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Growth, rhizome yield and biochemical components of turmeric (*Curcuma longa* L.) as influenced by mycorrhizae, poultry droppings and chemical fertilizers

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Abstract

The work aimed at contributing to the agricultural valuation of poultry droppings and mycorrhizal biofertilizers in order to increase the productivity of *Curcuma longa* L. (turmeric). Thereby, the effect of some amendments on the growth, the composition of some biochemical and mineral components of *C. longa* was studied during 23 weeks of cultivation in a greenhouse. The experimental device was a completely randomized block with five treatments (T_0= control; T_1= poultry droppings; T_2= NPK; T_3= mycorrhizae; T_4= poultry droppings + mycorrhizae). Concerning growth parameters, the T4 improves the height of the stems (38.1 ± 2.8cm), the basal stem diameter (9.2 ± 1.0mm) and the dry biomass of the leafy stems (16.2 ± 2.3g). Concerning the roots, the best results were obtained by T1 (4.3 ± 1.6g). The best results on the productivity of fresh rhizomes of *C. longa*, have been obtained to the T4 (308.8 ± 43.2g). According to biochemical components, the contribution of mycorrhizae and droppings increased the carotenoids content with the respective values of 2.63 ± 0.1g/l and 2.75 ± 0.1g/l. For the mineral constituents, the contribution of the T4 and T1 obtained better results in minerals such as: N and Fe inmg/100g DW. The results show the importance of using the droppings of laying hens in combination with mycorrhizae. It was found to be very promoting the turmeric plants and therefore can be used as an alternative to replace chemical fertilizer usually employed for cultivation.

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Introduction

According to WHO, about 80% of the world's population uses plants as a source of medicine. These plants have been used in the traditional pharmacopeia in response to various diseases (Hamill *et al.*, 2003). These plants have their therapeutic power to the active substances they contain. Among these plants commonly used around the world, there is *Curcuma longa* L. (Turmeric). It is widely imported and exported across the world, generating profits to several countries like India, Indonesia, China and Ethiopia (FAO/OMS, 2018). Fresh or dry, *C. longa* is used as spice, medicinal plant, or dye in food, textile, cosmetic and pharmaceutical industries. As a spice, it is used to enhance the flavor and to preserve food (Duta *et al.*, 2016). As a medicinal plant, *C. longa* is used in the treatment of several diseases including skin diseases such as scabies, indigestion, inflammatory diseases, liver problems, bone diseases such as rheumatism, metabolic diseases such as obesity and diabetes (Bouzabata and Nihed, 2013).

Turmeric powder is used as a dye for textiles such as cotton, foods such as mustard, and cosmetics such as soap, traditional beauty masks (Bouzabata and Nihed, 2013). This effectiveness of *C. longa* is due to its components, the major one being curcumin, which has a remarkable pharmacological activity.

Although *C. longa* is widely imported and exported across the world, generating huge profits and contributing to increase the economy of some countries, in Cameroon, its cultivation is still little known and is largely done in association in household gardens and sometimes in the fields.

In addition, Cameroon climate seems to be favorable to the growth of Curcuma like India climate (Bouzabata and Nihed, 2013). Moreover, the small quantity produced Curcuma in Cameroon is undervalued. It is therefore clear that there is a huge shortfall for Cameroon not to produce on a large scale and not to value and better exploit this rhizome. Thus, in Cameroon in particular and in the other countries of Central Africa, there is little scientific data on the cultivation of *C. longa*. According to the surveys made between 2010 and 2011 on several crops, there is a lack of information on the cultivation, exploitation and cost of *Curcuma longa* (DESA, 2015).

We note that *C. longa* is a plant that the importance is well established. In order to popularize, properly exploit, it is necessary to avoid or limit widely used practices such as slash-and-burn agriculture which reduces up to 30% of crop yields, uncontrolled and irrational use of chemical fertilizers which are one of the causes of the impoverishment and increase soils acidity (Ndonda, 2018). It will therefore be necessary to use fertilizers with the capacity to increase yields while maintaining the ecological balance. Organic fertilizers have the role of improving the structure of the soil, enriching it with fertilizing elements and supplying plants with the elements necessary for their growth and development (Asma *et al.*, 2018). In the same way, organic farming offers new perspectives with the use of organic fertilizers such as animal waste that include poultry droppings (Tchabi *et al.*, 2012). Also, biofertilizers such as mycorrhizal fungi are proving to be a very effective solution for increasing crop yields and reducing the need for chemical inputs (Hamel and Plenchette, 2007).

The aim of this work is to evaluate the effect of organic, mineral, mycorrhizal and organo-mycorrhizal amendments on the growth, the productivity, and some biochemical components of *curcuma longa*.

Material and methods

Study station

The experiment took place in the greenhouse of Faculty of Science, University of Douala located in the district of Ndogbong, Littoral Region, Cameroon (4°02'53 N, 9°42'15 E, 19 m altitude, Tropical-type climate, average temperature 26.2°C, annual average precipitations 3174mm).

Material

C. longa rhizomes were obtained from a producer in Douala market. Mature ones have been carefully selected for their ease of budding. Mineral amendments consisted of NPK (in proportion 20-10-10) obtained in the local market. Organic amendments were poultry droppings obtained from a farm in Bafoussam, West Region. Mycorrhizal fungi used consisted of a mixture of propagules of arbuscular mycorrhizal fungi of the *Acaulospora tuberculata* (50%) and *Gigaspora margarita* (50%) from the Applied Microbiology and Biological Control Laboratory of IRAD of Nkolbisson. Sterile sand was used as a growing medium in 3l plastic pots suitable for greenhouse work (Pizano, 2002). Wacquant's nutrient solution was prepared by dissolving 2 g of KNO_3, 2.4 g ofmgSO_4 and 0.55 g of $CaCl_2$ and 1.35 g of KH_2PO_4 for 5 l solution (Wacquant, 1974).

