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Response of Biostimulants Based on Native Arbuscular Mycorrhizal Fungi of the Glomeraceae on Maize Yield in a Farming Environment

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Abstract: In the face of persistent soil degradation in Benin caused by poor agricultural practices, including excessive use of chemical fertilizers, it is urgent to find solutions that take into account the microorganisms of interest. This study aimed to assess the effect of combining three strains of indigenous arbuscular mycorrhizal fungi (AMF) on maize production in northern Benin. The study involved 34 growers in Ouénou, Bagou and Kokey. The experimental setup consisted of three elementary plots with three treatments. Growth parameters were measured every 15 days, from the 15^{th} to the 60^{th} day after sowing, on ten plants per plot. Plant nutritional status, grain yield and mycorrhization were measured. The results showed that biostimulant + 50% NPK_Urea (N = nitrogen, P = phosphorus and K = potassium) had similar positive effects on growth parameters to those induced by the application of 100% NPK_Urea. Gains of 30.25% to 36.35% were recorded in plant height at Kokey. On the other hand, biostimulant+ 50% NPK_Urea induced a better phosphorus uptake of 21.08% to 27.77%. In addition, the grain yield of mycorrhizal plants was 8.37% higher than that of plants receiving 100% NPK_Urea at Ouénou. These results show that this technology could be integrated into the agricultural system to promote sustainable maize growing in Benin.

Keywords: maize; sustainable production; arbuscular mycorrhizal fungi; inoculum; yield

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1. Introduction

In Benin, the total area available for farming is 6,863,378 ha, of which 43.9% is exploited [1]. Agriculture is an important sector for the Beninese economy, accounting for 27% of gross domestic product and 72.1% of total exports [2]. It is also preponderant in the fight against poverty and food insecurity, both through the self-consumption of agricultural households, as well as through the supply of food products to local and urban markets [3].

Among the food products, maize (*Zea mays L.*) is the main cereal used in the diet of the population [4]. Currently, it is the cereal that benefits from special attention and its demand is constantly growing [5]. According to the Directorate of Agricultural Statistics (2021) [6], the sown area has increased from 1,000,361 ha in 2016 to 1,349,543 ha in 2021, an increase of 34.90%. Unfortunately, average yields dropped from 1376 to 1206 kg/ha during the same period. The increasing demand for maize and its declining productivity could lead to a tripling of maize imports by the developing world by 2050, at an annual cost of USD 30 billion [7].

Soil degradation poses a serious threat to food production and rural livelihoods [8]. A recent study conducted in Benin by the departments of Zou, Borgou and Alibori indicates that 90% of the land has a low level of fertility [9]. The land has suffered severe degradation due to poor farming practices that destroyed the soil's flora, organic matter, fauna and microfauna [10]. Soil degradation is, therefore, a serious threat to food production and rural livelihoods [8,11,12].

This continuous production, coupled with inadequate use of mineral fertilizers [13], has resulted in increased rates of nutrient extraction from the soil and contributed to soil infertility [14] and contamination of groundwater and surface water [15].

As a result, the emphasis in recent years has been on reducing high-input farming systems [16]. The application of microbial biostimulants, which take advantage of symbiotic relationships, is a long-term strategy for improving plant productivity and performance [17]. Implementing reliable and sustainable agricultural technology without adverse effects on soil health and the environment to meet food needs remains the major concern of agricultural research [18].

Several strategies have been researched to reduce the effects of mineral fertilizers, including the association of arbuscular mycorrhizal fungi (AMF) with plants. AMF form symbiotic associations with most crop species and are recognized as one of the most important groups of soil microorganisms for increasing food security in sustainable agriculture [19]. AMF are biofertilizers/biostimulants to improve soil nutrient availability and uptake [20], although they are neither nutrients nor pesticides [21].

Indeed, AMF allow the plant to acquire mineral elements, in particular, elements that are not very mobile in the soil, such as phosphorus, copper and zinc [22]. They increase plant tolerance to environmental stress and induce plant resistance to pathogens [23], water stress [24] and salinity [25]. The value of using and preserving AMF, for use as a biofertilizer for sustainable agriculture, is becoming increasingly evident, as proper management of these symbiotic fungi could decrease the use of chemical fertilizers that harm the environment and the health of living organisms (including our own) [26].

