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Evaluation of Vigour and Oil Quality in Cottonseed during Accelerated Ageing

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ABSTRACT

Cottonseeds were subjected to accelerated ageing treatment for 1, 2, 3, 5, 7, 15 and 20 days at 40°C and 95-100% relative humidity. These artificially aged seeds were compared with control for evaluation of vigour and biochemical changed in seed oil induced by these treatments. Accelerated ageing of cottonseed upto three days had no significant effect on germination percentage, however, further increase in ageing period decreased germination percentage linearly and there was not germination at 5, 7, and 20 days of accelerated ageing. There was concomitant decrease in the seedling length, seedling fresh weight and seedling dry weight with reduction of germination percentage. Oil analysis revealed gradual increase in free fatty acidity and lipid peroxidation. Peroxide value of artificially aged seeds showed a gradual increase but saponification value, iodine value and ester value showed decreasing trend with increasing the period of ageing. The results reveal that accelerated ageing caused depression of germination ability and oil quality in cottonseed.

Key Words: Cottonseed; Accelerated ageing; Fatty acid; Lipid peroxidation

INTRODUCTION

Rapid and uniform germination is as important for better crop production, as is the total germination. In order to get a successful stand establishment the seed must reach farmers with high quality, i.e., maximum germination capacity and vigour. A uniform stand, followed by timely management of cultural practices throughout the season, is really the nuts and bolts of cotton production that results in profitable yield at harvest time. It has been well documented that high-quality seed will perform well even under less than favorable conditions (Heydecker, 1977).

Seed possesses maximum germination ability and highest vigour when it is at its maximum dry weight, a stage known as physiological maturity in most crops (TeKrony & Egli, 1997), however, like any other form of life, they cannot retain this identity indefinitely. Seed is seldom planted immediately after harvesting; it is stored for a certain period of time before sowing. After harvest, seeds start deteriorating, moving inexorably towards death (Gregg *et al.*, 1994). During deterioration, vigour is the first component of seed quality, which is lost, this is followed by a loss of germination capacity and viability (Trawatha *et al*., 1995). Delayed emergence, increased seedling disease, and reduced stands are documented effects of low cottonseed quality (Bourland *et al.*, 1988).

Cottonseed like other oilseeds being rich in oil is highly prone to seed deterioration if improperly stored. High temperature, ambient relative humidity, and seed moisture content are the main factors influencing seed storage capacity (Abdul-Baki, 1980). These factors can be combined variously during accelerated ageing technique to obtain seed lots of different degree of vigour, and to relate the physiological parameters of germination with biochemical indices of vigour (Delouche & Baskin, 1973; Woodstock, 1973; Dell'Aquila, 1994). The accelerated ageing test is one of the most popular seed vigour test due to its simplicity, ease of standardization (TeKrony, 1995), and applicability to a wide range of crops (McDonald, 1995). Low-quality seeds deteriorate more rapidly than high-quality seeds under these conditions. The accelerated ageing test is considered standardized and correlates with field emergence under a variety of seedbed conditions (Egli & TeKrony, 1996). Accelerated ageing test has been widely used to investigate the mechanisms of seed ageing during storage (Sun & Leopold, 1995; Baily *et al*., 1996; Sung, 1996; McDonald, 1998). Therefore, accelerated ageing test can be performed to assess the physiological and biochemical changes during seed ageing of a crop seed.

Biochemical markers like free fatty acid contents may provide insight into the aging process. These markers may be helpful for assessing seed quality and aging (Staus & Hopper, 1983). Free fatty acid act as detergents and can damage lipid bilayer especially of mitochondria leading to reduced energy production (Booth *et al*., 1999). Another cause of seed deterioration is production of free radicals by non-enzymatic peroxidation of polyunsaturated free fatty acids (Stewart & Bewley, 1980; Wilson & McDonald, 1986). These free radicals have potential to damage membrane, enzymes, protein and ultimately cellular repair mechanism. Lipid peroxidation produces highly reactive free radical intermediates and lipid peroxides. Lipid peroxidation products have pronounced effects on other important cellular systems, and can also damage DNA (Wilson & McDonald, 1986). Copeland and McDonald (1995) reported that the loss of phospholipids in deteriorated seeds is due to lipid peroxidation. These peroxidative changes in the fatty acid composition of membrane lipids

will lead to massive dysfunction, increase in membrane viscosity, enhanced bilayer permeability, and swelling of mitochondria (Priestley, 1986). Changes in membrane lipids, therefore, could account for the increase in solute leakage (Sung, 1996).

