

Impact of the Mineral Fertilization on the Germination and Growth of *Acacia auriculiformis* A. Cunn. ex Benth by the Soilless Technique in Nursery

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To cite this article:

Chauvelin Douh, Clément Sosthène Oko, Ayessa Lekoundzou, Armain Louis Hollat, Mercia Niméliane Mouandza, Félix Koubouana. Impact of the Mineral Fertilization on the Germination and Growth of *Acacia auriculiformis* A. Cunn. ex Benth by the Soilless Technique in Nursery. *American Journal of Agriculture and Forestry*. Vol. 11, No. 3, 2023, pp. 74-81. doi: 10.11648/j.ajaf.20231103.11

Received: December 8, 2022; **Accepted:** January 3, 2023; **Published:** May 24, 2023

Abstract: The growing demand in wood energy requires mechanisms of production of the seedlings with a view to proceed reforestation to answer the needs of the populations. The study evaluates the germination and growth of *Acacia auriculiformis* seedlings in two types of substrates by the soilless technique. The seeds of *A. auriculiformis* species have been collected in Democratic Republic of Congo and sent at the Bateke Brazzaville Forest Plantations Society (SPF2B-nurseries God willing). They have been kept cold for 20 days in a refrigerator at an temperature of about 5°C. A complete device of two substrates (substrate fertilized with NPK 11-7-6, and unfertilized substrate, i.e. 1152 seeds per substrate) has been set up at the Batéké Brazzaville Forest Plantations Society. For NPK fertilization, we have taken 1000 grams of NPK (i.e. a 1 liter cup soaked in a 10 liter watering can). Seeds have been sown individually in each alveolus after treatment. The results reveal germination rates of 20.8% to 23.7%, respectively for the unfertilized substrate and the fertilized substrate. However, the mortality rates of 76.3% to 79.2% have been respectively recorded in the fertilized substrate and the unfertilized substrate. The Kolmogorov-Smirnov test demonstrated that there are significant differences (p-value = 0.86 and p-value = 0.200) on seed germination. As far as that goes, NPK 11-7-6 fertilization had a significant effect (p-value= 0.307) on seedlings growth. The effect of the fertilization on the mean number of phyllode demonstrated that the Shairo-Wilk test values are significant (p-value = 0.243 for the unfertilized substrate and p-value = 0.436 for the fertilized substrate). In so far as the industrial production of the seedlings of *A. auriculiformis* requires mineral fertilizer contribution in nursery, the study suggests large-scale reforestation of *A. auriculiformis* with seedlings from nurseries to ensure the sustainability of the plant considering of importance it plays with nitrogen-fixing bacteria in the soil.

Keywords: *Acacia auriculiformis*, Mineral Fertilization, Seeds, Germination, Seedlings, Nursery, Republic of Congo

1. Introduction

Forests ensure several important ecosystem services whose the production of wood. But, these services expose them to strong anthropogenic pressures which accelerate deforestation and degradation [1]. These anthropogenic pressures directly affect human beings, the environment and local economies [2,

3]. Consequently, reforestation in open environnement with seedlings from seeds collected at the base of the trees and conducted in nursery seems necessary, even essential [4, 5].

Reforestation is therefore an option that could settle these problems faced the populations. Also, to answer effectively to the needs of firewood, industrial wood, lumber and line poles, a few species of fast-growing trees were introduced from the 1950s in the Congo. These include *Pinus oocarpa*, *Eucalyptus*

spp., *Araucaria cunninghamii*, *Araucaria hunsteinii*, *Acacia* sp., *Cordia alliodora* and *Terminalia montalis* [6, 7].

However, the genus *Eucalyptus*, compared to other species, has benefited many research works focused on the genetics and vegetative propagation [8, 6, 9, 10]. For almost half a century, the *Eucalyptus* genus seems used much more in reforestation programs in Republic of Congo, with about 40.000 hectares of the forests reforested [11, 12]. Nonetheless, in the dynamics of sustainable development recommended by the reduction of the emissions from deforestation and forest degradation (REDD+) to countries that abound in natural forests, the production in wood energy is another opportunity to reduce anthropogenic pressure on these and participate at the protection of the biodiversity [13].

