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# Relative Germination Rapidity in Acacia Nilotica under Different Sulfuric Acid Concentrations and Treatment Duration

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***Abstract:*** *Dormancy is a serious germination inhibitory factor especially in tree species, while various seed pre-treatment methods exist for breaking dormancy, but acid treatment has proved the most effective rapid means. A laboratory study was carried out to compare the effects of five sulphuric acid concentrations (40, 50, 60, 70 and 80%) and five treatment durations (1, 5, 10, 15 and 20 minutes) on germination in A. nilotica. Results from 3 – 7 days after planting (DAP) consistently showed that the effects of acid concentration, treatment duration and their interaction on germination were generally significant (p<0.01). The result for acid concentration revealed that germination increased with each successive increase in acid concentration in the order 80% > 70% > 60% > 50% ≥ 40%, depicting that seed pre-treatment with 80% acid concentration was the best for germination of A. nilotica. Furthermore, there was positive linear increase in germination, in which daily germination rates widely varied from 0.3857 – 9.5571%, which expressed that germination was more rapid at higher (80 – 70%) than lower (60 – 40%) concentrations. Similarly, results in respect of treatment duration showed significantly higher germination at 20 minutes, followed by 15 minutes than at other durations. The highly significant (p<0.01) linear regression showed that germination occurred at a daily rate of 1.6857 – 6.7857% among treatment durations, with more rapid germination in 20 – 10 minutes than 5 and 1 minutes. Results on acid concentration x treatment duration interaction indicated that seed pre-treatment with 80% $H_2SO_4$ for 20 minutes, consistently gave the best percentage germination, and was closely followed by 80% $H_2SO_4$ for 15 minutes and 70% $H_2SO_4$ for 20 minutes. The regression coefficients expressed that every 5 min increase in treatment duration resulted in germination increase by 1.10, 1.40, 7.30, 12.1 and 11.6% in the 40%, 50%, 60%, 70% and 80% $H_2SO_4$ concentrations at 7 DAP, respectively. In conclusion, combination of 80% $H_2SO_4$ for 20 minutes gave the best and most rapid percentage germination and is therefore it is recommended as the best pre-treatment method for Acacia nilotica.*

***Keywords:*** Acacia nilotica, Concentrations, Germination, & Rapidity

## INTRODUCTION

Dormancy impairs germination of many plants and is especially a problem in trees with hard seed, thereby necessitating seed pre-treatment in raising tree seedlings in nurseries for establishment of plantations, windbreaks and shelterbelts. Trees offer succour to mankind in the form of food, shelter and fuel, and also have environmental benefits in the hydrologic cycle, soil conservation, climate change moderation and preservation of biodiversity, while maintaining soil structure and microbial balance (Ali *et al*., 2012; Nwokocha *et al*., 2015; Olagunju, 2015). It is estimated that forest resources serve as sources of livelihood to over 1.6 billion people globally, and 90% of the 1.2 billion people in the developing world (FAO, 2001; World Bank, 2004). However, there are indications that the tropical forest resources on which millions of rural people depend is rapidly being lost over the past century, and 350 million hectares have been deforested, while 500 million hectares are degraded (ITTO, 2002; Ogbonaya, 2002). In general, desertification has been mainly attributed to deforestation, as the result of tree logging for firewood and timber, land clearance for agricultural land expansion due to population pressure, overgrazing and bush burning (Acosta-Michlik *et al*., 2005; FAO, 2006; Lester, 2006; UNCCD, 2011; WEP, 2011; Jigawa, 2012; FME, 2013; Mohammed *et al*., 2013; Olagunju, 2015).

