

# Applying Rhizobacteria on Maize Cultivation in Northern Benin: Effect on Growth and Yield

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## Abstract

The aim of the study was to investigate the effects of five plant growth-promoting rhizobacteria (PGPR) (*Bacillus panthothenicus*, *Pseudomonas Cichorii*, *Pseudomonas Putida*, *Pseudomonas syringae* and *Serratia marcescens*) on the growth and yield of maize on a ferruginous soil under field condition. Maize seeds were inoculated with 10 ml of bacterial suspension. Study was conducted in a completely randomized design with fifteen treatments and three replicates. A half-dose of recommended (13, 17, 17 kg·ha<sup>-1</sup>) NPK was applied 15 days after emergence. The results show that the *Serratia marcescens* + 50% NPK treatment yielded the best results for height, fresh underground biomass, dry aboveground biomass, dry underground biomass, and grain yield with respective increases of 41.09%, 217.5%, 213.34%, 93.82%, and 39.05% compared to the control. Maximum stem diameter (increases of 49.65%) was recorded in the plants treated with 100% NPK (full dose NPK) while the highest leaf area (466.36 ± 9.57 cm<sup>2</sup>), obtained on plant treated with *Pseudomonas putida* + 50% NPK was 32.08% greater than in the non-inoculated control. Our results suggest the use of these rhizobacteria as biological fertilizers for enhancing the growth and maize seed yield in ferruginous soil in the North of Benin.

## Keywords

Rhizobacteria PGPR, Ferruginous Soil, Corn, Growth, Yield

## 1. Introduction

Maize (*Zea mays* L.), the basis of food in West Africa [1], occupies nearly 17% of the few 200 million hectares sown [2]. In Benin, it is cultivated by 85% of households engaged in agriculture [3]. It is a strategic cultivation in terms of food security, economic profitability and constitutes for the Government of Benin the main speculation to intensify to achieve food self-sufficiency [4] [5]. Most of Benin's land and that of other countries in sub-Saharan Africa are in a state of advanced degradation and deficient in nutrients including nitrogen, phosphorus and potassium [6] [7]. The decline in agricultural productivity caused by the poverty of cultivated soils has led the extension services to set up a fertilization program every year which recommends the doses of 150 kg/ha of NPK fertilizer (14-23-14) and 50 kg/ha of urea for maize cultivation. The intensive use of mineral fertilizers further exposes crops to attacks by pathogenic microorganisms, resulting in soil acidification and in a decline in fertility. In addition, the deterioration of soil fertility is also related to the loss associated with beneficial microbial diversity for plants [8]. Several studies have also identified a relatively small response of crops for exclusive use and for the duration of mineral fertilizers [9] [10]. In this context, to feed the population of Benin, which, according to [11] should double every 20 years if the current trend continues, it would require very fertile soils for sustainable and environmentally-friendly agricultural production. The use of technologies that integrate beneficial soil microorganisms especially rhizobacteria promoters of vegetative growth of plants called Plant Growth Promoting Rhizobacteria (PGPR) represent a solution sustainable alternative to ensure long-term soil fertility and increase agricultural productivity [12]. Indeed, since their first description in 1978 by [13] the PGPR have particularly attracted the attention of scientists. These are soil bacteria that colonize the root surface and have beneficial effects on plant growth through the secretion of control chemicals at the rhizosphere level [14]. They are involved in several biogeochemical processes including mineralization, soil solubility of inorganic compounds in soil [15] and are known for their ability to promote growth and yield even in the most stressed environments in many parts of the world [16] [17]. The safety of several strains of PGPR for humans [18] [19], the environment and their positive effects on the bioavailability of nutrients including nitrogen, phosphorus in soil, and restoration of soil fertility have been reported in numerous studies [20] [21]. It is in this context that the present study aims to evaluate the effects of five rhizobacteria (PGPR) isolated and identified in central and northern Benin on the growth and yield of maize on ferruginous soils in northern Benin.

## 2. Material and Methods

### 2.1. Material

**Strains of PGPR:** Five (05) strains of selected on the basis of their ability to

promote germination parameters *in vitro* and the growth parameters of maize under the greenhouse [22] are those used. These are: *Bacillus panthothenicus*; *Pseudomonas Cichorii*; *Pseudomonas Putida*; *Pseudomonas syringae* and *Serratia marcescens*. They were isolated and characterized from the rhizosphere of maize from different agro-ecological zones of central and northern Benin by [23] and stored at  $-20^{\circ}\text{C}$  in Muller Hinton broth with glycerol (10%) At the laboratory of Biology and molecular typing in microbiology of the University of Abomey- Calavi.

