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Evaluation of Certain Storage Conditions for Okra (*Abelmoschus esculentus* (L.) Moench) Seeds Against Potential Fungal Pathogens

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ABSTRACT

Okra variety Arka Anamika although a most popular, high yielding variety in India, it is susceptible to several fungal pathogens which results in the reduction of yield and nutritional quality. Healthy, moderately and severely infected seeds obtained from local market were evaluated for their storability for a year using different storage containers (cotton, polyethylene and paper bags) at varied temperatures. Biochemical parameters as well as pathogenic status were evaluated as a measure of seed quality. Results indicated that the moisture content play a key role in amplifying fungal biomass during storage period. Cotton bag at 28°C appeared to be the best suited for long storage of seeds. Thus stored seeds showed significant germination efficiency, seedling vigour without further deterioration of its biochemical constituents. With respect to fluctuation in moisture content cotton bags offer greater protection than that of polyethylene or paper bags.

Key Words: Okra; Storage containers; Seed germination; Seedling vigour; Biochemical changes

INTRODUCTION

Okra (*Abelmoschus esculentus* (L.) Moench) is an important summer/rainy season potential vegetable crop extensively used globally for its nutritional and health benefits. Okra constitutes minerals, vitamins, proteins, carbohydrates, enzymes and very high quantities of mucilages (Anonymous, 1959). Mucilaginous extract of green stems of okra are commonly employed in India for clarifying sugarcane juice and a similar preparation from the capsule has found application as a plasma replacement of blood volume expander. Besides these, okra has been known to enhance memory in growing children and adult due to its content. Okra therefore has been placed as a priority item in nutrition and health. In India 4.31 lakh tonnes of okra is produced every year and retained higher position in okra production (Anonymous., 2003). The crop losses have been attributed to various reasons such as the use of unhealthy seeds, agroclimatic conditions, pathogenic status etc. Recent studies indicated greater loss in the yield mainly due to pathogenic attacks. Lots of efforts are hence being put to improve the yield and growth of the crop. A major constraint has been the infectivity status. Okra is attacked by several fungal pathogens, which not only reduces the potency of seed, but also degrades the health beneficial and nutritional quality components (Anonymous., 2003). Further, seeds obtained in monsoon / summer has to be stored, prior to its usage in the next season. Indeed the storage becomes very crucial to avail the benefit of okra (Anonymous, 2003). Therefore, in the present investigation, emphasis has been given to evaluate the effect of storage of both healthy and infected seeds with respect to viability and other biochemical parameters such as total protein, phenols and carbohydrates.

MATERIALS AND METHODS

Collection of seeds. Okra seed samples viz., Arka anamika, Arka abhy, Pusa sawani, Pusa makhmali, Punjab padmini, Phabha kranti, including local varieties were collected from Indian Institute of Horticultural Research and Private Seed Companies at Bangalore, Karnataka State, India, and examined for fungal infection through incubation tests.

Based on the rate of infection, three different samples of a popular variety Arka anamika, namely, Arka anamika - Adik, Arka anamika - Sungro and Arka anamika - Multiplex were considered for storage studies and were respectively recognized as healthy, moderately infected and highly infected samples.

Seed storage. The seed, 1 kg of each of Arka anamika - Adik, Sungro, Multiplex were stored separately in cotton, paper and polyethylene bags and were maintained at different temperatures 10 ± 2^{o}C, 28 ± 2^{o}C and 45 ± 2^{o}C for a period of one year. The stored samples were drawn at regular intervals of 4, 8 and 12 months and were evaluated for the moisture content, incidence of mycoflora, percentage germination, vigour index and biochemical parameters such as total protein, carbohydrates, phenol and reducing sugars.

Seed samples stored in cotton, paper and polyethylene bags were placed in a BOD incubator, separately at 10 ± 2, 28 ± 2 and 45 ± 2° C for a period of one year. On 0th, 120th, 240th and 360th days of storage, these samples were analyzed for seed moisture content, mycoflora, germination, seedling, vigour and other biochemical parameters.

