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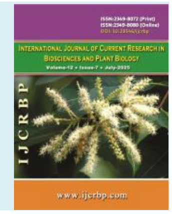

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Provenance, Storage Condition and Diverse Pre-Treatment Effects on Seed Germination Parameters in *Afzelia africana* Sm. ex Pers. (Caesalpiniaceae) – A Threatened Tree Species Collected from the Lama Forest in the Southern of Benin Republic

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Abstract

Assessing and monitoring forest species are essential for implementing effective conservation strategies that support biodiversity. Since Beninese *Afzelia* populations are threatened by regression, we analyzed as a preliminary study the germination behaviour of the species to plan subsequent programs for preservation and rehabilitation. In this context and to optimize *Afzelia africana* seed germination as well as to overcome and remove the obstacle of tegumentary inhibition, we have in the first preliminary attempt tested several chemical pretreatments (tap and distilled water, sodium hypochlorite 5% solution, potassium nitrate solutions KNO_3 2 and 5 gL^{-1}) for different seed ages and storage temperatures in three provenances. Thus, we could determine the optimal germination conditions that can be used afterward for evaluating the germination capacity and speed of the species. Germination capacity, speed and kinetics were analyzed, and the data was submitted to ANOVA. Kruskal-Wallis nonparametric tests were performed to identify and classify the seed ages, storage conditions, treatments and accessions. The solution of KNO_3 5 gL^{-1} allowed the best germination capacity (75-100%) in all genotypes during the first six months-ages of the seeds. The control (tap water) permitted the lowest seed germination capacity (50-65%). The germination capacity diminished to around 50% by the twelfth-months old seeds. Genotype 'Koto' delivered better results than Massi NC and Tchétou. The germination speed was generally weak (0.050 J^{-1}). The same trends for the seed germination speed as for the germination capacity were observed. Both germination capacity and speed decreased with age. Seeds stored at low temperatures (8°C) germinated better than those stored at high temperatures (37°C). Interactions: Accession × Treatment, Seed age × Treatment as well as Seed storage conditions × Treatment were very significant for all estimated parameters, indicating a relatively important contribution of the seed ages, storage temperatures, treatments and provenances to the phenotypic expression.

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Introduction

Plants have been used for centuries for different purposes such as for consumption as food and spices, for healing medicinal purposes, for purification of water, to ward off pest and disease-carrying insects and for ornamental purposes. There is a wide distribution of biologically active constituents in nature, particularly in plants used as feedstuff and human nutrition (Adeniji et al., 2019; Obayomi et al., 2019, Shimelis et al., 2013). Knowledge of these compounds has given rise to several investigations on their possible physiological implications in various biological systems (Shimelis et al., 2013, Becker, 2011) as some plants are toxic and yet may contain medicinal properties that may be utilized for therapeutic purposes (Chukwuma et al., 2019; Sofowora et al., 2013). Anti-nutritional factors are compounds that reduces the nutrient utilization and food intake of plant or plant products used as human food or animal feed. They are otherwise known as secondary metabolites in plants. A large segment of the human population and animals in developing countries suffer from protein malnutrition (Obayomi et al., 2019; Semba, 2016). Legumes such as soybean contain high concentrations of proteins, carbohydrates and dietary fibers and make an important contribution to the human diet in many countries (Chukwuma et al., 2019; Bashir et al., 2016).

Biological diversity is the set of all shapes of a living organism and is usually subdivided in three levels: genetic, specific and ecosystemic (Essou et al., 2017; Aoudjit Hayet, 2006). Beninese biodiversity is labelled by great species richness, but also a big variability of landscapes, ecosystems and habitats (Sinsin and Owolabi, 2001). Desertification, deforestation and genetic erosion are the most important factors responsible for the loss of biodiversity as well as the deterioration of soils in the savannah regions. The resulting negative effects can be minimized thanks to reforestation programs. However, to reach that objective, collection and seed germination issues must be taken into consideration since any seed germination process is often mainly very difficult for numerous useful species due to the physiological phenomenon of the levee of dormancy. Germination refers merely to a set of changes by which an embryo pushes in a young plant (Essou et al., 2017; Bello and Gaba, 2015; Raven et al., 2005). Regardless the genotype as well as the ecological conditions of the natural habitat of plants, three other conditions remain necessary for germination: humidity, temperature and atmospheric oxygen (Essou et al., 2017; Baskin, 2001). The lack of biological and ecological knowledge of most native tree species constitutes a limitation and a major handicap for the management and conservation of tropical forest genetic resources (Deboux, 1998). Given current trends in deforestation and degradation of tropical woody formations (Food and Agriculture Organization of the United Nations [FAO], 1992; Gilarowski, 2002), it is interesting to assess the efforts made to create forest plantations for either industrial timber production, i.e., "industrial plantations," or fuelwood and timber for service or protection, i.e., "non-industrial plantations" (Edondoto et al., 2020).

The West African rainforest areas are endowed with wonderful vegetation unequaled by any other continent of the world. Most of the vegetative species are either underutilized or are not utilized at all. Some of these plant species are used as vegetables, wooden species and as browse to livestock species and the seeds or nuts are attributed only as less value to human utilization. These vast species have added a wide range of animal feed resources for which the West African rainforest areas are blessed with in the form of 'grains', oil seeds and agro-industrial products which could be used in the formulation of good quality livestock feeds. Some of these plant products (seeds, leaves and barks) are allowed to waste and are underutilized or unharnessed (Sobola, 2023; Chukwuma et al., 2019). The efforts to determine the biosafety status of some of these plant species have attracted the attention and interest of Nutritionists and Biochemists on possible treatments such as, heat treatment (cooking, roasting and toasting), the use of enzymes and probiotics to inactivate some anti-nutritional substances to enhance their utilization (Chukwuma et al., 2019). Tropical ecosystems, particularly African forests and savannahs, play a significant socio-economic role for local communities by providing vital natural resources. However, these ecosystems are currently facing severe degradation due predominately to human activities including the uses of different plant parts (fire woods, medicinal provisions, feedstuff for livestock, utilization of woods in carpentry, etc.) (Calvin et al., 2025; Waya et al., 2022; Adeniji et al., 2019; Soulama et al., 2015; Mama et al., 2013). This degradation, which also affects associated protected areas, is occurring at an alarming rate, raising concerns among scientists and development stakeholders (Foutse, 2017; Avakoudjo et al., 2014). Protected areas are primarily established to conserve biodiversity and maintain ecosystem services (Konan et al., 2015). Nonetheless, the classification and protection levels of various protected areas in Africa remain inadequate to ensure their long-term viability (Alassane, 2018). The existence of these areas often leads to significant changes in resource access for nearby communities (Calvin et al., 2025).

Afzelia africana Sm. ex Pers. known as African mahogany or oak is a large deciduous tree. It is a leguminous tree that belongs to the family Caesalpiniaceae, and is found in humid and dry forests, especially in the forest-savannah borders or semi-deciduous forests (Adeniji et al., 2019; Obayomi et al., 2019; Ahouangonou and Bris, 1997). Several studies on *A. africana* showed that its seeds and leaves contain vital nutrients such as proteins, fats, and micro as well as macro minerals. Anti-nutrients such as haemagglutinins, oxalate, phytic acid, saponins, tannins and trypsin inhibitors have been found in the tree foliage (Chukwuma et al., 2019). Every part of the plant is of great importance (Obayomi et al., 2019). Wood from *Afzelia africana* is used in carpentry, canoe and house building, furniture making, flooring and heavy construction, wood carvings and other traditional uses (Obayomi et al., 2019). Seeds are milled into flour which serves as soup thickener (Bamigboye et al., 2024; Adeniji et al., 2019; Igwenyi and Akubugwo, 2010) or substitute for wheat flour in biscuits and doughnuts (Orwa et al., 2009). It is fodder: the leaves, fruits and seeds are browsed by wildlife, particularly before the regrowth of grasses in the early rainy season, also wild animals browse the arils, and antelopes eat the young shoots while flying foxes and bats eat the flowers (Ogbimi and Sakpere, 2021; Adeniji et al., 2019; Orwa et al., 2009). The seeds contain about 31% fat and may be a source of seed oil for domestic and industrial uses. *Afzelia africana* leaf is one of the non-conventional vegetables obtained from forests and supplements the conventional ones obtained from farms and home gardens. The seeds of the plant contain extractable oil (Ogbimi and Sakpere, 2021). The physicochemical properties of methyl ester derivatives of oils from *Afzelia africana* show their potentials in biodiesel production. The oil can release a high amount of heat during combustion and the cetane index shows that the oil can ignite easily in a combustion engine. The iodine and peroxide values show increased stability of the oil during storage and transportation (Igwenyi and Akubugwo, 2010). As a result of the usefulness of this plant, it has been over-exploited, owing to population explosion, urbanization and the resultant degradation of natural forests and the preference of farmers to produce conventional vegetables (Ogbimi and Sakpere, 2021; Adeniji et al., 2019).

Throughout the humid tropics, especially in Africa, numerous woody species have provided the indigenous people with food, medicine, construction wood and various other services (Olujobi et al., 2024; Adebooye et al., 2005). Currently, the majority of these products particularly medicinal plants are exploited extensively from the wild. Despite their numerous roles (provision of employment, income, food and rural health), their natural population has been diminishing both in size and gravity due to natural and human-induced factors (Odetola and Etumnu, 2013). Though the high value of these plants, their propagation has been inhibited. By several factors, the natural regeneration of those woody species is very limited, so those species are threatened to extinction. However, for plants to efficiently propagate, germination is a requirement. Effective and sustainable germination of seeds is usually affected by seed structures and environmental factors, a condition referred to as seed dormancy (Olujobi et al., 2024; Chahooki and Ali, 2010). Though seed dormancy is often considered an impeding factor, many plants use it as a survival mechanism that ensures that germination occurs only during favourable conditions. Many forms of seed dormancy have been identified with the degree of dormancy varying depending on the species, genome and type of dormancy. These include: physical, chemical, physiological, photo- or thermo-dormancy (Olujobi et al., 2024; Essou et al., 2017; Zoghi et al., 2011; Mwase and Mvula, 2011). Seed germination and early seedling growth phases are considered critical for raising a successful crop as they directly determine the crop stand density and the yield of the resultant crop.

It is a known fact that seed germination, seedling growth, and seedling survival percentage, are governed by internal and external factors and are species-specific (Hossain et al., 2005). Many trees have been identified as fast-growing and categorized as high biomass yielders, while many other trees have seeds that possess hard coats that are nearly impermeable to water; thus, they cannot germinate or have delayed germination under normal conditions (Olujobi et al., 2024).

Afzelia africana is propagated by seeds and vegetative techniques via budding. Natural regeneration is poor because seed predation by animals is usually high

(Ogbimi and Sakpere, 2021; Sobola, 2023; Adeniji et al., 2019; Amusa, 2011). *Afzelia africana* seeds are dormant and they become recalcitrant on storage (Orwa et al., 2009). The rate of seed germination in the wild is low and its seedlings rarely develop into saplings. Although treatment with Tetraoxosulphate (VI) acid to overcome dormancy in *Afzelia africana* seeds has been reported by Amusa (2011), no report is documented for pretreatment with sodium hydrochloride and potassium nitrate.

