Bangladesh J. Bot. **35**(1): 55-61, 2006 (June)

TECHNIQUE FOR STORAGE LONGEVITY OF MUNG BEAN AND SUNFLOWER SEEDS USING SODIUM DIKEGULAC AND *EUCALYPTUS* OIL

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Key words: Storage longevity, Sunflower, Mung bean, Sodium dikegulac, *Eucalyptus* oil

Abstract

An investigation was performed to analyse the efficacy of sodium dikegulac (Na-DK, 2,3:4-6-di-O-isopropylidine-α-L-xylo-2-hexalofuranosate) and *Eucalyptus* oil on maintenance of storage potential as well as enhancement of longevity of seeds of a mung bean (*Vigna radiata* L. Wilczek) and a sunflower (*Helianthus annuus* L.) having viability problems. For obtaining expeditious and more or less accurate results accelerated ageing technique was adopted. Pretreatment of mung bean and sunflower seeds with Na-DK (500 μg/ml) for 8 hours before accelerated ageing treatment (99.5% RH, 32 ± 2^0 C) for different durations (0 to 30 days) or continuous treatment of the seeds with *Eucalyptus* oil vapour for 30 days under the same ageing condition slowed down the ageing-induced decline of germination percentage and TTC (2,3,5-triphenyl tetrazolium chloride) stainability of both the seed species. The chemicals also significantly arrested the reduction of protein level and activity of peroxidase enzyme in seed kernels during the forced ageing period. Correspondingly, ageing-induced progressive increase of amino acid and soluble carbohydrate levels as well as activities of amylase and protease enzymes in seed kernels was remarkably checked in seed lots pretreated with the test chemicals. The promising effects of Na-DK and *Eucalyptus* oil on storage potential and viability extension of mung bean and sunflower seeds under adverse storage situation are apparent in this investigation.

Introduction

Deterioration of seeds and other plant propagules is a normal natural catabolic process which terminates their life span resulting in complete loss of viability. The process may be accelerated by some pathogenic attack and/or by adverse environmental conditions. Otherwise, the deteriorative events follow their normal course culminating in the production of nonviable seeds. This inevitable natural detrimental process, particularly pathogen and adverse environment-induced accelerated ageing, leading to quicker deterioration of seeds is a matter of serious concern to the seed technologists, crop growers and seedsmen associated with seed industry. Nowadays some strategies are being adopted to prolong the storage potential of seed by using some physical and chemical manipulative methods (Basu 1976, Bhattacharyya and Basu 1990, Chhetri *et al.* 1993, Basu 1994, Rai *et al.*1995, Maity *et al.* 2000, Chakrabarti *et al.* 2005).

Keeping in mind the problem of seed storing in India, an attempt was made in this investigation to prolong the storage life of a proteinaceous (mung bean) and a fatty (sunflower) seed species having viability problems using Na-dikegulac (Na-DK) and *Eucalyptus* oil. Although seed hardening effect of Na-DK under adverse storage condition is recently being reported (Rai *et al.* 1995; Bhattacharjee *et al.* 1999, Das *et al.* 2003, Kanp and Bhattacharjee 2003) from a few seed species, its role on storage potentiatial and viability extension of seeds needs to be clearly established using a wide range of seed species, particularly low vigour ones, which often cause

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hazards in Indian agriculture and reduce crop yield. The effect of volatile oil on the manipulation of seed longevity is also scanty in the literature (Chhetri, *et al.* 1993, Chakrabarti *et al.* 2005).

Thus, the prime objective of this investigation was to explore the efficacy of Na-DK and *Eucalyptus* oil on enhancing the storage potential and longevity of seeds of a selected mung bean and a sunflower cultivar under accelerated ageing condition. Accelerated ageing treatment, in fact, provides a powerful manipulative tool which makes it possible to study the process of seed deterioration over a short period (Heydecker 1972) and this mimics the natural ageing process.

Materials and Methods

Experiments were carried out with certified seeds of mung bean (*Vigna radiata* L. Wilczek cv. PS-16) and sunflower (*Helianthus annuus* L. cv. Morden).

After surface sterilization (0.1% $HgCl_2$ for 90 sec.) the seed samples of each cultivar were separately presoaked in the aqueous solution of Na-DK (500 μg/ml) or distilled water for 8 h and then dried back to the original dry weight of the seeds. The pretreated seed lots (200 g each) were taken in separate cloth bags and then stored in a desiccator in which 99.5% relative humidity (RH) was preimposed by keeping 250 ml 1.57% H_2SO_4 within it. This experimental set up was kept in laboratory and seeds were allowed to experience forced ageing treatment (99.5% RH, 32 ± 2°C), and H_2SO_4 was changed at 7-day intervals to restore the desired RH within the desiccator for 30 days.

