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Abstract— Despite their socio-economical potential, edible Annonaceae are underused in Senegal. The misuse of their various organs associated with the adverse effects of drought and seasonal bush fires reduce drastically natural regeneration. The limiting factors intrinsic to the seeds also lessen the renewal of stands in the wild. This study was undertaken to evaluate the in vitro germination capacity of Annonaceae seeds and to define the optimal conditions favorable to their germination process. Viability study and germination capacities were undertaken with a focus on seeds stored at 6 °C for one month. Various pretreatments such as with bleach, sulfuric acid (95%) solution and complete husking of seeds, were carried out at different temperature levels, in various lightening conditions or in darkness. The best germination percentages were recorded with both seeds scarified mechanically and chemically. Whatever the species, the germination is improved when the pretreatment duration with concentrated sulfuric acid increases. The highest germination rates were obtained at 30 °C for Annona muricata (61%) and A. squamosa (74%) and at temperature levels of 27 °C and 30 °C for A. senegalensis (59%). The A. squamosa seeds, previously scarified with absolute sulfuric acid for 60 min, better germinated in darkness (69%) than under illumination (46%) in contrast to those of A. senegalensis (58% in the light and 44% in the dark) and of A. muricata (63% in the light and 32% in the dark).

Keywords: seeds; Annonaceae; germination; viability; scarification; temperature; photosensitivity.

I. INTRODUCTION

The Annonaceae are trees or shrubs found in tropical regions [1]. Annona muricata L. and Annona squamosa L. are nowadays cultivated in warm climates [2]. They are among the few Annonaceae species with edible fruits that are domesticated today [3]. Annona senegalensis Pers. is found in the wild, in the underwood of Sudanese savanna and in the para-sand beaches in West Africa [4]. They have a major socio-economic and pharmacological importance. Their fleshy fruits are edible [5] and are highly valued by local people, while their leaves, bark and roots are used in traditional medicine to treat various diseases such as dermatitis, diarrhea, malaria, etc. [6]; [4]; [7]. In countries where they are grown, they are subject to a large industrial operation both in the food and in the pharmacological or chemical field.

In Sub-saharan Africa, the Sahel region is undergoing profound mutations and, with the drought raging there for several decades, there has been a gradual degradation of ecosystems [8]. Despite their economic importance, these species are experiencing real problems in sahelian Africa. According to Deroin [9], Annona senegalensis is a species subject to savanna fires. During the dry season, fires occur frequently in the Sudano-sahelian savannah [10] where this species is very common. Moreover, drought has been persistent in Senegal over the last thirty years [11]. It is characterized by its duration, its intensity and exponential expansion [12]. It seriously affects the
environment of the Ferlo region extended to the Lower Casamance [13] and increases the frequency and intensity of bushfires. The drought thus acts directly and indirectly on this species, seriously compromising its regeneration and consequently its sustainability. A. squamosa and A. muricata are introduced species in Africa and their cultivation is not well developed despite their usefulness. They are very under-utilized. The subject of food crop cultivation intended only to supply the local market. Due to the fact that all the organs of these plants are used (fruits, leaves, roots, bark, seeds, etc.), high pressure is applied to these species, which became endangered. Their propagation is done in Senegal by conventional methods such as planting seeds [14]. This method is very slow and expensive though the germination percentage (90%) remains significant for Annona squamosa [15]. The regeneration of these species thus requires a better understanding of their different methods of propagation including germination.

Germination is the first stage or transitional stage of plant development; it corresponds to the passage of inert seed to seedling [16]. But, in natural ecosystems, seed germination may be limited by intrinsic factors such as the seed dormancy or extrinsic one such as climate changes, predation or infestation of seeds by pathogens. Seed-coat dormancy is widespread in the dry tropical zones and is, in most cases, due to the impermeability of the coats [17]. Indeed, a seed can germinate if the embryo has the opportunity to soak but the presence of impermeable cell layers prevents the progress of the critical process of imbibition. These seeds with impermeable seed-coats are called hard seeds [16]. However, there are several ways to lower coat inhibitions. In the environment, the infestation of seeds and the involvement of soil microorganisms without damage to the embryo may increase the permeability of the seed-coat to water and thus promote the germination of hard seeds [17].

Seed germination is also a critical step in the life cycle of plants because the seeds are often subject to high mortality rates [18]. Although several factors may affect seed germination, light and soil moisture are the most important ones. In the laboratory, the most common methods to overcome seed-coat dormancy are to partially destroy the protective envelopes so that they become permeable to water and oxygen without harming the embryo.

The light acts as an important environmental signal that regulates seed germination of desertic or Sahelian zones. They can undergo a transition and move from a primary dormancy to a secondary one and thus develop a need for light to trigger germination [19]. The need for light is a genetic trait mediated by phytochrome [20]. This last one stimulates the synthesis of substances like growth promoters that initiate the germination process [21]. Light only affects germination in some species while in other ones the response to light depends on the temperature [22].

