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Pelleting and priming effect on biochemical parameters of fennel (*Foeniculum vulgare*) seeds

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ABSTRACT

The experiment was carried out to extrapolate the effect of seed pelleting and priming on biochemical traits of fresh and stored fennel (*Foeniculum vulgare* Mill.) seeds in the Laboratory of Department of Seed Science and Technology, CCS Haryana Agricultural University, Hisar during 2017–19 under ambient conditions on cultivar Hisar Swarup (HF 33). Results showed that the biochemical parameters, viz. catalase, peroxidase, dehydrogenase and superoxide dismutase decreased significantly as storage period increased in all the treatments. Prior to storage in case of seed pelleting, biochemical activity was recorded highest in seeds pelleted with Captan (3 g/kg) + Imidacloprid (2 g/kg), followed by neem leaf powder (100 g/kg seeds) and KNO_3 (1.0%), except peroxidase activity. Whereas, in case of priming, maximum biochemical activity was observed in seeds primed with *Trichoderma viride* (8 g/kg seed), followed by KNO_3 (1%), neem leaf extract (10%) and *Trichoderma harzianum* (8 g/kg seed). At the end of experiment, biochemical activity was highest in seeds pelleted with Captan (3 g/kg) + Imidacloprid (2 g/kg) after 18 months of storage followed by KNO_3 (1%), except in peroxidase activity. In case of catalase and dehydrogenase activity, seed pelleting with neem leaf powder (100 g/kg seeds) showed at par value with Captan (3 g/kg) + Imidacloprid (2 g/kg).

Keywords: Biochemical activity, Fennel, Pelleting, Priming, Storage

Fennel (*Foeniculum vulgare* Mill.) is an important commercial cash crop of arid and semi-arid region of India. It is a biennial plant widely cultivated for its edible, strongly flavoured leaves and fruits particularly in Northern India as a *rabi* crop. The fennel seed, which is actually the dried fruit of the fennel plant, is used as a spice, either whole or ground. Quality seed is a basic input in crop production. Successful agriculture programme largely depends on the quality of seeds used for sowing. Thus, the seed producers hold greater responsibility in maintaining genetically pure seeds and preserving the quality of seeds from harvest to next sowing. Seed deterioration is a serious problem in seed spices including fennel, especially in tropical countries like India, where seed attains higher moisture at higher temperature, thereby increasing the respiration and causing biochemical and physiological deterioration during seed ageing. Seed ageing also resulted in higher reduction in germination characteristics associated with catalase activity and ascorbate peroxidase activity decreased significantly (Yadollhhi and Mashayekhi 2013). In view of the fact that seed is a living entity, it is subjected to various environmental stresses, which affect their quality particularly during storage. The viability and vigour of the seeds in storage is regulated by many physico-chemical factors like moisture content, relative humidity, temperature, initial seed quality, physical and chemical composition of seed, gaseous exchange, packaging materials, storage structure, etc. (Doijode 1988). Some physiological and biochemical changes leading to seed deterioration have been related to increased activity of enzymes catalase, peroxidase, *etc*., lipid autoxidation (Koostra and Harrington 1969) and accumulation of toxic metabolites, free radicle damage, decreased protein synthesis, breakdown in mechanism triggering germination, reduced respiration, ultra structural damage to cell and its organelles, accumulation of cytotoxic and mutagenic compounds, etc. (Roberts 1972). With this endeavor the present study on effect of pelleting and priming of fresh and stored fennel seeds with relation to biochemical changes was undertaken.

MATERIALS AND METHODS

The experiment was conducted at the Laboratory of Department of Seed Science and Technology, CCS Haryana Agricultural University, Hisar during 2017–19 for a period of 18 months under ambient conditions on cultivar Hisar Swarup (HF 33) of fennel. Seeds were collected from Department of Vegetable Science with standard

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germination above Indian Minimum Seed Certification Standard (IMSCS). Pelleting of seed was done with three botanicals and four chemicals. Gum arabica was used as binder/adhesive. Wood ash was used as filler material for pelleting of treatments P_5 to P_8. Fourteen different seed priming treatments were given to fennel seeds that involve three botanicals, four chemicals, one hydration-dehydration, three bio-agents each with two different concentrations and a control. The pelleted and primed seeds were stored under ambient conditions and observations were taken at three months interval for 18 months or till the germination falls below IMSCS. Seeds were stored in polythene bags of 700 gauge. Immediately, polythene bags were heat sealed. The enzyme activity was recorded for fresh seeds and at the time of last sampling. The experiment was laid in completely randomized block design and the number of treatments was 22 (7 for pelleted seeds + 14 for primed seeds + control). The statistical method described by Panse and Sukhatme (1961) was followed for the analysis and interpretation of experimental results. The treatment details of the experiment are as follows:

P_1- Control; P_2 - *Pongamia* leaf powder (100 g/kg seed); P_3 - Turmeric leaf powder (100 g/kg seed); P_4 - Neem leaf powder (100 g/kg seeds); P_5 - KH_2PO_4 (2.0%); P_6 - KNO_3 (1.0%); P_7 - K_2SO_4 (1.0%); P_8 - Captan (3 g/kg) + Imidacloprid (2 g/kg); T_2 - Hydration- dehydration; T_3 - $CaCl_2$ (1%); T_4 - NaCl (1%); T_5 - KH_2PO_4 (1%); T_6 - KNO_3 (1%); T_7 - Turmeric leaf extract (10%); T_8 - *Pongamia* leaf extract (10%); T_9 - Neem leaf extract (10%); T_{10} - *Trichoderma viride* (4g/kg seed); T_{11} - *Trichoderma viride* (8g/kg seed); T_{12} - *Pseudomonas fluorecens* (4g/kg seed); T_{13} - *Pseudomonas fluorecens* (8g/kg seed); T_{14} - *Trichoderma harzianum* (4g/kg seed); T_{15} - *Trichoderma harzianum* (8g/kg seed).

The biochemical traits were estimated using following procedures-

Catalase activity test (mg/protein/min): The catalase activity was estimated as per the method followed by Aebi (1983), based on the reduction of potassium dichromate to chromic acetate by hydrogen peroxide. For this, two hundred milligram of imbibed fennel seeds (soaked in water at 30ºC for 24 h) were grounded in a chilled pestle mortar by adding 10 ml phosphate buffer (*p*H 7.8) and a pinch of corning sand. Then the homogenate was centrifuged at 12000 rpm for 20 min at 4ºC. The supernatant so obtained was then again centrifuged at 15000 rpm for 10 min. The clear supernatant was obtained and that is used for estimating the activity of different enzymes. In a side mouthed test tube, 0.5 ml of enzyme extract, dichromate acetic acid reagent (5% potassium dichromate + glacial acetic acid in the ratio of 1:3), 0.5 ml of 0.3 M H_2O_2 and 1.0 ml of 0.1 M buffer phosphate (*p*H 7.0) was added. The reaction mixture was mixed rapidly, incubated at 37ºC for 5 minutes and then 4 ml of dichromate acetic acid reagent was added. These were then heated for 10 min in a boiling water bath and the colour changed into greenish due to the formation of chromic acetate. After cooling of the reaction mixture, absorbance was measured by Systronic Spectrophotometer 169 at 570 nm. The activity of catalase enzyme has been expressed as the amount of enzyme required to bring about a change in absorbance by 0.01 per min.

Peroxidase activity test (mg/protein/min): Peroxidase activity was determined by the method of Shannon *et al*. (1996) following the oxidation of O-dianisidine in the presence of hydrogen peroxide (H_2O_2). For the estimation of peroxidase activity, 2 ml of 0.1 M acetate buffer (*p*H 4.5) and 0.1 ml of the O-dianisidine solution (10 mg O-dianisidine dissolved per 2 ml of methanol) were added to 0.05 ml of enzyme extract. Then 0.1 ml of 0.2 M hydrogen peroxide also added to start the reaction. The absorbance was taken at 470 nm wavelength after every 15 second for 1 min and enzyme unit was expressed as mg/protein/min.The amount of enzyme required to bring about a change in absorbance of 0.01 per min.

Dehydrogenase activity test (OD/g/m): Dehydrogenase activity test was done by using the method given by Kittock and Law (1968). One gram seed of each seed lot replicated thrice was ground to pass through 20 mesh screen. Then the 200 mg flour was soaked in 5 ml of 0.5% tetrazolium solution at 38°C for 3–4 hr, centrifuged at 10000 rpm for 3 min. The supernatant was poured off and the formazan was extracted with 10 ml acetone for 16 h, followed by centrifugation and absorbance of the solution was determined by Systronic spectrophotometer 169 at 480 nm. The observations were expressed as change in OD/g/m.

