Indian Journal of Agricultural Sciences **92** (3): 397–401, March 2022/Article
https://doi.org/10.56093/ijas.v92i3.122704

Physiological and biochemical responses of okra seed (*Abelmoschus esculentus*) to fungicides and containers

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Received: 21 October 2021; Accepted: 04 January 2022

ABSTRACT

The present study was carried out at Department of Seed Science and Technology, CCS Haryana Agriculture University, Hisar, Haryana during 2018–20, that comprised of seven fungicides used as seed treatment @2 gm/kg, viz. carbendazim, tebuconazole, difeconazole, flusilazole, chlorothalonil, azoxystrobin and vitavax power along with control. The freshly harvested okra seeds treated with these fungicides were stored in three different containers, viz. polythene bag, hermetic bag and metal box under ambient conditions up to a period of 18 months and their quality was assessed at a regular interval for three months. A significant decline in seed quality was observed in all the treatments as the period of ageing increased. Seeds treated with azoxystrobin and stored in metal box recorded significantly higher germination per cent (74.9%), shoot length (8.8 cm), root length (6.8 cm), seedling dry weight (0.230 g), vigour index-I (1171), vigour index-II (17.22), catalase (198.9 mg/protein/min), superoxidase dismustase (132.2 mg/protein/min), dehydrogenase (0.46 OD/g/ml) and peroxidise (676 mg/protein/min) and lower electrical conductivity (1.036 μS/cm/g) after 18 months of storage as compared to control. Hence, the study recommends that use of appropriate packaging material and seed treatment would leads to prolong the longevity and health of okra seeds.

Keywords: Containers, Fungicides, Okra, Seed quality, Seed storage

Vegetables are important component of human diet for maintenance of good health. China is the leading producer of fresh vegetables with a production volume of nearly 549 million metric tonnes, followed by India with approximately 128 million metric tonnes in 2018 (FAO-Statistica 2021). Okra is one of the most commonly known and utilized species of the family Malvacae, an economically important vegetable crop grown in tropical and sub-tropical parts of the world. The centre of origin of okra is Ethiopia (Satish and Eswar 2013), thereafter it was propagated in different parts of world and India by the 12th century BC. India is the global leader in the production with cultivation area of 5.1 lakh ha and annual production of 61.26 lakh tonnes (FAOSTAT 2018). The crop is grown over wide range of soils and climatic conditions, both in summer and rainy seasons. The importance of seed in agriculture is very well known in developing countries like India, where the majority of the population and GDP significantly depend upon agriculture. Deterioration of seed is associated with ageing phenomenon which is stated as an irreversible degradation change in the quality of a seed. Among all deteriorative changes, membrane degradation has been proposed as the primary event in ageing, which effects mainly by leaching of compounds; particularly electrolytes, high level of lipid peroxidation, chromosomal damage, loss of various enzymes, degradation of respiratory system and loss of ATP production. Good storage is an essential requirement in seed programme for the maintenance of high germination and vigour from harvesting to next planting season.

In storage condition seeds are to be mainly protected from insects and pathogens. Seed treatment serves as first line of defence which provide prohylactic protection aginst storage fungi and insects thus improving germination, crop stand, seedling emergence and plant vigour. The phenomena of seed deterioration is irreversible but the rate could be slowed down by packing the seeds in controlled conditions (Yalamalle and Kuchlan 2016). Looking into above facts, the present study was carried out to know the biochemical and physiological responses of okra seed to fungicides and containers.

MATERIALS AND METHODS

The present investigation was conducted during 2018–20 at Department of Seed Science and Technology, CCS Haryana Agricultural University, Hisar, Haryana. The location of the city is at 29^o 10 North Latitude and 75^o 46 East Latitudes and at an altitude of about 215.2 m

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amsl. The climate of region is semi-arid with hot and dry desiccating winds accompanied by frequent dust storms of high velocity in summer, severe cold during winter and warm humid conditions during rainy season. The mean monthly maximum and minimum temperature showed a wide range of fluctuations during both the years. The mean monthly maximum temperature of 43–45^{o}C was common during the summer months of May to June while minimum temperature during the winter months of December and January sometimes went as low as 0^{o}C.

