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# Prolongation of Seed Viability by Some Selected Plant Extracts

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## A B S T R A C T

Pretreatment of sunflower (*Helianthus annuus* L. cv. MRSF-1051) seeds with rhizome extract of mango ginger (*Curcuma amada* Roxb.) and leaf extracts of basil (*Ocimum sanctum* L.) and lemon (*Citrus media* L.) revealed that all the plant extracts significantly reduced the loss of germinability under accelerated ageing condition (99.5% RH, 30 ± 2°C) during 0, 15, 30 and 45 days. TTC (2,3,5-triphenyl tetrazolium chloride) stainability gradually decreased in seeds which underwent accelerated ageing, and partial alleviation in loss of stainability was noted in the seed samples pretreated with the plant extracts. The plant extract-induced changes of percentage seed germination and TTC staining were associated with a proportional shift in germinating seed metabolism. Substantial increase of amino acids and soluble carbohydrates in seed leachates with the progress of seed ageing was considerably checked by plant extracts. The plant extracts also more or less arrested the rapid loss of protein and RNA as well as the activities of dehydrogenase and catalase enzymes in seed kernels during the process of accelerated ageing. The beneficial effect of the plant extracts, particularly rhizome extract of mango ginger, on prolongation of seed viability of sunflower under accelerated ageing condition is discussed.

***Key words*:** Accelerated ageing, Basil, Germination %, Lemon, Mango ginger, Plant extract, Protein, RNA, Soluble carbohydrate, Sunflower seed

Sunflower seeds suffer from the drawback of retaining a sound vigour and viability at ambient storage conditions prevailing in West Bengal State of India where high temperature and high relative humidity (RH) impair seed health by accelerating the harmful physiological and biochemical processes associated with seed ageing phenomenon. These inevitable detrimental processes, caused by natural ageing (physiological deterioration) and/or by pathogenic attack (pathological deterioration) lead to make seeds nonviable causing problem to the crop growers. To get rid of this handicap, strategies are now being undertaken to improve the storage potential of seeds for enhancing their life span [1-2]. In the present investigation, an attempt is made to evaluate the efficacy of rhizome extract of mango ginger and leaf extracts of basil and lemon on enhancing the storage potential of sunflower seeds under accelerated ageing condition.

## MATERIALS AND METHODS

All experiments of the present investigation were carried out with freshly harvested, fully viable seeds of sunflower (*Helianthus annuus* cv. MRSF-1051). Plant extracts were prepared by thoroughly homogenizing 25 g rhizome of mango ginger, 25 g fresh leaf of basil and lemon in distilled water and subsequent straining of the homogenates using fine cloth followed by centrifugation of aqueous extracts. The total volume was made up to 500 ml with distilled water and these were taken as seed pretreating agents. After surface sterilization (0.1% $HgCl_2$ for 90 sec.) the seeds were separately presoaked with the rhizome extract of mango ginger and leaf extracts of basil and lemon or distilled water for 6 hours and then sundried. This was repeated twice and the seed samples were then allowed to experience accelerated ageing treatment in a desiccator in which 99.5% relative humidity (RH) was preimposed by keeping 250ml 1.57% $H_2SO_4$ [3]. This experimental set-up was kept under laboratory condition (30±2°C) for 45 days allowing the seeds to experience forced ageing treatment and $H_2SO_4$ was changed at 7-day intervals to restore the desired RH with in the desiccator for 45 days. Data were recorded after 0, 15, 30 and 45 days of seed ageing. To analyze percentage seed germination, the individual 100 seed samples in four lots (4×100 seeds) were transferred to Petri dishes containing filter paper moistened with 10 ml distilled water. Germination data were recorded following International Rules for Seed Testing [4]. Samples were taken from seeds which underwent accelerated ageing from zero to 45 days at 15-day intervals.

