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CONTENTS

Impact of <i>Zea nicaraguensis</i> introgression on Kernel Trait Variability in maize lines	231
SENTHILKUMAR V., PRIYA GARKOTI., THOTLA NARESH, MAYANK TIWARI, ANIRUDH T. V. and NARENDRA KUMAR SINGH	
Improving <i>Brassica juncea</i> performance through hybrid breeding strategies: a focus on combining ability and heterosis analysis	244
ANU SINGH, USHA PANT, PREETI LOHANI, A. S. JEENA and ANIL KUMAR	
Study of Nano Urea application under graded n rates on growth, productivity and nitrogen use efficiency of transplanted rice (<i>Oryza sativa</i> L.)	251
S.K.YADAV , D.K.SINGH, PRATIMA ARYA and YUVRAJ SINGH	
Isolation, screening and characterization of Drought tolerant Plant Growth Promoting bacteria from Indian Himalayas	261
PRIYANKA KHATI, PANKAJ KUMAR MISHRA and LAKSHMI KANT	
Impact of Glomalin-Related Soil Proteins on <i>in vitro</i> Finger Millet (<i>Eleusine coracana</i> (L.) Gaertn.) seed germination	272
AMIT SINGH RANA, SUGANDHA PANT, ASHOK KUMAR VERMA and ASHUTOSH DUBEY	
Rating scale of pedological development in humid moisture regime of guava growing soils in north-east region of Haryana	279
DHARAM PAL and DINESH	
Coating micronized elemental sulphur powder on prilled urea: process and product evaluation	286
P. O. SURESH, N. R. PATEL, R. JAT, R. A. PANIA, A. K. MISHRA, P. B. VAISHNAV	
Multi-year temporal analysis of sheath blight incidence in rice using geostatistical technique	297
AMIT BIJLWAN, RAJEEV RANJAN, MANENDRA SINGH, RAJ KUMAR SINGH, RAJEEV KUMAR SRIVASTAVA, KRISHNA PRATAP SINGH and RAVINDRA KUMAR SINGH RAJPUT	
Efficiency assessment of classifiers for sugarcane area mapping: A machine learning approach with Google Earth Engine	305
POOJA YADAV, AJEET SINGH NAIN and SHIVANK DEVLİYAL	
Calibration and performance evaluation of the APSIM and CERES-Wheat model in the foot hills of Western Himalayas	319
NEHA PAREEK, A.S. NAIN, P. K. SINGH, HEMANT KUMAR, SHRUTI V. SINGH, MANJARI SINGH, PRIYANKA SWAMI and SANTOSH KUMAR	
Population dynamics of major insect pests of sesame and their correlation with meteorological factors	330
BHUMIKA RAWAT, M. S. KHAN, ASHUTOSH and DEEPIKA JEENGAR	
<i>In-vitro</i> screening of <i>Trichoderma</i> isolates for their antagonistic potential against <i>Rhizoctonia solani</i> causing aerial blight of Soybean	335
ARUNKUMAR, BHUPESH CHANDRA KABDWAL and ROOPALI SHARMA	
Physiological and biochemical responses of okra seed (<i>Abelmoschus esculentus</i> L.) to botanicals and containers during storage	350
SUNIL KUMAR, S. S. JAKHAR, ANIL KUMAR MALIK and AXAY BHUKER	
Effect of integrated weed management practices on growth parameters in vegetable pea (<i>Pisum sativum</i> L.)	357
NEELIMA RAWAT, MANOJ RAGHAV, DHIRENDRA SINGH, ALKA VERMA, NAVNEET PAREEK, HITAIISHI KURIYAL and IMAMUDDIN SHAH	

