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RESEARCH ARTICLE

EFFICACY OF DRY AND WET PHYSIOLOGICAL TREATMENTS FOR THE MAINTENANCE OF VIGOUR, VIABILITY AND YIELD POTENTIAL OF STORED MUNG BEAN SEEDS (*VIGNA RADIATA* (L.). R. WILCZEK)

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ABSTRACT

Dry dressing treatments in harvest fresh mung bean (*Vigna radiata* (L.). R. Wilczek) seeds (cv. chaiti) with chemicals (viz., bleaching powder, ferulic acid, Iodinated calcium carbonate, Calcium carbonate), pharmaceutical formulations (viz., aspirin), finely powdered crude plant materials (viz., red chilli powder, amla powder) and wet treatment (viz., soaking-drying, moist sand conditioning drying and moist sand conditioning soaking drying) effectively controlled the loss of seed deterioration after subsequent storage under ambient conditions. The field performance and productivity of dry and wet treated seeds were also significantly improved than the untreated control. Among the treatment, amla, red chilli powder and ferulic acid have shown better results in extending longevity and increasing field performance and productivity. Physiological and biochemical studies revealed that a significantly reduced leakage of electrolytes and sugar along with lower volatile aldehyde production and higher dehydrogenase enzyme activity by the dry and wet treated seeds than the untreated control after subsequent accelerated ageing. On the basis of the results, pre-storage dry treatments with amla powder @ 2g/kg of seed, red chilli powder @ 1g/kg of seed and ferulic acid @ 500mg/kg of seed may be suggested for improved storability and field performance of mung bean seeds.

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INTRODUCTION

Green gram (*Vigna radiata* (L.).R. Wilczek) is one of the important pulse crop of India and serve as a major source of dietary protein for the vast majority of people. It has been grown in India since ancient times. It is still widely grown in Southeast Asia, Africa, South America and Australia. India is the largest producer of mung bean. Mung bean is a high source of protein, fibre, antioxidants and phytonutrients. Although in most parts of the world they are less popular than other bean varieties, like Chick peas. Mung beans are a high source of nutrients including – Mn, Mg, K, Folate, Cu, Zn and various B Vitamins. Seed is a crucial factor and basic input in agriculture to increase crop yield per unit area and to improve agricultural economy of country. But, in Eastern India, the progress in the cultivation of mung bean is really very slow. The problem of seed storage in our country is due to high humidity and high temperature. In Eastern India, the average relative humidity is about 80% and temperature around 30^0C and as a result, storage of seed in moisture pervasive containers under ambient conditions is really problematic to maintain high vigour and viability of the seeds for the next sowing season.

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In West Bengal, mung bean seed harvested in April - May and stored in moisture impervious containers under ambient conditions showed a rapid fall in germinability and at the time of sowing during February (in the next year), the viability of the seeds may go down below 20%. During monsoon season i.e., June – July, the ambient relative humidity increases and as a result, the seeds stored in moisture pervasive containers absorb a lot of moisture from the humid atmosphere and coupled with high temperature hastens the ageing process of the seed. Occasional rain during harvesting and threshing also accelerate the seed deterioration. Studies conducted in the present laboratory by Mandal, Basu and other co-workers have developed a number of inexpensive methods of dry seed treatments for the maintenance of vigour, viability and productivity of a large number of agricultural and horticultural crop seeds (Mandal and Basu, 1983; 1986; De *et al*., 2005, Guha and Mandal, 2011; Majumder *et al*., 2015). High humidity and high temperature not only accelerate the physiological deterioration of seeds but also make it susceptible to mould growth and attack by storage grain pests and storage microflora (Grewal and Kapoor, 1966). Therefore, proper storage of seed is a very serious problem in our country and has not yet received due attention of the farmers. In such situations, the development of easy and inexpensive method of seed treatment for the maintenance of vigour, viability and

field performance of stored seeds would be of great advantage to our cultivars and seed merchants. In the present study, major emphasis has been given towards standardization of suitable inexpensive pre-storage seed invigoration treatments with chemicals, pharmaceutical formulation and crude plant preparations for improved post-storage germinability and field performance and productivity of mung bean seeds. Besides, the physiological and biochemical changes in the seeds have been studied to elucidate the mode of action of seed invigoration treatment.

