Chhetri, D. R., Rai, A. S. and Bhattacharjee, A. (1993), *Seed Sci. & Technol.*, 21, 31–44

Chemical manipulation of seed longevity of four crop species in an unfavourable storage environment

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(Accepted August 1992)

Summary

Accelerated ageing treatment (98.2% relative humidity, RH) on French bean (*Phaseolus vulgaris*), pea (*Pisum sativum*), lentil (*Lens culinaris*) and millet (*Panicum miliaceum*) seeds for two weeks reduced the germinability of seeds. The rate of seed germination was also slowed down by this rapid ageing treatment. On the other hand, the moisture level in seeds and leaching of soluble carbohydrates and electrolytes from seeds started increasing with the advancement of the ageing process under an unfavourable storage environment of 98.2% RH. Accelerated ageing treatment also reduced chlorophyll and protein contents in leaves of seedlings raised from the aged seeds and decreased the percentage of TTC-stained seeds as well as the total dehydrogenase activity of the seeds. Such changes in metabolism were associated with a proportional shift of seedling growth. The aged seeds produced some abnormal and/or subnormal seedlings upon germination and the effect was age-dependent. Concomitantly, root length and shoot length of seedlings were reduced proportionately. Pretreatment of the seeds with sodium-dikegulac (Na-DK, 2, 3:4-6-di-O-isopropylidene-α-L-xylo-2 hexalofuranosate) and ascorbic acid for 10 hours before accelerated ageing treatment or treatment of the seeds with basil oil vapour for two weeks under accelerated ageing conditions substantially alleviated all of the deleterious effects of ageing. No such effect on the changes in seed germination, seedling growth and metabolism was evident when the seeds were pretreated with IAA or with distilled water. The beneficial effects of Na-DK, ascorbic acid and basil oil with respect to maintenance of the storage potential of seeds are discussed.

Introduction

Economically, seed deterioration is a major problem in agricultural production. The climatic conditions of India greatly accelerate the seed ageing phenomenon under the ambient storage environment, causing consequent deterioration and loss of viability of seeds (Basu, 1976). The problem of retention of seed vigour in Darjeeling and surrounding areas is much more acute because of the extremely high relative humidity which is very conducive to the growth of microorganisms. Hence, agriculturists and horticulturists of this region are often handicapped with respect to maintenance of standard seed vigour under ambient storage conditions. Keeping this problem of seed storage in mind, an attempt was made to enhance the storage potential of four seed species which undergo rapid deterioration under the adverse ambient storage environment.

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Although some reports exist in the literature that the lifespan of seeds can be enhanced by applying some physical manipulative methods like X-ray irradiation, hydration-dehydration etc. or by pretreating seeds with diverse chemicals like phenols, salts, organic acids, hormones, vitamins before storage (Basu, Chattopadhyay and Pal, 1974; Dharmalingam and Basu, 1978; Coolbear, Francis and Grierson, 1984), this field of seed physiology still remains relatively unexplored. Previous observations of Bhattacharjee (1984), that some growth retardants particularly sodium-dikegulac (Na-DK) had a strong delaying effect on plant senescence, have prompted the present investigation to observe the efficacy of a few chemicals on retardation of seed senescence under storage. Antioxidants (Dey and Jana, 1988), and some phytohormones (Leopold and Kriedemann, 1975), are also thought to have a role in delaying senescence, and an essential oil of a basil species (*Ocimum sanctum*) has antimicrobial properties (Dey and Choudhuri, 1984). Therefore, ascorbic acid, IAA and basil oil were used along with Na-DK as chemical manipulative agents for the deferral of seed senescence. Thus, the major objective of this work was to test the efficacy of a growth retardant (Na-DK), a phytohormone (IAA), an antioxidant (ascorbic acid) and a volatile oil (basil oil) on the alleviation of seed deterioration under storage.

Materials and methods

Experiments were carried out with freshly harvested crop seeds. Pea (*Pisum sativum* L. cv. B22) and lentil (*Lens culinaris* Medic. cv. B256) seeds were collected from the Oil Seeds and Pulses Research Station, Berhampore and the French bean (*Phaseolus vulgaris* L.) and millet (*Panicum miliaceum* L.) seeds were obtained from the Principal Agricultural Officer, Darjeeling. All experiments were carried out under accelerated ageing conditions following the technique of Halder and Gupta (1980).

