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RESEARCH ARTICLE

Seed treatments and storage conditions on vigour and viability of sesame seeds

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Abstract

Sesame, referred as Queen of Edible Oil Seeds, has an excellent nutritional content and therapeutic properties. Presence of high oil content accelerates rapid deterioration turning them rancid and perish quickly than conventional seeds. In addition, oilseeds are vulnerable to peroxidation of polyunsaturated fatty acids, which produces free fatty acids and free radicals that harm cells. The present study was undertaken to analyse the effect of seed treatments and storage conditions on vigour and viability of this economically important oil seed crop. Sesame seeds were treated with dry halogen (6 g kg^{-1} seeds) and halo polymer (8 g kg^{-1} seeds), packaged in 700-gauge polythene bags and kept in ambient and cold storage settings for a total of six months, along with untreated control. Seeds were evaluated initially and at monthly intervals for physiological, biochemical, anatomical and seed health parameters. Higher germination (94 %), vigour index (1493), oil content (46.84 %), catalase activity (1.089 mmol H_2O_2 min^{-1} g^{-1}) were observed in halo polymer-treated sesame seeds. Also seeds treated with halo polymer recorded minimum pathogen infection (4 %), in contrast to control 7 %). Halo polymer-treated sesame seeds also demonstrated lower cellular deterioration and loss of turgidity than untreated seeds. When compared to ambient storage, seeds kept in cold storage retained seed quality better for both the physiological and biochemical parameters.

Keywords: germination; halo polymer; longevity; seed treatment; sesame

Introduction

Sesame (*Sesamum indicum* L.), one of humanity's oldest cultivated crops, is frequently referred to as the "Queen of Edible Oil Seeds" (1) due to its rich nutritional content and therapeutic properties. This is the third most important oilseed crop in India with a production of 0.71 million metric tons (seednet.gov.in). The principal sesame growing nations are India, Myanmar, Sudan and China. Sesame seeds, which typically contain 50 % oil, 25 % protein and 15 % carbohydrates, are utilised in the food sectors, nutraceutical, pharmaceutical and other industries around the world (2). Furthermore, the crop holds significant ritualistic, religious and cultural value. Sesame oils have 40 % oleic and 40 % linoleic acid content and is utilised in margarine, salad dressings and cooking. Due to the presence of antioxidant, sesamol, sesame oil and dishes cooked in it have long shelf life (3). The oil can be used to make soap, paint, fragrances, medicines and pesticides. For poultry and animals, sesame meal provides a high-grade protein (40 %) feed. Sesame seeds are a powerhouse of energy and rich in minerals including iron, calcium, magnesium, phosphorus, zinc, copper and potassium as well as vitamins A, B and E complex. It is the valuable alternative to breast milk, particularly infants with milk allergies. Exceptional amounts of tryptophan, methionine and other amino acids with countless advantages can be found in sesame seeds. The high oil content and rapid cellular respiration in sesame seeds contribute to accelerated deterioration. High moisture in seeds and storage temperatures are favourable to fungus growth (4). Unwanted consequences of fungal activity in stored seeds include heating, seed discoloration, shrink-age, damage, reduced nutritional value and lower germination rate.

Seed is the essential and crucial input in crop production. Uninterrupted supply of high-quality seeds along

with necessary inputs can raise yields by 10-12 %. The storage of seeds for the future generation is crucial since crop cultivation is depending on the seasons. Farmers, breeders and businessman's all depend on the availability of good quality seeds. However, due to rapid viability loss, maintaining the quality of seed during storage is a serious challenge. Oil seeds deteriorate more quickly than other conventional seeds because of their high oil content, which makes them probe to rancidity and spoilage. In addition, oilseeds are vulnerable to polyunsaturated fatty acid peroxidation, leading to the production of free fatty acids and free radicals that harm cells. As a result, the oil content of seed is inversely connected to its ability to resist deterioration.

Although seed deterioration is unavoidable, it can be significantly slowed down by using the right technology, such as storage techniques, storage environments, containers and seed treatments. A seed's inherent characteristics, such as its amount of moisture, can be affected by the environment and the seed's chemical makeup (5). Therefore, by utilising 700-gauge polythene bags (moisture-vapour-proof) and storing under cold storage conditions, moisture level variations brought on by climatic changes can be kept at a control (6). Numerous studies have shown that halogen compound equilibration of the seeds prior to storage can significantly reduce the deterioration sequence. Halogens are quickly transferred to the vapour phase at room temperature, allowing them to bond to the fatty acid chain and prevent the generation of free radicals (7). Halogenation is therefore an affordable and practical storage method that may be employed by both large and small-scale farmers to extend the shelf life of seeds. The present study was carried out with the objective to study the physiological, biochemical and anatomical manifestations of seed deterioration during storage and also evaluating the effect of seed treatments and storage conditions on maintaining seed vigour and viability in sesame.

