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PRE-STORAGE DRY AND WET SEED TREAMENTS ON VIGOUR, VIABILITY AND FIELD PERFORMANCE OF STORED FIELD PEA (*Pisum sativum L.*)

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ABSTRACT

The efficacy of pre storage dry dressing treatments with different chemicals viz.calcium carbonate @ 2g/kg, iodinated calcium carbonate @ 2 g/kg , bleaching powder @ 2g/kg of seed ,pharmaceutical product viz. aspirin @ 50 mg/kg of seed , crude plant materials viz. red chilli powder @ 1 g/kg , amla fruit powder @ 2 g/kg of seed and wet treatments on the maintenance of storability and field performance of stored field pea seeds were evaluated under accelerated ageing conditions. It was observed that after 24 days of accelerated ageing at 98% R.H. and 40^{0}c temperature, seeds treated with redchilli powder and amla fruit powder proved to have better germinability and vigour index over untreated control . Pre storage soaking drying treatment did not show any encouraging results in improving storability and field performance might be due to soaking injury. Field performance specially in terms of yield of the dry dressed bottle stored (8 months) seeds especially with red chilli powderand amla fruit powder was significantly higher. Biochemical studies revealed reduced leakage of electrolytes, free soluble sugars and free amino acids and increased dehydrogenase enzyme activity of the treated seeds over untreated control. Based on the results, the use of crude plant materials like red chliili powder and amla fruit powder may be suggested for effective seed storage ,field performance and productivity of field pea.

INTRODUCTION

Field pea (*Pisum sativum var arvense*) under the family fabaceae is a self pollinated cool season crop. Field pea is primarily used for human consumption or as a livestock feed and have great nutritional importance due to high content of protein, complex carbohydrates, dietary fiber, minerals, vitamins and antioxidant compound. It thrives well in places with cool climate and hence is grown in almost all the temperate region of the world. In tropical countries it is grown during the cold months. The major producing countries of field pea are Russia, China followed by Canada, Europe, Australia and United States. In India it is mainly grown in Uttarpradesh, Bihar, Madhyapradesh and Maharastra.

Good quality vigorous seed is a prime parameter behind a successful harvest. Farmers of our country mostly have to depend on their own stored seed for cultivation in the ensuing season. But due to erratic atmospheric condition like high humidity, rainfall and temperature prevailing in most of the field pea growing areas of the country accompanied with poor household storing facilities poses a great concern to the health of the stored field pea seed which in turn affect the germinability and the ultimate field performance of those seeds.

Two important factor which determine the rate of ageing are temperature and moisture content at which seeds are stored (walter 1998). Basu and his coworker reported that pre-storage dry treatment and mid-storage hydration-dehydration treatments effectively slowdown the seed deterioration of many leguminous and non-leguminous crop under subsequent storing condition (Basu,1976; Mandal and Basu,1983; Mandal *et al.*, 2000). Esurnoso (2010) reported that redchilli powder and trigonella seed powder each at 1 g/kg of seed showed significant improvement in post storage germinability as well as yield of kenaf crops over untreated control. Dry dressing treatments of high vigour leguminous and non-leguminous crop seeds with halogenated compounds such as bleaching powder and iodinated calcium carbonted greatly slow down the seed deterioration under ambient storage condition (Basu and Rudra pal, 1980; Mandal and Basu, 1986).

Keeping the above references in mind the present study was taken up to standardize a suitable eco-friendly and an affordable but effective seed invigoration treatment for the maintenance of germinability and field performances of stored field pea seed.

MATERIALS AND METHODS

Field pea cultivar Rachna was procured from Agricultural Experimental Farm of Calcutta University and sundried properly to a safe moisture content (9%) before storing in rubber stoppered glass bottle until the treatments were carried out. In this experiment 250 g of seeds were taken for each treatment.

Harvest fresh (15 days old) field pea seeds were dry dressed with crude plant materials like red chilli powder (active ingradient capsicin) @ 1g/kg and amla (active ingradient phyllemblin) @ 2g/kg, chemicals like calcium carbonate @ 2g/kg, iodinated calcium carbonated @ 2 gm/kg, bleaching powder @ 2g/kg of seed and pharmaceutical product viz. aspirin @ 50 mg/kg of seed in the rubber stoppered glass bottles under ambient condition. Wet treatments like Soaking for 2 hours followed by drying, Moist sand conditioning for 24 hours followed by drying and moist sand conditioning for 24 hours followed by soaking 2 hours then drying back to its original moisture content were also give at the same time. In case of wet treatments, seeds were kept in the over fused calcium chloride for 7 days to stabilize moisture content to a low and uniform level and stored in rubber stoppered glass bottle. After treatments, all the bottles were shaked twice a day for seven days for uniform mixing of chemicals, crude plants materials and pharmaceutical products with the seeds.