Maximizing Chrysanthemum (<i>Dendranthema gradiflora</i>T.) growth and yield: Unveiling the superiority of Black Polythene Mulch	360
HARSHITA BORA, MAMTA BOHRA and K. C. SINGH	
Utilization of ultrasonicated edible coating to prolong shelf life of fresh cut- onion	368
NEHA RAWAT, SATISH KUMAR SHARMA, ANIL KUMAR, NAVIN CHANDRA SHAHI, ASHUTOSH DUBEY, CHARU BISHT, ARCHANA GANGWAR	
Effect of cooperative societies on food security status of cassava farming households in delta state, Nigeria	378
IZEKOR, O.Band OKOROR O.T.	
Strategies for Improving Agricultural practices: A case study of tomato growers from Uttarakhand	388
TAMANNA JOSHI and ASHUTOSH SINGH	
Physico-functional and sensory qualities of instant custard powder incorporated with resistant starch from Grand Naine banana	398
SRUTHY. P. M., SHARON. C. L., SEEJA THOMACHAN PANJIKKARAN, A. N. JYOTHI, ANEENA E. R.and LAKSHMI P. S.	
Development and quality evaluation of rice-based meal replacer with chocolate flavour for adults	404
ATHIRA RAJ, SUMAN K.T., BEENA A. K., SEEJA THOMACHAN PANJIKKARAN, SHARON C. L., LAKSHMY P. S., DELGI JOSEPH C.and SREELAKSHMI A. S.	
Effect of bleaching on optical properties of <i>dhaincha</i> (<i>Sesbania aculeata</i>) pulp	411
SURABHI DAS, ANITA RANI, MANISHA GAHLOT, SAKSHI and NIDHI SISODIA	
Evaluation of genetic and non-genetic factors affecting first lactation traits in crossbred cattle	421
NAYLA FRAZ, B. N. SHAHI, R. S. BARWAL, C. V. SINGH and A. K. GHOSH	
Mushroom (<i>Agaricus bisporus</i>) waste as a replacement for deoiled rice bran and its impact on immunocompetence against Ranikhet (Newcastle) disease virus in Rhode Island Red Chicken	426
MANAS ARORA, R. KUMAR, A. TEWARI, A. KUMAR, J. PALOD and B.C MONDAL	
Effect of <i>Aloe vera</i> leaf extract on pathological lesions of <i>Escherichia coli</i> infected broiler chickens	433
MAMTA KUMARI, RAJENDAR P. GUPTA, DEEPIKA LATHER, PREETI BAGRI, RENU SINGH, SARVAN KUMARand KOMAL	
Effect of metronidazole on hematological parameters in Common Carp (<i>Cyprinus carpio</i>)	443
ANIKA SHARMA, MADHU SHARMA, TARANG SHAH and PRASANJIT DHAR	
Reproductive and productive performances of Japanese Quails (<i>Coturnix japonica</i>) under agro-climatic conditions of Assam	449
DEBAJIT DEKA, ARFAN ALI, ASHIM KUMAR SAIKIA, MRIDUL DEKA, UTPAL JYOTI SARMA, MANORANJAN NEOG and RANJIT KUMAR SAUD	
Performances of Turkey birds under backyard system in agro-climatic condition of Assam	454
DEBAJIT DEKA, ARFAN ALI, ASHIM KUMAR SAIKIA, MRIDUL DEKA, MANORANJAN NEOG, RANJIT KUMAR SAUD and UTPAL JYOTI SARMA	
Nutraceutical supplements for managing pain and inflammation: A special focus on palmitoylethanolamide and astaxanthin	459
AKHTER RASOOL, DIVYA CHAVAN, PULI VISHNUVARDHAN REDDY, JAN MOHD MUNEEB and IRTIQA MANZOOR	
Characterization and use of hydrochars from wheat straw, fruit peels, and sewage sludge: A potential biofuel source	470
KARAN SATHISH and SHWETA SARASWAT	
Battery assisted single wheel weeder for medicinal plants	479
SANDEEP KUMAR SAROJ and JAYANT SINGH	
Chat GPT: Perception of students towards AI tool	486
ARPITA SHARMA KANDPAL and POOJA GOSWAMI	

Physiological and biochemical responses of okra seed (*Abelmoschus esculentus* L.) to botanicals and containers during storage

