



## Germination Characteristics of Seeds of Maize (*Zea mays* L.) Exposed to Magnetic Fields under Accelerated Ageing Condition

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### ABSTRACT

Temperature and relative humidity in combination cause rapid decline in seed quality during storage. To maintain the seed quality in storage a number of physical and chemical treatments are used. Magnetic field treatment is one such physical treatment practiced to improve the seed quality. An experiment was conducted to investigate the efficacy of this treatment to improve the storability of seeds by subjecting the treated seeds to accelerated ageing conditions and periodically evaluating the deterioration. Our earlier experiments showed that exposure of fresh seeds of maize (*Zea mays* L) to specific static magnetic fields enhanced germination characteristics, field emergence traits and reduced seed leachate conductivity. In the present study, seed lots of maize (cv. Ganga Safed-1) were exposed to 100 mT for 2 h and 200 mT for 1 h respectively and subjected to accelerated ageing. Results showed that seed viability and vigour with days of artificial ageing was partially ameliorated in magnetically exposed seeds but showed significantly less reduction as compared to untreated controls. Similarly, magnetically treated seeds showed lower leachate conductivity than in unexposed control seeds during ageing. Ageing reduced the activities of antioxidant enzymes, viz., superoxide dismutase, catalase and peroxidase and level of soluble protein. However, seeds exposed to magnetic field maintained greater activity of these enzymes and higher levels of soluble proteins compared to untreated controls. Exposure of seeds to magnetic field reversed to some extent the deterioration caused by ageing as indicated by greater germination percent, seedling length and seedling dry weight than aged unexposed control.

**Key words:** Germination characteristics, Seed leachate conductivity, Antioxidant enzymes, Seedling vigour

### Introduction

Temperature and relative humidity together cause rapid decline in seed quality during storage. The pattern of seed longevity and storage potential of different species have been studied by several workers both for long and medium term under ambient and controlled storage conditions and for short term under accelerated ageing conditions. The accelerating ageing test, in which seed is incubated for a short period (a few days) under high humidity and high temperature conditions, was first developed by Delouche and Baskin (1973) for predicting the storability of seed.

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Likhatchev *et al.* (1984) concluded that physiological changes in seeds subjected to accelerated ageing were largely similar to those during natural ageing with the main difference being the rate at which they occur. The possible sequence of events, occurring during the seed ageing has been described by (McDonald, 1999). Generally it is believed that the damage to cellular membranes is one of the key changes, followed by impairment of cellular repair and biosynthetic processes, reduction in the speed of germination and the rate of seedling growth increased susceptibility to abiotic and biotic stresses, poor field emergence and finally the loss of viability. Deteriorative reactions proceed more readily in seeds at higher moisture content and subsequently,

the high moisture conditions constitute threat to the longevity of seed survival (Vertucci 1990).

Increased membrane permeability has been reported to result in an increase in electrical conductivity of seed leachate in different crop species with ageing (Dadlani and Agrawal, 1983; Ferguson *et al.*, 1990 and Kalpana and Rao, 1996). Electrical conductance of seed leachate is low in fresh seeds which increase as the ageing period increases, due to loss of membrane integrity in ageing process and leading to more loss of electrolytes into the imbibing medium. The main cause for membrane deterioration would be lipid peroxidation (Parrish and Leopold 1978). High temperature and high seed moisture further accelerates seed deterioration (Abba and Lovato, 1999). A large number of reactive oxygen species are generated in the seed during ageing which causes lipid peroxidation (McDonald, 1999). This free radical induced non-enzymatic peroxidation, has the potential to damage membrane and is the major cause of seed deterioration in storage. Some protective mechanisms involving free radical and peroxide scavenging enzymes, such as catalase (CAT), peroxidase (POD), glutathione reductase (GR) and superoxide dismutase (SOD) are evolved within the seed (McDonald, 1999). Loss of seed viability has also been associated with decreases in superoxide dismutase, catalase and glutathione reductase activities.

