CHAPTER – 3

BIOCHEMICAL CHANGES IN *JATROPHA CURCAS* L. SEEDS ON STORAGE

3.1. INTRODUCTION

Jatropha curcas L. (physic nut) of the Euphorbiaceae family is a plant whose oil-rich seeds contain 21% saturated and 79% unsaturated fatty acids (King et al., 2009). Being rich in unsaturated fatty acids, the cost of oil production from the seeds is relatively low and its oil has good oxidation stability for biodiesel production. This makes *J. curcas* oil better suited for fuel production than other vegetable oils (Sarin et al., 2007). However, before the seeds are used for oil extraction, there is a long storage period during which loss of seed quality occurs. There is a lack of information on the distinct changes taking place in *J. curcas* seeds on storage. In this chapter the biochemical changes that happen during seed storage are reported.

Seeds are said to be viable if they retain their genetic and physiological quality and are able to germinate. Seed longevity depends on many factors predominant among them is-the way in which the seeds are stored. Seeds are sensitive to the environmental conditions during storage and these conditions act as a key determinant of seed quality on storage (Arc et al., 2011). Prolonged seed storage generally leads to seed aging with the manifestation of irreversible metabolic and cellular changes such as oxidation of proteins, nucleic acids and lipids; loss of enzyme activity, membrane perturbation and impairment of RNA and its biosynthesis (Kibinza et al., 2006; El-Maarouf-Bouteau et al., 2011; Hu et al., 2012).

Even if an optimal environment is provided, seed deterioration is inevitable and this is a major hurdle for agriculture and *ex situ* seed germplasm conservation (Li et al., 2017). Storage of oilseeds is a greater challenge than that of cereal grains as oilseeds are more susceptible to seed deterioration.

High moisture content and temperature during storage are the prime causes of seed deterioration (Kibinza et al., 2006). Stored lipids within the seeds deteriorate mainly due to (i) oxidation caused by high temperature and moisture content, (ii) hydrolysis, wherein fat is degraded into fatty acids and (iii) contamination (Abdellah and Ishag, 2012). Lipid peroxidation is widely reported as one of the main causes of seed deterioration. A major initiator of these deleterious changes is the group of molecules known as reactive oxygen species (ROS).

The intracellular mitochondrial electron transport chain is the main source of production of ROS in harvested dry seeds. Superoxide, a major free radical is dismutated by Super Oxide Dismutase (SOD) or non-enzymatically converted at low pH into H_2O_2. This H_2O_2 can diffuse through membranes and induce cell damage by oxidizing lipids, proteins and nucleic acids (Roach et al., 2010). The OH^- radical is the most potent and reactive ROS, and oxidizes any biomolecule in its proximity (Sahu et al., 2017). Elevated endogenous levels of ROS on prolonged storage of orthodox seeds like *Helianthus annuus* (El-Maarouf-Bouteau et al., 2011) and *Arabidopsis thaliana* (Rajjou et al., 2008) has been reported.

The polyunsaturated fatty acid (PUFA) of the membrane becomes the ultimate target of free radicals. Lipid peroxidation that causes oxidative damage to PUFA is specifically destructive because it functions through chain reaction which is self-perpetuating (Park et al., 2009).

Lipid peroxidation proceeds by targeting PUFA or other unsaturated fatty acids present. $R^•$ is the initiator free radical. A fatty acid free radical ($L^•$) is initially formed. Because of the presence of lone pair of electron present in fatty acid radical, it easily attracts oxygen to form fatty acid peroxy radicals ($LOO^•$). These newly formed peroxy radicals are the agents of further new chain reaction of oxidation of the unsaturated fatty acids generating lipid hydroperoxides (LOOH). These lipid hydroperoxides further get broken down to aldehydes, ketones etc, while producing numerous free radicals as by-product (Esterbauer et al., 1990).

$$LH + R^• \rightarrow L^• + RH$$

$$L^• + O_2 \rightarrow LOO^•$$

$$LOO^• + LH \rightarrow LOOH + L^•$$

$$LOOH \rightarrow LO^• + LOO^• + \text{aldehydes.}$$

These formed aldehydes, from the site of production can easily diffuse to other parts of the cells because they are biologically active compounds causing tissue injuries

(Devasagayam et al., 2003). Malondialdehyde (MDA) and volatile aldehydes are the products of lipid peroxidation. Determination of MDA is a convenient and conventional method to measure the extent of lipid oxidation. Thiobarbituric acid (TBA) is used to estimate the amount of MDA present in a given sample.

In plant tissues hydrogen peroxide (H_2O_2) is one of the major reactive oxygen species and it has been shown to function either as a signalling molecule or as a deleterious molecule (Bailly et al., 2008). Some of the basic processes such as development, defense and acclimation in plants are regulated by hydrogen peroxide (Ślesak et al., 2007). Contrary to superoxide, H_2O_2 being non-radical in nature carries no net charge (Halliwell, 2006). Due to its diffusibility through membranes and relative stability, hydrogen peroxide is considered to be a long-distance signalling molecule (Vranová et al., 2002), acting as a translocating second messenger triggering Ca^{2+} fluxes, gene expression and protein modifications (Bienert et al., 2006).

In plants, the generation of H_2O_2 is increased due to various types of stresses (Neill et al., 2002). In maize seedlings, endogenous H_2O_2 production has been shown to increase as a result of the chilling stress (Prasad et al., 1994). It is produced through the process of oxidation where oxygen is reduced to superoxide which further gets dismuted either spontaneously or by the action of superoxide dismutase to form hydrogen peroxide. Chloroplast and mitochondria are the major source of production of H_2O_2 (Asada, 1999; Moller, 2001). In plants, even peroxisomes can contribute to hydrogen peroxide production by the action of glycolate oxidase (Noctor et al., 2002). Oxalate oxidase (Hu et al., 2003) and membrane bound NADPH oxidase (Jiang and Zhang, 2003) are the other two enzymes which catalyse the generation of H_2O_2. Plants also have abundant expression of membrane bound amine oxidases which are responsible for H_2O_2 in the apoplast (Joseph and Srivastava, 1995 and Joseph et al., 1996). Oxidative damage by H_2O_2 peaks when plants encounter biotic and abiotic stress (Asada, 1999). The increased amount of H_2O_2 can function as signalling molecule to induce the plants to respond to the stress, such as excessive radiation, ozone and wounding, extreme temperature, high humidity, pathogen attack and drought (Neill et al., 2002; Foyer et al., 1997; Prasad et al., 1994; Orozco-Ca´rdenas et al., 2001; Wohlgemuth et al., 2002). When oil seeds are subjected to either extreme temperature and moisture content or prolonged storage, then the formation of H_2O_2 crosses the thresh hold value of functioning as a signalling molecule and then acts as a harbinger of destruction. The amount of H_2O_2 produced during prolonged period of storage mounts to a deleterious level causing severe membrane damage, loss of DNA integrity and inactivation of enzymes etc.

One quick parameter to assess the extent of seed deterioration, especially for oil seeds could be by estimating the amount of H_2O_2 present within the seeds. Methods like fluorometry (Genfa and Dasgupta, 1992), chemiluminescent (Pe´rez and Rubio, 2006) and spectrophotometry (Patterson et al., 1984) are used to determine H_2O_2. Zhou et al., (2006) developed a novel protocol to estimate the amount of H_2O_2 in plants which is easy and rapid. This novel method utilizes the basic principle of glucose determination in blood (Trinder, 1969). In the presence of glucose oxidase, glucose is converted to H_2O_2 and gluconic acid. 4-aminoantipyrine and phenol react with H_2O_2 to form a red product, catalysed by peroxidase. This red product formed is measured using spectrophotometer at 505 nm.

