

Sunflower Seed Invigoration by Chemical Manipulation

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Abstract: Sunflower (*Helianthus annuus* L. cv. Morden) seeds lose viability at a rapid pace under accelerated aging condition. Pretreatment of the seeds with salicylic acid and ascorbic acid for 8 h before imposition of Accelerated Aging (AA) treatment in desiccator (99.1% RH and 32±2°C) or continuous treatment of the seeds with eucalyptus oil vapour for 60 days in a separate desiccator under the same Aging condition slowed down the Aging-induced rapid loss of germination. Plant performance was found to be much better when they were developed from seeds which underwent chemical pretreatment measured in terms of field emergence capacity, root length, shoot length, fresh weight and dry weight of plants. Plant potential was also higher in the pretreatments as evidenced from the treatment-induced higher chlorophyll, protein, DNA and RNA levels as well as activities of catalase and peroxidase enzymes in spite of adverse storage situation. Yield attributes like diameter and fresh weight of capitulum, seed number per capitulum, 1000 seed weight were found significantly higher in plants raised from pretreated seed samples. Results therefore, pointed out that in spite of experiencing accelerated Aging treatment, the chemical-pretreated seeds retained higher seed vigor and produced healthier plants which resulted in enhancement of crop yield.

Key words: Accelerated aging, ascorbic acid, eucalyptus oil, salicylic acid, seed viability, sunflower, yield

INTRODUCTION

Storing of seeds is a serious problem in tropical and subtropical countries where high temperature and high relative humidity greatly accelerate seed aging phenomenon causing consequent deterioration and non-viability of seeds. The problem of retention of seed vigor in many states of India is much more acute because of its semiarid climate where high relative humidity prevailing during the major part of a year is very conducive to the growth of microorganisms, particularly fungi (Christensen and Kaufmann, 1965; Halder, 1981; Aziz and Shahin, 1997). As most crop seeds require storage for either one or several planting seasons, agriculturists and horticulturists of this region are often handicapped with respect to maintenance of standard seed vigor under ambient storage environment.

The problem becomes more acute as seeds experience drought and other stress periodically under storage. Seed vigor and viability are two interrelated characters and generally loss of vigor precedes loss of viability. As per the principles proposed by Copeland and Mc-Donald (2001), Roozrokh *et al.* (2002), Sengupta *et al.* (2005) and Barman (2006), we may look upon vigor as the quality of

the seed responsible for rapid and uniform germination, extended storability and perform a good field emergence. Keeping this problem of seed storage in mind, an attempt was made in this investigation to prolong the storage life of a sunflower cultivar having viability problems. Present experiment was performed under Accelerated Aging (AA) condition by imposing high relative humidity with a view to maintaining a uniform adverse storage condition and also to obtain expeditious results. In fact, AA treatment provides a powerful manipulative tool which makes it possible to study the process of seed deterioration over a very short period (Heydecker, 1972) and this mimics the natural aging process (Deluche and Baskin, 1973; Perl *et al.*, 1978; Halder, 1981).

Although, efficacy of several classes of chemicals viz., hormones, retardants, redox chemicals, phenols, vitamins and salts on maintenance of seed health under storage has been established (Bhattacharyya and Basu, 1990; Chhetri *et al.*, 1993; Basu, 1994; Rai, 2000; Richa and Sharma, 2003), this field of seed physiology still remains relatively less explored.

Thus, the major objective of this research was to test the efficacy of a growth retardant salicylic acid, an antioxidant (ascorbic acid) and a volatile oil

(eucalyptus oil) on the alleviation of deterioration and viability extension of sunflower seeds under storage.

MATERIALS AND METHODS

After surface sterilization (0.1% HgCl₂ for 90 sec) certified hybrid seeds of sunflower (*Helianthus annuus* L. cv. Morden) were separately presoaked in the different concentration grades of aqueous solutions of salicylic acid, ascorbic acid or distilled water for different durations. And the seeds were then dried back to original dry weight by intense sun drying followed by measuring of the moisture level. After initial screening to allow maximum penetration of the experimental chemicals, it was found that the concentrations of salicylic acid (200 µg mL⁻¹) and ascorbic acid (200 µg mL⁻¹) at 8 h is more effective to the present experiment. The pretreated seed lots (200 g each) were taken in separate porous cloth bags and thus, stored in a desiccator in which 99.1% Relative Humidity (RH) was preimposed by keeping 250 mL 3.03% H₂SO₄ within it (Rao *et al.*, 2003). This experimental set-up was kept at 32±2°C for 60 days allowing the seeds to experience forced aging treatment and H₂SO₄ was changed at 7 days intervals to restore the desired RH within the desiccator for 60 days.