Methods

Treatments and experimental design

Five treatments were applied as follows: control treatment without amendment (T_0), treatment with poultry droppings (T_1), treatment with NPK (T_2), treatment with mycorrhizae (T_3) and treatment with a mixture of ½ droppings + ½ mycorrhizae (T_4).

- *Chicken droppings treatment*

The droppings were dried in an oven at 60°C for 48 hours, then crushed to obtain a powder. The treatment consisted of four applications of 30 g for the entire cultivation period (Karthikeyan *et al.*, 2009). The first dose was applied as basal dressing fertilizer when planting the rhizomes and the others at 30, 60 and 90 days after planting.

- NPK Treatment

A single dose of 20 g of NPK was applied for the entire cultivation period. The application was done 22 days after planting in a circular band around each seedling so as to avoid any contact with the stem (Wamba *et al.*, 2012).

- Mycorrhizae treatment

A single dose of 50 g of mycorrhizal inoculum was applied for the entire cultivation period. The application was done in sandwich on the same day of the planting of rhizomes (Ngonkeu, 2003).

- Treatment with association of poultry droppings and mycorrhizae

A dose of 15g of poultry droppings powder associated with 25g of mycorrhizal inoculum strain was applied at the base in sandwich with the planting of rhizomes. Afterwards, three other applications of poultry droppings (15g) were done 30, 60 and 90 days after planting (Karthikeyan *et al.*, 2009).

The experimental design was a completely randomized one-block device (Fig. 1). A total of 6 repetitions were done per treatment. The gaps between the pots were 20 and 10cm respectively (Bomisso *et al.*, 2018).

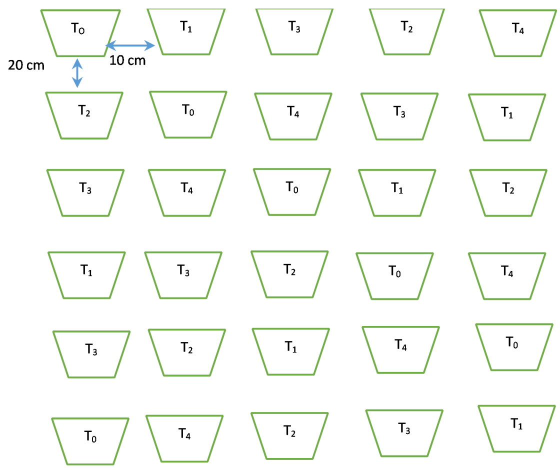

Fig. 1. Experimental design.

T_0 = Control, **T_1** = Treatment with poultry droppings, **T_2** = Treatment with NPK, **T_3**= Treatment with mycorrhizae, **T_4** = Treatment with association of poultry droppings and mycorrhizae

Greenhouse cultivation

The seeds (rhizomes) of *C. longa* were pregerminated for 4 weeks and emergence of buds was observed (Bouzabata and Nihed, 2013). Germination being effective, these seeds were planted to a depth of 5 to 10cm (one rhizome per pot) on pots previously perforated and filled at 2/3 with sand. The seedlings with treatments T_0, T_1, T_3 and T_4 were supplied with Wacquant's nutrient solution every 3 days during 22 days (Wacquant, 1974) for the absorption of the minerals Na^{2+}, K^+, Ca^{2+} andmg^{2+}. As for the seedlings receiving the treatment T_2, they were supplied with distilled water.

Data collection

Growth parameters

In each trial, growth parameters for number of leaves, plant height, collar diameter, leaf area were assessed each week from 6th to 17th week. The chlorophyll content of leaves was assessed only on the 6th week. The number of leaves was determined by simple counting, while plant height, collar diameter, length and width of leaf were measured using a graduated ruler and a caliper (Flowers and Yeo 1989). Leaf area was calculated from length and width of leaf (Murray, 1960; Kumar *et al.*, 2002). For chlorophyll content measurement, fresh leaves were cleaned to remove soil and other contaminants, and 0.1 g of fresh leaves was used to extract chlorophyll using 95% ethanol. The chlorophyll content (chlorophyll a and chlorophyll b) of the filtered solution was measured using the classical spectrophotometric method with a spectrophotometer (Biobase, China). Optical densities were read at 645 nm (X) and 663 nm (Y); 645 nm and 663 nm being absorption wavelengths of chlorophyll a and b respectively. Chlorophyll contents inmg/l were calculated using the following formulas (Taffouo *et al.*, 2008).

Chlorophyll a = 12,7Y - 2,69X
Chlorophyll b = 22,9X - 4,68Y
Chlorophyll (a + b) = 20,21X + 8,02Y

Fresh and dry biomass

Twenty-three weeks after planting, the plants were harvested. The clumps were pulled out, then cleared of the sand, and the stem was cut at the level of the collar. The rhizomes were carefully washed, separated and then spread out. After separation of the leaf, stem, rhizome and root parts, the fresh biomass was determined using a precision balance (KERN EMB 600-2). Dry biomass of these parts was determined after drying at 105°C for 24 hours for leaf, stem and root, and 72 hours for the rhizomes. Moisture content was deduced from dry and fresh matter (Savouré, 1980).

Biochemical parameters

Biochemical composition of rhizomes was determined on samples dried at 45°C for 72 hours. The parameters analyzed were total carotenoids, total polyphenols and some minerals.

Total carotenoids content

Total carotenoids content was evaluated by photometry (iChek™ Carotene; BioAnalyt GmbH, Teltow, Germany) at 446 nm according to protocol used previously by Dongho *et al.* (2014). For carotenoids extraction, approximately 0.10 g of *C. longa* powder was added to a tube containing 10ml of the mixture of ethanol/hexane (1:1). The mixture was shaken vigorously and allowed to stand for 15 hours, and then centrifuged at 3000 revs/min for 5 min, the hexane phase was transferred to another tube. The procedure was repeated until the total discoloration of the residue. The volume of the hexane phases obtained was completed at 20ml and 2ml was used to read carotenoids content in a photometer which gives the carotenoids content of solution in mg/l.