Okra seed of cv. Varsha Uphar harvested in November 2017 was procured from Department of Vegetable Science, CCS HAU. The seed lots were treated with the following fungicides, viz. carbendazim 75% WP, tebuconazole 2 DS, difeconazole 25% EC, flusilazole 40% EC, chlorothalonil 78.2% WP, azoxystrobin 23% SC and vitavax power (Carboxin 37.5% + Thiram 37.5% DS) along with Untreated (control) and stored in three different containers, viz. polythene bag, hermetic bag and metal box. The okra seeds and fungicides were weighed 42 g and 0.084 g respectively, The seeds and fungicides were mixed in beakers and shaked for some time for uniform dressing all over the seeds. Total numbers of treatments (8×3=24) were kept in different containers in the laboratory under ambient conditions. The experiment consisted of two factors (three different packing materials as storage container were used as level factor "C" and the eight fungicides treatments were used as level factor "T") and was laid out in completely randomized design (CRD). Seeds were taken from each of the different containers at quarterly interval up to 18 months (October 2018 to March 2020) and observations were recorded for seed technological parameters.

S*tandard germination test (%) as per ISTA (2011)*: Four hundred seeds of each crop for each treatment were placed in three replications in between the germination paper and placed in germinators at 25±1°C. The germination was checked on 10th day and normal seedlings were considered for per cent germination.

$$\text{Seed germination (\%)} = \frac{\text{Number of seeds germinated}}{\text{Total number of seeds placed for germination}} \times 100$$

Shoot and Root length (cm): Ten normal seedlings per replication were selected at random at the time of final count of standard germination. Shoot and root length was measured using a measuring scale and average length was recorded.

Dry seedling weight (g): Seedling dry weight was assessed after the standard germination test. The ten seedlings of each treatment replicated thrice were taken. Seedlings were dried in hot air oven for 24 h at 80±1°C. The dried seedlings were weighed and average seedling dry weight of each treatment was calculated.

Vigour indices: Seedling vigour indices (I & II) were calculated according to the standard method suggested by Abdul-Baki and Anderson (1973).

Electrical conductivity test (µS/cm/g): Electrical conductivity of the seed leachates was measured to know the status of membrane permeability as per ISTA (2011).

Enzyme activity tests: The catalase activity was assessed by using the method described by Aebi (1983), which was based on the reduction of potassium dichromate to chromic acetate by hydrogen peroxide. POD activity was determined by the method of Shannon *et al.* (1966) following the oxidation of O-dianisidine in the presence of hydrogen peroxide (H_2O_2). Dehydrogenase activity was measured by method suggested by Kittock and Law (1968) and SOD activity was determined by the method suggested by Giannopolitis and Ries (1977).

RESULTS AND DISCUSSION

The seed quality attributes declines with the passage of time. The hygroscopic nature of seed affects its quality by change in environmental conditions, viz. relative humidity, temperature, moisture content, gaseous exchange, packaging material, etc (Yalamalle and Kuchlan 2016). Seed lot utilized in experiment was having initial germination (91%) above the Indian Minimum Seed Certification Standards (IMSCS). The result mentioned below under each parameter was recorded at quaterly intervals. However, the results explained below are for last observation recorded at 18 months of storage (Table 1 and 2).

Data showed (Table 1) that the germination percentage declined gradually with the passage of storage time. All the treatments showed better germination as compared to control. Significantly higher germination was recorded in treatment T_7 (74.9%) followed by treatment T_5 (73.4%) and lowest germination was recorded in control T_1 (66.9%) was might be due to the seed deterioration. Containers effect was found significant and the highest value was showed in metal box. The best interaction (75.7%) was observed in seeds treated with Azoxystrobin (T_7) and stored in metal box (C_3). The decline in germination was may be due to increase in moisture content which ultimately leads to increase in seed respiration, membrane leakage due to increased temperature and relative humidity. The results are in accordance with Arif *et al.* (2006) as they concluded that germination percentage decreased gradually as storage period increased. The results are in conformity with earlier findings of Yalamalle and Kuchlan (2016) in onion seeds.

