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Retention of Sunflower Seed Vigour and Viability using Medicinal Herbs

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Abstract

Sunflower (*Helianthus annuus* L. cv. Morden) seeds lost viability rapidly under accelerated ageing condition. Pretreatment of the seeds with aqueous solution 1:25 (W/V) of two excellent medicinal herbs of leaf extract of basil (*Ocimum sanctum* L., Lamiaceae) and tuber extract of safed musli (*Chlorophytum borivilianum* Sant. *et* Fernand, Liliaceae) for 8 hours before accelerated ageing treatment, (99.5% RH and 32±2°C) for different durations (0 to 30 days) slowed down the ageing-induced rapid loss of germination and reduced the time required for 50% seed germination (T_{50}). TTC stainability was maintained to a considerable extent in seeds, which received, pre-treatment with the indigenous plant extracts. The reduction of protein and insoluble carbohydrate levels as well as activities of catalase and dehydrogenase enzymes of the seed kernels during forced ageing period was ameliorated to a significant extent in the plant extracts pre-treated seed lots of sunflower. On the other hand, ageing-induced progressive increase of levels of soluble carbohydrates and amino acids in control samples were remarkably arrested in seed lots pretreated with all the plant extracts. Considering the changes of all the biochemical parameters, the treatments were found to be effective for enhancing the storage potential with concomitant extension of viability of sunflower seeds. There have a lot of chemicals which was used for the purpose of seed viability extension but our pretreated agents are absolutely herbal and it is a modern research in indigenous technology to make the environment cleaned.

Keywords
Accelerated ageing, basil leaf, plant extracts, safed musli, seed viability, sunflower seed.

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INTRODUCTION

Seed ageing is one of the most intriguing and challenging scientific problems of universal concern. It is of particular interest in India where high temperature and high relative humidity greatly accelerate seed ageing phenomenon [1, 2, 3] causing consequent deterioration and nonviability of seeds. The problem of retention of seed vigour in Midnapore and surrounding areas of West Bengal state in India appears to be much more acute because of high relative humidity associated with hot climatic condition prevailing during the major part of a year 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 in mind this problem of seed storing in this region an attempt was made in this investigation to prolong the storage life of seeds of a sunflower, which are prone to microbial attack at high atmospheric moisture. Although, now-a-days, some strategies are being adopted to prolong the storage potential of seeds by using some physical and chemical manipulative methods [2, 4, 5, 6a, 6b] in the present investigation an attempt was made to enhance storage potential of sunflower seeds by using leaf extract of basil (*Ocimum sanctum* L.) and tuber extract of safed musli (*Chlorophytum borivilianum* Sant. et Fernand). These two plants are tremendous medicinal herb. First one is very much common and found in locally and second one safed musli commonly called 'root of gold' found in forest now a day it is cultivated as a cash crop. Natural products available in such plants particularly essential oil like – α-pinene, α-thujene, α-camphane, α-myrcene, β-pinene, eucalyptol, sabinene, camphor, eugenol, cubeno, selinene, phytol, oleic acid, ethyl cyclohexenal ketone, heptanol and saponin are reported to have some antimicrobial property [7] which is expected to check or slow down pathological deterioration of seeds under adverse storage situations.

Thus, the major objective of this study was to explore the efficiency of the leaf extract of basil and tuber extract of safed musli on seed potentiation and prolongation of seed storage of a low vigour sunflower seeds under artificially imposed highly adverse storage environment. 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 [8] and this mimics the natural ageing process.

MATERIALS AND METHODS

The experiments were carried out with freshly harvested, 100% viable seeds of sunflower (*Helianthus annuus* L. cv. Morden).

Seed samples were allowed to experience artificially imposed adverse environmental conditions called accelerated ageing to obtain a relatively uniform and expeditious results.

Plant extracts were separately prepared by thoroughly homogenizing 10 g freshly harvested leaf of basil and tuber extract of safed musli in distilled water and subsequent straining followed by centrifugation of the aqueous extracts. The total volume was made up to 250 ml using distilled water and these were taken as seed pretreating plant extracts.

