CHAPTER – 4

EFFECT OF STORAGE OF *JATROPHA CURCAS* L. SEEDS ON GERMINATION

4.1. INTRODUCTION

Growing attention towards the use of the primary energy source due to the impending shortage of fossil fuel has become the priority for humanity. In designing new technologies to develop power plants, energy efficiency and ecological efficiency has taken precedence. In reducing the greenhouse gas emission, carbon credits create a market giving a monetary value to the cost of air pollution (Lozano-Isla et al., 2018). In this regard, *Jatropha curcas* L. comes to forefront because of its special properties including presence of highest oil content within seeds. *J. curcas* through carbon credits creates an opportunity to achieve future financing projects (Lozano-Isla et al., 2018). An orthodox seed like *Jatropha* is prone to deterioration during storage. Due to the presence of high amount of unsaturated fatty acids lipid peroxidation becomes an easy means to bring about rancidity. Hence storage of these seeds either for the purpose of overcoming dormancy or germplasm or preserving plant genetic diversity is always a risk filled endeavour. Controlling the loss of the physiological properties and seed deterioration during storage is important (González et al., 2012). Seed storage has received a very little attention which is a major concern in agriculture (Tekrony, 2006). In tropical region, seed deterioration is both inevitable and irreversible due to high temperature and moisture content (Pukacka et al., 2009). Longer period of seed storage or the inability of the seed to withstand negative influence of storage both may result in loss of seed vigour and viability. Such seeds culminate either in producing poor seedlings or fail to germinate. If seeds like *Jatropha curcas* L. are to be utilised for the purpose of biodiesel then plantation of such plants also need to be in larger scale to produce excessive amount of seeds for oil extraction to be used as feedstock. Hence seed viability, vigour and germinability of *Jatropha curcas* L. need to be both preserved and enhanced as well.

Germination is one of the best and traditional methods to qualify the seed vigour and viability and to evaluate the physiological quality of the seed. Imbibition of the quiescent seed is the beginning of germination and elongation of the embryonic axis is final stage of germination. Reserves in the seed are utilized to supply energy to the developing of the radicle (Almansouri et al., 2001). Lipid rich seeds subjected to long term storage results in loss of seed germination. Considerable reduction in seed germination also takes place if seeds are not properly dried before subjecting them to storage. Lipid peroxidation and free radicals are the primary causes of reduction or loss in seed germination for oil rich seeds. Thus, storage period and germination are negatively correlated.

Subjecting the seeds to induced artificial aging helps us to understand changes seen on long durations in a short period of time. Fantazzini et al., (2018) studied the association between artificial aging test and natural storage of coffee seeds. It was observed that as the storage period and artificial aging treatment gets prolonged there is a decrease in seed germination in the five cultivars of coffee seeds.

Reactive oxygen species accumulate during aging and the ongoing and unstoppable lipid peroxidation damages the membrane lipids causing structural and functional deleterious changes and this results in membrane leakage (Simon, 1974). When there is a loss of membrane integrity there is seed leaching bringing about viability loss in several species (Ratajczak and Pukacka, 2005). Loss in seed viability will reflect in poor emergence of seedlings or complete loss of germination.

Mira et al., (2011) presented the findings on germination capacity and vigour in four wild Brassicaceae species seeds during aging. Environmental condition of 45°C and 90% relative humidity created during aging, brought about lipid peroxidation and changes in membrane integrity. Aging reduced the percentage of seed germination and extended the time period taken to achieve 50% of germination (T_{50}). Increased membrane permeability was negatively correlated to vigour loss and germination reduction in this study.

Xia et al., (2015) designed an experiment to determine a relationship between lipid peroxidation and the ultrastructural changes of the embryo cells. Fluctuating the moisture content between 4 to 16%, they subjected oat seeds to 0, 8, 16, 24, 32 and 40 days of aging in 45°C. They reported higher the moisture content greater the changes at the ultrastructural level and greater the germination loss. Decline in the integrity of the ultrastructural cells and germination were closely associated with the accumulation of H_2O_2 and this was correlated to the decrease of the antioxidant enzymes activity in these aged seeds. During early stages of germination, the orthodox seed species possess relatively lesser levels of ATP and ADP than the recalcitrant seed species making them more susceptible to the ill-effects of aging (Pasquini et al., 2012).

