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Use of the Biostimulant Based on the Mycorrhizae Consortium of the *Glomeraceae* Family in the Field to Improve the Production and Nutritional Status of Maize (*Zea mays* L.) Plants in Central Benin

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Abstract

Excessive use of mineral fertilizers in maize farming negatively affects farmers' income and impacts long-term soil health. This study aims to appreciate the effectiveness of biostimulant based on native *Glomeraceae* arbuscular mycorrhizal fungi on the production and uptake of phosphorus, nitrogen and potassium of maize (*Zea mays* L.) plants in central Benin. The trials were set up in a farming environment with thirty-four producers. The experimental design was composed of three treatments installed at 34 producers. Three growth parameters were evaluated on 60 ème days after sowing. Grain yield, nutritional status of maize plants and mycorrhization parameters were determined at harvest. The results showed that the *Glomeraceae* + 50% NPK (NPK: azote-phosphore-potassium)_Urea treatment improved the height, the crown diameter and the leaf area by 17.85%, 21.79% and 28.32% compared to the absolute control and by 0.41%, 1.11% and 1.46% compared to the 100% NPK_Urea treatment, respectively. Similarly, grain yield improved by 45.87% with the use of *Glomeraceae* + 50% NPK_Urea compared to the absolute control and by 3.96% compared to the 100% NPK_Urea treatment. The *Glomeraceae* + 50% NPK_Urea significantly improved the phosphorus and potassium uptake of maize plants. With respect to nitrogen uptake, no statistical difference was observed between treatments. The mycorrhizae strains used improved root infection in the maize plants. We recorded 66% frequency and 40.5% intensity of mycorrhization. The biostimulant based on indigenous *Glomeraceae* combined with 50% NPK_Urea can be used as a strategy to restore soil health and improve maize productivity in Benin.

Keywords

Ecological Resilience, Microorganism, Plant Nutrition, Sustainable Agriculture, *Zea mays* L.

1. Introduction

In Benin, maize occupies an important place in dishes and is commonly consumed in several forms [1]. However, maize yield remains low [2]. The low maize yield is justified by attacks by maize pathogens, poor rainfall distribution and declining soil fertility [3]. Thus, to alleviate this problem, producers resort to using mineral inputs but in an abusive manner, which causes adverse effects on the environment and human health [4] [5]. It is, therefore, imperative to find palliative measures to make agriculture environmentally friendly [6]. Thus, several technologies are considered, including the involvement of microorganisms in agriculture, to reduce the consequences of the use of mineral fertilizers on soil health and the economy of producers [6] [7].

Soil-plant-microorganism interactions are known in ecological studies [8] [9] [10]. But the recent aspect of molecular biology has contributed to the knowledge about the functions of microorganisms present in the soil-root environment, a case study of Arbuscular Mycorrhizal Fungi (AMF) [9]. Thus, among the plant-fungal associations, the most frequent is the arbuscular mycorrhizal symbiosis: the majority of plant species are associated, at the root level, with fungi of the *Glomeromycetes* group [11] [12]. These fungi spread in the soil and infect plant roots through propagules, such as spores, hyphae, vesicles, and arbuscules [13]. Once in association with the roots, AMF gives up soil nutrients to the plant, which they explore beyond the capacity of the plant roots, and benefit in return from carbonaceous substances derived from photosynthesis [14]. Similarly, AMF also develops mycelia that have the property of excreting glomalin. Glomalin is a substance that is hormonal in nature. It acts as a glue that binds the finest soil particles together to form aggregates essential for the absorption of water, mineral elements and gas exchange by plants [14]. In addition, the improvement of soil structure by mycorrhizae leads to better water penetration of about 25%, thus slowing down the effect of erosion and stabilizing the soil [15] [16]. In addition, AMF has proven their effectiveness in agronomic and ecological contexts by limiting mineral inputs such as pesticides, nitrogen-phosphorus-potassium (N, P, K) and conferring to plants better mineral nutrition and tolerance to biotic and abiotic stresses [17] [18] [19] [20]. This is the case in Indonesia [21] with maize productivity and in Algeria [22] with wheat production where the benefits of symbiotic relationships of AMF and these different crops have been proven. Mycorrhizal inoculation dependence and nutrient uptake tested by [23] were significantly increased by five different AMF species used to inoculate vegetable crops, such as green bell pepper, tomato and eggplant. According to [24], symbiosis between AMF and plants significantly increases root colonization, which consequently has different effects on root and plant growth.

In Benin, a lot of work has been done with focusing interest on the use of inoculation of different exogenous [25] [26] [27], as well as indigenous [28] [29] [30] strains of AMF on the productivity of maize (*Zea mays* L.) with beneficial results. Growing maize with AMF is more profitable for producers with a net margin of 63,830 fcfa compared to 43,730 fcfa for the conventional practice of using mineral inputs in Benin [29].

Most of these studies were conducted on small areas. The present study envisages a larger area with a view to popularizing the mycorrhizal inoculation technology in Benin. Furthermore, no study has been carried out to assess the combined effect of three indigenous strains of Glomeraceae: Glomus caledonius, Rhizophagus intraradices and Funneliformis geosporum in Benin. Similarly, there is no information on the effect of indigenous Arbuscular Mycorrhizal Fungi on the nutrition of maize plants (Phosphorus, Potassium and Nitrogen) in Benin.

The fact that the application of these biological strains in a cropping system is becoming urgent to achieve profitable and sustainable agriculture [31], there is a need for further research. It is in this context that our work consists to study the efficacy of the biostimulant formulated based on the combination of three indigenous Arbuscular Mycorrhizal Fungi (*Glomus caledonius, Rhizophagus intraradices and Funneliformis geosporum*) on the parameters of growth, yield and nutritional status of maize plants in a peasant environment on ferruginous soil in central Benin.

2. Materials and Methods

2.1. Study Area and Materials

Post-harvest activities as well as those related to mycorrhization tests were carried out at the Laboratoire de Biologie et de Typage Moléculaire en Microbiologie de l'Université d'Abomey-Calavi. In addition, activities related to seeding and collection of growth variables were carried out on three sites in three different municipalities in Central Benin, namely: Bantè (8˚25'0" North, 1˚52'60" East), Ouèssè (8˚28'60" North, 2˚25'60" East) and Dassa-Zounmè (7˚41'33" North, 2˚13'25" East) (**Figure 1**).