In a separate experiment, a seed lot (200 g) was kept in a smaller desiccator in which 5 mL eucalyptus oil extracted from mature leaves, procured from Shri Mahavir eucalyptus Oil Company, Tamil Nadu, India was taken in a small petri dish in addition to 250 mL 3.03% H₂SO₄. Here the seeds underwent treatment with the vapour of eucalyptus oil along with accelerated aging treatment (99.1% RH) throughout the experimental period. From the seed lots of both the experiment, germination and field emergence capacity of seeds were made after 0, 20, 40 and 60 days of accelerated aging treatment.

To analyse the percentage germination, four groups of 100 seeds (total 400 seeds) were transferred to separate petri dishes containing filter paper moistened with distilled water. Percentage germination data were recorded after 168 h of seed soaking following the International Rules for Seed Testing (ISTA, 1976). Percentage field emergence capacity were recorded after emerging of radicle and plumule from the treated seed samples sown in the experimental field after 10 days of seed sowing (Bhattacharjee, 1984; Pati, 2007).

Some growth and biochemical parameters as well as yield attributes were recorded from the plants raised from the accelerated aged (0 and 20 days only) seeds. Plants were established and data were recorded at two developmental stages viz., pre-heading stage (30 days old plants) and post-heading stage (60 days old plants) from

10 uniformly grown plants of each treatment. Extraction and estimation of chlorophyll and protein from mature leaves of each treated samples were done by the method of Arnon (1949) and Lowry *et al.* (1951), respectively. Extraction of nucleic acids was made from the same leaves following the method described by Cherry (1962) and quantitative estimation was done as per the method of Markham (1955) modified by Choudhuri and Chatterjee (1970).

Activity of Catalase (CAT) was analysed following the method of Snell and Snell (1973) as modified by Biswas and Choudhuri (1978) and that of Peroxidase (POD) was analysed as per the method of Kar and Mishra (1976). For assaying of the enzymes, the blank was taken as zero time control and the activity was expressed as:

$$\text{Activity} = \frac{\Delta A \times T_v}{t \times v}$$

Where:

ΔA = The absorbance of the sample after incubation minus the absorbance of the zero time control

T_v = The total volume of the filtrate

t = The time (min) of incubation with the substrate

v = The volume of the filtrate taken for incubation (Fick and Qualset, 1975)

Yield attributes recorded include diameter and fresh weight of capitulum, seed number per capitulum and 1000 seed weight. Statistical analysis of the data was done in terms of Least Significant Difference (LSD) which was calculated at 95% confidence limits (Panse and Sukhatme, 1967).

RESULTS AND DISCUSSION

Results showed that pretreatment of sunflower seeds with salicylic acid, ascorbic acid and eucalyptus oil significantly alleviated the aging-induced loss of germination and enhanced field emergence capacity under accelerated aging environment (Table 1). Reduced seed germinability and field emergence capacity are considered to be the important visible criteria for the evaluation of poor seed vigor (Anderson, 1970; Halder *et al.*, 1983; Rai, 2000). In this investigation, the chemical-induced cessation of loss of seed germination and field emergence capacity are indicative of storage potential enhancement property of the test chemicals.

Seeds were presoaked with salicylic acid, ascorbic acid or distilled water for 8 h and then sun dried or seeds were given continuous treatment with the vapour of eucalyptus oil. The seed samples were then separately allowed to experience AA treatment (99.1% RH) in a

Table 1: Effect of seed pretreatment with Salicylic Acid (SA, 200 µg mL⁻¹) and Ascorbic Acid (AA, 200 µg mL⁻¹) and treatment with Eucalyptus oil (E. oil) on germination and field emergence capacity of sunflower seeds stored under AA condition for 60 days

Treatments	Percentage germination AA (days)				Field emergence capacity (%) (AA, days)			
	0	20	40	60	0	20	40	60
Control	100	48.6 ^d	27.5 ^a	NA	87.9 ^a	38.5 ^d	12.4 ^a	NA
SA	100	56.6 ^c	39.9 ^d	NA	90.2 ^a	40.1 ^d	18.0 ^c	NA
AA	100	54.4 ^{bc}	35.2 ^c	NA	91.2 ^a	36.6 ^{bc}	14.1 ^b	NA
E. oil	100	51.8 ^b	33.5 ^{bc}	NA	88.5 ^a	31.5 ^a	12.3 ^a	NA
LSD (p = 0.05)	NC	2.05	1.81	-	NS	2.90	1.20	-