Germination test of both treated and untreated seeds were conducted after 10 days of treatment by taking 200 seed with 3 replications following the method of Punjabi and Basu (1982) with minor modification. Data on germination percentage, root length and shoot length were recorded after 7 days 20 $\pm$ 1°C.

After 2 months of bottle storage, equal number of seeds from each treatment were taken separately in perforated paper packet containing equal number of holes and kept in a glass dessicator for accelerated ageing at 98% relative humidity and 40°c temperature for 24 days and then germination test were performed following the above mentioned method.

Biochemical analysis like leaching of electrolytes, free soluble sugar, free amino acid and dehydrogenase enzyme activity of both treated and untreated seeds were carried out immediately after treatment (after 10 days) and another after having accelerated ageing for 24 days at 98% R.H. and 40°c temperature.

Membrane permeability of seed and amount of free soluble sugar was measured following the method of Anderson *et al.* (1964) and Mc. Cready *et al.* (1950) respectively. Four field pea seeds from each treatment including control were soaked in 30 ml of distilled water in separate test tube for 30 minutes at 30°c. Seed steep water was used to measure the elrectrical conductivity in systronics conductivity meter. To measure the free soluble sugar, 4 ml of ice cold freshly prepared anthrone reagent (0.2% anthrone in 98% sulphuric acid) was added to 2 ml of pre cooled seed leachate in a hard glass test tube and kept in cold for 30 minutes . The intensity of bluish green colour was measured in systronics sprectophotometer at 580 nm. To measure the dehydrogenase enzyme activity of seed (kittock and law 1968), 4 sprouted embryo of field pea seed were dipped in 3 ml of 0.2% tetrazolium chloride solution in a small glass vial and kept in dark for 3 hour at 30°c temperature. After 3 hours, the solution was decant off and the embryos were thoroughly washed with distilled water followed by addition of 3 ml of methoxy ethanol and kept in dark for overnight for synthesis of red colour formazan. The absorbance of the red coloured solution was measured in systronics sprectophotometer at 470 nm. For estimation of Free amino acid (Moore and stein 1948), 6 field pea seeds were soaked in 20 ml of distilled water for 6 hour . Then 0.1 ml of seed steep water was taken in a test tube to which 0.9 ml of water and 1 ml of freshly prepared ninhydrin solution was added . The solution was boiled for 10 minutes at 100°c covering the top of the test tube by glass lid . After cooling the solution, 5 ml of diruent (equal volume of n-propanal and water) was added and the solution kept for 15 minutes. Intensity of purple colour was measured in systronics sprectophotometer at 570 nm.

Field experiment

After 8 months of bottle storage the field experiment of treated and untreated seeds were carried out in the experimental farm of Calcutta University using randomized block design with a individual plot size of 6 sq. meter (3x2 sq m.). Seeds were sown @ 60 kg/ha by giving a spacing of 30 cm between rows and 10 cm between plants. The crop received a fertilizer dose of N: P_2O_5: K_2O at the rate of 40:40:20 , apart from 3 irrigation and other cultural practices from time to time. Data on plant population per square meter was recorded 2 weeks after sowing. Other parameters like plant height, pod per plant, seed per pod, pod length ,seed yield and 1000

seed weight was recorded replication wise at different stages of cropping period. Data obtained from laboratory and field were statistically analysed to evaluate the treatment effect on storability and field performance following the analysis of variance technique (Fisher, 1984).

RESULTS AND DISCUSSION

Germination test conducted immediately after treatment did not show any significant improvement on germinability over untreated control. But the germination test conducted after 24 days of accelerated ageing at 98% R.H. and 40°c showed that most of the dry treatments were significantly better in terms of germinability over untreated control (Table 1). Among the treatments in respect to storability red chilli powder followed by amla fruit powder proved to be the best treatment. Pre storage wet treatment, soaking drying showed adverse affect on storability over the untreated control possibly due to soaking injury in harvest fresh seed. Moist sand conditioning drying and Moist sand conditioning soaking drying treatment showed marginally better germinability over the control (Table 1).

Regarding the field performance, it was observed that the germination count, plant height ,seed yield per unit area and 1000 seed weight was significantly higher in most of the dry treated seeds (Table 2). But out of the 9 selected treatments, red chilli powder followed by amla fruit powder gave the highest field performance.

