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Activities of Beneficial Microorganisms to Stimulate the Development of Papaya Plant (*Carica Papaya*)

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Abstract

This study aims to evaluate the effectiveness of beneficial microorganisms on the development of papaya (*Carica papaya*). Thus, *Bacillus cereus* and arbuscular mycorrhizal fungi (AMF) were applied in pots to this plant. The experimental setup was a factorial-block design with three treatments consisting of *B. cereus* (T1), mycorrhizal fungi (T2), a mixture of *B. cereus* plus AMF (T3), and a negative control (T0), all repeated three times. The analysis of the results shows that the study soil has a sandy-clay-silt texture, a slightly acidic pH (6.2), and a very high C/N ratio of 39. Regarding the effect of microorganisms on the photosynthesis of the plants, it was observed that the treatments induced a significant increase, reaching 40 mg/l with treatment T3 compared to 19 mg/l in the control (T0). Their effects on the phosphorus content in the plants

followed the same trend, 24.42 mg/kg with T3 compared to 17.44 mg/kg in T0. On the other hand, it is treatment T2 that shows the best performance in terms of nitrogen content (899.85 mg/g), followed by T1 (824.92 mg/g), whereas the value obtained for the control (T0) is around 549.01 mg/g. Moreover, plants treated with microorganisms have a significantly larger size compared to the control, measuring 28 cm with T3, followed by T2 (23 cm) versus 18.5 cm for the control. The same trend is observed for total biomass, where the values recorded with T3 reach 37.3 g compared to 13.2 g for T0. Therefore, from all these results, it appears that the application of beneficial microorganisms (*Bacillus cereus* and mycorrhizal fungi) stimulates the development of papaya, and this beneficial effect is stronger with the combination of these two microorganisms.

Keywords: Papaya, Beneficial Microorganisms, Stimulate, Development

Introduction

Papaya, often called the "fruit of the angels," is a fruit that aids digestion, improves skin health, and strengthens the immune system, particularly due to its exceptional content of calories (43 kcal), vitamin C, vitamin A (rich in beta-carotene), folate (vitamin B9), fiber (helps digestion), enzymes (notably papain, which helps break down proteins), and antioxidants (such as lycopene, which helps reduce oxidative stress) (Bomo *et al.*, 2022) [2]. However, despite its importance, its production in Cameroon remains low, at 38 tons per year (EUROSTAT, 2020) with a yield of 28 tons/ha. Kouam *et al.* (2024) [4] explain that the low crop yields in Cameroon are related to various constraints, including drought, post-harvest losses, poor soil quality, and poor agricultural practices (slash-and-burn, excessive use of chemical inputs, etc.). At the same time, it has been shown that the excessive use of synthetic chemical pesticides leads to an accumulation of residues in agricultural soils, which disrupts local ecosystems, pollutes rivers and lakes, and jeopardizes human and environmental health (Ngweme *et al.*, 2023) [11]. It is therefore urgent to explore fertilization strategies that are environmentally friendly and safe for human health. In this regard, the use of beneficial microorganisms is a good example (Tobolbai *et al.*, 2021) [12]. These beneficial microorganisms are known for their ability to enhance water, phosphorus, and nitrogen uptake in host plants and to strengthen their resistance to pathogen attacks (Inna *et al.*, 2023). This study therefore aims to investigate the effect of beneficial microorganisms on the growth of papaya.

Materials and Methods

Study Site

This study was conducted in the city of Yaoundé, in the Central Region of Cameroon, from October 2022 to July 2023. The area has a Guinean-type climate, characterized by bimodal rainfall, with two rainy seasons and two dry seasons. Annual rainfall ranges between 1600 and 1900 mm; the average temperatures are 22°C for the minimums and 42°C for the maximums. The site is located within the campus of the University of Yaoundé 1, specifically at the Botanical Garden of the Soil Microbiology Laboratory, situated opposite the building of the Institute of Population Training and Research (IFORD). Furthermore, the soil substrate used for the experiments was sampled under aseptic conditions in the EMANA district at a site with the geographical coordinates 4°16'0''N; 11°19'60''E 546. This soil sample was mixed with sterilized sand in a 3:1 ratio in order to aerate it and optimize microbial activity.

