PHYSIOLOGICAL AND BIOCHEMICAL ASPECTS OF SEED STORAGE AND VIABILITY IN *SYZYGIUM* SPECIES.

Thesis submitted to the University of Calicut
for the Degree of
DOCTOR OF PHILOSOPHY IN BOTANY

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CERTIFICATE

This is to certify that the dissertation entitled "PHYSIOLOGICAL AND BIOCHEMICAL ASPECTS OF SEED STORAGE AND VIABILITY IN *SYZYGIUM* SPECIES" submitted by Anil Kumar, C. in part fulfilment of Ph.D. Degree in Botany, University of Calicut, is a bonafide record of research work undertaken by him in this Department under my supervision during the period 1993-1998 and that no part of it has been submitted before for the award of any degree.

Dr. NABEESA SALIM

DECLARATION

I, Anil Kumar C, do hereby declare that this thesis entitled "PHYSIOLOGICAL AND BIOCHEMICAL ASPECTS OF SEED STORAGE AND VIABILITY IN *SYZYGIUM* SPECIES" is a bonafide record of research work done by me under the guidance of Dr. Nabeesa Salim, Reader, Department of Botany, University of Calicut.

I also declare that this thesis has not been submitted by me fully or partially for the award of any Degree, Diploma, Title or Recognition before.

ANIL KUMAR C

ACKNOWLEDGEMENTS

The research work presented in this dissertation was undertaken under the guidance of Dr. Nabeesa Salim, Reader in botany, University of Calicut and I express my deep indebtedness and gratitude to her.

I am thanking Dr. K. Unnikrishnan, Professor and Head of the Department of Botany, University of Calicut, for providing facilities.

Thanks are due to Dr. P. Pushpangadan, Director and Dr. P.N. Krishnan, Scientist, Tropical Botanic Garden and Research Institute, Palode, Thiruvananthapuram for their encouragements and facilities provided.

Finally I am thanking my parents, family and friends for all the helps rendered during the course of this study.

CONTENTS

GENESIS

Despite their compact and apparently conservative morphology, seeds display great structural and biochemical diversity as well as physiological responses to environmental signals. There is still a need to collect primary information on many aspects of seed biology, particularly for tropical and/or recalcitrant species.

Seeds have been classified into two categories with regard to their responses to water - orthodox or recalcitrant (Roberts, 1973). Orthodox seeds can be dried without damage to low moisture content. Recalcitrant seeds on the other hand contain relatively high moisture content and are highly susceptible to desiccation injury and thus are not storable under normal conditions. Recalcitrant seeds, therefore, are more like the normal tissues of plants.

Ellis (1991) stated that in many recalcitrant seeds, development and germination appear to be more of a continuum. Since germination continues immediately after shedding from the mother plants, the metabolism is expected to be similar to that observed in orthodox seeds in which germination has progressed after imbibition (Pammenter *et al.*, 1984, Farrant *et al.*, 1985, 1986). It has been proved that like imbibed orthodox seeds, recalcitrant seeds are very sensitive to low temperatures (Woodstock, 1988; Chin, 1988; Ellis, 1991) and at high temperature are highly sensitive to desiccation (Farrant *et al.*, 1988, 1992; Berjak *et al.*, 1990; Pammenter *et al.*, 1994; Pritchard *et al.*, 1995) which is directly related to water potential of cells (Priestly, 1986).

Ultrastructural and biochemical studies have shown that recalcitrant seeds progress towards visible germination during storage (Berjak *et al.*, 1989, 1993; Farrant *et al.*, 1989, 1992; Finch-savage, 1992). The radicle emergence depends on both the moisture status of the seed and on the storage temperature. Most of the characters and storage behaviour of recalcitrant seeds clearly indicate that the seeds cannot be stored for long time despite several methods have been suggested by plant physiologists. Moreover when some specific storage condition like optimum temperature and RH are applied, the longevity of recalcitrant seeds is found to be increased which varies from species to species. Nevertheless, these conditions stimulate germination (mostly radicle growth) along with viability retention.

Syzygium aromaticum and *Syzygium cumini*; tropical and subtropical respectively in habitat bear highly recalcitrant seeds. The seeds of both species can be stored at low temperature and radicle growth is found to be very prominent in storage condition. So the present study is proposed to investigate metabolic changes occurring in the viable / (germinating) seeds. The metabolism analyzed at specific interval reflects the viability loss of both *S.aromaticum* and *S.cumini* which enable to draw a correlation of the metabolic events of these stored seeds related to germination process.

However, the time taken for visible germination to be manifested differs among species and the degree of desiccation and chilling sensitivity are similarly variable and may be correlated with the native environment of the species (Farrant *et al.*, 1988). So a comparative study of two economically important species of the genus *Syzygium* is undertaken.

ABSTRACT

Syzygium aromaticum, native to Moluccas, is a medium sized tree grown on small scale in India, Sri Lanka, Malaysia and Haiti. In India clove is thriving well in certain parts of Kerala and Tamil Nadu where the climate is somewhat warm, humid and soil loamy with a good supply of water. Suggestions have been formulated for trial cultivation as second storey crop, in plantation based agroforestry systems among the hill ranges of Madhya Pradesh, valleys of Orissa, Bengal, Assam and Andaman and Nicobar islands. In Kerala, flowering starts from the month of December and continue upto February. All the fertilized flowers do not develop into viable seeds as they fall down prematurely. Clove is propagated from seeds by raising seedlings. Nursery men and farmers relay on the viable seeds that are brought from outside the state particularly from Tamil Nadu. Conventional practice is that the seeds are sown immediately after dehusking by spreading over wet sheets or floors. But during transportation of seeds from distant places to nursery sites, there is a high chance for some seeds at stake due to drying. This results in the reduced germinability, in some case only below 70%. Since the large scale propagation of this economic plant is feasible only through seeds, the aim of this study was to standardize the collection, storing and sowing of clove seeds for establishing and stabilizing this species sustenance.

Pulpy seeds remained viable only about half a month period in all the experimented conditions while seeds de-pulped prior to storage were free from any infections and also with the longer storage period. As the storage temperature increased, a corresponding increase in the seed longevity was

observed. At 0°C seeds become dead within a day while at 30°C seeds remained viable upto 270 days. De-pulped clove seeds at natural conditions (25±5°C and 70% RH) lost their viability at open room conditions within four days as their moisture content become lowered below 38%. Maintaining the initial seed moisture content (approximately 48%) is also found to be very essential and influencing physical factor for viability maintenance under storage. Sprouting of seeds under storage, especially at higher temperature and humidity is an uncontrollable natural event.

Biomolecular estimations conducted with the stored seeds revealed dry weight percentage reduced in conditions except at room, where dry weight percentage increased along with the loss of viability. Generally the sugar content remained unchanged while the phenolic content was significantly increased along with the loss of viability. This was evidenced by browning and then blackening of seeds which morphologically manifested complete loss of viability.

Syzygium cumini is a large evergreen tree native to India growing upto an altitude of 1,800 metres. In Kerala, this tree grows well in areas with loamy well drained soils. From the flowers of January-February, fruits mature by May-June. Oblong or somewhat ellipsoidal fruits, blackish when fully ripe contains seed set in juicy pink coloured pulp.

Propagation of *S.cumini* is mainly through seeds, though inarching, grafting and budding can be tried. Natural reproduction is from self sown seeds with polyembryos which germinate on the ground during rainy seasons. Seeds dispersed to areas with water scarcity become dead within two weeks. In this experiment, the relationship between seed moisture content and germination percentage were studied. Results indicate that fresh seeds have moisture content and germination percentage, approximately 50 and 100 respectively; when dried at open conditions, the moisture content was reduced to 34% with a corresponding germination percentage of 66. *S.cumini* seeds when stored with pulp remained viable only upto 15 days in all the experimental conditions. When de-pulped, seeds remain viable even upto two years at 10°C in polyethylene bags. Regarding the results on storage temperatures, *S.cumini* seeds are susceptible to freezing temperature but not to chilling temperatures as evidenced by their storability even upto 720 days at 10°C. At higher temperatures like 20 and 30°C, stored seeds sprouted from first month onwards, particularly at 30°C, the degree of sprouting was fast and hence their storage becomes impossible beyond 180 days.

Regarding the results of biochemical estimations on stored seeds, the dry weight percentage showed a decrease in all conditions of 10, 20 and 30°C while at room conditions, due to desiccation dry weight percentage was considerably enhanced along with loss of viability. Protein content was reduced gradually side by side with loss of viability under storage. Starch content was reduced along with the loss of viability of stored seeds at respective conditions i.e., after three months at 30°C, while in seeds stored at 10°C, the lowest value was reported only after 24 months of storage. Sugar content of de-pulped seeds showed an insignificant reduction in all conditions while the pulpy seeds generally showed a slight enhancement of sugar content in all experimental conditions. Chlorophyll content was reduced along with the loss of viability while phenolics were significantly increased in line with loss of viability in all the experimental conditions as evidenced by the blackening of aged seeds. The data are interpreted in terms of metabolic changes occurring during germination which takes place under storage condition at various temperatures By evaluating the results of present study it becomes clear that the clove seeds are sensitive to chilling temperature and desiccation. To conclude, it was experimentally found that the viability of de-pulped seeds can be extended to 270 days if stored in polyethylene bags kept at 30°C / 80% RH without light.

From the evaluation of all results on desiccation, effect of storage temperatures and biomolecular changes of stored seeds, it may be concluded that the best storage method for maintaining viability of *S.cumini* seeds is by storing them in polyethylene bags kept at 10°C / 80% RH, without light.

INTRODUCTION

Anil Kumar. C "Physiological and biochemical aspects of seed storage and viability in Syzygium species." Thesis. Department of Botany, University of Calicut, 1998

INTRODUCTION

Seed development and germination are distinct physiological stages in the life cycle of plant during which metabolic events, particularly those related to the stored reserves, differ markedly. During development, there is extensive synthesis and sequestering of reserves-proteins, carbohydrates and lipids. Mobilization of these reserves occurs during germination. These distinct metabolic events suggest the necessity for a switch to effect the transition from the developmental anabolic mode to catabolic post-germinative mode. Since most seeds undergo desiccation during the final stages of maturation, it is logical to consider loss of water as a causative event in this change in metabolic direction.

Roberts (1973) recognised and classified seeds into 'orthodox' and 'recalcitrant' types according to their behaviour during storage. Orthodox seeds which shed from the parent plant at low moisture content can tolerate desiccation considerably and so the period of viability may be extended by lowering their moisture content in the range of 1-5% (Ellis, 1991). Recalcitrant seeds do not undergo maturation drying and are shed from the mother plant at relatively high moisture content and are desiccation intolerant (Roberts, 1973; Chin, 1988). They can only be stored in wet medium to avoid dehydration injury and at relatively warm condition to avoid chilling injury. As a result, recalcitrant seeds live for only short duration and are difficult to store successfully because their high moisture content encourages microbial contamination and result in rapid deterioration (King and Roberts, 1979, Roberts *et al.*, 1984).

Plant species with recalcitrant (homoiohydrous) seeds are usually woody and mostly favour moist tropical or aquatic environments. Embryo is well protected against desiccation by seed coat or the pericarp. As recalcitrant seeds are predominantly large, they are often characterized by extensive vascularization in seed coat or cotyledons. King and Roberts (1979) noted the greater size of recalcitrant seeds. Tompsett (1992) in his review of previous studies on seeds of Dipterocarpaceae pointed out the association of recalcitrance with greater seed size, moist tropical habitat, shorter seed longevity and slow desiccation.

There is a wide range of morphological and structural variation among recalcitrant seeds. The seeds or nuts are often surrounded by thick endocarp while many tropical fruits are covered with a fleshy or juicy arilloid structure. Most of the recalcitrant seeds are spherical or oval in shape while some of them are flattened and very light weighted as in the case of *Lophopetalum wightianum*. Roberts *et al* (1984) reported that species with recalcitrant seeds are mainly of two types (a) Riparian (b) large seeded types. It is quite obvious why riparion species produce recalcitrant seeds due to the easy availability of water for germination and their subsequent establishment to their habitat before being washed away. The average weight and volume of recalcitrant seeds usually far exceed those of orthodox seeds. Seeds of some recalcitrant species such as mango, *syzygium* are polyembryonic (Maheswari, 1971).

The characteristics of recalcitrant seeds have been discussed by King and Roberts, (1979); Roberts and King, (1980); Roberts and King, (1982), and many longevity improving approaches have been made by

King and Roberts (1979) and King and Roberts (1980). Kaul (1979) studied the period of survival of seeds of a large number of recalcitrant species after drying them to 15±3% moisture content and then storing them at laboratory temperature. Some of them are *Aegle marmelos, Agathis palmerstonii, Ailanthus fordi, Aleurites fordii, Artocarpus integrifolia, Azadirachta indica, Dipterocarpus alatus, Eugenia jambolana, Eugenia cymosa, Eugenia grandis, Hopea oderata, Mangifera indica and Shorea robusta.*

Recalcitrant seeds having elevated moisture content on shedding deteriorate more or less rapidly under all storage conditions (Chin and Roberts, 1980; Farrant, *et al*, 1988; Berjak *et al*, 1990). Majority of the recalcitrant seeds are sensitive to chilling injury at low temperatures (King and Roberts, 1979). Recalcitrant seeds of *Shorea talura* are intolerant to 4°C (Sasaki, 1976) and *Theobroma cacao*, do not even tolerate temperatures of 15°C (Hor *et al*, 1984). As recalcitrant seeds are desiccation sensitive, only short term storage is feasible, and that too if they are kept in moist media. Moist or imbibed storage has been employed in a number of crops including rambutan, (Chin, 1975), rubber (Ang. 1976), Cocao (King and Roberts, 1982; Hor *et al.*, 1984).

According to Chin and Roberts (1980) species producing recalcitrant (homoiohydrous) seeds usually occur naturally where conditions are generally continuously favourable for seedling establishment. Once shed, such seeds initiate germination with no further requirement of water (Farrant *et al.*, 1986, 1988, 1989; Berjak *et al.*, 1990; Ellis, 1991). It is not easy to define and identify precisely the transition from developmental growth to germination in recalcitrant seeds (Tompsett and Pritchard, 1993).

For recalcitrant seeds, the rate of moisture loss and periods of time for which seeds are exposed to drying conditions can be of importance (Berjak *et al.*, 1989; Pritchard, 1991). However according to (Berjak *et al.*, 1990) the time taken for visible germination differs among homoiohydrous seed species. The degree of desiccation and chilling sensitivity is similarly variable and may be correlated with the native environment of the species (Farrant *et al.* 1988).

Difference in the type of seed storage physiology, among species of the same genus may be associated with aspects such as type of reserves, seed size, seed developmental status, environmental conditions, desiccation rate and tolerance, interwoven with moisture content and maturation drying (Tompsett, 1984, 1992; Hong and Ellis, 1990).

The major problems usually met in moist storage of recalcitrant seeds are the germination of stored seeds and profuse fungal growth. Several attempts have been made to store seeds within the fruit or fruit juice but all become futile as in the case of rambutan (Chin, 1975). But positive results were obtained when partially dried seeds were stored with the application of fungicide. After treated with adequate amounts of a fungicide, semi-recalcitrant coffee seeds were stored in polyethylene bags at 15°C (Vossen and vander, 1979) and citrus seeds in polyethylene bag at 0°C to 4°C (King and Roberts, 1979) and their seeds were found to be viable for long time.

Information on critical moisture content values below which dehydration injury occurs has been reported for many recalcitrant seeds of various families (Pritchard, 1991; Tompsett, 1992, 1994). According to Berjak *et al.* (1989) and Farrant *et al.* (1989) the recalcitrant seeds after progress towards visible germination during storage and the speed of radicle emergence depends both on the moisture status of the seed and on the storage temperature. Water status changes during development in relation to the germination and desiccation tolerance of *Aesculus hippocastanum* L. seeds was studied by Tompsett and Pritchard (1993). Pammenter *et al.*(1994) suggested that, in storage, recalcitrant seeds are exposed to an initially mild, but increasingly severe, water stress leading to the death due to uncontrolled free-radical mediated oxidative damage. The effect of moisture content on the low temperature responses of recalcitrant *Araucaria hunsteinii* seed and excised embryos was investigated by Pritchard *et al.* (1995) in relation to germination storage.

Seed storage studies on tropical forest fruit species mostly of with recalcitrant seeds is very meagre (Hanson, 1984) and there are many storage problems yet to be solved in tropical plantation crops (Chin, 1978). In spite of all the ongoing research programmes regarding the standardisation of an efficient germplasm conservation, recalcitrant seed storage still remains as a challenge of seed science.

After the classification of seeds into orthodox and recalcitrant, (Roberts, 1973; Ellis *et al.*, 1986) suggested the negative logarithmic relation between longevity and their moisture content in air-dry storage of orthodox seeds. An international recommendation for the long term seed storage for genetic conservation of orthodox seeds at 5±1% moisture content and at 20°C with 10-13% RH (Ellis *et al.*, 1988, 1989) was revised and confirmed by Ellis *et al.* (1995). However, recalcitrant seeds pose serious problems in germplasm storage as these seeds are intolerant to desiccation and freezing. This provides a formidable task in developing appropriate conservation protocols for recalcitrant seeds.

According to Farrant *et al.* (1988), Ellis (1991), Pammenter *et al.* (1994) and Chien and Lin (1997) the current view is that, if recalcitrant seeds are maintained under storage condition that prevent water loss, they will ultimately loss viability and hydrated recalcitrant seeds are metabolically active and undergo germination associated changes in storage.

Syzygium aromaticum is an important spice, growing for its dried unopened flower bud, the clove of commerce while *Syzygium cumini* is a native tree species growing for its edible fruits with many medicinal values. Considerable research has been conducted on aspects of cultivation (Rao, 1982; Krishnamoorthy and Rema, 1994); chemical constituents (Mangala Kumari and Mathew, 1985; Gopalakrishnan *et al.*, 1988; Menon and Narayanan, 1992; Vernin *et al.*, 1994); pharmacology (Yamahara *et al.*, 1983; Kurokawa *et al.*, 1995; Deans *et al.*, 1995; Kurokawa *et al.*, 1998). Studies on seed germination in *Syzygium aromaticum* seeds (Nayar *et al.*, 1979) suggested that sprouted seeds are superior for planting than fresh seeds. Effect of different concentrations of Gibberelic acid on clove seed germination (Dhalimi, 1983) resulted in increased percentage of germination as well as speed of germination, especially of 30 to 60 ppm concentrations. According to Sabale *et al.* (1992), considerable loss of seed germination percentage of seedling vigour in *S.aromaticum* seeds was observed when stored seeds were used. Possibility of increasing seed germination and seedling growth of clove by application of NAA and Gibberelic acid has been discussed by Bagade and Shinde (1994). Investigations on seed storage in *S.aromaticum* and *S.cumini* have been done by a few authors. Sutarno and Utami (1984) studied the storage viability. Sabale *et al.* (1992) reported that considerable loss of germination and seedling vigour occurred in clove when seven to fourteen days stored seeds were sown . Notwithstanding, seed behaviour under prolonged storage is not studied in detail.

Similarly in *S.cumini* also several investigations have been conducted on various aspects like embryology (Janick and Moore, 1975; physiology (Antoszewski *et al.*, 1989; Rao *et al.*, 1989; Geetha *et al.*, 1992; Ponnammal *et al.*, 1992; Krishnamurthy *et al.*, 1997); pharmacology (Prince *et al.*, 1997). Germination studies are conducted on *S.cumini* (Shanmugavelu, 1967; Sultan Sing and Singhrot, 1984) and found that deeper sowing resulted in earlier and higher percentage of germination. Viability loss in *S.cumini* has been reported by Shanmugavelu (1967). All these studies revealed some aspects of storability, but storage temperature, duration etc. are not clearly mentioned. Notwithstanding, physiological studies on seed storage, viability and germination are lacking in the seeds of these species. Studies on correlation that exist in the storage temperature, moisture content and extend of germination are considered worth for further study. Moreover, *Syzygium aromaticum* and *Syzygium cumini* trees possess recalcitrant seeds, the viability of which is found to be very short under normal environmental conditions. The seeds of these species are highly sensitive to desiccation and rapidly loose viability at normal temperature. Preliminary studies on the seeds of *Syzygium* species confirmed that storage for prolonged period is possible at some specific temperature and relative humidity and germination process (radicle elongation only) is progressing continuously under storage. Moreover, considerable difference exists between *S.aromaticum* and *S.cumini* in their habitat and seed behaviour under storage.

There has been little comprehensive research in the physiology of seed germination in recalcitrant seeds, even though some aspects has been described for few species (Ellis, 1991; Berjak *et al.*, 1984, 1990; Pritchard *et al.*, 1995; Copeland and McDonald, 1995). The present study identifies the optimum condition of temperature and relative humidity for seed storage, the extend of germination (radicle elongation only) occurring in the storage and the biochemical changes of metabolites in the seeds during the period of nine months in *S.aromaticum* and twenty four months in *S.cumini*.

In the present study, germination pattern and desiccation tolerance of *S.aromaticum* and *S.cumini* seeds under natural conditions and longevity of these seeds under storage of various temperatures compared to room temperature are proposed to undertaken. Furthermore, the behaviour of seeds under different conditions of storage is also proposed to be investigated on the basis of germination analysis as well as metabolic changes occurring in them.

Since recalcitrant seeds are highly sensitive to temperature, they were stored 10, 20, 30°C and at room temperature. Metabolites analysed during storage and germination included protein, starch, sugars, phenolics and chlorophyll contents.

MATERIALS AND METHODS

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MATERIALS AND METHODS

Syzygium aromaticum (L.) Merril & Perry and *Syzygium cumini* (L.) Skeels are two of the economically important species of the family Myrtacease. Of these, *Syzygium aromaticum* is an important spice crop for its dried unopened flower buds, the clove of commerce, while *Syzygium cumini* is a fruit and timber yielding tree with many medicinal values. Normal propagation of these two species are through seeds, which are of recalcitrant type with short viability period.

Flower to fruits development of *Syzygium aromaticum*

Phenological and developmental studies on *Syzygium aromaticum* are conducted among 30 year old trees growing at an altitude of 500 m in a clove plantation at Nagercoil, Tamil Nadu. Trees begin to flower during the month of August-September. The floral primordia develops gradually during the month of October-November and by December-January they become mature flower buds. It is these flower buds that are dried and used as the clove of commerce. February and March are the months during which highest percentage of anthesis and fertilization occur.

All the pollinated flowers exhibits a pronounced swelling of the calyx tube while those flowers in which pollination has been unsuccessful will abscise. Fruits attain harvesting maturity during June-July and this was represented by fully mature harvestable deep purple coloured pulpy oblong fruits.

Flowering and Fruiting of *Syzygium cumini*

Syzygium cumini flowers twice a year, of which the first one being during November-December followed by a second with much more profuse bloom of January-February. Fruits developed out of the bloom of January-February attain harvesting maturity by May-June and become deep purple, afterwards the increased rate of fruit fall indicates the full attainment of physiological and harvesting maturity.

Mode of collection

Fruiting season of *Syzygium aromaticum* spreads between the months of June-July. Ripened fruits were hand-harvested during the month of June from a tree growing at an altitude of 500 m in a plantation of Nagercoil. Fruits oblong, blackish when fully ripe contains single seed set in juicy pink pulp. Each seed has an average weight, length and breadth of 1.113±0.08 gm, 1.8±0.7 cm and 0.867±0.6 cm respectively. Cotyledons are chlorophyllous and covered by a papery testa. Embryo which is tugged in between the cotyledons has a prominent pink radicle.

Syzygium cumini sets fruits twice in a year, of which the first set is during April-May while the second set is during May-June. Fruits ripened during May-June was superior in both quality and quantity wise and so were hand harvested from a tree growing at an altitude of 150 m amongst semi-evergreen forest of the Western Ghats (Latitude 8°45' & 8°47' N and Longitude 77°1' and 77°4' E).

Oblong or somewhat ellipsoidal fruits, blackish when fully ripe contains single seed set in juicy pink pulp. Each seed has an average weight, length and breadth of 1.55±0.23 g, 2±0.35 and 0.93±0.06 cm respectively. Cotyledons are chlorphyllous and are covered by a papery testa. Number of embryos per seeds varies from 2 to 10 relatively with the fruit size.

Soon after collection, fruits of *Syzygium aromaticum* and *Syzygium cumini* were brought from field to laboratory where the following studies were conducted.

Sampling

From the collection, randomly selected 2,500 fruits were equally divided into five lots and were stored separately in five polyethylene bags. Another 3000 fruits were de-pulped by smearing with saw dust and then washed with water. Cleaned seeds were spread evenly over a bloating paper and fan dryed for half an hour. These fresh seeds were equally divided into six lots, from which five lots were packed and kept separately in individual polyethylene bags. The sixth lot comprising 500 seeds were used for studying the relationship between seed moisture content and viability.

Experimental Studies

Effect of De-pulping

Seeds were sowed with and without pulp for studying the effect of pulp on seed germination. For storage studies pulpy and de-pulped seeds were stored separately at different conditions to study the effect of pulp on seed viability.

Effect of Seed Moisture Content

About 500 de-pulped seeds were allowed to dry under normal room conditions and at an interval of 12 hours and 24 hours for *Syzygium aromaticum* and *Syzygium cumini* respectively. Seed sample were analysed for corresponding germination percentage and moisture content. Seed moisture content was determined according to high constant temperature oven method (ISTA rules, 1985) by drying at 130°C for one hour in an oven and were expressed as percentage of fresh weight. These experiments were replicated five times, each with randomly selected three seeds and average value taken.

Effect of Storage

Seeds with and without pulp were stored separately in polyethylene bags (200 gauge, size 60 x 30 cm). Preliminary experiments showed that when the storage bags were completely filled with pulpy and depulped seeds, within a few days seeds became brown and dead. Therefore in all the experimental conditions, half portions of the polyethylene bags were kept empty to facilitate enough air supply to stored seeds. The conditions of storage were as follows -

(1) Open room, 60% RH and 25±5°C

(2) Polyethylene bags kept in room, 60% RH and 25±5°C

(3) incubator, 80% RH and 30°C

(4) incubator, 80% RH and 20°C

(5) incubator, 80% RH and 10°C

(6) refrigerator, 45% RH and 0°C

Polyethylene bags were opened for seed retrieval only at definite intervals decided as per preliminary experimental results. Preliminary data on storage indicated that *Syzygium aromaticum* and *Syzygium cumini* seeds behaved almost similarly upto 15 days of storage, but afterwards the deterioration of *Syzygium aromaticum* was much speedier than *Syzygium cumini* seeds. So the case of *Syzygium aromaticum*, the intervals were 24 hours, 4 days, 15 days, 1 month, 2 months, 3 months, 6 months and 9 months while in the case of *Syzygium cumini*, the intervals were 24 hours, 4 days, 15 days, 1 month, 3 months, 6 months, 12 months, 18 months and 24 months.

Germination Studies

Preliminary observations showed that the optimum temperatures for seed germination were between 30 to 32°C and that light was not an influencing factor initially. Pulpy and depulped seeds were sowed separately in the field and laboratory conditions for studying their germination behaviour. In the field, the germinating medium was pure sand taken in earthen seed pots which were watered twice a day and the conditions were 30 / 20°C and 60% RH. In the laboratory germination studies were conducted in paper towels kept in a seed germinator set at 30°C and 85% RH without light. Each experiments of germination studies in field and laboratory, consisted of 50 seeds each in 5 replicates. All experiments were conducted a minimum of 5 times separately.

Biochemical Studies

Biochemical studies were conducted to elucidate the biomolecular transformations of metabolites taking place simultaneously with the change in seed viability under different conditions of storage. Estimation of metabolites like starch, sugars, protein, phenolics and chlorophyll were conducted side by side with germination studies by using random tissue samples from respective stored seed stocks. The intervals of sampling in *S.aromaticum* and *S.cumini* were same as described under *effect of storage*.

Dry weight

When the sampling was done for each experiment at all intervals, one sample in duplicate was kept for dry weight determination. Choped seed samples as replicates of five with three seeds each, were kept at 100°C for one hour in a hot air oven and the temperature was kept constant at 60°C until constancy in dry weight was obtained.

Estimation of Protein

Residue of the chlorophyll estimation was flocculated for 5 minutes with 5 ml 0.1 N NaOH. By centrifuging at 2000 rpm for 5 minutes, the supernatent was stored in a glass tube. By diluting the supernatent 10 times with distilled water, aliquot was prepared to which 5 ml of the alkaline copper reagent (mixture of 2% Sodium Carbonate in 0.1 N NaOH and 0.5% Copper Sulphate solution in 1% Sodium Potassium tartarate solution) was added and kept the tubes at room temperature for 15 minutes; finally added 0.5 ml of Folin-Ciacalteau reagent. Reaction mixture was kept in dark for 20 minutes and the absorbance at 650 nm was measured spectrophotometrically against a reagent blank (Lowry *et al*, 1951).

Extraction Procedure for Sugars, Phenolics and Starch:

Hundred milligram tissue was homogenised with 5 ml 80% alcohol. This mixture was taken in a test tube and placed for 5 minutes in a water bath maintained at 70°C. By centrifuing at 2,000 rpm for 3 minutes, the supernatent was collected in a glass test tube. The residue was again activated with 5 ml of 80% alcohol in a water bath maintained at 70°C for 5 minutes and thus complete extraction of sugars and phenols were ensured. This was again centrifuged and supernatent obtained was pooled with the previous supernatent and made to 10 ml with 80% alcohol.

Estimation of Starch

The residue was boiled with 5 ml of distilled water for 10 minutes in a water bath and then centrifuged at 1500 rpm for 10 minutes to collect the supernatent. Second residue was again boiled with 5 ml distilled water as described previously and this supernatent was pooled and made up to 10 ml with distilled water.

To 4 ml of the aliquot prepared from the supernatent made out of distilled water. 1 ml of 20 times diluted Lugol's reagent was added and read spectrophotometrically at 630 nm against a reagent blank (Mc Cready *et al.* 1950).

Estimation of Sugar

An aliequot of alcoholic supernatent was taken to which 4 ml of 2% anthrone reagent (10 - Keto 9, 10 - dihydro anthracene) was added and kept the tubes in a boiling water bath for 10 minutes. Then the absorbance was measured at 620 nm spectrophotometrically against a reagent blank (Packer, 1967).

Estimation of Phenolics

Total phenolics estimation was estimated by the method of Swain and Hillis (1959). The reaction mixture was prepared in the following manner:

To 0.9 ml of the aliquot of the alcoholic supernatent, 0.1 ml of Folin - Ciocalteau reagent was added and kept for 5 minutes. Then 5 ml of 20% Na_2CO_3 was added to the mixture and kept for 5 minutes in a water bath maintained at 70°C. Then the absorbance was measured at 660 nm spectrophotometrically against a reagent blank (Swain and Hillis, 1959). Total phenolics in the sample was calculated from a standard curve prepared with Tannic acid.

Estimation of Chlorophyll

Estimations were conducted in ice cool temperature by homogenising 100 mg of tissue in 80% acetone solution. Centrifuged at 3.000 rpm for 5 minutes, the supernatent was stored in closed test tubes. Then the residue was washed 3 times, each with 2.5 ml of 80% acetone and final supernatent solution was made upto 10 ml. Taking 80% acetone as blank, the supernatent was read in a spectrophotometer at 663 and 645 nm. Chlorophyll a and chlorophyll b were calculated according to Arnon's method (1949). The amount of chlorophyll present in the extract:- milligram chlorophyll per gram tissue was calculated using the formula:

Milligram chlorophyll a/g tissue:- 12.7 (A_{663}) - 2.69 (A_{645}) x (V/1000) x W

Milligram chlorophyll b/g tissue:- 22.9 (A_{645}) - 4.68 (A_{663}) x (V/1000) x W

Milligram total chlorophyll/g tissue:- 20.2 (A_{645}) + 8.02 (A_{663}) x (V/1000) x W

where A = absorbance at specific wavelengths

V = final volume of chlorophyll extract in 80% acetone

W = fresh weight of tissue.

All estimations were repeated a minimum of 6 times using samples collected at different times. Average values and standard deviation were calculated and standard error was given along with mean values in Tables. Test of significance was done where ever necessary using 't' test.

REVIEW OF LITERATURE

Anil Kumar. C "Physiological and biochemical aspects of seed storage and viability in Syzygium species." Thesis. Department of Botany, University of Calicut, 1998

REVIEW OF LITERATURE

From pre-historic time man has understood the role of seeds for harvesting their next crop. Maintenance of viability in storage is of prime importance and majority of seeds retain their viability if they are first dried and then stored at low temperature in sealed containers. Roberts (1973) classified seeds into 'Orthodox' and 'recalcitrant' types based on the physiology of their response to desiccation and storage behaviour. Orthodox seeds can tolerate desiccation and freezing temperatures while recalcitrant seeds are killed if their moisture content is reduced below some critical value (12-31%). According to Hanson (1984) it was more accurate to call orthodox seeds 'desiccation-tolerant' and recalcitrant seeds 'desiccation-sensitive'. Species producing recalcitrant seeds usually occur naturally where conditions are generally continuously favourable for seedling establishment (Chin and Roberts, 1980). Several investigators like Farrant *et al.* (1986, 1988, 1989) and Berjak *et al.* (1990) are of opinion that once shed, such seeds initiate germination with no further water requirement. Generally recalcitrant seeds germinate with a short time in wet conditions as their germination process has already started even from the time of shedding.

Plant species with recalcitrant (homoiohydrous) seeds are usually woody and mostly favour moist tropical or aquatic environments. Embryo is well protected against desiccation by seed coat or the pericarp. Unlike orthodox (Poikilohydrous) seeds, recalcitrant seeds lack pronounced maturation drying as they are usually shed at a high moisture content.

Observation of Hofmann and Steiner (1989), von Teichman and van Wyk, (1991; 1994) and Berjak *et al.* (1993) illustrated that highly recalcitrant seeds are an adaptation of woody taxa particularly to humid tropical forest environments. while minimally recalcitrant seeds tend to occur in taxa distributed in more temperate. or tropical, but mainly seasonally dry regions. These authors also suggested that recalcitrance constitutes a primitive character state in the dicotyledons. Berjak *et al.* (1989) viewed recalcitrance as a possible evolutionary 'hang over' acquired subsequently to the evolution of the seed. Convincing evidences has been put forth by Lamont *et al.* (1991) to state that delayed seed release is an advanced condition over spontaneous release of seeds when they become mature.

According to Farrant *et al.*, (1992) only a few detailed studies are available on the pre-shedding developmental events in recalcitrant seed type. Nevertheless a few studies have been conducted in *Durio zibethinus* Murr., (Soepadmo and Eow, 1977); *Erythroxylum coca* Lam., (Bosewinkel and Geenem, 1980); *Nephelium lappaceum* L., (Lan, 1984); *Mangifera indica* L. (von Teichman *et al.*, 1988); *Litchi chinensis*, (Steyn and Robbertse, 1992); *Persea americana* (Steyn *et al.*, 1993).

The seeds of following species were also reported to be recalcitrant. *Agathis laranthifolia* (Suriamihardja, 1979), *Dipterocarpus humeratus, Shorea parvifolia* (Maury-Lechon *et al.*, 1981), *Dipterocarpus bandii, Shorea curtisii, Shorea sumatrona* (Yap, 1981), *Eugenia brasiliensis* (Goldbach, 1979). *Myristica fragrans* Hautti an evergreen dioecious taxon native to Moluccas and widely cultivated throughout tropics for the two valuable spices nutmeg and mace, possesses recalcitrant seeds (Chin *et al.*, 1984).

King and Roberts (1979) noted that recalcitrant seeds are predominently large, and are often characterised by extensive vascularization. Tompsett (1992) in his excellent review of earlier studies on seeds of Dipterocarpaceae pointed out the association of recalcitrance with greater seed size, moist tropical habitat, shorter seed longevity and slow desiccation. Boesewinkel and Bouman (1984) stated that when considering seed size among angiosperms in general state, seeds of more primitive families on the whole has relatively more and extensively developed vascular system. Advanced taxa (like Sympetalae) often have simple and small seeds with poorly differentiated vascular bundles. All these indicate that in recalcitrant species, greater seed size is a relatively ancestral character. As part of his durian theory. Corner (1949) postulated that the immediate ancestors of modern flowering plants might have been woody plants or tropical and subtropical rain forests with large desiccation intolerant seeds. Observations made by von Teichman and van Wyk (1994) with 45 families with recalcitrant seed showed that the exalbuminous state is significantly (68%) associated with the presence of seed recalcitrance.

Many of the previously considered recalcitrant seeds are now shown to be orthodox ones. Mumford and Grout (1979) found that lemon seeds lost their recalcitrant behaviour if the testae were removed and thus prevented desiccation injury. King *et al.* (1981) suggested that the seeds of citrus genus can be stored at -20°C and 5% moisture content for longer periods. The orthodox nature of recalcitrant seeds of *Elaeis guineensis*, oil palm seeds, was reported by Grout *et al.* (1983) since their embryos suffer little damage when dried to 10.4% moisture content and stored for eight months at –196°C.

