EFFECT OF STORAGE CONDITIONS AND DURATION OF STORAGE ON SEED GERMINATION OF *TETRAPLEURA TETRAPTERA* (Schum and Thonn) Taub AND *ANNONA MURICATA* (Linn.)

ORIGINAL RESEARCH ARTICLE

Authors' Contribution: "Author 'A' designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Author 'B' manage the analyses and literature searches of the study. All authors read and approved the final manuscript.

ABSTRACT

Tetrapleura tetraptera and *Annona muricata* are medicinal plant species with a great medicinal values. Despite their usefulness, preservation of their seed from harvest time to the time when they are required for planting has not been given adequate attention. Therefore, this study investigated the effect of storage conditions and duration of storage on seed germination of *T. tetraptera* and *Annona muricata.* Fifty (50) seeds each of *Tetrapleura tetraptera* and *Annona muricata* were planted in top soil to determine their germination percentages before subjected to storage conditions, while *Tetrapleura tetraptera* has 94% germination, *Annona muricata* has 72% germination. Four storage conditions viz: cold room, refrigerator, room air conditioned and laboratory were used and the experiment was carried out in Ekiti State University, Ado – Ekiti. Seeds stored at different temperatures were collected and planted in polythene nylons filled with top-soil and watered daily, the experiments were replicated four times to test for the germinability on monthly basis for six months. In *Tetrapleura tetraptera*, highest percentage germination (50%) was recorded in seeds stored in the air conditioned room at 1 month. This was followed by seeds store in the refrigerator with 47% at 2 month. Seeds stored in the room air conditioned had percentage germination of 39% at 2 months, 30.50% at 3 months and 10.70% at 4 month of the experiment. No further germination in seeds under this treatment was observed at the last two months of the experiment. While seeds stored in refrigerator recorded 5.20% at 1 months of the experiment. Seeds stored in cold room and those in laboratory recorded no emergence throughout the period of the experiment. Also in *Annona muricata*, Highest percentage germination (58.00%) was observed in seeds stored in the room air conditioned at 1 month of

the experiment, this was followed by the seeds in this same temperature at two month of the experiment with mean plumule emergence value of 39.00%, seeds under this temperature (air condition) did not respond to germination at 5th and 6th months of the experiment. Seeds of *Annona muricata* stored in the cold room responded to germination with unappreciable value of 2.05% only at third month of the experiment. Only 1.6% of the seeds stored in the laboratory emerged at 5th month of experimental time, seeds stored in the refrigerator did not respond for the first two months, last fifth and sixth month of the experiment but had 15.53% and 35.00% plumule emergence at third and fourth month of the experiment respectively. Thus, storage of seeds of *T. tetraptera* and *A. muricata* in air conditioned room with temperature of -20^{0}C might enhance germination in these species.

Keywords –Medicinal plant, Storage Conditions, Duration of Storage, *Tetrapleura tetraptera*, *Annona muricata*

INTRODUCTION

Tetrapleura tetraptera (Schum and Thonn.) Taub. Commonly known as Aridan in South Western Nigeria is a medicinal plant of the family Mimosaceae. The plants is locally known as Uyayak in Ibiobio; Edeminang in Efik; Osakirisa or Oshosho in Igbo and Dawo in Hausa (all in Nigeria) (Ojewole and Adewumi, 2004). Its fruit is used for the management of convulsion, leprosy, inflammation, and rheumatism (Okwu, 2003; Ojewole and Adewumi, 2004; Jamwal *et al*., 1972).

Tetrapleura tetraptera is one of the highly valued medicinal plants in Nigeria. The plant was reported to have both cardiovascular and neuromuscular activities. Pharmacologically, *T. tetraptera* is found to be anti-hypotensive, anti-ulcerative, anti-inflammatory and anti-microbial (Ojewole and Adewumi, 2004).

Annona muricata, called soursop due to the sweet and sour flavor of the large fruit, is a lowland tropical fruit-bearing tree in the Annonaceae family. Related species include cherimoya (*A. cherimola*) and sugar-apple (*A. squamosa*). Other common names include graviola and guanábana (sometimes shortened to guanába). The genus name, 'Annona' is from the Latin word 'anon', meaning 'yearly produce', referring to the production of fruits of the various species in this genus (NRCS, 2008). *Annona muricata* is a small, upright, evergreen tree. Its young branches are hairy.

Based on chemotaxonomy approach, some plants of the Annonaceae family have anticancer activity (Sismindari, *et al.,* 2008). *Annona montana* contains monotetrahydrofuranic

acetogenins which have toxicity to liver cancer (Roesker, *et al.,* 2007). *Annona squamosal,* containing ribosome-inactivating protein (RIP), an immunotoxin for the treatment of cancer (Sismindari *et al*., 2008). Based on this chemotaxonomy studies, plant that have close kinship for example, *Annona muricata* are likely to contain similar compounds (Roesker, *et al.,* 2007).