After surface sterilization (0.1% $HgCl_2$ for 90 sec.) the seed samples were separately soaked in the aqueous solutions of leaf extract of basil or tuber extract of safed musli (1:25 w/v) or distilled water for 8 h and then dried back to the original dry weight of the seeds. The pretreated seed lots (100 g each) were taken in separate porous cloth bags and thus stored in a desiccator in which 99.5% RH was preimposed by keeping 250 ml 1.57% H_2SO_4 within it [6a, 9]. This experimental set-up was kept at 32 ± 2^0C for 30 d allowing the seeds to experience forced ageing treatment and H_2SO_4 was changed at 10 d intervals to restore the desired RH throughout the 30 days period. Data on germination behaviour, TTC-stainability and metabolism of seeds were analysed after 0, 10, 20 and 30 d of accelerated ageing.

Percentage germination of seeds was assessed following the ISTA rules [10]. The time required for 50% germination of seeds (T_{50}) was determined following the method of Coolbear *et al.* [11].

For recording TTC (2, 3, 5-triphenyl tetrazolium chloride) stainability, dehusked seeds of each treatment (in 4 groups of 100 seeds) were allowed to imbibe 0.5% (w/v) TTC solution in Petri dishes and kept overnight in dark. Percentage TTC stained embryonal axes (deep red) was calculated from the total number of seeds of each treatment.

Protein and amino acid levels were analysed from seed kernels following the method of Lowry *et al.* [12] and Moore and Stein [13] respectively. Both Insoluble and soluble carbohydrate levels were analysed from seed kernels following the method of McCready *et al.* [14].

The activity of total dehydrogenase of intact seeds was analysed by the reaction of tetrazolium chloride according to the method of Rudrapal and Basu [15]. The hydrogen atoms released by the total dehydrogenase enzymes which are involved in the respiration processes of living tissue, reduces tetrazolium to red coloured formazan [16]. To analyse dehydrogenase (total) activity the TTC-stained embryonal axes were homogenized in 10 ml methoxy ethanol, centrifuged at 10,000 *g* and OD values of the extracted formazan (red colour) was recorded at 520 nm.

Extraction and estimation of the enzyme catalase was done as per the method described by Snell and Snell [17]. For the assay of this enzyme 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 [18].

All the data were statistically analysed at the treatment and replication levels, the least significant difference (LSD) values were calculated at 95% confidence limits [19].

RESULTS

Effect on germinability (Table 1). Percentage seed germination started declining with the advancement of accelerated ageing duration in all the seed samples irrespective of the treatments as well as in distilled water control. However, the magnitude of the fall of seed germination was found to be significantly less in seed lots pretreated with leaf extract of basil and tuber extract of safed musli.

Effect on T_{50} value (Table 2). Concomitantly, the leaf extract of basil and tuber extract of safed musli remarkably reduced the time required for 50% germination of seeds. In seed lots pretreated with distilled water (control) 50% seed germination was not at all attained after 10 days of forced ageing treatment.

Effect on TTC-stainability (Table 3): TTC-stainability of the embryonal axes of sunflower seeds decreased at all the treatments as the seeds experienced accelerated ageing and the degree of stainability was found to be distinctly ageing dependent. Seed pretreatments with the plant extracts were ameliorative with respect to retention of TTC staining. The effect of safed musli was recorded to be most significant in this regard

Effect on changes of protein and amino acid levels in seed kernels (Table 4). As regards the changes in levels of these two, a diametrically opposite trend was recorded. Under accelerated ageing condition, protein level decreased and amino acid level increased progressively with the advancement of ageing duration. Pretreatment of the seeds with basil and safed musli extracts significantly arrested the ageing-induced loss of protein level and increase of amino acid level.

