DISCUSSION
Ageing is a common phenomenon in all the living entities, which results in progressive decline in all vital events ultimately leading to death. Seeds, which are the elements of propagation of a plant species for generations, also have specific and definite life spans which may vary in different genera, species and accessions or genotypes. Extensive research in the area of seed biology and seed technology during the last few decade has unraveled the mechanism of the degenerative processes which bring down the quality of seeds only to some extent due to immense variability among various kinds of seed. Extreme examples of ancient seed that had longevities of thousands of years such as the Arctic lupine seeds, which remained in the dormant viable state for more than 10000 years, exist in the literature, such seeds fall in the category of desiccation tolerant or orthodox seeds. On the other hand, seeds of several genera belonging to the same category such as soybean, onion and groundnut do not have such extended longevities. Many factors, both intrinsic and extrinsic, play important roles in keeping the quality of seeds. Several complex changes occur in seeds during storage, and the rate of these changes depend on the conditions of storage. Regardless of the causal agents, changes in some quantifiable traits occur when seeds deteriorate, which can be used to estimate the rate of deterioration. An understanding of the mechanism of seed deterioration and the various physiological, biochemical and molecular changes associated with it is important to devise methods to control the rate of such deterioration in order to extend their longevity, and also develop reliable indices for measuring the ageing process.

Cowpea is a crop mainly propagated through seeds, which are orthodox in nature. It plays a critical role in the lives of millions of people in Africa and other parts of the developing world, including India, where it is a major source of dietary protein that nutritionally complements staple low-protein cereal and tuber crops, and is a valuable and dependable commodity that produces income for farmers and traders (Singh et al. 2002, Langyintuo et al. 2003). All the plant parts that are used for food are nutritious, providing protein, vitamin and minerals. Cowpea grain contains on an average 23-25% protein and 50-67% starch. Going beyond its importance for food and feed, cowpea can arguably be regarded as the fulcrum of the sustainable farming in semiarid lands. Research on seed storage and mechanism of ageing of cowpea seeds is
limited and warrants attention. The present work was therefore undertaken to develop some predictable indicators of seed quality loss in three cowpea varieties with different seed coat colours and seed sizes. The varieties selected for this study were V 240, V 578 and V 585. Two of these varieties had non-pigmented seed coat while one (V 240) had dark brown seed coat. Deteriorative changes were investigated by subjecting the seeds to artificial ageing by controlled deterioration method and preparing seeds of three vigour categories viz. high, medium and low. These were analysed for various biochemical and cytological changes including the nutritional and antinutritional factors.

In this chapter an attempt has been made to discuss the significant findings and offer possible explanations and evidences, wherever necessary, with a view of finding out the cause and effect relationship among the different seed vigour categories and varieties with respect to the parameters studied.

5.1. Effect of seed ageing on physiological parameters

5.1.1. Germination per cent

The rate of seed ageing depends on the physiological status and genetic constitution of the seeds as well as on the capacity of a cultivar to withstand ageing conditions (Anderson and Gupta 1986, Priestly 1986, Roberts and Ellis 1989, Kalpana and Madhava Rao 1994). In the present study, it is evident that two cultivars V 240 (brown seed coat) and V 585 (cream seed coat) exhibited very good stress-withstanding capacity, while variety V 578 (very bold seeded and cream seed coat) exhibited least tolerance. The increase in germination and vigour index after 24 h of ageing in V 240 and V 585 could be due to an effect similar to priming during the first 24h when the metabolic events had commenced within a few hours of exposure to high temperature and proceeded till the seeds were removed after a day and the deterioration had not set it yet. Such seeds can be expected to show enhanced seed quality in terms of germination and vigour due to repair processes that might have occurred during the initial stages of ageing. The studies of Ali et al. (2003) in rice, Lush et al. (1981) in wheat, Ward and Powell (1983) in onion and Naylor and Syversen (1988) in Italian ryegrass corroborate this.

Progressive loss in seed-quality attributes occurred with ageing in all varieties but the inter varietal differences were highly significant. Similar observations have been made by others.
Discussion in several other crops viz. pea (Jatoi et al. 2001), wheat (Siddiqui et al. 2008, Sterlec et al. 2010), pearl millet (Sundareswaran et al. 2009) and rice (Seshu and Krishnaswamy 1987, Ramanadane and Ponnu.swamy 2004, Ali et al. 2003). This kind of variability in response to ageing can be attributed to genetic constitution and the inherent capacity of a cultivar to withstand stresses.

A reduction in germination percentage has been observed further in higher durations of ageing in all the varieties. Our results are in agreement with those of Ruzrokh et al. (2003), Bradford et al. (1993), Thornton and Powell (1995) Lewis et al. (1998) and Verma et al. (2003). Failure of aged seeds to germinate might be due to lipid peroxidation, mitochondrial dysfunction and less ATP production (Basra et al. 2003, Sung 1996, McDonald 1999). Alternatively, the reduction in germination might be due to degradation of mitochondrial membrane leading to reduction in energy supply necessary for germination (Gidrol et al. 1998).

Demirkaya et al. (2010) suggested that decrease in the activity of several enzymes in the seed lowers the respiratory potential of seeds, which causes lowering of both the energy (ATP) and the supply of assimilates to the germinating seed. Thus, several changes in the enzyme macromolecular structure may contribute to the lowered germination ability of seeds.

Woltz and Tekrony (2001) suggested that the accelerated ageing could predict seed vigour better than the standard germination test. Genetic damage and loss of membrane integrity might have resulted in undesirable changes in protein synthesis during germination (Gidrol et al. 1990), which possibly was responsible for delayed germination, abnormal growth and finally, loss of germinability (Ellis and Roberts 1981).

5.1.2. Mean Germination Time (MGT)

When the seed metabolism is disturbed, initial changes occur resulting in slowing down of germination process leading to late emergence of the seedlings. Increase in the mean germination time is the earliest symptom of ageing (Eski and Demir 2011). Basak et al. (2006) observed in pepper seed lots that decrease in germination percent was associated with an increase in emergence time of seeds. Seed deterioration expresses itself in a reduction in germination rate and uniformity, reduced tolerance to environmental stresses and consequently inferior seedling emergence and growth (Hampton and Tekrony 1995). In our study on cowpea
Discussion

A highly significant negative correlation between per cent germination and MGT was obtained indicating that germination rate is an important indicator of seed-quality loss.

