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Effect of integrated nutrient management on seed quality of fennel (*Foeniculum vulgare* mill.)

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Abstract
The field experiment was conducted during *Rabi* season of 2016-17 and 2017-18 at Research Farm of Department of Vegetable Science, CCS Haryana Agricultural University, Hisar. It comprised of sixteen treatment levels of fertilizer in randomized block design with three replications. Seed quality parameters of fennel crop were significantly affected by the organic and inorganic fertilizer treatments. Maximum test weight (9.11g), seed density (1.58 g/cc), standard germination (91.43%), seedling length (22.42cm), seedling dry weight (4.06g), vigour index- I & II (2053&317.6), accelerated ageing at 120 h (27.17), enzyme activity DHA & SOD (1.232 & 0.930), field emergence index (8.14) and seedling establishment (81.67) were found significantly highest in treatment T_9: (100%) RDN through vermicopost + *Azotobacter* + PSB. The lowest value found in treatment T_{16}: control (7.33, 1.14, 75.12, 15.75, 2.60, 1184, 222.2, 18.33, 1.127, 0.703, 6.43 and 66.67). The least electrical conductivity of 118.79 μS cm^{-1} g^{-1} was observed under T_9: 100% RDN through vermicompost + *Azotobacter* + PSB which was significantly lower than all their treatment. Under control condition were no chemical, organic and biofertilizer were applied, the electrical conductivity of the seeds was highest (167.31) μS cm^{-1} g^{-1}.

Keywords: Vermicompost, test weight, seedling length, vigour index, electrical conductivity

Introduction
Fennel (*Foeniculum vulgare* L.) is a biennial medicinal and aromatic plant belonging to the family *Apiaceae* (*Umbelliferaceae*). It is a hardy, perennial-umbelliferous herb with yellow flowers and feathery leaves. The flowers are produced in terminal compound umbels. The fruit is a dry seed 4–10 mm long. The seeds of fennel have an active substance, which is called essential oil and most important constituent is anethole that is used in pharmaceutical, food, perfumery and favoring industry (Miraldi, 1999). Fennel essential oil possesses valuable antioxidant, and has antibacterial, anticancer and antifungal activity (Lucinewton *et al.*, 2005; El-Alwadi and Esmat, 2010) [15, 6]. Mature fennel fruits are used as flavoring agents in food products such as pickles, liqueurs, bread, pastries and cheese (Zoubiri *et al.*, 2014) [33]. Fennel fruits are used in diseases like cholera, nervous disorders, constipation, dysentery and colic pain. Fennel also contains minerals and vitamins like calcium, potassium, sodium, iron, phosphorus, thiamine, riboflavin and vitamin C. By virtue of its finest quality and high vitamin content, demand of export due to its high quality seed is increasing steadily. The quality seed is prerequisite to enhance the production and productivity. Use of quality seeds increased productivity of crop by 15-20% (Sidhawani, 1991) [25]. The quality of seed is mainly measured by its genetic purity and capacity to develop into a healthy plant. Further, due to high value of seed spices, the quality of seeds becomes more important. This is mainly measured by its high genetical and physical purity, free from insect-pest and diseases, high vigour, germination percentage and uniformity in appearance. The advantages of high vigour seed are most often associated with rapid and high rate of emergence and stand establishment. There is also a need to have some more reliable parameters that evaluate the seed quality before it is sown in the field. Therefore, an attempt has been made to evaluate the fennel genotypes for the seed quality and vigour.

Materials and Methods
The present experiment was carried out during 2016-17 and 2017-18 at Chaudhary Charan Singh Haryana Agricultural University, Hisar. The field experimental site was located at between 29.15°N latitude 75.69°E longitude with a mean altitude of 215 m above msl. The crop were sown (10th November 2016 for first year and 5th November 2017 for second year) with Randomize block design having three replications with sixteen fertilizer treatment in each replication and having plot size of 3.0 m × 2.4 m with spacing of 45 cm × 20 cm. All recommended agronomic practices were followed timely for successful raising the crop.

Seed harvesting (15 May, 2017 for first year and 12 May 2018 for the second year) was done after full maturity and seeds were sun dried for 3 to 4 days in the field. After proper drying, cleaning and attaining the optimum moisture content the seeds were collected and the completely randomized design (CRD) was followed to conduct laboratory testing for the seed quality parameters in the seed testing laboratory, Department of Seed Science and Technology, CCS Haryana Agricultural University, Hisar.

