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Seed invigoration treatments in different seed sizes of sunflower (*Helianthus annuus* L.) for maintenance of vigour, viability and yield potential

D. Saha and A.K. Mandal*

Department of Plant Physiology, Institute of Agricultural Science,
University of Calcutta, 51/2 Hazra Road, Kolkata – 700 019, India.

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ABSTRACT

Pre-storage dry treatment of freshly harvested (high-vigour) sunflower (*Helianthus annuus* L.) seeds in different seed sizes (*viz*. composite, large, medium and small) with red chilli powder (@ 1 g/kg of seed) and bleaching powder (@ 2g/kg of seed) significantly slowed down seed deterioration under natural ageing conditions and increased field performance and productivity along with reduced leakage of electrolytes, lower volatile aldehyde production with higher dehydrogenase enzyme activity over their respective untreated control. Among the seed sizes, large seeds showed higher post-storage germinability as well as field performance and productivity than the other categories of seeds. Interaction effects were non-significant which indicated that the treatment effects are independent of seed sizes. On the basis of the present investigation, pre-storage dry treatments in large sized seeds with red chilli powder and bleaching powder may be suggested for improved germinability and yield potential of stored high-vigour sunflower seeds.

Key words: Aldehyde production, Field performance, Lipid peroxidaion, Membrane permeability, Seed treatment, Viability, Vigour.

INTRODUCTION

Storage of seeds in sunflower oil seed crop is an acute problem in many states of sub-tropical India (e.g. West Bengal) because of its prevailing high temperature and high relative humidity during the major part of a year which is very conducive to the growth of microorganisms, particularly fungi. In eastern parts of India, sunflower seeds are generally grown in the *rabi* (December-March) season and then stored in moisture pervasive containers, germinability of seeds falls very rapidly in the monsoon months due to absorption of moisture from the humid atmosphere. So, maintenance of vigour and viability of the high-vigour (freshly harvested) seeds for planting in the next season is very difficult.

Pre-storage dry treatments in freshly harvested high-vigour seed of several crop plants are effective in slowing down seed deterioration under subsequent storage conditions (Bhattacharya *et al.*, 2015). Guha *et al.* (2012) have also reported that dry-dressing treatment in high-vigour seed with finely powdered halogenated compounds (bleaching powder), pharmaceutical formulations (aspirin) and crude plant materials (red chilli powder) were effective in controlling seed deterioration of wheat and okra.

The vigour of the seedling is an important factor in crop improvement and it is already shown that large sized seeds would give higher germination percentage (Arnott, 1975), field emergence, crop growth and biological yield (Baalbaki and Copeland, 1997) than small and medium sized seeds.

Keeping the problem of seed storage in mind, in the present study, major emphasis has been given towards standardization of suitable method of seed invigoration treatments in different sizes (composite, large, medium and small) of high-vigour sunflower seeds for improved storability and field performance and productivity.

MATERIALS AND METHODS

Freshly harvested high-vigour sunflower (cv. Morden) seeds were cleaned and sun dried to a moisture content of 8 % for safe storage and then graded into large, medium and small size of seeds by using 3.5 and 2.5 mm oblong sieves along with a composite category (mixture of large, medium and small seeds).

High-vigour seeds of different seed sizes were dry dressed with finely powdered aspirin (active ingredient, *ortho*-acetyl salicylic acid) @ 50 mg/kg of seed, bleaching powder (calcium hypochlorite) @ 2 g/kg of seed and red chilli powder (active ingredient, capsaicin) @ 1g/kg of seed in rubber stoppered glass bottles at room temperature (28±1 ºC) under ambient conditions, which were shaken once daily for seven days to mix thoroughly with the powdered ingredients.

After 7 days of treatment, treated and untreated seeds of different sizes were taken separately in perforated paper packets (containing same amount of seed with equal number of holes) and then all the packets were subjected to natural ageing in a cloth bag under ambient conditions

*Corresponding author's e-mail: akmcu2002@gmail.com.

(RH 76±3.2 % 30±1.6 °C) for 180 days. The packets were shaken at a regular interval for uniform ageing. Germination tests (minimum 400 seeds for each treatment as specified by ISTA, 2009) were done immediately after treatment (before ageing) and after natural ageing for six months following the method of Punjabi and Basu (1982) with minor modifications. Data on germination percentage and seedling length were recorded after 7 days of germination at 23±1 ºC temperature.

