Journal of Medicinal Plants Research Vol. 5(17), pp. 4108-4111, 9 September, 2011
Available online at http://www.academicjournals.org/JMPR
ISSN 1996-0875 ©2011 Academic Journals

Full Length Research Paper

Alleviation of seed deterioration and enhancement of plant potential of a pea species using selected medicinal plant extracts

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Accepted 18 July, 2011

Pea (*Pisum sativum* L. cv. Azad P1) seeds lost viability at a rapid pace under accelerated ageing condition. Pretreatment of the seeds with leaf extracts of bel (*Aegel marmelos*) and kalmegh (*Andrographis paniculata*) 50g in 250ml distilled water of each for 6 hours before accelerated ageing treatment (100% RH and 30±2°C) for different durations for 45 days under the accelerated ageing condition slowed down the ageing-induced rapid loss of seed germination. Plant performance was found to be much better when they were developed from seeds which underwent plant extract pretreatments. This was measured in terms of field emergence capacity, root length, shoot length, fresh weight and dry weight of the plants. Again, plant potential was also higher in the pretreatments as evidenced from the treatment-induced higher chlorophyll and protein levels as well as activities of catalase and peroxidase enzymes. Results, therefore, pointed out that in spite of experiencing accelerated ageing treatment, the plant extract-pretreated seeds retained higher seed vigour and produced healthier plants.

Key words: Pea, accelerated ageing, bel, kalmegh, seed germination, plant potentiation.

INTRODUCTION

Seed storage is a serious problem in tropical and subtropical countries like India where high temperature and high relative humidity greatly accelerate seed ageing phenomenon causing consequent deterioration and non-viability of seeds. The problem of retention of seed vigour in India is much more acute because of extremely high relative humidity prevailing during the major part of the year and 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 standard seed vigour under ambient storage environment.

Keeping this problem of seed germination, storage and production of healthier plants in mind, an attempt was made in this investigation for the retention of the seed viability and enhancement of plant potential of a pea seed species having viability problems. Present experiment was performed under accelerated ageing condition by imposing high relative humidity with a view to maintaining the uniform adverse storage condition and also to obtain expeditious results. In fact, accelerated ageing treatment provides a powerful manipulative tool which makes it possible to study the process of seed deterioration over a very short period and this mimics the natural ageing process (Heydecker, 1972).

Although efficacy of several classes of chemicals viz; hormones, retardants, redox chemicals, phenols, vitamins and salts on maintenance of seed health under storage has been established (Bhattacharyya and Basu, 1990; Chhetri et al.,1993; Basu, 1994; Rai, 2000), this field of seed physiology still remains relatively less explored. Thus, the major objective of this work was to test the efficacy of a leaf extracts of bel (*Aegel marmelos*) and kalmegh (*Andrographis paniculata*) on the alleviation of deterioration and viability retention and enhancement of plant potential of a pea seed species.

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Table 1. Effect of seed pretreatment with leaf extracts of bel and kalmegh (50 g in 500 ml of water of each) on germination and field emergence capacity of pea seeds stored under accelerated ageing condition for 45 days. Seeds were presoaked with leaf extracts of bel and kalmegh or distilled water for 6 h and then sun dried. The seed samples were then separately allowed to experience accelerated ageing treatment (100% RH) in a desiccator. Data were recorded after 0, 15, 30 and 45 days of accelerated ageing seeds.

Treatments	Accelerated ageing (days)							
	Percentage germination					Field emergence capacity (%)		
	0	15	30	45	0	15	30	45
Control	100	89.0	77.5	39.10	92.9	77.5	64.4	31.0
Bel	100	95.7	87.3	52.20	94.2	84.1	76.0	46.0
Kalmegh	100	93.4	84.9	51.12	93.2	80.2	75.1	43.4
LSD (P=0.05)	NC	3.15	5.21	2.03	NS	5.80	4.53	2.22

NC: Not calculated, NS: Not significant.