Roberts *et al.* (1984) reported that species with recalcitrant seeds are mainly of two types (a) riparian (b) large seeded types. It is quite obvious why riparian species produce recalcitrant seeds because of the easy availability of water for germination and their subsequent establishment to their habitat before being washed away. However, most of the tropical plants and plantation crops such as rubber, cocao and coconut, tropical fruit crops such as mango, mangostein, jack fruit as well as tropical timber species belonging to the family Dipterocarpaceae are with recalcitrant seeds (Chin *et al.*, 1984).

According to Boroughs and Hunter (1963) most of the recalcitrant seeds of tropics which are adapted to moist warm forest habitat are killed at subambient temperatures. A typical example is that of *Theobroma cacao* seeds which abruptly looses their viability at or below 15°C. These authors reasoned the loss of *Theobroma cacao* seed viability below 15°C is due to (1) the presence of some temperature – dependent reaction, the cessation of which leads to lethal metabolic disruption, (2) the absence of some protective substance present in seeds which are not susceptible to chilling and (3) the liberation of some toxic material owing to cold induced changes in membrane permeability.

King and Roberts (1979) reported that majority of the recalcitrant seeds are sensitive to chilling injury at low temperatures. Recalcitrant seeds of *Theobroma cacao,* do not even tolerate temperatures of 15°C (Hor *et al.*, 1984), or 4°C for *Shorea talura* (Sasaki, 1976) while most of the seeds, freezing temperature is found to be detrimental.

Seeds of tropical and sub-tropical origin suffer chilling injury when exposed to temperatures above the freezing point of tissue but below approximately 15°C. The chilling injury may be exhibited as loss of viability or reduced growth during germination at favourable temperatures (Simon, 1979; Wolk and Herner, 1982).

Developmental studies of recalcitrant seeds indicate that desiccation tolerance is not lost, but rather than full desiccation tolerance never develops in recalcitrant type of seeds, which do not dry to low moisture content before shedding (Probert and Brierley, 1989; Hong and Ellis, 1990). However, these authors are of opinion that like orthodox seeds, the desiccation tolerance of recalcitrant seeds can increase during seed development in plants like *Acer pseudoplatanus* and the increased recalcitrance may then be progressively lost during germination, which may occur close to, or even before, shedding as in *Avicennia marina*: (Farrant *et al.*, 1986).

Limited desiccation can stimulate germination in recalcitrant species, such as *Avicennia marina* (Farrant *et al.*, 1985), *Shorea robusta* (Nautiyal and Purohit 1985a) despite their inability to survive desiccation below relatively high moisture content. Developmental studies conducted by Finch-Savage (1992) on *Quercus robur* L. revealed large variation in the time of germination of fruits and seeds. This was unrelated to size or moisture content, but was affected by the time of shedding of fruits, but not seeds where the pericarp-induced restriction of germination affected by light related to the date of shedding.

The characteristics of recalcitrant seeds have been discussed by King and Roberts, (1979); Roberts and King, (1980, 1982) and Roberts (1983). Many longevity-improving approaches also have been made by these authors.

According to Berjack *et al.* (1989) the recalcitrant seeds never show dormancy but instead continue their development and progress towards germination. At physiological maturity recalcitrant seeds are much higher in moisture content (50-70%) than orthodox seeds. Recalcitrant seeds having elevated moisture content on shedding deteriorate more or less rapidly under all storage conditions (Chin and Roberts, 1980; Farrant *et al.*, 1988 and Berjak *et al.*, 1989).

According to Berjak *et al.* (1984) recalcitrant seeds are shed from the parent plants at high moisture content usually after a limited degree of maturation. Large seed size faces greater problems of internal transport of water than with smaller seeds. Water in seeds is found as free water and bound water. The free water is necessary for the movements of molecules from one centre of metabolism to another when dryed, this free water is removed and loss of weight is expressed as moisture content of the seeds. The bound water maintains the stability of macromolecules, membranes and functioning of multi-enzyme systems in desiccated orthodox seeds unlike that of recalcitrant seeds.

Roberts (1972) suggested that the low moisture content is an important factor in maintaining the viability of stored orthodox seeds but, recalcitrant seeds cannot be dried below a high critical moisture content value (Roberts, 1973). According to Roberts (1979) loss in membrane integrity, denaturation of larger molecules, accumulation of toxic substances are some changes known to cause loss of viability. Information on critical moisture content values below which dehydration injury occurs have been reported for both temperate and tropical recalcitrant seeds of species in Fagaceae (Tamari, 1978; Pritchard, 1991) in Dipterocarpaceae (Tompsett, 1992) in Meliaceae and Araucariaceae (Tompsett, 1994).

The response of recalcitrant seeds to desiccation and chilling depends on the nature of seeds and varies from species to species. Seeds of *Theobroma cacao* are damaged below 27% moisture content (Hor *et al.*, 1984) whereas *Nephelium lappaceum* seeds are damaged at moisture content less than 20% (Chin, 1975). The critical moisture content as described as the lowest safe moisture content by Tompsett (1987) varies from species to species and is a relatively high value ranging from 12 to 31% (Roberts, 1973). King and Roberts (1979) suggested two ways for the detrimental effects of dehydration on recalcitrant seeds i.e., either death occurs rapidly at or below some critical moisture content (critical moisture content hypothesis) or loss of viability occurs at a rate which is negatively related to the moisture content or a wide range of moisture contents (non critical moisture content hypothesis).

Avicennia marina seeds starts to germinate on shedding and become more sensitive to desiccation with the onset of cell division and vacuolation (Bewley, 1979). As *Avicennia* seeds withstand the loss of 18% of their initial moisture content and remain viable, Farrant *et al.* (1988) described their behaviour similar to that of imbibed orthodox seeds. Studies on the effect of desiccation and chilling on tea, cocoa and jack fruit revealed that these seeds survived desiccation to 24, 35 and 31 percentage moisture content respectively (Chandel *et al.*, 1995).

For attaining the goal of long term storage of seeds, new techniques such as cryogenic storage of embryos, are to be practiced. Roberts *et al.* (1984) described seed storage in liquid nitrogen as one of the most promising method, though there are several technical problems to be overcome. If seeds are to survive, they must be dried prior to freezing (Fedosenko, 1974; Sakai and Noshiro, 1975) and only at a very low moisture content they can survive at $-196^{\circ}C$ (Stanwood and Bass, 1978). Chin (1988) reported that International Board for Plant Genetic Resources (IBPGR) funded a project on techniques for the dehydration and conservation of recalcitrant seeds at University Pertanian Malaysia. This project investigated the possibility of drying embryos below the high moisture freezing limits (HMFL) and the use of colligative substitution of a cryoprotectant in the liquid nitrogen storage of embryos. Chin (1988) also found that the embryos of *Artocarpus heterophyllus* can be dried to 11% moisture content and survive in culture media.

Tissues were found to be survived from dried and cryopreserved *Araucaria hunsteinii* embryos (Pritchard and Prendergast, 1986). Critical moisture content of rubber seeds was found to be between 15 and 20% (Chin *et al.*, 1981) but Normah *et al.* (1986) found that the rubber embryos with moisture content between 14 and 20% survived cryopreservation for 24 hours and formed seedlings with normal roots and shoots when cultured *in vitro*. Chandel *et al.* (1995) suggested that excised embryonic axes of tea and jack fruit after drying to 14% of moisture content survived cryopreservation. Cryopreservation experiments with *Coffea arabica* by Dussert *et al.*(1998) indicated that greater damage to endosperm than to embryo during freeze/thaw cycle could be overcome by slow precooling of seeds which had beneficial effect on their survival.

Seeds of tropical and sub-tropical origin suffer chilling injury when exposed to temperatures above the freezing point of tissue but below approximately 15°C. Injury caused by chilling during the initial hours of imbibition in cotton was manifested in an aborted radicle tips plus a proliferation of latteral roots which leads in cortex damage during later period (Christiansen, 1963, 1967). Chilling during hydration of cacao seed resulted in destruction of cotyledonary tanning cells (Ibanez *et al.*, 1965). Chilling temperature magnifies the stress of imbibing seeds (Pollock and Toole, 1966). Gradual increase of seed moisture by exposure to humidified air prior to imbibition of liquid water avoids imbibitional injury (Pollock, 1969). Damaging the seed coat decreases the barrior to rapid water uptake and increases the susceptibility to chilling (Tully *et al.*, 1981; Taylor and Dickson, 1987). Spaeth (1989) has attributed damage and leakiness to physical stress resulting from rapid water uptake. Bedi and Basra (1993) in their review article emphasized the need to distinguish imbibitional chilling injury, chilling injury of previously hydrated tissues and hydration damage related to low-water concentrations when referring to mechanisms of seed leakage during imbibition.

In recalcitrant seeds such as that of *Aesculus hippocastanum* (Tompsett and Pritchard, 1993) the storage tissue serve as a water reservoir for the axes and water moves from cotyledons to the axes during germination. Viability of *Quercus robur* seeds were controlled by the water content of cotyledons rather than that of the axes (Finch-Savage, 1992).

Bonner (1978) reported that *Quercus* species seeds are difficult to store longer than one winter without serious loss of viability. As in other large, recalcitrant tree seeds, the naturally high moisture content must be maintained to assure viability in the seeds of *Quercus robur* L. and when maintained above their critical moisture content value, approximately at 40-50% moisture content, their longevity was enhanced (Blomme and Degeyter, 1986). Effect of drying *Quercus robur* acorns to different moisture content followed by storage, either with or without imbibition was studied by Gosling (1989) and reported that these seeds are found to be both short lived and desiccation sensitive during storage at 2°C over the similar moisture content range of 25 to 45%.

Anil Kumar *et al.* (1996) correlated the moisture content and germinability in *Aporusa lindleyana* (Wight) Baillon. Seeds with 40.3%

moisture content were kept open at room conditions (30/20°C and 60% RH) and after 24 hours the moisture content became 33% with a corresponding germination percentage of 98%. But after 36 hours of room drying, the seed moisture content and germination became 30 and 32% respectively. Seeds dried for 48 hours with moisture content 25% failed to germinate.

Mature seeds of *Machilus thunbergii* with 43% moisture content and 90% germinability when allowed to dry at 25°C / 73% RH for 30 days, their moisture content became 36% with 60% germination and because of this desiccation sensitivity at water content higher than 30%, Lin and Chen (1995) considered them as recalcitrant seeds.

Regarding the effect of high seed moisture and moderate storage temperatures, the storability of cocoa seeds (Hor *et al.*, 1984) at 33.5% moisture content was not found significantly different from that at 35% and 32%. Critical temperature for cocoa is between 15°C to 17°C. Results of this study show that cocoa seeds required temperature above 17°C with a minimum moisture content of 33.5%.

Seeds of tea, *Camellia sinensis* became damaged when dryed below 28% moisture content and the best storage temperature is found to be 5 - 7°C (Sebastiampillai and Anandappa, 1979). Suszka and Tylkowski (1980) reported that acorns of the English Oak (*Quercus robur*) can be stored upto 4 years with 40% moisture content under aerobic conditions at -1°C. High moisture content of recalcitrant seeds enhances the chance to germinate under storage and according to Goldbach (1981) this problem of sprouting under storage can be checked hormonally by using abscissic acid or osmotically with polyethylene glycol (PEG).

As reported earlier, recalcitrant seeds are desiccation sensitive, only short term storage is feasible, and that too if they are kept in moist media. Moist or imbibed storage has been employed in a number of crops including rambutan, (Chin, 1975), rubber (Ang. 1976), Cocao (King and Roberts, 1982; Hor *et al.*, 1984). The major problems usually met in moist storage are the germination of stored seeds and profuse fungal growth. Several attempts have been made to store seeds within the fruit or fruit juice but all become futile as in the case of rambutan (Chin, 1975). But positive results were obtained when seeds treated with adequate amount of fungicide, semi-recalcitrant coffee seeds were stored in polyethylene bags at 15°C (Vossen and Vander, 1979) and citrus seeds in polyethylene bags at 0°C to 4°C (King and Roberts, 1979) and partially dried seeds were stored with the application of fungicide. Hor et al. (1984) stored treated Cocoa seeds with 0.2% 'Benlate'/ 'Thiram' mixture in polyethylene bag for 24 weeks and found 50% of the seeds were germinable.

The relationship between seed development and storage life was assessed by evaluating seedlings from seeds collected at different stages of maturity and stored at 15°C or 28°C for 65 days. (Sutarno and Utami 1984). They found that the optimum seed harvesting time for these storage conditions was from 78 to 91 days after fruit set. Chaniago *et al.* (1981) evaluated the seedling establishment of clove seeds mixed with charcoal dust, saw dust and coconut husk then stored for 7, 14, 21, 28 and 35 days. Results showed that saw dust was the best storage media while the charcoal dust was least effective for maintaining the viability of stored seeds. Hassanah *et al.* (1984) evaluated the effect of urea (10 g / 100 seeds) and ABA (0.0001 M) on the viability of stored clove seeds. Urea significantly reduced the percentage and rate of germination as well as length of root compared to control. ABA increased percentage and rate of germination, reduced length of root and increased the quality of seedling growth. However these effects were influenced by temperature and package. They also found that the viability in air conditioned room ($16^{\circ}C$) was significantly higher than that in ordinary room (25-30 degrees).

Seeds of *Trichilia dregeana* lost viability within three weeks of storage at $25^{\circ}C$ and 40% RH. But when these seeds were stord in polyethylene bags at 2 to $15^{\circ}C$ and 100% RH maintained 90% germination for five weeks (Choinski, 1990). These water sensitive seeds lost their viability when dried to 30% moisture content, which indicated the recalcitrant nature.

The major problems associated with moist seed maintenance is germination during storage. King and Roberts, (1982) found that Cocoa seed with initial moisture content 45.2% with 92% viability suffered a decline in viability when compared to fresh seeds. It was found that storage of seeds at 81% RH or at moisture contents below 36.7% is incompatible with survival. It is possible that at 40.6% moisture content some cellular disruption ~~had~~ may occured ~~that~~ which would have been avoided at a slightly higher moisture content and relative humidity. Although germination during storage was meagre, if seeds were not dried for 3 hours, considerable germination took place during storage. Broad evaluation of the effect of seed moisture and storage temperature by Hor., *et al.* (1984), showed that cocao seeds with 32% seed moisture stored best equally at 30°C or 22°C storage temperatures. But drying to 27% moisture content was found to be detrimental.

Recalcitrant seeds of the *Dipterocarpus oblongifolius* (Yap, 1981); *Shorea parvifolia* (Maury-Lechon *et al.*, 1981) remained viable for longer period in nitrogen than in air when stored in closed bags. Villiers (1975) theoretically explained how the storage life of fully - hydrated orthodox seeds of *Lactuca sativa* and *Fraxinus excelsior* were enhanced than the stored dried seeds. He opined that in hydrated seeds, all the metabolic activities are in full swing so that quick repair occurs at sub cellular levels.

Kaul (1979) studied the period of survival of seeds of a large number of species after drying them to 15±3% moisture content and then storing them at laboratory temperature. Some of them are *Aegle marmelos, Agathis palmerstonii, Ailanthus fordi. Aleurites fordii, Artocarpus integrifolia, Azadirachta indica. Dipterocarpus alatus, Eugenia jambolana, Eugenia cymosa. Eugenia grandis. Hopea oderata, Mangifera indica. Shorea robusta* etc.

In *Araucaria hunsteinii* there is evidence to suggest that seed longevity may be improved at moisture content above the critical value of 32% (Arentz, 1980; Tompsett, 1982). Tompsett (1983) studied about the influence of gaseous environment on the storage life of *Araucaria* *hunsteinii* seeds and when seeds were stored in 10% oxygen concentration, 31% germination was obtained after 4 months, whereas in nitrogen all seeds became dead within a month. Hor. (1984) found a very sharp reduction of cocao seed viability when storage temperature was reduced from 17 to 15°C. He found a three-fold increase in leachate conductivity and lower [^{14}C] leucine incorporation which manifested a number of physiological, biochemical and ultrastructural changes occurred at 2°C.

Clove seeds retained viability only for a limited period and therefore impose a problem to collect seed material for sowing within short period (Purseglove, 1981). Chaniago *et al.* (1982) reported that seedling survival and growth decreased significantly in 21 days of storage after harvesting. Sabale *et al.* (1992) stored de-pulped seeds in polyethylene bags containing sawdust as a medium after treating them with 0.05% Bavistin solution for 5 minutes. Cent percent germination was noticed in freshly harvested seeds but after 7, 14, 21, 28, 35 and 42 days of storage the germination percentage was reduced to 91.6, 86, 63.8, 44, 38.8 and 36 percentages respectively. These authors also noticed a reduction in the vigour of seedlings arised from seeds stored beyond 42 days.

Corbineau and Come (1986) studied the effect of desiccation upon loss of viability of *symphonia globulifera* seeds. Seeds can be stored only in wet media at a temperature not lower than 15°C and at this temperature they keep well for two months after which germination occurs gradually and all seeds germinate after 10 months storage.

Recalcitrant seeds ultimately lose viability if maintained in a hydrated state, even if microbial contamination is controlled. As rule of thumb, Harrington (1972) the warmer and wetter the natural habitat, the shorter the longevity. Recalcitrant seeds cannot withstand the loss of non-freezable water (Pammenter *et al.*, 1991) as this is a membrane - associated phenomenon. Viability of *Hancornia speciosa* during storage was short and best condition for retaining viability was by keeping seeds in polyethylene bags and that too with moisture content above 30%. According to Oliveira and Valio (1992), low temperature did not improve longevity and dehydration below 25% moisture content resulted in a rapid loss of viability.

Madhusudanan and Babu, (1994) attempted to understand the relationship between moisture content and viability of nutmeg seeds when stored under different conditions. According to these authors nutmeg seeds lose viability when 20 percent of their moisture content is lost within a short period of seven days under tropical conditions. Germination studies showed that the intact seeds took 30-40 days for germination while the decoated seeds germinated within 7-10 days. The best seed collection period for sowing was standardized as the day of natural shedding.

Hevea brasiliensis seeds are classified as recalcitrant (Roberts, 1973) and hence their production and genetic conservation remains problematic (Chin, 1978). Detailed studies by Chin *et al.* (1981), on rubber seeds about the effects of different methods of drying at various temperatures, revealed the critical lethal moisture level and the associated changes in cell ultrastructure with loss of viability. Seeds stored with their original moisture content of 36% at mean temperatures of 22 and 28°C remained viable while those stored at -5 or 45°C lost viability. Irrespective of the method of drying such as air condition drying, sun drying and oven drying at 45°C, the dehydration of seeds resulted in the loss of viability at the critical moisture level which was found to be 15 - 20%. Ultrastructural changes involved in the seeds killed by drying were found to be the damages to cell membrane and the absence of a distinctive nucleus with necleolus. According to Chin *et al.* (1981) seeds of many tropical plants contain high concentrations of phenolic compounds and of phenolic oxidases. The compounds are normally compartmentalized within cells; on desiccation the cell membranes are damaged and the phenolic compounds are released. They are then oxidized and protein/phenol complexes are formed with a consequent loss of enzyme activity.

According to Corbineau and Come (1988) seeds of *Shorea* and *Hopea* dried to moisture content of 17%, *Mangifera* seeds dried to 30% and *Symphonia* seeds dried to 37% lost their viability when stored at 20°C/55%RH. Storage studies revealed that these seeds and their seedlings quickly dried at 5°C. At 15°C, *Symphonia* seedlings could be stored for longer period than those of *Mangifera* because growth of root and stem was much slower. Ultimately temperature induced seedling death involved necrosis of root and then stem of seedlings. At higher temperatures like 20, 25 and 30°C growth is usually too fast and so cannot be stored for longer periods.

Pammenter *et al.* (1984) reported that in *Avicennia marina*, after 10 days of storage seeds lost their viability because of the continuation of germination process after shedding, though this is not a case of structural vivipary. These changes which occur during desiccation of these recalcitrant seeds are not analogous to those associated with maturation drying of orthodox seeds of the parent plant (Berjak *et al.*, 1984). When *Avicennia marina* seeds were stored for 10 days in a dry air stream viability was retained and certain ultrastructural changes similar to germination occurred in embryonic root primordium. (Farrant *et al.*, 1986)

Farrant *et al.* (1988) observed that, the recalcitrant seeds of *Avicennia marina* starts germination even at the time of shedding and can withstand a loss of 18% of their initial moisture content. But as germination proceeds these seeds become more and more sensitive to desiccation. These results showed that it is better to desiccate seeds when fresh, before being stored for extending their storability. This new approach of harvesting seeds prior to seed fall and drying them partially may help to lengthen their storage life, thus ensuring the survival of genetic material collected in the field and also for planting the next season's crop.

Experiments conducted about the effect of gaseous environment on seed viability in onion by Ibrahim, (1982) revealed that oxygen is deleterious to survival below 15% moisture content, but above it is essential to sustain longevity. Villiers (1975); Villiers and Edgcumbe (1975) postulated that the extended survival of fully hydrated lettuce seeds above 15% moisture content is due to the operation of subcellular repair aided by oxygen. Experiments on *Beta vulgaris* (Basu and Dhar, 1979), *Oryza sativa* (Basu and Pal, 1978, 1979, 1980), *Gossypium hirsutum* L. (Dharmalingam and Basu, 1978) showed that the temporary wetting can prolong the viability by some partial repair and turnover of damaged subcellular systems. Basu and Dasgupta, (1978); Basu and Rudrapal, (1979)claims better results from hydration in the presence of antioxidative chemicals such as sodium phosphate, sodium chloride, sodium thiosulphate, oxalic acid and potassium iodide for extending seed longevity.

Pritchard *et al.* (1995) found the relationship between longevity and moisture content of *Araucaria hunsteinii* seeds stored at 6°C. Seeds with 46±5% moisture content retained 84% germinability even after 175 days of storage at 6°C. When moisture content was reduced from 46% to 31% before storing, a progressive reduction of germinability to 40% occured with 50 days of storage. Regarding the effect of storage temperature on seed longevity of *Araucaria hunsteinii* seeds, these authors found that the seeds with moisture content 46% when stored at 21°C, became dead after 120 days. They recommended a ventilated storage of *Araucaria* seeds with 40% moisture content at 2°C for a maximum short-term storage.

A perusal of literature showed that germination studies on recalcitrant seeds are scanty. However *S.aromaticum* and *S.cumini* have been subjected to some investigations on their germination. In *Syzygium aromaticum* seeds Nayar *et al.* (1979) found that transferring the sprouted seeds to nursery bags was superior than direct sowing. The effect of gibberellin at concentrations of 15, 30, 45 and 60 ppm and seed maturity on the germination of clove seed was evaluated by Dhalimi (1983). Results showed that gibberellin treatment at concentrations of 30 to 60 ppm increased the speed of germination, but not the percentage of germination. The immature seeds gave the highest percentage of germination while the fully mature showed the lowest. Seed maturity did not affect the speed of germination. Sutarno and Utami (1984) studied morphogenesis of fruit and seed storage ability in clove. When 7 to 14 days stored seeds were sowed, Sabale *et al.* (1992) found that there occurred a considerable loss of germination percentage and seedling vigour, compared to that of seedlings arised from fresh seeds. Clove seed germination in Shevroys was observed by Kumar *et al.* (1992) while effect of growth regulators on seed germination and seedling growth of clove was studied by Bagade and Shinde (1994). Possibility of increasing seed germination and seedling growth of clove by application of NAA and GA has been discussed by Bagade and Shinde (1994). The foliar application of GA 200 ppm was found to help in early seedling vigour which helps in transplanting as well as good seedling establishment.

Shanmugavelu (1967) reported loss of *Syzygium cumini* seed viability within a month. Germination studies conducted by Sultan Sing and Singhrot (1984) and found that the deeper the sowing, the earlier and higher the percentage of germination. They opinioned that the poor germination in shallow sowing was due to more fluctuation in seed environment in the seed bed. The more tap root growth in deeper sowing may be due to congenial conditions like etiolation. Results of this germination study advocates the sowing of *Syzygium cumini* seeds in a depth of 4-5 cm for good germination and seedling growth. In another experiment Sultan Sing and Singhrot (1985) found that seeds sowed during July came up with a higher germination percentage of 75.9 within 70-80 days while there was no germination of seeds in October. Since the seedling height and length of tap root were greater in plants arised from seeds sown in July and August, they standardized this as the suitable sowing season. Arjunan and Ponnammal (1994) conducted preliminary studies on the phenology and germination of *Syzygium cumini*.

According to Berjak *et al.* (1989) the time taken for visible germination to be manifested differs among homoiohydrous seed species. It is generally believed that recalcitrant seeds never go into dormancy but, instead continue the development and progress towards germination immediately after shedding. Germination characteristics of *Symphonia globulifera* studied by Maury-Lechon *et al.* (1980) found that the germination is slow covering two to eight weeks at the optimal temperature of 25 or 30°C. When germination starts, one to ten roots usually appear at the radicular pole. The gemmular pole gives to a stem covered with scales. Then one or many adventitious roots soon differentiate at the stem base which grow rapidly to form the final root system.

Chin *et al.* (1984) observed that the seeds of *Shorea acuminata*. *Shorea leprosula* and *Elaeis guineensis* germinated readly even without a medium, when kept in polyethylene bags. They also reported that the seeds of *Artocarpus heterphyllus* were killed on drying even to a still high level of 43% moisture content, a decrease of 10% from the original 53%

moisture content. As per the germination studies conducted by Corbineau and Come (1988) on seeds of *Hopea odorata* and *Managifera indica*, they germinated only at or above 5°C whereas seeds of *Shorea roxburghii* and *Symphonia globulifera* germinated at or above 10 and 15°C respectively. Optimum temperature for the germination of almost all these seeds were found to be between 25 and 30°C. Studies on *shorea* seeds by Nautiyal and Purohit (1985b) revealed that the fresh seeds with 100% germinability containing 54% moisture content become dead as the moisture content was reduced to 2% within 12 days of open storage. 25% moisture content of the whole seed or 30% in the embryo seems to be critical moisture content value.

The minimum temperature for germination in *Quercus robur* seed has been estimated to be about 1°C (Pritchard and Manger, 1990). Recalcitrant seeds are seldom found in the soil seed bank and they germinate mostly below the canopy with the seedlings surviving in a seedling bank (von Teichman and van Wyk, 1991). It is not easy to define and identify precisely the transition from developmental growth to germination in recalcitrant seeds (Tompsett and Pritchard, 1993). Vertucci (1993) reported that a five percent reduction of moisture content (38 to 33%) in *Machilus kusanoi* seeds decreased germination from 97 to 26% though 33% moisture content is high enough to provide free water to stabilize membrane structures and macromolecules.

During germination in a shady forest habitat, nutrients stored in the recalcitrant embryo itself, from cotyledons, are immediately available for the growth of the radicle and plumule than from the surrounding endosperm. Hence von Teichman and van Wyk (1994) proposed that in large recalcitrant seeds, the transfer of the main storage function from endosperm to embryo was probably an ancestral development.

Viability loss of *Machilus kusanoi* seeds occurred when artificially dried from 51.5% to 41.4% of moisture content within two months and when these seeds were stored wet at 5°C without drying, a 50% reduction of germination percentage occurred within four months (Lin and Chen, 1993).

Lin and Chen (1995) suggested a wet storage of *Machilus thunbergii* seeds for 10 months at 4°C on the basis that their radicle protrusion requires an incubation period of 14 days at 22°C and also another 35 days to complete the course of germination. Chien and Lin (1997) interprets that the loss of *Machilus kusanoi* seed germinability may be due to desiccation sensitivity as well as physiological ageing within seeds.

Pritchard *et al.* (1995) reported that at 2°C, sprouting of *Araucaria hunsteinii* seeds was prevented and germination was occurred during storage at 6°C.

Rubinstein *et al.* (1977) suggested that there are two components to the water stress brought about by germinative metabolism during hydrated storage of recalcitrant seeds, i.e., the level of stress and duration of stress. A mild osmotic stress can reduce the electrical potential difference across the plasmalemma and a mild water stress could adversely affect the cytoskeleton, which is involved in the spatial organization of multi-enzyme systems (Hrazdina and Jensen, 1992). According to these authors differential effects of mild water stress on various enzyme system could cause problems in the control of the relative rates of individual reactions.

Evidence is accumulating for the involvement of free-radical-based process in dehydration damage of desiccation-sensitive seeds. This has been demonstrated on dehydration of germinating *Zea mays* seeds (Leprince *et al.*, 1990) and on drying of recalcitrant Quercus rober seeds (Hendry *et al.*, 1992). Pammenter *et al.* (1994) suggested that in storage, recalcitrant seeds are exposed to an initially mild, but increasingly severe, water stress. Deleterious events associated with a water stress of considerable duration are suggested to lead ultimately to the death of the tissue.

In newly shed quiescent seeds, during early germination metabolic changes enhanced mitochondrial organization and activity of respiratory enzymes like succinic dehydrogenase along with increased Golgi activity and accumulation of starch in plastids. (Farrant *et al.*, 1985) These authors also suggested that an enhanced protein synthesis is indicated by an increased levels of cytoplasmic and membrance bound polysomes and an increased rate of leucine. According Farrant *et al.* (1985) for the continuation of germination process, additional water is required and this stage appears to coincide with the onset of cell division and increased vacuolation. As these germination - related events proceed, the seeds become increasingly sensitive to desiccation and finally even though the seeds are stored at a constant moisture content, water becomes a limiting factor and viability is lost.

Farrant *et al.* (1988) suggested that as more and more metabolic pathways are initiated as germination proceeds, an increasing proportion of the water present would have structure imposed up on it by the newly synthesized subcellular components. Lack of adequate amount or even loss of structured water would result in distortion of certain metabolic pathways. Hypocotyl extension and the ability for phasic root production of highly recalcitrant *Bruguiera gymnorrhiza* seeds which are shed into an intertidal wet land environments characterized by total inundation to complete exposure (Farrant *et al.*, 1988). This viviparity nature helps the seed to overcome dehydration during germination and seedling establishments. But on prolonged desiccation, tissue damage is initiated at the distal tip of the hypocotyl and in such cases root primordia are initiated from hypocotyl cambium at loci far removed from the distal area of necrosis (Farrant *et al.*, 1988).

Lyons-Raison hypothesis by Lyons (1973) states that the primary cause of chilling injury is due to the physical responses of membrane lipids to low temperature. It is postulated that membrane lipids of chilling sensitive species undergo a phase change (decrease in fluidity) at the chilling temperatures. Revised version of the hypothesis suggests that phase changes may occur in only 2-3% of the lipids, in particular the phosphatidyl glycerol fraction (Murata, 1983; Raison and Wright, 1983). This lipid is located mainly in the chloroplast and is perhaps the only plant lipid which has a low enough degree of unsaturation to undergo a phase change at a temperature between 0 and 30°C.

Many biochemical changes have been detected in seeds as they deteriorate in storage (Roberts, 1972), but a few data are available for recalcitrant seeds. In *Quercus borealis* seeds, Szezotka (1974) found that amylotic activity was very high at the start of storage, but quickly declined to non detectable levels in eight month. The white oak group seeds exhibit epicotyl dormancy i.e., they put down a root system in winter and shoot emerges in the following spring. Szezotka (1975, 1978) reported that both decreased proteins synthesis and oxygen uptake paralleled the decline in germination of *Quercus robur* and *Quercus borealis* seeds in storage for eight months at –1°C.

Simon *et al.* (1976) have indicated that protein denaturation may be a causative factor for chilling injury in seeds. The response to chilling of soluble, non-membranous enzymes are also important. Pyruvate orthophosphate dikinase (Shirakashi *et al.*, 1978) and phosphenol pyruvate carboxylase (Graham *et al.*, 1979) from tropical plants are particularly chilling sensitive. The irreversible loss of activity and the decreased enzyme affinity for substrates are possible through weakening of the hydrophobic bonds and enzyme dissociation into subunits at the chilling temperature. Maeshima *et al.* (1980) have reported that membrane proteins such as cytochrome C oxidase are intrinsically affected by chilling temperatures. Microtubules which are composed of proteins, the tubulins, are also chilling-sensitive (Sakiyama and Shibaoka, 1990).

In series of papers on *Shorea robusta* (Nautiyal and Purohit, 1985a, 1985b, 1985c) investigated physiological and biochemical aspects of seed development, physiological and biochemical aspects of ageing and membrane disruption in ageing seeds. Nautial *et al.* (1985) reported protein changes accompanying loss of viability in *Shorea robusta.* According to these authors, non-synchronised growth of seed parts during development is found to be the cause of rapid loss of moisture content and seed viability in *Shorea robusta.* Further the sequential loss of moisture content from seed coat, cotyledon and embryo results in loss of permeable character of membranes and increase in solute leachates from ageing seeds. In these seeds of *Shorea robusta* the declain in germination percentage and viability is positively correlated with increased concentration of seed leachates like sugars, inorganic ions etc. and these results are found to be due to membrane disruption probably as a result of desiccation below the critical moisture level in storage condition. Nautiyal *et al.*, (1985d) found variations in protein profile of seed parts of *Shorea robusta* and suggested that deterioration of proteins occurs in nonviable seeds and in general the proteins with higher mobility (electrophoresis) get denatured during the process of ageing.

Clatterbuck and Bonner (1985) studied the relative changes of reserve food during eight months of storage at 2°C among the seeds of four North American species of *Quercus*; *Q.nigra* L., *Q.falcata* var. *Pagodaefolia* Ell., *Q.shumardii* of the red oak group and *Q.alba* L. Moisture content increased during storage for all the four *Quercus* species. Crude fat contents decreased significantly during storage while an increase of soluble carbohydrates noted. Insoluble carbohydrates, probably starches increased through three or four months of storage and declined afterwards when radicle emergence occurred in the storage containers. This indicates that starch is broken down to supply the simple carbohydrates for the radicle growth. Priestly (1986) stated that during seed storage, the deterioration which preceede death is due to the changes in macromolecules like protein, nucleic acid, lipid etc. and this occurse at very low water potential. So the seed deterioration is not due to metabolic changes but directly related to water potential (Roberts and Ellis, 1989). According to Roberts and Ellis (1989) recalcitrant seeds are like normal plant tissues. It would seem that at some particular water potential, death occurs immediately. Above this critical value longevity increases with increase in moisture content to the highest water potential attainable commensurate with preventing germination.

Among the biological mechanisms attributable to loss of viability during desiccation, the role of membrane damage has been shown to be significant, based on the measurement of conductivity, lipid peroxidation and composition of the leached electrolytes and sugars from the seed (Roberts, 1979; Mc Kersie and Stinson, 1980; Ruhl *et al.*, 1988). A protective role has been assigned to sugars in several anhydrous systems (Crowe *et al.*, 1984; Leopold and Vertucci, 1986). According to Koster and Leopold (1988) changes in soluble sugar contents in the axes of Pea, Corn and Soybean have been correlated with loss of desiccation tolerance during germination. When desiccated, the soluble carbohydrate increased six fold in cocoa and 2 tc 4 fold in tea (Chandel *et al.*, 1995) due to the degradation of starch into simple sugars. According to these authors a significant decline in viability associated with desiccation and freezing and cocoa and freezing alone in jack fruit was found to be accompanied by an increase in electrical conductivity and lipid peroxidation. Lipid peroxidation is mediated by free radicals.

In the case of *Avicennia marina* seeds, Farrant *et al.* (1992) found that, the lower the amount of starch presents, the more the chance for the soluble sugars to function principally as an immediate available carbohydrate reserve in seed.

An earlier review and comprehensive list of recalcitrant seed references prior to 1979 is provided by King and Roberts (1979), Chin and Roberts (1980) and Thomson (1983). Later an excellent review of bibliography on recalcitrant seeds by Chin (1988) highlights the characteristics and identification of recalcitrant seeds, their moisture contents, desiccation and chilling sensitivity, storage and germination behaviour.

According to Chin *et al.* (1989) at physiological maturing, recalcitrant seeds are much higher in moisture content (50-70%) than orthodox seeds. It is generally believed that recalcitrant seeds never go into dormancy, but instead, continue their development or progress towards germination (Berjak *et al.*, 1989).

Ellis (1991) described some characteristics of seeds which are intermediate to orthodox and recalcitrant. However their storage and germination behaviour are not fully investigated.

Seed technological aspects like storage under specific media of recalcitrant seeds are briefly described by Copeland and Mc Donald

(1995). According to these authors, the storage of recalcitrant seeds is possible using endogenous seed inhibitors such as abscisic acid or replacing the high water content with other substances such as sugars or ethylene glycol to permit successful storage.

In orthodox seeds, by definition germination incorporates these events that commence with the uptake of water by the quiescent dry seeds and terminate with the elongation of embryonic axis (Bewley and Black, 1994). According to Bewley (1997) germination commences with imbibition and is completed when radicle extends to penetrate the structures that surround it. However, the definition of germination is not applicable to recalcitrant seeds since, the radicle elongation is found to be a continuous process of seed development and during storage under favourable conditions like low temperature or high humidity the radicle elongation proceeds.

