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Physiological and Biochemical Changes in Mustard Seeds during Storage

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ABSTRACT: Mustard is a recent introduction to Tamil Nadu and the thermo sensitivity of the crop is the major limitation for its spread unlike other oilseeds crop. Seed production and storage activity is to be initiated/started for the quality seed production and maintenance. The present investigation is an attempt to study the effect of different storage treatment and containers in mustard cv. GM2. The experiment conducted at Tamil Nadu Agricultural University during 2007. The Seeds treated with Halogen m0ixture @ 4 g kg^{-1}, Bavistin @ 2 g kg^{-1}, Halogen mixture @ 4 g kg^{-1} and Bavistin @ 2 g kg^{-1} along with untreated seeds were stored in cloth bag and 700 gauge polythene bag for 10 months under ambient storage conditions. The moisture content varied from 7.0 to 7.5 per cent as the period of storage increased. The germination (86.7 %), shoot (8.54 cm) and root length (18.58 cm), vigour index (2398) and dry matter production (22.1 mg) decreased as the period of storage advanced. Seeds treated with Halogen mixture @ 4g kg^{-1} + Bavistin @ 2g kg^{-1} of seed and stored in 700 gauge polythene bag maintained higher germination (88.7 per cent) and vigour index of 2463.The biochemical parameters like a-amylase activity, electrical conductivity, oil content and free fatty acid varied due to period of storage, treatments and containers. The study thus concluded that seed treated with Halogen mixture @ 4g kg^{-1} + Bavistin @ 2g kg^{-1} stored in polythene bag maintained better vigour and viability up to 10 months compared to other treatments under ambient condition.

Keywords: Mustard, Germination, Vigour index, Halogen mixture, Storage, Polythene bag, Cloth bag

Indian mustard (*Brassica juncea* L.) is one of the important oilseed crops contributing 25 per cent of the oilseed production of the country. It occupies a prominent place next to groundnut in meeting the oil requirement of about 50 per cent population. Physiological deterioration of seeds during storage is considered to be one of the major factors preventing seeds from normal germination and vigorous growth. Mustard is a recent introduction to Tamil Nadu and the thermo sensitivity of the crop is the major limitation for its spread unlike other oilseeds crop. Seed production and storage activity is to be initiated / started for the quality seed production and maintenance. Therefore, studies to evolve technologies for the production and storage of quality seeds would be a pre requisite. The deterioration of seeds in storage is aided by adverse storage environment, moisture content of seed and the containers used for storage besides it's susceptibility to fungal invasion [1]. Hence, an improved storage strategy to prolong shelf life of seeds under ambient storage condition is to be evolved for maintenance of seed quality.

MATERIALS AND METHODS

A laboratory experiment was conducted to study the effect of seed treatments and storage containers on seed viability during storage. Mustard cv. GM-2 seeds were cleaned and dried to uniform moisture content of seven per cent. The seeds were graded using 1.75 mm sieve and seeds after treatment were stored under ambient conditions for 10 months. The different treatments includes Control (Untreated) (T_1), Bavistin @ 2g kg^{-1} of seed (T_2), Halogen mixture @ 4 g kg^{-1} of seed (T_3) and Halogen mixture @ 4 g kg^{-1} of seed + Bavistin @ 2 g kg^{-1} of seed (T_4) and stored in two types containers *viz*., Cloth bag and 700 gauge polyethylene bag. The seed samples were drawn at bimonthly intervals and the observations were recorded.

Germination test was conducted using roll towel method. One hundred seeds of each variety in four replications were in roll towel paper and kept at 25°C in seed germinator. The final count was taken on 7th day and normal seedlings were considered for calculating

per cent germination [2]. Seedling length (root +shoot) was measured on ten randomly selected normal seedlings taken from four replication of germination test and recorded in centimeters. Average of ten seedlings was taken for final calculation. These ten seedlings whose length was measured were dried in hot air oven for 24 hours at 80 ±1°C. The dried seedlings of each replication were weighed and average seedling dry weight of each genotype was calculated and expressed in milligrams. The seedling vigour index was calculated [3] as Vigour index = germination percentage (%) x Average seedling length.

Electrical conductivity of the seed leachates was measured to know the status of membrane permeability. Four replicates of 25 seeds each were taken at random from each treatment, initially rinsed with deionised water and then soaked in 25 ml of deionised water for 8h at room temperature. The seed steep water decanted and referred as seed leachate. The electrical conductivity of seed leachate was measured in digital ELCO conductivity meter [4] with a cell constant of one and expressed as μdSm^{-1}.

