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Polymer seed coating with antioxidants and biochemical performance of soybean seed

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Abstract
The present investigation was carried out to explore the effect of polymer coating and chemical antioxidants on the soybean seed biochemical performance during storage. Soybean seed of JS 335 was treated with four antioxidants at three concentrations of 100 ppm, 300 ppm and 500 ppm and stored in airtight containers. The polymer with chemical antioxidant showed non-significant influence on seed leachates and superoxide dismutase but showed a significant effect on malondialdehyde. Six months after storage, seeds treated with polymer + ascorbic acid @ 300ppm followed by tocopherol showed significantly lowest electrical conductivity and high superoxide dismutase compared to untreated control. Lower MDA was recorded in the seeds treated with tocopherol @ 100 ppm + polymer as compared to the untreated control. Soybean seed coated with tocopherol and ascorbic acid, with or without polymer recorded less increase in MDA compared to untreated control.

Keywords: Antioxidants, polymer coating, biochemical, soybean seed quality

Introduction
Soybean (*Glycine max* L.) is an important oil seed crop (22%) rich protein (42-45%). Soy bean seed typically a poor storer which loses its quality faster rate. Soybean short storage life is the result of its high lipid content and high levels of polyunsaturated linolenic and linoleic acids (Joshi *et al*., 2014) [11]. Soybean seed deteriorates at rapid rate results poor plant stand in the field. Autoxidation of lipids and increase in the content of free fatty acids during storage period are the main reasons for rapid deterioration of soybean seed. Deterioration of seeds has been defined as "irreversible degenerative changes in the quality of a seed after it has reached its maximum quality" (Abdul Baki and Anderson, 1973) [1]. The consequence of seed deterioration is the production of aged seeds with symptoms of reduced vigour, possibly loss in germinability (the ability to produce a normal seedling) and ultimately losses in viability (Bewley and Black, 1982) [3]. It is inexorable and the best that can be done is to control its rate, by following recent seed quality enhancement technologies like seed treatment with chemical antioxidants and polymer coatings. The polymer coat provides protection from the stress imposed by accelerated ageing. The coat is thin (8 μ m), simple to apply, diffuses rapidly and non-toxic to the seedling during germination. By encasing the seed within a thin film of biodegradable polymer, the adherence of seed treatment to the seed is improved, ensures dust free handling, making treated seed both useful and environment friendly. The polymer film may act as physical barrier, which has been reported to reduce the leaching of inhibitors from the seed coverings and may restrict oxygen diffusion to the embryo. Keeping in view of the importance of the enhancement of seed longevity in soybean and to make the farming community self-sufficient in the availability of quality seed, the present investigation was carried out to study the effect of polymer coating in combination with antioxidants on seed quality parameters throughout the seed storage period.

Material and Methodology
The present study was carried out during *Rabi* 2014-15 at Department of Seed Science and Technology, College of Agriculture, Professor Jayashankar Telangana State Agricultural University, Rajendranagar, Hyderabad. Soybean seed of JS 335 were obtained from the Agricultural Research Station, PJTSAU, Adilabad, Telangana.
Eighteen kilograms of soyabean variety JS 335 seed was treated with 4 antioxidants, namely ascorbic acid, β carotene, reduced glutathione and tocopherol as alone and in combination with green polymer @ 8 ml kg^{-1} with the help of a polymer coating machine. Antioxidants were

used at three different concentrations of 100 ppm, 300 ppm and 500 ppm. All the treated seed were shade dried, packed in air tight containers, separately in three replications using CRD (Completely Randomized Design) techniques, as described by Panse and Sukhatme (1985) [16] and stored under ambient conditions for six months. Observations on seed biochemical parameters were recorded at bimonthly intervals for a period of six months. The electrical conductivity (µmhos cm^{-1}50 $seeds^{-1}$) test was conducted as per ISTA rules (2009).

Superoxide dismutase (SOD) was estimated according to the method of Dindra *et al.* (1986) [5]. The principle involved is that the assay was based SOD activity was determined by measuring its ability to inhibit the photo-reduction of Nitro Blue Tetrazolium chloride (NBT), and formation of blue coloured formazon. Which absorb at 560 nm and the enzyme SOD decreases this absorbance due to reduction in the formation of O^{-}_{2} radical by enzyme. The enzyme extract was prepared by taking three day old soybean seedlings (10 seedlings) and homogenized in potassium-phosphate buffer (100mM, pH 7.8) amended with (Ethylene di bromide tetra acetic acid) EDTA (3mM). The homogenate was centrifuged at 12000 rpm for 10 min. The supernatant was collected and centrifuged once again at 12000 rpm for 10 min. This supernatant was taken for SOD assay. Procedure for the enzyme estimation was done by taking three ml of reaction mixtures contains 200 mM methionine, 2.25 mM (Phenazine Nitroblue tetrazolium) NBT, 3 mM EDTA, 100 mM phosphate buffer (pH 7.8), 1.5 M sodium bicarbonate (Na_2CO_3) and 0.1 ml crude enzyme. The reaction was started by the addition of 60 µM riboflavin into reaction mixture and incubated at 30ºC for 25 min under a 100W florescent bulb. Two enzyme blanks were run parallel to the reaction mixture. One blank was irradiated under light called as positive control, while another was kept in dark called as negative blank. Irradiated enzyme blank developed maximum colour. Reaction was stopped by switching off the light. Color intensity of the formazen in the reaction mixture was measured at 560 nm against non-irradiated blank with the help of UV spectroscopy. One unit of SOD activity (U) was taken as that amount of enzyme, which reduced the absorbance reading to 50% in comparison with irradiated blank and the results were expressed as µmol min^{-1} mg^{-1} protein. The enzyme activity was quantified by using the following formula.