Biochemical analysis carried out immediately after treatment did not show any significant difference between treated and untreated seeds on membrane integrity as measured by electrical conductance, leaching of sugar and amino acid along with dehydrogenase enzyme activity (Table 3). But after ageing it was noticed that most of the treated seed showed lower electrical conductance, leaching of free soluble sugar, free amino acid and higher dehydrogenase enzyme activity over untreated control (Table 4) the leaching of electrolytes, sugar and amino acid are lower in soaking-drying treatment inspite of lower germination percentage and dehydrogenase enzyme activity, probably, due to initial leakage of metabolites at the time of soaking treatment. Among the treatment, red chilli powder followed by amla fruit powder has shown greater membrane integrity and higher enzyme activity.

Seed deterioration is an irreversible process. Once seed deterioration has occurred , this catabolic process cannot be reversed (Copeland and Mc. Donald, 1985). The most widely accepted single criteria of seed deterioration is reduced germinability. However, many test for measuring the loss of viability have been developed based on the physiological effect of ageing (ISTA, 1993). Among them the most important method is accelerated ageing which is done by subjecting the seeds to elevated temperature and high relative humidity. It provides a simple and good process of deterioration over a short period. In our present study accelerated

Table 1.

Effect of pre-storage seed invigoration treatments for the maintenance of germinability of field pea seed (*cv. Rachna*) after accelerated ageing at 98% Relative humidity and 40°c temperature for 24 day

Treatments	Germination		Mean Root Length (mm)	Mean Shoot Length (mm)	Vigour Index
	Percentage	Arc Sin Value			
Control	75	60	54	12	3960
Red Chili Powder	91.6	73.15	63	15	5705.7
Bleaching Powder	83.3	65.88	62	13	4941
Soaking Drying	50	45	43	12	2475
Moist Sand conditioning drying	83.3	65.88	54	11	4282.2
Moist Sand Conditioning soaking drying	83.3	65.88	47	13	3952.8
Aspirin	83.3	65.88	47	14	4018.68
Calcium Carbonate	75	60	58	14	4320
Iodinated Calcium Carbonate	83.3	65.88	54	13	4479.84
Amla fruit Powder	91.6	73.15	59	15	5413.3
LSD at 0.05 p	–	8.260	3.455	1.915	4.956
LSD at 0.01 p	–	11.867	4.964	NS	7.120

Treatments were given to 15 days old seed. Vigour index was calculated by multiplying the germination percentage with seedling length. Abbrebiation : NS –Not significant

Table 2.

Effect of pre- storage seed invigoration treatments on yield and yield attributes of field pea seed (cv. Rachna).

Treatments	Plant Population/m^2	No. of Pod/ Plant	No. of seed/Pod	Total yield (g/ m^2)	1000 seed weight (g)
Control	26	7	5	171.99	189
Red Chili Powder	28	8	6	270.01	201
Bleaching Powder	26	7	6	211.84	194
Soaking Drying	25	6	6	169.2	188
Moist Sand conditioning drying	26	7	6	207.48	190
Moist Sand Conditioning soaking drying	27	7	5	178.65	189
Aspirin	26	7	6	208.5	191
Calcium Carbonate	25	8	6	218.4	182
Iodinated Calcium Carbonate	26	8	6	229.63	184
Amla fruit Powder	27	8	6	254.01	196
LSD at 0.05 p	0.472	NS	NS	2.133	3.035
LSD at 0.01 p	0.678	NS	NS	3.064	4.360

Treatments were given to 15 days old seed. Vigour index was calculated by multiplying the germination percentage with seedling length. Abbrebiation : NS –Not significant

Table 3.

Effect of pre- storage seed invigoration treatments on membrane permeability and dehydrogenase enzyme activity of field pea seed (*cv. Rachna*) just after treatment i.e. before ageing condition

Treatments	Leaching of sugar (µg glucose/ ml of leachate)	Free Amino acid (µg leucin/ ml of leachate)	Electrical conductance (μs^{-cm2})	Dehydrogenase activity (n mole/ embryo/min.)
Control	14.62	22.85	22.04	71.66
Red Chili Powder	14.17	21.42	23.15	73.3
Bleaching Powder	14.62	24.28	19.20	73.7
Soaking Drying	15.82	28.57	24.25	72.45
Moist Sand conditioning drying	14.62	25.71	20.05	71.5
Moist Sand Condition Soaking Drying	13.88	20.71	26.17	72.1
Aspirin	14.62	23.57	20.10	74.6
Calcium Carbonate	13.73	27.14	26.18	72.9
Iodinated Calcium Carbonate	14.62	20	23.45	72.9
Amla fruit Powder	14.47	26.42	21.22	73.2
LSD at 0.05 p	NS	NS	NS	NS
LSD at 0.01 p	NS	NS	NS	NS

Treatments were given to 15 days old seed. Vigour index was calculated by multiplying the germination percentage with seedling length. Abbrebiation : NS –Not significant

ageing was one of the mechanism to judge the treatment effect on seed deterioration. Peroxidation of membrane lipid by free radical would increase permeability (Van stadess *et al.*, 1976) and decrease membrane fluidity (Dobretsovel *et al.*, 1977). Villers (1972) suggested that the deterioration of biomembrane in aged seed is due to peroxidation of phospholipid. The effectiveness of seed invigoration treatment on improvement of germinability , storability and field performance of seed have been reported by several research worker (Mandal and Basu, 2002. Adebisi *et al.*, 2003; Adebisi and oyekale, 2005). In the present studies encouraging responses have been noticed in terms of storability and field performances of field pea seed when treated with several dry dressing

Table 4.