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ABSTRACT: The present investigation was carried out on okra seeds during 2018-20, which comprised of three botanicals as seed dressers @ 100g/kg viz., *Pongamia pinnata*, *Azadiracta indica* and *Curcuma longa* leaf powders along with control. The seeds coated with the above botanicals were stored in three different containers viz., polythene bag, hermetic bag and metal box under ambient conditions and their quality was assessed at a regular interval of three months. A significant decline was observed in all the treatments as the period of ageing was augmented. Seeds coated with *Azadiracta indica* powder and stored in a metal box recorded significantly higher germination percentage (71.9%), shoot length (8.2 cm), root length (6.0 cm), seedling dry weight (0.195 g), vigour index-I (1021), vigour index-II (14.03), catalase (198.9 mg/protein/min), superoxidase dismutase (132.2 mg/protein/min), dehydrogenase (0.46OD/g/ml), peroxidase (676 mg/protein/min) and lower electrical conductivity (1.036 μ S/cm/g) after 18 months of storage as compared to control. The control showed germination (66.9%), shoot length (6.2 cm), root length (4.2 cm), seedling dry weight (0.166 g), vigour index-I (694), vigour index-II (11.09), catalase, superoxidase dismutase and peroxidase (134.8, 71.0 and 446.0 mg/protein/min) and higher electrical conductivity (1.100 μ S/cm/g) at the end of storage period.

Key words: Botanicals, containers, okra, seed quality, seed storage

Vegetables are an important component of the human diet for the maintenance of good health. China is the leading producer of fresh vegetables with a production volume of nearly 549 million metric tons, followed by India with approximately 128 million metric tonnes in 2018 (FAO-Statistica, 2021). Okra is one of the most commonly known and utilised species of the family Malvaceae, an economically important vegetable crop grown in tropical and sub-tropical parts of the world. The centre of origin is Ethiopia (Satish and Eswar, 2013). Afterward, it was propagated in different parts of the world and India by the 12th century BC. India is the global leader in production, with a cultivation area of 5.1 lakh ha and an annual production of 61.26 lakh tonnes (FAOSTAT, 2018). The crop is grown over a wide range of soils and climatic conditions, both in the summer and rainy seasons.

The importance of seeds 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 the ageing phenomenon, which is defined as an irreversible change in the quality of a seed. Among all the deteriorative changes, membrane degradation has been proposed as the primary event in ageing, which is mainly affected by chromosomal damage, loss of various enzymes and deterioration of membrane integrity. Good storage is an essential requirement in a seed programme for the maintenance of high germination and vigour from harvesting to the next planting season.

Seeds that are treated with botanicals have fungicidal toxicity or antagonistic effects, which eliminate most of the seed borne micro-flora and maintain viability and vigour for longer periods during storage (Basavegowda *et al.*, 2013). Botanicals have been used as seed treatments for the invigoration of seeds due to its biologically active molecules possessing antimicrobial properties (Ramya *et al.*, 2011). They are eco-friendly and cheaper in nature when compared to chemicals. Looking into the above facts, the present study was undertaken to determine the

physiological and biochemical responses of okra seeds to botanicals and containers.

MATERIALS AND METHODS

The present investigation was conducted from 2018 to 2020 in the laboratory of the Department of Seed Science and Technology, CCS Haryana Agricultural University, Haryana, India. The location of the city is at 29° 10 North Latitude and 75° 46 East Latitude, at an altitude of about 215.2 m above mean sea level. The climate of the 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 the rainy season. The mean monthly maximum and minimum temperatures showed a wide range of fluctuations during both years. The mean monthly maximum temperature of 43-45°C was common during the summer months from May to June, while the minimum temperature during the winter months from December and January sometimes went as low as 0°C.

Okra seeds of *cv.* Varsha Uphar were coated with the following botanicals @100g/kg *viz.*, T₂:*Pongamia pinnata*, T₃:*Azadiracta indica* and T₄:*Curcuma longa* along with T₁: Untreated (control).