So, the objective of this study is to evaluate the germination capacity of seeds of some Annonaceae species under axenic conditions. More specifically, it is to assess the viability of seed lots and the influence of two methods of scarification (mechanical and chemical) on seed germination. The influence of two important external factors for germination, i.e. temperature and light, was also evaluated to determine the temperature most favorable for germination and type of photosensitivity. Indeed, light can be an important factor for seed germination. This sensitivity to light can have a significant impact in the environment on the germination of seeds.

II. MATERIALS AND METHODS

A. Plant materials

The fruits of the Annonaceae were collected using random sampling technique from parcels located in the Dakar suburb for Soursop (A. muricata L.) and Apple Cinnamon (A. squamosa L.). The seeds are mainly extracted from fruits harvested from 25 plant trees, with equivalent trunk diameter and height while the seeds of the “Dugor” fruits (A. senegalensis Pers.) were harvested from 25 other randomized plant trees issued from natural population of the Louga region (North of Senegal). The harvest of fruits was carried out from August to September, period which corresponds to the species fructification. The seeds have been collected from individuals. After dehulling the ripe fruits, equal samples of seeds were combined to give one bulk population sample from which sub samples were taken for germination tests. The seeds were preserved to dry in the ambient air on a laboratory bench for 7 days. Subsequently, they were kept in jars stored in a cold room at a temperature of 6 ± 1°C. The germination tests were performed on intact seeds which pericarp had been removed.

B. Viability test

Before performing the tests for the seed germination, the viability of seed lots was assessed using the tetrazolium chloride test at 1%. For each species, 20 seeds were used to associate numerical values on the scale of Moore [23]. To apply the treatment, the seed coat is fully husked and put to soak in water for 24 hours. Subsequently, a longitudinal incision is made along the endosperm to separate the two cotyledons. The cotyledons with the embryo axis attached are immersed in a complex solution of 1% triphenyl 2, 3, 5 tetrazolium chloride (TTC) for 9 h at 35°C in the dark. Batches of 20 seeds of each species were peeled, distributed in Petri dishes with 5 seeds per box. The evaluation of the topographic coloring of the seed constituents (embryo, cotyledons, radicle and endosperm) was carried out (Table 1) according to the Moore protocol [23].

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C. Mechanical Scarification

To compare the germination potential of different types of seed, batches were subjected to mechanical or chemical treatments. Mechanical scarification was performed by peeling off entirely the seed coats, while respecting the kernel integrity. A completely randomized design with five replications for each treatment was used to evaluate seed germination performance. For each species and for each repetition, a batch of 20 unpeeled seeds and another batch of 20 peeled seeds were used.

Before culturing, non-scarified and scarified seeds were surface-sterilized with bleach (NaOCl, 8° chlorometric) for 30 min and 8 min, respectively, and then rinsed 3 times with sterile distilled water (120°C for 20 min). The jars were then incubated at a constant room temperature of 30°C ± 1°C, with a photoperiod of 16 daytime hours and 8 nighttime hours. The cumulative percentage germination was calculated daily.

D. Chemical Scarification

For this treatment, seeds of different species have undergone a chemical scarification by soaking in a 95% solution of concentrated sulfuric acid (H$_2$SO$_4$) for variable durations, with 20 seeds per treatment, followed by one rinse with running tap water and 6 rinses with sterile distilled water. Starting with the longest time, the jars containing the seeds are filled with the same 25 ml amount of sulfuric acid, with gentle stirring, to ensure proper immersion of the seeds. This procedure allows us to appreciate the immersion time required to achieve optimal germination. Thus, 12 treatments, lasting from 5 to 60 min, and associated with 5 replicates for each treatment time, were performed under aseptic conditions. The seeds are then soaked for 24 h in sterile distilled water before culturing.

E. Influence of Temperature

The effect of temperature on the seed germination of Annonaceous was studied with seeds having undergone the best pretreatment with sulfuric acid, i.e. for a 60 min duration. The seeds of each species were then germinated in an incubator at six different temperatures: 17°C, 23°C, 27°C, 30°C, 38°C and 42°C. For each species and each temperature, a batch of 20 seeds per replication was used. Five repetitions were performed for each temperature. The seeds were surface disinfected with sodium hypochlorite (8° chlorometric) for 8 min and rinsed 3 times with sterile distilled water. The treated seeds were aseptically cultivated and incubated in a constant temperature with a photoperiod of 16 daytime hours and 8 nighttime hours. The number of germinated seeds was recorded every 24 hours for each temperature.