With the exception of genus *Eucalyptus*, the genus *Acacia* species are fast-growing legumes and have been preferred in most studies due to their ability to form forest ecosystems fast enough [14-16].

In addition, they enrich the soil with nitrogen and allow optimize agricultural yields and farmer's incomes in the agroforestry production system [17, 18]. Among the species introduced in Republic of Congo, only *Acacia auriculiformis* and *Acacia mangium* have been better able to adapt in the climatic conditions [19, 16]. Unfortunately, these species have not benefited from relatively sustainable and sustained research programs compared to the genus *Eucalyptus*. According to the Bateke Brazzaville Forest Plantations Society (SPF2B), the production of the seedlings intended to support the plantation programs is done for the most part by generative means, that's to say an extensive production which proceeds i.e. by seedlings directly in phytocells, i.e. through the germinator for a maximum duration of 120 days. This process is subject to several technical, logistical and professional constraints.

In order to contribute to a maximum production in wood energy, the generative method does not seem possible. Consequently, the method of industrial production soiless using mineral fertilization to accelerate the growth of the seedlings seems a possible and little explored track. In addition, to optimize the production conditions of *Acacia* seedlings, this study proposes to evaluate the germination and growth of *Acacia auriculiformis* seedlings in two types of substrates using the soiless technique in nursery of the Bateke Brazzaville Forest Plantations Society (SPF2B).

To achieve this, the following assumptions are issued: (i) mineral fertilization significantly influences the duration, germination rate and mortality of *Acacia auriculiformis* seeds; (ii) mineral fertilization has a significant effect on the height growth of *Acacia auriculiformis* seedlings, and (iii) the leaf biomass of the seedlings of fertilized substrate increases gradually compared to unfertilized substrate.

2. Materials and Methods

2.1. Study Site

Acacia auriculiformis seeds have been collected in Democratic Republic of Congo (DRC) in December 2020. They have been transported then received at the Bateke

Brazzaville Forest Plantations Society (SPF2B) and kept cold for 20 days in a refrigerator at an temperature of around 5°C. The experimental device was installed within of the Bateke Brazzaville Forest Plantations Society (SPF2B-nurseries God willing) located about 100 km from Brazzaville on the plateau of the cataracts whose altitude varies of 400 to 550 m. SPF2B (God willing nurseries) is located between 3°10' and 3°20' South latitude and 15°50' East longitude (Figure 1), and extends on a ferrallitic soil of sandy type, blackish color and depleted in mineral matter [20]. It covers an area of 6ha, which only 3ha are used. The SPF2B (God willing nurseries) and its surroundings have a climate contrasted by two distinct periods: a long rainy season from September to May and a short dry season from June to August marked by low temperatures [21]. Temperatures are between 21°C and 24°C [20]. The climate changes from tropical to subequatorial with an annual rainfall ranging from 1400 to 2200 mm [21].

2.2. Experimental Device, Germination Tests and Counting of the Phyllodes

The seeds of *Acacia auriculiformis* A. Cunn. Ex Benth received totalized 2304 seeds. A complete device of two blocks (9 cell boxes per block at the rate of 128 seeds per cell box, i.e. 1152 seeds per block) has been set up at the SPF2B (nurseries God willing). The seeds of *Acacia auriculiformis* being dormant, they have undergone a pretreatment to break the dormancy with boiling water at 90°C [4, 5]. Each block, composed of 1152 seeds, has been installed on a previously sterilized substrate made up of 80% black earth (essential backing for plant root fixation), 4% crushed charcoal backing of trace elements to the plant, a excellent bactericide) and 16% sawdust (backing of organic matter obtained after slow decomposition). Then, block 2 composed of 1152 seeds was fertilized with NPK (11-7-6).

Block 1, also composed of 1152 unfertilized seeds, has been considered as a control block. For the NPK treatment, we have took 1000 grams of NPK (i.e. a 1 liter cup soaked in a 10 liter watering can). After stirring and making sure the NPK was dissolved, Block 2 was deemed to have been fertilized. We have then filled the substrate into the cell boxes by arranging them in parallel rows on the shelves.

The choice of the fertilization repose on the previous studies on seeds of *Acacia auriculiformis* species [18, 15]. The germination tests have been carried out at the SPF2B (God willing nurseries) at an average temperature of 22°C and an average relative humidity of 60%. The seeds have been individually and immediately sown in each cell after treatment January 15, 2021.