Fungal inoculum composed of 3 genera in the family *Glomeraceae* (*Glomus caledonius*, *Rhizophagus intraradices* and *Funneliformis geosporum*), isolated

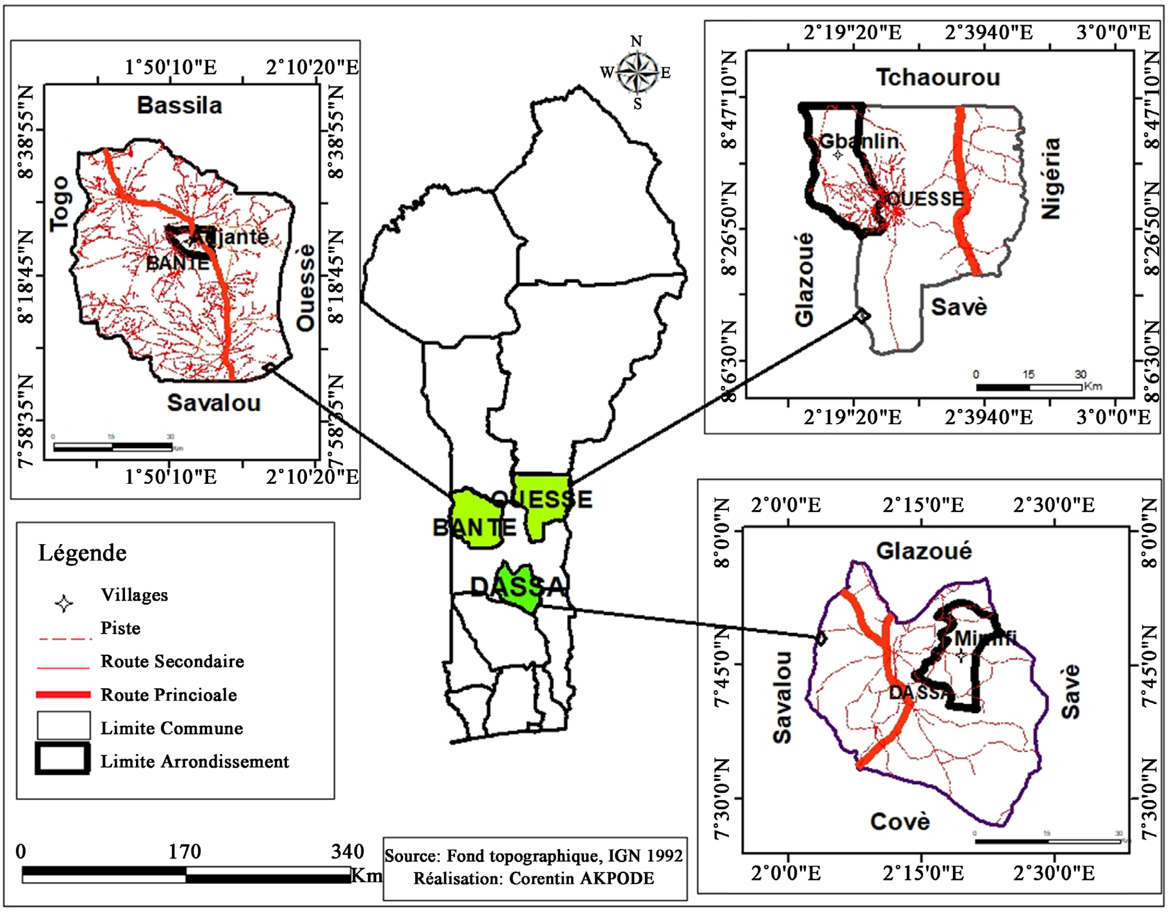


Figure 1. Geographic location of the experimental sites.

and identified from maize rhizosphere soils in Central Benin by [28] was used. These spores were isolated from maize rhizosphere soils in central Benin, examined under a photomicroscope, identified and characterized on the basis of morphological criteria (diameter, color, ornamentation, spore wall thickness once collected in Petri dishes and mounted between slide and slide in a drop of Polyvinyl-lactoglycerol (PVLG). The description sites (http://invam.caf.wvu.sedu/Myc_Info/Taxonomy/species.htm) were consulted. The morphology of the collected spores was compared and identified based on established reference data [28].

The seed used was the maize variety (SYNEE-W BENIN 2000) supplied by the International Institute of Tropical Agriculture (IITA) and the National Institute of Agricultural Research of Benin (INRAB). It is an extra early variety of 8 0 days, with a potential grain yield of 2.5 t/ha in a farming environment [32].

2.2. Methods

2.2.1. Chemical Analysis of Soils

Soil samples were taken from 0 - 20 cm depth using an auger before sowing according to the method described by [33]. They were sent to the Laboratory of Soil, Water and Environmental Sciences of the Agricultural Research Center of

Agonkanmey. The soil pH-water was determined by the electrometric method using a pH (water). For organic matter and carbon, the [34] method was used. The extraction of exchangeable cations was done by ammonium acetate pH-7, described by [35]. Calcium, magnesium, and sodium were determined by atomic absorption spectrophotometer. Assimilable phosphorus was determined by the method of [36]. Total nitrogen was determined by the method of [37].

2.2.2. Preparation of Biostimulant Based on *Glomeraceae* Strains

The biostimulant based on strains of the *Glomeraceae* family was formulated at the Laboratoire de Biologie et Typage Moléculaire en Microbiologie of the Faculté des Sciences et Techniques of the Université d'Abomey-Calavi. It consists in the inoculation of sorghum plants (*Sorghum bicolor* L.) with indigenous spores of Glomeraceae. Sorghum seeds were previously soaked for 2 minutes in a 0.024% sodium hypochlorite (NaClO) solution and rinsed 5 times with distilled water under vortex agitation [38]. Sorghum plants were grown in greenhouse in pots containing the sterilized substrates consisting of a mixture of clay and peat (2:1v/v). Plants were watered daily with water (from the tap) to maintain a soil capacity similar to that in the field for 6 weeks and to ensure good sporulation of the strains. The root biomass and substrate were then ground to obtain the biostimulant [39].

2.2.3. Seed Coating

The dose of biostimulant used was one kilogram (1 kg) for ten kilograms (10 Kg) of maize seeds. In fact, 600 ml of sterile distilled water was added to 1 kg of biostimulant to make a mixture into which the 10 kg of maize seeds were poured. After mixing, the maize seeds thus coated were spread out in the air for drying before sowing [40].

2.2.4. Experimental Device and Field Maintenance

The trials were set up in central Benin at the Research and Development (R&D) sites of Miniffi, Adjantè and Gbanlin at a total of 34 producers. At each farmer's level, the experimental set-up was the same: three elementary plots with three treatments (T0: Absolute control (no CMA, no chemical fertilizer); T1: Glomeraceae + 50% NPK_Urea and T2: 100% NPK_Urea. Each elementary plot was 500 m^2 in size (25 m * 20 m). Sowing was done with two (02) seeds per plot at a spacing of 0.4 m × 0.8 m *i.e.* a distribution of 1562 plants per elementary plot [41].

