Efficacy of Biostimulants Formulated With *Pseudomonas putida* and Clay, Peat, Clay-Peat Binders on Maize Productivity in a Farming Environment in Southern Benin

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Maize plays a vital role in Benin's agricultural production systems. However, at the producer-level, yields are still low, although the production of this cereal is necessary for food security. The aims of this study were to assess the efficacy of solid biostimulants formulated from the rhizobacteria *Pseudomonas putida* and different binders on maize cultivation in the farming environment in three (03) study areas in South Benin. For this purpose, three (03) biostimulants were formulated based on *Pseudomonas putida* and the clay, peat and clay-peat combinations binders. The experimental design was a randomized block of four (04) treatments with 11 replicates per study area. Each replicate represented one producer. The trials were set up at 33 producers in the study areas of Adakplamè, Hayakpa and Zouzouvou in Southern Benin. The results obtained show that the best height, stem diameter, leaf area as obtained by applying biostimulants based on *P. putida* and half dose of NPK and Urea with respective increases of 15.75, 15.93, and 15.57% as compared to the full dose of NPK and Urea. Regarding maize yield, there was no significant difference between treatments and the different study areas. Taken together, the different biostimulants formulations were observed to be better than the farmers' practice in all the zones and for all the parameters evaluated, with the formulation involving *Pseudomonas putida* on the clay binder, and the half-dose of NPK and Urea showing the best result. The biostimulant formulated based on clay + *Pseudomonas putida* could be used in agriculture for a more sustainable and environmentally friendly maize production in Benin.

Keywords: biostimulants, clay, formulations, peat, PGPR, *Zea mays* L.

INTRODUCTION

In most West African countries, particularly in Benin, maize (*Zea mays* L.) *is* emerging as a staple food for food security. It is one the major cereal crops that undergoes more than a hundred different modes of processing (Adjadi et al., 2015). In terms of production, high nutritional value has been attributed to its grains. The grains have been reported to be a notable source of protein, lipids, fiber and sugar (Ignjatovic-Micic et al., 2015). Maize is the most traded cereal on the domestic and subregional market (Gandonou et al., 2019). Despite the importance of this speculation and its increasing demand, its productivity faces many constraints, including the constant decline in the fertility of cultivated soils due to their degradation (Igué et al., 2013). The land is subjected to severe degradation as a result of poor farming practices that destroy the flora, organic matter and soil fauna and microfauna. Cultivated land is being depleted at an accelerating rate, and crop yields are continually declining, thereby dangerously compromising the productivity and sustainability of the entire agricultural system (Alamri et al., 2016). In modern agricultural systems, thousands of millions of synthetic agrochemicals are used to achieve high crop yields. After application, these synthetic chemicals are not entirely used by plants, but persist in the soil in different forms. In addition, excessive use of synthetic agrochemicals, declining soil nutrients, and water-use issues, amongst others, are threats to the ecosystem (Omomowo and Babalola, 2019). These chemicals seep into the soil, and thus disrupt the diversity and performance of the rhizosphere (Ai et al., 2012) and human health via the food chain (Ayala and Rao, 2002). The use of synthetic fertilizers is therefore not considered as good practice because of the high costs and acute environmental risks (López-Bellido et al., 2013). In order to reduce the use of toxic chemicals, one of the safe management options is the use of environmentally friendly solutions (Adesemoye et al., 2009). These alternatives include microbial biostimulants. Biostimulants are substances or microorganisms applied to plants with the aim to enhance nutrition efficiency, abiotic stress tolerance and/or crop quality traits, regardless of its nutrients content (du Jardin, 2015). Those containing microorganism's sus as those containing Plant Growth Promoting Rhizobacteria (PGPR) can directly stimulate plant growth through the production of phytohormones (Kang et al., 2019; Ahmed et al., 2020), biological nitrogen fixation (Glick, 2014; Kumar et al., 2020), production of siderophores (Glick, 2014) and phosphate solubilization (Alori et al., 2017; Agbodjato et al., 2018). As biocontrol agents, PGPRs suppress plant pathogens (Bajracharya, 2019). Some rhizobacteria play an important role in improving soil fertility and plant growth by providing various unavailable nutrients. Rhizobacteria secrete organic acids that reduce the pH of the rhizosphere and thus freely produce phosphate available to plants (Kashyap et al., 2020). Alori and Babalola (2018) mentioned that the use of microbial inoculants is a reliable alternative to the use of chemical inputs because these microbial inoculants can act as biofertilizers, bioherbicides, biopesticides and biocontrol agents. The development of plant biostimulants has become the focus of much research interest. Plant biostimulants are diverse substances and microorganisms used to enhance plant growth. Plant biostimulants also designate commercial products containing mixtures of such substances and/or microorganisms (du Jardin, 2015). In recent years, there has been increasing use of biostimulants (Schisler et al., 2004; Viswanathan and Samiyappan, 2008; Gu et al., 2014). In Benin, several studies have been carried out on microbial biostimulants based on native PGPRs from rhizospheric soils (Adjanohoun et al., 2012; Noumavo et al., 2013; Agbodjato et al., 2015; Amogou et al., 2019; Adoko et al., 2020). Most of this work was carried out with PGPR-based biostimulant suspensions. The work carried out with solid biostimulants formulated based on different binders in Benin has proved the effectiveness of the biostimulant clay + *P. putida* in greenhouse conditions on ferralitic and ferruginous soil. The aim of the study was to evaluate the efficacy of solid biostimulants formulated from the rhizobacteria *Pseudomonas putida* and different binders on maize cultivation in the farming environment of South Benin.

MATERIALS AND METHODS

Study Areas

The trials were set up with 33 producers in three zones of South Benin: 11 producers in Adaplamè (Kétou), 11 producers in Zouzouvou (Djakotomey) and 11 producers in Hayakpa (Torri Bossito) (**Figure 1**). The sites were flat land with a maximum 2% slope, not flooded, and declining soil fertility is a priority constraint (source). The producers were at least 500 m apart from each other.

Characteristics of the Bacterial Inoculant and Maize Seeds

- The rhizobacteria *Pseudomonas putida* used was isolated and characterized from the maize rhizosphere in southern Benin by Adjanohoun et al. (2011) and preserved at −85°C in Muller Hinton broth with added glycerol (10%) at the Laboratoire de Biologie et de Typape Moléculaire en Microbiologie (LBTMM) of the Université d'Abomey-Calavi (UAC). It is recognized as a producer of indole acetic acid and capable of solubilizing phosphate (Noumavo et al., 2015).
- Maize seeds of the variety 2000 SYN EE W were used during the study. They are provided by the Center de Recherche Agricole Nord (CRA-Nord) of the Intstitut National de Recherches Agricoles du Bénin (INRAB). It is an extra-precocious variety with a vegetative cycle of 80 days. It is resistant to breakage, streak, American rust and blight. It is moderately resistant to drought (MAEP, 2016).