Materials and methods

Sesame var. TMV 7 has been used as the base material for storage study since it is a ruling variety with high productivity, released at state level during 2009 and play a major role in seed chain. Halo polymer (black) was obtained from M/s: Hilton Halo polymer company, Coimbatore. A pilot study was conducted to determine the dosage of halo polymer and dry halogen for treating sesame seeds. In dry halogen treatment, the seeds did not need to be redried to bring back its original moisture content since it's at dried state. But t a dusting off problem while handling was observed. In contrast, the Halo polymer seed treatment provided a smooth coating with no dusting off problem, but it needed shade drying to dry the seeds. Hence, these two products were compared along with untreated control (T_0) to find out its effectiveness on seed storability.

Storage of seeds

Treated and control seeds were filled in 700-gauge polythene bags and stored in ambient conditions (temperature of 28-38^{0}C and relative humidity of 30-60 %) and the cold storage unit (temperature of 50 °C with 40 % relative humidity) for six months. Four replicates of sesame seed samples were analyzed initially and at monthly intervals to find out the influence of seed treatments and storage conditions on storability of sesame seeds. Parameters observed were germination (%) (8), vigour index (9), dehydrogenase enzyme activity (OD value) (10), oil content (11), catalase activity (12) and lipid peroxidation (13).

Treatment details

Seed treatment

T_0 - Control

T_1 - Seed treatment with iodine impregnated halo polymer @ 8 g kg^{-1} of seed

T_2 - Seed treatment with calcium hypochlorite @ 6 g kg^{-1} of seed

Period of storage

P_0 - Initial

P_1 - One month after storage

P_2 - Two months after storage

P_3 - Three months after storage

P_4 - Four months after storage

P_5 - Five months after storage

P_6 - Six months after storage

Anatomical analysis

The scanning electron microscope FEI QUANTA 250 was used for anatomical examination. The embryonic axis of the seed was removed and cuts were made laterally and transversely. To view the exterior surface, the sectioned embryonic axis was oriented upward. Prior to imaging, the samples were sputter-coated with a thin layer of gold (approximately 10 nm) using a sputter coater to enhance conductivity and reduce charging under the electron beam. The axis was then positioned on a double-sided adhesive carbon conducting tape fixed to an 8 mm diameter aluminium stub. The Embryonic Axis Surface was seen with an Everhart-Thornley detector at 10 kV and 60 Pa pressure, under 500X and 4000X magnification.

Seed Health Test (ISTA 2010)

Twenty-five seeds were placed on the wetted blotter paper with 0.2% 2,4-D solution and kept for seven days at 20 ±2 °C with 12-hrs light (UV) and 12-hrs dark. Then seeds were checked for the presence of fungal infection on the eighth day and the values were expressed in percentage. The data collected during this study were statistically analysed using Agres software (14).

Results and Discussions

At six months following storage, sesame seeds treated with halo polymer had a higher germination rate of 88 %, while control seeds had 83 % (Fig. 1). However, only the interaction between halogen seed treatments and storage times was shown to be important, while other interactions were found insignificant. These findings are consistent with previously reported results for sesame (15). The greater germination percentage in seeds treated with halogens may be due to a quicker conversion of insoluble stored materials into a readily accessible soluble condition (15). Storing soybean seeds under controlled conditions improved germination rates compared to seeds stored in ambient conditions. Similar findings for soybean were also reported (16). The performance of the seeds

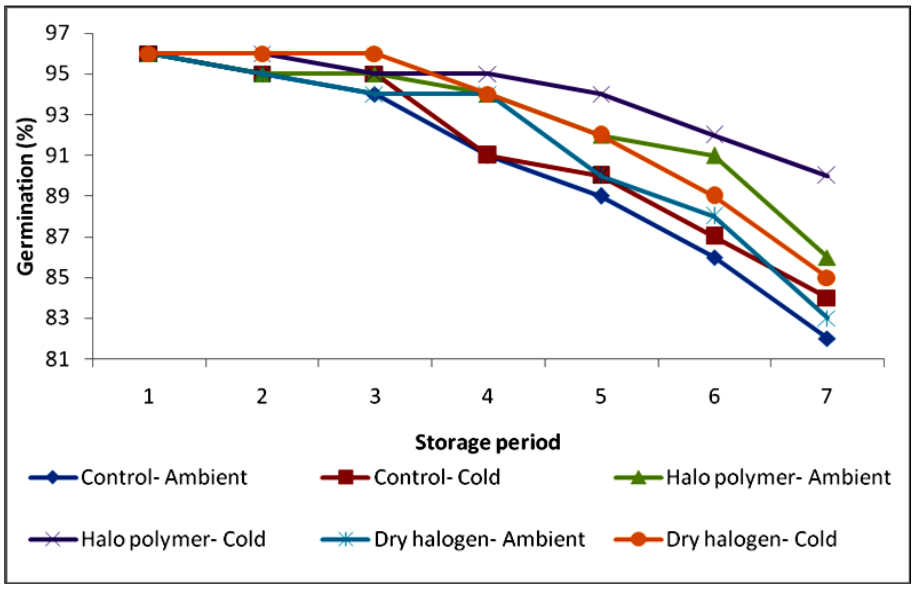

Fig. 1. Effect of halogen seed treatment, storage period and, storage condition on seed germination (%) of sesame var. TMV 7 seeds during storage.