MATERIALS AND METHODS

The experiments were carried out in three consecutive seasons (2015-2018). Seeds of mung bean (cv. chaiti) were collected from the Calcutta University Agricultural Experimental Farm at Baruipur, 24 Parganas (South), West Bengal. After collection, seeds were cleaned and dried properly in a drying cabinet for 7 days at 35±1ºC to a moisture content of about 8% and stored in the rubber stoppered glass bottles (300 ml capacity) under ambient conditions in the laboratory till further use. The initial germination percentage of the seed lot was 100%. Wet treatment (viz. soaking-drying, moist sand conditioning drying and moist sand conditioning soaking drying) as well as dry-dressing treatments were given to one month old mung bean seeds. Seeds were dry dressed with finely powdered chemicals viz., bleaching powder @ 2g/kg of seed, ferulic acid @ 500mg/kg of seed, iodinated calcium carbonate @ 2g/kg of seed and calcium carbonate @ 2g/kg of seed; pharmaceuticals viz., aspirin @ 50 mg/kg of seed, and crude plant materials viz., red chilli powder @ 1g/kg of seed and amla powder @ 2g/kg of seed in the rubber-stoppered glass bottles under ambient conditions and then kept on the shelf in the laboratory at room temperature (29±1ºC). After treatment, glass bottles were shaken once daily for 7 days to mix the chemicals and plant materials with the seeds properly. Soaking-drying treatments were also given to the same seed lot by soaking the seeds in water for half an hours at room temperature (29±1ºC) followed by drying to its original moisture content. In case of moist-sand conditioning-drying (MSC-D), seeds were preconditioned by a slow and progressive rise in the moisture content which were achieved by keeping the seeds (250 g) in moist sand (sand: seed:: 3 : 1). For this purpose, sand was sterilized with concentrated sulphuric acid to kill the microbes and then washed thoroughly and finally dried.

Air-dried sand was moistened with water @4% (750 g sand + 45 ml water) at room temperature. Seeds were then thoroughly mixed with the moist sand and kept covered for 12 h at room temperature (29± 1°C). After the stipulated period, seeds were sieved to remove the sand followed by drying in the cabinet over a current of dehumidified air at 35± 1°C for about 72 hours to dry back the seeds to their original moisture content. In case of Moist-sand conditioning followed by soaking-drying (MSC-S-D), seeds (250 g) were pre-conditioned with moist sand for 12 hours following the above mentioned method and then soaked in water for half an hour at room temperature (29± 1°C) followed by drying to its original moisture content (Saha *et al.,* 1990). The untreated control seeds were not soaked or dry dressed but kept in the rubber stoppered glass bottles along with the treated seeds. After 7 days of treatment, seeds were subjected to accelerated ageing at 100% RH and 40˚C temperature for 22 days.

Germination test of the treated and untreated seeds (minimum 400 seeds for each treatment as specified by ISTA, 1996) were done before and after accelereted aging. Data on germination percentage, root and shoot length were recorded after germination for 5 days at 29±1ºC temperature. Field performance and productivity of treated and untreated mung bean seeds were studied at the Agricultural Experimental Farm, University of Calcutta, during the pre-kharif season (February-May) using randomized block design with 3 replications for each treatment. A fertilizer dose of N: P_2O_5: K_2O was given @ 40:20:40 kg/ha respectively. During land preparation, 50% of the total nitrogen and the whole amount of phosphate and potassium were added. The rest of the nitrogen was supplied in two split doses, one at the time of thinning and another at flowering. Seeds were sown @ 20kg/ha giving a spacing of 30 cm between the rows and 15 cm between the plants. Apart from the post sowing irrigation, the crop received 3 more irrigations and usual culture practices were followed throughout the cropping period. Data on field emergence was recorded after 15 days of sowing. The plant height (cm), number of pods per plant, length of pods (cm), number of seed per pod, total seed yield per unit area (g) and 1000-seed weight (g) were recorded replication-wise after harvesting of the crop. To study the membrane permeability as evidenced by electrical conductance and leaching of sugar were done following the method of Anderson *et al*., (1964) and McCready *et al.,* (1950) respectively. The dehydrogenase enzyme activity was measured following the method of Kittock and Law (1968). The volatile aldehyde productions of treated and untreated seeds were also studied following the method of Wilson Mc Donald (1986) with a modified aldehyde trapping device (Sur and Basu, 1990a). Data collected on various parameters were statistically analyzed (Fisher 1948) to evaluate the treatment effects on germinability and field performance of mung bean seed. Germination percentage data were transformed to their respective arc-sin before analysis and vigour index data were calculated as germination percentage multiplied by seedling length.