Loss of Viability by desiccation is caused by membrane rupture and a rapid increase in leachate are considered as the main characters of recalcitrant seeds (Nautiyal and Purohit , 1985c; Farrant *et al.* , 1988 and Pammenter *et al.* , 1994). Kinetin is suggested to induce tolerance to desiccation-sensitive seeds by retaining the membrane integrity. Several reports (Wilson and Mc Donald, 1986; Hendry *et al.*, 1992 and Pammenter *et al.* , 1994) revealed that a close correlation exists between membrane permeability and lipid peroxidation. In *Shorea robusta*, kinetin induced lowering of lipid peroxidation and hence viability was prolonged.

Free radical mediated damage to macromolecules, as well as membrane lipids in seeds is proposed by Pan and Yau (1991) and this free-radical will interact with membrane phospholipids and lead to their de-esterification resulting in accumulation of fatty acids and incresed membrane deterioration.

Kinetin (10 ppm) treated seeds of *Shorea robusta* showed only decreased leakage from the seeds and leachate conductivity also was very low in kinetin treated seeds (Chaithanya and Naithani , 1998). Lipid peroxidation shown by thiobarbituric acid reactive substances showed that accumulation of TBRS was rapid in control than in kinetin treated seeds. Superoxide liberation also was delayed by kinetin treatment. Superoxidedismutase activity was enhanced by kinetin and higher rate of SOD activity showed a peak on 10^{th} day in treated seeds, while in control on third day.

RESULTS

RESULTS

Syzygium aromaticum

Seed germination

Seeds of *Syzygium aromaticum* consist of green cotyledons and bright red radicle wrapped in white filmy testa which is automatically removed when the fruit is de-pulped. So the 'seed' is only the embryo consisting of exposed radicle and cotyledons with hidden plumule between them.

Though viviparity is absent, the pattern of germination in *Syzygium aromaticum* favours a rapid seedling establishment as its seeds are desiccation sensitive and are of recalcitrant type which has an ecological preference to native island habitat. Naturally frugivors de-pulp and disperse seeds to wet and well drained soil with high humidity.

De-pulped seeds, when imbibed enough water, at the very first day itself cotyledons move apart exposing bright red radicle. Radicle elongation and root hair initiation can be seen within two days of sowing and further elongation of roots occurs at a rapid rate so that within a week they grow up to 10 cm in length. By this time several lateral secondary rootlets are developed and thus the seedling can anchor well in the soil to avoid being washed away.

In *Syzygium aromaticum*, even after 10 days of seed germination with fully developed roots and several lateral secondary rootlets, plumule growth occurs only under the influence of light. This character is evident with the observations made at lab as well as field germination tests. When seeds are tested for germination in petry dishes kept in a seed germinator set at 30°C / 80% RH without light, only radicle growth occurred. While these 'seeds' / 'seedlings' were exposed to light, plumule growth started within a week time. Even after a prolonged storage of nine months, these seedlings with fully developed root system in the germinator were devoid of plumule growth.

At field, when seeds were sowed in sandy medium taken in earthen pots, at a depth twice as their own size, even after one month only radicle growth occurred. On the other hand when seeds are sowed with their one-third exposed to soil surface, hypocotyl elongated within one month rendering cotyledons to spread apart above soil and plumule initiation started thereafter. It was observed that seeds sowed to the ground deeper than its own size suffered from germination inhibition presumably by lack of light and gaseous exchange. Experiments conducted by the present author with various depth of seed sowing showed that it is better to expose one-third of seed size above the sandy medium. Similarly initial seed moisture content should also be maintained as the seeds are of recalcitrant type. Pulp is found to retard the germination percentage as well as speed of germination probably by encouraging insect and microbial infections.

Germination is epigeal type since the cotyledons come up above soil surface and starts assimilatory function. In due course of time cotyledons are withered away as the apical bud grows and differentiates into primary leaves under the influence of light.

Desiccation responses

Moisture content of fresh seeds with green cotyledons and bright red radicle was 48.9% (Table 1, Figure 1). These seeds registered 100% germination in five days. When seeds are allowed to dry for 12 hours at open room conditions, 3% reduction of moisture content occurred and consequently end portions of cotyledons become slightly brown while radicle retained its original appearance. When germination tested, 94% germination resulted in 10 days. As the seeds are dried for 24 hours, their moisture content become 42%, black spots appeared over green cotyledons and radicle lost its brightness and become red. Corresponding germination percentage of 86 occurred within 24 days. On further drying for 36 hours, the moisture content was drastically reduced (10% reduction from the initial moisture content) and only 72% of seeds germinated, that too took 24 days for the completion of germination. By this time cotyledons become pale green with more brown areas and black spots extending while the radicle appeared as yellow red and wilted. When seeds were dried for 48 hours at open room conditions, their cotyledons become brown, radicle become brown red and when tested, the moisture content was found to be as low as 33% with a corresponding germination of only 20% reported within 15 days.

Effect of de-pulping

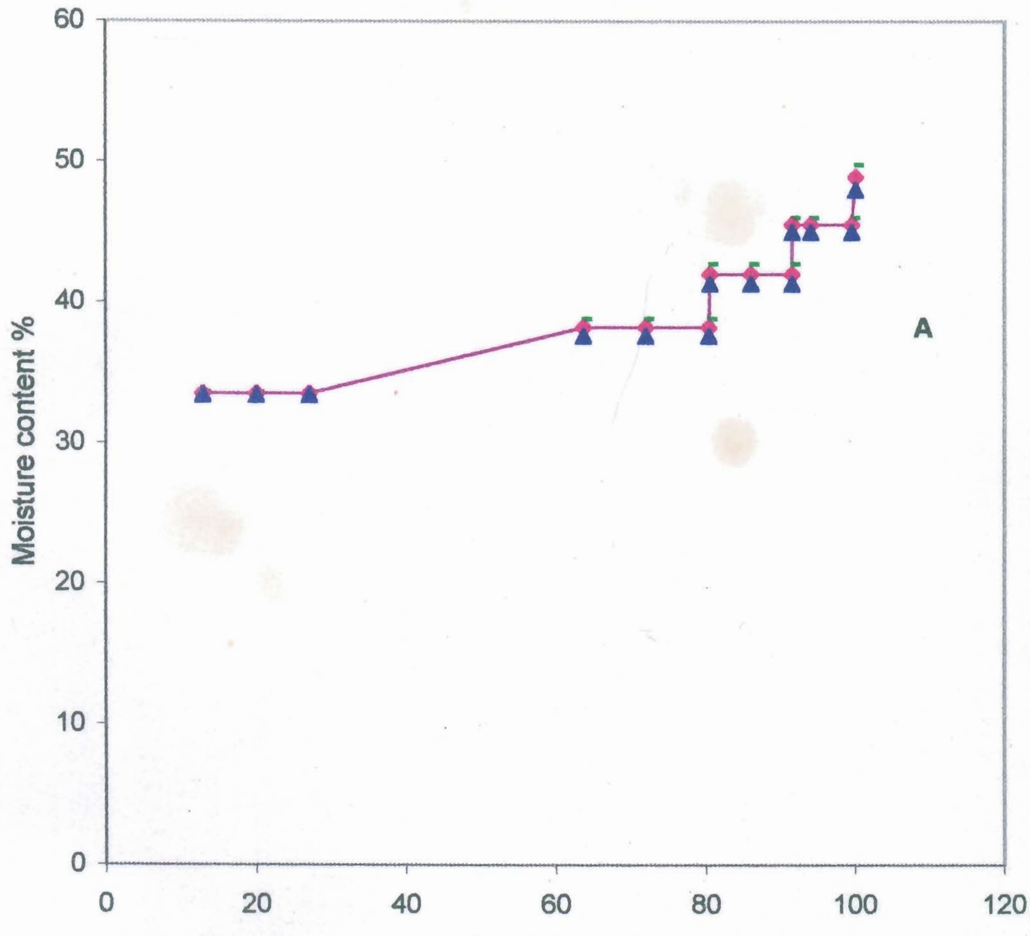
Pulpy seed denotes a fruit while seed means a de-pulped fruit, word de-pulped is used only when a comparison between pulpy and de-pulped seeds are attempted. De-pulped seeds registered 100% germination while the percentage germination of pulpy seeds was only 40%. Irrespective of

Table 1 Desiccation responses of *Syzygium aromaticum* seeds

Period of drying (hrs)	Moisture content (%±SD)	Germination (%±SD)	Time for completion of germination (days±SD)	Morphological features
0	48.9±0.7	100	5±2.6	Cotyledon - Green Radicle - Bright red
12	45.5±0.5	94±5.5	10.4±2.9	Cotyledon - Green with slightly brown ends Radicle - Bright red
24	42.0±0.7	86±5.5	24.0±6.5	Cotyledons - Pale green with brown areas and black spots Radicle - red
36	38.2±0.6	72±8.4	23.6±2.7	Cotyledons - Pale green with more brown areas and black spots. Radicle - Yellow red
48	33.5±0.1	20±7.1	15.6±2.9	Cotyledon - Brown Radicle - Brown red

58B

Fig. 1: Relationship between germination and moisture content of Syzygium seeds



A: *Syzygium aromaticum*

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the difference in storage conditions, pulpy seeds become yellow green and dead in 15 days while the de-pulped seeds retained their viability for longer periods depending on the storage conditions as shown in Table 3, figure 3a and 3b.

Effect of storage and temperature on germination

The moisture content of fresh seed was 48.9% with a corresponding 100% germination (Table 3). After one day of storage, at open room conditions, the moisture content was reduced to 42% with a corresponding germination of 86% in de-pulped seeds. Pulpy seeds retained their original moisture content as well as cent percent germinability. After four days of storage, in the de-pulped seeds moisture content was reduced to half and a complete loss of viability was observed. By this period, moisture content of pulpy seeds also was only one half and germination percentage showed only slight decrease.

De-pulped seeds stored in polyethylene bags kept at room conditions retained their original moisture content and germination percentage up to four days of storage. But pulpy seeds showed a reduction in germination with only 76% after four days of storage, though the moisture content was as same as that of fresh seeds. After 15 days of storage, the moisture content of pulpy and de-pulped seeds did not show any significant change while the germination percentage remained unchanged in de-pulped seeds though pulpy seeds lost their viability. Depupled seeds produced 0.8 cm of radicle under storage but no plumule initiation was noticed. Cotyledons of pulpy seeds became yellow green and radicle in dull red colour. After 30 days of storage, the moisture content of de-pulped seeds was reduced

Table 3
Effect of storage and temperature on germination of *Syzygium aromaticum* seeds

Temperature °C	Duration days	Moisture Content %±SD	Germination %±SD	Morphological Features
0	1	48.9±0.7 (48.9±0.7)	0 (0)	Cotyledons green and radicle bright red (Cotyledons green and radicle bright red)
10	1	48.9±0.6 (48.9±0.7)	100 (100)	Cotyledons green and radicle bright red (Cotyledons green and radicle bright red)
	4	48.8±0.4 (48.7±0.9)	92.0±4.5 (90.0±7.1)	Cotyledons green and radicle bright red (Cotyledons green and radicle bright red)
	15	47.6±0.5 (47.1±0.2)	90.0±7.1 (72.0±4.5)	Cotyledons green and radicle bright red (Cotyledons yellow green and radicle red)
	30	48.2±0.7	78.0±8.4	Cotyledons green and radicle bright red
	60	48.3±0.4	32.0±8.4	Cotyledons green and radicle bright red
20	1	48.9±0.4 (48.9±0.6)	100 (100)	Cotyledons green and radicle bright red (Cotyledons green and radicle bright red)
	4	48.6±0.2 (48.9±0.3)	100 (84.0±5.5)	Cotyledons green and radicle bright red (Cotyledons green and radicle bright red)
	15	47.8±0.3 (50.8±0.3)	100 (0)	0.3±0.4 cm of radicle growth (Cotyledons yellow green)
	30	48.1±0.3	100	0.8±0.5 cm or radicle growth
	60	48.3±0.3	98.0±4.5	2.0±0.6 cm of radicle growth
	90	48.7±0.3	88.0±4.5	3.4±0.4 cm of radicle growth
	180	49.4±0.4	62.0±4.5	3.4±0.7 cm of radicle with lateral roots of 1.8±0.2 cm
30	1	48.9±0.7 (48.9±0.7)	100 (100)	Cotyledons green and radicle bright red (Cotyledons green and radicle bright red)
	4	48.5±0.4 (48.8±0.5)	100 (86.0±5.5)	Cotyledons green and radicle bright red (Cotyledons green and radicle bright red)
	15	48.1±0.2 (47.1±0.1)	100 (0)	2.3±0.3 cm of radicle growth (Cotyledons yellow green and radicle red)
	30	48.4±0.2	100	2.7±0.1 cm of radicle growth
	60	48.9±0.2	98.0±4.5	4.5±1.1 cm of radicle growth
	90	50.3±0.6	96.0±5.5	7.0±2.4 cm of radicle growth
	180	54.0±0.2	72.0±4.5	6.1±3.2 cm of radicle and 1.4±1.0 cm of lateral root growth
	270	55.1±1.6	65.0±5.5	Cotyledons dark green with sporadic black spots
Room*	1	48.9±0.6 (48.7±0.2)	100 (100)	Cotyledons green and radicle bright red (Cotyledons green and radicle bright red)
	4	48.9±0.5 (48.9±0.4)	100 (76.0±5.5)	Cotyledons green and radicle bright red (Cotyledons green and radicle bright red)
	15	47.0±0.2 (49.0±0.4)	98.0±4.5 (0)	0.8±0.2 cm of radicle (Cotyledons yellow green and radicle red)
	30	44.9±0.7	88.0±4.5	1.0±0.1 cm of radicle growth, cotyledons became dark green & wilted
	60	35.30	0	Cotyledons become black and hard
Control	0	48.9±0.7	100	Cotyledons green and radicle bright red
Room**	1	42.0±0.7 (48.9±0.7)	86.0±5.5 (100)	Cotyledons green and radicle bright red (Cotyledons green and radicle bright red)
	4	25.7±0.6 (50.3±0.6)	0 (82.0±4.5)	Cotyledons became black, hard and plumule became black and brittle (Fruit wall shrunken and cotyledons green)

Values and data in parentheses are of pulpy seeds; * - At 25±5°C in polyethylene bags; ** - At 25±5°C and 70% RH in open trays

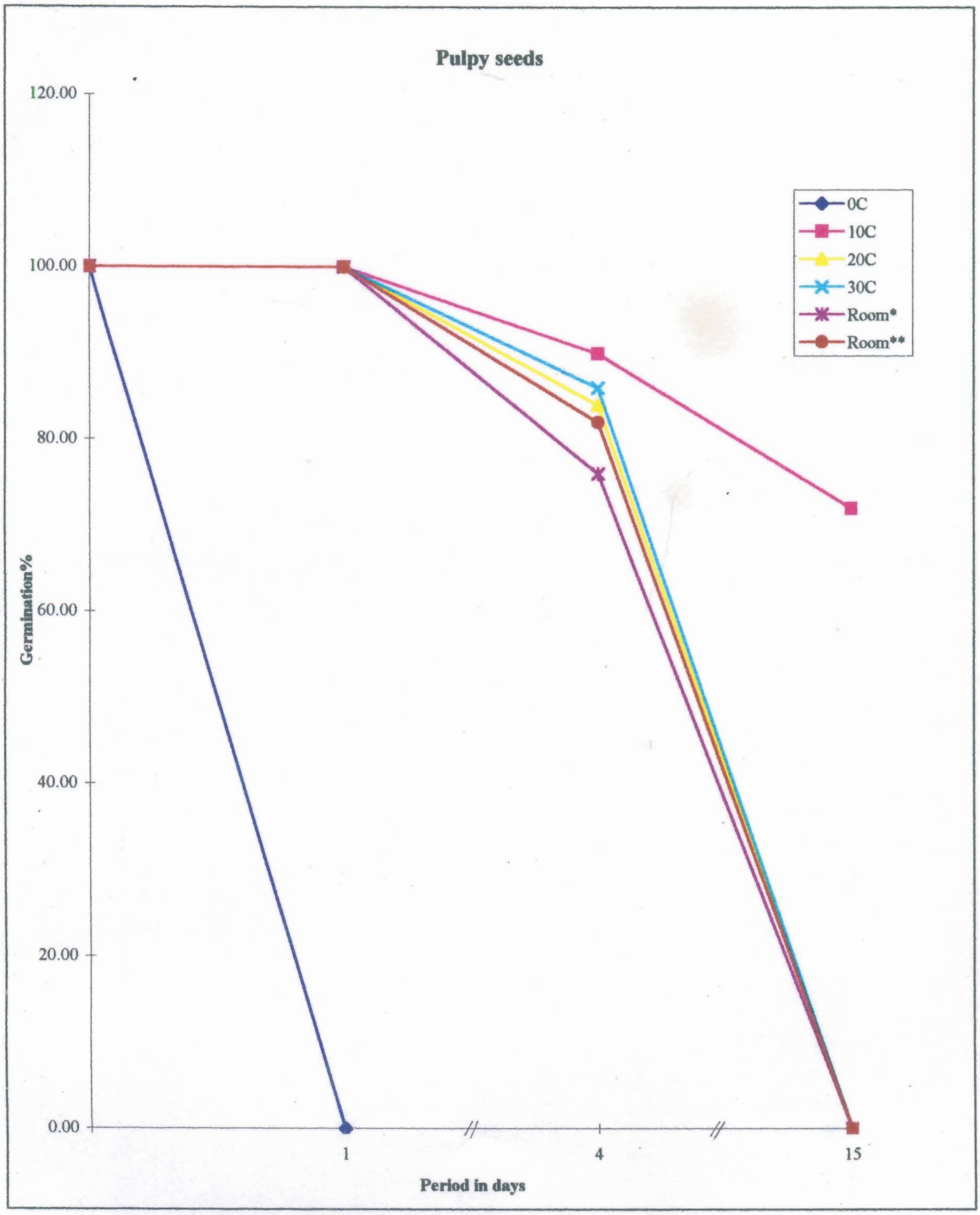

Fig. 3a Germination percentage of *Syzygium aromaticum* seeds during storage under different conditions.

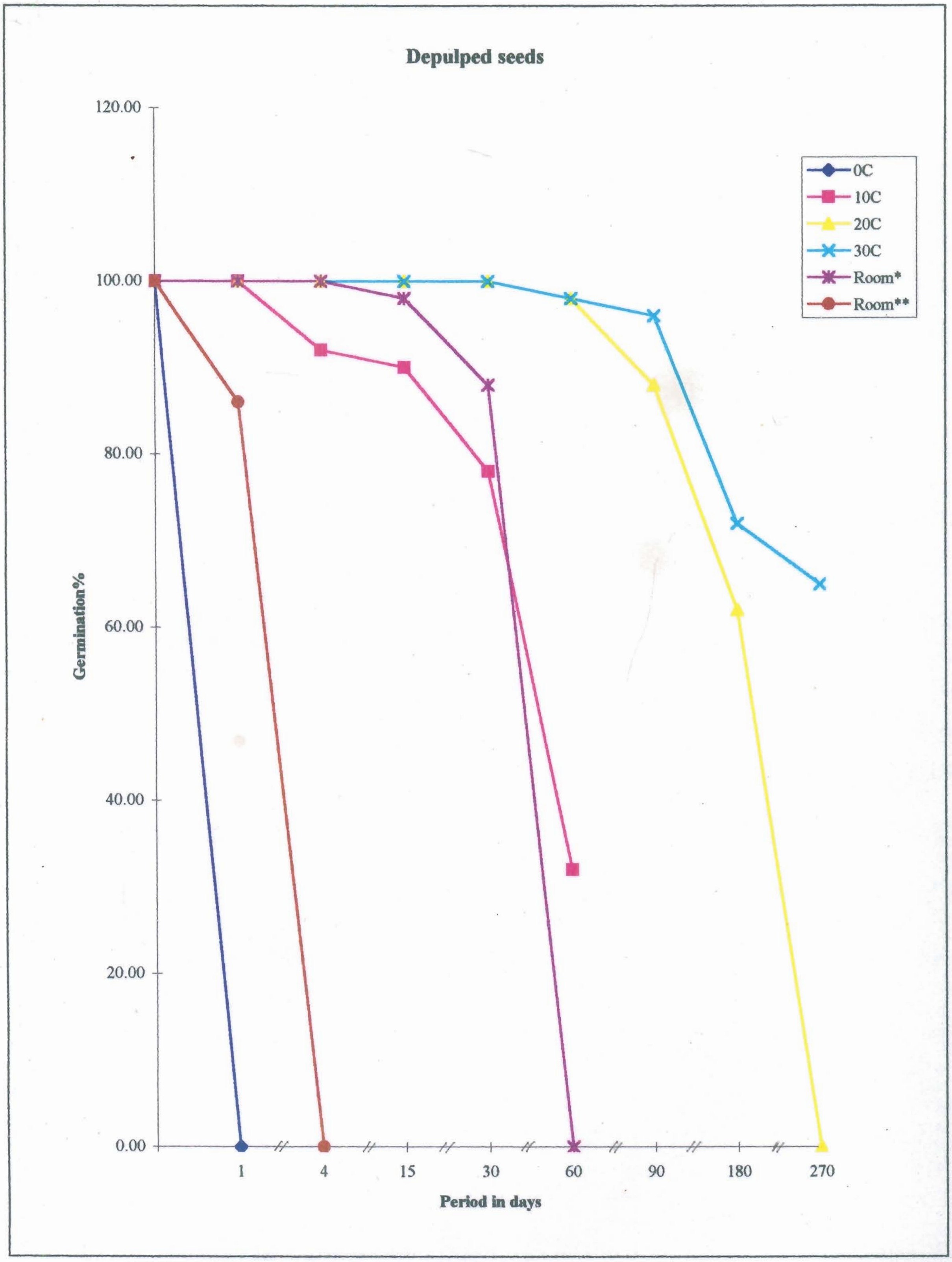

Fig. 3b Germination percentage of *Syzygium aromaticum* seeds during storage under different conditions.

(insignificant) with a corresponding decrease in germination percentage also. The radicle length of sprouted seeds under storage was 1.0 cm. Cotyledons became more dark green and showed a wilted nature.

Pulpy and de-pulped seeds maintained their initial moisture content after 24 hours of storage at 0°C but lost their viability though they looked as if when fresh.

At 10°C, one day storage had no impact on moisture content and germination percentage but after four days of storage, though pulpy and de-pulped seeds retained their appearance and initial moisture content, but their germination percentage was reduced significantly. After 15 days of storage, the moisture content of pulpy and de-pulped seeds did not show significant change while the germination percentage remained unchanged in de-pulped seeds, but in pulpy seeds occurred a significant reduction. Though de-pulped seeds had no change in morphology, cotyledons of pulpy seeds became yellow green and radicle dull red. Thirty days of storage did not change the appearance of de-pulped seeds and their moisture content but germination percentage showed considerable reduction. Though de-pulped seeds maintained their moisture content and morphology as that of the previous stage, their germination percentage was again reduced significantly after 60 days of storage.

At 20°C, both pulpy and de-pulped seeds stored up to four days remained unchanged regarding their moisture content and morphological appearance, thought the germinability of pulpy seeds became reduced to 84% compared to the control. Viability of pulpy seeds was completely lost after 15 days of storage. Cotyledons became yellow green and the moisture content showed insignificant increase. De-pulped seeds retained their original moisture content, morphology as well as 100% germination at this interval. These seeds started to sprout under storage showing 0.3 cm of radicle length during 15 days of storage. Plumule initiation was absent even after 30 days of storage but the radicle growth was doubled at this interval. Observations after 60 days showed that the moisture content and germination percentage did not change. The radicle length under storage at this stage was 3.4 cms showing a significant increase. On 90th day the moisture content remained same as the previous interval but the germination percentage was decreased significantly. During this period moisture content remained more or less same with no plumule growth. Germination percentage of de-pulped seeds reduced significantly though there occurred no change in the moisture content of the seeds within 180 days of storage. Though the radicle length was found to be the same as that of the previous sample, with many lateral roots of 1.8 cm length, no plumule initiation was observed.

At 30°C, the moisture content and germination percentage of both pulpy and differential equation-pulpy seeds were the same as the fresh seeds after one day. On fourth day also the stored pulpy and de-pulped seeds at 30°C retained their morphology, moisture content and germinability as that of fresh seeds. After 15 days of storage, moisture content of pulpy seeds did not change at all though seeds lost their viability. De-pulped seeds also did not exhibit any change in moisture content but showed 100% germination. The seeds started sprouting under storage at this stage with 2.3 cm of radicle growth. Thirty days of storage did not change the moisture content and germinability of de-pulped seeds, but their radicle length increased slightly. Sixty days of storage resulted in no change in moisture content but a slight reduction in germination percentage and the radicle length was considerably increased. The seeds of 90 days of storage with radicle length of 7 cms showed an increase in their moisture content (insignificant) though the germination was reduced to 96%. After 180 days of storage at 30°C, the moisture content again showed slight increase and a significant reduction in germinability was observed in de-pulped seeds. The radicle elongation was arrested, but lateral roots up to the length of 1.4 cm were developed from the radicle. The last sample (270 days) of de-pulped seeds did not show any change in moisture content but their germination percentage was reduced significantly. At this stage their cotyledons became dark green with sporadic black spots and radicle in dull red colour.

Germination percentage of stored seeds

Initial cent percent germination of both pulpy and de-pulped seeds became zero when stored for one day at 0°C (Table 3, Figure 3a and 3b). One day storage in conditions like 10, 20, 30 room and open and room plyethylene bags maintained their original germinability of both pulpy and de-pulped seeds.

Observations of four days stored pulpy seeds showed a decreases in the germination in all seed samples, with a minimyum decrease in seeds stored at 10°C, folowed by 30°C, 20°C, room open and, maximum decrease of germination percentage in seeds stored at room polyethylene bags. Fifteen days storage of pulpy seeds at 10°C reduced the germination percentage significantly while those seeds stored at 20, 30°C room open and room polyethylene bags completely lost their viability.

De-pulped seeds stored at open room condition for 4 days lost their viability, but all other seed samples maintained their original cent percent germination except the seeds stored at 10°C showed a reduction in germination percentage while seed samples stored at 20, 30°C an room polyethylene remained unchanged. Compared to the previous storage, the germination percentage of seeds stored at 10°C was further reduced drastically and that at room polyethylene lost their viability, but at 20 and 30°C, seed viability remained the same after 60 days of storage. After 90 days of storage the germinability of seeds stored at 20°C was reduced but at 30°C, the germination percentage was slightly reduced One eighty days of storage resulted in further reduction of germination of both the seed types stored at 20 and 30°C. After 270 days, seeds stored at 20°C completely lost their viability while seeds stored at 30°C registered considerable rate of viability.

Biochemical aspects of stored seeds

Dry weight percentage

Dry weight distribution of *Syzygium aromaticum* is given in Table 5, Fig. 5a and 5b. Dry weight percentage of fresh seeds accounts approximately half of their fresh weight (51.10). After one day and four days of storage, significant increase ($P<0.01$ and $P<0.01$) of dry weight percentage, 57 and 66 respectively, was observed in the de-pulped seeds stored at open room conditions. In all other conditions, namely 0, 10, 20 and 30°C both pulpy and de-pulped seeds maintained almost their original dry weight at

Table 5 Dry weight percentage of *Syzygium aromaticum* seeds during storage and germination under different conditions

Treatment		Control	Interval							
			24 hs	4 day	15 day	1 month	2 month	3 month	6 month	9 month
0°C	P		51.40 ±0.74							
	D		50.80 ±0.65							
10°C	P		52.22 ±0.49	51.90 ±1.12	51.78 ±0.73					
	D		52.72 ±0.56	52.60 ±0.66	52.45 ±0.62	51.80 ±0.71	51.71 ±1.33			
20°C	P		52.30 ±0.68	49.20 ±0.96	49.20 ±0.97					
	D		52.50 ±0.99	52.30 ±0.65	52.20 ±0.43	51.90 ±0.57	51.67 ±0.74	51.3000 ±0.8670	50.60 ±0.78	
30°C	P		51.20 ±0.55	52.40 ±0.74	52.90 ±0.82					
	D		52.30 ±0.77	52.10 ±0.47	51.90 ±0.58	51.60 ±0.79	51.10 ±0.85	49.7000 ±1.0120	46.00 ±1.74	44.90 ±0.83
Room*	P		51.70 ±0.91	51.10 ±0.64	51.00 ±0.41					
	D		51.92 ±0.77	52.40 ±0.69	53.00 ±0.45	55.10 ±0.66	64.70 ±1.75			
Room**	P		52.56 ±1.41	54.40 ±0.80	56.62 ±1.46					
	D	51.10 ±0.82	57.81 ±0.87	66.48 ±1.44						

P - Pulpy seeds; D - Depulped seeds; * - At 25±5°C in polyethylene bags; ** - At 25±5°C and 70% RH in open trays
The values given are the mean of six replicate ± Standard error

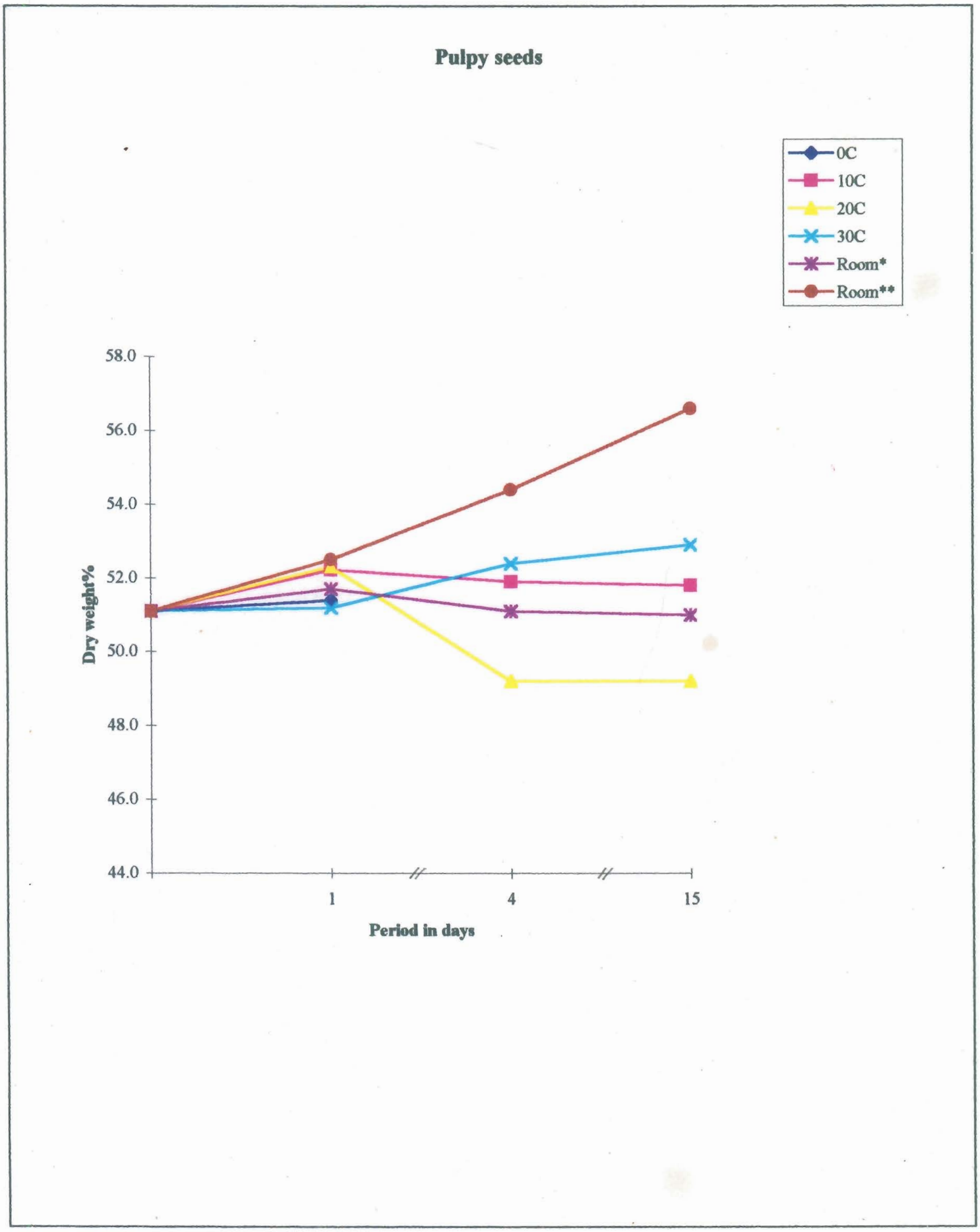

Fig. 5a Dry weight percentage of *Syzygium aromaticum* seeds during storage and germination under different conditions

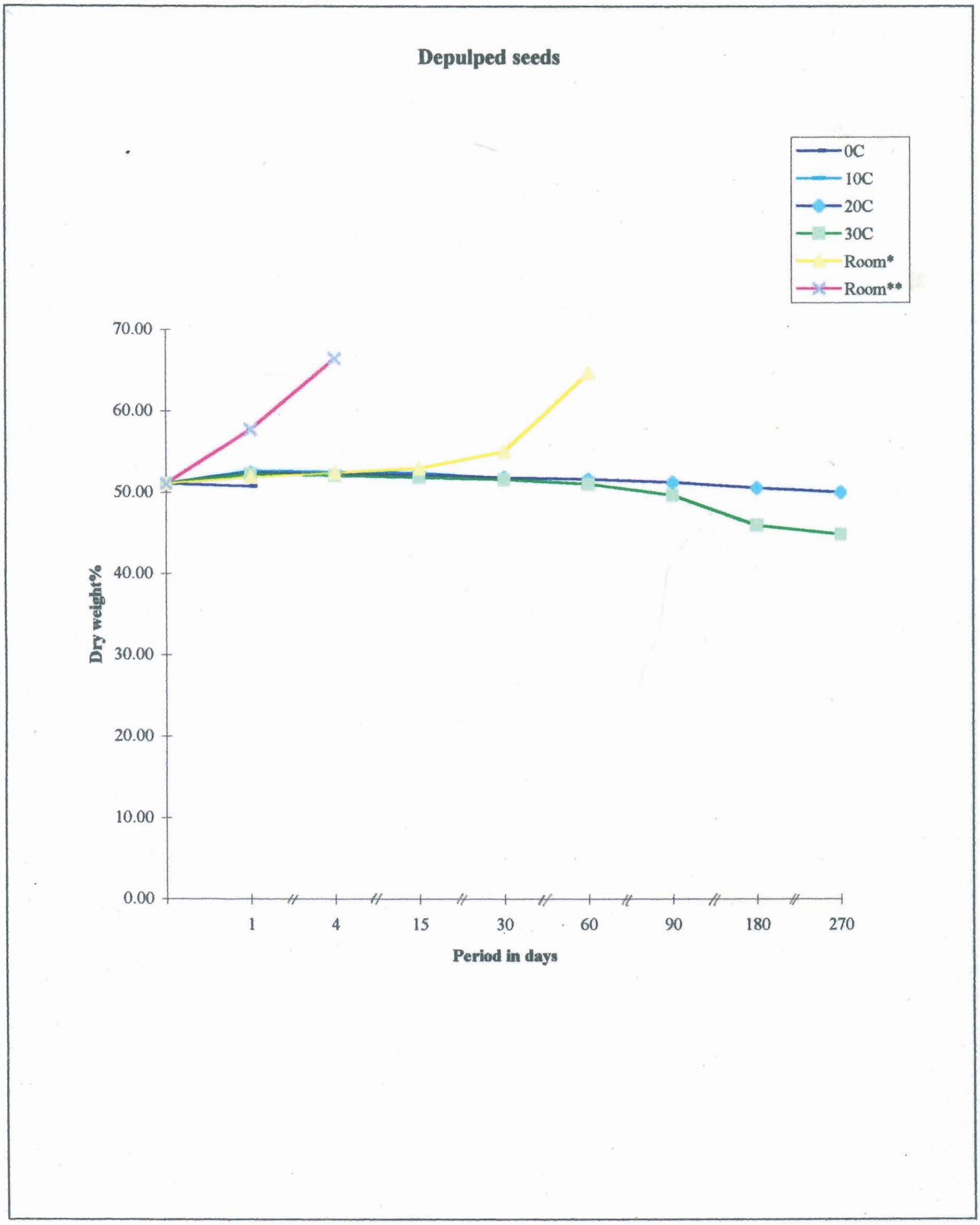

Fig. 5b Dry weight percentage of *Syzygium aromaticum* seeds during storage and germination under different conditions

all intervals up to six months of storage. A gradual but insignificant (P<0.2) increase in dry weight was observed in pulpy seeds during storage at open room condition up to 15 days. But pulpy seeds stored in polyethylene bags at room conditions did not show any change in dry weight. Among the de-pulped seeds, those stored in polyethylene bags kept at room conditions showed a gradual increase and insignificant (P<0.05) hike in their dry weight percentage up to one month of storage. This was followed by a significant increase in dry weight percentage (64.7) on completion of two month storage. The seeds stored at 30°C gradually lost their dry weight percentage to 44.9% over nine months of storage.

