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Oxidative enzymes activity in cowpea (*Vigna unguiculata*) seeds simultaneous effect of sowing dates, seed treatments and nutrient spray

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ABSTRACT

Oxidative enzyme activity in plants is affected by innumerable direct as well as indirect ways. Sowing dates, nutrients in the form of foliar sprays and pre-seed treatments are the most common paths that can enhance the activity of oxidative enzymes in plants. The present field study was carried out during rainy (*kharif)* seasons of 2019–20 and 2021–22 at the experimental field of Chaudhary Charan Singh Haryana Agricultural University, Hisar, Haryana to evaluate the seed quality and yield of cowpea [*Vigna unguiculata* (L.) Walp] affected by the date of sowing, foliar application and seed treatment. The experiment was laid out in split-split plot design (SSPD) having 3 sowing dates, viz. 30 March (S_1); 30 May (S_2); and 30 June (S_3) and 3 different seed treatments along with control i.e. T_0, Control (untreated seeds); T_1, Captan @2 g/kg; T_2, Vitavax @2 g/kg; and T_3, Bavistin @2 g/kg, replicated thrice. Nutrient spray, viz. FS_0, No spray; FS_1, NPK (18:18:18) @2%; FS_2, NPK (17:44:0)-Urea phosphate @2% and FS_3, NPK (0:0:50)- Sulphate of potash @2% were also done at at 35–45 DAS (days after sowing) and 55–65 DAS. The results reported that delayed sowing time reduced the oxidative enzyme activity and thus affected the productivity of quality cowpea. Captan was observed to be the most efficient and preventive, seed treatment agent. Out of the three-nutrient spray NPK @18:18:18 resulted in the maximum oxidative enzyme activity. The best quality seeds having highest values of oxidative enzyme activity including Catalase, Peroxidase, Dehydrogenase and Superoxide Dismutase were harvested from captan treated seed of second sowing with two sprays of NPK (18:18:18).

Keywords: Captan, Catalase, Legume, Nutrient spray, Peroxidase, Superoxide dismutase, Seeds

Cowpea [*Vigna unguiculata* (L.) Walp.] native to Africa is an annual fodder legume and vegetable crop having good protein content (25%), high fiber content (6.3%) and starch content (50–60%). It is sensitive to low temperature but well adapted under warm and humid climatic conditions (Kassab *et al.* 2012, Shaheen *et al.* 2013, Pandino *et al.* 2013 and Mahrous *et al.* 2015). It is an important pulse crop which is grown in diversified agro-ecological conditions and soils (Jaleel *et al.* 2007, Pandey and Girish 2011). The green cowpea fodder is rich in protein and is considered as balance diet to animals for higher productivity and have a potential to boost farmer's income (Roy *et al.* 2011). For instance, farmers in Nigeria, witnessed a 25% boost in their yearly revenue when they sold dried cowpea fodder during the peak of the dry season (Singh *et al.* 1997).

Although the area seeded for pulse crops is growing steadily, but yield is declining annually (Joshi and Rao 2017). Deficient soil fertility (macro and micronutrients) is most likely the cause of lower productivity of pulses. Apart from this experiment, it was determined that several factors contribute to the lower yield of pulse crops. These include improper fertilizer application, physiological issues such as inefficient assimilate distribution, inadequate pod formation, excessive flower shedding, and insufficient nutrients at crucial stages of crop development. These issues lead to nutrient-related stress, hindered growth, and diminished overall productivity. Due to the fast transfer of nutrients from leaves to growing pods, synchronized flowering in pulse crops has altered the source-sink relationship, making nutrients assimilation significant (Chandrasekhar and Bangarusamy 2003). Another important consideration for harvesting high-quality seed is the time of sowing. To get over these limitations, foliar spray which provides extra nutrition is essential for pulse production because it promotes root growth, nodulation, energy conversion, and other metabolic processes (Maheswari and Karthik 2017). It also increases pod setting, which raises yield. With these considerations in mind, an experiment was conducted to determine the ideal seed treatment, optimal

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date of planting, and optimal nutrient levels for producing higher-quality seed.

