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SHORT COMMUNICATION

Prolongation of seed viability under storage

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ABSTRACT

Keeping in mind the problem of seed storing in tropical and subtropical countries where high temperature and high relative humidity (RH) greatly accelerate seed ageing phenomenon causing consequent deterioration and non-viability of seeds, an investigation was carried out on maintenance of storage potentiation of a green gram species by using selected plant extract. Pretreatment of green gram (*Phaseolous mungo* L.) seeds with aqueous solution of leaf extract of neem (*Melia azadarch*) 50g in 500 ml distilled water for 2 hours and then dried back to the original dry weight of the seeds before accelerated ageing treatment (99.1% RH and 32±2 ºC) for different durations (0 to 30 days) slowed down the rapid loss of germination and reduced the time (h) required for 50% seed germination (T_{50}) of the seeds. The plant extract also significantly arrested the reduction of protein, insoluble carbohydrate, DNA and RNA levels as well as activity of catalase enzyme of seed kernels during forced ageing period. Ageing-induced stimulation of the activity of amylase enzyme was also alleviated by the seed pretreating agent.

***Keywords*:** Green gram, *Melia azadarch*, *Phaseolous mungo*, seed potentiation, seed viability, accelerated ageing

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1. INTRODUCTION

Deterioration of seed is a natural catabolic process which results in serious impairment of seed viability and consequent termination of life span. This process may be accelerated by some pathogenic attack and/or by adverse environmental conditions. Maintenance of vigour and viability of seeds in tropical and subtropical countries like India is a matter of serious concern to the crop growers because of high temperature and high relative humidity (RH) prevailing in major parts of the country almost throughout the year. These two environmental factors strongly impair seed and seedling health and cause to reduce percent seed germinability and seedling performance at a rapid rate [1-2].

Modern agriculture, with its bias for technology and precision, demands that seeds should be of high vigour and can germinate expeditiously to produce vigorous seedlings and healthy plants for ensuring higher productivity. Post-harvest storage of seeds under ambient agroclimatic conditions prevailing in West Bengal state of India is a matter of serious concern to agriculturists because high temperature and high relative humidity (RH) impair seed health by accelerating the harmful physiological and biochemical processes associated with seed ageing phenomenon. It has been reported that simply by using standard vigour seeds, yield could be remarkably increased [3-5]. Keeping in mind the problem of seed storing, an investigation was carried out on prolongation of seed viability of a green gram species with leaf extract of neem (*Melia azadarch*) under storage.

Experiments of this investigation were carried out under accelerated ageing condition to obtain more or less uniform and expeditious results. In fact, accelerated ageing treatment, as imposed by high temperature and high relative humidity (RH), provide a powerful tool for studying the process of seed deterioration over a very short period and this mimics the natural ageing process [6-9]. Thus, the prime objective of this work was to probe the efficacy of the test leaf extract on enhancement of seed potential of green gram species by analysing germination behavior and metabolic status of seeds.

2. MATERIALS AND METHODS

Experiments of this investigation were carried out with freshly harvested healthy seeds of green gram (*Phaseolous mungo* L.).After surface sterilization (0.1% $HgCl_2$ for 90 seconds) the seed sample was presoaked in the aqueous leaf extract of neem (*Melia azadarch*) 50g/500ml or distilled water for 2 hours (1+1 h in two installments) and then dried back to the original dry weight of the seeds. This was repeated twice allowing maximum penetration of the chemicals present in the aqueous solution. The pretreated seed lot was taken and then stored in a desiccator in which 99.1% relative humidity (RH) was preimposed by keeping 3.03% H_2SO_4 within it. This experimental set up was kept at 32±2 ºC for 30 days allowing the seeds to experience forced ageing treatment and H_2SO_4 was changed at 15 day intervals to restore the desired RH within the desiccator for 30 days.

Data on germination behaviour and metabolism of seeds were analysed after 0, 15 and 30 days of accelerated ageing. Analysis of seed potential, measured in terms of some reliable biochemical variables were critically analysed from samples grown from 0, 15 and 30 days of accelerated ageing seeds.