$$\text{One Unit of Enzyme} = \frac{\text{Positive Control} - \text{Test Sample}}{\text{Positive Control}/2}$$

The extent of lipid peroxidation was estimated through the quantification of Malondialdehyde (MDA) according to the method of Okhawa *et al.* (1979) [15]. Principle involved is that the Malondialdehyde (MDA) was formed from the breakdown of polyunsaturated fatty acids serves as a convenient index for the determination of the extent of peroxidation reaction. MDA, a product of lipid peroxidation reacts with TBA (thiobarbituric acid) to give a pink coloured product. Procedure for the enzyme estimation was done by taking three day old soybean seedlings (10 seedlings) and were homogenized with 7 ml of distilled water. To this homogenate, 7 ml of thiobarbituric acid (TBA; 0.5%) and tri chloro acetic acid (TCA; 20%) reagents were added. The above reaction mixture was incubated at 95 ºC in a hot air oven for 30 minutes in capped reaction tubes. The extract was cooled in an ice bath for half an hour and centrifuged at 5000 g for 10 min. Supernatant was collected and the colour intensity was recorded at 532 nm, 600 nm and also at 450 nm. The enzyme activity was quantified by using the following formula.

C_{MDA} (µ mol mL^{-1}) = 6.45 x (D_{532}-D_{600}) -0.56 X D_{450}

Mean data collected on biochemical parameters (EC, SOD and MDA) were analyzed statistically as per CRD design. The results indicated that polymer with antioxidant and only antioxidants treatments were significant at 5% level of significance for the parameters studied at bimonthly intervals for six months of storage period.

Results and Discussion

Autoxidation of lipids and increase in the content of free fatty acids during storage period are the main reasons for rapid deterioration of soybean seed (Hartman *et al*., 1994) [9]. Oxidation is a chemical reaction that transfers electrons or hydrogen from a substance to an oxidizing agent. Oxidation reactions can produce free radicals. In turn, these radicals can start chain reactions. When the chain reaction occurs in a cell, it can cause damage or death to cell. Antioxidant is a molecule that terminates these chain reactions by removing free radical intermediates and inhibits other oxidation reactions. Therefore, the use of chemical antioxidants may have the potential to be a cost-effective mechanism for prolonging storage life (Wilson and McDonald, 1986) [24].

Electrical conductivity (EC) (µmhos cm^{-1}50 $seeds^{-1}$)

The results on electrical conductivity as influenced by antioxidants in combination with polymer coating with storage period were presented in Table 1. Electrical conductivity increased progressively with the advancement of storage period with a mean increase of 1.89 µmhos cm^{-1}50 $seeds^{-1}$. Soybean seed coated with polymer + antioxidant showed no significant effect on the EC up to five months of storage. After six months of storage, significantly lowest electrical conductivity was recorded in seeds treated with polymer + ascorbic acid @ 300ppm (1.94 µmhos cm^{-1}50 $seeds^{-1}$) compared to untreated control (2.23 µmhos cm^{-1}50 $seeds^{-1}$). Among the treatments (Figure 1), seed coated with ascorbic acid with or without polymer recorded less solute leakage (EC of 1.75 and 1.91, µmhos cm^{-1}50 $seeds^{-1}$, respectively). And this is followed by tocopherol treatment. Among the concentrations of ascorbic acid, 100 ppm and 300 ppm recorded less solute leakage (Figure 2). It was reported that ascorbic acid protects plants and animals from oxidative stress (Lobo *et al.*, 2010) [13]. This might be due to the fact that ascorbic acid is having the ability to donate electrons and acts as a free radical scavenger and reduce higher oxidation states (Nicholas Smirnoff, 2018) [14].

Polymer coated seed showed less EC over control. This might be due to the reason that the polymer film might have acted as a physical barrier, which has been reported to reduce the leaching of inhibitors from the seed coverings and may restrict oxygen diffusion to the embryo (Shelar, 2002 and Turnipseed, 1993) [17, 22] and there by restricting the ageing effect in stored soybean seeds. High vigour seeds have the ability to recognize or repair membrane structure to a greater extent and they give a lower measure of conductivity or electrolyte leakage (Hampton and Tekrony, 1995) [8].