In addition to their application as a biofertilizer, discoveries about AMF that could help sustain agricultural development include AMF's roles in controlling soil erosion, enhancing phytoremediation and eliminating other organisms that may be harmful to crops through a shared mycelium network [27]. The plant symbiotic association involving these fungi is a subject of scientific debate [28].

In the subregion, many research works have been conducted by different researchers on AMF-based biofertilizers. Examples include studies by Ndoye et al. [29] ; Haro et al. [30] and Zoungrana et al. [31] on the effect of mycorrhizal inoculation with strains of arbuscular mycorrhizal fungi on *Digitaria exilis, Mucuna pruriens var. utilis* and *Sesamum indicum*, respectively.

In Benin, several studies have also been carried out, including those of Aguégué et al. [32] on the effects of combining mineral fertilizers with a biological fertilizer based on *Rhizophagus intraradices* on a small-scale farming environment on maize production in the south, center and north of Benin. The study showed an improvement in the growth of maize plants and an increase in yields of 28%, 38.21% and 13.21%, respectively, in the south, center and north of Benin compared to the farmers' practice.

The synergistic effect of a co-inoculation of AMF in comparison to mono-inoculation would possibly improve maize production. It is from this perspective that the fungal inoculum used in the present study is composed of three species of Glomeromycota (*Glomus caledonius, Rhizophagus intraradices* and *Funneliformis geosporum*), isolated from the soil of the maize rhizosphere in Benin by Aguégué and al. [33]. We hypothesized that using biostimulants based on arbuscular mycorhizal fungi would reduce the application of chemical fertilizers and become an alternative and eco-friendly approach to sustainable agriculture. The objective of this current study therefore was evaluating the combined effect of Glomus fungi on maize growth and yield at three research and development (R&D) sites in northern Benin.

2. Materials and Methods

2.1. Experimental Sites

The study was carried out in the field with 34 producers, 11 in Ouénou, 11 in Bagou and 12 in Kokey. The trials were set up in northern Benin (Figure 1), on the research and development (R&D) sites of the Institut National des Recherches Agricoles du Bénin (INRAB), where the decline in soil fertility is a priority constraint. In addition, they are non-flooded, flat lands with a maximum slope of 2% and are at least 1 km apart.

