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Effects of Soil Substrate and Nitrogen Fertilizer on Growth Rate of *Acacia senegal* and *Acacia sieberiana* in North Eastern Uganda

Moses Otuba[1,*], Martin Weih[2]

[1]Natural Resource Management Programme, Nabuin Zonal Agricultural Research and Development Institute, Soroti, Uganda
[2]Department of Crop Production Ecology, Swedish University of Agricultural Sciences, Uppsala, Sweden

Abstract Low tree production as a result of soil nutrient depletion is a major challenge in Uganda. Trees are often planted in marginalized land characterized with very low soil nutrients or absolutely no soil nutrients. The farmers practice poor farming methods such as continuous cultivation and monoculture. The farmers especially smallholders rarely use inorganic fertilizers due to their associated high cost involved. An experiment was set up in North Eastern Uganda to investigate the effects of soil substrate and nitrogen fertilizer on the growth rate of *Acacia senegal* and *A. sieberiana*. It aimed at determining the effects of soil substrate and nitrogen fertilizers on the growth rate of *A. senegal* and *A. sieberiana* seedlings. A multi-factorial experiment design was used for collecting the data to address the objective of this study. Soil N was determined by Kjeldahl method, P by spectrophotometric method and K by Flame photometry method. The seedlings were subjected to four rates of N treatments (0, 50, 100, 150 mg per plant) as a single dose of ammonium nitrate at the start of experiment and using a randomized complete block design. Two-way analysis of variance (ANOVA) was used to test if the magnitude of effect of soil substrates on growth rate and leaf nitrogen concentration varies between the two acacia species. Pearson correlation analysis was used to test if the leaf N concentration functionally relates to the growth. There was a significant effect ($P \leq 0.05$) of the soil substrate and the species- soil interaction on the growth rate between two acacia species. The mean relative leaf length of *A. sieberiana* (0.013 mm mm^{-1} d^{-1}) in unfertilized (N0) soil A was higher compared to those in the soils treated with N fertilizer. While there also was no significant effect ($P \leq 0.05$) of species, soil substrate and species-soil interaction on the relative stem, leaf biomass growth and the relative root biomass growth at final harvest, there was a significant species effect ($P \leq 0.05$) on the leaf N concentration. There was no statistically significant effect ($P \leq 0.05$) of the leaf N concentration on the growth traits of the two acacia species in all treatments. It can be concluded that unfertilized soil substrates increased the growth rate of *A. senegal* and *A. sieberiana* seedlings more than N fertilizer treatments.

Keywords Trees, Production, Ammonium nitrate, Nutrients, Seedlings, Semi-arid areas

1. Introduction

Acacia tree resources are widely distributed in the arid and semi-arid parts of tropical Africa, from Senegal and Mauritania in the west to Eritrea and Ethiopia in the North-East and to South Africa [22]. The acacia species grow well on the dry and rocky hills, and in low-lying dry savannas with annual rainfall of approximately 250 - 350 mm. They also tolerate a maximum temperature of 50℃ and a minimum temperature close to 0 ℃ and soil pH of approximately between 5.0 and 8.0 [5].

Despite the unfavorable growth conditions for most plants in the arid and semi-arid areas, these acacia species are appreciated in these areas because of their specific morphological and physiological attributes enabling them to cope with those conditions. These trees are used by the rural communities and manufacturing industries in many ways, for example *A. senegal* to restore soil fertility in rain-fed sorghum-producing areas consisting of clay soils in the Blue Nile region, Sudan [19]. According to [19] also state that these trees can be used for rotational bush – fallow systems. The rotational system consists of relatively short periods of crop cultivation followed by longer periods of fallow under mainly naturally regenerated *A. senegal* trees when soil fertility declines.

The gum exudates tapped from *A. senegal* and *A.sieberiana* also contribute to environmental rehabilitation and desertification control through stabilization, reduction of surface run-offs and sheet erosion and soil micro-climate improvement in the Kordofan region of Sudan [6]. In addtion the trees are used as fencing materials, fuel wood, poles,

* Corresponding author:
mosesotuba@yahoo.com (Moses Otuba)
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fibre, crafts, medicine and tannins in Uganda.

Given the major role of *A. senegal* and *A. sieberiana* in the development of local communities in Uganda specifically and globally in general, there are plans to invest into these two species with the aim to increase growth rate and the production of gum exudates [7]. Increased use of the two species could be an opportunity for alleviating poverty in the rural areas particularly in the semi-arid areas.