MATERIALS AND METHODS

After surface sterilization (0.1% $HgCl_2$ for 90 s) the seeds of pea (*Pisum sativum* L. cv. Azad P1) were separately presoaked in the aqueous solutions of leaf extracts of bel (*A. marmelos*) and kalmegh (*A. paniculata*) 50 g in 250 ml distilled water of each for 6 h and then dried back to the original dry weight of the seeds. The pretreated seed lots (250 g each) were taken in separate porous cloth bags and thus stored in a desiccator in which 100% relative humidity (RH) was preimposed by keeping distilled water within it. This experimental set-up was kept at 30±2°C for 45 days allowing the seeds to experience forced ageing treatment and distilled water was changed at 15-day intervals to restore the desired RH within the desiccators for 45 days.

From the seed lots germinability and field emergence capacity of seeds were made after 0, 15, 30 and 45 days of accelerated ageing treatment.

To analyse the percentage germination, four groups of 100 seeds (total 400 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 (ISTA, 1976) and field emergence capacity was recorded after 15 days of seed sowing. Some growth and biochemical parameters were recorded from the leaves of the 30 and 60 days old plants raised from the 0 and 45 days of accelerated ageing seeds.

Extraction and estimation of chlorophyll and protein from leaves were done by the method of Arnon (1949) and Lowry et al. (1951), respectively. Activity of catalase was analysed following the method of Snell and Snell (1971) as modified by Biswas and Choudhuri (1978) and that of peroxidase was analysed as per the method of Kar and Mishra (1976).The assaying of the enzymes were done as per the methods of Fick and Qualset (1975).

Statistical analysis of the data was done in terms of least significant difference (LSD) which was calculated at 95% confidence limits and as per the method of Panse and Sukhatme (1967).

RESULTS AND DISCUSSION

Results showed that pretreatment of pea seeds with bel and kalmegh leaf extracts significantly alleviated the ageing-induced loss of germination and enhanced field emergence capacity under accelerated ageing environment (Table 1). Reduced seed germinability and field emergence capacity are considered to be the important visible criteria for the evaluation of poor seed vigour (Anderson, 1970; Halder et al., 1983; Rai, 2000). In this investigation, the plant extracts induced arrestation of loss of seed germination and field emergence capacity are indicative of retention of seed viability property of the experimental medicinal plant extracts.

Accelerated ageing treatment impaired field performance of experimental plants as evident from the reduction of root length and shoot length (Table 2), fresh weight and dry weight (Table 3) levels of chlorophyll and protein (Table 4) as well as activities of catalase and peroxidase enzymes (Table 5). The plant extracts-induced alleviation of the deleterious effects of ageing on the overall growth and metabolism of experimental plant thus indicates the retention of potential status of the experimental plants by leaf extracts used in this experiment.

Chlorophyll, protein, catalase and peroxidase are regarded as reliable indices of vigour status of plants. In this investigation, comparatively better plant health and higher metabolic status of plants, raised from the plant extracts treated seeds, are indicative of invigouration of seeds under storage. The invigourated seeds subsequently exhibited better field performance which was recorded in terms of plant growth and metabolism. Superior performance of plants raised from high vigour seeds is available in the literature (Rai, 2000). In this investigation, herbal-induced retention of seed viability, plant growth and metabolism clearly indicate the hardening or invigouration property of the pretreating agents and such hardening effect on seed was reflected in plant growth and metabolism. In fact, the magnitude of the loss of the chlorophyll and protein (Table 4) as well as catalase and peroxidase (Table 5) activities were found to be significantly less in plants developed from seeds which underwent pretreatment with the leaf extracts of the bel and kalmegh plants.

Loss of some vital cellular components occurred during the process of seed deterioration are available in

Table 2. Effect of seed pretreatment with leaf extracts of bel and kalmegh (50 g in 500 ml of water of each) followed by accelerated ageing treatment for 45 days on changes of root length and shoot length of pea plants. Data were recorded from 30 and 60 days old uniformly grown plants raised from the 0 and 45 days of accelerated ageing seeds.