MATERIALS AND METHODS

The present field study was carried out during rainy (*kharif*) seasons of 2019–20 and 2021–22 at the experimental field of Chaudhary Charan Singh Haryana Agricultural University, Hisar, Haryana. Seeds of the cowpea variety CS-88, freshly harvested, were obtained from Chaudhary Charan Singh Haryana Agricultural University, Hisar, Haryana. The soil of the experimental field had a sandy loam belonged to the Alfisols order with cation exchange capacity (CEC), exchangeable sodium percentage (ESP) and base saturation percentage (BSP) ranged from 10.5–14.4 cmol (p+)/kg, 0.95–4.2% and 84.3–114.7%, respectively. The DTPA-extractable Zn, Fe, Cu and Mn in mg/kg varied from 0.22–1.80, 1.04–2.49, 0.23–3.21, and 0.94– 3.27, respectively. The experiment was laid out in split-split plot design (SSPD) having 3 sowing dates, viz. 30 March (S_1); 30 May (S_2); and 30 June (S_3) and 3 different seed treatments along with control i.e. T_0, Control (untreated seeds); T_1, Captan @2 g/kg; T_2, Vitavax @2 g/kg; and T_3, Bavistin @2 g/kg, replicated thrice. Nutrient spray, viz. FS_0, No spray; FS_1, NPK (18:18:18) @2%; FS_2, NPK (17:44:0)-Urea phosphate @2%; and FS_3, NPK (0:0:50)- sulphate of potash @2% were also done at at 35–45 DAS (days after sowing) and 55–65 DAS.

Sowing: For every treatment, the crop was sown in a 12 m^2 (4.0 m × 3.0 m) gross plot with a row-to-row distance of 30 cm, according to all approved package instructions.

Test for the oxidative enzyme activity: The seeds obtained from the experiment underwent testing to assess their oxidative enzyme activity. The enzyme extraction process for Superoxide dismutase (SOD) followed the described method, while the calculation of Catalase (CAT) activity was carried out using Sinha's approach with slight modifications (Sinha 1972).

Catalase: The outlined procedure is depicted in Fig. 1, detailing the sequential steps. Initially, 0.4 ml of 0.2 M H_2O_2 and 50 µl of enzyme extract were combined with 0.55 ml of 0.1 M potassium phosphate buffer (*p*H 7.0). Subsequently, 3.0 ml of a 5% potassium dichromate: acetic acid (1:3) solution was added. A control, consisting of 0.6 ml assay buffer and 0.4 ml of 0.2 M H_2O_2 without enzyme extract, was run alongside the samples. Following a 10 min boiling water bath, all tubes were cooled, and the absorbance at 570 nm was measured using a blank of dichromate acetate solution. For calculations, the absorbance of samples was initially subtracted from the control, and the quantity of H_2O_2 was determined using the standard curve. A single CAT unit is defined as the amount of enzyme required to consume one µmol of H_2O_2/min or per mg of protein.

Superoxide dismutase: All phases of the SOD enzyme extraction procedure occurred within a temperature range of 0–4°C. The tissue underwent maceration in a chilled mortar and pestle using 5 ml of 0.1 M phosphate buffer (*p*H 7.5), containing 1 mM EDTA, 10 mM β-mercapto ethanol, and 5% (w/v) polyvinylpyrrolidone (PVP). Following homogenization, the sample was centrifuged for 20 min at 4°C at 10,000 rpm. The crude enzyme preparation was obtained by carefully pouring off the supernatant. The assessment of SOD's ability to inhibit the photochemical reduction of nitro blue tetrazolium (NBT) was conducted using the Beauchamp and Fridovich (1973) method.