Preparation of the Inoculum and of the Various Formulationts

Preparation of the Inoculum

The inoculum was obtained by culture in a nutrient medium (liquid MH) for 24 h at 30°C. The concentration of the bacterial culture was adjusted to about 10^8 CFU/ml (OD 0.45 at 610 nm) with a spectrophotometer according to the method described by Govindappa et al. (2011).
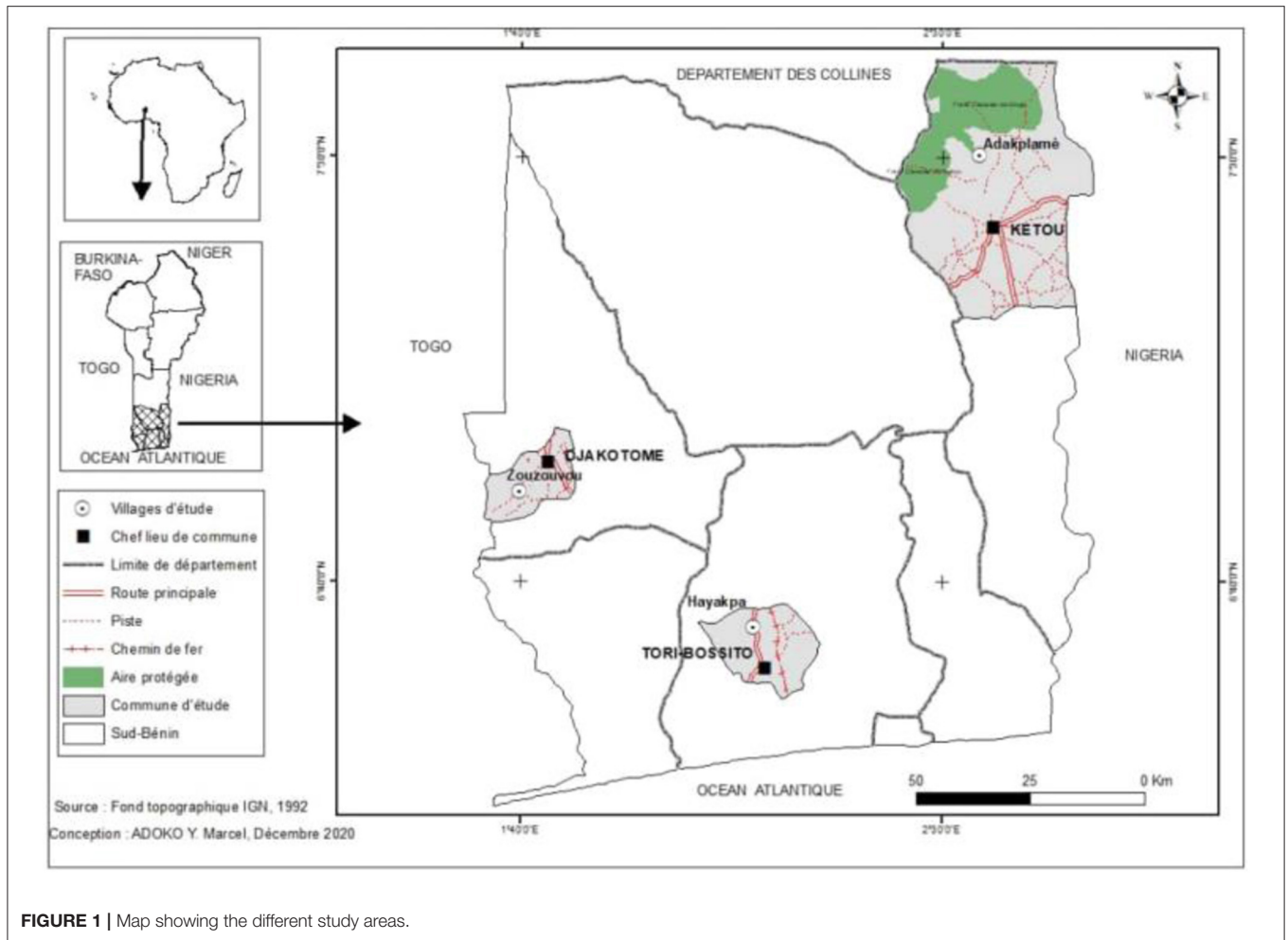
FIGURE 1 | Map showing the different study areas.

Preparation of the Various Formulations

The modified method of Connick et al. (1991) was used for the preparation of the formulation. Clay, peat and maize flour were separately sterilized for 15 min at 120°C. Thirty-two gram maize flour, 6 g binder (clay, peat and clay- peat), 2 g sucrose and 30 ml bacterial suspension (10^8 CFU/ml) of *Pseudomonas putida* were considered as a ratio for the preparation of the biostimulant. After cooling, the appropriate amounts of each component were mixed with gloved hands under aseptic conditions until a soft paste was obtained. The latter was spread on aluminum foil for 2 days at room temperature (25°C). After 2 days of drying, the paste was crushed in mortar then sieved.

Soil Sampling and Analysis Prior to Installation of the Tests

Thirty-three (33) composite soil samples were taken at a depth of 0–20 cm from the fields of the various producers. These samples were sent to the Laboratoire des Sciences du Sol Eau et Environnement (LSSEE) of the INRAB for the determination of chemical characteristics. The analyses consisted of the determination of organic carbon by the method of Walkley and Black (1934); total nitrogen by the Kjeldahl (1883) method;

pH water and pH KCl using a pH meter with (1/2.5) as a soil-water ratio; Assimilable phosphorus, by the Bray and Kurtz method (1945); Exchangeable cations (Ca, Mg, K and Na), by the ammonium acetate method using atomic absorption spectrophotometry (Thomas, 1982).

Installation of the Trials in a Farming Environment and Fertilization

Experimental Design

The experimental design was a randomized block of four (04) treatments with eleven (11) replicates per study area. In a study area, each replicate represented one producer. Each elementary plot had a surface area of 40 m² and was made up of 5 lines of 10 m long with 0.80 m spacing. The distance separating each plot was 5 m. Sowing was done at a spacing of 0.80 × 0.40 m, i.e., a density of 31,250 plants/ha (Yallou et al., 2010a). The treatments defined as follows:

   T0: peasant practice (100% NPK and Urea);
   T1: clay + *P. putida* + ½ NPK and Urea;
   T2: peat + *P. putida* + ½ NPK and Urea;
   T3: clay-peat + *P. putida* + ½ NPK and Urea.

With: 100% NPK and Urea is recommended dose of mineral fertilizer, and ½ NPK and Urea is half of recommended dose of mineral fertilizer.

Seed Sowing and Application of the Formulated Biostimulant and Mineral Fertilizer

Three (03) seed holes of about 5 cm of depth and 2 cm apart were realized and 2 maize seeds were put in the central hole. Then, 5 g of formulated biostimulant and half a dose of NPK were applied separately in the other two holes on the day of sowing and the holes were immediately closed. The urea doses were applied on the 46th day after sowing according to each treatment. For the T0 treatment, application of the recommended dose of NPK was made according to the practice popularized to the producers on the 15th day after sowing. Note that the recommended dose of mineral fertilizer (NPK and Urea) for maize cultivation in Benin is 200 kg/ha of NPK and 100 kg of Urea (INRAB, 1995). Note that the NPK used in our study is of formula is $N_{13}P_{17}K_{17}$. As for urea, it contains 46% of nitrogen (N).

Data Collection

At 60 days after sowing, the height was measured with a tape measure. The diameter at the collar of the plants was measured using a caliper, and the leaf area was estimated by multiplying the length and width of the leaves by a coefficient of 0.75 (Ruget et al., 1996). At harvest (80 days after sowing), the ears of the maize plants were harvested. After shelling, the total weight of the maize grains was measured with a precision balance (Highland HCB 3001, Max: 3000 × 0.1 g), and the moisture content was taken with a moisture meter (LDS-1F). Maize grain yield values were obtained using the formula (Valdés et al., 2013):

$$R = \frac{P \times 10.000}{S \times 1.000} \times \frac{14\%}{H} \qquad (1)$$

Where: R is the maize yield, expressed in T/ha; P is the maize mass per calculated elemental area, expressed in kg; S is the useful parcel area in m^2; H is the grain moisture rate, in %.

Statistical Analysis

The various tests were carried out using R 4. 0. 2 software (R Core Team, 2020). These analyses required the use of the dplyr and DescTools packages for the calculation of descriptive statistics, the ggplot2 and ggpur packages for the creation of mustache boxes, the stats package for the shapiro-Wilk and levene tests, the car package for the ANOVA and the multcomp package for the post-hoc pair comparison test. The effect of the experimental area and the treatments applied on the growth and yield performance of the plants was assessed by means of a two-factor.

ANOVA Test

The normality and the homogeneity of the data variances were verified (Glèlè Kakaï et al., 2006). As the experimental design was unbalanced, the type III ANOVA test was adopted. Once the ANOVA test was significant, a pair-wise comparison

post hoc test using the Tuckey post hoc test (Douglas and Michael, 1991) was carried out to assess statistical differences in the means. Besides, descriptive statistics were calculated for each measured parameter. The significance threshold used was 5%.