2. 1. Analyses of germination behaviour of seeds after accelerated ageing

Percentage seed germination: To analyse the percentage germination 100 seeds were transferred to separate Petri dishes containing filter paper moistened with distilled water. Germination data were recorded after 120 h of seed soaking following the International Rules for Seed Testing [10].

T_{50} of seed germination: The time for 50% germination of seeds (T_{50}) was determined following the method described by Coolbear *et al.* [11].

2. 2. Analyses of biochemical changes of seeds after accelerated ageing

Protein from seed kernels: Hundred mg samples from seed kernel of each treatment were taken and homogenized with 0.1M NaOH solution. The protein was solubilized by treating with 0.1M NaOH at 80 ºC for 1 h. After boiling the sample was cooled and centrifuged at 6000g for 10 min. Then supernatant was taken and a definite volume (10 ml) was made with the extraction medium. It was then estimated by reacting the protein solution with folin phenol reagent and subsequent measuring of the OD values at 650nm as per the method of Lowry *et al*. [12].

Insoluble carbohydrates from seed kernels: To estimate insoluble carbohydrate, the residue of soluble carbohydrates in the centrifuge tube was extracted by using 25% H_2SO_4 and this was boiled at 80 ºC for 30 min. Estimation and quantification of insoluble carbohydrates was done following the methods of McCready *et al*. [13].

Nucleic acids from seed kernel: Extraction of nucleic acids (both DNA and RNA) was made from 100mg seed kernels following the method described by Cherry [14]. The levels of DNA and RNA were estimated from a common stock employing the method of Markham modified by Choudhuri and Chatterjee [15-16].

Catalase activity from seed kernels: To analyse catalase activity 500 mg seed kernels of each sample was taken. Extraction and estimation was done as per the method Snell and Snell modified by Biswas and Choudhuri [17-18].

Amylase activity from seed kernels: Activity of the enzyme amylase was done following the method described by Khan and Faust with necessary modification, 500 mg of each sample from seed kernels was homogenized with 10 ml 0.1M phosphate buffer (pH 6.5). The homogenate was centrifuged at 5000*g* for 10 minutes. The supernatant was taken as the crude source of the enzyme [19].

For assaying the enzyme, a blank was taken as zero time control and the activity was expressed as $(\Delta OD \times T_V)/(txv)$, where ΔOD is the difference of OD of the blank and sample, T_V is the total volume of the filtrate, t is the time (min) of incubation with the substrate and v is the volume of filtrate taken for incubation [20].

2. 3. Statistical Analysis

Data were statistically analysed at the treatment and replication levels and least significant difference (LSD) values were calculated at 95% confidence limits [21].

3. RESULTS AND DISCUSSION

Seed senescence or seed deterioration under ambient storage conditions is an internal programmed 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 specific seed type. Like plant senescence the process of seed senescence is complicated and the pattern varies with the broad classes of seeds like orthodox and recalcitrant seeds and even with the seed species of different plant taxa [22-24]. Accelerated ageing treatment, as imposed by high temperature and high relative humidity (RH) provide a powerful tool for studying the process of seed deterioration over a very short period and this mimics the natural ageing process [25-29]. The results of the present study showed that high RH treatment accelerated the ageing and deterioration of green gram seeds as would be evident from the progressive fall of germination percentage and higher T_{50} hours.

Table 1. Effect of seed pretreatment with leaf extract of *Melia azadarch* (50 g in 500 ml of water) on percentage seed germination and T_{50} value of green gram seeds.

Seeds were presoaked with the aqueous solution of the plant extract or distilled water for 2h and then dried back to original seed weight. This was repeated twice. Pretreated seed samples were kept under 99.1% RH and data were recorded after zero (0), 15 and 30 days of accelerated ageing.

Seed sample	Treatment	Percentage seed germination			T_{50} of germination		
		Days after accelerated ageing					
		0	15	30	0	15	30
Green gram	Control	100	72	41	12	30	NA
	Melia sp.	100	78	56	12	24	78
	LSD (P = 0.05)	NC	1.25	3.10	NC	2.01	5.12

NC: Not calculated; NA: Non attainment of 50% germination.

Table 2. Effect of seed pretreatment with leaf extract of *Melia azadarch and* (50 g in 500 ml of water) on protein (mg/g fr. wt.) and insoluble carbohydrates (mg/g fr. wt.) levels of green gram seeds.