Effect on superoxide dismutase (SOD) (µmol min^{-1} mg^{-1} protein)

Superoxide dismutase is an important indicator of lipid peroxidation. It is an antioxidant defense in nearly all living

cells exposed to oxygen. This enzyme has been quantified to assess the effect of polymer coating on soybean longevity during seed storage. The data on the effect of chemical antioxidants in combination with polymer coating on superoxide dismutase and storability is presented in Table 2. Superoxide dismutase (SOD) decreased progressively with the advancement of storage period with an average decrease of 0.87 µmol min^{-1} mg^{-1} protein (24%). The mean superoxide dismutase recorded at initial and 6th month of storage period was 3.6 µmol min^{-1} mg^{-1} protein and 2.73 µmol min^{-1} mg^{-1} protein, respectively. Statistically no significant effect of polymer + antioxidant treatment was recorded on the SOD over a period of six months of storage. Six months after storage, high superoxide dismutase (2.99 µmol min^{-1} mg^{-1} protein) was recorded in the soybean seeds treated with ascorbic acid @ 300 ppm with polymer compared to the untreated control (2.6 µmol min^{-1} mg^{-1} protein). Among the treatments (Figure 3) soybean seed coated with ascorbic acid with or without polymer recorded less decrease in SOD (0.77 and 0.88, µmol min^{-1} mg^{-1} protein, respectively). Among the concentrations of ascorbic acid, 100 ppm recorded less reduction in SOD (Figure 4).

The result indicates the role of chemical antioxidants and polymer coating in the control of seed deterioration by promoting the SOD enzyme activity and quenching of free radicals. Decrease in the enzyme activity of superoxide dismutase was reported during storage of sunflower seed within 12 months in comparison with fresh seed (Tubic *et al*., 2005) [21]. And the enzyme activity in aged seed was decreased due to harmful changes in their structure during seed ageing. Free radical quenching activity of tocopherol has been reported in quenching singlet oxygen and scavenging of various ROS and ROS by-products, including lipid peroxyl radicals (Girotti, 1998 and Angelo and Achim, 2000.) [6, 2]. Reduced glutathion is also a cofactor for several detoxifying enzymes and showed counter effect on lipid peroxidation (Gregory *et al*., 2004) [7].

Malonyldialdehyde (MDA) (µ mol ml^{-1})

Malondialdehyde is also another indicator of lipid peroxidation which activity will be increased with the advancement of seed storage period. Lipid peroxidation leads to decrease in vigour and seed germination. The final product of lipid peroxidation is lipid hydroperoxide (ROOH) from which aldehydes are formed such as malonyldialdehyde (MDA). Therefore, determination of MDA content is considered as an indirect measure of lipid peroxidation (Sung and Jeng, 1994) [19]. The results on MDA as influenced by chemical antioxidants in combination with polymer coating treatments and storage period were presented in Table 2. The mean malonyldialdehyde recorded at initial and 6th month of storage period was 2.09 µ mol ml^{-1} and 4.13 µ mol $ml,^{-1}$ respectively with an average increase of 2.04 over a period of six months. Soybean seed coating with antioxidants and polymer showed statistically significant effect on MDA throughout the storage period of six months. Lower MDA was recorded in the seeds treated with tocopherol @ 100 ppm + polymer (3.89 µ mol ml^{-1}) as compared to the untreated control (4.15 µ mol ml^{-1}) over a period of six months of storage. Among the treatments (Figure 5) soybean seed coated with tocopherol and ascorbic acid, with or without polymer recorded less increase in MDA. Among the concentrations of 500 ppm of tocopherol and ascorbic acid recorded less increase in MDA (Figure 6).

Among the treatments, seed treated with tocopherol @ 100 ppm and 300 ppm with polymer were recorded lower MDA, all other treatments, treated with chemical antioxidants are on par with these treatments. Seed of untreated control were recorded higher MDA. This may be due to the reason that the chemical antioxidants were involved in the quenching of free radicals or neutralizing the free radicals that are released from the lipid peroxidation.

It was reported that the tocopherols scavenge and quench various ROS and lipid oxidation products, stabilize membranes, and modulate signal transduction (Brigelius-Flohe and Traber, 1999; Wang and Quinn, 2000; Yamauchi *et al*., 2001; Tian *et al.*, 2008 and Li *et al.*, 2008) [4, 23, 25]. It was also reported that the malondialdehyde (MDA), a product of lipid peroxidation, and total peroxide levels increased 3-fold within 18 months of storage of artificially and naturally aged cotton seeds (Sheoran *et al*., 2003) [18].