The data (Table 1) revealed that shoot and root length of okra seeds was also recoreded in decreasing trend starting from initial to last month of storage. All the treatments recorded higher shoot and root length as compared to control (T_1). The highest shoot and root length was observed when seed treatment was done with T_7 (8.8 cm and 6.8 cm) and lowest was observed in control T_1 (6.2 cm and 4.2 cm). The containers effect was observed significant and metal box (C_3) proved superior. The best interaction was found in seeds treated with Azoxystrobin (T_7) and kept in metal box (C_3) (9.5 cm and 7 cm) for shoot and root length respectively. Seedling characters like shoot and root length was found varied over storage period (Monira *et al.* 2012). The results are corroborated with Raiker *et al.* (2011) who stated that rice seeds stored in polythene bag recorded

Table 1 Effect of fungicides and containers on physiological parameters of okra seed

Treatment	Germination (%)				Shoot length (cm)				Root length (cm)				Seedling dry weight (g)			
	C_1	C_2	C_3	Mean	C_1	C_2	C_3	Mean	C_1	C_2	C_3	Mean	C_1	C_2	C_3	Mean
T_1	66.0	67.0	67.7	66.9	5.5	6.3	6.7	6.2	4.2	4.1	4.2	4.2	0.159	0.163	0.176	0.166
T_2	72.0	72.3	73.0	72.4	7.5	7.6	8.4	7.8	5.4	5.2	5.3	5.3	0.171	0.175	0.184	0.176
T_3	72.3	70.7	72.7	71.9	6.3	6.6	7.2	6.7	4.5	4.4	4.7	4.5	0.174	0.181	0.186	0.181
T_4	71.7	70.7	71.0	71.1	7.2	7.6	7.5	7.4	6.5	6.5	6.4	6.4	0.199	0.202	0.193	0.198
T_5	73.7	73.7	73.0	73.4	6.6	6.6	6.7	6.6	5.6	5.3	5.8	5.6	0.179	0.183	0.187	0.183
T_6	68.3	70.7	71.0	70.0	6.2	6.5	6.6	6.4	5.9	5.8	6.4	6.0	0.191	0.199	0.198	0.196
T_7	74.3	74.7	75.7	74.9	8.3	8.6	9.5	8.8	6.7	6.8	7.0	6.8	0.224	0.236	0.230	0.230
T_8	70.3	71.3	71.0	70.9	6.5	6.7	6.8	6.7	5.2	5.6	6.0	5.6	0.179	0.187	0.188	0.185
Mean	71.1	71.4	71.9		6.8	7.1	7.4		5.5	5.5	5.7		0.185	0.191	0.193	
CD (5%)	T	C	TXC		T	C	TXC		T	C	TXC		T	C	TXC	
	0.80	0.49	1.39		0.31	0.19	0.53		0.202	0.124	0.350		0.004	0.002	0.007	
S.E (m)	0.28	0.17	0.49		0.10	0.06	0.18		0.071	0.043	0.123		0.001	0.001	0.002	