Very little work had been conducted on causes of seed deterioration in cottonseed. The aim of the present research was to investigate the possible causes of seed deterioration and to explore in the biochemical mechanisms of cottonseed deterioration during accelerated ageing.

MATERIALS AND METHODS

An upland cotton cultivar NIAB-78 (*Gossypium hirsutum* L.) was used in the study. Seeds were delinted by thorough mixing of seeds with commercial grade H_2SO_4 in plastic containers @ 1 L H_2SO_4/10 kg cottonseed. Immediately after delinting the seeds were washed with excessive water, rinsed with deionised water and dried under shade by forced air for 2 h.

Accelerated ageing. The seeds were aged following the method of Sung (1996) with minor modifications. A plant growth chamber was used as an automatic temperature and humidity controlling chamber (Model: LPH-300-RD, Nk System Biotran, Nippon Medical & Chemical Instruments Co. Ltd., 22-38, 1-Chome Edobori, Nishi-ku, Osaka, Japan 550). The seeds were spread in a single layer on plastic trays. At a time, 3 kg of seeds were aged. The seed lots were aged for 1 day, 2, 3, 5, 7, 10, 15 and 20 days at 45°C and 95-100% relative humidity. Following each ageing treatment the seeds were allowed to air dry at room temperature until their original weight was restored (Sung & Jeng, 1996) and sealed in polythene bags in a refrigerator until use.

Seed vigour evaluation. The aged seeds were compared with control ones following the standard germination test in filter paper sheets (AOSA, 1991) with minor modifications as described by Basra *et al.* (2002). To record data, the sheets were unrolled 4, 8 and 12 days after planting, and the seeds that had produced normal seedlings were counted and recorded. At this time, one count of normal seedlings that had a combined hypocotyl and root length of 3.81 cm in or longer was made (AOSA, 1991). Following observations were recorded after 12 days of planting.

Seed oil analysis

Oil extraction. Oil was extracted by solvent extraction using diethyl ether as solvent. A weighed quantity (30 g) of dried seed of ground seeds was placed in a cellulose thimble, covered with cotton and placed in Soxhlet apparatus. The extraction was carried upto 6-8 cycles for complete extraction. The solvent was recovered by distillation. Percentage of oil was determined according to AOCS, Aa 4-38 (1990).

The oil obtained was filtered and used for the determination of the following parameters employing the official methods of AOCS (1990), (i) Acid value (Cd 3a-63); (ii) percentage of free fatty acids (Aa 6-38); (iii) saponification value (TI 1a-64); (iv) iodine value (cd 1b-87) and (v) peroxide value (Cd 8-53).

Statistical analysis. Germination test was arranged in a completely randomized design. The data were analyzed using a statistical package, MSTATC. The recorded data were analyzed statistically using Fisher's analysis of variance techniques and LSD test was applied at 5% probability level to compare the differences among treatment means (Steel & Torrie, 1984).

RESULTS

Standard germination test:

Germination. Accelerated aging had a significant effect on germination. During the first three days of aging the seeds remained viable and there was no significant effect on germination percentage. Further increase in aging period had a suppressive effect on germination. Practically no normal seedlings developed at 5 or more days of ageing. There was a complete loss of germination at 20 days of accelerated aging. Unaged seeds exhibited average germination of 86.67% (Table I).