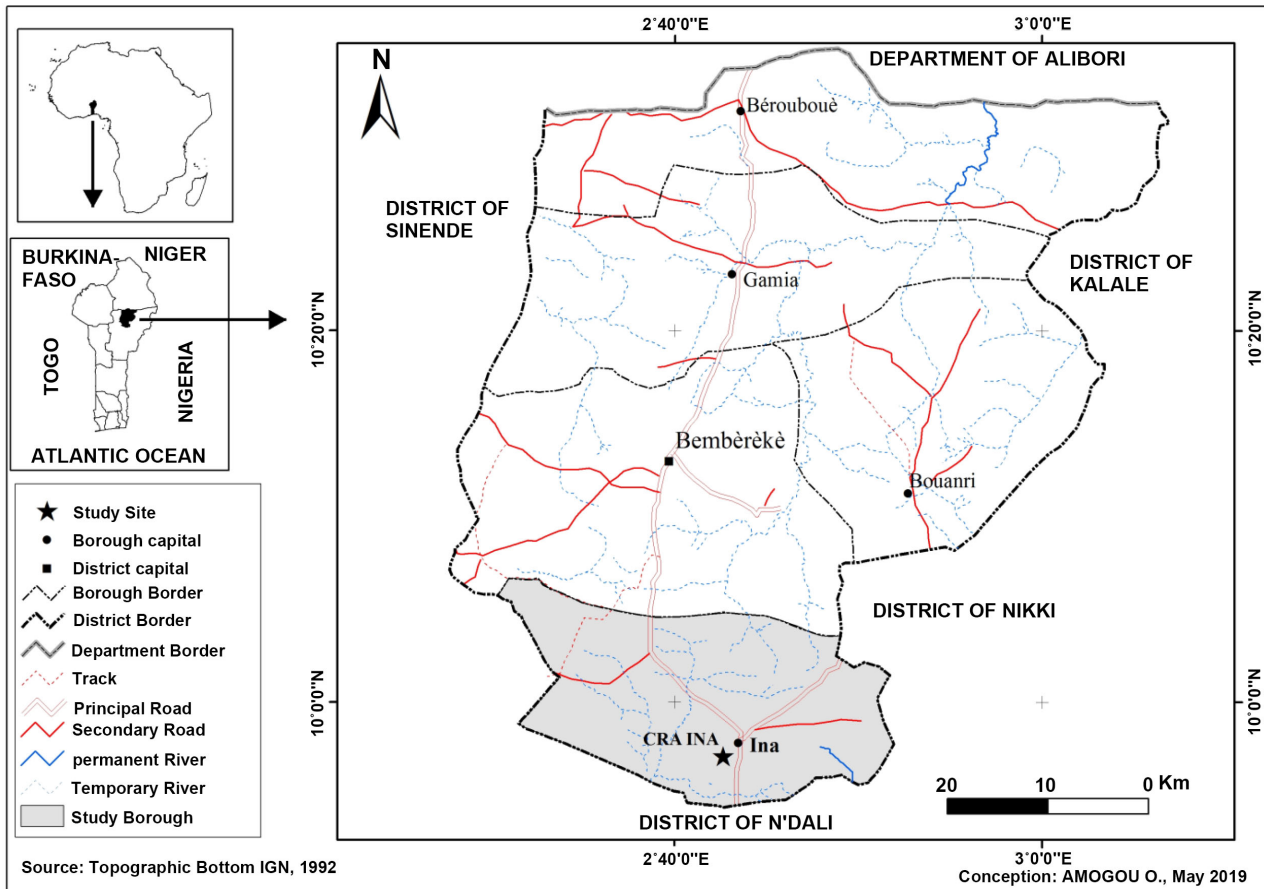
**Seed of Maize:** Maize seeds of the variety 2000 SYNEE-W were used during the study. They are provided by the Northern Agricultural Research Centre (CRA-Nord) of Benin's National Agricultural Research Institute (INRAB). It is an extra-precocious variety with a vegetative cycle of 80 days and a potential yield of 4 t/ha in the plant and a peasant is 2.5 t/ha. It is resistant to breakage, streak, American rust and blight. It is moderately resistant to drought. It tolerates pests [24].

## 2.2. Description of the Study Site

The study was conducted on a leached tropical ferruginous soil at the station of the North Agricultural research Centre located at Ina at an altitude of 358 metres between latitude  $9^{\circ}58'\text{N}$  and longitude  $2^{\circ}44'\text{E}$  in the municipality of Bembèrèkè, Department of Borgou at north-east of Benin (Figure 1). There is a tropical climate of the Sudanese type characterized by a dry season from October to April and a rainy season from May to September. Rainfall varies between 1100 and 1200 mm with an evapotranspiration (ETP) of 1600 mm [25].

## 2.3. Experimental Device and Fertilization

The experimental design was a complete random block of 15 treatments. Each treatment was repeated 3 times on elementary plots of 5 lines of 4 m with 8 pockets. The distance is 0.80 m (between the lines) and 0.40 m (between the pockets). The treatments compared are defined as follows: CTL: Control (no bacteria, no mineral fertilizers); half of the recommended dose of  $\text{N}_{13}\text{P}_{17}\text{K}_{17}$  fertilizers (50% NPK); recommended dose of  $\text{N}_{13}\text{P}_{17}\text{K}_{17}$  fertilizers (100% NPK); T1: *Bacillus pantothenicus*; T2: *Pseudomonas cichorii*; T3: *Pseudomonas putida*; T4: *Pseudomonas syringae*; T5: *Serratia marcescens*; T6: *Serratia marcescens* + *Pseudomonas Putida*; T1 + half of the recommended dose of  $\text{N}_{13}\text{P}_{17}\text{K}_{17}$  fertilizers (T1 + 50% NPK); T2 + half of the recommended dose of  $\text{N}_{13}\text{P}_{17}\text{K}_{17}$  fertilizers (T2 + 50% NPK); T3 + half of the recommended dose of  $\text{N}_{13}\text{P}_{17}\text{K}_{17}$  fertilizers (T3 + 50% NPK); T4 + half of the recommended dose of  $\text{N}_{13}\text{P}_{17}\text{K}_{17}$  fertilizers (T4 + 50% NPK); T5 + half of the recommended dose of  $\text{N}_{13}\text{P}_{17}\text{K}_{17}$  fertilizers (T5 + 50% NPK); T6 + half of the recommended dose of  $\text{N}_{13}\text{P}_{17}\text{K}_{17}$  fertilizers (T6 + 50% NPK). For the fertilization of the experimental plots,  $\text{N}_{13}\text{P}_{17}\text{K}_{17}$  (maize fertilizer) and Urea (46% N) of the recommended doses were applied 15 days after emergence (15 DAE) and 45 DAE, respectively, depending on the treatments.



**Figure 1.** Map of the study area showing the experimental site (AMOGOU O., 2019).

Both urea and NPK fertilizer were buried in the soil at a depth of about 3 to 5 cm followed by the closure of the hole to prevent losses by leaching and volatilization. Pockets were made near each foot of corn plant.