4.2. RESULTS

4.2.1. Determination of germination

Germination experiments were set up as described by Kibinza et al., (2006) (Chapter 2). A significant decrease in germination was noted in 3-month, 6-month, 9-month and 12-month old seeds of natural aging compared to control (Figure 4.1). As storage period prolonged beyond 12 months the seeds failed to germinate. Seeds of AA12hr, AA1d, AA2d, AA3d, SSAA12hr, SSAA1d, SSAA2d and SSAA3d (Figures 4.2 and 4.3) showed germination, but the values are significantly low compared to control. Seeds that were exposed to more than four days of either accelerated aging or saturated salt accelerated aging treatment never showed any germination. Percentage of germination reported in AA12hr and AA1d is same as the percentage noted in NA9m and NA12m. Similarly, germination percentage observed in SSAA1d is nearly same as the percentage calculated in NA12m (Table 4.1).

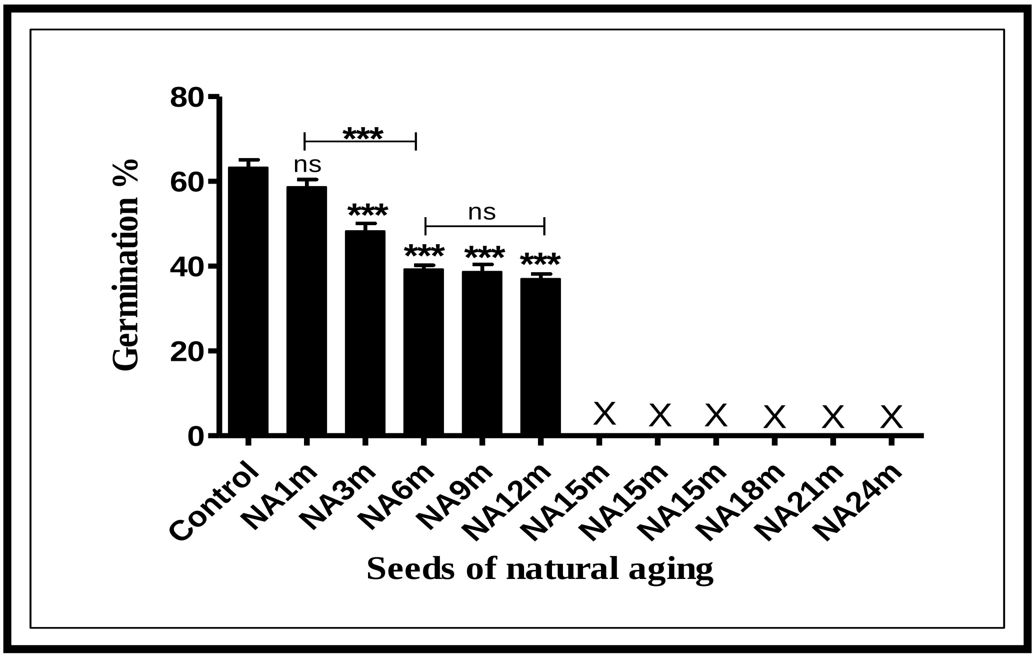

Figure 4.1: Percentage of germination in control seeds and in seeds of natural aging of 1, 3, 6, 9, 12, 15, 18, 21 and 24 months. Values are mean ± SE; *, **, * indicates significantly different at P<0.05, P<0.01 and P<0.001 as compared to the control, NA1m and NA6m, n=3.**