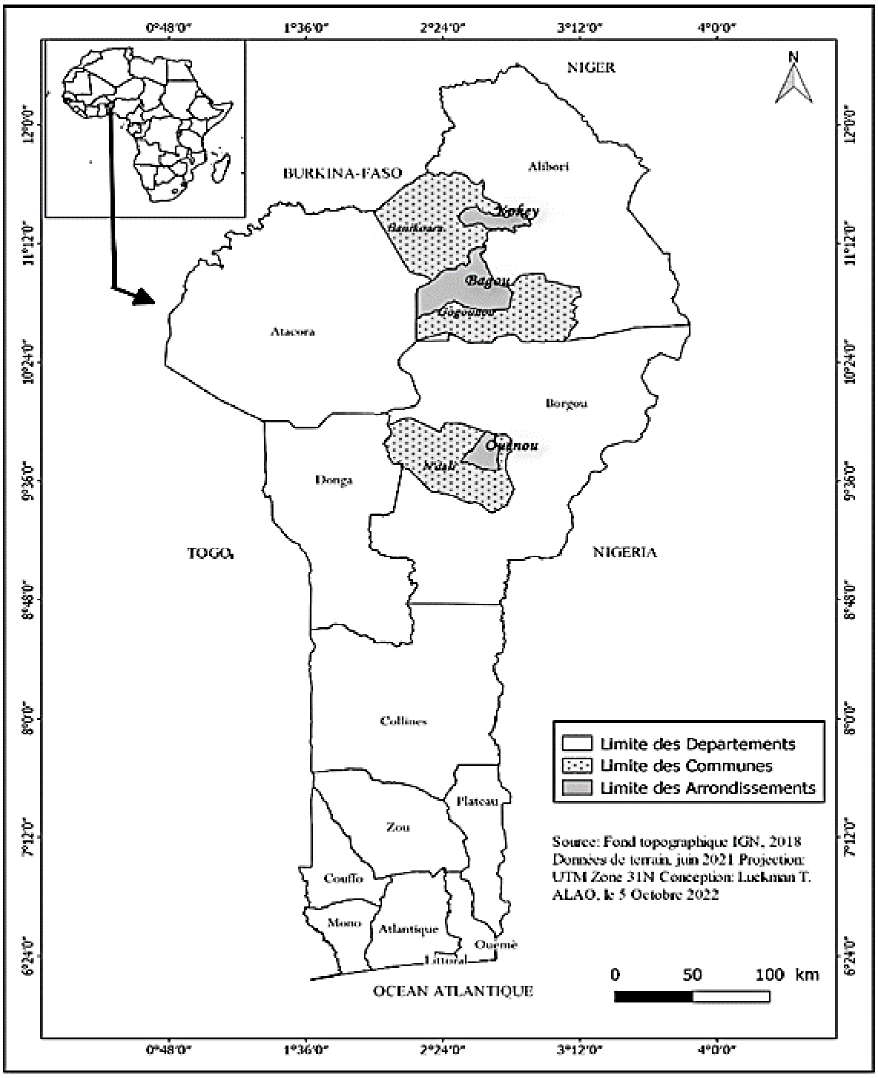

Figure 1. Map showing study areas.

The experiment began in July 2021 and lasted 105 days. During the trial period, mean temperatures recorded in Ouénou, Kokey and Bagou were 26.32 °C, 28.25 °C and 27.6 °C, respectively. Average rainfall was 177.97 mm, 152.55 mm and 237.9 mm in Ouénou, Kokey and Bagou, respectively. However, Ouénou recorded a good rainfall distribution during the trial period.

2.2. Materials

The maize variety QPM FAABA, with an intermediate cycle of 105 days, was used. It was supplied by the International Institute of Tropical Agriculture (IITA) and the National Institute of Agricultural Research of Benin (INRAB). It was white in color and rich in amino acids essential to the organism (lysine and tryptophan) with a potential yield of

3.5 t/ha in the field [34]. Fungal inoculum composed of three species of Glomeromycota (*G. caledonius, R. intraradices and F. geosporum*), isolated from maize rhizosphere soils in Benin by Aguégué and al. [33], was used.

2.3. Methods

2.3.1. Preparation of the Inoculum

Maize seed inoculation was performed as described by Fernandez et al. [35]. The amount of inoculum applied was 10% of the weight of the corn seed with a quantity of distilled water equivalent to 600 $ml.kg^{-1}$ of inoculum. The seeds were coated with biostimulant and left to dry in ambient air for 12 hours before sowing.

2.3.2. Experimental Setup

The experimental setup at each producer was made up of three elementary plots with three treatments. The area allocated to each elementary plot was 500 m^2 (25 m × 20 m). The different treatments were T0 (control without inoculum, representing the farmer's practice); T1 (AMF + ½ NPK_Urea) and T2 (100% NPK_Urea of the recommended rate). Sowing was at a spacing of 0.80 m × 0.40 m with 31.250 plants/ha [36]. The recommended dose of mineral fertilizer was 200 Kg/ha and that of urea was 100Kg/ha. The mineral fertilizer $N_{13}P_{17}K_{17}S_6B_{15}Zn0_5$ was applied as a bottom dressing on the day of sowing in the different treatments. In addition, urea was applied as a maintenance fertilizer on the 45th day after sowing in the different treatments. On the control plots, each grower adopted his cultivation practices. These practices included the date of fertilizer application, dose of fertilizer applied and method of application and varied from one site to another.

2.3.3. Collection of Soil Samples

Soil samples were collected in diagonal order. Sampling was performed before planting at 0–20 cm depth at each producer's site. A 200g composite soil sample was taken from the different research sites for analysis. The method of Boudoudou al. [37] was used to determine soil pH, while the method of Bray and Kurtz [38] was used for the determination of assimilable phosphorus and the method of Thomas [39] was used for the determination of exchangeable cations (Ca, Mg, K and Na). In addition, organic matter and organic carbon were determined according to the method of Walkley and Black [40], while the cation exchange capacity (CEC) was determined by the method of Metson [41]. Finally, total nitrogen was determined by the method of Kjeldahl [42].

2.3.4. Evaluation of Growth Parameters

At each elementary plot, the height and stem neck diameter of maize plants were measured from ten (10) selected plants from the two central lines. Measurements were taken every 15 days from the 15th to the 60th day after sowing (DAS) at the different sites. Only the data related to the calculation of the leaf area of the plants were measured on the 60th DAS. The height of maize plants was measured with a tape measure, while plant diameter was measured with a caliper at the collars of the plant. The leaf area was estimated by the product of leaf length and width with a coefficient of 0.75 [43].