Abnormal seedlings. Controlled ageing increased the number of abnormal seedlings (Table I). Control and aged for one day seeds produced 100% normal seedlings. Cottonseeds aged for two and three days produced statistically similar abnormal seedlings. Seeds subjected to accelerated aging for 5 and 7 days did not produce any normal seedling and produced 33.33 and 26.66% abnormal seedlings, respectively. Rest of the seeds did not germinate. With further increase in accelerated ageing period up to 10 days there was a significant increase in abnormal seedlings (33.33%). Seeds aged for 15 days produced the maximum abnormal seedlings (40%). A longer ageing treatment of 20 days was lethal and resulted in complete failure of germination (Table I).

Seedling length. Accelerated aging significantly inhibited seedling growth (Table I). Ageing up to three days and control produced statistically similar seedling length. No seedlings were produced by seeds of 5, 7 and 20 days of aging, while significantly weak and shorter seedlings were produced by seeds aged for 10 and 15 days.

Seedling weight. Maximum shoot fresh weight was recorded in one day accelerated aging treatment (Table I). Control and aged for two and three days seeds exhibited significantly less seedling weight than one day aging treatment. Further increase in aging period significantly reduced the seedling fresh weight. The seeds those did not germinate produced weak and abnormal seedlings. The seedling dry weight significantly affected by accelerated ageing (Table I). Rapid ageing up to three days produced similar seedling weight. However, beyond this period ageing damaged the seedlings.

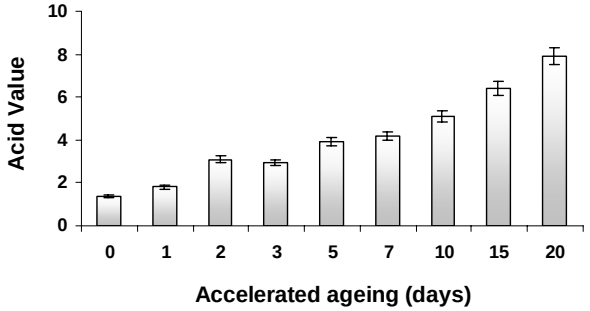
Table I. Influence of accelerated ageing on germination, seedling length, seedling fresh, and dry weight of cottonseed cv. NIAB-78 sown in filter paper sheets

Accelerated ageing (days)	Germination (%)	Abnormal seedling (%)	Seedling length (cm)	Seedling fresh wt (g)	Seedling dry wt (g)
Control	86.67 a	0e	15.50 ab	0.197 b	0.072a
1	93.33 a	0e	19.53 a	0.4 a	0.077a
2	80.00 a	6.667d	13.96 ab	0.216 b	0.068ab
3	73.33 a	6.667d	12.10 b	0.2 b	0.055b
5	0.00 b	33.33b	0 c	0 d	0d
7	0.00 b	26.67c	0 c	0 d	0d
10	20.00 b	33.33b	4.9 c	0.11 c	0.037c
15	20.00 b	40a	3.6 c	0.1 c	0.01d
20	0.00 b	0e	0.00 c	0 d	0d
LSD	22.87	3.647	6.909	0.0268	0.013

Oil analysis of cottonseed oil

Acid value. The acid value of accelerated aged seed oil under investigation increased with an increase in time period of accelerated ageing and reached to a maximum value of 15.71 after 20 days aging (Fig. 1). The minimum acid value was attained by the control. Increase in accelerated aging period resulted in gradual increasing trend in acid value.

Fig. 1. Influence of accelerated ageing on acid value oil of cottonseed cv. NIAB-78. Means±SE



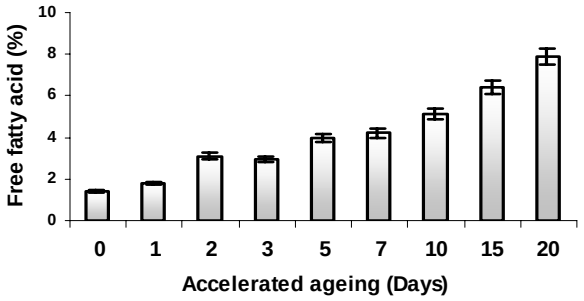
Free fatty acids. The free fatty acid content of one day accelerated aging was similar to that of control. The increase in the time period of aging increased the free fatty acid contents linearly (Fig. 2).