H_2O_2 is relatively a long-lived (half-life of 1 ms) reactive oxygen species due to its ability to react moderately with other molecules. From the production site it can diffuse to some distance damaging the molecules that it encounters (Bhattacharjee, 2005). During aging such detrimental molecule causes unwanted damage and fosters seed deterioration.

Veselova et al., (2015) tried to study the deterioration mechanisms during early aging in air-dry pea seeds. Their results revealed that besides the lipid peroxidation, non-enzymatic hydrolysis of sucrose and oligosaccharides is another reason for early aging in air-dry pea seeds. Primary aging is also caused by non-enzymatic glycosylation of amino acids and proteins (Murthy et al., 2003). Interaction of the carbonyl group of glucose with the free amino group of amino acids in protein to form glycosylamine is known as the Amadori Maillard reaction. Advanced end product of glucosamine causes membrane injury. During non-enzymatic hydrolysis of oligosaccharides there is a formation of glucose. This happens prior to glycosylation (the first step of the Amadori Maillard reaction) because this reaction requires the linear form of glucose with a free carbonyl group (H-C=O) (Stepanenko, 1977). Accumulation of glucose arising from the degradation of non-enzymatic hydrolytic oligosaccharides could be considered a possible factor that induces early deterioration of seeds. This is true of seeds like pea or wheat that are rich in carbohydrates but in case of lipid rich seeds the lipid peroxidation and free radical formation predominantly imparts more membrane damage and seed deterioration than glucose accumulation or Amadori Maillard end products (Veselova et al., 2015).

The loss of membrane integrity and increase of membrane permeability gives way to electrolyte leakage. Due to electrolyte leakage there is an increased electrical conductivity due to seed leaching which is measured by electrical conductivity meter. The extent of seed leaching can be used as a determinant of seed viability and seed vigour (Aiazzi et al., 1997; Ratajczak and Pukacka, 2005). The conductivity test has been validated as a vigour test for peas (ISTA, 2009), and Brassicaeae seeds stored at high humidity (Lazar et al., 2014). The electrical conductivity is measured by electrical conductivity meter.

3.2. RESULTS

3.2.1. Estimation of lipid peroxidation

The levels of malondialdehyde (MDA) in *J. curcas* seeds were monitored to estimate lipid peroxidation. MDA levels in *Jatropha curcas* L. seeds start increasing significantly after 3 months of natural aging compared to control and same trend is found thereafter in consecutive months of natural aging up to 24 months (Figure 3.1).

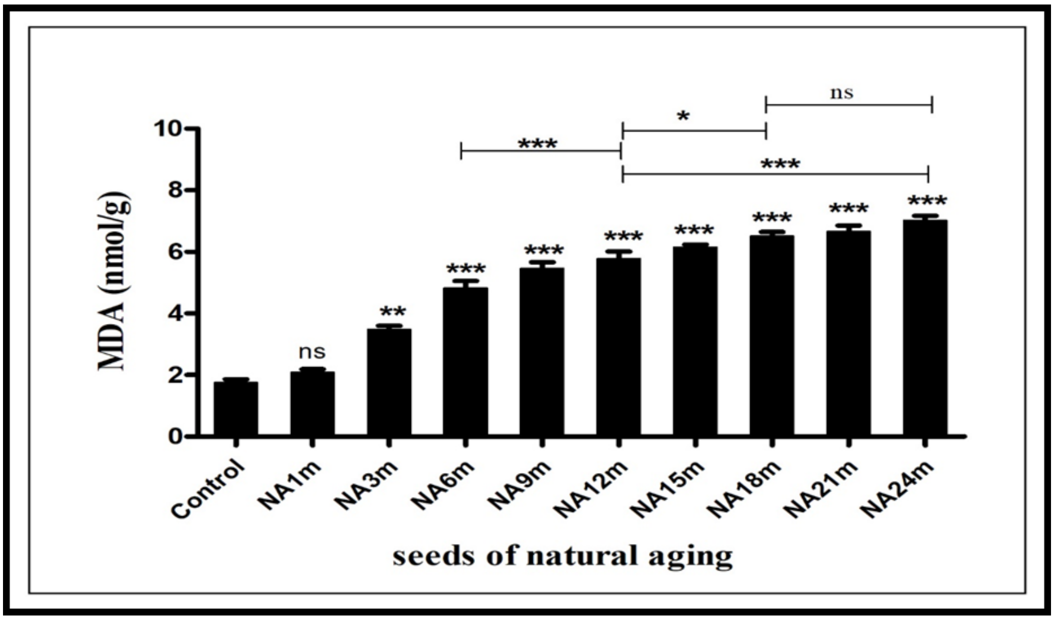

Figure 3.1: MDA levels in control seeds and in seeds of natural aging of 1, 3, 6, 9, 12, 15, 18, 21 and 24 months.Values are mean ± SE; *, **, * indicates significantly different at P<0.05, P<0.01 and P<0.001 as compared to the control, NA6m, NA12m and NA18m, n=3.**

A similar kind of significant increase in MDA level was observed in all the seeds of accelerated aging (AA) and saturated salt accelerated aging (SSAA). A mere 12 hours of accelerated aging and saturated salt accelerated aging treatment brought about a significant increase in MDA content in seeds and these levels were comparable to NA 3m (Figures 3.2 and 3.3). A four-fold increase in MDA levels were seen in 15 d old AA and SSAA.