However, recent studies show that the productivity and fertility of soils in the world are declining due to degradation and intensive use of soils without a consideration of proper soil-management practices [11, 5]. It is estimated that around 60 % of cultivated soils limit plant production [5].

Several factors such as soil type, farmers' practice, crop residues and mineral fertilizer management influence plant growth and yield [3]. Among those factors, soil texture and the chemical composition of soils remain the major constraints to crop production in large scale in the tropical regions of Africa [18].

This shows that in the coming decades, plant-nutrition -related research will be a high- priority research area contributing to crop production and sustaining soil fertility. Nitrogen, P, and K are the most limiting nut rients to plant growth and crop yield [17]. Nitrogen is primarily required for increasing plant growth and crop yield more than any other nutrients. The absence of N in the plants is often associated with slow growth, reduced leaf size, yellowing; short branches, premature fall colour and leaf drop, and increase the likelihood of some diseases.

On the other hand, high amounts of these nutrients often result in excessive shoot and foliage growth, reduced root growth, reduced fruit quality, low plant food reserves, and increased susceptibility to environmental stresses and some plant diseases [8]. Excess N can also lead to an accumulation of nitrate in the edible foliage of plants such as spinach and forage.

Although the productivity of most trees are well documented, studies on effects of soil substrate and nitrogen fertilizer on growth rate of *A.senegal* and *A. sieberiana* under the operational nursery conditions in Uganda are scanty. To bridge these gaps in knowledge, it was necessary to conduct this study so that more knowledge is generated on the nutrient requirements of these two species. An assessment of soil substrate and nitrogen fertilizer therefore will contribute to equip the stakeholders with the relevant information for improved silvicultural management practices under the nursery operations. It was also hypothesized that (i) the magnitude of effect of soil substrates on the growth rate and leaf nitrogen concentration varies between the two acacia species and (ii) the leaf N concentration is functionally related to the growth traits of the two acacia species.

2. Materials and Methods

Seeds of *A. senegal* and *A. sieberiana* and soil samples were collected from Moroto and Kotido districts in North Eastern Uganda, East Africa (Figure 1). The region is located between latitude 1° 30'– 4° N, longitude 33° 30'– 35° E at an altitude; of 1400 m above sea level [21]. The National Environment Management Authority, reports Moroto and Kotido districts in Karamoja region as semi-arid with distinct wet and dry seasons [7].