2.3.5. Grain Yield Assessment

Maize grain yield data collection was assessed at harvest (105 DAS). Maize cobs were harvested, spathes were removed and maize kernels were shelled per unit plot. Their mass was determined using a precision balance (Highland™ HCB 302, Max: 300g × 0.01g), and then moisture content was determined using a moisture meter (Wile DIGITAL CHOPIN Technologies). The average grain yield of maize plants was determined according to the formula described by Valdès et al [44], as follows,

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

where R = average seed yield of maize plants, t/ha; P = seed mass of maize plants, kg; S = harvest area, m²; H = percentage grain moisture, %.

2.3.6. Assessment of the Nutritional Status of Corn Plants

The evaluation of the nutritional status of the maize plants consisted of the determination of the N, P and K contents. Indeed, after mineralization of the plant material (whole maize plant) and their distillation by the method of Bray and Kurtz [38], the nitrogen content was determined by titration, phosphorus by the method of Metson [41] and potassium by atomic absorption spectrophotometer [39].

2.3.7. Determination of Mycorrhization Frequency and Intensity

Maize root samples were collected at harvest. Evidence of endo mycorrhizal infection was obtained by staining fine plant roots according to the method described by Phillips and Hayman [45]. The observation was made by light microscopes (Motic® MORE THAN MICROSCOPY). The roots were first cut into 1 cm long pieces, and 0.2 g of these roots was introduced into test tubes. After the addition of 10% KOH, the contents were put in an oven at 90 °C for 1 h. Afterwards, the KOH solution was discarded and the roots were rinsed thoroughly with tap water. This operation emptied the cells of their cytoplasmic contents. Subsequently, 0.05% trypan blue solution was added to the roots and heated in the oven at 70 °C for 15 min, followed by a light rinse with water to remove excess dye. This allows staining of the fungal structures.

After staining, preparations were made and mounted between the slide and coverslip for observation. The fragments were observed under a light microscope (Motic® MORE THAN MICROSCOPY) at magnification (G × 100). The mycorrhization rate was estimated by two parameters of arbuscular mycorrhizal infections, namely:

Mycorrhization frequency (F) was determined by the following formula, which reflects the degree of infection of the root system [46,47]:

$$\mathrm{F}(\%) = \frac{(N - n_0)}{N} \times 100 \tag{1}$$

where N is the number of fragments observed and no is the number of fragments without a trace of mycorrhization.

Mycorrhization intensity: I (absolute mycorrhization intensity) expresses the portion of the colonized cortex in relation to the whole root system [46,47] according to the following formula:

$$\mathrm{I}(\%) = \frac{(95 n_5 + 70\, n_4 + 30\, n_3 + 5\, n_2 + n_1)}{(N - n_0)} \tag{2}$$

In this formula, n_5, n_4, n_3, n_2 and n_1 are the numbers of fragments respectively noted in the five (05) infection classes marking the importance of mycorrhization, namely: 5 = more than 95%, 4 = from 50 to 95%, 3 = 30 to 50%, 2 = 1 to 30%, 1 = 1% of cortex.

2.3.8. Statistical Analysis

A two-factor analysis of variance (ANOVA) test was performed to assess the effect of the experimental location (Bagou, Kokey and Ouénou) and the treatments applied (T0, T1, and T2) on the growth and yield performance of the plants. A post hoc test of pairwise comparisons using the Tukey post hoc test [48] was performed to capture the statistical differences in means when the ANOVA test was significant. The different tests were carried out in the R 4. 1. 3 software [49] and required the use of the dplyr and DescTools packages for the calculation of descriptive statistics, the ggplot2 and ggpur packages for generating the whisker boxes, the ‘car′′ package for the ANOVA and the multcomp package for evaluating the post hoc test of comparison by pairs. The threshold of significance was 5%. Moreover, using the ggpubr package, a dot chart was plotted to evaluate the frequency and intensity of mycorrhization of the plants at each experimental site.

A principal component analysis (PCA) was performed using the PCA function of the FactoMineR package after data standardization. Then, the factoextra and corrplot packages were used to produce a visualization of the PCA results [50,51]. This analysis was performed on a matrix where the rows represented different treatments and the columns the measured variables.

To refine the analysis, cross-validation was performed to assess the robustness of the principal components selected. In addition, a varimax rotation was applied to improve the interpretability of the axes using the psych package. The eigenvalues of the principal components were analyzed using the Kaiser criterion (eigenvalue greater than 1) to decide on the optimal number of components to retain. The first two axes retained more than 50% of the information and were therefore used to identify the relationships existing between these different variables, taking into account the quality of their representation on these two axes.