Effect on changes of insoluble and soluble carbohydrates in seed kernels (Table 5). Almost an identical trend like the changes of protein level was recorded when insoluble carbohydrate level was analysed. So far the overall changes of soluble carbohydrate level are concerned, a clear reverse picture was noted. In basil and safed musli extracts treated seeds the rate of increase of soluble carbohydrates was much less than control sample.

Effect on changes of dehydrogenase and catalase activities in seed kernels (Table 6). Activities of the enzymes dehydrogenase and catalase declined with seed ageing process from zero to 30 d both in control and in herbal extracts pretreated seed samples. However, the rate of decreasing in activities were found to occur slowly in seeds which received pretreatment with leaf extract of basil and tuber extract of safed musli. The data relating to the beginning of the ageing (0-d) showed no statistical significance in all the treatments.

Table 1. Effect of seed pretreatment with leaf extract of basil and tuber extract of safed musli on percentage germination of sunflower seeds stored under accelerated ageing condition for 30 days.

Seeds were presoaked with leaf extract of basil and tuber extract of safed musli or distilled water for 8 hours and then dried back to original seed weight. The pretreated seed samples were then allowed to experience accelerated ageing treatment (99.5% RH, 32±2°C temperature) in a desiccator. Data were recorded after 0, 10, 20 and 30 days of seed ageing.

Treatments	Percentage germination			
	Days after accelerated ageing			
	0	10	20	30
Control	100	24.1	11.6	NA
Basil	100	52.4	23.4	3.8
Safed musli	100	54.8	32.2	12.4
LSD (*P*=0.05)	NC	2.44	1.21	0.35

NA: Non-attainment of germination; NC: Not calculated.

Table 2. Effect of seed pretreatment with leaf extract of basil and tuber extract of safed musli on time (hours) to 50% germination (T_{50}) of sunflower seeds stored under accelerated ageing condition for 30 days. Treatments are the same as in Table 1. Data were recorded after 0, 15, 20 and 30 days of seed ageing.

Treatments	T_{50} values of germination			
	Days after accelerated ageing			
	0	10	20	30
Control	25.1	NA	NA	NA
Basil	22.4	70.8	NA	NA
Safed musli	22.6	59.3	NA	NA
LSD (*P*=0.05)	NS	5.75	-	-

NA: Non-attainment of 50% germination; NS: Not significant.

Table 3. Effect of seed pretreatment with leaf extract of Basil and tuber extract of safed musli on percentage TTC-stainability of sunflower seeds stored under accelerated ageing condition for 30 days.

Treatments are the same as in Table 1. Data were recorded after 0, 10, 20 and 30 days of seed ageing.

Treatments	TTC stainability (%)			
	Days after accelerated ageing			
	0	10	20	30
Control	100	43.2	23.6	13.2
Basil	100	59.8	41.6	23.7
Safed musli	100	68.9	48.4	29.0
LSD (*P*=0.05)	NC	4.27	2.33	1.15

NC: Not calculated.

Table 4. Effect of seed pretreatment with leaf extract of Basil and tuber extract of safed musli on changes of protein and amino acid contents in the kernels of sunflower seeds stored under accelerated ageing condition for 30 days.

Treatments are the same as in Table 1. Data were recorded from the seed kernels after 0, 10, 20 and 30 days of seed ageing.

Treatments	Protein (mg/g fr. wt.)				Amino acid (mg/g fr. wt.)			
	Days after accelerated ageing							
	0	10	20	30	0	10	20	30
Control	156.2	104.9	86.1	76.7	12.08	16.54	19.10	23.32
Basil	158.1	120.5	109.3	97.6	12.08	12.68	14.63	18.11
Safed musli	160.6	146.3	134.5	125.0	12.07	12.20	13.12	16.10
LSD (*P* = 0.05)	NS	9.92	8.22	6.69	NS	1.20	1.36	1.67

NS: Not significant.

Table 5. Effect of seed pretreatment with leaf extract of Basil and tuber extract of safed musli on changes of insoluble carbohydrates and soluble carbohydrates contents in the kernels of sunflower seeds stored under accelerated ageing condition for 30 days.