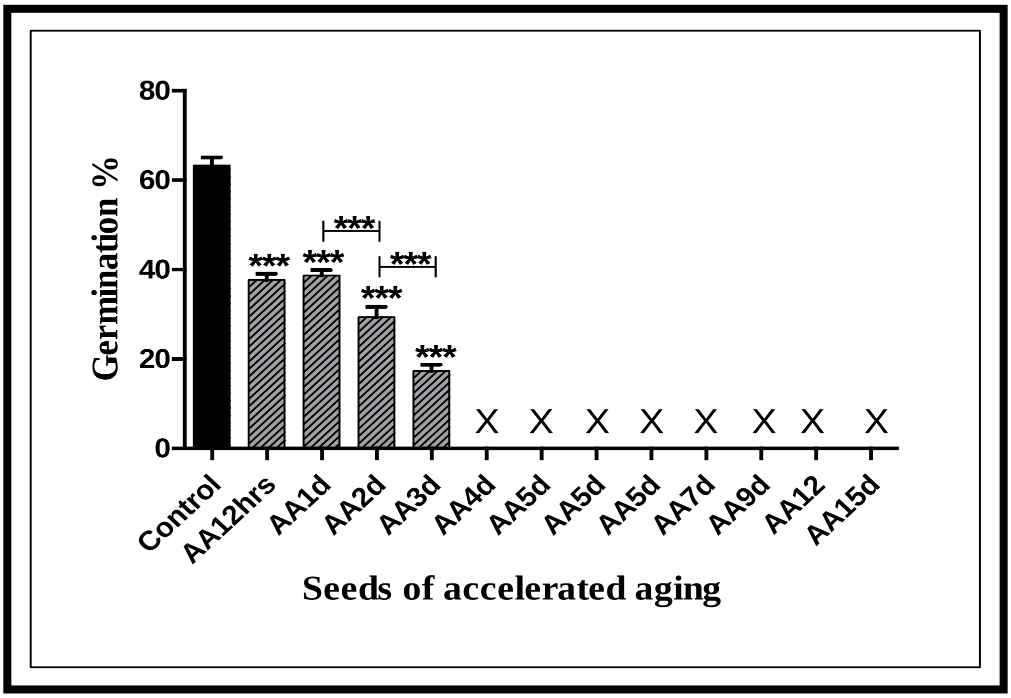

Figure 4.2: Percentage of germination in control seeds and in seeds of accelerated aging of 12hours, 1, 2, 3, 4, 5, 7, 9, 12 and 15 days. Values are mean ± SE; *, **, * indicates significantly different at P<0.05, P<0.01 and P<0.001 as compared to the control, AA1d and AA2d, n=3.**

