

# DISCUSSION



## **DISCUSSION**

Seed deterioration is an undesirable attributes of agriculture which can be defined as deteriorative changes occurring with time involving cytological, physiological, biochemical and physical changes in seed resulting in reduced vigour and ultimately causes of death of the seed. Deterioration of seed starts in the field itself before harvest when the seed reaches physiological maturity which extends till the seeds are harvest. Pre harvest factors like exposure to hot and humid conditions, rainfall, photoperiod, after ripening are pre-harvest factors, cause seed quality loss after the seed attains physiological maturity. Khatun et al. (2009) revealed that seed after physiological maturity if retain on the mother plant will deteriorate and also causes physiological changes in seed which might be due to formation of rigid seeds or off colour seeds in pulse crop. Storability of seed is greatly affected by the quality of the seed at the time of storage , pre-storage history of seed , moisture content of the seed, relative humidity, temperature of storage environment and biotic agents ( Khatun et al. , 2009 ; Biabani et. al., 2011). In this experiment effort was made to minimize the deterioration of seeds of field pea during storage by employing different dry dressing and wet treatments, the findings of which is discussed below.

### **Effect of Storage container on maintenance of germinability**

Seed storage is an integral part of seed production programme since seeds of many field crops are produced with greater care and cost. Hence a good storage is essential to keep them alive and vigorous until required for subsequent sowing season .The packaging materials used are decided by kind and quality of seed to be packed, the type of package, duration of storage, storage temperature and relative humidity of the storage etc.

Seed must be properly stored in order to maintain acceptable level of germination and vigour until the time of planting. The storage period may vary from as little as six months, if the seeds are to be planted in the next season or longer if the seeds are to be carried over for one or more season.

Rao et al., (2006) opined that packaging container and storage duration significantly affected viability and seedling vigour. Sushma(2003) noticed that garden pea seeds stored in 700 gauge polythene bag recorded significantly less percent of seed infestation, moisture content and more hundred seed weight than those in cloth bag at the end of 10 month of storage period. Haque et al., (2007) stored the jute seeds in the tin pot, poly bag and gunny bag at room temperature and relative humidity for six month and found that among three containers, seed stored in tin pot showed highest 80 % germination. While examining the pattern of decline in seed vigour and viability of field pea seed stored in different types of containers, it was observed that the bottle storage of seed gave the highest germination percentage and vigour index till the next sowing time followed by tin and poly bag storage. The findings are in conformity with the research outcome of Talwar and Sathyarajan (1974) who found sealed glass bottle as an effective storage container for jute seed. Singh and Singh (1990) also observed that onion seeds stored in sealed glass bottle remains viable for longer period than stored in cloth bag, sealed single paper, double and triple paper.

#### **Effect of seed invigoration treatment on germinability and field performance**

Ageing which induces seed deterioration could be expressed as the loss of vigour and viability. The rate of ageing however, depends on the physiological status and genetic constitutions of the seeds as well as on the resistance capacity of a cultivar to withstand ageing conditions (Anderson and Gupta, 1986). There are several changes associated with ageing that depends upon ageing conditions, differences among cultivars (Kalpana and Rao, 1995; Jain et al., 2006) or heredity conditions (Shepherd et al., (1995). During ageing, seed vigour is the component of seed quality that deteriorates first followed by reduced germinability and seed survival (Trawatha et al., 1995). According to Walter (1988), Seed ageing is recognized by some parameters viz. delay in germination and enzymes, slow growth and increasing susceptibility to environmental stresses. Ghasemi golezam et al., (2010) noticed that decrease in germination percentage and other parameters in aged seed is due to the biochemical and physiological changes associated with aging.

There are number of report that hydration-dehydration treatments as well as treatments of seeds with chemicals with diverse nature viz. salts, phenols, organic acids anti-oxidants, essential oils, fungicides, plant growth regulators, nano particles etc. can favorably influence the viability status of seeds (Kanp and Bhattacharjee, 2003; Sengupta et al., 2005; Pati and Bhattacharjee, 2011, Mandal et al., 2011).

In the present study seeds were treated with different dry crude plant materials, chemicals, pharmaceutical product and several wet treatments were also employed to evaluate the efficacy of those treatment on germinability and field performance.