Determination of seed moisture. Moisture content of the seed samples was assessed through hot air oven method at low constant temperature. 5 g of powdered samples were separately dried at 103 ± 2° C for 17 h. The loss in weight was finally recorded and for each sample based on the average of two replicates the percent seed moisture content was determined.

Evaluation of seeds for mycoflora. 400 seeds of each stored samples were separately plated on 3 layers of wet blotters in plastic plates and incubated for a period of one week under alternate cycles of light and darkness. Further each and every seeds were examined under stereomicroscope for the occurrence of mycoflora (ISTA, 1996). The incidence of mycoflora was assessed and the data were tabulated.

Evaluation of seed germination and seedling vigour. Four replicate of 100 seeds each were incubated in wet blotter towels for a period of 21 days in okra seedlings should be considered for germinating test according to ISTA under standard conditions of light, temperature and humidity. On 21st day the incubated towels were unrolled and the root and shoot length of the normal seedlings were measured. Percentage of seed germination was also recorded. The vigour index of the seedlings was calculated using the formula of Abdul Baki and Anderson (1973).

Estimation of carbohydrate, protein, phenol and reducing sugars. Okra samples stored in different bags at different temperatures were drawn at specified period of storage such as 0, 120, 240 and 360 days and were used for the extraction. 1 g seeds of each sample was separately drawn and finely powdered using mini grinder. 10 mL of distilled water was added and ground thoroughly using mortar and pestle. The extract was centrifuged at 2500 rpm and the supernatant was further used for the estimation of total carbohydrates, protein, phenols and reducing sugars.

1 g of each sample was powdered and extracted in 10 mL of distilled water. Carbohydrate estimation was carried out using phenol sulphuric acid method (Dubois *et al*., 1956). Aliquots were used for the total sugar content using a calibration curve developed with glucose (0-50 μg) 10-50 μL of the extracts were used for the estimation of protein content based on Lowry's procedure. Bovine serum albumin (10-100 μg) was employed to develop a calibration curve as described by Lowry *et al.* (1951). Total phenol content of the samples were estimated with Gallic acid as standard (0-50 μg) (Malick & Singh, 1980). Reducing sugar was estimated by Dinitro salicylic acid (DNS) method, against the glucose as standard at the range of 0-50 μg (Miller, 1959).

RESULTS AND DISCUSSION

Variation in moisture content, germination, vigour index etc., at 28°C has been depicted in Table I. Moisture content of healthy seed sample was low compared to the moderately infected and highly infected seed samples. At the time of storage, the moisture content of the healthy samples was comparatively lower. Irrespective of the sample, the moisture content was increased with increase in storage period. Highly infected samples showed high moisture content. At 10 and 28° C also all samples irrespective of the container, showed higher moisture level. Percentage of seed germination and vigour index was found to be higher in healthy seeds compared that of moderately infected and highly infected samples (Fig. 1).

Healthy seed samples with respect to the different containers at 10° C showed good germination percentage in cotton bag and polyethylene bag. The same was poor the samples stored in paper bags. Similar result was also observed with respect to the seedling vigour. Moderately infected seeds at 10°C, stored in cotton bags showed better germination percentage and vigour index compared to that of paper bag.

Fungi like *Fusarium verticilloides, Macrophomina phaseolina* and other *Aspergillus* species appeared to be predominated in both moderately infected as well as highly infected samples. However, severely infected seeds showed 40% fungal incidence, initially and was increased to 82% on 360 days of storage (Table II). The increase in fungal incidence could be attributed to rapid multiplication under congenial conditions. It is interesting to note that although, there was two fold increase in fungal load in 360 days stored samples compared to 0 day assessed samples. Biochemical constituents did not vary in the samples stored in cotton bag, however it showed only minimum changes (Fig. 2 to 5). The data may suggest the consumption of seed protein for multiplication of fungi.