Exposure of dry seeds to both static and pulsed magnetic field has improved the germination and vigour of a number of crops (Galland and Pazur, 2005). In our earlier experiment we had found significant increase in germination and vigour characteristics in maize (*Zea mays* L.) seeds subjected to magnetic fields of 100 mT for 2 h and 200 mT for 1 h. Among various combinations of magnetic field (MF) strength and duration, best results have been obtained with MF of 100 mT for 2 h and 200 mT for 1 h exposure (Vashisth and Nagarajan, 2007). The present investigation was carried out to evaluate the efficacy of this treatment in ameliorating the adverse effect of ageing in maize seeds.

## Materials and Methods

### *Seed Viability and Vigour of Seeds Exposed to Magnetic Fields during Accelerated Ageing*

Maize (*Zea mays* L.) seeds (Var. Ganga Safed-2) were obtained from National Seed Cooperation, New Delhi. The moisture content of the seed was  $7.55 \pm 0.018\%$ . Seeds were exposed to the MF of 100 and 200 mT for 2 and 1 h respectively using electromagnetic field generator (Vashisth and Nagarajana, 2008). Magnetically treated and untreated seeds were subjected to accelerated ageing as per the procedure described by Deoulouche and Baskin (1973). The effect of magnetic field on the viability of seeds was investigated. Seeds in muslin cloth bags were placed at high temperature (40°C) and high humidity (95-100%) by placing over sieve in desiccators having water at the bottom. Seed moisture content, seed leachate conductivity and germination characteristics were measured in the seeds sampled on alternate days till they lost their viability completely. Seed moisture content was determined by oven drying seeds at 95°C to constant weight (Walters, 1998). Moisture content (%) was calculated as  $[(W_1 - W_2) / W_2] \times 100$  where  $W_1$  was the initial weight of the seed and  $W_2$  was the final weight of the seed after drying. Seed germination was determined by following the method of ISTA (1985). Four replications each with 25 seeds were placed between two layers of moist germination papers, rolled carefully and wrapped in a sheet of wax paper to reduce surface evaporation. They were placed in the germination incubator at 25°C in an upright position. After 7 days, germinated seeds were grouped as normal, abnormal seedling, fresh ungerminated and dead seeds. Germination percentage was calculated based on normal seedling. Ten such seedlings from each replicate were randomly taken for measuring shoot and root length. Subsequently, they were dried overnight in an oven at 90°C and the dry weight of these seedlings was measured. Seedling vigour was calculated following Abdul Baki and Anderson (1973) as;

Vigour index I = Germination % x Seedling length (Root + Shoot)

Vigour index II = Germination % x Seedling dry weight (Root +Shoot)

Four replications each with 25 seeds were soaked in 25 ml of distilled water at 25°C for 24 hours. A control with distilled water (but without seeds) was also taken. The electrical conductance of the seed leachate decanted in a 50 ml beaker was measured at room temperature (ISTA, 1985) using digital conductivity meter (Systronics, model 304, India).

#### ***Antioxidant Enzymes in Aged Seeds Treated with Magnetic Field and Untreated Seeds***

Treated and untreated seeds were subjected to accelerated ageing condition. The effect of magnetic field on the activity of antioxidant enzymes of seeds was assessed from the loss of viability by about 30%. This happened on 6<sup>th</sup> day of accelerated ageing treatment. SOD (Yu and Rengel, 1999), catalase (Aebi, 1984) and peroxidase activities (Castillo *et al.*, 1984) were estimated.