F. Influence of Illumination

To study the incidence of photosensitivity on germination, particularly the role of light on seed germination for the three species, batches of 20 seeds were used (2 batches per species and per replication). Those batches had previously undergone the best pretreatment to the sulfuric acid followed by an 8 min sodium hypochlorite (8° chlorometric) treatment. For each experimental condition, a batch is put to germinate in an incubator under continuous light, another in continuous darkness, while a third consignment had undergone an alternating cycle of 16h light / 8h dark. The experiments were conducted at 30° ± 1°C. The number of germinated seeds was also recorded every 24 hours. Daily counts were then expressed in germination percentage.

For tests performed under continuous light or alternating light-dark, the light source consists of fluorescent tubes (white type Industry, Phillips brand). The counting of germinated seeds placed in continuous darkness was achieved with actinic green light.

G. Culture media and in vitro Germination

The culture medium used is sterilized sea sand previously washed with tap water for 24 h and then with distilled water for 24 h as well. Sterilization of the substrate was performed by autoclaving at 120°C for 1 hour. In each jar, containing 3 cm$^2$ of sterilized sand, 10 seeds were sown aseptically. The jars are then placed in an incubator where the temperature was set at 30 ± 1°C for the mechanical and chemical scarification and the study of the influence of light. The number of germinated seeds was logged every 24 hours for 30 days. Daily counts were translated into germination percentages. The cumulative rates of germination were calculated.

The breakthrough of the radicle from the seed coat was used as the criterion for germination. The seeds were scored daily for germination. Therefore, parameters of germination, listed below, were chosen as defined by Evenari [24], Timson [25] and Côme [26]:
- the lag time, that is the time taken from sowing to the first appearance of the radicle;

<table>
<thead>
<tr>
<th>Scale</th>
<th>Description</th>
<th>Viability</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Uniform red color of the embryo and radicle</td>
<td>Very high probability of germination</td>
</tr>
<tr>
<td>2</td>
<td>Pale pink color of the embryo and radicle</td>
<td>High probability of germination</td>
</tr>
<tr>
<td>3</td>
<td>Half of the cotyledons unstained</td>
<td>Low probability of germination</td>
</tr>
<tr>
<td>4</td>
<td>Radicle unstained or damaged</td>
<td>No germination</td>
</tr>
<tr>
<td>5</td>
<td>No color</td>
<td>No germination</td>
</tr>
</tbody>
</table>

Table 1. Color scale of the different parts of the seed (Moore, 1985)
- The germination time, which is the time between the first and last germination during the observation period;  
- The mean percentage of seed germination is calculated using the Timson formula [25]:
  \[ G(\%) = \left(\frac{a}{h}\right) \times 100, \]
  where \( a \) being the number of germinated seeds for a given time, and \( h \) the total number of seeds sown.

The percentage of the cumulative number of germinated seeds was determined daily. Scatter plots are established and express the rate of germination depending on the number of days after sowing. In general, the variation observed is logarithmic of the form:
  \[ T = b \ln(N) - b_0, \]
  where \( T \) is the germination rate and \( N \) the number of days after sowing. This model is fitted to the data of germination rate and number of days after sowing to determine the parameters \( b_0 \) and \( b \). In this equation, the parameter “\( b_0 \)” is related to the initial germination rate (the day from which germination was observed after sowing) and “\( b \)” gives an estimate of the germination speed.

**H. Statistical Analysis**

The data was subjected to the analysis of variance. A one-criterion variance analysis was then performed on the percentage of germination and the \( b \) parameter to compare the effect of different treatments on the germination speed of the different species. The variation of “\( b \)” being significant depending on the treatment and species, a structuration of the average values was performed using the Student, Newman and Keuls test.

**III. RESULTS**

**A. Viability Test**

The test with 1% tetrazolium chloride was 100% positive for the seeds of *Annona squamosa* and *A. muricata*. All batches of 20 treated seeds belonged to group 1 of the Moore scale (Figures 1&2). There is a uniform purplish red color of the embryo and the radicle. They thus seem viable and constitute seeds with very high probability of germination. For the control batches, no seed germination was recorded. Therefore, these seeds were able to germinate when they were subjected to favorable conditions.

For *A. senegalensis*, 15% of seeds belong to group 1 and 60% of them to group 2 (Figure 3). These seeds represent 75% of our sample; therefore, they have a high probability of germination. 5% of the seeds tested have half of their cotyledon unstained; these seeds belong to group 3. Among seed batches of *A. senegalensis*, 20% of them have an unstained radicle. They belong to group 4 according to the scale of Moore and therefore cannot germinate.

**B. Influence of mechanical scarification**

The results shown in Table 2a reveal a significant effect of mechanical scarification on disinfection and germination of seeds. Indeed, the entire seed husking changes significantly the average of both the infection and germination rates. This reduces considerably in comparison to those of the control groups (whole seeds in coats, not disinfected) and batches of whole seeds in coats and disinfected.