Therefore, the main objective of the current study is to analyze the factors that could lead to the better conservation of the seed genetic resources existing in *Afzelia africana* in Benin. The specific objectives of the study are to evaluate the effects of: (i) three provenances known as accessions or genotypes on germination parameters; (ii) different pre-germination treatments on seedling emergence and (iii) seed storage conditions (e.g. temperature of storage place) on long-term conservation of *Afzelia africana* genetic resources. A successful field emergence is vital for crop production from seeds and maximum yield is often very dependent on successful germination of seeds and early growth. *Afzelia africana* seeds are covered in hard coats which make them shallowly dormant. They germinate slowly and over a narrow range of conditions if planted without undergoing a pre-treatment method. Because of this, pre-treating *Afzelia africana* seeds would increase the rate of germination, which in return would maximize its yield.

Materials and Methods

Study area

The study was carried out in the Benin Republic covering 114763 km^2. It is located between the latitudes 6°15'N and 12° 25'N, the longitudes 0°40'E and 3°45'E, and is constituted of three main climatic zones: the Guinean, Guineo-Sudanese and Sudanese zones (MEPN, 2008).

The climate is of type subequatorial in the South, tropical humid of transition in the Middle part and tropical dry in the North of the country. The study was particularly undertaken in the Lama forest (latitudes 6^{o}55’ to 7^{o}N, longitudes 2^{o}04’-2^{o}12’E).

Plant materials

Afzelia africana seeds were harvested from their natural habitat in the Lama forest representing a band of natural vegetation across Benin Republic from Eastern to Western of the land in the Southern. The fresh seed harvesting was achieved from November to December. A water floatation test was conducted on the seed of *A. africana* to authenticate the viability of the seed before the germination experiments. A similar method for viability test was used by Sobola (2023). Accessions or provenances (seeds) have been harvested in the localities of Koto, Massi (“Noyau Central”) and Tchétou. The seeds of Koto constitute accession Koto, whereas those of Massi and Tchétou represent accessions Massi NC and Tchétou, respectively. Seed samples have been collected in plastic bags, were divided into two sets and were stocked in conditioned chambers (8°C and ambient temperature 37°C) since their harvest.

Methodology

Experimental protocol

The germination test was carried out under a controlled environment in the Laboratory of Plant Sciences and Pharmacopeia at the University Abomey-Calavi, Benin. The experimentation lasted 12 months. Each month (30 days), 50 seeds from each provenance and storage condition were deposited on Canson filter papers moistened and/or previously dampened in a solution corresponding to a given treatment in Petri dishes. Pretreatments consisted of immersing the seeds in tap, distilled water, sodium hypochlorite (NaClO) 5% and potassium nitrate solutions (KNO_3) 2 gL^{-1} and 5 gL^{-1} during 1, 40, 24 and 24 hrs for the four later, respectively. The experimental design is a complete randomized block with three repetitions per accession in each treatment as well as each seed storage condition. Germinated seeds were counted every five days to determine the parameters characterizing the physiological process of germination. Seeds are considered to have germinated whenever the radicle emerges from the tegument. The estimated germination parameters were: initial (TG1), second, third, fourth, fifth and sixth dates numbering of germinated seeds (TG2, TG3, TG4, TG5 and TG6), germination rates, and germination speed (GS) inversely related to the period of germination (PG). The germination capacity (GC) corresponds to the final germination rate, either TG6. To analyze the kinetics of germination, the germination rates were averaged over the six first months. The different parameters are given below by the formulas:

$$GC\ (\%) = \frac{TNSG}{TNST} \times 100$$

$$GS\ (J^{-1}) = \frac{\sum n}{\sum n \times J_n}$$

Where,

GC (%) = germination capacity (percentage of seeds capable of germinating in the conditions of the experimentation until its end); GS (J^{-1}) = germination speed (characterizing the time taken by the seed to germination); TNSG = total number of seeds germinated; TNST= total number of seeds tested; n = number of seeds germinated in a given day Jn; Jn = number of days from the start of experimentation to a given day.

Statistical analysis

Statistical analysis of the results was performed and achieved by the software JMP version 7 (SAS Institute NC, 2007) and MINITAB 19 (2019). Germination parameters were subjected to one- and two-way analyses of variance at 5%. The Fisher-Snedecor test was applied to analyze the relative importance of the factors 'Seed age' (1 to 12 months), 'Seed storage condition' (8°C and 37°C), 'Accession (Provenance)' and 'Treatment' as well as of their interactions in the observed variability. Parameter means were compared between accessions and treatments by the test of Tukey-Kramer implemented in either JMP or MINITAB 19.

The interactions between the various provenances (accessions), seed ages, storage temperatures and the treatments were completed using the software MINITAB version 19. Kruskal-Wallis test implemented in MINITAB 19 was used for the comparison of the seed ages, seed storage conditions treatments and accessions studied *vis-à-vis* the germination parameters of interest. This test permitted to identify the best age after harvest, storage temperature, treatment as well as the best accession driving to the higher rates and speed of germination in the threatened species of concern in the current survey.

Results and Discussions

Kinetics of germination

Average germination rates vary significantly between seed ages, storage conditions, accessions and treatments applied including the control represented by the tap water (Fig. 1). For the control, the maximum mean of germination rate reached is equal or very close to 100.00, 75.00 and 80.00% for Koto, Massi NC and Tchétou, respectively (Fig. 1). The first accession to reach the maximum of germination with the tap water as pretreatment and in thirty (30) days is Massi NC (65%) followed by Koto (62%) and Tchétou (60%). With distilled water, NaClO 5% and KNO_3 solutions 2 and 5 gL^{-1}, the three provenances undergo some increase in the germination rate as well as its speed (Figs. 1-3). Indeed, the curves displaying the germination kinetics of the seeds treated in different solutions indicate values superior to the control.

Otherwise, according to the kinetics curves or histograms (Fig. 1), differences in the response to the different treatments are expressed. No significant difference was noted between the provenances. However, Tchétou seeds germinated lower and slower than Koto and Massi NC. The average germination rate remains moderate and varies little during the whole 15 days before showing an exponential acceleration in all accessions, even though results seem better in Koto than Massi NC and Tchétou (Fig. 1).

The highest germination rate (>95%) is recorded in Koto, followed by Tchétou (65-80%), while Massi NC (65-73% between the distilled water and the solution of KNO_3 5 gL^{-1}, respectively) showed a smaller trend.

KNO_3 5 gL^{-1} showed better germination rates followed by KNO_3 2 gL^{-1}, distilled water, sodium hypochlorite 5% and the control, respectively. Seeds stored at 8°C germinated better than those kept at ambient temperature (37°C). Seed age lowered the germination rates after 6 months whichever the storage condition was, and also independently of the treatment (Fig. 1).

Germination capacity

Results show that the main sources of variation of the germination capacity are the seed age, seed storage condition and the accession (Tables 1 and 2, Fig. 2 and 4). It is followed respectively by the effect of the treatment and the interactions Accession × Treatment, Seed age (SA) × Treatment and Seed storage condition (SSC) × Treatment. The seeds of Koto reacted better than the two other accessions. Compared to the three other treatments, KNO_3 solutions 5 gL^{-1} and 2 gL^{-1}

presented significant positive effects on the germination capacity (Figs. 2-4). Interactions Accession × Treatment showed the same trend in importance regardless of the accession considered (Table 2, Figs 3,4). Results from the statistical analysis presented in Tables 1 and 2 show that the germination rates of all studied accession seeds are affected significantly and positively across all other treatments apart from the control represented by the tap water. However, Massi NC presents a germination capacity (>90%) higher than Koto and Tchétou, in particular when their seeds are treated in the KNO_3 solutions 5 and 2gL^{-1} (Fig. 3). The main effect of genotype on GC is positively significative for Massi NC and Koto, and negative for Tchétou (Fig. 4).

Seeds stored at 8^{o}C germinated better than those conserved at ambient temperature (37^{o}C) (Fig. 5). Furthermore, after six months, seeds stored at 37^{o}C lost drastically their germination capability (Fig. 6). Interactions SSC × Treatment and SA × Treatment were also significant (Figs 5-8). The seed germination capacity diminished with age independently of the treatment (Fig. 6). The main effect of SSC resulted in a lower GC whenever the seeds were conserved at 37^{o}C than at 8^{o}C. The solutions KNO_3 5 and 2 gL^{-1} had significant positive effects on GC, whereas NaClO 5% and the control (tap water) acted negatively on GC (Fig. 7). Six months aged as well as older seeds germinated lower than seeds freshly harvested until 7 months (Fig. 8).

Instead of comparing mean values of the three populations analyzed using one-way or two-way ANOVA, the Kruskal-Wallis non-parametric test was applied to detect the best factor (seed age, seed storage condition, treatment and provenance) concerning the most common germination parameters: germination capacity and speed. According to this test, H’ indicates if the median values of the given factor are equal or not, and allows so their classifying.

The sample medians for the 12 seed ages were 78.00, 81.00, 75.00, 69.00, 78.00, 81.00, 75.00, 69.00, 54.00, 47.00, 37.00 and 40.00%, respectively. The z value for SA 11-months is -7.12, the smallest value of z. This size indicated that the mean rank for this treatment differs least from the mean rank for all observations. Moreover, the mean rank of this SA (50.9) was lower than the mean of the observation set (180.5), and the z value is negative (z = -7.12). The mean rank for the SA 2 and 6 was higher than in the whole experiment, with a positive z value (z = 4.10) (Table 3). The test statistics (H = 17.33) had a *p*-value of 0.000, when it is adjusted or not for ties, indicating that the null hypothesis can be rejected at levels higher than 0.000 in favour of the alternative hypothesis of the existence of at least one difference among the SA groups. SA 2 and 6 appeared the most significant to a good seed germination capacity in *Afzelia africana* collected in the Lama forest of Benin.

The sample medians observed for both seed storage conditions were 72.00 and 60.00%, respectively. The z value for SSC 2 is -4.16, the smallest value of z. This size indicated that the average rank for this SSC differs least from the mean rank for all observations. Moreover, the mean rank of this SA (157.7) was lower than the mean of the observation set (180.5), and the z value is negative (z = -4.16). The mean rank for the SSC 1 (203.5) was higher than in the whole experiment, with a positive z value (z = 4.16) (Table 3). The test statistics (H = 17.33 or 17.35) had a *p*-value of 0.000, when it is adjusted or not for ties, showing that the null hypothesis can be rejected at levels higher than 0.000 in favour of the alternative hypothesis of the existence of at least one difference among the Seed Storage condition (SSC) groups. The SSC 1 (8^{o}C) was the most significant to a relatively good seed germination capacity in *Afzelia africana* collected in the Lama forest of Benin Republic

The sample medians for the three accessions were 71.00, 68.00 and 62.00% for Koto, Massi NC and Tchétou, respectively as delivered by the Kruskal-Wallis test. The z value for Tchétou is -3.16, the smallest absolute value of z. This size shows that the mean rank for this accession is the one that differs least from the mean rank across all observations. Furthermore, the mean rank of this provenance (156.0) was lower compared to the average over the whole study (180.5), and the z value is negative (z = -3.16). Koto’s mean rank is higher, with a positive z value (z = 2.09) (Table 3). The test statistics (H = 10.35 or 10.36) had a *p*-value of 0.006 when it is adjusted or not, showing that the null hypothesis can be rejected at levels superior to 0.006 in favour of the alternative hypothesis of the existence of at least a difference among the groups of accessions. The germination capacity of Koto proved to be significantly the best whatever the treatment in *Afzelia africana*..