RESULTS AND DISCUSSION

Pre-storage dry and wet treatments of stored mung bean seeds did not show any significant improvement on germination percentage and seedling growth over untreated control when tested immediately after treatment. But after accelerated ageing, most of the dry treated seeds showed significant improvement on germination percentage and seedling length as measured by root and shoot length over untreated control (Table 1). The vigour index was also higher in all the dry and wet treated seeds than the control. Among the dry treatment, amla, red chilli powder and ferulic acid showed considerable improvement on storability. The field performance and productivity, especially, seed yield per unit area, plant population per unit area, length of pod, number of pod per plant, number of seed per pod and 1000-seed weight were also significantly higher in most of the pre-storage dry treatments than the untreated control (Table 2). Among the treatment, red chilli powder, amla powder and ferulic acid has shown better results in improving field performance and productivity. The membrane integrity and enzyme activity of the seed as determined by leaching of electrolytes, leakage of sugar and dehydrogenase enzyme activity along with volatile aldehyde productions did not show any significant difference between treated and untreated seeds when tested immediately after treatment (Table 3).

Table 1. Effect of pre storage seed invigoration treatments for the maintenance of vigour and viability of Mung bean seeds before and after accelerated ageing at 100% RH and 40˚C temperature for 22 days

Treatment	Before Ageing					Accelerated Ageing				
	Germination		Mean root length	Mean shoot length	Vigour Index	Germination		Mean root length	Mean shoot length	Vigour Index
	%	Arc Sin value				%	Arc Sin value			
Control	100	90.00	45	84	12900	48	43.85	30	61	4368
Amla	100	90.00	52	89	14100	64	53.13	45	76	7744
Red Chilli Powder	100	90.00	54	81	13500	64	53.13	42	74	7424
Bleaching Powder	84	66.42	49	78	10668	52	46.15	36	69	5460
Aspirin	100	90.00	44	83	12700	52	46.15	39	72	5772
Ferulic Acid	100	90.00	46	85	13100	60	50.77	39	76	6900
Calcium Carbonate	92	73.57	47	67	10488	48	43.85	38	63	4848
Iodinated Calcium Carbonate	100	90.00	39	76	11500	56	48.45	33	71	5824
Moist Sand Conditioning - Drying	84	66.42	44	63	8988	52	46.15	39	61	5200
Moist Sand Conditioning Soaking - Drying	92	73.57	48	74	11224	60	50.77	34	67	6060
Soaking - Drying	72	58.05	43	53	6912	48	43.85	32	60	4416
L.S.D. (P= 0.05)	-	NS	NS	NS	-	-	4.4	3.2	4.0	-
L.S.D. (P= 0.01)	-	NS	NS	NS	-	-	6.1	4.4	5.5	-

Table 2. Effect of pre storage seed invigoration treatments on field performance and productivity of Mung bean

Treatment	Plant Population / m²	Plant Height (cm)	No. of Pods / Plant	Length of pod (cm)	No. of seed / pod	Seed Yield (g/m²)	1000 seed weight (g)
Control	36	30.13	10	6.12	10	104.30	20.15
Amla	45	37.00	15	6.89	11	122.00	21.46
Red Chilli Powder	47	40.93	17	7.08	12	127.92	21.86
Bleaching Powder	37	31.40	12	6.73	10	106.23	20.25
Aspirin	37	32.58	13	6.84	10	109.45	20.46
Ferulic Acid	43	35.00	15	6.80	11	117.32	21.40
Calcium Carbonate	38	30.98	15	6.61	10	111.59	20.53
Iodinated Calcium Carbonate	39	32.43	17	6.66	11	119.35	20.66
Moist Sand Conditioning - Drying	37	31.29	13	6.43	11	110.60	20.87
Moist Sand Conditioning Soaking - Drying	40	33.37	15	6.79	11	117.60	21.11
Soaking - Drying	36	30.27	11	6.21	10	105.63	20.34
L.S.D. (P= 0.05)	2.0	2.0	0.9	NS	NS	3.9	1.3
L.S.D. (P= 0.01)	2.3	3.0	1.4	NS	NS	5.6	2.0

Table 3. Effect of pre storage seed invigoration treatments on membrane integrity, dehydrogenase enzyme activity and volatile aldehyde production of mung bean seeds before and after accelerated ageing at 100% RH and 40˚C temperature for 22 days