Total phenolic content

Total phenolic content (TPC) was determined by spectrophotometric method (Saeed *et al.*, 2012). Approximately 1g of *C. longa* powder was macerated in 10ml of methanol (70% v/v) for 1 hour and then filtered using a No.4 Wattman filter paper. The filtrate obtained was macerated a second time for 1 hour to maximize the extraction, then all of the two homogenates were centrifuged at 3000 rpm for 20 min. The supernatant containing the polyphenols was recovered and the volume noted. Afterwards, 1ml of this supernatant was mixed with 1ml of Folin-Ciocalteu's phenol reagent.

After 5 min, 10ml of a 7% Na_2CO_3 solution was added to the mixture followed by the addition of 13ml of deionized distilled water; then all were thoroughly mixed. The mixture was kept in the dark for 90 min at 23°C, and the absorbance was read at 750 nm. The TPC was deducted from extrapolation of the calibration curve which was made by preparing gallic acid solution as standard. The TPC was expressed as micrograms of gallic acid equivalents per g of extract (μgGAE/g).

Minerals content

Minerals analyzed in turmeric powder were total nitrogen, total phosphorus, potassium, sodium, magnesium, calcium and iron. They were determined according to the international methods recommended by Pauwel *et al.* (1992), and respecting the ISO, AFNOR NF and EN standards.

- Total nitrogen by mineralization sulfuric acid attack of 0.1g sample, in the presence of copper (II) and a catalyst (titanium oxide) then distillation by steam distillation and dosage with sulfuric acid (Kjeldahl method, NF ISO 11261 standard);

- Total phosphorus by colorimetric with ammonium phosphomolybdate yellow after calcination of 1g of dry matter at 450°C and extraction with 1N nitric acid. Reading at 420nm wavelength (Standard NF EN 14672);

- Potassium and sodium by flame emission spectrometry by direct reading in the extract (AFNOR NF T 90-019 standard);

- Calcium and magnesium by complexometry and titrimetric method in the extract. (AFNOR NF U 44-146 standard);

- Iron by colorimetry with potassium thiocyanate after complexation. Reading at 530 nm wavelength (AFNOR NFU 44-041 standard). All the results were expressed in mg/100 g of *C. longa* powder.

Statistical Analysis

Raw data were tabulated on MS Excel spreadsheet and organized for statistical analysis, then exported to GRAPHPAD PRISM version 5.9 (GraphPad Software, La Jolla California USA, www.graphpad.com). The statistical significance was assessed using one-way ANOVA (analysis of variance) followed by Duncan post hoc tests for pairwise separation and comparison of means. The data were subjected to descriptive statistics and the results were expressed as mean ± standard error of mean (SEM). The values of $P < 0.05$ were considered significant.

Results

The above methodology enabled to highlight the effects of different fertilization on three important aspects on *C. longa* that are the growth parameters, the production yield, and the biochemical components of rhizomes.

Effect of amendments on the growth parameters of Curcuma longa

Effect of amendments on the growth parameter of *C. longa* was evaluated using the evolution of the number of leaves, the height of the stems, the collar diameter, the leaf area, chlorophylls contents.

Evolution of number of leaves

The number of leaves of *C. longa* increase from week 6 to week 11 for all treatments (Fig. 2). Then, it starts to drop for the plants amended with bio fertilizer and later the control without amendment. It was noted a significant increase ($P<0.05$) of the number of leaves starting at week 7, on the plants amended with NPK and association of poultry droppings + mycorrhizae compared to the control. From the 13th week of culture, the mycorrhizae amendment decreases the number of leaves compared to the association droppings + mycorrhizae and the control. At week 16, the number of leave of *C. longa* amended with NPK (17 ± 2) and by the association poultry droppings + mycorrhizae (16 ± 5) were significantly higher ($P<0.05$) than the control (11 ± 4) and the ones amended with poultry droppings (14 ± 5cm) and mycorrhizae (10 ± 4).

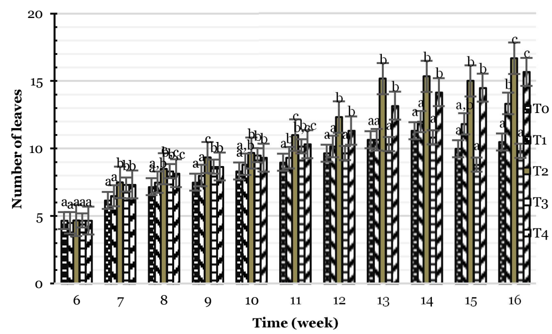

Fig. 2. Number of leaves of *Curcuma longa* according to the treatment and time after planting. **T_0** = Control, **T_1** = Treatment with poultry droppings, **T_2** = Treatment with NPK, **T_3** = Treatment with mycorrhizae, **T_4** = Treatment with association of poultry droppings and mycorrhizae.

For a given week, histograms with the same letters are not statistically different according to Duncan's test at $P < 0.05$; Error bar = Standard deviation

Evolution of the height of stems

The evolution of the height of *C. longa* stems according to the treatments and the duration of the culture is showed in fig. 3.

The height of the stems increased with time and is affected by amendment. It was noted that the mycorrhizae and the combination poultry droppings + mycorrhizae significantly affected (P<0.05) the stem height compared to the control. The same observation was made with poultry droppings started at week 8. Nevertheless, from the 14th week, it was noted a slowing down of the elongation of the stems of plants amended with NPK. At week 16, the height of the stems of *C. longa* amended with poultry droppings (37.4 ± 3.7cm), mycorrhizae (31.6 ± 5.5cm) and by the association poultry droppings + mycorrhizae (38.1 ± 2.8cm) were significantly higher (P<0.05) than the control (32.5 ± 3.7cm) and the one amended with NPK (34.2 ± 3.8cm).