5.1.3. Seed Viability

A good correlation between the viability calculated from TZ test with the germination test as well as seedling vigour index existed, indicating that this test can be used as a substitute for germination test in case quick results are desired. These findings are in agreement with those of Bittencourt et al. (2012) in aged corn seeds and by Santos et al. (2007) in tomato seeds. In tetrazolium staining, deteriorated tissues tended to appear flaccid and develop weak or mottled colour, which represents intermediate stages between normal and dead tissue. The decline in seed viability, whether by natural or accelerated ageing, has long been associated with damages to macromolecules and cellular membranes, probably initiated by the action of activated oxygen radicals (Berjak and Villiers 1972, Osborne 1980, Wilson and McDonald 1986b, Ponquett et al. 1992 and Hendry 1993). Roberts (1972) concluded that depletion of essential metabolites, including loss of food reserves, is one of the important factors responsible for loss in seed viability. Bailly et al. (1996) performed TZ test in aged sunflower seeds and reported their non-viability by TZ staining. Aiazzi et al. (1996) also reported viability to be sensitive to accelerated ageing and decreases according to time of exposure to ageing conditions.

The information obtained here with respect to the assessment of cowpea seed vigour is similar to the findings of Santos et al. (2007) with tomato, Dias and Barros (1995) with maize, Vieira and Bittencourt (1999) with peanut and Dias et al. (2001) with pumpkin, which indicated that the separation of stained seeds in three classes instead of eight classes (Franca-Neto et al. 1999) is sufficient to determine seed vigour by the tetrazolium test. This procedure for interpretation, involving a few categories, is preferred to achieve an accurate standardization among seed analysts.

5.1.4. Seedling Vigour and Vigour index

A decline in seedling vigour and vigour index, as ageing proceeded, was observed for all the three varieties in the present study. While an initial increase for these parameters could be found of the three varieties, V 585 recorded maximum seedling vigour and vigour index followed by V 240 and V 578. Differences in seedling vigour between seed lots are attributed
to the rate of germination and seedling growth. There is evidence from comparison of seed lots
of onion (Wheeler and Ellis 1991) and artificially aged seeds of wheat (Guy and Black 1998)
that seeds, which germinate earliest, have an early start in growth and produce longer seedlings.
Iqbal and Smith (1996) observed a negative impact of seed ageing on root vigour in pea
seeds. According to Powell (1988), seed deterioration during storage in general resulted in
decreased seedling vigour. Seed ageing not only affected the emergence percentage but also
decreased the speed of germination and seedling-growth rate, resulting in reduced seedling
vigour in cucumber, which was cultivar dependant (Al-Maskri et al. 2002). Similar results
were reported in peanut (Sung and Jeng 1994, Nautiyal et al.1997). In our experiments we
found that seedling vigour was highly positively correlated with germination percentage and
highly negatively correlated with MGT. Decrease in seedling vigour index due to ageing has
been reported by earlier workers, viz. Agrawal and Kharlukhi (1985) in wheat and chickpea
seeds, Pallavi et al. (2003) in artificially aged sunflower seeds. The possible reason for this
loss of vigour induced by ageing might be due to delayed and non-synchronous germination,
which in turn is due to altered sequence of metabolic events eventually leading to seed death.
Since seedling vigour index is the product of germination and seedling vigour; increase or
decrease in any one of the components or both can influence its value greatly.

With the fall in germination of cowpea, seedling dry weight also recorded a drastic
decline with increased duration of ageing and hence vigour index II. Theses results are in
accordance with Varier and Dadlani (1992) in cotton and pearl millet and Singh and Dadlani
(2003) in soybean. Accelerated ageing also decreased seedling length, seedling-vigour index
and seedling shoot and root lengths. Similar results were reported in peanut (Sung and Jeng
1994), cotton (Iqbal et al. 2002), chickpea (Kapoor et al. 2010) and Rice (Kapoor et al.
2011). The possible reason of this reduction might be the lowering of biochemical activities in
seeds. The decline in seedling-vigour index (vigour index I and vigour index II) might be
attributed to DNA degradation with ageing, which leads to impaired transcription causing
incomplete or faulty enzyme synthesis essential for earlier stages of germination (Kapoor et al.
2010). Ageing is reported to produce damaging effect on enzymes that are necessary to convert
reserve food in the embryo to usable form and ultimately production of normal seedling (Iqbal
et al. 2002).

Discussion
At higher durations of ageing seed deterioration results in decreased rate of germination and decreased percentage of normal seedlings. Seedling growth and the fraction of seed-reserve mobilization in terms of reserve-mobilizing enzymes indicated a significant decrease with the advance of deterioration. Overall, the results obtained in this study show that seed ageing resulted in reduced seedling growth and this could have been due to decline in the amount of mobilized seed reserve as reported by Mohammadi et al. (2011).

Differences in the storage potential of cultivars within a species have also been reported in grain legumes as being associated with seed-coat pigmentation. In our study, cowpea cultivar V 240 with dark brown seed coat had a better storage potential than the other two varieties which had light coloured/unpigmented seed coat. This was in accordance with the findings of Abdullah et al. (1991) in long bean, Abdullah et al. (1993) in Phaseolus vulgaris and Mugnisjah et al. (1987) in soybean, where cultivars having unpigmented seed coats deteriorated more rapidly during storage than cultivars with pigmented seed coats. The poor storage potential of unpigmented cultivars could be partly attributed to the greater increase in their seed moisture content that occurred when the seeds were held at high humidities (Kueneman 1983, Abdullah et al. 1991).

5.2. Effect of seed ageing on biochemical parameters

5.2.1. Electrical conductivity and UV absorbance of leachates

The reduction in the Electrical Conductivity (EC) has frequently been reported in the literature as one of the main results of seed ageing. EC has always been found to be a better indicator in leguminous and oil seeds where the cotyledons are made up of living cells, which undergo simultaneous deterioration with the embryo when the seed is subjected to storage at adverse environmental conditions.

In the present study, there was a concomitant rise in seed leachate EC with decrease in germination, suggesting that membrane integrity was affected. Our observations revealed that as the aging period increased, leachate conductivity also increased. The EC of the leachate was significantly higher in the medium and low vigour seed lots. Previous workers have also reported similar results in low quality seeds of musk melon (Pesis and Timothy 1983), tomato (Coolbear et al. 1984), pearl millet (Yadav et al. 1986) and wheat (Tajbakhsh 2000). The correlation between seed deterioration and leakage of electrolytes from seeds may be related to...
to the hypothesis of some researchers that the increase of electrical conductivity is due to the loss of ability to re-organize cellular membranes completely and rapidly during early imbibition. Vieira et al. (1999) also opined that higher EC is an indication of low vigour due to an increase in membrane permeability of the low vigour seeds. Several researchers have reported that EC provided the best estimate of seed vigour and that higher EC of seed lot results in poor germination due to low vigour. Moreover, this test detects the seed deterioration process since its early phase (Khan et al. 2010, Samarah and Al-Kofahi 2008, Powell 1998, Fessel et al. 2006 and Tajbakhsh 2000). The reduction in germination for aged seeds is probably due to ageing induced inhibition in membrane repair (Senaratna et al. 1988, Bewley 1986, Pukacka 1991). This corollary is indirectly supported by increased electrolyte leakage of aged seeds.