Seeds sown (NGS, number of seeds sown) have been watered using a sprayer every other days, and germination has been monitored daily. The inventory number of phyllodes of the seedlings was done every other day during the experiment. The experiment ended April 16, 2021, i.e. after 3 months of monitoring. At the end of experiment, the number of the seeds having effectively germinated (NGG), the number of phyllodes of the seedlings have been estimated for

The germination percentage (TG%) has been then calculated [26, 27]. The counting of the phyllodes has began one week after the first emergences.

Data have been entered in the Excel spreadsheet to generate graphs. After checking the normal distribution of the data by the Kolmogorov-smirnov and Shapiro-wilk tests, the Pearson correlation test has been performed to assess the effect of the fertilization on germination and then verify the link between

To verify the influence of NPK 11-7-6 treatment on the growth and number of phyllodes of the seedlings, the Student's t test has been chosen to compare the mean groups of the two blocks. Thus, the Kolmogorov-smirnov and Shapiro-wilk tests showed that our data followed a normal distribution. Consequently, the conditions were required for Student's t test. Student's t test has been calculated at the 5% level. These analyzes have been performed using SPSS version 17.0 software.



Figure 1. Location of the Bateke Brazzaville Forest Plantations Society (red circle = nurseries God willing) [22].

3. Results

3.1. Duration, Germination Rate and Mortality of the *Acacia auriculiformis* Seeds

Table 1 demonstrates that during 10 weeks of monitoring, 307 seeds germinated among the 1152 seeds sown of the block 1 (unfertilized substrate), with an relative germination time of 23 days. On the other hand, the block 2 (fertilized substrate) displays 276 germinated seeds with an relative duration of germination of 27 days (table 1).

Nonetheless, figures 2 and 3 illustrate the germination and mortality rates of the two blocks in terms to the type of substrates. In the block 2 (fertilized substrate), we observe a germination rate of 23.7%, which differs slightly from block 1 (unfertilized substrate) whose germination rate is 20.8%

(Figure 2). However, mortality rates of 76.3% to 79.2% have been respectively recorded in the block 2 (fertilized substrate) and the block 1 (unfertilized substrate) (Figure 3).

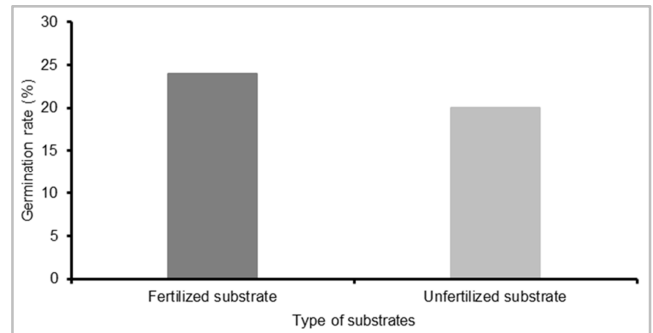


Figure 2. Germination rate in terms of the type of substrates.

Table 1. Overall result of the entire device.

Bloc	Type of substrates	Number of seeds sown	Number of germinated seeds	Duration of germination (days)
Bloc 1	unfertilized substrate	1152	307	23
Bloc 2	fertilized substrate	1152	276	27
Total	-	2304	583	50

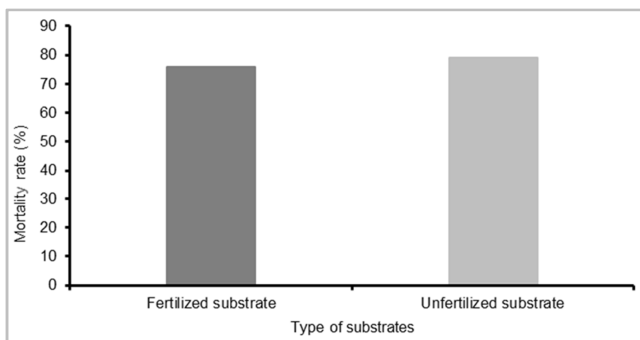


Figure 3. Mortality rate in terms of the type of substrates.