In Nigeria, the vulgar of weather is much more prominent in the desert prone semi-arid environment of Nigeria where excessive cultivation, deforestation and over grazing is a progressive serious problem, thus the need for replenishment of the deforested woodlot and plantations is more critical in the northern part of the country where several interventions have taken place in the past. Deforestation poses a serious threat to the vast fragile sahelian environment, hence the need for rapid multiplication methods for raising seedlings of adapted tree species for use in afforestation programmes. The nigerian Sahel is characterized by consistent gradual decline in vegetation, partly due to low rainfall, poor regeneration of tree species and consequently low vegetation cover, exacerbated by deforestation activities of humans for fuel and farmland expansion due to population explosion. Over exploitation of tree resources has resulted serious desertification in northern part of the country, encroaching rapidly at an alarming rate of 0.6 km annually, thus 351,000 $km^2$ of the landmass is already lost to desertification, while 15% still remains vulnerable (Abahussain *et al*., 2002; Jaiyeoba, 2002; Nwafor, 2006; Emodi, 2013; Tercula, 2015; Olagunju, 2015). Reforestation and afforestation programs are sure means of arresting the problem of desert encroachment (Le *et al*., 2011). In this realization, there were several international, national, regional, state, local and individual interventions. Ibrahim and Muhammad (2015) offered an extensive review covering the 78 years afforestation programmes in Nigeria. That begun with tree planting campaign in the 40s, became expansive from 60s - 80s with over 236,500 hectares of shelterbelts established, and subsequent gigantic projects from 90s – 2000, such as The Nigerian Forest Action Programme in 1997, The Green Belt Project in 2000 and The Great Green Wall.

Trees are largely impacted by human activities, this coupled their low coppicing ability and dormancy, which has imposed significant barrier to the rapid multiplication of hard seed coat tree species, calls for deliberate re-forestation efforts starting with tree nurseries. This calls for a thorough investigation and knowledge of the entire germination process, such as dormancy breaking methods, especially the best acid concentrations and time of treatment for ecologically adapted and economically important tree species with food, health and industrial values. *Acacia nilotica* is one of the important tree species of high multipurpose environmental, economic and medicinal values in the semi-arid sudano-sahelian agro-ecology (Malviya *et al*., 2011; Ali *et al*., 2012; Auwal *et al*., 2014). However, its germination is impeded by dormancy owing to the hard seed coats, which render slow germination and therefore need seed pre-treatment to enhance germination and subsequent growth of seedlings. Researchers have extensively explored pre-treatment method such as dry heat (Denton *et al*., 2013), cold water (Owonubi *et al*. 2005; Ibrahim and Otegbeye, 2004; Zare *et al*., 2011) and hot water treatments (Ibrahim and Otegbeye, 2004; Owunobi *et al*., 2005; Wang *et al*., 2007; Abubakar and Maimuna, 2013). The other effective pre-treatment method include sulphuric acid (Baskin *et al*., 2000; Muhammed and Amusa, 2003; Ren and Tao, 2004; Wang *et al*., 2007; Lorenzo and Souza, 2008; Saikou *et al*., 2008; Pego *et al*., 2016) and abrasion of the seed coat with sand paper for breaking dormancy in hard seeds (Aref *et al.*, 2011). However, most studies offered simple comparison of methods and were fragmentary using single acid concentration or temperature regime regardless of species, while harmonizing treatment time and force applied for abrasion with sandpaper posed difficulty in comparing results. Therefore, the aim of this study is to thoroughly explore sulphuric acid as means of breaking seed dormancy, as to permit specific recommendation as to the best concentration and treatment duration for effective germination of *A. nilotica*, an important xerophytic tree of the sahelian region of Nigeria.

## MATERIALS AND METHODS

### Experimental Procedure

The germination test was carried out using sterile cotton-wool in Petri-dishes, and was watered daily using 5 mls of distilled water with syringe. All glasswares were subjected to autoclave sterilization at 121 °C for 15 minutes. Germination of seeds in Petri-dishes were observed daily for the period of two weeks. Number of germinating seeds were counted and recorded, by observing the emergence of the radicle, and at the end of germinating period, percentage germination was calculated by the method of Nikoleave (1997):

$$\text{Germination (\%)} = \frac{\text{Number of seeds germinated}}{\text{Number of seeds planted}} \times 100$$

### Pre-treatment methods

Pre-treatment with sulphuric acid ($H_2SO_4$) at different concentrations and durations were tested in *Acacia nilotica*, using sample size of 10 seeds per Petri-dish, and each of the treatments was replicated ten times. The procedure by Zencirkiran *et al*. (2008) was followed, in which the 400 seeds were treated with 40, 50, 60, 70 and 80% sulphuric acid for 1, 5, 10, 15, and 20 minutes. The seeds were rinsed with distilled water and then immediately planted for germination to take place and the control sown directly without applying any treatment.