Superoxide dismutase activity test (mg/protein/min): Superoxide dismutase activity was measured as per method given by Giannopolitis and Ries (1977), by measuring the ability of the enzyme extract to inhibit the photochemical reduction of nitroblue tetrazolium (NBT). For this, 0.1 ml of enzyme extract was added in 3.0 ml of 0.1 M phosphate buffer (*p*H 7.0) containing 1.3 µM riboflavin, 13 mM methionine and 63 µM NBT. Glass tubes containing the mixture were exposed to light for 10 min (two 15 Watt fluorescent lamps). Similar tubes, which were not illuminated, were taken as blank. After illumination the tubes were covered with black cloth and absorbance was measured at 560 nm. Long A-560 was plotted as a function of volume of enzyme extract used in the reaction mixture. From the resultant graph, volume of enzyme extract corresponding to 50% inhibition of the photochemical reaction was obtained and considered as one enzyme unit (Beauchamp and Fridovich 1971).

RESULTS AND DISCUSSION

A decline in the catalase activity can be noticed (Table 1) as the storage period progresses among all the treatments. Prior to storage, catalase activity was found maximum (0.189 mg/protein/min) in seeds pelleted with Captan (3 g/kg) + Imidacloprid (2 g/kg), which was statistically at par with seeds pelleted with KNO_3 (1%), i.e. 0.188 mg/protein/min and neem leaf powder (100 g/kg seeds), i.e. 0.183 mg/protein/min. In case of seed priming, before storage catalase activity was found maximum (0.187 mg/protein/min) in seeds primed with *Trichoderma viride* (8g/kg seed), which

Table 1 Effect of seed pelleting and priming on biochemical parameters in fresh and stored fennel seeds

Treatment	Catalase activity test (mg/protein/min)		Peroxidase activity test (mg/protein/min)		Dehydrogenase activity (OD/g/m)		Superoxide dismutase activity (mg/protein/min)	
	Fresh seeds	Stored seeds	Fresh seeds	Stored seeds	Fresh seeds	Stored seeds	Fresh seeds	Stored seeds
P_1	0.165	0.125	0.377	0.144	0.676	0.540	1.84	0.87
P_2	0.173	0.144	0.453	0.267	0.756	0.617	1.85	0.88
P_3	0.174	0.145	0.459	0.259	0.766	0.610	1.86	0.90
P_4	0.183	0.153	0.499	0.286	0.783	0.670	1.94	0.95
P_5	0.171	0.143	0.442	0.18	0.732	0.615	1.76	0.92
P_6	0.188	0.152	0.475	0.272	0.770	0.667	1.89	1.01
P_7	0.175	0.149	0.460	0.257	0.766	0.613	1.83	0.85
P_8	0.189	0.157	0.521	0.29	0.799	0.673	1.96	1.02
T_2	0.172	0.138	0.444	0.233	0.737	0.610	1.79	0.79
T_3	0.179	0.141	0.480	0.239	0.766	0.616	1.72	0.75
T_4	0.176	0.135	0.475	0.211	0.766	0.603	1.71	0.74
T_5	0.167	0.133	0.416	0.204	0.687	0.603	1.82	0.77
T_6	0.185	0.146	0.493	0.269	0.789	0.619	1.85	0.95
T_7	0.169	0.133	0.437	0.200	0.706	0.580	1.82	0.95
T_8	0.167	0.129	0.391	0.181	0.682	0.567	1.79	0.93
T_9	0.181	0.136	0.485	0.224	0.769	0.610	1.80	0.94
T_{10}	0.172	0.128	0.443	0.166	0.736	0.567	1.82	0.91
T_{11}	0.187	0.148	0.503	0.269	0.791	0.620	1.91	0.97
T_{12}	0.166	0.133	0.417	0.188	0.705	0.577	1.78	0.95
T_{13}	0.168	0.127	0.389	0.157	0.681	0.543	1.81	0.89
T_{14}	0.180	0.14	0.452	0.229	0.739	0.609	1.80	0.90
T_{15}	0.182	0.141	0.484	0.247	0.763	0.611	1.83	0.87
SEm±	0.003	0.003	0.008	0.004	0.010	0.018	0.03	0.017
CD at 5%	0.008	0.007	0.022	0.012	0.029	0.052	0.08	0.040

was statistically at par with KNO_3 (1%), i.e. 0.185 mg/protein/min, whereas minimum catalase activity (0.165 mg/protein/min) was observed in case of control. Catalase activity decreased significantly as storage period increased in all pelleting treatments (Table 1). At the end of experiment, maximum catalase activity (0.157 mg/protein/min) was found in seeds pelleted with Captan (3 g/kg) + Imidacloprid (2 g/kg) followed by KNO3 (1.0%), i.e. 0.152 mg mg/protein/min and Neem leaf powder (100 g/kg seeds), i.e. 0.153 mg/protein/min, while minimum was found in control (0.125 mg/protein/min).

