higher lipid contents in cotyledon does not allow accumulation of water as lipids are hydrophobic.
Strong relationship exists between germination capacity and vigour of the neem seeds. Determinations like tetrazolium test, germination index, rate of germination (MGT) and seedling vigour index etc. performed to quantify the vigour showed that the decline in these parameters preceded the loss of germination. Though, the data clearly points out significant vigour loss in dehydrating seeds when the viability was still 100%, enhanced desiccation below LSMC resulted in pronounced decline in seed vigour attributes.

The loss of viability in neem seeds seems to be closely influenced by irreparable membrane perturbations as enhanced efflux of cellular constituents into seed leachates was resulted immediately after dehydration below LSMC. Leachate conductivity is considered a reliable marker of seed deterioration and its close correlation with drying suggest dehydration induced membrane damage in these seeds. The severe leakage loss during loss of viability may be attributable to the architectural impairment of membrane lipids due to desiccation/low water content. Leakage loss of sugar, protein and other inorganic constituents like K⁺, Zn²⁺ and Ca²⁺ indicates that their loss into seed leachates plays a detrimental role in the loss of seed viability especially after desiccation below 10.9% (LSMC). These organic and inorganic constituents are vital for basal metabolism of cell.

Desiccation of seeds induced increased rates of peroxidation of membrane lipids, mediated by increased liberation of superoxide radicals. Reactive oxygen species and its derivatives have been implicated in oxidative damage. Increased production of superoxide radical in dehydrating seed is suggested to be responsible for enhanced lipid peroxidation. The free radical damage to cellular membrane phospholipids and the resultant fatty acids would lead to increased membrane dysfunction. Formation of MDA, one of the sensitive
markers of lipid peroxidation vis-a-vis membrane damage, exhibited a significant correlation with the drying of seeds. Thus, it is proposed that the loss of viability in neem seeds after 15 da, may be due to the cumulative toxic effect of peroxidized products of the stored PUFA's and membrane lipids, in the embryonic axes and cotyledons as well. Substantial production of superoxide in the cotyledons of desiccating neem seeds indicate the contributory participation of cotyledonary tissue in desiccation intolerance. It is concluded that enhanced synthesis of superoxide triggers increased rates of lipid peroxidation, both in the embryonic axes and cotyledon which cumulatively deteriorate the membrane leading to loss of viability in desiccating intermediate neem seeds.

The desiccating neem seeds are protected against oxidative injury to definite critical moisture content by enzymic antioxidants, viz., superoxide dismutase, catalase, ascorbate peroxidase and guaiacol peroxidase, the combined action of which, converts the potentially dangerous superoxide radical and hydrogen peroxide into water and molecular oxygen, thus averting cellular damage. A differential expression of SOD and CAT/AsPOD/GPOD was observed in neem seeds. Increased SOD with simultaneous reduction in CAT, AsPOD and GPOD activities immediately following the drying of seed below critical moisture content at one place favours dismutation of $\cdot O_2^-$ whereas on the other hand, may favour accumulation of ROS. Impairment of AsPOD, GPOD and CAT may lead to unabated production and accumulation of ROS including highly reactive hydroxyl radicals via the transition metal (such as iron and copper) catalyzed Haber-Weiss cycle resulting, ultimately in loss of viability in neem seeds when desiccated below critical moisture content. The desiccation-tolerance in orthodox seeds is linked notably with higher activities of SOD, CAT and POD whereas, their massive loss was closely attributable to viability loss in desiccation-sensitive
seeds. Therefore, it is suggested that collapse of CAT/AsPOD and GPOD and not of SOD activity per se in response to desiccation below critical moisture content is critical in determining intermediate storage physiology in neem seeds.

The protein content of the seeds is often associated with viability and vigour of the seed. Enhanced protease activity led to corresponding decline in protein content in dehydrating neem seeds. Decline in protease activity in the non-viable seeds was perhaps due to reduced protein synthesis shown in other seeds (Osmund et al., 1975). The increase in protease activity, right from the beginning (in the viable seeds) is similar to the enhanced protease activity in germinating orthodox seeds.

As the neem seeds could be dried down to intermediate moisture contents, the possibility of its cryoconservation was evaluated. The desiccated excised neem seeds (7.5 % moisture) have been shown to tolerate LN2 temperatures for 12 months with 60% survival, hence can be categorised as moderately cryotolerant. These seeds also exhibited sufficiently high vigour although loss was evident along with increased membrane perturbations at later stage of LN2 storage. Compared to non-desiccated seeds, the desiccated excised neem seeds showed longer period of survival at LN2 temperatures because of significant levels of antioxidant enzymes. Higher levels of these enzymes protected the seeds from damaging effects of superoxide and its mediated lipid peroxidation. The cryoinjury in non-desiccated neem seeds was resulted majorly due to the enhanced accumulation of superoxide with corresponding loss and failure of its processing enzymes. Protective role of free radical damage of cellular membranes and failure of these antioxidant enzymes leads to impairment of cellular metabolism.
In the present study, active metabolism exhibited by desiccated excised neem seeds constantly for one year at cryotemperatures overrule the hitherto regarded view that cryopreservation offers the opportunity of significantly reducing the damage of the tissue by suppressing all the metabolic activities (Stanwood and Bass, 1981). Gradual increase in antioxidant enzymes activity as well as free radical production from 12 h to 12 months is clearly an indicator of active metabolism in these seeds.

Therefore, we warrant short-term (1 h to 1 month) studies performed to evaluate the suitability of cryostorage at least for desiccation-sensitive recalcitrant and intermediate seeds as these seeds may exhibit altered viability and vigour due to active metabolism even at cryotemperatures during long term storage. Therefore monitoring of viability and vigour in desiccation-sensitive seeds for longer periods (6 months to 8 months) is recommended for germplasm conservation of seeds at cryotemperatures in seed banks.