NPK mineral fertilizer was applied as a basal dressing on the day of sowing for treatments with the AMF biostimulant and plots with the recommended NPK rate. In addition, Urea was applied as a maintenance fertilizer on 45 ième days after sowing (DAS). The recommended dose of NPK was 200 Kg/ha and that of Urea was 100 Kg/ha [42].

2.2.5. Determination of Growth Parameters and Grain Yield of Maize Plants

The height and the diameter at the collar of the plants were measured on the 60^{th}

day of the month using a tape measure and a caliper on the plants of the two (02) central lines that constitute the useful plot to limit the border effects. The leaf area was estimated on the 60th day of the season using the method of [43]. It consists in multiplying the product of the length and width of the leaves by a coefficient of 0.75.

Then, the maize cobs were harvested from the two (02) center rows of each elementary plot at each producer level. These ears were dehusked, shelled, and weighed per elementary plot using a precision range scale (Highland HCB 3001. Max 3000 g × 0.1 g). Grain moisture percentage was taken using a moisture meter (55 Wile DIGITAL). Yield values were determined by the following formula [44]:

$$R = \frac{P \times 10,000}{S \times 1000} \times \frac{14}{\%H}$$

where: R: Maize grain yield, expressed in t/ha; P: Mass of maize grain per elementary plot of calculation, expressed in kg; $\%H$: Percentage of moisture; S: Harvest area in m^2.

2.2.6. Assessment of the Nutritional Status of Maize Plants

The evaluation of the nutritional status of corn plants consists in the determination of the phosphorus, nitrogen and potassium contents of corn plants. Thus, after mineralization of plant material (whole maize plant) by dry method and distillation by the method of [36], Phosphorus (P) was determined by the method of [45], Potassium (K) by atomic absorption spectrophotometry [35] and Nitrogen (N) by [37].

2.2.7. Evaluation of Mycorrhizal Infections

Root samples were collected at harvest. These samples were collected from the plants in the central rows of each plot that received the AMF biostimulants treatment. The staining of root fragments was done by the method of [46] while the quantification of mycorrhizal infections was done by the technique developed by [47]. Two hundred and fifty (250) milligrammes of maize roots previously washed with potable water, dried in ambient air and cut into small pieces to obtain root fragments of about 1 cm were weighed and then introduced into test tubes. A 10% KOH solution was added to the root fragments and placed in an oven at 90˚C for one hour. The roots were then carefully rinsed with tap water, drained and put back into the test tubes. Then, a 0.05% Trypan blue solution was added and incubated at 70˚C for 15 minutes. The reading was done by binoculars using [48]. The mycorrhization infection variables calculated were frequency and intensity.

Mycorrhization frequency (F%): the degree of infection of the root system.

$$F(\%) = \frac{(N - n_0) \times 100}{N}$$

where: N: number of observed fragments; n_0: number of fragments with no trace of mycorrhization.

The mycorrhization intensity (m: absolute mycorrhization intensity) expresses the portion of the cortex colonized in relation to the whole root system:

$$m(\%) = \frac{95n_5 + 70n_4 + 30n_3 + 5n_2 + n_1}{N - n_0}$$

In this formula, n_5, n_4, n_3, n_2 and n_1 are the numbers of fragments marking respectively the importance of mycorrhization, namely: 5 = more than 90%, 4 = 50 to 90%, 3 = 10 to 50%, 2 = 1 to 10%, 1 = 1% of the cortex. This parameter better reflects the degree of mycorrhization. The scale ranges from 0 (no mycorrhization) to 5 (at least 90% of the fragment was mycorrhized) [48].

2.2.8. Statistical Analysis of Data

The effect of the experimental zone (Adjantè, Gbanlin and Miniffi) and the treatments applied (T0, T1 and T2) on the growth and yield performance of the plants was assessed using a two-factor ANOVA test. A posthoc test of pairwise comparisons using the Tuckey post hoc test [49] was performed to assess the statistical differences in means for significant ANOVA tests. The different tests were performed on each variable measured in the software R 4.1.3 [50]. These analyses required the use of the packages dplyr and DescTools for the calculation of descriptive statistics, the packages ggplot2 and ggpur for the realization of the boxes with moustaches, the packages "car" for the ANOVA and the packages multcomp for the realization of the post hoc test of comparison by pairs. The threshold of significance is 5%. By means of the R package ggpubr a dotchart (lollipop) was realized in order to appreciate the frequency and intensity of mycorrhization of the plants at each experimental site.

3. Results

3.1. Chemical Characteristics of the Study Soils

Table 1 illustrates the chemical characteristics of the soils at each research site where the experiments were conducted. The PHeau is 6.79 at Adjantè; 6.74 at Gbanlin and 6.62 at Minifi. Organic matter (OM) varies from 1.20 to 2.26. It is 2.26 in Adjantè, 1.25 in Gbanlin and 1.20 in Minifi. Carbon (C%) is 1.31 in Adjanté; 0.72 in Gbanlin, and 0.69 in Minifi, while the nitrogen content is 0.10 in Adjantè; 0.07 in Gbanlin and Minifi. As for exchangeable cations, values of 9.69, 5.90 and 5.67 were recorded in Adjantè, Gbanlin and Minifi, while for assimilable phosphorus, values of 18.36 in Adjantè, 21.27 in Gbanlin and 10.91 in Minifi were also recorded.