After surface sterilization with 0.1% (v/v) $HgCl_2$ for 90 seconds all the crop seeds (250 g each) were separately presoaked in the aqueous solutions (500 ml) of Na-DK (2000 and 1000 µg/ml), ascorbic acid (1000 and 500 µg/ml), IAA (200 and 100 µg/ml) or distilled water for 10 hours and then dried back to their original weight. Subsequently, the pretreated seed lots were taken in separate cloth bags and stored in a desiccator in which an environment of 98.2%, RH was imposed by keeping a beaker containing 250 ml 5.96% H_2SO_4 in the base of the desiccator. This experimental set-up was kept at room temperature (20 ± 2°C) allowing the seeds to experience a rapid ageing treatment and the H_2SO_4 was changed periodically by quick replacement of the beaker to maintain the desired RH within the desiccator for two weeks. In a separate experiment, another four lots of the same seeds were kept in a smaller desiccator in which 5 ml of essential oil of basil (*Ocimum sanctum*) was taken in a small Petri dish in addition to 250 ml 5.96% H_2SO_4. Here, the seeds underwent treatment with the vapour of basil oil along with the accelerated ageing treatment of 98.2% RH throughout the experimental period. From the seed lots of both the experiments, some physiological, biochemical and growth analyses were made after 0-, 7- and 14-days of accelerated ageing. However, in tables other than Tables 1, 8 and 10 the data recorded after 7 days of accelerated ageing were not presented, since no further changes were observed.

To analyse the percentage germination of the individual seed samples, four groups of 50 seeds were transferred to separate Petri dishes containing filter paper moistened with 10 ml distilled water. Germination data were recorded after 7 days of germination following the International Rules for Seed Testing (ISTA, 1976). The time for 50% germination of seeds (T_{50}) was determined following the method described by Coolbear, Francis and Grierson (1984).

Moisture content of the seeds was determined following ISTA rules (1976). To measure electrical conductivity, 5 g of each seed type were immersed in 25 ml deionised distilled water for 16 h at room temperature. Leakage of electrolytes was measured from the pooled seed leachate of control and treated samples of three replications each by a direct reading conductivity meter. For analysing TTC stainability, dehusked seeds of each treatment (in four groups of 40 seeds) were allowed to imbibe 1% (w/v) TTC (2, 3, 5-triphenyl tetrazolium chloride) solution in Petri dishes and kept overnight in the dark. The percentage TTC-stained (deep red) seeds was calculated from the total number of seeds of each treatment.

The soluble carbohydrate level was analysed from the same seed leachate as that used for measuring electrical conductivity, following the method of McCready, Guggloz, Silviera and Owens, (1950). The activity of total dehydrogenases of intact seeds was analysed by the reaction of TTC according to the method of Rudrapal and Basu (1979). Samples for analysing chlorophyll and protein contents were taken from leaves of five uniformly grown 10-day-old seedlings, raised from accelerated ageing seeds of each treatment. Chlorophyll and protein levels of leaves were estimated following the methods of Arnon (1949) and Lowry, Rosebrough, Farr and Randall (1951), respectively.

The growth behaviour of the French bean, pea, lentil and millet seedlings was analysed in terms of the percentage of normal seedlings, as well as root length and shoot length of seedlings, developed from the rapidly aged seeds. Twenty seedlings of each treatment were grown in Petri dishes and data were recorded from 10-day-old uniformly grown seedlings of each treatment.

All the data recorded in this investigation were statistically analysed at the treatment and replication levels (Panse and Sukhatme, 1967). LSD (least significant difference) values at the 5% level were incorporated in the tables.

Results

The germinability of French bean, pea, lentil and millet seeds declined with increased duration of accelerated ageing. However, this decline in germinability occurred at a slow rate for seeds treated with Na-DK, ascorbic acid and basil oil vapour. The effect of seed treatment with distilled water or IAA was found to be least significant or insignificant in this regard (Table 1). The rates of germination (T_{50}) were also high in all seed lots treated with Na-DK, ascorbic acid and basil oil vapour in comparison

Table 1. Effect of seed pretreatment with different concentrations of sodium-dikegulac (Na-DK, 2000 and 1000 µg/ml), ascorbic acid (AA, 1000 and 500 µg/ml) and IAA (200 and 100 µg/ml) as well as treatment with essential oil of *Ocimum* on percentage seed germination of four crop species stored under accelerated ageing condition.