#### ***Antioxidant Enzymes Assay***

Enzyme extract for SOD, CAT, GR and POX was prepared by grinding 1 g seed sample in 10 ml of 0.1 M potassium phosphate buffer, pH 7.5 containing 0.5 mM EDTA. Extract was centrifuged at 15000 g at 4 °C for 20 min and supernatant was used as enzyme. For superoxide dismutase estimation reaction mixture consisted of 20 mM methionine, 2.25 mM nitroblue tetrazolium chloride, 3 mM EDTA, 60 µM riboflavin, 50 mM sodium carbonate, 100 mM phosphate buffer pH 7.8 and 100 µl enzyme extract. Tubes containing reaction mixture were kept under two 15 W fluorescent lamps for 15 min. Reaction mixture without enzyme was used as control. A sample containing complete reaction mixture kept in dark was used as blank. Absorbance was recorded at 560 nm (Yu and Rengel 1999). Catalase activity was estimated following the method described by Aebi (1984). Reaction mixture contained 50 mmol /L phosphate buffer (P<sup>H</sup>=7.0), 15 mmol /L H<sub>2</sub>O<sub>2</sub> and 50 µl enzyme extract. Reaction was monitored after each 30 min at 240 nm in double beam UV-

spectrophotometer and using pure buffer as blank. Peroxidase assay the reaction mixture contained 10 mM phosphate buffer pH 6.1, 12 mM hydrogen peroxide, 96 mM guaiacol and 50 µl enzyme extract. Blank contained complete reaction mixture without H<sub>2</sub>O<sub>2</sub>. Absorbance was recorded at 470 nm (Castillo *et al.* 1984).

#### ***Magnetic Treatment of Partially Aged Seeds***

Maize seeds were subjected to accelerated ageing at high temperature (40°C) and high humidity (95-100%) until they lost about 30% of their initial viability. This happened in 6 days. Then they were exposed to magnetic fields of 100 mT for 2 h and 200 mT for 1 h respectively. The germination characteristics and seed leachate conductivity of fresh seeds (exposed and unexposed to magnetic field) and partially aged and then exposed to magnetic field along with unexposed aged controls were measured.

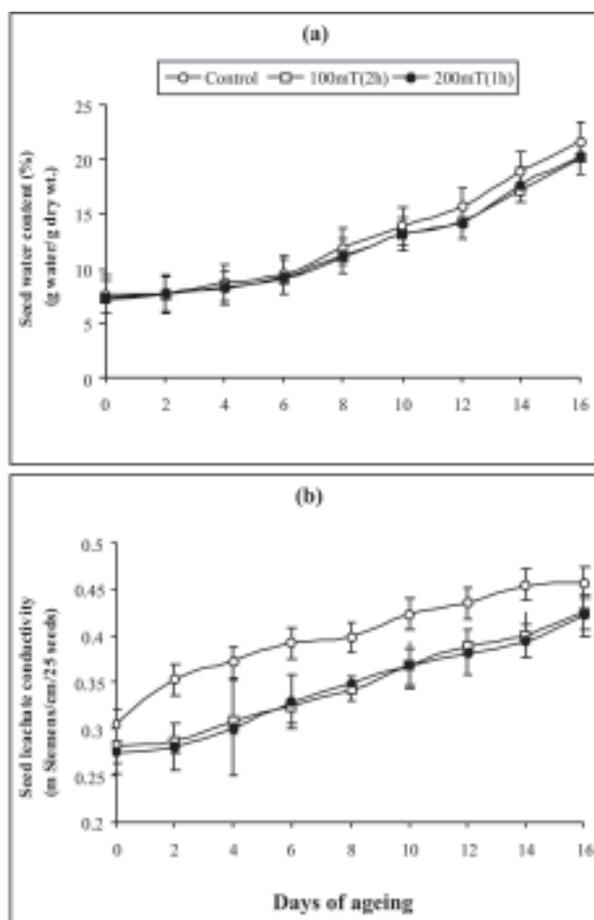
### **Results**

#### ***Seed Water content and Seed Leachate Conductivity during Accelerated Ageing***

Changes in the seed water content during accelerated ageing process and the corresponding changes in the conductivity of seed leachate for magnetically exposed and unexposed seeds are given in Fig.1a&b. Initially there was no difference between the treated and untreated seeds and from 8<sup>th</sup> day onwards, seed water content of control was greater than that of treated seeds. Leachate conductivity increased with days of ageing and unexposed seed had significantly more leachate conductivity than seeds exposed to magnetic field under accelerated ageing condition throughout the period of ageing. Seed leachate conductivity had the lowest value for seeds treated with 200 mT magnetic field for 1 h among all treatments.