As regards the effect of the fertilization on the germination, table 2 demonstrates that in the blocks 1 (unfertilized substrate)

and 2 (fertilized substrate), the values of the Kolmogorov-Smirnov test are respectively p-value=0.86 and p-value=0.200. On the other hand, the values of the Shairo-Wilk test have showned themselves more significant with values lower to than of the first test and which rejects the hypothesis of normality of block 2 (fertilized substrate) (p-value = 0.035) and of block 1 (unfertilized substrate) (p-value = 0.077). Based on the Kolmogorov-Smirnov test, there are significant differences on the seeds germination, whence the assumption that the data follow the normal law is accepted (Table 2). Nonetheless, table 3 also shows that there is a significant difference (p-value= 0.307) between the two groups of samples highlighted. Consequently, NPK11-7-6 fertilization had a significant effect on the germination and/or seedling growth (Table 3).

Table 2. Normality test of the data distribution on the germination.

Normality test						
Type of substrates	Kolmogorov-Smirlov ^a			Shapiro-Wilk		
	Statistics	ddl	p-value	Statistics	ddl	p-value
Unfertilized substrate	0.228	12	0.86	0.848	12	0.035
Fertilized substrate	0.199	12	0.200*	0.876	12	0.077

Table 3. Group comparison of the means of fertilized block (block 2) and unfertilized (block 1).

		Levene's test on the equality of variances		Means equality test					
		F	p-value	t	ddl	p-value	Means difference	Standard Error	95% confidence interval Inferior Superior
Height (cm)	Hypothesis of equal variances	1.089	0.307	0.334	24	0.741	4.357142857	13.04063668	-22.5574084 31.27169414
	Hypothesis of unequal variances	-	-	0.330	21.936	0.744	4.357142857	13.19621234	-23.0147374 31.72902310

3.2. Average Size of the *Acacia auriculiformis* Seedlings

Figure 4 illustrates the evolution of the average size of the seedlings from fertilized and unfertilized substrates in terms of time. For the fertilized substrate, the evolution of the curve shows that in the first week, the seedlings have an average size of 2 cm for to stabilize relatively to the weeks 5 and 6 with an average size of 5 cm. Finally, to the last week, the seedlings reached an average height of 9 cm in all the fertilized substrate (Figure 4).

However, the evolution of the curve of the unfertilized substrate demonstrates an average size of 2 cm aware of the first and second week of observation (Figure 4). At the fifth and ninth week, the seedlings did not reach one cm more, they remained relatively constant at an average height of 4 cm for to grow slightly to 5 cm at the tenth week (Figure 4).

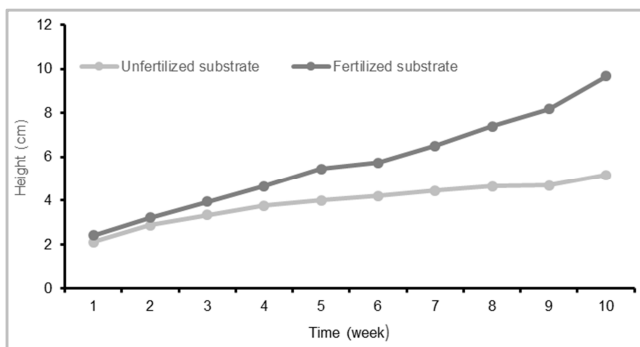


Figure 4. Evolution of the average size of seedlings in terms of time.

Nonetheless, from the point of view of average number of the phyllodes of *Acacia auriculiformis* seedlings, figure 5 demonstrates the evolution of average number of the phyllodes during the 10 weeks of growth of the seedlings from devices with fertilized and unfertilized substrate in nursery. The curve of fertilized substrate illustrates that during the first and second week, the seedlings developed only one phyllode for to obtain a average number of six phyllodes at the seventh week. Finally, at the ninth and tenth week, the seedlings developed average numbers of 10 and 12 phyllodes, respectively (Figure 5).

However, from the first to the third week of observation, the

curve of the unfertilized substrate did not indicate any emergence of the phyllodes (Figure 5). It is only at the fourth week that we observe the emergence of a single phyllode. Nonetheless, the seventh and eighth week distinguished themselves by the average number of 3 phyllodes for all seedlings. Finally, the ninth and tenth week displayed the average numbers of 4 and 5 phyllodes respectively (Figure 5).