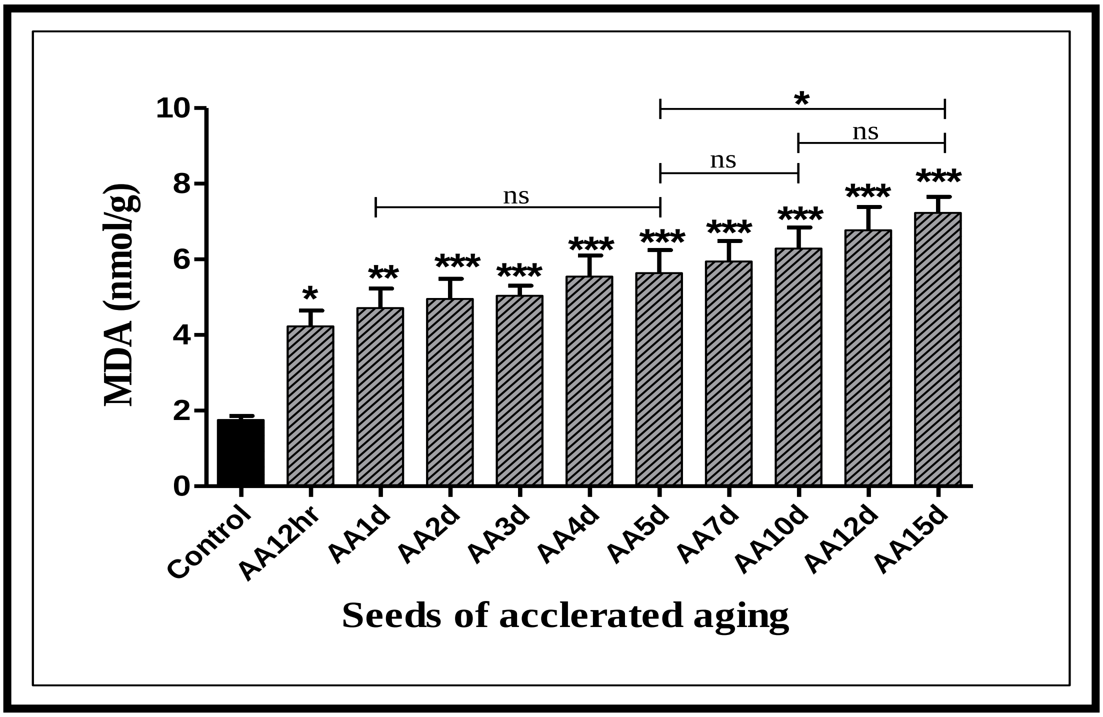

Figure 3.2: MDA levels in control seeds and in seeds of accelerated aging of 12hours, 1, 2, 3, 4, 5, 7, 10, 12 and 15 days.Values are mean ± SE; *, **, * indicates significantly different at P<0.05, P<0.01 and P<0.001 as compared to the control, AA1d, AA5d and AA10d, n=3.**

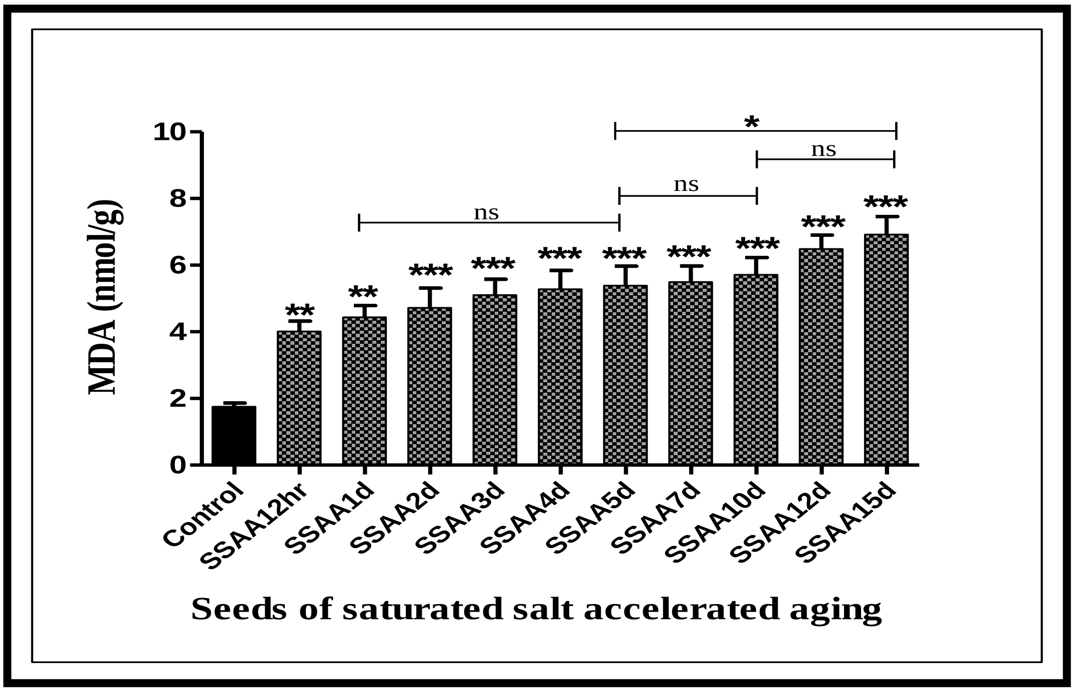

Figure 3.3: MDA levels in control seeds and in seeds of saturated salt accelerated aging of 12hours, 1, 2, 3, 4, 5, 7, 10, 12 and 15 days.Values are mean ± SE; *, **, * indicates significantly different at $P<0.05$, $P<0.01$ and $P<0.001$ as compared to the control, SSAA1d, SSAA5d and SSAA10d, n=3.**

MDA content in seeds after 1, 5, 7, 12 days of AA and 2, 10, 12, 15 days of SSAA is the same as the MDA content present in seeds kept for 6, 12, 18 and 24 months of natural aging respectively (Table 3.1).

Table 3.1: Comparative analysis of MDA in natural aging with accelerated aging and saturated salt accelerated aging.

Level of MDA in seeds of natural aging (NA)	Levels of MDA in seeds of accelerated aging (AA)	Levels of MDA in seeds of saturated salt accelerated aging (SSAA)
Mean ± SE	Mean ± SE	Mean ± SE
Control – 1.76 ± 0.110		
NA6m – 4.83 ± 0.228	AA1d – 4.71 ± 0.513 (ns)	SSAA2d – 4.72 ± 0.596 (ns)
NA12m – 5.78 ± 0.223	AA5d – 5.64 ± 0.605 (ns)	SSAA10d – 5.72 ± 0.514 (ns)
NA18m – 6.51 ± 0.113	AA7d – 6.25 ± 0.537 (ns)	SSAA12d – 6.48 ± 0.421 (ns)
NA24m – 7.02 ± 0.152	AA12d – 6.77 ± 0.617 (ns)	SSAA15d – 6.92 ± 0.535 (ns)

Values of MDA in NA6m, NA12m, NA18m and NA24m found equivalent (~) with those groups in AA and SSAA. ns - Non-significant when AA1d and SSAA2d, AA5d and SSAA10d, AA7d and SSAA12d, and AA12d and SSAA15d are compared to NA6m, NA12m, NA18m and NA24m respectively. Values are mean of ± SEM, n=3.

3.2.2. Estimation of electrolyte leakage

Seeds undergoing physiological and biochemical changes during storage are characterized by membrane damage at the cellular level caused by the robust production of free radicals that attack the membrane. The severity of membrane damage is determined by measuring the electrolyte leakage in the form of electrical conductivity. Electrical conductivity is expressed as millisiemens/cm (ms/cm). In the present study, electrical conductivity in seeds significantly increased after 3 months of natural aging and continued to increase thereafter in the consecutive months of natural aging (Figure 3.4). Similar kind of significant increase was also found in seeds of all groups of accelerated aging and saturated salt aging, starting from AA12hr and SSAA12hr (Figure 3.5 and 3.6).