A significant decrease of seed infection from 19% to 6% (F = 1111.857, \( p = 0.000 \)) for *A. squamosa*, from 21% to 3% (\( F = 1597.400, p = 0.000 \)) for *A. muricata*, and 22% to 2% (\( F = 88.889, p = 0.000 \)) for *A. senegalensis* is recorded. The mean percentage of germination increased from 29% to 73% (\( F = 82.383, p = 0.000 \)) for *A. squamosa*, from 21% to 60% (\( F = 214.333, p = 0.000 \)) for *A. muricata* and 16% to 60% (\( F = 430.222, p = 0.000 \)) for *A. senegalensis*. Comparison of “\( b \)” value between treatments applied to *Annona* species revealed also that ASET1 is equivalent to AMT1 but is slightly different from AST1 (Table 2b). Comparing T2 treatment for the three species shows that the mean value of “\( b \)” is similar. In contrast, there is a significant difference between *Annona* species with treatment T3. The highest speed is registered for AST3, followed respectively by ASET3 and AMT3 (Table 2c). The germination percentage of seeds is too low in the absence of mechanical pretreatment. Indeed, in these seed batches, the germination rate is 25% for *A. squamosa*, 13% for *A. muricata* and 6% for *A. senegalensis*, respectively. The time lag period is also longer: it lasts 8 days for control seeds of *A. squamosa* (Fig. 4) and 10 days for those of *A. muricata* (Fig. 5), and *A. senegalensis* (Fig. 6). However, for batches of husked and disinfected seeds, germination begins in the third day and reaches a maximum rate of 73% for *A. squamosa* and 60% for *A. senegalensis*. Germination starts on the 5th day for *A. muricata* (Fig. 5) and reaches an optimal rate at day 15 (60%). The maximum percentage of germination for all control batches did not exceed 20% for all species and the germination process is a lot longer than for those of treated seeds.

**C. Influence of chemical scarification**

Pretreatment with the absolute sulfuric acid has a very beneficial effect, both on the disinfection and germination seeds of the three species. The longer the application time of the pretreatment, the higher the proportional increase of the rate of seed germination and the lower the rate of infection, regardless of the species. Indeed, in *A. squamosa* (Table 3a), the germination rate (\( F = 198.764, p = 0.000 \)) equivalent to 9% for the control batch of seeds, reaches 70% when seeds were scarified with absolute sulfuric acid for 60 min. This value is not significantly different from the values obtained at T55 min (67%) and T50 min (69%) duration of pretreatment. Regarding the average rate of infection (\( F = 211.578, p = 0.000 \)), it is equivalent to 100% for the control batches but it decreases with the scarification processing time. The effect of seed treatment with sulfuric acid begins to be significant at T20 min with an infection rate of 78%. With 55 min pretreatment, the infection rate...
is only 3%, an average that is very close to the one obtained at T60 min (2%). The optimal soaking duration for the A. squamosa seeds is 60 min with a 70% germination percentage, recorded on the seventh day after sowing. The latency period prior to germination is only 3 days (Fig. 7).

**TABLE 2a.** Infection and germination rates of disinfected or not and shelled or not (control groups) of Annonaceae seeds after 30 days of culture on sterile sand at 30 °C.

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Treatments</td>
<td>Infection (%)</td>
<td>Germination (%)</td>
<td>Infection (%)</td>
</tr>
<tr>
<td>Control seeds</td>
<td>100 c</td>
<td>25 a</td>
<td>100 c</td>
</tr>
<tr>
<td>Whole seeds</td>
<td>19 b</td>
<td>29 a</td>
<td>21 b</td>
</tr>
<tr>
<td>Shelled seeds</td>
<td>6 a</td>
<td>73 b</td>
<td>3 a</td>
</tr>
</tbody>
</table>

In column, for the same species and for the same parameter, values followed by the same letter are not significantly different according to the Newman-Keuls test (P ≤ 0.05)
TABLE 2b. "b" Parameter expressing the germination speed following different disinfection and mechanical scarification treatments of Annona seeds.

<table>
<thead>
<tr>
<th>Species</th>
<th>Treatments</th>
<th>Mean values of b</th>
<th>CV (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A. muricata</td>
<td>T1</td>
<td>3.25a</td>
<td>51.53</td>
</tr>
<tr>
<td>A. muricata</td>
<td>T2</td>
<td>5.20a</td>
<td>52.36</td>
</tr>
<tr>
<td>A. muricata</td>
<td>T3</td>
<td>15.34b</td>
<td>50.44</td>
</tr>
<tr>
<td>A. squamosa</td>
<td>T1</td>
<td>4.97a</td>
<td>67.44</td>
</tr>
<tr>
<td>A. squamosa</td>
<td>T2</td>
<td>7.47b</td>
<td>49.02</td>
</tr>
<tr>
<td>A. squamosa</td>
<td>T3</td>
<td>33.12c</td>
<td>18.45</td>
</tr>
<tr>
<td>A. senegalensis</td>
<td>T1</td>
<td>2.08a</td>
<td>30.28</td>
</tr>
<tr>
<td>A. senegalensis</td>
<td>T2</td>
<td>4.965bc</td>
<td></td>
</tr>
<tr>
<td>A. senegalensis</td>
<td>T3</td>
<td>18.066e</td>
<td></td>
</tr>
</tbody>
</table>

T1: none disinfected and unshelled seeds; T2: unshelled and disinfected seeds; T3: shelled and disinfected seeds; CV: coefficient of variation.