While examining the UV absorbance values of leachates from seeds of different vigour categories, it was observed that as vigour decreased, the UV absorbance increased. Pandey (1989a) observed that UV absorbing materials in aged french bean seeds increased several folds as compared to the non-aged control. A highly significant positive correlation between EC and UV absorbance at both \( A_{260} \) and \( A_{280} \) nm as well as a highly significant negative correlation between germination and EC and germination and UV absorbance at both wave lengths was obtained. Similar positive and negative correlations between electrical conductivity and UV absorbance and between germination and UV absorbance respectively were reported by Prusinski et al. (1999). The leakage of substances absorbing UV light during imbibition, according to Duke et al. (1983), is due both to a passive diffusion of low molecular weight solutes and the macromolecules released from ruptured cells.

The effects of various methods of priming on partially aged seeds of mustard were studied by Srinivasan et al. (1998) and Pandey (1989b) in french bean. In both the studies the authors observed a reduced UV absorbance in aged seeds, which were primed, than in aged unprimed seeds, indicating that this biochemical test can be used as a good indicator for seed vigour.

### 5.2.2. Lipid Peroxidation Studies

Several reports explain the relationship between seed ageing, changes in the lipid and fatty acid contents as well as the extent of lipid peroxidation but most of these are pertaining to oil seeds where changes are well expressed. Peroxidative changes in the fatty acid composition

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*Discussion*
Discussion

of membrane lipids lead to enhanced bilayer permeability, mitochondrial swell and lysis occur in severe cases (Priestley 1986). Oxygen can add to the fatty acid chain to form peroxides and hydroperoxides. Peroxides undergo cleavage to produce bad smelling aldehydes, ketones and acids. The peroxide value is a measure of the amount of these products (Basra et al. 2003). The fatty acid composition of different lipid fractions was almost unchanged during the ageing of seeds of peanuts (Pearce and Abdel-Samad 1980). According to these authors, loss of control over sub-cellular compartmentation or intracellular concentrations of metabolites, resulting from loss of membrane lipids, might have been the cause of the loss of viability. Theories of mechanisms of lipid oxidation indicate that fatty acids with two or more unsaturated bonds should be more labile and prone to form free radicals than more saturated acids.

There is a high probability that the reduction in the lipid content was brought about by active peroxidation of the membrane lipids in the present study. According to Linn and Pearce (1990), when seeds are damaged by improper storage conditions, lipid degradation reaction occurs and lipase and lipoxygenase are the two principle enzymes involved in the degradation of lipid in seeds. Lipid peroxidation has been shown as the most important factor causing loss of seed viability by many workers (Harman and Mattick 1976, Stewart and Bewley 1980, Lima et al. 2010, Kaewnaree et al. 2011 and Wang et al. 2012). However, there was no direct relationship between lipid peroxidation and deterioration in membrane integrity in sunflower seeds, as per the reports of Hussein et al. (2011).

The magnitude of changes observed in the germinability was substantial that these cannot be equated to small but significant changes observed in the lipid or fatty acid contents and more often the medium and poor vigour seeds seldom differed in their fatty acid content. This could be due to the fact that cowpea seed is a non-oily seed and due to low lipid content, the free radical attacks may not be occurring at proportionate frequencies to cause corresponding quantitative changes in the lipids. Similar trend in fatty acid changes with vigour decrease has been reported by Thapliyal and Connor (1997) in Dalbergia sissoo, with very low lipid content. The increased lipid peroxidation value taken in conjunction with reduced germination or vigour index indicate that increased lipid peroxidation might be one of the causes of the low vigour of the poor quality seeds, as has been observed by Kaewnaree et al. (2011) in sweet pepper seeds. In the present study, lipid peroxidation and oil content and lipid peroxidation and
unsaturated fatty acids correlated negatively. But the actual observed changes in the physiological parameters were several times greater than those occurring in the oil and/or fatty acid content and therefore pinpointing these as exclusive causal factors for seed quality loss may not be logically correct. There may be other factors simultaneously acting on the seed to bring down the germinability and vigour of cowpea seeds, ultimately resulting in complete loss of quality. In artificially aged husk tomato seeds, where the linoleic acid content was more than 80%, significant reduction in the germination and seedling emergence rate was observed without any effect on the fatty acid content, while respiratory rates correlated with loss in seed quality thus indicating that the loss of physiological quality in deteriorated seeds can be attributed to the reduction in respiratory activity (Gonzalez et al. 2010).

The study indicates that in cowpea, lipid peroxidation is closely associated with membrane disintegration as revealed by increased electrical conductivity of seed steep water as the vigour of the seeds declined accompanied by a simultaneous increase in the lipid peroxidation value.

5.2.3. Soluble Protein

In the present study, the content of proteins decreased to half of the initial value in variety V 578 and V 585 in low vigour seeds, whereas a significant reduction was observed in the variety V 240. Among the three varieties, the protein content in V 240 was the highest and that of V 578 the lowest. The same trend was found in various vigour categories of all the varieties. These results are in agreement with those of Ayyappan et al. (2006) who found that the total protein decreased to half of the initial content in cucumber seeds on the 8th day of ageing and was considerably reduced after ageing in Dendrocalamus, as reported by Ravikumar et al. (2002). A reduction in the total content of storage components such as proteins and carbohydrates with ageing has also been reported by other workers (Agnieszka et al. 2010). Rashed et al. (2010) recorded highly significant differences in protein content of watermelon seeds between untreated and aged seeds. The possible reason for this reduction could be the increased enzymatic degradation of proteins by proteases leading to a sharp increase in total free amino acids, as recorded by Ravikumar et al. (2002), Coolbear et al. (1984), Nautiyal et al. (1985) and Basavarajappa et al. (1991). Ghosh et al. (1981) demonstrated leaching of amino acids into the imbibing medium during ageing of rice seeds.
suggesting that proteins were hydrolysed during ageing. The protein loss depended on the severity of ageing conditions.

The loss in seed viability and protein content are due to the fact that the cellular membranes are composed mainly of proteins and lipids. During the seed deterioration process, disorganization of proteins and lipid-phase transition influence the membrane structure and integrity and consequently seed viability (Mc Donald 1999). As seeds age, plasma membrane becomes leaky, enzymes lose catalytic activity, seed food reserves deplete and the byproducts of catabolic reactions become toxic (Walters 1998). Other time-dependent changes like the loss of protein content and increase in soluble sugar contents may lead to irreversible degradation of important cellular machinery (Mc Donald 1999).