Treatments and recording of data as in Table 1.

Seed samples	Treatments	Protein			Insoluble carbohydrates		
		Days after accelerated ageing					
		0	15	30	0	15	30
Green gram	Control	70.10	50.11	21.96	34.10	18.50	10.19
	Melia sp.	70.15	58.82	33.11	34.16	30.19	27.07
	LSD (P = 0.05)	NS	4.20	2.17	NS	1.01	1.08

NS: Not significant.

Table 3. Effect of seed pretreatment with leaf extract of *Melia azadarch* (50 g in 500 ml of water) on DNA (mg/g fr. wt.) and RNA (mg/g fr.wt.) levels of green gram seeds.

Treatments and recording of data as in Table 1.

Seed samples	Treatments	DNA			RNA		
		Days after accelerated ageing					
		0	15	30	0	15	30
Green gram	Control	40.9	45.5	28.2	120.0	70.9	30.6
	Melia sp.	40.7	49.2	35.2	120.0	88.2	49.9
	LSD (P = 0.05)	NS	4.05	2.25	NS	6.50	2.20

NS: Not significant.

Table 4. Effect of seed pretreatment with leaf extract of *Melia azadarch* (50 g in 500 ml of water) on activities of enzyme catalase (ΔODxTv/txv) and amylase (ΔODxTv/txv) of green gram seeds.

Treatments and recording of data as in Table 1.

Seed samples	Treatments	Catalase			Amylase		
		Days after accelerated ageing					
		0	15	30	0	15	30
Green gram	Control	42.0	29.0	18.8	38.9	48.9	69.2
	Melia sp.	42.1	38.1	32.2	38.0	41.2	50.0
	LSD (P = 0.05)	NS	1.17	1.50	NS	3.28	4.39

NS: Not significant.

However, the pretreatment of the seed species with leaf extract of neem (*Melia azadarch*) 50g in 500 ml distilled water significantly alleviated the ageing-induced loss of germination and reduced T_{50} hours (Table 1), alleviated the loss of protein and insoluble carbohydrates (Table 2), nucleic acids (Table 3) and catalase enzyme (Table 4). The pretreating agents also alleviated the ageing-induced rapid increase of the activity of amylase in seed kernels (Table 4). Metabolic status of the seeds during accelerated ageing was altered as evident from the declining of some important macromolecules within seeds such as protein, insoluble carbohydrates, nucleic acids (both DNA and RNA), activities of catalase and amylase. However, the magnitude of reduction was much less in the herbal pretreated seed samples. On the other hand, protein, insoluble carbohydrates, and activity of catalase level progressively deteriorated in seeds up to 30 days of their ageing and such declining trend was arrested in seeds which received pretreatment with leaf extract of neem (*Melia azadarch*).

The proposal that a decrease in membrane lesions might play a significant role in deterioration of seeds has been supported by the work on solute leakage accompanying a loss

in germinability and viability [30-33]. The ability of seeds to recognize its membrane rapidly as the desiccated tissue rehydrates is a crucial factor for successful germination and this is clearly documented in the literature [34].

Thus, in the present study ageing-induced reduction of seed germinability is indicative of damage of seed membrane and consequent loss of seed vigour and viability. The plant extract-induced substantial amelioration of all the deleterious effects are indicative of seed potentiation under adverse storage environment. Available reports show that during seed ageing a loss of some vital cellular components including protein, carbohydrates, nucleic acids and enzymes like catalase, amylase etc. occurred [35-37]. In this investigation, the plant extract-induced arrestation of rapid loss of the enzyme activity is indicative of strengthening the defense mechanism by the herbal extract under adverse storage condition. The results therefore point out that although deterioration is a common phenomenon in treated and control samples of the seed species, the catabolic processes within the treated seed sample remained somewhat subdued, thereby rendering them tolerant against unfavorable storage environment.

4. CONCLUSIONS

It can be concluded that the experimental leaf extract is most effective in enhancing storage potential of green gram seeds. If this apparent beneficial action of leaf extract of neem (*Melia azadarch*) is established in future researches from a wide range of crop seeds, the practice of conventional methods of seed storing may be suitably substituted by the experimental herbal agent.

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