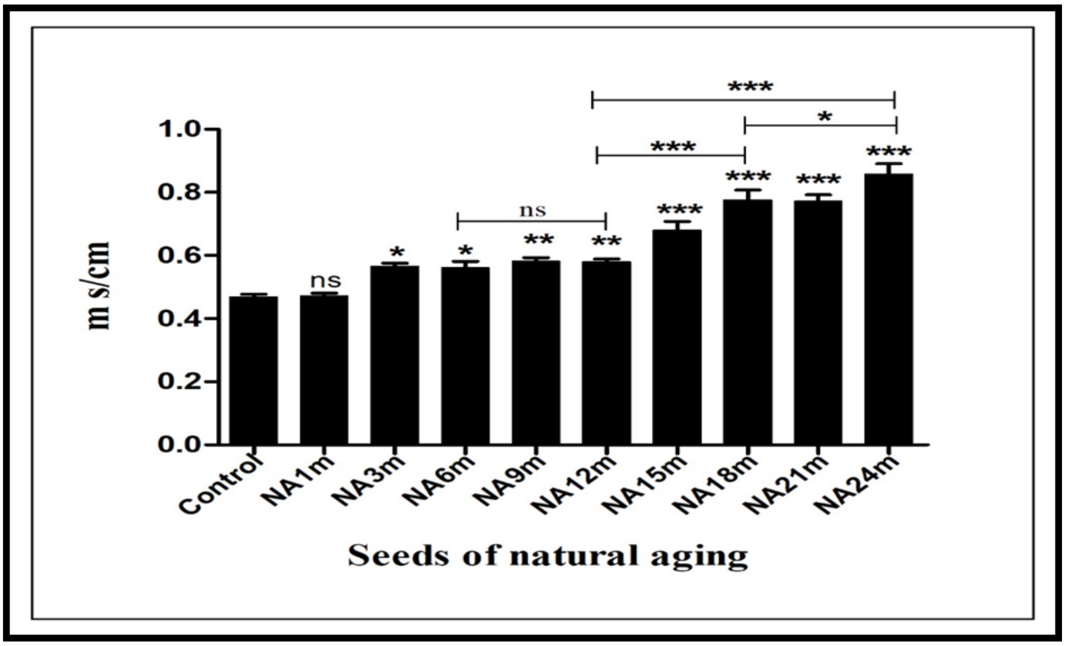

Figure 3.4: Electrical conductivity found in control seeds and in seeds of natural aging of 1, 3, 6, 9, 12, 15, 18, 21 and 24 months.Values are mean ± SE; *, **, * indicates significantly different at P<0.05, P<0.01 and P<0.001 as compared to the control, NA6m, NA12m and NA18m, n=3.**

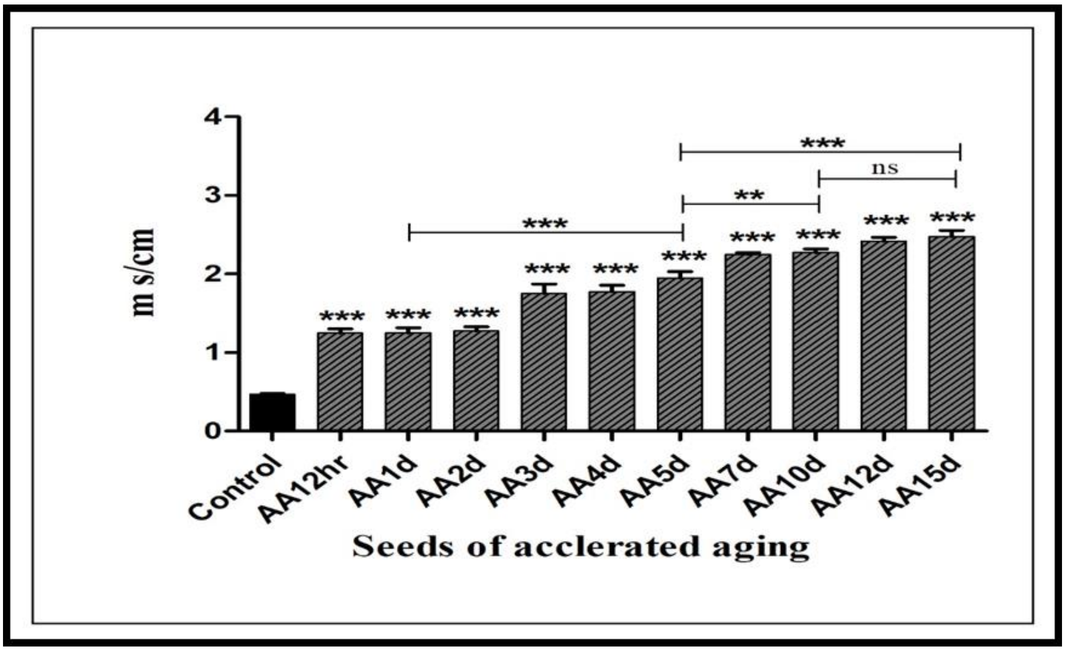

Figure 3.5: Electrical conductivity found in control seeds and in seeds of accelerated aging of 12hours, 1, 2, 3, 4, 5, 7, 10, 12 and 15 days.Values are mean ± SE; *, **, * indicates significantly different at P<0.05, P<0.01 and P<0.001 as compared to the control, AA1d, AA5d and AA10d, n=3.**

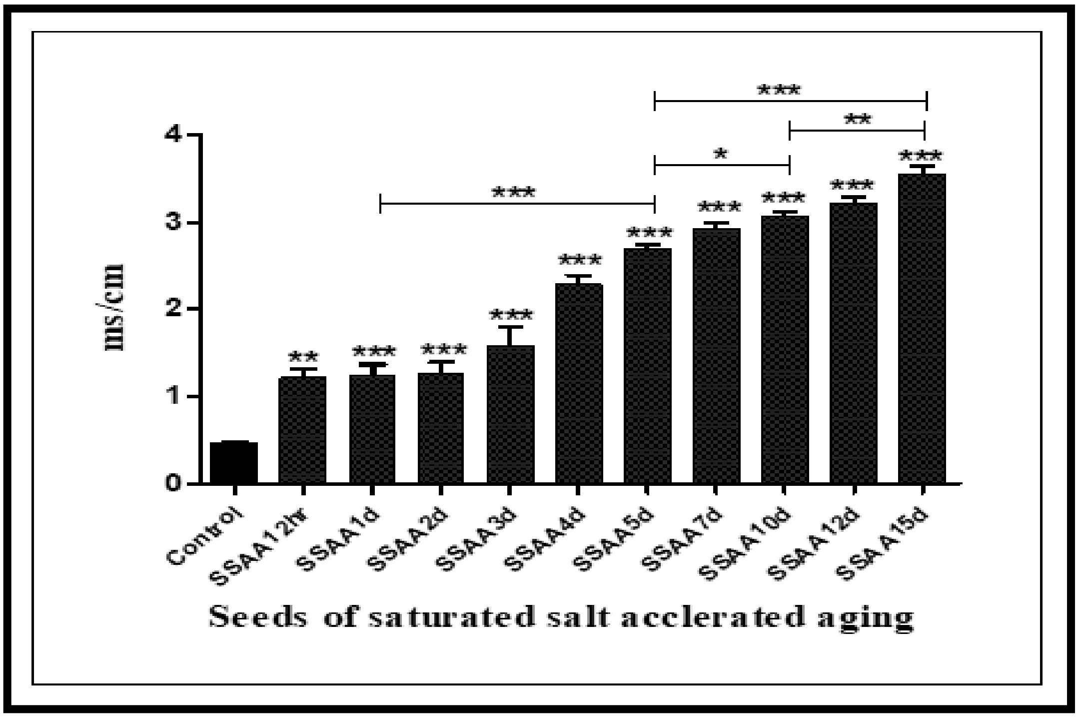

Figure 3.6: Electrical conductivity found in control seeds and in seeds of saturated salt accelerated aging of 12hours, 1, 2, 3, 4, 5, 7, 10, 12 and 15 days.Values are mean ± SE; *, **, * indicates significantly different at P<0.05, P<0.01 and P<0.001 as compared to the control, SSAA1d, SSAA5d and SSAA10d, n=3.**

Correlation study revealed a positive correlation between MDA content and electrolyte leakage in seeds of natural aging (r= 0.878***) (Figure 3.7), accelerated aging (r= 944***) (Figure 3.8) and saturated salt accelerated aging (r= 0.921***) (Figure 3.9).

