Annals of R.S.C.B., ISSN:1583-6258, Vol. 25, Issue 2, 2021, Pages. 4389- 4399
Received 20 January 2021; Accepted 08 February 2021.

Viability Extension of Sunflower Seeds by Chemical Manipulative Method

Uttam Kumar Kanp[1], Aloke Bhattacharjee[2] and Mahmudul Haque [3*]

[1]Assistant Professor, Department of Botany, Narajole Raj College, Narajole - 721 211, Paschim Medinipur, West Bengal., INDIA. E. mail: kanpuk2008@gmail.com

[2]Retired Professor and HOD (Former), UGC-Centre for Advanced Study, Department of Botany, The University of Burdwan, Burdwan-713104, West Bengal., INDIA. E. mail: allokebc@yahoo.co.in

[3]Research Scholar, Department of Botany and Forestry, Vidyasagar University, Midnapore – 721102, West Bengal, INDIA. Email: mhaque179@gmail.com.

SUMMARY

Sunflower (*Helianthus annuus* L. cv. Morden) seeds lost viability at a rapid pace under accelerated ageing condition. Pretreatment of the seeds with sodium-dikegulac (Na-DK; 2,3:4-6-di-O-isopropylidine-α-L-xylo-2-hexalofuranosate) and ascorbic acid for 8 hours before accelerated ageing treatment (99.5% RH and 32±2^{o}C) for different durations (0 to 60 days) or continuous treatment of the seeds with *Eucalyptus* oil vapour for 60 days under the same ageing condition slowed down the ageing-induced rapid loss of germination. Plant performance was found to be much better when they were developed from seeds which underwent chemical pretreatment. And this was 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 ageing treatment, the chemical-pretreated seeds retained higher seed vigour and produced healthier plants which resulted in enhancement of crop yield.

Key words: Accelerated ageing, ascorbic acid, biochemical test, Eucalyptus oil, germination %, Na-dikegulac, plant growth, seed viability, sunflower, yield.

***Corresponding author:** Mahmudul Haque, Research Scholar, Department of Botany and Forestry, Vidyasagar University, Midnapore – 721102, West Bengal. Phone: +918910190779; Email: mhaque179@gmail.com.

INTRODUCTION

Storing of seeds is a serious problem in tropical and subtropical countries like India where

Annals of R.S.C.B., ISSN:1583-6258, Vol. 25, Issue 2, 2021, Pages. 4389- 4399
Received 20 January 2021; Accepted 08 February 2021.

high temperature and high relative humidity greatly accelerate seed ageing phenomenon causing consequent deterioration and non-viability of seeds. The problem of retention of seed vigour in India is much more acute because of extremely high relative humidity prevailing during the major part of a year and which is very conducive to the growth of microorganisms, particularly fungi. 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 a standard seed vigour under ambient storage environment. 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 ageing condition by imposing high relative humidity with a view to maintaining a uniform adverse storage condition and also to obtain expeditious results. In fact, accelerated ageing 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 ageing process (Delouche 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, Kanp and Bhattacharjee 2003, Richa and Sharma 2003), this field of seed physiology still remains relatively lessexplored. Thus, the major objective of this work was to test the efficacy of a growth retardant sodium dikegulac, 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% $HgCl_2$ for 90 seconds) certified seeds of sunflower (*Helianthus annuus* L. cv. Morden) were separately presoaked in the aqueous solutions of Na-dikegulac (Na-DK, 1000 μg/ml), ascorbic acid (1000 μg/ml) or distilled water for 8 hours and then dried back to the original dry weight of the seeds. The pretreated seed lots (200 g each) were taken in separate porous cloth bags and thus stored in a desiccator in which 99.5% relative humidity (RH) was preimposed by keeping 250 ml 1.57% H_2SO_4 within it (Maity *et al*. 2000, Das *et al*. 2003, Rao *et al.* 2003). This experimental set-up was kept at 32 ± 2^0C for 60 days allowing the seeds to experience forced ageing treatment and H_2SO_4 was changed at 7-day 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 5ml *Eucalyptus* oil was taken in a small Petri dish in addition to 250 ml 1.57% H_2SO_4. Here the seeds

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underwent treatment with the vapour of *Eucalyptus* oil along with accelerated ageing treatment (99.5% RH) throughout the experimental period (Chakrabarti 2003). From the seed lots of both the experiment, germinability and field emergence capacity of seeds were made after 0, 20, 40 and 60 days of accelerated ageing 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 10 ml distilled water. Germination data were recorded after 168 h of seed soaking following the International Rules for Seed Testing (ISTA 1976) and field emergence capacity was recorded after 14 days of seed sowing.

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 in the research field of Vidyasagar University 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 leaves were done by the method of Arnon (1949) and Lowry *et al*. (1951) respectively. Extraction of nucleic acids was made from the 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 was analysed following the method of Snell and Snell (1971) as modified by Biswas and Choudhuri (1978) and that of peroxidase 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 $(\Delta A \times T_v)/ (t \times v)$, where ΔA is the absorbance of the sample after incubation minus the absorbance of the zero time control, T_v is the total volume of the filtrate, t is the time (minutes) of incubation with the substrate and v is 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. Average results of three consecutive years were considered for analysis of yield data.