For analyzing TTC stainability four 100-seed samples of dehusked sunflower seeds from each ageing period were allowed to imbibe 0.5% TTC (2, 3, 5-triphenyl tetrazolium chloride) solution (w/v) in Petri dishes for 16 hours in dark condition. TTC stainability was analyzed from the embryonal axes of the seed samples aged for 0, 15, 30 and 45 days. To

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analyze leachable free amino acids level, 1 ml of the seed leachate and 3 ml of 0.1% ninhydrin solution (in 90% ethanol) were placed in test tubes (glass marbles at the top) and kept in a water bath for 15 minutes. When the reaction mixture turned to violet colour, the test tubes were taken out, cooled and the volume was completed to 4 ml 80% ethanol. The absorbance of the solution was measured at 580 nm by a spectrophotometer. The quantitative estimation was made by comparing the optical density (O.D.) values from a standard curve prepared from glycine. This method was adopted from Moore and Stein [5] after necessary modification. The soluble carbohydrate level was determined from the leachate stock following the colorimetric method of McCready *et al.* [6] using anthrone reagent. Actual content was evaluated from a previously prepared standard curve with glucose. Protein level was analysed from the seed kernels. Samples (100mg each) were crushed in a mortar with 80% ethanol and centrifuged at 6000*g* for 10 minutes. The residue was then solubilized with 0.5N NaOH at 80°C for 1 hour. A definite volume (4 ml) was made with the extraction medium. It was then estimated by reacting the protein solution with Folin-phenol reagent and measuring the O.D. values at 650 nm according to the method of Lowry *et al.* [7]. Extraction of RNA was made from 100mg seed kernels following the method described by Cherry [8] and quantitative estimation was done as per the method described by Markham [9], modified by Choudhuri and Chatterjee [10], based on a colorimetric method using orcinol reagent. RNA level was calculated from a standard curve prepared from yeast RNA.

To analyze dehydrogenase (total) activity, dehusked seeds of each treatment were immersed in 1% TTC solution in test-tubes and incubated for 12 hours in dark. The hydrogen atoms released by the total dehydrogenase enzymes which are involved in the respiration process of the living tissue, reduced tetrazolium to red coloured formazan [11]. This formazan, produced after incubation, was extracted with 10 ml of 2-methoxyethanol and O. D. values of the solutions were recorded at 520 nm. This method was adopted after Rudrapal and Basu [12] with slight modification. To analyze catalase activity 500 mg seed kernel of each sample was homogenized with 8 ml of chilled 0.1M phosphate ($Na_2HPO_4$/ $NaH_2PO_4$) buffer (pH 6.8). The homogenate was centrifuged at 3000 g for 20 minutes. The enzyme activity was determined following the method of Snell and Snell [13] modified by Biswas and Choudhuri [14]. For assaying of the enzymes, the blank was taken as zero-time control and the activity was expressed as $(\Delta A \times Tv)/(t \times v)$, where $\Delta A$ is the difference of the absorbance of the sample after incubation and 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 [15]. All the data mentioned above were analyzed at the replication and treatment level; the least significant difference (LSD) was calculated at 95% confidence limits [16].

## RESULTS AND DISCUSSION

*Effect on germinability and TTC stainability of seeds*

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 rhizome extract of mango ginger and leaf extracts of basil and lemon. 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 pretreatment with all the plant extracts was ameliorative with respect to retention of TTC staining. The effect of mango ginger was recorded to be most significant in this regard (Fig 1).

