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BIOFERTILIZER BASED ON *ECHINOCHLOA PYRAMIDALIS* (LAM.), *PANICUM MAXIMUM* L., *TITHONIA DIVERSIFOLIA (*HEMSEL.) A. GRAY AND COW DUNG, AN ALTERNATIVE TO THE USE OF CHEMICAL FERTILIZERS IN URBAN AND PERI-URBAN AGRICULTURE IN BRAZZAVILLE, REPUBLIC OF CONGO

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ABSTRACT

Rural agricultural production that feeds urban areas is supplemented by products from urban and peri-urban agriculture, which, to compensate for low yields, resort to practices that are not very environmentally friendly. The market belts of Brazzaville are no exception to these practices. Chemical fertilizers are used to raise production levels. The present study was undertaken to contribute to the mitigation of the excessive use of chemical fertilizers in these vegetable markets, through biofertilizers that could optimize crop yields. Two completely randomized blocks with six treatments each of *E. pyramidalis, P. maximum, T. diversifolia* and cow dung were set up. Each treatment had 30 plants in the first set-up and 81 plants in the second. The first set-up was first cultivated with *Amaranthus hybridus L.*. Mineral fertilizer NPK 15-15-15 and NPK 20-10-10 was used as control. Growth parameters of crown Height and Diameter were measured every seven days until harvest. The dry biomass was estimated with 35 plants per treatment and per replication for the second experimental design. Results obtained show case low overall mortality rates of about 1.1% for the first set-up and 2.05% for the second set-up. The variance analysis at the 5% threshold, during harvest, revealed a treatment impact on height ($P<0.001$), crown diameter ($P<0.001$) for both experimental designs, and total dry biomass ($P<0.001$). The combinations *E. pyramidalis* + *T. diversifolia*, *E. pyramidalis* + *P. maximum* + *T. diversifolia* + Cow dung and *P. maximum* + *T. diversifolia* were the best.

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INTRODUCTION

The world's population is growing so exponentially that between 9.4 and 12.4 billion people will inhabit the earth by 2050 (Buettner, 2020). Most of this increase will occur in Africa and Asia. Furthermore, the proportion of the world's population living in urban areas is expected to reach about 60% by the year 2050 (Véron, 2007).
Population increase coupled with urbanization threatens food security in urban areas (Owusu, 2017; Singh and Yang, 2021). Due to rapid population growth in most African cities, food security in urban areas has become a non-negligible alternative to rural agriculture (Koc *et al*., 1999). Since the 1990s, urban agriculture has become a major issue in sustainable development, urban planning and the fight against food insecurity (Boulianne, 2016). In Brazzaville, urban agriculture is dominated by market gardening, which brings together 1,200 to 1,500 market gardeners working an annual area of 400 to 1,000 m2 (Mfoukou-Ntsakala *et al*., 2006). It is a female-dominated sector, although the presence of men has increased over the last two decades (Bizibandoki, 1996). The current population of the Republic of Congo is estimated at 5,780,460, of which 2,893,478 are male and 2,886,982 are female. The Congo is ranked among the most urbanized countries in Black Africa (Kalassa, 1996; Mfoukou-Ntsakala, 2006). It is the most urbanized country in Sub-saharan Africa (Mfoukou-Ntsakala, 2006). To feed this population, it is imperative to increase agricultural production in rural, urban and peri-urban areas to ensure food security. The use of organic manure, due to its beneficial effects on the various physio-chemical and biological properties of the soil,

makes it possible to achieve sustainable agriculture (Kaho *et al.*, 2011). The success of this strategy depends on the one hand, on the quality of the organic material used and on the other hand, on the quantity of nutrients that this material contains (Bado, 2002; Kaho *et al.*, 2011; Kasonga *et al.,* 2013; Mokuba *et al*., 2013). According to Lunze *et al*. (2007), the best practice to improve soil fertility remains the use of organic manure. The general objective of this study is to define a biofertilizer capable of optimizing yields in the vegetable markets of Brazzaville. Specifically, the aim was to (i) determine the proportions of different fertilizer plants as a component of the biofertilizer, (ii) estimate the yields of a control plant from different mixtures of fertilizers, (iii) evaluate the amount of biomass induced by the use of the biofertilizer on *Amaranthus hybridus* L.