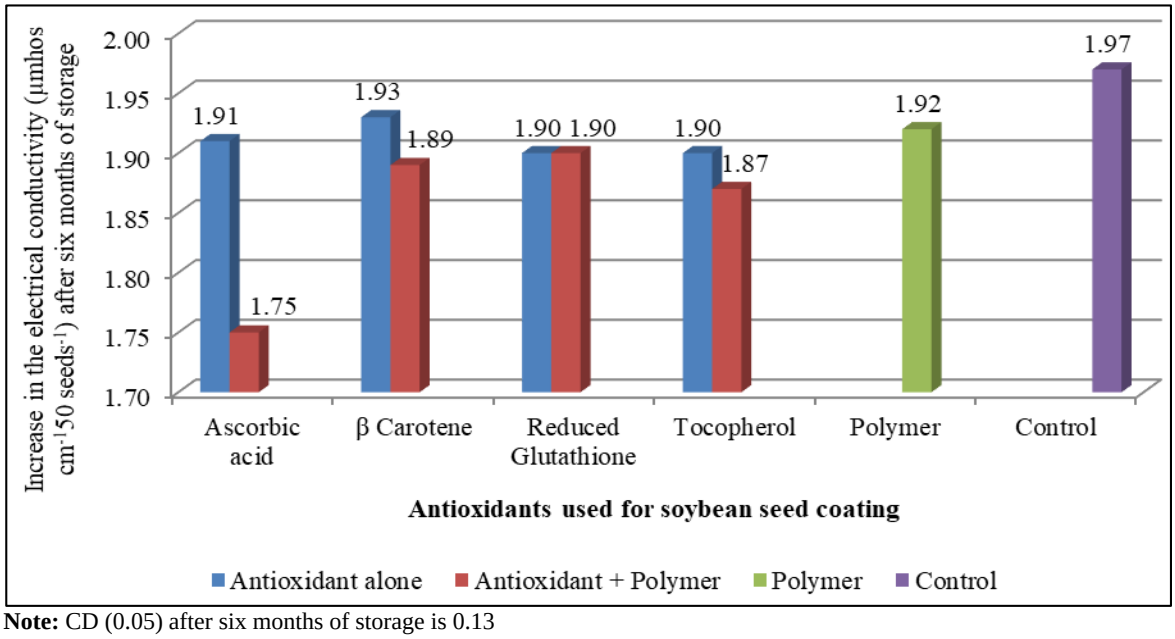
Table 1: Effect of antioxidants and polymer coating on electrical conductivity and longevity in soybean

No.	Treatments	Electrical Conductivity (µmhos cm^{-1}50 $seeds^{-1}$)							Increase
		Initial	1 MAS	2 MAS	3 MAS	4 MAS	5 MAS	6 MAS	
T1	Ascorbic acid @ 100 ppm	0.25	0.32	0.55	0.99	1.40	1.81	2.16	1.91
T2	Ascorbic acid @ 100 ppm + Polymer	0.25	0.34	0.57	1.01	1.42	1.66	1.96	1.71
T3	Ascorbic acid @ 300 ppm	0.25	0.34	0.56	1.01	1.42	1.83	2.18	1.93
T4	Ascorbic acid @ 300 ppm + Polymer	0.27	0.34	0.56	1.01	1.42	1.73	1.94	1.67
T5	Ascorbic acid @ 500 ppm	0.25	0.33	0.56	1.00	1.41	1.82	2.13	1.88
T6	Ascorbic acid @ 500 ppm + Polymer	0.25	0.33	0.56	1.00	1.41	1.72	2.12	1.87
T7	β Carotene @ 100 ppm	0.25	0.34	0.56	1.01	1.42	1.60	2.18	1.93
T8	β Carotene @ 100 ppm + Polymer	0.26	0.34	0.57	1.01	1.42	1.86	2.19	1.93
T9	β Carotene @ 300 ppm	0.26	0.35	0.57	1.02	1.43	1.78	2.19	1.93
T10	β Carotene @ 300 ppm + Polymer	0.26	0.34	0.57	1.01	1.42	1.83	2.18	1.92
T11	β Carotene @ 500 ppm	0.26	0.34	0.56	1.01	1.42	1.86	2.18	1.92
T12	β Carotene @ 500 ppm + Polymer	0.27	0.35	0.57	1.02	1.43	1.84	2.10	1.83
T13	Reduced Glutathione @ 100 ppm	0.26	0.33	0.55	1.00	1.41	1.77	2.09	1.83
T14	Reduced Glutathione @ 100 ppm + Polymer	0.26	0.35	0.57	1.02	1.43	1.81	2.11	1.85
T15	Reduced Glutathione @ 300 ppm	0.25	0.34	0.57	1.01	1.42	1.83	2.19	1.94
T16	Reduced Glutathione @ 300 ppm + Polymer	0.27	0.35	0.57	1.02	1.43	1.89	2.19	1.92
T17	Reduced Glutathione @ 500 ppm	0.25	0.34	0.57	1.01	1.42	1.83	2.18	1.93
T18	Reduced Glutathione @ 500 ppm + Polymer	0.26	0.34	0.56	1.01	1.42	1.83	2.18	1.92
T19	Tocopherol @ 100 ppm	0.26	0.33	0.56	1.00	1.41	1.82	2.18	1.92
T20	Tocopherol @ 100 ppm + Polymer	0.25	0.35	0.58	1.02	1.43	1.91	2.05	1.80
T21	Tocopherol @ 300 ppm	0.26	0.34	0.57	1.01	1.42	1.83	2.18	1.92