Saponification value. Saponification value of seed oil increased by accelerated ageing up to three days, however then onward linearly decreased (Fig. 3) at minimum level of 153.75 as against 194.8 of control.

Iodine value. Accelerated aging significantly decreased the iodine value of the oil except increasing trends in 3 and 10 days of ageing (Fig. 4). Accelerated ageing for three days exhibited highest iodine value (103.98) whereas with the ageing of 20 days iodine value decreased to 86.62.

Peroxide value. The peroxide value increased rapidly with increase in accelerated aging period and reached maximum

Fig. 2. Influence of accelerated ageing on free fatty acid (%) of oil of cottonseed cv. NIAB-78. Means±SE



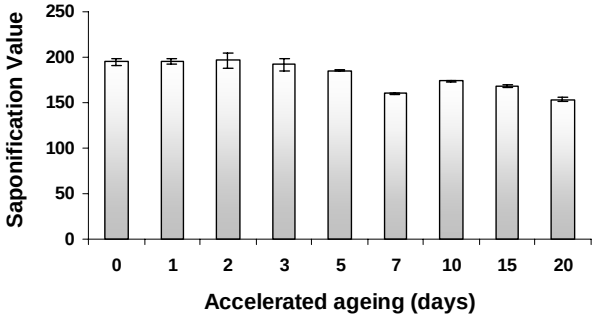
after 20 days. The value obtained after 20 days of accelerated aging was 21.99 as compared to 6.10 by the oil of untreated seeds (Fig. 5).

DISCUSSION

Cottonseed with a germination of 80% or more is considered to be of high quality (A.O.A.C., 1991). The cultivar used in this experiment, NIAB-78, showed good germinability (86.67%) which indicates its high vigour and quality.

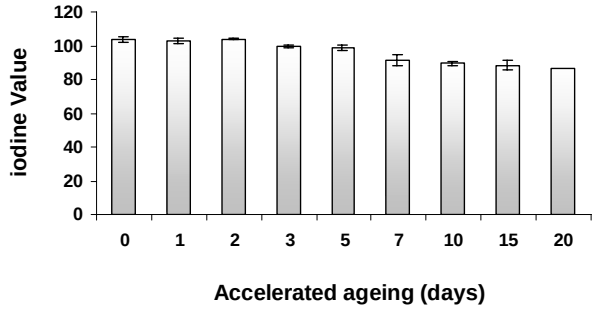
Accelerated aging reduced the cottonseed germination (Table I) under this study. Seeds aged upto three days showed improved germination percentage. This may be due to slow hydration, as in priming, which is a control hydration technique that alters germination character and improves germination percentage (Tilden & West, 1985). Increasing the period of artificial ageing upto five days produced deleterious effects on seeds and germination ability was reduced significantly. Low germination percentage is related to low seed quality (Bourland *et al.*, 1988). Accelerated ageing of seeds for 5, 7, and 20 days did not show germination. This severity is related to seed deterioration caused by ageing.

Fig. 3. Influence of accelerated ageing on saponification value oil of cottonseed cv. NIAB-78. Means±SE



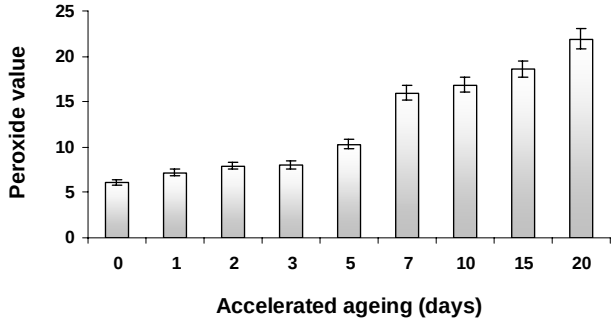
Decrease in germination percentage is related to reduction in seed vigour results in the production of abnormal seedlings. Only control and one day aged seeds did not produce any abnormal seedling. All other seeds showed high rate of abnormal seedling production (Table I). Accelerated ageing also decreased seedling dry and fresh weights, and seedling length. Similar results were reported in peanut (Sung & Jeng, 1994) and groundnut (Nautiyal *et al*., 1997). The possible reason of this reduction might be the lowering of biochemical activities in seeds. Ageing have damaging effect on enzymes that are necessary to convert reserve food in the embryo to usable form and ultimately production of normal seedling.