will decline with longer storage times, changes in the environment's temperature and humidity and the depletion of food reserves. DNA degradation, which results in faulty transcription and inactive enzyme production, are two factors that negatively affect the ageing of seeds (17).

The Halo polymer seed treatment recorded the higher vigour index (1493), followed by the dry halogen treatment (1435) over the untreated control seeds (1393) (Fig. 2). With longer storage periods, vigour index reduced. In cotton and in sunflower similar results were reported (18, 19). The findings in seeds of paddy (20) experiment support the above results. Increased free radical activity and lipid peroxidation cause membrane disintegration and decreased enzymatic activity, which decreases vigour. This effect is crucial for the stabilisation of cell lipoprotein membranes and the saturation of unsaturated fatty acids (7).

Regardless of crops, halogen seed treatments, or storage conditions, dehydrogenase enzyme activity decreased as storage time increased. The rate of decrease from initial to final was from 0.657 to 0.440 OD value. The results of previous findings in sunflower (21) and brassica (22) are in agreement with this. They claimed that as seeds became older, the enzyme responsible for respiration of the seed became less active. With increasing storage time or seed entering the senescence phase, decrease in dehydrogenase enzyme activity was noted in soybean and cowpea (23). In aged cabbage seeds, degradation of the respiratory pathway (at cytochrome C) leading to fermentation and high ethanol production, resulting in reduced dehydrogenase activity, was reported (24), which may be the cause of the inability of the seed tissues to reduce tetrazolium chloride into insoluble formazon. The interaction effect demonstrated that after six months of storage, seeds treated with halo polymer and stored in cold circumstances maintained higher dehydrogenase activity (0.503 OD value) than control seeds stored in ambient conditions (0.383 OD value) (Table 1). This was consistent with previous research findings (25). By preventing enzyme degradation, the halogen treatments lessened the effects of ageing and kept the viability intact for a longer duration.

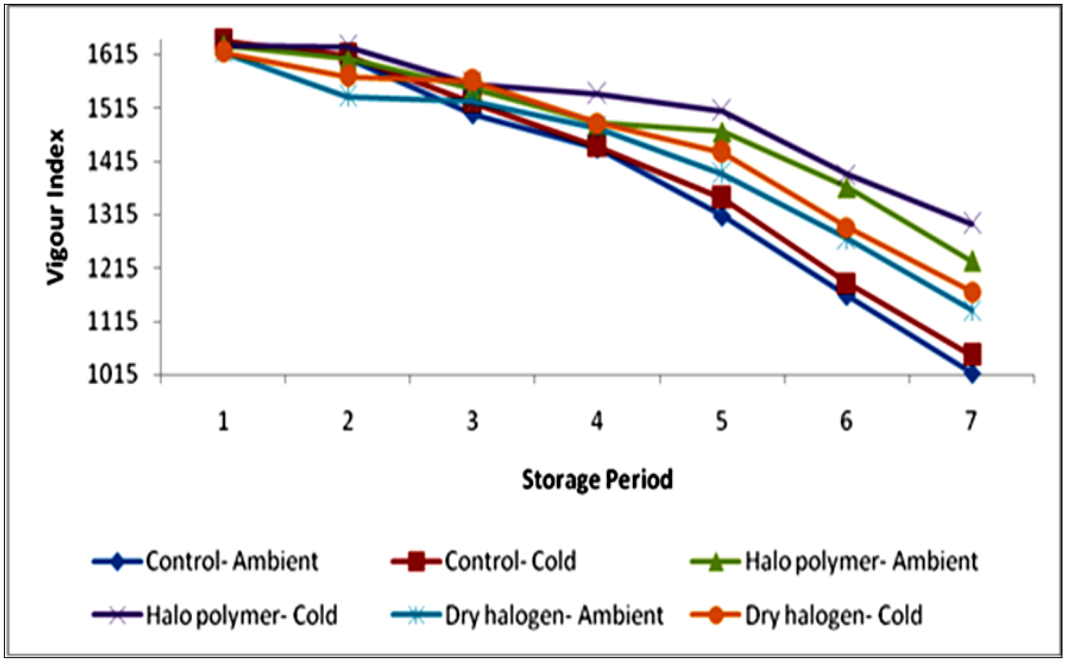

Fig. 2. Effect of halogen seed treatment, storage period and, storage condition on vigour index of sesame var. TMV 7 seeds during storage.