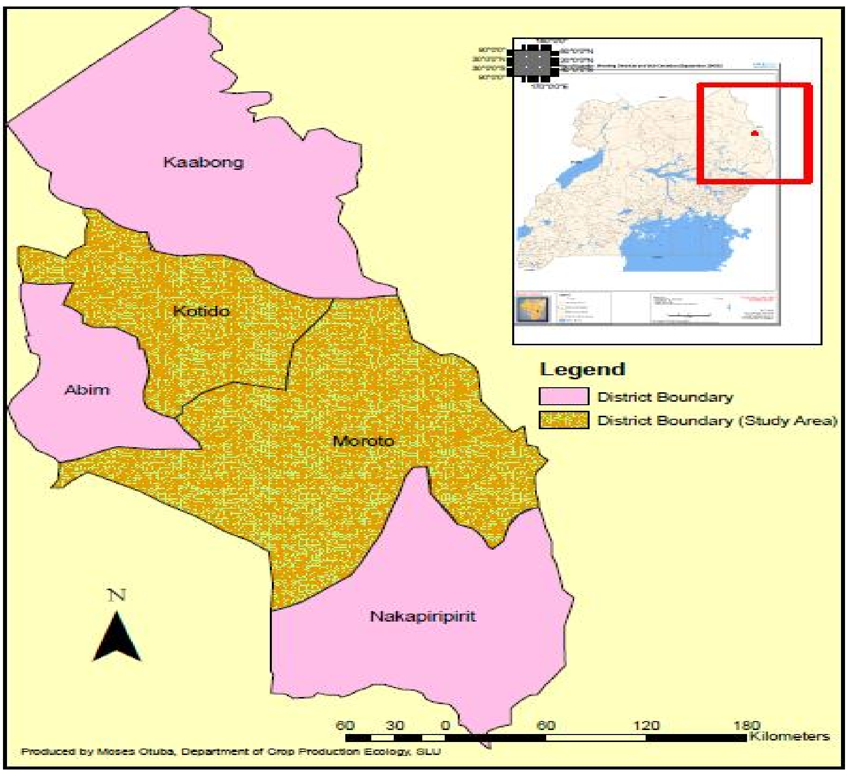

Figure 1. Location of Kotido and Moroto districts in Uganda

The amount of rainfall is inadequate, not evenly distributed and unreliable [23, 6, 7]. The mean annual rainfall is about 600 mm per year with the higher amount in the surrounding mountain ranges. The average annual temperatures range from 30℃ to 35℃ [10]. The soils are classified as vertisol and some are sandy easy to dig while others are heavy difficult to dig. The vegetation has faced gradual degradation since the 1960s because of deforestation, overgrazing, fires and mono cropping.

The seeds of the two acacia species and soil (0 - 25 cm) were collected randomly from a sample plot measuring 30 m x 30 m in each district and a multi-factorial experiment was set up. Soil A and B from Moroto and Kotido district, respectively, were air dried and sieved (2 mm) to remove large debris like leaves and stones. Soil A was lighter than soil B, i.e it was easier to excavate soil sample A than soil sample B. However, both of them were crushed into small particles, then mixed separately and filled in 160 larger U-plastic pots; of 2 L volume for establishing the experiments in the greenhouse. Small quantity of the soil was removed from each pot before and after sowing seeds to determine initial and final amounts of Nitrogen (P), Phosphorus (P) and Potassium (K). This was to rule out if the growth of seedlings is influenced by the available soil nutrients or through nutrients fertilisation during the experiment. N was determined by Kjeldahl method, P by Spectrophotometric method [14] and K by Flame photometry method [1].

Before sowing the seeds into the larger plastic pots, they were scarified with concentrated sulphuric acid, H_2SO_4 for 20-30 min to break the dormancy and then rinsed several times with tap water. The seeds were sown 2-3 per pot to reduce risks of gap filling, topped with sand to maintain equal volume and then watered daily throughout the entire experiment duration of 8 weeks. To avoid nutrient leaching, the pots were placed on plastic polythene sheet. After 1-2 weeks from the start of experiment, seedling number per pot was reduced, leaving only a single healthy seedling per pot.

A total of 160 seedlings were raised in the nursery shade for one month, of which 80 seedlings of *A. senegal,* 40 each were grown in soil A and B, respectively, and another 80 seedlings of *A. sieberiana,* 40 each grown in soil A and B.

Prior to the start of ammonium nitrate (NH_4NO_3) treatments, 32 seedlings were harvested, i.e. 8 replicate seedlings for each soil type (2) and species (2) to determine initial shoot height, leaf length, shoot and root biomass (i.e. biomass allocation), as well as leaf N content. During the experiment, the seedlings were grown in the greenhouse with humidity of 78 % and temperature of 30℃ at National Semi Arid Resources Research Institute in Uganda. The seedlings were subjected to four rates of N treatments (0, 50, 100, 150 mg per plant) as a single dose of ammonium nitrate at the start of experiment and using a randomized complete block design. Eight replicate seedlings were grown in each species (2), soil type (2) and N fertilizer treatment (4) combination for one month.

At the end of the N fertilization experiment, the remaining 128 seedlings were harvested to determine the final shoot height and leaf length as well as leaf N content. The seedlings were also irrigated with demonized water to reduce the concentration of ammonium nitrate immediately after its exposure to seedlings.

2.1. Measurements

The shoot heights and leaf length of the two acacia species in the soil A and B substrates were measured before and after treatment of the seedlings with N fertilizer. Seedling heights were measured from the soil surface in the pots to the end of the shoot while the length of three leaves at bottom of each plant were measured from axillary bud by use of a tape measure.

Leaf N concentration was determined from 80 acacia samples i.e 16 samples analyzed after first phase of seedling harvesting and then 64 after second phase using a Kjeldahl method [14] at the Department of Agricultural Production, Makerere University, Kampala in Uganda .

2.2. Statistical Data Analysis

The functional approach to plant growth analysis [20] was used to compare seedling growth rates in various species and treatment combinations, by means of relative growth rates. Thus, the relative height growth RGR_{HT}, and relative leaf length RGR_{LL} were computed according to the following;

$$RGR_{HT}\ (cm\ cm^{-1}\ d^{-1}) = (InHT_2 - InHT_1) / (t_2.t_1) \quad (1)$$

$$RGR_{LL}\ (mm\ mm^{-1}\ d^{-1}) = (InLL_2 - InLL_1) / (t_2\text{-}t_1) \quad (2)$$

Where t_2: final time (weeks), t_1: initial time, HT_2: final seedling height (cm), HT_1: Initial seedling height (cm), LL_2: final leaf length (mm), LL_1: initial leaf length (mm).