Fig. 4. Influence of accelerated ageing on iodine value oil of cottonseed cv. NIAB-78. Means±SE



Oil analysis revealed an increase in acid value (Fig. 1) and percentage of free fatty acid (Fig. 2) in aged seeds as compared to control. Free fatty acids are important constituents of cottonseed and cottonseed products (Bourland *et al.*, 1988). Acid value is a useful parameter for characterizing the quality of oil. It measures the extent to which hydrolysis has librated the fatty acids from their ester linkage to the present triglyceride molecule. Acidity is extensively quoted as free fatty acids, which is percentage of free fatty acids in the oil or fat. Our findings that loss of vigour, poor germination, weak and abnormal seedling is associated with increases in the free fatty acids which

Fig. 5. Influence of accelerated ageing on peroxide value oil of cottonseed cv. NIAB-78. Means±SE



produce deleterious effects on cell (Priestly *et al.*, 1985).

Peroxide value of cottonseed was higher than control and reached at maximum value at 20 days of ageing (Fig. 5). Peroxide value is an important parameter of oil, which is used to access the autoxidation of oil and fats. Increase in peroxide value is related to non-enzymatic reactions, which lead to production of free radicals which combine with oxygen to form hydroperoxides lead to enzyme inactivation and denaturation of proteins and nucleic acids. Cell membranes have large surface areas and a high proportion of unsaturated fatty acids, which makes the lipids particularly susceptible to peroxidative damage. Accelerated aging increased the peroxide value under the present study (Fig. 5). Lipid peroxidation produces highly reactive free radical intermediates and lipid peroxides. Lipid oxidation products have pronounced effects on other important cellular systems and can damage DNA (Wilson & McDonald, 1986). The increase in percentage of abnormal seedling with accelerated aging confirms the DNA denaturation. The interaction of lipid hydroperoxides and proteins at relatively high moisture content leads to protein-protein cross linking; at lower levels of hydration scission of protein is favoured (Priestley, 1986). So, lipid peroxidation can be considered as the main cause of seed ageing.

Elevated levels of free fatty acids, which are toxic to most cells, are not present in healthy seed tissues (Priestley, 1986). So, it can be concluded that both lipid peroxidation and free fat acidity contributes to seed deterioration through disruption of the membranes.

The iodine value is a measure of the unsaturation of fatty acids and is expressed in terms of the number of grams of iodine absorbed by 100 g of oils/fats. The higher the iodine value, the greater the unsaturation or larger the number of double bonds present in the fatty acids. It also indicates the stability of the oil towards oxidation. Accelerated ageing caused in the reduction in the iodine value (Fig. 4) indicating lower number of double bond, which is apparently not related to seed ageing.

Saponification value of cottonseed oil revealed a gradual decrease. Saponification value is the number of mg of KOH that is required to saponify the 1 g of oil to

neutralize the fatty acids and fatty acids present as acyl glycerol. It is measure of molecular weight of components of oil and is related to molecular mass of oil. In the present study the decrease in saponification value (Fig. 3) might be due to accelerated ageing which resulted in the bond breakage in oil and resulted in the reduction in average molecular weight of fatty acids present in the oil and lowered the oil quality.

CONCLUSION

It is concluded that accelerated ageing up to three days under the present experimental conditions had no significant effect on cottonseed quality. However, ageing beyond three days reduces the cottonseed vigour. The extended period of accelerated ageing up to 20 days resulted in complete of loss of germinability. The main cause of seed deterioration by accelerated ageing might be membrane disintegration and inactivation of enzymatic systems mainly due to lipid peroxidation and increase in free fat acidity. These findings can help understand the processes leading to cottonseed deterioration while undergoing natural ageing process.

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