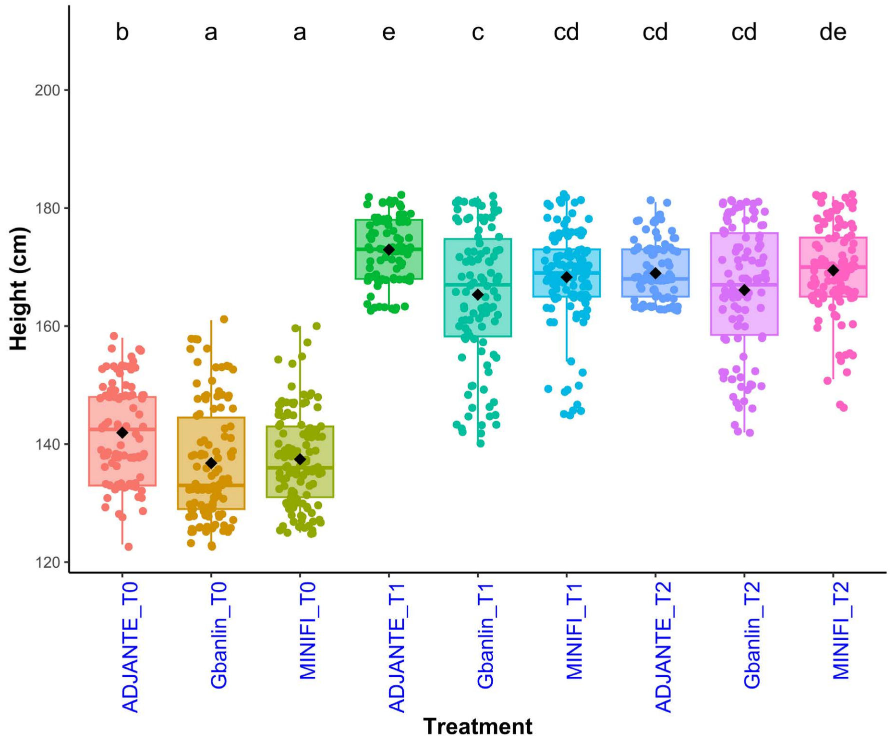
3.2. Effects of Biostimulant on Maize Plant Growth Variables

The histogram in **Figure 2** illustrates the height of maize plants at 60 ème days after sowing. The smallest plant height values were obtained with treatment T0 in all study areas, with the smallest in Gbanlin (136.78 ± 0.92), while the most significant height values were obtained with treatments T1 and T2. The greatest value in the height of maize plants was recorded in Adjantè with treatment T1

Table 1. Chemical characteristics of soils.

Locations	C (%)	N (%)	C/N	M.O. (%)	pH_{water} (1/2.5)	pH_{KCl} (1/2.5)	Ca-ech (meq/100g)	Mg (meq/100g)	K-ech (meq/100g)	Na ech. (meq/100g)	Sum. cations (meq/100g)	CEC	P. Ass. (mg/Kg)
Adjantè	1.31	0.10	12.82	2.26	6.79	6.57	7.76	1.21	0.55	0.50	10.02	9.69	18.36
Gbanlin	0.72	0.07	10.36	1.25	6.74	6.61	4.88	0.81	0.30	0.36	6.37	5.90	21.27
Minifi	0.69	0.07	10.75	1.20	6.62	6.29	5.13	1.04	0.37	0.58	7.12	5.67	10.91

MO: organic matter; Pass: assimilable phosphorus; %: percentage; ppm: part per million; meq/100g: milliequivalent per 100 g of soil; C: carbon; N: nitrogen; CEC: cation exchange capacity; K-ech: exchangeable potassium.



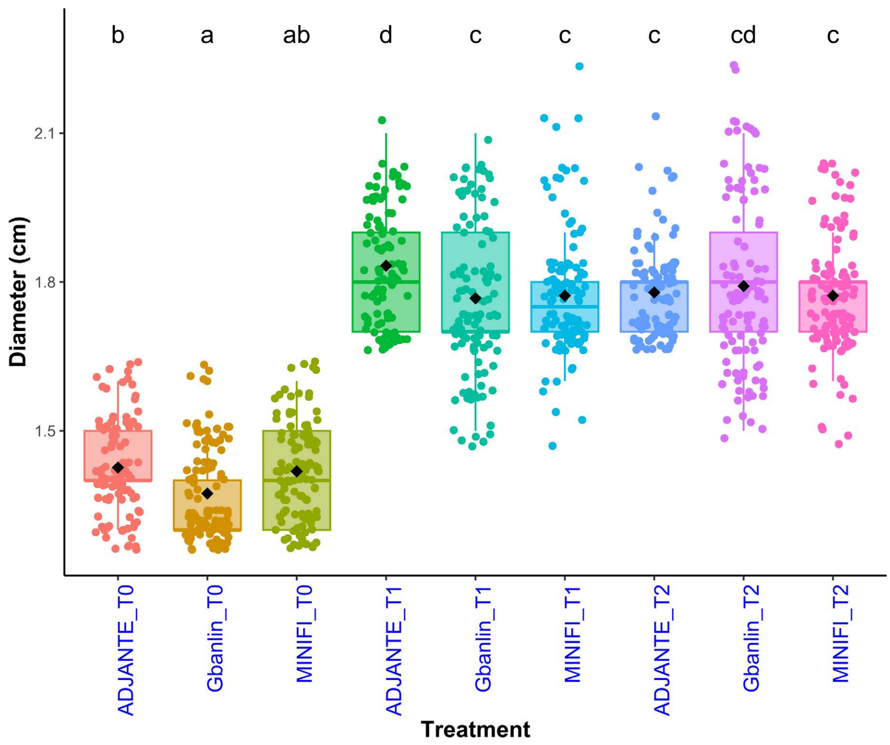
T0: Absolute control (no AMF, no chemical fertilizer); T1: Glomeraceae (AMF) + 50% NPK_Urea and T2: 100% NPK_Urea.

Figure 2. Effects of treatments on maize plant height at 60 ème JAS.

(172.95 ± 0.54), exceeding the absolute control T0 by 20.91%. These results were similar in the different localities. Indeed, in Adjantè, the height of the T1 treatment plants exceeds that of the T0 treatment by 17.93% followed by the T2 treatment by 15.99%. However, in Gbanlin and Minifi, it is the T2 treatment that expressed more and induced an increase of 17.66% and 18.89% compared to the absolute control, followed by the T1 treatment that exceeded the T0 absolute control by 17.27% and 18.33%, respectively. The smallest values in the height of maize plants were recorded in Gbanlin while the largest values were observed in Adjantè followed by Minifi. Plant height development was significantly influenced by the zone and especially by the treatment applied ($p < 0.001$). This shows an interaction between zones and treatments. The T1 treatment was more expressed in height growth in the Adjantè locality. The same T1 treatment increased height performance (168.86 cm) by 17.85% compared to the absolute control (138.72 cm) and by 0.41% compared to the T2 treatment (168.16 cm), considering the three study zones.