Treatments	Root length (cm)				Shoot length (cm)			
	Plants age (days)							
	30		60		30		60	
	Accelerated ageing (days)							
	0	45	0	45	0	45	0	45
Control	5.15	1.23	15.11	1.29	37.92	12.91	77.82	18.31
Bel	7.92	2.93	18.99	3.10	59.30	14.61	98.27	21.54
Kalmegh	7.52	2.09	18.39	2.99	57.63	15.71	97.67	20.51
LSD (P=0.05)	2.21	0.64	1.83	1.17	1.73	1.17	5.22	1.81

Table 3. Effect of seed pretreatment with leaf extracts of bel and kalmegh (50 g in 500 ml of water of each) followed by accelerated ageing treatment for 45 days on changes of fresh weight and dry weight of pea plants. Data were recorded from 30 and 60 days old uniformly grown plants raised from the 0 and 45 days of accelerated ageing seeds.

Treatments	Fresh weight (g)				Dry weight (g)			
	Plants age (days)							
	30		60		30		60	
	Accelerated ageing (days)							
	0	45	0	45	0	45	0	45
Control	26.3	09.5	298.9	10.0	5.7	0.97	94.4	1.8
Bel	31.7	10.8	367.7	19.3	7.0	1.9	118.3	2.6
Kalmegh	30.2	10.3	354.6	16.1	6.8	1.3	113.1	2.3
LSD (P=0.05)	2.55	0.83	26.76	2.08	1.15	0.63	7.16	0.73

Table 4. Effect of seed pretreatment with leaf extracts of bel and kalmegh (50 g in 500 ml of water of each) followed by accelerated ageing treatment for 45 days on changes of chlorophyll and protein contents in leaves of pea plants. Data were recorded from 30 and 60 days old uniformly grown plants raised from the 0 and 45 days of accelerated ageing seeds.

Treatments	Chlorophyll (mg/g fr. wt.)				Protein (mg/g fr. wt.)			
	Plants age (days)							
	30		60		30		60	
	Accelerated ageing (days)							
	0	45	0	45	0	45	0	45
Control	2.33	1.06	3.87	0.89	17.49	14.25	28.25	16.32
Bel	3.43	1.29	5.20	1.24	21.37	19.89	47.60	22.33
Kalmegh	3.37	1.18	5.10	1.13	20.22	18.51	45.52	21.63
LSD (P=0.05)	0.68	0.17	0.13	0.19	1.60	1.15	1.83	1.66

literature (Abdul-Baki and Anderson, 1972; Kole and Gupta, 1982; Rao et al., 2003). Catalase (Abdul-Baki and Anderson, 1972; Yadav et al., 2003) and peroxidase (Bhattacharjee and Choudhuri, 1986; Yadav et al., 2003) activities are generally used as very reliable indices for the evaluation of seed viability. High level of catalase activity in high vigour seeds have also been reported (Bhattacharjee et al., 1999; Pati, 2007). So, from the present observations of higher metabolic status of the leaf extracts of bel (*A. marmelos*) and kalmegh (*A. paniculata*) pretreated pea seeds, it seems quite apparent that the seed pretreating agents considerably hardened the seeds and such hardening is effected at the metabolic level which subsequently resulted in retention of seed vigour and consequent extension of seed viability with concomitant enhancement of plant potential.

Table 5. Effect of seed pretreatment with leaf extracts of bel and kalmegh (50 g in 500 ml of water of each) followed by accelerated ageing treatment for 45 days on changes of catalase and peroxidase activities in leaves of pea plants. Data were recorded from 30 and 60 days old uniformly grown plants raised from the 0 and 45 days of accelerated ageing seeds.

Treatments	Catalase (unit/h/g fr. wt.)				Peroxidase (unit/h/g fr. wt.)			
	Plants age (days)							
	30		60		30		60	
	Accelerated ageing (days)							
	0	45	0	45	0	45	0	45
Control	33.4	10.9	31.6	11.7	53.8	29.7	50.9	17.9
Bel	48.3	12.6	45.2	12.2	68.2	34.6	66.7	21.4
Kalmegh	43.0	12.3	42.0	12.0	66.1	28.5	64.6	19.7
LSD (P=0.05)	2.01	1.11	1.40	1.43	1.08	1.17	3.05	1.19

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