Table 1. Effect of halogen seed treatment, storage condition and storage period on dehydrogenase activity (OD value) of sesame var. TMV 7 seeds during storage

Seed treatment (T)	Storage condition (S)	Period of storage in months (P)							Mean
		P_0	P_1	P_2	P_3	P_4	P_5	P_6	
Control	Ambient storage	0.658	0.650	0.638	0.621	0.541	0.472	0.383	0.566
	Cold storage	0.658	0.650	0.640	0.624	0.549	0.480	0.391	0.570
	Mean	0.658	0.650	0.639	0.622	0.545	0.476	0.387	0.568
Halo polymer(8 g kg^{-1})	Ambient storage	0.654	0.652	0.641	0.625	0.588	0.554	0.496	0.601
	Cold storage	0.654	0.649	0.642	0.627	0.590	0.559	0.503	0.603
	Mean	0.654	0.650	0.641	0.626	0.589	0.556	0.499	0.602
Dry halogen (6 g kg^{-1})	Ambient storage	0.658	0.649	0.637	0.620	0.563	0.523	0.431	0.583
	Cold storage	0.658	0.652	0.639	0.624	0.567	0.527	0.439	0.587
	Mean	0.658	0.650	0.638	0.622	0.565	0.525	0.435	0.585
	Period Mean	0.657	0.650	0.640	0.623	0.566	0.519	0.440	0.585

Storage Condition mean	Ambient storage					Cold storage	
	0.583					0.587	
	T	S	P	T × S	S × P	T × P	T × S × P
SEd	0.009	0.008	0.014	0.013	0.021	0.025	0.035
CD (P=0.05)	0.019**	NS	0.029**	NS	NS	NS	NS

The storage environment and duration significantly impact the qualitative characteristics of seed, particularly the quantity and quality of oil. Seeds rich in lipids tend to have a short shelf life due to their unique chemical composition, such as high oil content that causes loss of germinability and seed viability, (26). In the current investigation, halo polymer-treated sesame seeds had an estimated oil content that was 1.2 percentage higher than the control seeds (Table 2). Seeds stored under cold conditions retained higher oil content, compared to seeds stored in ambient environmental conditions. This is likely because, at elevated temperature, lipids are used for respiration, leading to notable reduction in oil content (27). With longer periods of storage, oil content decreased by 3.1 % in sesame. In sunflower, also similar findings were reported (28). Storage fungus increased free fatty acid, causing oil to become rancid and resulting in a drop in oil content (29). The aforementioned results are consistent with research conducted on several oilseed crops (29). Triglycerides are transformed into free fatty acids during the oxidative process that occurs when seeds are stored and an accumulation of free fatty acids causes seeds to lose viability. Oilseeds are extremely sensitive to the environment and the oil content easily oxidises, causing the seeds to degrade while being stored (30).

Catalase and peroxidase are key antioxidant enzymes that scavenge the free radicals generated during the lipid peroxidation process. Seeds, naturally have evolved antioxidant enzymes including catalase (CAT) and superoxide dismutase (SOD) to stop the generation of free radicals (31). Sesame seeds treated with halo polymer (1.089 mmolH_2O_2 min^{-1} g^{-1}) had higher catalase activity than the control (0.984 mmol H_2O_2 min^{-1} g^{-1}) (Fig. 3). Because halogens have a detoxifying effect, it is possible that seeds treated with halo polymers in sesame retained

Table 2. Effect of halogen seed treatment, storage condition and storage period on oil content (%) of sesame var. TMV 7 seeds during storage

Seed treatment (T)	Storage condition (S)	Period of storage in months (P)							Mean
		P_0	P_1	P_2	P_3	P_4	P_5	P_6	
Control	Ambient storage	47.80	47.40	47.00	46.32	45.73	44.78	43.93	46.14
	Cold storage	47.80	47.50	47.20	46.40	45.90	45.02	44.00	46.26
	Mean	47.80	47.45	47.10	46.36	45.81	44.90	43.96	46.20
Halo polymer (8 g kg^{-1})	Ambient storage	47.80	47.58	47.30	46.90	46.53	45.90	45.32	46.76
	Cold storage	47.80	47.60	47.40	47.00	46.70	46.20	45.70	46.91
	Mean	47.80	47.59	47.35	46.95	46.61	46.05	45.51	46.84
Dry halogen (6 g kg^{-1})	Ambient storage	47.80	47.50	47.20	46.68	46.20	45.47	44.80	46.52
	Cold storage	47.80	47.50	47.30	46.90	46.60	45.80	44.20	46.59
	Mean	47.80	47.50	47.25	46.78	46.40	45.64	44.50	46.55
	Period Mean	47.80	47.51	47.23	46.70	46.28	45.53	44.66	46.53