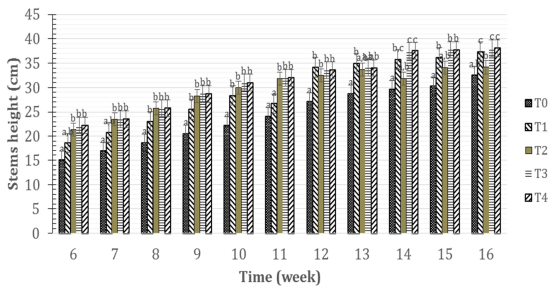

Fig. 3. Stems height of *Curcuma longa* according to the treatment and time.

T_0 = Control, **T_1** = Treatment with poultry droppings, **T_2** = Treatment with NPK, **T_3** = Treatment with mycorrhizae, **T_4** = Treatment with association of poultry droppings and mycorrhizae;

For a given week, histograms with the same letters are not statistically different according to Duncan's test at P < 0.05; Error bar = Standard deviation

Evolution of the stem diameter

The stem diameter increases as the cultivation time increases and is affected by amendment (Fig. 4). Poultry droppings + mycorrhizae association and NPK had a significant effect (P < 0.05) on the collar diameter of *C. longa* compared to the control. Poultry droppings and mycorrhizae increases, but not significant (P < 0.05), the collar diameter compared to the control, except at week 9, 10 and 13. On the other hand, the effects of droppings and mycorrhizae treatments were compared to the control.

Poultry droppings + mycorrhizae association increase significantly (P < 0.05) the collar diameter at week 15, compared to all the other treatments. At week 16, the collar diameter of *C. longa* amended with poultry droppings (7.9 ± 1.2mm) and the association poultry droppings + mycorrhizae (8.2 ± 1.0mm) were significantly higher (P<0.05) than the control (6.6 ± 1.0mm) and the plants with biofertilizer (6.8 ± 2.4mm); it was not found a significative difference with those amended with NPK (7.3 ± 0.7mm).

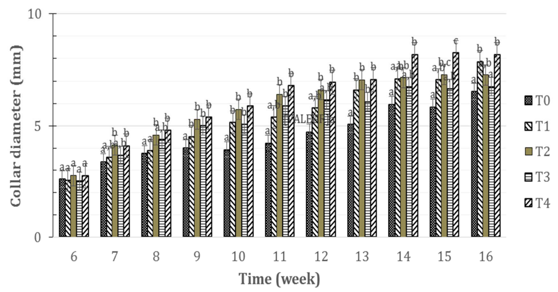

Fig. 4. Collar diameter of *Curcuma longa* according to the treatment and time.

T_0 = Control, **T_1** = Treatment with poultry droppings, **T_2** = Treatment with NPK, **T_3** = Treatment with mycorrhizae, **T_4** = Treatment with association of poultry droppings and mycorrhizae;

For a given week, histograms with the same letters are not statistically different according to Duncan's test at P < 0.05; Error bar = Standard deviation

Evolution of leaf area

The leaf area of *C. longa* increases as the cultivation time increases while treated with Poultry droppings + mycorrhizae association (Fig. 5). Poultry droppings + mycorrhizae association and NPK had a significant effect (P < 0.05) on the leaf area of *C. longa* compared to the control started at week 9. The association of droppings-mycorrhizae significantly increases the leaf area *C. longa* in the 10th week compared to the addition of mycorrhizae, but not significantly compared to the control; droppings and NPK. Nevertheless, it was not found a significant effect (P<0.05) of the contribution of chicken droppings on the leaf area compared to the control, except at week 16. At week 16, the leaf area of *C. longa* amended with NPK (601.2 ± 174.9cm^2), mycorrhizae (598.7 ± 49.8cm²) and the association poultry droppings + mycorrhizae (654.7 ± 347.1cm²) were significantly higher (P<0.05) than the control (cm²); it was not found a significant difference with the one amended with poultry droppings (513.2 ± 243cm²).

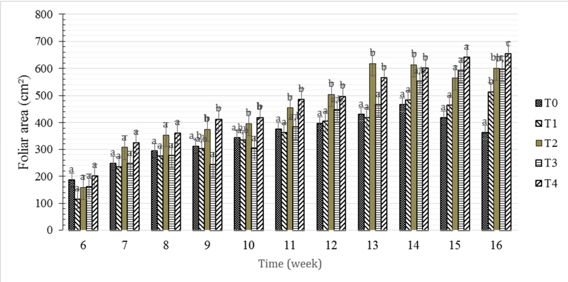

Fig. 5. Leaf area of *Curcuma longa* according to the treatment and time.

T₀ = Control, **T₁** = Treatment with poultry droppings, **T₂** = Treatment with NPK, **T₃** = Treatment with mycorrhizae, **T₄** = Treatment with association of poultry droppings and mycorrhizae;

For a given week, histograms with the same letters are not statistically different according to Duncan's test at P < 0.05; Error bar = Standard deviation

Chlorophylls contents

A significant effect (P<0.05) had not been observed on the contents of a, b and total chlorophylls of *C. longa* with the amendments compared to the control (Fig. 6). The value found varied from 22.5 ± 0.1 to 23.6 ± 1.9mg/L extract for chlorophyll a, 49.0 ± 1,6 to 51,4 ± 0,2mg/L extract for chlorophyll b, and 71,5 ± 3,3 to 73,7 ± 0,2mg/L extract for the total chlorophyll.

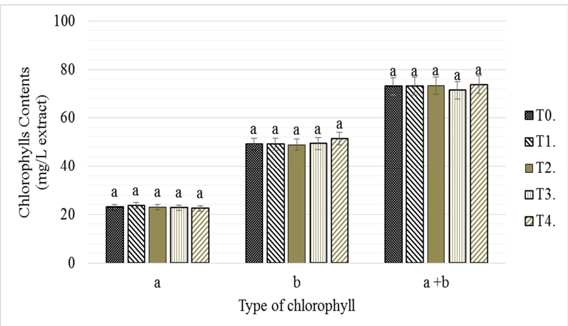

Fig. 6. Chlorophylls contents of *Curcuma longa* according to the treatment.