The experiment was analyzed by general linear models with the species and substrates (soil types) as the main factors using Microsoft Office Excel 2007. Two-way analysis of variance (ANOVA) was used to test if the magnitude of effect of soil substrates on growth rate and leaf nitrogen concentration varies between the two acacia species.

The least square means were used to detect significance of on growth rate and leaf nitrogen concentration of the two acacia species. An effect was considered to be significant if its p-value was ≤0.05. On the other hand, Pearson correlation analysis was used to test whether the leaf N concentration functionally relates to the growth, i.e. height and leaf length of the two acacia species. An effect was considered to be significant if its correlation coefficient was positive value and if its p-value was ≤0.05.

3. Results and Discussion

3.1. Growth Rate

Analysis of variance of data on growth rate showed that there was a significant effect ($P \leq 0.05$) of soil substrate on relative height growth (RGR_{HT}), as well as significant species (S) effect and species-soil (S x SN) interaction on relative leaf length (RGR_{LL}) between the two acacia species

(Table 1).

This suggests that height growth was significantly greater in soil A compared to soil B, regarding leaf length growth: *A. sieberiana* had a much greater leaf length growth than *A. senegal*, and leaf length growth of *A. sieberiana* was lower in soil B compared to soil A, whereas in *A. senegal*, leaf length growth was greater in soil B compared to soil A. This supports the hypothesis that the magnitude of effect of soil substrates on growth rate varies between two acacia species.

Soil A was more porous and lighter than soil B, possibly indicating some differences between the soil substrates used here. Lighter soil possibly facilitates atmospheric N fixation by microorganisms that frequently are associated with acacia roots. Soil A being lighter than soil B could also have allowed water to more easily infiltrate in it, thereby enhancing the growth of the two acacia species.

The RGH_{HT} of *A. sieberiana* and *A. senegal* were significantly higher in the N0 treatments compared to those of N50, N100 and N150 treatments. (Figure 2a). This suggests that amount of N fertilizer applied in this study may not be suitable for biomass production of these acacia seedlings especially at the age of 2 months. Nevertheless, this needs further investigations on a complete and balanced nutrient solution with small quantities of N fertilizers less than the rates used in this study (Table 3). The age of acacia seedlings can as well as be taken into consideration under nursery conditions.While *A. siberiana* had the highest RGR_{HT} in soil B of the N50, N100 and N150 treatments, it had very low RGR_{HT} in soil A (Figure 2a).

The RGR_{LL} of *A. senegal* and *A. sieberiana* in this study varied depending on the soil substrates and levels of N treatments. The results show that RGR_{LL} of *A. sieberiana* (0.013 mm mm^{-1} d^{-1}) in unfertilized soil A (N0 treatments) was higher compared to those in the soils treated with N fertilizer (N50, N100 and N150) (Figure 2b). *A. senegal*, however, had higher RGRLL (0.012 mm mm^{-1} d^{-1}) in soil B treated with N50 mg / plant compared to those treated in soil A and B with N100 and N150 mg / plant.

This suggests that the growth conditions for these two acacia species were altered by the treatment with single N fertilizer doses. In the experiments of this study, addition of large quantities of single nutrients, i.e nitrogen without the proportional addition of other nutrients could have resulted in very strong growth limitations by other nutrients (most likely e.g. phosphorus) (Table 3). The addition of large quantities of ammonium nitrate applied as a salt solution might have exposed these plants to a "salt chock" and hampered their growth. Consequently, there was a very poor growth of all plants exposed to any of the N treatments, except the unfertilised treatments (N0), which did not receive any additional nitrogen.

In addition, the results agree with other findings of [9] and [4] that application of N in excess to the plants does not increase yield or vegetative growth but negatively affect their derived products. This may also cause underground contamination if leached with excess irrigation or rainfall [2].