NA: No germination, NC: Not Calculated, NS: Not Significant

Table 2: Effect of seed pretreatment with Salicylic Acid (SA, 200 µg mL⁻¹) and Ascorbic Acid (AA, 200 µg mL⁻¹) and treatment with Eucalyptus oil (E. oil) followed by AA treatment for 20 days on changes of root length and shoot length of sunflower plants

Treatments	Plant age (days)							
	Root length (cm)				Shoot length (cm)			
	30		60		30		60	
	0*	20*	0*	20*	0*	20*	0*	20*
Control	5.09 ^a	4.23 ^a	14.18 ^a	10.01 ^a	28.9 ^b	12.0 ^a	82.5 ^a	69.5 ^a
SA	5.36 ^a	5.12 ^c	15.68 ^{bc}	12.83 ^b	25.2 ^a	15.3 ^c	100.2 ^c	83.5 ^{cd}
AA	5.20 ^a	5.08 ^c	16.89 ^c	12.99 ^b	32.4 ^c	18.2 ^d	110.6 ^d	87.5 ^d
E. oil	5.13 ^a	4.95 ^{bc}	15.40 ^{bc}	12.56 ^b	30.5 ^{bc}	13.3 ^b	91.2 ^b	76.8 ^{bc}
LSD (p = 0.05)	0.41	0.33	1.31	1.01	1.9	1.22	8.21	6.85

Table 3: Effect of seed pretreatment with Salicylic Acid (SA, 200 µg mL⁻¹) and Ascorbic Acid (AA, 200 µg mL⁻¹) and treatment with Eucalyptus oil (E. oil) followed by AA treatment for 20 days on changes of fresh weight and dry weight of sunflower plants

Treatments	Plant age (days)							
	Fresh weight (g)				Dry weight (g)			
	30		60		30		60	
	0	20	0	20	0	20	0	20
Control	36.00 ^a	32.50 ^a	398.90 ^a	321.00 ^a	12.70 ^a	7.5 ^a	52.40 ^a	41.9 ^a
SA	54.30 ^d	47.20 ^c	445.80 ^b	406.30 ^d	16.60 ^c	11.0 ^d	78.30 ^d	71.5 ^d
AA	51.10 ^{cd}	46.30 ^c	434.60 ^b	375.10 ^c	15.80 ^{bc}	10.1 ^c	72.10 ^c	67.1 ^c
E. oil	43.20 ^b	40.50 ^b	401.50 ^a	350.70 ^b	15.40 ^b	9.4 ^b	64.70 ^b	58.2 ^b
LSD (p = 0.05)	3.55	3.22	26.85	22.08	1.05	0.6	5.26	4.2

Table 4: Effect of seed pretreatment with Salicylic Acid (SA, 200 µg mL⁻¹) and Ascorbic Acid (AA, 200 µg mL⁻¹) and treatment with Eucalyptus oil (E. oil) followed by AA treatment for 20 days on changes of chlorophyll and protein contents in leaves of sunflower plants

Treatments	Plant age (days)							
	Chlorophyll (mg g ⁻¹ , wt.)				Protein (mg g ⁻¹ , wt.)			
	30		60		30		60	
	0	20	0	20	0	20	0	20
Control	1.20 ^a	0.96 ^a	1.89 ^b	0.88 ^a	15.49 ^a	13.25 ^a	18.25 ^a	16.32 ^a
SA	1.43 ^b	1.10 ^c	2.27 ^c	1.83 ^d	21.37 ^d	19.89 ^d	29.60 ^d	26.33 ^d
AA	1.26 ^a	1.07 ^{bc}	2.11 ^a	1.36 ^c	19.22 ^c	17.51 ^c	24.52 ^c	22.63 ^c
E. oil	1.23 ^a	1.00 ^a	1.93 ^b	1.02 ^b	17.33 ^b	15.25 ^b	20.19 ^b	19.16 ^b
LSD (p = 0.05)	0.10	0.05	0.13	0.10	1.60	1.33	1.83	1.64