T22	Tocopherol @ 300 ppm + Polymer	0.26	0.34	0.57	1.01	1.42	1.88	2.16	1.90
T23	Tocopherol @ 500 ppm	0.26	0.34	0.57	1.01	1.42	1.83	2.12	1.86
T24	Tocopherol @ 500 ppm + Polymer	0.26	0.34	0.57	1.01	1.42	1.83	2.18	1.92
T25	Polymer @ 8ml Kg^{-1} seed	0.26	0.34	0.57	1.01	1.42	1.83	2.18	1.92
T26	Untreated control	0.26	0.33	0.55	1.00	1.41	1.96	2.23	1.97
	Mean	0.26	0.34	0.57	1.01	1.42	1.82	2.15	1.89
	CD (0.05)	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	0.13	
	S.Ed	0.02	0.02	0.02	0.02	0.03	0.10	0.07	
	S.Em	0.01	0.01	0.01	0.01	0.02	0.07	0.05	
	CV%	7.24	7.03	4.00	2.36	2.19	6.54	3.69	

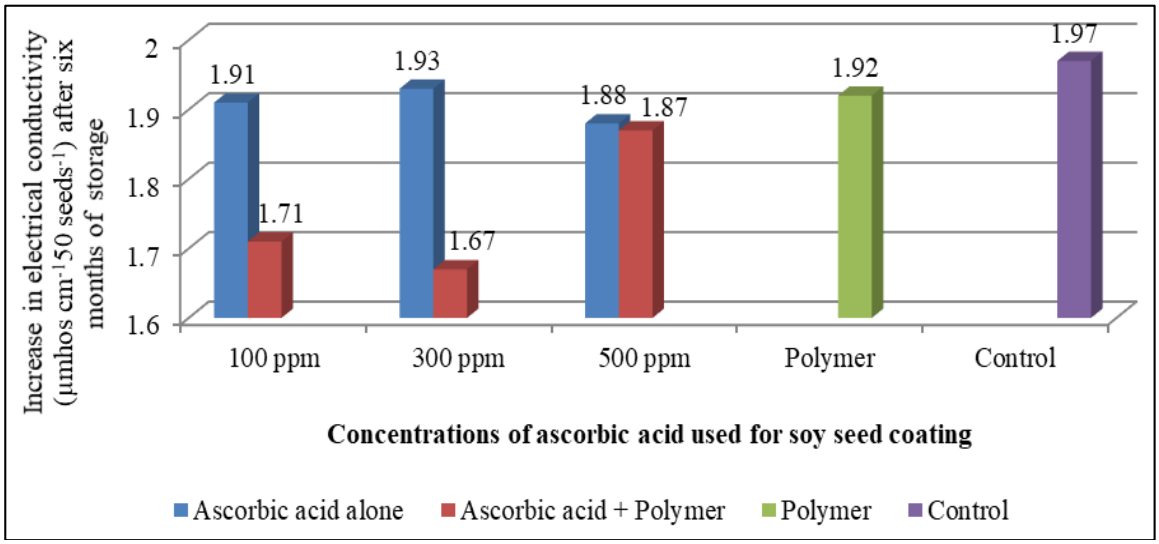
Table 2: Effect of polymer coating and chemical antioxidants on superoxide dismutase (SOD) and Malonyldialdehyde (MDA) and soybean seed longevity