Field Experiment: For the field experiment, treated and untreated sunflower seeds of different sizes were restored in rubber-stoppered glass bottles after 7 days of treatment and kept in the laboratory under ambient condition till the sowing in the field. Field experiment was carried out at the Agricultural Experimental Farm of Calcutta University at Baruipur, West Bengal during *rabi* (December-February) season using randomized block design (RBD) with three replications for each treatment. After final land preparation, seeds were sown in the pit @ 10 kg/ha giving a space of 40 cm between the pits and 40 cm between the rows. The plot size was 10 m^2 (4 m×2.5 m) for each replication. A fertilizer dose of N: P_2O_5: K_2O was given @ 80: 40: 40 kg/ha respectively. The entire amounts of phosphate and potash and 50 % of nitrogen were added at the time of sowing along with an immediate post-sowing irrigation for proper field establishment. The rest 50% of nitrogen was top dressed in two equal split doses; one at one month after sowing and another at the flower initiation stage. The crop has received a total of four irrigations and periodical intercultural practices were made during the cultivation period.

Data on plant population/m^2 was taken after 15 days of sowing. After thinning, plant population was fixed at 5 per square meter. The number of seeds per head, yield per m^2 and 1000-seed weight were recorded replication-wise for each treatment after harvest. The experiments were carried out in two consecutive seasons (2012-13 and 2013-14).

Biochemical study: Biochemical studies were conducted immediately after treatment (before ageing) and six months' natural ageing. The membrane permeability of treated and untreated high-vigour sunflower seeds of different sizes were measured by leakage of electrolytes following the method of Anderson *et al.* (1964) and dehydrogenase enzyme activity was measured following the method of Kittock and Law (1968). The chemical assay of volatile aldehyde production by the treated and untreated seed was studied following the method of Wilson and McDonald (1986). Data collected on various parameters were statistically analyzed (Fisher, 1948). Germination percentage data were transformed to their respective angles (arc-sin) before analysis.

RESULT S AND DISCUSSION

Germination test conducted immediately after treatment in different seed sizes (*viz*. composite, large, medium and small) did not show significant differences on germination percentage and seedling vigour between treated and their respective untreated control. However, after natural ageing at 76±3.2 % RH and 30±1.6 ºC temperature for 180 days, red chilli powder and bleaching powder followed by aspirin treated seeds showed significant improvement on germinability and total seedling length over untreated control (Table 1).

TABLE 1: Effect of pre-storage seed invigoration treatments on different seed sizes of sunflower (cv. Morden) on germination percentage and total seedling length (mm) after natural ageing at 76±3.2 % RH and 30±1.6 ºC temperature for 6 months

	Seed sizes (S)									
Treatments	Germination percentage					Total seedling length (mm)				
Physiological Treatments (T)	Composite	Large	Medium	Small	Mean	Composite	Large	Medium	Small	Mean
Control	43 (40.98)	58 (49.60)	40 (39.23)	23 (28.66)	41 (39.82)	110.38	132.63	103.45	73.94	105.10
Aspirin (50 mg/kg of seeds)	48 (43.85)	60 (50.77)	45 (42.13)	28 (31.95)	45 (42.13)	114.38	134.33	109.31	76.38	108.61
Bleaching powder (2 g/kg of seeds)	53 (46.72)	78 (72.03)	53 (46.72)	35 (36.27)	55 (47.87)	152.99	174.46	144.93	88.41	140.20
Red chilli powder (1 g/kg of seeds)	65 (53.73)	80 (63.43)	60 (50.77)	43 (40.98)	62 (52.94)	164.69	184.8	149.93	98.78	149.56
Mean	52 (46.15)	69 (56.17)	50 (45.00)	32 (34.45)		134.86	156.05	128.17	84.38	

	CD value at 0.05P
Pre-storage treatments (T)	4.97
Seed size (S)	4.97
Interaction (T×S)	NS

	CD value at 0.05P
Pre-storage treatments (T)	11.77
Seed size (S)	11.77
Interaction (T×S)	NS

The crop raised from the pre-storage dry treated and untreated seeds showed that red chilli powder and bleaching powder significantly improved yield per unit area, 1000 seed weight and number of seeds per head in all the four categories of seed sizes of sunflower over untreated control (Table 2 and 3).