### Germination Success (%)

Germination of seeds in each of Petri dishes was observed daily for a period of seven days. Number of germinating seeds in each dish was counted by observing the emergence of the radicle and then recorded. At the end of germinating period, germination success was calculated for each treatment by the method of Nikoleave (1997).

$$\text{Germination success (\%)} = \frac{\text{Number of germinated seeds}}{\text{Number of seeds sown}} \times 100$$

**Experimental Design and Treatments**

The two-factor experiment was conducted using Randomized Complete Block Design (RCBD) with factorial layout (arrangements) of treatments. The factors and treatments were sulphuric acid concentrations (40%, 50%, 60%, 70% and 80%) and treatment durations (1 min, 5 min, 10 min, 15 min and 20 min).

**Data Collection and Analysis**

Data generated were subjected to statistical analysis, using the software, Staistix Version 8.0 (Microsoft, 2003). F-test analysis of variance (ANOVA) was computed and the level of significance was pegged at 5% probability, and means were compared using Least Significant Difference (LSD) at 5% level of probability. Charts were drawn using Microsoft Excel (2003), and used to depict the pattern that indicates the best regression model to compare daily percentage germination in seeds of the *A. nilotica* under different concentrations and treatment time. Regression analysis was employed to compute the best-fit regression equations, in which the coefficient of determination ($r^2$) shows the effects of treatments on germination and the regression coefficient (b-value) gives the rate of increase in germination.

**RESULTS**

Table 1 shows the effects of different sulphuric acid concentrations and treatment duration on the germination of *Acacia nilotica* over one week period. There was no germination at 1 and 2 DAP in respect of both acid concentration and treatment duration. Subsequently, however the effects of acid concentration and treatment duration on germination, from 3 – 7 DAP were both highly significant ($p<0.01$). Thus, germination in respect of the five acid concentrations significantly varied from 0 – 6.4%, 1.8 – 22.2%, 1.8 – 31.8%, 1.8 – 42.8% and 1.8 – 52.2% at 3, 4, 5, 6 and 7 DAP, respectively. In general, germination increased with each successive increase in acid concentration, in which 80% $H_2SO_4$ consistently gave significantly better effects, followed by 70% with significantly higher germination than 60%. However, percentage germination in acid concentrations from 60 – 80%, were all significantly higher than 40 – 50% that were statistically at par. Thus, results consistently expressed that germination significantly increased with each successive increase in acid concentration in the order: 80% > 70% > 60% > 50% ≥ 40%, depicting that seed pre-treatment with 80% acid concentration was the best for germination of *A. nilotica*. Fig. 1 shows the effects treatment durations on pattern and rate of germination in *A. nilotica*, over the seven days period. In general, there was highly significant ($p<0.01$) positive (direct) linear increase in germination regardless of concentration. The coefficient of determination ($r^2$) shows that effects of the acid concentrations varied from 75.0 – 96.44%, indicating that effects increased with increase concentration and was higher in 60 - 80% $H_2SO_4$ concentration. The regression coefficient depicts that the daily rate of increase in germination for the 80, 70, 60, 50 and 40% concentrations of $H_2SO_4$ were 9.5571, 6.7857, 2.5714, 0.5143 and 0.3857%, respectively. This expressed more rapid germination at higher concentrations, thus 80% > 70% > 60% > 50% > 40%.