For estimating oil content, the seeds from each sample were dried at 105 ± 2°C in a hot air oven for 16 h and cooled in desiccator for half an hour. From this, 5 g of seeds were ground in a porcelain mortar and properly packed in Whatman No.1 filter. After weighing (A), this was transferred to an extraction thimble. The thimble was then placed inside the Soxhlet extractor to which sufficient quantity of petroleum ether solvent with boiling point of 40-60°C was added and heated for 6 h until 6-8 siphonings were completed. Then the filter paper packet was taken out and dried in a hot air oven maintained at 105 ± 2°C for 6h, cooled in desiccator for half an hour and weighed (B). The oil content [5] was calculated using the following formula and expressed as percentage.

$$\text{Oil content (\%)} = \frac{(A - B)}{5} \times 100$$

The free fatty acid content [6] is inversely proportional to oil content. The free fatty acid content is estimated as a known quantity of oil (1g) was mixed with 25 ml of neutralized 95 per cent alcohol. The mixture was heated to boiling and titrated while hot against 0.02N NaOH solution to a faint pink end point using phenolphthalein as an indicator. The total free fatty acid content was calculated as per cent oleic acid using the following formula.

$$\text{Per cent of free fatty acid content} = \frac{28.2 \times (\text{N}) \text{of alkali} \times \text{ml of alkali used}}{\text{Weight of oil (g)}}$$

To study about a- amylase activity [7], two grams of agar shreds and one gram of potato starch mixed together in water to form a paste and the volume was made up to 100 ml. The homogenous solution of agar starch mixture after boiling was poured into sterilized petridishes and allowed to settle in the form of a gel agar after cooling. The presoaked (72 h) and half cut mustard seeds (with their embryo portion intact) were placed in the petridishes. Then it was incubated in dark at 30°C, after 24 h, the petridishes were uniformly poured with potassium iodide solution (0.44 g of iodine crystal + 20.008 g potassium iodide in 500 ml of distilled water) and excess solution was drained off after few minutes clear zone will be formed. The diameter of halo (clear zone formed around the seed) was measured in mm and reported as alpha amylase activity. The vigorous seeds formed larger halo zone than seed possessing low vigour. The factorial experiment in Completely Randomized Design (CRD) has been conducted and the data obtained from experiment were analysed as per standard methods [8].

RESULTS AND DISCUSSION

In the present study the progressive decline in germination from 90 per cent to 78 per cent was observed during storage period, irrespective of treatments and containers (Figure 1a; 1b; 1c). The decline in germination during storage may be due to depletion of food reserves, decline in synthetic activity as reported by [9] and [10] or may be due to the physiological ageing process accelerated by the interaction effect of increased seed moisture and storage period [11]. The superiority of polythene bags in maintaining higher germination

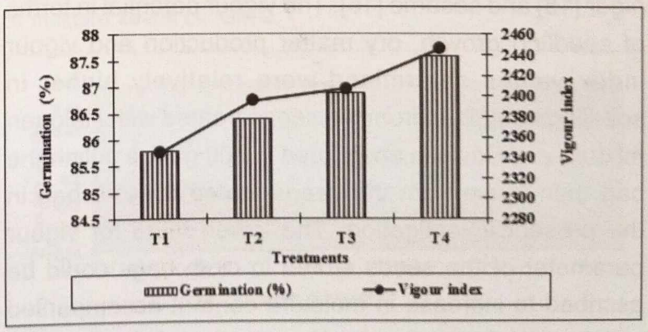

Figure 1a. Influence of seed treatment on germination percentage and vigour index in mustard seeds cv. GM-2

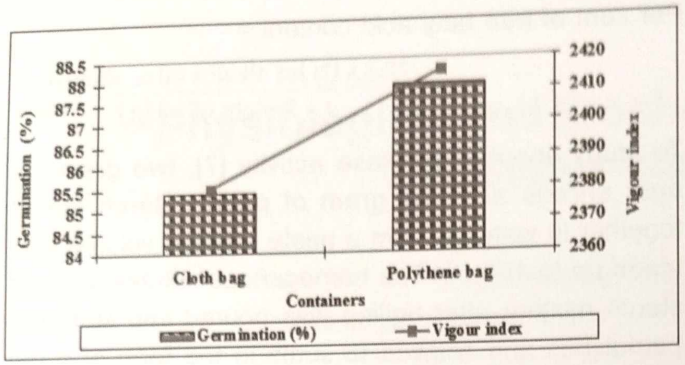

Figure 1b. Influence of storage containers on germination percentage and vigour index in mustard seeds cv. GM-2