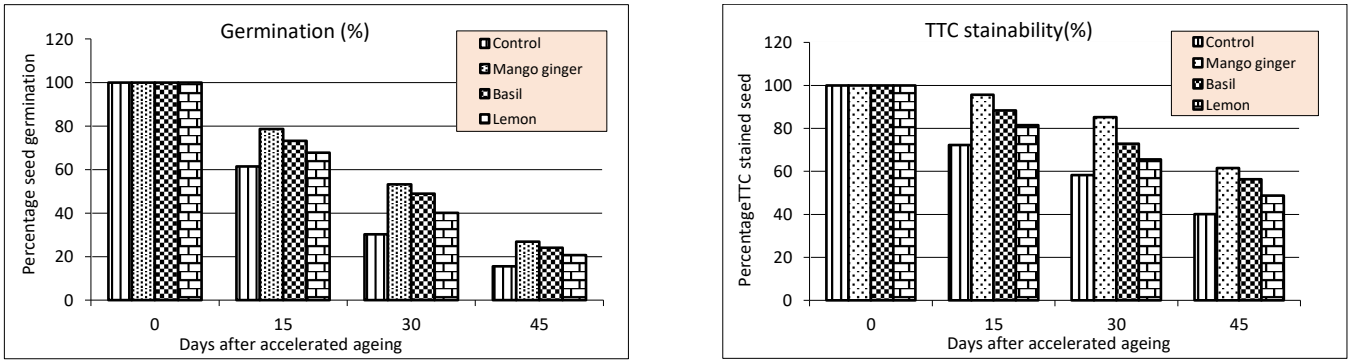


Fig 1 Effect of seed pretreatment with rhizome extract of mango ginger and leaf extracts of basil and lemon on seed germinability and TTC stainability of sunflower seeds stored under accelerated ageing condition for 45 days

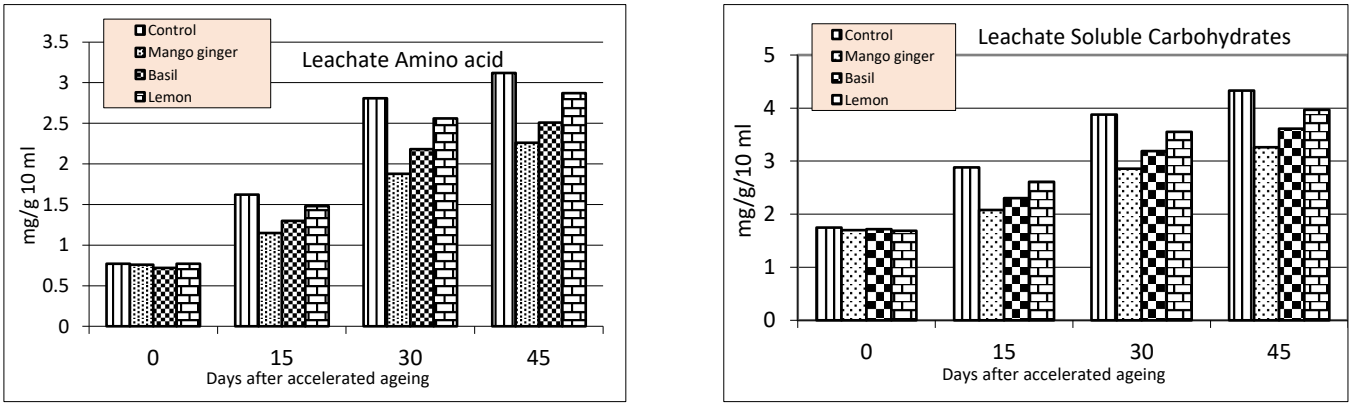


Fig 2 Effect of seed pretreatment with rhizome extract of mango ginger and leaf extracts of basil and lemon on leaching of amino acids and soluble carbohydrates from sunflower seeds stored under accelerated ageing condition for 45 days

*Effect on leaching of amino acids and soluble carbohydrates*

Leaching of amino acids and soluble carbohydrates went on increasing keeping pace with the ageing duration regardless of the samples analyzed. However, alarming increase of seed leaching was alleviated by seed pretreatments with all plant extracts and the effect was found significant at later observation periods (Fig 2).

*Effect on changes of protein and RNA levels in seed kernels*

Protein content in seed kernels gradually declined keeping pace with the period of accelerated ageing in control as well as in seed samples pretreated with plant extracts. But the plant extracts were found to maintain the protein level to a considerable extent at later periods of observations. Overall changing pattern of RNA level followed an identical trend that of protein (Fig 3).