T0 = Control, T1 = Treatment with poultry droppings, T2 = Treatment with NPK, T3 = Treatment with mycorrhizae, T4 = Treatment with association of poultry droppings and mycorrhizae;

For a given week, histograms with the same letters are not statistically different according to Duncan's test at P < 0.05;

Error bar = Standard deviation

Effect of amendments on production yield of Curcuma longa

The fresh weight and the dry biomass measures were used to compare the effect of amendments on production yield of *C. longa*. The fresh weight of *C. longa* rhizomes for the plants amended with the association of droppings + mycorrhizae (308,8 ± 43,2g) was significantly higher (P<0.05) than those of the control (140,4 ± 39,6g), NPK (208,6 ± 41,7g) and mycorrhizae (178,6 ± 98,6); It was not found significant difference compared to poultry droppings (277,8 ± 53,4g) (Fig. 7). Moreover, droppings increase, but not significantly (P<0.05), the rhizomes' fresh weight compared to the control NPK and mycorrhizae. The amendment with NPK and mycorrhizae do not show a significant (P<0.05) effect on the rhizomes' fresh weight of *C. longa*.

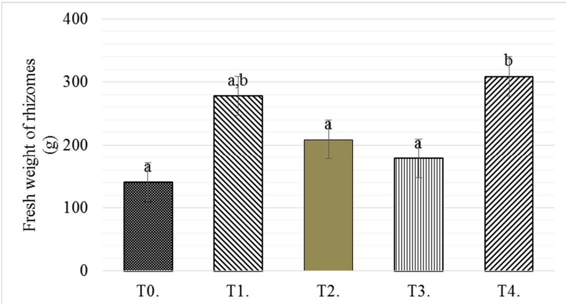

Fig. 7. Fresh weight of *Curcuma longa* rhizomes according to the treatment.

T0 = Control, T1 = Treatment with poultry droppings, T2 = Treatment with NPK, T3 = Treatment with mycorrhizae, T4 = Treatment with association of poultry droppings and mycorrhizae;

For a given week, histograms with the same letters are not statistically different according to Duncan's test at P < 0.05;

Error bar = Standard deviation

It appears that the treatments with poultry droppings and the combination of droppings + mycorrhizae significantly increased (P<0.05) the dry biomass of stems, leaves, roots, and rhizomes of *C. longa* compared to the control (Fig. 8). In the same way, NPK and biofertilizer significantly increased ($P<0.05$) the dry biomass of rhizomes of *C. longa* compared to the control. On the other hand, a significant difference ($P<0.05$) was not found between control, NPK and mycorrhizal regarding their effects on the dry biomass of the stems and leaf as well as roots of *C. longa*.

Poultry droppings presented higher values of the dry biomass of the roots, statistically different ($P<0.05$) to all the other treatments. The same observation was made with the droppings + mycorrhizae on rhizomes. The dry biomass of *C. longa* varied from 8.5 ± 2.4g to 16.2 ± 2.3g for stems and leaves, 1.2 ± 0.7 to 3.1 ± 1.6g for roots, and 5.5 ± 2.3 to 9.8 ± 3.5g for rhizomes.

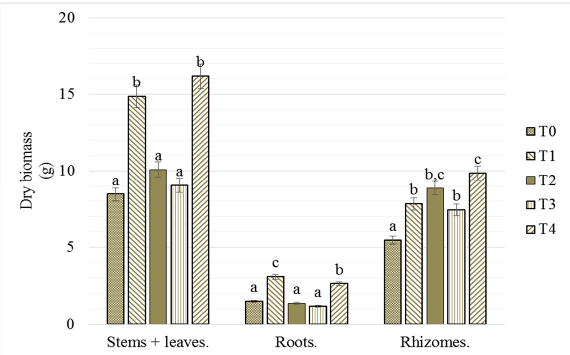

Fig. 8. Dry biomass of *Curcuma longa* organs according to the treatment.

T0 = Control; T1 = Treatment with poultry droppings; T2 = Treatment with NPK; T3 = Treatment with mycorrhizae; T4 = Treatment with association of poultry droppings and mycorrhizae;

For a given part, histograms with the same letters are not statistically different according to Duncan's test at $P < 0.05$;

Error bar = Standard deviation

Effect of amendments on biochemical components of Curcuma longa rhizomes

The biochemical components used for the study were the moisture, the total carotenoids, the total phenolic and the minerals content of *C. longa*.

Moisture content

The amendments did not show a significant difference ($P<0.05$) on moisture content of *C. longa* compared to the control (Fig. 9). The moisture content of *C. longa* varied from 89.1 ± 1.2 while amended with NPK to 91.1 ± 0.9% while amended with poultry dropping.

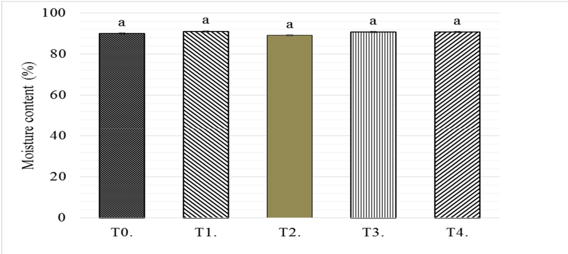

Fig. 9. Moisture content of *Curcuma longa* according to the treatment.

T0 = Control, T1 = Treatment with poultry droppings, T2 = Treatment with NPK, T3 = Treatment with mycorrhizae, T4 = Treatment with association of poultry droppings and mycorrhizae;

Histograms with the same letters are not statistically different according to Duncan's test at $P < 0.05$;

Error bar = Standard deviation

Total carotenoids content

The total carotenoid content of *C. longa* for plants amended with poultry droppings (2,7±0,1g/l) and mycorrhizae (2,6±0,1g/l) significantly higher ($P<0.05$) compared to control (1,4 ± 0,1g/l), NPK (1,6±0,1g/l) and the combination of droppings + mycorrhizae (1,3±0,3g/l) (Fig. 10). It was also noted that NPK increased, but not significantly, the carotenoid content compared to the control. Moreover, the combination of droppings + mycorrhizae seem to have a negative effect on the total carotenoid content of *C. longa* compared to all the other treatments.

