Response of Cucurbitaceae seedlings, *Cucurbita mochata*, *Cucurbita pepo* and *Lagenaria siceraria* to the fertilization by pig *dejecta* at Brazzaville, Congo

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ABSTRACT
*Objective***:** To assess the main effects of three "Species" of Cucurbitaceae and two "Status" of their seedlings, individually taken, their interactions related to three variables measured on stem, three variables measured on leaf and one variable measured on flower were tested.
Methodology and Results: Cucurbita moschata, Cucurbita pepo and *Lagenaria Siceraria* were used as genotypes. They were planted according to a completely randomised design. Growth variables were measured on the stem, leaf and flower. The General Linear Model combining the comparison of means according to the Student Newman-Keuls test as well as the Student t or Z test with two samples at the risk threshold of 5% were applied. With regard to the first of the 3 interactions, the variations of the diameter at the collar (DC), diameter of the rod (DT) and length of the rod (LT) were independent of the variations of "Species" and "Status "authorizing the classifications of the variants of the aforementioned factors. For the second of the 3 interactions, the fluctuations in leaf length (LF), blade width (IL) and blade length (LL2) were dependent of the simultaneous fluctuations of the species and the Prohibiting Status any classification. Regarding the third interaction, the variations in the number of flowers produced (NFP) were independent of those concomitant with the Species and Status, allowing classification of the same variants of the above factors. For the first of the 3 interactions mentioned above, concerning the main effects "Species" and "Status", *Lagenaria siceraria* recorded both the highest diameter at the collar and the longest stem as well as a greater growth of seedlings treated with pig *dejecta.* . This suggests that growth in Cucurbitaceae is genotype dependent. It is also influenced by pig *dejecta*. Concerning the same main effects "Species" and "Status", linked to the third interaction, Cucurbita pepo and the seedlings treated with organic *dejecta* from pigs expressed the greatest cumulative effective number of flowers (EFFCUM).
Conclusion and application of the results: Factors "Species" and "Status" can be studied simultaneously, because they do not interact on the DC, DT, LT and EFFCUM. *Lagenaria siceraria* grows very well relative to the first 3 variables mentioned above while *Cucurbita pepo* expresses a high cumulative number of flowers. Three kilograms of pig *dejecta* are necessary to allow good growth of the stem and diameter of *Largenaria siceraria*. The latter should be used for the production of aboveground biomass while *Cucurbita pepo* should be used for the production of fruits.
Key words: Growth variables, GLM of Anova, pig *dejecta*, interaction.

RESUME

Objectif **:** Pour évaluer les effets principaux, de trois "Espèces" et deux "Statuts" des plants de semis, pris individuellement, leurs interactions liées à trois variables mesurées sur la tige, trois variables mesurées sur la feuille et une variable mesurée sur la fleur ont été testées.

Méthodologie et Résultats : Cucurbita moschata, Cucurbita pepo et Lagenaria Siceraria ont été utilisés comme génotypes. Ils ont été plantés selon un dispositif en bloc complètement aléatoire. Des variables de croissance ont été mesurées sur la tige, la feuille et la fleur. Le Modèle Géneral Linéaire associant la comparaison de moyennes selon le test de Student Newman-Keuls ainsi que le test t ou Z de Student à deux échantillons au seuil de risque de 5 % ont été appliqués. En ce qui concerne la première des 3 interactions, les variations du diamètre au collet (DC), diamètre de la tige (DT) et longueur de la tige (LT) ont été indépendantes des variations de "l'Espèce" et du "Status" autorisant les classifications des variantes de facteurs pré-cités. Pour la deuxième des 3 interactions, les fluctuations de la longueur de la feuille (LF), de la largeur du limbe (IL) et de la longueur du limbe (LL2) ont été dépendantes des fluctuations simultanées de l'Espèce et du Statut, interdisant toute classification. Concernant, la troisième interaction, les variations du nombre de fleurs produites (NFP) ont été indépendantes de celles concomitantes de l'Espèce et du Status, permettant la classification des mêmes variantes de facteurs sus-cités. Pour la première des 3 interactions sus-mentionnées, concernant les effets principaux "Species" et "Status", *Lagenaria siceraria* a enregistré à la fois le plus haut diamètre au collet et la tige la plus longue ainsi qu'une plus grande croissance de plants de semis traités avec la fumure de porc. Ceci suggère que la croissance chez les Cucurbitaceae est genotype dépendant. Elle est aussi influencée par la fumure de porc. Concernant les mêmes effets principaux "Species" et "Status", liés à la 3e interaction, *Cucurbita pepo* et les plants de semis traités avec la fumure organique de porc ont exprimé le plus grand nombre effectif cumulé de fleurs (EFFCUM).