### **Pre storage seed invigoration treatments**

In the present experiment it was observed that seed treated with red chilli powder @ 1gm/kg of seed and amla fruit powder @ 2 gm/kg of seed exhibit better capacity to withstand seed deterioration as they showed highest vigour index and field performance than others including untreated control while soaking-drying have least tolerance. Esuronso (2010) reported that red chilli powder and trigonella seed powder each at 1g/kg of seed showed significant improvement in germinability as well as yield of Kenaf crop over untreated control. Mandal and co-worker reported that dry physiological treatments with crude plant materials and household preservatives are effective for maintenance of germinability and field performance of wheat, soybean and blackgram(De et al., 2003,2004; Mandal et al.,2000). Our results are in conformity with the findings of earlier worker in this field. Pati et al., (2011) observed that pretreatment of the pea seeds with leaf extract of bel(*Aegel marmelos*)and Kalmegh(*Andrographis paniculata*) 50 gm in 250 distilled water for six hour before accelerated ageing treatment(100% R.H  $30 \pm 2^\circ\text{C}$  temperature) for 45 days gave much better plant performance than untreated control. Our findings are also similar to that of Mishra et al.,(2005) who observed that pre-storage dry seed treatments with red chilli powder @ 1 gm/kg of seed and aspirin @ 50 mg/kg of seed were effective in controlling seed deterioration of sunflower during subsequent storage under ambient conditions.

### **Imbibitional injury during wet treatment**

In legume seed-soaking injury due to rapid water uptake is a well documented phenomenon. Imbibitional injury causes changes in vigour in legume seeds in a temperature range from temperate to tropical climate (Powell et al., 1984). Imbibition damages the seed due to rapid entry of water into the cotyledons during water uptake leading to cell death and high solute leakage from the seeds (Powell and Mathews, 1978a). Soaking injury might be happened from a lack of oxygen during imbibitions, harmful effect of pure water on imbibitions tissues, subtropical temperature during soaking, cellular disorganization(membrane integrity) because of dry seed, leaching out of essential compounds and bacterial action or any combination of these(Larson,1968). Simon and Raja Hrun(1972) opined that as seeds dry out, cell membrane lose their integrity when such dry seeds imbibe water there is a short period before membrane integrity is reformed during which solute can leak out the cell. It was proposed that in the dry pea seed, membrane phospholipids adopt the porous hexagonal organization so that leakage occurred rapidly when the dry seed first placed in water but further leakage is depressed by reforming the normal bi layer structure of membranes following rehydration, also solutes from inner cells may be limited by the increased length of diffusion path( Simon,1974).Bramlage et al.(1978) suggested that leakage period is a time of membrane reorganization and a greater solute leakage during imbibitions may deplete the tissues of soluble food reserves resulting in delaying germination as retarding growth of soybean seedling. Moreover, solute leakage may stimulate growth of microorganism. In this study reduction in germination percentage and vigour index has been observed in the seed lots treated with soaking drying treatment. Use of osmoticum, polyethene glycol very successfully overcomes soaking injury by reducing water uptake ((Wood stock and Tao, 1981). In our experiment; we used moist-sand conditioning drying and moist-sand conditioning- soaking drying treatment so as to reduce the pace of entry of water in the seed to minimize the effect of imbibitional injury and it was observed that mid-storage moist-sand conditioning soaking-drying and moist sand conditioning drying treatments were effective in controlling further deterioration of field pea seed under storage condition. Our results in this regards are in accordance with the findings of the Mandal and Basu 1984, 1987).

### **Mid storage treatment**

Progressive loss in seed quality occurred with ageing in all the treatments under mid storage conditions but the inter treatment variations as well as difference between the performance of treated and untreated control were very significant. Mid storage dry seed invigoration treatment with amla fruit powder @ 2 g/kg of seed exhibit the best performance in terms of germinability and field performance followed by red chilli powder @ 1 g/kg of seed. The present results are in accordance with the findings of Mandal and Basu ,(1983) ; Mandal et al., (2000;2008) and Guha et al.(2011), observed that mid storage wet as well as dry dressing treatments in high medium vigour seeds are effective in maintaining the germinability and productivity.

The present findings also revealed that mid storage moist-sand conditioning soaking-drying treatments were much effective in controlling the seed deterioration might be due to the scavenging of free radicals and counteraction of lipid peroxidation reactions.