Table II represents the percentage incidence of fungi at different temperature condition like 10, 28 and 45° C with respect to storage period of 120, 240 and 360 days in different container like cotton, polyethylene and paper bags. At 10 and 28° C in healthy seeds were without pathogenic fungi compared to moderately and highly infected seeds. At 45° C, irrespective of the storage containers healthy, moderately infected and highly infected seeds showed heavy fungal colonization.

Results indicated the lack of fungal colonies in healthy seeds. While moderately infected and heavily infected seeds showed 20 and 80-90% incidence of fungi, respectively. Further, 1.5 to 3 fold decrease in protein, carbohydrate and phenol levels and 2-3 fold increase in reducing sugar was observed in moderately infected and highly infected seeds compared to healthy seeds (Fig. 2 to 5). The results therefore indicate the degradation of protein and carbohydrates and accumulation of reducing sugar due to degradation of carbohydrates, by the pathogen.

Table I. Effect of storage condition on the seed moisture, germination and seedling growth of okra (var. Arka Anamika)

Seed sample	Storage containers	Duration (Days)	Moisture content (%)	Seed germination (%)	MRL (cm)	MSL (cm)	Vigour index
	Nil	0	4.08	80	6.4 ± 0.3	11.5 ± 0.5	1368
Healthy	Cotton bag	120	6.9	75	5.1 ± 0.3	9.8 ± 0.2	1080
		240	7.0	72	4.8 ± 0.3	9.2 ± 0.2	972
		360	7.2	68	4 ± 0.1	9 ± 0.2	863
	Polythene bag	120	7.2	70	6.2 ± 0.2	14.8 ± 0.5	1421
		240	7.5	70	6 ± 0.2	14 ± 0.5	1351
		360	7.8	68	5.5 ± 0.3	12.5 ± 0.5	1169
	Paper bag	120	7.1	60	4.2 ± 0.1	8.8 ± 0.4	750
		240	7.2	60	4 ± 0.1	8 ± 0.4	690
		360	7.8	60	4 ± 0.1	8 ± 0.4	690
	Nil	0	5.7	60	4 ± 0.1	10 ± 0.5	804
Moderately infected	Cotton bag	120	6.5	60	4 ± 0.1	9 ± 0.5	744
		240	6.5	58	4.2 ± 0.1	8.3 ± 0.5	690
		360	7	55	4 ± 0.1	8 ± 0.4	632
	Polythene bag	120	7	58	4.6 ± 0.1	7.7 ± 0.3	690
		240	7.6	53	4 ± 0.1	7 ± 0.3	561
		360	7.9	53	4 ± 0.1	6 ± 0.3	508
	Paper bag	120	7.2	58	4.9 ± 0.2	10.3 ± 0.5	841
		240	7.4	52	4.5 ± 0.2	10 ± 0.5	717
		360	7.5	50	4.2 ± 0.1	9.5 ± 0.5	655
	Nil	0	9.2	40	4.0 ± 0.1	9.5 ± 0.5	576
Highly infected	Cotton bag	120	9.5	38	6 ± 0.2	9.2 ± 0.4	554
		240	10	35	6 ± 0.2	9 ± 0.4	511
		360	10	32	5.5 ± 0.3	8.5 ± 0.4	425
	Polythene bag	120	10	37	4.7 ± 0.2	12.1 ± 0.5	595
		240	10.5	36	4 ± 0.1	10 ± 0.5	482
		360	10.8	33	4 ± 0.1	9.5 ± 0.5	425
	Paper bag	120	10	35	6 ± 0.3	9.2 ± 0.5	504
		240	10	32	6 ± 0.3	9 ± 0.5	460
		360	10	30	5.5 ± 0.3	8.5 ± 0.5	396

Data were based no the samples of 28°C storage

Negligible deterioration of protein was observed in all the healthy and infected seed samples. However, polyethelene and paper bags showed 38, 48 and 65% degradation of protein in healthy, moderate infected and highly infected samples, respectively.

