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Effect of Some Germination Substrates on the Emergence Rate of Pregerminated Beans in Water and the Growth of Seedlings in Cocoa (*Theobroma cacao* L.) Nursery, under Kisangani Conditions

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Abstract. In an attempt to determine the best substrate for germination and growth in cocoa nurseries, a study was conducted using a randomized complete block design with three replications of three stubble treatments. The treatments tested corresponding to the substrates were forest soils (T_1), decomposed pig manure (T_2) and a mixture of the two substrates (soils and manure). Observations were made on the emergence rate and growth in the nursery. Growth was assessed at three by measuring the diameter at the collar and the height of the seedlings as well as by counting the number of leaves.

The results obtained indicated that decomposed pig manure was the best germination substrate (100%), followed in decreasing order by the mixture (forest soil and decomposed pig manure) (57.8%) and forest soil (44.4%). The growth was generally more favorable in the mixture although there were no significant differences between the three substrates, so the substrates have similar effects in the growth of cocoa seedlings in nursery. The mean values varied from 5.51 to 5.83 mm for diameter; 32.9 to 34.45 cm for height and 12 to 15 leaves unfolded over a period of three months.

Key words: substrate; emergence rate; pregerminated beans; growth; seedlings; nursery

Introduction

Issues

Agriculture is and has always been the basis of the economy of all countries in general and the Congolese economy in particular. Its share of national income reached up to 50% in the 1990s, partly due to the collapse of other sectors of the economy (mining in particular). Since the restoration of peace in 2002, this share has gradually declined, but the agricultural sector still provided 40.3 percent of GDP (compared to about 13 percent for the mining sector) and employed three-quarters of the labor force in 2006 (Jean-Paul *et al*., 2012).

The Democratic Republic of Congo has exceptional agronomic potential and an area of agricultural land unmatched in Africa, with only 5% of its 80 million hectares of arable land in use. The International Food Policy Research Institute (IFPRI) estimates that the DRC has the potential to produce enough food for 3 billion people. Despite this exceptional potential, the DR Congo is unable to feed its approximately 70 million inhabitants (Jerome, 2015).

Cocoa cultivation is expected to grow significantly in the DRC due to the excellent agro-climatic conditions and the fact that the crop is not very labor-intensive or requires special cultivation methods. While in the vast majority of producing countries, production comes essentially from family plantations, in the Congo it has been and still is largely the result of commercial plantations. At independence, production was 5200 tons and was largely the work of agro-industrial companies established in the provinces of Equateur and Bas-Congo. The Unilever Company accounted for 45% of total production and five other large plantations for 40%. Village production (10%) was developed around these agro-industries, which offered plants and outlets to village planters, and through specific projects such as the

Bengamisa Cocoa Project (CABEN) in the Eastern Province. Production has never exceeded a very low level, especially when compared to other African countries such as Côte d'Ivoire (1.4 million t.), Ghana (750,000 t.), Nigeria and even neighboring Cameroon. In the 1980s, it reached a maximum of 6,300 tons before slowly declining to around 3,500 tons per year in the mid-1990s. It was 2,000 tons in 2006 (Jean-Paul *et al*., 2012).

It is difficult to have a precise idea of the current state of the sector, both in terms of production (plantation areas, yields, production) and marketing channels. Production is essentially located in Bas-Congo, Equateur (north) and Oriental Province, with small amounts still produced in Bandundu, Maniema, Kasai (north), South and North Kivu. Actual statistics are not available, and much of the production that leaves the country illegally enters the data of neighboring countries such as Uganda and Rwanda.

Currently, there is talk of reviving cocoa and coffee farming. This revival must be supported by various structures such as the production of homogeneous, unharmed and quality seedlings, the development of modern cultivation methods that are efficient and appropriate, etc.

It is in this perceptive that we carried out this investigation based on the study of the influence of some substrates on the rate of emergence of the pregerminated seeds in water and the juvenile growth of the seedlings of Cacaoyer (*Theobroma cacao* L) in the conditions of Kisangani. This study attempts to answer the following questions:

What is the germination capacity of pre-germinated seeds in water and nursery growth of cocoa seedlings obtained under forest soils, decomposed pig dung and their mixture?

What is the best germination and nursery substrate for use in seedling production in cocoa?

Hypothesis

The present work is conducted under the following research hypotheses:

- Forest soils, decomposed pig manure and their mixture have different effects on the emergence of pre-germinated seeds and nursery growth of cocoa seedlings, given that each substrate has its own physical, chemical and biological characteristics;
- The mixture of forest soils and decomposed pig dung is the best germination and nursery substrate compared to their separate use for the production of planting material in cocoa.