Effect of pre storage seed invigoration treatments on membrane permeability and dehydrogenase enzyme activity of field pea seed (*cv. Rachna*) after accelerated ageing at 98 % relative humidity and 40°c temperature for 24 days

Treatments	Leaching of sugar (µg glucose/ ml of leachate)	Free Amino acid (µg leucin/ml	Electrical conductance (μs^{-cm2})	Dehydrogenase activity (n mole/ embryo/min.)
Control	28.22	204.39	120.90	12.68
Red Chili Powder	19.17	127.70	71.08	19.40
Bleaching Powder	22.58	137.19	88.90	17.77
Soaking Drying	34.21	190.61	92.06	14.60
Moist Sand conditioning drying	24.44	160.79	136.75	16.04
Moist Sand Condition Soaking Drying	32.12	182.18	85.76	15.08
Aspirin	23.52	146.65	110.75	17.00
Calcium Carbonate	24.02	143.55	94.25	16.33
Iodinated Calcium Carbonate	20.69	138.42	92.7	17.19
Amla fruit Powder	19.32	129.42	76.25	18.63
LSD at 0.05 p	6.34	11.22	1.686	0.355
LSD at 0.01 p	9.10	16.12	2.422	0.510

treatments. Basu and coworkers (Rudrapal and Basu,1980; Mandal and Basu, 1986) demonstrated that dry dressing of seed with halogenated compounds such as bleaching powder or iodinated calcium carbonate effectively slowed down the deterioration of seed. Basu and Rudrapal (1980) attributed the beneficial effect of iodination to the stabilization of the unsaturated fatty acid component of lipoprotein membrane. Protein protective role of ortho-acetyl salicylic acid (aspirin, the active ingredient of aspro) might be responsible for maintenance of viability of stored seed (Mandal *et al.*, 2000). Mode of action of beneficial effect of dry treatments on viability maintenance is yet to be elucidated. Capsicin an active ingradient of red chilli powder has been established as an inhibitor of lipid peroxidation(Brand *et al.*,1990; Dey and Ghosh,1993). Amla (active ingredient phyllemblin) are effective broad spectrum antioxidant and free radical scavengars, helping to reduce disease and slow ageing process. The beneficial effect of amla as an antioxidant have been examined by a number of author (Kumar *et al.*, 2006; Rao *et al.*, 2005). In legume seed , soaking injury due to rapid water uptake is a well documented phenomenon. Use of osmoticum polyethylene glycol very successfully overcame soaking injury by reduced water uptake in soybean (Wood stock and Tao, 1981; Saha and Basu,1982). The present results confirm the earlier findings on soaking drying treatment in leguminous seed. The overall results obtained in the present study also are in accordance with the Mandal and coworker who reported that dry physiological treatments with crude plant materials and house hold preservatives are effective for the maintenance of storability and field performance and productivity of wheat, soybean, black gram and okra (De *et al.*, 2003, Mandal *et al.* 2000; Guha and Mandal, 2013).

On the basis of the present findings, dry dressing treatment with easily procurable, eco-friendly and inexpensive crude plant materials like red chilli powder and amla fruit powder are suggested to improve storability and field performance of high vigour field pea seeds.

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EFFICACY OF BOTANICALS, PHARMACEUTICAL FORMULATION AND WET TREATMENTS FOR EXTENDED STORABILITY, ENZYME ACTIVITY AND IMPROVED FIELD PERFORMANCE OF FIELD PEA (*Pisum Sativum* L., CV. RACHNA) SEED

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ABSTRACT

Studies were conducted to evaluate the effect of dry dressing treatments with different chemicals, pharmaceutical products and crude plant materials and wet trteatments on greminability, different biochemical parameters and field performances of field pea. Treated along with untreated seeds were subjected to natural ageing for three months in cloth bag under ambient condition. Morphological test conducted after three months of natural ageing revealed that dry dressing of seed with red chilli powder @ 1g/kg of seed, amla fruit powder @ 2g/ kg of seed and iodinated calcium carbonate @ 2g/ kg of seed showed significantly higher germination percentage and vigour as measured by root and shoot length of the seedlings over control. Field performance in terms of germination count, 1000 seed weight and yield of dry dressed bottled stored (8 months) seeds especially with red chilli powder and amla fruit powder were significantly higher than the control. Most of the treated seeds showed lower leakage of sugar along with higher enzymatic activity (peroxidase and catalase) than control. Total soluble seed protein banding pattern of different treated and untreated seed lot revealed that there has been reduction in band intensity, band number or even loss of some bands with the progress of ageing.