Biological Material

Plant Material

The plant material consists of papaya seeds of the 'solo no. 8' variety obtained from affiliated commercial centers.

Microbiological Material

Two groups of beneficial microorganisms were used in this study:

- An inoculum of *Bacillus cereus* catb1 bacteria, which was obtained from the Soil Microbiology Laboratory of the Biotechnology Center of the University of Yaoundé1 (in Nkolbisson) with the following biochemical characteristics: cellulase-, protease+, urease+, catalase+, amylase-, glucose+, lactose+, maltose+, fixes nitrogen, solubilizes phosphorus, produces Iodine ACC deaminase;
- An inoculum of arbuscular mycorrhizal fungi that was obtained from the Soil Microbiology Laboratory of the Biotechnology Center at the University of Yaoundé 1 (in Nkolbisson) and is distributed by the Organic Agriculture GIC of Cameroon based in Yaoundé (NGOUSSO district). The characteristics of this inoculum are as follows: a mix of *Glomus hoi*, *Gigaspora margarita*, *Rhizophagus intraradices*, *Scutellospora gregaria*. The spore concentration is 30 ± 2 g/soil.

Methods

1. Physico-chemical analyses of the soil

The physico-chemical analyses were carried out at the Soil Analysis and Environmental Chemistry Laboratory of the Faculty of Agronomy and Agricultural Sciences at the University of Dschang. The main parameters to be analyzed were:

1. The pH was measured using a pH meter in a soil-water suspension at a 1:2.5 ratio according to the MC Lean (1982) protocol;
2. Organic carbon was determined by digestion with chromic acid followed by spectrophotometric analysis;
3. Available phosphorus was analyzed using the molybdate blue method according to the procedure described by Murphy and Riley (1962) [7];
4. Soil texture was determined using the hydrometer method with hexametaphosphate (Sciltner, 1994).

2. Pot evaluation of the effect of beneficial microorganisms on papaya development

Experimental setup

The setup implemented is a randomized complete block design with a single factor. It consists of four treatments: a negative control (T0), an inoculum of *Bacillus cereus* (T1), an inoculum of a mycorrhizal fungus consortium (T2), and a mixture of *B. cereus* and mycorrhizal fungi (T3). The experimental unit is a pot with a capacity of 10 liters containing sterilized soil, in which a single papaya plant is sown and to which a treatment is applied. Each experimental unit is repeated three times.

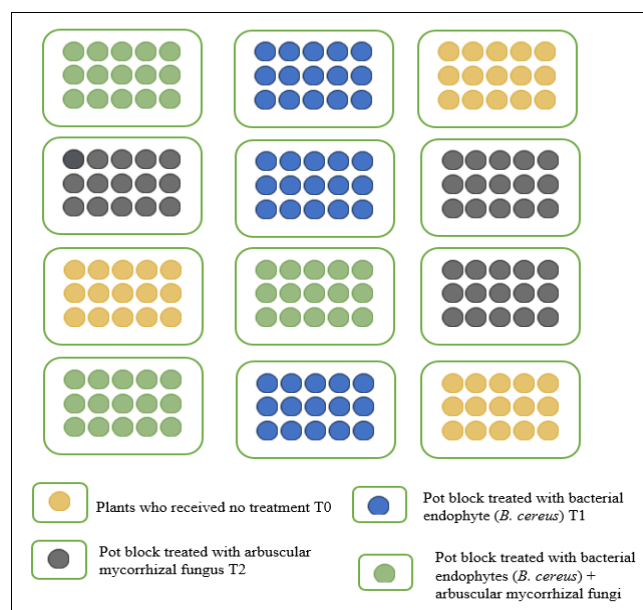


Fig 1: Experimental setup

3. Data Collection

Chlorophyll Assay

It was carried out according to the method of LORENZEN *et al.* (1967), where 0.5 g of freshly harvested leaf is finely cut and ground dry in a mortar with a pinch of pre-treated sand and calcium carbonate. The homogenate is mixed with 2 ml of petroleum ether and tetrahydrofuran. Then 3 ml of petroleum ether are added again, and the homogenate is filtered through filter paper. The obtained filtrate was used to measure chlorophyll a, chlorophyll b, and β -carotene using a spectrophotometer. Chlorophyll a and chlorophyll b were measured at wavelengths of 645 and 655 nm; β -carotene at wavelengths of 480 and 495 nm. Each measurement was repeated three times, and the pigment content was calculated using the formula by Bulda *et al.* (2008):