A reaction mixture was prepared by combining 2.5 ml of 60 mM Tris-HCl (*p*H 7.8), 0.1 ml of L-methionine (420 mM), 1.80 mM NBT, 90 µM riboflavin, 3.0 mM EDTA, and 0.1 ml of enzyme extract in a total volume of 3.0 ml for enzyme estimation. Following the addition of riboflavin, the tubes were gently shaken and positioned 30 cm below a light source comprising three 20 W fluorescent bulbs. The light was switched on to initiate the reaction, and after 40 min of incubation, it was turned off. To protect the tubes from light after completion of the reaction, they were covered with black cloth. As a control, a reaction mixture that is not exposed to radiation was maintained and it did not change colour. The reaction mixture that lacked the enzyme extract reached its peak shade. At 560 nm, the absorbance was measured. The quantity of enzyme needed to prevent one µmol of NBT from being

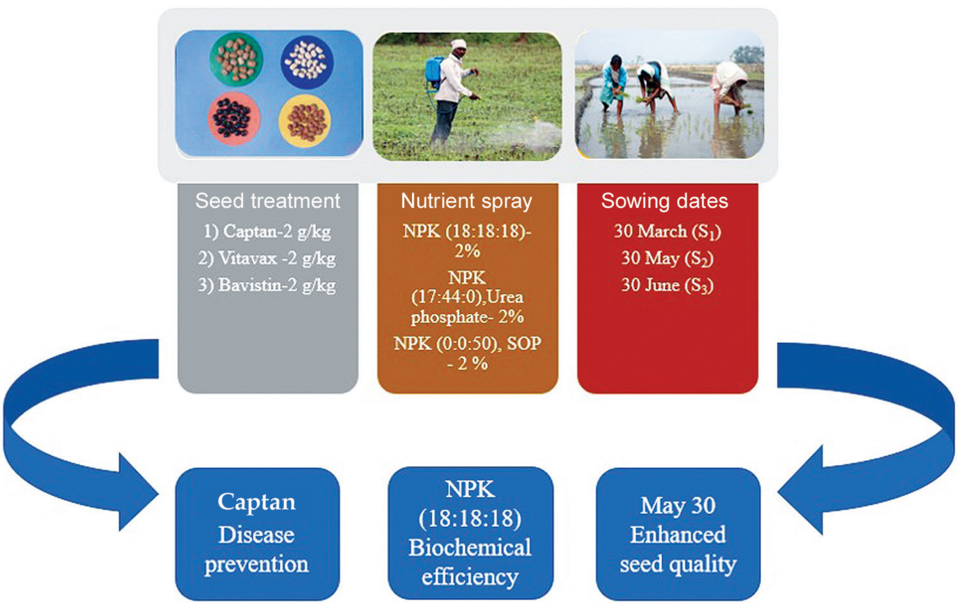

Fig. 1 Graphical abstract of the sequential steps taken during the experiment.

photo-reduced is known as an enzyme unit. The enzyme activity is given here in units/g fresh weight for kinetic and regulatory purposes, and it was as (Giannopolitis and Ries 1977):

SOD units =V-v divided by v

where V, Rate of assay reaction in absence of SOD; v, Rate of assay reaction in presence of SOD.

Percentage inhibition was calculated as (Asadaand Nagate 1976):

Per cent inhibition = V-v divided by V × 100.

Peroxidase: The enzyme extraction procedure for peroxidase (POX) activity followed a similar protocol to that of superoxide dismutase (SOD) and catalase (CAT), as outlined by Rao *et al.* 1998. To determine POX activity, the speed of guaiacol oxidation in the presence of H_2O_2 at 470 nm was calculated. In the peroxidase enzyme estimation process, a cuvette was filled with 2.15 ml of 0.1 M Tris-HCl buffer (*p*H 7.0), 0.6 ml of 1% guaiacol, and 0.1 ml of the enzyme extract. The solution was thoroughly mixed and adjusted to 100% transmission at 470 nm. Subsequently, 0.15 ml of 100 mM H_2O_2 was added, and the initial reading was recorded at 470 nm. The increase in absorbance was monitored at 15-second intervals for up to 3 min.