In addition, a biplot of individuals and variables was generated to simultaneously visualize the distribution of treatments and the influence of variables. All tests were performed using R 4.2.2 [52].

3. Results

3.1. Chemical Characteristics of the Study Soils

The chemical characteristics of the soil at the R&D sites are presented in Table 1. Soil water pH values at Ouénou (pH = 6.6), Bagou (6.4) and Kokey (6.7) were acidic. Organic matter ranged from 1.2 to 1.5%, while assimilable phosphorus was 6.2 mg/kg at Ouénou, 7.7 mg/kg at Bagou and 4.8 mg/kg at Kokey. The estimated exchangeable bases ranged from 5.6 to 7.1 meq/100 g soil, while cation exchange capacities at Ouénou, Bagou and Kokey were 6.5, 10.3 and 7.8, respectively. Soils were acidic (pH < 7) and showed similar CEC and levels of nutrients and organic matter (Table 1).

Table 1. Chemical characteristics of soils in the study areas.

Parameters/Locality	C/N	M.O. %	pH $_{(H2O)}$ (1/2.5)	pH$_{KCl}$ (1/2.5)	P-ass (mg/kg)	Ca meq/100g	Mg meq/100g	K meq/100g	Na meq/100 g	Sum of Cations meq/100g	CEC	%V = S/T ×100
Ouénou	15.5	1.5	6.6	6.3	6.2	4.3	0.7	0.2	0.4	5.6	6.5	84.3
Bagou	13.4	1.3	6.4	5.9	7.7	5.5	1.0	0.2	0.4	7.1	10.3	71.1
Kokey	12.0	1.2	6.7	6.2	4.8	4.0	0.9	0.4	0.3	5.6	7.8	72.8

pH (water); pH (kcl); OM: organic matter; P-ass: available phosphorus; Ca: calcium; Mg: magnesium; K: potassium; Na: sodium; C: carbon; N: nitrogen; CEC: cation exchange capacity; %V: volume.

3.2. Effect of CMA-Based Biostimulant on Growth Parameters

3.2.1. Height of Maize Plants

Table 2 shows the results of the maize plant height measurements. The plant heights obtained varied between 133.20 ± 3.42 cm and 212.72 ± 1.98 cm. It was observed that regardless of the treatment applied to the plants, the vegetative development in height of these plants was lower in the Ouénou locality, whereas the best developments in height (203.19 ± 2.15 cm and 212.72 ± 1.98 cm) were observed in the Kokey locality. Moreover, regardless of the locality, the best performance in height (190.77 ± 2.14 cm at Bagou; 212.72 ± 1.98 cm at Kokey and 169.20± 2.62 cm at Ouénou) was observed in the plants subjected to treatments T1 (CMA+ ½ NPK_Urea) and T2 (100% NPK_Urea). The analysis of the variance test showed that both the experimental locality and the treatment had a significant effect on the vegetative development in the height of the plants (p-value = 6.289×10^{-6}).

Table 2. Average values of height, crown diameter and leaf area of plants.

Area	Treatment	Height of the Plants (cm)		(cm)	Leaf Area of the Plants (cm²)	
		Mean	Standard Error	Standard Error	Mean	Standard Error
Bagou	T0	156.01 [b]	5.19		1279.60 [a]	89.34
	T1	190.77 [d]	6.42		1659.88 [a]	98.61
	T2	188.62 [d]	5.97		1702.36 [a]	94.02
Kokey	T0	156.00 [b]	9.90		1339.36 [a]	88.92
	T1	203.19 [e]	6.45		1652.78 [a]	84.57
	T2	212.72 [e]	5.94		1796.85 [a]	76.14
Ouénou	T0	133.20 [a]	10.26		1329.83 [a]	108.15
	T1	165.79 [bc]	6.63		1567.12 [a]	110.76
	T2	169.20 [c]	7.86		1660.69 [a]	122.76
p Value		6.289×10^{-6} ***			0.11130	

Values that are not followed by the same letters in the same column are significantly different according to the Tukey test ($p < 0.05$). *** Very significant. In statistical analyses, *p* values less than 0.001 are given three asterisks.