Treatments are the same as in Table 1. Data were recorded from the seed kernels after 0, 10, 20 and 30 days of seed ageing.

Treatments	Insoluble carbohydrates (mg/g fr. wt.)				Soluble carbohydrates (mg/g fr. wt.)			
	Days after accelerated ageing							
	0	10	20	30	0	10	20	30
Control	176.3	129.4	118.2	98.3	23.2	52.1	64.3	83.0
Basil	174.1	160.8	142.5	130.4	22.2	32.5	48.4	61.4
Safed musli	173.0	168.1	156.9	137.8	22.8	25.6	38.1	44.7
LSD (*P* = 0.05)	NS	11.57	10.14	8.22	2.18	2.48	3.63	4.50

NS: Not significant.

Table 6. Effect of seed pretreatment with leaf extract of Basil and tuber extract of safed musli on changes of dehydrogenase and catalase activities in the kernels of sunflower seeds stored under accelerated ageing condition for 30 days.

Treatments are the same as in Table 1. Data were recorded from the seed kernels after 0, 10, 20 and 30 days of seed ageing.

Treatments	Dehydrogenase (ΔOD/g wet wt./5 ml)				Catalase (unit/h/g fr. wt.)			
	Days after accelerated ageing							
	0	10	20	30	0	10	20	30
Control	0.46	0.25	0.20	0.14	90.2	71.2	53.3	38.0
Basil	0.45	0.33	0.27	0.21	89.6	84.0	68.5	47.7
Safed musli	0.47	0.42	0.38	0.27	91.9	88.8	73.6	55.4
LSD (*P* = 0.05)	NS	0.06	0.05	0.02	NS	5.86	4.62	3.26

NS: Not significant.

DISCUSSION

Deterioration of seeds under ambient storage conditions is an internal programme phenomenon which leads to loss of vigour followed by loss of viability and consequent death and decay 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 type.

Results showed that pre-treatment of sunflower seeds with leaf extract of basil and tuber extract of safed musli significantly averted the ageing-induced fall of germination (Table 1) as well as reduced the time required for 50% germination (Table 2), increased TTC stainability (Table 3), alleviated the loss of protein and increase of amino acid (Table 4) as well as check the increase of soluble carbohydrates and loss of insoluble carbohydrates (Table 5) content and arrested reduction of dehydrogenase and catalase activities (Table 6).

Reduced seed germinability are considered to be the important visible criteria for the evaluation of poor seed vigour [2, 6a, 20, 21a, 21b, 22]. In this investigation, the basil and safed musli extracts-induced arrestation of loss of seed germination and lowering of T_{50} hours are indicative of storage potentiation property of the plant extracts.

The influence of the basil and safed musli extracts on maintaining storage potential of the seeds can also be substantiated from the data on TTC stainability and dehydrogenase activity of the embryonal axes of the seeds. The plant extract-induced substantial restoration of TTC staining as well as dehydrogenase activity is indicative of enhanced storability of the plant extracts under adverse storage situation. There are reports that as seeds age, they lose vigour as evaluated by counting percentage TTC-stained seeds

and/or by observing the pattern of TTC staining [23, 24]. Again, dehydrogenase activity can be considered to be a reliable index for evaluation of seed viability [25, 26]. The present data thus pinpoint that in spite of accelerated ageing, seed pretreatments with the leaf extract of basil and tuber extract of safed musli retained higher seed vigour than the control samples.

The plant extracts-induced substantial retention of seed health can also be strongly supported from the changes in protein and insoluble carbohydrate levels as well as activities of dehydrogenase and catalase enzymes. The plant extracts significantly alleviated the ageing-induced reduction of protein and insoluble carbohydrate contents as well as catalase activity of seed kernels. Concomitantly, alarming increase in the levels of the amino acid and soluble carbohydrate were kept subdued by the pretreating plant extracts. Protein and insoluble carbohydrate are the vital cellular macromolecules which maintain normal functional life of living organs or organisms. Again, dehydrogenase and catalase activities are used as reliable indices for the evaluation of viability and general health status of seeds [2, 9, 27, 28, 29a, 29b]. Higher activity of the H_2O_2 scavenger enzyme catalase [30] was also shown in plants having higher potential and maintaining a vigorous growth.