The observations recorded on seed quality parameters were test weight, seed density, standard germination, seedling length, seedling dry weight, vigour index-I and Vigour index-II, Accelerated ageing, electrical conductivity, Enzyme activity (Dehydrogenasse activity and superoxidase dismutase activity), field emergence and seedling establishment. Test weight: From each genotype seed lot, 1000 seeds were counted and weight was calculated in gram. Standard germination (%): Hundred seeds per replication for individual genotypes were placed separately between two layers of moist germination paper (BP) and then kept in seed germinator at 200C. The final count of normal seedlings was made on the 21st day and expressed as percent germination. Seedling length (cm): The seedling length was measured for ten randomly selected normal seedlings taken from three replications of standard germination test and recorded in centimeter. At last, average of ten seedlings was taken for final calculation. Seedling dry weight (mg): Ten normal seedlings selected for measuring seedling length were further kept in hot air oven for taking dry weight. These are dried at 80 ºC for 48 h and then seedling dry weight was recorded in milligram. The average weight of ten seedlings was taken for further calculations. Vigour indices: The vigour indices were calculated according to the following formulae suggested by Abdul Baki and Anderson (1973). Vigour Index-I: Standard germination (%) x Average seedling length (cm). Vigour Index-II: Standard germination (%) x Average seedling dry weight (mg). Accelerated ageing test: Sufficient number of seeds in a single layer from each genotype were taken on wire mesh tray fitted in plastic boxes having 40 ml of distilled water. The boxes were placed in ageing chamber after closing their lids. The seeds were aged at 40±1° C temperature and about 100 percent relative humidity for 120 hour and tested for germination in three replications of 100 seeds for each genotype. Then seeds were evaluated in terms of standard germination only. Electrical conductivity test: To measure the electrical conductivity, 50 normal and uninjured seeds in three replications were soaked in 75 ml deionized water in 100 ml beakers. Seeds were immersed completely in water and beakers were covered with foil. Thereafter, these samples were kept for 24 h at 25°C. The electrical conductivity of the seed leachates was measured using a direct reading conductivity meter. The conductivity was expressed in µS/cm/ gram (ISTA, 1999).

Dehydrogenase activity: In Dehydogenase activity test (Kittock and Law, 1968), reduction of 2,3,5-triphenyl tetrazolium chloride to red formazan by dehydrogenase enzyme in seed embryo is the basic principle for topographical tetrazolium test for seed viability. It is a quantitative method, which may be used to determine varying dehydrogenase activity between seeds of similar viability, and therefore, it is a measure of seed vigour. Sample of 1 g of each genotype was grounded to pass through a 20-mesh screen to obtain 200 mg flour. The flour was soaked in 5 ml of 0.5 % tetrazolium solution at 38ºC and was centrifuged after 3-4 h at 10,000 rpm for 3 minutes and supernatant was poured off. Formazan was extracted with 10 ml acetone for 16 h followed by centrifugation and then absorbance of the solution was determined by Systronic spectrophotometer 169 at 520 nm. Superoxide dismutase activity: In 3.0 ml of 0.1M phosphate buffer (pH 7.0) containing 1.3 µM riboflavin, 13 mM methionine and 63 µM nitroblue tetrazolium, 0.1 ml of enzyme extract was added. SOD activity was assayed by measuring the ability of the enzyme extract to inhibit the photochemical reduction of NBT. Glass tubes containing the mixture were exposed to light (two 15 W florescent lamps) identical tubes, which were not illuminated, served as blanks. After illumination for 10 min., they were covered with black cloth and absorbance was measured at 560 nm. Long A560 was plotted as function of volume of enzyme extract used in 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). Units: One unit of SOD was defined as the enzyme activity which inhibited the photo reduction of NBT to blue formazan by 50% and expressed as units SOD mg protein^{-1}. Field emergence index: The number of seedlings emerged were counted on each day from 1st day to 30th day and the field emergence index (speed of emergence) was calculated as described by Maguire (1962).

$$\text{Field emergence index} = \frac{\text{No.of seedlings emerged}}{\text{First day of count}} + \ldots\ldots + \frac{\text{No.of seedlings emerged}}{\text{Day of last count (30th)}}$$

Seedling establishment: The seedling establishment was determined by counting the total number of seedlings when the emergence was completed or when there was no further addition in the total emergence *i.e* on 30th day.