### **Efficacy of Seed Size**

Seed size is one of the main factors that affect seedling rate. Environmental variation affects the seed size culminating in production of smaller seed under unfavourable conditions. Seed thus produced affect the germination, seed vigour, field stand and processing recovery (Dighe and Patil, 1981). Seed size one of the important yield components which has an effective role on cultivar adaptation to different condition with affecting the seed vigour (Morison and Xue, 2007). Large Seed size significantly increased survival of seedling compared to small seed by 25% (Rohita et al., 2004). Similar study was reported by Stougarrd et al. (2005) who inferred that large seed size improve the grain yield by 16% in wheat. In our study it was observed that large size seed when treated with red chilli powder@ 1 gm/kg of seed gave the best performance both in terms of germinability and productivity. However the treatment effects are independent of seed size. Our observations are similar to that of many research workers in this field. Dharmalingam and Basu (1987) demonstrated that large and medium size seeds of mung bean (*Vigna radiata*) were superior to small or ungraded seed in terms of field emergence, crop growth and yield. Perry (1980) reported the correlation between seed

size and seed nutritional resources and an increase in seed size has a positive role in seedling size has a positive role in seedling growth and subsequently increasing the seed yield in favourable germination and growth condition.

### **Compatibility of dry dressing treatment with pesticidal formulation**

Infection of plants or seeds in the field or in storage by various plant pathogens, fungal, bacterial or virus can reduce seed vigour directly through such mechanisms as enzymatic degradation, toxic production and growth regulation. Several workers have applied various pesticidal treatments both organic and inorganic to minimize the effect of pathogen attack during seed storage. In the present experiment it was noticed that dry dressing of seed with red chilli powder @ 1g/kg of seed and amla fruit powder @ 2g/kg of seed in combination with thiram @1g/kg of seed improved storability and field performance of the resultant crop . The result support the work of Shekheramurthy et al. (1994) who demonstrated that thiram treatment could delay the seed deterioration process under adverse storage condition of sorghum seed. Seed treated with organic pesticide viz. *trichoderma viridii* also slowed down seed deterioration than other treatment combinations and untreated control. Mastouri et al. (2010) found that seed treated with *trichoderma harzianum* increase seedling vigour and ameliorates biotic and abiotic stress by inducing physiological protection in plants against oxidative damage. Adebisi et al. (2004) reported that the soybean seed when dry dressed with fungicides and or insecticides viz. Apron plus (Carboxin + furathiocarb), Fernazan D (Thiram), Almithio (lindane + thiram) showed significantly longer storage life than untreated seeds. Mandal and co-workers reported that dry treatments in combination with pesticidal formulations significantly improved storability and field performance of several orthodox crop seeds [(Guha et al., (2011); De et al., (2004); Biswas et al., (2010)] which further strengthen our work.

### **Lipid peroxidation activity and volatile aldehyde production in seed deterioration**

Lipid peroxidation is an oxidative damage of cell membranes, lipoproteins and other molecules containing lipids, caused by oxidative stress. Once initiated, reaction of lipid peroxidation continues auto-catalytically and progressively leads structural and functional substrate changes (Popovic, 2001). The mechanism of lipid peroxidation is often initiated by oxygen around unsaturated or poly unsaturated fatty acids such as oleic acid and linoleic acids commonly found in seed membranes which terminate into release of secondary toxic product like malondialdehyde, hexaldehyde and other volatiles causing damage to the seed membrane. Many research workers supported the fact that Lipid peroxidation is one of the important factors causing loss in seed viability (Harman and Mattick 1976; Stewart and Bewley, 1980; Lima et al., 2010, and Wang et al., 2012). The initiation of membrane leakiness and consequent loss of intracellular compartmentalization predominantly due to lipid damage are distinguishing factors of seed ageing. Lipid peroxidation is commonly used as an indicator of oxidative damage by free radical accumulation in plants (Smirnoff 1993). Kaewnaree et al. (2011) observed an increase in lipid peroxidation activity resulting in reduced germination indicating that increased lipid peroxidation might be one of the causes of the low vigour of poor quality seed in sweet pepper. Wei et al. (2011) observed in *Jatropha curcas* that protective enzymes of seeds were destroyed by membrane lipid peroxidation resulting in a decrease in superoxide dismutase activity and an increase in the malondialdehyde activity.