The seed proteins, when denatured by heating in the presence of excess Sodium Dodecyl Sulphate (SDS) and 2-mercaptoethanol bind with SDS in constant weight ratio, as if they have essentially identical charge density. This complex migrates in polyacrylamide gel of the correct porosity according to polypeptide size. Separation of cowpea soluble proteins of various vigour categories along with control, using this method, revealed that the total number of protein bands were 23, 21 and 25 for V 240, V 578 and V 585 respectively. In V 240 and V 585, the intensity as well as the number of bands decreased with decrease in seed vigour, while in V 578 the number of bands did not differ among the various vigour categories. Comparing proteins of control seeds with the high, medium and low vigour seeds by the banding profile showed differences in band staining for all the varities. Our results are in accordance with those of several other workers (Kehinde et al. 2013) in amaranth, (Vasudevan et al. 2012) in peanut, (Vishwanath et al. 2007) in tomato, (Das et al. 2010, Raghunathachari et al. 2000 and Rao et al. 2002) in rice, who reported similar changes in number and intensity in protein banding of low vigour seeds. Machado et al. (2001) also reported changes in the banding pattern of protein profile of naturally and artificially aged french bean seeds. The loss of bands observed in the medium and low vigour categories of V 240 and V 585 correspond to the decline in the soluble protein content due to protein degradation during moderate or severe ageing as explained above. Consistent loss of band intensity indicates losses in protein functions and suggests that the seeds lost genetic integrity at this point of seed ageing, as
documented by Kehinde et al. (2013). On the contrary, Basavarajappa et al. (1991) in maize, Varier and Dadlani (1992) as well as Vieira (1996) in cotton found a higher number of bands in the electrophoretic profile of proteins of artificially aged seeds compared to non-aged seeds, while Krishnaswamy and Yugasandhya (2002) did not notice any difference in protein profile of aged and non-aged seeds of maize. Das et al. (2010) reported the absence of some protein bands in aged seeds in some wild species of rice. Their finding indicated that different species can be distinguished on the basis of the total number of protein bands.

Ageing-induced deterioration increases the extent of protein oxidation, thus inducing the loss of function of all the properties of proteins and enzymes (Loic and Dominique 2008). Freitas et al. (2006) reported that in naturally aged bean seeds the physiological quality tests were more sensitive than the electrophoretic analyses to monitor seed deterioration, while in the artificially aged seeds, electrophoretic profiles were more efficient. These results suggest that different protein degradation patterns occur during the artificial and natural ageing, probably driven by different mechanisms (Machado et al. 2001).

5.2.4. Effect of seed ageing on stored reserves

5.2.4.1. Total Carbohydrates

The content of total carbohydrate decreased as the vigour of seed lots decreased and was the maximum for the control seed lots. Among the control seed lots the carbohydrate content was maximum in variety V 240 and minimum in V 578. Although there was a germination and vigour enhancement in the high vigour seed lots of all the varieties, a conspicuous decrease in the carbohydrate content was noted. The findings of the present study corroborate the earlier observations of Basavarajappa et al. (1991), Bernal-Lugo and Leopold (1992) and Sammour (1989). They noticed a decreasing trend in the content of carbohydrates during accelerated ageing. The decrease could be due to utilization of carbohydrate in respiration of seeds. Depletion of essential metabolites, including loss of food reserves, is one of the important factors responsible for loss in seed viability (Roberts 1972). Madhava Rao and Kalpana (1994) also reported a reduction in starch and soluble sugar contents during accelerated ageing of pigeonpea seeds and suggested that this reduction might be attributed to the action of hydrolytic enzymes and respiration. On the contrary, results of studies on Brassica campestris by Verma et al. (2003) showed that the levels of total soluble carbohydrates in seeds increased significantly.
with age.

Nevertheless, no simple relationship exists between seed vigour and seed carbohydrate contents. Bernal-Lugo and Leopold (1992) observed a decrease in the content of monosaccharides in aged corn, while Narayana and Sun (2000) observed increase of glucose content in deteriorating mung bean seeds and related it with increase of Maillard reaction products (Monnier 1989). Horbowicz (1997) described various changes of mono and oligosaccharides contents in vegetable seeds during ageing. On the other hand, no correlation between seed germination and oligosaccharide content was observed by Kataki et al. (1997).

The nonaccumulation of galactose and the disappearance of each of the other simple sugars observed in the studies conducted by Feeney and Whitaker (1982) could possibly be accounted for by their participation in the Amadori reaction, and its successor, the Maillard reaction. These protein-degrading reactions are known to occur in seeds. The presence of reducing sugars may affect the viability of seeds through the Amadori and Maillard reactions, which occur more rapidly under accelerated ageing conditions (Wettlaufer and Leopold 1991). Galactose is among the most reactive of the hexose sugars with regard to the Amadori reaction. Bernal-Lugo and Leopold (1992) suggested that these nonenzymatic reactions would be expected to result in lowered activity of enzymic components of the embryo. They reported a decline in several soluble carbohydrates with the decline in seed vigour.

The Amadori reaction involves a simple non-enzymic attack by reducing sugars on amino groups that are usually but not always within proteins, to form fructosyl derivatives or glycated Amadori products to form polymeric brown coloured compounds. These reactions have been reported to cause protein inactivation and DNA damage (Nursten and O’ Reilly 1986). Thus there may be accumulation of reducing sugars in cowpea seeds during accelerated ageing, which could have led to the occurrence of Amadori and Maillard reactions and their product, which in turn might have resulted in protein inactivation, nucleic acid damage, thereby reducing the viability of seeds.

Seed vigour has also been associated with carbohydrate content. In corn, the decline of sucrose and raffinose contents during ageing was closely correlated with the loss of ability to germinate and the rate of germination (Bernal-Lugo and Leopold 1992).

\textit{Discussion}
Furthermore, the quantity of oligosaccharides larger than sucrose ($C_{12}H_{22}O_{11}$) decreased as the period of imbibition during the germination process increased (Koster and Leopold 1988). For prolonged seed storability the presence and mutual proportions of several basic carbohydrates may be even more important, than the total carbohydrate content (Chen and Burris 1990, Bernal-Lugo and Leopold 1992). The decrease of raffinose content in stored seeds has been correlated with decreased germinability (Horbowicz and Obendorf 1994, Piotrowicz-Cieslak 2005). Sucrose, raffinose and its homologues are supposed to be the key factors stabilising the cell structure during dehydration, and dry storage.

In cowpea, great variations in sugar composition namely sucrose, raffinose and stachyose, has been reported among the cultivars by Shahidul et al. (2008). Black Crowder had the highest sucrose, raffinose and stachyose contents. This cultivar also exhibited high germination percentage. The lowest content of sucrose, raffinose and stachyose was in Mississippi Purple, which had low germination percentage. The sucrose, content in ungerminated seed was highest in Black Crowder, followed by Texas cream 40 and lowest in Mississippi Purple. In our study also, it was observed that the cowpea variety V 240 exhibiting the highest germination had the highest carbohydrate content in the control, followed by variety V 585. Variety V 578, had the lowest germination, depicting the lowest carbohydrate content among all the varieties.

