Summary and Conclusion

Increasing concern over depletion of fossil fuel and environmental hazards caused by such fuel, necessitate a search for renewable energy. Biodiesel is considered to be an excellent alternative fuel with its added advantage of being eco-friendly marked with less emission of greenhouse gases. Of the many putative plant sources for biodiesel, *Jatropha curcas* L. seed is way ahead. Besides being a vital alternative source for biodiesel, *J. curcas* is also identified for other beneficial properties varying from herbal medicine to horticulture to cosmetics. Oil-rich seeds generally have a short shelf life, are sensitive to desiccation and lose their vigour and viability on storage. Humidity, temperature and duration of storage play a negative role on seed health during seed storage. The purpose of this study was to elucidate the biochemical, physiological and molecular changes that occur during the storage of *J. curcas* seeds. Accelerated aging and salt-accelerated aging models are valuable in understanding the changes seen over long periods of storage in short time intervals. We successfully mimicked the changes seen during natural aging in our accelerated aging models in a short duration. Knowledge of the behaviour of seeds during storage is important in order to increase their longevity and to prevent significant loss in their genetic and physiological quality. We subjected *J. Curcas* seeds to natural aging (NA) (naturally stored), accelerated aging (AA) and saturated salt-accelerated aging (SSAA) and studied the associated biochemical and physiological changes. Detrimental changes were brought about mainly by high moisture content and temperature. The uncontrolled production and accumulation of reactive oxygen species (ROS) during seed aging caused oxidative stress in oil seeds. It was noted that there was an increase in MDA content from the third month of natural aging, indicating the start of the inevitable process of lipid peroxidation. 12 hours of accelerated aging and saturated salt accelerated aging was sufficient enough to mimic this increase in the MDA level. Significant increase of electrical conductivity and hydrogen peroxide observed in these groups of seeds were the evidences of deleterious manifestation of increased MDA content. Though, hydrogen peroxide could also be the cause, in case of artificial aging treatment, non-significant value of hydrogen peroxide content noted in SSAA12hr and SSAA1d may denote the protective role of alkaline condition created in saturated salt accelerated aging. However, in correlation analysis, when MDA content was correlated with that of electrical conductivity and hydrogen peroxide, it yielded a positive correlation which supported lipid peroxidation to be the primary cause bringing about membrane leakage and formation of reactive oxygen species during *J. curcas* seed storage. Studies on comparison of germination of control seeds to NA12m, AA12hrs to AA3d and SSAA12hrs and SSAA1d proves that the *J. curcas* seeds possess the inherent ability to withstand the negative effect of storage and artificial aging. Progressively declining germinability and radicle length observed in natural aging, accelerated aging and saturated salt accelerated aging indicated the ongoing loss of seed vigour and viability in *J. curcas* seeds. Seeds that failed to germinate were the dead seeds with complete loss of vigour and viability. The results on DPPH free radical scavenging capacity supported the fact that seeds kept for 3 months to 12 months of storage (natural aging) exhibited significantly increased free radical scavenging activity. Similar trend was identified from AA2d to AA4d and SSAA2d to SSAA4d. This increase can be correlated to the significantly increased superoxide dismutase, catalase and peroxidase activity found in these groups of seeds. This accelerated level of free radical scavenging activity and antioxidant enzymes activity ensured the seed protection from free radicals and hence maintenance of seed vigour and viability. Decreased level of free radical scavenging activity and antioxidant enzymes activity seen in later part of storage and artificial aging was evident of breakdown of antioxidant pool in these seeds. Non-enzymatic antioxidants like gamma tocopherol followed a slightly different trend of progressive decline compared to enzymatic antioxidants. The non-significant decrease in gamma tocopherol noted from NA1m to NA12m once aging supported the finding of this study that *Jatropha curcas* seeds can be used as for feedstock for biodiesel even after one year of storage. In this present investigation of *J. curcas* seed storage, there was a significant reduction in content of oil in seeds and oleic acid methyl esters of oil when seeds were stored beyond the point of one year. However elevated levels of free fatty acids, saponification value and peroxide value of the oil were found to be unchanged well after one year of storage. In tropical country like India, *Jatropha curcas* L. seeds emerge out to be one of the best feedstocks well suited for biodiesel as they maintain optimum oil quantity and quality even up to one year of storage withstanding high temperature and moisture. Results obtained from microscopic studies revealed that distorted shape and size of oil bodies and shrinkage of the same were evident in aged seeds of beyond 12 months. This can be correlated to the decrease in oleosin – oil body specific protein level, observed from NA15m. Those seeds that possessed significantly decreased level of oleosin also failed to germinate, thus establishing a positive correlation between oleosin level and germinability. The same can be said of accelerated and salt accelerated aging with regard to oleosin level. DAPI staining to understand nuclear morphology indicated cell death in aged seeds. Studies on genomic DNA show that seeds aged up to 12 months of natural aging and 3 days of both accelerated and saturated salt accelerated aging maintained their DNA integrity. But pushing the seed for further aging caused loss of DNA integrity and DNA damage.

The seed has its own destiny of deterioration from the time of its harvest, that is, before being subjected to further treatment for different purposes. In this study of kinetics of seed deterioration of economically important biodiesel plant, we have established the time points of various changes occurring during aging in *J. curcas* seeds. We found that with prolonged storage, molecules like ROS have a deleterious effect on seeds subjected to normal aging and accelerated aging. Increased lipid peroxidation, membrane damage, impairment of antioxidant enzymes, loss of viability and decreased germination were the negative consequences as seen in previous studies on orthodox seed deterioration. A parallel was drawn between changes seen in natural aging and accelerated aging, indicating the exact time points and events of the progressive and irreversible seed deterioration. From the results obtained with accelerated aging (AA) and saturated salt-accelerated aging (SSAA), we can predict the trajectory of changes with prolonged natural aging. Seeds belonging to the natural aging group up to 12 months emerged as best suited for both oil extraction and seed germination since they were found to retain their fidelity to genetic and physiological characters and were able to withstand the deleterious effects of seed storage.

This present study reiterates the prudence in selecting *J. curcas* seeds as feedstock for biodiesel. For optimum yield, *J. curcas* seeds should not be stored beyond one year. The results of this study will also help in designing methods to protect the seeds from the ill effects of prolonged aging. Further studies can be undertaken to look at the changes in mRNA in seeds with aging.