In a separate experiment, fresh seed lots (200 g) of each cultivar were kept in a smaller desiccator in which 5 ml *Eucalyptus* oil (E. oil) was taken in a small Petri dish in addition to 250 ml 1.57% H_2SO_4. Here the seeds underwent treatment with the vapour of *Eucalyptus* oil along with accelerated ageing treatment (99.5% RH, 32 ± 2°C) throughout the experimental period. From the seed lots of both the experiments some physiological and biochemical analyses were made after 0, 15 and 30 days of accelerated ageing treatment.

To analyse the percentage germination, four groups of 50 seeds (*i.e.* 200 seeds) of each treatment were transferred to separate Petri dishes containing filter paper moistened with 10 ml distilled water. Germination data were recorded after 7 days of seed soaking following the rules of International Seed Testing (ISTA 1976).

For recording TTC-stainability, dehusked seeds of each treatment (in 4 groups of 50 seeds) were allowed to imbibe 0.5% (w/v) TTC solution in Petri dishes and kept overnight in dark. Percentage TTC-stained (red coloured) embryonal axes of seeds were calculated from the total number of seeds of each treatment.

Protein and amino acid levels were analysed from the seed kernels following the method of Lowry *et al.* (1951) and Moore and Stein (1948), respectively. Quantification of soluble carbohydrate from seed kernels was done following the method of McCready *et al.* (1950).

Extraction and estimation of peroxidase and amylase were made following the method of Kar and Mishra (1976) and Khan and Faust (1967), respectively and that of protease enzyme was done as per the method of Snell and Snell (1971) modified by Biswas and Choudhuri (1978). For the assay of the activities of these enzymes, the blank was taken as zero time control. The activity of each enzyme was expressed as $[(\Delta A \times Tv)/(t \times v)]$; where ΔA is the absorbance of the sample after incubation minus the absorbance of the zero time control, Tv 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).

Results and Discussion

Under accelerated ageing environment seed germination percentage progressively decreased with the duration of ageing regardless of treatments in both the seed species, but the magnitude of decrease was less pronounced in seed lots treated with Na-DK and *Eucalyptus* oil (Fig. 1). Concomitantly, TTC-stainability (per cent) of the embryonal axes of mung bean and sunflower

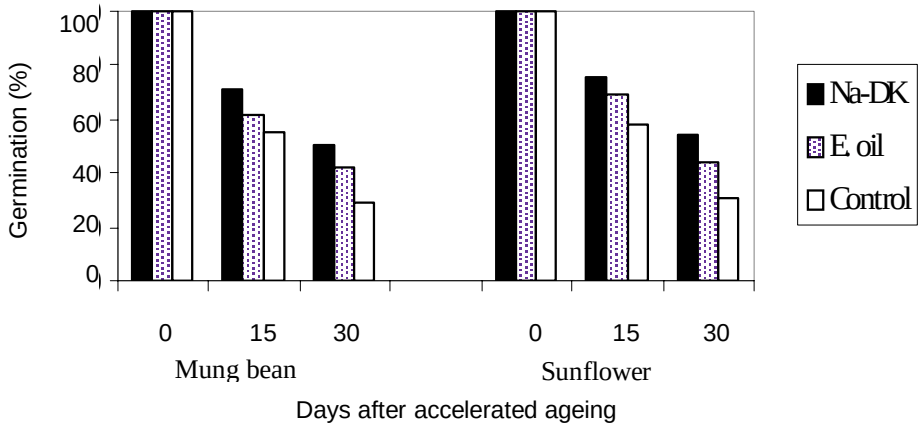

Fig. 1. Effects of seed pretreatment with Na-DK and *Eucalyptus* oil on percentage germination of mung bean and sunflower seeds stored under accelerated ageing condition for 30 days.

seeds decreased at all the treatments as the seeds experienced accelerated ageing, and the degree of stainability was distinctly ageing dependent. Seed pretreatments with the chemicals alleviated the ageing-induced loss of the percentage TTC-staining, and the effect of Na-DK was recorded to be most significant in this regard (Fig. 2).