Two research hypotheses were formulated in relation to this study:

- Treatments influence *Amaranthus hybridus* L. growth and;
- The mineralization of the different treatments influences *Amaranthus hybridus* L. growth.

MATERIALS AND METHODS

The plant material used consists of *Amaranthus hybridus* L., *Echinochloa pyramidalis* (Lam.), *Panicum maximum* L. and *Tithonia diversifolia* (Hemsel) A. gray. Two experimental designs were set up. The first one was previously cultivated with *A. hybridus in* order to evaluate the number of crop cycles that could be achieved with biofertilizer burial. The first experimental set-up consists of six experimental units of 1 m² in a completely randomized block with three replications. Each experimental unit has thirty plants installed at a density of 444,444 stems/ha at a spacing of 0.15 m x 0.15 m, i.e., 180 plants for the entire block and 540 plants for the entire experimental design. The distance between two experimental units is 0.40 m; the distance between two blocks is also 0.40 m. The usable area is of 0.0225 m². This experimental design was subjected to two production cycles with *A. hybridus* using only biofertilizer in the first cycle. The composition of the experimental units or treatments is reported in the following table I:

Table 1. Treatment composition

Components	Treatments					
	T1	T2	T3	T4	T5	T6
E. pyramidalis (Lam.)	50%	50%			33.33%	25%
P. maximum L.	50%		50%		33.33%	25%
T. diversifolia (Hemsel) A. gray		50%	50%		3.33%	25%
Cow dung						25%
Mineral fertilizer				100%		

The second experimental design is a completely randomized block with six experimental units and three replications. Each experimental unit has an area of 2.25 m^2 and has eighty-one plants installed at a density of 444,444 stems/ha at 0.15 m x 0.15 m spacing. The whole set-up has four hundred and eighty-six plants. The experimental set-up had one thousand four hundred and fifty-eight plants. The distance between the experimental units is 0.40 m for the whole set-up and 0.40 m separates two blocks, each with a usable area of 0.0225 m².

Biofertilizer manufacture and treatment composition: The collection of fresh plant material was done in the flooded areas bordering the Congo River, in the eighth district of Madibou, Brazzaville, using a machete or a mower. After collection, this plant material was transported to the milling room to be crushed, species by species. After grinding, each species was stored in 50 kg plastic bags which were then sealed with a wire. After 2 days, the biofertilizer started to emit a strong heat and ooze. On the fourth day, the colonization of the different components (species) by insects was observed. At 6 days, the insect colony as well as the heat decreased. At this stage, the different components were mixed to form the different biofertilizer treatments and buried in the soil. After burial, the system was irrigated for two days with 20 liters of water from a watering can per experimental unit.

Biofertilizer burial: The different components of the biofertilizer treatments were then mixed accordingly and buried. An experimental unit of 1m^2 received 10.1kg of the biofertilizer mixture and 22.53 kg was used for the 2.25 m^2. These quantities were then divided by the number of biofertilizer components. The ploughing was done two days before transplanting at a depth of 5 cm. The beds were then watered for two days with 20 liters of water per day per experimental unit before sunrise. On the other hand, mineral fertilizer was applied on the same day at the same depth of 5 cm. 112.5 g of NPK 15- 15-15 were applied per experimental unit in the first set-up and 50 g of NPK 20-10-10 per experimental unit in the second set-up.

Transplantation of the 3-week-old seedlings was done when they reached the 5-6 leaf stage of development, 15 days after sowing. Transplanting was done in three stages:

- Watering: the plants were first watered to avoid traumatizing or disturbing the roots of the plants during the uprooting;
- The uprooting of the plants: it was done by hand for additional control to avoid repotting the sick or attacking plants. This was done by choosing mature, vigorous and healthy plants;
- Transplanting: it was done after 15 hours to avoid wilting.