RESULTS

Chemical Characteristics of Soil

Soil chemical properties of the sites before the tests were set up (**Table 1**) generally showed that the soils at the different sites in South Benin were slightly acidic (5.7 ≤ pH ≤ 6.4). All soils had low fertility 12.46 ≤ C/N ≤ 15.61 characterized by high C/N ratios. The soils had low levels of organic carbon (8.9 ≤ C ≤ 10.6) (g/Kg), total nitrogen (0.57 ≤ N ≤ 0.72) (g/Kg), exchangeable bases (3.3 ≤ Ca^{2+} ≤ 5.14 (g/Kg); 2.3 ≤ Mg^{2+} ≤ 3.72 (cmol/Kg) and 0.7 ≤ K^+ ≤ 1.9) (cmol/Kg). Generally speaking, assimilable phosphorus (28.38 ≤ P ≤ 36.8) (mg/Kg), was lower in the soils of the different sites.

Effect of Biostimulants on Maize Plant Height

The histogram in **Figure 2** illustrates the variation in average maize plant height as a function of treatments at DAS 60 in the different study areas. In the Hayakpa and Zouzouvou zones, the biostimulant clay + *P. putida* + ½ NPK and Urea gave the best result with respective increases of 4.18 and 12.41% compared to the peasant practice (100% NPK and Urea). In the Adakplamè area, the peat biostimulant + *P. putida* + ½ NPK and Urea was the highest with an increase of 15.75% compared to 100% NPK and Urea. The results of the analysis of variance showed a significant difference in the effects of the treatments ($p = 0.01$) and the experimental area ($p < 0.001$) on maize plant height. Plants in the Adakplamè zone induced the best performance (15.75% increase) for most treatments than plants in the other zones (**Figure 3**). Moreover, the interaction between the different treatments and the study areas was significant ($p < 0.05$).

Effect of Biostimulants on the Stem Diameter of Maize Plants

The histogram in **Figure 4** shows the variation in the stem diameter of maize plants as a function of the treatments at 60th DAS in the different study areas. In the Hayakpa and Adakplamè zones, the biostimulant clay + *P. putida* + ½ NPK and Urea were in the lead, with an overrun of 0.78 and 9.32%, respectively, compared to the recommended dose of NPK and Urea. In the Zouzouvou area, the peat biofertilizer + *P. putida* + ½ NPK and Urea resulted in a better collar diameter. This better treatment exceeded the recommended dose of NPK and Urea by 15.93%. The results of the analysis of variance showed a significant difference in the effects of the treatments ($p = 0.01$). On the other hand, no difference was recorded between the experimental areas ($p = 0.12$) on the stem diameter of the maize plants. It was also noted that the interaction between treatment and area was also non-significant ($p = 0.20$), indicating that the variation in maize plant crown diameter per treatment does not depend on the experimental site. From the analysis of **Figure 4**, it appears

Sites	Villages	pH (water)	C-org (g/Kg)	N-total (g/Kg)	C/N	P_{ass}-Bray1 (mg/Kg)	B.E (cmol/kg)		
							Ca^{2+}	Mg^{2+}	K^+
Kétou	Adakplamè	6.4	8.10	0.65	12.46	36.8	33.3	2.3	1.9
Tori	Hayakpa	5.9	10.6	0.72	14.72	33.92	5.14	3.72	0.7
Djakotomey	Zouzouvou	5.7	8.9	0.57	15.61	28.38	5.02	3.39	1.08

C-org, organic carbon; N-total, Azote total; P-Bray1, Phosphorus available; B.E, Base Exchangeable.
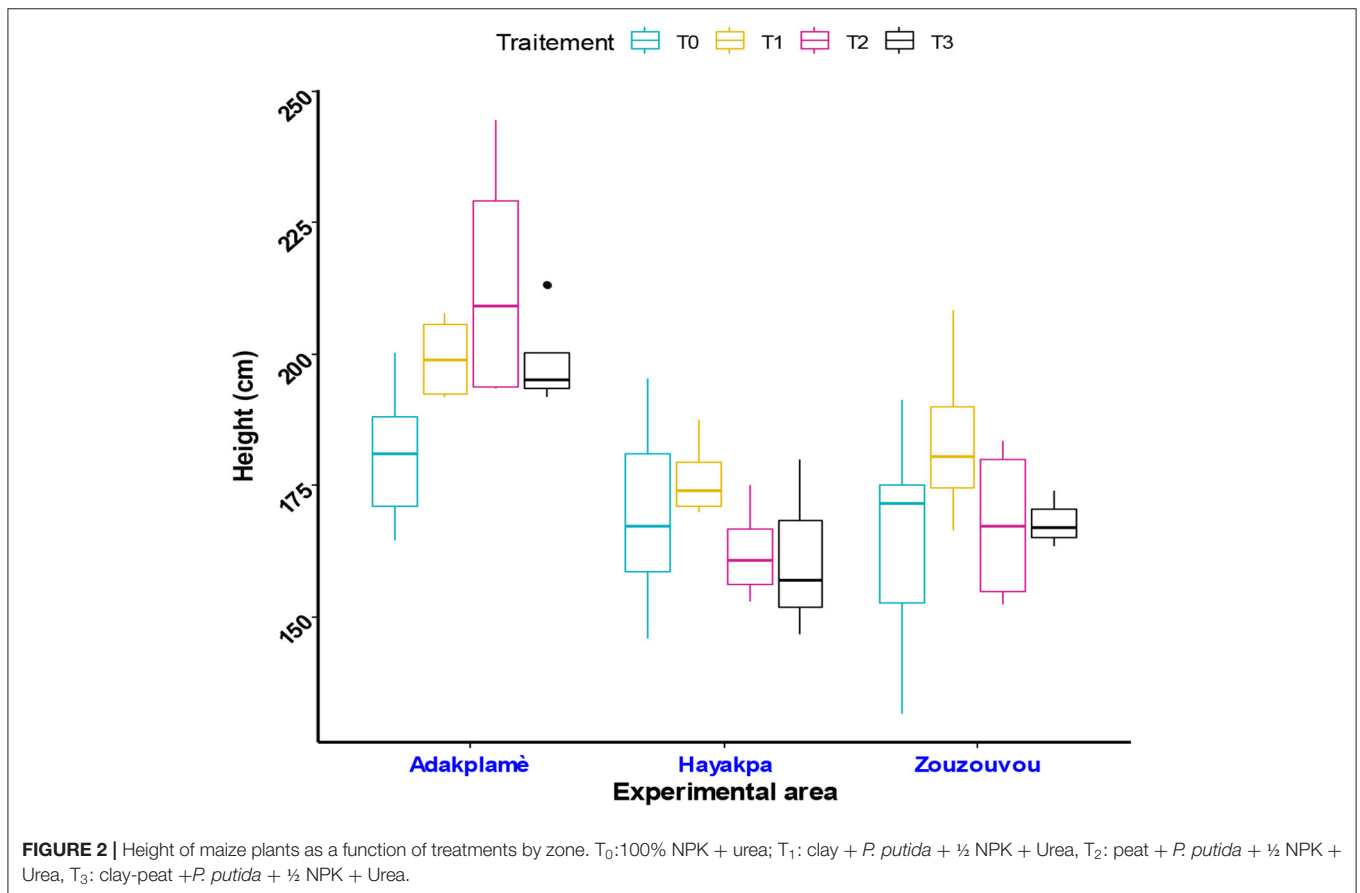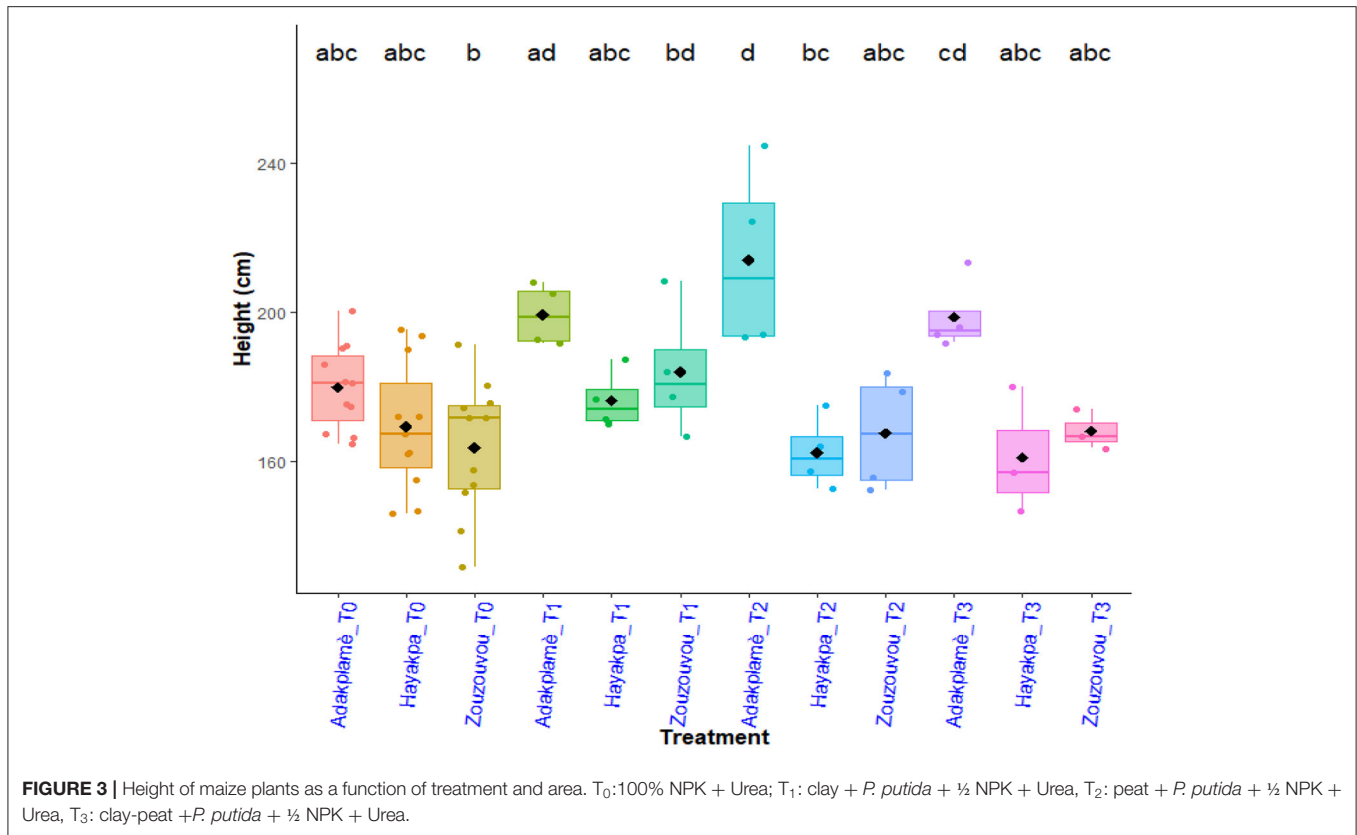