Results in Fig. 2 indicated stable concentration of protein in healthy seed, irrespective of the storage condition. 2 to 3% reduction in protein levels in 3 samples stored at 10 and 45° C may be due to protein degradative activated and be enzymes which is responsible for degradation.

As proteins, total bound carbohydrates as well as phenols were not affected much at 28° C in healthy sample, while 2-3 fold reduction was observed within 100 days of storage in cotton bags. Reducing sugars concentration was increased during prolonged storage indicating the degradation of polymers of sugars to smaller degraded sugar.

Information on storage of okra seeds to maintain the viability and vigour from harvest to next planting season and for carry over purposes is of prime importance in any seed production program. An understanding of how best the seeds can be stored under ambient temperature in various packing materials at relatively low cost, with minimum deterioration in quality, for periods extending over a year constituting one or two seasons will be of immense use to seed industry and farming community. Therefore, an attempt has been made to determine suitable storage conditions for okra seeds under different temperatures with various packing materials.

Seeds were hygroscopic and the exchange of moisture from the atmosphere tend to come to equilibrium state with the relative humidity of the atmosphere at given temperature. Christensen and Lopez (1965) reported that various fungi associated with such losses are influenced by container during storage and its moisture content Christensen and Kaufman (1965) also reported that seeds absorb ambient moisture continuously during storage. Cloth bag being pervious, might have allowed free flow of air from atmosphere Christensen (1982).

The fact that pre storage seed treatment helps to improve the shelf life of seeds and checks proliferation of seed mycoflora during storage was earlier observed by Asalmol and Patil (1993), Gutpa and Sing (1988). The seed germination is adversely affected by various seed borne fungi associated with, it was supported by the earlier work of Subha Raju (1973), Asalmol and Zade (1998).

Pathogenic status, germination efficiency and biochemical studies as well as seedling vigour have been incorporated in the study to determine the potentiality of seed protection to obtain high yielding and nutritionally rich

Table II. Effect of storage conditions on seed mycoflora of okra (var. Arka Anamika)