Treatment	Before Ageing						Accelerated Ageing					
	Germination		Electrical Conductance (dSm^{-1})	Leaching of Sugar (O.D)	Dehydrogenase Enzyme Activity (O.D)	Assay of Volatile Aldehyde (O.D)	Germination		Electrical Conductance (dSm^{-1})	Leaching of Sugar (O.D)	Dehydrogenase Enzyme Activity (O.D)	Assay of Volatile Aldehyde (O.D)
	%	Arc Sin value					%	Arc Sin value				
Control	100	90.00	34.73	0.060	0.660	0.080	48	43.85	102.6	0.227	0.190	0.221
Amla	100	90.00	31.70	0.045	0.738	0.076	64	53.13	71.82	0.221	0.203	0.193
Red Chilli Powder	100	90.00	36.43	0.055	0.689	0.080	64	53.13	68.04	0.216	0.212	0.205
Bleaching Powder	84	66.42	50.63	0.075	0.591	0.096	52	46.15	90.72	0.339	0.170	0.231
Aspirin	100	90.00	45.33	0.055	0.550	0.093	52	46.15	75.60	0.221	0.156	0.210
Ferulic Acid	100	90.00	39.41	0.063	0.612	0.086	60	50.77	75.60	0.224	0.188	0.219
Calcium Carbonate	92	73.57	57.17	0.070	0.570	0.091	48	43.85	98.28	0.271	0.187	0.216
Iodinated Calcium Carbonate	100	90.00	55.58	0.080	0.657	0.095	56	48.45	94.50	0.309	0.190	0.227
Moist Sand Conditioning – Drying	84	66.42	46.84	0.058	0.652	0.082	52	46.15	83.16	0.223	0.195	0.239
Moist Sand Conditioning Soaking – Drying	92	73.57	49.59	0.079	0.705	0.092	60	50.77	90.72	0.321	0.182	0.225
Soaking - Drying	72	58.05	44.85	0.050	0.523	0.074	48	43.85	90.72	0.275	0.197	0.234
L.S.D. (P= 0.05)	-	NS	NS	NS	NS	NS		4.4	5.2	0.02	0.02	0.02
L.S.D. (P= 0.01)	-	NS	NS	NS	NS	NS		6.1	7.4	0.03	0.03	0.01

But soaking-drying treatment showed reduced leakage of electrolytes and sugar than the control and other treatments because of initial leakage during soaking for half an hour at the time of treatment (Table 3). But after accelerated ageing, the membrane integrity as measured by leakage of electrolytes and sugars were significantly lower with a higher dehydrogenase enzyme activity in the treated seeds then the untreated control (Table-3). The volatile aldehyde productions were also lower in the treated seeds than the untreated control of mung bean seeds (Table 3). Among the dry treatment, red chilli powder, amla, ferulic acid and aspirin has shown reduced leakage of electrolytes and sugar along with higher dehydrogenase enzyme activity and lower volatile aldehyde production. In this experiment, use of dry-dressing treatments with chemicals, pharmaceutical formulations, crude plant materials and wet treatment showed greater germinability than the untreated control. They have also significantly improved field performance and productivity over untreated control. Dry-dressing of seeds with halogenated compound like bleaching powder has been successful in a number of harvest fresh non-leguminous and leguminous seeds (Mandal and Basu, 1986; De *et al*., 1998; Mandal *et al*., 2000). The role of bleaching powder, (a source of chlorine) in viability maintenance of mustard and wheat seed has earlier been suggested to be due to stabilization of unsaturated fatty acid components of lipoprotein biomembranes (Basu and Rudrapal, 1980; Mandal and Basu, 1986). There is also the possibility of halogens acting as free radical quenchers or scavengers (Pryor and Lasswell, 1975). Regarding the mode of action of crude plant preparations, capsaicin, an active ingredient of red chilli fruits is acknowledged inhibitors of lipid peroxidation (Brand *et al*., 1990; Dey and Ghosh, 1993). The effects of the natural plant preparations have been basically physiological in nature because the volatile aldehyde productions were lower in the treated seeds (De *et al*., 1998). The protein protective role of acetyl salicylic acid (aspirin, the active ingredient of aspro) might be partly responsible for viability maintenance of stored seed (De *et al*., 2004). Aspirin, a non-steroidal anti-inflammatory drug is related chemically in that they are weak organic acids. They may also decrease the production of free radicals and superoxide and may interact with adenyl cyclase to alter the cellular concentration of cAMP (Bertram, 1998). Further, Takaki and Rosim (2000) have reported that aspirin application to *Raphanus sativus* L. seed would increase the tolerance to high temperature and synchronize seed germination. Another pharmaceutical product, celin containing Vitamin-C (ascorbic acid, used as an antioxidant) also effectively controlled seed deterioration during storage (De *et al*., 2004; Guha *et al*., 2012).

Conclusion

Whatever may be the exact mechanism operative in the viability maintenance, pre-storage dry treatments with amla powder @ 2g/kg of seed, red chilli powder @ 1g/kg of seed and ferulic acid @ 500mg/kg of seed may be suggested for the improvement of germinability and field performance and productivity of mung bean seed.

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