Dehydrogenase: The dehydrogenase activity (DHA) estimation test was conducted using Kittock and Law's (1968) methodology. Every seed lot was replicated three times, and 1g of the seeds was broken down so they could go through a 20-mesh size screen. For a duration of 3 h, 200 mg of flour underwent immersion in 5 ml of a 0.5% tetrazolium solution at a temperature of 38±1°C. Following a 3 min centrifugation at 10,000 rpm, the supernatant was removed. The formazan extraction involved soaking in 10 ml of acetone for 16 h, followed by centrifugation, and the absorbance spectrum of the resulting solution was measured at 480 nm using the Eppendorf Biospectrometer. The obtained results were expressed as the variance in OD/g/ml. Statistical analysis of the data collected during the investigation was performed using the OPSTAT statistical software.

RESULTS AND DISCUSSION

Catalase enzyme activity (µmoles/mg dry weight): Out of the three sowing dates, maximum catalase activity was recorded in second sowing (30 May) (44.87), captan treated seeds (49.09 in 2019 and 48.47 in 2021) (Table 1). All the three seed treatments were found significant over control. Maximum catalase activity (43.90) was reported in captan treated seeds, followed by vitavax (40.29), bavistin (38.69) and untreated seeds (36.13). During year 2021, the order of catalase activity observed was (43.45) in captan treated seeds>vitavax (39.58)>bavistin (37.67). Minimum (35.70) catalase activity was recorded in untreated seeds.

Among the different foliar spray, maximum catalase activity (42.37) during 2019 was recorded in NPK (18:18:18) spray treatment which was significantly higher than sulphate of potash (40.64), urea phosphate (39.47) and control (36.53). During 2021, same trend was observed with maximum catalase activity (41.82) in NPK (18:18:18) treatment followed by sulphate of potash (40.03), urea phosphate (38.94) and control (35.62).

The minimum catalase activity (32.95) was found in third sowing in year 2019. In year 2021, the maximum catalase activity (44.36) was found in second sowing and minimum catalase activity (32.62) was found in third sowing. It can be concluded that in contrast to untreated areas, the application of the fungicide bavistin resulted in a noteworthy elevation in the concentrations of total phenols, flavonoids, antioxidant enzymes, reduced glutathione, lipid peroxidation, and hydrogen peroxide. Conversely, when compared to untreated plants, fungicides like Topsim (Thiophanate-methyl) and Monceren (Pencycuron) increased all the mentioned parameters, except for phenols, MDA (malondialdehyde), and H_2O_2 (hydrogen peroxide). These results are in accordance with Zolfaghari *et al.* (2019).

Superoxide dismutase (SOD) enzyme activity (µmoles/mg dry weight): Superoxide dismutase activity was recorded (Table 2) in second sowing (30 May) in captan treated seeds, 68.77 in 2019 and 63.77 in year 2021 and during year 2019, the results ranged as-captan (62.45)>vitavax (58.58)>bavistin (56.67) treated seeds. During year 2021, maximum superoxide dismutase activity (58.45) was found in captan treated seeds which was significantly higher than vitavax (54.58) and bavistin (52.67) treated seeds.

Also, maximum SOD activity (60.82) during first year was recorded in NPK (18:18:18) spray treatment which was significantly higher than sulphate of potash (59.03) and urea phosphate (57.94) and control (54.62) and in second year maximum SOD activity (56.82) was recorded in plot sprayed with NPK (18:18:18) followed by sulphate of potash (55.03) and urea phosphate (53.94).

Furthermore, maximum SOD activity was recorded in second sowing (64.36) followed by first sowing (59.32) and least (50.62) was found in third sowing in year 2019. In year 2021, the maximum superoxide dismutase activity (59.36) was found in second sowing and minimum SOD activity (47.62) was observed in third sowing. The highest accumulation of hydroxyl cinnamates and to a lower extent flavonoids during the first sowing may be explained by the results that plants vary the production of their secondary metabolites in response to climatically induced variations in temperature and solar exposure. The results are in accordance with Jabeen and Ahmad (2011).

