

Effects of Poultry Manure, Kitchen Ashes and Mycorrhizae on the Growth, Chlorophylls and Carotenoids Contents of Banana (*Musa* spp) Plants Coming from Stem Fragments

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Abstract: The aim of this study was to find alternatives to the use of chemical fertilizers to help improve the availability in the nursery of plants of the Batard and Big ebanga cultivars of plantain. The trial was set up at the IRAD Agricultural Research Station in Njombe using a completely randomized Fisher block system. The study assessed the impact of three alternative fertilizers (Poultry manure, kitchen wood ash, mycorrhizal fungi) on the evolution of morpho-physiological parameters (number of leaves, pseudostem height, collar circumference and leaf area) and biochemicals (chlorophylls and carotenoids) of the plants with NPK treatment serving as a positive control. Ten weeks after treatments, results show that the poultry manure and the NPK have a significant impact on the morphological and biochemical parameters compared to the other treatments. The average heights of the pseudostem and that of the circumferences at the collar of the plants treated with NPK are respectively 20.1 cm and 5.7 cm for the Batard variety and 22 cm and 6 cm for the Big ebanga variety; those of plants treated with poultry manure are 17.4 cm and 5 cm for Batard and 19.2 and 5.6 for Big ebanga. The chlorophyll contents of the leaves of the plants treated with NPK and poultry manure are respectively 21.1 and 15.1 for Batard and 26.9 and 23.3 µg/ml for Big ebanga. A mass of 7g per plant of poultry manure appear as an alternative to the use of chemical fertilizers for producing plants from stem fragments.

Keywords: Mycorrhiza, Fertilization, Growth, Plantain

1. Introduction

Thanks to its richness in carbohydrates, the plantain contributes to the fight against hunger since it constitutes a basic product of great consumption in Cameroon both in the countryside and in the cities. Plantain is consumed in more than twenty forms (steamed plantain, crisps, fries, pounded plantain flour, etc.) nationally according to regions and ethnic groups [20]. Parasitic pressures and the use of already contaminated shoots for the establishment of a banana plantation limit the duration of exploitation and consequently

reduce the availability of good quality plant material. Today, one of the possible solutions for obtaining many healthy plantain banana plants is the PIF (plant from stem fragment) technique implemented by the African Plantain Research Center (CARBAP) located in Njombe in Cameroon [11]. The depletion of cultivable soils reduces production yield, which is why fertilization is used.

Organic and biological fertilizers also improve the physical, chemical and biological properties of the soil [1, 7]. They provide the soil with humus and all the nutrients necessary for plant growth. However, the use of chemical fertilizers is limited among small farmers given the high cost and

accessibility [29]. In a global context marked by the rising cost of mineral fertilizers and their adverse side effects on the environment, the use of organic and biological fertilizers appears as a panacea in the agriculture of less developed countries like Cameroon. Hence the present study: The specific objectives were (i) to evaluate the effect of mycorrhizae, poultry manure, NPK and kitchen ashes on the growth parameters of plantain PIF plants in the nursery; (ii) Analyze some biochemical parameters (chlorophylls and carotenoids) of the leaves of the PIF plants resulting from the different parameter treatments responsible for the expression of the morphological parameters.

2. Materials and Methods

2.1. Plant Material

Plant material used consists of live plantain plants of the Big ebanga and Batard cultivars produced by the PIF technique (plants from stem fragments) in the IRAD seedbeds.

2.2. Study Site

The work was carried out under shade on the research sites of the Agricultural Research Institute for Development (IRAD), Research station of Njombe-Cameroon located in the monomodal rainforest area (4°35'00" North, 9°40'00" East).