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).

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TABLES

Table 1. Effect of seed pretreatment with Na-dikegulac (Na-DK, 1000 μg/ml) and ascorbic acid (A. A., 1000 μg/ml) and treatment with *Eucalyptus* oil (E. oil) on germination and field emergence capacity of sunflower seeds stored under accelerated ageing condition for 60 days.

Seeds were presoaked with Na-DK, ascorbic acid or distilled water for 8h 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 accelerated ageing treatment (99.5% RH) in a desiccator. Data were recorded after 0, 20, 40 and 60 days of accelerated ageing seeds.

Treatments	Percentage germination				Field emergence capacity (%)			
	Accelerated ageing (days)							
	0	20	40	60	0	20	40	60
Control	100	41.3	7.5	NA	87.9	28.5	NA	NA
Na-DK	100	58.6	19.9	NA	90.2	40.1	8.0	NA
A. A.	100	54.4	15.2	NA	91.2	36.6	4.1	NA
E. oil	100	51.8	13.5	NA	88.5	31.5	2.3	NA
LSD (P=0.05)	NC	4.05	0.81	-	NS	2.90	0.20	-

NA: Nonattainment of germination, NC: Not calculated, NS: Not significant

Table 2. Effect of seed pretreatment with Na-dikegulac (Na-DK, 1000 μg/ml) and ascorbic acid (A. A., 1000 μg/ml) and treatment with *Eucalyptus* oil (E. oil) followed by accelerated ageing treatment for 20 days on changes of root length and shoot length of sunflower plants.

Data were recorded from 30 and 60 days old uniformly grown plants.

Treatments	Root length (cm)				Shoot length (cm)			
	Plant age (days)							
	30		60		30		60	
	Accelerated ageing (days)							
	0	20	0	20	0	20	0	20
Control	4.09	3.23	13.08	10.01	28.9	12.0	82.5	69.5
Na-DK	5.66	5.32	15.68	12.83	25.2	15.3	100.2	83.5
A. A.	6.20	5.68	16.89	13.99	32.4	18.2	110.6	97.5
E. oil	5.11	4.15	14.40	11.56	30.5	13.3	91.2	76.8
LSD (P=0.05)	0.41	0.33	1.31	1.01	1.90	1.22	8.21	6.85

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Table 3. Effect of seed pretreatment with Na-dikegulac (Na-DK, 1000 μg/ml) and ascorbic acid (A. A., 1000 μg/ml) and treatment with *Eucalyptus* oil (E. oil) followed by accelerated ageing treatment for 20 days on changes of fresh weight and dry weight of sunflower plants.

Data were recorded from 30 and 60 days old uniformly grown plants.

Treatments	Fresh weight (g)				Dry weight (g)			
	Plant age (days)							
	30		60		30		60	
	Accelerated ageing (days)							
	0	20	0	20	0	20	0	20
Control	37.0	32.5	268.9	221.0	10.5	7.5	52.4	41.9
Na-DK	56.3	51.2	445.8	406.3	16.6	11.0	78.3	71.5
A. A.	50.1	46.3	414.6	375.1	15.7	10.1	72.1	67.1
E. oil	41.2	36.5	342.5	300.7	15.2	9.4	64.7	58.2
LSD (P=0.05)	3.65	3.20	25.85	22.08	1.05	0.60	5.25	4.20

Table 4. Effect of seed pretreatment with Na-dikegulac (Na-DK, 1000 μg/ml) and ascorbic acid (A. A., 1000 μg/ml) and treatment with *Eucalyptus* oil (E. oil) followed by accelerated ageing treatment for 20 days on changes of chlorophyll and protein contents in leaves of sunflower plants.

Data were recorded from 30 and 60 days old uniformly grown plants.

Treatments	Chlorophyll (mg/g fr. wt.)				Protein (mg/g fr. wt.)			
	Plant age (days)							
	30		60		30		60	
	Accelerated ageing (days)							
	0	20	0	20	0	20	0	20
Control	0.86	0.46	1.29	0.88	15.49	13.25	18.25	16.32
Na-DK	1.43	1.10	2.07	1.83	21.37	19.89	29.60	26.33
A. A.	1.21	0.93	2.01	1.36	19.22	17.51	24.52	22.63
E. oil	1.03	0.74	1.73	1.02	17.33	15.25	20.19	19.16
LSD (P=0.05)	0.10	0.05	0.13	0.10	1.60	1.33	1.83	1.64

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Table 5. Effect of seed pretreatment with Na-dikegulac (Na-DK, 1000 µg/ml) and ascorbic acid (A. A., 1000 µg/ml) and treatment with *Eucalyptus* oil (E. oil) followed by accelerated ageing treatment for 20 days on changes of DNA and RNA levels in leaves of sunflower plants.