Treatment	Vigour index-I				Vigour index-II				Electrical conductivity (µS/cm/g)			
	C_1	C_2	C_3	Mean	C_1	C_2	C_3	Mean	C_1	C_2	C_3	Mean
T_1	643	699	740	694	10.50	10.90	11.89	11.09	1.100	1.106	1.094	1.100
T_2	929	926	1000	952	12.28	12.63	13.41	12.78	1.063	1.072	1.069	1.068
T_3	783	777	865	809	12.61	12.82	13.54	12.99	1.057	1.053	1.049	1.053
T_4	979	992	985	985	14.26	14.28	13.73	14.09	1.054	1.045	1.041	1.046
T_5	896	875	913	894	13.16	13.48	13.65	13.43	1.056	1.051	1.044	1.051
T_6	827	869	925	874	13.05	14.09	14.08	13.74	1.058	1.057	1.049	1.055
T_7	1110	1150	1253	1171	16.68	17.60	17.38	17.22	1.046	1.033	1.029	1.036
T_8	821	873	912	868	12.61	13.34	13.32	13.09	1.068	1.069	1.061	1.066
Mean	873	895	949		13.14	13.64	13.87		1.063	1.061	1.055	
CD (5%)	T	C	TXC		T	C	TXC		T	C	TXC	
	28	17	48		0.28	0.17	0.49		0.004	0.002	0.007	
S.E (m)	9	6	17		0.10	0.06	0.17		0.001	0.001	0.002	

*T_1, Untreated; T_2, Carbendazim; T_3, Tebuconazole; T_4, Difenoconazole; T_5, Flusilazole; T_6, Chlorothalonil; T_7, Azoxystrobin; T_8, Vitavax Power. *C_1, Polythene bag; C_2, Hermetic bag; C_3, Metal box.

Table 2 Effect of fungicides and containers on enzymatic activities of okra seed

Treatment	Catalase activity (mg/protein/min)				Superoxidase activity (mg/protein/min)				Dehydrogenase activity O(D/g/ml)				Peroxidase activity (mg/protein/min)			
	C_1	C_2	C_3	Mean	C_1	C_2	C_3	Mean	C_1	C_2	C_3	Mean	C_1	C_2	C_3	Mean
T_1	128.0	131.7	144.7	134.8	65.0	69.0	79.0	71.0	0.20	0.19	0.20	0.20	430	446	463	446
T_2	139.7	143.7	152.7	145.3	82.7	86.7	94.0	87.8	0.32	0.30	0.31	0.31	602	622	642	622
T_3	143.3	150.3	155.3	149.7	86.0	88.3	95.3	89.9	0.23	0.22	0.25	0.23	537	551	561	550
T_4	168.0	171.0	162.3	167.1	102.7	103.0	97.0	100.9	0.43	0.43	0.42	0.42	598	615	623	612
T_5	147.7	152.0	156.0	151.9	91.7	95.0	96.7	94.4	0.34	0.31	0.36	0.34	504	512	510	508
T_6	160.0	168.3	167.3	165.2	90.7	101.0	101.0	97.6	0.32	0.31	0.37	0.33	454	474	481	470
T_7	193.3	204.7	198.7	198.9	126.7	136.0	134.0	132.2	0.45	0.46	0.48	0.46	672	677	680	676
T_8	148.3	156.0	156.7	153.7	86.0	93.3	93.0	90.8	0.23	0.27	0.31	0.27	569	581	587	579
Mean	153.5	159.7	161.78		91.4	96.5	98.8		0.31	0.31	0.34		546	560	568	
C.D (5%)	T	C	TXC		T	C	TXC		T	C	TXC		T	C	TXC	
	3.90	2.39	6.77		2.81	1.72	4.87		0.020	0.012	0.035		5.63	3.44	9.75	
S.E (m)	1.37	0.83	2.37		0.98	0.60	1.70		0.007	0.004	0.012		1.97	1.20	3.52	

*Treatment details given in footnote of Table 1.

significantly higher shoot and root length. Seedling dry weight also followed the same pattern of decreasing values as observed in germination, shoot length and root length (Table 1). The highest seedling dry weight was recorded in treatment T_7 (0.230 g) and lowest was in control T_1 (0.166 g). Container C_3 (metal box) was superior than other two containers; hermetic bag (C_2) and polythene bag (C_1). The maximum dry weight (0.280 g) was recorded in seeds treated with Azoxystrobin (T_7) and kept in metal box (C_3). The results found similarity with findings of Monira *et al.* (2012) in soybean.