#### 2.4. Soil Chemical Analysis of the Experimental Site

A composite sample (300 g) of soil collected (0 - 20 cm) from the experimental site prior to the installation of the test was analyzed at laboratory of soil Sciences, water and Environment (LSSEE) of the National Institute of Agricultural Research of Benin (INRAB). The analyses consisted of the determination of nitrogen according to the method of [26], phosphorus assimilated by the extraction method of Bray 1 [27], carbon according to the method of [28]. The exchangeable bases (Ca, Mg, Na, K) [29] as well as the pH in proportion of 2:1 (Soil: Solution) [30] were also determined.

#### 2.5. Preparation of the Inoculum of PGPR Strains

After revivification of *Pseudomonas* strains on King B medium [30] and those of *Bacillus* and *Serratia* on nutrient agar [31] [32], a pure colony of each strain was cultured in nutrient broth (Muller Hinton Broth) medium and incubated at

30°C for 24 h. The bacterial suspensions obtained were adjusted to approximately  $1 \times 10^8$  CFU/ml (OD 0.45 to 610 nm) using the spectrophotometer (BioMATE 3S, Thermo scientific) as described by [33].

## 2.6. Sowing, Inoculation of Maize Seeds and Maintenance of Plots

Two seeds of each treatment was sown at the depth of 5 cm. The seeds were inoculated with 10 ml of bacterial suspension in accordance with the treatments and the hole was immediately closed. Two weeding towers were carried out 12 days after sowing (DAS) and 42 DAS respectively for the maintenance of the various plots. Thinning to one plant per hil was done at 15 DAS.

## 2.7. Data Collection

### 2.7.1. Parameters Related to the Growth of Maize Plants

The height and stem diameter of nine plants maize selected on the three central row of each plot were measured at every 7 days, respectively, using a ruler tape and the caliper from the thinning of the plants until the end of the trial. In addition, the leaf area of plants was estimated at 63th DAS by the product of length and width of leaves affected by coefficient 0.75 [34].

### 2.7.2. Parameters Related to Yield

Nine plant maize per field hole were harvest 70 DAS and data was recorded for fresh biomass (by weighing using a precision scale), shoot and root dry weight after drying in an oven at 100°C for three days to constant weight. Maize cobs were shelled and weighed in accordance with the treatment. The moisture percentage of the grain was evaluated using a moisture meter (LDS-1F). On each elemental parcel, the average grain yield was calculated using the formula below used by [35].

$$R = \frac{P \times 10.000}{SI \times 1.000} \times \frac{14\%}{H}$$

whereas R is the average yield of maize, expressed in t/ha; P is the fresh weight of maize per basic calculation area, expressed in kg; SI is the surface interpretable in (m<sup>2</sup>); H is the moisture content of the grains, expressed %; 10,000 represents the conversion of ha in m<sup>2</sup>; 1000 represents the conversion of tone (t) in kg.

## 2.8. Determination of the Nutritional Status of Maize Plants

Samples of two whole maize plants removed from each elemental parcel were formed. Dry matter obtained after drying followed by grinding was digested according to the wolf method. The assessment of the nutritional status of the seedlings consisted to the determination of the phosphorus (P) content by flame emission and by Colorimetry [36], nitrogen (N) using the Kjeldahl method as described by [37] and potassium by the atomic absorption Spectrophotometer [38]. In terms of calcium (Ca) and magnesium (Mg), they were evaluated using the [39] method.

## 2.9. Statistical Analysis

The data were analyzed with R 3.4.4 software and required the use of packages such as agricultural and graphics. Data have been previously transformed into a logarithm and square root and then subjected to an analysis of variance with the exception of data on height, fresh aerial biomass, grain yield and calcium (Ca) content for which the Kruskal-Wallis ( $p < 0.05$ ) test has been used. The choice of this non-parametric test was motivated by the fact that the averages of these variables are not normally distributed. The differences among various treatment means were achieved using the student-Newman-Keuls test at the 5% ( $p \leq 0.05$ ) probability level. Finally, an ascending hierarchical classification (CHA) was performed on the average of the different parameters by treatment to group them into homogeneous classes. The graph Pad software Prism version 7.00 has been used to plot graphs.

## 3. Results

### 3.1. Soil Chemical Characteristics of the Experimental Site

The analysis in **Table 1** reveals that the organic matter ( $9.5 \text{ g}\cdot\text{kg}^{-1}$ ), nitrogen ( $0.8 \text{ g}\cdot\text{kg}^{-1}$ ) and the content ( $3 \text{ mg}\cdot\text{kg}^{-1}$ ) of phosphorus are low in the soil of the experimentation site. The sum of the bases ( $1.82 \text{ Cmol}\cdot\text{kg}$ ) and cation exchange capacity ( $4.64 \text{ Cmol}\cdot\text{kg}$ ) are also low. pH values (pH-water = 5.54 and pH-KCl = 5.03) showed that the study soil was moderately acidic. These chemical properties reflect a limited fertility of the study soil because of its low stored nutrient reserves.

### 3.2. Effects of PGPR on the Growth Parameters of Maize Plants

The effect of inoculation of PGPR on the height and stem diameter of maize plants was very highly significant at  $p < 0.001$  for stem diameter and highly significant at  $p < 0.05$  for height (**Table 2**). Seedlings inoculated with *S. marcescens* + 50% NPK exhibited the highest heights with an improvement of more than 41.09% compared with un-inoculated plants and 3.14% compared to plants receiving 100% NPK. The lowest heights were recorded at the level of the control plants. As for the stem diameter, the highest mean values were 2.14 cm, 2.06 cm and 2.01 cm, respectively, with 100% NPK, *S. marcescens* + 50 NPK, and *P. putida* + 50% NPK, which induced improvement in the same order of 49.65%, 43.35% and 39.86% per comparison to the control plants. On the other hand, no positive effects were observed with *B. pantothenicus* treatment and the *S. marcescens* + *P. putida* combination for this parameter. For the leaf area of the seedlings, the inoculation treatment with the PGPR Rhizobacteria showed a very highly significant difference with the un-inoculated controls ( $p < 0.001$ ). It is observed from the analysis of the results (**Table 2**) that the largest leaf area values were obtained with the plants treated with *P. putida* + 50% NPK ( $466.38 \text{ cm}^2$ ) followed by *S. marcescens* + 50% NPK ( $452.08 \text{ cm}^2$ ) with a percentage improvement rate of 32.08% and 28.04% compared to control plants.