Results also indicated highly significant ($p<0.01$) differences in the effects of treatment duration on germination, which ranged 0.6 – 4.4%, 3.0 – 18.4%, 4.8 – 24.8%, 7.4 – 30.8% and 9.4 – 36.0% at 3, 4, 5, 6 and 7 DAP, respectively (Table 1). Results consistently showed that percentage germination increased with increase in treatment duration, with significantly higher germination at 20 min than at other durations, except at 3 DAP in which 15 min gave statistically similar effects. Fig. 2 shows the effects of treatment durations on pattern and rate of germination in *A*. *nilotica*, over the seven days. The chart consistently depicts highly significant ($p<0.01$) positive linear increase in germination irrespective of concentration. Consequently, the coefficient of determination ($r^2$) expressed that the effects of treatment duration on germination varied from 93.83 – 95.65%, and the effects increased with increase in treatment duration (Fig. 2). Furthermore, the regression coefficient indicated increase in germination rate as treatment duration increased, giving daily rates of increase of 6.7857, 5.0571, 4.0500, 2.2357 and 1.6857% for 20, 15, 10, 5 and 1 minutes treatment duration, respectively.

Interaction effects of concentration x treatment duration on germination were also highly significant ($p<0.01$) on days 3, 4, 5, 6 and 7, and results are presented in Table 2. Percentage germination among treatment combinations on these respective days of assessment ranged from 0 – 10.0%, 0 – 34.0%, 0 – 49.0%, 0 – 64.0% and 0 – 76.0%. Thus, treatment combinations of 80% $H_2SO_4$ for 20 min consistently gave the best percentage germination, however 80% $H_2SO_4$ for 15 min was equally as effective at 3, 4 and 6 DAP, as the case also was for 70% $H_2SO_4$ for 20 min at 4 and 5 DAP. Fig. 3 shows the interactive effects acid concentrations and treatment durations on pattern and rate of germination in *A*. *nilotica* at 7 DAP, in which effects ($r^2$) varied from 91.57 – 99.47%. Results generally indicated highly significant ($p<0.01$) positive linear increase in germination over treatment durations among acid concentrations. The regression coefficient rates the germination for different concentrations over treatment durations, in which every 5 min increase in treatment duration resulted in germination increase by 1.10, 1.40, 7.30, 12.1 and 11.6% in the 40%, 50%, 60%, 70% and 80% $H_2SO_4$ concentrations at 7 DAP, respectively.

## DISCUSSION

Dormancy is a serious germination inhibitory factor especially in tree species, while various seed pre-treatment methods exist for breaking dormancy, but acid treatment has proved the most effective rapid means. In the present study, the significant improvement in germination at higher acid concentration and longer treatment duration has confirmed existence of dormancy in *A*. *nilotica*. Similarly, the absence of germination in the control and at lower acid concentrations of 40 and 50%, as against rapid germination within three days at higher acid concentrations (60 - 80%) and treatment durations (10 - 20 min) further attest to high dormancy in the tree species. These results concur with the findings of Satti *et al*. (2016) who observed germination within initial three days in the same tree species and concentrated (100%) sulphuric acid, while Yousif *et al*. (2019) reported higher dormancy in *Acacia nilotica* (81%), in comparison to *A. seyal* (74%), *A. mellifera* (15%) and *A. senegal* (5%).

Acid scarification has been used for long in breaking the dormancy of hard seeds, but effects differed remarkably among different species, concentrations and treatment durations.

In the present study, result for the five acid concentration treatments showed that 80% $H_2SO_4$ consistently gave the best germination, and thus the optimum concentration for germination of *A*. *nilotica*. Physical dormancy has been attributed to impermeability of the seed coat, owing to lignin, phenolic deposits, lipids, suberin and wax (Kolattukudy, 1981; Argel and Paton, 1999; Baskin *et al*., 2000; Baskin, 2003; Agbagwa *et al*., 2004). Inter-species germination differences had been attributed to variable physical and biochemical seed characteristic, but in the present study with single species, the differences could be due to sole effects of acid concentrations and treatment duration. Thus, the increased effectiveness of sulphuric acid at higher concentrations (60 - 80%) in the present could be attributed to quicker softening of the hitherto impermeable cuticle and elimination of the water-resistant compounds in the seed testa layers. Supportive literatures exist in which scarification using acid generally enhanced germination capacity by increasing the leaching of growth inhibitors from the seed (Baskin, 1998; Kolattukudy, 1981; Argel and Paton, 1999; Baskin *et al*., 2000; Baskin, 2003; Agbagwa *et al*., 2004). However, according to Nasroun and Al-Mana (1992) species that did not suffer from any dormancy problems, did not respond to any of the acid and other pre-treatment methods. Consequently, the present result on *A*. *nilotica* with high dormancy expressed that germination significantly increased with successive increase in acid concentration. The superiority of the present study is that it has determined the rate of germination in each acid concentration, in order to compare the rapidity of germination among different acid concentrations, and the relative order was 80% > 70% > 60% > 50% > 40%, judging by the respective germination rates of 9.5571, 6.7857, 2.5714, 0.5143 and 0.3857% per day.