The sample medians for the five treatments were 50.00, 66.00, 60.00, 76.00 and 84% for the control, distilled water, sodium hypochlorite 5% solution, and the KNO_3 solutions 2 and 5 gL^{-1}, respectively. The z value for the control is -5.90, the smallest value of z. This size indicated that the mean rank for this treatment differs least from the mean rank for all observations. Moreover, the mean rank of this treatment (115.8) was lower than the mean of the observation set (180.5), and the z value is negative (z = -5.90). The mean rank for the treatment KNO_3 5 gL^{-1} is higher than in the whole experiment, with a positive z value (z = 6.26) (Table 3).

The test statistics (H = 77.5 or 77.6) had a *p*-value of 0.000, when it is adjusted for ties or not, indicating that the null hypothesis can be rejected at levels higher than 0.000 in favour of the alternative hypothesis of the existence of at least one difference among the treatment groups. The treatment KNO_3 5 gL^{-1} appeared the most significant to a good seed germination capacity in *Afzelia africana* collected in Benin.

Speed of germination (GS)

Tables 1 and 2 indicate the results of the analysis of variance for the germination speed in *Afzelia africana* seeds conserved under two different conditions, of diverse ages and submitted to the above-mentioned treatments. The same tendencies as for the germination capacity are revealed. However, the germination speed was low (0.050 J^{-1}). Furthermore, similar observations are made while referring to the effects of the different factors evolved, as well as about their interactions (Table 2, Figs 9-15).

For all accessions, the seed germination period was longer and smaller when the seeds were not treated with tap water. Statistical analysis indicated that the effect of the treatments presented in Fig. 9 resulted in a significant reduction of the germination period regardless of the seed age, storage condition and the genotype examined. However, Massi NC showed a better tendency than Tchétou which was greater than Koto (Figs. 9-15). The main effects of genotypes, SSC, SA and Treatments as well as their interactions on GS delivered the same trends as for GC. Nonetheless, 11-months seeds showed greater interactions ‘SA × Treatment’ on GS (Fig. 13). In general, younger seeds germinated slower than older ones (Fig. 15).

For the seed germination speed (GS), the sample medians for the 12 seed ages were 0.05094, 0.05000, 0.04941, 0.04947, 0.05094, 0.05000, 0.04941, 0.04947, 0.05039, 0.05187, 0.05328 and 0.05130 J^{-1}, respectively. The z value for SA 4- and 8-months is -3.29, the smallest value of z. This size indicated that the mean rank for this treatment differs least from the mean rank for all observations. Moreover, the mean rank of these SA (120.8) was lower than the mean of the observation set (180.5), and the z value is negative (z = -3.29). The average rank for the SA 11 was higher than in the whole experiment, with a positive z value (z = 7.22) (Table 3). The test statistics (H = 105.40) had a *p*-value of 0.000, when it is adjusted or not for ties, indicating that the null hypothesis can be rejected at levels higher than 0.000 in favour of the alternative hypothesis of the existence of at least one difference among the SA groups. The SA 11 appeared the most significant to a good seed germination speed in *Afzelia africana* collected in the Lama forest of Benin.

The sample medians observed for both seed storage conditions were 0.05026 and 0.05055 J^{-1}, respectively. The z value for SSC 2 is -0.65, the smallest value of z.

This size indicated that the average rank for this SSC differs least from the mean rank for all observations. Moreover, the mean rank of this SA (176.9) was lower than the mean of the observation set (180.5), and the z value is negative (z = -0.65). The mean rank for the SSC 1 (184.1) was higher than in the whole experiment, with a positive z value (z = 0.65) (Table 3). The test statistics (H = 0.42) had a *p*-value of 0.516, when it is adjusted or not for ties, showing that the null hypothesis can be rejected at levels higher than 0.516 in favour of the alternative hypothesis of the existence of at least one difference among the Seed Storage condition (SSC) groups. The SSC 1 (8°C) was the most significant to a good seed germination speed in *Afzelia africana* collected in the Lama forest of Benin Republic

The sample medians for the three accessions were 0.05049, 0.05087 and 0.05000 J^{-1} for Koto, Massi NC and Tchétou, respectively as delivered by the Kruskal-Wallis non-parametric test. The z value for Tchétou is -2.04, the smallest absolute value of z. This size shows that the mean rank for this accession is the one that differs least from the mean rank across all observations. Furthermore, the mean rank of this provenance (164.7) was lower compared to the average over the whole study (180.5), and the z value is negative (z = -2.04).

Table.1 One-way ANOVA (Seed age, Seed storage condition, Provenance or Treatment effect) for seed germination capacity (GC) and speed (GS) in *Afzelia africana* collected from Benin

Parameter	Source of variation	Degree of freedom	Sum of square	Mean square	F Ratio	Prob>F*
GC	Seed age effect	11	81570	7415	36.09	0.000
	Residuals	348	71520	206	-	-
	Total variance	359	153090	-	-	-
	Seed storage condition effect	1	7971	7971	19.66	0.000
	Residuals	358	145119	405	-	-
	Total variance	359	153090	-	-	-
	Provenance effect	2	4180	2090	5.01	0.007
	Residuals	357	148910	417	-	
	Total variance	359	153090	-	-	
	Treatment effect	4	32727	8182	24.13	0.000
	Residuals	355	120363	339	-	-
	Total variance	359	153090	-	-	-
GS	Seed age effect	11	0.0004836	0.0000440	14.26	0.000
	Residuals	348	0.0010733	0.0000031	-	-
	Total variance	359	0.0015569	-	-	-
	Seed storage condition effect	1	0.0000016	0.0000016	0.37	0.543
	Residuals	358	0.0015553	0.0000043	-	-
	Total variance	359	0.0015569	-	-	-
	Provenance effect	2	0.00000272	0.0000136	3.18	0.043
	Residuals	357	0.0015297	0.0000043	-	-
	Total variance	359	0.0015569	-	-	-
	Treatment effect	4	0.0002712	0.0000678	18.72	0.000
	Residuals	355	0.0012857	0.0000034	-	-
	Total variance	359	0.0015569	-	-	-

* (Prob < 0.05): Significant effect according to F-test at 5% threshold.

Table.2 Two-way ANOVA for seed germination capacity (GC) and speed (GS) in three provenances of *Afzelia africana* collected from Benin

Parameter	Source of variation	Degree of freedom	Sum of square	Mean square	F Ratio	Prob>F*
GC	Total variance	359	153090	-	-	-
	Seed age effect (1)	11	81570	7415.43	65.39	0.000
	Seed storage condition effect (2)	1	7971	7971.21	24.83	0.000
	Provenance effect (3)	2	4180	2090.14	6.21	0.002
	Treatment effect (4)	4	32727	8181.68	72.14	0.000
	Interaction (1 × 4)	44	4770	108.42	0.96	0.555
	Interaction (2 × 4)	4	32	7.91	0.02	0.999
	Interaction (3 × 4)	8	110	13.78	0.04	1.000
	Residuals	341	34023	113.41	-	-
GS	Total variance	359	0.0015569	-	-	-
	Seed age effect (1)	11	0.0004836	0.0000440	18.64	0.000
	Seed storage condition effect (2)	1	0.0000016	0.0000016	0.44	0.508
	Provenance effect (3)	2	0.0000272	0.0000136	3.82	0.023
	Treatment effect (4)	4	0.002712	0.0000678	28.74	0.000
	Interaction (1 × 4)	44	0.0000943	0.0000021	0.91	0.640
	Interaction (2 × 4)	4	0.0000004	0.0000021	0.03	0.998
	Interaction (3 × 4)	8	0.0000303	0.0000038	1.07	0.383
	Residuals	341	0.0007078	0.0000024	-	-

* (Prob < 0.05): Significant effect according to F-test at 5% threshold.

Table.3 Kruskall-Wallis non-parametric test of germination traits (GC and GS) comparisons in three *Afzelia africana* provenances of 1 to 12 months ages (SA) under 2 different seed storage temperatures (SSC) and 5 pretreatments

Parameter	Factor		N*	Median	Average rank	z
GC	SA (months)	1	30	78.00	249.8	3.81
		2	30	81.00	255.0	4.10
		3	30	75.00	217.5	2.04
		4	30	69.00	197.4	0.93
		5	30	78.00	249.8	3.81
		6	30	81.00	255.0	4.10
		7	30	75.00	217.5	2.04
		8	30	69.00	197.4	0.93
		9	30	54.00	126.4	-2.98
		10	30	47.00	92.2	-4.85
		11	30	37.00	57.3	-6.77
		12	30	40.00	50.9	-7.12
		Overall	360	-	180.5	-
	SSC	8°C	180	72.00	203.5	4.16
		37°C	180	60.00	157.7	-4.16
		Overall	360	-	180.5	-
	Provenance	Koto	120	71.00	196.7	2.09
		Massi NC	120	68.00	188.8	1.08
		Tchétou	120	60.00	156.0	-3.16
		Overall	360	-	180.5	-
	Treatment	Control	72	50.00	115.8	-5.90
		Distilled water	72	66.00	176.8	-0.34
		NaClO 5%	72	60.00	143.3	-3.39
		KNO_3 2gL^{-1}	72	76.00	217.4	3.37
		KNO_3 5gL^{-1}	72	84.00	249.2	6.26
		Overall	360	-	180.5	-
GS	SA (months)	1	30	0.05094	207.5	1.48
		2	30	0.05000	159.2	-1.17
		3	30	0.04941	120.8	-3.28
		4	30	0.04947	120.7	-3.29
		5	30	0.05094	207.5	1.48
		6	30	0.05000	159.2	-1.17
		7	30	0.04941	120.8	-3.28
		8	30	0.04947	120.7	-3.29
		9	30	0.05039	185.6	0.28
		10	30	0.05187	226.0	2.50
		11	30	0.05328	311.8	7.22
		12	30	0.05130	226.2	2.51
		Overall	360	-	180.5	-
	SSC	8°C	180	0.05026	184.1	0.65
		37°C	180	0.05055	176.9	-0.65
		Overall	360	-	180.5	-
	Provenance	Koto	120	0.05049	177.3	-0.42
		Massi NC	120	0.05087	199.6	2.46
		Tchétou	120	0.05000	164.7	-2.04
		Overall	360	-	180.5	-
	Treatment	Control	72	0.04907	116.0	-5.88
		Distilled water	72	0.05003	161.5	-1.74
		NaClO 5%	72	0.04984	164.3	-1.48
		KNO_3 2gL^{-1}	72	0.05070	218.5	3.46
		KNO_3 5gL^{-1}	72	0.05128	242.3	5.63
		Overall	360	-	180.5	-