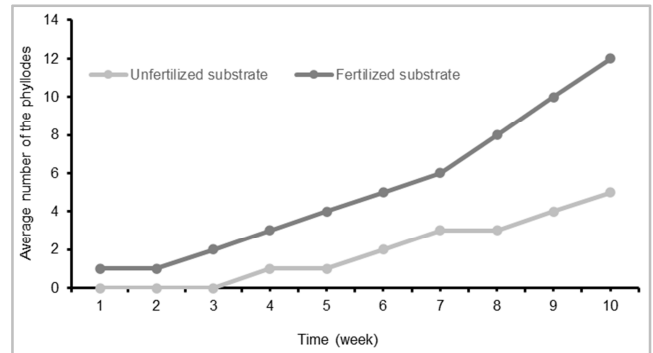


Figure 5. Evolution of average number of the phyllodes in terms of time.

3.3. Effect of the Fertilization on the Average Number of the Phyllodes

Table 4 demonstrates that in unfertilized and fertilized substrates, the values of the kolmogorov-smirlov test are relatively low (p -value = 0.200). On the other hand, the values of the Shapiro-Wilk test illustrated themselves more significant (p -value = 0.243 for the unfertilized substrate and p -value = 0.436 for the fertilized substrate). Consequently, there are significant differences on the mean number of phyllodes, whence the hypothesis that the data follow the normal distribution is accepted (Table 4).

On the other hand, Table 5 shows that there is no significant difference ($t = -2491$; p -value = 0.038) between the two groups of samples highlighted despite the variations of the curves observed (Figures 4 and 5). Consequently, the Student's t -test let believe that the NPK11-7-6 fertilization did not have a significant effect on the average phyllodes number of *Acacia auriculiformis* seedlings (Table 5).

Table 4. Normality test of the distribution of data on the mean number of the phyllodes.

Normality tests						
Type of substrates	Kolmogorov-Smirlov ^a			Shapiro-Wilk		
	Statistics	ddl	p-value	Statistics	ddl	p-value
Unfertilized substrate	0.196	10	0.200*	0.904	10	0.243
Fertilized substrate	0.134	10	0.200*	0.929	10	0.436

Table 5. Comparison of groups of the means of fertilized and unfertilized substrates.

		Levene's test on the equality of variances		Means equality test						
		F	p-value	t	ddl	p-value	Means difference	Standard Error	95% confidence interval	
									Inferior	Superior
Height (cm)	Hypothesis of equal variances	5.004	0.038	-2491	18	0.023	-3.40000000	1.365175338	-6.26612696	-0.531873043
	Hypothesis of unequal variances	-	-	-2491	12.706	0.027	-3.40000000	1.365175338	-6.35622904	-0.443770964

4. Discussion

4.1. The Germination of the *Acacia auriculiformis* Seeds Varies in Terms Mineral Fertilization and Time

In the present study, the duration of germination observed extends on an duration of 23 days on the unfertilized substrate and 27 days on the fertilized substrate. Thus, a difference of three days was observed between the two types of substrates. This difference could result from relatively inappropriate storage conditions of the seeds, resulting in the gradual loss of their germination power. These germination durations are widely superior to those demonstrated in other works who reported a germination duration of 21 days with *Acacia auriculiformis* seeds [28].

The germination rate observed in the present study was relatively low below 50% and has been recorded at 21 days in the both types of substrates. Thus, with regard to the unfertilized substrate, we recorded a germination rate of 23.7% against a mortality rate of 76.3%. On the other hand, the fertilized substrate displayed a germination rate of 20.8% against a mortality rate of 79.2%. In addition, the low rate of germination on the fertilized substrate could be link to the composition of substrate whose contribution of basic fertilizer NPK (11-7-6) certainly influenced negatively on the process of germination of the seeds.

The low germination rate could be explained by several points, in particular: (i) the storage conditions of the seeds after harvest; (ii) the age of the seeds at harvest and (iii) the storage duration of the seeds before sowing [29].