Agnieszka et al. (2010) observed that accelerated ageing clearly affected carbohydrate levels in cereals seeds. However the pattern of these changes strongly depended on plant species/cultivar and ageing conditions, thus no simple universally valid hypothesis is available to explain the changes in carbohydrates related to seed-quality loss.

**5.2.4.2. Oil Content**

There was an overall decrease in the oil content of seeds of all the three varieties as the seed vigour decreased. The study involved analysis of oil and fatty acids extracted from whole seeds. Lipid contents have been related to seed vigour in many seeds (Perl et al. 1987). Decrease in total seed-lipid content with germination and vigour loss has been reported by many workers, viz. Worang et al. (2008) in Jatropha seeds, Pukacka and Kuiper (1988) in Acer seeds, Gidrol et al. (1989) in sunflower, Koostra and Harrington (1969) in cucumber, Powell and Matthews (1981) in pea, Francis and Coolbear (1984) in tomato,
Stewart and Bewley (1980) in soybean and by Freitas et al. (2006) in cotton seeds. A significant decrease in total lipid content with duration of storage has been reported for red gram and green gram varieties by Gopinath et al. (2011). Our findings also confirm such a decrease in total lipid content with decrease in seed vigour. On the contrary, increase in total oil was detected due to accelerated ageing by Maqsood et al. (2000). Oil constituents show that cowpea cultivars did not differ significantly in composition of seed oil. Moreover, the quantity of free fatty acids in seed oil of all cultivars are very low, indicating that seed oil from all cowpea cultivars is of high quality. Fatty-acid profile of all cowpea cultivars reveals the lipids as a good source of saturated and unsaturated fatty acids as palmitic and stearic acids. The presence of high levels of unsaturated fatty acids, in all the presently studied cultivars, is nutritionally desirable and results are comparable to previously published works (Adebooye and Singh 2007).

Thapliyal and Connor (1997) reported positive and substantial correlation of seed vigour with lipid content. Seeds of broad bean showed a drop of approximately 20 to 30% in the lipid fraction when subjected to accelerated ageing, however pea seed, showed an opposite trend (Shahidul et al. 2008).

5.2.4.3. Fatty acid profile

High temperature and high moisture content during storage of seeds are known to accelerate the oxidation of unsaturated fatty acids present in the membrane lipid enzymatically or non enzymatically, depending on the adversity of condition. However, the reports regarding loss of vigour and corresponding increase or decrease of the fatty acids are highly contradictory. Harman and Mattick (1976) found that the decrease in germination rate in aged pea seed was paralleled by a pronounced decline in linoleic and linolenic acid, whereas saturated and monoenoic fatty acids remained unchanged. Increase in oleic acid content as well as decrease in linoleic, linolenic acid contents were observed over a period of six months of storage of red gram and green gram varieties by Gopinath et al. (2011). When seeds of soybean were subjected to ageing, the amount of total lipid from whole seed increased, while the amount of unsaturated fatty acids showed a decline (Priestley and Leopold 1983). On the other hand, detectable changes were not observed in the fatty-acid composition of storage lipids from whole seeds and axes of two soybean cultivars despite a considerable decline.
Discussion in seed vigour (Ferguson et al. 1990a, 1990b).

Corebeneau et al. (2002) found that loss of viability in sunflower, was accompanied by an increase in the total lipid content, which corresponded to a similar increase in the saturated as well as unsaturated fatty acids. The cause of such an increase has been hypothesized by them as due to relative stability of many of the enzymes involved in lipid biosynthesis. Besides, Douglas and Palag (1981) have observed that lipogenesis continues in various plant organs subjected to various stresses. The increase in the saturated fatty acids as well as less saturated oleic acids observed in the present study is probably due to selective synthesis of saturated fatty acids, although unsaturated fatty acids showed a clear trend of decrease due to loss of vigour. Significant negative correlation between the saturated and unsaturated fatty acid content indicates the proportional shifts in the ratio of the two kinds of acid during quality changes in seeds of cowpea.

Gidrol et al. (1989) have observed a conspicuous decrease in the contents of both saturated and unsaturated fatty acids with loss of viability in sunflower. Also there was accelerated formation of conjugated dienes at stressful condition but the viability remained high. In cowpea, even in high vigour seeds irrespective of the varieties used, the lipid peroxidation values were significantly higher than control seeds. This increase could have been due to high-temperature-induced oxidation of non-specific lipids present in parts other than the membranes. Gidrol et al. (1989) have raised questions as to whether the peroxidation of the lipids occurs as a consequence of seed ageing or as a factor causing seed ageing. Perhaps investigating the ageing phenomenon and determining the products of peroxidation at frequent and shorter intervals might throw more light on these processes.

The breakdown products of peroxidation of unsaturated fatty acids, particularly the volatile aldehyde compound (VAC), have been sited to cause denaturation of DNA, altered base sequencing, translation and transcription processes, cross linking of sugars with the polypeptides and oxidation of certain amino acids. While superoxide anion is produced by autoxidation of hydroquinones, flavains and thiols, singlet oxygen is generated in the termination step of lipid peroxidation. Hydroxyl radicals are formed from superoxide radicals. According to Trawatha et al. (1995), soybean seeds with highest linolenic acid
content and lipoxygenase lost their physiological quality fastest during storage in unfavourable or extreme environmental (high temp and high moisture) conditions. It is in support of our findings where in the variety V 578 having the highest content of linolenic acid showed the fastest drop in seed quality.

5.2.4.4. Mineral Contents

The mineral content of harvested seeds and after storage can impact food and feed quality. There are not many studies evaluating micronutrients with decreasing seed vigour or quality. The present research has also taken this specific objective under consideration to find out the influence of decreasing seed vigour on quality parameters like mineral composition and antinutritional factors in cowpea seeds.

A general decrease in the ash content with decrease in the vigour was observed for all the varieties. A similar observation has been reported in chilli by Deepa et al. (2011), in watermelon by Opeyemi (2012), in stored grape fruits by Kayisoglu and Demirci (2006). On the contrary, Fagbohun and Lawal (2011) have reported an increase in ash content in stored watermelon seeds.

The iron content decreased significantly in all the varieties with decrease in vigour, and this reduction was more in the variety V 578. Similar decrease in iron content was observed in the studies by Deepa et al. (2011) in chilli seeds stored in jute bags. However, Kayisoglu and Demirci (2006) did not find any significant difference in the iron content in grape fruits stored for prolonged period. The decline may be attributed to its sensitivity to temperature, as reported by Deepa et al. (2011) in chilli seeds. Uma Chitra (1994) has reported that heating processes noticeably decreased iron content in several legumes.