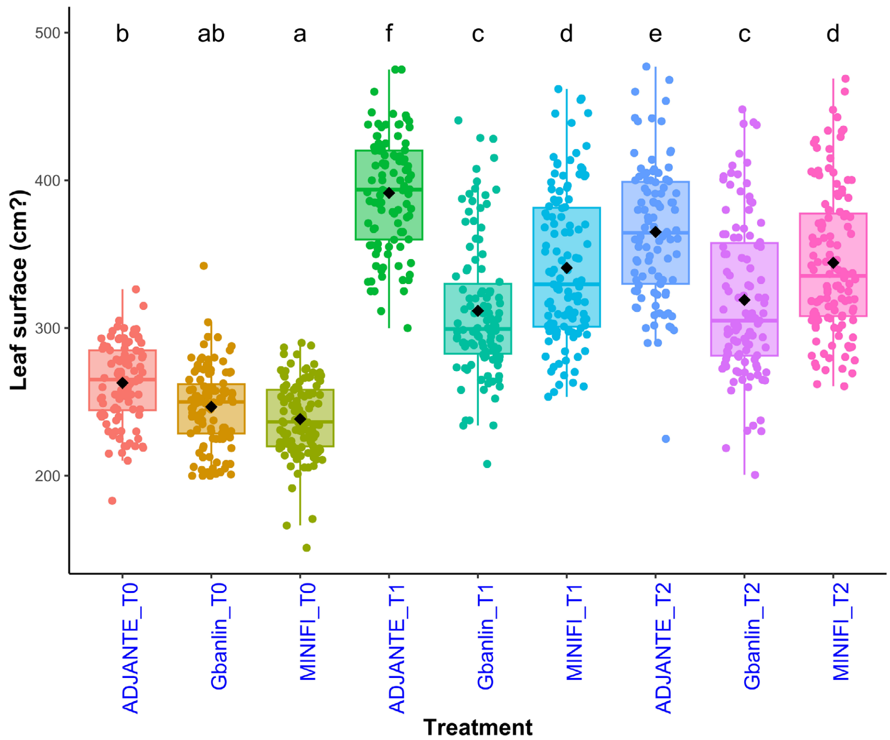
Figure 3 illustrates that the smallest values of diameter at the neck of maize plants were observed in Gbanlin with treatment T0 (1.37 ± 0.00). In comparison, the large values of diameter were obtained with treatments T1 and T2 with the largest value in Adjantè for treatment T1 (1.83 ± 0.01) exceeding treatment T0 by 25.14%. From Adjantè, Gbanlin to Minifi, the values of diameter at the collar of maize plants obtained with treatment T1 exceeded those of the control plants by 22.08%; 22.42% and 19.89% respectively. However, it is the T2 treatment that expressed itself the most in Gbanlin, exceeding the control by 23.34%. As for Minifi, the same diameter value (1.77 ± 0.01) was obtained with treatment T1 and T2. The observation remains the same regarding the influence of the zones and treatments on the vegetative development of the maize plants with a statistically significant effect ($p < 0.001$). Of the three experimental zones, the T1 treatment expressed itself better (1.78) by improving the diameter at the collar by 21.79% compared to the absolute control. The T2 treatment came with 1.77 of diameter at the collar. It should be noted that the diameter at the collar of the plants obtained with treatment T1 in Minifi and that of treatment T2 in the same locality were intermediate to those of Adjantè and Gbanlin, respectively.

Figure 4 presents the effects of treatments on the leaf area of maize plants at



T0: Absolute control (no AMF, no chemical fertilizer); T1: Glomeraceae (AMF) + 50% NPK_Urea and T2: 100% NPK_ Urea.

Figure 3. Effects of treatments on maize plant diameter at 60 ème JAS.



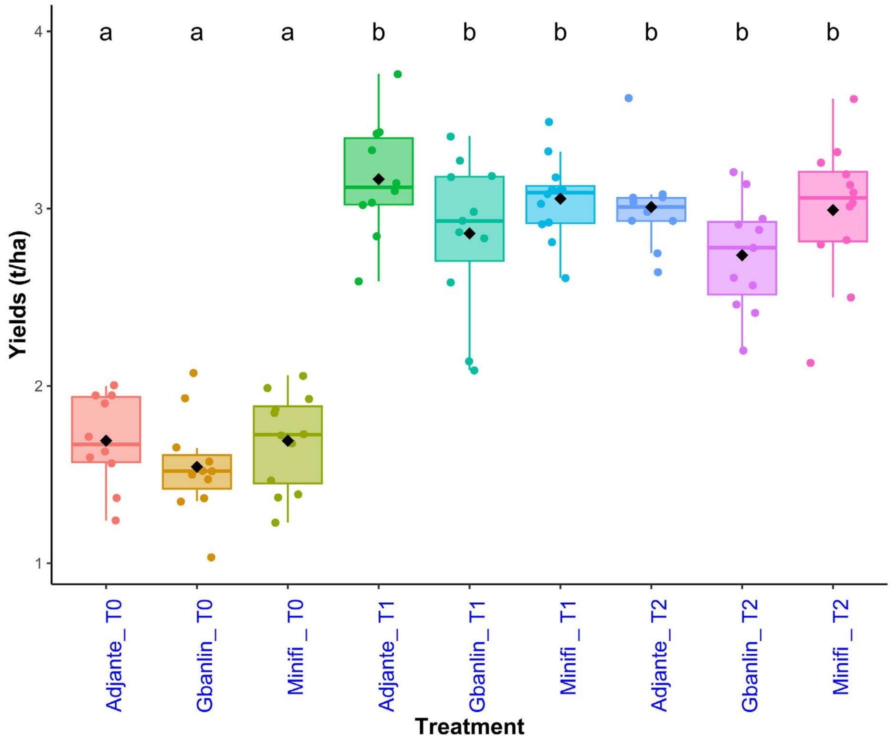
T0: Absolute control (no AMF, no chemical fertilizer); T1: Glomeraceae (AMF) + 50% NPK_Urea and T2: 100% NPK_Urea.

Figure 4. Effects of treatments on leaf area of maize plants at 60 ème JAS.

60 ème days after sowing (DAS). An improvement in leaf area was obtained at Adjantè with plants treated with the biostimulant combined with 50% NPK (T1) (391.43 ± 3.86 cm^2) exceeding the smallest (238.42 ± 2.30 cm^2) by 39.09% at Minifi with the T0 absolute control treatment. This difference was statistically significant ($p < 0.001$). At the zonal level, at Adjantè, treatment T1 induced an improvement in leaf area and exceeded treatment T0 absolute control by 32.83% while at Gbanlin and Minifi the greatest value of leaf area was obtained with treatment T2 exceeding treatment T0 absolute control by 22.68% and 30.73% followed by treatment T1 by 20.86% and 30.04%, respectively. However, no significant difference was found between the performance of T1 and T2 treatments, either at the level of each zone or as a whole. It should be noted that T1 treatment improved the leaf area (347.85) by 28.32% compared to the T0 absolute control treatment (249.33) and by 1.46% compared to T2 treatment (342.76) considering the three study areas.