Conclusion et application des résultats **:** Facteurs "Species" et "Status" peuvent être simultanément étudiés, car ils n'interagissent pas sur le DC, DT, LT ainsi que l'EFFCUM. *Lagenaria siceraria* croit très bien relativement aux 3 premières variables sus-citées alors que *Cucurbita pepo* exprime un haut effectif cumulé de fleurs. Trois Kilogrammes de fumure de porc sont nécessaires pour permettre une bonne croissance de la tige et du diamètre de *Largenaria siceraria*. Ce dernier devrait être utilisé pour la production de la biomasse aérienne alors que *Cucurbita pepo* devrait l'être pour la production de fruits.

INTRODUCTION

Cucurbitaceae is family of dicotyledonous plants used in the human food. They are used as food, source of proteins and fats yielding as well as secondary metabolites (Toshihiro *et al*., 2001). World squash yielding reaches 16 million tonnes obtained from 1.3 million hectares (Robinson, 1997). In Congo (Brazzaville), the Pool, Bouenza and Plateaux regions yield squash (Dupriez *et al*., 1987). The species of Cucurbitaceae such as *Cucurbita mochata*, *Cucurbita pepo* and *Lagenaria siceraria* are cultivated for food and medicinal purposes. Nonetheless, no statistics is available. Likewise, no information is available about the agro-morphological description of local Congolese cultivars. Nevertheless, ethno-botanical and biochemical studies were carried out (Moutsamboté *et al*., 1988; Mampouya *et al*., 2012). Thus, the characteristics related to the growth of stem, leaf and flowers are unknown. Indeed, on-station and on-farm trials evidencing the characteristics of stem, leaf and flowers have never been achieved in Congo-Brazzaville. Likewise, no information is available about interaction between two factors implied in an on-station and on-farm studies. The achieving of these trials in the field could allow good description of locally cultivated species. Likewise, factors such as "Species" accounting for genotypes and "Status" of seedlings corresponding to the adding or not of the organic pig *dejecta* were never tested in the form of interaction and main effects. The test of effects of these factors might allow us to know whether we can study them simultaneously. For that, we postulate that the variations of diameter at collar, diameter of stem and length of stem as well as cumulative count of flowers are independent of those from factors "Species" and "Status" of seedlings. The work aimed to study the response to the fertilisation by pig's *dejecta* of the Cucurbitaceae seedlings of *Cucurbita mochata*, *Cucurbita pepo* and *Lagenaria siceraria* at Brazzaville, Congo

