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Influence of plant extracts of *Asteracanthus longifolia* and safed musli on viability extension of sunflower seeds under artificial stress storage environment

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Abstract
Seeds of sunflower (*Helianthus annuus* L. cv. Morden) lost potentiality rapidly under accelerated ageing situation. Seeds treated with aqueous solution 1:20 (W/V) of two very important medicinal plants of leaf extract of *Asteracanthus longifolia* Nees. (Acanthaceae) and tuber extract of safed musli (*Chlorophytum borivilianum* Sant. *et* Fernand, Liliaceae) for 8 h preceding accelerated ageing treatment, (99.5% relative humidity and 32±2°C) for diverse periods (0 to 45 days) slowed down the ageing-influence quick damage 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, pretreatment with the indigenous plant extracts. The reduction of protein and insoluble carbohydrate levels as well as catalase and dehydrogenase enzyme activities of the seed kernels during forced ageing period was ameliorated to a significant extent in the plant extracts pretreated 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, *Asteracanthus longifolia* leaf, plant extracts, safed musli tuber, seed viability, sunflower seed

1. Introduction
Seed ageing is one of the most intriguing and challenging scientific problems of universal concern. It is of particular interest in India where temperature and relative humidity is very high, which helps to accelerate seed ageing (Kanp and Bhattacharjee, 2003) [15], (Rai, 2000) [25], (Kundu *et al*., 2015) [17] as a result seeds goes to degeneration and nonviability. Holding of seed vigour is a great enigma in Midnapore and surrounding areas of West Bengal state in India appears to be abundant keen caused by extremely relative humidity associated with hot climatic condition. This environmental situation exist greater part of a year which is very helpful to raise of microorganisms and various fungi. As maximum crop seeds need storage for either one or more implanting periods, agriculturists and horticulturists of this region are often disabled with respect to maintenance of a level of quality seed viability under ambient storage situations. Detaining in mind this question for solution of seed storing in this region an effort was prepared 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 (Basu, 1994) [3], (Das *et al.*, 2003) [8], (Das *et al.*, 2003) [9] in the present investigation an attempt was made to enhance storage potential of sunflower seeds by using leaf extract of *Asteracanthus longifolia* Nees. 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 days it is cultivated as a cash crop. Natural products available in such plants particularly lupeol, stigmasterol, butelin, β-sitosterol, saponin, eucalyptol, curcumin and zinziberene are reported to have some antimicrobial property (Kaufman *et al*., 1993) [16] which is expected to check or slow down pathological deterioration of seeds under adverse storage situations.

Thus, the major goal of this study was to explore the efficiency of the leaf extract of *Asteracanthus longifolia* 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. In fact, accelerated ageing treatment, as imposed by high temperature and high relative humidity (RH) provided a powerful tool for studying the process of seed deterioration over a short period (Heydecker, 1972) [12] and this mimics the natural ageing process.

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

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 *Asteracanthus longifolia* 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 200 ml using double 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 singly soaked in the watery solutions of leaf extract of *Asteracanthus longifolia* or tuber extract of safed musli (1:20 w/v) or distilled water for 8 h after that these extract soaked seeds sun dried and restored its original dry weight. 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 prearranged by keeping 250 ml 1.57% H_2SO_4 within (Maity *et al*., 2000) [19]. This experimental set-up was remained at 32 ± 2^0C for 45 d allowing the seeds to experience forced ageing treatment and H_2SO_4 was changed at 15 d intervals to give back the desired RH throughout the 45 days period. Data on germination behaviour, TTC-stainability and metabolism of seeds were analysed after 0, 15, 30 and 45 d of accelerated ageing.

Percentage germination of seeds was assessed following the ISTA rules (1976) [13]. The time required for 50% germination of seeds (T_{50}) was determined following the method of Coolbear *et al.* (1984) [7].

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.* (1951) [18] and Moore and Stein (1948) [22] respectively. Both Insoluble and soluble carbohydrate levels were analysed from seed kernels following the method of McCready *et al.* (1950) [20].

The activity of total dehydrogenase of intact seeds was analysed by the reaction of tetrazolium chloride according to the method of Rudrapal and Basu (1979) [26]. 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 (Moore, 1973) [21]. To analyse dehydrogenase (total) activity the TTC-stained embryonal axes were homogenized in 10 ml methoxyethanol, 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 (1971) [27]. For the assay of this enzyme the blank was taken as zero time control. The activity of each enzyme was expressed as [(ΔA x Tv)/ (t x 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) [10].

All the data were statistically analysed at the treatment and replication levels, the least significant difference (LSD) values were calculated at 95% confidence limits (Panse and Sukhatme, 1967) [23].

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 *Asteracanthus* and tuber extract of safed musli.

Effect on T_{50} value (Table 2). Concomitantly, the leaf extract of *Asteracanthus* 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 15 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 *Asteracanthus* 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 is concerned, a clear reverse picture was noted. In *Asteracanthus* 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 45 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 *Asteracanthus* 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 *Asteracanthus* and tuber extract of safed musli on percentage germination of sunflower seeds stored under accelerated ageing condition for 45 days. Seeds were presoaked with leaf extract of *Asteracanthus* 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±2ºC temperature) in a desiccator. Data were recorded after 0, 15, 30 and 45 days of seed ageing.