INTRODUCTION

Field pea (*Pisum sativum* L.) is a popular pulse crop of India. It is a cool season crop requires an optimum mean temperature of 13°C to 18°C for its growth. Pea seeds are good source of protein. The crop is grown for their succulent or dry seeds. Peas are used as fresh or processed. The processed form include freezing, canning and hydrated. Mature seed may be used as dal and prepared in various ways for human consumption. Field pea contributes 3 % in total pulse area and about 5% in the total pulse production of India. Uttarpradesh is the major field pea growing state in India followed by Madhyapradesh and Bihar. One of the major bottleneck in increasing the area of field pea in India is the lack of availability of good quality seed. Due to inadequate storage facilities, the farmers are forced to use the deteriorated seed for sowing in the next season causing reduction in yield. Several workers have applied different dry and wet treatments on the seed prior to storage to minimize the deterioration of seed during storage. Pre-storage dry dressing treatments and mid-storage hydration-dehydration treatments of stored orthodox seeds of wheat, soybean and sesamum has been reported to be very effective for maintenance of vigour, viability and productivity (Mandal and Basu , 1983 ; Mandal *et al.*, 2000; 2008)

Pati *et al.* (2011) demonstrated that the pre-treatment of pea seeds with leaf extract of bel (*Aegel marmelos*) and kalmegh (*Andrographis panniculata*) 50 g in 250 ml of distilled water for six hours before accelerated ageing treatment for (100% relative humidity and 30 ± 2°C temperature) 45 days found much better plant performance than untreated control.

More recently infusion of fungicides, growth regulators, pesticides, bio-products, bio-ingredients, agro-chemicals and herbicides into the seeds prior to germination is reported to alleviate the impact of adverse factors on seed quality and performance (Janmohammadi *et al.*, 2008).

Present study was taken up with a view to development of some suitable seed invigoration treatments so that storability and field performance could be maintained keeping the atmospheric condition of eastern zone in mind.

MATERIALS AND METHODS

Seed Material

To carryout the present experiment, properly dried seeds of current season were collected from the Agricultural Experimental Farm of Calcutta University and were stored in the 2.5 lit. capacity rubber stoppered glass bottle. Pre-storage treatments were given to one month old seeds.

Method of pre storage seed treatment:

a) *Dry treatment :* Dry seed treatment was employed using crude plant materials like red chilli powder (active ingredient, capsaicin) @ 1 g/kg of seed and amla (active ingredient, phyllemblin) 2 g/kg of seed, chemicals like calcium carbonate @ 2 g/ kg of seed , bleaching powder @ 2 g/kg of seed and pharmaceutical product viz, aspirin @ 50 mg/kg of seed separately with the seeds in the rubber stoppered glass bottle and kept under ambient condition. Treated seeds were gently shaken for seven days for thorough mixing of the materials with seed.

b) *Wet treatment :* Seed priming like soaking in double volume of water for one hour followed by drying back to its original moisture content, moist sand conditioning for 24 hours followed by drying and moist sand conditioning for 24 hours followed by soaking in double volume of water for one hour and then drying for 3-4 days until the seeds regain its original moisture content were also done simultaneously.

After wet treatment, seeds were kept in the perforated paper packet and kept in the dessicator containing fused calcium chloride for 4-5 days to stabilize the moisture content to an uniform level and then kept in the small glass bottle and stored under ambient condition.

Natural Ageing: Treated seeds were placed separately in the perforated paper packets having equal number of holes and kept in a cloth bag before subjecting them to natural ageing for various period under ambient condition to evaluate the efficacy of treatments effect on the viability and vigour of seeds.

Seed performance test:

a) *Germination test :* Germination test were carried out immediately after 10 days of treatment and after three months of natural ageing under ambient condition following the method of Punjabi and Basu (1982) with minor modification employing 400 seeds for each treatment. Data were recorded after germination for 7 days at 20 ± 1°C.

b) Bio-chemical test : Biochemical analysis viz. leaching of sugar, enzyme activity viz. catalase and peroxidase and SDS-PAGE gel of the total soluble protein was carried out to judge the treatment effect on the seed quality.