MATERIALS AND METHODS

Study site, plant material, soil state, trial monitoring, installing and experimental design: Experiment was installed and monitored at the garden of Faculty of Sciences and Techniques from Marien Ngouabi University. This one is located at Bacongo area, particularly at 15°15'17.3'' West longitude, 4°17'1.7'' North latitude and 291 metres above sea level. This experiment was carried out from 3nd January to 30th May 2018. Climate is sub-equatorial type with small dry and minor rainy seasons as well as long rainy and long dry seasons. Structure of soil is particulate type with clay sand texture. Plant material consisted of three species, known as *Cucurbita moschata*, *Cucurbita pepo* and *Lagenaria siceraria* whose seeds were used in experiment. Experiment area measuring 120 m^2 brought six ridges of 11 metres in length and 1.20 metre width each. Each ridge brought two rows of plantation of 10 seedlings each. Gap of 1 metre was maintained among seedlings from the same row. Organic pig *dejecta* used as basal fertilisation was added in seed pocket due to 3 seeds per pocket. Three ridges received organic fertilisation while three others did not receive it. A two-complete factor 3 x 2 scheme in a completely randomised design was used. Factors "Species" and "Status" with 3 and 2 variants respectively were tested. Here, treatment was defined as a combination of variants of factors "Species" and "Status" of seedlings. Six treatments were tested. Each treatment being in triplicate, in all 18 treatments were installed in the experimental design. Density of plantation was 1.51 seedling/m^2. Two weeks after the sowing, germinated seeds and having been regenerated in seedlings were separate. The surplus was conserved onto ridge for potential replacement. Seedlings were watered with 50 litres of water per ridge. Weeding and harrowing were done lightly every two weeks. Measurements were carried out one month later. Two groups of variables were measured. These are growth and flowering variables. The former were composed of stem and leaf variables whereas the latter consisted of cumulative flowers count (EFFCUM). Variables of stem were accounted for by diameter at collar (DC), diameter of stem (DT) and length of stem (LT). Variables of leaf were constituted of length of leaf (LF), width of blade (IL) and length of blade (LL2). Variable of flowering consisted of cumulative count of flowers yielded (EFFCUM)

Statistical data analysis: Xlstat and SPSS software, versions 2007 and 22.0, respectively were used. Means comparison incorporated in Anova with or without GLM as well as Student's parametric two-sample t or Z test at 5% likelihood were applied. For Anova, the following model, related to completely randomised design was applied: Y = μ + Species + Status + Species x Status + ε. Where, Y is response variable. μ is general mean. Species is the first factor comprising three variants. Status is the second one counting two variants. Species x Status represents the interaction between the two above factors cited. ε is the error. Error eij is supposed to be normally distributed with null mean and variance σ^2, that is to say, eij ~ N (0, σ^2).

RESULTS

Impact of the three tested species and *dejecta* addition, on the expression of the DC, DT and LT: The examination of interaction Species x Status revealed that the variations of diameter at collar (DC), diameter of stem (DT) and that of length of stem (LT) were independent of concomitant variations of Species and Status (p-values DC = 0.296; p-values DL = 0.358; p-values LT = 0.444).. This examination shows that when passed from Species to Species or from Status to Status, such variations trigger the significant variations of diameter at collar (p-values DC = 0.000***) and those of length of stem (p-values LT = 0.000***). These variations can be structured in distinct sub-sets. In the same way, regarding the Status of seedlings. This allowed classifying the means of factors "Species" and "Status" (Table1).

Table 1: Assessment of the effects of factors "Species" and "Status" of seedlings on the expression of three measured variables through Anova.

Source	Dependent variable*	SS*	df*	MS*	F*	p-value*
Corrected model	DC	6.034	5	1.207	28.707	0.000
	DT	100.759	5	20.152	1.3	0.262
	LT	2164614.520c	5	432922.904	12.05	0.000
Intercept	DC	199.497	1	199.497	4745.31	0.000
	DT	341.675	1	341.675	22.1	0.000
	LT	14348907	1	14348907	399.29	0.000
Species	DC	5.133	2	2.566	61.05	0.000
	DT	44.279	2	22.14	1.432	0.241
	LT	1605897.54	2	802948.77	22.344	0.000
Status	DC	0.799	1	0.799	18.997	0.000
	DT	24.573	1	24.573	1.589	0.208
	LT	500208.333	1	500208.333	13.919	0.000
Species x Status	DC	0.103	2	0.051	1.224	0.296
	DT	31.907	2	15.953	1.032	0.358
	LT	58508.647	2	29254.323	0.814	0.444
Error	DC	12.36	294	0.042		
	DT	4545.891	294	15.462		
	LT	10565330.5	294	35936.498		
Total	DC	217.892	300			
	DT	4988.325	300			
	LT	27078852	300			
Corrected total	DC	18.394	299			
	DT	4646.65	299			
	LT	12729945	299			

Legend. Dependent variable*: DC: Diameter at collar. **DT:** Diameter of stem. **LT:** Length of stem. **SS*:** Sum of squares. **df*:** Degree of freedom. **MS*:** Mean of square. **F*:** Fisher-Snedecor's test statistics. **P-value***: Critical value.