Other studies report that fertilizer salts usually build up when plants are irrigated and affect plants indirectly by changing soil permeability, water and nutrient availability, and directly by ion toxicity [17]. According to [16], salt tolerance of acacias is affected by the genetics and seed sources of the materials used since plant tolerance to salinity is controlled by genetics. Nevertheless, high doses of N fertilization may be important in building up internal nutrient reserves of seedlings by inducing luxury nutrient consumption [24]. The higher reserves are a readily available source of nutrients for remobilization and retranslocation to new growth soon after planting, a critical period of plantation establishment. The partly very high leaf N concentration in the seedlings exposed to N fertilization in this study indicates some evidence for luxury consumption in those seedlings. Nutritional stress during seedling establishment would be characterized by limited root development in the soil and increased exploitation of internal nutrient reserves [24].

Table 1. Summary of analysis of variance on the effects of species, soil substrate and their interaction on relative growth height, relative leaf length of *A.senegal* and *A. sieberiana*

Source	SS	df	MS	F-value	P-value	LoS
RGR_{HT} (cm cm^{-1} d^{-1})						
Species (S)	3.511×10^{-6}	1	3.51×10^{-6}	0.1015	0.7524	N.S
Soil substrate (SS)	0.00016	1	0.00016	4.6311	0.0402	*
(S x SS)	1.326×10^{-5}	1	1.32×10^{-5}	0.3833	0.5408	N.S
Error	0.00097	28	3.46×10^{-5}			
RGR_{LL} (mm mm^{-1} d^{-1})						
Species (S)	0.00017	1	0.00017	6.0490	0.0204	*
Soil substrates (SS)	3.4×10^{-7}	1	3.4×10^{-7}	0.0121	0.9133	NS
(S x SS)	0.00027	1	0.00027	9.4772	0.0046	*
Error	0.0046	28	2.82×10^{-5}			

LoS: Level of Significance, *: significant at P≤0.05, N.S; Not Significant

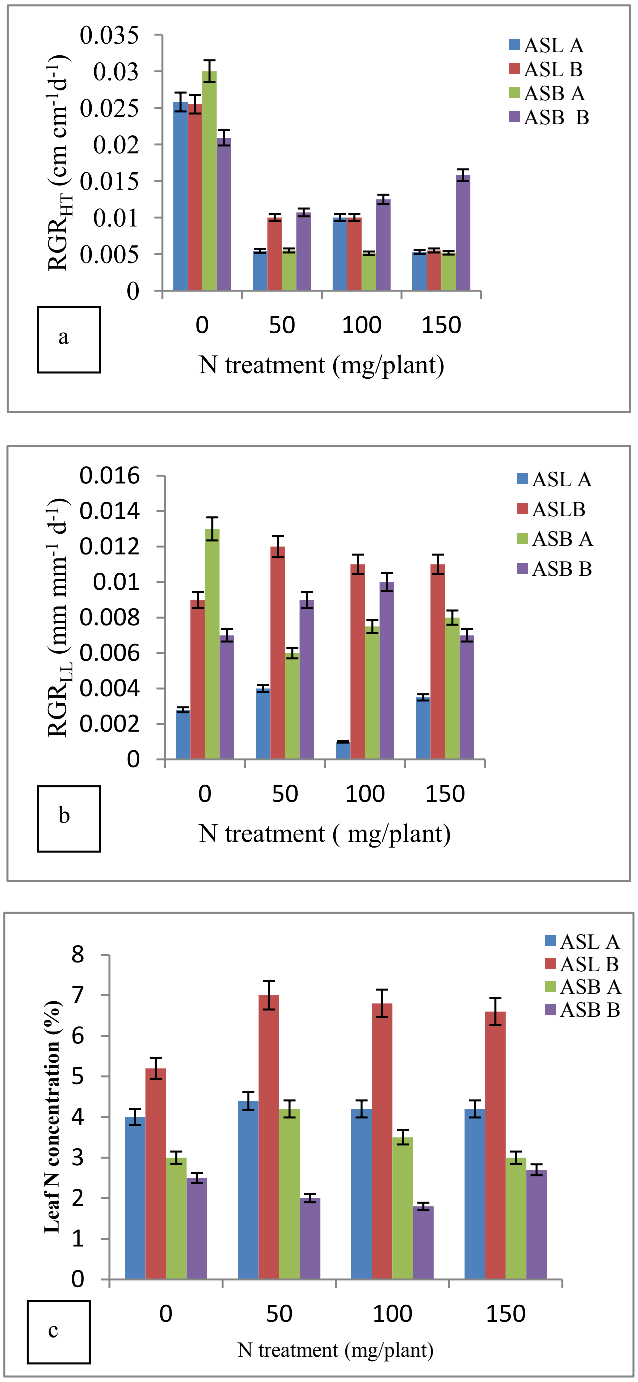

Figure 2. (a) The influence of nitrogen (N) fertilizer on mean relative growth height (RGRHT) A. senegal (ASL) and A.sieberiana (ASB) seedlings in soil A and B, (b) A comparison of height of *A.sieberiana* seedlings in the pots treated with N fertilizer with those of N0 treatments in greenhouse. (c) The influence of nitrogen (N) fertilizer on mean relative leaf length (RGR_{LL}) of *A.senegal* (ASL) and *A.sieberiana* (ASB) seedlings in soil A and B)