2.3. Trial Device

Under a shade house, a completely randomized block device (Fisher) was selected for this experiment. For each of the varieties, two blocks (repetitions) were formed. Total randomization was done in each block for the five treatments selected. Each treatment had an average of ten plants. For the 'Big Ebanga' and 'Batard' a total of 200 plants (100 plants per variety and 45 plants per block) were used.

$$\text{Chlorophyll a} = 12.25 (\text{OD Chlb}) - 2.79 (\text{OD Chla})$$

$$\text{Chlorophyll b} = 21.5 (\text{OD Chla}) - 5.1 (\text{OD Chlb})$$

$$\text{Chlorophyll a} + \text{chlorophyll b} = 7.15 (\text{OD Chlb}) + 18.71 (\text{OD Chla})$$

$$\text{Carotenoids} = ((1000 \times \text{OD Carotenoids}) - ((1.82 \times \text{Chla}) + (85.02 \times \text{Chlb}))) / 198$$

2.6. Statistical Analysis of Data

Statistical analysis were performed using R version 2020 software. The data collected was subjected to an analysis of variance. The comparison of the means, in the event of significant results, was made by Duncan's test at the 5% level.

3. Results and Discussion

3.1. Substrate and Fertilizers Chemical Characteristics

The results of the analyzes of the particle size, the contents

2.4. Fertilization

2.4.1. Mycorrhiza Inoculation

The strains of mycorrhizal fungi come from the regional laboratory for applied biological and microbiological control of IRAD located in Yaoundé (Nkolbisson). The inoculum consisted of a 50% mixture of propagules of *Acaulospora tuberculata* and *Gigaspora margarita*. 50g of inoculum were introduced two weeks after transplanting the seedlings as a single application [21].

2.4.2. Fertilizer Spreading

Poultry manure from a local farm, Kitchen ashes obtained by burning firewood and butternut tree branches and NPK (21-8-12+2MgO+2.7S+2.5CaO) sold locally were applied per plant in a circular band every two weeks for ten weeks as 4g of ash [9], 1g of NPK [17] and 7g of poultry manure [16].

2.5. Assessment of Growth Parameters and Pigments

2.5.1. Growth Parameters

The growth parameters measured are those of Anno [2] and Herrera and Aristizabal [14]. Every two weeks, observations made it possible to measure the circumference of the plant at the level of the collar, the height of the pseudostem, the number of leaves and the leaf area of the last leaf. This leaf area (S) was calculated as followed: $S = 0.8 \times L \times l$, in which L and l are respectively the length and width of the youngest leaf [8]. Ten weeks after inoculation, shoots and roots fresh weights were assessed.

2.5.2. Assessment of Chlorophyll and Carotenoids Contents

The chlorophyll and carotenoid contents were determined according to the Lichtentaler method [19]. This determination is based on the principle that acetone is a solvent that will extract chlorophyll pigments from plant cells. The concentrations estimated in µg/ml of pigments are calculated according to the method described by Lichtentaler.

of the mineral elements and the chemical properties are presented in table 1. The data reveals that the substrate (control) used has a sandy-clay texture, a neutral pH and 6.9% organic matter.

It appears from table 1 that the poultry manure used are richer in nitrogen and phosphorus, and less rich in potassium than kitchen ashes. Regarding trace elements (sodium, magnesium, calcium) kitchen ash is richer than poultry manure.

Table 1. Chemical characteristics of the treatments.

Elements	T3	T5	T2
Clay (%)	22.5± 0.7	-	-
Silt (%)	1.5± 0.1	-	-
Sand (%)	76±1	-	-

Elements	T3	T5	T2
pH-H ₂ O	6.9±0.3	10.9±0.17	9 ±0.1
OC%	4±0.3	0.5 ±1.7	26 ±0.1
OM%	6.9±0.1	1 ±0	52 ±1.73
CEC pH7 (meq/100)	42±2	63 ±1	64 ±1
C/N	7±0.2	2±1	18 ±0
N (%)	0.56±0.030	0.83 ±0.11	1.43± 0.05
P (%)	0.01±0.006	0.05 ± 0.1	0.6±0.01
K (%)	0.0001±0.00	6.1±0.17	2.4 ±0.1
Ca (%)	0.002±0.001	3.4 ±0.2	3.7±0.1
Mg (%)	0.0007	6.2 ±0.17	1.2 ±0.05
Na (%)	0.88±0.1	3.13 ±0.05	2.33 ±0.05

T3: Control (soil); T5: Kitchen ashes; T2: Poultry manure.