FIGURE 2 | Height of maize plants as a function of treatments by zone. T_0:100% NPK + urea; T_1: clay + *P. putida* + ½ NPK + Urea, T_2: peat + *P. putida* + ½ NPK + Urea, T_3: clay-peat +*P. putida* + ½ NPK + Urea.

that the plants in the Adakplamè area performed best. The Tukey test carried out confirmed the trend (**Figure 5**). Thus, the clay + *P. putida* + ½ NPK and Urea treatment in the experimental areas gave the best performance in terms of diameter at the crown, with an increase of 15.93% compared to the extended practice.

Effect of Biostimulants on the Leaf Area of Maize Plants

The effect of biostimulants on the leaf surface as a function of the treatments and by zone was illustrated by the histogram in **Figure 6**. In the Hayakpa zone, the biostimulants clay + *P. putida* + ½ NPK and Urea induced a large leaf area. This application resulted in a 5.77% growth rate in relation to the popularized dose of NPK and Urea. In the Zouzouvou area, the same treatment was better, with an increase of 18.31% in relation to the recommended dose of NPK and Urea. In Adakplamè, with the biostimulants formulated with peat + *P. putida* + ½ NPK and Urea, an increase of 15.57% in relation to the recommended dose of NPK and Urea was recorded. The results of the analysis of variance indicated a non-significant difference in the effects of the treatments ($p = 0.051$) in the same locality. However, a highly significant difference between experimental areas ($p < 0.001$) was observed. It is noted that the treatment-area interaction was non-significant ($p = 0.08$), indicating that the variation in the leaf area of maize plants does not depend on the treatments but on the experimental area. From the analysis in **Figure 6**, it appears that the plants in the Zouzouvou area performed best. The Tukey test carried out confirms the trend (**Figure 7**). Thus, the Zouzouvou zone comes first, followed by Hayakpa and Adakplamè.

FIGURE 3 | Height of maize plants as a function of treatment and area. T_0:100% NPK + Urea; T_1: clay + *P. putida* + ½ NPK + Urea, T_2: peat + *P. putida* + ½ NPK + Urea, T_3: clay-peat +*P. putida* + ½ NPK + Urea.

Effect of Biostimulants on Maize Grain Yield

Maize grain yields as a function of treatment and area were illustrated by the histogram in **Figure 8**. In the Hayakpa zone, the biostimulants clay-peat + P. putida + ½ NPK and Urea performed better in maize grain yield. This treatment has an increase of 2.17% compared to the recommended dose of NPK and Urea. In the Zouzouvou area, the peat + *P. putida* + ½ NPK and Urea application was better with an increase of 3.24% concerning the recommended dose of NPK and Urea. In Adakplamè it is the biofertilizer clay + *P. putida* + ½ dose of NPK and Urea was better with an increase of 10.96% in relation to the recommended dose of NPK and Urea. The results of the analysis of variance revealed that there were no significant differences in the effects of the treatments ($p = 0.92$) and between the experimental areas ($p = 0.14$) on maize grain yield. Treatment and zone interactions were also non-significant ($p = 0.81$), indicating that maize grain yield variations do not depend on treatments and experimental zones.
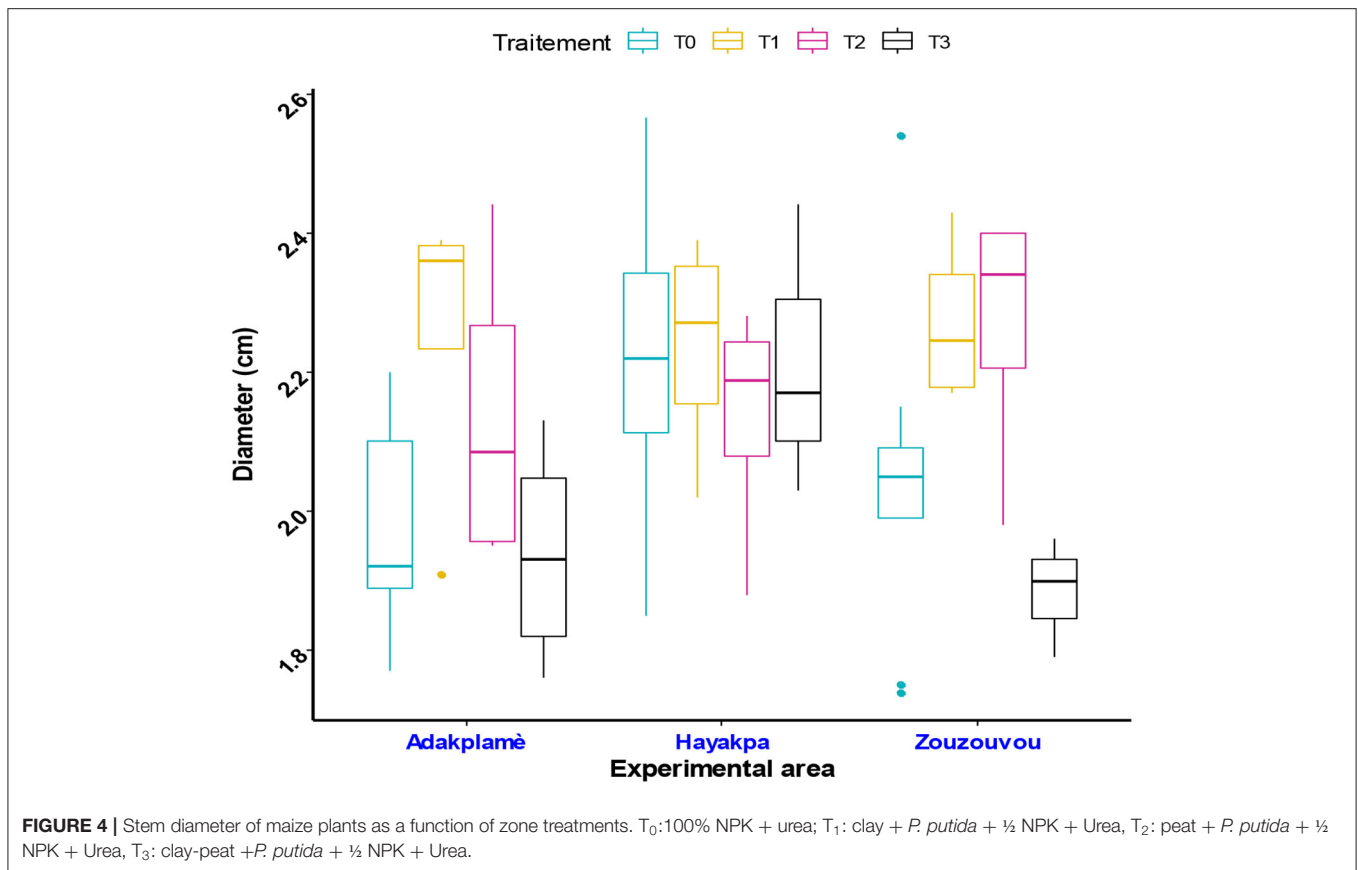
Correlation Between Growth and Yield Parameters