Protein

Fresh seeds of *Syzygium aromaticum* contained 52.9 mg/g proteins on dry weight basis (Table 7, Fig. 7) of proteins. After 24 hours, protein remained unchanged in all samples of various treatments. Only de-pulped seeds exhibited a significant decrease in their protein content (30.2 mg/g) within four days of storage at open room conditions. No change in protein content occurred in any sample at this interval. In the case of all stored seed lots, both pulpy as well as de-pulped irrespective of their storage conditions up to 15 days, maintained a constancy in their protein content which has an insignificant reduction compared to control. After one month, significant decrease in the protein content of de-pulped seed samples become evident, (28 mg/g dry weight) in seeds stored at 10°C and room temperature (29.56 mg/g dry weight). De-pulped seeds stored at 20 and 30°C registered a gradual but insignificant reduction in the protein content after one month of storage. During the storage of two

Table 7 Protein content of *Syzygium aromaticum* seeds during storage and germination under different conditions

Protein mg/g Dry weight

Treatment		Control	Interval							
			24 hs	4 day	15 day	1 month	2 month	3 month	6 month	9 month
0°C	P		52.32 ±0.36							
	D		52.89 ±0.21							
10°C	P		52.91 ±0.07	51.34 ±0.59	49.20 ±0.38					
	D		51.91 ±0.32	49.18 ±1.21	46.87 ±0.69	28.44 ±0.76	26.60 ±0.15			
20°C	P		52.69 ±0.73	51.87 ±0.98	44.51 ±1.51					
	D		50.87 ±0.62	49.64 ±1.17	46.85 ±0.31	46.60 ±0.13	47.17 ±0.58	44.39 ±0.45	33.83 ±0.18	
30°C	P		52.13 ±0.67	50.91 ±0.53	49.57 ±0.17					
	D		51.24 ±0.38	49.60 ±0.44	48.85 ±0.89	46.47 ±0.57	40.99 0.16	34.95 ±0.57	33.95 ±0.19	32.24 ±0.16
Room*	P		52.41 ±1.07	51.29 ±1.12	50.94 ±0.18					
	D		52.19 ±0.82	51.62 ±0.73	49.22 ±0.36	29.56 ±0.45	22.74 ±0.44			
Room**	P		52.28 ±0.98	52.13 ±1.04	49.64 ±0.33					
	D	52.90 ±0.14	52.21 ±0.62	30.20 ±0.52						

P - Pulpy seeds; D - Depulped seeds; * - At 25±5°C in polyethylene bags; ** - At 25±5°C and 70% RH in open trays
The values given are the mean of six replicate ± Standard error

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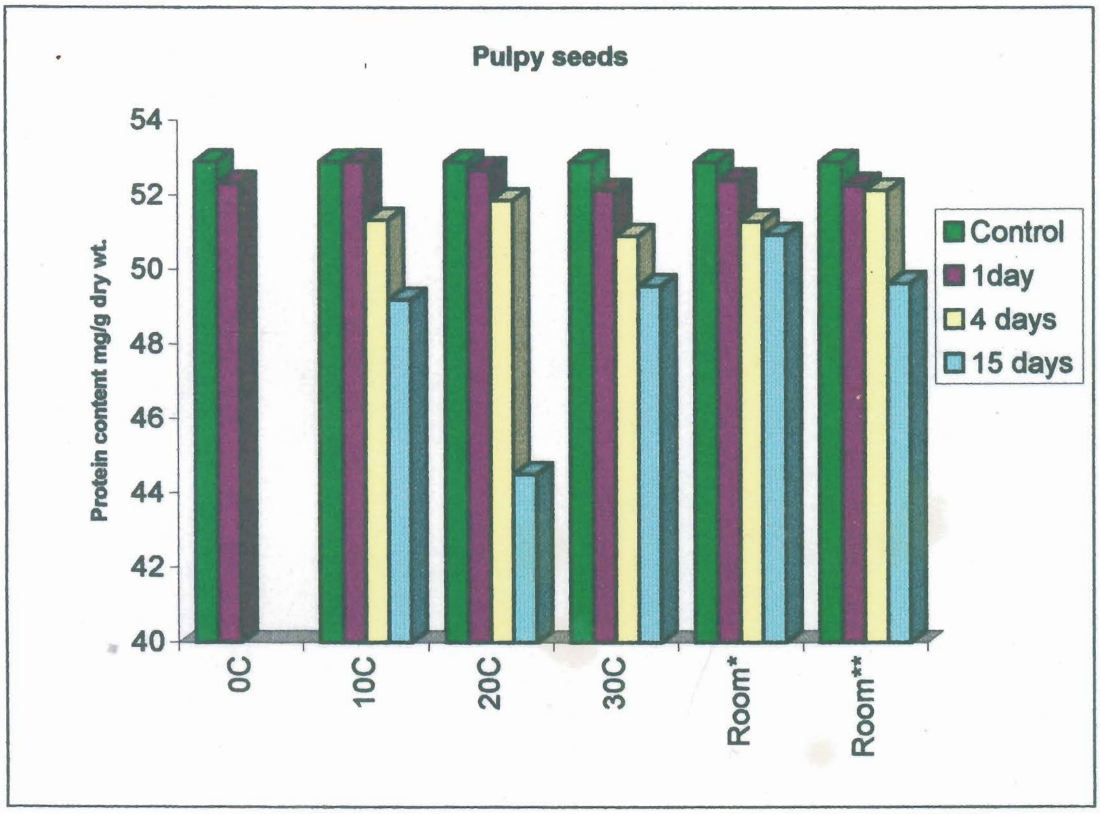


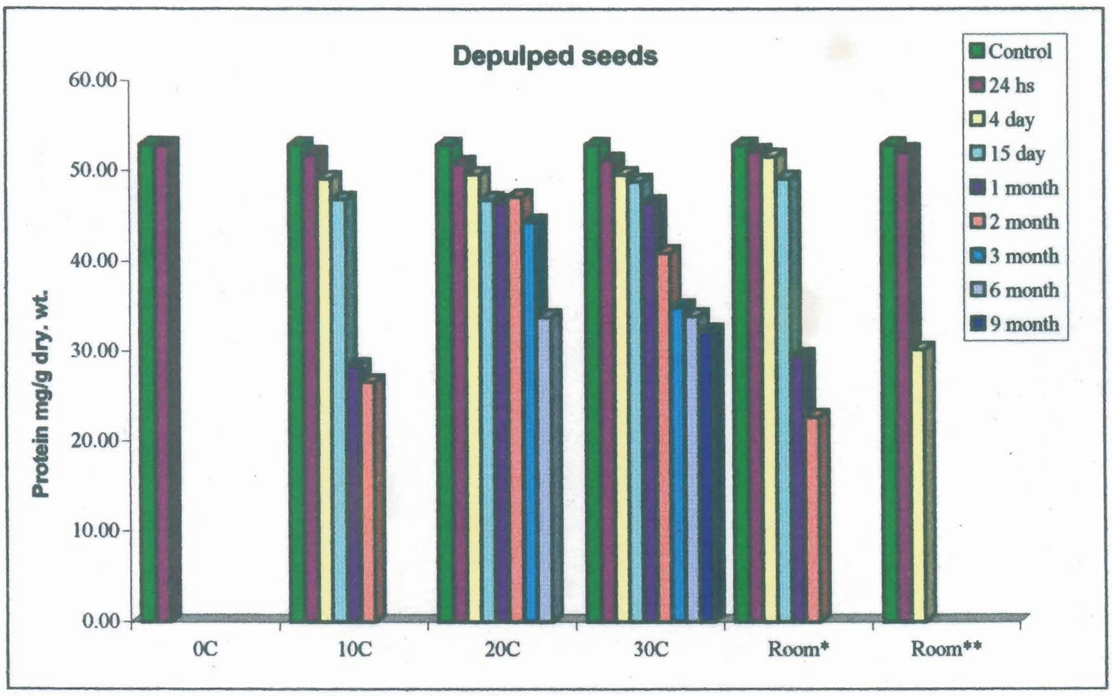


Fig. 7 Protein content of *Syzygium aromaticum* seeds during storage and germination under different conditions

months, de-pulped seeds at 20°C registered no change in their protein content in comparison with the previous stage. But at 30°C a significant (P<0.01) decrease was observed. At this interval, the seeds at room temperature (in polyethylene bags) exhibited a significant reduction than the previous stage. At this stage the protein content is reduced to less than half of the initial protein content (fresh seeds). Seeds stored at 20°C showed only insignificant reduction after three months while that of 30°C exhibited a highly significant reduction (34.9 mg/g). After six months storage, seeds kept at 20 and 30°C showed same amount of protein with significant reduction only at 20°C. The protein content of nine month stored seeds at 30°C remained unchanged compared to the previous stage. All these observations indicate that the final protein values, when compared to that of the control, showed a significant reduction of 26.6, 33.8, 32.2 and 22.7 mg/g respectively among the seeds stored for two months at 10°C, six months at 20°C, nine months at 30°C and two months at room (polyethylene) conditions.

Starch

Fresh seeds contained 254 mg/g of starch on dry weight basis (Table 9, Fig. 9). Twenty four hours of storage at different conditions did not changed the starch content of both pulpy and de-pulped seeds. After four days of storage, there was no significant change of starch with the seeds stored at 10°C, but at 20°C both pulpy and de-pulped seeds showed an insignificant (P<0.01, P<0.01 respectively) increase in their starch content (264 and 272 mg/g on dry weight basis respectively). Seeds stored at 30°C, both pulpy and de-pulped seeds, registered a significant (P<0.01, P<0.01) starch content increase while at room polyethylene

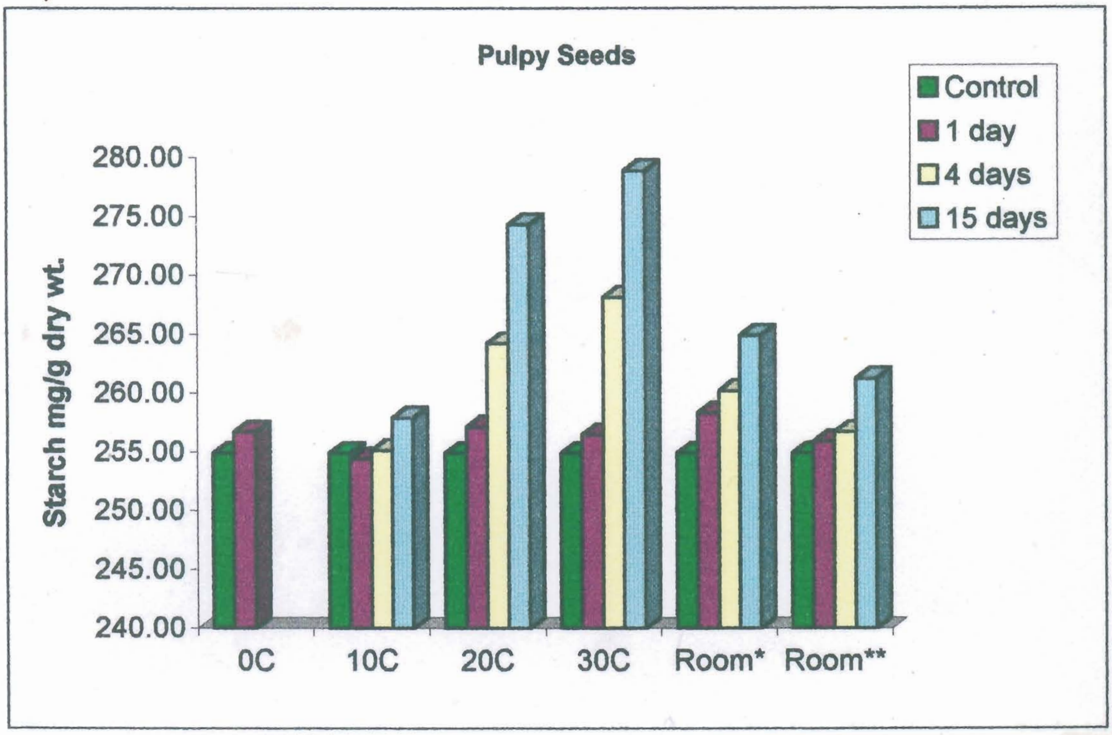
Table 9 **Starch content of *Syzygium aromaticum* seeds during storage and germination under different conditions**

Starch mg/g Dry weight

Treatment		**Control**	**Interval**							
			24 hs	**4 day**	**15 day**	**1 month**	**2 month**	**3 month**	**6 month**	**9 month**
0°C	P		256.70 ±1.78							
	D		263.50 ±0.74							
10°C	P		254.42 ±0.92	255.13 ±1.22	257.90 ±0.80					
	D		255.92 ±0.93	256.13 ±0.81	259.70 ±2.34	262.46 ±0.80	275.37 ±0.85			
20°C	P		257.13 ±0.78	264.21 ±1.16	274.30 ±1.36					
	D		256.42 ±0.91	272.24 ±0.83	287.50 ±1.27	296.10 ±0.78	283.90 ±0.90	278.50 ±0.64	269.80 ±1.78	
30°C	P		256.47 ±1.24	268.12 ±0.73	278.90 ±1.90					
	D		254.38 ±0.92	271.18 ±1.13	306.20 ±1.60	310.80 ±1.23	299.80 ±1.27	298.10 ±0.47	288.50 ±1.12	251.90 ±1.18
Room*	P		258.32 ±1.41	260.21 ±0.75	264.90 ±0.59					
	D		258.42 ±1.44	262.12 ±0.93	287.30 ±0.67	282.10 ±0.86	229.00 ±2.66			
Room**	P		255.90 ±1.47	256.71 ±0.78	261.25 ±0.66					
	D	254.90 ±0.78	253.72 ±0.13	256.29 ±0.48						

P - Pulpy seeds; D - Depulped seeds; * - At 25±5°C in polyethylene bags; ** - At 25±5°C and 70% RH in open trays

The values given are the mean of six replicate ± Standard error



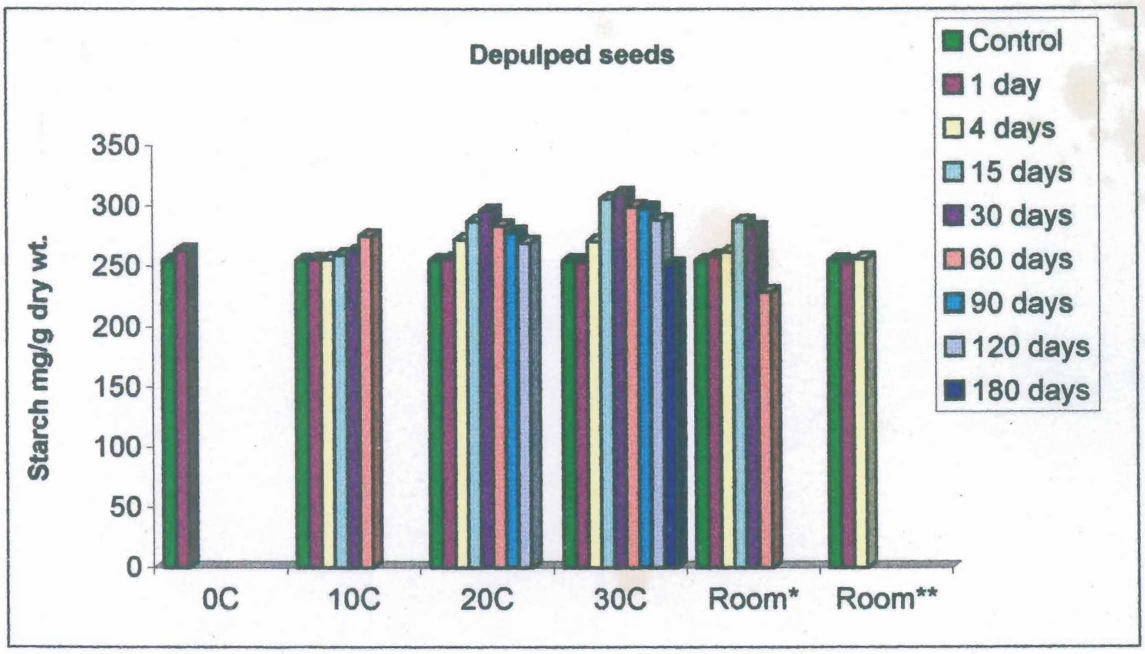


Fig. 9 Starch content of *Syzygium aromaticum* seeds during storage and germination under different conditions

bags, the values were slightly increased in pulpy and de-pulped seeds in comparison with the control. Seeds that are stored at open room conditions for four days showed no significant change in their starch content. Observations after 15 days of seed storage revealed the following results: at 10°C, starch content of both pulpy and de-pulped seeds remained steady; at 20°C, both pulpy and de-pulped seeds showed a significant hike in their starch content by 274 and 287 mg/g dry weight respectively; at 30°C, pulpy and de-pulped seeds registered a significant ($P<0.01$, $P<0.01$) increase of starch compared to the control. At room polyethylene bags pulpy seeds contained 264 mg/g of starch while at open room conditions pulpy seeds contained 261.3 mg/g of starch. De-pulped seeds at room conditions (polyethylene) registered a significant hike in starch content at this interval. After one month of storage, de-pulped seeds stored at 10°C showed an insignificant increase of starch content while at 20 and 30°C, the starch content was significantly rised up to 296 and 310 mg/g respectively. De-pulped seeds stored in room polyethylene bags showed a decrease ($P<0.01$) of starch content than the previous stage. After two months of storage, de-pulped seeds at 10°C registered a significant ($P<0.2$) increase in starch content when compared to previous stage. At the same time, de-pulped seeds stored at 20, 30°C and room polyethylene showed a significant decrease ($P<0.01$, $P<0.01$ and $P<0.01$ respectively) in their starch value, when compared to the previous stage. Three months of storage showed no significant change in starch value while observations after six months of storage revealed a significant ($P<0.01$, $P<0.01$) decrease of starch value at 20 and 30°C respectively. After nine months of storage, the starch content of de-pulped seeds at 30°C become significantly low when compared to the previous stage. This starch content is almost similar to that of the control.

Sugars

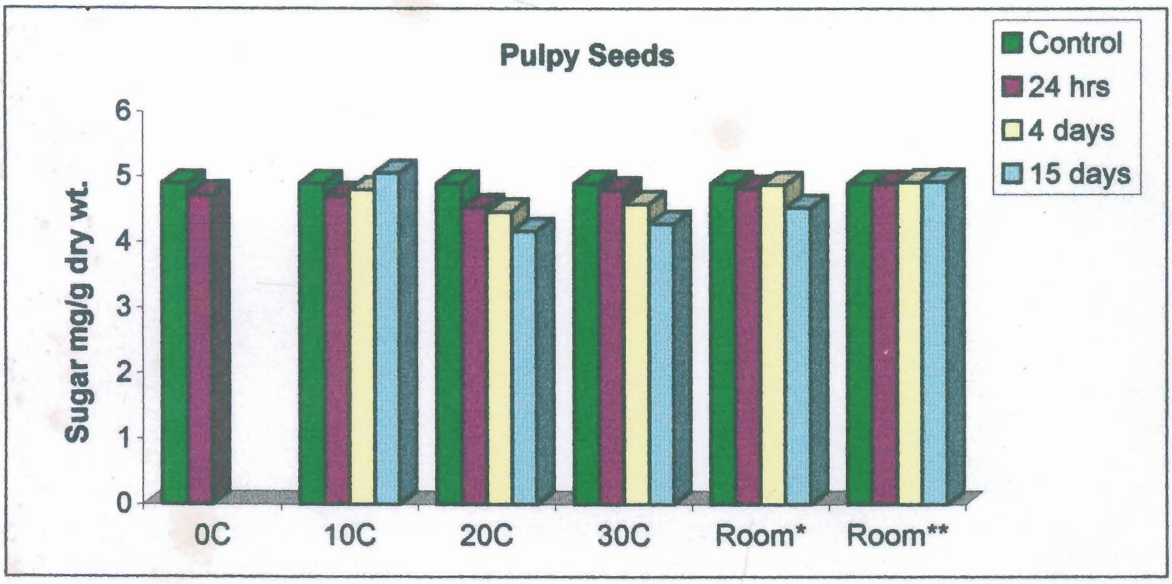
Fresh seeds contained 4.9 mg/g sugars on dry weight basis (Table 11, Fig. 11). Observations after 24 hours of storage showed a slight but insignificant decrease in sugar content both in pulpy and de-pulped seeds stored at 0, 10 and 20°C temperatures while it remained steady in seed stored at 30°C and room conditions. Pulpy seeds stored for four days kept at 20 and 30°C showed an insignificant reduction in sugar content while in all other cases, no change was observed at this interval. On 15th day, pulpy seeds at 20 and 30°C temperatures, registered only insignificant reduction, but de-pulped seeds, showed significant decrease., The seeds stored at room temperature (polyethylene bags) did not show any change in sugar content. After one month of storage, the sugar content of de-pulped seed remained same as the previous stage at 10°C and 20°C. At 30°C, compared to control, a significant reduction was observed at this interval ($P< 0.01$) while at room (polyethylene) conditions a significant increase ($P<0.01$) of sugar content was noted. Observations made on de-pulped seeds stored in polyethylene bags from first month onwards revealed that at 10, 20, 30°C, and room conditions, the loss of seed viability at respective periods were 2, 6 and 9 months with a more or less same sugar content (5.6, 5.3, 5.7 and 5.5 respectively) at these intervals. But these values did not show any significant increase of sugar in comparison with that of fresh seeds (Control).

Table 11 Sugar content of *Syzygium aromaticum* seeds during storage and germination under different conditions

Sugar mg/g Dry weight

Treatment		Control	Interval							
			24 hs	4 day	15 day	1 month	2 month	3 month	6 month	9 month
0°C	P		4.73 ±0.01							
	D		4.58 ±0.02							
10°C	P		4.72 ±0.01	4.81 0.05	5.06 ±0.06					
	D		4.79 ±0.03	4.99 0.06	5.05 ±0.02	5.32 ±0.09	5.60 ±0.02			
20°C	P		4.54 ±0.08	4.47 0.01	4.17 ±0.03					
	D		4.82 ±0.05	4.79 0.07	3.92 ±0.01	4.038 ±0.05	4.24 ±0.08	4.70 ±0.01	5.27 ±0.06	
30°C	P		4.80 ±0.03	4.59 0.10	4.29 ±0.01					
	D		4.89 ±0.03	4.87 0.10	3.67 ±0.11	3.951 ±0.07	4.17 ±0.04	4.80 ±0.01	5.26 ±0.06	5.72 ±0.40
Room*	P		4.82 ±0.01	4.88 0.01	4.53 ±0.08					
	D		4.91 0.05	4.81 0.06	4.85 ±0.06	5.20 ±0.03	5.50 ±0.01			
Room**	P		4.90 ±0.04	4.92 0.01	4.93 ±0.06					
	D	4.90 ±0.02	4.91 0.05	4.84 ±0.01						

P - Pulpy seeds; D - Depulped seeds; * - At 25±5°C in polyethylene bags; ** - At 25±5°C and 70% RH in open trays
The values given are the mean of six replicate ± Standard error



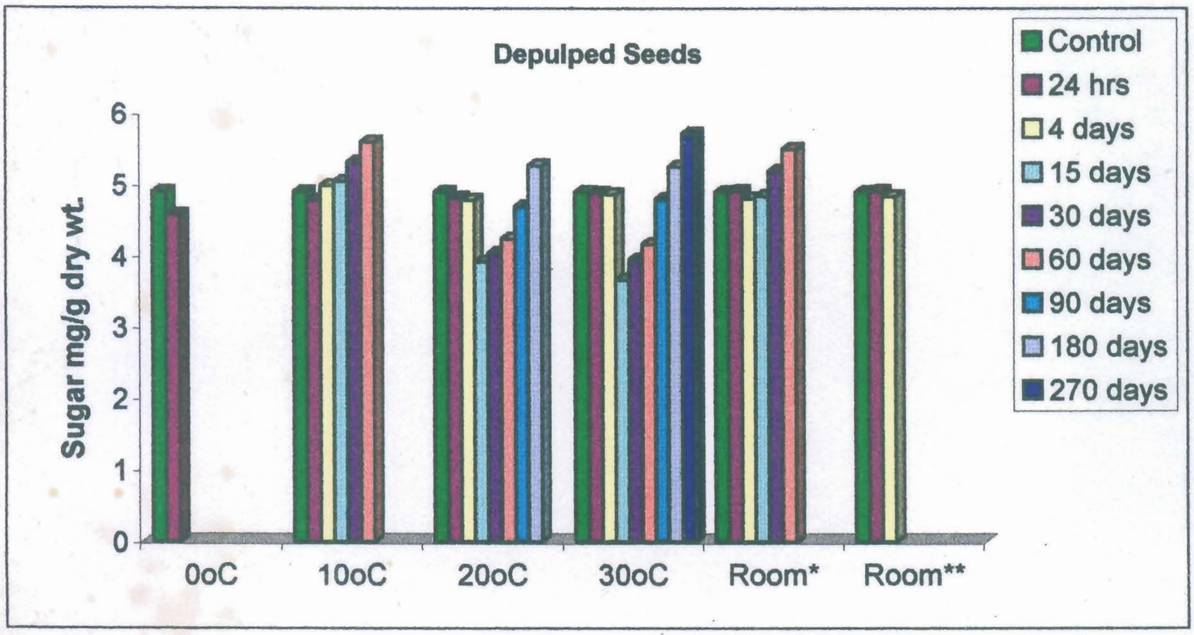


Fig. 11 Sugar content of *Syzygium aromaticum* seeds during storage and germination under different conditions

Phenolics

Initially *Syzygium aromaticum* seeds contained 13 mg/g dry weight of phenolics (Table 13, Fig. 13). Pulpy and de-pulped seeds stored at 0°C for 24 hours showed a significant increase ($P< 0.01$, $P<0.01$) in their phenolic contents while the phenolic content of both pulpy and de-pulped seeds stored at rest of the conditions remained more or less same. When examined after four days of storage a significant increase ($P<0.01$) of phenolics was noticed with the de-pulped seeds at open room conditions while with the other stored seeds, only an insignificant rise in phenolic content occurred. After 15 days of storage, no significant change in phenolics was noticed in comparison with that of previous stage. Observations made on first month of storage revealed that the de-pulped seeds stored at 10, 20 and 30°C as well as room conditions experienced a slight increase in their phenolics (approximately 18±1 mg/g dry weight) compared to the previous stage. From second month of storage onwards, the phenolic content gradually increased reaching maximum in their final stages of storage. The phenolic content become as high as 29 mg/g with the de-pulped seeds stored for nine months at 30°C. Even though the increase of phenolics from stage to stage was not significant, the final samples of all treatment registered a significant hike in comparison with the control.

Chlorophyll

Fresh clove seeds contained 1.9 mg Chl_a, 2.9 mg Chl_b, 4.8 mg/g total chlorophyll and Chl_a/Chl_b ratio of 0.66/gm tissue (Table 15, Figures 15a, 15b, 15c, 15d, 15e and 15f). After 24 hours of storage a significant decrease of the chlorophyll content was observed only in room open de-

Table 13 Phenolics content of *Syzygium aromaticum* seeds during storage and germination under different conditions

Phenolics mg/g Dry weight

Treatment		Control	Interval							
			24 hs	4 day	15 day	1 month	2 month	3 month	6 month	9 month
0°C	P		18.66 ±0.05							
	D		17.44 ±0.06							
10°C	P		13.22 ±0.04	14.56 ±0.03	15.50 ±0.04					
	D		13.33 ±0.02	13.98 ±0.04	14.52 ±0.02	18.25 ±0.02	22.82 ±0.01			
20°C	P		13.55 ±0.06	15.34 ±0.06	17.28 ±0.03					
	D		13.32 ±0.02	13.43 ±0.05	15.00 ±0.03	18.92 ±0.05	19.46 ±0.03	20.72 ±0.02	22.44 ±0.05	
30°C	P		13.43 ±0.05	15.62 ±0.06	16.54 ±0.04					
	D		13.62 ±0.02	14.94 ±0.05	15.84 ±0.02	17.90 ±0.06	21.52 ±0.04	23.80 ±0.03	26.53 ±0.06	29.41 ±0.05
Room*	P		13.77 ±0.06	16.22 ±0.06	18.35 ±0.05					
	D		13.62 ±0.04	16.37 ±0.06	17.22 ±0.03	19.32 ±0.04	21.60 ±0.04			
Room**	P		13.94 ±0.07	16.69 ±0.05	17.79 ±0.05					
	D	13.213 ±0.034	13.47 ±0.07	20.19 ±0.03						

P - Pulpy seeds; D - Depulped seeds; * - At 25±5°C in polyethylene bags; ** - At 25±5°C and 70% RH in open trays

The values given are the mean of six replicate ± Standard error

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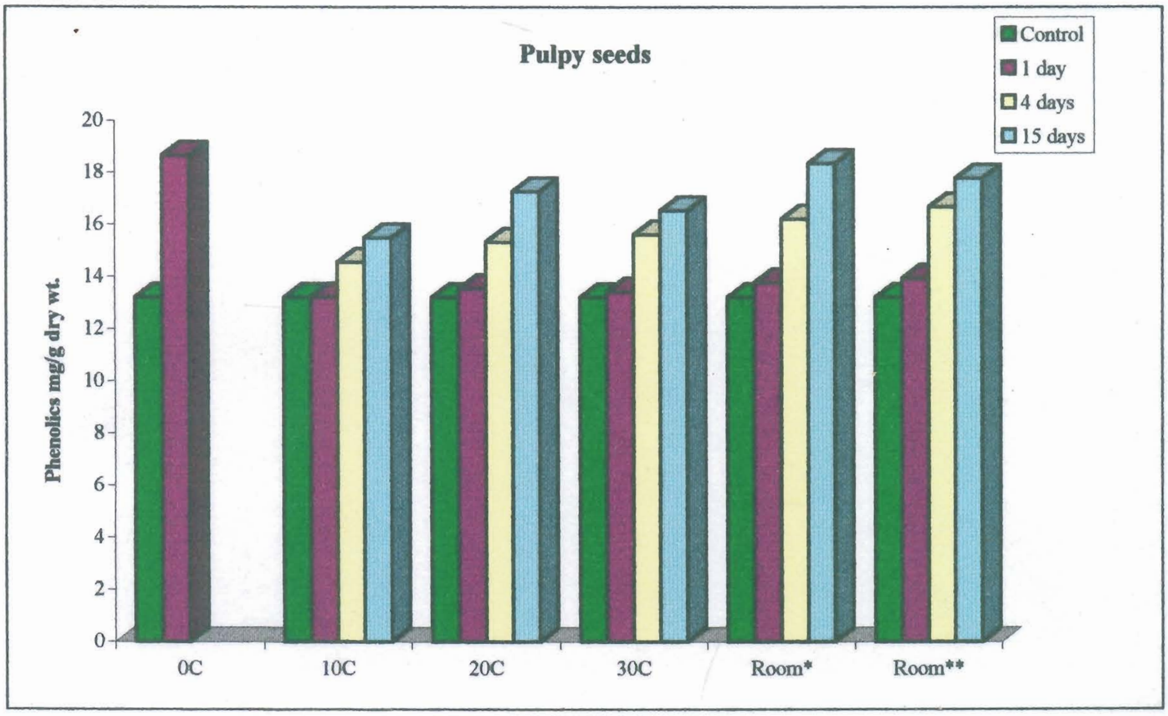

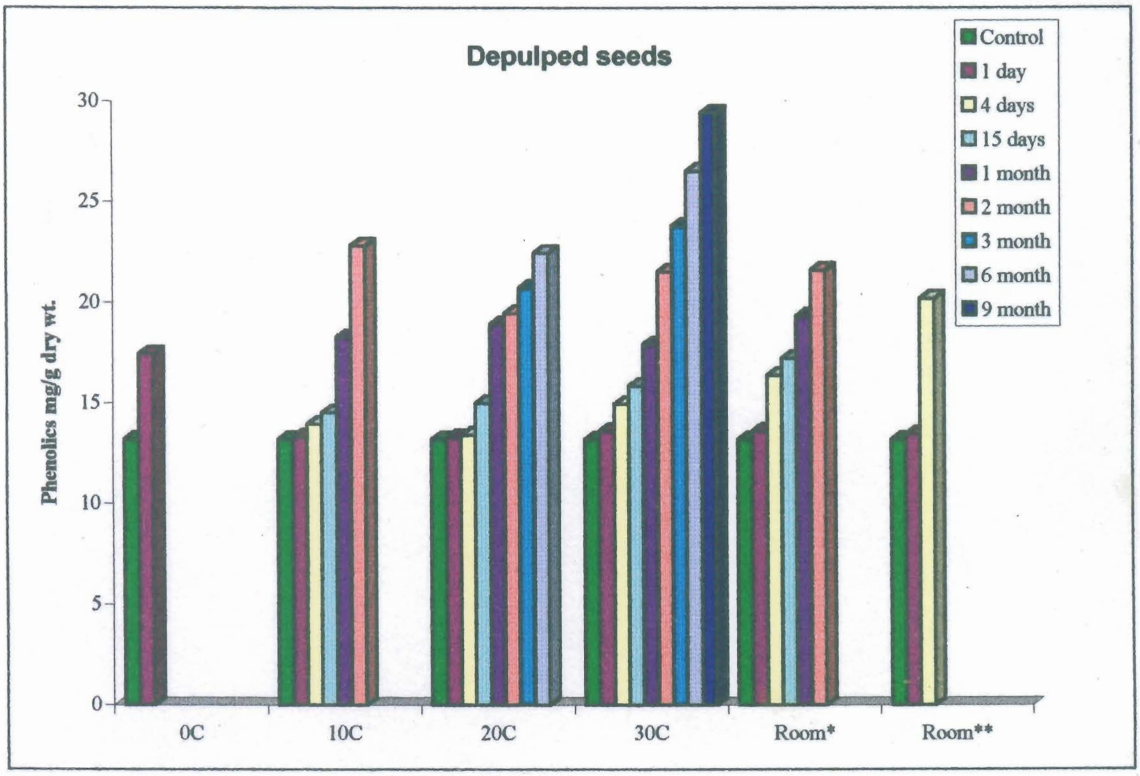

Fig. 13 Phenolics content of *Syzygium aromaticum* seeds during storage and germination under different conditions

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Table 15 Chlorophill Content of *Syzygium aromaticum* seeds during storage and germination under different conditions

Treatment			Chlorophyll on mg/g dry weight								
			Interval								
			Control	24 hours	4th day	15th day	1st month	2nd month	3rd month	6th month	9th month
0°C	P	a		1.58 ± 0.009							
		b		1.86 ± 0.093							
		t		3.46 ± 0.018							
		a/b		0.85 ± 0.096							
	D	a		1.65 ± 0.008							
		b		1.96 ± 0.094							
		t		3.68 ± 0.008							
		a/b		0.84 ± 0.085							
10°C	P	a		1.84 ± 0.004	1.71 ± 0.007	1.60 ± 0.008					
		b		2.15 ± 0.045	1.90 ± 0.063	1.79 ± 0.007					
		t		4.00 ± 0.017	3.65 ± 0.023	3.60 ± 0.019					
		a/b		0.86 ± 0.089	0.89 ± 0.111	0.89 ± 0.082					
	D	a		1.84 ± 0.003	1.73 ± 0.002	1.45 ± 0.004	1.42 ± 0.008				
		b		2.36 ± 0.066	2.33 ± 0.028	1.98 ± 0.095	1.76 ± 0.091				
		t		4.31 ± 0.027	4.07 ± 0.007	3.43 ± 0.019	3.25 ± 0.008				
		a/b		0.78 ± 0.045	0.89 ± 0.071	0.73 ± 0.042	0.80 ± 0.087				
20°C	P	a		1.86 ± 0.007	1.84 ± 0.003	1.65 ± 0.008					
		b		2.44 ± 0.088	2.46 ± 0.031	2.24 ± 0.081					
		t		4.31 ± 0.017	4.31 ± 0.013	3.93 ± 0.008					
		a/b		0.76 ± 0.080	0.75 ± 0.097	0.74 ± 0.098					
	D	a		1.86 ± 0.007	1.73 ± 0.005	1.66 ± 0.006	1.63 ± 0.009	1.54 ± 0.003	1.45 ± 0.007	1.33 ± 0.004	
		b		2.29 ± 0.072	2.14 ± 0.062	2.08 ± 0.089	2.04 ± 0.094	1.99 ± 0.087	1.89 ± 0.089	1.76 ± 0.068	
		t		4.49 ± 0.006	3.96 ± 0.014	3.75 ± 0.005	3.69 ± 0.008	3.59 ± 0.008	3.36 ± 0.013	3.14 ± 0.006	
		a/b		0.81 ± 0.097	0.81 ± 0.081	0.80 ± 0.067	0.79 ± 0.095	0.78 ± 0.034	0.77 ± 0.078	0.75 ± 0.058	
30°C	P	a		1.89 ± 0.072	1.53 ± 0.004	1.30 ± 0.005					
		b		2.73 ± 0.026	2.25 ± 0.037	2.02 ± 0.079					
		t		4.46 ± 0.008	3.88 ± 0.013	3.48 ± 0.015					
		a/b		0.69 ± 0.077	0.68 ± 0.108	0.64 ± 0.063					
	D	a		1.87 ± 0.010	1.72 ± 0.007	1.57 ± 0.004	1.50 ± 0.006	1.39 ± 0.006	1.34 ± 0.005	1.33 ± 0.007	1.27 ± 0.006
		b		2.79 ± 0.089	2.68 ± 0.064	2.39 ± 0.089	2.31 ± 0.079	2.15 ± 0.075	2.09 ± 0.078	2.08 ± 0.074	1.99 ± 0.071
		t		4.69 ± 0.023	4.44 ± 0.017	4.32 ± 0.027	3.85 ± 0.024	3.69 ± 0.022	3.45 ± 0.018	3.44 ± 0.008	3.29 ± 0.007
		a/b		0.67 ± 0.112	0.64 ± 0.109	0.63 ± 0.045	0.65 ± 0.075	0.65 ± 0.080	0.64 ± 0.064	0.64 ± 0.094	0.64 ± 0.084
Room*	P	a		1.87 ± 0.006	1.62 ± 0.004	1.42 ± 0.007					
		b		2.56 ± 0.057	2.23 ± 0.063	1.98 ± 0.088					
		t		4.51 ± 0.021	3.85 ± 0.019	3.51 ± 0.020					
		a/b		0.73 ± 0.105	0.73 ± 0.063	0.71 ± 0.079					
	D	a		1.87 ± 0.006	1.84 ± 0.007	1.74 ± 0.001	1.51 ± 0.008	1.04 ± 0.005			
		b		2.59 ± 0.072	2.55 ± 0.065	2.39 ± 0.068	2.12 ± 0.094	1.45 ± 0.077			
		t		4.62 ± 0.018	4.44 ± 0.008	4.20 ± 0.009	3.71 ± 0.009	2.65 ± 0.018			
		a/b		0.72 ± 0.083	0.72 ± 0.108	0.73 ± 0.014	0.71 ± 0.085	0.72 ± 0.065			
Room**	P	a		1.83 ± 0.004	1.62 ± 0.002	1.29 ± 0.006					
		b		1.93 ± 0.062	1.73 ± 0.026	1.54 ± 0.073					
		t		3.87 ± 0.021	3.37 ± 0.018	2.86 ± 0.019					
		a/b		0.95 ± 0.065	0.94 ± 0.077	0.84 ± 0.082					
	D	a	1.91 ± 0.002	1.37 ± 0.008	1.00 ± 0.003						
		b	2.92 ± 0.075	2.18 ± 0.076	1.46 ± 0.061						
		t	4.85 ± 0.010	3.59 ± 0.017	2.55 ± 0.028						
		a/b	0.66 ± 0.026	0.63 ± 0.105	0.68 ± 0.049						

P - Pulpy seeds; D - Depulped seeds; * - At 25 ± 5°C in polyethylene bags; ** - At 25 ± 5°C and 70% RH in open trays; a - Chlorophyll$_a$; b - Chlorophyll$_b$; t - Total chlorophyll; a/b - Chlorophyll$_a$/chlorophyll$_b$ ratio.
The values given are the mean of six replicates ± standard error.