The interaction of locality and treatment was used to establish the height performance groups of the plants. The height performance of the plants subjected to the T0 treatment (control, not inoculated) was the lowest. However, the plants subjected to the T0 treatment in the Ouénou locality had a statistically different average performance (133.20 ± 3.42 cm) from those in the Kokey (156.00 ± 3.30 cm) and Bagou (156.01 ± 1.73 cm) localities.

Plants treated with T1 (CMA+ ½ NPK_Urea) and T2 (100% NPK-Urea) in Ouénou locality had equally low performances, respectively (165.79 ± 2.21 cm and 169.20 ± 2.62 cm), but performed better than the plants subjected to the control treatment (T0).

In the Bagou locality, the plants subjected to the treatments T1 (CMA+ ½ NPK_Urea) and T2 (100% NPK_Urea) had relatively intermediate responses that were statistically different from the low performance of the plants subjected to T1 and T2 in the Ouénou locality and the control treatment (T0) in all localities. The average heights of the plants subjected to the T1 and T2 treatments in the Bagou locality constituted the second-best performance obtained, namely 190.77 ± 2.14 cm for T1 and 188.62 ± 1.99 cm for T2.

The best average heights were observed in plants subjected to treatments T1 (CMA+ ½ NPK_Urea) and T2 (100% NPK_Urea) in the Kokey locality. The average height of the T1 treatment plants in this locality was 203.19 ± 2.15 cm, and the average height of the T2 treatment plants was 212.72 ± 1.98 cm. These mean heights were statistically similar for both treatments in this locality.

These results show that the height development of the plants was significantly influenced by the treatment applied but especially by the locality.

3.2.2. Neck Diameter of Maize Plants

Table 2 shows the results of the mean values for the diameter at the crown of maize plants. The diameters of plants subjected to treatment T1 (AMF + ½ NPK_Urea) increased by between 20.58 and 43.05% compared with the control treatment (T0). Regardless of the treatment applied to the plants, vegetative development in terms of crown diameter was lowest in Ouénou, while the best development in terms of crown diameter was (2.06 ± 0.02 cm and 2.15 ± 0.03 cm) observed in Kokey (Table 2). Irrespective of the locality, the best performances in diameter at the crown (1.91 ± 0.04 cm at Bagou; 2.15 ± 0.03 cm at Kokey and 1.36 ± 0.04 cm at Ouénou) were observed in plants subjected to T2 treatments (100% NPK_Urea). The analysis of the variance test showed that experimental locality and treatment had a significant effect on vegetative development in terms of crown diameter (*p*-value = 7.706×10^{-9}).

The diameter performance of T0-treated plants was the lowest. However, T0-treated plants in Ouénou had a mean diameter (1.02 ± 0.03) statistically different from those in Kokey (1.44 ± 0.05) and Bagou (1.48 ± 0.04).

Plants treated with T1 (AMF + ½ NPK_Urea) and T2 (100% NPK_Urea) in the Ouénou locality also performed poorly (1.23 ± 0.03 and 1.36 ± 0.04), respectively, compared with plants subjected to the control treatment (T0) in the other localities.

At Bagou, plants subjected to treatments T1 (AMF + ½ NPK_Urea) and T2 (100% NPK_Urea) were statically different. The average diameters of plants subjected to treatments T1 and T2 in this locality represented the second-best performances obtained, i.e., 1.80 ± 0.03 cm for T1 and 1.91 ± 0.04 cm for T2.

In the Kokey locality, the highest average diameters were observed in plants subjected to the T2 treatments (2.15 ± 0.03 cm). Plants subjected to treatment T1 (AMF + ½ NPK_Urea) in this locality had an average diameter development of 2.06 ± 0.02 cm. These mean diameters were statistically different for the two treatments in this locality. Therefore, as observed with plant heigh, plant diameter development was also significantly influenced by both treatment and locality.

3.2.3. Leaf area of Maize Plants

Average leaf area values for maize plants are shown in Table 2. The leaf areas of the plants obtained ranged from 1279.60 ± 29.78 cm^2 to 1796.85 ± 25.38 cm^2. The lowest vegetative development in the leaf area was obtained in the Bagou locality (1279.60 ± 29.78 cm^2), while the best vegetative development in the leaf area was observed in Kokey (1796.85 ± 25.38 cm^2). Regardless of location, the best leaf area performances (1702.36 ± 31.34 cm^2 at Bagou, 1796.85 ± 25.38 cm^2 at Kokey and 1660.69 ± 40.92 cm^2 at Ouénou) were observed in plants subjected to T2 treatments (100% NPK_Urea). Leaf area performance was lowest in plants subjected to treatment T0 (control, non-inoculated). However, statistical analysis showed that experimental location and treatment had no significant effect on vegetative development in plant leaf areas.