Seven days after transplanting, replanting was carried out to guarantee the homogeneity of the growth conditions of the plants. Only these replanted plants were no longer considered in the measurements.

Maintenance operations: Weeding and hoeing operations were carried out twice during this study, on the 7th and 14th days after transplanting. The experimental systems were watered with 10 liters of water per experimental unit, i.e. 60 liters per replication in the first week, i.e. a total of 180 liters per day, and 1,260 liters per week. Thus, during the whole vegetative cycle, the same quantities of water were brought for the whole system. During the first week of transplanting, watering was done every day (twice a day) with a 10-liters watering can at a rate of 120 liters on each replication per day, i.e., a total of 2,520 liters. Two weeks after transplanting, only one watering was done at sunset. Fifteen liters of water were added per experimental unit, i.e., 1,890 liters, as well as in the second and third.

Biomass Estimation: It was done manually on each experimental unit of the second set-up only by removing each seedling with its roots. Each experimental unit consisted of 81 plants and each plant was identified with a number at transplanting. 35 plants per treatment, per experimental unit and per block were randomly removed for biomass estimation. The plants were then transported to the laboratory and sectioned by area (roots, stem, leaves, and flowers).

Measured Parameters and Statistical Processing of the Data: The measured parameters were the height and diameter of *Amaranthus hybridus* L. over a period of three weeks. The data were recorded on Excel files. The averages obtained during the treatment period on the two systems were compared using the software "R" at the threshold of 5%. The averages of the different parameters are presented in the form of histograms or curves accompanied by confidence intervals.

RESULTS AND DISCUSSION

Effect of the experimental design on the mortality

The variance analysis showed a treatment effect on the mortality rate (P=0.007) only after 21 days of cultivation, i.e. at the maturity age, although between the intermediate ages, the highest mortality values were obtained with the second experimental design. The optimal value at the end of the cycle reached 2.06% for experimental design 2 and 1.11% for experimental design 1. Moreover, 9.53% of the plants of the second experimental design needed to be replanted on the 7th day of culture against 4.07% of the first experimental design (Figure 1).

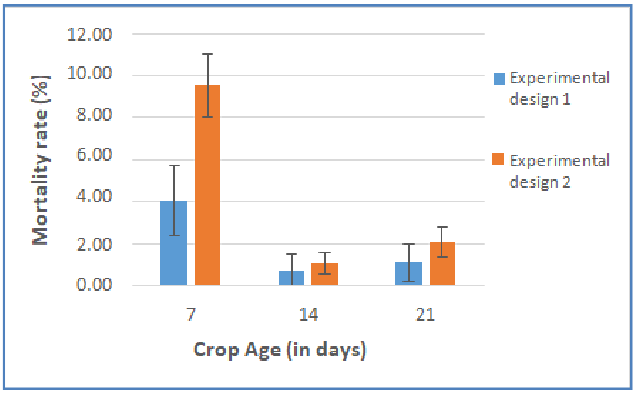

Figure 1. Influence of the experimental design on the mortality rate. Bars represent confidence intervals at P0= 5% level

Treatment effect on mortality rate: The variance analysis did not show a treatment effect on the mortality rate at all ages, for experimental design 1 (P-value> 0.05). On the other hand, for experimental design 2, a treatment effect on mortality was noted at 21 days (P-value=0.0012). The results by experimental designs, are presented in table II below.

The diameter growth of the two experimental designs is reported in Figure 3. The experimental design x treatment interaction was moderately significant at 7 days and highly significant at 14 and 21 days (P<0.001).