Principal Component Analysis (PCA) on the different maize plant growth and yield parameters showed that the first two axes retain 80.01% of the total variance (**Figure 9**). Height and grain yield were positively correlated with axis 1, while the area was negatively correlated with the same axis. The collar diameter is

strongly associated with axis 2 as shown by the projection of the variables in the first two axes.

Classification of Treatments According to Their Performance

The analysis of the projection of the individuals indicates three classes of grouping of treatments (**Figure 10**) discriminated by the variable's height, stem diameter, leaf area, grain yield. The first class (C1) is made up of three practical peasant treatments (100% NPK and Urea); clay + *P. putida* + ½ NPK and Urea; peat-clay + *P. putida* + ½ NPK and Urea from the Zouzouvou zone. The plants maintained under these treatments are characterized by an average height of 171.91 cm ± 10.68 and an average grain yield of 2.14 T/ha ± 0.04. The second class (C2) is made up of five treatments, including the four (04) treatments of Hayakpa, a 100% NPK and urea peasant practice; clay + *P. putida* + ½ NPK and Urea; peat + *P. putida* + ½ NPK and Urea; clay-peat + *P. putida* + ½ NPK and Urea and the peat + *P. putida* + ½ NPK and Urea treatment of Zouzouvou. The plants having benefited from the treatments of this class (C2) have an average height of 167.30 cm ± 6.09 and an average grain yield of 2.27 T/ha ± 0.06. The third class (C3) consisted of the four treatments of Adakplamè 100% NPK and Urea; clay + *P. putida* + ½ NPK and Urea; peat + *P. putida* + ½ NPK and Urea; clay-peat + *P. putida* + ½ NPK and Urea. The plants subjected to these treatments have an average height of 197.95 cm ± 14.01 and an

FIGURE 4 | Stem diameter of maize plants as a function of zone treatments. T_0:100% NPK + urea; T_1: clay + *P. putida* + ½ NPK + Urea, T_2: peat + *P. putida* + ½ NPK + Urea, T_3: clay-peat + *P. putida* + ½ NPK + Urea.

average grain yield of 2.38 T/ha ± 0.12. The class (C3) gave the best performance in both height and grain yield of maize.

DISCUSSION

The biostimulants are substances and or microorganisms that contain living microorganisms and have no toxic effects on the soil. Their use would be inexpensive compared to mineral fertilizers (Amutha et al., 2014). The application of mineral fertilizers in combination with biostimulants could be an effective strategy to improve soil health and nutrient availability for crops. The aim of the study is to test solid biostimulants formulated with the rhizobacteria *P. putida* and various binders in a farming environment in southern Benin. The trials were set up in three different areas in southern Benin on ferrallitic soils.

Analysis of the initial chemical properties of the test soils shows that the soils at the three sites are slightly acidic. The C/N ratio (12.46–15.61) was high in the topsoil. The level of assimilable phosphorus was lower. In general, in the soils of the study areas, the sum of exchangeable bases and the cation exchange capacity are low, which reflects their low fertility (Adjanohoun et al., 2011). In the soils of the three study zones, potassium was globally deficient in relation to calcium and magnesium. Better still, imbalances between calcium, magnesium and phosphorus were noted. These results, which were in line with those reported by Igué et al. (2013), showed that it was

necessary to provide nutrients to the soil because these quantities are insufficient to meet maize's nutritional requirements (Yallou et al., 2010b). Regarding the growth parameters of maize plants on ferrallitic soil, the formulated biofertilizers were expressed differently.

In all three zones, the best height and stem diameter were obtained with biostimulants formulated with clay and peat supports. Significant differences were recorded between these applications and the farming practice on these growth parameters. The same observations were made by different authors in Benin when they combined liquid microbial biostimulants with mineral fertilizers (Agbodjato et al., 2015; Amogou et al., 2019; Adoko et al., 2020). These increases can be explained by the growth stimulating effect of the rhizobacteria *P. putida* (Noumavo et al., 2015) under study, on the one hand, and, on the other hand, the effect of conservation binders (clay and peat) which maintain the bacterial concentration for a long time and which would better protect the PGPR strains against abiotic factors (Brar et al., 2012).