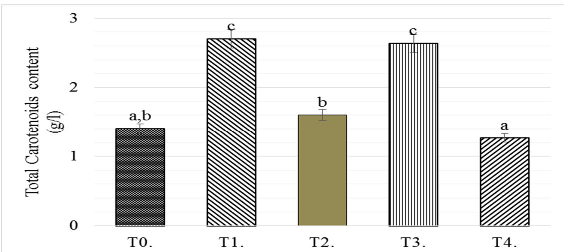

Fig. 10. Total Carotenoids content of *Curcuma longa* according to the treatment.

T0 = Control, T1 = Treatment with poultry droppings, T2 = Treatment with NPK, T3 = Treatment with mycorrhizae, T4 = Treatment with association of poultry droppings and mycorrhizae;

Histograms with the same letters are not statistically different according to Duncan's test at P < 0.05;
Error bar = Standard deviation

Total phenolic content

It was found that the total polyphenol content of the rhizomes of *C. longa* treated with NPK (0,551 ± 0,042 µgGAE/g) is significantly higher (P<0.05) than the control (0,509 ± 0,031µgGAE/g) and all the other treatments (Fig.11). Moreover, the total polyphenol content of the rhizomes with association of droppings + mycorrhizae (0,381 ± 0,033µgGAE/g) is the smallest. Nevertheless, this content with poultry droppings (0,456 ± 0,004µgGAE/g) was not significantly different (P<0.05) compared to the combination of droppings + mycorrhizae and with biofertilizer (0,489 ± 0,074µgGAE/g).

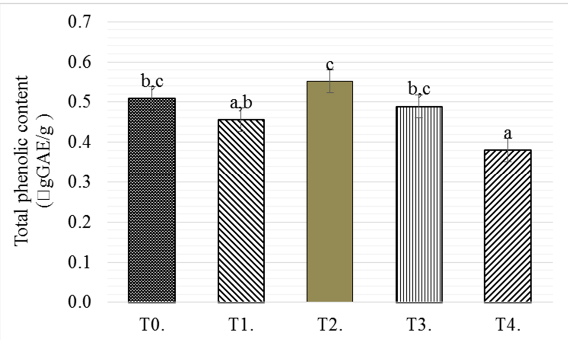

Fig. 11. Total phenolic content of Curcuma longa according to the treatment.
T0 = Control, T1 = Treatment with poultry droppings, T2 = Treatment with NPK, T3 = Treatment with mycorrhizae, T4 = Treatment with association of poultry droppings and mycorrhizae;
Histograms with the same letters are not statistically different according to Duncan's test at P < 0.05;
Error bar = Standard deviation

Minerals contents

The contents of total phosphorus total, total nitrogen, sodium, potassium, calcium, magnesium and iron of the *C. longa* rhizomes according to the treatments is presented in table 1. Its note that organic fertilizer, mycorrhizal and chemical fertilizer had variable effects on these contents. The association of poultry droppings + mycorrhizae significantly increased the contents of various minerals (total nitrogen, total phosphorus, calcium, magnesium and iron) compared to the control and NPK.

Plants amended with droppings provided significantly higher levels of total phosphorus, iron, calcium and total nitrogen than those of the control, NPK and mycorrhizae. But not significant compared to the association poultry droppings + mycorrhizae. Furthermore, biofertilzer significantly increased the levels of iron, calcium and total phosphorus of rhizomes compared to the control. On the other hand, this amendment increased, but not significantly, the magnesium content compared to the control.

Regarding the NPK ferilizer, it's significantly increased the iron content of *C. longa* rhizomes compared to the control. But in the case of calcium, magnesium and total phosphorus, the contribution of NPK increased, but not significantly, their contents compared to the control. Poultry droppings, NPK, mycorrhizae and the combination of droppings + mycorrhizae did not show a significant effect (P<0.05) on the sodium and potassium contents of *C. longa* rhizomes.

Table 1. Minerals content of *Curcuma longa* according to the treatment.

	Minerals contents inMG/100g dry weight				
	T0	T1	T2	T3	T4
Ca	1,250 ± 0,950a	3,010 ± 0,415b,c	2,127 ± 0,375a,b	2,650 ± 0,450b,c	3,650 ± 0,550c
Fe	0,807 ± 0,006a	0,900 ± 0,000c	0,870 ± 0,010b	0,857 ± 0,015b	1,004 ± 0,006d
K	0,280 ± 0,210a	0,340 ± 0,190a	0,300 ± 0,060a	0,520 ± 0,020b	0,440 ± 0,090a,b
Mg	4,050 ± 2,350a	6,400 ± 1,000a,b	6,300 ± 1,050a,b	6,483 ± 0,029a,b	7,883 ± 1,774b
N total	11,550 ± 0,050c	12,200 ± 0,100d	11,273 ± 0,075b	10,050 ± 0,050a	12,673 ± 0,075c
Na	0,050 ± 0,035a	0,060 ± 0,020a	0,057 ± 0,006a,b	0,050 ± 0,036a	0,080 ± 0,010b
P total	0,500 ± 0,030a	0,613 ± 0,015c	0,543 ± 0,015a,b	0,570 ± 0,040b,c	0,583 ± 0,038b,c

Total phosphorus total (P), total nitrogen (N), sodium (Na), potassium (K), calcium (Ca), magnesium (Mg), iron (Fe);
T0 = Control, T1 = Treatment with poultry droppings, T2 = Treatment with NPK, T3 = Treatment with mycorrhizae, T4 = Treatment with association of poultry droppings and mycorrhizae;

For a given mineral, histograms with the same letters are not statistically different according to Duncan's test at $P < 0.05$;