(*): Number of observations

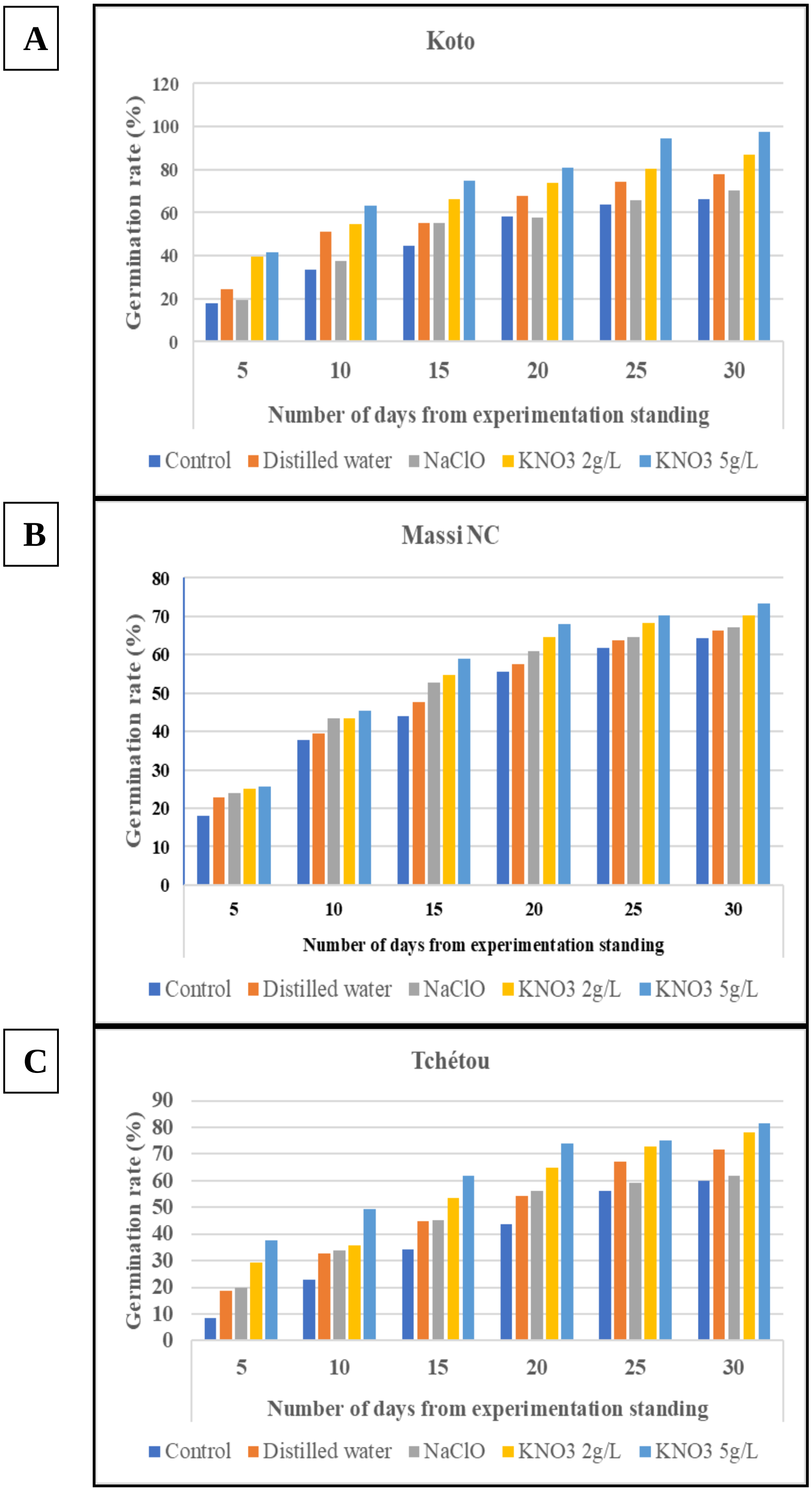

Figure.1 Seed germination kinetics of three *Afzelia africana* accessions (Koto, Massi NC and Tchétou) collected in Benin after 30 days experimentation under 5 treatments (Control = tap water; distilled water; KNO_3 solutions 2 and 5 gL^{-1}). A-Koto. B- Massi NC. C- Tchétou.

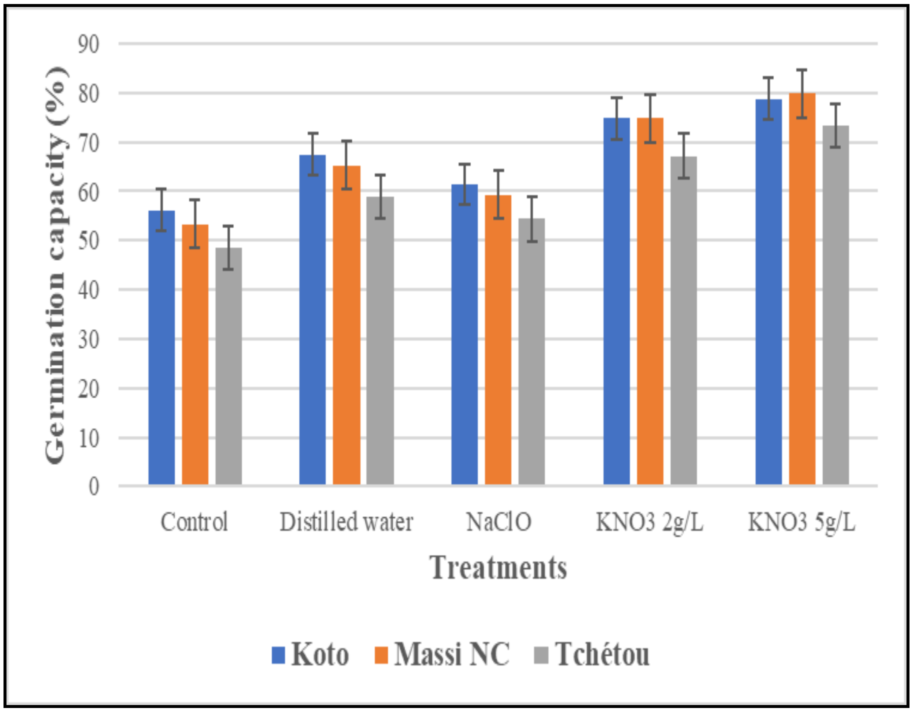

Figure.2 Seed germination capacity of three *Afzelia africana* accessions (Koto, Massi NC and Tchétou) collected in Benin after 30 days experimentation under 5 treatments (Control = tap water; distilled Water; NaClO 5%; KNO_3 solutions 2 and 5 gL^{-1}).

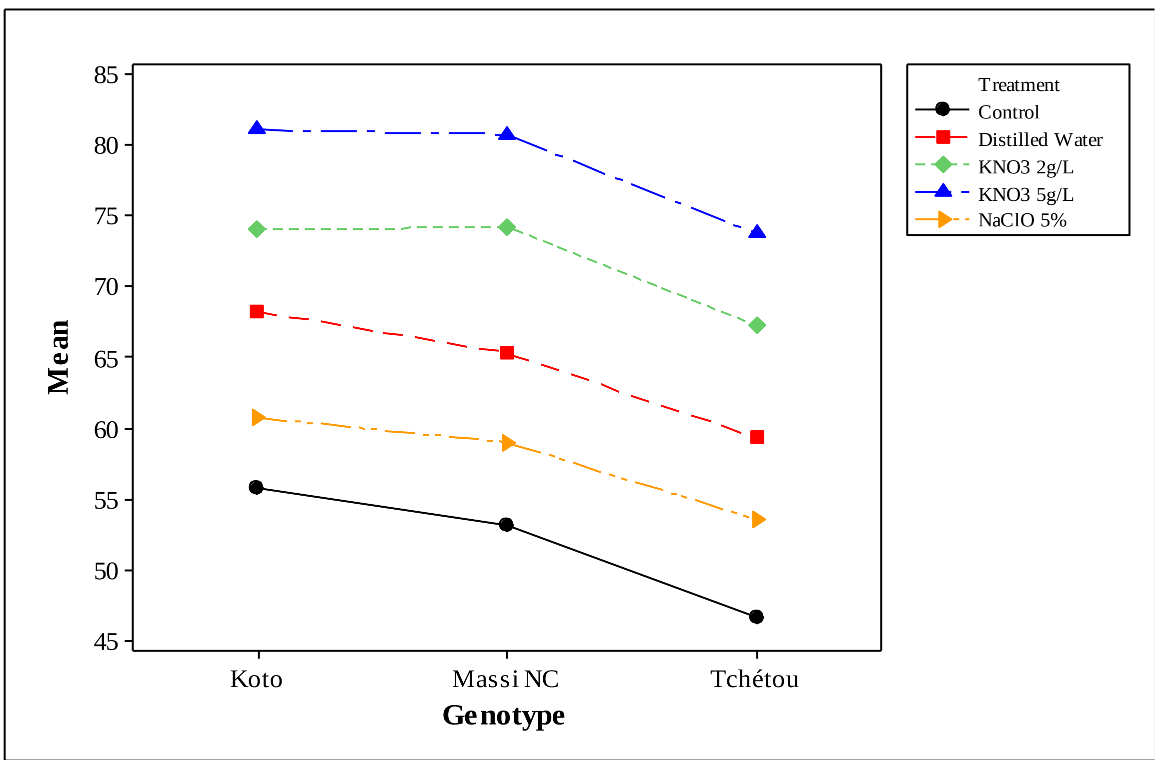

Figure.3 Interactions between Provenances (Genotypes) and Treatments for seed germination capacity (GC) in *Afzelia africana* collected in the Lama forest in Benin.

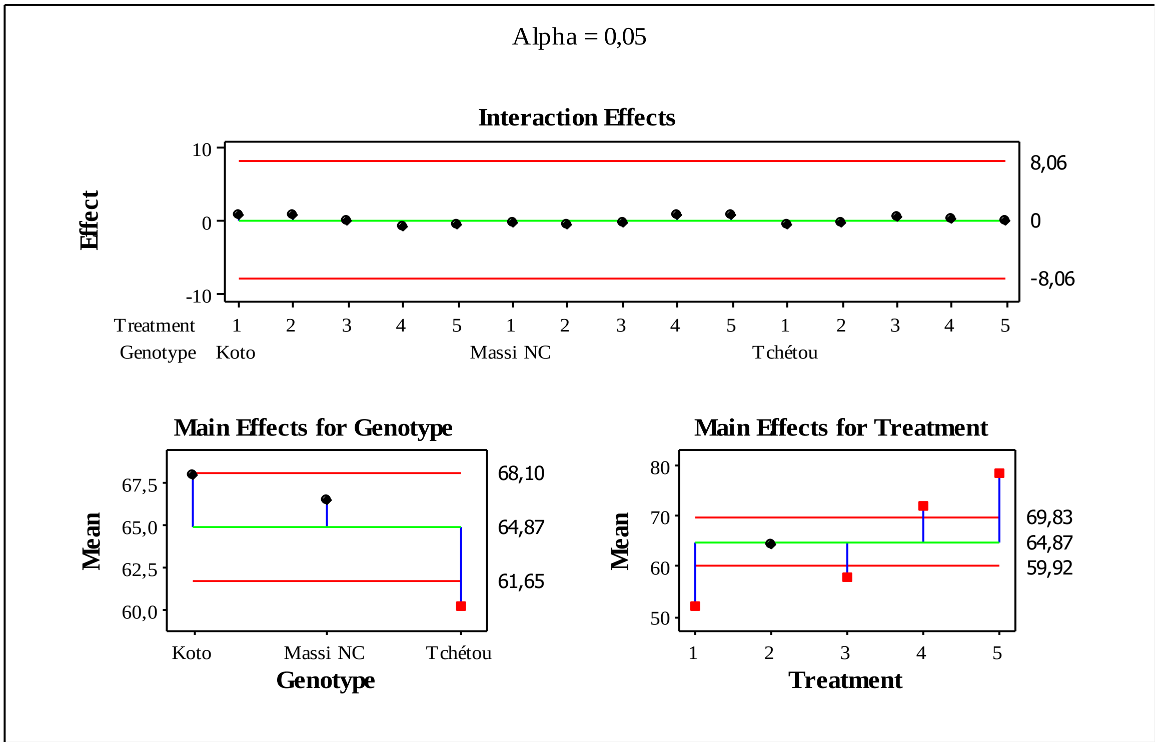

Figure.4 Provenance (Genotype) and treatment (Control = tap water; distilled water; sodium hypochlorite NaClO 5%; KNO_3 solutions 2 and 5 gL^{-1}) main effects and interactions on germination capacity (GC) of *Afzelia africana* collected from 3 ecozones in the Lama forest of Benin Republic.