Treatments (µg/ml)	Seed types											
	French Bean			Pea			Lentil			Millet		
	Days after accelerated ageing											
	0	7	14	0	7	14	0	7	14	0	7	14
*Control	100	70.2	45.4	100	62.2	51.4	95	55.2	42.2	90	42.5	36.3
*Water control	100	74.8	54.4	100	68.0	55.2	95	59.3	47.6	90	50.2	42.1
Na-DK 2000	100	80.0	60.7	100	78.0	63.1	95	68.7	60.5	90	72.7	60.5
Na-DK 1000	100	80.1	65.9	100	81.9	66.1	95	72.3	64.2	90	77.6	63.1
AA 1000	100	89.4	75.6	100	72.6	60.6	95	72.1	65.4	90	70.3	59.9
AA 500	100	85.2	77.4	100	80.5	69.4	95	71.9	63.6	90	74.7	63.6
IAA 200	100	70.3	47.3	100	65.3	56.2	95	50.5	40.2	90	52.4	45.4
IAA 100	100	71.0	50.1	100	69.5	54.4	95	57.1	46.1	90	57.3	46.2
Ocimum Oil	100	90.1	60.6	100	87.6	72.6	95	70.7	59.6	90	78.1	65.7
LSD (P = 0.05)	NC	4.85	4.02	NC	5.02	4.85	NC	5.88	5.01	NC	6.01	5.30

* Control = Untreated seeds; Water control = seeds pretreated with distilled water. NC = Not calculated.

Table 2. Effect of seed pretreatment with different concentrations of sodium-dikegulac (Na-DK, 2000 and 1000 μg/ml), ascorbic acid (AA, 1000 and 500 μg/ml) and IAA (200 and 100 μg/ml) as well as treatment with essential oil of *Ocimum* on time (hour) to 50% seed germination (T_{50}) of four crop species stored under accelerated ageing condition.

Treatments (μg/ml)	Seed types							
	French Bean		Pea		Lentil		Millet	
	Days after accelerated ageing							
	0	14	0	14	0	14	0	14
Control	18.0	72.1	26.0	71.2	22.0	66.5	36.0	92.0
Water control	18.9	69.5	26.3	67.5	23.0	64.5	34.2	88.7
Na-DK 2000	20.2	58.8	27.2	54.9	23.8	49.4	38.4	68.5
Na-DK 1000	19.9	57.9	26.9	50.5	24.0	44.5	37.0	68.2
AA 1000	18.5	54.4	25.8	52.2	22.5	50.5	36.9	65.2
AA 500	18.9	50.8	25.5	51.0	22.3	48.8	36.7	64.5
IAA 200	18.0	66.1	25.0	72.0	21.8	60.6	35.0	112.0
IAA 100	18.2	75.0	24.9	70.9	21.6	66.5	34.4	100.8
Ocimum Oil	18.0	53.8	27.0	53.5	22.7	46.2	35.8	64.9
LSD (P = 0.05)	NS	3.70	NS	3.90	NS	4.02	NS	3.88

NS = Not significant.

to untreated control value, as indicated by less time (h) required for 50% germination (Table 2).

The moisture level in seeds increased after 14 days ageing irrespective of seed samples and treatments used. In seed lots treated with basil oil vapour the moisture level remained significantly low in comparison to untreated seeds regardless of the seed species used. On the other hand, a significant increase of the same was noted only in French bean seeds pretreated with Na-DK 1000 and 2000 μg/ml, ascorbic acid 1000 μg/ml and IAA 200 μg/ml (Table 3).

A large increase in the leaching of soluble carbohydrates (Table 4) and in the electrical conductivity (Table 5) of seed leachates was found in all treated and untreated samples after 14 days ageing. However, the magnitude of the increase was found to be remarkably low in all seed types treated with Na-DK, ascorbic acid and basil oil vapour. A similar but smaller increase in both values along with the same effect of treatment was observed after 7 days (data not presented).