Seed Sample	Storage container	Duration of storage (days)	% incidence of fungi in stored okra seeds at different temperature conditions (10, 28, 45°C) 10°C																28°C																45°C															
			a	b	c	d	e	f	g	h	i	j	k	l	m	n	o	p	a	b	c	d	e	f	g	h	i	j	k	l	m	n	o	p	a	b	c	d	e	f	g	h	i	j	k	l	m	n	o	p
Healthy Arka anamika (Adik)		0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	Cotton bag	120	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
		240	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
		360	0	0	0	0	0	0	0	0	0	0	2	0	2	0	0	0	0	0	0	0	0	0	0	0	0	0	2	0	2	0	0	0	0	0	0	0	1	0	1	0	0	0	2	0	2	0	0	0
	Polythene bag	120	0	0	0	0	0	0	0	0	0	0	2	0	2	0	1	1	2	3	3	0	0	0	0	0	0	0	4	0	4	0	0	0	0	0	0	0	1	0	1	0	0	0	4	1	4	0	0	1
		240	2	0	0	0	0	0	0	0	0	0	2	0	2	0	1	1	2	4	3	0	0	0	0	0	0	0	4	0	4	0	0	0	0	0	0	0	1	0	1	0	0	0	8	2	8	0	0	1
		360	2	0	0	0	0	0	0	0	0	0	4	0	5	0	2	2	2	3	3	0	0	0	0	5	0	5	0	0	0	0	0	0	0	0	0	0	1	0	1	0	0	0	8	2	8	0	0	1
	Paper bag	120	1	0	0	0	0	0	0	0	0	0	2	0	2	0	1	2	2	3	4	0	0	0	0	0	0	0	6	0	8	1	9	0	0	0	0	0	1	0	1	0	0	0	4	3	4	0	0	1
		240	2	0	0	0	0	0	0	0	0	0	3	0	3	0	2	2	3	4	5	0	0	0	0	0	0	0	8	0	8	1	9	0	0	0	0	0	1	0	1	0	0	0	5	3	6	0	0	1
		360	2	0	0	0	0	0	0	0	0	0	4	0	4	0	3	4	3	4	5	0	0	0	0	0	0	0	8	0	8	1	9	0	0	0	0	0	1	0	1	0	0	0	5	3	6	0	0	1
Moderately infected Arka anamika (Sungro)		0	1	0	0	0	0	0	1	0	0	0	1	0	1	0	0	1	2	3	3	1	0	0	0	0	0	0	3	0	2	0	0	0	0	0	0	0	1	0	1	0	0	0	3	2	3	0	0	0
	Cotton bag	120	2	0	0	0	0	1	2	0	0	0	2	0	2	1	1	2	4	5	6	2	0	0	0	0	0	0	5	0	4	1	1	0	0	0	0	0	2	0	2	0	0	0	6	3	6	0	0	1
		240	0	0	0	0	0	1	2	0	0	0	4	0	4	1	2	2	4	5	6	2	0	0	0	0	0	0	6	0	4	1	1	0	0	0	0	0	2	0	2	0	0	0	8	4	8	0	0	12
		360	0	0	0	0	0	1	2	0	0	0	4	0	4	2	2	2	4	5	6	2	0	0	0	0	0	0	6	0	4	1	1	0	0	0	0	0	2	0	2	0	0	0	8	4	8	0	0	1
	Polythene bag	120	2	0	0	0	0	1	2	0	0	0	4	0	4	1	2	4	6	6	2	1	1	2	1	2	2	1	1	6	1	2	0	0	0	0	0	0	2	0	3	0	0	0	8	4	8	0	0	1
		240	2	0	0	0	0	2	2	0	0	0	4	0	4	4	1	2	4	6	6	2	1	1	2	2	1	2	2	1	7	2	2	0	0	0	0	0	2	0	3	0	0	0	8	4	8	0	0	1
		360	2	0	0	0	0	2	2	0	0	0	4	0	4	4	1	2	5	8	8	3	1	2	3	3	2	3	3	1	7	3	2	0	0	0	0	0	2	0	3	0	0	0	8	4	8	0	0	1
	Paper bag	120	2	0	1	0	1	2	2	0	0	0	4	0	4	5	2	4	5	5	8	3	1	2	2	3	2	3	3	1	7	3	2	0	0	0	0	0	2	0	3	0	0	0	8	4	8	0	0	1
		240	2	0	1	0	1	2	2	0	0	0	5	0	5	5	2	4	5	5	8	3	1	2	3	3	2	3	3	1	7	3	2	0	0	0	0	0	2	0	3	0	0	0	8	4	8	0	0	1
		360	2	0	1	0	1	2	2	0	0	0	5	0	5	6	3	5	5	5	8	3	1	2	3	3	2	2	3	1	7	3	2	0	0	0	0	0	2	0	3	0	0	0	8	4	8	0	0	1
Highly infected Arka anamika (Multiplex)		0	2	0	0	0	1	2	0	1	0	0	3	0	3	3	2	0	1	3	1	1	2	0	0	0	0	2	3	2	2	0	0	0	0	0	0	0	2	0	2	0	0	0	4	2	4	0	0	1
	Cotton bag	120	3	0	1	1	2	3	0	1	0	0	6	0	5	6	3	0	2	3	1	2	3	0	0	0	0	3	5	3	4	1	1	1	0	0	0	0	3	0	3	0	0	0	8	4	8	0	0	1
		240	3	0	2	2	2	3	0	1	0	0	6	0	6	6	3	0	2	4	1	2	3	0	0	0	0	3	5	3	4	1	1	1	0	0	0	0	3	0	3	0	0	0	8	4	8	0	0	1
		360	4	0	2	2	2	3	0	1	0	0	6	0	6	6	4	0	2	4	1	2	4	0	0	0	0	4	5	3	4	1	1	1	0	0	0	0	3	0	3	0	0	0	9	2	7	0	0	1
	Polythene bag	120	5	2	3	4	2	4	3	2	3	4	6	2	8	7	4	5	2	4	2	3	4	2	2	2	1	4	5	3	4	1	2	2	1	0	0	0	3	0	4	0	0	0	9	2	7	0	0	10
		240	6	2	3	4	2	4	4	2	3	4	6	2	8	7	5	6	2	4	3	4	5	5	6	3	2	5	7	3	8	1	2	2	1	0	0	0	3	0	4	0	0	0	10	4	9	0	0	10
		360	8	2	3	4	2	4	4	2	3	4	6	2	8	7	5	6	2	4	3	4	5	6	6	3	2	6	8	3	8	2	4	2	1	0	0	0	3	0	4	0	0	0	10	4	9	0	0	10
	Paper bag	120	8	2	3	4	2	4	4	5	5	4	8	3	9	8	6	8	2	5	4	5	6	8	8	6	3	7	9	4	9	3	5	2	1	0	0	0	3	0	4	0	0	0	12	4	10	0	0	10
		240	10	2	3	4	2	4	4	5	5	4	8	3	9	8	6	8	2	6	5	5	6	8	9	8	4	10	10	4	11	4	6	2	1	0	0	0	3	0	4	0	0	0	12	4	12	0	0	10
		360	10	2	3	4	2	4	4	5	5	4	8	3	9	8	6	8	3	7	6	6	6	9	10	10	15	5	18	4	6	2	2	1	1	0	0	0	3	0	1	0	0	0	15	4	12	0	0	10