During the storage of the melon seeds, Opeyemi (2012) observed that mineral contents (Na, K, Ca, Mg, Zn, Fe and Cu) in the freshly-shelled watermelon seeds decreased conspicuously at the twentieth week of storage. Similar observation was made by Ekundayo and Idzi (2005), who reported decrease in the mineral content of melon seeds after two weeks of storage. However, this is in contrast to the findings of Fagbohun and Lawal (2011), who reported an increase in the minerals of sundried soybean stored for twenty weeks. Variation in the mineral content between the different vigour lots could be attributed to their redistribution in seeds and a possible microbial contamination (Bognar et al.)
The reduction observed in various minerals in the present study, especially in the medium vigour/low vigour seeds, could be due to the destruction and conversion into an undetectable form due to the high temperature to which the seeds were exposed while subjecting to controlled deterioration. The increase in mineral content, particularly in low vigour seeds could be due to the mineral-ion contribution by fungal growth due to decrease in vitality of seeds.

5.2.5. Effect of seed ageing on enzyme activity

5.2.5.1. Dehydrogenase activity

Dehydrogenases are the enzymes playing crucial role in the transfer of electrons during the respiratory process (TCA cycle). Decrease in the activity of dehydrogenases indicates a decrease in the respiratory rate and metabolism and hence a decline in seedling vigour. Our results are in agreement with the findings of Vasudevan et al. (2012) in peanut, Narwal (1995) in okra, Verma et al. (2003) in mustard and Pallavi et al. (2003) in sunflower seeds. Krishnaswamy and Seshu (1989) reported a positive correlation between oxygen uptake and seed germination rate in rice.

Similar results were also observed in natural and artificially aged wheat varieties by Chauhan et al. (2011). Sundareswaran et al. (2009) reported the existence of genotypic differences in dehydrogenase activity between pearl millet lines subjected to accelerated ageing. Dehydrogenase activity decreased at a faster rate to about 50 per cent of its initial level in the seeds of some genotypes, while it was slow in others. In our study also, dehydrogenase activity was found to be the maximum for high vigour seed lots after which it declined in terms of absorbance in all the varieties. The possible reason of this reduction might be the differential rate of cellular damage that occurred to the sensitive embryo cells during ageing.

5.2.5.2. Oxidative Enzymes

Free radicals and other active derivatives of oxygen are inevitable by-products of biological redox reactions. Seed deterioration has been found to be associated with an accumulation of active forms of oxygen, including superoxide radical (O²⁻), hydrogen peroxide (H₂O₂), and hydroxyl radical (OH) (Noctor and Foyer 1998). Antioxidant defense systems in plants include free radical and peroxide-scavenging enzymes (Song et al.)
Protection against free-radical damage is the major means of preventing oxidative stress predominantly through enzymes. Catalase, Peroxidase and Superoxide dismutase are important active free-radical-scavenging enzyme reported in literature (Bailly et al. 1996, Goel et al. 2003).

In the present study, the activity of various anti-oxidant enzymes has been assayed so as to find their role in cowpea seed deterioration under ageing. Catalase and peroxidase activities decreased as the vigour decreased. In general, decrease in enzyme activity in seed lowers its respiratory potential, which in turn lowers both the energy (ATP) and food supply to the germinating seed. Several changes in the enzyme macromolecular structure may contribute to their lower effectiveness. They may undergo compositional changes by losing or gaining certain functional groups, by partial folding or unfolding of ultrastructure, by condensation to form polymers and degradation to sub units, by oxidation of sulf-hydral groups or by conversion of amino acids within the protecting structure.

Our results are in agreement with several other researchers who have reported similar decrease in the activity of catalase and peroxidase; Scialabba et al (2002) in radish. Pallavi et al. (2003) in sunflower and Bao et al. (2011) in pear stock seeds. Peroxidase and catalase activities were relatively higher in young seeds of Chenopodium rubrum (Mitrovic et al. 2005).

During ageing, peroxidative changes may be the major cause of seed deterioration (Goel and Sheoran 2003). Protective mechanisms that could scavenge the liberated free radicals and peroxides have been reported in soybean (Sung 1996). Demirkaya et al. (2010) reported that a high level of correlation existed between the loss in seed viability and the decrease that occurred in catalase activity in onion. The decreased germination ability of aged seed was also correlated with decrease in activity of antioxidant enzymes by several workers (Cakmak et al. 2010, Bhanuprakash et al. 2010). On the contrary, Sung and Jeng (1994) did not find any decrease in the catalase enzyme activity during ageing of peanut seeds.

Natural ageing not only stimulated lipid peroxidation but also depressed the activity of radical and peroxide-scavenging enzymes. It is possible that accelerated ageing of cowpea seeds denatured catalase and peroxidase-enzyme proteins to different degrees. Among the cultivars, V 585, the cultivar relatively resistant to ageing treatment, showed higher
levels of both catalase and peroxidase, when compared to the other two cultivars. However, all the varieties demonstrated a continuous decline in catalase and peroxidase activities as the vigour decreased. Catalase activity dropped substantially with ageing and the percentage declines recorded at the end of ageing treatment were the maximum in V 578, followed by V 240 and V 585. These findings thus agree with those of Bailly et al. (1996), who showed that loss of seed viability was associated with a decrease in catalase activity during accelerated aging of sunflower seeds. In contrast, Pukacka and Ratajczak (2005) observed that the catalase activity of embryo axes and cotyledons in beech (Fagus sylvatica L.) seeds increased during storage. Merritt et al. (2003) found that catalase activity was not related to seed viability, when seeds of Acacia bivenosa, Anigozanthos manglesii, Banksia ashbyi and Mesomelaena tetragona were stored at different temperatures and humidities.

Peroxidase showed an initial rise in activity in one variety V 240 with ageing, followed by a continuous fall in activity. Similar findings were reported by Kalpana and Madhava Rao (1994). Our findings on catalase and peroxidase activities are consistent with those of Samshery and Banerji (1979), Bhattacharjee and Bhattacharya (1988), Saxena et al. (1987) and Nkang (1988). The loss in enzyme activity may result from age-induced protein deterioration due to Maillard reaction, as hypothesized by Sun and Leopold (1995). It is thus apparent that the reduced enzyme activity of peroxidase metabolism occurring due to ageing may lead to a less protective situation for the seeds during storage at unfavorable conditions.

In all the varieties, however, increased peroxidase activity was recorded immediately after 24 h of ageing. The improved germination and increased activity of 24 h aged seeds may therefore, be attributed to the counteraction of free radicals and resynthesis of membrane-bound enzymes, as reported in other seeds (Basu and Dasgupta 1978, Saha et al. 1990). A further decrease in peroxidase enzyme activity has been observed in the medium-vigour and low-vigour seed lots. Decline of peroxidase activity with increasing storage time was reported by Nkang (1988) and Basavarajappa et al. (1991) in maize, Scialabba et al. (2002) in radish, Gholami and Golpayegani (2011) in rice and Goel et al. (2003) in cotton.