The chlorophyll level in leaves of 10-day-old seedlings declined as seedlings were raised from the rapidly aged seeds, and this declining rate was much less in the case of seedlings which were raised from seeds pretreated with Na-DK, ascorbic acid and with basil oil vapour (Table 6). However, the chemicals were found least effective on lentil in this regard. The changes in the protein level of leaves were found to be almost identical with those of chlorophyll level regardless of the seed types, treatments and their concentrations used (Table 7).

Table 3. Effect of seed pretreatment with different concentrations of sodium-dikegulac (Na-DK, 2000 and 1000 μg/ml), ascorbic acid (AA, 1000 and 500 μg/ml) and IAA (200 and 100 μg/ml) as well as treatment with essential oil of *Ocimum* on seed moisture content (%) of four crop species stored under accelerated ageing condition.

Treatments (μg/ml)	Seed types							
	French Bean		Pea		Lentil		Millet	
	Days after accelerated ageing							
	0	14	0	14	0	14	0	14
Control	7.0	14.2	8.5	15.7	6.0	13.0	5.9	10.5
Water control	7.3	13.8	8.8	14.5	6.5	12.8	6.1	9.8
Na-DK 2000	7.0	15.8	8.4	15.6	6.6	13.2	6.2	10.7
Na-DK 1000	7.8	15.4	8.4	15.9	6.4	13.5	6.0	10.5
AA 1000	7.2	14.5	8.3	15.7	6.7	13.4	6.1	10.2
AA 500	7.0	15.0	8.5	15.0	6.7	13.5	6.0	10.4
IAA 200	7.2	15.6	8.8	16.0	6.3	13.8	6.2	11.0
IAA 100	6.8	14.8	9.0	15.9	6.4	13.4	6.4	10.9
Ocimum Oil	6.9	12.0	8.7	13.0	6.5	11.9	6.6	8.9
LSD ($P = 0.05$)	0.70	1.01	NS	0.92	NS	0.95	NS	0.79

NS = Not significant.

Table 4. Effect of seed pretreatment with different concentrations of sodium-dikegulac (Na-DK, 2000 and 1000 μg/ml), ascorbic acid (AA, 1000 and 500 μg/ml) and IAA (200 and 100 μg/ml) as well as treatment with essential oil of *Ocimum* on leaching of soluble carbohydrate (mg/g/25 ml) from the seeds of four crop species stored under accelerated ageing condition.

Treatments (μg/ml)	Seed types							
	French Bean		Pea		Lentil		Millet	
	Days after accelerated ageing							
	0	14	0	14	0	14	0	14
Control	7.8	19.9	3.0	14.6	2.5	13.1	9.5	24.2
Water control	6.9	17.0	2.8	14.3	2.3	12.9	9.0	23.8
Na-DK 2000	7.5	14.8	2.7	10.8	2.1	9.5	9.2	18.4
Na-DK 1000	7.6	14.7	2.7	10.5	2.3	10.0	9.4	17.0
AA 1000	7.2	15.0	2.9	11.4	2.4	10.7	9.0	18.0
AA 500	7.3	13.9	2.8	11.8	2.3	9.9	8.9	17.5
IAA 200	7.0	17.8	2.9	14.0	2.3	13.4	9.6	22.9
IAA 100	7.1	18.0	3.0	14.2	2.4	13.0	9.4	24.0
Ocimum Oil	7.3	13.4	3.1	10.2	2.6	9.9	9.3	16.9
LSD ($P = 0.05$)	0.42	0.65	0.31	0.80	0.35	0.70	0.61	1.30

Table 5. Effect of seed pretreatment with different concentrations of sodium-dikegulac (Na-DK, 2000 and 1000 µg/ml), ascorbic acid (AA, 1000 and 500 µg/ml) and IAA (200 and 100 µg/ml) as well as treatment with essential oil of *Ocimum* on electrical conductivity (m mhos/25 ml) of pooled seed leachate of four crop species stored under accelerated ageing condition.