3.3. Effects of Biostimulant on Maize Grain Yield

The effects of treatments on maize grain yield were shown in Figure 5. The analysis of variance revealed a significant difference ($p < 0.001$) between treatments. The best maize grain yield was obtained from plants treated with the combination of biostimulant plus 50% NPK (T1). Treatment T1 (3.03 t/ha) improved grain yield by 45.87% over the absolute control T0 (1.64 t/ha) and by 3.96% over



T0: Absolute control (no AMF, no chemical fertilizer); T1: Glomeraceae (AMF) + 50% NPK_Urea and T2: 100% NPK_Urea.

Figure 5. Effects of treatments on maize plant yield.

treatment T2 (2.91 t/ha) considering the results from all study areas. Regardless of the study area, treatment T1 dominated. It induced an increase in grain yield of 46.52% in Adjanté, 46.15% in Gbanlin and 44.59 in Minifi compared to the absolute control treatment T0. The lowest maize grain yield performance of the three study areas was obtained in Gbanlin with the T0 treatment (1.54 ± 0.08) while the highest performance was obtained in Adjantè with the T1 treatment (3.16 ± 0.10) and exceeded that of the T0 absolute control treatment by 51.27%.

3.4. Effects of Arbuscular Mycorrhizal Fungi on the Nutritional Status of Maize Plants

According to the analysis of variance (Table 2), the effect of the biostimulant on nutrient uptake in combination with the half-dose of azote-phosphore-potassium (NPK) and urea fertilizer was significant ($p < 0.05$) on phosphorus and potassium content of maize plants compared to plants that did not receive the biostimulant (T0 and T2). The best phosphorus (P) accumulations were induced by the biostimulant (T1) in Adjantè and Minifi while the highest potassium (K) content was obtained with the same treatment in Gbanlin. On the other hand, no statistical difference was recorded for nitrogen (N) content between the treatments.

3.5. Mycorrhizal Colonization Rate of Maize Plant Roots

Table 3 shows the variation in mycorrhizal infections by experimental area. It is

Table 2. Effect of treatments on the nutritional status of maize plants.

Area	Treatment	Nitrogen (N) %		Phosphorus (P) %		Potassium (K) %	
		means	Standard error	means	Standard error	means	Standard error
Adjantè	T0	1.70a	0.028	1.817b	0.017	1.73	0.012
	T1	1.85a	0.028	2.98f	0.012	1.88	0.017
	T2	1.80a	0.006	1.85bc	0.017	1.79	0.006
Gbanlin	T0	1.68a	0.011	1.74ab	0.017	1.65	0.012
	T1	1.97a	0.017	2.2e	0.058	2.06	0.012
	T2	1.89a	0.017	1.95cd	0.012	1.87	0.006
Minifi	T0	1.81a	0.011	1.64a	0.01	1.87	0.006
	T1	2.16a	0.127	2.93f	0.040	1.96	0.012
	T2	1.91a	0.011	2d	0.012	1.89	0.006
Pr (>F)		0.1341408		3.524e−12***		1.186e−10***	

*** = $p < 0.001$ (highly significant). Values that are not followed by the same letters in the same column are significantly different according to Tukey test ($p < 0.05$).

Table 3. Variation in mycorrhizal infections by experimental area.

Site_RD	Parameter	Average (%)	Min (%)	Max (%)
"Adjantè"	Frequency	33.1	20	51
	Intensity	21.9	12.4	35.5
"Gbanlin"	Frequency	41.5	20	66
	Intensity	26.4	10.7	40.5
"Minifi"	Frequency	32.6	10	45
	Intensity	18.5	10	30.5

noted that the mycorrhization frequency rate varies from 10% to 66% and the mycorrhization intensity rate varies from 10% to 40.5%. Variations in the average mycorrhization frequencies and intensities of maize plant roots show that the highest average mycorrhization frequency was found in Gbanlin (41.5%) and the lowest in Minifi (32.6%). Similarly, the highest average intensity was obtained in Gbanlin (26.4%) and the lowest in Minifi (18.5%).

Figure 6 illustrates the influence of study environments on mycorhization of maize plant roots. The maximum frequencies and intensities were obtained in Gbanlin 66% and 40.5% followed by Adjantè 51% and 35.5% respectively. It was no statistically significant difference in these different results between the three study areas ($p = 0.16$ for frequency and $p = 0.09$ for intensity). The strains of native Arbuscular Mycorrhizal Fungi used therefore have a good mycorhizogenic capacity.

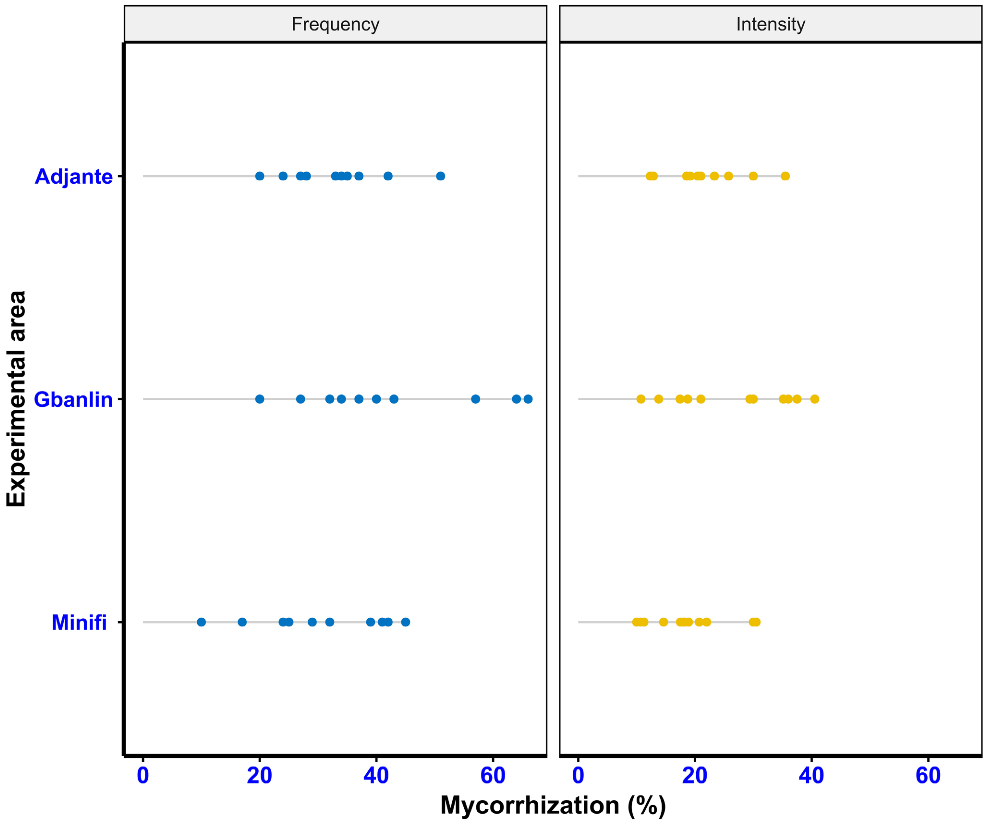


Figure 6. Average of influence of study environments on mycorrhization of maize plant roots: Frequency % and Intensity %.