$Ca = 19.00 A_{655} - 7.61 A_{645}$; $Cb = 21.45 A_{645} - 5.92 A_{655}$, where a is the absorption of the solution at 645 and 655 nm. Ca is therefore the concentration of chlorophyll a in mg/l, Cb the concentration of chlorophyll b (mg/l), and the total chlorophyll content is the sum of chlorophyll a and chlorophyll b (mg/l).

$$C\beta\text{-car} = 17.16 A_{495} - 3.96 A_{480}$$

$Clut = 11.51 A_{480} - 20.62 A_{495}$ where A480 and A495 are the absorbance of the solution at 480 and 495 nm. C is the beta-carotene concentration (mg/l), C in lutein (mg/l), and

the total carotenoid content was the sum of beta-carotene and lutein (in mg/l).

Measurement of plant height and collar diameter

Plant height measurements were taken using a ruler marked in centimeters, from the collar to the last node where the uppermost leaf of the stem is attached. The collar diameter was measured using a tape ruler;

Number of leaves

The number of leaves was counted every four weeks, excluding fallen leaves.

Determination of dry biomass

Aboveground and belowground biomass were weighed using a precision electronic balance after drying in an oven until a constant weight was achieved.

Data Analysis

The data and the description of the analyses obtained were carried out using Microsoft Excel 2013. The comparison of the means for each parameter studied was performed using a one-way analysis of variance (ANOVA) with the statistical software SPSS around 2016. The Tukey test allowed us to compare the means with each other at the 5% threshold, while ANOVA indicated the significant differences.

Results

1. Physico-chemical analyses of the soil

The analysis of the results (Table 1.) shows that the study soil has a silt-sandy-clay texture (a slightly acidic pH (6.2), and a very high C/N ratio of 39.

Table 1: Physico-chemical analyses of the soil

Parameters	Values	
Organic Carbon (%)	2,21	
Organisces matters (%)	3,81	
total nitrogen (g/kg)	0,57	
C /N report	39	
Available phosphorus (mg/Kg)	5,56	
CEC pH 7 (méq/100g)	18,48	
Water pH	6,2	
Organic nitrogen (%)	0,057	
pH-KCl	5,1	
Total phosphorus (mg/kg)	13,18	
Texture	Sand	35
	Clay	23
	Silt	42

2. Effect of the beneficial microorganisms on chlorophyll content

Fig 2 illustrates the variation in the effects of beneficial microorganisms on the chlorophyll content in papaya plants. It appears that the combination of *B. cereus* and AMF (T3) shows the best effect (40 g/l), followed by AMF (38 g/l). On the other hand, the addition of *B. cereus* alone seems to negatively influence this chlorophyll content, 15 g/l (T2) compared to 19 g/l in the control (T0).

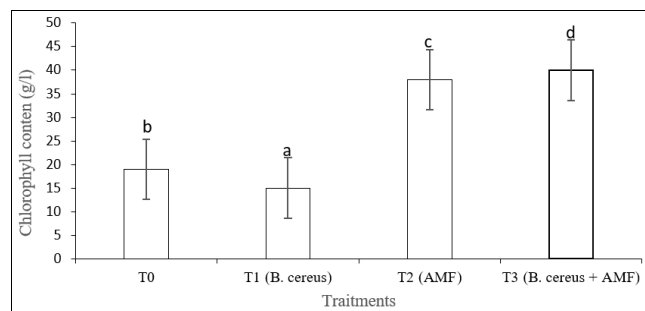


Fig 2: Effect of the beneficial microorganisms on chlorophyll content

3. Effects of beneficial microorganisms on phosphorus content

The variation in the impact of beneficial microorganisms on the phosphorus content in papaya plants is illustrated in Fig 3. In general, all treatments applied resulted in an improvement in the phosphorus content of papaya, with treatment T3 (24.42 g/kg) being the most prominent. Furthermore, no significant difference was observed between treatments T1 (21.81 g/kg) and T2 (22.1 g/kg).