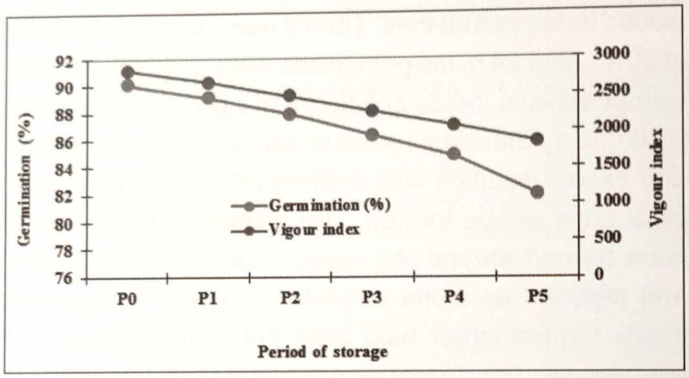

Figure 1c. Influence of period of storage on germination percentage and vigour index in mustard seeds cv. GM-2

(87.9 %) compared to cloth bag (85.4 per cent) in storage was due to its moisture vapour proof nature which besides offering protection against invasion of pathogen and insects. The maintenance of higher germination (87.6 %) of seeds treated with Halogen mixture may be due to stabilization of double bonds in unsaturated fatty acid and reduction of lipid peroxidation. Suggested that the role of chlorine in the stabilization of double bonds of unsaturated fatty acid moieties of lipoprotein bio-membranes as a possible reason for viability extension, besides the possibility of acting as a free radical controlling agent [12] Similar results were reported in sorghum [13], groundnut [14], niger [15] and sesame [16]. The vigour potential in terms of seedling growth, dry matter production and vigour index values determined were relatively higher in seedlings obtained from the seeds treated with Halogen mixture and Bavistin and stored in 700 gauge polythene bag than those from the seeds stored in cloth bag in the present investigation. The lower value for vigour parameter of the seeds stored in cloth bags could be ascribed to increase in moisture content accompanied with fungal infection resulted in reduction of physiological stamina of seeds.

Similar trend was followed in a – amylase activity (Figure 2a; 2b; 2c). The activity of the enzyme reduced from 1.4 mm to 0.9 mm, which might be due to the inability of seeds to synthesize the enzymes due to ageing.

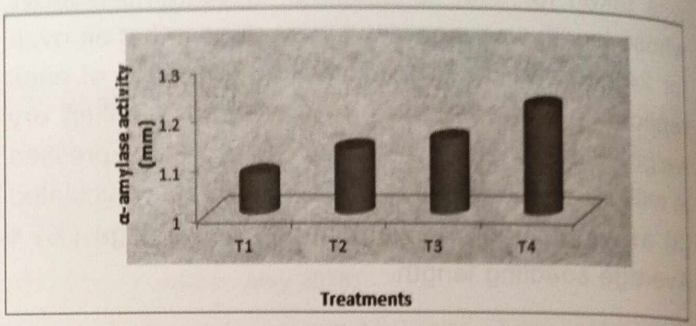

Figure 2a. Influence of seed treatment on α-amylase activity in mustard seeds cv. GM-2

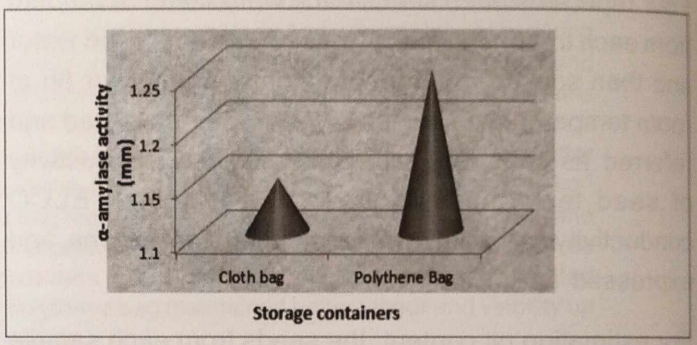

Figure 2b. Influence of storage containers on α-amylase activity in mustard seeds cv. GM-2