Storage condition mean	Ambient storage					Cold storage	
	42.98					43.04	
	T	S	P	T × S	S × P	T × P	T × S × P
SEd	0.074	0.061	0.114	-	-	0.197	-
CD (P=0.05)	0.147**	NS	0.229**	NS	NS	0.394**	NS

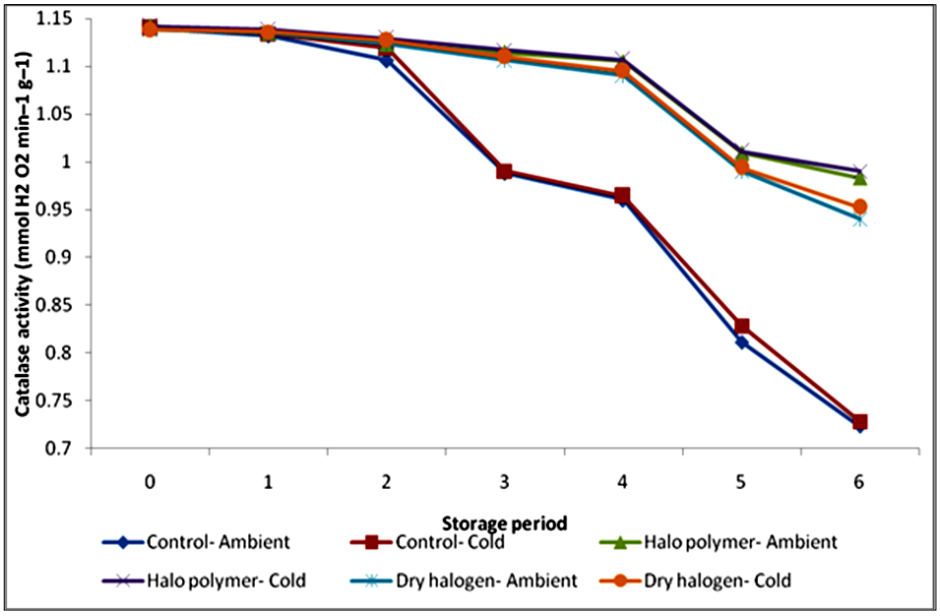

Fig. 3. Effect of halogen seed treatment, storage period and, storage condition on catalase activity (mmol H_2O_2 min^{-1} g^{-1}) of sesame var. TMV 7 seeds during storage.

higher peroxidase activity of 0.224 at the conclusion of six months of storage. In comparison to cold storage settings, the antioxidant enzyme activity and levels in seeds decreased after 6 to 12 months of ambient storage (40 °C). Halogens sustain the action of the antioxidant enzymes and impede the degradation process., The catalase activity of seeds that were being preserved reduced as storage time increased (32). Reduced catalase activity in cotton seeds implies a decline in seed viability (33). and In peas the cause of reduced catalase was found due to a drop in protein content (34). Seeds were harmed by hydrogen peroxide build up that occurred as seeds neutralised free radicals, which led to decreased peroxidase and catalase activity (35). In black gram and cotton depletion of antioxidant enzyme activity was also noted during seed ageing (36, 33).

Lipid peroxidation is a process in which lipids undergo oxidation, leading to the formation of non-radical compounds that contribute to seed deterioration. According to the current study and other biochemical quality assessments, lipid peroxidation increased as storage time increased. After six months of storage, sesame seeds treated with halo polymer exhibited minimal lipid peroxidation activity, measured at 1.495 mols MDA g^{-1} (Table 3). In contrast, untreated castor bean seeds showed the highest peroxidation activity at 1.927 mols MDA g^{-1} FW. Similarly, untreated castor bean seeds showed high lipid peroxidation level supported (37), which reported that calcium oxychloride reduces lipid auto oxidation and suppresses the generation of free radicals and aldehydes. According to (7) it might be attributed to interaction between the C=C double bond of polyunsaturated fatty acids, making

Table 3. Effect of halogen seed treatment, storage condition and storage period on lipid peroxidation (µmols MDA g^{-1}) of sesame during var. TMV 7 seeds storage