Treatments (µg/ml)	Seed types							
	French Bean		Pea		Lentil		Millet	
	Days after accelerated ageing							
	0	14	0	14	0	14	0	14
Control	0.46	0.98	0.18	0.66	0.50	1.20	0.25	0.80
Water control	0.40	0.88	0.16	0.62	0.47	0.90	0.24	0.73
Na-DK 2000	0.42	0.74	0.15	0.48	0.45	0.80	0.22	0.56
Na-DK 1000	0.38	0.70	0.15	0.48	0.47	0.82	0.22	0.52
AA 1000	0.44	0.68	0.17	0.52	0.48	0.86	0.24	0.55
AA 500	0.14	0.68	0.16	0.50	0.48	0.85	0.23	0.50
IAA 200	0.40	0.95	0.18	0.66	0.50	1.15	0.24	0.75
IAA 100	0.40	0.90	0.17	0.62	0.51	1.25	0.24	0.72
Ocimum Oil	0.42	0.70	0.16	0.50	0.48	0.79	0.22	0.58
LSD (P = 0.05)	NS	0.09	NS	0.05	NS	0.08	NS	0.06

NS = Not significant.

Table 6. Effect of seed pretreatment with different concentrations of sodium-dikegulac (Na-DK, 2000 and 1000 µg/ml), ascorbic acid (AA, 1000 and 500 µg/ml) and IAA (200 and 100 µg/ml) as well as treatment with essential oil of *Ocimum* on chlorophyll content (mg/g fresh weight) in leaves of seedlings, developed from the seeds of four crop species stored under accelerated ageing condition.

Treatments (µg/ml)	Seed types							
	French Bean		Pea		Lentil		Millet	
	Days after accelerated ageing							
	0	14	0	14	0	14	0	14
Control	1.28	0.65	1.05	0.50	0.95	0.42	1.01	0.52
Water control	1.15	0.70	1.01	0.55	0.92	0.45	0.98	0.55
Na-DK 2000	1.15	0.87	0.95	0.74	0.89	0.49	0.91	0.68
Na-DK 1000	1.19	0.90	0.98	0.78	0.93	0.49	0.93	0.72
AA 1000	1.25	0.97	1.00	0.75	0.90	0.52	0.95	0.65
AA 500	1.23	0.97	1.04	0.70	0.92	0.50	0.94	0.68
IAA 200	1.30	0.66	1.07	0.52	0.97	0.40	1.10	0.54
IAA 100	1.29	0.68	1.07	0.54	0.94	0.43	1.06	0.53
Ocimum Oil	1.26	0.92	1.07	0.77	0.96	0.55	1.00	0.70
LSD (P = 0.05)	0.09	0.06	0.08	0.04	0.09	0.04	0.08	0.06

Table 7. Effect of seed pretreatment with different concentrations of sodium-dikegulac (Na-DK, 2000 and 1000 μg/ml), ascorbic acid (AA, 1000 and 500 μg/ml) and IAA (200 and 100 μg/ml) as well as treatment with essential oil of *Ocimum* on protein content (mg/g fresh weight) in leaves of seedling developed from the seeds of four crop species stored under accelerated ageing condition.

Treatments (μg/ml)	Seed types							
	French Bean		Pea		Lentil		Millet	
	Days after accelerated ageing							
	0	14	0	14	0	14	0	14
Control	52.5	34.0	39.2	20.5	43.0	22.8	32.0	17.2
Water control	49.8	36.8	38.1	22.5	41.0	32.0	32.0	19.3
Na-DK 2000	45.5	39.8	38.0	30.2	40.8	32.5	30.5	24.7
Na-DK 1000	47.2	41.2	38.1	29.8	40.5	33.0	30.9	25.6
AA 1000	50.9	40.9	39.0	29.0	41.8	30.2	31.8	25.0
AA 500	52.0	42.2	39.2	28.7	42.0	31.9	32.6	24.9
IAA 200	54.5	37.1	40.0	23.8	43.3	23.4	33.0	18.2
IAA 100	54.9	36.2	41.8	24.5	43.5	24.0	30.2	18.7
Ocimum Oil	52.0	43.3	40.0	32.0	43.2	32.0	32.5	25.5
LSD (P = 0.05)	4.08	3.69	NS	2.52	NS	2.99	NS	2.33

NS = Not significant.

The percentage of TTC-stained seeds decreased as the seeds experienced prolonged accelerated ageing treatment and this decreasing trend with ageing was partially arrested by seed treatment with Na-DK, ascorbic acid and basil oil vapour (Table 8). Concomitantly, total dehydrogenase activity steeply declined after 14 days of accelerated ageing. The magnitude of fall was however, substantially checked in seeds which received pretreatment with Na-DK, ascorbic acid and basil oil vapour (Table 9).