a-Actinomycetes sp.; b-*Alternaria alternata*; c-*Aspergillus flavus*; d-*Aspergillus fumigatus*; e-*Aspergillus columnaris*; f-*Aspergillus niger*; g-*Botryodiplodia theobromae*; h-*Colletotrichum dematium*; i-*Cladosporium cladosporioides*; j-*Chaetomium globosum*; k-*Fusarium verticilloides*; l-*Fusarium solani*; m-*Macrophomina phaseolina*; n-*Rhizoctonia solani*; p-*Trichothecium roseum.*

seeds. Biochemical studies can also be correlated with the pathogenic status of crops and seeds. Among the seed samples, healthy, moderately infected and highly infected seeds are observed with the fungal load of 0, 10-30 and 45-80%, respectively.

The viability of seeds stored in cotton bags maintained stability till one year. Further, cotton bags offered more protection than polyethylene and paper bags, as indicated by retention of protein as that of 0 day storage. The inefficiency of paper and polyethylene and efficacy of cotton bags may be due to varied water retention in the samples during storage. Polyethylene bags on the other hand, though have reported to protect fruits and vegetables, the use of such bags are prohibited to avoid biohazardous environmental threat. Also poor maintenance of keeping quality of seeds in polyethylene bags, suggests its demerits for storing okra seeds. Regarding the effect of temperature, both in healthy and infected samples, 28^{o} C did not reduce the total protein, carbohydrate and phenolic contents. However, deterioration of proteins was observed with in 120 days of storage at 45^{o} C, 10^{o} C storage. Instability at 45^{o} C is probably due to activation of enzymes especially proteases and the same may be responsible for hydrolysis of biomolecules. Contrarily, instability at 10^{o} C may also due to reduction in elimination of moisture content encouraging the growth of the fungi. Present results therefore suggested that moisture content is crucial to safe guard the seeds under storage over a period of one year. Packing material or temperature manage the growth of fungi by altering moisture content in seed and hence protect the seeds against fungal pathogens,

Fig. 1. Variation in seedling vigour of different samples of okra (Arka anamika) due to varied incidence of fungi