Seed treatment (T)	Storage condition (S)	Period of storage in months (P)							Mean
		P_0	P_1	P_2	P_3	P_4	P_5	P_6	
Control	Ambient storage	1.270	1.290	1.375	1.456	1.590	1.780	1.962	1.532
	Cold storage	1.270	1.282	1.373	1.455	1.551	1.643	1.892	1.495
	Mean	1.270	1.286	1.374	1.455	1.570	1.711	1.927	1.514
Halo polymer (8 g kg^{-1})	Ambient storage	1.270	1.281	1.311	1.360	1.431	1.467	1.505	1.375
	Cold storage	1.270	1.270	1.315	1.384	1.403	1.425	1.486	1.365
	Mean	1.270	1.275	1.313	1.372	1.417	1.446	1.495	1.370
Dry halogen (6 g kg^{-1})	Ambient storage	1.250	1.290	1.354	1.393	1.458	1.491	1.636	1.410
	Cold storage	1.250	1.284	1.342	1.386	1.424	1.464	1.605	1.394
	Mean	1.250	1.287	1.348	1.389	1.441	1.447	1.620	1.402
	Period Mean	1.263	1.283	1.345	1.406	1.476	1.545	1.681	1.428
		Ambient storage					**Cold storage**		
Storage condition mean		1.439					1.418		
		T	**S**	**P**	**T × S**	**S × P**	**T ×P**	**T × S × P**	
SEd		0.024	0.04	0.037	0.035	-	0.02	-	
CD (P=0.05)		0.048**	NS	0.073**	NS	NS	0.04**	NS	

them less prone to oxidation. No significant difference in lipid peroxidation was observed between storage settings. However, values increased from 1.263 to 1.681 mols MDA g^{-1} FW over the course of storage. Lipid oxidation, which is the direct cause of the seed deterioration during storage, results in the destruction of the membrane systems at the cellular level. Polyun-saturated fatty acids, such as linoleic and linolenic, are especially vulnerable to oxidative degradation by enzyme-tic and non-enzymatic processes. Seed aging is further accelerated by enzyme inactivation, protein degradation, cell membrane disintegration and genetic damage (36).

In the current investigation, *Aspergillus* sp. was the most common fungus that infected the sesame seeds during storage. Infection by *Aspergillus* species reduces the amount of fat and protein content of seeds. Storage fungi not only reduces nutritional components like fat, protein and sugar in the seeds, but also produce aflatoxin which inhibit the germination and reduce plant growth (38, 39). After six months of storage, sesame seeds treated with halo polymer showed 8 % lesser pathogen infection than the control (16 %) (Fig. 4). Compared to seeds stored in ambient storage conditions, seeds stored in cold storage experienced a 1 % lower pathogen infection rate. By the end of the storage period, the overall pathogen infection rate had increased by 11 %.

Anatomical changes in sesame seeds after six months of storage were examined using a scanning electron microscope (SEM) (FEI QUANTA 250). The anatomical alterations in control and halogenated fresh and aged sesame seeds were observed. Under a scanning electron microscope, the seed of embryo, cotyledon and seed coat were examined. Untreated sesame seeds showed shrunken parenchymatic cells in the embryo and endosperm. Over the course of storage, control sesame seeds showed a significant reduction in cell turgidity and stiffness. In terms of sesame seed coat integrity, untreated seeds exhibited evident structural damage, but seeds treated with halo polymer showed less damage. Similar seed coat and palisade damage was also recorded in onion seeds (40). The reduced cellular damage observed in halogenated seeds may be attributed to halogens' ability to slow down the deterioration process and stabilise membrane integrity. Cell shrinkage in sesame seeds may be caused by a lack of cell turgidity. All seeds contain "oil bodies," which are small, spherical intracellular organelles that typically have a diameter between 0.5 and 2.0 mm and were used to store lipids (Fig. 5, 6). These oil bodies consist of a triacylglycerol matrix, a phospholipid monolayer and a group of interfacial proteins known as "oleosins". Oleosins plays a key role in maintaining oil body stability but do not specifically protect against desiccation. A notable finding of this study was reduced presence or relative absence of oleosins in the oil bodies of untreated sesame seeds, suggesting a breakdown oil body structure. Ultrastructural analysis confirmed increased membrane deterioration with prolonged storage (41). alterations take place as seed storage times increase. The main alterations in the seeds, include abnormalities of the mitochondria, loosening of the nuclear envelope, breakdown of the vacuole boundary membrane and protein bodies (41).

Conclusion

Sesame seeds treated with halo polymer (8 g kg^{-1}seeds) and stored under cold conditions retained higher physiological and biochemical qualities throughout, the storage period exhibiting minimal anatomical changes and reduced fungal infection. Hence treating the seeds with dry halogen polymer is highly recommended to increase the storage potential. Future studies could explore the long-term effects of halo polymer treatments on seed longevity across different oilseed crops, as well as the potential for scaling up this technology for commercial seed storage solutions. Additionally, investigating the environmental sustainability of such treatments could enhance their application in organic farming.