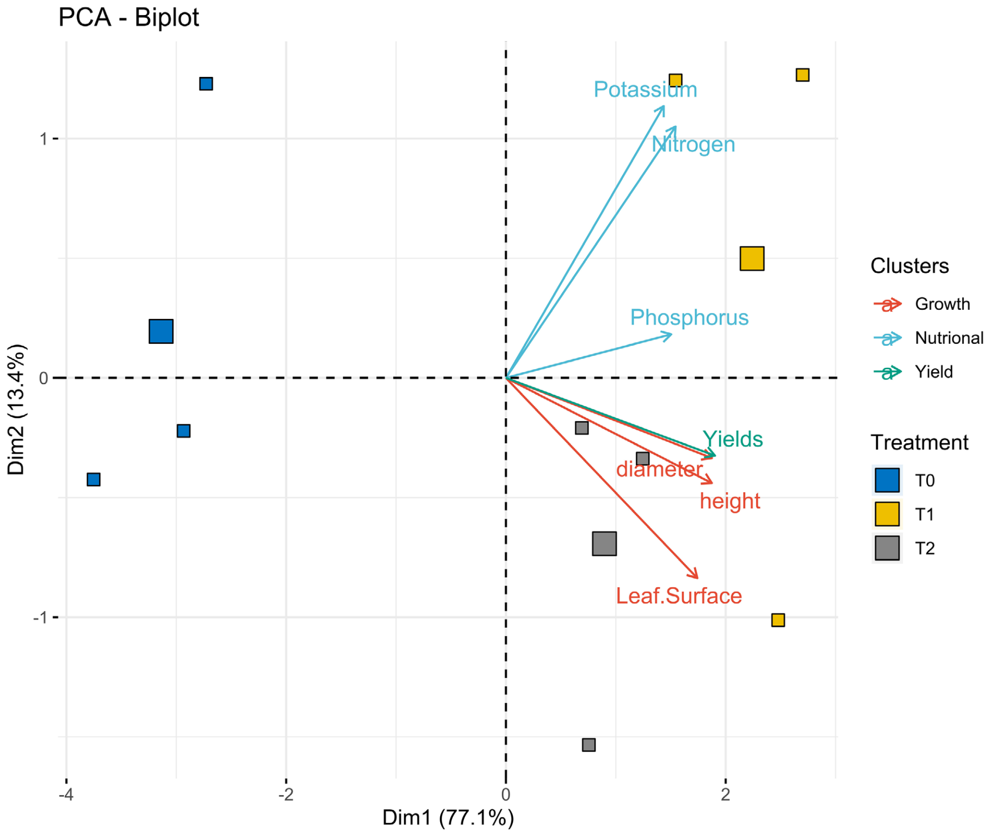
3.6. Correlation between Growth, Yield and Nutritional Status Parameters of Maize Plants

A principal component analysis (PCA) was carried out to establish the relationship between the parameters of growth, yield and nutritional status of maize plants (**Figure 7**). The analysis shows that the first two axes retain 90.42% of the cumulative variance and can thus be used to interpret the results. Indeed, all the evaluated parameters are positively correlated with axis 1 (Dim 1). Variables such as Phosphorus, Yields, Diameter and Height are those that have the best representation quality and are positively correlated with each other. Thus, the better the plant's capacity to absorb nutrients, especially phosphorus, the better the plant growth and grain yield.

4. Discussion

The control of the natural and optimal management of soils in the tropics for a sustainable and economic agriculture is the major concern of any research and development actor. It is within this framework that this study was initiated and aims to improve the growth and grain yield of maize by a mycorrhizal symbiosis while considerably reducing the contribution of mineral fertilizer.

The chemical characteristics of the soils in experiments showed that the soils studied were weakly acidic. The PH water varies from 6.62 to 6.79 (**Table 1**). In



T0: Absolute control (no AMF, no chemical fertilizer); T1: Glomeraceae (AMF) + 50% NPK_Urea and T2: 100% NPK_ Urea.

Figure 7. Principal Component Analysis (PCA) of the relationship between height, diameter, leaf area, yield and nutritional status of maize plants.

addition, the soils of the experimental sites have average fertility in Adjantè but low fertility in Gbanlin and Minifi. This justifies the use of mineral fertilizers by farmers in the study area to improve their productivity. In fact, according to authors [51], criteria for evaluating soil fertility classes, the classes of limitations of the soils of Gbanlin and Minifi vary globally between "medium limitations" and "severe limitations" while those of the soil of Adjantè vary between "no limitations" and "severe limitations". According to the work of [52], the soils of central Benin have very low Cation Exchange Capacity (CEC). Thus, they recommended sustainable production systems with low mineral inputs to restore soils in central Benin. However, the different chemical parameters highlighted, the soils under study being neither too weak nor too fertile, are therefore suitable for the activity of Mycorrhizal Fungi strains because the symbiotic relationship between the maize plant and the Arbuscular Mycorrhizal Fungi arises from the reciprocal (bidirectional) exchange of needs [14]. The plant thus needs the minimum possible nutrients from the soil to be able to make sugar (carbon element) which it will share with the Arbuscular Mycorrhizal Fungi to receive in return the mineral elements from the soil that were not accessible to it [14].

The suitability of the soils under study for mycorrhization was confirmed by the results of the mycorrhization tests carried out during the work. Indeed, the mycorrhization frequencies of the roots vary from 10% to 66% and the mycorrhization intensities of the roots vary from 10% to 40.5% on a total of one hundred (100) root fragments observed (**Table 3**). Mycorrhization (frequency and intensity) varies according to environmental factors of the localities and as a function of time [21] and not necessarily of spore abundance in the soil [53]. And beyond 12% of mycorrhization intensity, the benefits of the relationship become interesting in the plant according to [54].