Seedling abnormality (Table 10) and reduction of root length (Table 11) and shoot length (Table 12) occurred as a result of accelerated ageing treatment. Here also, Na-DK, ascorbic acid and basil oil vapour reduced the degree of deterioration during ageing to a significant extent. Again the above treatments reduced the extent of the reduction in length of seedlings, raised from seeds which experienced accelerated ageing for 14 days.

Discussion

The results of this investigation showed that high RH treatment enhanced the ageing process of French bean, pea, lentil and millet seeds, as evident by reduced seed vigour evaluated by a number of reliable physiological and biochemical parameters used in this investigation. Pretreatment of the seeds with Na-DK and ascorbic acid and also treatment of seeds with basil oil vapour during the storage period significantly reduced the loss of germinability over untreated control samples (Table 1). The loss of germina-

Table 8. Effect of seed pretreatment with different concentrations of sodium-dikegulac (Na-DK, 2000 and 1000 µg/ml), ascorbic acid (AA, 1000 and 500 µg/ml) and IAA (200 and 100 µg/ml) as well as treatment with essential oil of *Ocimum* on percentage of TTC-stained (deep red) seeds of four crop species under accelerated ageing.

Treatments (µg/ml)	Seed types											
	French Bean			Pea			Lentil			Millet		
	Days after accelerated ageing											
	0	7	14	0	7	14	0	7	14	0	7	14
Control	100	70.5	55.0	100	60.9	49.0	90	62.5	53.0	80	58.0	46.6
Water control	100	73.2	57.0	100	62.5	51.8	90	63.0	54.2	80	59.5	50.0
Na-DK 2000	100	80.1	69.5	100	70.8	63.0	90	75.0	62.5	80	66.5	59.0
Na-DK 1000	100	78.8	70.2	100	72.0	64.2	90	70.9	59.8	80	63.1	61.2
AA 1000	100	82.0	73.0	100	73.8	61.2	90	74.1	63.0	80	65.2	58.7
AA 500	100	81.3	74.5	100	71.1	63.7	90	74.9	62.8	80	65.8	58.9
IAA 200	100	71.0	55.5	100	83.0	50.5	90	63.0	52.0	80	57.0	49.0
IAA 100	100	70.5	56.0	100	63.4	52.0	90	63.9	55.0	80	61.2	50.9
Ocimum Oil	100	79.2	72.0	100	69.5	59.7	90	70.8	61.1	80	66.6	57.8
LSD ($P = 0.05$)	NC	6.98	6.08	NC	5.92	5.12	NC	7.01	5.92	NC	5.55	5.02

NC = Not calculated

Table 9. Effect of seed pretreatment with different concentrations of sodium-dikegulac (Na-DK, 2000 and 1000 μg/ml), ascorbic acid (AA, 1000 and 500 μg/ml) and IAA (200 and 100 μg/ml) as well as treatment with essential oil of *Ocimum* on dehydrogenase activity (ΔOD/g/ml) of the seeds of four crop species stored under accelerated ageing conditions.

Treatments (μg/ml)	Seed types							
	French Bean		Pea		Lentil		Millet	
	Days after accelerated ageing							
	0	14	0	14	0	14	0	14
Control	0.80	0.40	0.48	0.25	0.62	0.26	0.25	0.05
Water control	0.78	0.39	0.45	0.24	0.62	0.28	0.25	0.07
Na-DK 2000	0.77	0.54	0.44	0.31	0.59	0.48	0.22	0.15
Na-DK 1000	0.79	0.59	0.45	0.31	0.59	0.38	0.24	0.16
AA 1000	0.80	0.52	0.47	0.33	0.60	0.38	0.23	0.16
AA 500	0.81	0.56	0.49	0.30	0.58	0.35	0.25	0.17
IAA 200	0.82	0.45	0.48	0.26	0.61	0.27	0.26	0.06
IAA 100	0.78	0.45	0.47	0.25	0.58	0.27	0.26	0.06
Ocimum Oil	0.82	0.55	0.46	0.30	0.62	0.40	0.23	0.17
LSD (P = 0.05)	NS	0.04	NS	0.03	NS	0.04	NS	0.008