CONCLUSIONS

So, from the present observation of higher metabolic status in the plant extract-pretreated seed samples, it seems quite apparent that the present experimental seed pretreating substances considerably hardened the seeds and such hardening was affected at the metabolic level. Also it can be speculated that the accelerated ageing-induced adverse effects were also nullified, at least to some extent, by the seed pretreating medicinal herbs.

Thus, the basil and safed musli extract-induced metabolic alterations positively influenced seed health and resulted in substantial retention of seed vigour and viability.

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REFERENCES

1. Agarwal PK, Siddique MN., Influence of storage temperature and seed moisture on germination, free fatty acid content and leaching of sugars of soybean seeds during storage. *Seed Res*, 1: 75 – 82, (1973).
2. Rai AS. *An investigation into the problems of maintenance of seed vigour and viability under adverse climatic conditions of Darjeeling hills*. Doctoral thesis, North Bengal University, India. 2000.
3. Kanp UK, Bhattacharjee A. Influence of sodium-dikegulac, ascorbic acid and *Eucalyptus* oil on ageing of sunflower seeds. *Ind J Plant Physiol, (Special Issue):* 240-243, (2003).
4. Basu RN., An appraisal of research on wet and dry physiological seed treatments and their applicability with special reference to tropical and sub-tropical countries. *Seed Sci. & Technol*, 22: 107-126, (1994).
5. Dahiya BS., Rai KN., Seed Technology. Kalyani Publishers, New Delhi. (1997).
6. a. Das RK, Kanp UK, Bhattacharjee A., Influence of plant Extracts of *Eucalyptus*, Turmeric and Ginger on seed potentiation of a Mung Bean Cultivar. *Environ & Ecol,* 21 (3): 599-606, (2003 a).
6. b. Das RK, Kanp UK, Bhattacharjee A., Influence of plant extracts on storage potential of seeds and field performance of seedlings of pea and horse gram. *J Bot Soc*, 57: 17-23, (2003 a).
7. Kaufman PB, Cseke LJ, Warber S, Duke JA, Brielmann HL., Natural Products from plants. CRC Press, Washington, DC, (1993).
8. Heydecker W. In: Roberts E.H. (Ed.) Vigour and Viability of Seeds. Chapman and Hall Ltd. London, 1972, pp. 209 – 252.
9. Maity S, Banerjee G, Roy M, Pal C, Pal B, Chakraborti D, Bhattacharjee A., Chemical induced prolongation of seed viability and stress tolerance capacity of mung bean seedlings. *Seed Sc & Technol,* 28: 155-162, (2000).
10. International Seed Testing Association. International rules for seed testing. *Seed Sc & Technol*, 4: 51-177, (1976).
11. Coolbear P, Francis A, Grierson D. The effect of low temperature pre-sowing treatment on the germination performance and membrane integrity of artificially aged tomato seeds. *J Exptl Bot*, 35: 1609-1617, (1984).
12. Lowry OH, Rosebrough NH, Farr AL, Randall RJ., Protein measurement with the folinphenol reagent. *J Biol Chem,*193: 265-275, (1951).
13. Moore S, Stein WW., Photometric ninhydrin method for use in the chromatography of amino acids. *Journal of Biological Chemistry*, 176: 367-388, (1948).
14. McCready RM, Guggloz J, Silviera V, Owens HS. Deterioration of starch and amylase in vegetables. *Analytical Chem*, 22: 1156-1158, (1950).
15. Rudrapal AB. Basu RN. Physiology of hydration-dehydration treatment on the maintenance of seed viability in wheat (*Triticum aestivum* L.). *Indian Journal of Experimental Biology,* 17: 768-771, (1979).
16. Moore RP. Tetrazolium staining for assessing seed quality. In Heydeker W. (Ed.), *Seed Ecology,* Butterworths, London.1973, p. 347.
17. Snell FD, Snell CT., Colorimetric methods of analysis. Van Nostrand Reinhold Co., New York.1971. Vol. IV AAA pp. 7-145.