Superoxide dismutase (SOD) plays a key role in inhibition of oxidative stress (Scandalios.
Ageing coincides with protein denaturation and degradation, inactivation of enzymes, breakdown of phospholipids and depository lipids, lipid peroxidation and alteration of membrane permeability. Among the three varieties studied, the highest initial SOD activity was recorded for V 578 and the lowest for V 240. The enzyme activity in cowpea seeds was observed to decrease with ageing in the present study. This is in corroboration with the results obtained in other seeds subjected to artificial ageing such as in beech (Pukacka and Ratajczak 2005, 2007), cotton (Goel et al. 2003), sunflower (Bailly et al. 1996, Kibinza et al. 2006) and soybean (Sung 1996, Murthy et al. 2002 and Balasevic-Tubic et al. 2011). These results support the hypothesis of Bailly et al. (1996) that a decrease in antioxidant enzymes is linked to increased lipid peroxidation and accelerated ageing. Subsequently, Bailly et al. (2000, 2002) proposed a positive relationship between antioxidant enzyme activity and the vigour of the seed. On the contrary, there are reports in literature pertaining to increase in the activity of antioxidant enzymes during accelerated ageing. (Li et al. 2010, Kalpana and Madhava Rao 1994). Gidrol et al. (1994) analysed changes in protective enzyme activities of the high viability and low viability seeds along with monitoring the superoxide anion and \( \text{H}_2\text{O}_2 \) and cytokinin contents. They concluded that in low viability seeds (subjected to ageing), superoxide radicals accumulate in the second phase of germination, which causes induction of SOD and inhibition of catalase. This, in turn, led to accumulation of \( \text{H}_2\text{O}_2 \). The accumulation of superoxide radicals in low viability seeds was presumed to have occurred during seed ageing. The membrane damage, particularly of mitochondria, was identified to be at least partially responsible for the over production of superoxide radicals. Their results indicated that like biotic or abiotic stress related changes, low-viability seeds experience an oxidative stress and hence, the induction of SOD is a measure to combat the above.

It was significant to note that in spite of a decline in the activity of SOD enzyme during ageing, there was no alteration in the banding pattern of various isoforms of SOD and catalase could be observed and therefore no definite relationship could be derived between isozyme banding pattern and vigour of cowpea seeds.

5.2.5.3. Reserve mobilizing enzymes

In the present study, cowpea seeds exhibited reduced activity of \( \alpha \) and \( \alpha \) amylase
with decrease in the seed vigour. In the variety V 240, α amylase activity increased in the high-vigour seed lots. A similar trend was observed for α amylase activity in variety V 585. Reserve mobilization is an important prerequisite for seed germination, which was reflected in improved germination and vigour in high vigour seed lots. These results are in agreement with previous workers in aged seeds of Bambusa (Ravikumar et al. 1998), in wheat (Chauhan et al. 2011). Vasudevan et al. (2012) reported a significantly higher α amylase activity in fresh seeds of Peanut and the values progressively decreased over the period of natural ageing and reached the lowest values at 9 months of storage. Similar to natural ageing, accelerated ageing also depressed the enzyme activity.

A decrease in the α amylase activity in onion seeds due to ageing was reported by Bhanuprakash et al. (2006). Since enzymes are also proteins, cross linking would seriously disturb their functioning. So, there is a direct correlation between loss of viability and decline in enzyme activity (Abdul-Baki and Anderson 1972). On the contrary, increased activities of α and β amylases were reported by Ravikumar et al. (2002) in Dendrocalamus strictus and by Nkang (1988) in Guilfoylia monostylis seeds.

Phosphatases are important reserve mobilizing enzymes providing inorganic phosphates which in turn play important roles in the energy metabolism of growing seedlings. Basavarajappa et al. (1991) showed that activity of acid phosphatase decreased after ageing treatment in maize seeds. In the present study, the activities of acid phosphatase and alkaline phosphatase were found to exhibit decreased activity in different vigour lots for all the varieties. Since acid phosphatase is involved in the maintenance of constant cellular pool of phosphate, this condition might affect the maintenance of a cellular pool of phosphate as well as phosphate metabolism in seeds during ageing. In the light of the aforesaid facts, it is concluded that seed deterioration may be due to marked changes in the activities of enzymes involved in degradation of stored reserves.

Initial higher phosphatase enzyme activity in control seed of cowpea varieties except V 240 indicates the active mobilization of reserves during germination. As the vigour of the seeds decreased, the activity declined in all the varieties in different vigour lots, at different rates. The reason for such differences is not known. These results suggest a positive relationship between reduced acid phosphatase activity and seed vigour. Association
between reduced acid phosphatase activity and reduced seed vigour has been reported by earlier workers in maize seeds (Basavarajappa et al. 1991, Spinola et al. 2000) in wheat seeds (Vieira 1996, Ram and Wiesner 1988) and in cotton (Freitas et al. 2006). Acid phosphatase catalyzes monoesters phosphate hydrolysis (Aoyama et al. 2001) and can be related to lipid peroxidation (Spinola et al. 2000). On the other hand, Rajagopal and Sen-Mandi (1992) found high activity of this enzyme in the embryo of artificially aged rice seed, which was also confirmed by Brandao Jr. (1999) in the corn seeds after 96 h of artificial ageing. Dias et al. (2010) did not observe any association between alterations in acid phosphatase activity and viability loss in stored papaya seeds.

In the present study, the activity of alkaline phosphate significantly decreased in the medium and low vigour seed lots in all the varieties. Aged (naturally and artificially) embryos of rice exhibited a gradual decline in alkaline phosphatase activity. Unaged (99%) embryos exhibited highest alkaline phosphatase activity in dry as well up to 12 h of germination (Rajagopal 1992). Similar findings have been reported by Ravikumar et al. (1998, 2002) in bamboo and Dendrocalamus, respectively.

5.2.6. Effect of ageing on antinutritional factors

Research on changes of antinutritional factors of cowpea seeds carried out by many investigators, but mostly conducted to find the effect of thermal processing, cooking, boiling, dehulling, sprouting and germination. Reports are well documented to find the effect of antinutritional factors in domestically processed cowpea (Modgil et al. 2012), variations in nutrients and antinutrients (Sreerama et al. 2008, Celestine et al. 2012), Water soaking, boiling, roasting, microwave cooking, autoclaving and micronization (Khattab and Arnfield 2009, Wang et al. 1997, Ogun et al. 1989), thermal processing (Udensi et al. 2007), isolation and characterization of trypsin inhibitor (Benjakul et al. 2000, Richa 2009), dehulling (Noubissie et al. 2012, Ogun et al. 1989), traditional processing (Akinyele 1989). However, literature on the effect of seed ageing on the antinutritional factors, viz. trypsin inhibitor, phytic acid and phenols, is totally lacking. Therefore, an attempt has been made in the present study to track the changes in the above mentioned antinutritional factors with moderate and severe loss of vigour in the three cowpea cultivars.