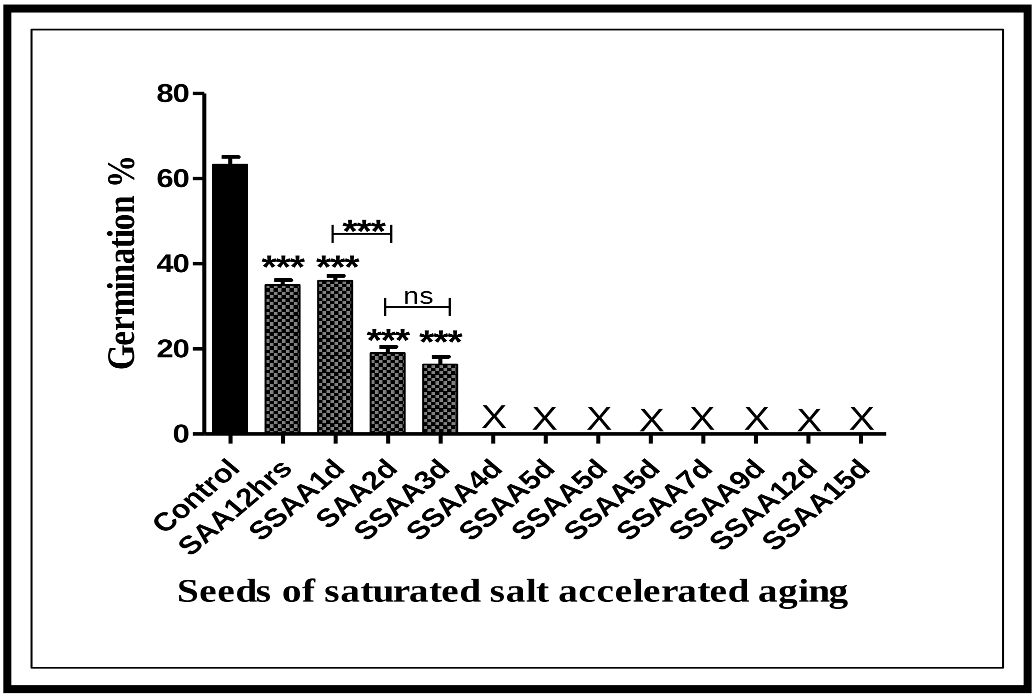

Figure 4.3: Percentage of germination in control seeds and in seeds of saturated salt accelerated aging of 12hours, 1, 2, 3, 4, 5, 7, 9, 12 and 15 days. Values are mean ± SE; *, **, * indicates significantly different at P<0.05, P<0.01 and P<0.001 as compared to the control, SSAA1d and SSAA2d, n=3.**

Table 4.1: Comparative analysis of percentage of germination in natural aging with accelerated aging and saturated salt accelerated aging.

Percentage of germination in seeds of natural aging (NA)	Percentage of germination in seeds of accelerated aging (AA)	Percentage of germination in seeds of saturated salt accelerated aging (SSAA)
Mean ± SE	Mean ± SE	Mean ± SE
Control - 63.3 ± 1.76		
NA9m - 38.7 ± 1.76	AA12h - 37.7 ± 1.45 (ns)	
NA12m - 37.0 ± 1.15	AA1d - 38.7 ± 1.20 (ns)	SSAA1d - 36.0 ± 1.15 (ns)

Percentage of germination in NA6m, NA9m and NA12m found equivalent (~) with those groups in AA and SSAA. ns - Non-significant when AA12h and AA1d are compared to NA9m and NA12m respectively and SSAA1d is compared with NA12m. Values are mean of ± SEM, n=3.

Correlation analysis reveal a negative correlation between MDA content and percentage of germination in seeds of natural aging (r = - 0.905***) (Figure 4.4), accelerated aging (r = - 0.830**) (Figure 4.5) and saturated salt accelerated aging (r = - 810**) (Figure 4.6).

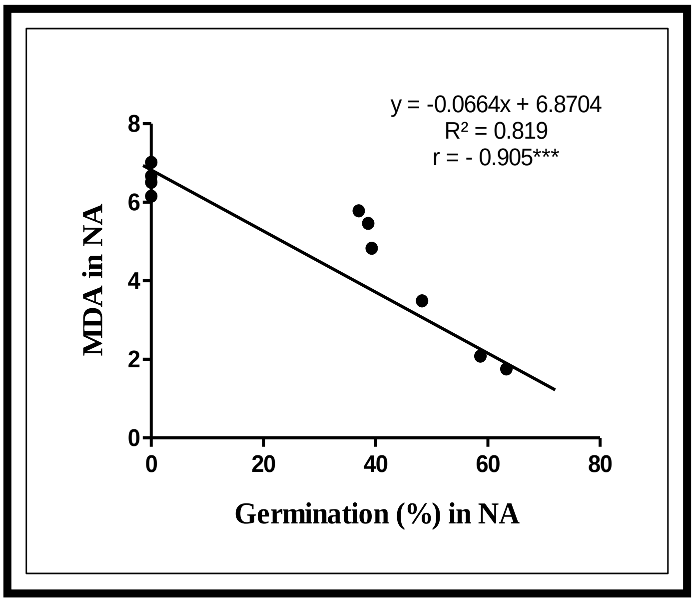

Figure 4.4: Correlation between percentage of germination and MDA content found in seeds of natural aging. Values are mean ± SE; *, **, * indicates significantly different at P<0.05, P<0.01 and P<0.001.**