**Table 1.** Soil chemical characteristics of the experimental site.

Soil	pH						B.E (meq/100g)															
	C		N		C/N		MO		P .ass		Ca		Mg		K		Na		SB		CEC	
	Eau	Kcl	%	%	%	%	ppm%											%	meq/100 g			
	5.54	5.03	0.55	0.08	6.88	0.95	3.00	0.94	0.54	0.15	0.17	1.82	4.64									

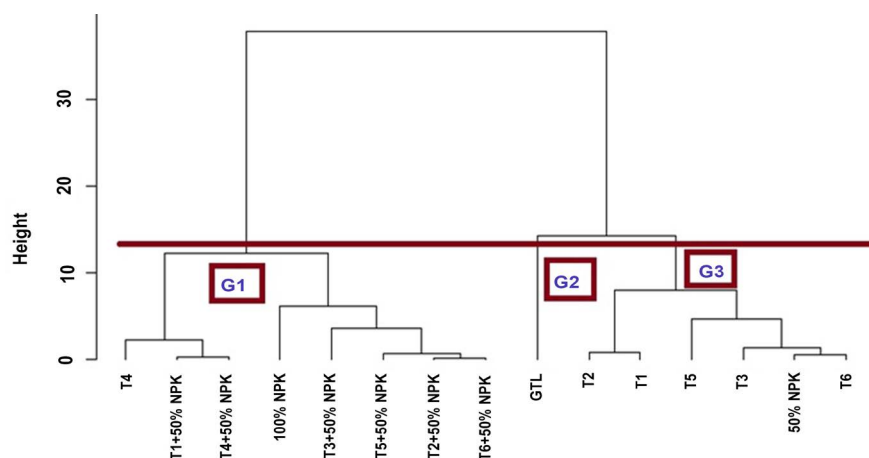
**Table 2.** Effects of PGPR on maize plant growth parameters.

Treatments	Height (cm)	Stem Diameter (cm)	Leaf Area (cm <sup>2</sup> )
	m ± sd	m ± sd	m ± sd
CTL	116.94 ± 24.54a	1.43 ± 0.10e	353.10 ± 21.72c
50% NPK	138.16 ± 12.01a	1.83 ± 0.07abcd	361.13 ± 30.66c
100% NPK	161.33 ± 15.88a	2.13 ± 0.15a	413.79 ± 9.37b
T1	135.83 ± 30.42a	1.4 ± 0.14e	372.82 ± 27.22bc
T2	131.88 ± 15.15a	1.74 ± 0.01abcde	391.92 ± 14.88bc
T3	136.22 ± 30.34a	1.7 ± 0.30bcde	361.98 ± 24.4c
T4	132.61 ± 19.20a	1.46 ± 0.14de	394.18 ± 6.18bc
T5	135.94 ± 11.11a	1.56 ± 0.06cde	374.84 ± 13.72bc
T6	132.22 ± 8.21a	1.41 ± 0.06e	363.46 ± 4.36c
T1 + 50% NPK	149.83 ± 23.50a	1.83 ± 0.10abcd	443.13 ± 1.85a
T2 + 50% NPK	158 ± 8.81a	1.89 ± 0.07abc	398.83 ± 8.04bc
T3 + 50% NPK	158.16 ± 7.22a	2 ± 0.20ab	466.36 ± 9.57a
T4 + 50% NPK	158.72 ± 9.84a	1.77 ± 0.29abcde	364.78 ± 19.42c
T5 + 50% NPK	165 ± 7.62a	2.05 ± 0.04ab	452.12 ± 10.10a
T6 + 50% NPK	154.61 ± 6.61a	1.91 ± 0.113abc	453.92 ± 13.50a
<i>p-value</i>	<b>0.04743</b>	<b>&lt;0.001</b>	<b>&lt;0.001</b>
<i>Signification</i>	<b>**</b>	<b>***</b>	<b>***</b>

Value: mean ± standard deviation, NS =  $p > 0.05$ : not significant. CTL: control (no bacteria, no mineral fertilizers); T1: *Bacillus pantothenicus*; T2: *Pseudomonas cichorii*; T3: *Pseudomonas putida*; T4: *Pseudomonas syringae*; T5: *Serratia marcescens*; T6: *Serratia marcescens* + *Pseudomonas putida*.

### 3.3. Classification of Treatments on the Basis of Growth Parameters

In order to consolidate the treatments into sufficiently homogeneous groups, an ascending hierarchical classification was carried out (Figure 2). The dendrogram below shows three as the optimal number of groups to be retained for classification of treatments. Group 1 (G1) whose heights, leaf surfaces and stem diameters were significantly improved, consisting of four (04) treatments (T1 + 50% NPK, T3 + 50% NPK, T5 + 50% NPK, and T6 + 50% NPK). Group 2 (G2) has seven (07) treatments (CTL, T4 + 50% NPK, T5, T1, T6, 50% NPK and T3) and all plants fertilized with 100% NPK as well as those inoculated with other rhizobacteria are classified in Group 3 (G3).



**Figure 2.** Dendrogram of groups Hierachiques. CTL: controle (no bacteria, no mineral fertilizer); T1: *Bacillus pantothenicus*; T2: *Pseudomonas cichorii*; T3: *Pseudomonas putida*; T4: *Pseudomonas syringae*; T5: *Serratia marcescens*; T6: *Serratia marcescens*+*Pseudomonas putida*.