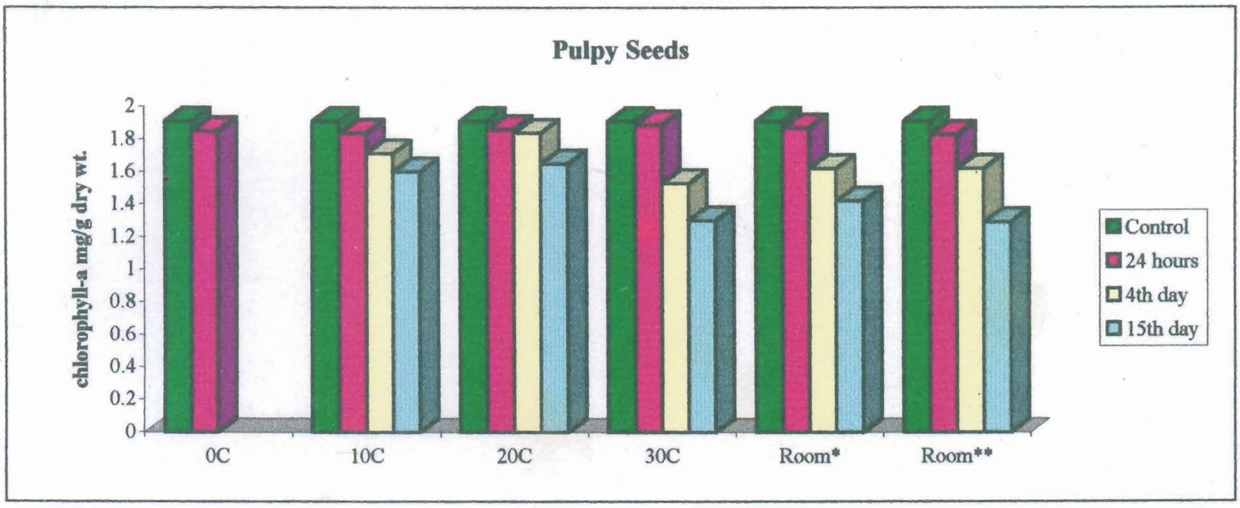

Fig. 15a Chlorophill-a content of *Syzygium aromaticum* seeds during storage and germination under different conditions

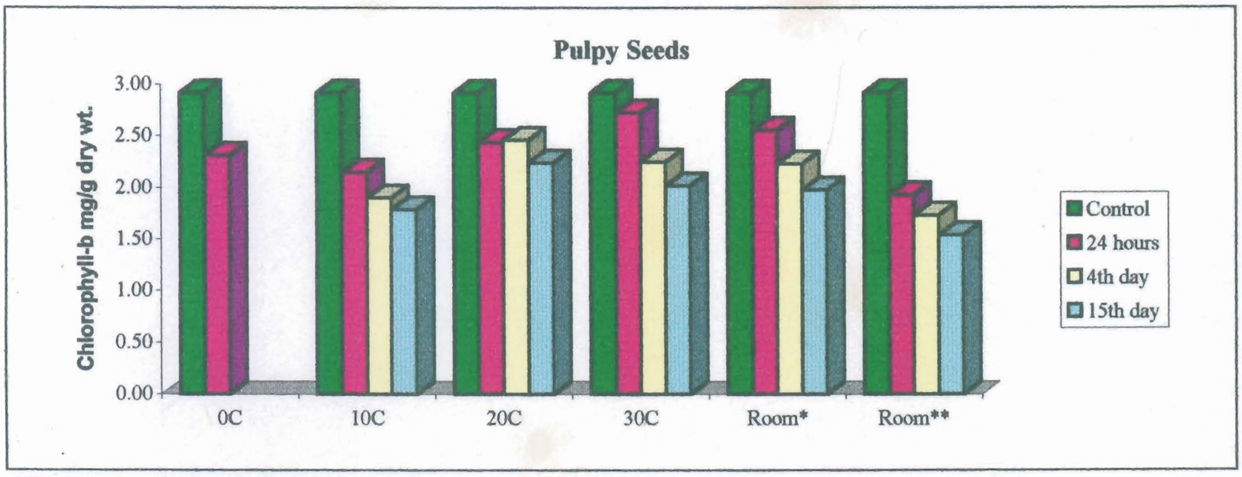

Fig. 15b Chlorophill-b content of *Syzygium aromaticum* seeds during storage and germination under different conditions

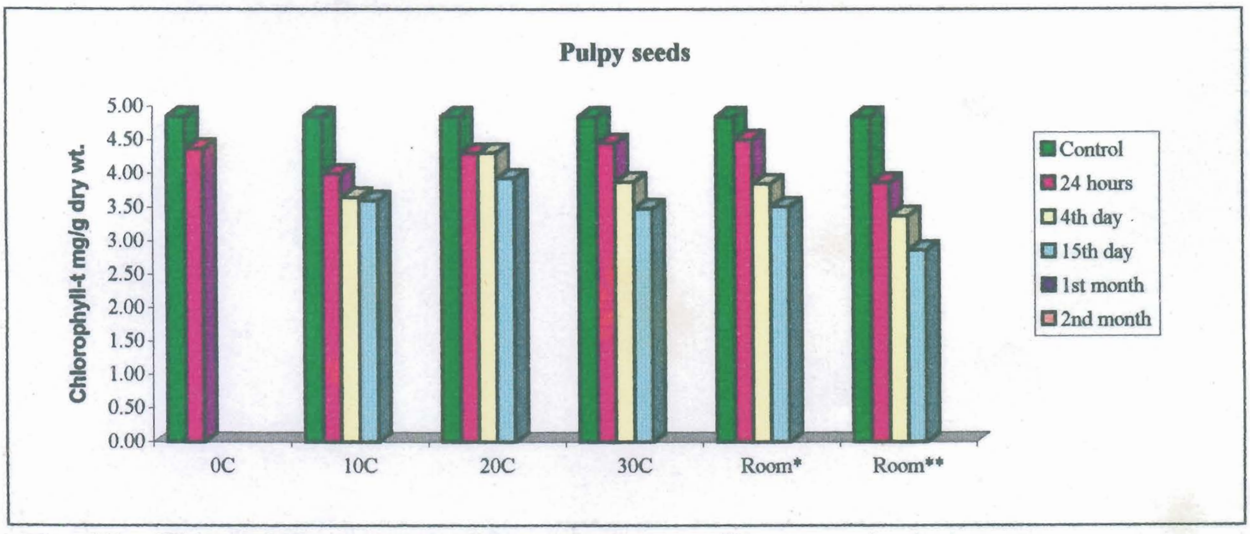

Fig. 15c Chlorophill-t content of *Syzygium aromaticum* seeds during storage and germination under different conditions

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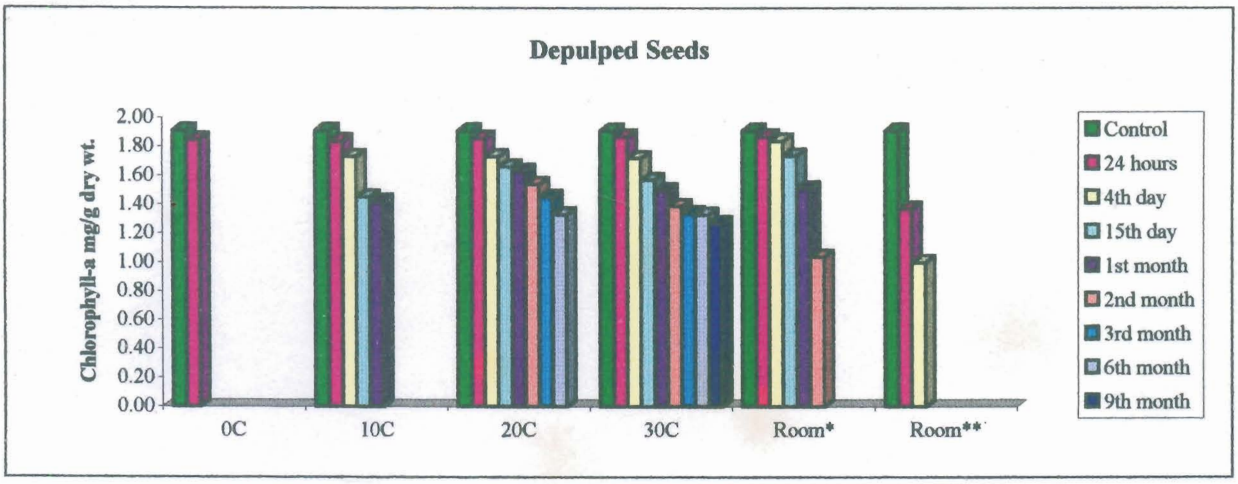

Fig. 15d Chlorophill-a content of *Syzygium aromaticum* seeds during storage and germination under different conditions

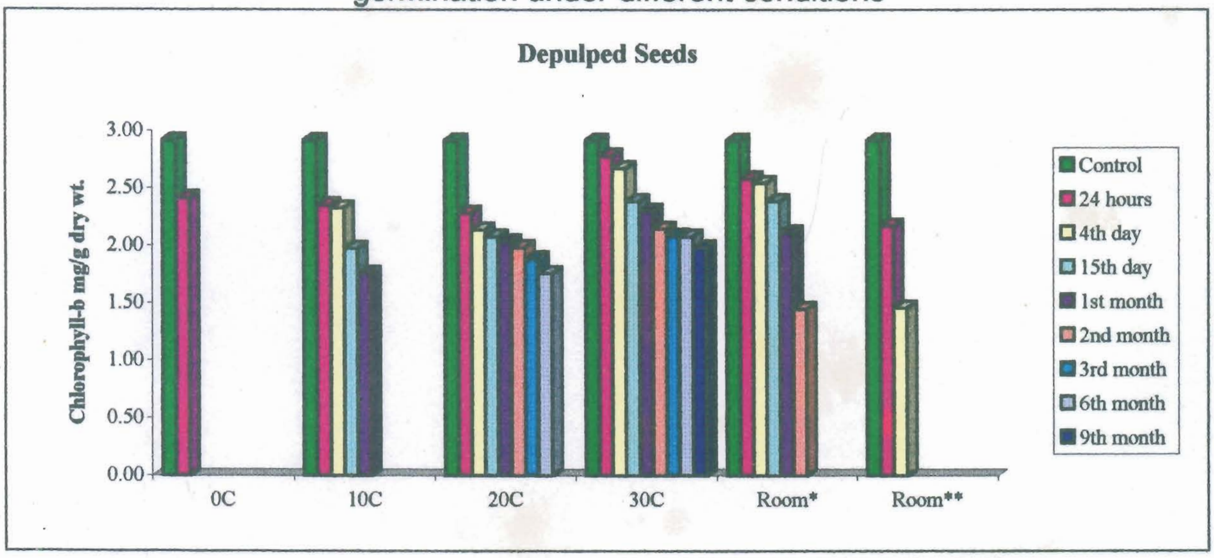

Fig. 15e Chlorophill-b content of *Syzygium aromaticum* seeds during storage and germination under different conditions

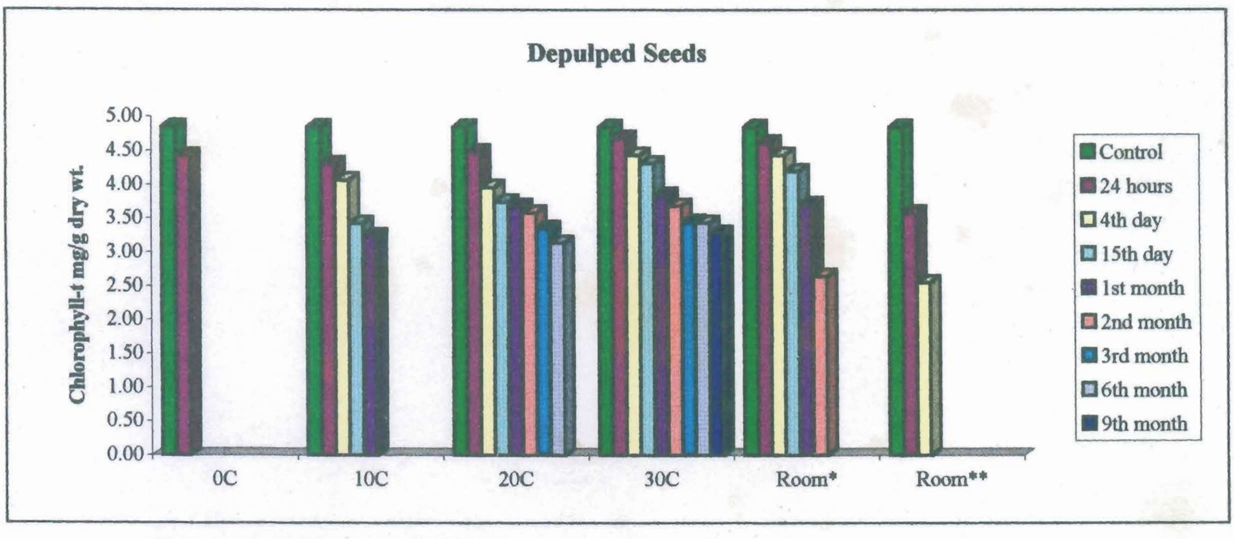

Fig. 15f Chlorophill-t content of *Syzygium aromaticum* seeds during storage and germination under different conditions

pulped conditions while in all the other conditions chlorophylls remain unchanged. Observations on four day stored pulpy and de-pulped seeds in polyethylene bags showed an insignificant decrease of the chlorophylls compared to previous stage. During this period, de-pulped seeds stored at open room conditions showed significant decrease of their chlorophyll values (Chl_a, Chl_b, total chlorophyll and Chl_a/Chl_b ratio to 1 mg/g, 1.5 mg/g, 2.5 mg/g and 0.7 respectively). Among the pulpy seeds stored after 15 days, those seeds stored at 30°C and room lost more chlorophylls than those seeds stored at 10 and 20°C particularly room pulpy seed's Chl_a, Chl_b, total chlorophyll and Chl_a/Chl_b ratio become as low as 1.3 mg/g, 1.5 mg/g, 2.9 mg/g and 0.08 respectively. De-pulped seeds stored at 10°C lost more chlorophyll than those of de-pulped seeds stored at 20, 30°C and room conditions after 15 days of storage. Observations after one month of storage revealed that the de-pulped seeds stored at 10°C lost more chlorophylls than those seeds stored at 20°C and above. Chlorophyll content of de-pulped seeds stored at 20 and 30°C were insignificantly reduced after two months of storage while a significant reduction of Chl_a, Chl_b, total chlorophyll and Chl_a/Chl_b ratio to 1.04 mg/g, 1.5 mg/g, 2.7 mg/g and 0.7 respectively were noticed in seeds stored at room conditions. During the third month, seeds stored at 20 and 30°C recorded an insignificant reduction in the chlorophylls compared to previous stage and over a storage period of six months, values remained more or less same. At 30°C, de-pulped seeds registered 1.3 mg/g Chl_a, 1.9 mg/g Chl_b, 3.3 mg/g total chlorophyll and Chl_a/Chl_b ratio of 0.64 after nine months of storage.

Syzygium cumini

Seed germination

Seeds of *Syzygium cumini* are sensitive to desiccation and are of recalcitrant type, ecologically suited for wet evergreen to semi-evergreen regions. Frugivors disperse the seeds to distant places after deliberate de-pulping. Under natural conditions, clumps of seedlings, from a single seed may be emerged as the seeds are polyembryonic. Consistent observations indicated that when these seedlings faced water stress due to drought, many of them perishes while the more competent ones survived.

Though appears as a single seed, on removal of the enveloping testa seeds which are many in number due to polyembryony are exposed. Number of embryos per seed is directly proportional to seed size and varies from 2 to 10. Embryonic axis is slightly pink in colour, and size varies from 1 to 3 mm in length. When germinated majority of the embryos started germination within 2 to 4 days under lab conditions, germination tests were conducted with seeds placed in petry dishes kept in a seed germinator set at 30°C / 85% RH, dark indicated that the radicle and plumule initiation occurs almost simultaneously and for this light was not an influencing factor.

Observations on the pattern of germination revealed that the radicle which is elongated and wide coloured at the initial stage comes up within 2 to 3 days of sowing. Lateral roots are produced within 5 to 7 days of sowing and this was coincided with the plumule initiation which marks the full attainment of the germination process.

In the field conditions, germination pattern is somewhat 'semi-hypogeal' type as the hypocotyl never elongates and so the chlorophyllous cotyledons lie at soil surface with their tip portions more green than the regions of attachment to embryonic axis. But if the seeds are sowed deep in the soil, cotyledons remain underground typical as in the case of hypogeal type of germination. The plumule which is green in colour grows out and primary leaves attains assimilatory functions within two weeks of sowing. By this time the root system become fully established for an effective seedling anchorage.

Desiccation responses

Fresh seeds of *Syzygium cumini* with moisture content 49.5% registered 100% germination in seven days (Table 2 and Figure 2). Testa envelopes many white embryos which are hidden between green cotyledons. When seeds are dried at open room conditions (25±5°C and 70% RH) for 24 hours, the testa become papery and the moisture content was lowered to 44% while all other features including germination percentage remains unaltered. Moisture content was reduced to 40% when seeds are dried for 48 hours and a corresponding germination percentage of 96 occurred within 17 days of sowing. Testa become remarkably wrinkled though the cotyledons and embryos look as if when they are fresh. Germination percentage was lowered to 66 and that too in 28 days of sowing, when the seeds are dried at open room conditions for a period of 72 hours. Moisture content was 34% and seed samples were characterized with wrinkled testa which is separated from the green, wilted cotyledons. After 96 hours of open room drying, the moisture content was further lowered

Table 2 Desiccation responses of *Syzygium cumini* seeds

Period of drying (hrs)	Moisture content (%±SD)	Germination (%±SD)	Time for completion of germination (days±SD)	Morphological features
0	49.5±0.5	100	6.8±0.5	Testa appressed to green cotyledons. Embryo white
24	44.0±0.9	100	6.8±0.8	Testa become papery. Cotyledons green. Embryo white
48	40.6±0.7	96±5.5	16.6±0.9	Testa wrinkled. Cotyledons green. Embryo white
72	34.0±0.7	66±8.9	28.4±1.1	Testa become separated from green and wilted cotyledons
96	32.2±0.2	48±8.4	31.0±4.0	Testa become more brittle. Cotyledons dull green. Embryo white
120	24.1±0.3	24±5.5	42.6±2.5	Testa brittle. Cotyledons black and hard. Embryo blackened

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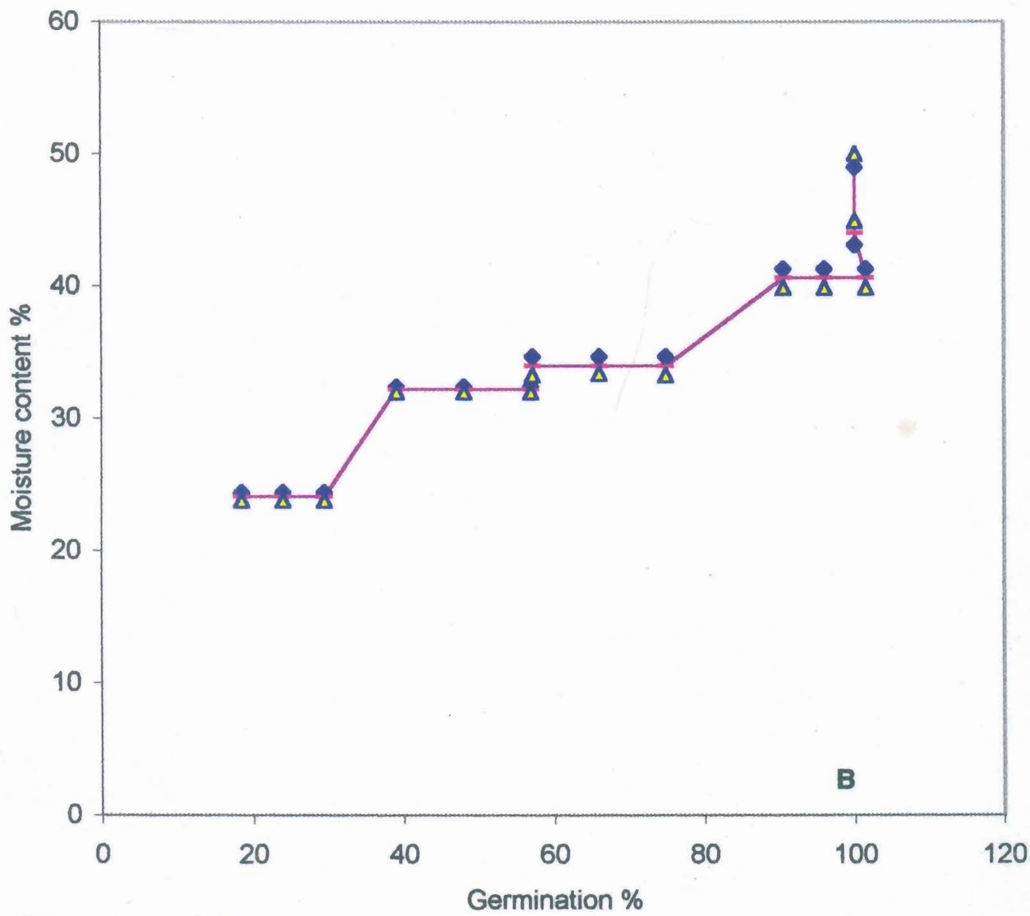
Fig. 2: Relationship between germination and moisture content of Syzygium seeds



B: *Syzygium cumini*

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to 32% with a corresponding germination percentage of 48 within 31 days. By this time, the testa become more brittle, cotyledons dull green though the embryos retained their original appearance. Cotyledons and embryos become hard and black after 120 hours of open room drying. Moisture content was reduced to half of the original with only 24% of germination which was reported in much more prolonged period of 43 days.

Effect of de-pulping

Pulpy seed denotes a fruit while seed means a de-pulped fruit, word de-pulp is used only when a comparison between pulpy and de-pulped seeds are attempted. De-pulped seeds registered 100% germination within a week while the pulpy seeds when sowed got insect and fungal infection which reduced the germination percentage to 40. Regarding the effect of pulp and seed storage, it has been observed that the cotyledons of the pulpy seeds become yellow green and dead within 15 days irrespective of storage conditions. De-pulped seeds retained their viability for extended periods depending on the storage conditions as shown in Table 4.

Effect of storage and temperature on germination

Moisture content of fresh seed was 49.5% and when tested, cent percent germination recorded up to a week (Table 4, Figure 4a and 4b). When fresh de-pulped seeds were allowed to dry at open room conditions, a reduction in moisture content and no change in morphology and germination percentage occurred within a day while after four days the moisture content became 34% with a corresponding germination

Table 4
Effect of storage and temperature on germination of *Syzygium cumini* seeds

Temperature °C	Duration days	Moisture Content %±SD	Germination %±SD	Morphological Features
0	1	49.5±0.5 (49.5±0.5)	0 (0)	Cotyledons green (Cotyledons green)
10	1	49.5±0.5 (49.5±0.5)	100 (100)	Cotyledons green (Cotyledons green)
	4	49.5±0.5 (49.5±0.5)	100 (98.0±4.5)	Cotyledons green (Cotyledons green)
	15	52.3±0.5 (51.2±0.4)	100 (0)	Cotyledons green (Cotyledons yellow green)
	30	53.5±0.5	100	Cotyledons green
	90	53.6±0.2	100	Cotyledons green
	180	54.2±0.5	98.0±4.5	0.4±0.1 cm of radicle growth. Cotyledons green
	360	55.4±0.4	94.0±5.5	Testa become dry, yellow brown and got separated from cotyledons
	540	59.0±0.6	68.0±8.4	Black spots appear all over the green cotyledons.
	720	63.2±0.5	22.0±4.5	Portions of cotyledons become black
20	1	49.5±0.5 (49.5±0.5)	100 (100)	Cotyledons green (Cotyledons green)
	4	49.5±0.5 (49.5±0.5)	100 (92.0±4.5)	Cotyledons green (Cotyledons green)
	15	54.5±0.5 (54.4±0.5)	100 (0)	0.6±0.3 cm of radicle and 0.3±0.1 cm of plumule growth (Cotyledons yellow green)
	30	55.5±0.5	100	1.4±0.1 cm of radicle and 0.8±0.2 cm of plumule growth
	90	56.6±0.3	100	1.6±0.4 cm of radicle and 0.9 cm of plumule growth
	180	60.8±0.7	90.0±7.1	2.2±0.6 cm of radicle and 1.2±0.3 cm of plumule growth
	360	61.1±0.7	66.0±8.9	2.4±0.4 cm of radicle and 1.3±0.4 cm of plumule growth. Black spots appear all over green cotyledons
	540	61.6±0.2	28.0±8.4	Portions of cotyledons became black
30	1	49.5±0.5 (49.5±0.5)	100 (100)	Cotyledons green (Cotyledons green)
	4	49.5±0.5 (49.5±0.5)	100 (100)	Cotyledons green (Cotyledons green)
	15	52.2±0.4 (55.5±0.3)	100 (0)	3.4±1.5 cm of radicle and 0.8±0.2 cm of plumule growth (Cotyledons yellow green)
	30	54.0±0.2	100	3.4±1.8 cm of radicle and 1.6±0.7 cm of plumule growth
	90	56.9±0.5	100	6.6±1.1 cm of radicle and 2.8±0.2 cm of plumule growth
	180	62.3±0.4	100	15.6±3.3 cm of radicle with 10 to 12 nos. of lateral roots of ≈ 2.4±1.6 cm and 2.4±1.6 cm of plumule growth
Room*	1	49.5±0.5 (49.5±0.5)	100 (100)	Cotyledons green (Cotyledons green)
	4	49.5±0.5 (49.5±0.5)	100 (88.0±4.5)	Cotyledons green (Cotyledons green)
	15	52.7±0.2 (52.0±0.4)	100 (0)	2.5±0.5 cm of radicle and 0.98±0.3 cm of plumule growth (Cotyledons yellow green)
	30	47.4±0.3	100	2.9±1.1 cm of radicle and 1.1±0.3 cm of plumule growth
	90	36.3±0.3	70.0±7.1	3.2±1.2 cm of radicle and 1.4±0.4 cm of plumule growth. Cotyledons became dark green
	180	31.4±0.3	40.0±7.1	Seeds become more hard, dark green and reduced in size
Control	0	49.5±0.5	100	Cotyledons green
Room**	1	44.0±0.9 (49.5±0.5)	100 (100)	Cotyledons green (Cotyledons green)
	4	34.0±0.7 (49.5±0.5)	66.0±8.9 (100)	Testa separated from wilted cotyledons (Cotyledons green)
	15	20.8±0.5 (49.3±0.3)	0 (94.0±5.5)	Cotyledons became dark green (Fruit wall shrunken, Cotyledons green)
	30	0 (24.9±0.2)	 (38.0±8.3)	Cotyledons become hard and black (Cotyledons dark green and hard)

Values and data in parentheses are of pulpy seeds; * - At 25±5°C in polyethylene bags; ** - At 25±5°C and 70% RH in open trays

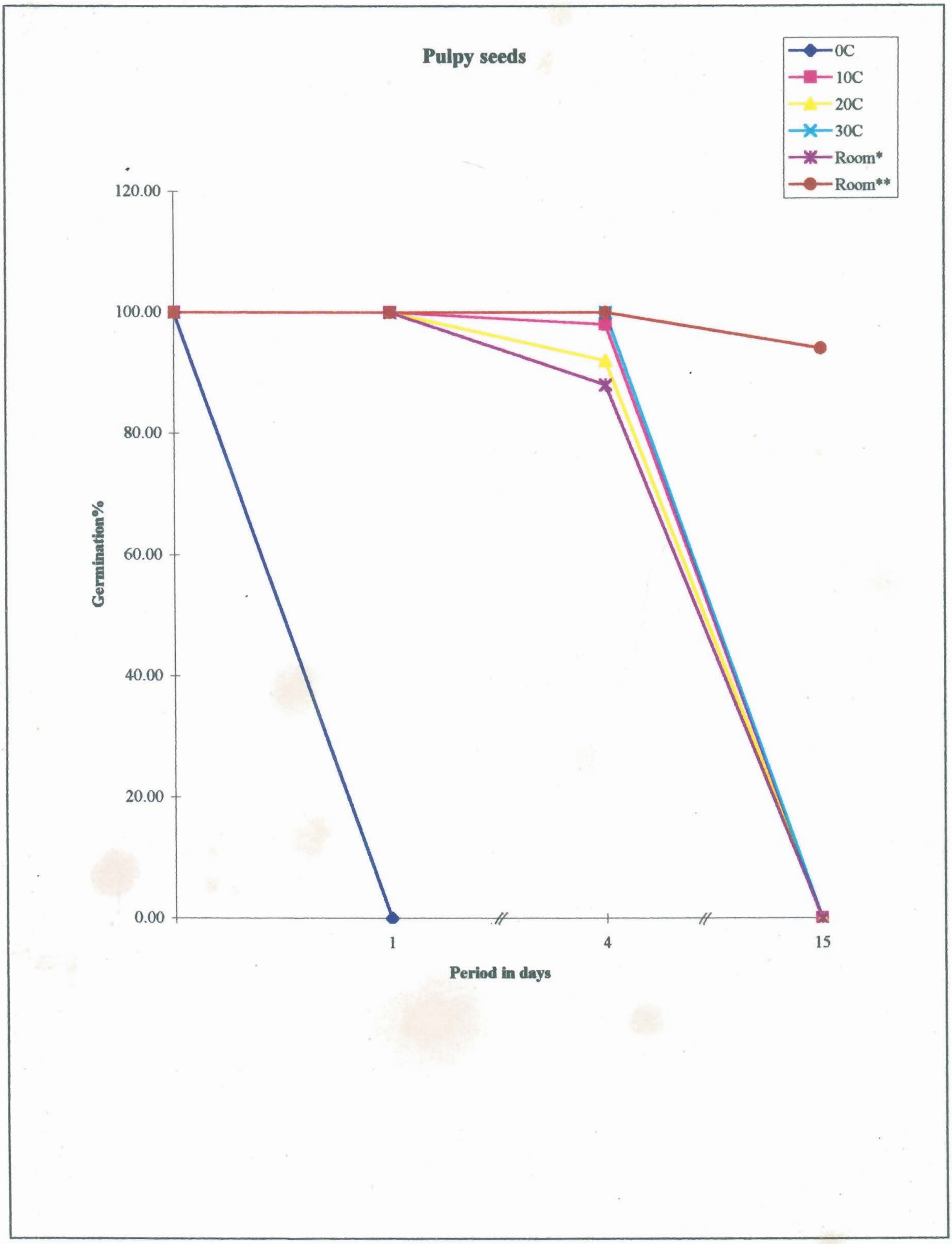

Fig. 4a Germination percentage of *Syzygium cumini* seeds during storage under different conditions.

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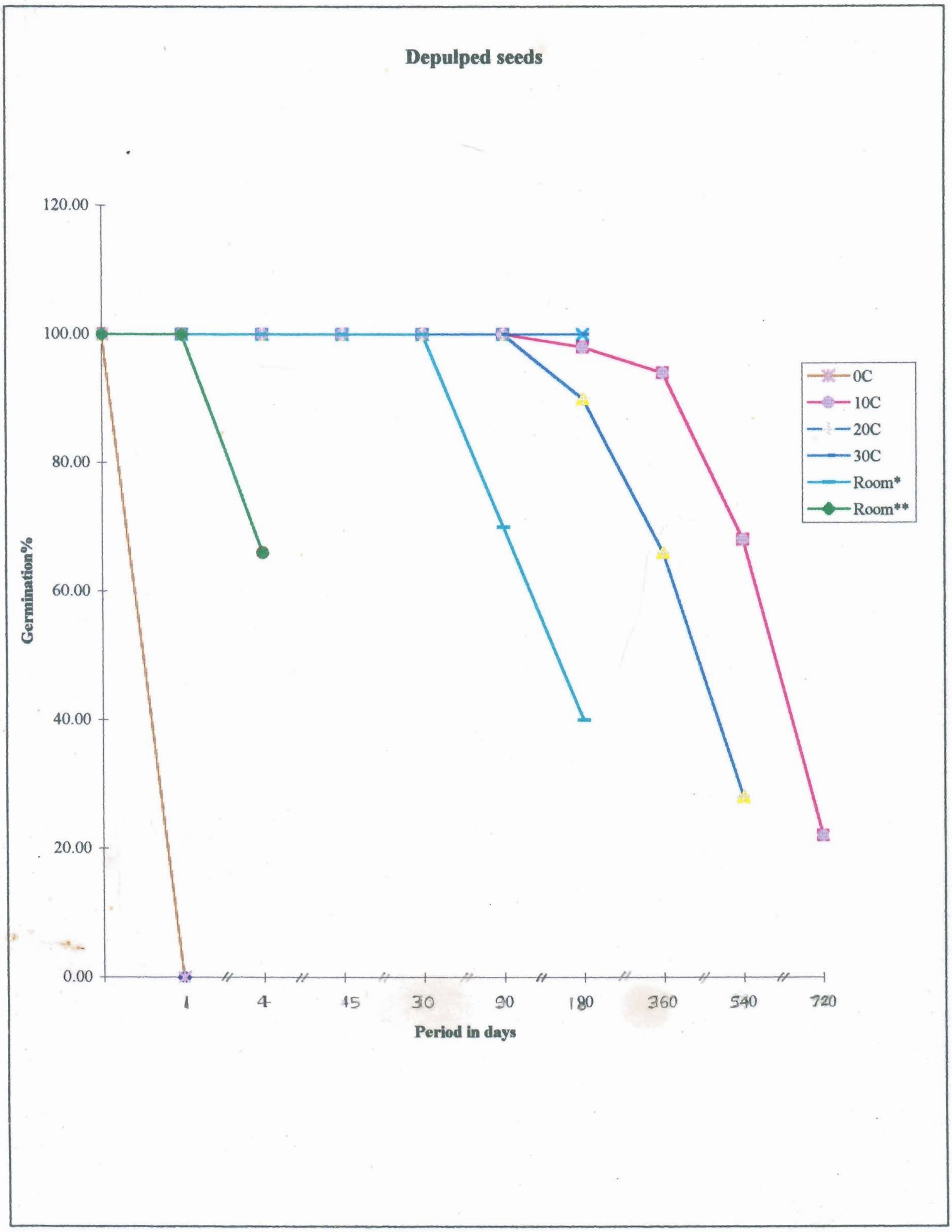

Fig. 4b Germination percentage of *Syzygium cumini* seeds during storage under different conditions.