#### ***Germination Characteristics of Seeds Exposed to Magnetic Field under Accelerated Ageing Condition***

Change in germination percentage of exposed and unexposed seeds of maize to magnetic field kept at accelerating ageing condition are shown



**Fig. 1.** Effect of accelerating ageing on maize seeds exposed to different magnetic field on (a) seed water content and (b) seed leachate conductivity

in (Fig. 2a). Both the exposed and unexposed seeds lost their viability by 16 days of ageing. Initial germination percentage was marginally higher for the exposed seeds as compared to the unexposed control and remained higher only up to 8 days of ageing, after which the germination percentage reduced sharply in the exposed seeds similar to control. Therefore the longevity of the seed viability does not change by the application of magnetic treatment. The change in shoot and root length of the magnetically exposed and unexposed seeds subjected to accelerated ageing are shown in the (Fig. 2b & c). Both shoot and root lengths of treated seeds were higher than untreated seeds until 12 days of ageing and a drastic decrease occurred 2 days earlier in control compared to the treated seeds. Seedling dry

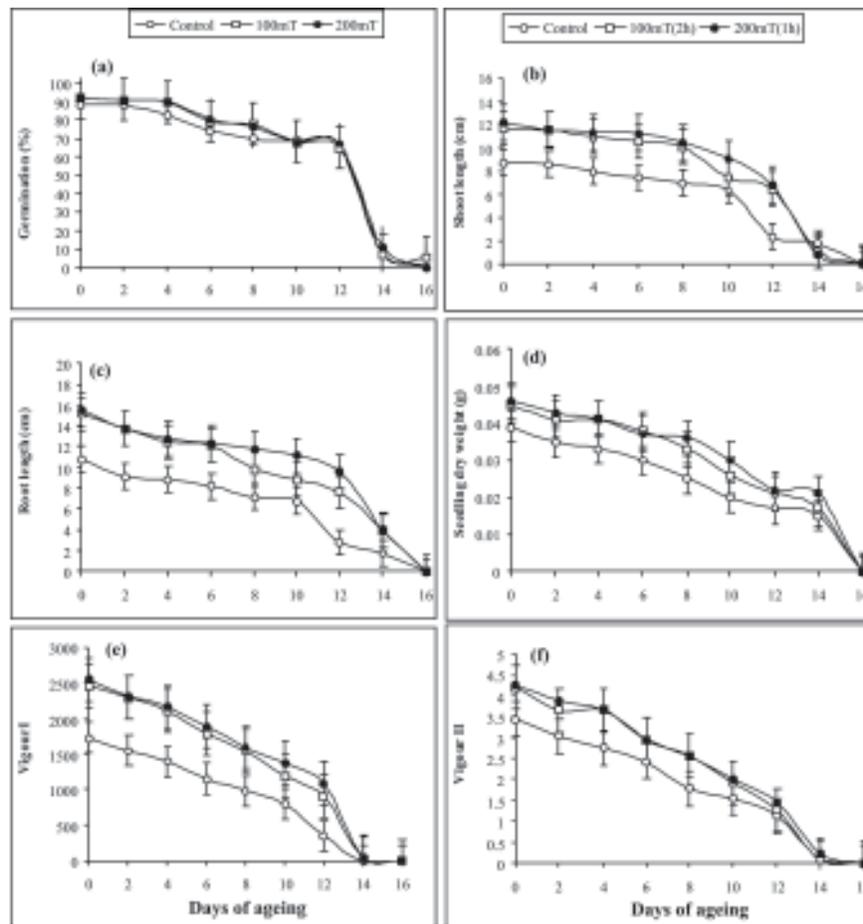
weight steadily decreased with ageing in all treatments and was greater for the exposed seeds until 12 days of ageing compared to the control seeds (Fig. 2d). The calculated seedling vigour I, based on seedling length and germination percent (Fig. 2e) and seedling vigour II, based on seedling dry weight and germination percent (Fig. 2f) showed similar trend as seedling length and seedling dry weight. Both 100 mT (2h) and 200 mT(1h) magnetic fields exhibited greater values compared to control till 12 days of observation.