### 3.4. Effects of PGPR on the Yield Parameters of Maize Plants

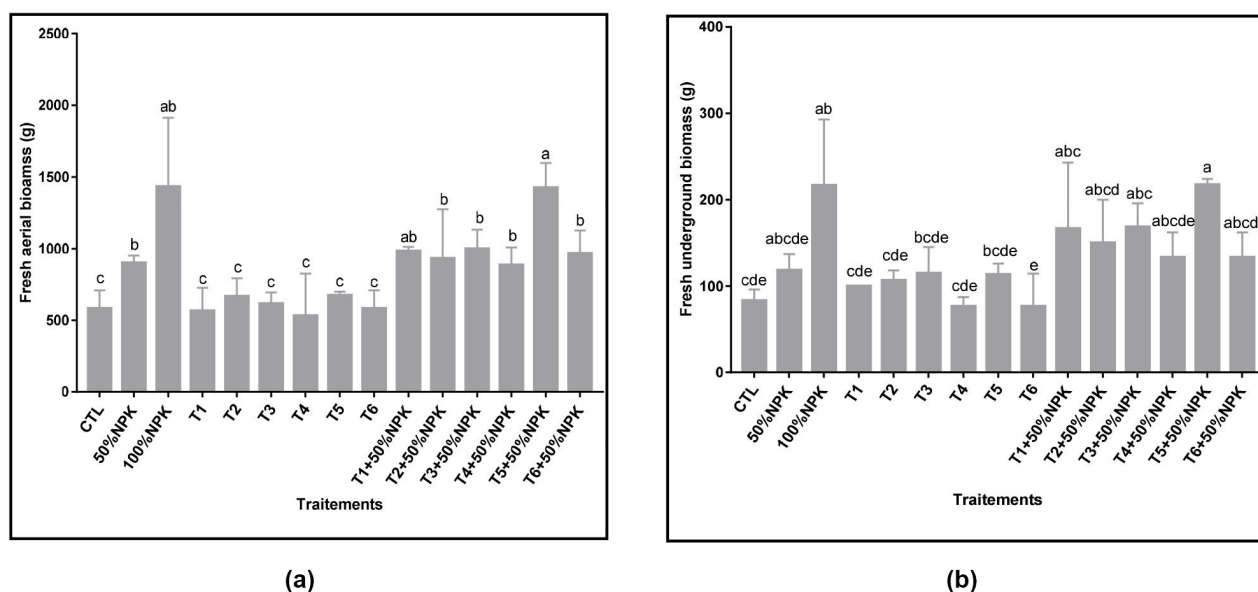
#### 3.4.1. Fresh Biomass of Maize Plants

The fresh weight of the aerial biomass (**Figure 3(a)**) and underground biomass (**Figure 3(b)**) of maize plants was significantly improved ( $p < 0.001$ ) for the majority of seedlings inoculated against controls. Among the inocula tested, the best results were observed in *S. marcescens* + 50% NPK with a percentage improvement of 144.28% for aboveground biomass and 117.5% for underground biomass followed by *P. putida* + 50% NPK with an improvement percentage of 71.45% for aboveground biomass and 66.66% for underground biomass. Fertilization of maize plants with 100% NPK resulted in the recording of 1433.33 g of aboveground biomass and 216.67 g of underground biomass. These results show that there is no difference between the effects induced by *S. marcescens* + 50% NPK and the 100% NPK treatment on the fresh biomass yield of maize plants. On the other hand, *B. pantothenicus* and *P. syringae* induced a decrease in aboveground fresh biomass compared with controls.

#### 3.4.2. Dry Biomass and Grain Yield Produced by Maize Plants

The influence of PGPR on the yield parameters (dry biomass and grain yield) produced by maize plants is illustrated in **Table 3**. Analysis of the variance applied to the averages of dry biomass and grain yield of maize plants showed a very highly significant difference ( $p < 0.001$ ) between the different treatments. The most important production of dry aerial biomass was recorded with the treatments *S. marcescens* + 50% NPK followed by *P. putida* + 50% NPK with the respective improvement percentages of 213.34% and 131.79% compared to the un-inoculated plants. All the isolated tested increased underground biomass, except for the combination of *P. putida* and *S. marcescens*. The most effective bacterial treatment (*S. marcescens* + 50% NPK) induced an increase of 5.41% and 140.38%, respectively, compared with 100% NPK treatment and un-inoculated





**Figure 3.** Effect of PGPR on fresh aerial biomass of maize plants (a) and fresh underground biomass of maize plants (b). CTL: control (no bacteria, no mineral fertilizer); T1: *Bacillus pantothenicus*; T2: *Pseudomonas cichorii*; T3: *Pseudomonas putida*; T4: *Pseudomonas syringae*; T5: *Serratia marcescens*; T6: *Serratia marcescens* + *Pseudomonas putida*.

plants. It was followed by *P. putida* + 50% NPK which obtained the similar yield (113.28 g) as that recorded at the level of the plants fertilized with 100% NPK with an improvement rate of 128.84% compared to the control plants. At the reading of **Table 3**, the efficacy of different isolates for grain yield was variable. All the inoculated treatments showed grain yield, significantly higher than control. Maximum grain yield ( $2.356 \pm 0.21$  ha/t), obtained with *S. marcescens* + 50% NPK was 39.05% greater than in the non-inoculated control. It followed by 100% NPK (full dose of NPK) and *P. putida* + 50% NPK which induced 35.5% and 34.91% compared to control, respectively.