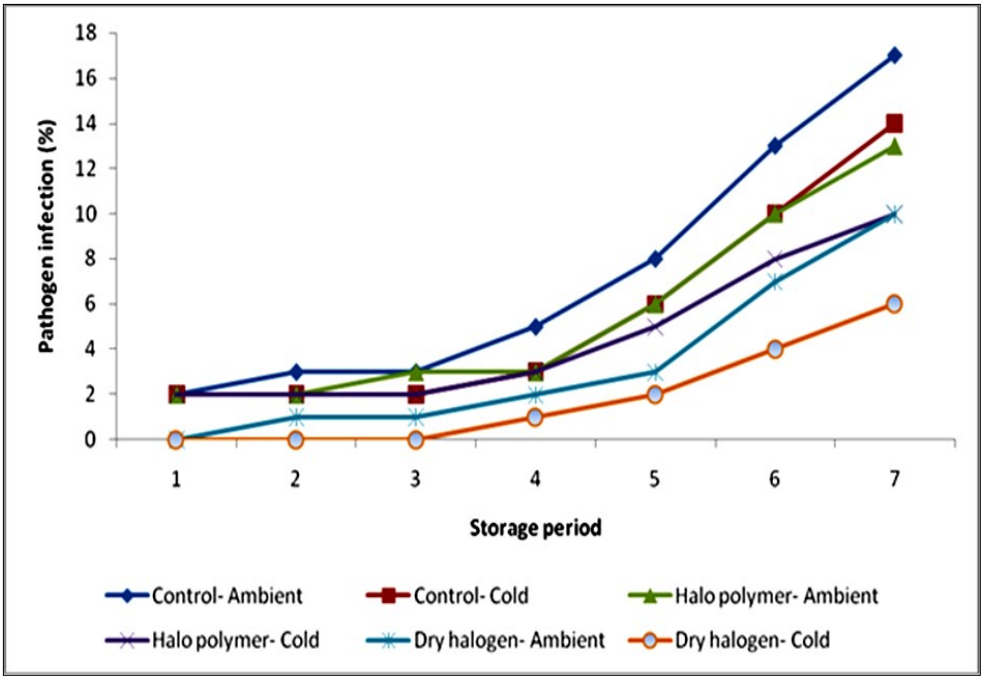

Fig. 4. Effect of halogen seed treatment, storage period and, storage condition on pathogen infection (%) of sesame var. TMV 7 seeds during storage.