Discussion

The objective of this work was to evaluate the effect of organic, mineral, mycorrhizal and organo-mycorrhizal amendments on the growth, the productivity, and biochemical components of *Curcuma longa*. For growth parameters, the results showed that NPK influenced the production of leaves. The nitrogen, phosphorus and potassium are essential macroelements for good growth and good plant development of cultivated plants. It therefore follows that NPK has a rapid mineralization and the high concentration of these minerals slowdown the growth and development of plants. Our results are not in agreement with those of Kouakou *et al.* (2019) on the cultivation of two varieties of cucumbers (*Cucumis sativus*) after application of animal manure in Ivory Coast. Regarding the amendment with poultry droppings and the association droppings + mycorrhizae, their effects were significant ($P<0.05$) on the production of the number of leaves of *C. longa*. It can be explained by the richness of droppings in macro and microelements demonstrated in several scientific works. Our results agree with those obtained by Outende (2016) working on the chemical and agronomic characteristics of five waste composts and their effect on the chemical properties of the soil, the physiology and the yield of maize and tomato in located in Togo, which had demonstrated that the phosphorus and potassium contained in animal waste are mainly involved in the growth of aerial organs such as leaves and stems.

As for stem height, the results showed that droppings and the combination of droppings + mycorrhizae improved the height of the stems of the *C. longa* from the 15th week of cultivation. This strong growth would be due to the richness of the droppings in nitrogen and phosphorus; to their permanent availability when the need for nutrients is essential during the critical phase of growth; and to the effective role of the mycorrhizae which facilitate the assimilation of phosphorus through their phosphatases. These results were similar to those obtained by Kouakou *et al.* (2019) with cucumber (*Cucumis sativus)*.

One also noted that the association droppings + mycorrhizae improved the collar diameters of *C. longa*. These results demonstrate that droppings associated with mycorrhizae would improve the circumference of *C. longa*. According to Galabi *et al.* (2016), organic manure (droppings) improves the quality of the soil, in particular the pH, the organic matter content and the cations exchange capacity, thus ensuring good hydromineral absorption which promotes good development of the apices, especially the collar. Also, Lukáš *et al.* (2021) demonstrates in previous study that hen manure is a substrate for the development of earthworms, bacteria and fungi that facilitate mineralization and the assimilation of the minerals necessary to good plant growth. On the other hand, NPK didn't increase the collar diameter unlike mycorrhizae. This result could be explained by the fact that NPK does not act on the formation of organic matter (humus), on hydromineral absorption such as mycorrhizae and does not improve cations exchanges.

As for leaf area, the results showed a positive impact of the association droppings + mycorrhizae from the 10^{th} week compared to the control and to the other treatments. But overall, all the treatments had the same effect. This could be due to the morphology of the plant because it has large leaves that are as long as they are wide (Jansen *et al.*, 2005). Hence organic amendments, mycorrhizae and chemical fertilizers did not have a significant effect ($P<0.05$) on the leaf area. Our results do not corroborate those of Asma *et al.* (2018), who demonstrated the positive effects of organic manures and biofertilizers on growth and quality of Beetroot (*Beta vulgaris*)

The results obtained on the foliar a, b and total chlorophyll contents of *C. longa* studied did not show a significant difference ($P<0.05$) with treatment. Plant photosynthesis consists of reducing carbon dioxide from the atmosphere by the water absorbed by the roots using solar energy captured by the leaves with the release of oxygen and sugars.

From our results, we can deduce that the chlorophyll content is closely related to the water content of the plant. Our results didn't agree with those obtained by Karthikeyan *et al.* (2009) who demonstrated in India that a sufficient source of potassium increased chlorophyll content of *Curcuma longa*.

As for the impact of amendment on *C. longa* productivity, the results showed that the dry biomass of the different organs varied according to the treatments. Regarding stem + leaf part, droppings and the combination of droppings + mycorrhizae significantly increased their dry biomass. A medium enriched with phosphorus, as is the case for plants amended with droppings, considerably increases the dry matter of stems as demonstrated by Kamdem *et al.* (2020) on soybean. Also, one noted that droppings and combination of droppings + mycorrhizae increased the dry biomass of *C. longa* roots. Our results are in line with those obtained by Kamal and Yousuf (2012) who demonstrated that animal faeces contained phosphorus and calcium which in large quantities promote root development. Finally for rhizomes, one noted that the various treatments did not have a significant effect ($P<0.05$) on their dry biomass compared to control. This could be due to the amount of *C. longa* the study was conducted on.

Moreover, the results showed that the association of droppings + mycorrhizae significantly increased the fresh weight of the rhizomes, as well as droppings compared to NPK and mycorrhizae. The productivity of *C. longa* is enhanced when organic and mycorrhizae are combined. This would be probably due to the richness of droppings in macro and microelements and to the role played by mycorrhizae which is to mobilize mineral elements and make them available for culture in mycorrhizal symbiosis and thus promote an increase in crop yield. Our results are similar to those of Ndonda (2018), who obtained, Kisangani RDC, important production yield of cassava amended with mycorrhizae combined with farmyard manure.

Concerning the impact of amendment on biochemical components of *C. longa*, one noted that the different treatments applied didn't affect water content. This could be due to the nature of the growing medium. A good substrate must fulfill four functions: promote rooting, contain nutrients, provide water and allow good gas exchange (aeration). In this study, the growing medium (sand) would have improved drainage, because for all treatments, plants had good water nutrition, hence had the same water content. This corroborates the study of Marta (2001) who showed with study on floriculture that sand is most often used as a rooting medium for its ability to improve drainage and aeration.