In the same locality, there was no significant difference between treatments for the leaf area of the maize. However, from one area to another, it was highly significant, with the Zouzouvou area leading the way. The best leaf area was obtained with the application of biostimulants T1: peat-clay + *P. putida* + ½ NPK and Urea. This could be explained by the lack of variability in the chemical composition of the soils of the different producers
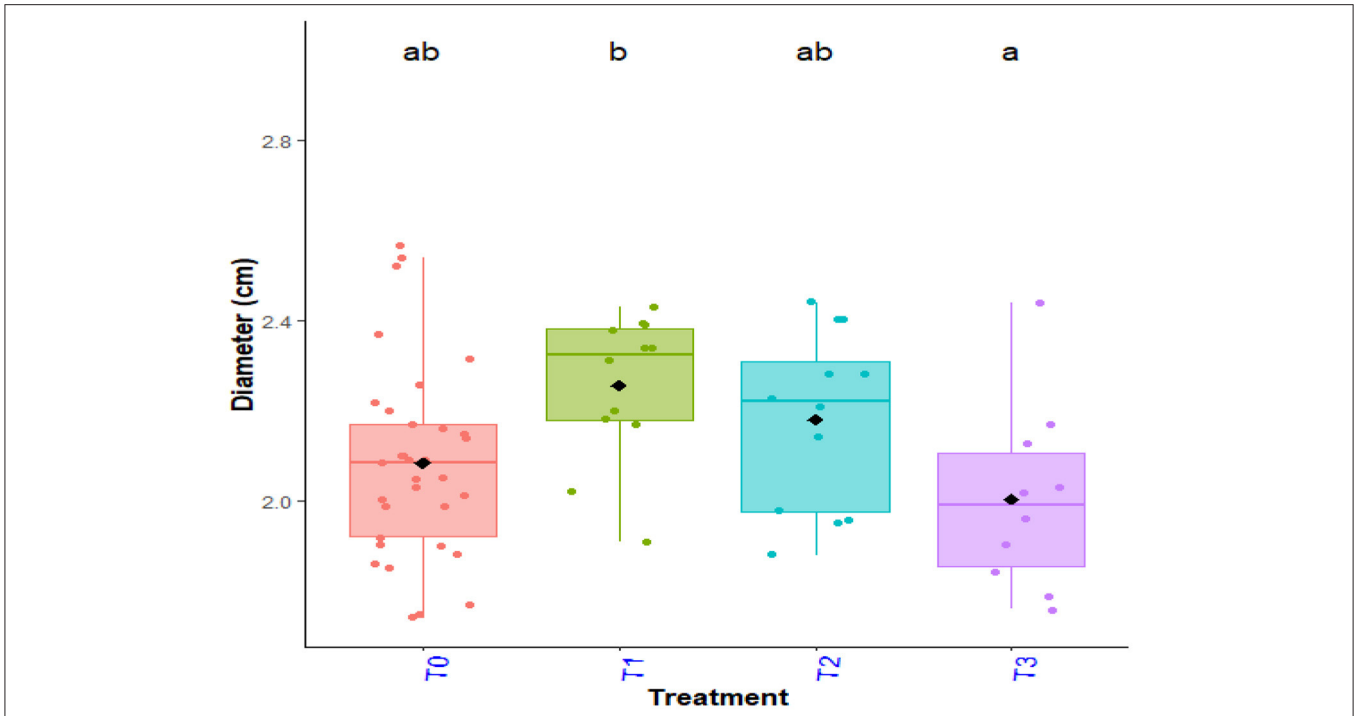
FIGURE 5 | Stem diameter of maize plants as a function of treatments. T_0:100% NPK + urea; T_1: clay + *P. putida* + ½ NPK + Urea, T_2: peat + *P. putida* + ½ NPK + Urea, T_3: clay-peat +*P. putida* + ½ NPK + Urea.
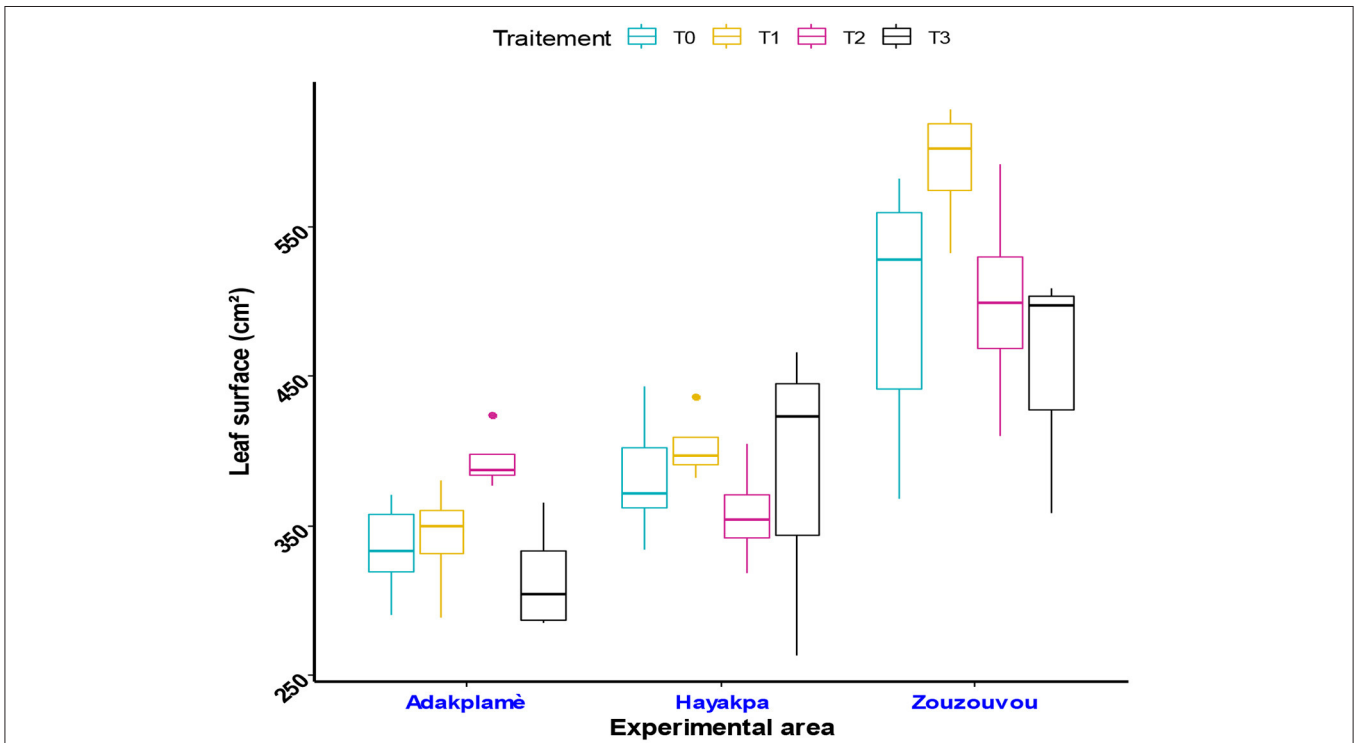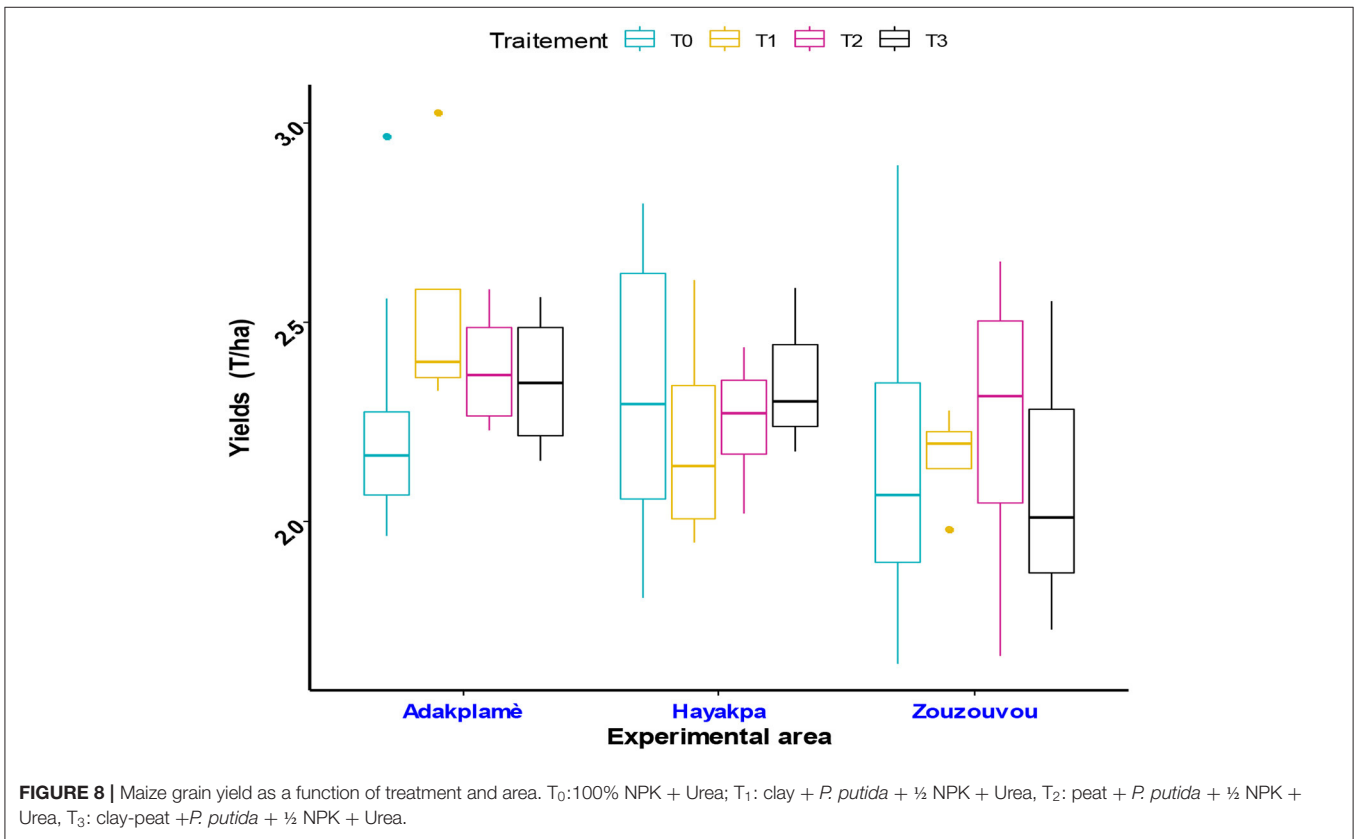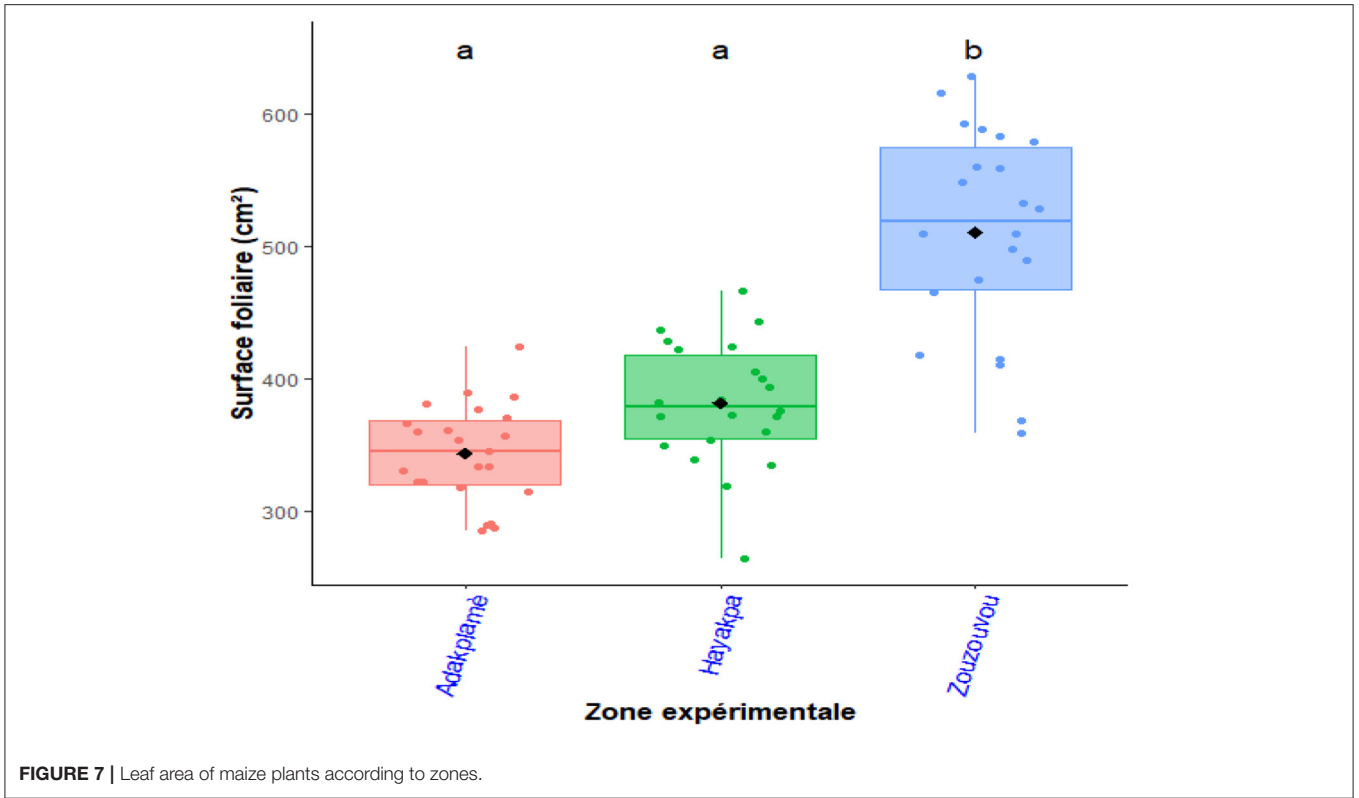