Data were recorded from 30 and 60 days old uniformly grown plants. Values in a vertical column followed by the same letter are not significantly different at 5% level; *AA (days)

desiccator. Data were recorded after 0, 20, 40 and 60 days of AA seeds. Values in a vertical column followed by the same letter are not significantly different at 5% level. Accelerated aging treatment for 20 days impaired field performance of sunflower plants as evident from the reduction of root and shoot length (Table 2), fresh and dry weight (Table 3), levels of chlorophyll and protein

(Table 4), DNA and RNA (Table 5) as well as activities of CAT and POD enzymes (Table 6). The chemical-induced alleviation of the deleterious effects of aging on the overall growth and metabolism of sunflower plants which indicates the effectiveness of pretreatment by salicylic acid, ascorbic acid and eucalyptus oil. The aging-induced adverse effects on overall growth and

Table 5: Effect of seed pretreatment with Salicylic Acid (SA, 200 $\mu\text{g mL}^{-1}$) and Ascorbic Acid (AA, 200 $\mu\text{g mL}^{-1}$) and treatment with Eucalyptus oil (E. oil) followed by AA treatment for 20 days on changes of DNA and RNA levels in leaves of sunflower plants

Treatments	Plant age (days)							
	DNA ($\mu\text{g g}^{-1}$, wt.)				RNA ($\mu\text{g g}^{-1}$, wt.)			
	30		60		30		60	
	0	20	0	20	0	20	0	20
Control	231.1 ^b	182.5 ^a	206.5 ^a	236.1 ^b	905.2 ^a	757.8 ^a	1100.4 ^a	867.0 ^{cd}
SA	238.7 ^c	200.9 ^b	294.8 ^d	244.4 ^b	928.7 ^b	789.4 ^c	1190.5 ^d	948.2 ^{bc}
AA	221.0 ^a	188.9 ^a	265.6 ^c	210.8 ^a	912.4 ^a	783.8 ^b	1168.3 ^c	887.3 ^d
E. oil	217.0 ^a	185.2 ^a	231.7 ^b	207.6 ^a	910.7 ^a	780.5 ^b	1148.7 ^b	815.4 ^a
LSD (p = 0.05)	4.01	11.26	18.74	12.88	10.47	4.72	18.54	30.48

Table 6: Effect of seed pretreatment with Salicylic Acid (SA, 200 $\mu\text{g mL}^{-1}$) and Ascorbic Acid (AA, 200 $\mu\text{g mL}^{-1}$) and treatment with Eucalyptus oil (E. oil) followed by AA treatment for 20 days on changes of catalase and peroxidase activities in leaves of sunflower plants

Treatments	Plant age (days)							
	Catalase (unit/h/g, wt.)				Peroxidase (unit/h/g, wt.)			
	30		60		30		60	
	0	20	0	20	0	20	0	20
Control	100.40 ^a	68.9 ^a	163.6 ^a	102.70 ^a	94.80 ^a	59.70 ^a	145.90 ^a	97.70 ^a
SA	112.30 ^b	82.0 ^a	184.2 ^b	122.20 ^a	109.20 ^b	74.60 ^a	168.70 ^b	135.40 ^a
AA	111.40 ^b	75.3 ^b	166.0 ^a	110.30 ^b	101.10 ^a	68.50 ^b	154.60 ^a	126.00 ^b
E. oil	108.70 ^b	69.8 ^a	162.3 ^a	103.50 ^a	98.70 ^a	63.10 ^{ab}	151.10 ^a	121.60 ^b
LSD (p = 0.05)	6.05	5.1	12.4	6.33	7.08	6.07	10.05	7.09

Data were recorded from 30 and 60 days old uniformly grown plants. Values in a vertical column followed by the same letter are not significantly different at 5% level; *AA (days)

Table 7: Effect of seed pretreatment with Salicylic Acid (SA, 200 $\mu\text{g mL}^{-1}$) and Ascorbic Acid (AA, 200 $\mu\text{g mL}^{-1}$) and treatment with Eucalyptus oil (E. oil) followed by AA treatment on yield attributes of sunflower plants