Objectives of the Work

The general objective of this work is to highlight the influence of some substrates on the emergence rate of pre-germinated seeds in water and the nursery growth of cocoa seedlings under Kisangani conditions.

To achieve this objective, the work has the following specific objectives:

- To evaluate the emergence rate of pre-germinated seeds as well as the growth in nursery of the seedlings obtained under various substrates;
- Determine the best germination and nursery substrate to use in order to obtain quality and homogeneous cocoa seedlings.

Interest of the Work

The present work has a double interest namely scientific and economic:

- On the scientific level, it constitutes a source of information for any researcher interested in pregerminative seed treatments in general and in the production of seedlings in the cocoa tree in particular;
- On the economic level, the mastery of a good technique of seedling production in nursery will be able to generate optimal incomes to farmers and nurserymen.

Environment, Materials and Methods

Environment

The experiment was carried out in the concession of the Institut Facultaire des Sciences Agronomiques de Yangambi (IFA-Yangambi) located on Avenue Abbé Munyororo, in the Plateau Médical district of the commune of Makiso in Kisangani. Kisangani, capital of the Tshopo Province, is located in the eastern part of the central Congolese basin at 0°31'N and 25°11'East, at an average altitude of 396 m.

The city of Kisangani which has a climate that belongs to the Af type of the Koppen classification. It is a hot and humid equatorial climate, with annual, monthly and daily temperatures varying around 25°C. Temperature variations are not very noticeable. Relative humidity persists throughout the year between 80 and 90%, rainfall is abundant. The average rainfall is high throughout the year, but the distribution is by no means uniform: 1728.4 mm (minimum 417.5 mm and maximum 1915.4 mm) interrupted by two short dry seasons. The soils of Kisangani are ferralitic of the ferra-soil type and belong to the oxisol order of the Soil Taxonomy, consisting of sands and clays and poor in nutrients and organic matter. They are deep, leached by rainwater and have a sandy-clay texture. This soil contains for 100g: 0,41 meq of exchangeable aluminium; 0,25 meq of exchangeable hydrogen; 7,75 meq of exchangeable bases; while the cationic exchange capacity amounts to 8,4 meq/100g. The clay fraction is very rich in kaolinite, the clear dominance of the latter translates the state of extreme alteration of the Kisangani soil (Mambani *et al.*, 2007). The vegetation of the city of Kisangani was formerly an evergreen rainforest. According to Lubini (1982), there were climatic formations of *Gilbertiodendron dewevrei* on ferrasols. Kisangani currently offers a profoundly altered vegetation. This modification is one of the causes of important climatic changes observed in the region.

Material Used

The biological material used during the experiment consisted of cocoa beans (seeds) of the Trinitario group of the Angolan variety (*Theobroma cacao* L) harvested in the Alibuku plantation located 56 km from the city. The pods used are shown in Figure 1.

Figure 1: Pods used in our trial

The technical equipment used in this work was constituted in particular by the watering can, wheelbarrow, machete; tape measure; polyethylene bags and foot-slide. In addition to these materials, we also used forest soils and decomposed pig manure.

Methodology

Experimental design and treatments

In our experiment, we adopted the randomized complete block design with three replications of three treatments each.

The experimental treatments consisted of three substrates: forest soil (T_1), pig manure (T_2) and a mixture of soil and pig manure (T_3). The experimental plots are 50 cm long and 30 cm wide, i.e. 1500 cm² of surface separated by 15 cm in all directions. The total area of the plot is 2.04 m².

Conduct of the trial

Our test was conducted in a shed (a shade), after the various works of clearing and leveling. The shade house has a length of 3 m, width of 2.20 m and height of 1.60 m. After the construction of the shed (a shade), we proceeded to the filling of the polyethylene bags. To allow a better arrangement under the shade, the pointed bases of the bags are pressed in order to obtain a more or less circular base when the bag is filled to 3/4 of their capacity. Then, the bags were filled with the different substrates leaving 1 to 2 cm of free height.

After extraction of the beans from the pods, we soaked the beans in water at room temperature for 3 days before sowing. This step constitutes the pre-germination.

The sowing was done in polyethylene bags at a rate of one seed per bag at a depth of about 2 cm. We sowed by depositing the seeds flat and we covered each seed with a small superficial layer of soil to facilitate germination and emergence because the seeds of cocoa tree are those with negative photosensitivity. A total of 135 pre-germinated seeds were sown at a rate of 45 seeds per treatment.