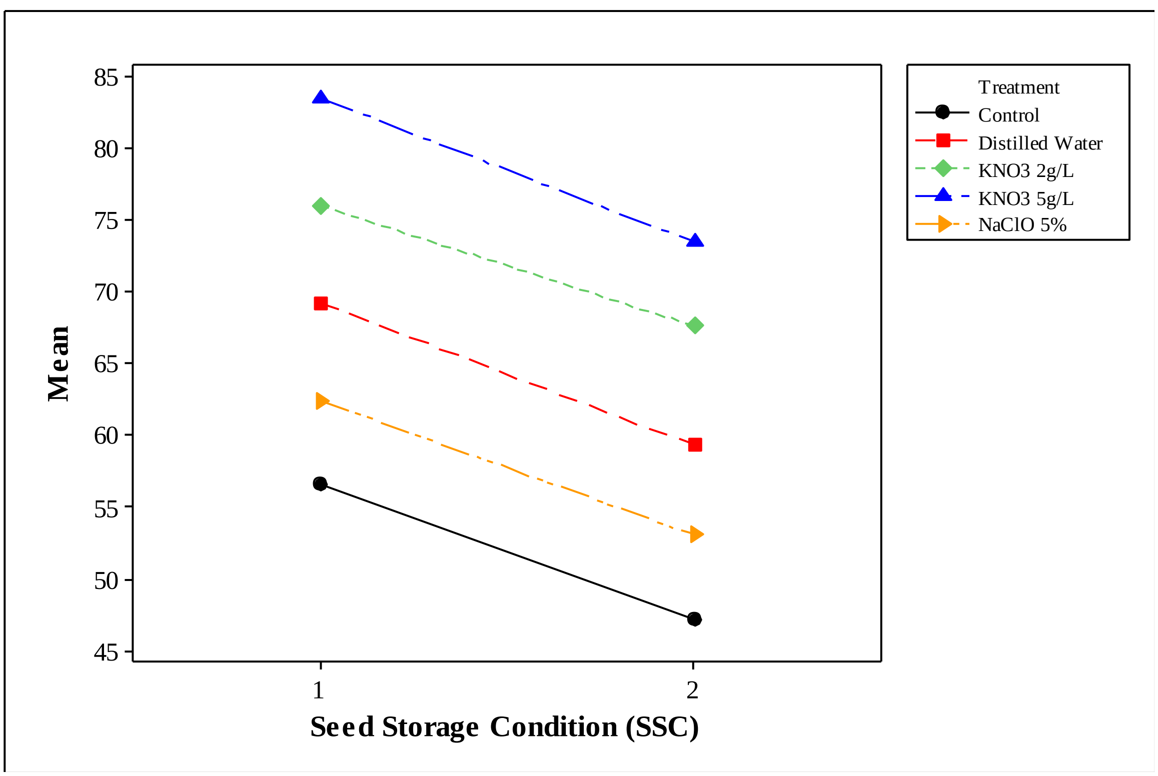

Figure.5 Interactions between Seed storage conditions (SSC) and Treatments for seed germination capacity (GC) in *Afzelia africana* collected in the Lama forest in Benin. SSC 1 = 8°C; SSC 2 = 37°C.

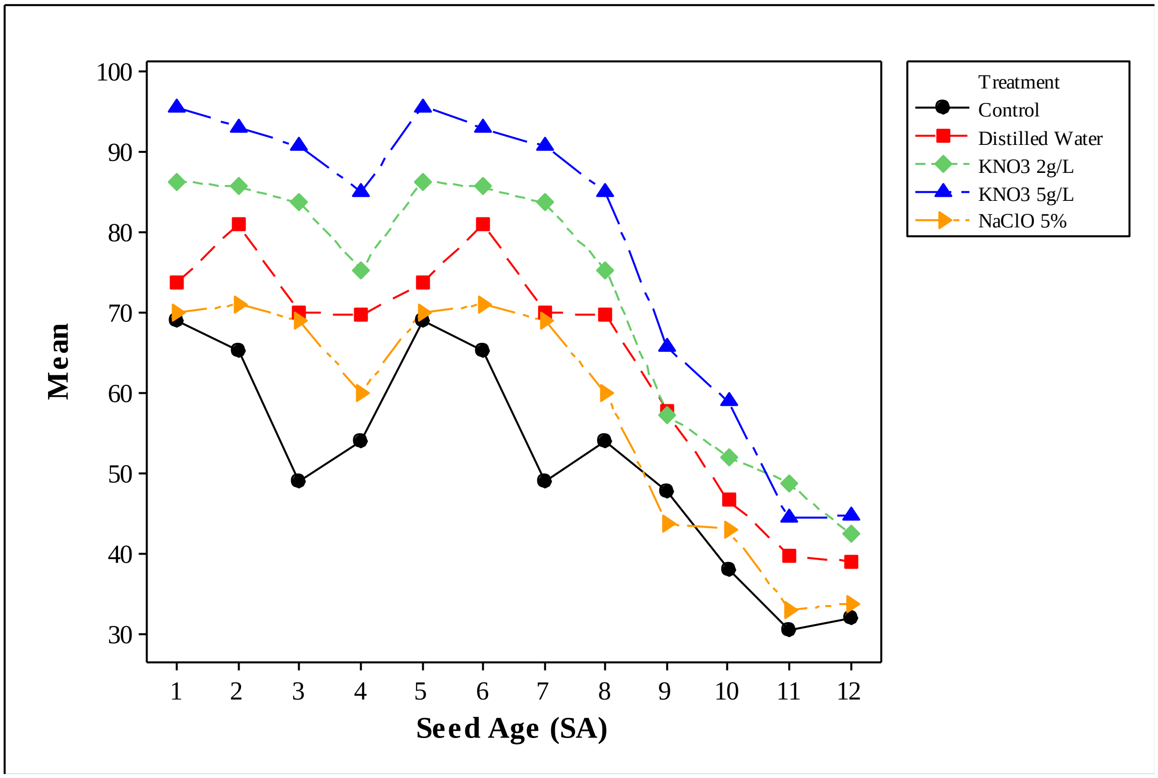

Figure.6 Interactions between Seed age and Treatments for seed germination capacity (GC) in *Afzelia africana* collected in the Lama forest in Benin.

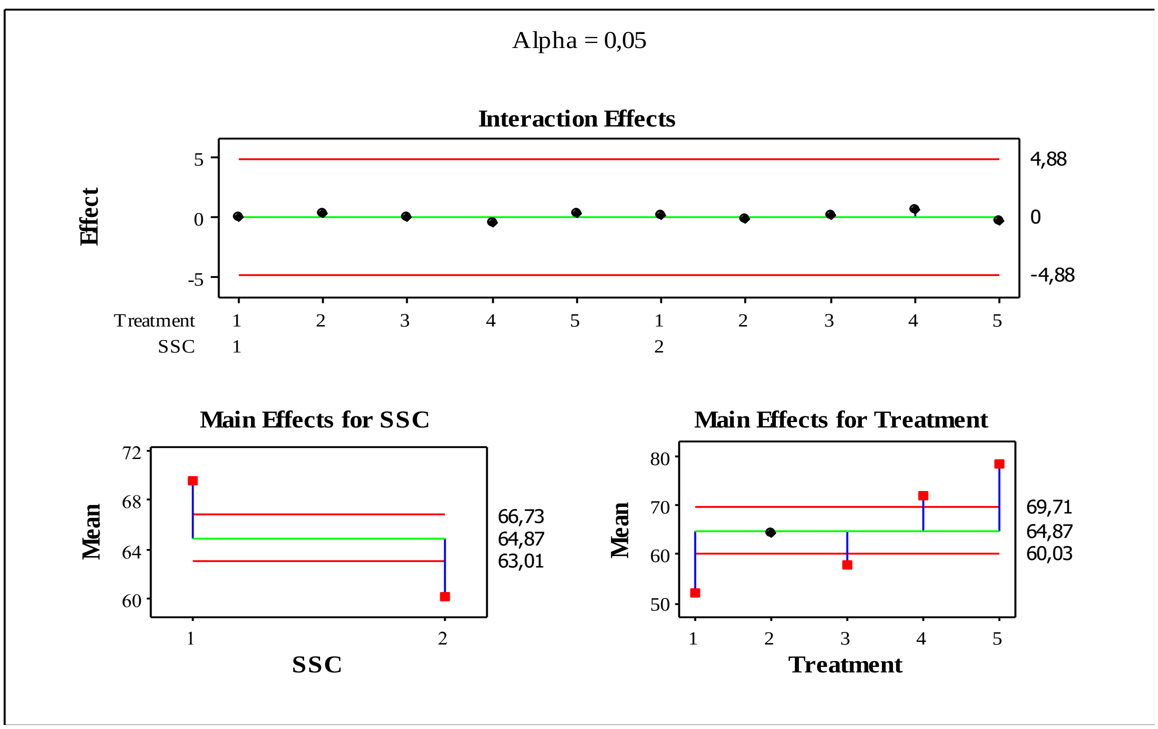

Figure.7 Seed storage condition (SSC) and treatment (Control = tap water; distilled water; sodium hypochlorite NaClO 5%; KNO_3 solutions 2 and 5 gL^{-1}) main effects and interactions on germination capacity (GC) of *Afzelia africana* collected from 3 ecozones in the Lama forest of Benin Republic.