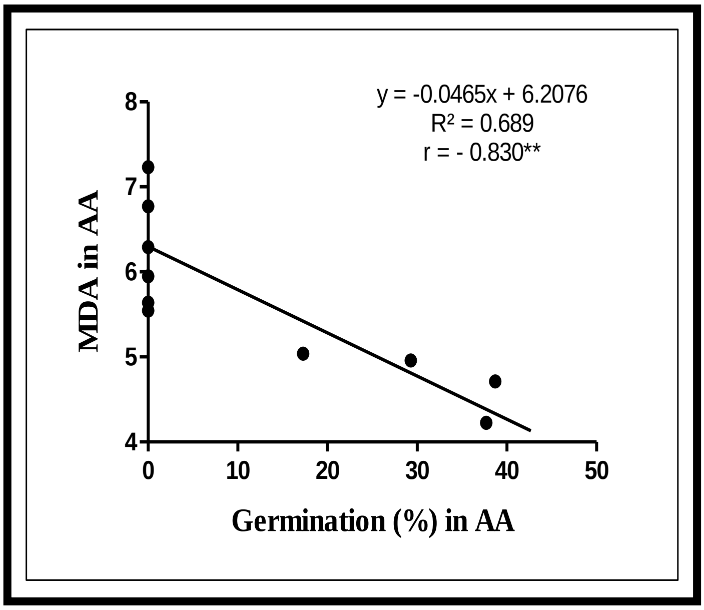

Figure 4.5: Correlation between percentage of germination and MDA content found in seeds of accelerated aging. Values are mean ± SE; *, **, * indicates significantly different at P<0.05, P<0.01 and P<0.001.**

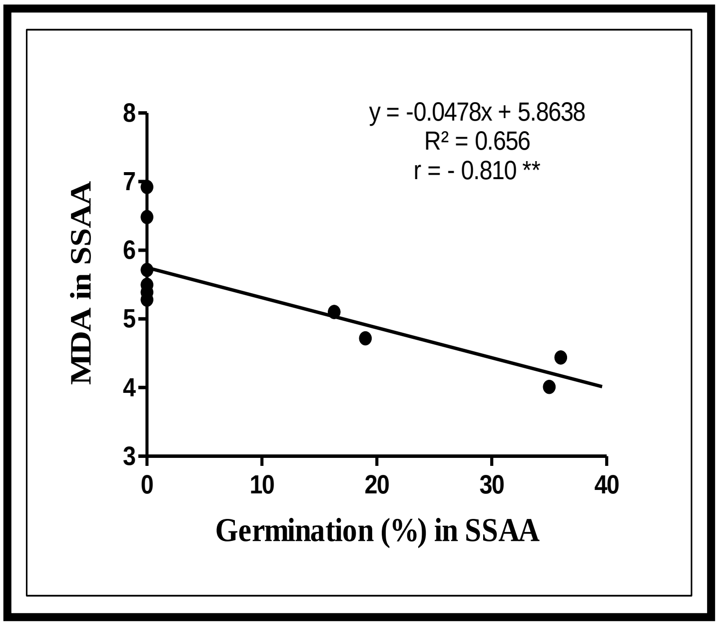

Figure 4.6: Correlation between percentage of germination and MDA content found in seeds of saturated salt accelerated aging. Values are mean ± SE; *, **, * indicates significantly different at P<0.05, P<0.01 and P<0.001.**

4.2.2. Measurement of radicle length

A significant decrease of radicle length was recorded from NA6m to NA12m compared to control (Figure 4.7). AA12hr, AA1d, AA2d, AA3d, SSAA12hr, SSAA1d, SSAA2d and SSAA3d were also reported to have decreased radicle length compared to control (Figures 4.8 and 4.9). As storage period of natural aging is lengthened and days of accelerated aging and saturated salt accelerated aging prolonged, the decrease in radicle length was evident compared to control.