Peroxidase (POX) enzyme activity (µmoles/mg dry weight): Maximum peroxidase activity was recorded in second sowing in captan treated seeds (Table 3). It was 0.369 and 0.319 in year 2019 and 2021, respectively. The effect of all the three seed treatments was found significant over control. Maximum peroxidase activity (0.332) was found in captan treated seeds, followed by vitavax (0.313), bavistin (0.303) and untreated seeds (0.294). During year the 2021, maximum peroxidase activity (0.292) was found

Table 1 Effect of date of sowing, seed treatment and foliar spray on catalase activity (μmoles/mg dry weight) of cowpea seed

2019–20

	S_1					S_2					S_3				
	FS_0	FS_1	FS_2	FS_3	Mean	FS_0	FS_1	FS_2	FS_3	Mean	FS_0	FS_1	FS_2	FS_3	Mean
T_0	31.54	43.28	36.36	40.98	38.04	36.68	43.53	39.75	43.81	40.94	23.24	33.01	29.24	32.16	29.41
T_1	41.28	51.21	45.46	44.79	45.69	46.73	51.65	49.86	48.10	49.09	32.25	39.19	38.28	37.95	36.92
T_2	39.29	44.10	40.16	42.70	41.56	45.33	45.39	44.36	46.64	45.43	31.59	36.37	32.62	34.91	33.87
T_3	37.67	42.47	40.66	40.91	40.43	42.72	44.68	44.93	43.76	44.02	30.02	33.49	31.91	30.99	31.60
Mean	37.45	45.27	40.66	42.34		42.87	46.31	44.72	45.58		29.28	35.52	33.01	34.00	

Overall mean						Factors	CD (P=0.05)	Factors	CD (P=0.05)
Seed treatment		Foliar spray		Date of sowing		Factor(S)	0.05	Interaction S × FS	NS
T_0	36.13	FS_0	36.53	S_1	41.43	Factor(T)	0.61	Interaction T × FS	1.41
T_1	43.90	FS_1	42.37	S_2	44.87	Interaction S × T	1.07	Interaction S × T × FS	NS
T_2	40.29	FS_2	39.47	S_3	32.95	Factor(FS)	0.70		
T_3	38.69	FS_3	40.64						

2021–22

	S_1					S_2					S_3				
	FS_0	FS_1	FS_2	FS_3	Mean	FS_0	FS_1	FS_2	FS_3	Mean	FS_0	FS_1	FS_2	FS_3	Mean
T_0	30.04	41.62	37.48	39.40	37.13	35.10	44.19	40.98	42.12	40.60	23.72	32.72	30.14	30.92	29.38
T_1	39.88	49.24	44.14	45.70	44.74	45.15	52.44	48.41	49.08	48.77	33.08	38.42	37.16	38.72	36.85
T_2	37.96	42.40	39.76	41.86	40.50	43.80	46.08	43.92	45.72	44.88	31.28	35.66	32.30	34.22	33.37
T_3	36.40	40.84	39.10	39.34	38.92	41.28	45.36	43.20	42.90	43.19	29.72	32.84	30.68	30.38	30.90
Mean	36.07	43.53	40.12	41.57		41.33	47.02	44.13	44.95		29.45	34.91	32.57	33.56	

Overall mean						Factors	CD (P=0.05)	Factors	CD (P=0.05)
Seed treatment		Foliar spray		Date of sowing		Factor(S)	0.57	Interaction S × FS	NS
T_0	35.70	FS_0	35.62	S_1	40.32	Factor(T)	0.66	Interaction T × FS	1.32
T_1	43.45	FS_1	41.82	S_2	44.36	Interaction S × T	NS	Interaction S × T × FS	NS
T_2	39.58	FS_2	38.94	S_3	32.62	Factor(FS)	0.66		
T_3	37.67	FS_3	40.03						

S_1, 30 March (summer); S_2, 30 May; S_3, 30 June (*kharif*); T_0, Control (untreated seeds); T_1, Captan @2 gm/kg; T_2, Vitavax @2 gm/kg; T_3, Bavistin @2 gm/kg; FS_0, No spray; FS_1, NPK (18:18:18) @2%; FS_2, NPK (17:44:0)-Urea phosphate @2%; FS_3, NPK (0:0:50)-SOP Sulphate of potash @2%.