3.2. Effects of Fertilizers on Plant Morphological Parameters

3.2.1. Pseudostem Height

Figure 2 presents respectively the absolute growth rates in height of the pseudostem of Batard and Big ebanga. The highest values were obtained with the NPK (20.1 cm) and poultry manure (17.4 cm) treatments for batard. Regarding Big ebanga, the height of the pseudostem varies from 12.4 cm to 22 cm and the highest average values are those of plants treated with poultry manure (19.2 cm) and NPK (22 cm). The Kitchen ash treatment is the one that induced the lowest growth rate.

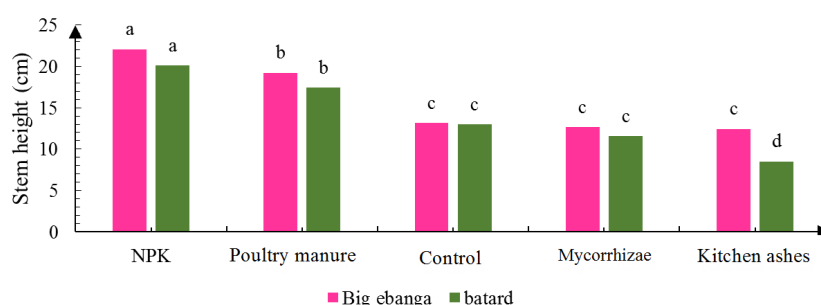


Figure 1. Pseudostem's heights of the treatments for the two varieties. The means with different letters in the figure are significantly different at 5% of average probability according to Duncan's test.

3.2.2. Collar Circumference

The average values relating to the circumference at the collar of the pseudostem varied from 3.7 to 5.7 cm for Batard and from 4.4 to 6 cm for Big-ebanga depending on the treatments applied highest circumferences were observed with plants treated with poultry manure and with NPK.

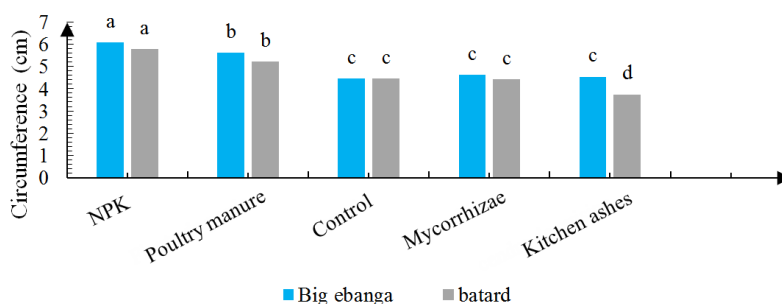


Figure 2. Collar circumference as a function of treatments and variety. The means with different letters are significantly different at the 5% level according to Duncan's test.

3.2.3. Leaf Area

70 days after fertilization, the leaf area varies from 45.8 to 258.6 cm² for the Batard variety and from 69 to 266.9 cm² for Big-ebanga. The leaves of the plants treated with NPK and chicken droppings developed well compared to the other treatments due to their very high average values.

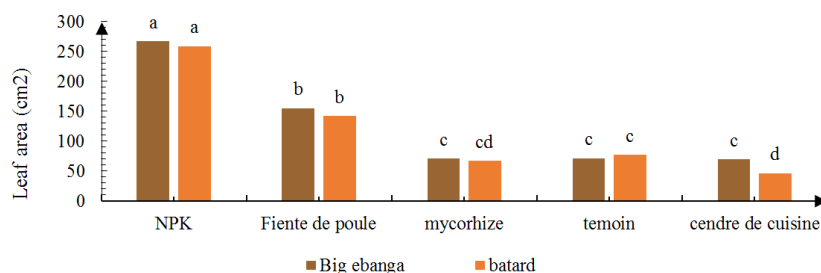


Figure 3. Leaf area of varieties as a function of treatment. The means with different letters are significantly different at the 5% level according to Duncan's test.

3.2.4. Leaves Number

NPK, poultry manure, kitchen ash and control treatments emitted more leaves than the mycorrhizae for the Batard. The average values of the number of leaves emitted by Big

ebanga plants are between 3.9 and 4.9. At the 5% threshold according to the Duncan test, the NPK and chicken droppings treatments are significantly different between them and the other treatments.