Preparation of botanical powder

The fresh leaves of different plants were plucked, cleaned properly and shade dried for 7-10 days at room temperature. After drying, leaves were grinded with an electric grinder to make a fine powder. These powders were sieved through muslin cloth and stored in airtight containers.

Coating of seeds with botanicals

The okra seeds, botanicals and gum were weighed at 50, 5 and 1 g, respectively; wearing gloves and using an appropriate weighing balance for each treatment. The seeds, botanicals and gum were mixed in beakers and shaken for some time for a uniform coating over the seeds. Then, the treated seeds were stored in different containers *viz.*, C₁: polythene bag, C₂: hermetic bag and C₃: metal box in the laboratory under ambient conditions.

The total numbers of treatments were twelve which were kept in different containers in the laboratory under ambient conditions. The experiment consisted of two factors (three different packing materials as storage containers were used as factor “C” and the three botanical treatments were used as factor “T”) and was laid out in a factorial completely randomized design (FCRD). Seeds were taken from each of the different containers at a quarterly interval up to 18 months (October 2018 to March 2020) and observations were recorded for seed quality parameters.

The seeds from each treatment were tested for germination by adopting between paper towel method kept in optimum conditions of temperature (25° C) in four replications of 100 seeds (ISTA, 2011). The number of normal seedlings counted at the end of 10 days. The number of seeds germinated x 100 gave the seed germination percentage. The shoot and root lengths were recorded on the 10th day by randomly selecting ten seedlings. The data were averaged and expressed in centimetres. These seedlings were then kept in a suitable paper packet and dried in a hot air oven at a constant temperature of 80°C for one day. The dried samples were then cooled in desiccators and weighed by using electronic balance and expressed in grams for obtaining data on dry weight. Seedling vigour index-I & II was calculated by multiplying germination percentage with seedling length and seedling dry weight, respectively (Abdul-Baki and Anderson, 1973). Electrical conductivity of the seeds 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. Peroxidase activity was determined by the method of Shannon *et al.* (1966) following the oxidation of O-dianisidine in the presence of hydrogen peroxide (H₂O₂). Dehydrogenase activity was measured by method suggested by Kittock and Law (1968) and Superoxidase activity was determined by the method

suggested by Giannopolitis and Ries (1977).

The statistical analysis of data obtained for character studied was done by OPSTAT software using completely randomized design as per standard method (Panse and Sukhatme, 1985).

RESULTS AND DISCUSSION

The seed lot utilised in the experiment had an initial germination of 91 per cent which was above Indian Minimum Seed Certification Standards (Anonymous, 2013). The result mentioned below under each parameter was recorded at quarterly intervals. However, the results mentioned below are for the last observation recorded at 18 months of storage.

Table 1 illustrates 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 with *Azadiracta indica* (71.9%) followed by treatment with *Curcuma longa* (71.1%), *Pongamia pinnata* (68%) and the lowest germination was recorded in control (66.9%). This might be due to the seed deterioration (Kumar *et al.*, 2022). Among containers, the highest value was achieved in metal box (72.7%). The best interaction (72.7%) was observed in seeds coated with *Azadiracta indica* and stored in metal box. It is assumed that some of the micronutrients were present in botanicals that are favourable for seed invigoration, as stated by Sasthriand Srimathi (2010). Botanicals act as catalysts for the production of reactive oxygen species (ROS) in a slow and sustained manner for the maintenance of viability. The perusal of data in Table 1 revealed that the shoot and root length of okra seeds were also recorded in a decreasing trend starting from the initial to the last month of storage. All the treatments recorded higher shoot and root lengths as compared to control. The highest shoot and root lengths were observed when seeds were coated with *Azadiracta indica* (8.2 and 6.0 cm) and the lowest were observed in control (6.2 and 4.2 cm). The best interaction was found in seeds coated with

Azadiracta indica and kept in metal box (8.2 and 6.4 cm) for shoot and root lengths respectively. The increased germination rate might have improved the shoot and root length. Seedling characteristics like shoot and root lengths were found to vary over storage period (Monira *et al.*, 2012). The results are corroborated by Meena *et al.* (2017) in groundnut and Natubhai *et al.* (2018) in onion.