### ***Antioxidant Enzymes (SOD, Catalase and Peroxidase) in Aged Magnetically Treated Seeds***

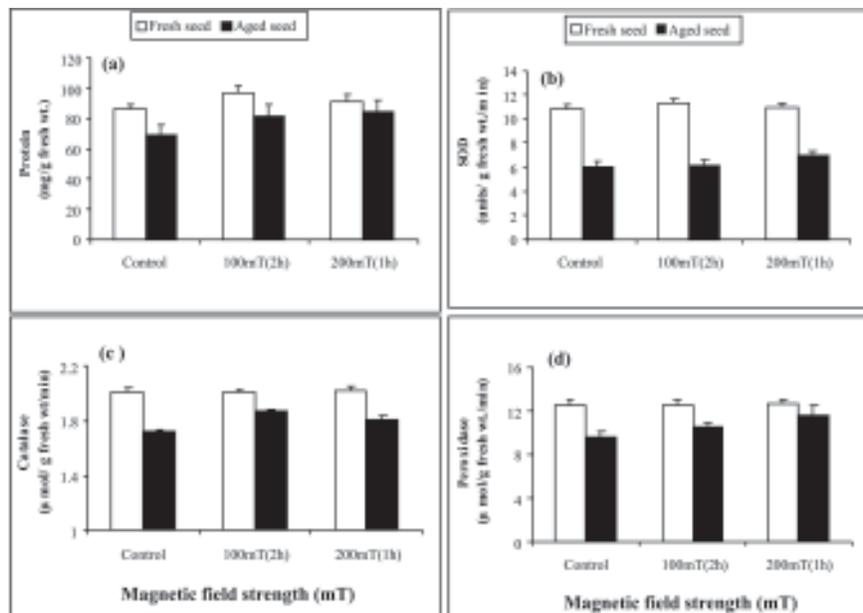
Twenty seeds each of the exposed and unexposed were subjected to accelerated ageing at high temperature of 40°C and humidity (100%) until they lost viability by about 30% from the initial value. The soluble protein content and activity of antioxidant enzyme measured in aged (until 8 days) and fresh seeds of magnetically treated and untreated seeds are given in Fig 3 (a, b, c & d). Soluble protein was observed to significantly decrease during artificial ageing. The magnetically exposed aged seeds had significantly higher protein (18-22%) than in the unexposed aged seeds. The activities of superoxide dismutase (SOD), catalase (CAT) and peroxidase decreased with ageing. The activities of SOD decreased significantly (37-46%) with ageing. Magnetically exposed aged seeds had marginally greater value (3-16%) than that of unexposed aged seed. The decrease in the activities of catalase (CAT) was (7-14%). Decrease in the activities of peroxidase was (8-31%). The magnetically exposed seeds showed slightly higher activity than the corresponding aged unexposed control.

### ***Effect of Magnetic Treatment of Partially Aged Seeds on Germination Characteristics and Seed Leachate Conductivity***

Magnetic treatment on partially aged seeds improved the germination by 10-12%, shoot length by 45-52%, root length by 58-68%, total seedling length by 55-57%, dry weight by 24-27% and hence the calculated vigour I and vigour II by 71-



**Fig. 2.** Effect of accelerating ageing on maize seeds exposed to different magnetic field on (a) percent germination, (b) shoot length, (c) root length, (d) seedling dry weight, (e) seedling vigour I and (f) seedling vigour II

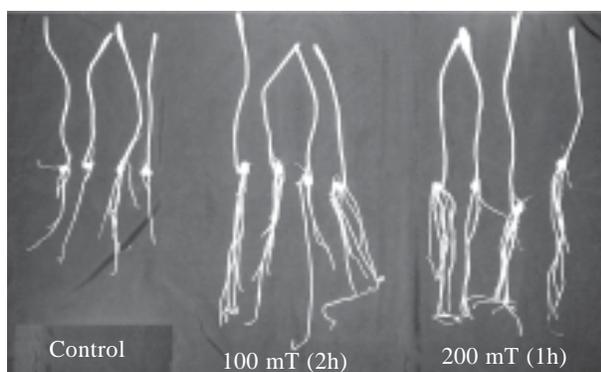


**Fig. 3.** Effect of magnetic field on (a) Soluble protein, (b) SOD, (c) Catalase and (d) Peroxides activities of fresh and aged seeds