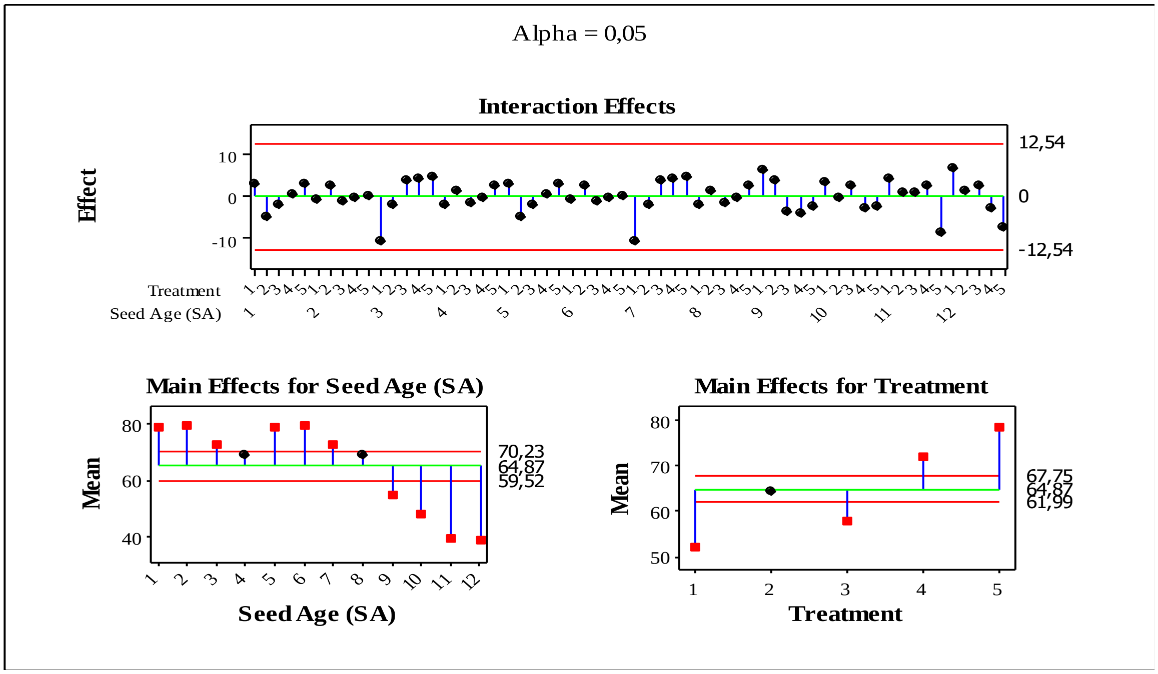

Figure.8 Seed age (SA) and treatment (Control = tap water; distilled Water; sodium hypochlorite NaClO 5%; KNO_3 solutions 2 and 5 gL^{-1}) main effects and interactions on germination capacity (GC) of *Afzelia africana* collected from 3 ecozones in the Lama forest of Benin Republic.

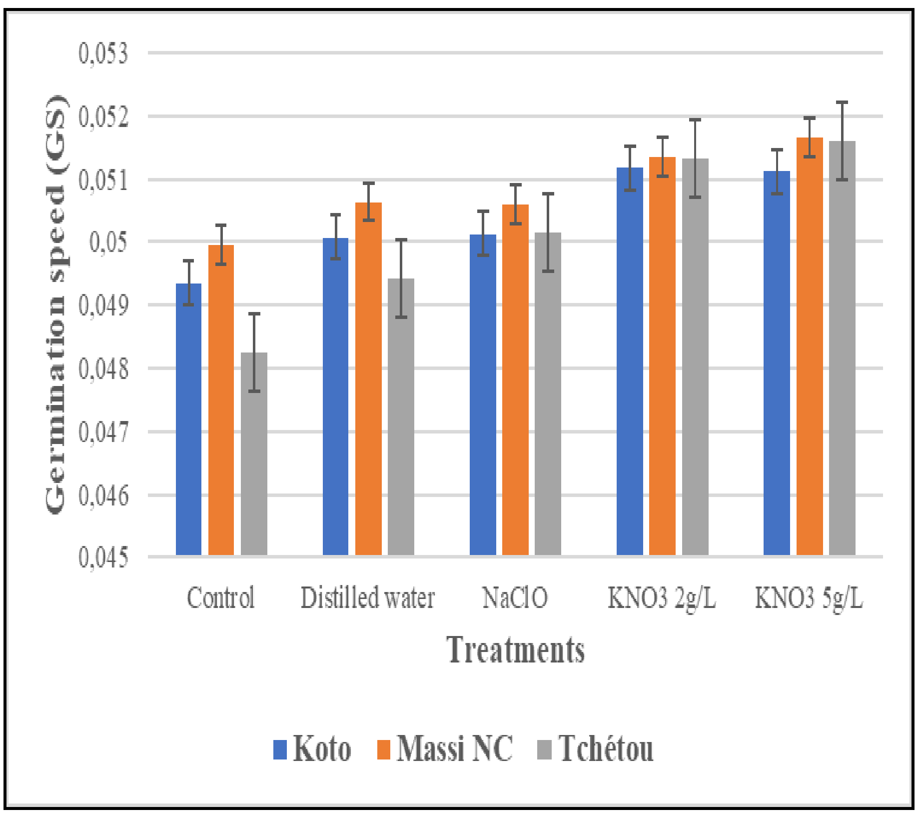

Figure.9 Seed germination speed of three *Afzelia africana* accessions (Koto, Massi NC and Tchétou) collected in Benin after 30 days experimentation under 5 treatments (Control = tap water; distilled Water; NaClO 5%; KNO_3 solutions 2 and 5 gL^{-1}).

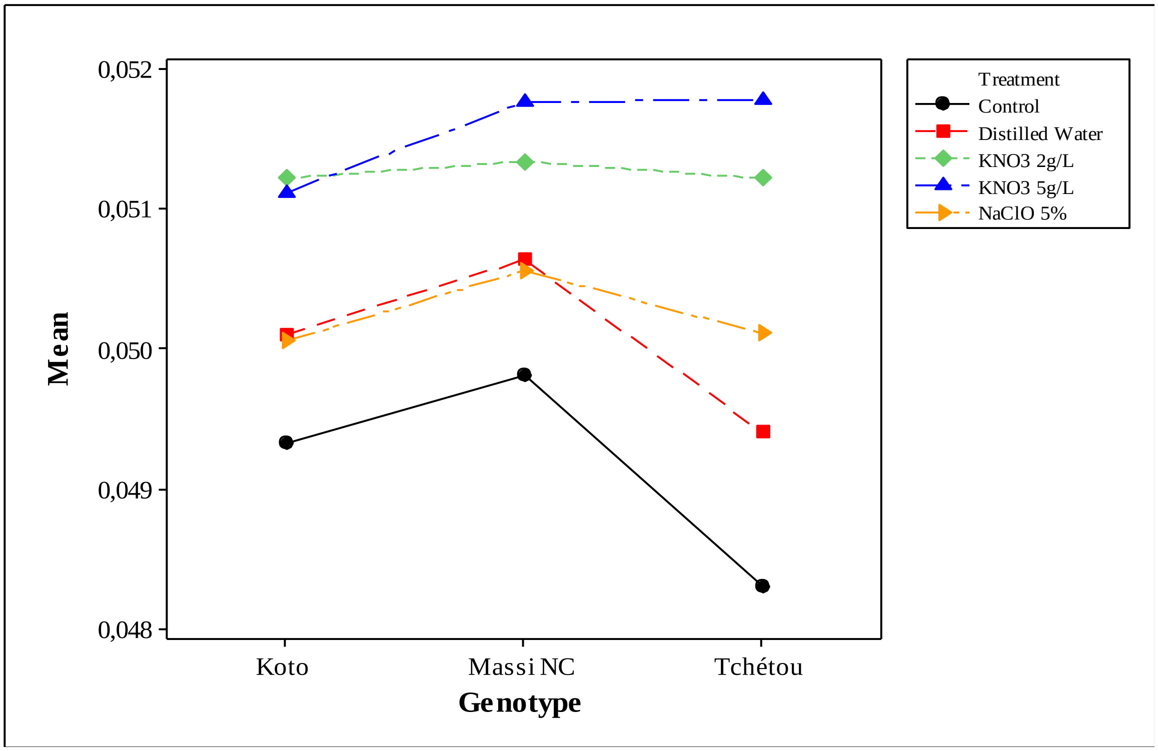

Figure.10 Interactions between Provenances (Genotypes) and Treatments for seed germination speed (GS) in *Afzelia africana* collected in the Lama forest in Benin.

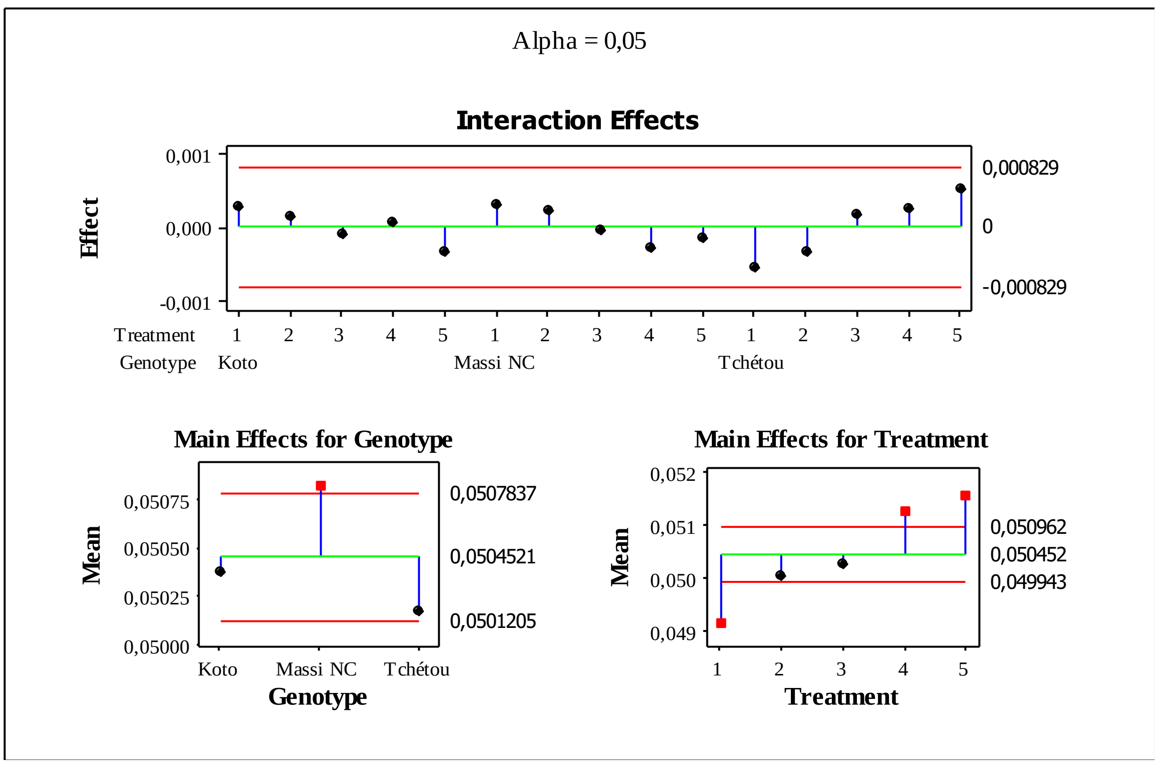

Figure.11 Provenance (Genotype) and treatment (Control = tap water; distilled water; sodium hypochlorite NaClO 5%; KNO_3 solutions 2 and 5 gL^{-1}) main effects and interactions on germination speed (GS) of *Afzelia africana* collected from 3 ecozones in the Lama forest of Benin Republic.