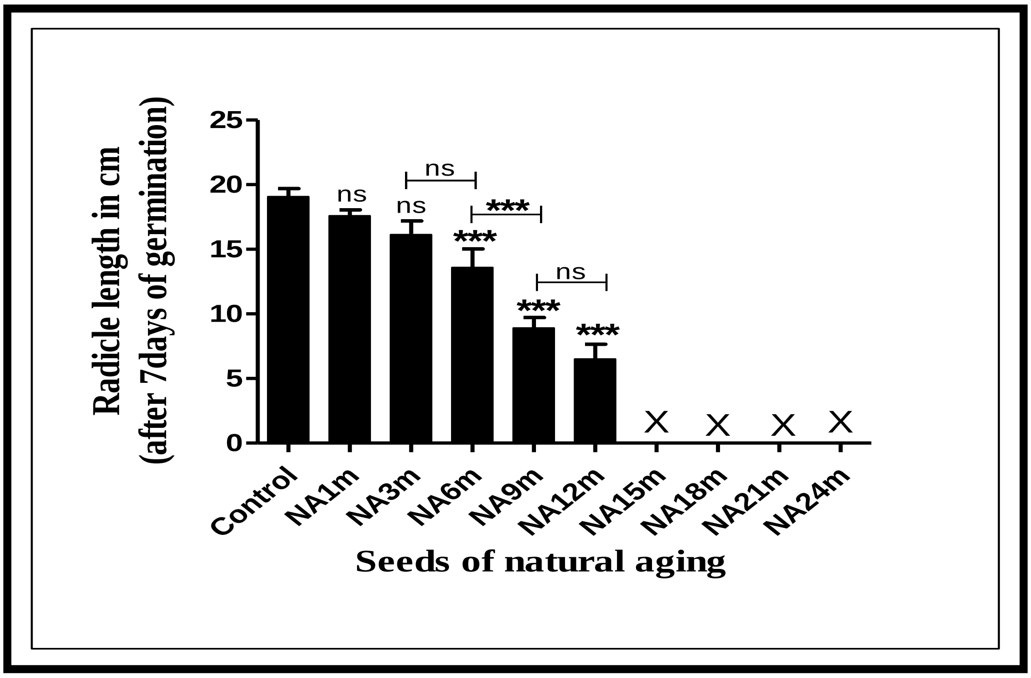

Figure 4.7: Radicle length found in germinated seeds of control and natural aging of 1, 3, 6, 9, 12, 15, 18, 21 and 24 months. Values are mean ± SE; *, **, * indicates significantly different at P<0.05, P<0.01 and P<0.001 as compared to the control, NA3m, NA6m and NA9m, n=3.**