The post-harvest process of medicinal plants have great importance in the productive chain, its direct influence on the quality and quantity of the active principles. Among the post-harvest processes, drying and storage are essential to maintain the product with the physical and chemical characteristics closer to those found in the fresh plant, and failures in any of these steps influence the quality of the final product. Drying, if not performed properly, can compromise the content of active ingredients while, incorrect storage can lead to loss of material, whether for physical or biological reasons (Martinazzo, 2006; Martinazzo *et al.,* 2007). Medicinal plants are often stored for long periods, before being used as raw material for the manufacture of various products (Masand *et al.,* 2014). However, improper storage can result in physical, chemical and microbiological changes (Peter, 2006; Mayuoni-Kirshinbaum,*et al*., 2013).

Seed viability is the ability of the embryo to germinate and it is affected by a number of factors including temperature, light, oxygen, water and species type. Germinability which is determined by germination percentage is the proportion of seeds that germinate from seeds subjected to the right conditions for growth while the germination rate is the speed with which the seeds germinate and is affected by seed viability, dormancy and environmental effects that impact on the seed and seedling (Zamora 2014). This is given as a percentage of germination over a certain amount of time, for example 90% germination within 20 days. Germination energy is the proportion of seeds in a given sample which germinate within the time of peak germination, generally taken as the highest number of germinations in a 24-hour period (Mrđa et al. 2011). The interest in germination energy is based on a theory that only those seeds which germinate rapidly and vigorously under favourable conditions are likely to produce vigorous seedlings in field conditions, whereas weak seeds or delayed germination is often fatal (Aldhous 1972).

Simple techniques have been adopted to maintain the seed viability in both domesticated and wild sources (Ellis et al. 1992, Vertucci and Roos 1991). Seed deterioration and loss of viability, is a natural phenomenon occurring during storage (Nasreen et al. 2000, Schmidt. 2002) and inappropriate storage medium such as room temperature storage (Hezewijk et al.

1993, Müller et al. 2011) has often resulted in low seed germination. Several factors, including temperature, nature of the seeds, seed moisture content, relative humidity influence the seed longevity during storage (Onyekwelua and Fayose 2007, Pradhan and Badola 2008). Proper storage conditions, may effectively retain substantial viability in seeds over a considerable storage period (Butalo and Badola 2004, Chen et al. 2007, Pradhan and Badola 2008).

A long-term storage of seeds, especially under unfavourable conditions, leads to loss of viability. The nature of this physiological damage is variable, e.g. short-term deterioration in the field is different from long term deterioration during storage, which in turn is different from mechanical damage (Mrđa et al. 2011). All parts of the seed such as the seed coat, which is of maternal origin and acts as a physical and chemical barrier, and the embryo are susceptible to physiological damage. Seeds are grouped based on their storage characteristics as orthodox and recalcitrant. Orthodox seeds are those that can be dried to moisture contents of 10% or less, in this condition they can successfully be stored at subfreezing temperatures (Onyekwelua and Fayose 2007). Recalcitrant seeds are seeds that do not survive drying and freezing during exsitu conservation and cannot resist the effects of drying or temperatures less than 10°C; thus, they cannot be stored for long periods like the orthodox seeds because they can lose their viability. Plants that produce recalcitrant seeds include avocado, mango, mangosteen, lychee, cocoa, rubber tree, some horticultural trees, and several plants used in traditional medicine such as species of Virola and Pentaclethra (Pradhan and Badola 2008).

Seeds of different plant species lose viability at varying degrees. For example, onion seed is very difficult to store while barley seed maintains good germination under a variety of storage conditions (Zamora 2014). Seeds and especially oilseeds including E. bussei contain large quantities of carbon stored as carbohydrate, lipid and protein (Bewley and Black 1985). The chemical composition of oilseeds which are rich in lipids causes specific oxidative processes to occur during storage and these are said to cause limited longevity depending on their specific chemical composition (Balešević et al. 2007).

Seed of these two plant species have been reported by the local farmers in Ekiti State, Nigeria to have a negative challenge after storage. Hence, the reason for this study.

MATERIALS AND METHODS

Seed Source

Fresh fruits of Tetrapleura tetraptera were obtained from Oba's market, Ado-Ekiti, Ekiti State, Nigeria and were taken to the herbarium of the Department of Plant Science and Biotechnology, AdoEkiti, Nigeria for authentication. While the mature fruits of *Annona muricata* were collected from the parent plant from its natural habitat in Ipoti-Ekiti located in Ijero Local Government Area of Ekiti State.

Viability Test

Seeds were subjected to viability test by using 2 3 5 Triphenyl Tetrasolium chloride at 1% according to Kruse et al., (2004).

Procedure

First Step

Fifty seeds each of *Tetrapleura tetraptera* and *Annona muricata* were divided into five (5) parts and sown into black polythene bags separately filled with top soil and subjected to natural condition favourable for germination. Germination initiation was observed for two (2) months when the total emergence was counted and recorded.