In the present study, intensity of lipid peroxidation increased during accelerated ageing. It was observed that malondialdehyde (MDA) content in seed under accelerated ageing condition was significantly increased in the untreated control seed compared to different treated seeds. The present study indicates that lipid peroxidation has a definite relationship with membrane disintegration and elevated volatile aldehyde production as exposed by increased electrical conductivity of seed steep water after accelerated ageing with simultaneous increase in the value of lipid peroxidation. From the present experiment it can be inferred that seeds treated with red chilli powder, amla fruit powder under pre-storage condition and seed treated with the same plant materials and moist sand conditioning soaking-drying treatment under mid-storage condition significantly reduced

lipid peroxidation activity. It has been shown that i.e. large size seed alone or treated with red chilli powder and amla fruit powder could significantly lower down the lipid peroxidation activity in field pea seed. The research findings of several workers like Brand et al., (1990); Dey and Ghosh,(1993) ,Mandal et al.,(1999;2000) further strengthen the present findings in this regard.

### **Enzyme activity in relation to seed deterioration**

The enzyme plays an important role in the progress of seed deterioration and changes in their activity can be an indication of quality loss. Almost all the enzyme activity is positively correlated with germination of seed , as ageing progressed germination also decreased and enzyme activity also decreased which showed significant deterioration in both accelerated as well as in natural aged seed lot(Merritt et al.,2003). Loss of seed viability in storage has been related to enzyme activity. Seed deterioration involves decrease in the activity of respiratory and associated enzymes viz. peroxidase, glutamic acid oxidase and catalase with simultaneous increase in the activity of hydrolytic enzyme viz. phytase, protease and phosphatase during storage Abdul-Baki (1980). Scialabba et al. (2002) reported that peroxidase and catalase activity decreased in aged seeds as compared to fresh seeds in radish. Pallavi et al. (2003) observed sharp decline in peroxidase and dehydrogenase enzyme activity during ageing in sunflower. Verma et al. (2003) observed that the dehydrogenase enzyme activity was reduced as the ageing progress and was found lowest after four year of storage of Brassica spp. Agarwal and Kharlukhi (1987) found decline in amylase activity in natural aged seed lot in natural aged gram, chickpea and wheat seed as time of aging increased. Baily (2004), reported decrease in germination percentage in aged seeds of barley due to reduction of  $\alpha$ -amylase activity and carbohydrate content. Dhaliwal et al. (1991) and Desikachar et al. (1960) reported significant decrease in the  $\alpha$ - and  $\beta$ -amylase activity of rough rice samples during storage. Hou-Long Yu et al. (2008) suggested that seed deterioration during accelerated ageing is closely related to the increase of electrical conductivity and the decrease of dehydrogenase and  $\alpha$ - amylase activities of the seeds of *Robini pseudoacacia* and *pinus tabulaeformis*.

In the present study progressive deterioration in the enzyme activity (catalase, peroxidase,  $\alpha$ -amylase,  $\beta$ -amylase, dehydrogenase) was observed after accelerated ageing which is in accordance with the finding of the earlier work. But out of the all treatment combinations, seed treated with red chilli powder @ 1gm/kg of seed as a pre-storage treatment and amla fruit powder @ 2g/kg of seed under mid storage treatment found better enzyme activity than others including control reflecting in better germinability and field performance which corroborate the findings of Mandal et al., (1986;2000;2003).

The enzymatic activity of red chilli powder (@ 1gm/kg of seed) and amla fruit powder (@ 2g/kg of seed) treated seed when formulated with thiram @ 1gm/kg of seed was higher than untreated control and other treatment combination. Our study report are in accordance with the findings of Kathiravan et al. (2007) reported the improvement of both physical and biochemical parameter of *Jatropha* seed when treated with Carbendazim @ 2gm/kg, halogen mixture (chlorine based) @3gm/kg of seed and carbendazim @ 2gm/kg + halogen mixture @ 3gm/kg of seed over untreated control. Treated seeds exhibit higher germination percentage, higher activity of peroxidase, dehydrogenase,  $\alpha$ -amylase and lower rate of electrical conductivity and lipid peroxidation. The findings of De et al. (2004) in wheat further confirms our result in this aspect.

Among several seed quality attributes, seed size is recognized as an important trait of seed vigour that influences storage potential and field performance in many crops. It also indicates the availability of reserve food materials during the time of germination field emergence and stress conditions. In the present experiment it was observed that, large size seed has better enzyme activity might be due to greater food reserves than medium and small size seeds. Further, it was noticed that large size seeds when treated with red chilli powder (@ 1gm/kg of seed) augmented the performance than other treatment. Earlier research work done by Roozrokh et al. (2005), shows large seeds of chick pea had high germination percentage, more seedling dry weight and better electrical conductivity in comparison to small seeds. The present works are also in line with that of Rise et al. (1976) who found that large seeds contain more protein and produced higher seedling weight than that of small seeds. Similar results have been detected on cowpea

(Franchis and Coolbera, 1984; Lush et al., 1980) and Barley (Roebuck and Trener, 1978).