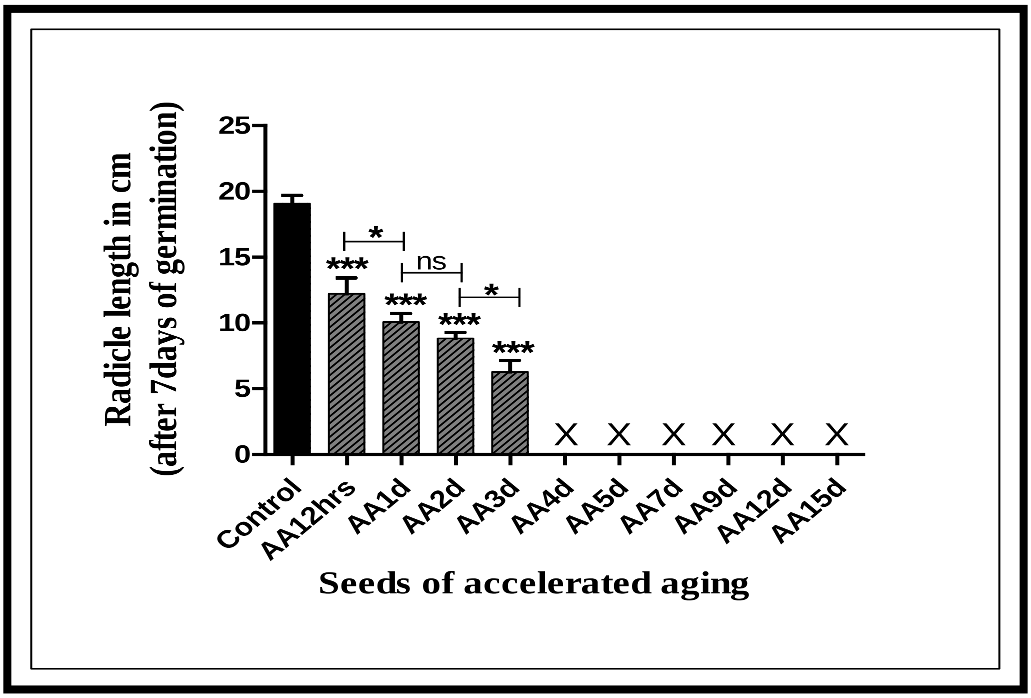

Figure 4.8: Radicle length found in germinated seeds of control and accelerated aging of 12hours, 1, 2, 3, 4, 5, 7, 9, 12 and 15 days. Values are mean ± SE; *, **, * indicates significantly different at P<0.05, P<0.01 and P<0.001 as compared to the control, AA12h, AA1d and AA2d, n=3.**

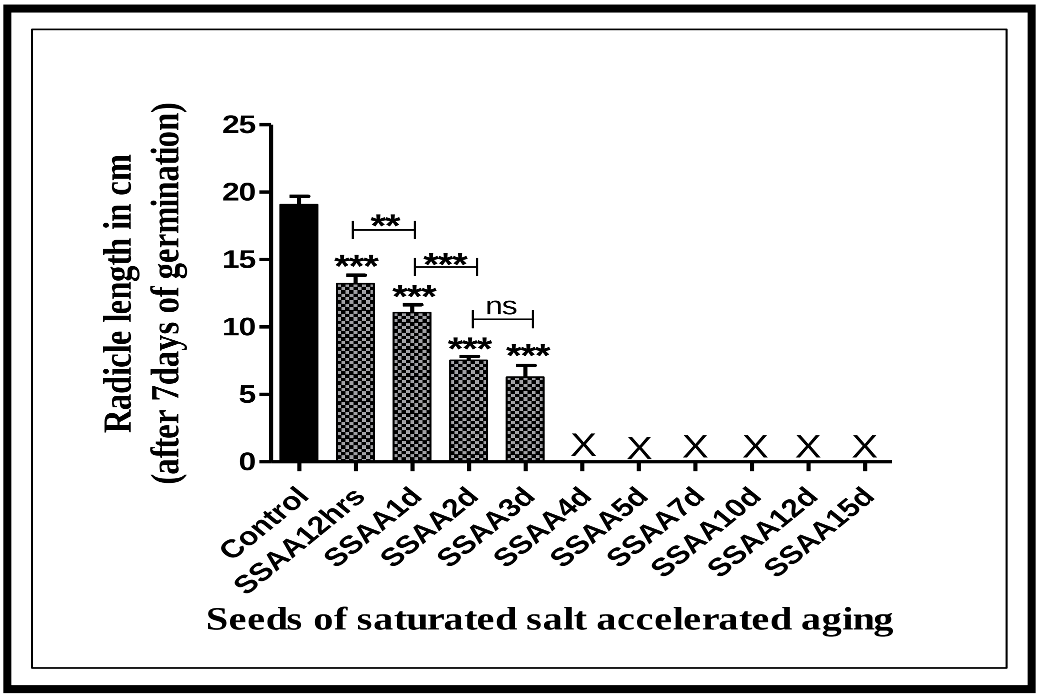

Figure 4.9: Radicle length found in germinated seeds of control and saturated salt accelerated aging of 12hours, 1, 2, 3, 4, 5, 7, 9, 12 and 15 days. Values are mean ± SE; *, **, * indicates significantly different at P<0.05, P<0.01 and P<0.001 as compared to the control, SSAA12h, SSAA1d and SSAA2d, n=3.**

4.3. DISCUSSION

4.3.1. Seed germination studies

A significant decrease of germination was seen to begin from NA3m to NA12 seeds indicating the inability of the seeds to combat the free radicals produced during storage. This decrease in germination and radicle length reflect the beginning of the loss of viability and vigour and the beginning of seed deterioration. Seeds failed to germinate after 12 months of natural aging indicate a complete loss of seed viability due to prolonged period of storage. The significant decrease observed in germination in AA12h, AA1d, AA2d, AA3d, SSAA12h, SSAA1d, SSAA2d, and SSAA3d seeds further confirms the detrimental effect of unfavourable temperature and moisture content made available during artificial aging. Complete failure in germination found from AA4d and SSAA4d onwards indicates severe seed deterioration and loss of vigour and viability.