In respect of treatment duration, result showed increase in germination as the duration increased, with significantly higher germination at 20 minutes than at other durations. This suggests that the efficacy of $H_2SO_4$ in breaking dormancy is time-dependent, thus longer time is needed for the elimination of germination inhibitory compounds, inherent in the highly lignified impermeable seeds of *A*. *nilotica*. However, it appears from the present result that that the optimum acid concentration was not reached, judging by the continuous germination increase up to the highest treatment time of 20 minutes. According to Yousif *et al*. (2019), with 98% sulfuric acid the best treatment effects occurred at 60 to 90 minutes for *A. nilotica*. Although time limits for achieving optimum germination widely varied from 0 – 120 minutes in literature, these depended on acid concentration and tree species (Nasr *et al*., 2013; Satti *et al*., 2016; Kheloufi *et al*., 2017; Yousif *et al*., 2019). In related species, pre-treatment with sulfuric acid at 98% concentration was effective at 10 - 90 minutes for *A. seyal* (Yousif *et al*., 2019), for 30 minutes for *Acacia salicina* (Rehman *et al*., 1999), 120 minutes for *A*. *cyanophlla* and *A*. *farnesiana* and 60 minutes for *A*. *decurrens* (Kheloufi *et al*., 2017). However, this result further found that germination rate was 6.7857, 5.0571, 4.0500, 2.2357 and 1.6857% daily, for treatment durations of 20, 15, 10, 5 and 1 minutes, respectively. Furthermore, treatment duration x concentration interaction revealed that every 5 minutes increase in treatment duration resulted in germination increase by 1.10%, 1.40%, 7.30%, 11.6 and 12.1% in the 40%, 50%, 60%, 70% and 80% $H_2SO_4$ concentrations at 7 DAP, respectively. In conclusion, combination of 80% $H_2SO_4$ for 20 minutes gave the best percentage germination in *Acacia nilotica*, and the rate of germination was faster.

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**Appendix**

Table 1. Effects of sulphuric acid concentrations and treatment duration on the germination of *Acacia nilotica*, 3 – 7 days after plating (DAP)

| Treatment | Germination (%) | | | | |
|---|---|---|---|---|---|
| | **3 DAP** | **4 DAP** | **5 DAP** | **6 DAP** | **7 DAP** |
| **Concentration (A)** | | | | | |
| 40% | 0 | 1.8 | 1.8 | 1.8 | 1.8 |
| 50% | 0 | 2.4 | 2.4 | 2.4 | 2.4 |
| 60% | 1.8 | 8.8 | 9.8 | 11.0 | 14.0 |
| 70% | 3.0\ | 15.6 | 23.4 | 32.0 | 35.2 |
| 80% | 6.4 | 22.2 | 31.8 | 42.8 | 52.2 |
| SE± | 0.40** | 0.90** | 0.80** | 1.09** | 1.30** |
| $LSD_{0.05}$ | 1.12 | 2.52 | 2.23 | 3.04 | 3.63 |
| $LSD_{0.01}$ | 1.47 | 3.32 | 2.94 | 4.01 | 4.79 |
| **Duration (B)** | | | | | |
| 1 min | 0.6 | 3.0 | 4.8 | 7.4 | 9.4 |
| 5 min | 0.8 | 5.4 | 7.0 | 9.0 | 12.8 |
| 10 min | 1.6 | 9.6 | 15.0 | 18.8 | 20.8 |
| 15 min | 3.8 | 14.4 | 17.6 | 24.0 | 26.6 |
| 20 min | 4.4 | 18.4 | 24.8 | 30.8 | 36.0 |
| SE± | 0.40** | 0.90** | 0.80** | 1.09** | 1.30** |
| $LSD_{0.05}$ | 1.12 | 2.52 | 2.23 | 3.04 | 3.63 |
| $LSD_{0.01}$ | 1.47 | 3.32 | 2.94 | 4.01 | 4.79 |
| **Interaction** | | | | | |
| A x B | ** | ** | ** | ** | ** |