3.2.4. Maize Yield Assessment

Table 3 shows the grain yields of maize plants. Grain yields of plants treated with T1 (AMF + ½ NPK_Urea) increased by 32.40% to 43.70% compared with the control treatment (T0). Regardless of the treatment applied to the plants, grain yields were lowest in Kokey, while the best grain yields (1.35 ± 0.45 cm, 1.94 ± 0.35 cm and 1.79 ± 0.4 cm) were recorded in Ouénou (table 3). The best grain yields of 1.59 ± 0.41 cm and 1.94 ± 0.35 cm, obtained respectively in the localities of Bagou and Ouénou, were observed in plants subjected to T1 treatments (AMF + ½ NPK_Urea), while the lowest yields were recorded in plants subjected to the control treatment (T0). On the other hand, at Kokey, grain yield for T2 (2.44 ± 0.40 t/ha) was slightly higher than for T1 (2.43 ± 0.33 t/ha). The ANOVA test showed that both experimental locality and treatment had no significant effect on the grain yield of maize plants.

Table 3. Grain yields of maize plants.

Zone	Treatment	Plant Yield (t/ha)		
		Mean	Standard Error	IC–95%
Bagou	T0	1.13 [a]	0.40	[0.86; 1.40]
	T1	1.59 [a]	0.41	[1.32; 1.87]
	T2	1.55 [a]	0.26	[1.37; 1.72]
Kokey	T0	1.08 [a]	0.36	[0.85; 1.31]
	T1	1.43 [a]	0.33	[1.22; 1.64]
	T2	1.44 [a]	0.40	[1.19; 1.70]
Ouénou	T0	1.35 [a]	0.45	[1.04; 1.65]

T1	1.94 a	0.35	[1.71; 2.18]
T2	1.79 a	0.40	[1.53; 2.06]

The letter "a" means that there is no significant difference between the values of according to ANOVA test. 95% CI = 95% confidence interval

The interaction between zone and treatment also had no significant effect on plant yield. However, zones in isolation had an impact on plant yield, and treatments in isolation also had an impact on plant yield.

3.3. Nutritional Status of the Plants

Table 4 shows the nutritional status of maize plants. The results of the analysis of variance showed that the area–treatment interaction had a significant effect (p-value = 0.005 **) on the N concentration of maize plants. Treatments T1 and T2 induced the best N uptake, irrespective of the experimental area. However, the highest average nitrogen values at Kokey (2 ± 0.05) and Bagou (1.98 ± 0.05) were obtained with the application of biostimulant + 50% NPK_Urea (T1). Analysis of variance showed a highly significant effect (p-value = 0.0002 ***) of the area–treatment interaction on the phosphorus content of maize plants.

Table 4. Nitrogen, phosphorus and potassium contents of the maize plants.

Area	Treatment	Nitrogen (N) %		Phosphorus (P) %		Potassium (K) %	
		Mean	Standard Error	Mean	Standard Error	Mean	Standard Error
Bagou	T0	1.63 ab	0.07	1.80 bc	0.08	1.71 ab	0.08
	T1	1.98 d	0.14	2.30 e	0.16	2.10 e	0.18
	T2	1.82 c	0.10	1.92 cd	0.11	1.88 cd	0.14
Kokey	T0	1.72 ac	0.06	1.66 ab	0.07	1.70 ab	0.06
	T1	2 d	0.17	2.01 d	0.14	1.94 d	0.08
	T2	1.81 c	0.10	2.00 d	0.14	1.93 d	0.13
Ouénou	T0	1.60 a	0.09	1.61 a	0.05	1.59 a	0.05
	T1	1.74 ac	0.04	1.98 d	0.14	1.83 bd	0.08
	T2	1.75 bc	0.05	1.81 bc	0.04	1.73 abc	0.05
p Value		0.005 **		0.0002 ***		0.023 *	

Values that are not followed by the same letters in the same column are significantly different according to the Tukey test (p < 0.05). * = Not very significant at 5%; ** = significant at 5%; *** = very significant at 5%.