**Table 1.** Effect of magnetic treatment on germination characteristics of mid storage (partially aged) seeds of maize

Treatment	Germination (%)	Shoot length (cm)	Root length (cm)	Dry wt. of seedling (g)	Vigour index I	Vigour index II
<b>Fresh seeds</b>						
Control	88 ± 3.65	8.73 ± 0.31	10.84 ± 0.32	0.039 ± 0.0019	1724.8 ± 97.87	3.46 ± 0.307
100mT(2h)	92 ± 2.83	11.63 ± 0.45	15.15 ± 0.45	0.046 ± 0.0013	2463.1 ± 97.32	4.22 ± 0.131
200mT(1h)	92 ± 2.83	12.11 ± 0.26	15.53 ± 0.38	0.046 ± 0.0022	2547.4 ± 29.13	4.27 ± 0.245
<b>Partially aged seeds</b>						
Control	74 ± 1.15	7.54 ± 0.35	8.20 ± 0.56	0.033 ± 0.0006	1163.7 ± 73	2.46 ± 0.076
100mT(2h)	82 ± 1.15	10.93 ± 0.41	13.79 ± 1.32	0.0411 ± 0.0006	2028.2 ± 97.85	3.37 ± 0.074
200mT(1h)	83 ± 1.00	11.48 ± 0.59	13.00 ± 0.70	0.0421 ± 0.0002	2031.7 ± 34.78	3.50 ± 0.171

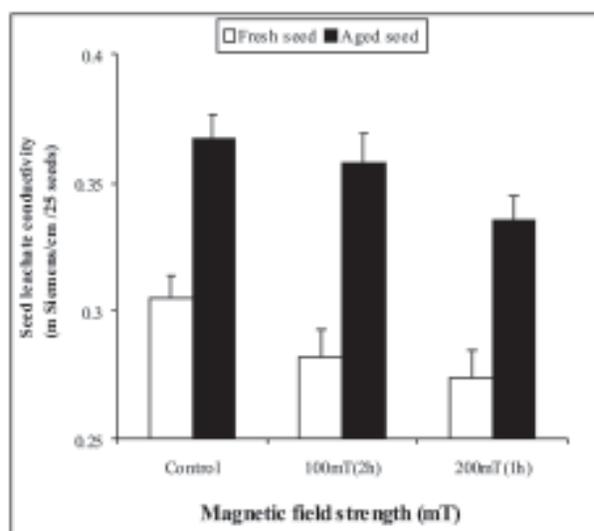
± Standard error

**Plate 1.** Effect of magnetic treatment on seedling growth of partially aged seeds of maize

74% and 37-42% respectively over the aged untreated control (Table 1). Plate 1 shows the effect of the treatment of partially aged seeds to magnetic field on germination characteristics of accelerated aged seeds. Leachate conductivities of aged seeds were significantly higher irrespective of magnetic treatment (Fig.4). In fresh seeds exposed to magnetic field the leachate conductivity was observed to decrease significantly. However, in seeds partially aged and then exposed to magnetic fields, seed leachate conductivity decreased in the treated seeds as compared to partially aged unexposed control.

## Discussion

Earliest symptoms of seed ageing are enhanced leakage of solutes (Jung and Sung, 1994 and McDonald, 1999) and such sharp increase in the solute leakage has also been seen in the present study due to ageing. This confirms that

**Fig. 4.** Effect of magnetic field treatment on seed leachate conductivity of partially aged seeds

solute leakage is a good indicator of the physiological status such as viability and vigour of the seeds (McDonald, 1999). The decrease in germination or viability related well with increased electrolyte leakage, thereby reflecting on the loss in membrane integrity. Seed viability loss is often attributed to the loss of integrity of the membrane (Bernal and Leopold 1998). This results in an increase in solute leachate from the seed. In all measurements of germination related traits, magnetic treatment was found to be superior to the control.