*** = F-test significant at 1% probability level*

**Table 2. Interaction effects of concentration x treatment duration on the germination of *Acacia nilotica*, 3 – 7 days after plating (DAP)**

| | Treatment time (min) | | | | |
|---|---|---|---|---|---|
| Concentration (%) | 1 | 5 | 10 | 15 | 20 |
| | | | **Day 3** | | |
| 40 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| 50 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| 60 | 0.0 | 0.0 | 0.0 | 4.0 | 5.0 |
| 70 | 0.0 | 0.0 | 2.0 | 6.0 | 7.0 |
| 80 | 3.0 | 4.0 | 6.0 | 9.0 | 10.0 |
| SE± | 0.90** | | | | |
| $LSD_{0.05}$ | 2.50 | | | | |
| $LSD_{0.01}$ | 3.29 | | | | |
| | | | **Day 4** | | |
| 40 | 0.0 | 0.0 | 2.0 | 3.0 | 4.0 |
| 50 | 0.0 | 0.0 | 3.0 | 4.0 | 5.0 |
| 60 | 0.0 | 6.0 | 9.0 | 12.0 | 17.0 |
| 70 | 5.0 | 9.0 | 10.0 | 22.0 | 32.0 |
| 80 | 10.0 | 12.0 | 24.0 | 31.0 | 34.0 |
| SE± | 2.02** | | | | |
| $LSD_{0.05}$ | 5.63 | | | | |
| $LSD_{0.01}$ | 7.42 | | | | |
| | | | **Day 5** | | |
| 40 | 0.0 | 0.0 | 2.00 | 3.0 | 4.0 |
| 50 | 0.0 | 0.0 | 3.0 | 4.0 | 5.0 |
| 60 | 0.0 | 6.0 | 11.0 | 13.0 | 19.0 |

| | | | | | |
|---|---|---|---|---|---|
| 70 | 8.0 | 9.0 | 25.0 | 28.0 | 47.0 |
| 80 | 16.0 | 20.0 | 34.0 | 38.0 | 49.0 |
| SE± | 1.79** | | | | |
| $LSD_{0.05}$ | 4.99 | | | | |
| $LSD_{0.01}$ | 6.58 | | | | |
| | | | **Day 6** | | |
| 40 | 0.0 | 0.0 | 2.0 | 3.0 | 4.0 |
| 50 | 0.0 | 0.0 | 3.0 | 4.0 | 5.0 |
| 60 | 0.0 | 6.0 | 11.0 | 13.0 | 25.0 |
| 70 | 18.0 | 18.0 | 28.0 | 40.0 | 56.0 |
| 80 | 19.0 | 21.0 | 50.0 | 60.0 | 64.0 |
| SE± | 2.44** | | | | |
| $LSD_{0.05}$ | 6.79 | | | | |
| $LSD_{0.01}$ | 8.96 | | | | |
| | | | **Day 7** | | |
| 40 | 0.0 | 0.0 | 2.0 | 3.0 | 4.0 |
| 50 | 0.0 | 0.0 | 3.0 | 4.0 | 5.0 |
| 60 | 0.0 | 6.0 | 16.0 | 17.0 | 31.0 |
| 70 | 18.0 | 18.0 | 29.0 | 47.0 | 64.0 |
| 80 | 29.0 | 40.0 | 54.0 | 62.0 | 76.0 |
| SE± | 2.91** | | | | |
| $LSD_{0.05}$ | 8.12 | | | | |
| $LSD_{0.01}$ | 10.70 | | | | |