Result and Discussion

Seed quality characters like test weight (9.11 g), seed density (1.58 g/cm^3), standard germination (91.43%), seedling length (22.42 cm), seedling dry weight (4.06 mg), vigour index-I (2053) and vigour index-II (317.6), accelerating aging after 120 hours (27.17), electrical conductivity (118.79 µs/cm/g) and enzymatic activity like DHA (1.232/g/ml) and SOD activity (0.930 mg/protein/minute) were significantly improved by T_9 (100% RDN through vermicompost + *Azotobacter* + PSB), over control (T_{16}) i.e. test weight (7.33 g), seed density (1.14 g/cm^3), standard germination (75.12%), seedling length (15.75 cm), seedling dry weight (2.60 mg), vigour index-I (1184) and vigour index-II (222.2), accelerating aging after 120 hours (18.33), electrical conductivity (167.31 µs/cm/g) and enzymatic activity like DHA (1.127/g/ml), SOD activity (0.703 mg/protein/minute).

Also the field emergence index and seedling establishment percentage was found highest in T_9 (8.14 and 81.67%) over T_{16}: Control (6.43 and 66.67%), respectively. The rate of germination might be due to bolder seeds that contain greater metabolites for consumption of embryonic growth during germination as reported by Kumar and Uppar (2007) in moth bean. It might also be attributed to the fact that combined application of biofertilizers and inorganic fertilizers led to the accumulation of more amount of food reserve material due to availability of adequate nutrients right from fertilization until maturity. The results are in close conformity with the findings of Anitha *et al*. (2015) [3] in fenugreek. Vermicompost might influence the physiological processes such as photosynthesis which resulted into in fully filled seeds (Valiki *et al.* 2015) [30]. The test weight was more due to higher leaf area for photosynthesis and effective utilization of these

photosynthates from source (leaves) to sink (reproductive parts) made the filling of seeds better as compared to controlled conditions. Seed density means higher mass per space available. More photosynthates were synthesized which helped in formation of bolder seeds due higher translocation of photosynthates in the seed. This might had increased the seed density. Bolder the seed, more reserve food present in the cotyledons, which resulted into higher seedling length and seedling dry matter during the early phase of the fennel plant. More bolder seeds may provide sufficient food which might resulted into better germination and better seed vigour index-I and II. The present results are in contrast to the results by Ievinsh (2011) [8] which reported that the seed germination of the vegetable crops (Garden beans, peas, beetroot, radish and two cultivars of both cabbage an Swedish turnip) was reduced. He proposed that vermicompost in solid and liquid form contains some phenolic compounds which are species dependent and can inhibit the seed germination. Nutrients such as nitrates, phosphates, and exchangeable calcium and soluble potassium in plant-available forms are present in vermicompost (Orozco *et al.* 1996) [24] which may led to lowering electrical conductivity of the seed and make the seed intact. Higher electrical conductivity in treatment $T_{16:}$ Control, depicted that the increased permeability of the membrane, which declined the compactness of the seeds, leading to excessive leaching of electrolytes, soluble sugars and free amino acids (Doijode, 1988) [5].

Makkawi *et al.* (2008) [17] also of the view that electrical conductivity of the seeds increased due to permeability of seed membranes during longer storability. After attaining the physiological maturity, the process of ageing of seed starts. It is the most challenging scientific problem and of universal concern (Moment, 1978) [20]. Increase in electrical conductivity of seed was due to leaching of electrolytes, nitrogen, and amino acids and cause lose in membrane integrity of aged seed and it ultimately resulted into less vigour and viability of seed (Singhal *et al.* 2017) [26] as found in the present study in treatment T_{16} *i.e.* in control. Germination of seeds is the resumption of active growth by the quiescent embryo in the seed, resulting in the rupture of the seed coat. This results into the emergence of the radicle and Plumule constituting a young plant. Actually, a seed is basically a kernel in which a small embryonic plant is covered by a hard seed coat. It contains some stored food, which promotes the growth of the embryo after receiving the signals from the appropriate climatic conditions (Thomas *et al*. 2006) [27].