Temperature, moisture, and period of storage are critical factors affecting genetic, physiological and biochemical changes that impact seed quality and properties (Chirchir et al., 2017). The stressful condition of storage or accelerated aging or saturated salt accelerated aging causes ultra-cellular changes (Moncaleano-Escandon et al., 2012) leading to membrane damage (Kapoor et al., 2010), solute leakage (Lin, 1990), DNA damage and impairment of transcription causing faulty or incomplete protein (enzyme) synthesis (Kapoor et al., 2010) essential for seed germination. Reduction in germination is also attributed to degradation of the mitochondrial membrane, leading to reduction in energy supply necessary for germination (Gidrol et al., 1998). The decline in germination (found in NA3m, NA6m, NA9m, NA12m, AA12h, AA1d, AA2d, AA3d, SSAA12h, SSAA1d, SSAA2d and SSAA3d) and termination of germination (found in NA15m, NA18m, NA21m, NA24m, AA4d, AA5d, AA7d, AA10d, AA12d, AA15d, SSAA4d, SSAA5d, SSAA7d, SSAA10d, SSAA12s and SSAA15d) are negatively correlated to the increased content of MDA and electrical conductivity found in the above mentioned groups of seeds. Results of the present investigation are similar to that of earlier studies done on macaw palm seeds (Sahu et al., 2017), sunflower (Kibinza et al., 2006) and soybean (Xin et al., 2014) where increased MDA content, electrical conductivity and hydrogen peroxide content were connected to loss of germination and reduction in radicle length. Partial degradation of DNA and RNA occurring in pea *(Pisum sativum)* seeds due to the artificial aging at 50°C and 60% relative humidity (Chen et al., 2013) was shown to weaken the seed viability and vigour which ultimately results in decreased germination. Changes in expression of genes associated with programmed cell death identified by transcriptome profiling in pea seeds that were artificially aged (Chen et al., 2013) is the strong evidence of loss of viability and vigour in aged seeds.

Mere treatment of 12 hours or one day of accelerated aging or saturated salt accelerated aging was sufficient enough to drastically bring down the germination percentage as noted in 12 months of natural aging (Table 4.1). This indicates the extreme sensitivity of *J. curcas* seeds to extreme condition of high temperature and moisture content. Percentage of germination and radicle length in *J. curcas* seeds seem to decrease with aging during natural aging up to 12 months of NA. A complete loss of germination in seeds subjected beyond the point of 12 months was evident through failure in germination. Accelerated decline in germination seen up to 3 days of accelerated and saturated accelerated aging and complete loss of germination beyond 4 days of artificial aging treatment also mimics the effect of natural aging. Declined level of germination and loss of germination are associated with lipid peroxidation brought about by the accumulation of hydrogen peroxide and inactivation of free radicals scavenging enzymes.

Our results are in conformity with the previous reports of loss of germination in aging seeds of sunflower (Kibinza et al., 2006), seeds of soybean (Xin et al., 2014), seeds of norway maple and sycamore (Pukacka and Ratajczak, 2007) and seeds of neem (Varghese and Naithani, 2008). Increased lipid peroxidation, membrane permeability and accumulation of hydrogen peroxide have enormous detrimental effect on lipid seeds thus decreasing the germinability of the seeds placed for natural aging and artificial aging.

Under the conditions reported in this study *Jatropha curcas* seeds started exhibiting detrimental effects from 3 months of NA indicating that for optimum yield of biodiesel it is of utmost importance to ensure proper storage conditions so as to maintain seed quality.