FIGURE 6 | Leaf area of maize plants as a function of treatments by zone. T_0:100% NPK + urea; T_1: clay + *P. putida* + ½ NPK + Urea, T_2: peat + *P. putida* + ½ NPK + Urea, T_3: clay-peat +*P. putida* + ½ NPK + Urea.

FIGURE 7 | Leaf area of maize plants according to zones.

FIGURE 8 | Maize grain yield as a function of treatment and area. T_0:100% NPK + Urea; T_1: clay + *P. putida* + ½ NPK + Urea, T_2: peat + *P. putida* + ½ NPK + Urea, T_3: clay-peat +*P. putida* + ½ NPK + Urea.
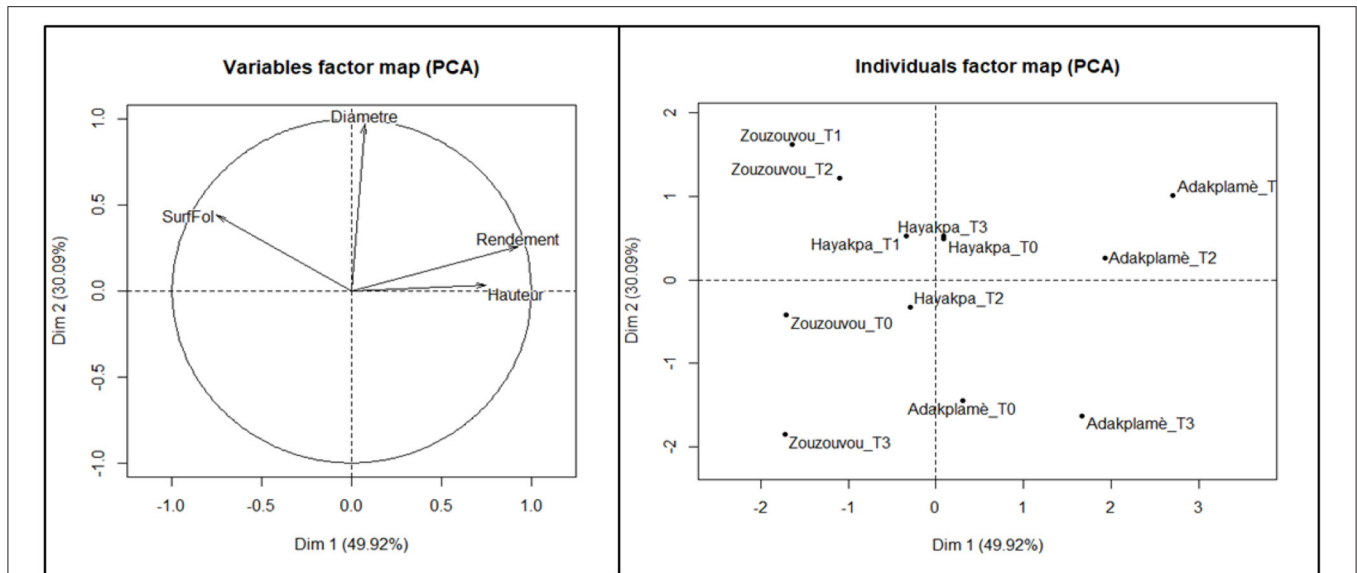
FIGURE 9 | Classification of different growth and yield parameters based on their performance. T_0:100% NPK + Urea; T_1: clay + *P. putida* + ½ NPK + Urea, T_2: peat + *P. putida* + ½ NPK + Urea, T_3: clay-peat +*P. putida* + ½ NPK + Urea.
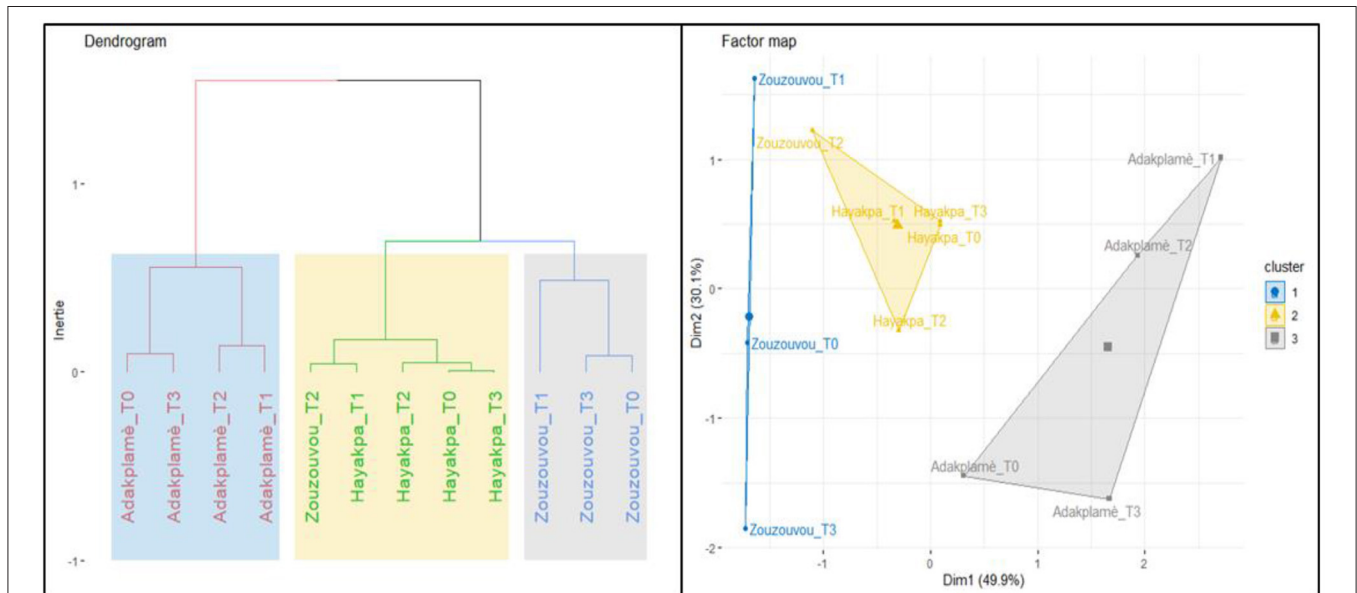