In case of vigour index- I & II, the values were found in descending order. The highest vigour index- I & II was observed with T_7 treatment (1171 and 17.22) and the lowest was in control T_1 (694 and 11.09). The superior interactions (1253 and 17.38) was recorded when seeds were treated with azoxystrobin (T_7) and stored in metal box (C_3). Balesevic-tubic *et al.* (2010) stated that differences in vigour indices during storage might be due to lipid changes of seed during storage and decrease in phospholipids and polyunsaturated fatty acids. The results are in conformity with findings of Raiker *et al.* (2011) in rice, Reddy and Biradarpatil (2012) in groundnut. Electrical conductivity was found increased by the passage of storage time and recorded highest after 18 months of storage. The lowest value was observed in T_7 treatment (1.036 μS/cm/g) followed T_4 (1.043 μS/cm/g) and highest was in control T_1 (1.100 μS/cm/g). Containers effect was found significant in all months of storage. The lowest electrical conductivity (1.029 μS/cm/g) was found when seeds were stored in metal box (C_3) followed by hermetic bag (C_2) and polythene bag (C_1). The lowest interaction value of electrical conductivity (1.029 μS/cm/g) was found in seeds treated with azoxystrobin (T_7) and stored in metal box (C_3). The increase in electrical conductivity was due to increase in solute leakage as membranes altered during ageing. The membrane weakens by damage of phospholipids causes exit of electrolytes and enzymes (Zamani *et al.* 2010) .

Table 2 depicts that the enzymatic activities, viz. catalase, superoxidase dismustase, dehydrogenase and peroxidase showed significant variation in response to various treatments during ambient room storage of okra seeds. They were found decreased with the advancement of ageing and the lowest was recorded at 18 months of storage in all the treatments. In case of dehydrogenase activity, it was found decreased with every interval of storage. The highest was observed in T_7 treatment (0.46 OD/g/ml) and the lowest was in control T_1 (0.20 OD/g/ml). The decrease in activity of dehydrogenase with the advancement of ageing was also stated by Kumar *et al.* (2019) in chilli and brinjal. The present study showed the decrease of antioxidant enzymes in seeds of okra. During storage catalase, superoxidase dismustase and peroxidase enzyme activities declined after three, six, nine, twelve, fifteen and eighteen months of storage respectively. The highest catalase activity, superoxidase dismustase activity and peroxidase activity was recorded in T_7 treatment

(198.9 mg/protein/min, 132.2 mg/protein/min and 676 mg/protein/min) and lowest in T_1 control (134.8 mg/protein/min, 71 mg/protein/min and 446 mg/protein/min) at the end of 18 months of storage. The decrease in antioxidant enzymes is attributed to increase in lipid peroxidation and ageing. The decrease in activity of enzymes during storage might be due to free radical production in the presence of traces of oxygen. In the absence of active enzymes, scavenging free radicals and degradation products of thermo-liable lipid peroxidation accumulate with the ageing of seeds and result in complete loss of viability (Rao *et al.* 2006). The enzymes go through configurational changes such as folding and unfolding of ultra-structure and polymer formation due to condensation and degradation to subunits i.e. absorbance of dehydrogenase enzyme declined with the progress of storage in sunflower. As study reveals that ageing coincides with protein denaturation or inactivation of enzymes, similar trend of decrease in catalase, SOD and peroxidase enzymes was reported by Rao *et al.* (2006) in Onion, Loycrajjou *et al.* (2008) in Arabidopsis.

The study revealed that seed treatment and proper storage containers can maintain high germination, vigour and biochemical parameters for longer period of time. At the end of 18 months of storage, all the seed quality parameters, viz. germination, shoot and root length, seedling dry weight, vigour index- I & II and enzymatic activities were found to be gradually decreasing except the electrical conductivity which was increasing with the advancement of ageing. Seed treatment with fungicides showed higher values as compared to control. The best interaction was recorded in azoxystrobin treated seeds stored in metal box. This could enhance the storage potential of okra seeds during natural ageing.

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