No.	Treatments	Superoxide dismutase (SOD) (µmol min^{-1} mg^{-1} protein)					Malonyldialdehyde (MDA) (µ mol mL^{-1})				
		Initial	2 MAS	4 MAS	6 MAS	Decrease	Initial	2 MAS	4 MAS	6 MAS	Increase
T1	Ascorbic acid @ 100 ppm	3.53	3.29	2.81	2.69	0.84	2.08	2.69	2.99	4.12	2.04
T2	Ascorbic acid @ 100 ppm + Polymer	3.76	3.47	3.08	3.01	0.75	2.13	2.86	3.16	4.09	1.96
T3	Ascorbic acid @ 300 ppm	3.61	3.39	3.06	2.67	0.94	2.07	2.94	3.24	4.11	2.04
T4	Ascorbic acid @ 300 ppm + Polymer	3.70	3.45	3.08	2.99	0.71	2.14	2.75	3.05	4.05	1.91
T5	Ascorbic acid @ 500 ppm	3.57	3.33	2.82	2.70	0.87	2.08	2.70	3.00	3.93	1.85
T6	Ascorbic acid @ 500 ppm + Polymer	3.53	3.38	2.94	2.68	0.85	2.13	2.82	3.12	4.05	1.92
T7	β Carotene @ 100 ppm	3.58	3.33	2.80	2.68	0.90	2.04	2.68	2.98	4.1	2.06
T8	β Carotene @ 100 ppm + Polymer	3.57	3.36	2.95	2.72	0.85	2.13	2.83	3.13	4.06	1.93
T9	β Carotene @ 300 ppm	3.58	3.30	2.78	2.66	0.92	2.04	2.66	2.96	4.02	1.98
T10	β Carotene @ 300 ppm + Polymer	3.64	3.23	2.73	2.61	1.03	2.14	2.61	2.91	4.12	1.98
T11	β Carotene @ 500 ppm	3.62	3.32	2.74	2.62	1.00	2.06	2.62	2.92	4.01	1.95
T12	β Carotene @ 500 ppm + Polymer	3.64	3.40	2.91	2.79	0.85	2.14	2.79	3.09	4.02	1.88
T13	Reduced Glutathione @ 100 ppm	3.63	3.35	2.80	2.68	0.95	2.05	2.68	2.98	4.1	2.05
T14	Reduced Glutathione @ 100 ppm + Polymer	3.61	3.39	3.06	2.94	0.67	2.14	2.94	3.24	4.11	1.97
T15	Reduced Glutathione @ 300 ppm	3.53	3.33	2.82	2.70	0.83	2.06	2.70	3.00	4.06	2.00
T16	Reduced Glutathione @ 300 ppm + Polymer	3.64	3.29	2.96	2.70	0.94	2.11	2.84	3.14	4.07	1.96
T17	Reduced Glutathione @ 500 ppm	3.61	3.37	2.94	2.68	0.93	2.12	2.82	3.12	4.05	1.93
T18	Reduced Glutathione @ 500 ppm + Polymer	3.69	3.34	2.89	2.77	0.92	2.11	2.77	3.07	4.00	1.89
T19	Tocopherol @ 100 ppm	3.59	3.40	3.00	2.65	0.94	2.05	2.88	3.18	4.11	2.06
T20	Tocopherol @ 100 ppm + Polymer	3.61	3.33	3.07	2.71	0.90	2.02	2.47	2.92	3.89	1.87
T21	Tocopherol @ 300 ppm	3.67	3.31	2.93	2.81	0.86	2.10	2.81	3.11	4.04	1.94
T22	Tocopherol @ 300 ppm + Polymer	3.61	3.42	2.89	2.77	0.84	2.01	2.48	2.98	3.96	1.95
T23	Tocopherol @ 500 ppm	3.56	3.31	2.89	2.77	0.79	2.05	2.77	3.07	4.00	1.95
T24	Tocopherol @ 500 ppm + Polymer	3.64	3.37	2.96	2.84	0.80	2.11	2.84	3.14	4.07	1.96
T25	Polymer @ 8ml Kg^{-1} seed	3.66	3.36	2.91	2.79	0.87	2.12	2.79	3.19	4.12	2.00
T26	Untreated control	3.48	3.29	2.73	2.60	0.88	2.08	2.73	3.33	4.15	2.07
	Mean	3.60	3.35	2.90	2.73	0.87	2.09	2.75	3.09	4.13	2.04
	CD (0.05)	N.S.	N.S.	N.S.	N.S.		0.05	0.25	0.23	0.22	
	S.Ed	0.10	0.12	0.13	0.16		0.02	0.12	0.12	0.11	
	S.Em	0.07	0.09	0.09	0.11		0.02	0.09	0.08	0.08	
	CV%	3.48	4.41	5.38	7.08		1.32	5.50	4.58	3.34	



Note: CD (0.05) after six months of storage is 0.13

Fig 1: Effect of polymer coating and antioxidants on the electrical conductivity (µmhos cm-150 seeds-1) and longevity of soybean.



Note: CD (0.05) after six months of storage is 0.13

Fig 2: Effect of concentrations of ascorbic acid on the electrical conductivity (µmhos cm^{-1}50 $seeds^{-1}$) and longevity of soybean.