NS = Not significant.

tion during ageing was in all cases associated with a reduced rate of germination reflected in an increase in the time to reach 50% germination. However, this reduction was less following Na-DK, ascorbic acid and *Ocimum* oil treatments. Thus, after 14 days accelerated ageing the time (h) to 50% germination was 68.5 h, 65.2 h and 64.9 h for Na-DK 2000, AA 1000 and ocimum oil respectively compared to 92 h for untreated control seeds of millet (Table 2). The chemicals also reduced the degree of leaching of soluble carbohydrates (Table 4) and electrolytes (Table 5) from rapidly aged seeds. Reduced seed germinability and slower rate of germination are considered to be the important visible criteria for the evaluation of poor seed vigour (Anderson, 1970; Halder, Koley and Gupta, 1983). In this investigation with four crop seeds, the observed chemical-induced alleviation of the rapid loss of germinability and the fall of the rate of germination under accelerated ageing condition, are indicative of the fact that Na-DK, ascorbic acid and basil oil vapour helped the seeds to tolerate the unfavourable storage environment. Partial arrest of the leaching of soluble substances suggests that the chemicals checked the damage to membranes during ageing which consequently reduced the level of leaching. There are numerous reports that seed membranes are highly affected in deteriorating seeds, resulting in increased permeability and decreased germination of seeds (Powell and Matthews, 1977; Francis and Coolbear, 1984). Thus, it seems possible that the chemicals used in this investigation, excepting IAA, have some potential for retaining membrane integrity at least for a certain duration.

Table 10. Effect of seed pretreatment with different concentrations of sodium-dikegulac (Na-DK, 2000 and 1000 µg/ml), ascorbic acid (AA, 1000 and 500 µg/ml) and IAA (200 and 100 µg/ml) as well as treatment with essential oil of *Ocimum* on percentage normal seedlings, developed from the seeds of four crop species stored under accelerated ageing condition.

Treatments (µg/ml)	Seed types											
	French Bean			Pea			Lentil			Millet		
	Days after accelerated ageing											
	0	7	14	0	7	14	0	7	14	0	7	14
Control	100	81.2	64.0	100	75.5	63.3	95	71.8	58.2	90	66.7	53.9
Water control	100	83.7	68.8	100	77.0	66.5	95	72.0	60.0	90	67.0	55.5
Na-DK 2000	100	88.5	77.0	100	86.1	75.9	95	83.8	76.2	90	77.5	66.2
Na-DK 1000	100	90.1	78.2	100	88.2	80.0	95	85.0	77.7	90	79.2	70.0
AA 1000	100	90.8	80.0	100	90.0	76.9	95	87.0	74.5	90	76.2	64.9
AA 500	100	92.9	80.0	100	89.5	80.0	95	87.8	76.0	90	77.9	68.2
IAA 200	100	82.0	60.9	100	71.1	64.5	95	69.5	59.2	90	64.5	56.0
IAA 100	100	82.8	63.8	100	78.7	67.7	95	71.1	60.6	90	66.3	59.1
Ocimum Oil	100	87.9	77.9	100	82.9	75.8	95	84.0	74.0	90	80.0	69.9
LSD ($P = 0.05$)	NC	7.05	6.88	NC	7.22	6.59	NC	7.12	6.52	NC	7.11	6.03

NC = Not calculated.

Table 11. Effect of seed pretreatment with different concentrations of sodium-dikegulac (Na-DK, 2000 and 1000 µg/ml), ascorbic acid (AA, 1000 and 500 µg/ml) and IAA (200 and 100 µg/ml) as well as treatment with essential oil of *Ocimum* on root length (cm) of seedling, developed from the seeds of four crop species stored under accelerated ageing condition.