Healthy **Moderately Infected** **Highly infected**

Fig. 2. Variation in Protein content of okra due to different storage conditions

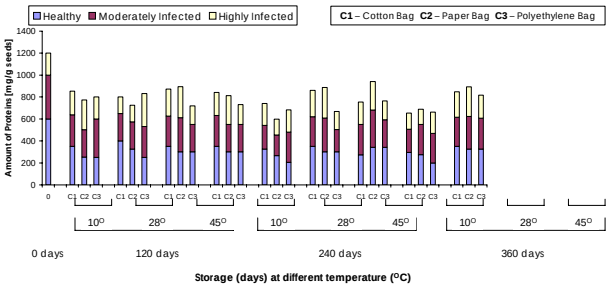


Fig. 3. Effect of storage conditions on Carbohydrate levels of okra

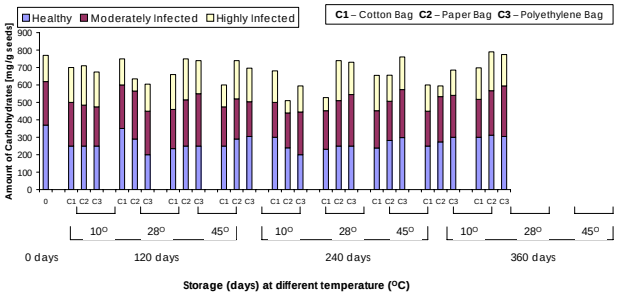


Fig. 4. Variation in Phenol content due to different storage conditions in okra

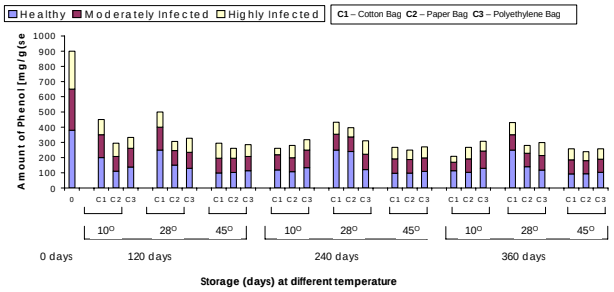
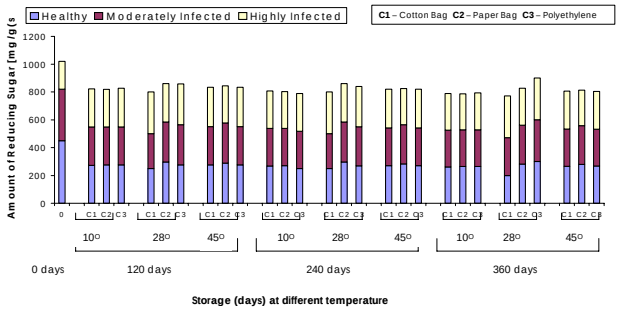


Fig. 5. Effect of storage conditions in Reducing Sugar levels of okra



which harbored in them. In this findings, changes in biochemical profiles confirms the faster deterioration in severely infected seed samples than healthy ones. Further, studies also indicated the predominance of fungal pathogens are in seed samples and they may get multiplied during storage. However, increased infectivity as of fungal pathogens did not affect the biochemical content. The results may raise a question as to whether fungal infected seeds to be treated with fungicides to eliminate fungal pathogens or to go for the use of beneficial packing material. Innumerable data from Lalithakumari *et al.* (1972) reported that thiram was best seed dressing fungicide. Savitri *et al.* (1998) employed the polyethylene and cloth bags for storage after the treatment of seeds with fungicides and insecticides. The toxicity of plastic, may be relieved by the use of cotton bags, which not only protect seeds against fungal infection, but also prevent a biohazards. Further hazardous fungicides are not recommended since they are toxic to human system. However, exclusive evidence for safety of anti-fungal agents should be provided and are being pursued in the laboratory. Hence, it would be advisable to treat infected okra seeds possessing high viability, with any one of the seed protectants of plant pathogen, namely neem extract or turmeric extract and pack them in cotton bags at 28°C for better maintenance of seeds to use in next planting season.

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