TABLE 2c. Comparison of "b" value between disinfection and mechanical scarification treatments applied to Annona species.

<table>
<thead>
<tr>
<th>Combination of treatments</th>
<th>Mean values of b</th>
</tr>
</thead>
<tbody>
<tr>
<td>ASET1</td>
<td>2.082a</td>
</tr>
<tr>
<td>AMT1</td>
<td>3.245ab</td>
</tr>
<tr>
<td>AST1</td>
<td>4.965bc</td>
</tr>
<tr>
<td>ASET2</td>
<td>5.052bc</td>
</tr>
<tr>
<td>AMT2</td>
<td>5.196bc</td>
</tr>
<tr>
<td>AST2</td>
<td>7.465c</td>
</tr>
<tr>
<td>AMT3</td>
<td>15.342d</td>
</tr>
<tr>
<td>ASET3</td>
<td>18.066e</td>
</tr>
<tr>
<td>AST3</td>
<td>33.124f</td>
</tr>
</tbody>
</table>

ASE: Annona senegalensis; AM: Annona muricata; AS: Annona squamosa; T: treatment; T1: none disinfected and unshelled seeds; T2: unshelled and disinfected seeds; T3: shelled and disinfected seeds.

Figure 4. Evolution of cumulative germination percentages Annona squamosa L. seeds, disinfected or not and mechanically scarified (shelled) or not, during 30 days of culture at 30 °C.

Figure 5. Evolution of cumulative germination percentages of Annona muricata L. seeds, disinfected or not and mechanically scarified (shelled) or not, during 30 days of culture at 30 °C.
Figure 6. Evolution of cumulative germination percentages of *Annona senegalensis* Pers. Seeds, disinfected or not and, mechanically scarified (shelled) or not, during 30 days of culture at 30 °C.

TABLE 3a. Infection and germination rates of scarified seeds or not with H$_2$SO$_4$ (95%) after 30 days of culture on sterile sand at 30 °C.

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Treatments (min)</td>
<td>Infection (%)</td>
<td>Germination (%)</td>
<td>Infection (%)</td>
</tr>
<tr>
<td>T0</td>
<td>100 h</td>
<td>9 a</td>
<td>100 g</td>
</tr>
<tr>
<td>T5</td>
<td>94 h</td>
<td>11 a</td>
<td>93 g</td>
</tr>
<tr>
<td>T10</td>
<td>95 h</td>
<td>11 a</td>
<td>95 g</td>
</tr>
<tr>
<td>T15</td>
<td>87 g</td>
<td>13 a</td>
<td>93 g</td>
</tr>
<tr>
<td>T20</td>
<td>78 f</td>
<td>15 a</td>
<td>76 f</td>
</tr>
<tr>
<td>T25</td>
<td>78 f</td>
<td>23 b</td>
<td>60 c</td>
</tr>
<tr>
<td>T30</td>
<td>69 e</td>
<td>25 b</td>
<td>52 d</td>
</tr>
<tr>
<td>T35</td>
<td>52 d</td>
<td>26 b</td>
<td>33 c</td>
</tr>
<tr>
<td>T40</td>
<td>38 c</td>
<td>48 c</td>
<td>29 c</td>
</tr>
<tr>
<td>T45</td>
<td>18 b</td>
<td>52 c</td>
<td>16 b</td>
</tr>
<tr>
<td>T50</td>
<td>14 b</td>
<td>69 d</td>
<td>6 a</td>
</tr>
<tr>
<td>T55</td>
<td>3 a</td>
<td>67 d</td>
<td>1 a</td>
</tr>
<tr>
<td>T60</td>
<td>2 a</td>
<td>70 d</td>
<td>0 a</td>
</tr>
</tbody>
</table>
With A. muricata and A. senegalensis seeds, the same significant effects of sulfuric acid on germination and infection are noted (Table 3a, Fig. 8 and 9). The germination rate varied in proportion to the seeds soaking duration in sulfuric acid. For seeds of A. muricata (Table 3a), a significant decrease in the average rate of infection (F = 407.660, p = 0.000), which is 6% at T50 and 1% at T55 min, is concomitantly accompanied by a significant increase in germination rate (F = 160.552, p = 0.000) with treatment time. No seed among those treated with concentrated H_2SO_4 for a period of 60 min was infected. Consecutively, 65% of them germinated (Fig. 8). The same proportion of germinated seeds is registered with a processing time of 55 min. With A. senegalensis seed batches (Table 3a), the lowest percentage of infected seeds is recorded at T55 min (1%) and T60 min (2%). The best germination percentage is noticed with 59% at T50 min. This value is not significantly different from those obtained at T55 min (57%) and at T60 min with 58%. So, for this species, we found a double beneficial effect of pretreatment with absolute sulfuric acid in both infection (F = 219.128, p = 0.000) and germination (F = 98.962, p = 0.000). Soaking period with absolute sulphuric acid exerted a significant positive effect on seed germination particularly after 55 and 60 min. Globally, it was observed that seed germination increased with increasing treatment time for the three species. These results are confirmed in Table 3b, where pretreatment times of 55 and 60 min, revealed that the speed of germination is significantly highest whatever the species.