Nonetheless, the results of the present study do not corroborate with the works carried out by Hamawa Y. *et al.* [30], who worked on the effect of pretreatments of the germination of *Acacia senegal* (L.) Wild seeds. in Cameroon. The authors demonstrated a germination rate of 87% for *A. senegal* at 21 days after sowing.

Also, other works on the effect of the different types of substrates on the intensive production of *Acacia auriculiformis* and *Acacia mangium* seedlings in nursery reports higher germination rates of 98.96% and 97.48%, respectively for *A. auriculiformis* and *A. mangium* [31]. These results are widely superior to those of the present study. Overall, we notice in the present study that the samples installed on the fertilized substrate displayed a low germination rate contrary to those installed on the unfertilized substrate.

This a priori surprising observation leads us to deduce that the differences observed between the two types of substrates could be explained by the contrast between the physico-chemical properties of the substrates used [32]. Consequently, in the case of the present study, the mineral fertilizer brought is a limiting factor in the industrial production of *Acacia*, because the nitrogen content in the clod of earth of the cells did not allow to the seeds to pursue their development normal. Indeed, we assume that at this stage of development, the seed is supposed to feed only on its own nutrients to accelerate the germination process.

4.2. Mineral Fertilization with NPK 11-7-6 Has Significantly Influenced Seedlings Height Growth

The results of the present study allowed us to note that the sample with the unfertilized substrate displayed a morphological delay because when the *A. auriculiformis* seedlings are still in nursery, they naturally need to be fertilized for better growth. These results corroborate with other works on the evaluation of the germination and growth monitoring of the *Moringa oleifera* seedlings in nursery [33]. The author demonstrates that the seedlings of *M. oleifera* presented a morphological delay and yellowing of the leaves due to NPK deficiencies.

Also, the seedlings regularly fertilized with NPK and installed on the fertilized substrate seem more vigorous with average sizes of 2.4 cm and 9 cm, respectively at the first and tenth week of observation. This could be explained by the fact that mineral fertilization would provide essential nutrients for the growth of the seedlings. On the basis of this observation, we could therefore conclude that the contribution of fertilizer has a positive influence on the height growth of the seedlings. These results are identical to other studies which have demonstrated that the supply of NPK has a beneficial effect on the growth of seedlings [31-33].

However, the seedlings unfertilized with NPK and installed in the unfertilized substrate demonstrate that the phyllodes of this sample have not been representative, and present a yellowish coloration. This trend could be explained by NPK deficiency caused by lack of fertilization. These results are identical to others on the effect of mineral fertilizer treatment on the branching and the development of vegetative organs in *Gnetum africanum* welw [34]. Likewise, other studies denote the restrained phyllodes presenting an yellow coloration [33]. Nonetheless, the results obtained in this sample are relatively appreciable due to the fact that the phyllodes have a greenish and very dark coloration. This could justify the regular application of mineral fertilization with NPK. These results corroborate the works of other authors [33, 31, 34]. On the other hand, this mineral fertilization had no significant effect on the development of phyllodes. This can be justified by the fact that the dose brought regularly was not sufficient enough for to allow an strong increase in the average number of phyllodes over time.

5. Conclusion

For the necessity to reforest face to the growing demand in timber, it was to assess the influence of mineral fertilizer on the duration of germination, the rate of germination, the growth in height and the development leaf biomass of *Acacia auriculiformis* in order to optimize the production yield. We have effectively showed that the application of mineral fertilizer on the seedlings is very beneficial for their growth after emergence, and accelerates their development by making them more vigorous. Consequently, the industrial production of *A. auriculiformis* seedlings soilless requires a

contribution mineral fertilizer in nursery. On the other hand, it is not possible to bring mineral fertilizer in the substrate at the time of sowing, because it tends to reduce the ability to germinate of the seeds. The study suggests large-scale reforestation of *A. auriculiformis* with the seedlings from nurseries for to ensure the sustainability of the plant in view of its importance as nitrogen-fixing tree through the relationship it plays with the nitrogen-fixing bacteria present in the soil.

Acknowledgements

The authors thanks Marien N'GOUABI University (Republic of Congo), the National Forest Research Institute (IRF), Department of Forest Ecology, the staff of the Bateke Brazzaville Forest Plantations Society (SPF2B-God willing nurseries) for their technical, scientific and financial supports.

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