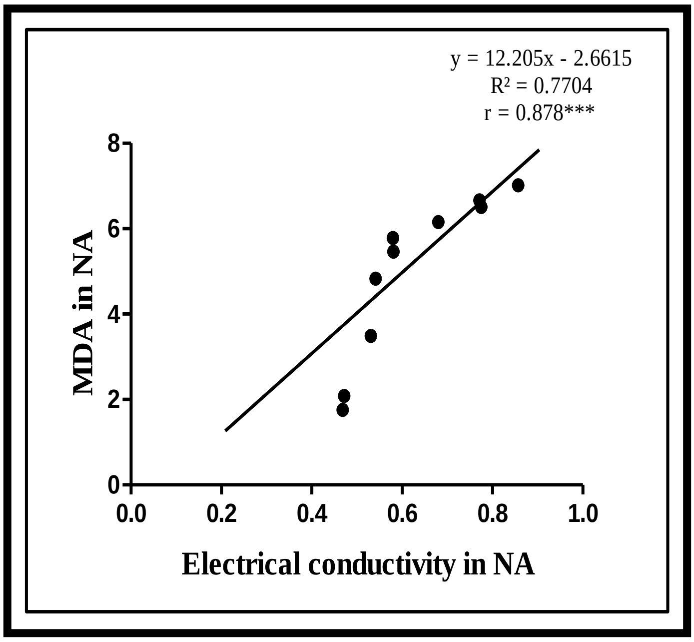

Figure 3.7: Correlation between electrical conductivity and MDA content found in seeds of natural aging. Values are mean ± SE; *, **, * indicates significantly different at P<0.05, P<0.01 and P<0.001.**

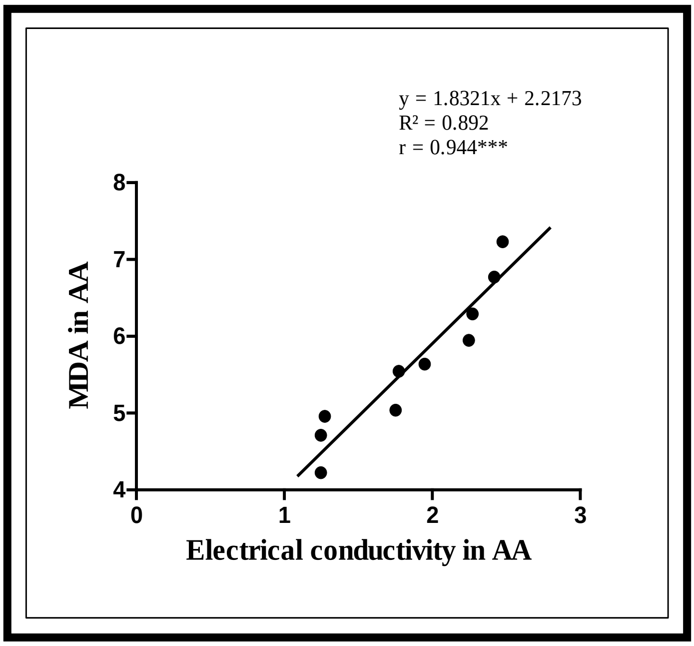

Figure 3.8: Correlation between electrical conductivity and MDA content found in seeds of accelerated aging. Values are mean ± SE; *, **, * indicates significantly different at P<0.05, P<0.01 and P<0.001.**

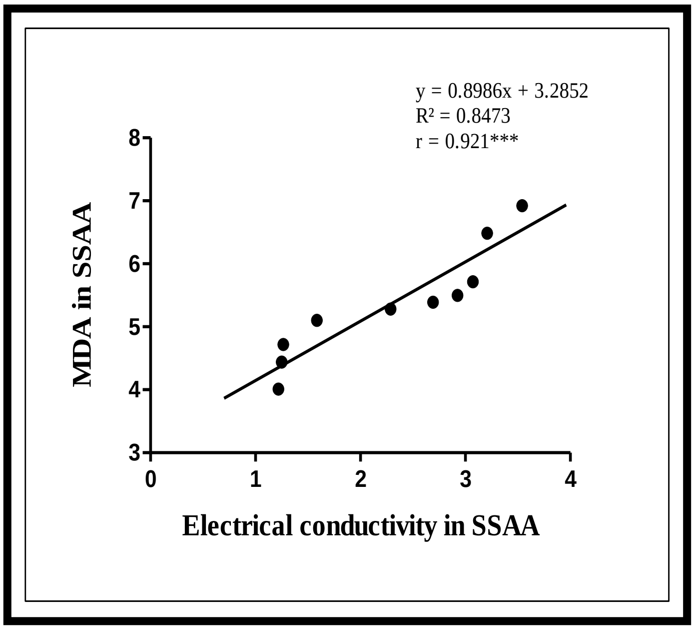

Figure 3.9: Correlation between electrical conductivity and MDA content found in seeds of saturated salt accelerated aging. Values are mean ± SE; *, **, * indicates significantly different at P<0.05, P<0.01 and P<0.001.**

3.2.3. Estimation of H_2O_2

Hydrogen peroxide content in *J. Curcas* seeds was estimated in naturally aged and seeds subjected to aging. As period of natural storage and artificial aging increased, hydrogen peroxide content increased.

A significant increase in hydrogen peroxide content was found from NA3m onward compared to control and there after gradual increase of hydrogen peroxide was evident in all the groups of seeds in natural aging compared to control as the period of aging extended (Figure 3.10).

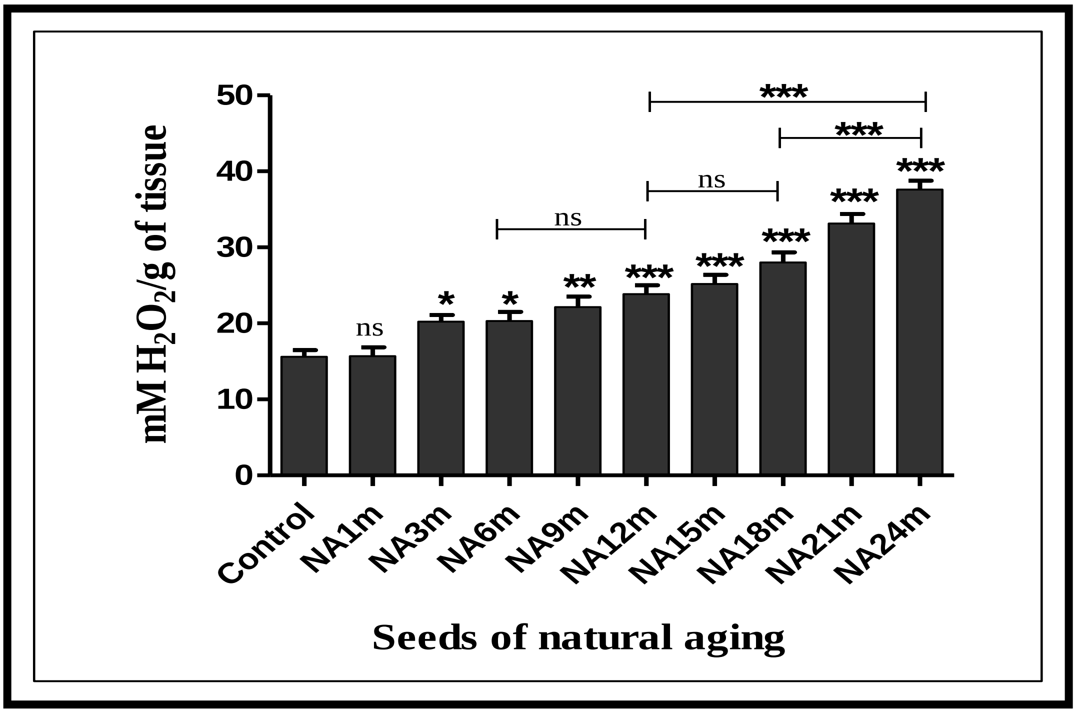

Figure 3.10: H_2O_2 level found in control seeds and in seeds of natural aging of 1, 3, 6, 9, 12, 15, 18, 21 and 24 months. Values are mean ± SE; *, **, * indicates significantly different at $P<0.05$, $P<0.01$ and $P<0.001$ as compared to the control, NA6m, NA12m and NA18m, n=3.**