*** = F-test significant at 1% probability level*

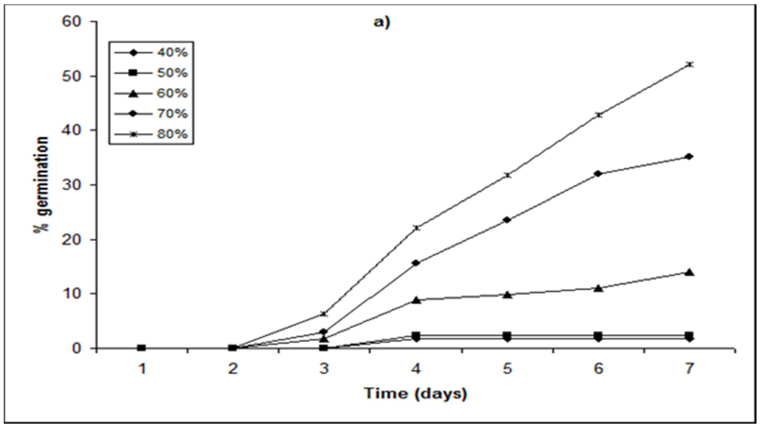


Fig. 1: Effects of different sulfuric acid concentrations on the a) pattern and b) rate of germination in A. nilotica

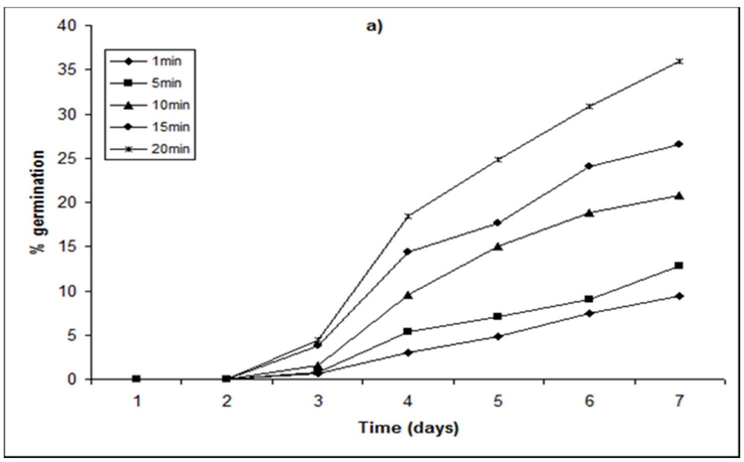


Fig. 2: Effects of different treatment duration with sulfuric acid on the a) pattern and b) rate of germination in A. nilotica

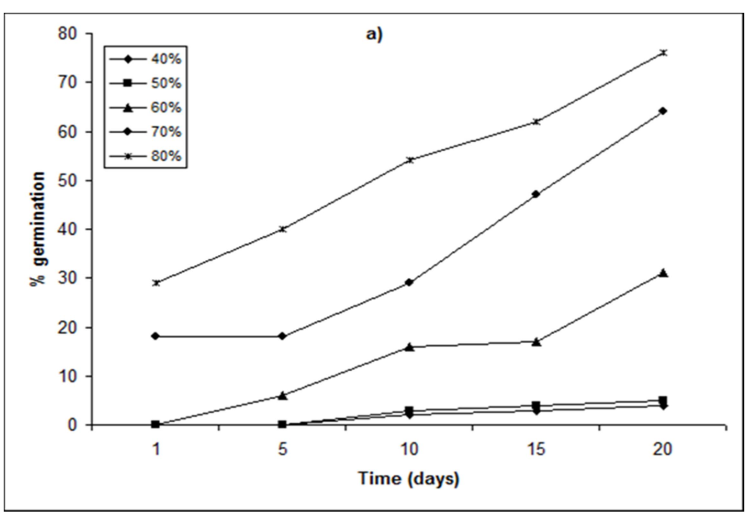

Fig. 3: Interactive effects of concentration x treatment duration with sulfuric acid on the a) pattern and b) rate of germination in A. nilotica