The leakage of sugar was measured following the method of Mc Cready *et al.* (1950) with minor modifications. Four millilitre of ice cold freshly prepared anthrone reagent (0.2% anthrone in 98% sulphuric acid) was added to 2 ml of pre cooled seed leachate in a hard glass test tube and kept in cold for 30 minutes. The intensity of bluish green colour was measured in systronics spectrophotometer at 580 nm. Catalase activity was measured following the method of Aebi (1984) with minor modification where the decomposition of H_2O_2 was measured by recording the decline in absorbance at 240nm for 3 minute. The reaction mixture contained 50mM phosphate buffer (Ph 7.0), 50mM H_2O_2 and 50 µl of enzyme extract in a measuring tube and volume was makeup to 3ml by adding buffer solution. A mixture without H_2O_2 served as blank. Catalase activity was calculated using mM extinction coefficient for H_2O_2 (39.4mM/cm) expressed as amount of H_2O_2 decomposed/min/g of fresh tissue. Peroxidase activity was estimated as per the protocol mentioned in the text book of Plant Physiology by Ghosh and Mukherjee. Hundred mg of 24 hour imbibed seed tissue was measured and crushed in mortar pestle with 10 ml of phosphate buffer (Ph 7.0). The extract is centrifuged at 10000 rpm for 20 minute and the supernatant was collected for estimation of peroxidase activity. Reaction mixture was prepared in the test tube by taking 5 ml buffer, 1 ml pyro catechol and 1 ml H_2O_2. The reaction was started by addition of 1 ml of enzyme extract. Absorbance was measured at 490nm just after the addition of enzyme with the reaction mixture and then after 3 minutes of incubation. The enzyme activity (Δ Abs.) is expressed in terms of difference in absorbance value per gram per minute.

SDS-PAGE of total soluble protein was carried out by using 12 percent acrylamide gel according to the method prescribed by Laemeli (1970). Hundred mg imbibed tissue was taken in mortar pestle and crushed in 2 ml of buffer containing 1.950 ml buffer and 50 ul betamercepto ethanol. The content was centrifuged at 14000 rpm for 30 minutes. Supernatant so collected was used for protein estimation and 50 µg protein was used

from each sample for loading on to the gel. A constant current of 20 mA was applied until the tracking dye crossed the stacking gel. Then a constant voltage of 80 volt was applied until the tracking dye reaches bottom of the resolving gel. Then the gel was stained using coomassie brilliant blue R 250 overnight and destained using a mixture of 100 ml methanol, 100 ml acetic acid and 800 ml of distilled water until the bands were clearly visible.

Field Experiment :

Treated and untreated seeds of field pea were sown in the field at Agricultural Experimental Farm of Calcutta University, Baruipur, 24 Parganas, West Bengal, during *rabi* season (November – March) using completely randomized block design with three replications for each treatment. After thorough land preparation, the fields were divided into 30 sub-plots, each sub-plots having a size of 6 square meter. Seeds were sown @ 60 kg/ hectare giving a spacing of 30 cm between the row and 15 cm between the plant. The fertilizer dose, N: P_2O_5: K_2O was applied @ 40:40:20 kg/ ha. Data on plant population, seed yield and 1000 seed weight was recorded replication-wise. Statistical analysis of the data obtained from laboratory and field was done following the analysis of variance technique (Fisher, 1948) to judge the treatment effect on storability and field performance. The experiments were carried out in two consecutive years (2013-2014 and 2014-2015).

RESULTS AND DISCUSSION

Germination test conducted immediately after treatment (10 days) implies that there was no significant effect in terms of germinability and vigour between treated and untreated seeds (control). But when the same test was conducted after 3 months of natural ageing, it was found that except soaking drying treated seed, most of the treated seeds showed significantly better results in maintaining the germinability than the untreated control. Among the different treatments, seeds treated with red chili powder and amla fruit powder proved to be the best in terms of germinability and vigour as measured by root and shoot length of the seedlings (Table 1).

Among the pre storage wet treatment, soaking-drying showed negative correlation with the germinability might be due to imbibitions injury in the harvest fresh seed. Soaking-injury in freshly harvested legume seeds is a well established fact, probably due to faster rate of imbibition during initial phase of soaking. Imbibitional injury could be minimize by the use of osmoticum polyethylene glycol as it could reduce the water uptake in the soybean (Woodstock and Tao, 1981, Saha and

Table 1.

Effect of pre-storage seed invigoration treatments for the maintenance of germinability of field pea seed (cv. Rachna) after natural ageing for 90 days under ambient condition (79.5± 2.1 % RH and 29.3± 1°C).