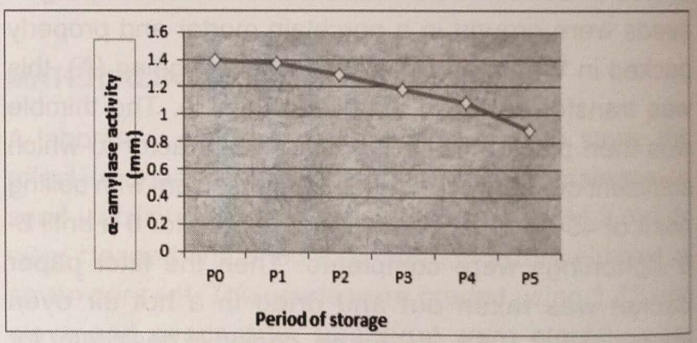

Figure 2c. Influence of period of storage on α-amylase activity in mustard seeds cv. GM-2

In the present study the electrical conductivity varied due to period of storage (Table 1). Electrical conductivity increased as period of storage advanced which might be due to the increased solute leakage as a result of loss of membrane integrity [17]. Similar results were reported in safflower [18] and rice [19].

With increased storage period, the oil content decreased and free fatty acid content increased (Figure 3a; 3b; 3c). This decrease and increase were less in Halogen treated seeds and stored in polythene bags.

Table 1. Influence of seed treatment, storage containers and period of storage on electrical conductivity (μ dSm^{-1}) in mustard seeds cv. GM-2

Treatments	Cloth bag (C)						Mean	Polythene bag (P)						Mean	G.Mean
(T)	P_0	P_1	P_2	P_3	P_4	P_5		P_0	P_1	P_2	P_3	P_4	P_5		
T_1	58.23	58.51	58.68	59.15	59.42	61.75	**59.29**	58.23	58.41	58.62	58.96	59.28	60.86	**59.06**	**59.18**
T_2	58.23	58.46	58.53	58.93	59.36	61.42	**59.16**	58.23	58.35	58.57	58.82	59.38	60.58	**58.99**	**59.07**
T_3	58.23	58.38	58.48	58.95	59.24	61.36	**59.11**	58.23	58.37	58.55	58.75	59.11	59.84	**58.81**	**58.96**
T_4	58.23	58.3	58.35	58.88	59.16	60.98	**58.98**	58.23	58.28	58.49	58.68	58.92	59.63	**58.71**	**58.84**
Mean	58.23	58.41	58.51	58.98	59.30	61.38	59.13	58.23	58.35	58.56	58.80	59.17	60.23	58.89	59.01
Mean (P)	P_0		P_1		P_2		P_3		P_4		P_5				
	58.23		58.38		58.56		58.89		59.23		60.80				
CD (p=0.05)	P		C		T		PxC		CxT		PxT		PxCxT		
	0.982		NS		NS		NS		NS		NS		NS		
SEd	0.494		0.285		0.404		0.699		0.571		0.989		1.399		

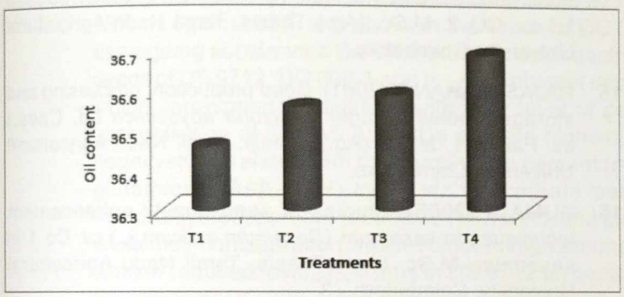

Figure 3a. Influence of seed treatment on oil content (%) in mustard seeds cv. GM-2

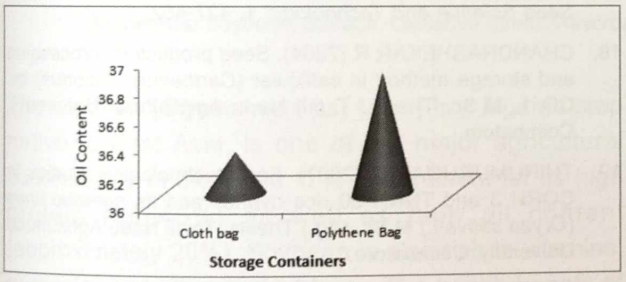

Figure 3b. Influence of storage containers on oil content (%) in mustard seeds cv. GM-2