Regarding factor "Species", three classes were both identified for diameter at collar and length of stem as against one for diameter of stem. Firstly, consisting of Species *Cucurbita moschata* was distinguished by small diameter at collar and length of stem. Secondly, constituted of Species *Cucurbita pepo* L. was marked by average diameter at collar and length of stem. Thirdly, composed of Species *Lagenaria siceraria*, was characterised by big diameter at collar and high length of stem (Table 2). With respect to "Status", here also, out of diameter of stem, two distinct sub-sets were revealed with respect to diameter at collar and length of stem. In the first, composed of seedlings at which no organic pig *dejecta* as basal fertilisation was added, was characterised by low diameter at collar and length of stem. In the second, consisted of seedlings for which organic pig fertiliser as basal fertilisation was brought, was singular in high diameter at collar and length of stem (Table 2).

Table 2: Means classification of the diameter at collar, diameter of stem and length of stem as a function of the species and *dejecta* adding.

Dependent variable*	Status*	Mean*	CV (%)*	Dependent variable	Species*	Mean	CV (%)
DC	Without *dejecta*	0.764a	2.56	DC	Cm	0.636a	3.30
	With *dejecta*	0.867b	2.32		Cp	0.653b	6.18
DT	Without *dejecta*	0.781a	2.33		Ls	0.868c	2.42
	With *dejecta*	1.353a	3.57	DT	Cm	0.943a	2.23
LT	Without *dejecta*	177.867a	7.45		Cp	0.970a	4.52
	With *dejecta*	259.533b	7.43		Ls	1.579a	24.89
				LT	Cm	136.260a	13.91
					Cp	205.770b	9.21
					Ls	314.070c	6.04

Legend. Dependent variable*: DC: Diameter at collar. **DT:** Diameter of stem. **LT:** Length of stem. **Treatment*: Without *dejecta*:** Control treatment. **With *dejecta*:** Tested treatment. **Mean*: Values** accompanied by the same letter are not significantly different according to Student's parametric two-sample Z test at 5% level. **CV (%)*:** Coefficient of variation. **Species*: Cm:** *Cucurbita moschata.* **Cp:** *Cucurbita pepo*. **Ls:** *Lagenaria Siceraria*.

Influence of the factors "Species" and "Status" of seedlings on the expression of three measured variables on leaves: The appreciating of interaction Species x Status of seedlings shown that the variations of length of leaf (LF), width of blade (IL) and length of blade (LL2) were dependent of simultaneous variations of Species and Status (p-value LF = 0.004**; p-value IL= 0.000*** ; p-value LL2 = 0.000***). This suggested the non-examination of main effects (Table 3).

Table 3: Assessment of the effects of factors "species" and "treatment" on the expression of length of leaf, width of blade and length of blade via Anova.