Treatments	Germination		Mean Root Length (mm)	Mean Shoot Length (mm)	Vigour Index
	Percentage	Arc Sin Value			
Control	83	65.88	49	11	3952.8
Red Chili Powder	100	90.0	55	16	6390.0
Bleaching Powder	63	65.88	54	15	4545.7
Soaking Drying	58	49.78	45	13	2887.2
Moist Sand conditioning drying	92	73.15	46	11	4169.5
Moist Sand Condition Soaking Drying	92	73.15	43	15	4242.7
Aspirin	92	73.15	54	13	4901.0
Calcium Carbonate	83	65.88	54	14	4479.8
Iodinated Calcium Carbonate	83	65.88	54	15	4545.7
Amla Powder	100	90.0	55	15	6300.0
LSD at 0.05 P	–	3.848	3.035	3.090	4.72
LSD at 0.01 P	–	5.529	4.360	NS	6.78

Treatments were given to one month old seeds. Data were recorded after germination for 7 days at 20± 1°C.
Abbreviation : NS = Not Significant
Vigour Index = Mean seedling length x Germination percentage

Basu, 1982). The results of the present study further consolidate the earlier findings.

Field performance of different treated and untreated seeds when scrutinized found that germination count, yield per square meter, 1000 seed weight was significantly higher in all the dry treated seeds than the untreated control (Table 2). Among the nine selected treatments, seed treated with red chilli powder, amla fruit powder and iodinated calcium carbonate experienced higher yield than others including control.

Table 2.

Effect of pre-storage seed invigoration treatments on yield and yield attributes of field pea seed (cv. Rachna)

Treatments	Plant Population /m^2	yield (g/m^2)	1000 seed weight (g)
Control	26	181.09	199
Red Chili Powder	28	282.24	210
Bleaching Powder	26	222.76	204
Soaking Drying	25	179	198
Moist Sand conditioning drying	26	219.32	200
Moist Sand Condition Soaking Drying	27	188.05	199
Aspirin	26	220	201
Calcium Carbonate	25	230.4	192
Iodinated Calcium Carbonate	26	242.81	194
Amla Powder	27	266.97	206
LSD at 0.05 P	0.472	2.133	3.035
LSD at 0.01 P	0.678	3.064	4.360

Treatments were given to one month old seeds. Data were recorded after germination for 7 days at 20± 1°C.

Table 3.

Effect of pre-storage seed invigoration treatments on the leaching of sugar, catalase and peroxidase enzyme activity immediately after treatment i.e. before ageing condition.

Treatments	Germination (%)	Leaching of sugar (µg glucose equiv./ml)	Catalase activity (n mole/min/g fresh tissue)	Peroxidase activity Δ(Abs/g tissue/min.)
Control	100	0.098	4.62	0.288
Red Chili Powder	100	0.095	4.75	0.299
Bleaching Powder	100	0.094	5.10	0.286
Soaking Drying	100	0.106	4.26	0.284
Moist Sand conditioning drying	100	0.099	3.94	0.302
Moist Sand Condition Soaking Drying	100	0.093	4.56	0.294
Aspirin	100	0.092	5.19	0.297
Calcium Carbonate	100	0.090	5.02	0.282
Iodinated Calcium Carbonate	100	0.089	4.87	0.304
Amla Powder	100	0.097	4.97	0.280
LSD at 0.05 P	–	NS	NS	NS
LSD at 0.01 P	–	NS	NS	NS

Treatments were given to one month old seeds. Data were recorded after germination for 7 days at 20± 1°C.
Abbreviation : NS = Not Significant

The beneficial effect of seed invigoration treatment on improvenment of germinability, storability and field performance of different other crop seeds have been observed by several research worker (Mandal and Basu, 2003, Adebisi *et al.*, 2003, Adebisi and Oyekale, 2005). Our present study is also in conformity with the findings of the earlier workers.

Lonelshrat *et al.*, (2014) observed that pre-treatment of the maize seeds with chemicals like aspirin , calcium carbonate and crude plant materials like trigonella seed powder and walnut shell powder gave better results on vigour and viability than control.

Biochemical analysis reveals that there was no significant difference between the treated and untreated seeds in terms of leaching of sugar as well as antioxidant enzyme activity viz. peroxidase and catalase when tested immediately after treatment (Table 3). But with the increase in storage period there was a increase in leaching of sugars with simultaneous decrease in enzyme activity (peroxidase and catalase). Most of the treated seeds showed superiority in all the enzyme activity and reduced leakage of sugar over control (Table 4). Among different treatments, red chilli powder, amla fruit powder and iodinated calcium carbonate treated seeds showed significantly lower leaching of sugar and higher peroxidase and catalase enzyme activity than control. Similar decrease in the activity of catalase and peroxidase enzyme was noticed in the aged seeds as compared to fresh seeds (Scialabba *et al.*, 2002, Chauhan *et al.*, 2011). Cakmak *et al.* (2010) also noticed decrease in germination ability of aged legume seeds which was correlated with decrease in enzymatic antioxidant activity. Zamani *et al.* (2010) pointed out that both natural and accelerated ageing reduced germination percentage, seed vigour and activity of catalase, peroxidase and ascorbate peroxidase (APX) with an increase in malondialdehyde (MDA) content and electrolyte leakage in safflower seed.