Table 2 Effect of date of sowing, seed treatment and foliar spray on superoxide dismutase (µmoles/mg dry weight) activity of cowpea

2019–20															
	S_1					S_2					S_3				
	FS_0	FS_1	FS_2	FS_3	Mean	FS_0	FS_1	FS_2	FS_3	Mean	FS_0	FS_1	FS_2	FS_3	Mean
T_0	49.04	60.62	56.48	58.40	56.14	55.10	64.19	60.98	62.12	60.60	41.72	50.72	48.14	48.92	47.38
T_1	58.88	68.24	63.14	64.70	63.74	65.15	72.44	68.41	69.08	68.77	51.08	56.42	55.16	56.72	54.85
T_2	56.96	61.40	58.76	60.86	59.50	63.80	66.08	63.92	65.72	64.88	49.28	53.66	50.30	52.22	51.37
T_3	55.40	59.84	58.10	58.34	57.92	61.28	65.36	63.20	62.90	63.19	47.72	50.84	48.68	48.38	48.91
Mean	55.07	62.53	59.12	60.58		61.33	67.02	64.13	64.96		47.45	52.91	50.57	51.56	
Overall Mean							Factors		CD (P=0.05)			Factors		CD (P=0.05)	
Seed treatment		Foliar spray		Date of sowing			Factor(S)		0.84			Interaction S × FS		NS	
T_0	54.70	FS_0	54.62	S_1	59.32		Factor(T)		0.97			Interaction T × FS		1.956	
T_1	62.45	FS_1	60.82	S_2	64.36		Interaction S × T		NS			Interaction S × T × FS		NS	
T_2	58.58	FS_2	57.94	S_3	50.62		Factor(FS)		0.98						
T_3	56.67	FS_3	59.03												

2021–22															
	S_1					S_2					S_3				
	FS_0	FS_1	FS_2	FS_3	Mean	FS_0	FS_1	FS_2	FS_3	Mean	FS_0	FS_1	FS_2	FS_3	Mean
T_0	45.04	56.62	52.48	54.40	52.14	50.10	59.19	55.98	57.12	55.60	38.72	47.72	45.14	45.92	44.38
T_1	54.88	64.24	59.14	60.70	59.74	60.15	67.44	63.41	64.08	63.77	48.08	53.42	52.16	53.72	51.85
T_2	52.96	57.40	54.76	56.86	55.50	58.80	61.08	58.92	60.72	59.88	46.28	50.66	47.30	49.22	48.37
T_3	51.40	55.84	54.10	54.34	53.92	56.28	60.36	58.20	57.90	58.19	44.72	47.84	45.68	45.38	45.91
Mean	51.07	58.53	55.12	56.58		56.33	62.02	59.13	59.96		44.45	49.91	47.57	48.56	
Overall Mean							Factors		CD (P=0.05)			Factors		CD (P=0.05)	
Seed treatment		Foliar spray		Date of sowing			Factor(S)		0.056			Interaction S × FS		NS	
T_0	50.70	FS_0	50.62	S_1	55.32		Factor(T)		0.846			Interaction T × FS		1.919	
T_1	58.45	FS_1	56.82	S_2	59.36		Interaction S × FS		NS			Interaction S × T × FS		NS	
T_2	54.58	FS_2	53.94	S_3	47.62		Factor(FS)		0.959						
T_3	52.67	FS_3	55.03												

S_1, 30 March (summer); S_2, 30 May; S_3, 30 June (*kharif*); T_0, Control (untreated seeds); T_1, Captan @2 gm/kg; T_2, Vitavax @2 gm/kg; T_3, Bavistin @2 gm/kg; FS_0, No spray; FS_1, NPK (18:18:18) @2%; FS_2, NPK (17:44:0)-Urea phosphate @2%; FS_3, NPK (0:0:50)-SOP Sulphate of potash @2%.