D. Influence of Temperature

The best germination percentage (Figure 10) is obtained at a temperature of 30 °C for seeds of A. squamosa (74%) and A. muricata (61%). In A. squamosa, this rate is higher than that obtained at 27 °C, at which temperature it was 65% (F = 130.236, p = 0.018). This rate decreases to 33% (p = 0.000) as the temperature rises to 38 °C with and decreases to 27% (p = 0.000) at 23 °C. It turns out that the results obtained at 23 °C and 38 °C are not significantly different (p = 0.103). As the temperature is raised to 42 °C, the germination rate drops from 33% to 16%, (p = 0.000). The same was done with the seeds of A. muricata. Indeed, for this species, the germination rate is 54% at 27 °C (F = 142.200, p = 0.012). The germination rate is equal to 33% at 23 °C and 37% at 38 °C, which is well below the value of 61% (p = 0.000) obtained at 30 °C. For A. senegalensis, the best germination percentage is obtained at 27 °C with 59% although this rate is not significantly different from that obtained at 30 °C which is 57% (F = 75.223, p = 0.604). In this species, the amount of seeds that germinated decreases considerably to 25% (F = 75.223, p = 0.000) if the incubation temperature is 33 °C and to 24% (F = 75.223, p = 0.000) at 42 °C. At 38 °C, 50% of seeds germinated in this species, which is important but is significantly less than the germination rate obtained at 27 °C (F = 75.223, p = 0.027). At 17 °C, no seed has germinated for each of the three species.

E. Effect of the illumination

The results are shown in Figure 11. The average germination percentage in Annona senegalensis and A. muricata is 58% and 63%, respectively, decreases to 44% (F = 26.133, p = 0.001) and 32% (F = 41.783, p = 0.000) in the light. The seeds of these species germinated better in light than in darkness. In contrast, the A. squamosa seeds germinated significantly better in the dark with an average percentage of 69%. The seed lots subjected to continuous illumination have a germination rate of 46% (F = 75.571, p = 0.000). However, a comparison with the germination of seeds that have been peeled, processed in the same conditions (mechanical scarification total) and put in germination at the same temperature but subjected to a photoperiod of 16 daytime hours and 8 nighttime hours shows there is no significant difference between the germination rate of the past with those seeds subjected to total darkness in A. squamosa and continuous light in A. senegalensis and A. muricata. With the photoperiod, germination rates are recorded at 73% for A. squamosa and 60% for A. muricata and A. senegalensis.

![Figure 7. Evolution of germination rates, depending on the soaking duration, of the Annona squamosa L. scarified seeds or not with H_2SO_4 (95%), after 30 days of culturing at 30 °C.](http://www.ijsat.com)

![Figure 8. Evolution of germination rates, depending on the soaking duration of the A. muricata L. scarified seeds or not with H_2SO_4 (95%) after 30 days of culturing at 30 °C.](http://www.ijsat.com)
Figure 9. Evolution of germination rates, depending on the soaking duration, of the *A. muricata* L. scarified seeds or not with H$_2$SO$_4$ (95%), after 30 days of culturing at 30 °C.

**TABLE 3b.** "b" Parameter expressing the germination speed following different pre-treatment time with concentrated sulfuric acid (95%) of *Annona* seeds.

<table>
<thead>
<tr>
<th>Treatments (min)</th>
<th>Mean values of &quot;b&quot;</th>
<th>CV (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td><em>A. muricata</em></td>
<td><em>A. squamosa</em></td>
</tr>
<tr>
<td>b0</td>
<td>1.30a</td>
<td>2.64a</td>
</tr>
<tr>
<td>b5</td>
<td>1.75a</td>
<td>2.94a</td>
</tr>
<tr>
<td>b10</td>
<td>2.64ab</td>
<td>2.97a</td>
</tr>
<tr>
<td>b15</td>
<td>4.42bc</td>
<td>4.42a</td>
</tr>
<tr>
<td>b20</td>
<td>4.75c</td>
<td>4.75a</td>
</tr>
<tr>
<td>b25</td>
<td>7.59d</td>
<td>8.14b</td>
</tr>
<tr>
<td>b30</td>
<td>8.14d</td>
<td>8.59b</td>
</tr>
<tr>
<td>b35</td>
<td>10.63e</td>
<td>8.84b</td>
</tr>
<tr>
<td>b40</td>
<td>11.10e</td>
<td>13.59c</td>
</tr>
<tr>
<td>b45</td>
<td>13.03f</td>
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</tr>
<tr>
<td>b50</td>
<td>19.05g</td>
<td>18.52d</td>
</tr>
<tr>
<td>b55</td>
<td>20.59g</td>
<td>21.03e</td>
</tr>
<tr>
<td>b60</td>
<td>23.75h</td>
<td>24.13f</td>
</tr>
</tbody>
</table>