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percentage of 66 (Table 2). Pulpy seeds exposed to open room conditions maintained their original moisture content and hence resulted in 100% germination. Observations made after 15 days of storage showed a drastic decrease in the moisture content of de-pulped seeds (21%) along with a complete loss of viability. By this time the cotyledons became dark green and hard with the testa completely separated. In the case of pulpy seeds, fruit wall was wrinkled with cracks all over. The moisture content of seed was 49% with a 94% germinability. When pulpy seeds were kept at open room conditions for a period of 30 days, pulp was completely dried and cotyledons became dark along with a significant loss of viability.

Pulpy and de-pulped seeds stored in polyethylene bags at room conditions maintained their initial moisture contents after one day as well as four days, but the germination percentage of pulpy seeds was reduced to 88. Within 15 days of storage, pulpy seeds lost their viability and the cotyledons became pale yellow green in colour. On the other hand de-pulped seeds started sprouting under storage showing 100% germination with their radicle and plumule growing up to 2.5 cm and 0.9 cm respectively. Both pulpy and de-pulped seeds exhibited an elevated moisture content of 52% each. After 30 days of storage, moisture content of de-pulped seeds was reduced considerably but cent percent germinability was observed. The moisture content was again lowered during 90 days of storage and the germination percentage became 70. At this stage, the seeds sprouted with radicle and plumule of 3.2 cm and 1.4 cm length respectively. By this time the colour of cotyledons became dark green. After 180 days of storage, the moisture content was lowered to 31.4% and only 40% of seeds germinated. Radicle and plumule length remained same compared to the previous stage while the seeds became darker, hard and reduced in size due to desiccation.

Pulpy and de-pulped seeds maintained their morphology and initial moisture content after 24 hours of storage at $0^{\circ}C$, but completely lost their viability.

At $10^{\circ}C$, the morphology, the moisture content and germination percentage of both pulpy and de-pulped seeds remained same up to 4 days of storage. After 15 days of storage, 3% increase in the moisture content was noted both with the pulpy and de-pulped seeds. It was found that in pulpy seeds the cotyledons became pale yellow green and lost their viability. Cotyledons of de-pulped seeds maintained their fresh appearance even after 30 to 90 days of storage and the moisture content remained almost same in both intervals with the corresponding germination of 100%. After 180 days, the moisture content started slightly increasing with a corresponding reduction in germination and seeds started sprouting under storage with 0.4 cm of radicle elongation but plumule initiation was completely absent. The same trend of change in moisture content and germination was followed after 360 days of storage showing 55.4 and 94 respectively. By this time the testa became dry, yellow brown colour and got separated from the green cotyledons.

The moisture content of seeds after 540 days of storage increased by 10% compared to the control but the related germination percentage was reduced to 68. Black spots appeared all over the green cotyledons. After 720 days of storage, certain portions of cotyledons became black which clearly indicated die back symptoms. It was found that the moisture content was again showed slight increase than the previous stage and the corresponding germination was only 22%.

At 20°C, the morphology, moisture content and germination percentage of both pulpy and de-pulped seeds remained more or less same up to four days of storage with a slight decrease of germination percentage in pulpy seeds. Observations after 15 days of storage revealed that the cotyledons of pulpy seeds became yellow green and lost their viability though their moisture content was elevated to 54%. In the case of de-pulped seeds, the moisture content was same with a related germination percentage of 100. The seeds began to sprout at this stage with radicle and plumule growth of 0.6 and 0.3 cms respectively. After 30 days, the moisture content remained more or less same as previous stage with a corresponding germination of 100%. The radicle and plumule length doubled by this interval. Samples of 90 days of storing showed an insignificant increase in the moisture content with cent percent seed germination. There occurred a negligible radicle and plumule growth when seeds were stored during 90 days. Moisture content became 60.8% after 180 days of storage with a corresponding germination percentage of 90 and the length of radicle and plumule were increased considerably. By 360 days of storage, the moisture content remained almost same as that of the previous stage and germination percentage became reduced significantly. The radicle and plumule did not show any change. Several black spots began to appear all over the green cotyledons. After 540 days of storage, the moisture content was not changed and germination percentage was again reduced to 28. By this time terminal portions of radicle and plumule as well as certain regions of cotyledons became black.

At 30°C, both pulpy and de-pulped seeds maintained a constancy as that of the control in their moisture content, morphology and germination percentage up to 4 days of storage. After 15 days of storage pulpy seeds lost their viability, and their cotyledons became pale yellow green in colour. De-pulped seeds on the other hand started to sprout under storage and the radicle and plumule growth was 3.4 and 0.8 cms respectively. The moisture content was found to increase slightly with cent percent germination. Thirty days of storage increased the moisture content insignificantly and the plumule growth doubled though their radicle growth was more or less same as that of the previous stage. After 90 days of storage, the moisture content became again increased insignificantly with a corresponding germination percentage of 100. Their radicle and plumule growth increased significantly than the previous stage. The moisture content was increased significantly after 180 days of storage while the radicle growth was very well elaborated with several lateral roots. The plumule growth did not change at all compared to the previous stage. Though these seeds were 100% regenerable, their further storage became impossible as a result of sporadic decay.

Germination percentage of stored seeds

Fresh seeds registered 100% germination (Table 4, Figure 4a and 4b). Irrespective of the difference in storage conditions seed samples maintained their initial germination percentage after one day storage except in the case of seeds stored at 0°C where the viability was completely lost in both pulpy and de-pulped seeds.

Observation on pulpy seeds after 4 days of storage revealed that the germination percentage of seeds stored at room open and 30°C remained unchanged while at 10°C, seeds experienced a slight decrease in their germination percentage. Significant reduction of germination percentage was resulted in seeds stored for 4 days at 20°C and room polyethylene bag. Fifteen days of storage showed only slight reduction in the germination percentage of pulpy seeds stored at open room condition while at rest of the conditions all the seed samples lost their viability.

Germination percentage of open room stored de-pulped seeds became significantly reduced within 4 days of storage while the viability of seed stored at rest of the conditions remain unchanged up to 15 - 30 days. After 90 days of storage, de-pulped seeds stored at room polyethylee experience a drastic reduction in their germination percentage while seeds stored at 10, 20 and 30°C remained unaltered. Germination percentage of seeds stored at 30°C after 180 days of storage was same as previous period, but a slight reduction of germination percentage was occurred with seeds stored at 10 and 20°C while the germination percentage of de-pulped seeds stored at room polyethylene bag was drastically reduced. Three sixty days of storage again reduced the germination percentage of seeds stored at 10°C but a significant reduction was resulted with the seeds stored at 20°C. After 540 days of storage, considerable loss of germination percentage was occurred in the seeds stored at 10°C but a drastic decrease was noticed with seeds stored at

20°C compared to its previous stage. De-pulped seeds stored at 30°C retained their viability after 720 days of storage at 10°C, though a reduction of germination percentage was observed at this stage.

Biochemical aspects of stored seeds

Dry weight percentage

Dry weight percentage of fresh seeds was found to be an average 50 percent of their fresh weight (Table 6, Fig. 6a & 6b). On storing for 24 hours at open room conditions, dry weight percentage of de-pulped seeds increased to 62 percentage, which indicated almost 10 percent loss of seed moisture content. Both the pulpy and de-pulped seeds stored for 24 hours at all other conditions registered only insignificant change in their dry weight percentage. After four days of storage at open room conditions, the dry weight percentage increased to 67.8 and simultaneously viability of seeds found to be lost. In all other samples, including those seeds with pulp that are stored at open room conditions showed an insignificant loss of dry weight than the previous stage as well as control. In the case of 15 days stored seeds, though pulpy seeds lost their viability, their dry weight percentage remained more or less same as in the case of de-pulped seeds and their values did not show any significant change in comparison with the previous sample. From first month onwards de-pulped seeds stored in room polyethylene bags experienced a significant hike in their dry weight compared to the previous stage while those seeds stored at 10, 20 and 30°C faced an insignificant decrease in their dry weight. Observations made on third month revealed a significant hike of dry weight percentage (58.4) only

Table 6 Dry weight percentage of *Syzygium cumini* seeds during storage and germination under different conditions

Treatment		Control	Interval								
			24 hs	4 days	15 days	1 month	3 months	6 months	12 months	18 months	24 months
0°C	P		51.20 ±2.84								
	D		50.70 ±1.83								
10°C	P		50.60 ±1.23	49.30 ±0.93	48.80 ±0.62						
	D		49.60 ±1.16	48.80 ±0.81	47.70 ±0.82	46.50 ±0.31	46.40 ±0.62	45.80 ±0.93	44.60 ±0.57	40.96 ±0.43	36.80 ±0.38
20°C	P		49.90 ±1.47	46.40 ±0.72	45.60 ±0.69						
	D		48.70 ±0.83	46.70 ±1.36	45.50 ±0.93	44.50 ±0.64	43.40 ±0.57	39.20 ±0.52	38.90 ±0.48	38.40 ±0.41	
30°C	P		49.30 ±1.22	45.80 ±0.79	44.47 ±0.84						
	D		50.30 ±1.11	49.10 ±1.32	47.80 ±0.58	45.96 ±0.92	43.10 ±1.12	37.70 ±0.63			
Room*	P		51.10 ±0.92	49.60 ±0.86	48.00 ±0.72						
	D		47.90 ±1.41	47.60 ±0.93	47.30 ±0.67	52.60 ±1.17	58.40 ±1.31	63.70 ±1.92			
Room**	P		48.40 ±1.48	46.10 ±1.74	50.70 ±1.23						
	D	50.50 ±0.82	62.60 ±1.71	67.80 ±1.26							

P - Pulpy seeds; D - Depulped seeds; * - At 25±5°C in polyethylene bags; ** - At 25±5°C and 70% RH in open trays
The values given are the mean of six replicate ± Standard error

78B

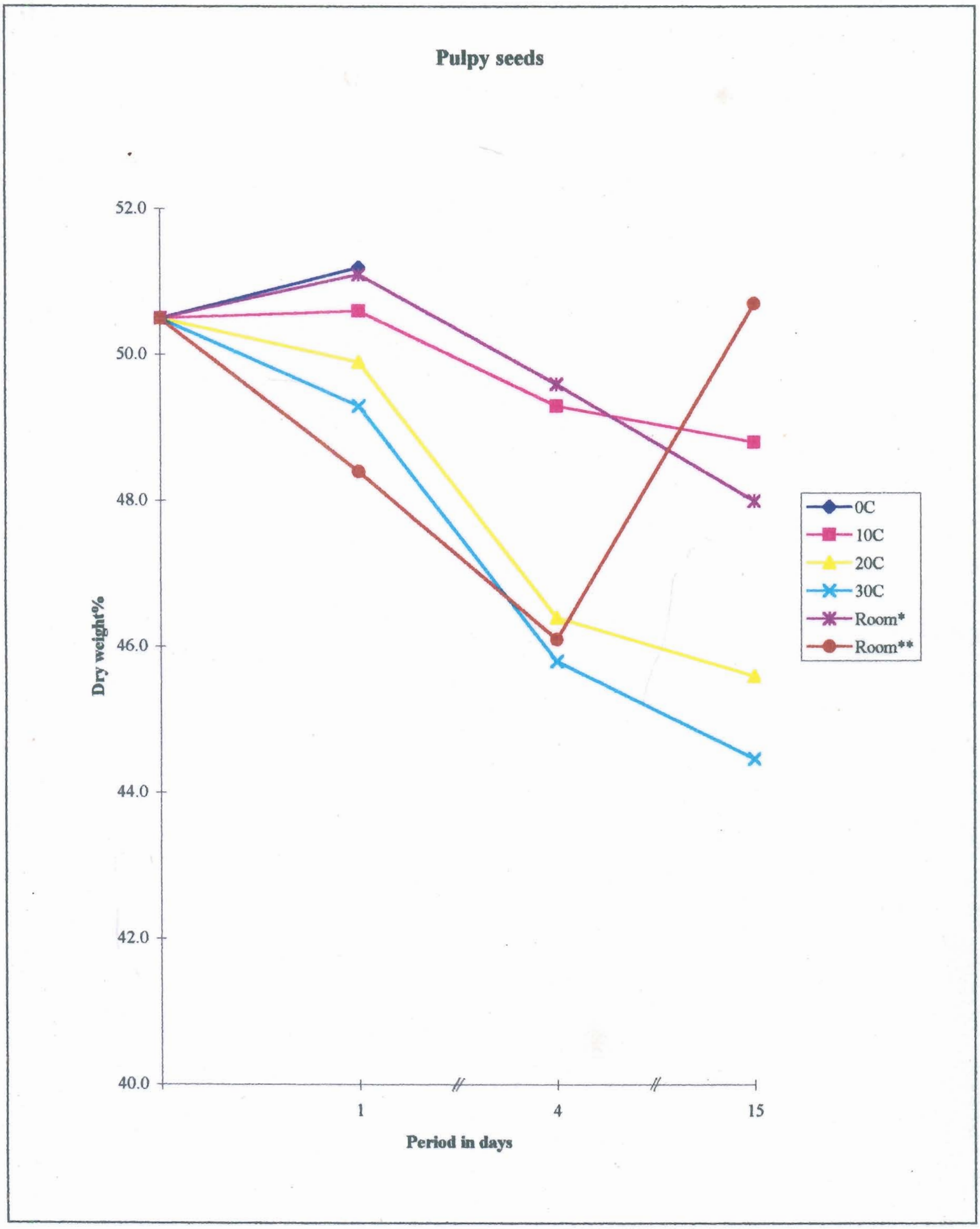


Fig. 6a Dry weight percentage of *Syzygium cumini* seeds during storage and germination under different conditions

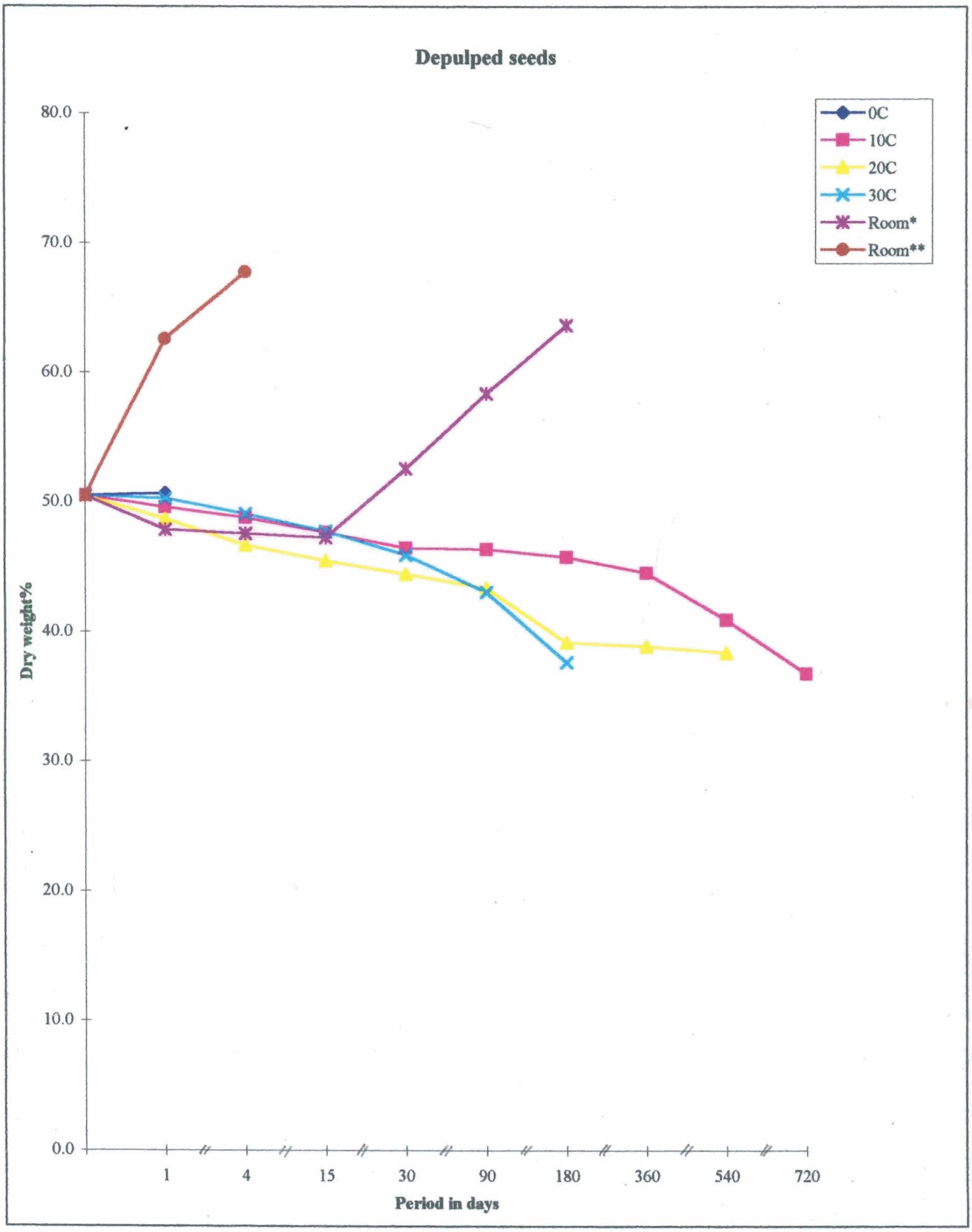


Fig. 6b Dry weight percentage of *Syzygium cumini* seeds during storage and germination under different conditions

with the seeds stored at polyethylene bags while the rest of the conditions, the values decreased slightly compared to the previous stage. By the sixth month of storage, reduction of dry weight percentage was meagre at 10°C, but at 20 and 30°C the values become significantly reduced to 39 and 38% respectively. In the case of seeds stored for six months in room polyethylene condition, they experienced a significant increase of dry weight along with a complete loss of their viability. Observations made from 12 months onwards with the de-pulped seeds stored at 10 and 20°C showed that their dry weight percentage went on decreasing gradually so that the values were 41 and 38 respectively after 18 months of storage. After 24 months of storage at 10°C, the dry weight percentage of de-pulped seeds become significantly reduced to 36.8% along with a loss of viability.

Protein

Protein content of both pulpy and de-pulped seeds remained same as that of fresh seeds up to 24 hours irrespective of the difference in storage conditions (Table 8, Figure 8). When observed on the fourth day of storage, it was found that all pulpy seeds showed reduction of their protein content particularly those stored at room conditions with the minimum of 144 mg/g protein ($P<0.01$). Regarding the de-pulped seeds stored for four days in polyethylene bags, protein content remained more or less same as that of the control but at open room conditions, it becomes significantly reduced to 102 mg/g and the seeds stored at the remaining conditions showed no change. After 15 days of storage, protein content of pulpy seeds become significantly reduced in all storage conditions

Table 8 Protein content of *Syzygium cumini* seeds during storage and germination under different conditions

Protein mg/g Dry weight

Treatment		Control	Interval								
			24 hs	4 day	15 day	1st month	3rd month	6 month	12 month	18 month	24 month
0°C	P		169.69 ±0.36								
	D		171.06 ±0.35								
10°C	P		171.56 ±0.48	158.45 ±1.32	130.41 ±1.49						
	D		170.97 ±0.54	167.28 ±0.73	161.07 ±0.18	157.01 ±0.18	146.12 ±0.59	143.45 ±0.365	108.59 ±0.19	104.39 ±0.52	87.52 ±0.21
20°C	P		171.54 ±0.65	157.34 ±1.20	128.30 ±0.21						
	D		171.67 ±0.56	170.01 ±1.33	162.45 ±0.52	156.58 ±0.14	154.84 ±0.26	142.20 ±0.25	101.42 ±0.22	77.00 ±0.56	
30°C	P		170.85 ±0.65	155.47 ±1.47	129.44 ±0.20						
	D		172.33 ±0.82	168.44 ±1.43	168.69 ±1.03	135.77 ±0.57	84.88 ±0.88	64.54 ±0.21			
Room*	P		172.47 ±0.47	159.57 ±0.67	134.04 ±0.45						
	D		171.98 ±0.74	170.12 ±0.65	169.24 ±0.37	146.66 ±0.17	116.16 ±0.79	68.90 ±0.45			
Room**	P		172.18 ±0.56	144.57 ±1.65	125.45 ±0.42						
	D	172.43 ±0.185	171.88 ±0.64	102.66 ±0.44							

P - Pulpy seeds; D - Depulped seeds; * - At 25±5°C in polyethylene bags; ** - At 25±5°C and 70% RH in open trays

The values given are the mean of six replicate ± Standard error

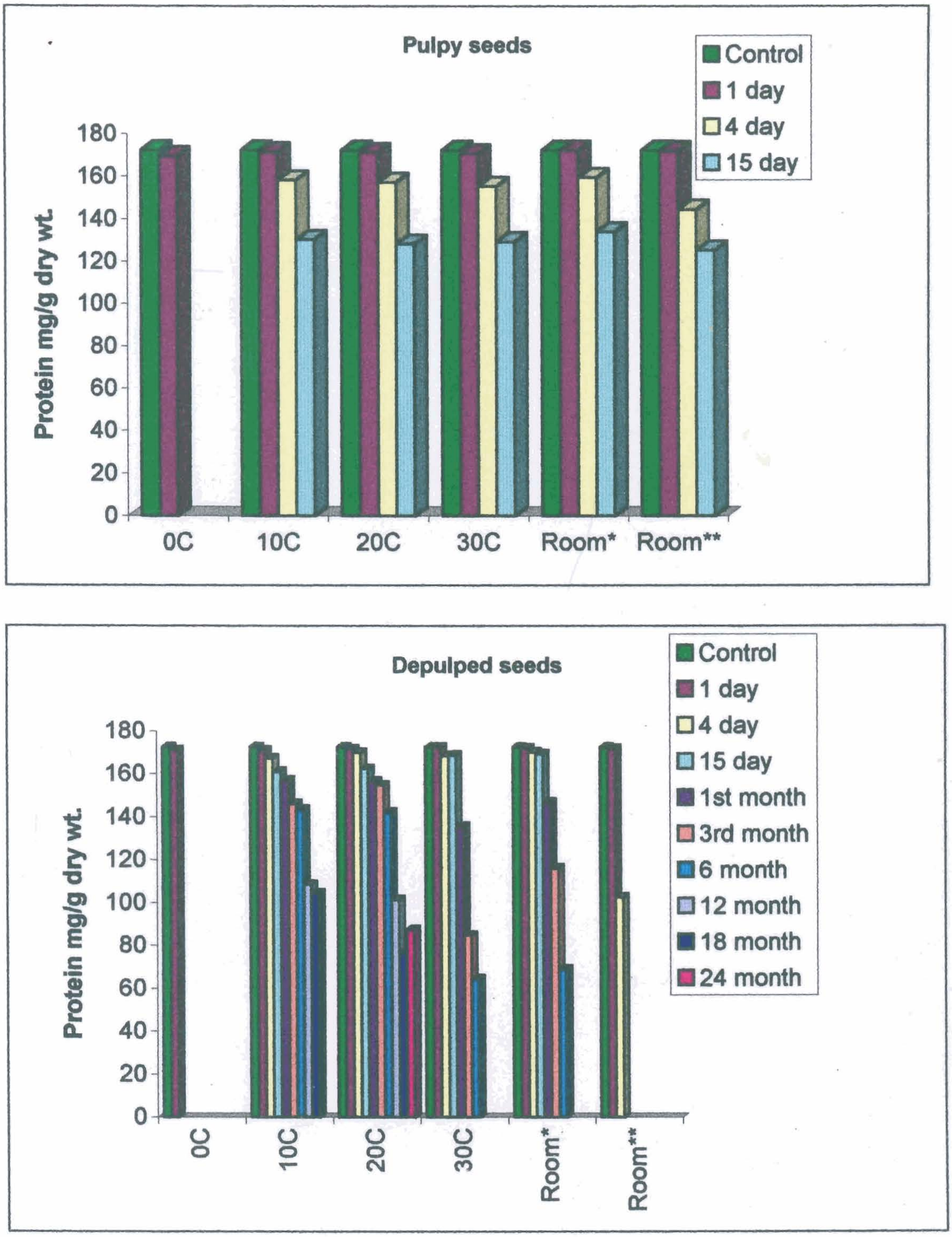
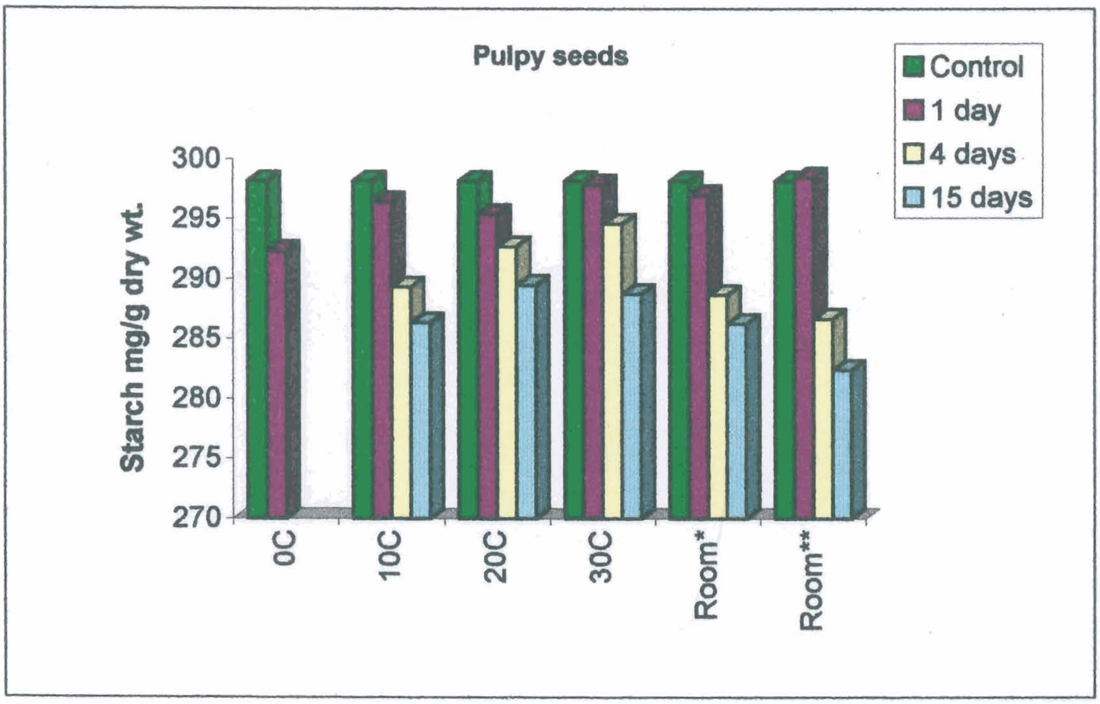


Fig. 8 Protein content of *Syzygium cumini* seeds during storage and germination under different conditions

compared to the control. De-pulped seeds stored at 10 and 20°C showed an insignificant reduction in protein content while seeds stored at 30°C and room polyethylene maintained their protein levels as compared to previous stage. Observations after first month of storage revealed a significant ($P<0.01$, $P<0.01$) reduction of protein content particularly those seeds stored at 30°C and room polyethylene bags. Seeds stored at 10 and 20°C showed only negligible reduction in protein after three months of storage, but seeds stored at 30°C and room (polyethylene) registered a significant ($P<0.01$, $P<0.01$) reduction. De-pulped seeds stored at lower temperatures like 10 and 20°C showed a gradual decrease in their protein content while at higher temperatures like 30°C and room conditions, a drastic reduction in protein content was evident after six month storage. Samples of 12 months of storage again showed significant reduction of protein content compared to previous stage. In the case of de-pulped seeds stored for 18 months at 10°C, showed no change in the protein content but samples of 20°C showed a significant reduction. At 10°C, the protein value of 87.5 mg/g dry weight was noticed along with the loss of viability after a period of 24 months.

Starch

When fresh, seeds contained 298 mg/g dry weight of starch (Table 10, Figure. 10). Twenty four hours of storage resulted in no change of starch content of seeds in all conditions. At 10°C, after four days of storage, pulpy and de-pulped seeds reported a slight reduction of starch. Similarly de-pulped seeds stored for four days at open room conditions reported a significant reduction in starch content. Observations on the 15th day of



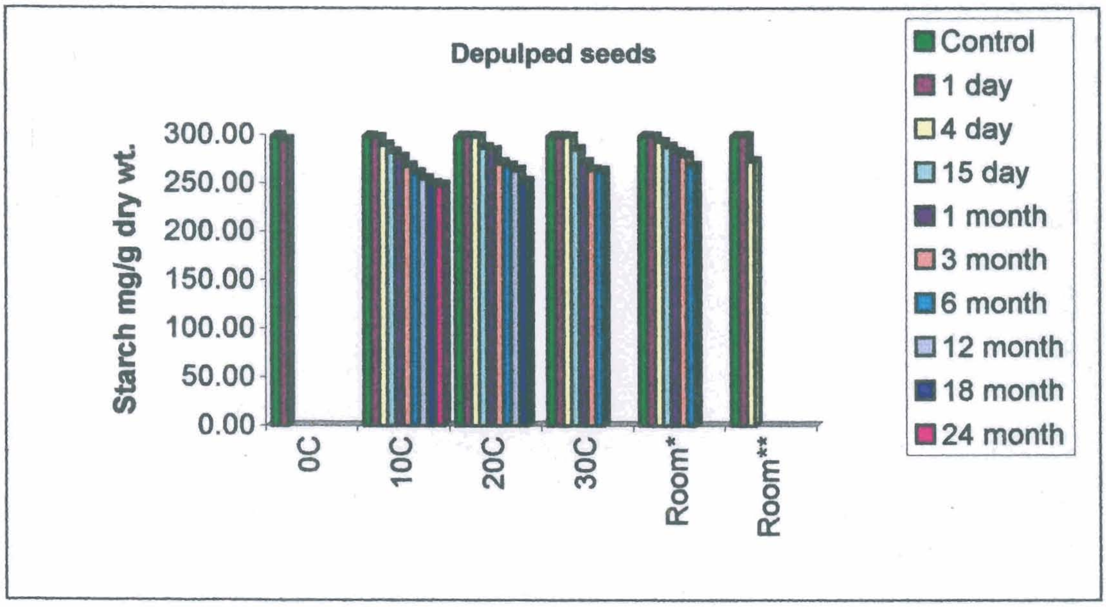


Fig. 10 Starch content of *Syzygium cumini* seeds during storage and germination under different conditions

Table 10 Starch content of *Syzygium cumini* seeds during storage and germination under different conditions

Starch mg/g Dry weight

Treatment		Control	Interval								
			24 hs	4 day	15 day	1 month	3 month	6 month	12 month	18 month	24 month
0°C	P		292.32 ±1.63								
	D		294.46 ±1.31								
10°C	P		296.43 ±1.47	289.32 ±0.72	286.33 ±0.81						
	D		296.57 ±0.68	288.94 ±0.70	281.68 ±0.60	276.58 ±0.90	267.65 ±1.68	260.28 ±1.59	254.46 ±0.88	249.50 ±2.40	247.94 ±1.95
20°C	P		295.41 ±0.86	292.69 ±1.33	289.46 ±2.36						
	D		298.24 ±0.76	297.68 ±0.79	286.66 ±0.87	283.71 ±1.22	270.94 ±1.45	267.52 ±2.38	263.08 ±1.88	251.80 ±1.38	
30°C	P		297.89 ±0.57	294.61 ±1.03	288.74 ±1.20						
	D		298.14 ±0.71	297.74 ±1.40	284.75 ±1.32	271.50 ±1.27	263.85 ±0.69	262.87 ±2.43			
Room*	P		296.98 ±1.45	288.65 ±1.84	286.25 ±2.05						
	D		297.57 ±1.38	292.47 ±1.80	287.44 ±1.83	282.13 ±1.56	277.89 ±0.79	267.79 ±1.69			
Room**	P		298.47 ±1.46	286.66 ±1.70	282.37 ±0.48						
	D	298.17 ±1.25	298.61 ±1.56	272.40 ±0.53							

P - Pulpy seeds; D - Depulped seeds; * - At 25±5°C in polyethylene bags; ** - At 25±5°C and 70% RH in open trays

The values given are the mean of six replicate ± Standard error

storage at all conditions revealed a slight reduction of starch content both in pulpy and de-pulped seeds. After one month of storage, the starch content of de-pulped seeds stored at 30°C became significantly ($P<0.01$) reduced while in other conditions the reduction of starch compared to previous stage was insignificant. The pattern of starch decline after three months of storage indicated more or less similar pattern in all seeds. Seeds stored at 30°C exhibited the least value for starch when compared to starch content of seeds stored at other conditions. Observations on six months of storage revealed the starch content in seeds stored at 30°C maintained stability while in other conditions like 10, 20°C and room a slight reduction of starch occurred. Storage for 12 to 18 months resulted in the further significant ($P<0.01$) decrease of starch within seeds stored at 20°C. Starch content of de-pulped seeds went on decreasing gradually at 10°C to a lower value of 248 mg/g on dry weight basis by the 24th month of storage.