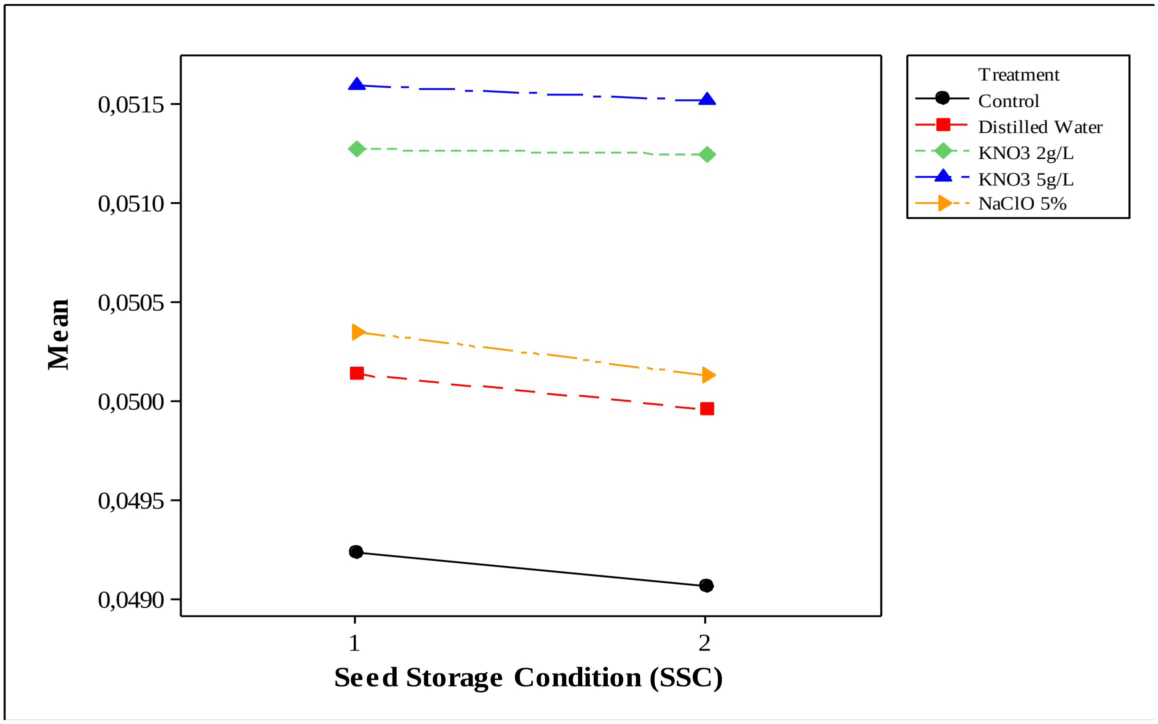

Figure.12 Interactions between Seed storage conditions (SSC) and Treatments for seed germination speed (GS) in *Afzelia africana* collected in the Lama forest in Benin. SSC 1 = 8°C; SSC 2 = 37°C.

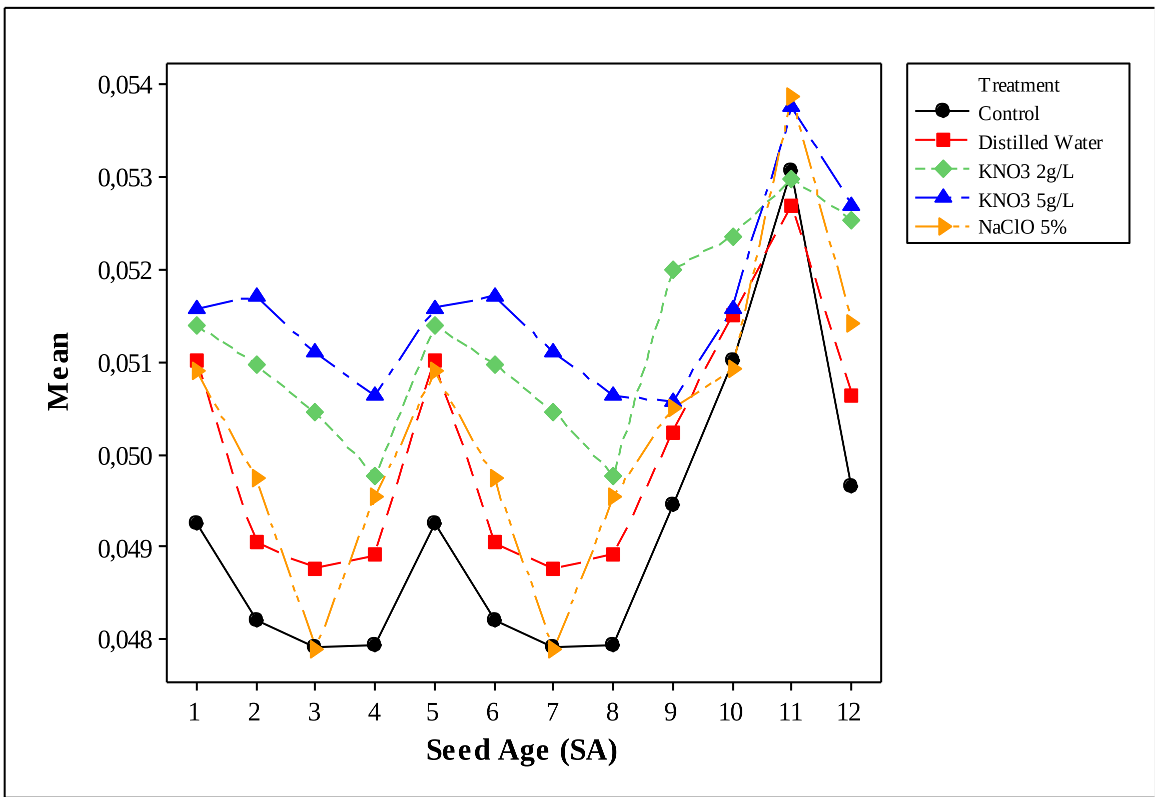

Figure.13 Interactions between Seed age (SA) and Treatments for seed germination speed (GS) in *Afzelia africana* collected in the Lama forest in Benin.

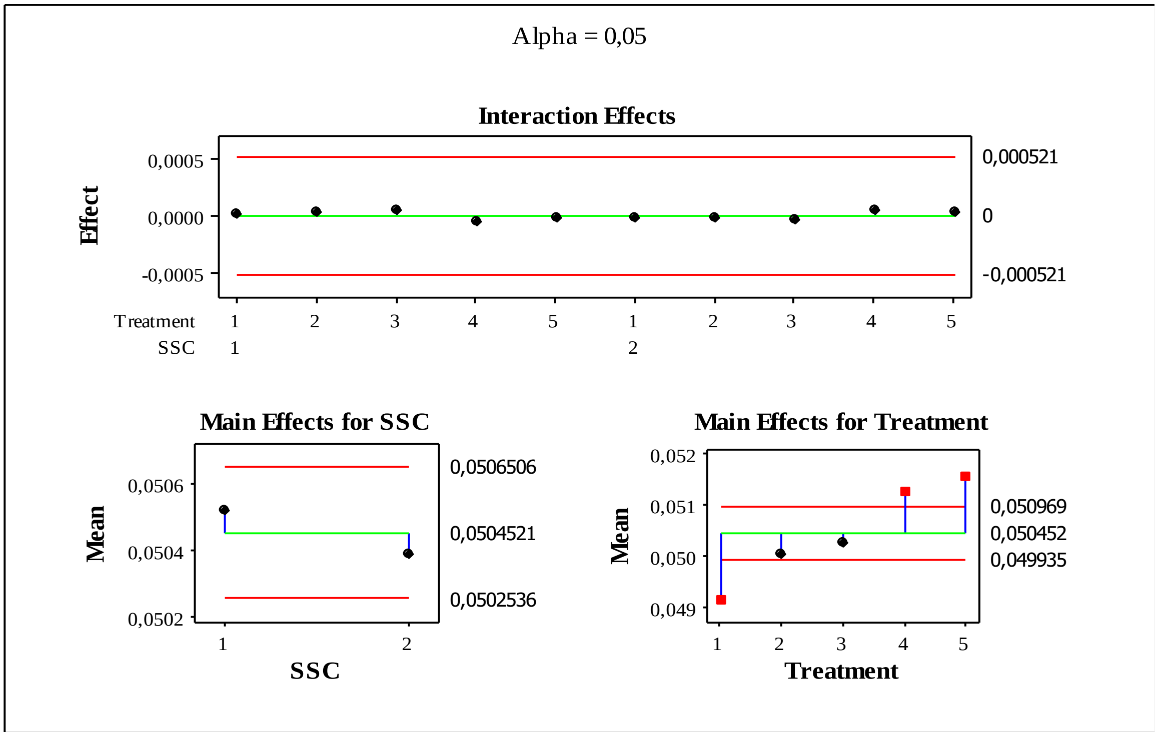

Figure.14 Seed storage condition (SSC) and treatment (Control = tap water; distilled water; sodium hypochlorite NaClO 5%; KNO_3 solutions 2 and 5 gL^{-1}) main effects and interactions on germination speed (GS) of *Afzelia africana* collected from 3 ecozones in the Lama forest of Benin Republic.

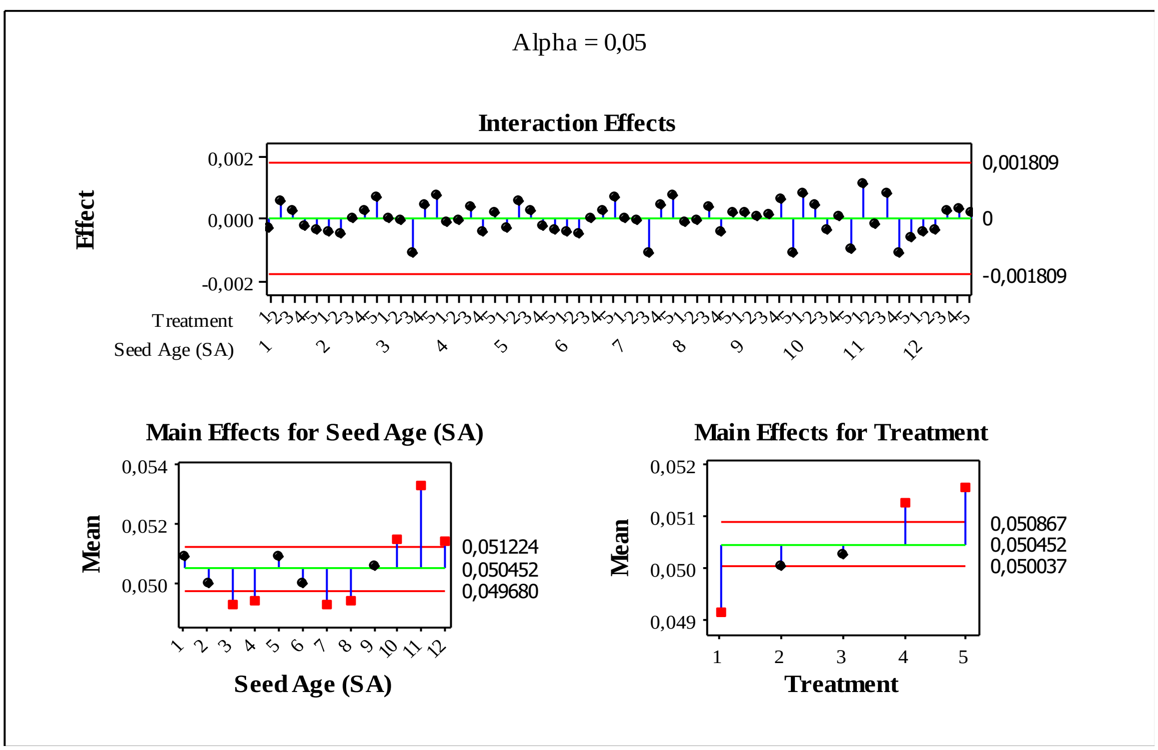

Figure.15 Seed age (SA) and treatment (Control = tap water; distilled water; sodium hypochlorite NaClO 5%; KNO_3 solutions 2 and 5 gL^{-1}) main effects and interactions on germination speed (GS) of *Afzelia africana* collected from 3 ecozones in the Lama forest of Benin Republic.