Figure 10. Effects of different thermal levels on germination rates of *Annonaceae* seeds.
IV. DISCUSSION

The results obtained with the *A. senegalensis* seeds confirmed those obtained during the viability test. These results were consistent with those obtained on *Maerua crassifolia* seeds [27], a sahelian woody forage, in which there was a decrease of seed viability over storage time. However, for two other species of *Annonaceae*, the germination rates were generally much lower than the percentages usually recorded in these species, which can reach 90 to 95% [28]; [29]. This difference in the percentages of viable seeds revealed by the viability test and germination rates could be explained by a progressive loss of seeds germination under the influence of age and the shelf life between harvest and implementation germination dates. The viability test was carried out about a week after harvest. According to some authors, the loss of viability would begin in some cases, for the *Annona* seeds, with the fruit picking in the fields. Setting germination must be made as soon as possible after harvest of ripe fruits [30]; [31]. The seeds shelf life for *A. squamosa* is 40 to 50 days while this period is 30 to 40 days for *A. muricata* [28]. This is a characteristic of recalcitrant seeds.

For many researchers, the irregularity of the seeds germination of *Annona* is due to different levels and types of dormancy [32]; [33]; [34]; [35]; [31]. This could explain the difference between the rates of viable seeds and the effective germination rates achieved even if this hypothesis is refuted by Sanewski [36]. In some species of *Annona* such as *A. muricata*, *A. squamosa* and *A. reticulata*, even in the absence of dormancy, seeds can take up to three months to reach the maximum germination [37]. So, beyond the presence of the integument, other factors, such as embryo dormancy, duration and mode of preservation may be the cause of the absence or the irregularity of seed germination process of these three species.

Treatment with sodium hypochlorite had a beneficial effect on the reduction of infections for the seeds of the three species. Thirty min treatment of whole seeds in sodium hypochlorite led to infection rates of 19%, 21% and 22% in *Annona squamosa*, *A. muricata* and *A. senegalensis*, respectively. These results confirmed the one obtained with the seeds of *Moringa oleifera* Lam. [38]. Disinfection with bleach was also used by several authors working on seed timber such as *Eucalyptus camaldulensis* [39]; *Prosopis chilensis* and *P. juliflora* [40]; *Acacia albida* [41] and provided unambiguous disinfection rate. Treatment with bleach used alone may be ineffective as shown with seeds of cowpea [42] or *Oxytenanthera abyssinica* [43]. Full peeling of the seeds and an 8-minute treatment with bleach allowed having average rates of infection of 6%, 3% and 2%, respectively, for *A. squamosa*, *A. muricata* and *A. senegalensis*.

These results also demonstrated that mechanical pretreatment of *Annonaceae* seeds significantly improved the germination capacity and speed, regardless of the species. Mechanical scarification as well as chemical treatment of seeds with concentrated sulfuric acid 95% increased significantly the speed and the germination rate of *Annonaceae* seeds, with respect to the entire seeds. These results attested to the existence of a seed coat inhibition in these species.
The main barrier to the Annonaceae seed germination would be associated with seedcoat dormancy. Indeed, all pre-treatments applied to the seeds led to the weakening of the integument and facilitated access to water and oxygen to the embryo on the one hand, and allowed elongation of the radicle on the other hand [16].

Mechanical scarification by a complete peeling of the seed-coat reduced significantly both the disinfecting treatment duration and the infection percentages. These results could be explained by the fact that the seeds of the three Annonaceae species are contaminated in depth. Indeed in these species, the seed has a small opening at its upper end. The long pretreatment duration with bleach seemed to disinfect only the seed surface but its action may be limited for deeper or endogenous contamination [13].

When the seeds have been mechanically scarified, 73% of A. squamosa seeds had germinated. With respect to the A. senegalensis and A. muricata seeds, the percentage of germinated seeds was 60%. In A. senegalensis species, scarification promotes the seed germination [44]. According to Hayat [37], it is the same for the A. squamosa seeds, except when they have dormant embryo. Total or partial mechanical scarification improves the germination percentage in many species with hard seeds [45]. So, these results indicated the existence of a seedcoat dormancy in Annonaceae.