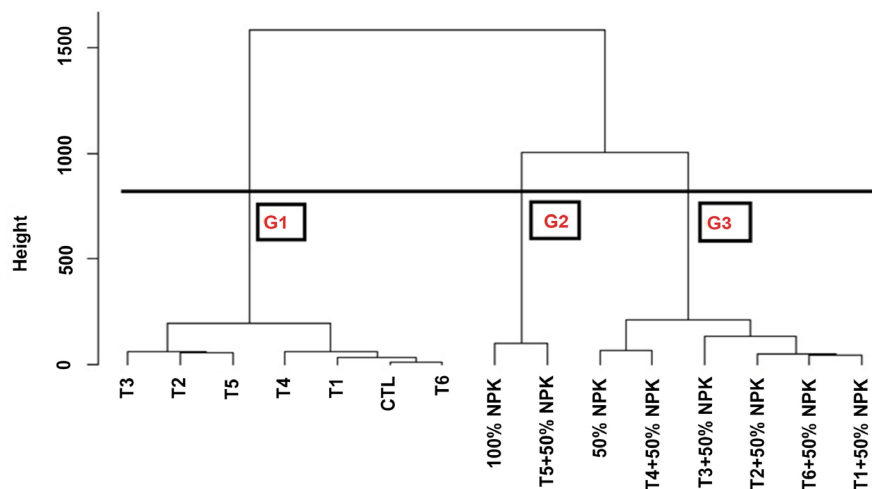
### 3.4.3. Classification of Treatments Based on Yield Parameters

Analysis of the dendrogram (**Figure 4**) shows that the 15 treatments were divided into three (3) groups. The first group (G1) includes treatments such as T2, T3, and T5 applied without the addition of chemical fertilizers, which induced improvement in performance parameters compared to control. The others (T4, T1, CTL, and T6) are characterized by their negative effect on grain yield compared to controls. Group 2 (G2) consists of two (02) treatments 100% NPK and T5 + 50% NPK, which induced the highest yields of biomass and in maize seeds. Group 3 (G3) is subdivided into two subgroups. Group 3 G3 (1) is composed of plants fertilized with 50% NPK and those inoculated with T4 + 50% NPK. The Group 3 G3 (2) was in turn left in two subgroups, one of which consists of the plants inoculated with T3 + 50% NPK characterized by yields of dry biomass and high seed in comparison with controls and the second in which the plants are found on influence of T2 + 50% NPK, T6 + 50% NPK and T1 + 50% NPK.

**Table 3.** Effect of PGPR on dry biomass and grain yield.

Treatments	Dry Aerial Biomass	Dry Underground Biomass	Grain yield
	m ± sd	m ± sd	m ± sd
CTL	306.13 ± 80.67d	49.50 ± 2.23bc	1.696 ± 0.156gh
50% NPK	504.14 ± 34.17bcd	73.80 ± 15.15abc	1.811 ± 0.024fgh
100 NPK	856.5 ± 173.74ab	112.88 ± 44.90a	2.299 ± 0.200ab
T1	305.82 ± 92.31d	61.39 ± 3.88abc	1.574 ± 0.157h
T2	342.18 ± 67.93d	64.60 ± 10.09abc	1.929 ± 0.088defg
T3	369.67 ± 156.64cd	63.53 ± 20.86abc	1.967 ± 0.144cdef
T4	310.19 ± 156.64d	66.44 ± 18.43abc	1.592 ± 0.139h
T5	395.99 ± 81.49cd	72.33 ± 17.69abc	1.996 ± 0.470def
T6	320.17 ± 97.31d	45.83 ± 7.29c	1.641 ± 0.125h
T1 + 50% NPK	595.85 ± 89.23abcd	99.94 ± 33.63ab	1.880 ± 0.055efg
T2 + 50% NPK	603.70 ± 221.99abcd	101.30 ± 31.62ab	2.132 ± 0.120abcd
T3 + 50% NPK	709.50 ± 124.40abc	113.28 ± 16.13a	2.284 ± 0.425abc
T4 + 50% NPK	559.18 ± 99.90abcd	93.88 ± 33.11abc	1.936 ± 0.065efg
T5 + 50% NPK	959.24 ± 259.78a	118.99 ± 25.51a	2.356 ± 0.210a
T6 + 50% NPK	617.60 ± 86.92abcd	87.35 ± 17.62abc	1.970 ± 0.063bcde
<i>p</i> -value	<0.001	<0.001	0.00183
Signification	***	***	**

Value: mean ± standard deviation, NS =  $p > 0.05$ : not significant. CTL: control (no bacteria, no mineral fertilizers); T1: *Bacillus pantothenicus*; T2: *Pseudomonas cichorii*; T3: *Pseudomonas putida*; T4: *Pseudomonas syringae*; T5: *Serratia marcescens*; T6: *Serratia marcescens* + *Pseudomonas putida*.



**Figure 4.** Dendrogram showing hierarchical classification of treatments. CTL: control (no bacteria, no mineral fertilizer); T1: *Bacillus pantothenicus*; T2: *Pseudomonas cichorii*; T3: *Pseudomonas putida*; T4: *Pseudomonas syringae*; T5: *Serratia marcescens*; T6: *Serratia marcescens* + *Pseudomonas putida*.

### 3.5. Effect of PGPR on the Nutritional Status of Maize Plants

The effect of PGPR on nutrient assimilation was highly variable from one strain to another (**Table 4**). Indeed, the best content ( $1.239\% \pm 0.09\%$ ) of nitrogen (N) absorbed by the seedlings was recorded with the inoculation of *S. marcescens*; an increase of 3.76% over the control plants. This content is followed by that ( $1.232\% \pm 0.05\%$ ) obtained at the level of the plants fertilized with the full dose of mineral fertilizers (100% NPK). In addition, the effect of *P. putida* and *P. cichorii* + 50% NPK resulted in a 25% improvement rate for phosphorus (P) uptake. The best absorption of calcium ( $0.180\% \pm 0.05\%$ ) was recorded at the level of plants that received *P. cichorii* + 50% NPK. The latter induced an improvement of 38.46% in comparison with the control plants. Calcium uptake was also stimulated by inoculation of *S. marcescens* and *P. putida*, which induced a similar increase of 20.45% in comparison with control plants. For potassium (K) and magnesium (Mg), their elevated levels were obtained with the inoculation of *P. cichorii*, an improvement of 12.35% and 10.85% in comparison with control plants. Analysis of the variance applied to mean N, P, K, Ca, and Mg concentrations indicated that there was no significant difference ( $p > 0.005$ ) between the different treatments (**Table 4**).