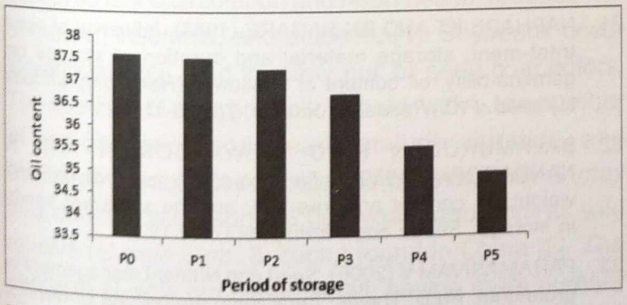

Figure 3c. Influence of period of storage on oil content (%) in mustard seeds cv. GM-2

This could be attributed to stabilization of double bonds in fatty acids and reduction of lipid peroxidation thereby minimizing the increase in free fatty acid content. Similar results were reported in soybean [20], sunflower [21, 22] and groundnut [23].

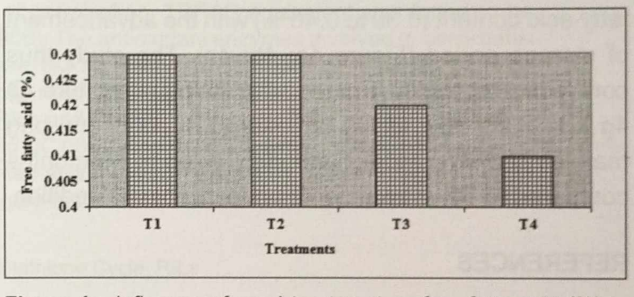

Figure 4a. Influence of seed treatment on free fatty acid (%) in mustard seeds cv. GM-2.

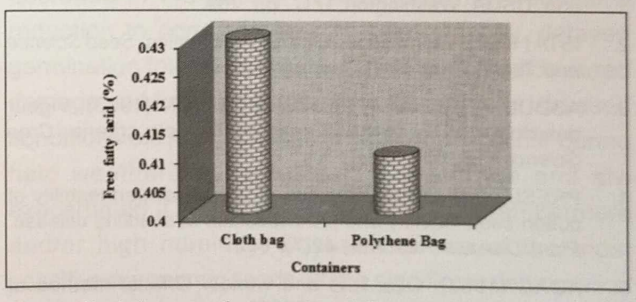

Figure 4b. Influence of storage containers on free fatty acid (%) in mustard seeds cv. GM-2.

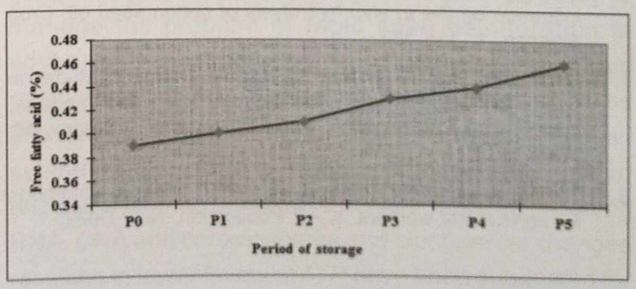

Figure 4c. Influence of period of storage on free fatty acid (%) in mustard seeds cv. GM-2.

CONCLUSION

The seeds treated with Halogen mixture @ 4 g kg^{-1}, Bavistin @ 2 g kg^{-1}, Halogen mixture @ 4 g kg^{-1} and Bavistin @ 2 g kg^{-1} along with untreated seeds were stored in cloth bag and 700 gauge polythene bag for 10 months under ambient storage conditions. The germination (86.7 %), shoot (8.54 cm) and root length (18.58 cm), vigour index (2398) and dry matter production (22.1 mg) decreased as the period of storage advanced. Seeds treated with Halogen mixture @ 4g kg^{-1} + Bavistin @ 2g kg^{-1} of seed and stored in 700 gauge polythene bag maintained higher germination (88.7 per cent) and vigour index of 2463. The biochemical parameters like a-amylase activity, electrical conductivity, oil content and free fatty acid varied due to period of storage, treatments and containers. The oil content decreased from 37.60 to 34.91 per cent with the corresponding increase in free fatty acid content (0.39 to 0.46 %) with the advancement of storage period (Figure 4a; 4b; 4c). The study thus concluded that seed treated with Halogen mixture @ 4g kg^{-1} + Bavistin @ 2g kg^{-1} stored in polythene bag maintained better vigour and viability up to 10 months compared to other treatments under ambient condition.

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