The dry weight of seedlings is a physiological manifestation of seed vigour, which is influenced by growth substances, metabolites and enzyme activity. Seedling dry weight (mg) also followed the same pattern of decreasing values as observed in germination, shoot length and root length as depicted in Table 1. The highest seedling dry weight was recorded in treatment with *Azadiracta indica* (0.195 g) and the lowest was in control (0.166 g). Container metal box was superior to the other two containers; hermetic bag and polythene bag. The maximum dry weight (0.199 g) was recorded in seeds coated with *Azadiracta indica* and kept in metal box. The decrease in dry weight might be due to hydrolysis of reserved metabolites, activation of endogenous enzymes and breakdown of food reserves during the period of storage as stated by Khatun and Bhuiyan (2011) in chickpea and Monira *et al.* (2012) in soybean. In case of vigour index-I and II, the values were found in descending order. The maximum vigour indices were observed with treatment *Azadiracta indica* (1021 and 14.03) followed by *Curcuma longa* (921 and 12.79), *Pongamia pinnata* (748 and 12.09) and the minimum was in control (694 and 11.09) respectively. The superior interaction (1059 and 14.18) was recorded when seeds coated with *Azadiracta indica* and stored in metal box. Balesevic-tubic *et al.* (2010) stated that differences in vigour indices during storage might be due to lipid changes in the seed during storage and a decrease in phospholipids and polyunsaturated fatty acids. The increase in vigour index was due to presence of micronutrients, vitamins, antioxidants, polyphenols and flavanoids in botanicals reported by Raiker *et al.* (2011) in rice, Reddy and Biradarpatil (2012) in groundnut.