5.2.6.1. Trypsin Inhibitory Activity (TIA)

In the present study a decline in trypsin inhibitor activity has been observed with decline in seed quality. There are no reports relating changes in TIA due to changes in seed
quality. In the present study, the various vigour categories of cowpea were created by increasing the moisture content of the seeds and subjecting them to controlled ageing. It is possible that during this process of ageing metabolic changes similar to those which occur during germination were triggered, but germination itself was prevented due to high temperature. This could have probably affected the TIA also resulting in its decrease. TIA of cowpea has been reported to decrease with increase in germination period (Kumar et al. 2006, Malomo et al. 2011). Moreover, the TI is a heat-labile protein (Wang et al. 1997), the decrease in activity is also expected due to exposure of the seeds to various periods to high temperature and high moisture content. This is evident from the fact that in the present investigation, the seed exposed to high temperature for longer duration (low vigour seeds) exhibited very low TIA as compared to the other seed lots as well as the control (Table 13 and 14).

5.2.6.2. Phenols study

There was an increase in total phenols of all the varieties in the medium and low vigour seed lots, while decrease to various magnitude was recorded in the high vigour lots. The increased concentration of total phenolics that was observed in the low vigour seed lots could be due to cellular deterioration during ageing leading to its greater permeability for the cellular and subcellular content (Roberts and Osborne 1973, Basavarajappa et al. 1991, Custodio and Marcos-Filho 1997, Dias et al. 1996, Hamman et al. 2001). In addition, Ramamurthy et al. (1992) showed that cellular-membrane damage led to synthesis of phenolic compounds as a part of the mechanism of curing. Seed has the capacity to maintain its germination unchanged for some time under conditions of high humidity and temperatures. However, after a certain period of time under conditions of high humidity and temperatures, a sigmoid loss of germination occurred, accompanied by cell membrane damage. As a result, the seed synthesized increased amounts of phenolic compounds. As per the hypothesis proposed by Simic et al. (2004), there were two opposite effects when seed germination decreased; on the one hand, synthesis of phenolics, as a part of the curing mechanism increases and on the other hand, the number of seeds that can synthesize phenolics decreases. As a result, the phenolic concentration increased at the beginning of ageing, but when the viability decreased, phenol concentration also decreased. The results of the present study on the phenol content of the cowpea cultivars corroborate this hypothesis.

In present study, variety V 240 with coloured seed coat recorded the highest content of phenol when compared to rest of the varieties which confirms the presence of more phenolic
content in the coloured seed coat as reported by earlier workers (Preet and Punia 2000, Nzaramba 2004, Shao et al. 2011 and Noubissie et al. 2012). It can be concluded from the present investigation that measurement of phenolic contents in cowpea seed exudates provides a fast and inexpensive method for evaluation of seed quality.

5.2.6.3. Phytic acid

Phytic acid seems to serve as an important reserve of phosphorus, which is generated by the action of phytase during seed germination, resulting in increase in available phosphorus and hence improving the mineral bioavailability (Beal et al. 1984, Hurrell et al. 1992, Davidsson et al. 1994). Phytate-phosphorus accounts for a large proportion of the total phosphorus existing in grains (Kikunaga et al. 1991). During germination, phytates are hydrolyzed by phytase to produce inorganic phosphorus needed in the metabolism, and therefore, the phytic acid content of the cowpea seeds decreased with germination (Ayet et al. 1997).

In the present investigation, a significant decline in the phytic acid content in the medium and low vigour seed categories was observed in all the varieties, while in the high vigour category V 240 and V 585 registered a non-significant decline. These findings are in accordance with the results of previous workers (Kon and Sanshuck 1981, Hernandez-Unzon and Ortega-Delgado 1989, Uma Chitra 1994). Kon and Sanshuck (1981) reported a 65% reduction in phytic acid content of California small white beans stored for 10 months at 32°C. Hernandez-Unzon and Ortega-Delgado (1989) observed that beans stored for 8 years had from 94% to 98% less phytic acid than the recently harvested common beans. In a warm, moist environment, there would be increased metabolic activity, phytase activation, and membrane degradation. Hydrolysis by phytase would result in conversion of phytate to inorganic phosphorus. Enzymatic hydrolysis was shown to increase the conversion of white bean phytate to inorganic phosphorus during storage at high temperatures and high humidities (Chang et al. 1977). The reduction in phytic acid during storage at high temperatures in the present study probably would have resulted from similar hydrolysis of phytate by phytase.

5.3. Cytological Studies

Mitotic index decreased with reduction in germination caused by increased ageing duration. A decreased mitotic index with decrease in seed-vigour lots confirms the earlier reports in other crops. Orlova and Soldamova (1975) found a decreased mitotic potential of cells during ageing in welsh onion (Allium fistulosum L.). The duration of the mitotic cycle was not increased during the course of seed ageing. They concluded that some of the cells of
embryo lose the capacity for division in proportion to ageing of seeds and thus manifested in lower germination potential. Murata et al. (1977) observed the first mitotic division to be delayed by 30 hours in pea seeds stored for longer periods and, as the length of storage period increased, the number of dividing cells and number of germinating seeds decreased. Chauhan and Swaminathan (1984) also observed a marked decrease in the mitotic index of barley cv. DL 3A and soybean cv. Alankar during accelerated ageing condition of 40± 2 °C and 100% RH. Kumar (1988) also observed decreased mitotic index in two varieties of pea and found more fluctuation in mitotic index of variety Bonneville compared to T-163.

There are some evidences of occurrence of chromosomal damage in accelerated ageing (Khan et al. 2003, Chauhan and Swaminathan 1984, Parrish and Leopold 1978), but literature particularly on controlled deterioration is meager. In the present study, the reduction in viability, appears to be correlated with occurrence of lower MI. Free radical damage and background radiation in the form of UV or microwaves are responsible for the chromosomal aberrations that take place during the process of ageing. For the controlled deterioration, the duration of exposure is short to have these kinds of aberration. DNA-repair mechanisms still is active in high viability seeds so any kind of damage that occurs is repaired. The reduction in mitotic index due to the ageing effect has been explained earlier. Seed ageing not only affects DNA integrity, but also alters the activity of the enzymatic machinery responsible for DNA synthesis (Osborne 1980) and limitation of intracellular resources for rapid turnover of DNA synthesis (Samis 1978). The limitation of these resources may perhaps be due to an increase in the leakage of sugars and electrolytes from aged seeds (Sen 1977). It has been observed that the rate of ATP synthesis and the rate of biosynthetic pathways (Nucleic acids, protein and polysaccharides) decline in deteriorated seeds (Anderson and Gupta 1986).

Hence in the present study, the decreased mitotic index observed may be due to the above reasons and a delay in emergence of root by many hours has been seen in aged seeds of the varieties tested.