For a good development of the seedlings, different maintenance operations were carried out periodically on the seedlings and in the shade house. Within the shadehouse, the maintenance work carried out is essentially the manual weeding of the shadehouse and its surroundings without forgetting the weeding of the bags containing the young plants. These weedings were made as soon as we notice a weediness.

The plants were watered periodically according to their age: from sowing to emergence, watering was done daily and after the effective emergence, watering was done every two days following the recommendations of Kebe *et al*. (2009).

We retained some parameters in our trial to evaluate the effects of the applied treatments: Emergence rate; height of seedlings from 1 to 3 months; number of leaves formed from 1 to 3 months; diameter at the collar of seedlings from 1 to 3 months.

The emergence rate was evaluated as a percentage. It was obtained by dividing the number of seedlings obtained by the number of beans sown, all multiplied by 100.

The height of the seedlings, the number of leaves formed and the diameter at the collar were taken every month for three months by the different measurements.

The effects of forest soils and decomposed pig manure on the growth parameters were assessed by one-way analysis of variance with Statgraphics software (version 5.0). The significance level was set at 0.05%.

Presentation of Results, Analysis and Discussion

Presentation of Results and Analysis

Seed emergence rate under different treatments

Data on cocoa seed emergence rate and emergence time are presented in Table 1.

Table 1: Cocoa seed emergence rate under different treatments (%)

Treatment	T_1	T_2	T_3
Number of seeds sown	45	45	45
Number of emerged seeds	20	45	26
Emergence rate (%)	**44,4**	**100**	**57,8**

Note: T_1: Topsoil; T_2: Decomposed pig manure; and T_3: Mixture of topsoil and pig manure

From the analysis of the results presented in Table 1, it appears that the rate of emergence varied according to the substrate used and this was in the range of 44.4 to 100% for all treatments. Based on the results thus obtained, we note that T_2 (decomposed pig manure) was characterized by a higher germination capacity compared to the other treatments.

Growth of seedlings in nursery

The growth data of cocoa seedlings produced in the nursery under various treatments are shown in Table 2. The raw data can be found in the Appendix.

Table 2: Mean collar diameter, height and number of leaves of cocoa seedlings at 3 months in the nursery

Parameters	T_1	T_2	T_3
Diameter at crown (mm)	5,51±0,61a	5,62± 0,20 a	5,83± 0,08 a
Height (cm)	32,9±9,971b	33,59±3,28b	34,45±0,58b
Number of leaves	**14,6 ±53.40 c**	**11,58 ±0,54c**	**13.4±0,86 c**

Note: T_1: Forest soils; T_2: Decomposed pig manure; and T_3: Mixture of forest soils and pig manure

From the results presented in Table 2, it appears that the growth of cocoa seedlings in the nursery varied from one treatment (substrate) to the other. There is a slight performance of the mixture of the two substrates (T_3) compared to the separate use for the three vegetative parameters measured. Statistical analysis revealed that there were no significant differences between treatments for the three parameters (*P*-value > 0.05) and also all data were clustered around the means.

Discussion of the Results

Seed emergence rate

The efficiency of the treatments is as follows: T_2 (100%) > T_3 (57.77%) > T_1 (44.44%). Despite this difference in emergence rate, it can be retained that they constitute a better substrate for germination of cocoa beans. Furthermore, the success of germination remains dependent on the physical, physiological and sanitary qualities of the seeds (N'Dri *et al*., 2011). Seed emergence is related to the qualitative composition of the substrates.

Comparing our results with those obtained by (Pamphile *et al*., 2019) in Gabon on the regeneration of Cacaoyer on a substrate based on *Jatropha curcas* L compost; using three substrates, substrate 1: 40% *Jatropha curcus* L compost added to 60% soil, substrate 2: 40% *Jatropha curcas* L compost mixed with sawdust added to 60% soil and substrate 3: 100% soil. The results show that substrates 1 and 2 have identical germination rates, i.e. 100%. On the other hand, substrate 3 has a lower germination rate of 85.71%. Thus, we see almost the same trends of influence of substrates. Indeed, these authors found germination rates in the range of 85.71% to 100%.

In a study on the improvement of the productivity of cocoa orchards for sustainable forest management in Togo by Ayi (2017) obtained an emergence rate of 95 to 96% in both types of pots using a substrate from a surface leached soil in the center, dried in crushed ambient air and sieved. Clearly, these results support the hypothesis that there are different

effects on the emergence rate of pre-germinated cocoa seeds between forest soils, pig manure and their mixture. These results show that the emergence rate of cocoa seeds is a function of the substrate used. These results indicate that decomposed pig dung has a positive influence on the germination capacity of cocoa seeds and can be designated as the best substrate for seed germination tests compared to others. These results support our second hypothesis that the mixture is the best substrate.