3.2. Leaf N Concentration and Growth Traits

The mean leaf N concentration of *A. senegal* and *A. sieberiana* in this study varied depending on the soil substrates and levels of the N treatments (Figure c). At no fertilization, A. senegal seedlings had higher leaf N concentration than A. sieberiana (Figure 2c). The seedlings of *A. senegal* in the soil substrates A and B treated with N fertilizers had higher leaf N concentration compared to those in soils with N0 treatments (Figure 2c). In all three N treatments (N50, N100 and N150), *A. senegal* had a higher leaf N concentration (7.1%, 6.6%, 6.3%) in soil B respectively compared to the N0 treatments.

On the other hand, *A. sieberiana* had a lower leaf N concentration (1.9%, 1.6%) in soil B treated with N50 and N100 respectively over the N0 treatments (Figure 2c).

Analysis of correlation of data on the growth traits indicated that there was no significant effect ($P \leq 0.05$) of the leaf N concentration on the growth height and leaf length of the two acacia species in all treatments (Table 2).

There was a negative correlation between the leaf N concentration and growth traits, i.e. growth height and leaf length of the two acacia species in all treatments (Table 2). The growth height of the two acacia species had a higher negative correlation coefficient (-0.43) in soil A and B treated with N150 mg / plant after final harvest compared to other treatments (Table 2). However, the leaf length of the two acacia species had a lower negative correlation coefficient (-0.001) in soil A and B treated with N100 compared to other treatments (Table 2).

This suggests that supply of nitrogen to the soil substrates increased its concentration in the leaves of Acacia seedlings although it did not lead to increased growth rates. This is also supported by the facts that there was negative correlation between the leaf N and the growth traits of the two acacia species (Table 2), which indicates that additional N reserves were stored in leaves ("luxury consumption") rather than used for growth, because growth was limited by other factors (cf. Other nutrient elements).

This further suggests that the seedlings were unable to translate the partly high leaf N concentrations into the growth in this study. These results are not consistent with other findings from studies using more balanced nutrient additions and indicating that the effects of concentration of N on photosynthesis of leaves may influence growth and partitioning of dry matter in trees [20] , thereby contributing to the increase in the leaf length as well as the plant height.

The results also showed that there was no statistically significant effect of the leaf N concentration on the growth traits of the two acacia species in all treatments (Table 2). This shows that the hypothesis that the leaf N concentration functionally relates to the growth traits of the two acacia species was rejected. Therefore, there was no impact caused the nutrient supply from both soil substrates and the N fertilizers on the growth rate of the two acacia species.

Table 2. Correlation Analysis between the leaf N concentration and the growth height and leaf length of two acacia species in soil A and B treated with N0, N50,N100 and N150 mg / plant after final harvest

DV	N0		N50		N100		N150	
	CE	PV	CE	PV	CE	PV	CE	PV
Growth Height	-0.121	0.656	-0.064	0.809	-0.124	0.647	-0.428	*0.098*
Leaf length	-0.317	0.232	-0.079	0.769	-0.001	0.999	-0.197	*0..466*

DV: Dependant Variable, CE: Correlation Efficiency, PV: P-Value, Significant at 0.05

Table 3. Mean chemical characterization of soil substrate before and after the experiment

Treatment (mg/plant)	Chemical content of soil before			Chemical content of soil after		
	N (%)	P (%)	K (%)	N (%)	P (%)	K (%)
0	0.156	0	0	0.05	0	0
50	0.145	0	0	2.95	0	0
100	0.155	0	0	3.00	0	0
150	0.135	0	0	3.23	0	0

4. Conclusions

Unfertilized soil substrates increased the growth rate of *A. senegal* and *A. sieberiana* seedlings more than the N fertilizer treatments.

The two soil substrates affected the growth traits of the acacia species.

The leaf N concentration also caused no impact on the growth traits of the two acacia species in all treatment.

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