## 4. Discussion

The use of PGPR is increasingly gaining its place as a widespread practice in agriculture to replace the excessive and uncontrollable use of chemical fertilizers [40]. The present investigation on ferruginous soil in northern Benin revealed the significant effect of inoculation of rhizobacteria on maize growth and yield and therefore a reduction in the dose of NPK fertilizer recommended by the services of extension. The low soil fertility status of the study environment (low nitrogen, phosphorus, organic matter) corroborates the results of the soil analysis (**Table 1**) and is also consistent with the finding made by [41]. This author has reported that organic matter has a major influence on the physical and chemical properties of soils. The study soil is of a weakly acidic nature (pH-water = 5.54 and pH-KCl = 5.03) as indicated [42] which asserted that soils characterized by pH values ranging from 5.1 to 5.5 are considered weakly acidic. Igué *et al.* [43] reported that the pH of such soils is favorable for good mineralization of mineral elements. It is also one of the main soil indicators that influences biological processes such as atmospheric nitrogen fixation and degradation of organic matter, which can limit the growth and yield of agricultural crops.

The different results of the test for growth parameters (**Table 2**) show that the plants that received *S. marcescens* + 50% NPK followed by *P. putida* + 50% NPK induced a significant increase in height, respectively, of 41.09% and 35.24% compared to control plants. The performance recorded for this parameter with *S. marcescens* + 50% NPK was 3.14% higher over 100% NPK treatment. These results are similar to those obtained in India by [44]. During two successive years of experimentation, the authors of this study reported significant improvements of

**Table 4.** Effects of PGPR on the nutritional status of maize plants.

Treatments	%N	%P	%K	%Ca	%Mg
	m ± sd	m ± sd	m ± sd	m ± sd	m ± sd
CTL	1.194 ± 0.16a	0.088 ± 0.01a	0.896 ± 0.04a	0.132 ± 0.00a	0.129 ± 0.00a
50% NPK	1.150 ± 0.19a	0.070 ± 0.03a	0.812 ± 0.11a	0.143 ± 0.00a	0.115 ± 0.00a
100% NPK	1.232 ± 0.05a	0.097 ± 0.02a	0.783 ± 0.09a	0.152 ± 0.01a	0.116 ± 0.01a
T1	1.052 ± 0.11a	0.083 ± 0.00a	0.763 ± 0.08a	0.146 ± 00	0.138 ± 0.01a
T2	1.224 ± 0.10a	0.091 ± 0.02a	1.005 ± 0.25a	0.147 ± 0.01a	0.144 ± 0.01a
T3	1.008 ± 0.16a	0.101 ± 0.00a	0.856 ± 0.13a	0.152 ± 0.01a	0.132 ± 0.01a
T4	1.149 ± 0.10a	0.098 ± 0.02a	0.763 ± 0.13a	0.149 ± 0.01a	0.129 ± 0.01a
T5	1.239 ± 0.09a	0.076 ± 0.01a	0.679 ± 0.01a	0.159 ± 0.03a	0.119 ± 0.04a
T6	1.067 ± 0.07a	0.075 ± 0.00a	0.749 ± 0.10a	0.143 ± 0.00a	0.114 ± 0.01a
T1 + 50% NPK	0.941 ± 0.11a	0.071 ± 0.01a	0.801 ± 0.11a	0.144 ± 0.00a	0.109 ± 0.01a
T2 + 50% NPK	1.083 ± 0.13a	0.109 ± 0.02a	0.816 ± 0.15a	0.180 ± 0.05a	0.115 ± 0.00a
T3 + 50% NPK	0.948 ± 0.01a	0.080 ± 0.00a	0.929 ± 0.15a	0.159 ± 0.03a	0.127 ± 0.01a
T4 + 50% NPK	1.008 ± 0.16a	0.089 ± 0.01a	0.806 ± 0.06a	0.146 ± 0.00a	0.124 ± 0.01a
T5 + 50% NPK	1.090 ± 0.06a	0.097 ± 0.02a	0.840 ± 0.08a	0.131 ± 0.01a	0.106 ± 0.00a
T6 + 50% NPK	1.037 ± 0.16a	0.066 ± 0.01a	0.681 ± 0.01a	0.162 ± 0.03a	0.135 ± 0.03a
<i>p-value</i>	0.058	0.226	0.118	0.608	0.457
<i>Signification</i>	NS	NS	NS	NS	NS

Value: mean ± standard deviation, NS =  $p > 0.05$ : not significant. CTL: control (no bacteria, no mineral fertilizers); T1: *Bacillus pantothenicus*; T2: *Pseudomonas cichorii*; T3: *Pseudomonas putida*; T4: *Pseudomonas syringae*; T5: *Serratia marcescens*; T6: *Serratia marcescens* + *Pseudomonas putida*.

20.83% and 14.28% compared to controls respectively of the height of wheat plants and that of maize with inoculation of *Pseudomonas plecoglossicida* (PSB-5) in the presence of rock phosphate as fertilizer. Other scientists also observed the increase in plant height by inoculation with *Serratia marcescens* on tea plant [45] and with *Azospirillum* on maize plant [46]. The effect of bio-fertilization of *S. marcescens* and *P. putida* and their combination in the presence of a half-dose of recommended mineral fertilizers was also demonstrated on the leaf area of the plants. Inoculation induced a highly significant increase in leaf area in the range of 28.55% to 32.08% compared with un-inoculated plants. Ahemad and Kiber [47] stated that mobilization of mineral nutrients like P and Fe in soil by rhizobacteria could be the main mechanism for increased growth and development of plants which makes these nutrients in more readily plant available forms. In comparison with control plants, the stem diameter of plants treated with *S. marcescens* + 50% NPK and *P. putida* + 50% NPK increased by 43.35% and 39.86%, respectively, after 70 days of cultivation. Such results were recently recorded in the work conducted by [48] with the inoculation of *Serratia sp.* on maize seeds grown on soil infested with *Fusarium*