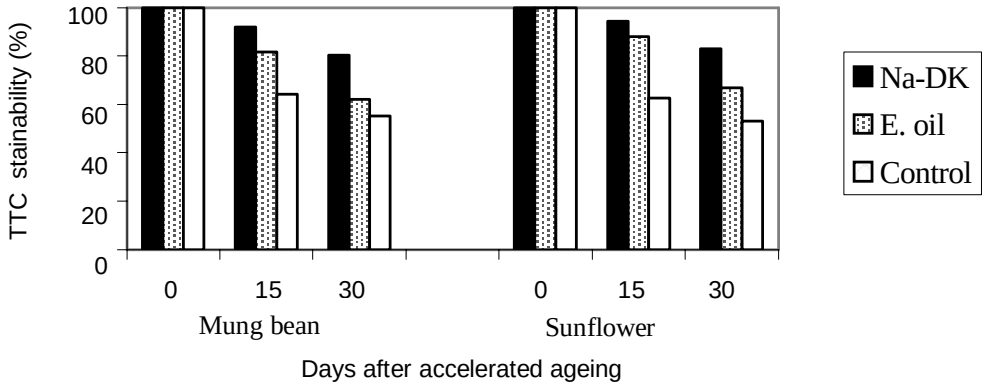

Fig. 2. Effects of seed pretreatment with Na-DK and *Eucalyptus* oil on percentage TTC stainability of mung bean and sunflower seeds stored under accelerated ageing condition for 30 days.

Changes on seed germinability and TTC stainability were associated with some biochemical changes in seed kernels. Protein content started decreasing along with seed ageing in all the samples analysed but Na-DK and *Eucalyptus* oil partially checked the rate of decrease in both the seed species (Fig. 3). On the other hand, levels of amino acids (Fig. 4) went on increasing in the control sample with the progress of ageing duration. The same trend was found in case of the chemical-pretreated seed samples, but here the rate of increase was considerably slowed down.

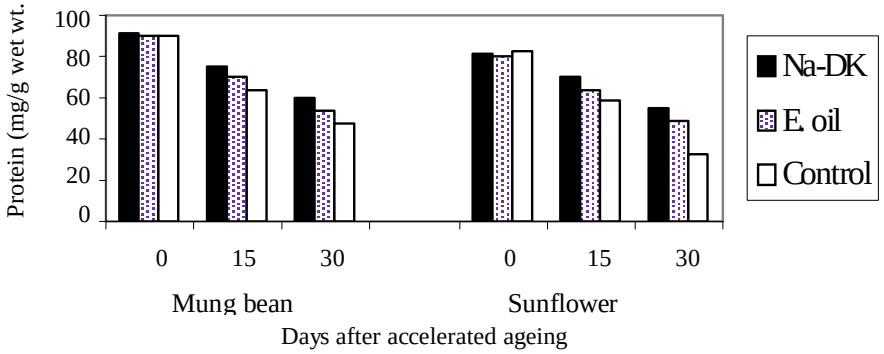

Fig. 3. Effects of seed pretreatment with Na-DK and *Eucalyptus* oil on protein level in seed kernels of mung bean and sunflower seeds stored under accelerated ageing condition for 30 days.

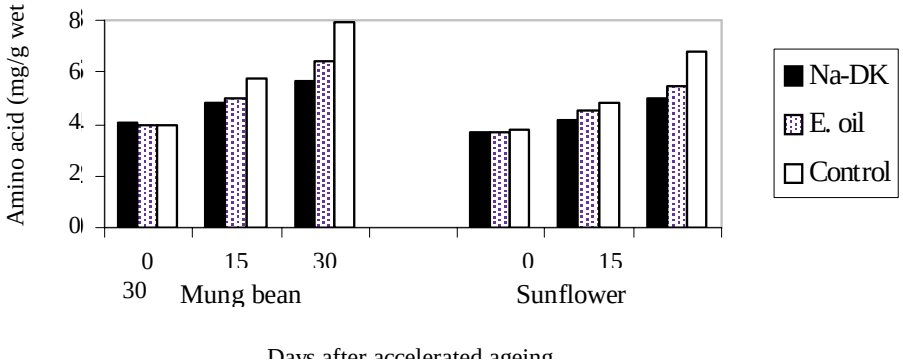

Fig. 4. Effects of seed pretreatment with Na-DK and *Eucalyptus* oil on amino acid level in seed kernels of mung bean and sunflower seeds stored under accelerated ageing condition for 30 days.

Table 1 shows that soluble carbohydrate level increased and peroxidase activity decreased with seed ageing process from 0 to 30 days both in control and in the chemical pretreated seed

samples. In Na-DK and *Eucalyptus* oil treated seeds the rate of increase of soluble carbohydrate and that of decrease of peroxidase were much less than control sample. Activities of both amylase and protease were found to increase progressively with the advancement of ageing duration; and here also, Na-DK and *Eucalyptus* oil checked the increasing drift in all the samples (Table 2).

Like plant senescence, seed senescence or deterioration is an internal programmed phenomenon which leads to nonviability or death of seeds. Depending upon the genetic make-up of seed species, the process of seed deterioration under storage is quickened or delayed determining the life span of a specific seed species.