### **Study of Soluble protein**

In the present study the quantity of free soluble protein decreased substantially due to accelerated ageing in all the treated seeds including control but inter treatment difference was remarkable and the difference with that of control was highly significant. These results are in agreement with those of Ayyappan et al. (2006) who observe that the total protein content was reduced to almost half of the initial content in cucumber seeds on the 8<sup>th</sup> day of accelerated ageing and was substantially decreased after ageing in *Dendrocalamus*, as also noticed by Ravi kumar et al. (2002). It was observed that seed treated with red chilli powder @ 1 g/kg of seed and amla fruit powder @ 2 g/kg of seed showed much higher free soluble protein contents than other treatments including untreated control. A reduction in the total content of storage components such as proteins and carbohydrate with ageing has also been observed 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. Lamphrang, (2013) reported that accelerated ageing in purple and yellow colour seed of maize reduce the soluble protein, total water soluble sugars and alpha amylase activity whereas amino acid, protease activity increased during seed deterioration. The possible reason for this decrease in protein content might be due to increase in enzymatic degradation of protein by protease leading to a sharp increase in total free amino acids as recorded in our present experiment and the same result was recorded by Ravi Kumar et al., (2002); Coolbear et al., (1984); Nautiyal et al., (1985) and Basavarijappa et al., (1991). Mc. Donald, (1999) described the reduction in seed viability and protein content are due to the fact that the cellular membranes are composed mainly of protein and lipids. As the process of seed ageing progresses, disorganization of proteins and lipid phase transition influence the membrane structure and integrity and subsequently the seed viability.

Separations of soluble proteins of seeds of field pea of various treatments including that of untreated control revealed that accelerated ageing resulted in decrease in the total

number of peptide band, intensity of band and total disappearance of some particular bands. Out of all treatment combination seeds treated with red chilli powder @1 g/kg of seed had reflected higher number of peptide band which is 14 as compared to the seed sample of control and soaking drying which had the lowest 9 and 8 numbers of peptide band respectively.

Several other research workers also found that as seed ageing progress there is reduction in protein quantity, number of peptide bands and intensity. Our results are in accordance with those other workers who have reported similar reduction in number and intensity in protein banding of low vigour seeds while working with amaranthus (Kehinde et al., 2013), peanut (Vasudevan et al., 2012), rice (Das et al., 2010). Vishwanath et al. (2007), working different seed lots of tomato reported that accelerated ageing culminate into decline in band intensity, band number or loss of some bands. Consistent loss of band indicates losses in protein function and suggested that the seeds lost genetic intensity at this point of ageing, as documented by Kehind et al. (2013).

Anuradha et al. (2014) also found that after accelerated ageing of 144 hours at  $40\pm 1^{\circ}\text{C}$  and 100% relative humidity there was disappearance of major protein band suggesting that besides the damaged caused by free radicals induced lipid peroxidation and protein denaturation also contributed towards seed deterioration in cotton seed.

### **Activity of Phenols and antioxidant during ageing**

Polyphenols are effective antioxidants which are classified to various groups according to their structure and attributes. Phenolics contains so called free radical scavenging effect which reduce the number of free radicals (Richard-forget et al., 1995), inhibit lipoxigenase and work as uncompetitive inhibitors. They have protective effect is against disease, pest and physical stress. High correlation between seed viability and total phenolic content has indicated their role in preventing seed ageing process (Pukacka and Ratajczak, 2007). Lachman et al. (2003) suggested an increase in phenolic acids with free hydroxyl group (caffeic acid and gallic acid) after accelerated ageing. Present study indicate that treated seed had more phenolic content than untreated control as ageing progress resulting in more DPPH free radical scavenging activity in those seeds. The

increased initial concentration of total phenolics which was observed in low vigor seed lots could be due to cellular degradation during ageing leading to its greater permeability for the cellular and sub cellular content (Robert and Osborne, 1973, Basavrajappa et al., 1991; Custodio and Marcos-Filho, 1997; Dias et al., 1996; Hamman et al., 2001)). Seed has the capacity to maintain its germination unchanged for some duration under adverse condition. 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 seed synthesizes more 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 curing mechanism increases on the other hand, the number of seeds that can synthesize phenolics decrease. As a result, the phenolic concentration increased at the beginning of ageing, but when the viability decreased, the concentration also decreased. The results shows that seeds treated with red chili powder @ 1g/kg of seed, amla fruit powder @ 2g/kg of seed had more total phenol and free phenol content resulting in higher DPPH free radical scavenging activity probably due to the fact that these treated seeds had higher vigour to resist the membrane degradation during accelerated ageing as compared to the seeds of untreated control.