Sugars

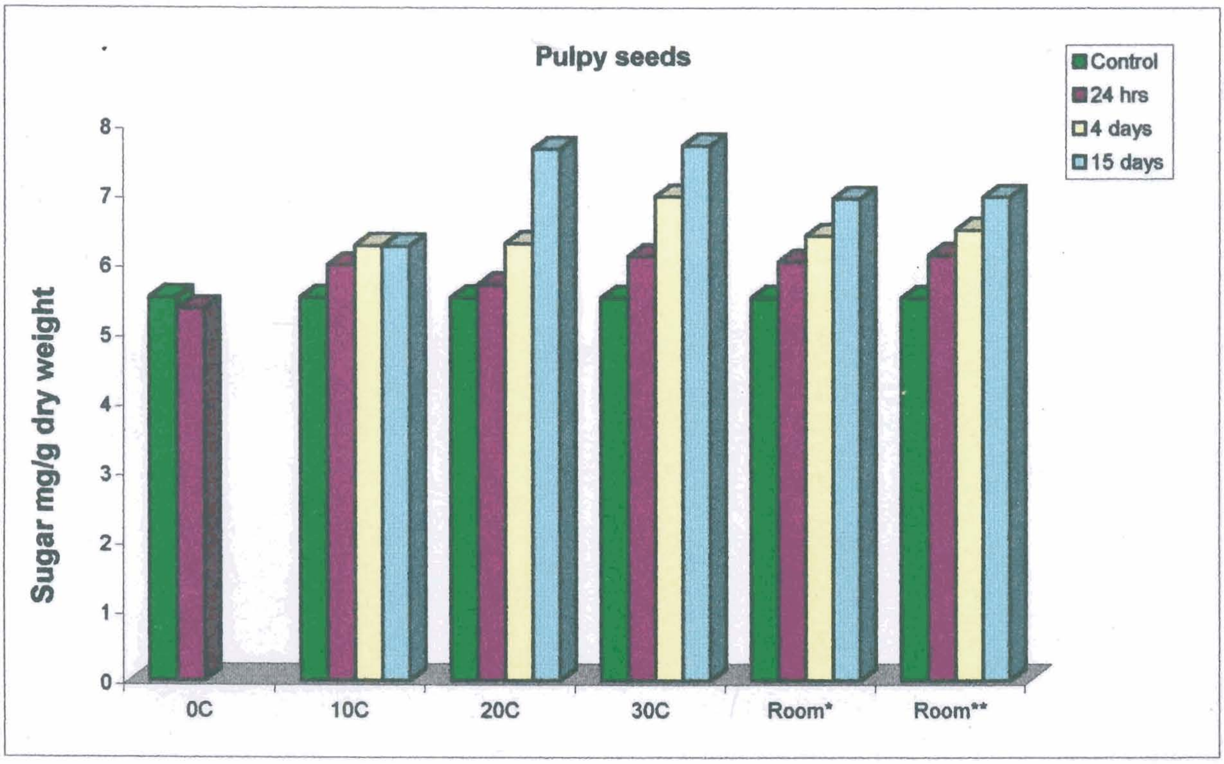
Fresh seeds contained 5.51 mg/g sugar on dry weight basis (Table 12, Figure. 12). After 24 hours of storage, except at 0°C, the pulpy seeds showed an increase in their sugar content particularly a significant ($P<0.01$, $P<0.01$, $P<0.01$) rise at higher temperatures like 30°C and room conditions. Sugar content of de-pulped seeds showed an insignificant reduction irrespective of the storage conditions. Four days of storage enhanced the sugar content of pulpy and de-pulped seeds at an insignificant rate compared to the previous stage in all storage conditions except at open room. De-pulped seeds stored at open room condition

Table 12 Sugar content of *Syzygium cumini* seeds during storage and germination under different conditions

Sugar mg/g Dry weight

Treatment		Control	Interval								
			24 hs	4 day	15 day	1 month	3 month	6 month	12 month	18 month	24 month
0°C	P		5.35 ±0.18								
	D		5.16 ±0.02								
10°C	P		5.98 ±0.16	6.26 ±0.12	7.25 ±0.07						
	D		5.13 ±0.02	5.16 ±0.05	5.20 ±0.04	5.72 ±0.13	5.87 ±0.02	6.37 ±0.06	6.86 ±0.09	7.61 ±0.02	9.02 ±0.08
20°C	P		5.7 ±0.02	6.29 ±0.13	7.65 ±0.02						
	D		5.42 ±0.18	5.52 ±0.08	5.69 ±0.01	6.01 ±0.02	6.30 ±0.04	7.52 ±0.16	8.32 ±0.02	9.09 ±0.03	
30°C	P		6.11 ±0.02	6.97 ±0.09	7.97 ±0.14						
	D		5.37 ±0.04	5.61 ±0.07	5.76 ±0.06	6.03 ±0.01	6.57 ±0.02	9.34 ±0.06			
Room*	P		6.03 ±0.01	6.40 ±0.18	6.94 ±0.09						
	D		5.13 ±0.10	5.21 ±0.01	5.42 ±0.13	5.42 ±0.05	5.59 ±0.01	5.64 ±0.055			
Room**	P		6.13 ±0.03	6.492 ±0.13	6.96 ±0.04						
	D	5.51 ±0.02	5.27 ±0.05	4.69 ±0.003							

P - Pulpy seeds; D - Depulped seeds; * - At 25±5°C in polyethylene bags; ** - At 25±5°C and 70% RH in open trays
The values given are the mean of six replicate ± Standard error



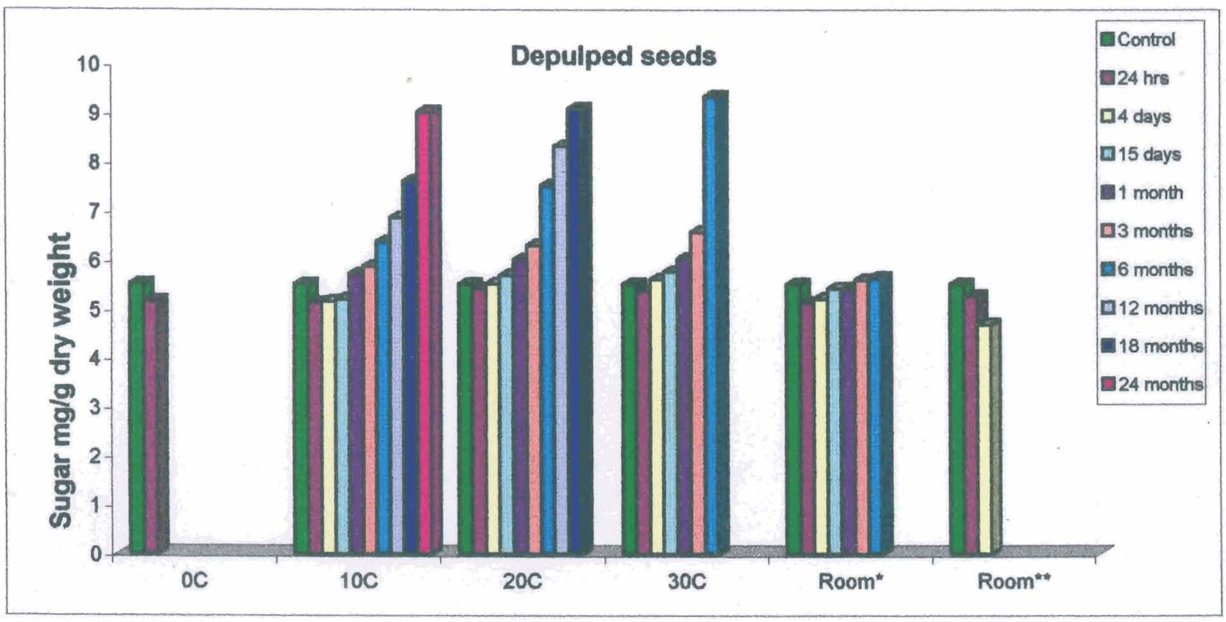


Fig. 12 Sugar content of *Syzygium cumini* seeds during storage and germination under different conditions

experienced a significant reduction of their sugar content simultaneous with the loss of viability. Significant increase of sugar content was observed with the pulpy seeds stored for 15 days at 10, 20 and 30°C ($P<0.01$, $P<0.01$, $P<0.01$ respectively) while at room conditions only an insignificant increase in sugar content was observed. The sugar value of de-pulped seeds was slightly higher than the previous value but was lower than those of pulpy seeds on fourth and 15th day. One month of storage did not change the sugar content of stored seeds at 10°C and room polythene while seeds stored at 20 and 30°C experienced an increase ($P<0.01$, $P<0.01$) of sugar value. Sugar content of de-pulped seeds remained steady as on third month. By six months of storage, a slight increase was noticed in the seeds stored at 20°C and a significant increase in sugar content was observed at 30°C. Seeds stored at 10°C and room polyethylene experienced an insignificant increase in their sugar content. Storage for 12 months resulted in a negligible increase of sugar content in seeds stored at 10 and 20°C compared to the previous stage. The same trend was followed in the next interval (18 months) also. However seeds stored at 10°C for 24 months registered a significant ($P<0.01$) increase.

Phenolics

Originally phenolic content of seeds was 19 mg/g on dry weight basis (Table 14, Figure. 14). Twenty four hours of storing at 0°C enhanced the phenolic content of both pulpy and de-pulped seeds ($P<0.01$, $P<0.01$). Phenolic content of both pulpy and depuplped seeds remained unaltered in all other conditions. By fourth day of storage phenolic contents of pulpy seeds were significantly increased irrespective of storage

Table 14 Phenolics content of *Syzygium cumini* seeds during storage and germination under different conditions

Phenolics mg/g Dry weight

Treatment		Control	Interval								
			24 hs	4 day	15 day	1 month	3 month	6 month	12 month	18 month	24 month
0°C	P		26.18 ±0.04								
	D		28.96 ±0.16								
10°C	P		22.35 ±0.24	25.62 ±0.02	34.75 ±0.30						
	D		19.13 ±0.63	19.12 ±0.23	19.13 ±0.61	20.26 ±0.78	21.10 ±0.18	22.54 ±0.68	25.31 ±0.62	29.65 ±0.67	34.57 ±0.64
20°C	P		19.28 ±0.56	26.68 ±0.32	34.42 ±0.21						
	D		19.18 ±0.41	20.18 ±0.22	22.30 ±0.18	24.56 ±0.29	26.13 ±0.19	30.88 ±0.46	32.81 ±0.19	34.29 ±0.22	
30°C	P		19.35 ±0.53	27.18 ±0.72	34.85 ±0.31						
	D		19.74 ±0.45	20.25 ±0.65	23.67 ±0.44	26.87 ±0.21	29.27 ±0.15	34.34 ±0.22			
Room*	P		20.17 ±0.14	28.25 ±0.18	34.71 ±0.18						
	D		19.03 ±0.61	20.08 ±0.53	21.98 ±0.17	27.76 ±0.19	29.81 ±0.16	29.12 ±0.04			
Room**	P		20.14 ±0.57	25.64 ±0.75	31.97 ±0.10						
	D	19.164 ±0.124	21.27 ±0.64	27.58 ±0.24							

P - Pulpy seeds; D - Depulped seeds; * - At 25±5°C in polyethylene bags; ** - At 25±5°C and 70% RH in open trays
The values given are the mean of six replicate ± Standard error

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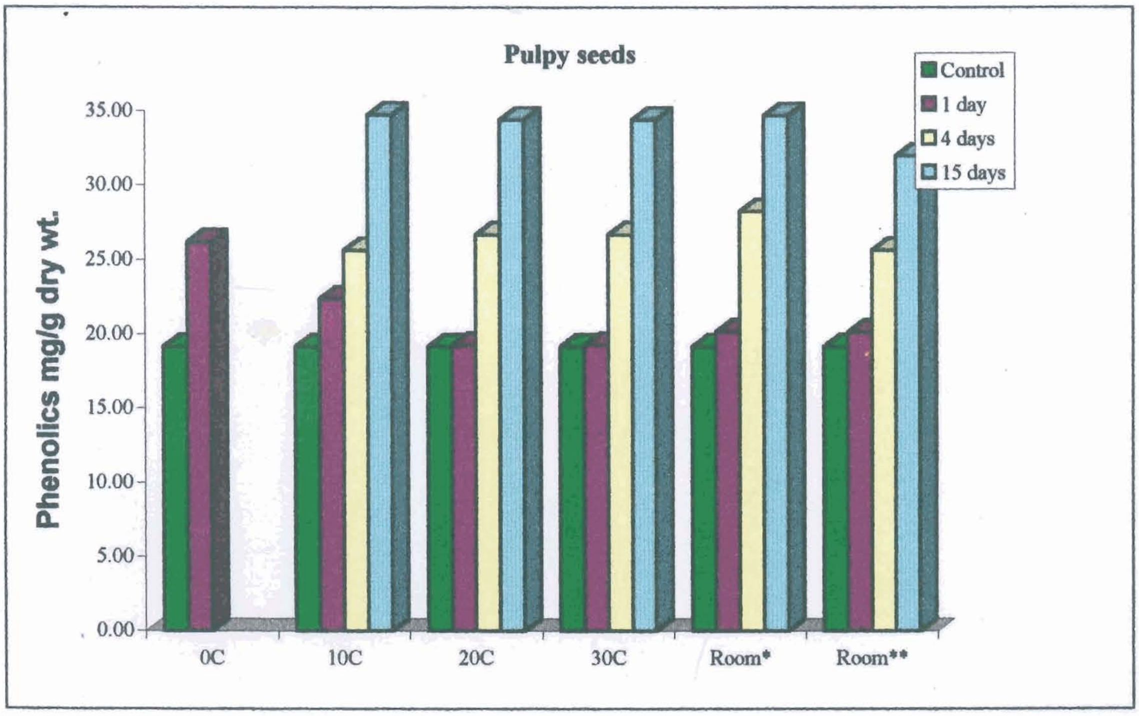

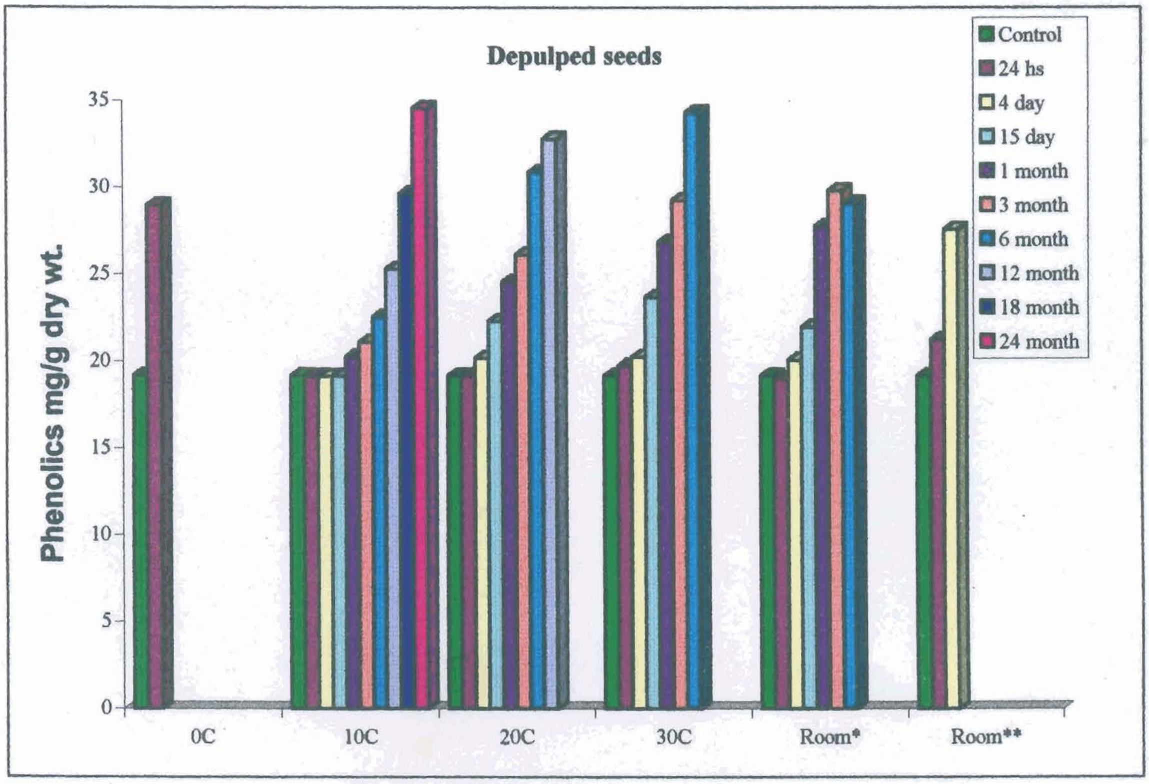

Fig. 14 Phenolics content of Syzygium cumini seeds during storage and germination under different conditions

conditions. De-pulped seeds stored at open room conditions exhibited a hike in their phenolic content while among rest of the storage conditions only an insignificant increase of phenolics was noticed. Fifteen days of storage resulted in a significant increase of phenolic content of pulpy seeds in all storage conditions while de-pulped seeds showed only insignificant increase compared to previous interval. Observations on de-pulped seeds after one month showed that at $0^{\circ}C$ the phenolic content remained unaltered, but at 20, $30^{\circ}C$ and room polyethylene a significant increase of phenolics was observed. During the third month of storage phenolic content of seeds were increased at an insignificant rate compared to the previous stage. By the sixth month of storage, phenolic content of seeds stored at room polyethylene remained the same as the third month, while seeds stored at 20 and $30^{\circ}C$ experienced a significant increase ($P<0.01$, $P<0.01$) but at $10^{\circ}C$ it was insignificant. After 12 months of storage the phenolics of seeds stored at 10 and $20^{\circ}C$ insignificantly increased. Observations made with de-pulped seeds stored for 18 months at 10 and $20^{\circ}C$ as well as with the de-pulped seeds stored for 24 months at $10^{\circ}C$ showed an insignificant increase of phenolic content compared to corresponding previous stages ($P<0.01$). An interesting point was that the final value of phenolic content with seeds stored at 0, 10, 20, $30^{\circ}C$, room polyethylene and room open were almost same and all were significantly at an increased rate than the control.

Chlorophyll

Fresh seeds contain 1.5 mg Chl_a, 1.3 mg Chl_b, 2.9 mg total chlorophyll and Chl_a/Chl_b ratio of 1.1 per gram tissue (Table 16, Figures. 16a, 16b,

Table 16 Chlorophill Content of *Syzygium cumini* seeds during storage and germination under different conditions

Treatment			Chlorophyll on mg/g dry weight									
			Interval									
			Control	24 hrs	4 day	15 days	1 month	3 month	6 month	12 month	18 month	24 month
0°C	P	a		0.751 ± 0.001								
		b		0.667 ± 0.016								
		t		1.579 ± 0.016								
		a/b		1.126 ± 0.0625								
	D	a		0.748 ± 0.002								
		b		0.672 ± 0.029								
		t		1.568 ± 0.021								
		a/b		1.113 ± 0.068								
10°C	P	a		0.751 ± 0.009	0.747 ± 0.007	0.723 ± 0.003						
		b		0.672 ± 0.083	0.663 ± 0.078	0.651 ± 0.047						
		t		1.407 ± 0.024	1.381 ± 0.014	1.391 ± 0.019						
		a/b		1.118 ± 0.108	1.127 ± 0.090	1.111 ± 0.064						
	D	a		0.756 ± 0.004	0.752 ± 0.003	0.747 ± 0.002	0.703 ± 0.008	0.678 ± 0.006	0.582 ± 0.003	0.495 ± 0.002	0.358 ± 0.003	0.276 ± 0.002
		b		0.676 ± 0.048	0.674 ± 0.054	0.671 ± 0.045	0.666 ± 0.061	0.657 ± 0.086	0.651 ± 0.091	0.645 ± 0.068	0.586 ± 0.054	0.432 ± 0.044
		t		1.442 ± 0.013	1.439 ± 0.007	1.434 ± 0.019	1.407 ± 0.020	1.378 ± 0.009	1.287 ± 0.021	1.145 ± 0.024	0.927 ± 0.019	0.807 ± 0.011
		a/b		1.118 ± 0.083	1.116 ± 0.056	1.113 ± 0.044	1.056 ± 0.131	1.032 ± 0.069	0.894 ± 0.033	0.767 ± 0.029	0.611 ± 0.055	0.639 ± 0.045
20°C	P	a		0.744 ± 0.007	0.722 ± 0.008	0.682 ± 0.008						
		b		0.663 ± 0.064	0.636 ± 0.097	0.577 ± 0.031						
		t		1.433 ± 0.022	1.366 ± 0.018	1.012 ± 0.008						
		a/b		1.122 ± 0.109	1.135 ± 0.082	1.182 ± 0.258						
	D	a		0.702 ± 0.006	0.748 ± 0.008	0.743 ± 0.006	0.717 ± 0.007	0.654 ± 0.008	0.577 ± 0.006	0.458 ± 0.005	0.347 ± 0.002	
		b		0.671 ± 0.063	0.671 ± 0.097	0.668 ± 0.039	0.649 ± 0.058	0.603 ± 0.009	0.597 ± 0.082	0.569 ± 0.074	0.555 ± 0.057	
		t		1.440 ± 0.007	1.406 ± 0.015	1.397 ± 0.005	1.346 ± 0.028	1.277 ± 0.012	1.175 ± 0.017	0.976 ± 0.021	0.802 ± 0.016	
		a/b		0.690 ± 0.095	1.115 ± 0.082	1.112 ± 0.154	1.105 ± 0.121	1.085 ± 0.862	0.966 ± 0.073	0.805 ± 0.067	0.625 ± 0.035	
30°C	P	a		0.743 ± 0.011	0.724 ± 0.007	0.634 ± 0.008						
		b		0.654 ± 0.099	0.621 ± 0.079	0.543 ± 0.069						
		t		1.427 ± 0.012	1.352 ± 0.018	0.914 ± 0.015						
		a/b		1.136 ± 0.111	1.166 ± 0.089	1.168 ± 0.115						
	D	a		0.758 ± 0.004	0.747 ± 0.007	0.741 ± 0.002	0.733 ± 0.006	0.697 ± 0.010	0.664 ± 0.006			
		b		0.670 ± 0.067	0.667 ± 0.073	0.663 ± 0.023	0.638 ± 0.087	0.576 ± 0.005	0.543 ± 0.097			
		t		1.432 ± 0.012	1.403 ± 0.008	1.397 ± 0.022	1.224 ± 0.021	1.021 ± 0.014	0.907 ± 0.011			
		a/b		1.131 ± 0.059	1.120 ± 0.096	1.118 ± 0.086	1.149 ± 0.069	1.021 ± 1.980	1.223 ± 0.105			
Room*	P	a		0.751 ± 0.006	0.717 ± 0.004	0.619 ± 0.009						
		b		0.669 ± 0.087	0.651 ± 0.086	0.556 ± 0.087						
		t		1.430 ± 0.014	1.373 ± 0.015	1.021 ± 0.014						
		a/b		1.123 ± 0.069	1.101 ± 0.047	1.113 ± 0.103						
	D	a		0.753 ± 0.007	0.707 ± 0.006	0.668 ± 0.008	0.611 ± 0.003	0.368 ± 0.012				
		b		0.667 ± 0.073	0.662 ± 0.089	0.648 ± 0.078	0.607 ± 0.075	0.572 ± 0.098				
		t		1.433 ± 0.011	1.381 ± 0.007	1.287 ± 0.012	1.023 ± 0.022	0.814 ± 0.022				
		a/b		1.129 ± 0.096	1.068 ± 0.067	1.031 ± 0.103	1.007 ± 0.040	0.643 ± 0.122				
Room**	P	a		0.749 ± 0.011	0.657 ± 0.007	0.586 ± 0.012						
		b		0.667 ± 0.075	0.620 ± 0.069	0.570 ± 0.077						
		t		1.440 ± 0.009	1.433 ± 0.017	1.254 ± 0.008						
		a/b		1.123 ± 0.147	1.060 ± 0.101	1.028 ± 0.156						
	D	a	0.758 ± 0.001	0.698 ± 0.003	0.317 ± 0.004							
		b	0.676 ± 0.014	0.569 ± 0.063	0.451 ± 0.087							
		t	1.442 ± 0.017	1.134 ± 0.018	0.873 ± 0.015							
		a/b	1.121 ± 0.071	1.227 ± 0.048	0.703 ± 0.046							

P - Pulpy seeds; D - Depulped seeds; * - At 25 ± 5°C in polyethylene bags; ** - At 25 ± 5°C and 70% RH in open trays; a - $Chlorophyll_a$; b - $Chlorophyll_b$; t - Total chlorophyll; a/b - $Chlorophyll_a$/$chlorophyll_b$ ratio.
The values given are the mean of six replicates ± standard error.

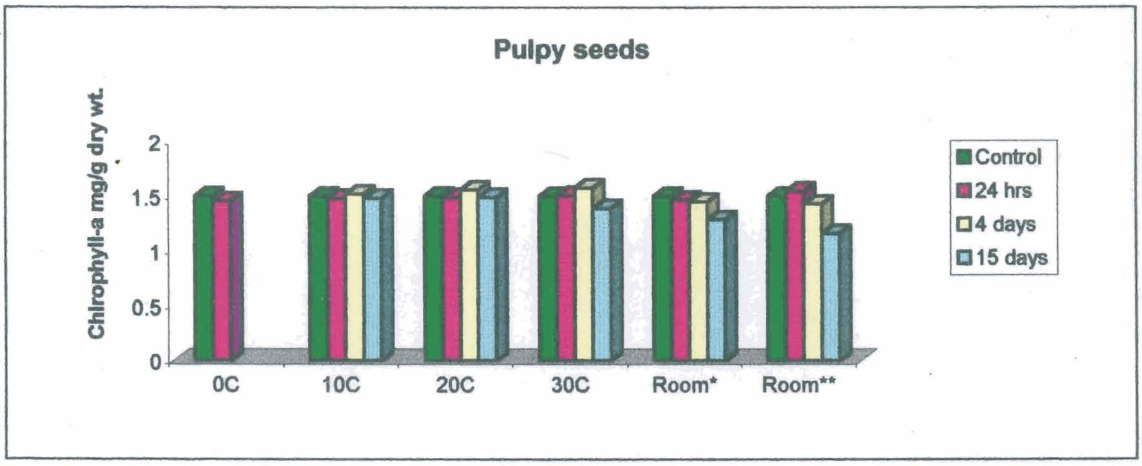

Fig. 16a Chlorophyll-a content of *Syzygium cumini* seeds during storage and germination under different conditions

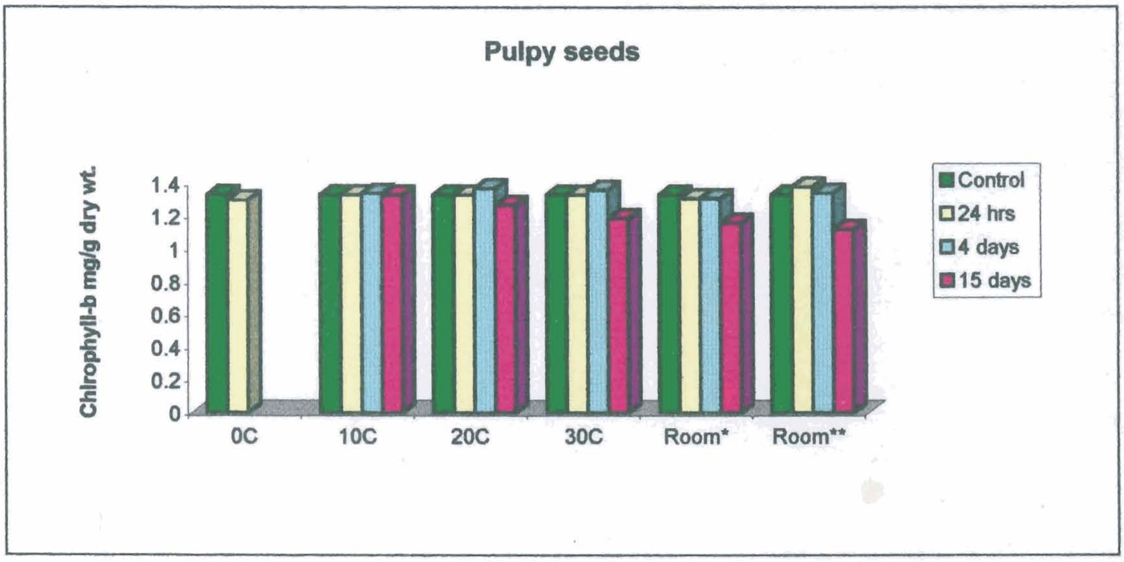

Fig. 16b Chlorophyll-b content of *Syzygium cumini* seeds during storage and germination under different conditions

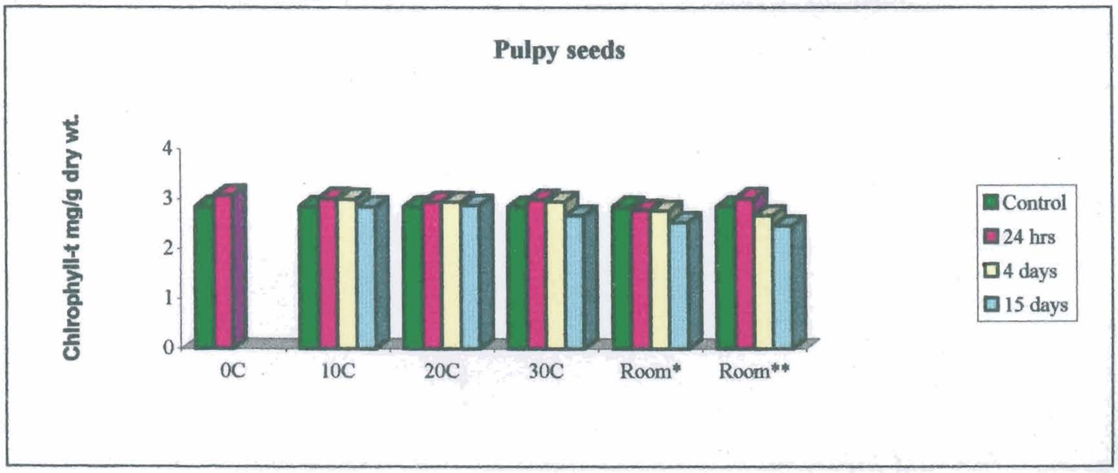

Fig. 16c Chlorophyll-t content of *Syzygium cumini* seeds during storage and germination under different conditions

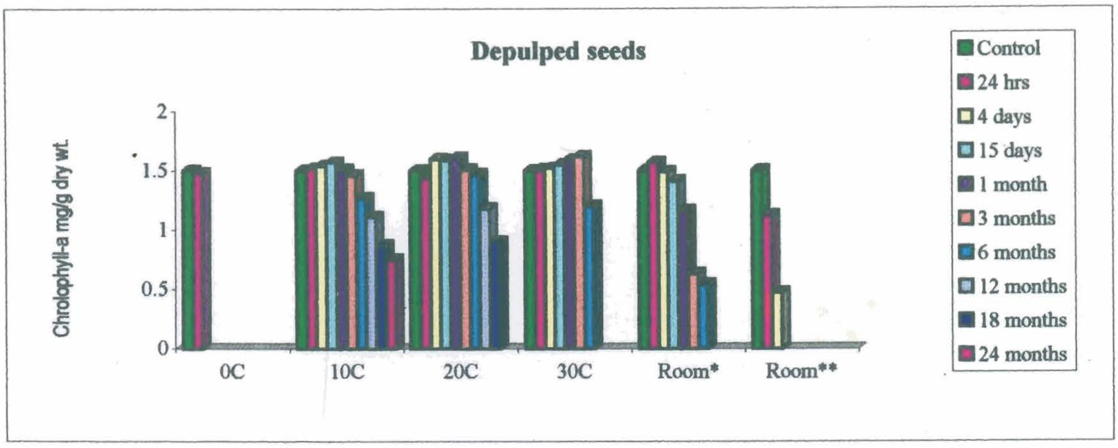

Fig. 16d Chlorophyll-a content of *Syzygium cumini* seeds during storage and germination under different conditions

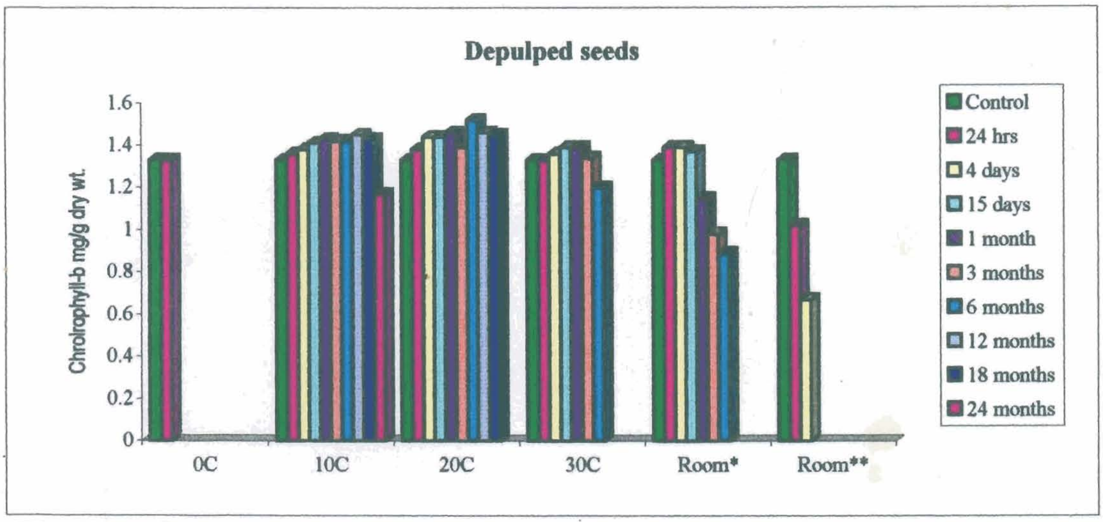

Fig. 16e Chlorophyll-b content of *Syzygium cumini* seeds during storage and germination under different conditions

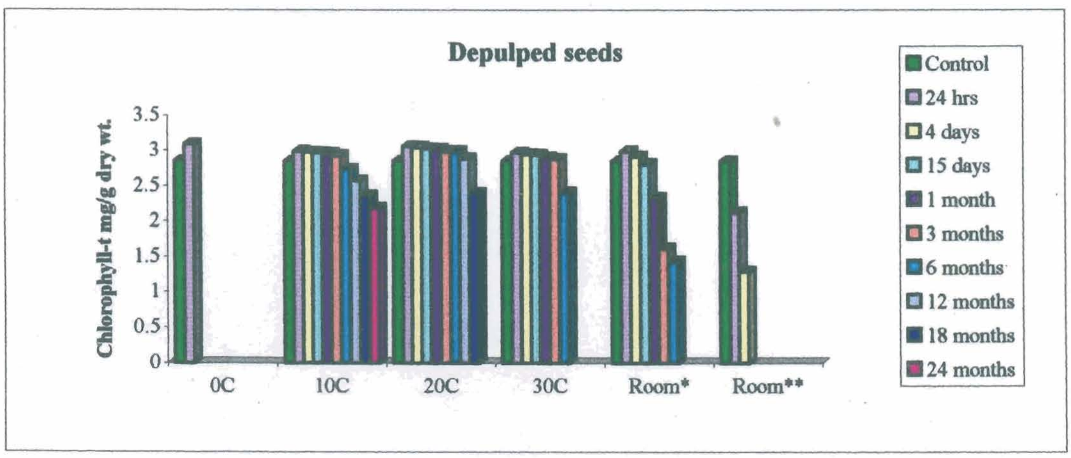

Fig. 16f Chlorophyll-t content of *Syzygium cumini* seeds during storage and germination under different conditions

16c, 16d, 16e and 16f). After 24 hours of storage, a decrease of the chlorophyll content was observed only in room open de-pulped condition while in all the other conditions the chlorophyls remained unchanged. Four days of storage at open room conditions resulted in significant reduction of chlorophyll content in de-pulped seeds. Observations by the 15^{th} day of storage revealed an insignificant decline of chlorophyll content of puplpy seeds, particularly those seeds stored at 30°C and room conditions. Irrespective of the difference in storage conditions, de-pulped seeds maintained their original chlorophyll values up to 15 days of storage. Chlorophyll contents remained the same after one month of storage at 10, 20 and 30°C while that at room conditions a significant reduction was noticed ($P<0.01$). This trend was repeated with the observations made after three months of storage but at room conditions a drastic reduction of chlorophyll were observed. Chlorophyll values obtained with six month stored de-pulped seeds found that at 10°C a negligible reduction of Chl_a occurred while Chl_b, total chlorophyll and Chl_a/Chl_b ratio remain unchanged compared to the previous values. Chlorophyll value with seeds stored at 20°C remained unchanged and at 30°C as well as at room conditions, six month stored seeds showed an insignificant reduction. Observations made during 12^{th} month showed an insignificant reduction of chlorophyll but by 18^{th} month a significant reduction in Chl_a was noticed ($P<0.01$) while Chl_b, total chlorophyll remained unchanged compared to the previous values. De-pulped seeds stored at 10°C experienced a reduction in chlorophyll at the slowest rate and thus by the 24^{th} month of storage, their Chl_a, Chl_b, total chlorophyll and Chl_a/Chl_b ratio become 0.75, 1.2, 2.2 and 0.6 mg/g respectively.

DISCUSSION

Anil Kumar. C "Physiological and biochemical aspects of seed storage and viability in Syzygium species." Thesis. Department of Botany, University of Calicut, 1998

DISCUSSION

There are many characteristics specific to recalcitrant seeds; perhaps the most documented of these is that they are desiccation sensitive. Fresh seeds of *S.aromaticum* containing about 50% moisture content show cent percent viability only up to 12 hours after harvest. The moisture content of recalcitrant seeds from tropical and temperate tree species is often observed to be between 40 and 60% (Nautiyal and Purohit, 1985a). The desiccation rate in *Syzygium aromaticum* seeds is very fast under room conditions and the critical moisture content is found to be about 40% (Table 1). After two days, the moisture content is only 33% and the viability is almost lost. The seeds cannot tolerate even a desiccation due to 3% reduction in moisture content occurring during a period of 12 hours. Earlier, Purseglove *et al.* (1981) reported that clove (*Syzygium aromaticum*) seeds retained viability only for a limited period.

In *S.cumini* also the moisture content is about 50% in fresh seeds and desiccation rate is comparatively slow. After 24 hours, under room conditions, the viability is cent percent, while six percent reduction in moisture content occurs. So these seeds are more desiccation tolerant than *S.aromaticum* seeds and they can tolerate the loss of about nine percent moisture content (Table 2). The viability of *S.cumini* seeds decreases gradually and after five days the rate of germination become very low. Drastic reduction of viability occurs between moisture content of 40 and 34% during a period of 3 days. So critical moisture content is about 35%. Information on critical moisture content below which dehydration injury (desiccation) have been reported in many recalcitrant plants like *Quercus* *rubra* (Pritchard, 1991; Finch-savage, 1992), Dipterocarp (Tompsett, 1992) and *Araucaria hunsteinii* (Pritchard *et al.*, 1995).

Considerable differences are observed in the critical moisture content and longevity under room conditions, between *S.aromaticum* and *S.cumini*. Nevertheless, seeds of both *S.aromaticum* and *S.cumini* are very sensitive to loss of moisture content so highly recalcitrant in nature. Many recalcitrant seeds have been reported to lose viability due to a slight fall in their moisture content. Cacao seeds which tend to lose viability if their moisture content falls below 27% (Hor *et al.*, 1984). Chien and Lin (1997) reported loss of viability in *Machilus kusanoi* seeds when moisture content reduces from 51.5 to 44.6%. In *Shorea robusta* (Nautiyal and Purohit, 1985a) the seeds lost viability when initial moisture content (50%) was reduced to 25%, *Camelia sinensis* seeds damaged due to desiccation when dried to 28% moisture content (Sebastiampillai and Anandappa, 1979). Similarly Anil Kumar *et al.* (1996) reported that *Aporusa lindleyana* seeds with 40% moisture content lost viability as the moisture content was reduced to 30% during a period of 36 hours. Like *Syzygium*, many recalcitrant seeds are reported to be highly sensitive to desiccation. Seeds of *Avicinnia marina* (Farrant *et al.*, 1988) and *Araucaria hunsteinii* (Pritchard *et al.* 1995) are reported as recalcitrant which are highly sensitive to desiccation. Nevertheless, the desiccation process has not yet been described in terms of water potential distribution. However, Probert and Brierley (1989) reported that recalcitrant seeds like *Zizania palustris, Spartina anglica* and *Portersia coarctata* are damaged by desiccation below a water potential about –1.5 to –3.0 MPa. Sensitivity of recalcitrant seeds towards desiccation and the critical moisture content have been reported in many plants and the desiccation and resultant viability loss have also been interpreted in terms of metabolic changes at ultra-structural levels. Chin *et al.* (1981) explained that at ambient temperatures, *Hevea* seeds undergo dehydration and deteriorate rapidly. During the deterioration, abundant fatty acids present in the seeds under go auto-oxidation of unstable fatty acids resulting in the release of free radicals which are highly reactive and are able to inactivate enzymes, denaturation of histones and damage of DNA and final death of seeds. Priestly (1986) stated that during seed storage the deterioration which precedes death is due to the changes in macromolecules like proteins, nucleic acids, lipids etc. and this occurs at very low water potential. So the seed deterioration is not due to metabolic change but directly related to water potential (Roberts and Ellis, 1989).