Table 3 Effect of date of sowing, seed treatment and foliar spray on peroxidase activity (µmoles/mg dry weight) of cowpea

	S_1					S_2					S_3				
2019–20															
	FS_0	FS_1	FS_2	FS_3	Mean	FS_0	FS_1	FS_2	FS_3	Mean	FS_0	FS_1	FS_2	FS_3	Mean
T_0	0.265	0.323	0.302	0.312	0.301	0.301	0.346	0.330	0.336	0.328	0.224	0.269	0.255	0.260	0.252
T_1	0.314	0.361	0.336	0.343	0.339	0.351	0.387	0.367	0.370	0.369	0.270	0.297	0.291	0.299	0.289
T_2	0.305	0.327	0.314	0.324	0.318	0.344	0.355	0.344	0.354	0.349	0.262	0.283	0.267	0.276	0.272
T_3	0.297	0.319	0.311	0.312	0.310	0.331	0.352	0.341	0.340	0.341	0.253	0.269	0.258	0.257	0.259
Mean	0.295	0.333	0.316	0.323		0.332	0.360	0.346	0.350		0.252	0.280	0.268	0.273	
Overall mean							Factors		CD (P=0.05)			Factors		CD (P=0.05)	
Seed treatment		Foliar Spray		Date of Sowing			Factor(S)		0.001			Interaction S × FS		NS	
T_0	0.294	FS_0	0.293	S_1	0.317		Factor(T)		0.006			Interaction T × FS		0.010	
T_1	0.332	FS_1	0.324	S_2	0.347		Interaction S × T		NS			Interaction S × T × FS		NS	
T_2	0.313	FS_2	0.310	S_3	0.268		Factor(FS)		0.006						
T_3	0.303	FS_3	0.315												
2021–22															
	S_1					S_2					S_3				
	FS_0	FS_1	FS_2	FS_3	Mean	FS_0	FS_1	FS_2	FS_3	Mean	FS_0	FS_1	FS_2	FS_3	Mean
T_0	0.225	0.283	0.262	0.272	0.261	0.251	0.296	0.280	0.286	0.278	0.194	0.239	0.225	0.230	0.222
T_1	0.274	0.321	0.296	0.303	0.299	0.301	0.337	0.317	0.320	0.319	0.240	0.267	0.261	0.269	0.259
T_2	0.265	0.287	0.274	0.284	0.278	0.294	0.305	0.294	0.304	0.299	0.232	0.253	0.237	0.246	0.242
T_3	0.257	0.279	0.271	0.272	0.270	0.281	0.302	0.291	0.290	0.291	0.223	0.239	0.228	0.227	0.229
Mean	0.255	0.293	0.276	0.283		0.282	0.310	0.296	0.300		0.222	0.250	0.238	0.243	
Overall mean							Factors		CD (P=0.05)			Factors		CD (P=0.05)	
Seed treatment		Foliar spray		Date of sowing			Factor(S)		0.001			Interaction S × FS		NS	
T_0	0.254	FS_0	0.253	S_1	0.277		Factor(T)		0.004			Interaction T × FS		0.010	
T_1	0.292	FS_1	0.284	S_2	0.297		Interaction S × T		NS			Interaction S × T × FS		NS	
T_2	0.273	FS_2	0.270	S_3	0.238		Factor(FS)		0.005						
T_3	0.263	FS_3	0.275												

S_1, 30 March (summer); S_2, 30 May; S_3, 30 June (*kharif*); T_0, Control (untreated seeds); T_1, Captan @2 gm/kg; T_2, Vitavax @2 gm/kg; T_3, Bavistin @2 gm/kg; FS_0, No spray; FS_1, NPK (18:18:18) @2%; FS_2, NPK (17:44:0)-Urea phosphate @2%; FS_3, NPK (0:0:50)-SOP Sulphate of potash @2%.

in captan treated seeds followed by vitavax (0.273) and bavistin (0.263).