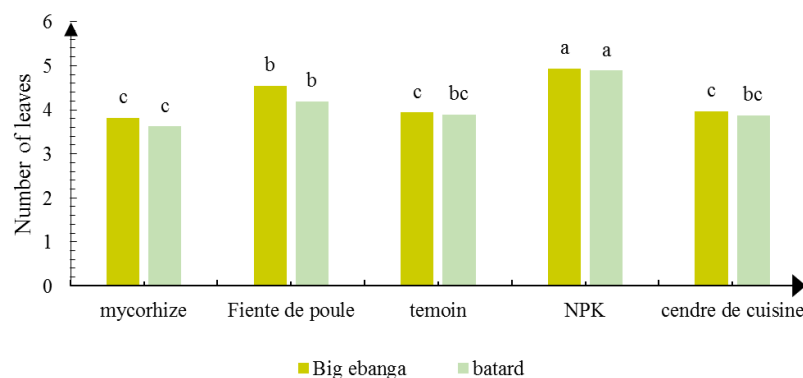


Figure 4. Number of leaves by treatments for the varieties. The means with different letters in the figure are significantly different at the 5% level according to Duncan's test.

The soil analysis showed that the mineral element contents of the treatments were higher than those of the soil values of the experimental site. This low level of soil nutrients thus caused a delay in the development of the plantain vivoplants, which justifies the low growth parameters for the control plants. Our results corroborate those of Hitha *et al.* [15] and Czarnecki and Düring [10] who showed that fertilization improves the quality of the soil in terms of mineral elements (nitrogen, potash, phosphorus and trace elements). These results could be explained by the fact that the use of fertilizer stimulates the leaf meristem and allows the plantain to grow and complete its crop cycle. Plants treated with chicken droppings (T2) and NPK (T1) have better growth than those treated with mycorrhizae (T4), cooking ashes (T5) in the control. This banana plants growth could be explained by the high composition in mineral elements of droppings (1.5% nitrogen, 0.6% phosphorus) and NPK (21% nitrogen, 8% phosphorus). Nutrients including nitrogen, phosphorus and potassium in quality and quantity are mainly involved in the growth of aerial organs (leaves and stem) [23]. They were therefore made available to banana vivoplants for their growth. The absorption of mineral elements by banana trees was facilitated by the pH of the soil. This growth can also be explained by the C/N ratio which is frequently used to evaluate the process of mineralization of organic matter [3]. On the agronomic quality aspect of the organic fertilizer, the C/N ratio between 15-20 is considered optimum. As a result, the C/N ratio of the chicken manure treatment being 18, it has better mineralization, so it quickly releases the main mineral elements for growth (nitrogen phosphorus and potassium). Plants treated with poultry manure and NPK had significantly higher growth for all the variables analyzed (circumference, height, number of leaves and leaf area of the last leaf emitted) compared to plants treated with kitchen ash mycorrhizae and the control for both varieties. These results could be explained by the high nitrogen content in these fertilizers, the main growth factor for green plants. Indeed, nitrogen is an essential

factor in plant growth, especially in the stems and leaves [5]. Poultry manure therefore improve the quality of the soil, in particular the pH, the organic matter and the cation exchange capacity, which promote development of the plants [12].

3.2.5. Plant Shoot and Root Biomass

Table 2 shows that only poultry manure and NPK had a strict influence on the biomasses for the two varieties. They allowed a significant improvement of the biomasses compared to the other treatments.

Table 2. Varieties biomass as a function of treatments.

	Batard		Big Ebanga	
	Shoot	Root	Shoot	Root
T1	153,35 ^a	59,19 ^a	150,28 ^a	37,44 ^a
T2	70,72 ^b	43,42 ^a	87,62 ^b	38,78 ^a
T3	34,47 ^c	19,58 ^b	24,7 ^c	11,47 ^a
T4	30,84 ^c	24,35 ^b	33,05 ^c	24,84 ^a
T5	26,71 ^c	19,45 ^b	30,86 ^c	19,81 ^a

T1: NPK; T2: Poultry manure; T3: Control (soil); T4: mycorrhizae; T5: Kitchen ashes. Means with the same letters are not significantly different at 5% of average probability through the Duncan test.