Massi NC mean rank is higher, with a positive z value (z = 2.46) (Table 3). The test statistics (H = 6.91) had a *p*-value of 0.032 when it is adjusted or not, showing that the null hypothesis can be rejected at levels superior to 0.032 in favour of the alternative hypothesis of the existence of at least a difference among the groups of accessions. The germination speed of Massi NC proved to be significantly the best whatever the treatment in *Afzelia africana*.

The sample medians for the five treatments were 0.04907, 0.05003, 0.04984, 0.05070 and 0.05128 J^{-1} for the control, distilled water, sodium hypochlorite 5% solution, the KNO_3 solutions 2 and 5 gL^{-1}, respectively. The z value for the control is -5.88, the smallest value of z. This size indicated that the mean rank for this treatment differs least from the mean rank for all observations. Moreover, the mean rank of this treatment (116.0) was lower than the mean of the observation set (180.5), and the z value is negative (z = -5.88). The mean rank for the treatment KNO_3 5 gL^{-1} is higher than in the whole experiment, with a positive z value (z = 5.63) (Table 3). The test statistics (H = 66.80) had a *p*-value of 0.000, when it is adjusted for ties or not, indicating that the null hypothesis can be rejected at levels higher than 0.000 in favour of the alternative hypothesis of the existence of at least one difference among the treatment groups. The treatment KNO_3 5 gL^{-1} appeared the most significant to a good seed germination speed in *Afzelia africana* collected in Benin.

Germination can be defined as the emergence and development from the seed embryo of those essential structures which, for the kind of seed in question are indicative of the ability to produce a normal plant under favourable conditions (Olujobi et al., 2024; Gunaga et al.,; 2011). In germination, the seed's role is that of a reproductive unit; it is the thread of life that assures the survival of all plant species. Furthermore, seed germination remains a key to forestry development because of its role in stand establishment. Thus, in a world acutely aware of the delicate balance between deforestation and world population, a fundamental understanding of seed germination and seedling growth is essential for maximum tree production and forest conservation (Olujobi et al., 2024). Some seeds germinate a few days after fertilization and some before their normal harvesting time. Others are dormant and require an extended rest period or additional development before germination occurs. Depending on the species and genotype, this period may last for only a few days or for as long as several years. *Afzelia africana* is one of the plants with a dense seed coat that usually takes a long time to germinate without some attempt to break through its exterior. Once the shell has undergone treatments, the germination success and speed greatly increase. Though sowing *Afzelia africana* seeds without pre-treatments may still result in seedlings, it is time-consuming. Therefore, a study on pre-treatment techniques that affect germination and early seedling growth of this tree species is required.

Seed germination requires the mobilization of reserves accumulated during its maturation. Degradation of those reserves brings the necessary energy to the young plant growth. This mobilization is the consequence of: the hydrolytic activities that release the nutriments stocked in the reserve, and the mechanisms of their transportation toward the embryo (Olujobi et al., 2024; Adeniji et al., 2019; Essou et al., 2017; Mihoub et al., 2005). Depending upon the species, these reserves can be majorly of carbohydrate, lipidic and/or protein nature (Olujobi et al., 2024; Adeniji et al., 2019; Essou et al., 2017; Khemiri et al., 2004). Respiration, hydrolysis of the reserves and many enzymatic activities stay under the dependence of the temperature. Indeed, all variations in the incubation temperature can affect in addition to the activity of enzymes, some processes in the control of germination such as the membrane permeability and the extensibility of the cell pecto-cellulosic cock (Calvin et al., 2025; Olujobi et al., 2024; Gul and Weber, 1999; Bewley and Black, 1994).

Seed tegument hardiness is an important factor that affects germination (Calvin et al., 2025; Olujobi et al., 2024; Adeniji et al., 2019; Aref et al., 2011). Seed dormancy is known in numerous tropical tree species (Ogbimi and Sakpere, 2021; Amusa, 2011). Uniyal et al., (2000) noted that the response of seeds to pretreatment is specific, genetic and no type of treatment may be considered as universally efficient. Aref et al., (2011) showed that all treatments applied to *Faidherbia albida* had significantly (p<0.05) affected germination parameters examined (germination percentage, average time and capacity) in all studied provenances. Olujobi et al., (2024) came to the same conclusions in *Acacia senegal*. *Afzelia africana* L. is one of the numerous multipurpose tree species in the tropics, particularly in Benin. However, it remains to date threatened because of weak natural regeneration (Calvin et al., 2025; Sobola, 2023; Adeniji et al., 2019).

Many woody species produce seeds that germinate thereafter with a lot of difficulties (Calvin et al., 2025; Adeniji et al., 2019; Niang et al., 2015). Seed germination issues in numerous species are bound among others to the physiological phenomenon named dormancy which appeared in various types. That phenomenon allows seeds to remain in slow life during a variable period of time according to the species or its taxonomical subdivisions and also its storage conditions (Aref et al., 2011; Aoudjit Hayet, 2006). Germination would be only possible if the dormancy is eliminated. Many studies on the seed germination process within the plant species have been undertaken by several authors. Thus, Niang et al., (2015) reported significant differences of the germination rate between various provenances of baobab in Senegal as seeds were pretreated in sulphuric acid extract. Ahoton et al., (2009) showed various tendencies on the seed germination rate and speed of *Prosopis africana* collected in Benin when submitted to four treatments having implied hot water, sulphuric acid, tap water and/or their combinations. Sobola (2023) and Olujobi et al., (2024) reported the effects of sulfuric acid on seed germination in *Afzelia africana* and *Acacia senegal*, respectively. Of these different works, germination rates of 90-100% were observed (Olujobi et al., 2024; Fredrick et al., 2016; Ahoton et al., 2009; Moussa et al., 1998; Njoukam, 1997).

In the present survey, freshly harvested seeds of *Afzelia africana*. from three agro-ecological zones in the Lama forest in Benin were subjected to germinate at the laboratory in Petri dishes filled with Canson paper moistened by tap (Control), distilled water, sodium hypochlorite 5% solution and after the pretreatment in KNO_3 solutions of 2 and 5 gL^{-1} followed by incubation on humid paper. Seed germination parameters have been estimated in one trial month and during 12 months. Seeds were kept under two temperature regimes (8 and 37°C). The capacity of germination remained generally high (more than 50%) during the whole experimentation period (12 months) (Figs. 1 and 2). However, the germination capacity declines from the sixth month to the end of experimentation. The germination speed stayed relatively weak generally and turned around 0.050 seed germinated daily. The germination spread out over the whole 30 days considered as the period of the single germination time. These results show that there is a high genotypic variability as well as great effects of seed age and storage temperature for the physiological parameters of germination in *Afzelia africana* as the surveys of Essou et al., (2017) and Fredrick et al., (2016) demonstrated in *Cleome gynandra* and *Faidherbia albida*, respectively. Salt solutions and nitrogenous compounds have often been used to break seed dormancy (Essou et al., 2017; Vandelook et al., 2008; Alboresi et al., 2005; Pérez-Fernandez and Rodriguez-Echeverria, 2003; Bell et al., 1999; Hendricks and Taylorson, 1972; Mayer and Polkjaoff-Mayber, 1963). In the present study, we used 2 and 5 gL^{-1} of KNO_3 solutions. Results obtained by soaking the seeds in KNO_3 solution 5 gL^{-1} for 24 hours were significant and permitted the most elevated germination rate (72%) (Fig. 4). These results remain in contrast to those presented by Zharare (2012) who reported that KNO_3 and K_2SO_4 are generally inefficient to unlock dormancy and to accelerate any germination process. Nonetheless, the results presented here are in accordance with those reported by Essou et al., (2017) in *Cleome gynandra*. Factorial tests combining the five treatments used here are in progress. Moreover, there is no study reported to date on the effects of seed ages and storage conditions on germination in *Afzelia africana*. Pretreatments as applied by different authors (Olujobi et al., 2024; Adeniji et al., 2019; Fredrick et al., 2016; Bello and Gada, 2015; Niang et al., 2015; Ahoton et al., 2009) combined with the methodology used in the present report are in progress and will permit to surround the various types of dormancy, and then to enhance the possibilities of their levee towards raising germination capacity and speed in *Afzelia africana*. This may be encouraging for better regeneration of the species and therefore its conservation. Seed source plays also an important role as factor as the treatment. This observation conforms with the findings of Uniyal et al., (2000), who noted the meaningful importance of seed source in the germination of *Grewia oppositifolia*. *Afzelia africana* provenances collected in different agro-ecological areas differed from one another in the expression of the germination parameters indicating that the source of seeds and therefore the genotype plays an important role in the seed response whenever they are submitted to a particular treatment. Furthermore, the seed age and storage condition play great roles in the germination capacity and speed. More results are expected from seeds of more than one year old and kept under various conditions to optimize the real conservation of *Afzelia africana* natural genetic resources. The weak germination speed observed might be due to the genetic heterogeneity of the accessions surveyed. More carefully collected provenances and factorial treatments may be investigated in future studies. Moreover, Hawker and Jenner (1993) suggested that the high temperatures inhibit the germination of the seeds while limiting the availability of energy and hydrolysates, resulting from a delay and an inhibition of the synthesis and/or the activity of the hydrolytic enzymes. Similarly, low temperatures entail a disruption and a delay of coordination at the time of the mobilization of the reserves (Nykiforuk and Johnson-Flanagan, 1994). From the present study, low-temperature storage of seeds permitted the conservation of the good high germination capacity even though the seed age and provenance (genotype) as well as their interactions with treatments play good roles too to improve and increase the germination parameters.

In conclusion, *Afzelia africana* constitutes a key element in its native area in Southern Benin and in the balance and maintenance of many ecosystems. The introduction of this species into reforestation programs offers a sustainable reforestation solution. However, the successful growth and development phases of this species require inevitably a thorough understanding of its germination characteristics, as well as its behaviour in relation to environmental conditions. The objective of this survey was to study the effect of different seed ages, storage conditions, genotypes and treatments on the seed germination of *Afzelia africana* collected in different agro-climatic zones in the Lama forest from Benin to determine the most suitable factors to improve and increase any expression of the germination parameters. The study revealed the existence of variation in germination rates, capacity and speed between the provenances, the factors 'seed age', 'seed storage temperature' and the treatments. Interactions Accession × Treatment, Seed age × Treatment and Seed storage condition × Treatment were significant. KNO_3 5 gL^{-1} and KNO_3 2 gL^{-1} were the most efficient treatments conducting to better results. Koto originated in the Western part of the Lama forest as well as Massi NC from the central area of the forest delivered the best results compared to the provenance Tchétou. Seeds conserved at low temperatures (8°C) kept the best germination capacity and speed. Seed age plays a great role in good germination capacity and speed. Further surveys on older seeds, diverse storage conditions as well as factorial treatments are ongoing. More genotypes may also be investigated. As seed germination control is crucial for avoiding the weak natural regeneration of *Afzelia africana* observed for some decades to date, such surveys will permit the maintenance and improvement of the genetic resources existing in *Afzelia africana* in Benin and the whole of Africa.

Conflict of interest statement

The Author declare that he has no conflict of interest.

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