Most important diagnostic feature of recalcitrant seeds is that they cannot be dried without damage and the high moisture that inspires the germination appears to vary considerably among different species. Dry weight distribution of both species of *Syzygium* seeds during storage is found to be correlated with storage conditions and seeds viability. The presence of pulp in these seeds is showing an additional effect compared to the de-pulped seeds under storage.

Both pulpy and de-pulped seeds of *S.aromaticum* and *S.cumini* lost viability in 24 hours without any change in dry weight percentage at $0^{\circ}C$. Since the seed contains about 50% moisture content, at $0^{\circ}C$ they are subjected to chronic freezing injury resulting in loss of viability. This is characteristic of almost all recalcitrant seeds (Chin, 1988; Ellis, 1991).

Hevea seeds become dead within three to four hours at 0°C (Chin *et al.*, 1981). Exceptionally *Quercus alba*, the most recalcitrant of *Quercus* species, seeds do not suffer chilling injury at 0 to 15°C (Clatterbuck and Bonner, 1985). Moreover, these seeds are tolerant to low temperatures and are capable of initiating germination at 2°C as evidenced by root protrusion after eight months in storage. According to Tompsett and Pritchard (1993) *Aesculus hippocastanum* seeds chilling causes no injury, but prolonged chilling is very essential to break dormancy, then only the seeds become germinable at 26°C.

One of the characters of recalcitrant seeds is response to chilling which depends on the nature and moisture content of seeds and varies from species to species. According to Lyons (1973) – Lyons – Raison hypothesis, the primary cause of chilling injury is due to the physical responses of membrane lipids at low temperature. These authors further explained that membrane lipids of chilling sensitive seeds undergo a phase change. Despite many reports of chilling effect on recalcitrant seeds (Chin *et al.*, 1981, 1989; Tompsett and Pritchard, 1993), the interpretation of chilling injury and resultant viability loss in seeds is found to be scanty, particularly the metabolic and ultrastructural changes. However, response of plan to low temperature, particularly metabolic changes are given by Graham and Patterson (1982) and many of their interpretations are applicable to chilling effect of recalcitrant seeds. According to these authors, the primary effect of cold temperature on plants is the effect on fluidity of membrane lipids which is likely to affect lipid associated enzymes of plant membranes. The membrane proteins, cytskeletal proteins and ion movement are found to be affected by chilling leading to injury and death. At low temperature, the enzyme Phenylalanine ammonia lyase (PAL) is found to increase resulting in an accumulation of phenolics. Superoxide dismutase (SOD) activity is reduced at chilling temperature. So superoxide is getting accumulated resulting in peroxidation of double bonds of lipids. All these changes lead to injury of cells exposed to chilling temperatures. Similar interpretation has been given by Bewley (1979) for the desiccation sensitivity of recalcitrant seeds.

As the temperature of storage increased to 10°C, viability of pulpy seeds in both species was found to be decreased gradually and finally lost by 15 days without any change in dry weight percentage. The cause of this viability loss is gradual chilling injury of the seeds at 10°C. Pulpy seeds of *S.aromaticum* stored at 20 and 30°C showed further reduction in viability without any marked change in dry weight percentage. Here the viability loss may be mainly due to inhibitory affect of pulp (Mayer and Poljakoff-Mayber, 1989) which is undergoing metabolic changes and tissue deterioration by fermentation and microbial infection, under these temperatures. According to Roberts (1972) fungi present in fruits are active at moisture content in equilibrium with 65% relative humidity and above, but not at moisture content lower than about 12%. In the present investigation the relative humidity at 20 and 30°C is 80% and hence optimum RH is prevailed for microbial growth. By the present author, microbial infection and their elaborated growth has been observed in the pulpy seeds of *Syzygium* (unpublished data). Removal of the pulp from the seed enhanced the germination in *Hancornia* (Parente and Machado, 1986). Stimulatory effect of de-pulping on seed germination in

Hancornia speciousa has been reported by Oliveira and Valio (1992).. A similar effect has been reported in *Quercus robur* in which the pericarp induces some restriction on germination (Finch-savage, 1992). However in *Syzygium cumini*, the inhibitory affect of pulp at 20 and 30°C is comparatively low.

De-pulped fruit is considered to be the actual seed in *Syzygium aromaticum* and *Syzygium cumini*, since the fruit is drupe (Purseglove *et al.*, 1981) containing a single seed and storage is possible only on de pulped seeds since pulp inhibits germination as described elsewhere. So the experimental studies on storage and germination were concentrated on de pulped seeds of *S.aromaticum* and *S.cumini*.

De-pulped seeds of both species cannot tolerate 0°C even for one day's storage (Table 3 and 4). Since the testa is very thin and papery, seeds which contain about 50% of moisture content, direct exposure to freezing temperature imparts chilling injury which is detrimental to viability, cessation of enzyme activity and injury to cell membranes and organelles. Because of elevated moisture content, recalcitrant seeds are unstorable by any conventional methods. Furthermore the seeds deteriorate rapidly under storage conditions (Berjak *et al.*, 1990; Farrant *et al.*, 1988, 1992). According to Pammenter *et al.* (1984) initiation of germination occurs in storage and the continuation of germination process immediately after shedding in *Avicennia marina* seeds is responsible for deterioration in storage. Notwithstanding, many recalcitrant seeds show progress in visible germination during storage (Berjak *et al.*, 1989; Farrant *et al.*, 1989; Finch-savage, 1992). Though recalcitrant seeds germinate at chilling temperatures in storage condition, at freezing temperature (0°C) no seed is reported germinating. However, the minimum temperature for germination in *Quercus robur* seed has been estimated to be 1°C (Pritchard and Manger, 1990).

Interaction between seed moisture content and storage temperature in cocoa (Hor *et al.*, 1984) showed that moisture content is more important than storage temperature that is storage at temperatures 30 and 22°C were found to be equally good for germination but when moisture content reduced from 32 to 27%, the germination was adversely affected at the same temperature. The moisture content and room temperature that is 25±5°C at (open condition) are found to be affecting germination in both species of *Syzygium* (Fig. 1 to 4b). *S.aromaticum* seeds which is considered as more recalcitrant shows the standard viability (germination percentage above 70) only for a short period during which only slight reduction of moisture content occurs. On the other hand, the seed technological viability of *Syzygium cumini* seeds is maintained for more days and during this period a significant reduction in moisture content was observed. This character can be correlated with the habitat of the species as mentioned earlier. However, under storage, viability and storage temperature are interrelated with species i.e., 10°C for *S.cumini,* 30°C for *S.aromaticum*.

Seeds of *S.aromaticum* kept at 10°C and 80% RH registered no change in dry weight percentage, but viability was getting reduced gradually and completely lost by two months storage, in contrast, the seeds of *S.cumini* exhibited gradual and significant decline in dry weight percentage. Full viability was retained up to three months and afterwards a gradual reduction and complete loss occurred only after 24 months of storage at 10°C.

Storage studies on *S.aromaticum* and *S.cumini*, identifies drastic difference in storage temperature. Seed longevity of *S.aromaticum* is (9 months) maximum at 20°C where as that of *S.cumini* (24 months) is at 10°C. Nevertheless, both species are chilling sensitive and viability loss is gradual and cumulative at 10 to 20°C respectively to *S.aromaticum* and *S.cumini*. In both species seeds under go a gradual chilling effect and viability loss is manifested earlier in *S.aromaticum* than *S.cumini*. It is recalled that desiccation sensitivity is more in *S.aromaticum* compared to *S.cumini*. Apart from this, in *S.cumini* the prolonged storage is possible at 10°C. So these seeds are comparatively less sensitive to chilling upto at least 6 months since the seeds / seedlings are actively growing (radicle alone) at this temperature, while in *S.aromaticum* the radicle growth is suppressed. The significant reduction of dry weight percentage at this storage condition is probably due to high metabolic activity and reserve utilization as the seeds/ seedling show profuse radicle elongation. Even after a storage period of two years, these stored *S.cumini* seeds / seedlings can be considered as stored germ plasm for retrieving healthy trees. Since the stored seeds are already sprouted under storage, germination rate or seedling vigour is not at all affected adversely. Contradictory to this findings Sabale *et al.* (1992) revealed that storability is possible, (even though the temperature is not specified) in clove seeds and according to them, the length of storage period has significant influence on germination percentage and the seedling vigour also is affected adversely in stored seeds. Contradictory to this, it has been proved that transferring the sprouted seeds to soil condition is superior than direct sowing (Sabal *et al.*, 1995).

At 20°C and 80% RH, *S.aromaticum* seeds are viable for six months without any change in dry weight percentage but 15 days onwards radicle growth started. In contrast with *S.aromaticum*, *S.cumini* seeds show storability up to 18 months at this temperature (20°C) and only radicle protrusion started but no further growth occurred. So the metabolic (respiration) changes are only feeble, resultantly no change in dry weight percentage.

At 30°C and 80% RH, the viability of *S.aromaticum* seeds was retained up to nine months. In contrast to that of 20°C, the seeds at 30°C registered a gradual as well as a significant decline in dry weight percentage after two months, maximum being at ninth month (Table 3). In this condition the seeds starts radicle elongation on 15th day onwards and lateral root formation up to ninth month. The vigorous growths of seeds / seedlings at 30°C resulted in a significant loss of dry weight.

In *S.cumini* at 30°C, the viability is retained up to six months. But dry weight percentage is significantly reduced at all intervals till sixth month. In contrast to *S.aromaticum*, the seeds of *Syzygium cumini* started radicle as well as plumule growth from 15th day onwards resulting high reduction in dry weight percentage (Table 4).

Many plants such as *Theobroma cacao* (Hor *et al*., 1984) possess recalcitrant seeds where the storage temperature for better storability has been reported on the basis of varying moisture content. The best storage condition was found to be with 33-35% of moisture content at 17°C. Generally the seeds of *Syzygium, S.aromaticum* in particular, are very sensitive to desiccation that even the loss of three percent moisture content is not tolerated by these seeds. So the best storage temperature could only be standardised in fresh seeds where the moisture content is about 50 % (Table 1).

Seeds of *S.aromaticum* stored in polyethylene bags at room temperature (25±5°C) retained their viability up to one month only and during this period there is a gradual increase in dry weight percentage contradictory to the other storage conditions because the desiccation is found to be occurred at this temperature.

S.cumini seeds kept in polyethylene bags at room temperature, prolonged their viability up to three month with a marked increase in dry weight percentage. The increased dry weight percentage is presumably due to desiccation (Fig. 6b). At room temperature (25±5°C) the RH is 65% which is comparatively lower than that of the chamber (at 30°C) where the relative humidity is 85% that prevents desiccation to some extent. So the viability loss is evidently due to desiccation in both species of *Syzygium* where seeds are stored at room temperature in polyethylene bags.

When a comparison is made on the *S.aromaticum* and *S.cumini* seeds stored in polyethylene bags at the room condition, the magnitude of longevity vary considerably (Table 3 and 4). Despite the same moisture content *S.cumini* seeds exhibited three times increase in longevity compared to *S.aromaticum*, may be due to some adaptation pertaining to their habitats. *S.aromaticum* belongs to tropical habitat characterised by warm and humid climate while *S.cumini* grows profusely at subtropical regions. In addition to the difference of the optimum temperature for better storage between the *Syzygium* species, significant difference in seed viability period was observed among *S.aromaticum* and *S.cumini* seeds under storage also (Table 3 and 4). According to Pammenter *et al.* (1994) recalcitrant seeds of tropical origin are metabolically very active especially during the early days of storage and the changes are similar to germination. Recalcitrant seeds of subtropical species also germinate in storage with a metabolism similar to tropical seeds but at a slower rate (Finch-savage, 1992). Pammenter et al. (1994) also stated that according to thumb rule the life span of recalcitrant seeds is related to their natural habitat. The warmer and wetter the natural habitat, the shorter the viability. So seeds of tropical plants are having shorter life span than that of subtropical plants.

According to Bewley (1979), generally recalcitrant seeds are highly susceptible to desiccation immediately after shedding from the mother plant. The seeds start radicle protrusion as a continuation of seed development. In many recalcitrant seeds, development and germination appear to be more of a continuum (Farrant *et al.*, 1986, 1988; Ellis, 1991) germination under storage at varying temperatures and relative humidity also have been reported in many recalcitrant seeds (Pammenter *et al.*, 1984; Berjak *et al.*, 1990, Farrant *et al.*, 1992). Once the radicle growth is started, the seeds / seedlings can withstand the desiccation at least for a short while. This character may be the reason of survival of *Syzygium* seeds stored in polyethylene bags at room temperature where the initial temperature as well as RH are optimum and/or not much deviated from the original condition of the fresh seeds. So the radicle protrudes and these seeds retained their viability for one and three months in *S.aromaticum* and *S.cumini* respectively. On the contrary, the seeds kept at room open conditions are subjected to immediate desiccation, so radicle protrusion is not occurring hence storage is not possible.

Another interesting interpretation of desiccation given by Bewley (1979) is centered around free radicals. In normally hydrated tissues, the free radical production is controlled by free radical absorbents or scavenging reactions. One of such scavenging reagent is superoxide dismutase (SOD) which converts the superoxide (O_{2}) to H_2O_2 and in turn this can be removed by catalase. According to Bewley (1979) in desiccation intolerant tissues, water loss induces more free radical synthesis, while the synthesis of SOD is inhibited. So this highly potential free radical react with metabolites like lipids, proteins etc. to kill the tissues.

However, seeds of *S.aromaticum* and *S.cumini* kept in open trays at room conditions retained their viability only up to four days. Seeds of both species experienced a significant increase in dry weight percentage due to sudden desiccation by drying in open air. Post-harvest drying of recalcitrant seeds and resultant desiccation causing viability loss has been reported in many species (Pritchard, 1991; Tompsett, 1992, 1994; Farrant *et al.*, 1992). Berjack *et al.* (1984) suggested that the increasing desiccation sensitivity of recalcitrant seed in storage may result from the initiation of germination associated events and may therefore be analogue to the desiccation sensitivity of imbibed / germinating seeds of orthodox species. As mentioned earlier, seeds of *S.aromaticum* and *S.cumini* stored at 10, 20, 30°C and room (polyethylene) are found to increase their storability for longer times (Table 3 and 4). In spite of sufficient quantity of water to initiate germination, during prolonged storage (e.g. 24 months for *S.cumini* and 9 months for *S.aromaticum*) the seeds are subjected to desiccation because further water is not supplied. Notwithstanding, the germination especially radicle elongation is progressing further in storage conditions. Hence it is seen that root growth is not much affected by desiccation. This character is in agreement with the view of Wu *et al.* (1994) according to whom root growth is often less inhibited that shoot growth at low water potential.

Starch content of pulpy seeds of *S.aromaticum* stored at 0, 10, 20, 30°C, room polyethylene and room open conditions exhibited no significant change up to 15 days and thereafter viability was lost indicating no utilisation of insoluble carbohydrates for metabolism (Table 9, Fig 9). De pulped seeds of *S.aromaticum* stored at 10°C contained more starch after two months and by that time viability was almost lost. So mobilisation of carbohydrate is not occurred. Similarly seeds stored at 20°C also showed a gradual increase of starch which is tend to be correlated with gradual decrease of viability during first, second, third and sixth month intervals. The same trend of relationship between starch content and viability was followed by de pulped seeds of *S.aromaticum* stored at 30°C up to six months. Starch content of seeds stored in polyethylene bags at room conditions showed an increase up to one month and the viability also was lost by this time.

This increase of starch is due to increased dry weight in desiccated seed at this storage condition. Distribution of starch in *S.aromaticum* seeds stored at room condition in polyethylene bags reveals an active metabolism of the seed for one month of storage upto which period these seeds are found to be viable. Since recalcitrant seeds start initiation of germination in storage as a continuation of developmental process (Pammenter *et al.*, 1984), active metabolic changes like high respiration rate are expected to occur in *Syzygium* seeds also during the first month of storage at room conditions. According to Chien and Lin (1997) seed respiration is the representative of gross metabolic activity of the seeds which is very low as 0.06 μ mol O_2 $min^{-1}g^{-1}FW$ in muskmelon (orthodox seed) and very high as 0.74 μ mol O_2 $min^{-1}g^{-1}FW$ in *Machilus* (recalcitrant). After one month considerable reduction of starch content was occured despite the drastic reduction in moisture content. Seeds of *S.aromaticum* kept at room open showed no change in starch. Corbineau and Come (1988) reported that at 15°C *Symphonia globulifera* seedlings could be stored for longer periods than *Mangifera indica* because growth of the root and stem was much slower in *Symphonia*. Similarly seeds of *S.aromaticum* stored at 30°C, *S.cumini* at 10°C shows maximum viablity nine months and 24 months respectively and during this period the radicle growth is very slow, and reduction in starch also is very slow since metabolic activity is found to be feeble. In an excellent review of recalcitrant seeds Farrant *et al.* (1988) suggested that in recalcitrant seeds, a short while after shedding, many changes are initiated which are similar to germination. In *S.aromaticum* and *S.cumini* during early days of storage starch content undergo negligible change probably due to an enhance respiration and starch synthesis presumably from lipids, resulting no net gain or loss of starch content. In *Quercus* species, Clatterbuck and Bonner (1985) reported very high amount of starch and fat, but during storage starch increased significantly upto four months and then declined, the peak coincided with radicle emergence under storage. Present study also showed that in *Syzygium* seeds, when radicle emergence occurred, a slight enhancement in starch level was noted (Table 9 and 10) which later declined as the radicle growth was continued in storage similar to that of *Quercus*.

S.cumini also exhibited no change in starch content of both pulpy and de pulped seeds kept at all storage conditions up to 15 days (Table 10, Fig. 10). Seeds kept at 10°C maximum storage period and the starch content showed a gradual reduction up to 24 months. The dry weight as well as viability also followed same pattern. Actually the starch content reduction is more prominent since the dry weight percentage reduction occurs in these seeds. This starch reduction is positively correlated with prolonged storage respiration. Similar trend in starch content was shown by seeds stored at 20°C up to 18 months and at 30°C up to six months. So starch reduction is a characteristic of seeds stored at 10, 20 and 30°C in *S.cumini* unlike in *S.aromaticum* probably due to more chilling injury for *S.aromaticum* at 10 and 20°C. Seeds of *S.cumini* kept in polyethylene bags at room showed only negligible change in starch. The slight decrease of starch in seeds kept at room open is due to increased dry weight by desiccation effect. Cell metabolism get arrested during desiccation but starch degradation also is found to be started by four days of storage and total viability is lost.

Sugar content of *S.aromaticum* seeds is very low and the changes during storage at various conditions also are negligible. At lower temperatures like 10 and 20°C, even though the seeds are viable, sugars are either increased or decreased. So no potential carbohydrate metabolism and dry weight loss takes place and the viability loss is due to gradual chilling effect. At 30°C, a slight increase in sugar is actually more significant since dry weight percentage was significantly reduced during this period. As mentioned earlier, these seeds undergo profuse proliferation of radicle and metabolisable sugar increase shows the reduced translocation probably due to nutritional stress that inhibit translocating form of sugars as the seeds depends solely on seed reserves as the source. In tea and cocoa seeds, an increase in soluble carbohydrate was occurred with desiccation and this increase could have been caused by degradation of starch into simple sugars (Chandel *et al.*, 1995). In *Quercus* species, soluble carbohydrates increased during storage and declined only after viability loss (Clatterbuck and Bonner, 1985). Farrant *et al.*, (1992) reported that sugars are present in *Avicennia* seeds and these sugars are readily available during immediate establishment of the seedling since germination occurs soon after abscission, as the continuation of development processes.

Seeds kept in polyethylene bags at room temperature also reported slight increase, but due to more dry weight in desiccated seeds in this condition net change is considerable. Seeds of open room condition contain sugars unaltered. Since metabolism is stopped due to drying / desiccation within a few days.

Studies on seeds (orthodox) of pea, corn and soyabean (Koster and Leopold, 1988) subjected to desiccation by drying imbibed seeds, showed that soluble sugars, oligosaccharides in particular, are utilized when radicle protrution takes place during imbibition. According to these authors, loss of desiccation tolerance in these seeds coincides with the loss of soluble sugars. A similar correlation can be drawn in the case of sugar content in *Syzygium* seeds kept at 30°C, room polyethylene and room open where seeds are subjected to desiccation. However, unfortunately, individual sugars are not identified in the present study, the quantity of oligosaccharides alone is not known. Notwithstanding, the distribution of sugar content in these seeds is presumably correlated with loss of desiccation tolerance. Apart from this the utilization of oligosaccharides prior to desiccation may be compensated by synthesis of glucose formed after desiccation (Koster and Leopold, 1988), resulting in no significant change in sugar. Non-reducing sugars especially sucrose play a protective role against desiccation damage (Chien and Lin, 1997).

However, the synthesis of sucrose can be ruled out since no net synthesis of sugars in desiccating seeds.

Sugar content of *S.cumini* is comparatively higher than *S.aromaticum*. Pulpy seeds when retained viability for 15 days in storage showed a slight increase in sugar. Since the pulp of *S.cumini* (Jamun fruit) contain large amount of sugars, that may be getting translocated during the storage under 10, 20 and 30°C whereas in room conditions, the sugar content is reduced when increase dry weight percentage is taken into account and also the reduction is correlated with the chance of gaseous exchange resulting in more prolonged viability compared to the seeds stored at low temperatures.

The de-pulped seeds of *S.cumini* kept at 10°C registered a gradual increase during storage up to 24 months, where the reduction in dry weight percentage is taken into consideration, these changes are more prominent. This accumulation of sugars in these seedlings can be correlated with lack of utilization for metabolism since the seeds / seedlings showed radicle sprouting up to six months and further growth rate was negligible probably due to nutritional stress and/or limiting factor and after 24 months the seeds become nonviable.

Similar interpretation of sugar metabolism can be applied to the seeds stored at 20°C also because more or less the same trend is seen in sugar distribution and this can also be correlated with dry weight percentage and viability period under this temperature.

The sugar content of seeds stored in polyethylene bags at room temperature (25±5°C) also did not change significantly up to six months. As mentioned earlier in this conditions seedlings show well developed radicle and slightly developed plumule also indicating active metabolism, but the seeds may be under nutrient stress due to long storage and profused radicle growth, both are depending only on seed reserves as source. The seeds kept at open room condition exhibited a reduction within four days and this may be due to the increase in dry weight during desiccation at open room condition. As interpreted above, the desiccation intolerance of *S.cumini* seeds also seemed to be correlated with soluble sugar content reduction.

Protein content of *S.aromaticum* is comparatively low (Table 7, Fig. 7). In pulpy seeds the protein did not show any change in storage up to 15 days. Irrespective of the difference in storage condition, the pulpy seeds of *S.aromaticum* lost viability without any marked change in total protein. Electrophoretic studies on *Shorea robur* seeds (Nautiyal *et al.*, 1985) showed that during storage / desiccation / ageing, proteins with high mobility are found to be denatured. So it is evident that total protein contents are need not be changed during viability loss whatever may be the cause in pulpy seeds of *S.aromaticum* and *S.cumini* under storage. De-pulped seeds stored at 10, 20 and 30°C exhibited a gradual as well as significant reduction. This reduction of total proteins during seed storage where the radicle growth is considerably increased especially in the stored seeds at 30°C, and 10°C for *S.aromaticum* and *S.cumini* respectively, is owing to the mobilisation of their reserve for metabolism.

At room temperature seeds stored in polyethylene bags contained only very low amount of protein after one month's storage. Desiccation is found to be the cause of this observation. As mentioned earlier, sugar content distribution remained almost unaltered in seeds stored at room open and room polyethylene where desiccation found to occur due to utilisation of oligosaccharides prior to desiccation and synthesis of glucose after desiccation (Koster and Leopold, 1988). According to these authors, glucose is synthesized after desiccation tolerance is lost and this reducing sugar can participate in the Millard reaction – a complex series of non-enzymic reactions and can lead to many end products. If this reaction occurs with an amino group of protein, the protein get in activated and damaged and this reaction can occur at low moisture content such as experienced by desiccating seeds. So the significant reduction in protein content of *S.aromaticum* and *S.cumini* seeds stored at room conditions can be correlated with desiccation imposed metabolism of sugars and resultant denaturation of proteins.

Many recalcitrant seeds like *Shorea robusta* (Nautiyal and Purohit, 1985b), are reported to suffer desiccation in storage and desiccation causes a significant reduction in proteins. However, this protein reduction alone cannot be taken as a single reason for viability loss due to desiccation. According to Nautiyal and Purohit, (1985c) desiccation results in loss of membrane permeability and hence more leachate constituting of sugars, proteins and inorganic phosphates are effluxed at a fast rate. So a concomitant reduction of protein in desiccated seeds of *S.aromaticum* and *S.cumini* is related with viability loss especially after one month's storage. In contrast with *S.aromaticum, S.cumini* seeds contained more protein (Table 8, Fig. 8). Pulpy seeds stored at 10, 20 and 30°C showed no change up to 4 days but later reduced significantly up to 15th day. De-pulped seeds stored at 10, 20 and 30°C showed a gradual and significant reduction during a period of nine months. The protein content reduction is proportional to the viability loss in all these conditions.

In seeds stored at room polyethylene bag, the reduction is very drastic after 15 days due to desiccation and loss of viability. A gradual reduction of protein content in de pulped seeds of *S.cumini* stored at 10, 20 and 30°C is similar to that of *S.aromaticum*, Presumably the metabolism of protein during storage will be alike. However, prolonged storability of *S.cumini* at 10°C is also found to be related to marked reduction in protein up to 24 months. Seeds stored in polyethylene bags at room conditions also exhibited sharp reduction during storage where desiccation found to be prevailed. In spite of the abundance of protein in *S.cumini*, the metabolism is found to be more or less similar to that of *S.aromaticum* because storage condition and storage duration and the resultant loss of viability in both species are more or less similar in pattern as the proteins also do.

Phenolics are heterogenous group of secondary metabolites which are originated in the endoplasmic reticulum and located in cell vacuoles (Parham and Kaustinen, 1977) and also located in cell walls (Zobel *et al.*, 1989).

Considerable amount of phenolics is present in fresh seeds of *S.aromaticum* (Table 13, Fig. 13). Polyphenols are reported to be present in chloroplast (Zobel *et al.*, 1989). Since the seeds of *S.aromaticum* and *S.cumini* are abundant in chlorophyll, phenolics are contributed by these pigments also. When stored with pulp, there occurred an increase in phenolics after four days and increased again after 15 days. This increase in phenol is found to be directly proportional to the viability loss.

De-pulped seeds stored at 10, 20 and 30°C showed gradual reduction in germination, and maximum amount of phenolics were present in seeds which are almost dead after nine months at 30°C. Seeds kept in polyethylene bag at room conditions also registered an increase in phenols especially in desiccated samples after four days. This character was more evident in seeds kept at open room conditions where viability was found to be lost after four days.

Phenolic content of *S.cumini* seeds are more in quantity when compared to that of *S.aromaticum* (Table 14, Fig. 14). The pattern of distribution of phenolic contents during storage under various conditions is almost similar in both species.

In general the metabolism of phenolics in recalcitrant seeds is very important because these compounds are involved in the activity of cell especially when water stress is occurring. According to Zobel *et al.* (1989) in cells with low water content, disorganisation of cell membranes and their dissembling lead to cell death. At low temperature, the enzyme phenylalanine ammonia lyase (PAL) is found to increase resulting in an accumulation of phenolics (Graham and Patterson, 1982). If the phenolics are leaking from the cells the cytoplasmic proteins are precipitated by phenols and cause cell death. As described earlier, in *S.aromaticum* and *S.cumini,* the seed viability is lost under storage either by chilling injury at 10 and 20°C or by desiccation at room temperature both in polyethylene bags and room open. Both chilling injury and desiccation are manifested in the recalcitrant seeds to culminate in viability loss and in the ultrastructural level, cause membrane disruption (Chandel *et al.*, 1995). So the phenol present in the vacuoles (Zobel *et al.*, 1989) get leaked to the cytoplasm and cell metabolism is inhibited by denaturing the protein with phenolics (Mosjidis, 1990). So seeds at this condition of storage become nonviable. However unlike in *S.aromaticum, S.cumini* seeds possessed more storability up to 24 months at 10°C, up to 18 months at 20°C and maximum phenolics are present in these samples. As reported earlier, the seeds stored at room conditions in polyethylene bag also lost viability due to desiccation and the phenolics increase is concomitant with viability loss. In *Voandzeia subterranea* germinability of seeds was lost during 12 moths storage and closely coinciding with this, there was an increase in total phenolic content (Sreeramulu, 1983). A correlation between phenolic content and seed viablity was presented by Taylor *et. al.* (1988) in cabbage seeds. According to them, leakage of a fluorescent compound namely Sinapine was observed around inbibed samples of heat killed cabbage seeds while not around the viable seeds.

Presence of chlorophyll in seeds is very rare. According to Mayer and Poljakoff-Mayber (1989) chlorophyll is occurring in gymnosperms and protochlorophyll in cucurbitaceae seeds. Seeds of *Arcenthobium occidentale* contain chlorophyll and this enables the seed to carry on photosynthesis which stimulates and enhances germination to some degree (Scharpf, 1970). The occurrence of chlorophyll in embryos was the subject of a survey by Yakovlev and Zhukova (1980) and Dahlgren (1980) who reported that chlorophylle embryos occur sporadically in many taxa but appear consistently in several families including convolvulaceae, dispsaceae and leguminosae. A few recalcitrant types of seeds like *Aporusa lindleyana, S.aromaticum, S.cumini* contain considerable amount of chlorophyll. However, in general little research has been done on the metablolism of seed chlorophyll in seeds. In *S.aromaticum* fresh seeds shows chlorophyll$_a$ and chlorophyll$_b$ (Table 15, Fig. 15a to 15f). Pulpy seeds stored at various temperatures lost viability within 15 days and a concomitant reduction of both chlorophyll$_a$ and chlorophyll$_b$ was observed. Since both chlorophyll$_a$ and chlorophyll$_b$ decrease was in the same rate, a/b ratio did not change during storage / viability loss.

De-pulped seeds stored at 10, 20 and 30°C also showed the same pattern of chlorophyll reduction and minimum amount is present in seeds which showed maximum viability loss after one month at 10°C, six month at 20°C and nine month at 30°C. In seeds which suffered desiccation when stored at room polyethylene bags showed reduction in both chlorophyll$_a$ and chlorophyll$_b$ and total chlorophyll content is minimum when desiccation was maximum with more dry weight. Considerable amount of chlorophyll loss occurs from desiccation intolerant recalcitrant seeds as a reflection of general cellular degradation (Bewley, 1979). So in *Syzygium* seeds the chlorophyll content reduction during storage is found to be one of the effects of desiccation to which the seeds are subjected. A correlation between chlorophyll content and desiccation has been put forth by Martin and Warner (1984) who stated that poikilohydrous mosses growing in exposed habitat reflect breakdown of chlorophyll with nil change in a/b ratio of chlorophylls, owing to water stress. Alberte *et al.* (1977) suggested that lower concentration of chlorophyll found in water stressed than well watered maize and this lower concentration of chlorophyll is the result of desiccation induced degradation of chlorophylls.

The total chlorophyll content of *S.cumini* seeds is low compared to that of *S.aromaticum* (Table 16, Fig. 16a to 16f). Similarly chlorophyll$_b$ is lesser than chlorophyll$_a$ and hence high a/b ratio where as in *S.aromaticum* the pattern is reverse. Pulpy seeds of *S.cumini* also showed similar pattern of reduction in chlorophyll distribution as that of *S.aromaticum* when kept under various conditions. De-pulped seeds also exhibited the same pattern of reduction in chlorophyll $_a$ and chlorophyll $_b$ during storage in all conditions, the minimum amount was present in seeds which lost their viability. The seeds stored at room polyethylene and open room conditions contained comparatively low amount of chlorophyll. Generally these seeds, open room stored in particular, lost viability due to desiccation as mentioned earlier. So that concomitant reduction of chlorophyll in these seeds is related to desiccation intolerance and this observations agree with the view of Bewley(1979) according to whom considerable amount of chlorophyll loss occurs in desiccation intolerant recalcitrant seeds as a result of general cellular degradation. But when the desiccation starts sugars protects membrane structure to some extent (Bewley, 1979). In the present study, sugar content upto one month in *S.aromaticum* and three months in *S.cumini* show slight increase (Table 11 and 12) probably protecting the chloroplast membranes as evidenced by the unchanged chlorophyll contents (Table 15 and 16) during these intervals. Thereafter, sugar and chlorophyll contents showed a decrease concomitant with desiccation intolerance.

CONCLUSION

CONCLUSION

Like many other recalcitrant seeds, *Syzygium aromaticum* and *Syzygium cumini* seeds do not show maturation drying or desiccation tolerance. Seeds of both species attain maturity almost at the same season i.e., during the rainy months of June-July. They are shed from the parent plant with high moisture content (about 50%). The recalcitrant nature of *Syzygium* seeds exclude all traditional methods of storage. The rapid loss of moisture content and concomittant loss of viability, particularly at very low relative humidity in recalcitrant seeds, has caused a great challenge in their storage

Morphologically fruits of these two species are alike in their appearance, dark violet colour and pulpy nature. The cotyledons of both seeds are chlorophyllous and are enveloped in papery testa which is comparatively more appressed to seed in the case of *S. cumini*. Fruits of *S. aromaticum* encloses a single seed with an embryonic axis tugged in between two massive cotyledons, while fruits of *S. cumini* encloses a combination of many seeds which appears to be a single seed each with its own embryonic axis and hence polyembryonic.

Since recalcitrant seeds are in a highly hydrated condition, they are metabolically active when harvested or shed from the mother plant and they under go immediate germination-associated changes. Recently, it has been proved that in many recalcitrant seeds development and germination appears to be more of a continuum. The tropical recalcitrant species of *Syzygium* (*Syzygium aromaticum*) and sub-tropical species (*Syzygium cumini*) produce seeds which cannot be dried at all since they are metabolically active and germination-associated changes are taking places soon after harvesting. It is experimentally proved that approximately 10% reduction of initial seed moisture content is fatal to both species. This desiccation sensitivity is an assertive of their recalcitrant nature.

Storage at low temperature enables the storability of recalcitrant seeds to some extent but at freezing temperature, most of them loose viability. Both pulpy and de-pulped seeds of *S. aromaticum* and *S. cumini* lost their viability when stored for one day at 0°C due to freezing injury. *Syzygium aromaticum* seeds can be stored beyond one month at 10°C while seeds of *S. cumini* are more tolerant to chilling injury and hence remained viable up to two years at 10°C. Higher temperatures like 20 and 30°C are also found to influence seed viability almost at the same level. Nevertheless, *S. aromaticum* seeds exhibit more longevity at 30°C while *S. cumini* at 20°C.

Another characteristic feature of recalcitrant seeds is sprouting under storage. At 10°C, *S. aromaticum* seeds never germinates while *S. cumini* seeds showed initiation of radicle growth. At 20°C, seeds of both species showed similar speed of germination while at 30°C, though both seed types sprouted profusely, speed and extent of germination was higher among *S. cumini* seeds.

Biochemical studies on stored seeds of both species indicate that degradation of metabolites is a common character for both pertaining to their high respiration rate and other germination related-changes under storage. But considerable increase in phenolics during storage is the manifestation of membrane damage by both chilling and desiccation.

Seed behaviour of *S. aromaticum* and *S. cumini* are biologically programmed to get in tune with their respective habitat requirements. Both of them exhibit characteristic features of recalcitrant seeds such as immediate loss of viability at natural atmospheric conditions, desiccation sensitivity, chilling injury, germination under storage and lack of storability. Despite the germination under storage, in order to prolong the metabolic changes associated with germination, condition of hydration (relative humidity) as well as temperature are very critical. In this study, optimum seed storage conditions such as 30°C / 80% RH in closed polyethylene bags for *S. aromaticum* and 10°C / 80% RH in closed polyethylene bags for *S. cumini* are standardised for an effective and extended germplasm storage.

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