This could be due to the improvement of soil conditions (humidity, water retention capacity, etc.) by poultry manure [13]. The action of mycorrhizae really begins 3 months after inoculation. Tsané *et al.* [28] demonstrated that after 2 months after inoculation, mycorrhizal symbiosis had no significant effect on the plants. The mycorrhizal fungi would not have had enough time to colonize the root spaces and induce their effect which is the extension of the root system, to make available the water and the mineral elements which are present in the soil. Since the effective role of mycorrhizae is to mobilize the mineral elements of the soil, make them available for the culture in mycorrhizal symbiosis of plants (facilitate the assimilation of phosphorus thanks to the phosphatases necessary for growth and development) and thus promote an increase in yield [26, 27, 4].

3.3. Effect of Fertilizers on Chlorophylls and Carotenoids Contents

The average values of chlorophyll a (Table 3) and carotenoids of the plants treated with NPK are higher than the other treatments.

Table 3. Chlorophylls and Carotenoids contents of the varieties as a function of treatments.

	Batard			Big Ebanga		
	Chl a (µg/ml)	Chl b (µg/ml)	Car (µg/ml)	Chl a (µg/ml)	Chl b (µg/ml)	Car (µg/ml)
T1	21,1 ^a	14,2 ^a	3405,7 ^a	26,9 ^a	14,57 ^a	3192,4 ^a
T2	15,1 ^b	9,9 ^{ab}	2313 ^b	23,3 ^a	9,89 ^a	2452,5 ^a
T3	11,9 ^b	9,2 ^b	1954,4 ^b	19,7 ^a	8,63 ^a	1993,6 ^a
T4	11 ^b	7,3 ^b	1714,7 ^b	21,2 ^a	8,07 ^a	2127,8 ^a
T5	11,6 ^b	6,4 ^b	1447,1 ^b	19,7 ^a	9,26 ^a	2100,8 ^a

T1: NPK; T2: Poultry manure; T3: Control (soil); T4: mycorrhizae; T5: Kitchen ashes. Means with the same letters are not significantly different at 5% of average probability through the Duncan test.

The green colouring of the plants is due to the presence of chlorophylls, pigments involved in the primary reactions of photosynthesis [22]. Nitrogen plays a fundamental role in the formation of vegetative tissues and reproductive organs [14, 18] therefore responsible for the vegetative growth of the plant. Chlorophyll is described as an early spectral signature for detecting nitrogen deficiency [24, 6]. According to Sinclair [25], variation in chlorophyll following nitrogen stress inhibits plant growth. This allows us to say that the quantity of pigments (chlorophylls and carotenoids) in the leaves should be greater for the fertilizer richer in nitrogen. From these results, it appears NPK followed by chicken droppings are the richest nitrogen treatments among the different treatments used, therefore their leaf pigments are also higher than the others. The chlorophyll a content is of decreasing order for the Batard variety: NPK, chicken droppings, control, mycorrhiza, kitchen ash. We can therefore say that the mycorrhizae have not colonized the roots of the plant enough to facilitate the acquisition of nutrients (mineral elements) in the soil, which is why the chlorophyll a content is low compared to the control and the ash of kitchen. Concerning the Big ebanga variety, the results of the chlorophyll content depend on the amount of nitrogen of each treatment. After the NPK and the chicken droppings, come the mycorrhizae, the kitchen ash and then the control.

4. Conclusion

Knowing the harmful effects of chemical fertilizers on the environment and their cost, this study has made it possible to highlight the effect of mineral fertilizers (NPK), organic fertilizers (poultry manure and kitchen ashes) biological fertilizers (mycorrhizal fungi) on the growth in the nursery of plants from plantain stem fragments of the Big Ebanga and Batard varieties. Poultry manure and NPK had the best results on growth parameters by increasing pseudostem height, collar circumference, leaf area and number of leaves and also chlorophyll and carotenoid contents of plants.

Poultry manure appear as an alternative to the use of synthetic fertilizers for the growth of plantain plants in nurseries.

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