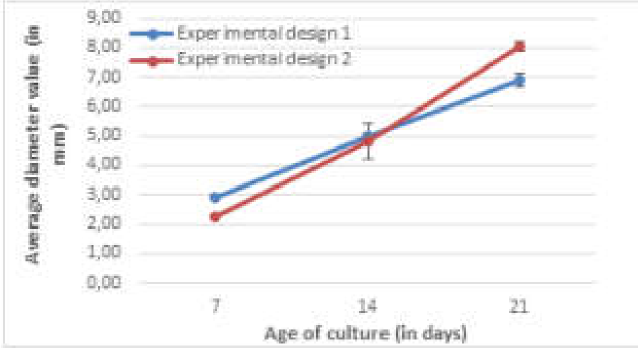

Figure 3. Impact of experimental design on diameter growth of *A. hybridus*. Bars represent confidence intervals at P0= 5% level

Treatment effect on the height growth of plants of *Amaranthus hybridus* L. : The variance analysis showed a highly significant treatment effect (P<0.001) on all ages in both the first and second experimental design. For the first set-up, the best treatments were T6 and T2, with respective mean heights of 34.67 ±1.97 cm and 32.32± 1.98 cm. On the other hand, for the second experimental design, treatments T3 (28.59 ±1.96 cm) and T4 (27.23±1.96 cm) are the best (Figures 4 and 5).

Table 2. Means values and confidence intervals at *p* = 0.05 of different treatments for the two experimental designs

Experimental designs	Treatments	Mortality rate at 7 days (%)	Mortality rate at 14 days (%)	Mortality rate at 21 days (%)
1	1	3.33±3.71	0.00±0.00	0.00±0.00
	2	6.67±5.15	2.22±0.32	3.33±3.20
	3	1.11±2.17	0.00±0.00	0.00±0.00
	4	5.56±4.73	0.00±0.00	0.00±0.00
	5	2.22±3.05	2.22±0.32	2.22±2.63
	6	5.56±4.73	0.00±0.00	1.11±1.87
2	1	7.00±3.21	1.65±0.10	2.88±1.81
	2	8.23±3.46	0.41±0.05	0.41±0.69
	3	11.93±4.08	0.41±0.05	0.82±0.98
	4	13.17±4.25	1.23±0.09	1.23±1.20
	5	7.14±3.29	1.23±0.09	1.65±1.38
	6	3.94±3.68	1.23±0.09	5.35±2.44

Experimental design effect on growth parameters: The variance analysis revealed a highly significant effect on height growth regardless of crop age (P<0.001) at all ages. For crown diameter, the influence of the experimental design was noted on the 7th day (P<0.001) and 21st day (P<0.001). At 14 days, on the other hand, the variance analysis did not reveal any effect (P=0.3168). The first experimental design was the best in terms of the height parameter, compared to the second experimental design (Figure 2).

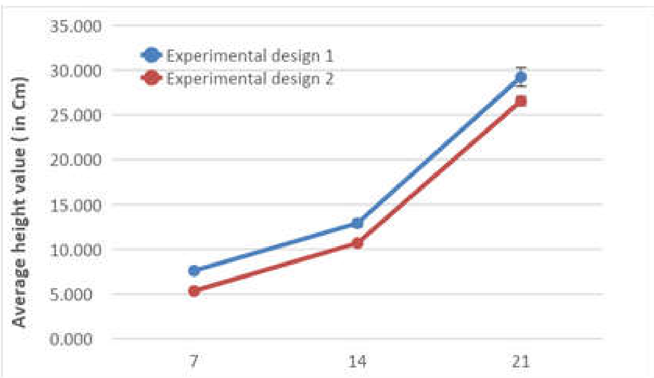

Figure 2. Impact of experimental design on height growth of *A. hybridus*. Bars represent confidence intervals at P0= 5% level.

Treatment effect on *A. hybridus* diameter growth: The variance analysis revealed a highly significant (P<0.001) treatment effect on diameter for all ages. The best treatments were T2 (8.25±1.97 mm), T3 (7.52±1.96 mm) for the first experimental design and T3 (8.37±1.96 mm) and T3 (8.25±1.96 mm) for the second experimental design.