Furthermore, the results showed that total carotenoids content was improved in *C. longa* amended with droppings and mycorrhizae compared to the control, NPK and combination of droppings + mycorrhizae. First of all, carotenoids are major determinants of the organoleptic and nutritional quality of fruits and vegetables. They are responsible for the color of fruits and vegetables (Fanciullino *et al.*, 2014). Synthesize and store in plastids, color is the characteristic element of these molecules (varies from yellow or red). The results obtained in this study could be due to the richness of droppings in nitrogen and phosphorus. They are comparable to those of Ndonda (2018) who showed with cassava that a nitrogen supply would allow a greater development of leaf biomass and thus contribute to a greater synthesis of carotenoids. Similarly, Fanciullino *et al.* (2014) in their studies on the enrichment of fleshy fruits with carotenoids, observed that phosphorus is essentially involved in the synthesis of carotenoids in the plant. Although chicken are rich in nitrogen and phosphorus, which are precursors of carotenoids in the plant, and according to Kamko *et al.* (2020), the Arbuscular Mycorrhizal Fungi appear as privileged sites for the absorption and accumulation of phosphorus, *C. longa* amended with the association of droppings + mycorrhizae had the lowest total carotenoid content. This could be explained by the fact that, during the cultivation of *C. longa* in the greenhouse, the plants receiving this treatment did not show morphological changes such as yellowing of the leaves, leaf spots, holes and fall of the leaves unlike the mycorrhizal treatment. It therefore follows that combining chicken droppings and mycorrhizae during cultivation ensures the protection of plants against biotic and abiotic factors. Subsequently, the *C. longa* plants treated with mycorrhizae also obtained a high carotenoid content. This would be due to the different morphological modifications observed on the leaves. Indeed carotenoids, secondary pigment collectors of light energy and antioxidants contribute to several major physiological functions and participate in the adaptation of plants to microclimatic variations with the ability to adjust their levels at the right time (Fanciullino *et al.*, 2014). Also, these results showed that NPK also improved the total carotenoid content compared to the control. The reason could be the low amounts of nitrogen and phosphorus provided by this treatment at the crucial moment of carotenoids synthesis in plant plastids, although this chemical fertilizer is very rich in nitrogen (20%), phosphorus (10%) and potassium (10%). Chemical fertilizers mineralize too quickly and are subject to risks of volatilization and leaching after watering, especially on a sandy substrate. Our results corroborate those obtained by Ewane *et al.* (2019), working on plantain cultivated on quaternary sands.

As for total polyphenols content, the results showed the positive impact of NPK. First of all, polyphenols are very common phytonutrients in fruits and vegetables (Renard *et al.*, 2014). They are obtained from the secondary metabolism of plants. Indeed, the high polyphenol content of *C. longa* treated with NPK could be due to the high mineralization (high concentration of nitrogen, phosphorus and potassium) thus making the culture medium acidic, thus favoring stress conditions. According to Serpantié and Ouattara (2001), excessively rapid mineralization promotes loss of absorbable minerals and acidification of the soil, especially on sand. In case of stress, the plant synthesizes polyphenols which play the role of defense, ensuring the growth and development of the plant. Our results corroborate those of Ndonda (2018) obtained with the culture of cassava in degraded soils of grassy fallows in Kisangani. With regard to *C. longa* plants treated with the association of droppings + mycorrhizae, the results obtained show that this treatment did not have a significant effect ($P<0.05$) on the content of total polyphenols. This undoubtedly reflects that the combination of these two fertilizers is very beneficial to ensure the protection of plants against the attack of pathogens and ensures good mineral nutrition. Similar results were obtained by Ndonda (2018), on cassava. On the other hand, these results are in contradiction with the assertion of Baranski *et al.* (2014), who showed that the vegetal products grown organically achieve levels of antioxidants such as polyphenols up to 69% compared to conventionally grown produce.

The results obtained on the mineral content of the *C. longa* rhizomes showed that combination of droppings + mycorrhizae effectively impacted the nutritional constitution of the rhizomes, as droppings which induce an excellent accumulation of minerals (nitrogen; phosphorus; calcium; magnesium and iron) in the rhizomes unlike the chemical synthesis product (NPK) which only improved the iron content in the rhizome. This could be due to the richness of droppings, which are highly concentrated in nutrients, and also to the effective role that mycorrhizae play in the association of organic and mycorrhizal amendments. Indeed, mycorrhizae act significantly in the decomposition and mineralization of organic matter and mobilize these nutrients for the benefit of the host plant (Ndonda, 2018).

As for potassium and sodium, one noted that the various amendments (droppings, mycorrhizae and combination droppings + mycorrhizae) as well as NPK didn't affect their contents. This could be explained by the fact that the plants were not under stress. However, previous studies such as done by Taffouo *et al.* (2006), showed that media enriched in potassium increase their sodium content in roots and tubers.

Furthermore, the results obtained for the plants amended with NPK showed that this fertilizer didn't contribute enough to the nutritional constitution of the rhizomes of the *C. longa* studied compared to the organic amendments and mycorrhizae. This could be due to the fact that synthetic fertilizers play a major role in improving the growth of cultivated plants, increasing crop yields to the detriment of the nutritional composition of fruits, vegetables, cereals and others (Bichel and Rossier, 2015).

Conclusion

This study showed that the best amendments positively affecting height of the stem, diameter at the collar, dry weight of leafy stems and roots of *C. longa* were droppings (30g per plant) and association droppings + mycorrhizae (in the proportion 15 and 25g respectively per plant). Combination of droppings + mycorrhizae was the best treatment regarding to the productivity of fresh *C. longa* rhizomes while mycorrhizal (50 g per plant) and droppings (30g per plant) were the best regarding total carotenoids content of rhizomes. Moreover, NPK (20g per plant) had a good impact on total polyphenol content while association droppings + mycorrhizae and droppings recorded the best results on minerals content. These results show the importance of combining organic fertilizers and mycorrhizal fungi to increase the productivity and improve nutritional composition of *C. longa*. To ensure the applicability and adoption by farmers of such amendments in their agricultural practices, it would be necessary to confirm these results in the field, to test several doses of fertilizers and to determine their influence on the physicochemical and biological characteristics of soil.

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Declaration of Interests

The authors declare that there are no conflicts of interests regarding the publication of this paper.

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