Prior to storage, highest peroxidase activity (0.521 mg/protein/min) was noticed in seeds pelleted with Captan (3 g/kg) + Imidacloprid (2 g/kg), followed by seeds pelleted neem leaf powder (100 g/kg seeds), i.e. 0.499 mg/protein/min, whereas, in case of priming, maximum peroxidase activity was recorded in seeds primed with *Trichoderma viride* (8g/kg seed), i.e. 0.503 mg/protein/min, which was significantly at par with KNO_3 (1%), i.e. 0.493 mg/protein/min, neem leaf extract (10%), i.e. 0.485 mg/protein/min and *Trichoderma harzianum* (8g/kg seed), i.e. 0.484 mg/protein/min, whereas minimum was recorded in control, i.e. 0.337 mg/protein/min. Higher peroxidase activity was noticed in seeds prior to storage as compared to that of 18 months of storage in all the treatments. At the end of experiment, the maximum peroxidase activity (0.29 mg/protein/min) was recorded in seeds pelleted with Captan (3 g/kg) + Imidacloprid (2 g/kg), while the minimum (0.144 mg/protein/min) was recorded in control.

Dehydrogenase activity decreased significantly with advancement in storability (Table 1). Prior to storage, maximum dehydrogenase activity (0.799 OD/g/m) was recorded when seeds were pelleted with Captan (3 g/kg) + Imidacloprid (2 g/kg), which was at par with neem leaf powder (100 g/kg seeds), i.e. 0.783 OD/g/m and KNO_3 (1.0%), i.e. 0.770 OD/g/m. Among the priming treatments, before storage maximum dehydrogenase activity (0.791 OD/g/m) was recorded when seeds were primed with *Trichoderma viride* (8g/kg seed)), followed by priming of seeds with KNO_3 (1%), i.e. 0.789 OD/g/m, and the minimum (0.676 OD/g/m) was obtained in control. At the end of experiment, maximum activity was found in seeds pelleted with Captan @ 3 g/kg + Imidacloprid @ 2 g/kg (0.673 OD/g/m), which was statistically at par with neem leaf powder (100 g/kg seeds), i.e. 0.670 OD/g/m and KNO_3 (1.0%) i.e. 0.667 OD/g/m, whereas minimum in control

(0.540 OD/g/m).

Before storage, the maximum superoxide dismutase activity (1.96 mg/protein/min) was recorded in seeds pelleted with Captan (3 g/kg) + Imidacloprid (2 g/kg), closely followed by neem leaf powder (100 g/kg seeds), i.e. 1.94 mg/protein/min and KNO_3 (1.0%), i.e. 1.89 mg/protein/min. Among priming treatments, maximum superoxide dismutase activity (1.91 mg/protein/min) was recorded in seeds primed with *T. viride* (8g/kg seed), KNO_3 (1%), i.e. 1.85 mg/protein/min and *Trichoderma harzianum* (8g/kg seed), i.e. 1.83 mg/protein/min, whereas minimum (1.71 mg/protein/min) was recorded in case of NaCl (1%). After 18 months of storage, maximum superoxide dismutase activity is observed in seeds pelleted with Captan @3 g/kg+ Imidacloprid @ 2 g/kg, i.e. 1.02 mg/protein/min followed by seeds pelleted with KNO_3 (1.0%), i.e. 1.01 mg/protein/min, whereas the minimum (0.87 mg/protein/min) was recorded in case of control. Hence, the catalase, peroxidise, dehydrogenase and superoxide dismutase activity decreased significantly as storage period increased in all the treatments. These might be due to acceleration of senescence in stored fennel seeds. The experimental results corroborates the findings of Goel and Sheoran (2003) in cotton, Kumar, (2004) in onion and Bhanuprakash *et al*. (2010) in bell pepper who stated that the decreased activities of peroxidase, catalase and superoxide dismutase were associated with accelerated ageing. Also, Singhal *et al*. (2017) and Chauhan *et al*. (2011) found that catalase and peroxidase activity decreased gradually with increased age and the rate of enzyme activity was decreased after 18 months of storage period in naturally aged seeds.

Based on results of the present study, it is concluded that biochemical activity was maximum in seeds pelleted with Captan (3 g/kg) + Imidacloprid (2 g/kg) after 18 months of storage followed by KNO_3 (1%), except in peroxidase activity. In case of catalase and dehydrogenase activity, seed pelleting with neem leaf powder (100 g/kg seeds) showed statistically at par value with Captan (3 g/kg) + Imidacloprid (2 g/kg).

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