While 12 hours of accelerated aging was sufficient enough to increase the hydrogen peroxide content of the seeds compared to control. Similar kind of significant increase was found in all the groups of seeds in accelerated aging compared to control (Figure 3.11). In case of saturated salt accelerated aging, only after 2 days there was a significant increase in hydrogen peroxide content compared to control (Figure 3.12). Similar trend of increase was observed in all the groups of seeds in saturated salt accelerated aging thereafter. Hydrogen peroxide content in seeds after 12hours, 4, 5, 10 days of AA and 12hours, 5, 7, 12 days of SSAA is the same as the hydrogen peroxide content in seeds kept for 6, 12, 18 and 24 months of natural aging respectively (Table 3. 2).

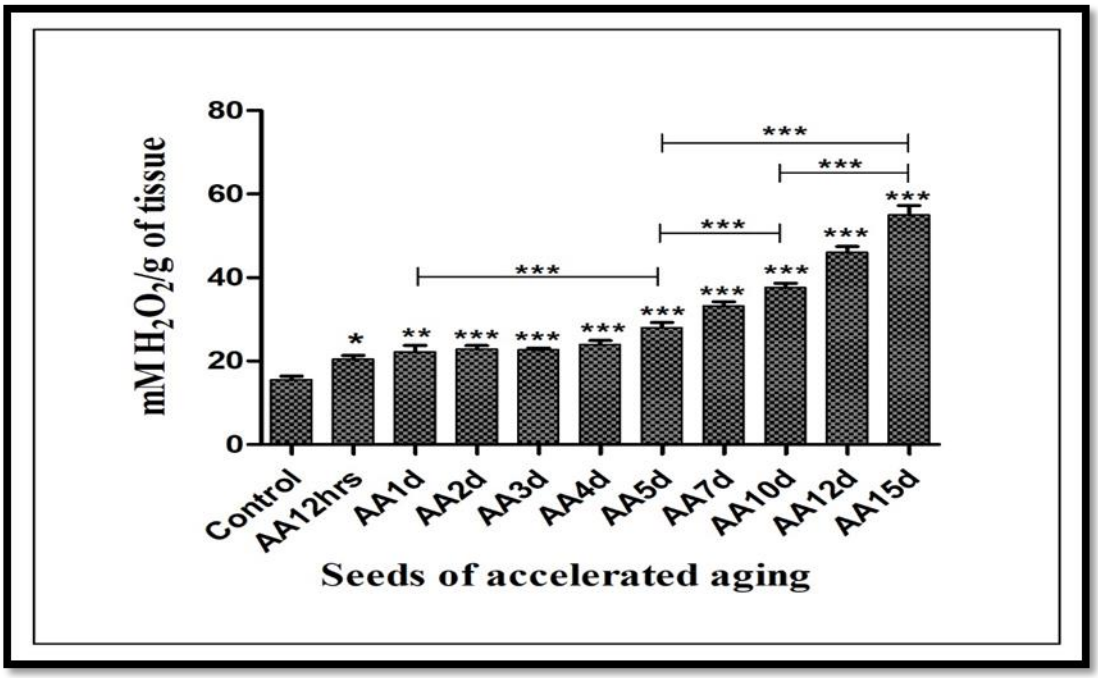

Figure 3.11: Hydrogen peroxide content found in control seeds and in seeds of accelerated aging of 12hours, 1, 2, 3, 4, 5, 7, 10, 12 and 15 days. Values are mean ± SE; *, **, * indicates significantly different at $P<0.05$, $P<0.01$ and $P<0.001$ as compared to the control, AA1d, AA5d and AA10d, n=3.**

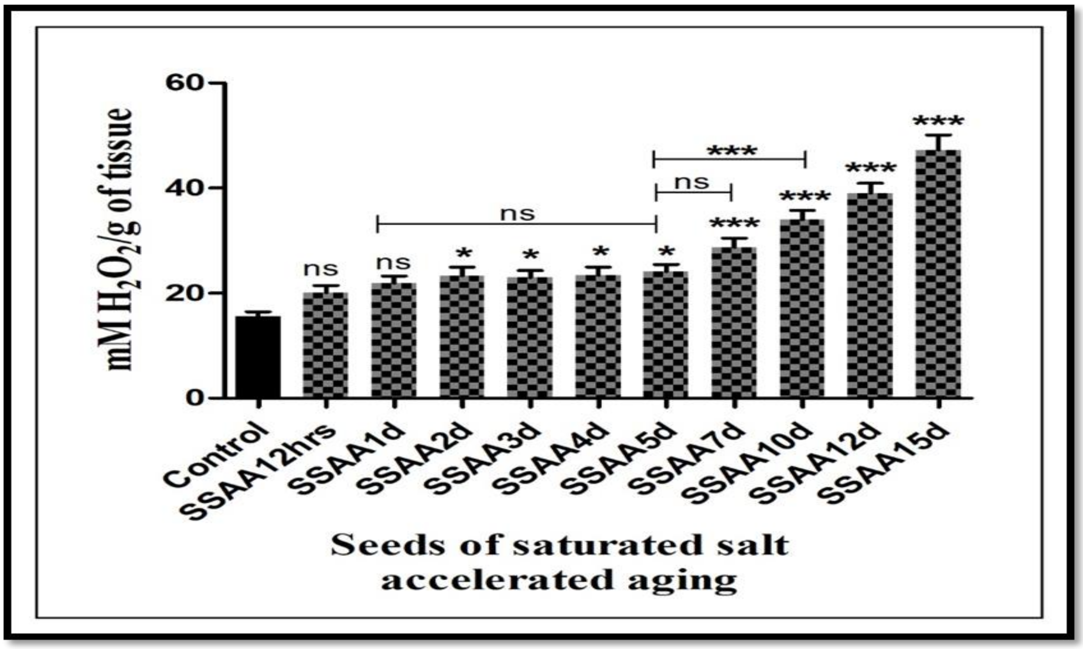

Figure 3.12: Hydrogen peroxide content found in control seeds and in seeds of saturated salt accelerated aging of 12hours, 1, 2, 3, 4, 5, 7, 10, 12 and 15 days. Values are mean ± SE; *, **, * indicates significantly different at $P<0.05$, $P<0.01$ and $P<0.001$ as compared to the control, SSAA1d, SSAA5d and SSAA10d, n=3.**

Table 3.2: Comparative analysis of levels of H_2O_2 in natural aging with accelerated aging and saturated salt accelerated aging.