The physiological and biochemical studies conducted immediately after treatment did not show significant differences between the treated and untreated seeds in different categories of seed sizes. But after natural ageing, red chilli powder followed by bleaching powder in different seed sizes significantly improved membrane permeability as measured by reduced leakage of electrolytes, higher dehydrogenase enzyme activity and lower volatile aldehyde production over their respective untreated control (Table 4).Large seeds showed increased leakage of electrolytes possibly due to larger surface area than small, medium and composite seed lot (Table 4). Large seeds showed significant improvement in field performance than small and medium sized seeds (Table 2 and 3). Interaction effect between seed sizes and pre-storage treatments on germinability, yield and other yield attributes were non-significant for all parameters which indicated that treatment effects were independent of seed sizes.

The literatures on seed size reveal that size of the seed considerably influences germinabilty as well as productivity in a number of crop species (Sadeghi *et al*., 2011). In the present experiment, use of large sized seeds showed greater germinability and better field performance and productivity than small, medium and composite seed lot. Dharmalingam and Basu (1987) demonstrated that large and medium sized seeds of mung bean were superior to small seeds in terms of crop growth and final seed yield. Similarly Sadeghi *et al*. (2011) suggested that the use of large seeds at planting was beneficial in terms of grain yield and seed quality in jute and safflower respectively. McDanial (1969) analysed the effects of seed size in barley on seedling vigour and suggested that mitochondrial efficiency, at least as a link in the metabolic chain was responsible for the vigour of seedling growth and since the specific mitochondrial activity

TABLE 2: Effect of pre-storage seed invigoration treatments on different seed sizes of sunflower (cv. Morden) on plant population/m2 and no. of seeds/head

	Plant population/m^2					No. of seeds/head				
	Seed sizes (S)					Seed sizes (S)				
Physiological Treatments (T)	Composite	Large	Medium	Small	Mean	Composite	Large	Medium	Small	Mean
Control	11	18	13	10	13	630	686	601	549	617
Aspirin (50 mg/kg of seeds	13	19	14	10	14	665	729	623	578	649
Bleaching powder (2 g/kg of seeds)	16	23	14	11	16	718	820	694	620	713
Red chilli powder (1 g/kg of seeds)	19	24	17	12	18	724	845	721	633	731
Mean	15	21	14	11		684	770	660	595	

	CD value at 0.05P		CD value at 0.05P
Pre-storage treatments (T)	NS	Pre-storage treatments (T)	65.38
Seed size (S)	NS	Seed size (S)	65.38
Interaction (T×S)	NS	Interaction (T×S)	NS

TABLE 3: Effect of pre-storage seed invigoration treatments on different seed sizes of sunflower (cv. Morden) on yield/unit area (g/m^2) and 1000 seed weight (g)

	Yield/unit area (g/m^2)					1000 seed weight (g)				
	Seed sizes (S)					Seed sizes (S)				
Physiological Treatments (T)	Composite	Large	Medium	Small	Mean	Composite	Large	Medium	Small	Mean
Control	98.75	121.75	93.25	61.64	93.85	32.02	32.04	30.38	17.77	28.05
Aspirin (50 mg/kg of seeds	99.17	125.25	96.53	65.5	96.61	32.08	33.60	31.86	18.34	28.97
Bleaching powder (2 g/kg of seeds)	113.58	135.5	105.55	83.82	109.61	35.50	38.72	33.91	22.04	32.54
Red chilli powder (1 g/kg of seeds)	115.81	144.16	106.3	91.67	114.49	36.82	40.30	35.73	22.71	33.89
Mean	106.83	131.67	100.41	75.66		34.11	36.17	32.97	20.22	

	CD value at 0.05P		CD value at 0.05P
Pre-storage treatments (T)	11.46	Pre-storage treatments (T)	3.10
Seed size (S)	11.46	Seed size(S)	3.10
Interaction (T×S)	NS	Interaction (T×S)	NS

TABLE 4: Effect of pre-storage seed invigoration treatments on different seed sizes of sunflower (cv. Morden) on the membrane permeability (measured by electrical conductivity of leachate in dsm^{-1}), dehydrogenase enzyme activity (nmole/hr/embryo) and volatile aldehyde production (O.D.) after natural ageing at 76±3.2 % RH and 30±1.6 °C temperature for 6 months

Electrical conductivity (dsm^{-1})