18. Fick NG, Qualset CO. Genetic control of endosperm amylase activity and gibberellin responses in standard height and short statured wheat. *Proceeding of National Academic Science.* USA, 72: 892-895, (1975).
19. Panse VG, Sukhatme PT. Statistical Methods for Agricultural Workers. Indian Council of Agricultural Research, New Delhi. 1967. 2nd edition, pp. 150-157.
20. Anderson JD., Metabolic changes in partially dormant wheat seeds during storage. *Plant Physiol,* 46: 605-609, (1970).
21. a. Mishra VK, Pati CK, Bhattacharjee A. Prolongation of seed viability of a medicinally important pulse crop horse gram (*Dolichos biflorus* L.). *Environment and Ecology,* 22: 115-119, (2004 a).
21. b. Mishra VK, Pati CK, Bhattacharjee A., Herbal regulations of plant growth and metabolism of two minor pulse crops. *Agricultural Science Digest*, 24: 295-297, (2004 b).
22. Bhattacharjee A, Pati CK, Kanp UK, Das RK, Chakrabarti D., Na-dikegulac, a novel chemical for enhancement of seed storage potential and seedling health of two pulse crops. *Proceedings - The BCPC International Congress, Crop Science & Technology,* Glasgow, Scotland, UK. 2005. vol. – 1, pp. 223-228.
23. Halder S. *Studies on viability, yield and associated biochemical changes in leaves during seed filling in sunflower (Helianthus annuus L. cv. EC 68414).* Doctoral thesis, Burdwan University, India, 1984.
24. Chhetri DR, Rai AS, Bhattacharjee A., Chemical manipulation of seed longevity of four crop species in an unfavourable storage environment. *Seed Sci & Technol,* 21: 31-44, (1993).
25. Abdul-Baki AA, Anderson JD., Physiological and biochemical deterioration of seeds. In Kozlowski, T.T. (Ed.), Seed biology, Academic Press, New York, USA.1972. vol. 2. pp. 203-215,
26. Rai AS, Chhetri DR, Bhattacharjee A., Influence of sodium-dikegulac on storage potential of selected seed species. *Seed Sci & Technol,* 23: 249-252, (1995).
27. Bhattacharjee A, Choudhuri MA., Chemical manipulation of seed longevity and stress tolerance capacity of seedlings of *Corchorus capsularis* and *C. olitorius. J Plant Physiol,* 125: 391-400, (1986).
28. Kanp UK, Bhattacharjee A., Chemical Manipulation of seed longevity and crop improvement of a sunflower cultivar. In: Biodiversity conservation: Fundamental and Applications. (eds. Saha, Ghosh, Gangopadhyay, Saha, Singh, Sarkar and Das) Published by Dum Dum Motijheel College, Kolkata, W. B., India. 2012. pp 110 – 114.
29. a. Kundu S, Nandi AK, Kanp UK, Bhattacharjee A., Invigouration of maize (*Zea mays* L. cv. Nilesh, NMH - 51) seeds during storage by using *Citronella* oil. *Ind Biol*, 47 (2): 51 – 55, (2015 a).
29. b. Kundu S, Nandi AK, Kanp UK, Bhattacharjee A., Influence of sodium dikegulac on storage potential of maize (*Zea mays* L. cv. Suvarna, NMH - 589) seeds. *Ind Agric,* 59(2): 109 – 115, (2015 b).
30. Fridovich I., Oxygen radical, hydrogen peroxide, and oxygen toxicity. In Free Radicals in biology, (Ed. W. A. Pryor) Academic Press, New York.1976. vol. 1. pp. 237-277.