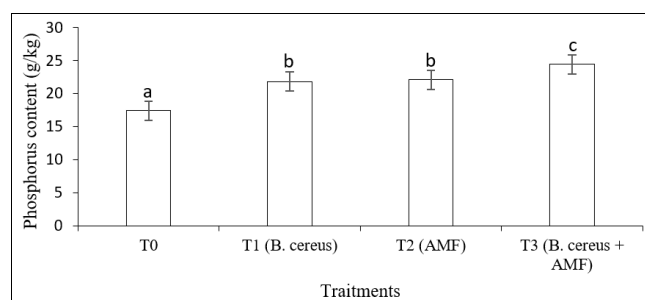


Fig 3: Effects of beneficial microorganisms on phosphorus content

4. Impact of beneficial microorganisms on nitrogen content in papaya plants

The application of beneficial microorganisms stimulates the total nitrogen content in papaya plants (Fig 4). However, the best score is recorded with the use of mycorrhizae alone (T2=899.85 mg/g), followed by treatment T2 (824.92 mg/g). The combination of microorganisms rather shows a slight improvement compared to the other two treatments (T3=711.48 mg/g).

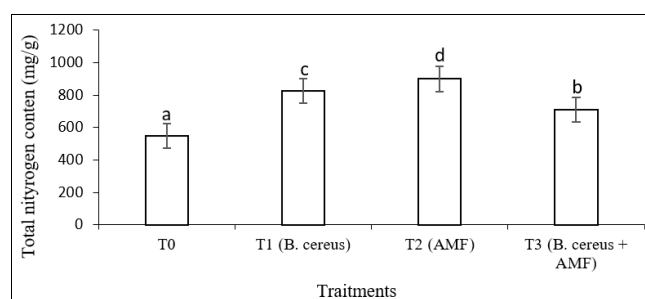


Fig 4: Impact of beneficial microorganisms on nitrogen content in papaya plants

5. Effect of beneficial microorganisms on plant size and neck diameter

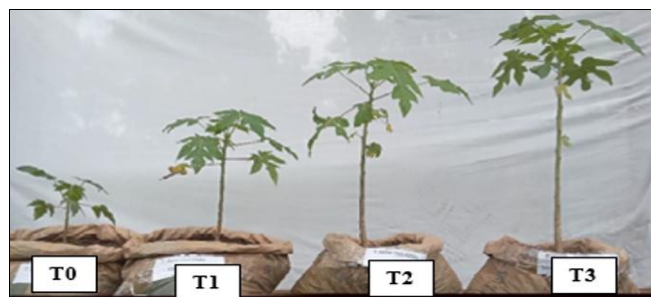


Fig 5: Papaya plant as affected by beneficial microorganisms

Table 2 summarizes the impact of beneficial microorganisms on the height and collar diameter of papaya plants. For height, the greatest improvement was induced by the combination of *B. cereus* and CMA (T3) with a score of 28 cm, followed by mycorrhizae (T2=23 cm) compared to the control (T0=9.5 cm). In contrast, the best collar diameter was observed in plants treated with mycorrhizae (T2=1.4 cm), followed by T3 (10.02 cm) compared to the control (T0=0.15 cm). The improvement induced by the application of *B. cereus* alone was comparatively the smallest (T1=0.87 cm).

Table 2: Effect of beneficial microorganisms on plant size and neck diameter

Traitments	Size (cm)	Neck diameter (cm)
T0 (nagatif control)	9,5	0,15
T1 (<i>B. cereus</i>)	18,5	0,87
T2 (AMF)	23	1,4
T3 (<i>B. cereus</i> + AMF)	28	1,02

6. Effects of beneficial microorganisms on biomass and leaf number

The contribution of beneficial microorganisms promotes the production of biomass and the number of leaves in papaya plants (Table 3). Regarding biomass, the combination of beneficial microorganisms shows a significantly higher performance compared to other treatments (T3=3.73g); this performance is followed by *B. cereus* applied alone (T1=2.40g) compared to 1.31g in the control that received no treatment. In contrast, the observation is rather mixed in terms of the number of leaves, where the application of mycorrhiza alone stands out (T2=15) compared to the other treatments, T0=9, T1=11, and T3=10.