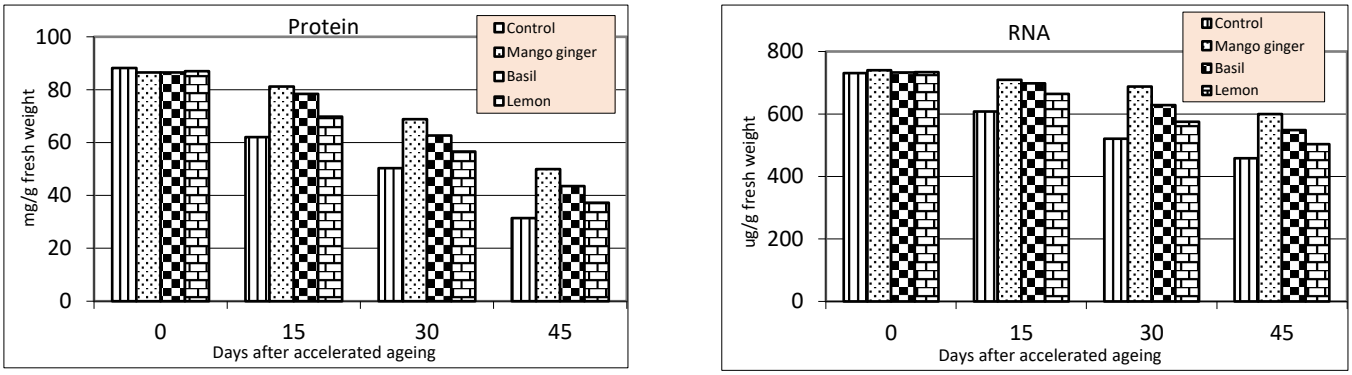


Fig 3 Effect of seed pretreatment with rhizome extract of mango ginger and leaf extracts of basil and lemon on protein and RNA contents in kernels of sunflower seeds stored under accelerated ageing condition for 45 days

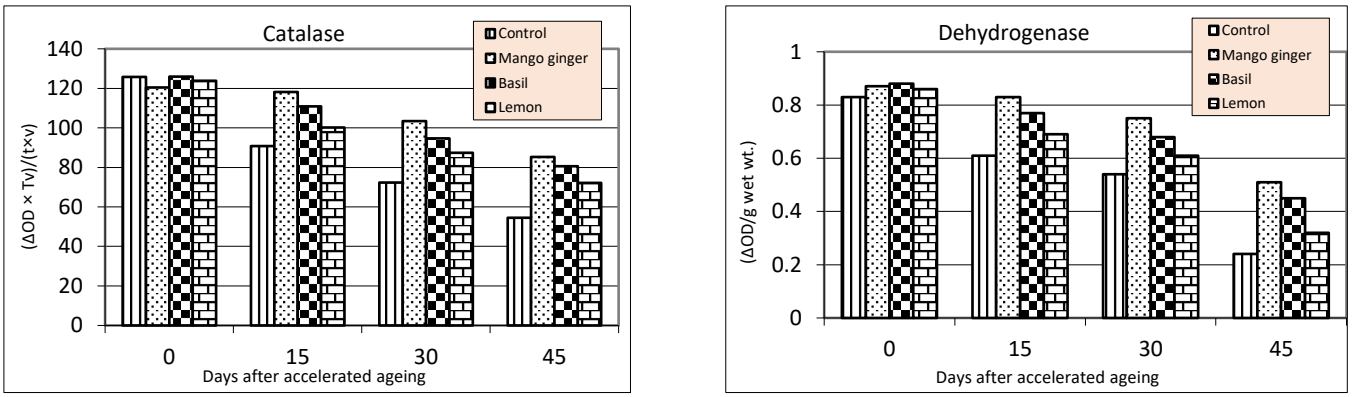


Fig 4 Effect of seed pretreatment with rhizome extract of mango ginger and leaf extracts of basil and lemon on the activities of catalase and dehydrogenase enzyme of sunflower seeds stored under accelerated ageing condition for 45 days

*Effect on changes of catalase and dehydrogenase activities in seed kernels*

Activities of the enzymes catalase and dehydrogenase declined with seed ageing process from zero to 45 days both in control and plant extracts pretreatment seed samples. However, the rate of decreasing in activity was found to occur slowly in seeds which received pretreatment with rhizome extract of mango ginger and leaf extracts of basil and lemon (Fig 4).