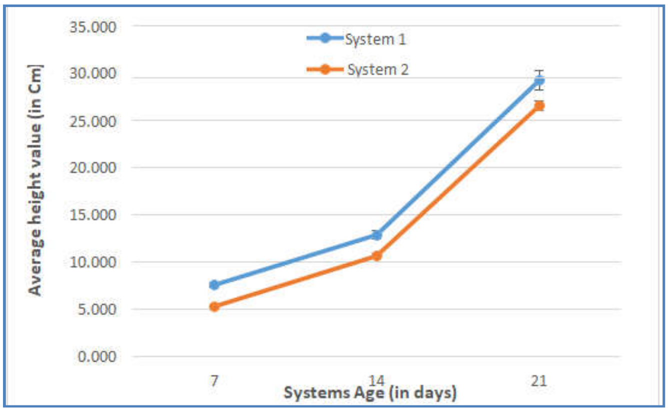

Figure 4. Experimental design effect on growth in height; Bars represent confidence intervals at *p* = 0.05

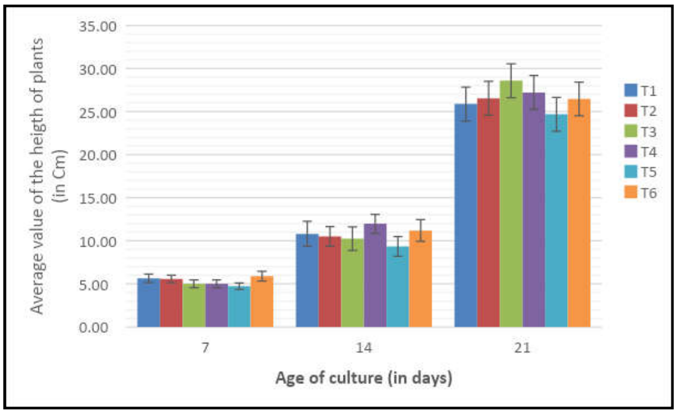

Figure 5. Treatment effect on the height growth for the experimental design 2. The bars represent the confidence intervals at P0= 5% level

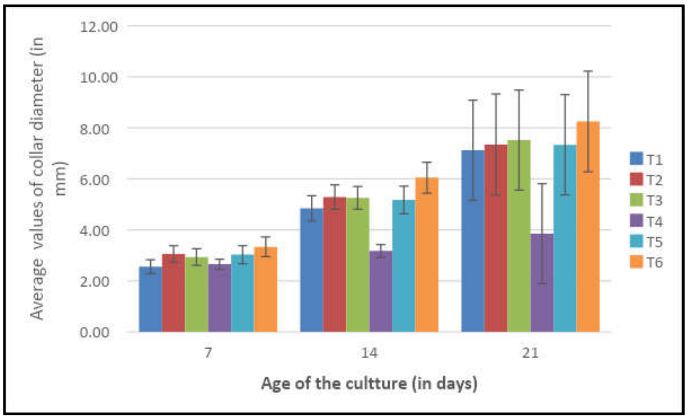

Figure 6. Treatment effect on diameter growth for the experimental design 1. Bars represent confidence intervals at P0= 5%

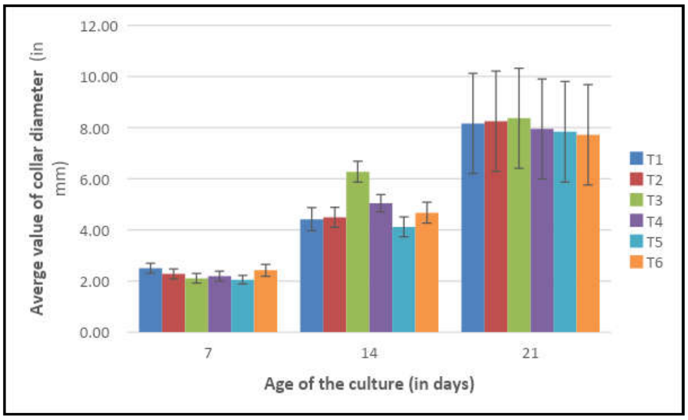

Figure 7. Treatment effect on diameter growth for the experimental design 2. Bars represent confidence intervals at P0= 5%level