Treatments	Percentage germination			
	Days after accelerated ageing			
	0	15	30	45
Asteracanthus	100	50.0	15.0	NA
Safed musli	100	51.0	18.0	5.0
Control	100	22.0	NA	NA
LSD (*P*=0.05)	NC	2.36	1.56	NC

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

Table 2: Effect of seed pretreatment with leaf extract of *Asteracanthus* and tuber extract of safed musli on time (hours) to 50% germination (T_{50}) of sunflower seeds stored under accelerated ageing condition for 45 days. Treatments are the same as in Table 1. Data were recorded after 0, 15, 30 and 45 days of seed ageing.

Treatments	T_{50} values of germination			
	Days after accelerated ageing			
	0	15	30	45
Asteracanthus	24.5	71.0	NA	NA
Safed musli	23.5	56.5	NA	NA
Control	24.6	NA	NA	NA
LSD (*P*=0.05)	NS	5.51	-	-

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

Table 3: Effect of seed pretreatment with leaf extract of *Asteracanthus* and tuber extract of safed musli on percentage TTC-stainability of sunflower seeds stored under accelerated ageing condition for 45 days. Treatments are the same as in Table 1. Data were recorded after 0, 15, 30 and 45 days of seed ageing.

Treatments	TTC stainability (%)			
	Days after accelerated ageing			
	0	15	30	45
Asteracanthus	100	56.0	21.0	9.0
Safed musli	100	65.0	28.0	12.0
Control	100	38.0	10.0	4.0
LSD (*P*=0.05)	NC	4.05	1.26	0.39

NC: Not calculated.

Table 4: Effect of seed pretreatment with leaf extract of *Asteracanthus* 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 45 days. Treatments are the same as in Table 1. Data were recorded from the seed kernels after 0, 15, 30 and 45 days of seed ageing.

Treatments	Protein (mg/g fr. wt.)				Amino acid (mg/g fr. wt.)			
	Days after accelerated ageing							
	0	15	30	45	0	15	30	45
Asteracanthus	161.5	115.8	103.7	89.3	11.11	13.57	14.67	21.12
Safed musli	162.3	138.4	122.4	123.2	11.09	13.22	16.14	19.08
Control	160.3	91.7	77.2	58.6	11.15	18.62	24.11	30.21
LSD (*P* = 0.05)	NS	8.93	7.53	6.01	NS	1.25	1.38	1.57

NS: Not significant.

Table 5: Effect of seed pretreatment with leaf extract of *Asteracanthus* 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 45 days. Treatments are the same as in Table 1. Data were recorded from the seed kernels after 0, 15, 30 and 45 days of seed ageing.

Treatments	Insoluble carbohydrates (mg/g fr. wt.)				Soluble carbohydrates (mg/g fr. wt.)			
	Days after accelerated ageing							
	0	15	30	45	0	15	30	45
Asteracanthus	168.6	157.6	128.6	117.7	21.3	42.4	60.6	74.5
Safed musli	168.2	163.2	139.1	128.7	22.6	31.9	45.3	55.6
Control	169.5	125.1	97.8	88.4	22.1	60.7	84.8	99.5
LSD (*P* = 0.05)	NS	11.21	8.97	7.81	1.93	2.75	4.49	5.47

NS: Not significant.

Table 6: Effect of seed pretreatment with leaf extract of *Asteracanthus* 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 45 days. Treatments are the same as in Table 1. Data were recorded from the seed kernels after 0, 15, 30 and 45 days of seed ageing.

Treatments	Dehydrogenase (ΔOD/g wet wt./5 ml)				Catalase (unit/h/g fr. wt.)			
	Days after accelerated ageing							
	0	15	30	45	0	15	30	45
Asteracanthus	0.46	0.32	0.26	0.20	101.4	82.2	45.5	37.5
Safed musli	0.48	0.37	0.26	0.21	102.2	87.9	57.3	40.9
Control	0.45	0.26	0.15	0.12	102.6	68.1	40.8	26.2
LSD (*P* = 0.05)	NS	0.03	0.03	0.02	NS	5.75	3.66	2.51

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 revealed that pretreatment of sunflower seeds with leaf extract of *Asterachanthus* 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 remembered to be the important visible measure for the evaluation of poor seed vigour (Anderson, 1970) [2], (Bhattacharjee *et al*., 2003) [4], (Bhattacharjee *et al*., 2005) [5]. In this investigation, the *Asterachanthus* 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 *Asterachanthus* 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 (Halder, 1984) [11], (Chhetri *et al*., 1993) [6]. Again, dehydrogenase activity can be regarded to be a certain index for evaluation of seed viability (Abdul-Baki and Anderson, 1972) [1], (Rai *et al*., 1995) [24]. The present data thus pinpoint that in spite of accelerated ageing, seed pretreatments with the leaf extract of *Asterachanthus* 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 dehydrogenase and catalase enzyme activities. The plant extracts significantly mitigated 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 (Rai *et al*., 1995) [24], (Kanp and Bhattacharjee, 2012) [14]. Higher activity of the H_2O_2 scavenger enzyme catalase (Rai *et al*., 1995) [24] was also shown in plants having higher potential and maintaining a vigorous growth. 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 much 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 *Asterachanthus* 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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