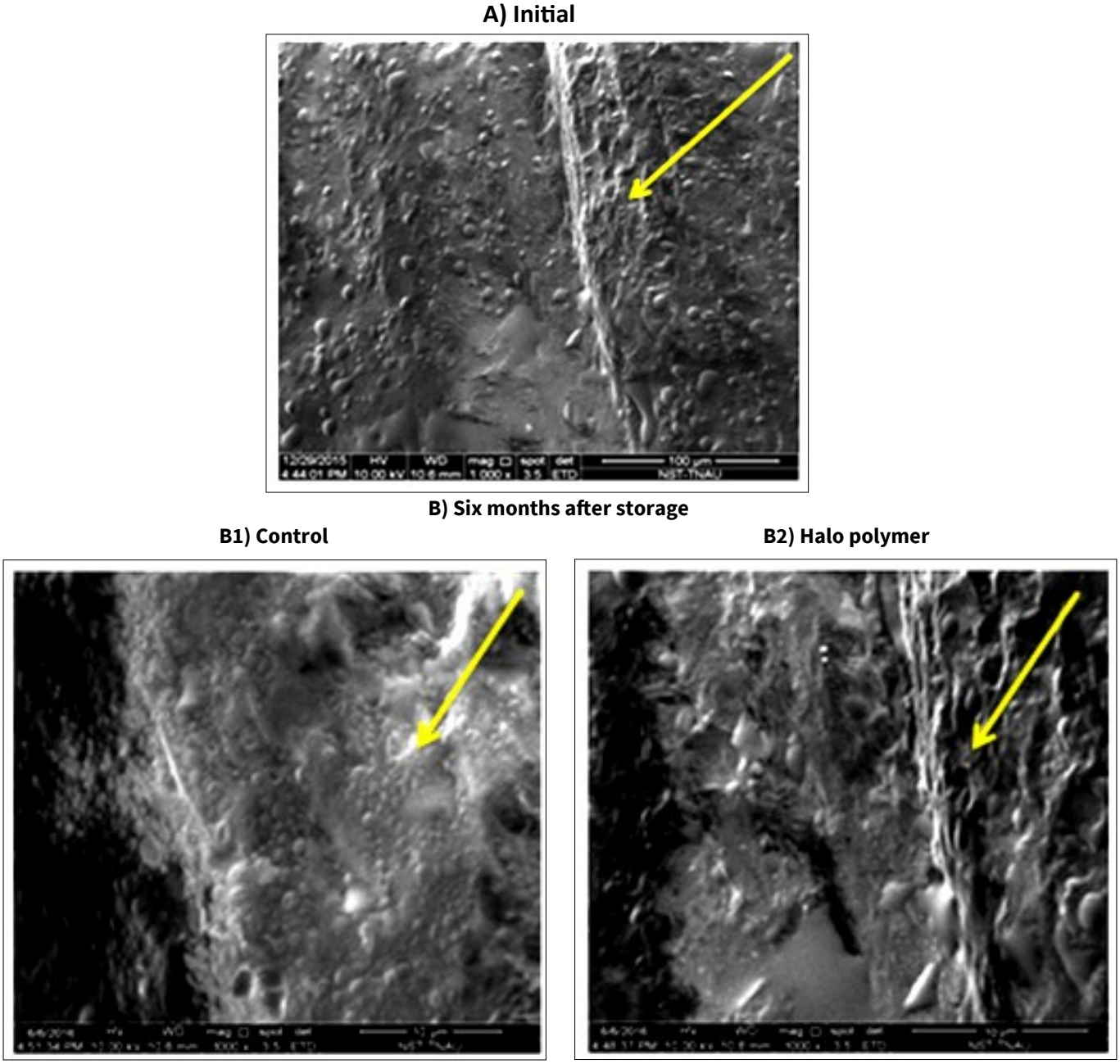

Fig. 5. Scanning Electron Microscope images of oil glands distribution in sesame var. TMV 7 seeds.

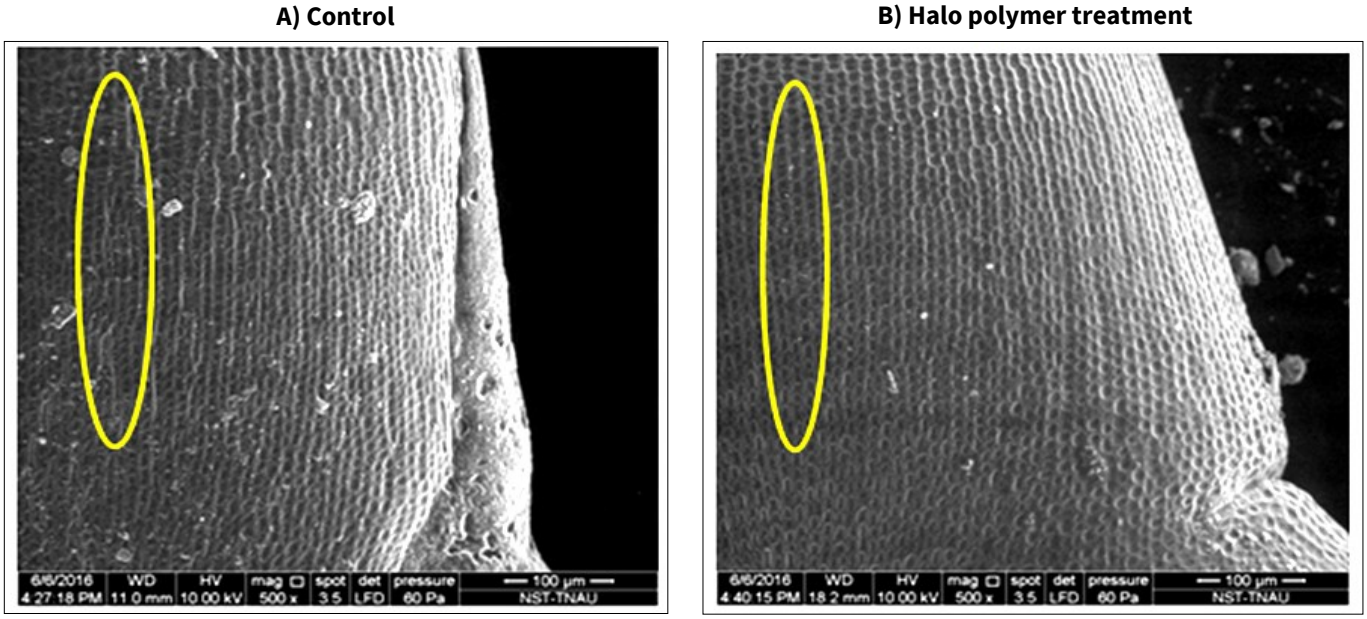

Fig. 6. Scanning Electron Microscope images of embryonic cells of sesame var. TMV 7 seeds at six months after storage.

Authors' contributions

KS and SPV carried out the Storage experiment, THA and VN done the dehydrogenase and oil content experiment. KPG and PC participated in the sequence alignment and drafted the manuscript. PK and VR carried out the lipid peroxidation and germination test. TSD and ET participated in the design of the study and performed the statistical analysis. All authors read and approved the final manuscript.

Compliance with ethical standards

Conflict of interest: Authors do not have any conflict of interests to declare.

Ethical issues: None

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