Source	Dependent variable*	SS	df	MS	F	p-value
Corrected model	LF	50260.475	5	10052.095	256.564	0.000
	IL	20259.746	5	4051.949	249.426	0.000
	LL2	4780.605	5	956.121	59.033	0.000
Intercept	LF	652737.857	1	652737.857	16660.127	0.000
	IL	345617.475	1	345617.475	21275.193	0.000
	LL2	188210.975	1	188210.975	11620.508	0.000
Species	LF	49397.873	2	24698.936	630.402	0.000
	IL	18786.351	2	9393.175	578.216	0.000
	LL2	2531.772	2	1265.886	78.158	0.000
Status	LF	425.221	1	425.221	10.853	0.001
	IL	930.273	1	930.273	57.265	0.000
	LL2	314.263	1	314.263	19.403	0.000
Species x Status	LF	431.019	2	215.510	5.501	0.004
	IL	567.073	2	283.537	17.454	0.000
	LL2	1896.972	2	948.486	58.561	0.000
Error	LF	58416.850	1491	39.180		
	IL	24221.432	1491	16.245		
	LL2	24148.907	1491	16.196		
Total	LF	771444.060	1497			
	IL	394956.750	1497			
	LL2	218590.500	1497			
Corrected total	LF	108677.325	1496			
	IL	44481.179	1496			
	LL2	28929.511	1496			

Legend. Dependent variable*: LF: length of leaf. **IL:** width of blade. **LL2:** length of blade.

Effect of the factors "Species" and "Status" on the expression of the cumulative count of flowers: The analysis of interaction Species x Status of seedlings shown that the variations of cumulative count of flowers independent of concurrent variations of Species and Status. This allowed the appreciating of main effects "Species" and "Status". Indeed, the variations of Species caused the variations of cumulative count of flowers suggesting the possibility to classify these Species in distinct classes. In the same way, the variations of Status of seedlings triggered the variations of cumulative count of flowers. These variations can be structured in distinct groups (Table 4). This authorised the means classification of cumulative count of flowers.

Table 4: Assessment of the effects of factors "Species" and "Status" of seedlings on the expression of the cumulative count of flowers by means of Anova.

Source	SS	df	MS	F	p-value
Corrected model	407024.377	5	81404.875	37.448	0.000
Intercept	1016986.96	1	1016986.96	467.838	0.000
Species	386757.547	2	193378.773	88.959	0.000
Status	16295.07	1	16295.07	7.496	0.007
Species x Status	3971.76	2	1985.88	0.914	0.402
Error	639097.66	294	2173.802		
Total	2063109	300			
Corrected total	1046122.04	299			

As far as the factor "Species" is concerned, three sub-sets were observed. First, accounted for by Species *Cucurbita moschata* was characterised by low cumulative count of flowers. Second, composed of Species *Lagenaria siceraria* was marked by average cumulative count of flowers. Third, constituted of Species *Cucurbita pepo* was singular in high cumulative count of flowers. Coefficient of variation varied from 4.9 to 19.12% (Table 5). As for "Status" of seedlings, two distinct groups were displayed. Firstly, constituted of seedlings at which no organic pig fertiliser as basal fertilisation was added, was marked by low cumulative count of flowers. Secondly, consisting of seedlings at which organic pig fertiliser as basal fertilisation was added was distinguished by high cumulative count of flowers. Magnitude of variation between the mean and each of modalities of the cumulative flowers count fluctuated from 5.8 to 7.49% (Table 5).

Table 5: Means classification of the cumulative count of flowers as a function of the species and status.

Dependent variable*	Factor	Treatment	Mean*	CV (%)
EFFCUM	Status	Without *dejecta*	50.853a	7.49
		With *dejecta*	65.593b	5.8
	Species	Cm	16.01a	19.12
		Ls	54.89b	8.49
		Cp	103.77c	4.49

Legend. Dependent variable*. EFFCUM: Cumulative count of flowers. **Treatment. Cm:** *Cucurbita moschata*. **Ls:** *Largenaria siceraria*. **Cp:** *Cucurbita pepo* L. **Mean*:** Values accompanied by different letters in column are significantly different according to Student's parametric two-sample Z and Student-Newman-Keuls' tests at 5% probability.