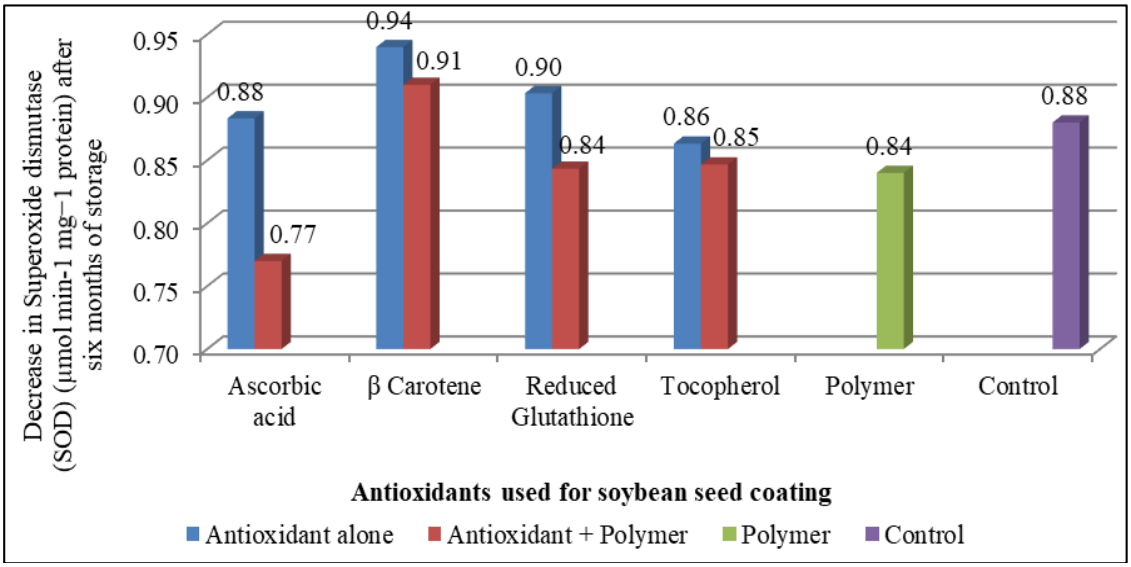
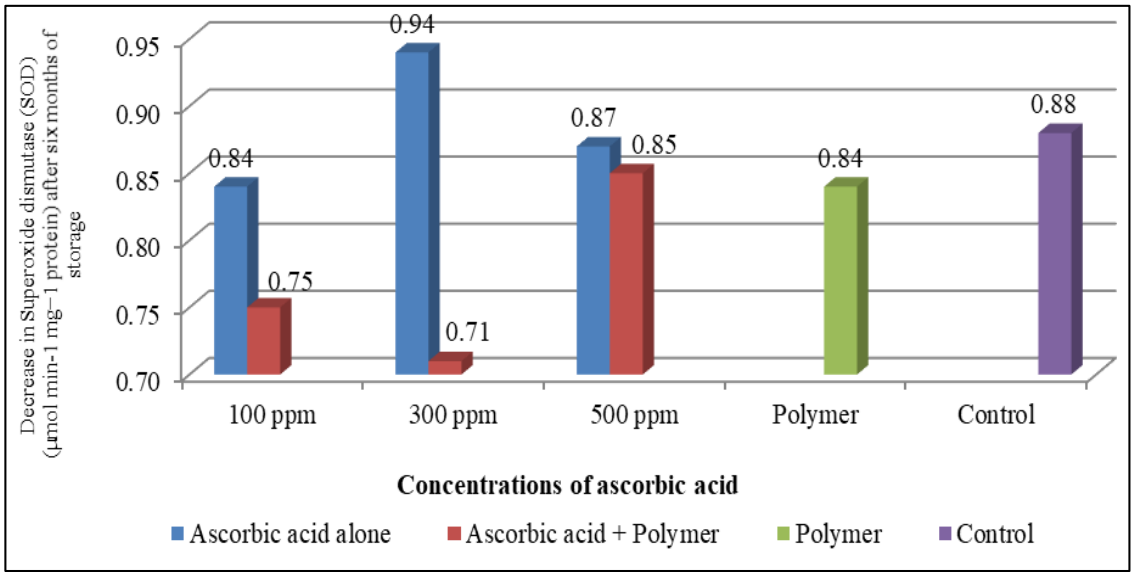
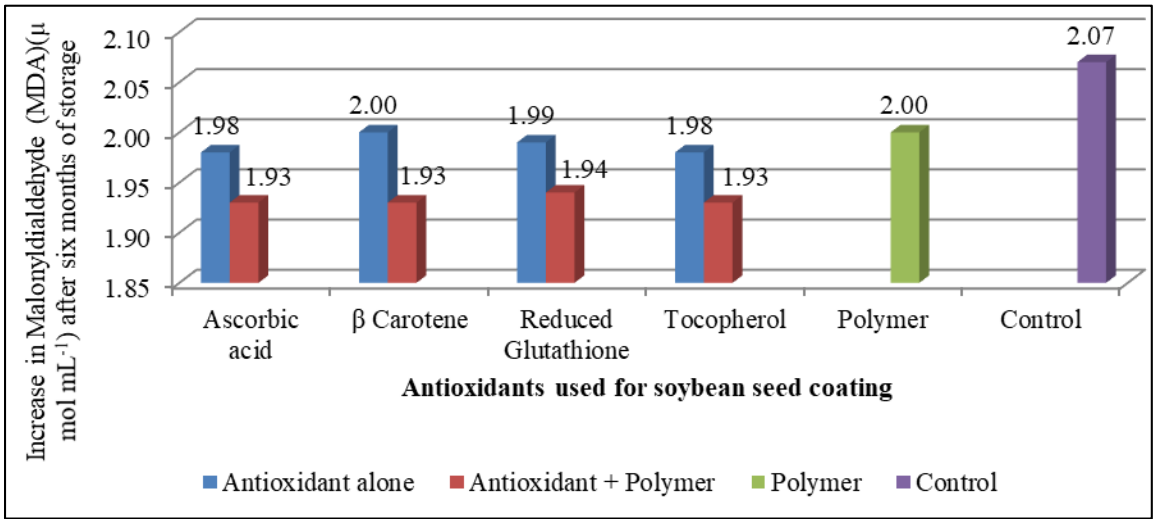


Fig 3: Effect of polymer coating and antioxidants on the Superoxide dismutase (SOD) (µmol min^{-1} mg^{-1} protein) and longevity of soybean.
Note: CD (0.05) after six months of storage is Non significant



Note: CD (0.05) after six months of storage is non-significant

Fig 4: Effect of concentrations of ascorbic acid on the superoxide dismutase (µmol min-1 mg−1 protein) and longevity of soybean.