Among the different foliar spray, maximum peroxidase activity (0.324) during year 2019 was recorded in NPK (18:18:18) spray treatment which was significantly higher than sulphate of potash (0.315), urea phosphate (0.310) and control (0.293). During year 2021 maximum peroxidase activity (0.284) was recorded in treatment sprayed with NPK (18:18:18) which was significantly higher than all the three treatments i.e., sulphate of potash (0.275), urea phosphate (0.270) and control (0.253).

During both the years of experiment, peroxidase activity was significantly affected by different sowing times. Maximum peroxidase activity was recorded in second sowing (0.347) followed by first sowing (0.317). The minimum peroxidase activity (0.268) was found in third sowing in year 2019. In year 2021, the maximum peroxidase activity (0.297) was found in second sowing and minimum peroxidase activity (0.238) was found in third sowing. Fungicides generally degrade the quality of seeds by changing the composition of seeds and causing undesirable biochemical and metabolic changes, even though they help reduce the occurrence of fungal infections and increase quantitative yield. So overall, it can be concluded that in the entire experiment, the treatment with captan was found best before sowing, and second time of sowing followed by NPK (18:18:18) were observed to be the optimum date of sowing, best nutrient, and seed treatment for higher quality seed production of promising varieties. The results are in line with Jabeen and Ahmad (2011).

Dehydrogenase enzyme activity (OD/g/ml): Maximum dehydrogenase activity was recorded in second sowing (30 May) in captan treated seeds (Supplementary Table 1). It was 2.83 in 2019 and 2.82 in year 2021. The effect of all the three seed treatments was found significant over control. Maximum dehydrogenase activity (2.56) was found in captan treated seeds, followed by vitavax (2.31), bavistin (2.21) and untreated seeds (2.04). During year 2021, maximum peroxidase activity (2.53) was found in captan treated seeds followed by vitavax (2.27) and bavistin (2.15). The effect of seed treatment was found significant over control. Minimum (2.04) peroxidase activity was recorded in untreated seeds. Among the different foliar spray, maximum dehydrogenase activity (2.46) during first year was recorded in NPK (18:18:18) spray treatment which was significantly higher than sulphate of potash (2.34), urea phosphate (2.26) and control (2.06). During second year maximum dehydrogenase activity (2.42) was recorded in treatment sprayed with NPK (18:18:18) which was significantly higher than all the three treatments i.e., sulphate of potash (2.30), urea phosphate (2.23) and control (2.01).

During both the years of experiment, dehydrogenase activity was significantly affected by different sowing times. Maximum dehydrogenase activity was recorded in second sowing (2.55) followed by first sowing (2.32). In the year 2021, the maximum dehydrogenase activity (2.52) was found in second sowing and minimum dehydrogenase activity (1.94) was found in third sowing. Similar findings reported by Nawaz *et al.* (2015) in wheat.

Based on present investigation, it is concluded that to produce quality seed of cowpea, the crop should be sown in last week of May. The better seed quality was found in second sowing (30th may), which was followed by early sowing S_1 (30th March) whereas lowest seed quality was found in late sowing S_3 (30th June). Different seed treatments have significantly affected the quality of cowpea seed. The seed treatment with captan fungicide before sowing reduces the disease incidence and enhances the seed quality. The foliar spray of NPK 18:18:18 has maximum effect on biochemical seed quality parameters. Nutritional foliar spray of NPK (18:18:18) helps in providing proper plant nutrition and getting higher yield and good quality seed.

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