DISCUSSION
Reciprocal influence Species x Status of seedlings was analysed with a view to appreciate their main effects, respectively, on the expression of seven measured variables. Works from Bertucci *et al*., (2018) revealed the lacking of interaction among rootstock, species and harvest date in cucurbits. Density x Season interaction tested in Dje-Bi *et al*., (2011) working onto Cucurbitaceae did not display the existence of interaction. Our works showed the non-existence of interaction with variables from stem and leaf but its existence with variables from the growth of leaf. Reciprocal effect Species x Status of seedling was non-existent with diameter at collar, diameter of stem and length of stem as well as length of leaf, width of blade and length of blade. However, this interaction was significant with cumulative count of flowers. The lacking of interaction might be found an explanation through the absence of competition for nutrients for the same cell

sites brought by organic pig *dejecta* for stem and leaf developments. This might be due to action of these nutrients on distinct genes activity on DNA. In opposite, the existence might find an explanation through the competition for nutrients for the same cell sites brought by organic pig *dejecta* for stem and leaf developments. In the same way, this interaction existence might be due to action of these nutrients on the genes activity brought by DNA. In sum, the lacking or existence of interaction Species x Status of seedlings might be due to action of nutrients on distinct or similar cell sites or action of these nutrients on distinct or identical genes from DNA. Works from Bertucci *et al.*, (2018) revealed the lacking of interaction among rootstock, species and harvest date in cucurbits. In contrast, Issali *et al.*, (2011) shown the existence of interaction between Staminodes x Petals in co-culture relatively to number of yielded callus. Consequently, in future, it will be necessary to continue measurements on stem and leaves when the 3 species of Cucurbitaceae when submitted to adding of organic pig *dejecta*. Likewise, it will be necessary to avoid measurement of cumulative count of flowers when the 3 species and 2 Status will be associated.

CONCLUSION

This study postulated that the variations of diameter at collar, diameter of stem and length of stem as well as cumulative count of flowers are independent of those from factors "Species" and "Status" of seedlings. Effectively, interaction was not significant relatively to diameter at collar and length of stem as well as cumulative count of flowers. Consequently, variants of factors whose interaction was not significant were classified. In future, it will be necessary to continue measurements on stem and leaves when the three species of Cucurbitaceae will be submitted to adding of organic pig *dejecta*. Likewise, it will be necessary to avoid measurement of diameter of stem when the 2

The adding of organic pig *dejecta* allowed better growth of diameter at collar, length of stem and cumulative count of flowers. Stems and flowers for their growth and development could directly use assimilated nutrients. Bembé *et al.*, (2010) and Salifou *et al.*, (2015) working without *dejecta*, shown that *Lagenaria* sp. recorded low values of length of stem than us. Indeed, organic farm *dejecta*s bring major nutrients such as N, P, K, Ca and Mg the minor ones such as Cu, Zn, Mn, Fe, B and Mo requested for growth of cultivated plants (Tran Sen *et al.*, 2007). Thus, 3 kilogrammes of organic pig *dejecta* should be brought in seed pocket before the sowing. They are necessary to improve the growth of stem and cumulative count of flowers   *Lagenaria siceraria* expressed good growth of diameter at collar as well as that of length of stem while *Cucurbita pepo* shown good yielding of flowers. The genes from species *Lagenaria siceraria* could be activated more easily for growth whereas those from *Cucurbita pepo* in flowers and fruits yielding. Bembé *et al.*, (2010) reported that *Largenaria siceraria* grown more than *Cucurbita moschata*. Zoro Bi *et al.*, (2003) shown that *Largenaria siceraria* yielded more flowers than *Cucurbita moschata*.

Status and 3 Species will be associated to gain the time. The adding of organic pig *dejecta* allowed better growth of diameter at collar, length of stem and cumulative count of flowers. Thus, 3 kilogrammes of organic pig *dejecta* should be brought in seed pocket before the sowing. They are necessary to improve the growth of stem and cumulative count of flowers. *Lagenaria siceraria* expressed good growth of diameter at collar as well as that of length of stem while *Cucurbita pepo* shown good yielding of flowers. Therefore, for more growth of stem, *Largenaria siceraria* should be used whereas for the yielding of higher number of flowers and fruit, *Cucurbita pepo* should be recommended.

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