Seed deterioration is a natural catabolic process which terminates their life span owing to serious impairment of seed viability. This process may be accelerated by some pathogenic attack and/or by adverse environmental condition. This inevitable deterioration of seeds is a matter of serious concern to the seed physiologists, and various strategies are developed to prolong the storage potential of seeds.

The present investigation shows that the pretreatment of sunflower seeds with the test plant extracts reduce the loss of germinability under accelerated ageing environment. Reduced germinability is considered to be the important visible criteria for the evaluation of poor seed vigour [17]. The efficacy of plant extracts of these three plant materials in maintaining storage potential of sunflower seeds can also be substantiated from TTC stainability in the seed samples which progressively declined with the advancement of accelerated ageing process. Seed pretreatment with the plant extracts partially averted the loss of stainability and consequently the seed vigour (Fig 1). This result is in corformity with the observation of Halder [18] who noted that seed ageing is associated with loss of vigour which is determined by counting the percentage TTC stained as well as low intensity of staining.

Damage of seed membrane during accelerated ageing is evident from the higher leaching of amino acids and soluble carbohydrates. Arrestation of leaching of these soluble substances by the pretreating plant extracts (Fig 2) indicates that they checked the damage of membrane which consequently relieved the deleterious effect of rapid leaching. Membrane is the most important site of a seed which appears to be affected first by any accelerated ageing treatment [19-20] and arguably any chemical purported to have an effect on seed viability must influence membrane integrity. The proposal that a decrease in membrane integrity and occurrence of membrane lesions might play a significant role in the deterioration of seeds has been supported by work on soluble leaching accompanying a fall in germinability and viability [21-22]. Francis and Coolbear [23] reported that under accelerated ageing condition, concomitant with the fall of germination, there is a decline in phospholipid and phosphatidyl choline which are important membrane components. The supporting references presented in membrane integrity and loss of seed storability, thus, indicate that the ageing-induced damage of sunflower seeds, as observed in this investigation, might possibly be a reason for their rapid loss of viability during storage. The significantly lesser leaching of soluble carbohydrates and amino acids from the pretreated seeds proved that the plant extracts of mango ginger, basil and

lemon rendered the seeds tolerant against storage deterioration by retaining the integrity of sunflower seed membrane.

Along with the changes in leaching of soluble substances during ageing, a proportional shift in metabolism of the germinating sunflower seeds was observed in the seed kernels. The efficacy of the plant extracts on maintenance of seed health can also be strongly supported from such biochemical data. Results showed that the level of protein and RNA (Fig 3), as well as activity of the enzymes catalase and dehydrogenase (Fig 4) gradually declined in control sample with ageing duration and this trend was considerably slowed down by the pretreating plant extracts. The results, therefore, point out that although deterioration is a common phenomenon in treated and control samples of sunflower seeds; the catabolic processes within the first one remained somewhat subdued, thereby rendering them tolerant against unfavourable storage environments. Available reports show that during seed ageing a loss of some vital cellular components occur [24-25] with concomitant increase of soluble substances such as amino acids and carbohydrates and decrease of nucleic acids [19-26]. Catalase and dehydrogenase activities are generally used as very reliable indices for the evaluation of seed viability. High level of dehydrogenase activity in high vigour seeds has also been reported [26]. So, from the present observations of high metabolic status of the plant extract-pretreated sunflower seeds, it seems quite apparent that the tested plant extracts considerably hardened the seeds and such hardening is affected at the metabolic level which subsequently resulted in retention of seed vigour.

## CONCLUSION

The results of this investigation can point out that seed pretreatment with rhizome extract of mango ginger and leaf extracts of basil and lemon can potentially alleviate storage deterioration of the present sunflower cultivar. And the promising responses of the investigation were recorded in the following order: mango ginger followed by basil and lemon.

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