The stored food in the seed consists of carbohydrates, proteins and lipids. The hard seed coat inhibits the liberation of this food due to impermeability to oxygen. After permeability to oxygen, the food will get soften and available to growing seed. But during germination, before the oxygen permeability, the availability of food to growing seed is available through the anaerobic respiration. The anaerobic respiration is carried by the activity of enzymes like dehydrogenases that help in catalyzing the catabolic chemical process in anaerobic conditions (Turner and Turner, 1975). Hence the higher dehydrogenase activity in the seed may be considered good for good germination. The dehydrogenase activity in the fennel seed under T_9 (100% RDN through vermicompost + *Azotobacter* + PSB) was found higher as compared to T_{16} (Control) i.e. in controlled conditions where no input was applied showed that T_9 (100% RDN through vermicompost + *Azotobacter* + PSB) improved the dehydrogenase activity. The more available substrate to act upon might be the reason for higher dehydrogenase activity as these enzyme works on the substrate which contains, proteins, lipids and carbohydrates and source of hydrogen. The pH of seed leachates in the fennel seed under T_9 (100% RDN through vermicompost + *Azotobacter* + PSB) was found higher (5.78) as compared to T_{16}: Control (4.76). The results are in confirmation with. Jacobson and Globerson, 1980) [10], Tripathy *et al*. (2009) [28], Vasudevan *et al*. (2008) [31], Valiki *et al*. (2015) [30], Narwal (1995) [22], Nishitkimi and Yagi, (1996) [26], Kumar and Verma (2008) [12], Ghorbanpour *et al*. (2011) [7], Mavi *et al*. (2010) [18], Kumari (2013) [14], Kumar (2015), Alhamdan *et al*. (2011) [2], Yadav & Dhankar (2001) [32] and Mor *et al*. (2009) [21].

Table 1: Effect of integrated nutrient management on test weight, seed density, germination, seedling length and dry weight in fennel

Treatments	Test weight (g)	Seed density (g/cc)	Standard germination (%)	Seedling length (cm)	Seedling dry weight (mg)
T_1: 100% RDN through FYM + *Azotobacter*	7.56	1.27	79.25	17.41	3.01
T_2: 75% RDN through FYM + *Azotobacter*	7.15	1.17	75.19	15.83	2.68
T_3: 100% RDN through Vermicompost + *Azotobacter*	8.62	1.49	87.31	20.68	3.71
T_4: 75% RDN through Vermicompost + *Azotobacter*	8.21	1.38	83.24	19.10	3.36
T_5: RDF (100%) + *Azotobacter*	8.20	1.37	83.33	19.08	3.35
T_6: RDF (75%) + *Azotobacter*	7.77	1.27	79.28	17.48	3.02
T_7: 100% RDN through FYM + *Azotobacter* + PSB	8.22	1.36	83.34	19.06	3.36
T_8: 75% RDN through FYM + *Azotobacter* + PSB	7.80	1.27	79.23	17.52	3.04
T_9: 100% RDN through Vermicompost + *Azotobacter* + PSB	9.11	1.58	91.43	22.42	4.06
T_{10}: 75% RDN through Vermicompost + *Azotobacter* + PSB	8.71	1.47	87.44	20.84	3.74
T_{11}: RDF (100%) + *Azotobacter* + PSB	8.66	1.47	87.39	20.68	3.70
T_{12}: RDF (75%) + *Azotobacter* + PSB	8.22	1.38	83.28	19.08	3.37
T_{13}: 100% RDN through FYM	7.16	1.18	75.22	15.86	2.70
T_{14}: 100% RDN through Vermicompost	8.23	1.40	83.26	19.14	3.39
T_{15}: Recommended dose of fertilizer (N 50: P 25 kg/ha)	7.79	1.29	79.32	17.52	3.04
T_{15}: Control	7.33	1.14	75.12	15.75	2.60
SEm±	0.09	0.02	0.84	0.34	0.08
C.D. at 5%	0.26	0.06	2.44	1.01	0.24

Table 2: Effect of integrated nutrient management on vigour index – (I&II), accelerated ageing E.C, and Dehydrogenase activity in fennel