Second step

Two hundred seeds of *Tetrapleura tetraptera* and *Annona muricata*) were divided into four groups consisting of 50 seeds each. Each batch were stored at four temperatures viz: cold room (22^0C), refridgerator (5^0C), air conditioner (-20^0C) and laboratory/room temperature (2^0C) as control. 20 seeds each of *Tetrapleura tetraptera* and *Annona muricata* from the four batches, stored at different temperatures were collected and planted to test for the germinability on monthly basis for six months. Planting was done in polythene nylons filled with top-soil and watered daily. The experiments were replicated four times. The number of seeds that germinate in each treatment was recorded and the percentage germination was calculated.

RESULTS

Percentage germination of seeds before subjected to storage conditions

Table 1 shows the percentage germination of both ***T. tetraptera*** and *A. muricata* before subjected to storage conditions. In *T. tetraptera,* 94% germination was observed while 72% germination was recorded in *A. muricata.*

Table 1: Percentage germination of seeds of *T. tetraptera* and *A. muricata*

Plants	**Percentage germination (%)**
T. tetraptera	94
A. muricata	72

Effect of Storage Conditions and Duration of Storage on Seed Germination of *T. tetraptera*

Table 2 shows that various storage condition and duration had different effect on seed germination of *T. tetraptera.* Results obtained revealed that seeds stored in cold room and those stored in the laboratory did not germinate throughout the period of experiment. Seeds stored in the refrigerator had mean germination of 5.20% and 47.00% for the first and second months experiment respectively and shows no further germination at 3rd, 4th, 5th and 6th months of the experiment. Highest seed germination (50%) was recorded in seeds under air condition at the first month of the experiment, mean germination of 39%, 30.50% and 10.70% were observed at 2nd, 3rd and 4th month of the experiment respectively with no further germination at 5th and 6th month in this same (air condition) treatment.

Table 2: Percentage Germination of *T. tetraptera* seeds stored under different storage conditions

Months	Percentage Germination (%)			
	Cold room	Refrigerator	Air conditioned	Room Laboratory
1st	0	5.20	50.00	0
2nd	0	47.00	39.00	0
3rd	0	0	30.50	0
4th	0	0	10.70	0

5th	0	0	0	0
6th	0	0	0	0

Effects of Storage Conditions and Duration of Storage on Seed Germination of *A. muricata*

Table 3 shows that seeds of *Annona muricata* stored in the cold room responded to germination with unappreciable value of 2.05% only at third month of the experiment, while no emergence was observed at first two months and the last three months of the experiment in this treatment. Only 1.6% of the seeds stored in the laboratory emerged at 5th month of experimental time, seeds stored in the refrigerator did not respond for the first two months, last fifth and sixth month of the experiment but had 15.53% and 35.00% plumule emergence at third and fourth month of the experiment respectively.

Highest percentage germination (58.00%) was observed in seeds stored in the room air conditioned at one month of the experiment, this was followed by the seeds in this same temperature at two month of the experiment with mean plumule emergence value of 39.00%, a decrease in the percentage plumule emergence in seeds under this same temperature was observed at 3rd and 4th months of the experiment with 10.50% and 9.82% respectively. While the seeds under this temperature (air condition) did not respond to germination at 5th and 6th months of the experiment.

Table 3: Percentage Germination of *A. mricata* seeds stored under different storage conditions

Months	**Percentage Plumule Emergence (%)**			
	Cold room	**Refrigerator**	**Air conditioned**	**Room** Laboratory
1st	0	0	58.00	0
2nd	0	0	39.00	0

3rd	2.05	15.53	10.50	0
4th	0	35.00	9.82	0
5th	0	0	0	1.6
6th	0	0	0	0

DISCUSSION

Results from this study emphasized the fact that appropriate storage conditions and duration of storage are of great necessity in preserving the viability of seeds from the time of collection to the time when they are needed for sowing. In fact, Genes and Nasreen (1995) observed that the use of seed is one of the propagation methods that can be used to multiply plants but the seeds need to be capable of germinating in the first place., hence the need for knowledge of its methods of germination. Since most plants produce seeds only once a year, knowledge of methods of seeds preservation for next season's generation was deemed important and so was the determination of how soon the seeds would lose its viability.

The results obtained in this study revealed that seeds of *T. tetraptera* and *A. muricata* stored in air conditioned room had the best percentage germination when compared with seeds in other storage conditions. Reason for this could be that temperature in other storage conditions might be too low or high to promote seed germination in this plant or that they were too high to enhance proper germination of this species. This finding agreed with previous observation of Lars (2000) that seeds germinate faster with increasing air temperatures up to a point. Extreme heat will slow germination and also increase moisture loss. The temperatures for optimal germination vary with the type of plant, some annual flowers and vegetables are extremely sensitive to cold, extremely hot or cold temperatures can hamper plant growth, as well as affect seed germination Lars (2000). Gamaene *et al.*, (1999) ascertained that under no storage condition would recalcitrant seed be kept for long as this tends to reduce its viability.

CONCLUSION

Storage conditions with extremely hot or cold temperatures does not encourage seed germination of these plant species. Therefore, storage of seeds of *T. tetraptera* and *A. muricata* in air conditioned room with temperature of -20^{0}C to keep its viability intact is recommended

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