Level of H_2O_2 in seeds of natural aging (NA)	Levels of H_2O_2 in seeds of accelerated aging (AA)	Levels of H_2O_2 in seeds of saturated salt accelerated aging (SSAA)
Mean ± SD	Mean ± SD	Mean ± SD
Control – 15.6 ± 0.908		
NA6m – 20.3 ± 1.21	AA12h – 20.5 ± 0.921(ns)	SSAA12h – 20.1 ± 1.31 (ns)
NA12m – 23.9 ± 1.15	AA4d – 24.0 ± 0.927 (ns)	SSAA5d – 24.1 ± 1.33 (ns)
NA18m – 28.0 ± 1.32	AA5d – 28.0 ± 1.18 (ns)	SSAA7d – 28.7 ± 1.60 (ns)
NA24m – 37.6 ± 1.20	AA10d – 37.9 ± 1.07 (ns)	SSAA12d – 39.0 ± 1.83 (ns)

Values of H_2O_2 in NA6m, NA12m, NA18m and NA24m found equivalent (~) with AA and SSAA. (ns) - Non-significant when AA12h and SSAA12h, AA4d and SSAA5d, AA5d andSSAA7d and AA10d and SSAA12d are compared to NA6m, NA12m, NA18m and NA24m respectively. Values are mean of ± SEM, n=3.

Correlation study revealed a positive correlation between MDA content and hydrogen peroxide content in seeds of natural aging (r= 0.877***) (Figure 3.13), accelerated aging (r= 0.950***) (Figure 3.14) and saturated salt accelerated aging (r= 0.927***) (Figure 3.15).

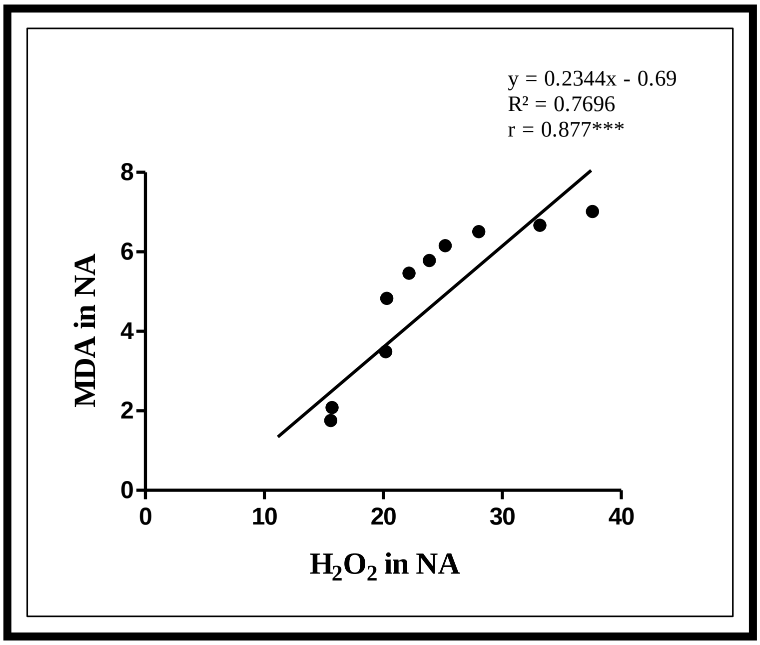

Figure 3.13: Correlation between hydrogen peroxide content and MDA content found in seeds of natural aging. Values are mean ± SE; *, **, *** indicates significantly different at $P<0.05$, $P<0.01$ and $P<0.001$.

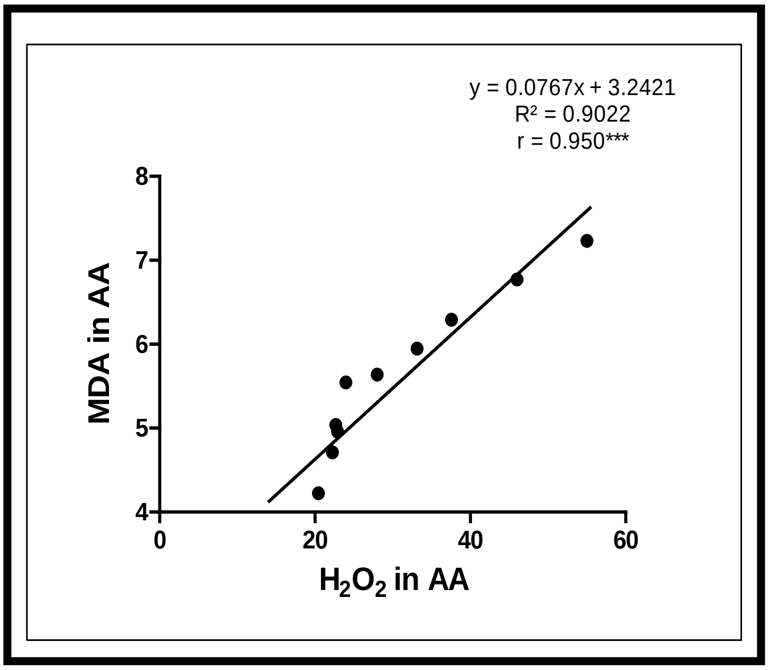

Figure 3.14: Correlation between hydrogen peroxide and MDA content found in seeds of accelerated aging. Values are mean ± SE; *, **, *** indicates significantly different at $P<0.05$, $P<0.01$ and $P<0.001$.

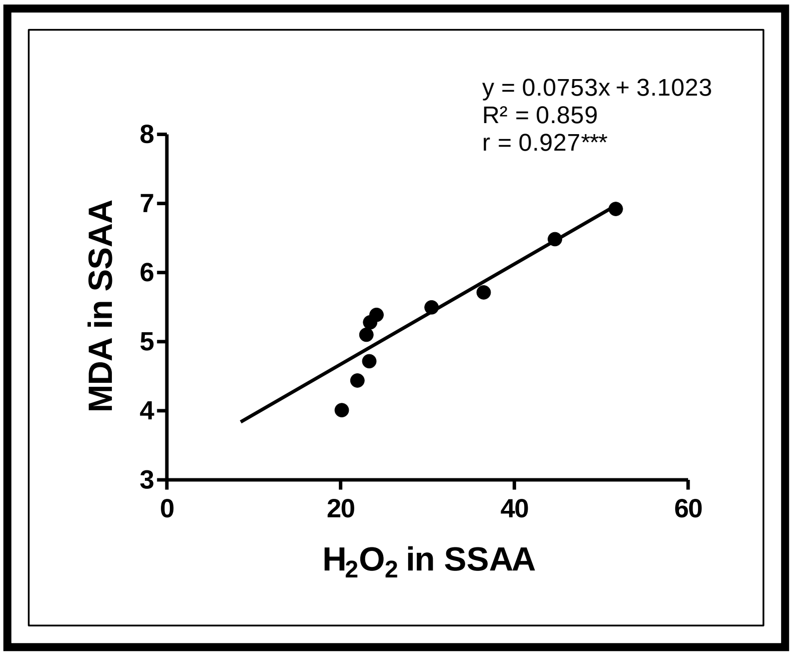

Figure 3.15: Correlation between hydrogen peroxide and MDA content found in seeds of saturated salt accelerated aging. Values are mean ± SE; *, **, * indicates significantly different at $P<0.05$, $P<0.01$ and $P<0.001$.**