The germination rate of unscarified seeds was not very low despite the presence of the integument. This could be explained by the presence of the micropylar opening at one end of the seeds. This one would facilitate access to a certain amount of water and oxygen to the embryo. Thus, the inhibitory effect of the seed coat was mitigated by the presence of this natural micropylar opening unlike seeds of Parkia biglobosa which absolutely need to be scarified properly to germinate [46].

The results obtained, on the behavior of seed germination of Annonaceae (Table 3a), showed that the germination of intact seeds (T0 min: 9%, 2%, 1%) was lower than those of seeds scarified with sulfuric acid, regardless of the pretreatment duration (T5 min: 11%, 4%). Thus, concentrated sulfuric acid acted both as a powerful scarifying chemical and as a disinfectant. This is corroborated by the significant differences in germination rates notified for different immersion time in sulfuric acid and the gradual increase in germination rate along duration of treatment applied. Indeed, the immersion of seeds in concentrated sulfuric acid is generally very effective in breaking dormancy intregumentary seeds of woody species [47]. Immersion for 60 min of seeds in sulfuric acid allowed having germination rate of 70% and 65% and infection rates of 2% and 0% respectively in A. s. The temperature is very high (greater than or equal to 45 °C), it is possible that the amount of oxygen that reaches the embryo is not sufficient to allow germination. However, the ability of seeds to germinate is retained up to 27 °C. These results are similar to those noticed with A. cherimola seeds germinating at 25 °C. This temperature is optimal for an ideal seed germination [52]. Optimal temperatures for germination of all the three species were between 27 and 30 °C. However, the ability of seeds to germinate is retained up to 42 °C although the germination rate decreased significantly down to 22% for A. muricata and A. senegalensis and to 18% for A. squamosa. Hence, a wider range of optimum temperature for seed germination is observed. In the sahelian region, the temperature does not constitute a major limiting factor for seed germination of Annonaceae as the ambient temperatures at the time of rising are between 25 and 42 °C [53]. According to Tybirk [47], high heat contributes in softening the seed coats and ensures a better permeability of the skin with water and air.

Indeed, the embryo needs more oxygen when the germination temperature rises [54]; [23]. In addition, high temperatures can kill the embryo and/or denature certain seeds, the germination rate was 59% for a treatment time of 50 min while the infection rate was 1% for a treatment time of 55 min. The sulfuric acid acts by gradual corrosion of the integument resulting in an increase of its permeability to oxygen and water, thus promoting imbibition of the embryo and the normal process of germination. Concentrated sulfuric acid has been used successfully to accelerate the seed germination of several species [48]. However, the duration of the seed imbibition varies among species and with the seed coat hardness. Parkia biglobosa seeds, treated 5 to 6 hours, germinate to 92% after 2 days [46] while a 10-minute pretreatment was enough for a 95 to 97% germination in Acacia flava syn. erhenbergiana [49].

Ultimately, the mechanical as well as the chemical scarifications have increased significantly the seed germination rate of the three species. For the chemical scarification with sulfuric acid, 95% germination rates were obtained according to the duration of the immersion of the seeds in acid. The best germination was obtained after a 60-minute immersion for A. muricata and A. squamosa and a 50-minute pre-treatment for A. senegalensis. The increase in seeds germination was proportional to the soaking time for periods of 5 to 60 min in A. squamosa and A. muricata and for periods of 5 to 50 min in the case of A. senegalensis seeds.
enzymes that are essential to the basic germination metabolism.

The results for the seeds germination subjected to continuous lighting, darkness or alternating light / dark revealed a significant difference between germination rates for all the species. This indicates that the light had a great influence on seed germination. The A. squamosa seeds germinated better in darkness than in light in contrast to A. senegalensis and A. muricata seeds for which the seeds germinated better in light. Chin & Mold Lassim [55] obtained similar results for A. muricata seeds. The seeds of many species such as strawberry need continuous light to properly germinate [56]; [57]. Most plant species have seeds that reveal this type of photosensitivity according to Côme et al. [50]. However, for the three species, a photoperiod of 16 daytime hours/8 nighttime hours was just as effective as total darkness for A. squamosa seeds or exposure to continuous white light for A. senegalensis and A. muricata seeds. Many factors can change the sensitivity to light of seeds. In this case, it is the spectral quality of the light provided (incandescent or fluorescent), its spectral composition, the duration of illumination as well as the shelf life of dry seeds [50]. For the strawberry, for example, the achenes germinate better in red light [58]. The appreciation of the seed photosensitivity type suggests that A. squamosa seeds had a negative photosensitivity while those of A. senegalensis and A. muricata would rather have a positive photosensitivity. The darkness had no effect on the viability of A. squamosa seeds but positively affected seed germination. However, further studies are needed to investigate the effects of the seeds storage conditions, the influence of the wavelength and the light-temperature interactions on the germination.

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