*oxysporum* and fertilized with  $N_{180}P_{140}K_{90}$  and then in Morocco with the inoculation of *Serratia proteamaculans* (2025-1) on tomato [49]. Duponnois *et al.* [50] indicated that the more robust the inoculated seedlings are than the un-inoculated plants, the more likely they are to survive by demonstrating a high capacity to withstand environmental conditions. These results obtained in our study are of great importance because they promote better resistance of maize plants and therefore an improvement in maize productivity in northern Benin in a context where anarchic exploitation of forests induces an acceleration of climate change [51]. On crop productivity (Figure 3), the study revealed a significant effect ( $P < 0.01$ ) of bacterial inoculation on the fresh biomass yields of maize plants in the presence or in absence of NPK. For the fresh biomass of plants, *S. marcescens* + 50% NPK induced an increase of 144.28% in the fresh weight of aboveground biomass and 213.34% of the fresh weight of roots compared with controls. It is followed by *P. putida* + 50% NPK. The latter induced an improvement of 131.79% of the underground biomass and 71.45% of the fresh weight of aboveground biomass compared with the control plants. The same trends were observed in the dry matter developed by plants (Table 3) where the efficacy of *S. marcescens* + 50% NPK resulted in a rate of increase of 11.90% and 5.41%, respectively, of dry aerial biomass and underground biomass dry compared to plants that received 100% NPK. Similarly, Isfahani and Besharati [52] have reported that *Pseudomonas sp.* + 50% NPK (B1 P50) significantly stimulated the dry weight of cucumber roots compared to controls, while the lowest dry weights were recorded at the level of maize plants that received the full dose of 100% NPK (P100B0) mineral fertilizers. Our results were also consistent with the findings of [53] who observed that inoculation of seeds with *Pseudomonas* DSMZ 13134 resulted in an improvement in the biomass of maize plants grown on phosphorus deficient acid soil. Data presented in Table 3 also showed that inoculation of PGPR had a significant impact ( $P < 0.01$ ) on grain yield. Taking into account the results of the classification of all the treatments shown in Figure 4, it is apparent that in the absence of mineral fertilizers, inoculation of *P. putida*, *S. marcescens*, and *P. cichorii* stimulates the improvement of maize grain yield, a corresponding increase of 17.68%, 15.97% and 13.73% in comparison with the witness. It is also noted that the addition of a half-dose of NPK increases the effect of these strains on yield. Indeed, the best yields of grains obtained were recorded with the treatments *S. marcescens* + 50% NPK followed by *P. putida* + 50% NPK which gave the respective rates of increase of 39.05% and 34.91% compared to the un-inoculated plants. These rates reach the 37% obtained by [54] with inoculation of *P. putida* 108 on wheat. Our results were also in agreement with the previous study where the yields of rice and sugar beet had been enhanced by inoculation with PGPR alone or in combination with low-dose mineral fertilizers [55] [56]. In Iran, Biari *et al.* [57] justified the performance of PGPR on maize growth and grain yield by better absorption of N, P, K, Fe, Zn, Mn, and Cu nutrients. In our trials, data on the effect of inoculation of PGPR on nutrient absorption (Table 4) indicate that there is no significant dif-

ference ( $p > 0.005$ ) between the different treatments. However, the most important levels of nitrogen and phosphorus in the dry matter of seedlings were recorded with inoculation of *P. putida*, *S. marcescens*, while calcium and magnesium assimilation was improved with treatment *P. cichorii* and *P. cichorii* + 50% NPK. The higher amount of N and P detected in the dry matter of inoculated plants by *P. putida*, *S. marcescens* as well as growth promotion and yield may be attributed to the bacterial-assisted growth enhancement phenomenon [58]. It has been previously shown that bacteria are able to mediate the acquisition of nitrogen from the air and delivering it to the plant may be used as bio-fertilizer to improve crop productivity and reduce synthetic nitrogen fertilizer application [59]. Overall, maize plants inoculated in our study showed relatively low levels of nutrients. These results could be explained on the one hand by the genetic profile of the strains tested. The same observations were made by [60], who reported that the nitrogen content of the plant depends on the nitrogenase activity of the nitrogen fixing bacteria contained in the maize rhizosphere. In their studies, Berger *et al.* [61] argued that rhizobacteria PGPR use one or more mechanisms to improve plant growth, yield and health. The good level of yield in biomass and seed corn obtained in our trials at the level of seedlings inoculated with rhizobacteria, despite the reduction in the dose of mineral fertilizers, can be explained by several factors. Indeed, the majority of the strains being studied were reported as isolates promoting the growth of plants with multiple PGPR attributes that could significantly influence crop growth and yield [62]. Auxin production has been proposed as a major mechanism in stimulating the direct growth of plants in some studies [63] [64]. The positive effect of observed PGPR may also be related to the ability of our strains to produce antifungal metabolites such as siderophores, hydrolytic enzymes and antibiotics [65] [66]. Inoculation of field crops with compatible strains showed better results than single inoculation [67]. The improving effect of seed inoculation with bacterial consortia on growth, nutrient content and grain yield of maize and rice plant was reported by several researchers [48] [68]. In the current study, (*S. marcescens* + *P. putida* + 50% NPK) treatment did not express a high stimulation capacity for certain parameters evaluated. The low performance recorded for this co-inoculation would be explained by the choice of microorganisms used. Rice *et al.* [69] had indicated that competition phenomena and antagonisms can be established between inoculated strains, and in some cases lead to reducing their intrinsic capacity.

## 5. Conclusion

The results of current study had clearly shown the power of our PGPRs on maize growth and productivity under field condition. Out of the strains tested, inoculation of *S. marcescens* and *P. putida* stimulates the most important levels of nitrogen and phosphorus in the dry matter of seedlings and better improvement of maize grain yield. The combined use of *S. marcescens* and *P. putida* and recommended half-dose of NPK was more economical in terms of crop yield, and it was also a sustainable crop production technology. Thus, this approach could

reduce over application of NPK fertilizer for the profit of farmers in northern Benin and should be an environmental friendly practice.

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## Conflicts of Interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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