Table 1. Effects of seed pretreatment with Na-DK and *Eucalyptus* oil on the changes of soluble carbohydrate level and peroxidase activity in the seed kernels of mung bean and sunflower seeds stored under accelerated ageing condition for 30 days.

Treatments	Days after accelerated ageing					
	Mung bean			Sunflower		
	0	15	30	0	15	30
	Soluble carbohydrates (mg/g fr. wt.)					
Na-DK	27.2	38.8	47.1	21.9	25.2	38.5
E. oil	28.3	45.5	54.5	20.1	30.0	45.4
Control	29.6	53.7	60.3	21.2	35.3	53.2
LSD (p = 0.05)	NS	4.01	4.75	NS	2.50	4.01
	Peroxidase activity (unit/h/g fr. wt.)					
Na-DK	78.2	63.5	48.7	85.7	73.2	50.6
E. oil	76.4	58.1	42.5	82.9	65.3	42.3
Control	76.2	44.9	28.1	83.0	58.7	31.5
LSD (p = 0.05)	NS	5.01	3.11	NS	5.75	3.20

NS = Non significant.

Table 2. Effects of seed pretreatment with Na-DK and treatment with *Eucalyptus* oil on the changes of amylase and protease activities in the seed kernels of mung bean and sunflower seeds stored under accelerated ageing condition for 30 days.

Treatments	Days after accelerated ageing					
	Mung bean			Sunflower		
	0	15	30	0	15	30
	Amylase activity (unit/h/g fr. wt.)					
Na-DK	27.2	33.1	37.9	26.6	30.5	35.6
E. oil	27.5	39.5	43.1	24.7	34.1	40.9
Control	28.1	44.4	49.7	24.8	38.6	46.1
LSD (p = 0.05)	NS	3.25	3.80	NS	3.01	3.60
	Protease activity (unit/h/g fr. wt.)					
Na-DK	1.5	1.9	3.1	1.2	1.5	2.1
E. oil	1.6	2.6	3.8	1.1	2.2	2.8
Control	1.7	3.6	4.6	1.4	3.0	3.9
LSD (p = 0.05)	NS	0.20	0.30	NS	0.15	0.22

NS = Non-significant.

The results of this investigation showed that high RH treatment enhanced the ageing process of mung bean and sunflower seeds as evident from reduced seed vigour which is determined by a number of reliable physiological and biochemical parameters used in this investigation. Pretreatment of the seed with Na-DK and *Eucalyptus* oil during the storage period slowed down the ageing-induced reduction of germinability over control samples. In fact, loss of germinability during seed ageing is associated with reduced speed of germination and these are reflected in the TTC-stainability of seeds (Rai 2000, Maity *et al.* 2000). Reduced seed germinability and TTC-stainability are considered as important visible criteria for the evaluation of poor seed vigour (Anderson 1970, Halder *et al.* 1983). In this investigation, the observed chemical-induced alleviation of the rapid loss of germinability and TTC-stainability are indicative of the fact that the chemicals helped the seeds to tolerate the unfavourable storage environment and thus such seed lots exhibited superior vigour status.

Available reports show that during seed ageing loss of some vital cellular components occur along with increase of some soluble substances (Ching and Schoolcraft 1968, Abdul-Baki and Anderson 1972, Kole and Gupta 1982, Bhattacharjee 1984, Rai 2000). In this investigation, decrease of protein (Fig. 3) with concomitant increase of soluble substances such as amino acids (Fig. 4) and soluble carbohydrates (Table 1) along with enzymes like amylase and protease corroborate the reported observations.

The chemical-induced substantial retention of seed health can also be strongly supported from the changes of the activities of peroxidase (Table 1). The chemicals significantly alleviated the ageing-induced reduction of peroxidase activity of seed kernels. Higher peroxidase and catalase activities are often used as reliable indices for the evaluation of higher viability and vigour status of seeds (Bhattacharjee and Choudhuri 1986, Rai 2000, Maity *et al.* 2000). Higher activities of these peroxidase and catalase (Fridovich 1976, Rai 2000) were also shown in plants having higher potential maintaining vigourous growth. The present results, therefore, point out that in spite of experiencing accelerated ageing treatment, the chemical-pretreated seed lots substantially retained their vigour.

From this investigation it may be concluded that both Na-DK and *Eucalyptus* oil are the potential chemical manipulative agents for storage invigouration and enhancement of longevity of seeds even under adverse storage situation.

Acknowledgements

The authors are indebted to Dr. R. Maag, Dielsdorf Ltd., Switzerland for generous supply of sodium-dikegulac.

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(Manuscript received on 3 April, 2006; revised on 17 April, 2006)