3.3. DISCUSSION

3.3.1. Estimation of lipid peroxidation

MDA serves as a molecular marker to estimate the extent of lipid peroxidation in lipid-rich seeds, especially those with a high content of unsaturated fatty acids (Priestley and Leopold, 1979; Goel and Sheoran, 2003). *Jatropha curcas* L. seeds contain 79% unsaturated fatty acids [oleic acid (47%) and linoleic acid (32%)] and the double bonds of unsaturated fatty acids are more susceptible to attack by free radicals. In the present study the elevated level of MDA was recorded from the 3rd month of natural aging (Figure 3.1) and a similar change could be achieved by 12 hours of accelerated and saturated salt accelerated aging (Figures 3.2 and 3.3). This is in conformity with previous studies done on cotton seeds (Goel et al., 2003), sunflower seeds (Balešević-Tubić et al., 2005) and sweet pepper (Kaewnaree et al., 2011). The results also indicate a complete rancidity of lipids and seed deterioration on prolonged period of seed storage and intense artificial aging. Lipid-rich seeds under ambient temperature and moisture content accumulate MDA beyond the level of tolerance. MDA has been widely used for many years as a convenient biomarker for lipid peroxidation because of its reactivity with thiobarbituric acid (TBA) (Pryor, 1989; Esterbauer and Cheeseman, 1990). Because MDA is a popular and reliable marker that determines oxidative stress and due to MDA's high reactivity and toxicity, it has been followed in studies undertaken for validating oxidative stress (Giera et al., 2012). The free radicals produced in excess do not allow the seed to maintain its vigour and viability during storage. Such deteriorated seeds either produce seedlings of low quality or do not germinate. Seeds subjected to 1, 5, 7, 12 of days accelerated aging and 2, 10, 12, 15 days of saturated salt accelerated aging treatment had MDA content equivalent to that found in seeds kept under 6, 12, 18 and 24 months of natural aging, respectively (Table 3.1). This comparison helps us to map the time points when similar changes can be expected in NA much earlier.

Barreto and Garcia, (2017) studied effect of accelerated aging on *Acrocomia aculeate* (macaw palm) seeds. They observed that during aging there is an increase in MDA content indicating the occurrence of the lipid peroxidation. Kumar and Mishra, (2014) concluded that when there is higher amount of MDA produced it results in rancidity of neem seed oil (*Azadirachta indica*) and seeds lose their viability and seed vigour and eventually die during natural aging. Bao et al., (2011) subjected pear stock seeds to 12 months of natural aging at room temperature. During and after 12 months of seed storage, MDA content in stored seeds increased indicating the onset of lipid peroxidation.

3.3.2. Estimation of electrolyte leakage

Increase in length of storage and exposure to accelerated aging treatment increased the electrical conductivity of *Jatropha curcas* L. seeds. A low but significant value of electrical conductivity was found in seeds kept under natural aging for 3 months to 12 months, which peaked at 15 to 24 months (Figure 3.4). This shows a lag in the membrane damage by free radicals in the first 12 months of storage. Peak in electrical conductivity seen in AA (AA12hr to AA15d) and SSAA (SSAA12hr to SSAA15d) (Figures 3.5 and 3.6) seeds is due to a surge in the production of free radicals which attack the membrane under high temperature and moisture content. Due to the extreme condition of temperature and moisture content there is an increased level of lipid peroxidation which is manifested as lipid damage causing increased electrical conductivity in these models of seed aging. Significant increase found in AA and SSAA compared to control indicated that seeds subjected to accelerated aging and saturated salt accelerated aging had followed the same kinetics of changes seen in NA. Positive correlation observed between electrical conductivity and MDA content clearly affirms the membrane integrity loss.

Our results are in conformity with that of an earlier report in cotton (Basra et al., 2000) and sunflower (Hussein et al., 2011) seeds. During storage, membrane lipids in the phospholipid bilayer are degraded by the sequential reaction of lipid peroxidation through the formation of ROS (Torres et al., 2006). In deteriorated seeds, loss of membrane integrity becomes irreversible because of the absence of a repair mechanism (Sung and Jeng, 1994), thus permitting the leaching of electrolyte in greater amounts (Sahu et al., 2017). The present study revealed that a significant increase in electrical conductivity accompanied by high MDA content qualifies the seeds as non-viable with no or low seed vigour. Comparison of values of electrical conductivity between NA and AA and SSAA seeds was not possible because the significance value of the AA and SSAA group was much higher than that of the NA group.

3.3.3. Estimation of H_2O_2 (Hydrogen peroxide)

Increase in length of storage and extended accelerated aging treatment lead to increase in the hydrogen peroxide content of *Jatropha curcas* L. seeds. This is evident from the significant increase found in NA3m and the hiked content of hydrogen peroxide in the rest of the groups of natural aging (Figure 3.10). Highly significant increase of hydrogen peroxide observed in AA (Figure 3.11) and SSAA (Figure 3.12) further supports the lipid peroxidation.

One of the key contributors to deterioration is accumulation of free-radicals produced by the metabolic process. On prolonged storage of seeds, its lipids undergo slow but consistent attack by oxygen, forming hydrogen peroxides, other oxygenated fatty acids and free radicals. The free radicals are unstable and may react and damage nearby molecules. Oxygenated fatty acids in the absence of enzymes activity in the dry seed would get accumulated and damage cellular components causing seed deterioration. Lipid peroxidation and free radical~~s~~ formation are the major causes for the deterioration of oil seeds in storage (Mahjabin et al., 2015). ROS formation and accumulation during the aging of the seeds has a negative correlation to that of seed viability (Pukacka and Ratajczak, 2007; Ratajczak et al., 2015). In *Arabidopsis* and sunflower seeds during aging, ROS like hydrogen peroxide seem to play a pivotal role in oxidizing the biomolecules present in the cellular structures (Bailly, 2004; Job et al., 2005; Kibinza et al., 2006). During aging of orthodox seeds there is a constant generation of OH radicals from H_2O_2 via Haber–Weiss reaction. This leads to the promotion of the formation of other free radicals subsequently (Ratajczak et al., 2015). In our study, increased level of hydrogen peroxide content found in NA (from NA3m to NA24m), AA (AA12h to AA15d) and SSAA (SSAA2d to SSAA15d) indicates that increased level of hydrogen peroxide will further lead to the formation of OH radicals thus promoting the generation of deteriorative causative free radicals in these group of seeds. Seeds of NA1m, NA3m, NA6m and NA9m show low level of deterioration. This can be attributed to the robust free radical scavenging capacity of the antioxidant enzymes in the young seeds which can keep the level of ROS below the detrimental level of deterioration. Similar kind of trend was observed in AA12h, AA1d, SSAA12h, SSAA1d, SSAA2d, SSAA3d, SSAA4d and SSAA5d. Positive correlation between MDA content and hydrogen peroxide content confirms the lipid peroxidation which is inevitable for the lipid rich seeds during prolonged seed storage. Our results are similar to that of earlier studies done on neem seeds (Sahu et al., 2017), where there was an increase in amount of OH radicals and H_2O_2 when the neem seeds were subjected to both natural aging of 100 days and controlled deterioration of 24 hours. Decline in germination was negatively correlated to the increased content of H_2O_2 during both natural aging and controlled deterioration of neem seeds.