Electrical conductivity has been increased over a

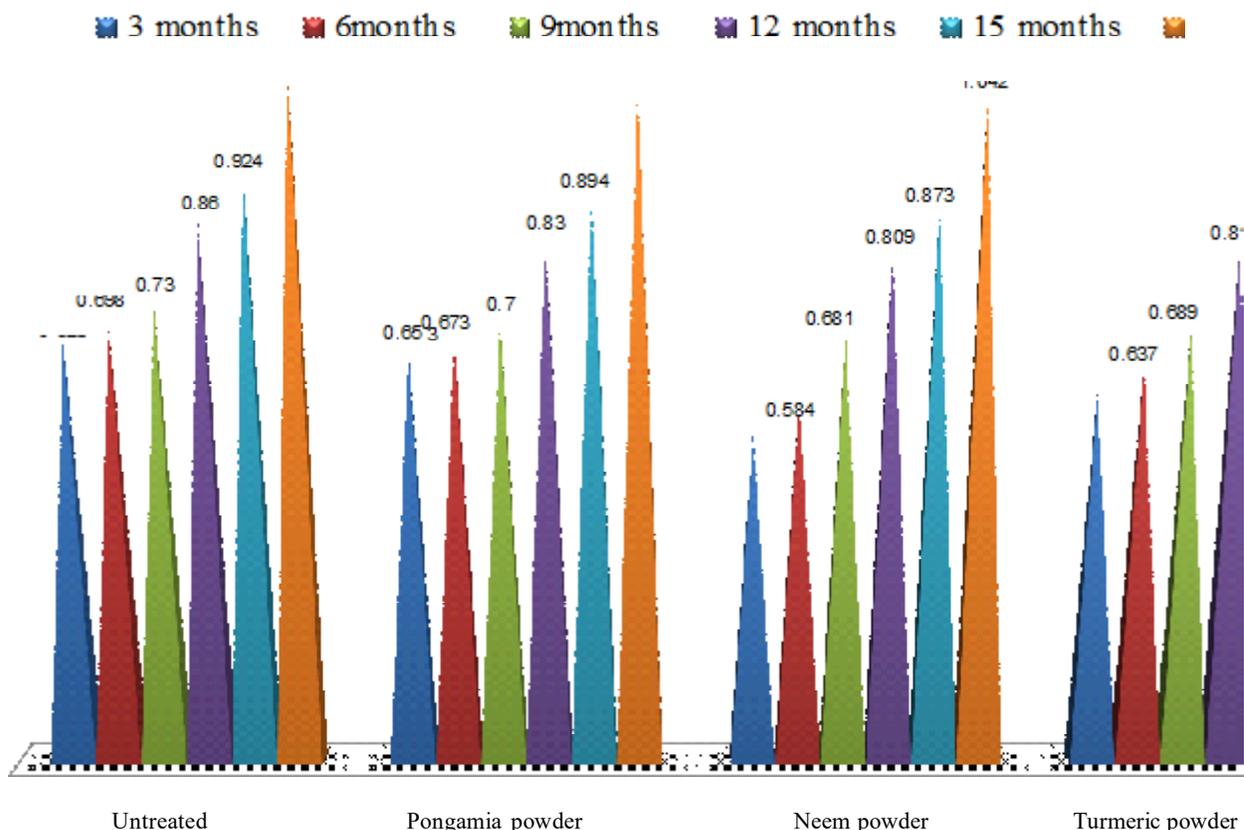


Fig. 1: Influence of botanicals and containers on electrical conductivity (µS/cm/g) of okra seeds stored under ambient conditions

Table 1: Effect of botanicals and containers on physiological parameters of seed quality of stored okra seeds

TREATMENT	Germination (%)				Shoot Length (cm)				Root Length (cm)			
	C1	C2	C3	MEAN	C1	C2	C3	MEAN	C1	C2	C3	MEAN
T1	66.0	67.0	67.7	66.9	5.5	6.3	6.7	6.2	4.2	4.1	4.2	4.2
T2	66.3	68.7	69.0	68.0	6.0	6.4	7.0	6.5	4.5	4.4	4.7	4.5
T3	72.3	70.7	72.7	71.9	8.1	8.1	8.2	8.2	5.9	5.8	6.4	6.0
T4	71.7	70.7	71.0	71.1	7.0	7.1	8.0	7.4	5.2	5.6	6.0	5.6
MEAN	69.1	69.3	70.1		6.7	7.0	7.5		5.0	5.0	5.3	
	T	C	TXC		T	C	TXC		T	C	TXC	
C.D (5%)	0.83	0.72	1.44		0.26	0.22	0.45		0.21	0.18	0.37	
S.E (m)	0.28	0.24	0.49		0.08	0.07	0.15		0.07	0.06	0.12	
TREATMENT	Seedling Dry Weight (mg)				Vigour Index-I				Vigour Index-II			
	C1	C2	C3	MEAN	C1	C2	C3	MEAN	C1	C2	C3	MEAN
T1	0.159	0.163	0.176	0.166	643	699	740	694	10.50	10.90	11.89	11.09
T2	0.171	0.178	0.183	0.178	701	737	807	748	11.36	12.25	12.65	12.09
T3	0.196	0.190	0.199	0.195	1013	992	1059	1021	13.83	14.06	14.18	14.03
T4	0.176	0.180	0.184	0.180	872	897	994	921	12.59	12.72	13.06	12.79
MEAN	0.176	0.180	0.183		807	831	900		12.16	12.48	12.86	
	T	C	TXC		T	C	TXC		T	C	TXC	
C.D (5%)	0.004	0.003	0.007		29	25	56		0.31	0.27	0.54	
S.E (m)	0.001	0.002	0.001		10	8	17		0.10	0.09	0.18	

*T1: Untreated T2: Pongamia powder T3: Neem powder T4: Turmeric powder, *C1: Polythene bag C2: Hermetic bag C3: Metal box

Table2: Effect of botanicals and containers on enzymatic activities of stored okra seeds