Diameter at the neck of the seedlings

For the diameter at the neck parameter the averages are: T_3 (5.83±0.08 mm), then T_2 (5.62±3.28 mm) on the one hand and T_1 (5.51±0.61 mm) on the other. The mixing of the two substrates was somewhat favorable to the vigor of the seedlings due to the synergism, probably because of the combined effects of the forest soils and decomposed pig manure.

Comparing our results with those obtained by (Pamphile *et al*., 2019), it is clear that there is a conformity. These results show that the substrates used over time have an influence on the diameter at the collar of cocoa seedlings and the averages present a significant difference on the different substrates used.

Height of seedlings

The average height of seedlings at three months is as follows: T_3 (34.45±0.58 cm) for the mixture of forest soil and decomposed pig dung, T_2 (33.59±3.28 cm) for pig dung and T_1 (32.91±9.97 cm). These results on the average height of seedlings 3 months after sowing are comparable to those obtained by Kouassi (2012) on the evaluation of different fertilizers on the growth and development of cocoa tree nursery raised on different substrates, he confirmed that from the ninth to the thirteenth week, the growth of seedlings is influenced by the substrate used. In the case of innovative cocoa farming systems (Raymond *et al*., 2010), a 6-month-old cocoa plant should be at least 60-70 cm tall with a stem diameter at its base of 0.8-1 cm or equivalent to the size of a pen.

Number of leaves formed per plantlet

Seedlings in T_1 developed more leaves 15 on average over the observation time compared to T_3 (13 leaves) and T_2 (12 leaves). During the three months of observation T_1 (forest soil) had a slightly larger numerical average compared to the other substrates.

Comparing our results with those obtained by (Pamphile *et al.*, 2019) in Gabon on the regeneration of Cacao tree on a substrate based on *Jatropha curcas* L compost. Using three substrates, substrate 1: 40% *Jatropha curcas* L compost added to 60% soil, substrate 2: 40% *Jatropha curcas* L compost mixed with sawdust added to 60% soil and substrate 3: 100% soil. They found that the plants of substrate 1 developed 6 leaves on average followed by substrate 2 (5 leaves) and the control (5 leaves).

Conclusion

The objective of the present work was to highlight the influence of some germination substrates on the emergence rate and juvenile growth of Cacaoyer seeds pre-germinated in water by soaking for six days in Kisangani. Thus, a trial was set up in a randomized complete block design with three replications of three treatments each in the Faculty Institute of Agronomic Sciences of Yangambi. The treatments consisted of three substrates: forest soil (T_1), decomposed pig manure (T_2) and a mixture of forest soil and pig manure (T_3). The parameters observed were the emergence rate, the diameter at the collar, the height and the number of leaves formed per plantlet.

The overall results showed that decomposed pig manure was the best germination substrate as it resulted in 100% emergence. From a growth point of view, the three substrates tested present an alternative in the production of good quality cocoa seedlings.

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APPENDIX OF RAW GROWTH DATA

Table 1: Average diameter at the neck (mm) of seedlings at 3 months after sowing

Treatments / Blocks	T_0	T_1	T_2
I	4,8	5,56	5,83
II	5,83	5,85	5,91
III	5,9	5,46	5,75
Sum	**16,53**	**16,87**	**17,49**
Mean	**5,51**	**5,62**	**5,83**
Standard deviation	**0,61**	**0,20**	**0,08**
CV (%)	**11,0**	**3,5**	**1,4**

Table 2: Average height of seedlings (cm) at 3 months after sowing

Treatments / Blocks	T_0	T_1	T_2
I	21,4	34,13	33,78
II	38,33	36,57	34,75
III	39	30,07	34,83
Sum	**98,73**	**100,77**	**103,36**
Mean	**32,91**	**33,59**	**34,45**
Standard deviation	**9,97**	**3,28**	**0,58**
CV (%)	**30,2**	**9,7**	**1,6**

Table 3: Average number of leaves formed at 3 months after sowing

Treatments / Blocks	T_0	T_1	T_2
I	11,2	11,33	14,22
II	18	12,21	12,5
III	14,6	11,21	13,5
Sum	**43,8**	**34,75**	**40,22**
Mean	**14,6**	**11,58**	**13,40**
Standard deviation	**3,4**	**0,54**	**0.86**
CV (%)	**23,2**	**4,6**	**6,4**