Magnetically exposed seeds showed decrease in seed water content and seed leachate conductivity with days of ageing as compared to

the control. The significantly lower values of leachate conductivity indicated better membrane integrity in treated seeds as compared to untreated controls during ageing process. This may be the reason for a marginally higher germination and seedling characteristics in magnetically exposed seeds. Similar improvement in germination and vigour characteristics had been observed with associated reduction in seed leachate conductivity in okra seed conditioned to low moisture levels (Nagarajan *et al.*, 2004).

In the presence of oxygen, ageing of seed can lead to peroxidative change in polyunsaturated fatty acid (PUFA) (Stewart and Bewley, 1980; Wilson and McDonald, 1986). This free radical inducing non-enzymatic peroxidation may lead to membrane damages and is likely to cause seed deterioration (Jung and chiu, 1995). Protective mechanism that could scavenge the peroxidatively produced free radicals and peroxides prevail within the cellular system to minimize seed deterioration reactions. Superoxide dismutase, peroxidase and catalase enzyme systems provide one such protective mechanism and inhibition of activities of these enzymes is reported to cause faster deterioration of seed (Jung and Chiu, 1995).

Loss of seed viability during accelerated ageing at 40°C and 100% RH was associated with a decrease in the activities of SOD, catalase and peroxidase in both magnetically treated and untreated control seeds. The decrease in activities of SOD, catalase and peroxidase were related to an increase in the seed leachate conductivity. Similar decrease in the activities of anti-oxidant enzymes have been reported in sunflower (Bailly *et al.*, 1996), soybean (Stewart and Bewley, 1980, Sung, 1996), peanut seeds (Sung and Jeng, 1994) and pigeon pea (Kalpana and Rao, 1994) seeds. The protein content in the aged seed decreased significantly, irrespective of magnetic treatment. This may possibly be due to the ageing induced deterioration in soluble proteins (Wettlaufer and Leopold, 1991, Sun and Leopold, 1995). SOD levels in fresh and aged seeds were similar in treated and untreated seeds. However, the levels of Catalase and Peroxidase enzymes were greater in treated than untreated seeds in aged seeds

even though they were similar in fresh seeds. It is possible that they might have played a role in controlling oxidative processes to some extent in magnetically exposed seeds.

Many workers have reported a improved seed vigour when seeds were pre-treated with antioxidants prior to accelerated and natural ageing in seeds of various crops (McDonald, 1999). Reduction in seed deterioration and improvement in seed vigour by the use of antioxidant like ascorbic acid is primarily due to quenching of free radicals, which prevents the peroxidative damage and enhances the activities of peroxide and radical scavenging enzymes (Hailstone and Smith, 1991). In the present study, magnetic exposure of seeds prior to ageing was observed to be more effective in improving the vigour of the seedlings than the percent germination as compared to unexposed control seeds. The activities of various antioxidant enzymes, which decreased during artificial ageing treatment, maintained marginally higher levels in magnetically exposed seeds as compared to unexposed controls. This may explain for the improved germination characteristics of magnetically exposed partially aged seeds.

## Conclusions

The decreased viability and vigour of seeds with artificial ageing was partially ameliorated in magnetically exposed seeds with a significant retention of viability as compared to untreated controls. Similarly, magnetically treated seeds had lower leachate conductivity than corresponding unexposed control during ageing process indicative of retaining greater membrane integrity. Activities of antioxidant enzymes, viz., superoxide dismutase, catalase and peroxidase were reduced and level of soluble protein was decreased in aged seeds. Seeds exposed to different magnetic fields strength maintained greater activity of these enzymes and higher levels of soluble proteins compared to unexposed aged and fresh controls. Exposure of partially aged seeds to magnetic field reversed to some extent the damage caused by ageing.

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