Treatments	Seed vigour index	Seed vigour index-II	Accelrated ageing at 120 h	Electrical conductivity (µS cm^{-1} g^{-1})	Dehydrogenase activity (OD $g^{-1}ml^{-1}$)
T_1: 100% RDN through FYM + *Azotobacter*	1382	248.4	21.17	147.11	1.161
T_2: 75% RDN through FYM + *Azotobacter*	1189	227.5	19.00	156.99	1.138
T_3: 100% RDN through Vermicompost + *Azotobacter*	1807	290.7	25.33	127.51	1.211
T_4: 75% RDN through Vermicompost + *Azotobacter*	1591	265.9	23.33	137.92	1.185
T_5: RDF (100%) + *Azotobacter*	1591	267.9	23.17	137.53	1.185
T_6: RDF (75%) + *Azotobacter*	1386	247.7	21.00	147.56	1.160
T_7: 100% RDN through FYM + *Azotobacter* + PSB	1589	267.8	23.17	138.29	1.180
T_8: 75% RDN through FYM + *Azotobacter* + PSB	1387	249.2	21.34	148.17	1.159
T_9: 100% RDN through vermicompost + *Azotobacter* + PSB	2053	317.6	27.17	118.79	1.232
T_{10}: 75% RDN through Vermicompost + *Azotobacter* + PSB	1825	299.6	25.33	128.32	1.212
T_{11}: RDF (100%) + *Azotobacter* + PSB	1809	297.4	25.17	128.23	1.205
T_{12}: RDF (75%) + *Azotobacter* + PSB	1588	278.3	23.17	138.00	1.186
T_{13}: 100% RDN through FYM	1193	230.5	19.34	146.37	1.141
T_{14}: 100% RDN through Vermicompost	1595	272.3	23.50	136.54	1.190
T_{15}: Recommended dose of fertilizer (N 50: P 25 kg/ha)	1389	250.4	21.33	155.99	1.166
T_{15}: Control	1184	222.2	18.33	167.31	1.127
SEm±	31.4	4.88	0.48	2.41	0.015
C.D. at 5%	99.9	14.12	1.39	6.97	0.005

Table 3: Effect of integrated nutrient management on SOD, field emergence, seedling establishment and pH of seed leachates in fennel

Treatments	SOD (mg^{-1} $protein^{-1}$ min^{-1})	Field emergence index	Seedling establishment (%)	pH of seed leachates
T_1: 100% RDN through FYM + *Azotobacter*	0.770	6.89	71.33	5.11
T_2: 75% RDN through FYM + *Azotobacter*	0.715	6.48	68.00	4.88
T_3: 100% RDN through Vermicompost + *Azotobacter*	0.875	7.74	78.67	5.58
T_4: 75% RDN through Vermicompost + *Azotobacter*	0.825	7.33	75.33	5.35
T_5: RDF (100%) + *Azotobacter*	0.825	7.31	75.00	5.35
T_6: RDF (75%) + *Azotobacter*	0.770	6.91	71.67	5.14
T_7: 100% RDN through FYM + *Azotobacter* + PSB	0.820	7.29	75.00	5.32
T_8: 75% RDN through FYM + *Azotobacter* + PSB	0.775	6.90	71.67	5.10
T_9: 100% RDN through Vermicompost + *Azotobacter* + PSB	0.930	8.14	81.67	5.78
T_{10}: 75% RDN through Vermicompost + *Azotobacter* + PSB	0.885	7.74	78.33	5.56
T_{11}: RDF (100%) + *Azotobacter* + PSB	0.870	7.72	78.33	5.55
T_{12}: RDF (75%) + *Azotobacter* + PSB	0.825	7.33	75.00	5.33
T_{13}: 100% RDN through FYM	0.725	6.50	68.33	4.89
T_{14}: 100% RDN through Vermicompost	0.830	7.36	75.67	5.36
T_{15}: Recommended dose of fertilizer (N 50: P 25 kg/ha)	0.780	6.92	72.00	5.14
T_{15}: Control	0.703	6.43	66.67	4.76
SEm±	0.011	0.13	0.99	0.06
C.D. at 5%	0.032	0.37	2.86	0.18

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