Treatment effect on the total Biomass of *Amaranthus hybridus L.*

The variance analysis showed a highly significant (P<0.001) treatment effect on MSR. Averages comparison indicated that treatments T2 (1.16 ± 0.20 g) and T6 (1.08 ± 0.17 g) did not differ significantly, but were different from treatments T4 (1.06 ± 0.15g), T3 (0.95 ± 0.17g), T5 (0.93 ± 0.16 g) and T1 (0.84 ± 0.16 g). Also, a highly significant repeat effect was noted on MSR (P<0.001). Repeats 1 (0.74 ± 0.09) and 2 (1.16 ± 0.14 g) as well as 1 (0.74 ± 0.09 g) and 3 (1.11 ± 0.12 g) differed significantly. Regarding stem mass, the variance analysis also showed a highly significant treatment effect (P<0.001). The highest MST means were obtained with treatments 2 (1.97 ± 0.6 g), 4 (1.86 ± 0.29 g) and 6 (1.82 ± 0.28 g). Treatments T1 (1.43 ± 0.29 g) and T5 (1.51±0.26 g) showed the lowest MST. The replicated effect was also noted. Repeats 1 (1.34 ± 0.17) and 2 (1.93 ± 0.25) as well as 1 (1.34 ± 0.17 g) and 3 (1.88 ± 0.21 g) were significantly different. The variance analysis done on the MSF showed very significant (P<0.001) treatment and replication effects. The highest mean MSF was obtained with treatment 4 (2.93 ± 0.37 g), the control treatment. This mean value differs significantly from those obtained in treatments 1, 2, 3, 5 and 6. The mean value of treatment 4 is followed by those obtained with treatments T6 (2.41 ± 0.29g) and T2 (2.41 ± 0.36 g). The lowest mean value was recorded on T1 (1.96 ± 0.34 g). Repetitions 1 (1.89 ± 0.19 g) and 2 (2.45 ± 0.24 g) as well as 1 (1.89 ± 0.19 g) and 3 (2.65 ± 0.24 g) were significantly different. Similarly, the variance analysis done on the parameter (MSFL) showed highly significant treatment effects (P<0.001). Comparison of the means showed that the dry mass of flowers on the treatments differed significantly (Table III). The mean MSFL values obtained from treatment 2 are significantly different from those obtained on treatments 1, 3, 4, 5 and 6. The highest MSFL is thus observed in the plants of treatment 2 (1.31 ± 070 g) followed by T6 (0.74 ± 0.23 g) and the control T4 (0.56 ± 0.37 g) while the lowest was recorded in treatment 1 (0.18 ± 0.26 g). Replications 1 (0.36 ± 0.41 g) and 2 (0.59 ± 0.39 g) as well as 1 (0.36 ± 0.41 g) and 3 (0.78 ± 0.22 g) were not significantly different.

Table 3. Variation in total *A. hybridus* biomass among treatments

Treatments	Biomasses			
	MSR	MST	MSF	MSFL
T1	0,84±0,16b	1,43±0,29b	1,96±0,34c	0,18±0,26b
T2	1,16±0,20a	1,97±0,36ab	2,41±0,36b	1,31±0,70a
T3	0,95±0,17ab	1,70±0,35ab	2,09±0,27bc	0,29±0,19b
T4	1,06±0,15ab	1,86±0,29ab	2,93±0,37a	0,56±0,37b
T5	0,93±0,16ab	1,51±0,26b	2,18±0,25bc	0,43±0,52b
T6	1,08±0,17a	1,82±0,28ab	2,41±0,29b	0,74±0,23b