Treatments	Plant age (days)							
	Capitulum diameter (cm)		Capitulum (wt., g)		Seed no./capitulum		1000 seed (wt., g)	
	0		0		0		0	
	0	20	0	20	0	20	0	20
Control	14.00 ^a	10.30 ^a	212.60 ^a	164.9 ^a	1001.5 ^a	764.1 ^b	38.4 ^a	32.2 ^a
SA	15.90 ^b	14.10 ^c	249.00 ^b	181.2 ^b	1103.0 ^b	821.0 ^a	48.0 ^c	43.0 ^d
AA	14.80 ^a	13.00 ^b	220.10 ^a	177.0 ^b	1012.0 ^a	816.7 ^a	46.2 ^c	38.1 ^c
E. oil	14.00 ^a	11.20 ^a	215.30 ^a	160.6 ^a	1006.9 ^a	800.5 ^a	42.7 ^b	35.6 ^b
LSD (p = 0.05)	1.03	1.01	15.33	12.36	68.51	65.20	2.63	2.10

Data were recorded after harvest. Values in a vertical column followed by the same letter are not significantly different at 5% level; *AA (days)

metabolism of sunflower plant were also reflected in some yield attributes. Reduced field performance of plants was associated with concomitant reduction of yield attributes leading to impairment of final seed yield (Table 7) of the plants which were developed from the forced aged seeds. Here also, salicylic acid, ascorbic acid and eucalyptus oil showed a promising role as the adverse effects on plant development and crop yield were alleviated to a considerable extent.

Chlorophyll, protein, DNA, RNA, CAT and POD are regarded as reliable indices of vigor status of plants. In this investigation, comparatively better plant health and higher metabolic status of plants, raised from the chemical-treated seeds are indicative of invigoration of seeds under storage. And the invigorated seeds subsequently exhibited better field performance which

was recorded in terms of plant growth and metabolism. Superior performance of plants raised from high vigor seeds is available in the literature (Rai, 2000). In this investigation, salicylic acid, ascorbic acid and eucalyptus oil induced enhanced seed germinability, plant growth and metabolism clearly indicate the hardening or invigoration property of the pretreating agents. Such hardening effect on seed was reflected in plant growth and metabolism.

Loss of some vital cellular components occurred (Abdul-Baki and Anderson, 1972; Kole and Gupta, 1982), along with decrease of nucleic acids (Bhattacharjee and Gupta, 1985; Pati, 2007) during the process of seed deterioration are available in literature. Catalase (Abdul-Baki and Anderson, 1972; Yadav *et al.*, 2003) and peroxidase (Bhattacharjee and Choudhuri, 1986;

Yadav *et al.*, 2003) activities are generally used as very reliable indices for the evaluation of seed viability. In fact, these two are considered as potential scavenger enzymes which can efficiently detoxify harmful metabolite like H_2O_2 and thus, help alleviating undesired toxic environment at the cellular level. Hence, higher activities of CAT, POD and superoxide dismutase, etc. are reported as strong defense elements in plant system (Yadav *et al.*, 2003; Mishra, 2006; Pati, 2007). High level of CAT activity in high vigor seeds have also been reported by Bhattacharjee, 1984 and Bhattacharjee *et al.* (1999). Eucalyptus oil releasing a number of volatile terpenes and it also contains one active component, eucalyptol which might have a direct role on antimicrobial activity. Eucalyptus oil induced enhancement of seed germinability and metabolism has also been reported and this result is in conformity with reported observation (Singh *et al.*, 1991; Bhattacharjee, 2001).

So from the present observations of higher metabolic status of the salicylic acid, ascorbic acid and eucalyptus oil-pretreated sunflower seeds, it seems quite apparent that the seed pretreating agents considerably hardened the seeds and such hardening is effected at the metabolic level which subsequently resulted in retention of seed vigor and consequent extension of seed viability with concomitant enhancement of plant potential.

CONCLUSION

From the overall results of this study, it can be concluded that salicylic acid, ascorbic acid and eucalyptus oil are not only effective in enhancing storage potential of sunflower seeds but they are equally efficient in improving plant vigor with concomitant augmentation of crop yield as evident from the results which clearly showed that the chemical-treated seeds were less sensitive than the control ones under adverse storage situations.

What ever might be the mechanism for the chemical manipulated seed invigoration, results of this investigation can at least indicate that the experimental chemicals has some efficacy for enhancement of storage potential of sunflower seeds and field performance of plants raised from such potentiated seeds of the test species.

The results also pointed out that the catabolic process in treated seeds remained partially subdued, there by rendering the seeds against unfavorable storage environment. And thus, the invigoration property of the present seed pretreating agents seems to be apparent from the experimental results.

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