TREATMENT	Catalase activity (mg/protein/min)			Superoxidase activity (mg/protein/min)			Dehydrogenase activity (OD/g/ml)			Peroxidase activity (mg/protein/min)					
	C ₁	C ₂	C ₃	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	65.0	69.0	79.0	71.0	0.20	0.19	0.20	0.20	430	446	463	446
T ₂	138.3	146.0	146.7	82.6	86.6	94.0	87.8	0.25	0.29	0.33	0.29	497	505	503	501
T ₃	155.3	173.0	176.0	103.3	105.6	106.0	105.0	0.43	0.40	0.45	0.43	562	574	580	572
T ₄	162.0	170.3	169.3	91.6	95.0	96.6	94.4	0.32	0.31	0.37	0.33	530	544	554	543
MEAN	150.3	156.0	157.0	91.4	96.5	98.8	94.4	0.30	0.30	0.34	0.33	546	560	568	560
C.D (5%)	4.0	3.4	6.9	T	C	TXC		T	C	TXC		T	C	TXC	
S.E (m)	1.3	1.1	2.3	1.3	1.1	2.3		0.021	0.018	0.037		6.0	5.1	10.25	
				1.3	1.1	2.3		0.007	0.006	0.013		2.0	1.7	3.5	

*T1: Untreated T2: Pongamia powder T3: Neem powder T4: Turmeric powder

*C1: Polythene bag C2: Hermetic bag C3: Metal box

period of storage time and was recorded to its highest after 18 months of storage (Figure 1). The lowest value was observed in the *Azadiracta indica* treatment (1.042µS/cm/g) followed by *Curcuma longa* (1.051µS/cm/g), *Pongamia pinnata* (1.064 µS/cm/g) and highest was in control(1.100µS/cm/g). The lowest interaction value of electrical conductivity (1.029 µS/cm/g) was found in seeds coated with *Azadiracta indica* and stored in metal box. The increase in electrical conductivity was due to an increase in solute leakage as membranes altered during ageing. The membrane weakens due to damage of phospholipids, which causes the exit of electrolytes and enzymes (Zamani *et al.*, 2010) in wheat.

As the time prolonged the seed quality attributes declined. The hygroscopic nature of seed affects its quality by changing environmental conditions *viz.*, relative humidity, temperature, moisture content, gaseous exchange, packaging material (Yalamalle and Kuchlan, 2016).

Table 2 shows that the enzymatic activities, *viz.* catalase, superoxidase dismutase, dehydrogenase and peroxidase showed significant variation in response to various treatments during ambient room storage of okra seeds. They were found to decrease with the advancement of ageing and the lowest was recorded at 18 months of storage in all the treatments. In the case of dehydrogenase activity, it was found decreased with every interval of storage. The highest was observed in *Azadiracta indica* treatment (0.43 OD/g/ml) and the lowest was in control (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 a decrease of antioxidant enzymes in the seeds of okra. During storage catalase, superoxidase dismutase and peroxidase enzyme activities were found to decline after three, six, nine, twelve, fifteen and eighteen months of storage respectively. At the end of 18 months of storage, the highest catalase, superoxidase dismutase and peroxidase activity was recorded in the *Azadiracta indica* treatment (172.1, 105.0 and 572.0 mg/protein/min) and the lowest in the control (134.8, 71.0 and 446.0 mg/protein/min). The interaction effect was found better in seeds treated with *Azadiracta indica* and stored in metal box. The decrease in antioxidant enzymes is attributed to an 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 even traces of oxygen. In the absence of active enzymes, scavenging free radicals and degradation products of thermo-labile 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., the absorbance of dehydrogenase enzyme declined with the progress of storage in sunflower. The study revealed that ageing coincides with protein denaturation or inactivation of enzymes. A similar trend of decrease in catalase, superoxidase and peroxidase enzymes was reported by Rao *et al.* (2006) in onion, Rajjou *et al.* (2008) in *Arabidopsis*.

CONCLUSION

The study revealed that 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 botanicals showed better results as compared to control. The best interaction was recorded in *Azadiracta indica* coated seeds stored in a metal box. This could enhance the storage potential of okra seeds during natural ageing.

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