Table 3: Effects of beneficial microorganisms on biomass and leaf number

Traitments	Biomass (g)	Leaf number
T0 (nagatif control)	1,31±0,59	9
T1 (<i>B. cereus</i>)	2,40±0	11
T2 (AMF)	2,24±0,47	15
T3 (<i>B. cereus</i> + AMF)	3,73±0,12	10

Discussion

It is widely accepted that soils are complex environments resulting from the weathering of rocks and interaction with living organisms. Their physico-chemical properties strongly affect their structures, fertility, and capacities to store and filter water and nutrients. Physico-chemical characteristics are important for understanding their

functioning and sustainable management. Our results indicate that the study soil has a silty-sandy-clay texture (42-35-23). These results are close to those of EDENE (2024), who, while working on soil from the Central Cameroon, obtained a clay-silty-sandy texture (49.40-45.25-5.35); the pH is slightly acidic (pH=6.2) with a C/N ratio of 39, indicating a low return of organic matter to the soil.

The data we obtained show that the contribution of beneficial microorganisms enhances the photosynthesis of papaya plants, with the best effects observed in plants that received the combination of *B. cereus* and mycorrhizal fungi, 40g/l compared to 19g/l in the control plants. These observations confirm those of Baibelpouya (2024), who, when applying beneficial microorganisms to tomatoes, recorded improvements in chlorophyll content reaching 9mg/g compared to 3mg/g in untreated plants. In fact, chlorophyll plays an important role in plants by enabling photosynthesis, the process by which plants convert sunlight into chemical energy.

Phosphorus and nitrogen are essential for the growth and health of plants. Phosphorus is crucial in the formation of DNA, cell membranes, and energy molecules, while nitrogen is a major component of proteins, amino acids, and DNA. Deficiencies in these elements compromise plant development. Our results indicate that the application of beneficial microorganisms increases the content of these two elements in papaya seedlings, with improvements in the range of 21.81-24.42 mg/g compared to 17.44 mg/g for phosphorus, and 711.48-899.85 mg/g compared to 549.01 mg/g for nitrogen. Mausin *et al.* (1997) explain that this increase in phosphorus and nitrogen content results from the use of organic or mineral forms of soil phosphorus by the symbionts. This ability is due to the excretion of enzymes (phosphatases) that break down organic phosphates, or to a modification of the physicochemical conditions of the rhizosphere by the symbionts and the presence of a synergistic microflora that solubilizes mineral phosphates.

The contribution of beneficial microorganisms significantly induced an increase in plant size (18.5-28 cm compared to 9.5 cm in the controls), stem diameter at the collar (0.87-1.4 cm compared to 1.15 cm in the untreated controls), biomass (2.24-3.73 g compared to 1.32 g in the untreated controls), and the number of leaves (11-15 leaves compared to 9 in the untreated control plants). These observations support those reported by Tobolbai *et al.* (2021) [12], Ngakou *et al.* (2007) [9], who, working on *Glycine max* and *Vigna unguiculata*, recorded gains of +40% compared to the untreated control. These improvements result from the benefits that microorganisms provide to their host plant, including phosphorus, nitrogen, water, synthesis of growth hormones, and protection against pathogen attacks (Nwaga *et al.*, 2010) [10]. Similarly, the fact that the best results come from the combination of microorganisms reflects a synergistic effect between these groups of beneficial microorganisms.

Conclusion

The purpose of this study was to determine the influence of beneficial microorganisms on the development of *Carica papaya*. Analysis of the results obtained shows that the soils on which the experiments were conducted had a silty-sandy-clay texture, with a slightly acidic pH and a low level of organic matter replenishment. Furthermore, the addition of beneficial microorganisms significantly improved the chlorophyll, phosphorus, and nitrogen content in the study

plants; this translated into significant improvements in plant morphometric parameters such as height, stem diameter, biomass, and number of leaves. However, it should be noted that, in general, the best results are achieved by combining arbuscular mycorrhizal fungi with endophytic bacteria (*B. cereus*), indicating a synergistic effect between these two groups of microorganisms.

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