Non-enzymatic anti-oxidant activity of amla fruit had been confirmed by a number of worker (Kumaran *et al.*, 2006). Amla (active ingredient, phyllemblin) is an effective antioxidant and free radical scavenger helps to reduce disease and slow down the ageing process.

Soluble protein banding pattern of different natural aged (3 months old) seed lot observed a decline in the band intensity, band number or disappearance of some bands as period of ageing advanced. In our present study a total of 19 polypeptide bands of diverse molecular weight ranging from 14.4 KDa to 116.25 KDa was observed. Seed treated with red chilli powder showed maximum peptide bands i.e. 19, while seed treated with soaking-drying had 15 peptide bands and the untreated

Table 4.

Effect of pre-storage seed invigoration treatments on the leaching of sugar, catalase and peroxidase enzyme activity after 90 days of natural ageing under ambient conditions (79.5± 2.1 % RH and 29.3± 1°C)

Treatments	Germination (%)	Leaching of sugar (µg glucose equiv./ml)	Catalase activity (n mole/min/g fresh tissue)	Peroxidase activity Δ(Abs/g tissue/min.)
Control	83	32.04	1.385	0.136
Red Chili Powder	100	21.19	3.43	0.269
Bleaching Powder	63	25.36	2.64	0.222
Soaking Drying	58	38.67	1.94	0.179
Moist Sand conditioning drying	92	27.4	2.45	0.212
Moist Sand Condition Soaking Drying	92	30.33	2.02	0.204
Aspirin	92	25.34	2.94	0.218
Calcium Carbonate	83	26.41	2.78	0.231
Iodinated Calcium Carbonate	83	23.41	3.01	0.246
Amla Powder	100	22.05	3.58	0.258
LSD at 0.05 P	–	1.48	0.487	0.025
LSD at 0.01 P	–	1.033	0.699	0.037

Treatments were given to one month old seeds. Data were recorded after germination for 7 days at 20± 1°C.

control had 15 peptide bands (Fig. 1). Bhanuprakash *et al.* (2006) reported alteration in banding pattern of protein profile of aged seeds compare to that of fresh seed. Vishwanath *et al.* (2007) revealed that due to accelerated ageing there has been a decline in soluble protein banding pattern in terms of band intensity, band numbers or even total loss of some bands.

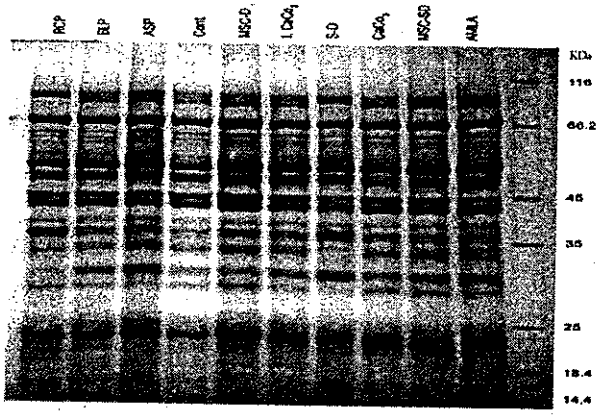

Fig. 1. Peptide profile of different treated and non-treated field pea seeds after 80 days of natural ageing

Abbreviation : RCP- red chilli powder, BLP- bleaching powder, ASP- Aspirin, Cont.- control, MSC-D- Moist sand conditioning drying, I.$CaCO_3$- iodinated calcium carbonate, S-D- soaking drying, $CaCO_3$- calcium carbonate, MSC-SD- moist sand conditioning soaking-drying, AMLA- amla fruit powder

Basu and coworker (Rudrapal and Basu, 1980; Mandal and Basu, 1986) reported that dry permeation of seeds with halogen compounds such as bleaching powder or iodinated calcium carbonate could slow down the deteriorative effect in seed due to ageing. The present study confirm the works of other researchers in this field which establish the fact that dry dressing with crude plant materials like red chilli powder, amla fruit powder and chemicals like aspirin, iodinated calcium carbonate are effective for maintaining the vigour, viability and field performance of different crop seeds (De *et al.*, 2003; Mandal *et al.* 2000; Guha and Mandal, 2013). Hence it is suggested to farmers and seed growers that dry dressing in harvest fresh seeds with red chilli powder @ 1g / kg of seed and amla fruit powder @ 2g/ kg of seed may be practiced for improved storability and field performance of field pea seeds.

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