Treatments (µg/ml)	Seed types							
	French Bean		Pea		Lentil		Millet	
	Days after accelerated ageing							
	0	14	0	14	0	14	0	14
Control	11.5	5.9	10.1	5.5	8.6	4.1	5.2	3.0
Water control	11.2	6.2	10.0	5.6	8.6	4.3	5.2	3.2
Na-DK 2000	10.0	6.8	9.2	6.7	7.8	5.2	4.5	3.5
Na-DK 1000	10.3	7.1	9.5	6.9	8.0	5.5	4.7	3.8
AA 1000	11.0	7.3	9.9	7.1	8.3	5.5	5.3	3.7
AA 500	11.3	7.3	10.2	7.1	8.5	5.3	5.3	3.7
IAA 200	11.5	6.2	10.1	5.5	8.7	4.2	5.1	2.9
IAA 100	11.6	6.2	10.3	5.7	8.7	4.2	5.3	2.8
Ocimum Oil	11.4	7.3	10.1	7.0	8.5	5.6	5.2	3.7
LSD ($P = 0.05$)	1.15	0.62	0.85	0.66	0.71	0.49	0.40	0.32

Table 12. Effect of seed pretreatment with different concentrations of sodium-dikegulac (Na-DK, 2000 and 1000 µg/ml), ascorbic acid (AA, 1000 and 500 µg/ml) and IAA (200 and 100 µg/ml) as well as treatment with essential oil of *Ocimum* on shoot length (cm) of seedlings, developed from the seeds of four crop species stored under accelerated ageing condition.

Treatments (µg/ml)	Seed types							
	French Bean		Pea		Lentil		Millet	
	Days after accelerated ageing							
	0	14	0	14	0	14	0	14
Control	14.5	6.5	12.0	6.0	8.8	3.2	4.2	1.8
Water control	14.2	6.9	11.8	6.3	8.3	3.5	4.0	2.0
Na-DK 2000	12.7	8.5	10.7	8.0	7.9	4.8	3.7	2.3
Na-DK 1000	13.0	8.8	11.0	8.2	7.9	5.1	3.9	2.5
AA 1000	13.8	9.0	11.5	8.5	8.5	5.3	4.0	2.5
AA 500	14.0	9.2	11.7	8.5	8.7	5.5	4.3	2.6
IAA 200	14.8	6.8	12.2	6.2	8.9	3.8	4.1	1.9
IAA 100	14.9	7.0	12.2	6.3	8.8	3.7	4.1	2.0
Ocimum Oil	14.3	9.2	12.0	8.8	8.5	5.6	4.2	2.5
LSD ($P = 0.05$)	1.30	0.82	1.02	0.78	0.62	0.48	0.35	0.25

The efficacy of Na-DK, ascorbic acid and basil oil on the maintenance of seed quality under storage was also supported from TTC-stainability (Table 8) and total dehydrogenase activity (Table 9) of the seeds under storage. Data showed that both the decline in dehydrogenase activity and percent TTC stained seeds during accelerated ageing were partially averted by the above treatments. Dehydrogenase activity is generally used as a reliable index for the evaluation of seed viability (Abdul-Baki and Anderson, 1972). There are also reports that as seeds age, they lose vigour, determined by counting the percentage of TTC stained seeds and/or by observing the pattern of TTC staining (Halder, 1981). Thus, in spite of experiencing accelerated ageing treatment, the chemically treated crop seeds retained higher vigour than the controls.

The beneficial effects of Na-DK, ascorbic acid and basil oil on the maintenance of the vigour of the crop seeds during storage were also reflected in seedling chlorophyll and protein content and in seedling growth. Thus the chlorophyll (Table 6) and protein (Table 7) contents in leaves of the seedlings, raised from the chemical-treated seeds, were comparatively high over untreated control values. Reduced seedling growth is also regarded as a sign of low seed vigour (Delouche and Caldwell, 1960; Woodstock and Grabe, 1967). In this investigation, the reduction in seed vigour was substantially alleviated by seed treatment.

Thus, the conclusion is made from this investigation that Na-DK, ascorbic acid and basil oil can potentially alleviate storage deterioration of seeds, and simple hydration-dehydration treatment as well as seed pretreatment with IAA are least effective or ineffective in this regard.

Acknowledgements

The authors are indebted to Dr. R. Maag Ltd., Dielsdorf, Switzerland for generous supply of sodium-dikegulac. Financial assistance by Hill Affairs Branch Secretariat, Govt. of West Bengal in the form of a Research Project is thankfully acknowledged. The authors are also grateful to Professor M. A. Choudhuri and Dr. K. Gupta of Burdwan University for their kind help on this work.

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