FIGURE 10 | Dendrogram of classes obtained and projection of treatments in the first two dimensions of PCA. T_0:100% NPK + Urea; T_1: clay + *P. putida* + ½ NPK + Urea, T_2: peat + *P. putida* + ½ NPK + Urea, T_3: clay-peat +*P. putida* + ½ NPK + Urea.

that hosted the trials in the same area. In the same way, the farming practices of a locality remain similar. But from one area to another, the soils do not have exactly the same chemical properties. Similar findings were made by Adoko et al. (2020) in their studies of the liquid biostimulants *P. putida* in a farming environment in Benin.

Maize grain yields obtained in this study from all treatments and in all areas were similar. The results of the statistical analysis did not reveal any significant differences between the various treatments and between the different zones. The formulated biostimulants combined with the half dose of NPK and Urea had comparable effects with the full dose of NPK and Urea in all study zones on maize grain yield. The same findings have been made by several authors who have applied liquid PGPR biostimulants in research stations (Noumavo et al., 2013; Agbodjato et al., 2015; Amogou et al., 2019) and then in farmers' fields (Adoko et al., 2020) in Benin and other countries (Amutha et al., 2014; Sagay et al., 2020). The rhizobacteria *P. putida*

contained in these formulated solid biostimulants was thus able to provide the plants with nutrients from the environment to increase their yield (Kashyap et al., 2020). This rhizobacteria was able to provide maize plants with the maximum nitrogen (N), phosphorus (P) and potassium (K) supplied or available in the soil, necessary for plant growth and yield (Ahmed et al., 2020). Ojuederie et al. (2019) also mentioned that multifaceted PGPRs are potential candidates for biofertilizer production to lessen the detrimental effects of drought stress on crops cultivated in arid regions.

In the present study, the correlation between growth and yield parameters showed that the biostimulant T1: clay + *P. putida* + ½ NPK and Urea expressed itself better than all the others. This biostimulant clay + *P. putida* was, therefore, the best in the farming environment. This result can be explained in part by the capacity of the preservative binder used for the bioformulation to maintain a good bacterial concentration in the rhizosphere for a long time. According to the work of Brar et al. (2012), the clay binder makes it possible to maintain a high population of PGPR for several months, which is favorable to the promotion of plant growth and yield. Earlier work by Noumavo et al. (2015) stated that *P. putida* used in this study is capable of producing growth phytohormones and solubilizing phosphate. The best growth and yield parameters recorded during this study can be explained by the combined effects of *P. putida* and the binder clay. Some strains of rhizobacteria of the genus pseudomonas are capable of producing ammonia, indole acetic acid (IAA), HCN, siderophores, solubilizing potassium (Verma and Pal, 2020), phosphate, zinc and increasing the bioavailability of nutrients for good plant development (Marra et al., 2012; Verma et al., 2015; Shahid et al., 2017; Singh and Jha, 2017; Ullah and Yusuf, 2019; Zaheer et al., 2019). The rhizobacteria *P. fluorescens* have also been reported to colonize the rhizosphere of wheat and sugarcane and stimulate plant growth (Verma et al., 2015). Oteino et al. (2015) attributed the efficacy of *P. fluorescens* on onion yield to its ability to produce indole acetic acid. Similarly, the biocontrol properties of this genus are well documented (Reetha et al., 2014; Khanghahi et al., 2018). PGPRs also secrete several growth phytohormones such as auxins, cytokinins, gibberellins and ethylene which improve both root growth and whole plant growth (Lugtenberg and Kamilova, 2009; Dodd et al., 2010; Wani et al., 2013). Furthermore, work carried out in Senegal by Diagne et al. (2020) has also shown that inoculation with PGPR and/or Arbuscular Mycorrhizal Fungi (AMF) can improve the salinity resistance of *Casuarina obesa* plants by increasing their growth parameters. The use of biologically active natural products and microbial extracts could be an important means of increasing soil nutritional status, absorption and improving the efficiency of nutrient use (NPK) by plants (De Pascale et al., 2017). Phosphorus, potassium and magnesium have been reported to improve root growth, resulting in improved water supply and drought tolerance. Cassán and Diaz-Zorita (2016) showed that the increase in crop yield was due to the ability of *Azospirillum sp* to provide the plant with nutrients. According to Zeffa et al. (2019) inoculation of maize seed with *Azospirillum brasilense* intensified plant growth and yield by improving nitrogen use in the event of nitrogen deficiency. It is in this same context that Fadiji and Babalola (2020) mentioned that the major benefit of embracing the beneficial microorganisms in the field of agriculture is to bring about a reduction in the use of different agrochemicals such as pesticides, chemical fertilizers, other artificial chemicals and this would make agriculture more productive and sustainable.

CONCLUSION

The results of the experiment show that *Pseudomonas putida*-based biostimulants combined with the half dose of NPK and Urea recommended (100 kg/ha NPK and 50 kg/ha) for maize cultivation in Benin gave the best performance both in terms of growth parameters and maize grain yield. The effects of these microbial biostimulants vary from region to region and according to the type of binder. The application of biostimulants formulated on the basis of clay or peat in combination with the half-dose of NPK and Urea in the different study areas is more favorable to corn plants than the recommended full dose (100% NPK and Urea). The *Pseudomonas putida* strain could be used as biofertilizers for environmentally friendly sustainable agriculture. It would be interesting to continue this study by repeating the trial on a larger area to assess the performance of this rhizobacteria to improve maize growth through the formulations made.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

AUTHOR CONTRIBUTIONS

NA, MA, OA, and FB carried out the experimental work and analysis. NA, MA, PN, AA, OB, and LB-M contributed to the design, supervision, and interpretation of the results. NA, MA, and OB revised the final draft. OB reviewed the final draft. All authors participated equally in the work and approved the final submission.

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