DISCUSSION

Although the study showed relatively low mortality rates for both experimental designs, it is important to note that the mortality rate of the second experimental design is higher at all ages and shows 2.06%, at 21 days, compared to 1.11% for experimental design 1. And the variance analysis revealed an effect at 21 days (P-value= 0.007). This difference in mortality rate between the experimental designs could be explained by the heat that accompanies the decomposition of the organic matter, assuming that for the first experimental design, the decomposition of organic matter, after a complete culture cycle of 30 days, is almost total. Considering the experimental designs, at 21 days, age of maturity of the control culture used, the highest mortality was obtained with treatment T2 (3.33 ± 3.20%) followed by treatment T5 (2.22 ± 2.63%), for experimental design 1 and, treatment T6 (5.33 ± 2.44%) followed by treatment T1 (2.88±1.81%) for experimental design 2. As for the growth parameters, the variance analysis showed a highly significant experimental design effect for height growth (P<0.001) at all ages, with the first experimental design being the best. The almost total decomposition of the organic matter, after one crop cycle, in the first experimental design would explain this better growth observed. These results are similar to those obtained by Koussihouede et *al*, (2016) who argue that the effect of organic fertilizers on different growth parameters depends on the doses applied and their duration in the soil. This would support that the supply of organic matter should be done well before the crop.

The best treatment, at 21 days, is for experimental design 1, the T6 treatment (34.37±1.97 cm); it is followed by the T2 (32.32±1.98 cm), T3 (31.97 cm) and T5 (31.55±1.96 cm) treatments. The last treatment is T4 (14.65±1.96 cm). Diameter at the collar shows the following trends: T6 (8.25±1.97 mm), followed by T3 (7.52±1.96 mm) and finally T5 (7.34±1.97 mm) and T2 (7.34±1.98 mm). For the second experimental design, the best treatment is T3 (28.59±1.96 cm) followed by T6 (26.48±1.96 cm), T2 (26.55±1.96 cm), and control T4 (27.23 ±1.96 cm). Regarding the diameter at the neck, the best treatments are T3 (8.37±1.96 mm), T1 (8.16±1.96 mm), T2 (8.25±1.96 mm) and finally T6 (7.72±1.96 mm). Regarding growth parameters, the best treatments are those associated with *Tithonia diversifolia.* Our results are similar to those obtained by Tshinyangu Kandanda et al, (2017), who used the green biomass of *Tithonia* *diversifolia,* a species capable of producing large amounts of easily decomposable and nutrient rich leaves (Muliele et al, 2017). With regard to root dry mass, the best treatment is T2. The average value of this treatment differs significantly with that of treatment 1 and not with the other treatments. The best mean values of MST were obtained with treatments, 2, 3, 4, 6. The lowest was obtained with T1. As far as leaf dry weight is concerned, the best treatment is T4, followed by T2 and then T6. The last treatment is T1. For this parameter, the mineral fertilizer treatment, T4 shows the best performance. For flower dry weight, the best treatment is T2. This treatment differs significantly with the other treatments; the last one is T1.

CONCLUSION

The results obtained on the two experimental designs made it possible to conclude on the significant effects of the treatments and the experimental designs on growth; the growth becomes significant when the biofertilizer is left to decompose well in the soil for at least one month. The mineralization of the biofertilizer helped obtain the best agronomic performance for the amaranth crop, from the second cycle. As we see, the treatments (T2, T3 and T6) that had as one of the components *Tithonia diversifolia* had the best growths. It would therefore be wise to recommend to growers to apply these different treatments in order to optimize the growth of *Amaranthus hybridus* L. instead of using chemical fertilizers that need to be applied at the beginning of each cycle. Thus, from these works, it follows that the associations *E. pyramidalis* + *Tithonia diversifolia*, *E. pyramidalis* + *P. maximum* + *T. diversifolia* + Cow dung and *P. maximum* + *T. diversifolia*, were the best biofertilizers.

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