### **Mode of Action of Wet treatment**

The beneficial effect of wet treatment was observed in field pea seed when employed as a mid storage treatment specially with moist sand conditioning soaking drying treatment and moist sand conditioning drying treatment in the maintenance of vigour, viability and yield potential of the crop.

Villiers (1974) and Villiers and Edgcumbe (1975) observed that in fully imbibed seeds, repair of vital bio-organelles would take place and as a result storage life of seed would be extended. Villiers (1974) showed that due to imbibitions of dry stored lettuce seed there was not only a reduction in the rate of senescence but also there was a definite reversal of damage to the chromosomes and membrane took place. Pan and Basu (1985), however, indicated the absence of *de novo* protein synthesis in moisture equilibrated

dried lettuce seed. But the involvement of pre existing enzyme proteins in the repair process can not ruled out.

The beneficial effect of hydration-dehydration treatment on seed might be due to enzymatic repair of biochemical lesions and quenching of free radical during hydration (Basu, 1976; Benjau, 1978; Buchvarov and Gantcheff, 1984). Vanitha et al.(2009) reported that pre storage hydration- dehydration treatment with chicory (*Cichorium intybus* L.) leaf extract improve the germination percentage, speed and total dry matter production. They also reported that treated seed showed lower electrical conductivity, leaching of amino acids and lipid peroxidation activities.

That seed hydration can bring about radical quenching has been shown in radiobiological experiments (Ehrenberg, 1961; Cook, 1963; Haber and Randolph, 1967). According to Ehrenberg (1961), the enhanced mobility of free radicals upon hydration would facilitate their recombination into harmless non-radical products. Scavenging of free radicals by superoxide dismutase (SOD) during seed hydration, as recorded in radiation studies (Krizala et al., 1983), may also lead to reduced free radical damage during subsequent storage.

Seed priming improved seed germination and seedling emergence and reduce seed deterioration. Restoration of seed quality; however, depends on the degrees of seed deterioration and the restoration could be achieved within a limited extent.

Seed priming could reduce the total peroxide and inhibit the evolution of malondialdehyde(MDA) . Boonsiri et al. (2007) observed that increase in germinability of primed seed was due to decrease in the total peroxide and malondialdehyde (MDA) and increase in total ascorbate, dehydroascorbate and catalase activity. In our experiment it was observed that mid storage moist sand conditioning soaking-drying and moist sand conditioning drying treatment could successfully reduce the total peroxide and MDA content in seed. Similar decrease in the total peroxide and MDA content as effective by seed priming was also reported in Maize (Ya-jing et al., 2009).

Priming also improves the germination of bitter gourd seeds and antioxidant activity (Wang et al., 2002; Hsu et al., 2003; Yeh et al., 2005). Boonmee et al. (2013) observed that seed priming could enhance the anti oxidant activity due to enhancement of scavenging enzyme activity such as superoxide dismutase, catalase etc. It could be suggested from the above findings that controlled priming can improve seed deterioration corresponding to the repair of the membrane which ultimately cause the reduction of lipid peroxidation and the increase rate of antioxidant system synthesis responsible for eliminating ROS from the cell in field pea seed.

#### **4.2 Mode of action of dry treatment**

Mandal, Basu and other co-workers tested a range of crude plant preparations which are generally used traditionally as spices and household preservatives. They observed that harvest-fresh (high vigour) wheat seed when treated with red chilli powder , turmeric rhizome powder (*Curcuma longa* L.), nisinda (*Vitex negundo*), neem(*Azadirachta indica* L.), bael (*Aegle marmelos*),mango (*Magnifera indica* L.), vinca(*Catharanthus roseus*) leaf powder could significantly slowed down the deterioration of seed in storage . (Mandal, De and Basu, 1999; Mandal ,De, Saha and Basu,2000, De and Mandal, 2012; Guha and mandal,2013). They also reported that the effect of natural plant preparations is basically physiological in nature because volatile aldehyde production was lower in the treated seed. Umarani et al. (1997) also noticed similar type of effect when they treated the seeds of *Casurina equisetifolia* seeds with different leaf powder viz. *Albizzia amara*, *Vitex negundo* and *Azadirachta indica* for the maintenance of vigour and viability during storage period. Present experimental findings further uphold the results of earlier mentioned worker in this regard.