Note: CD (0.05) after six months of storage is 0.22

Fig 5: Effect of polymer coating and antioxidants on the Malonyldialdehyde (MDA) (µ mol mL^{-1}) and longevity of soybean.

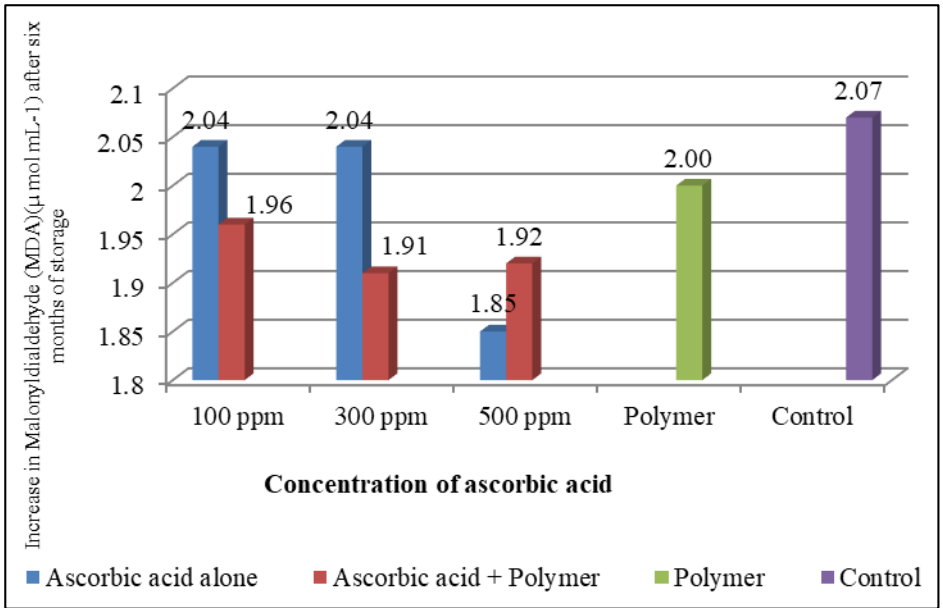


Fig 6a: Coating with ascorbic acid

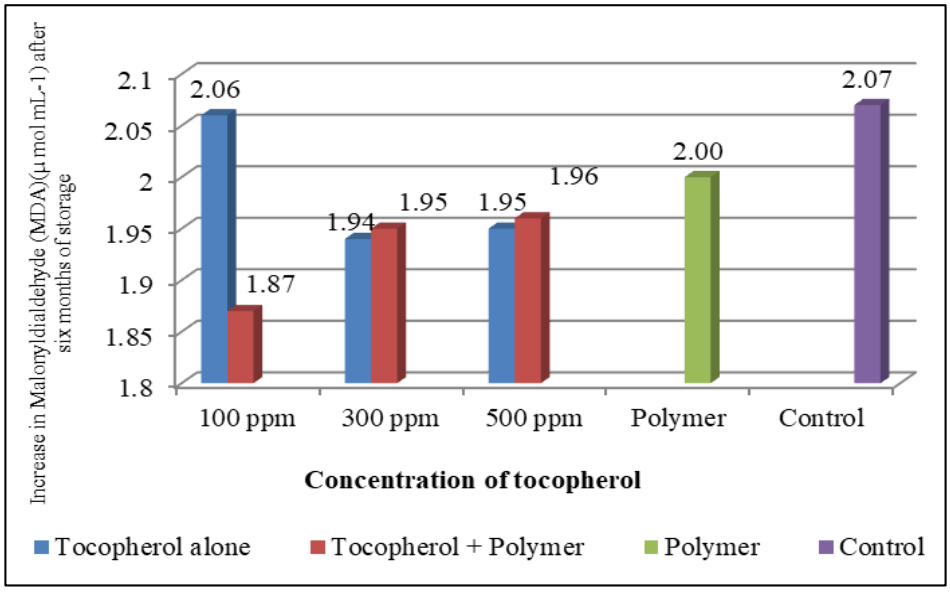


Fig 6b: Coating with tocopherol

Fig 6: Effect of coating with antioxidants on the Malonyldialdehyde (µ mol mL^{-1}) and longevity of soybean. Note: CD (0.05) after six months of storage is 0.22

Conclusion

It is to conclude that polymer coating with chemical antioxidants may enhance the soybean seed longevity by minimizing the process of oxidation during storage by decreasing the rate of leakage of seed leachates and malondialdehyde.

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