Data were recorded from 30 and 60 days old uniformly grown plants.

Treatments	DNA (µg/g fr. wt.)				RNA (µg/g fr. wt.)			
	Plant age (days)							
	30		60		30		60	
	Accelerated ageing (days)							
	0	20	0	20	0	20	0	20
Control	141.0	112.5	206.5	156.3	705.2	557.8	900.4	667.4
Na-DK	238.7	200.5	294.8	244.4	998.7	829.4	1230.5	948.9
A. A.	201.4	168.9	265.6	210.8	912.4	763.8	1168.3	887.3
E. oil	177.9	135.5	231.1	177.6	830.1	686.5	1048.7	715.4
LSD (P=0.05)	14.01	11.26	20.74	14.88	68.47	54.72	78.54	50.48

Table 6. Effect of seed pretreatment with Na-dikegulac (Na-DK, 1000 µg/ml) and ascorbic acid (A. A., 1000 µg/ml) and treatment with *Eucalyptus* oil (E. oil) followed by accelerated ageing treatment for 20 days on changes of catalase and peroxidase activities in leaves of sunflower plants.

Data were recorded from 30 and 60 days old uniformly grown plants.

Treatments	Catalase (unit/h/g fr. wt.)				Peroxidase (unit/h/g fr. wt.)			
	Plant age (days)							
	30		60		30		60	
	Accelerated ageing (days)							
	0	20	0	20	0	20	0	20
Control	61.4	48.9	133.6	62.7	73.0	49.9	125.9	87.7
Na-DK	112.3	80.9	184.6	122.2	109.2	78.6	168.7	135.4
A. A.	101.4	71.3	166.5	90.3	101.1	68.9	154.6	120.0
E. oil	88.7	63.1	152.3	81.5	92.0	60.1	141.1	111.6
LSD (P=0.05)	6.15	5.00	13.41	6.33	7.28	6.00	12.55	8.69

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Table 7. Effect of seed pretreatment with Na-dikegulac (Na-DK, 1000 µg/ml) and ascorbic acid (A. A., 1000 µg/ml) and treatment with *Eucalyptus* oil (E. oil) followed by accelerated ageing treatment on yield attributes of sunflower plants.

Data were recorded after harvest.

Treatments	Capitulum diameter (cm)		Capitulum fr. wt. (g)		Seed no./capitulum		1000 seed wt. (g)	
	Accelerated ageing (days)							
	0	20	0	20	0	20	0	20
Control	13.0	9.3	182.6	134.0	811.5	664.1	38.2	31.2
Na-DK	15.8	14.1	249.7	191.2	1103.2	911.1	48.8	43.2
A. A.	14.7	13.3	220.1	173.0	1015.6	846.2	46.2	38.1
E. oil	13.6	11.2	200.3	150.4	926.9	723.5	42.5	35.6
LSD (P=0.05)	1.13	0.92	17.33	13.36	78.51	65.20	3.63	3.10

RESULTS AND DISCUSSION

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

Accelerated ageing treatment for 20 days impaired field performance of sunflower plants as evident from the reduction of root length and shoot length (Table 2), fresh weight and dry weight (Table 3), levels of chlorophyll and protein (Table-4), DNA and RNA (Table-5) as well as activities of catalase and peroxidase enzymes (Table-6). The chemical-induced alleviation of the deleterious effects of ageing on the overall growth and metabolism of sunflower plants thus indicates the retention of potential status of the plants by Na-dikegulac, ascorbic acid and *Eucalyptus* oil. The ageing-induced adverse effects on overall growth and metabolism of sunflower plant was 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 plant which were developed from the forced aged seeds. Here also, Na-

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dikegulac, 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, catalase and peroxidase are regarded as reliable indices of vigour status of plants (Chakrabarti 2003). In this investigation, comparatively better plant health and higher metabolic status of plants, raised from the chemical-treated seeds, are indicative of invigouration of seeds under storage. And the invigourated seeds subsequently exhibited better field performance which was recorded in terms of plant growth and metabolism. Superior performance of plants raised from high vigour seeds and/or invigourated seeds is available in the literature (Rai 2000, Chakrabarti 2003). In this investigation, Na-dikuglac, ascorbic acid and *Eucalyptus* oil-induced enhanced seed germinability, plant growth and metabolism clearly indicate the hardening or invigouration property of the pretreating agents. And such hardening effect on seed was reflected in plant growth and metabolism. In fact, the magnitude of the loss of the chlorophyll and protein (Table 4), DNA and RNA contents (Table 5) as well as catalase and peroxidase (Table 6) activities were found to be significantly less in plants developed from seeds which underwent pretreatment with the chemicals.

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) 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. High level of catalase activity in high vigour seeds have also been reported (Bhattacharjee 1984, Bhattacharjee *et al*. 1999). So, from the present observations of higher metabolic status of the Na-dikegulac, 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 vigour and consequent extension of seed viability with concomitant enhancement of plant potential.

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Received 20 January 2021; Accepted 08 February 2021.

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