*Aspirin*, an active ingredient of ortho acetyl salicylic acid reported to have protein protective role which in turn might helps to maintain storability, field performance and productivity of wheat, soybean and black gram seed (De et al., 2003,2004; Mandal et al., 2000). Aspirin is an anti-inflammatory drug and chemically is a weak acid. It may decrease the production of free radical and superoxide and may interact with adenyl cyclase to alter the cellular concentration of cAMP (Bertram, 1998). Takaki and Rosim

(2000) have reported that aspirin application to *Raphanus sativus* L. seed would increase tolerance to high temperature and synchronize seed germination.

Vitavax (carboxin) , a fungicidal seed treatment has been utilized by several investigators not only to control fungal pathogens but also to improve percentage and rate of germination, to increase seedling growth and yielding in rough lemon seeds ( Sharma ,1989), rice seed (Mishra et.al.,1990), Chick peas (Zaidi et al.1991). Duan-Qiang et al., 2012 reported that maize seed when dry dressed with imidachloprid powder to have higher seedling height, germination potential, vigour index over untreated control.

The role of iodine in the stabilization of double bonds of unsaturated fatty acid moieties of lipoprotein bio membrane as a possible reason for viability extension was suggested by Basu and Rudrapaul (1980), besides the possibility of iodine acting as a free radical controlling agent (Pryor and lass well, 1975).

The beneficial role of chemicals dry-permeated into the seed on storability was recorded by Basu et al. (1979) in lettuce and Dey and Basu (1985) in Indian mustard. Woodstock et al. (1983) successfully minimized the deterioration of stored parsley (*Petroselinum crispum* L.) and onion (*Allium cepa* L.) seeds using the antioxidants vitamin E ( $\alpha$  – tocopherol) and butylated hydroxyl toluene (BHT) and suggested that lipid peroxidation was probably reduced by their application prior to storage.

The carrier, calcium carbonate even when used alone (not mixed with active chemicals) has been found to improve storability of number of seeds. In the present study , the chemicals, pharmaceutical products and crude plant materials were selected on the basis of the previous studies conducted in the present laboratory with seeds of wheat and other crop plants (Rudrapal and Basu,1980;Mandal and Basu,1986; Pal and Basu,1988;1993;De,Mandal and Basu,1998) for their possible effectiveness in controlling free radical reactions as antioxidants, antioxidant synergists and radio protective agents ( Heckley and Dimmick,1967; Demopoulos(1973b) ; Milvy,1973).

Mode of action of beneficial effect of dry treatments on viability maintenance is yet to be elucidated. Capsaicin an active ingredient of red chilli powder has been established as an

inhibitor of lipid peroxidation (Brand et al., 1990; Dey and Ghosh, 1993). Amla (active ingredient phyllembin) are effective broad spectrum antioxidant and free radical scavengers, helping to reduce disease and slow ageing process. The beneficial effects of amla as an antioxidant have been examined by a number of authors (Rao et al., 2005; Majumdar et al., 2015). Protein protective role of ortho-acetyl salicylic acid (active ingredient of aspirin) might be responsible for maintenance of viability of stored seed (Mandal et al., 2000). Kumaran et al. (2006) found that gallic acid and tannic were major phenolic compounds of *phyllanthus eblica* in protein fraction and the phenolic compounds are very important plant constituents because their hydroxyl groups confer scavenging ability.

A very important point which requires further research on the mechanism of entry of active ingredients of crude plant preparations or pharmaceuticals in the dry state into the dry-stored seeds. Surface application of dry powders on the outer surface of a seed with relatively high moisture content may cause a slow penetration of soluble materials but how the same would happen in a seed with 9-12% moisture content ( tightly hold water molecules which are not freely available for solubilization and diffusion of the solution ) is an interesting but debatable issue. The present findings and the findings of the earlier workers in this regard convincingly vindicate that the treatments significantly improve the seed performances. As the seed is not an absolutely sealed living unit, the cracks and crevices present in the seed coat might serve as a path of entry of these exogenously applied substances beside facilitating gaseous exchange.