Physiological Treatments (T)	Composite	Seed sizes (S) Large	Medium	Small	Mean
Control	0.078	0.114	0.071	0.069	0.083
Aspirin (50 mg/kg of seeds)	0.057	0.083	0.048	0.029	0.054
Bleaching powder (2 g/kg of seeds)	0.044	0.068	0.037	0.024	0.043
Red chilli powder (1 g/kg of seeds)	0.031	0.049	0.025	0.018	0.031
Mean	0.053	0.079	0.045	0.035	

	CD value at 0.05P
Pre-storage treatments (T)	0.032
Seed size (S)	0.032
Interaction (T×S)	NS

Dehydrogenase activity (nmole/hr/embryo)

Physiological Treatments (T)	Composite	Seed sizes (S) Large	Medium	Small	Mean
Control	7.94	8.64	8.12	5.76	7.62
Aspirin (50 mg/kg of seeds)	7.95	9.51	8.42	7.08	8.24
Bleaching powder (2 g/kg of seeds)	9.18	10.06	9.32	7.70	9.10
Red chilli powder (1 g/kg of seeds)	9.36	10.30	9.59	9.21	9.62
Mean	8.61	9.63	8.87	7.44	

	CD value at 0.05P
Pre-storage treatments (T)	0.70
Seed size (S)	0.70
Interaction (T×S)	NS

Volatile aldehyde production (O.D.)

Physiological Treatments (T)	Composite	Seed sizes (S) Large	Medium	Small	Mean
Control	0.692	0.668	0.697	0.718	0.694
Aspirin (50 mg/kg of seeds)	0.670	0.646	0.677	0.709	0.676
Bleaching powder (2 g/kg of seeds)	0.634	0.607	0.636	0.644	0.630
Red chilli powder (1 g/kg of seeds)	0.630	0.594	0.633	0.639	0.624
Mean	0.657	0.629	0.661	0.678	

	CD value at 0.05P
Pre-storage treatments (T)	0.027
Seed size (S)	0.027
Interaction (T×S)	NS

would remain constant over a wide range of seeds size within a given cultivar, the large seed would produce more vigorous seedling.

Peroxidation of membrane lipid by free radical would increase permeability and cause loss of membrane integrity (Goel and Sheoran, 2003) and reduced energy production (Booth and Bai, 1999) during ageing of seeds. Free radicals can also oxidize proteins (Stadtman, 1992) and nucleic acids (Reiss and Tappel, 1973). Beside the direct role of free radical and lipid peroxidation, the soluble sugars, aldehydes or ketones (by product of lipid peroxidation) non-enzymatically react with amino groups of proteins or nucleic acid to form advanced glycosylation end products. These types of glycation reactions attack enzymes and DNA (Taniguchi *et al.*, 1989).

Regarding the mode of action of the beneficial effect of the dry treatments on the viability maintenance is yet to be elucidated. Capsaicin (the active ingredient of red chilli powder) content in chili is 0.14 % (Cordell and Araujo, 1993). Capsaicin is an acknowledged inhibitor of lipid peroxidation (Dey and Ghosh, 1993) and acts as an anti-oxidant (Nascimento *et al.*, 2013) against free radicals like OH^o and peroxyl radical (Galano and Martinez, 2012). Capsaicin is shown to scavenge free radicals by hydrogen transfer from phenolic hydroxyl group (Galano and Martinez, 2012). Capsaicin also possesses anti-microbial properties which suggest its use as a potential natural inhibitor of pathogenic microorganisms in seed.

Vidyadhar and Singh (2000) observed that halogenation of mustard and maize seeds with chlorine (common bleaching powder) during storage resulting in stabilization of membrane lipid double bonds (Rudrapal and Basu, 1981) ensuring higher seed vigour and enhanced yield (Farooq *et al.*, 2008). Besides these possibilities, halogens like iodine also act as a free radical controlling agent (Pryor and Lasswell, 1975).

The present findings confirm the entry of active ingredients into stored seeds but the mechanism of their entry requires a critical elucidation. However, the cracks and crevices in the seed coat may possibly serve as entry point of exogenously applied substances. Channel proteins present in the membrane (permits entry of water soluble substances across the hydrophobic lipid bi-layer) possibly act as passage for the entry of the dry active ingredients inside cell.

On the basis of the above results, pre-storage dry seed treatment in large sized seeds with red chilli powder and bleaching powder may be suggested for extended storability and improved field performance and productivity of high-vigour sunflower seeds.

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