The results of maize plant height, crown diameter and leaf area showed that the *Glomeraceae* + 50% NPK_ Urea and 100% NPK_Urea treatments have a significant impact on the growth variables compared to the absolute control treatments. These plants treated with the *Glomeraceae* biostimulant + 50% NPK_Urea gave the best performances for the growth variables evaluated in all the experimental zones. As for the performance of the biostimulant in each zone, it can be classified from Adjantè, Minifi and Gbanlin according to the decreasing order of expression of the biostimulant on the variables (**Figures 2-4**). The highest values of root mycorrhizations were obtained in Gbanlin. This caused a reduction in the performance recorded in Gbanlin, although not significant, would be due to the initial quality of the Gbanlin soil because it is the poorest in C/N, exchangeable Ca, Mg, K, Na and cationic sum followed by the Minifi soil (**Table 1**). This observation was proved by [55] that the rational fertility of the soil is conducive to maximize the benefits of mycorrhization. This was explained by [56] that a nitrogen-mycorrhiza interaction makes the plant vigorous and naturally protected. The improvement of the results obtained in the plots treated with the biostimulant can be due to the symbiotic association of the maize plants and the biostimulant (AMF) [57] [58]. These performances obtained in the vegetative development of maize plants can be explained by the capacity of the AMF to improve plant nutrition by exploring the mineral elements from the topsoil that were not within the reach of the roots of the maize plants and that are essential for their developments [7] [59].

In Benin, the work of [60] with native AMF strains on ferruginous soil in Northern Benin confirms our results. They showed the ameliorative effect of these *Glomeraceae* strains on maize plant growth. Similar results were also published by [30] [61] [62] in Benin where the beneficial effects induced by indigenous Arbuscular Mycorrhizal Fungi combined with 50% mineral fertilizer were recorded. Better, [30] explained that *Glomeraceae* strains are effective on maize productivity in Benin.

In Togo, the experiments of [63] also proved the effectiveness of Arbuscular Mycorrhizal Fungi biostimulants on rice growth. Thus, research work conducted in Togo [64], Cameroon [65] [66] and India [67] have shown that symbiotic microorganisms (*mycorrhizae*) were potential alternatives for sustainable agricultural production. [4] also proved that the application of AMF spores improved the growth of wheat plants and increased the number of leaves and dry biomass. On the other hand, [20] explained that a mycorrhized plant has a greater capacity to absorb water, phosphorus and nitrogen present in the soil. They added that the uptake of phosphorus by plants is done up to 80% by mycorrhizal fungi and thus it increases the survival of plants by about 75%. According to [16], the effect of AMF is positively remarkable in growth stimulation and especially at root level and plant resistance to all stresses [68]. Thus, AMF could further stimulate the production of phytohormones (auxin and gibberellin) in plants that were treated with AMF for cell division and elongation [69].

Regarding maize grain yield, the variation in performance (3.03 t/ha for plants treated with the *Glomeraceae*-based biostimulant combined with 50% NPK_Urea of the recommended dose versus 2.91 t/ha for plants treated with 100% NPK_ Urea of the recommended dose) obtained during these experiments testifies to the interest of AMF in agriculture. Indeed, apart from the role that AMF play in mineral and water nutrition of plants [4] [70], AMF can be involved in the activation of abscisic acid, ethylene, auxin responsible for reproductive activities and seed maturation in plants. According to [69], it was these phytohormones that stimulate seed initiation and formation for proper maturation. Therefore, it can be said that this AMF -based biostimulant technology used in this study was effective in improving maize grain yield in the fields in central Benin. The same observations were made by [30] who demonstrated improved maize grain yield by using indigenous *Glomeraceae* strains combined with a reduced dose of mineral fertilizer in Benin. In addition, the results reported by [26] on grain yield of maize produced using exogenous AMF in a controlled environment in central Benin were beneficial but less glowing than those obtained in this study. It is therefore important to note that indigenous strains of AMF are better adapted to the conditions of the study environment. These results were supported by those obtained by [63] who showed the efficacy of arbuscular mycorrhizal fungi biostimulant on rice grain yield in Togo. In Tunisia, [31] proved that the use of AMF-based biofertilizer induces a significant increase in durum wheat (*Triticum durum Desf.*) yield and its nutritional quality. Note that, the application of AMF inoculum significantly increased the phosphorus content in the leaves and stems of wheat plants. According to [70] in Morocco, not only do AMF contribute to the improvement of agricultural yield, but also to good photosynthetic activity, good stomatal conductance and decrease soil PH. And thus, they prove to be a better ally for the improvement of soil quality by modifying its general conditions [71] [72].

In poor soils, plants adapt to nutrient deficiencies by establishing a symbiosis with arbuscular mycorrhizal fungi. These microorganisms are able to extend nutrient uptake beyond the level of development and expansion of the root system [73]. Although no significant difference was noted in nitrogen, the application of biostimulant + 50% NPK_Urea induced a better accumulation of phosphorus and potassium levels in maize plants. These results could be explained by the good mycorrhizal capacity expressed by the indigenous arbuscular mycorrhizal fungi strains used in Gbanlin and Adjantè. The same observations were made by [74] who reported a strong positive correlation between mycorrhizal root colonization and NPK content of bean plants. The advantage conferred for maize plants on nutrient uptake by Glomeraceae + 50% NPK_Urea in our study corroborates with several other works [75] [76].

5. Conclusion

The results of this work showed that the soils of the three localities, Adjantè in Bantè; Gbanlin in Ouessè and Minifi in Dassa-Zounmè are suitable for mycorrhization. The *Glomeraceae* strains used can be well adapted to the environmental conditions, which induced a good growth of the maize plants and considerably improved the maize grain yield. The performances obtained during this research prove that AMF can replace the recommended doses of NPK_Urea in the agricultural practice in the middle of Benin. Indeed, the results obtained with the *Glomeraceae*-based biostimulant combined with 50% NPK_Urea of the recommended dose and those obtained with the full conventional dose of NPK_Urea are statistically similar. This biostimulant is therefore of great interest for the establishment of a truly sustainable and environmentally friendly agricultural practice. Trials with this same biostimulant and under the same conditions as this one should be conducted in the North and South of Benin in order to better appreciate the potentiality of this biostimulant on maize production in the other agroecological zones of Benin. Also, it would be desirable to evaluate the aptitudes and post-harvest qualities of maize produced with the use of Arbuscular Mycorrhizal Fungi biostimulant in Benin.

Authors Contributions

C.A., S.A.A., M.R.A. and M.Y.A. performed the experimental work and analysis. C.A., S.A.A., M.R.A. and M.Y.A. participated to the designing and interpretation of the results. C.A., S.A.A., S.M.I.H., O.A., N.A.A., A.A. and L.B.-M. revised the final version of the manuscript. A.A. and L.B.-M. reviewed the manuscript article. All the authors have contributed to this scientific and intellectual work and have given their approval for its publication.

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Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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