Phosphorus uptake was significantly influenced by the area–treatment interaction. At Bagou (2.3 ± 0.05), Kokey (2.01 ± 0.05) and Ouénou (1.98 ± 0.05), the best phosphorus uptake was achieved by plants receiving biostimulant + 50% NPK_Urea (T1). The biostimulant induced phosphorus accumulation of the order of (21.08 to 27.77%) in maize plants.

As for potassium, the highest levels (2.1 ± 0.06and 1.94d ± 0.03) in plants were recorded with T1 (biostimulant + 50% NPK_Urea) at Bagou and Kokey, respectively. However, no difference was noted between the effect induced by T1 and T2 at Kokey.

3.4. Evaluation of Mycorrhization Frequency and Intensity

Table 5 shows the variation in mycorrhizal infections by experimental area. The table shows that mycorrhization frequency rate varied from 12% to 87%, and the mycorrhization intensity rate varied from 10% to 50.58%.

Table 5. Variation in mycorrhizal infections by experimental area.

Area	Parameters	Mean	Min	Max
Ouenou	Frequency	34	12	62
	Intensity	22.08	10	32.74
Kokey	Frequency	45.92	25	87
	Intensity	29.87	10	50.58
Bagou	Frequency	46.09	25	58
	Intensity	26.42	10	46.52

The highest average mycorrhization frequency (46.09%) was obtained in Bagou, while the highest average intensity (29.87%) was recorded in Kokey. Ouénou recorded the lowest average frequency (34%) and the lowest average intensity (22.08%).

Figure 2 shows the influence of the environments studied on mycorrhization of maize roots. The highest mycorrhization frequency (87%) was obtained in Kokey, while the lowest (12%) was recorded in Ouénou. Similarly, the highest intensity (50.59%) was recorded in Kokey, while the lowest (10%) was recorded in all three localities.

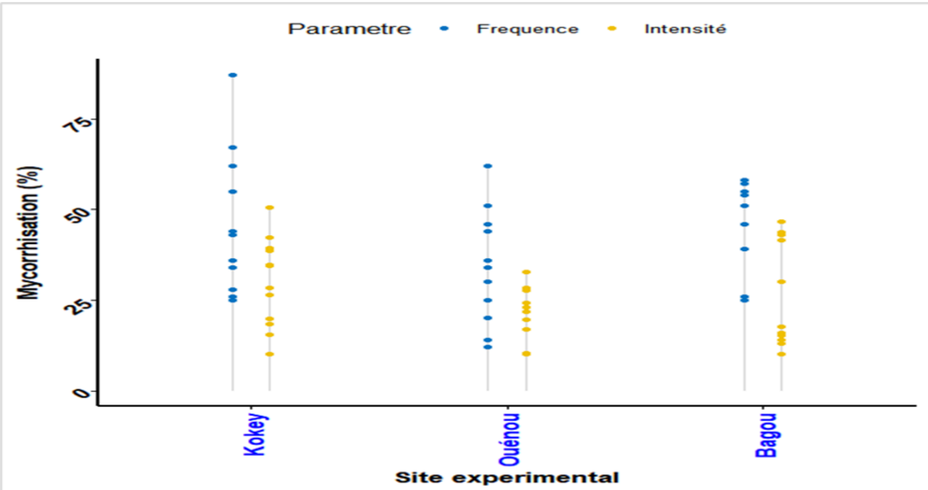

Figure 2. Average of influence of study environments on mycorrhization of maize plant roots: frequency % and intensity %.

3.5. Principal Component Analysis

A principal component analysis (PCA) was carried out to establish the relationship between growth parameters and the nutritional status of maize plants (Figure 3). The analysis showed that the first two axes retained 77.8% of the cumulative variance percentage, and can therefore be used to interpret the results. In fact, both plant nutritional status (nitrogen, phosphorus and potassium) and plant growth parameters (height, diameter and leaf surface) were positively correlated with axis 1 (Dim 1). Variables such as potassium, height and diameter had the best representation quality and were strongly positively correlated with each other. Thus, the better the plant's ability to absorb nutrients, especially potassium, the better the plant's growth, especially its height. Additionally, a projection of the different treatments applied to the plants in the axis system indicates that the plants subjected to treatment T0 did not perform better in terms of nutrient uptake and growth parameters, whereas the plants subjected to treatments T1 and T2 performed best in terms of uptake and growth. The plants subjected to treatment T1 (AMF+ ½ NPK_Urea) were those with the highest absorption rates and consequently the best height development. As for plants subjected to treatment T2 (100% NPK_Urea of the recommended dose), they were associated with greater development in diameter.

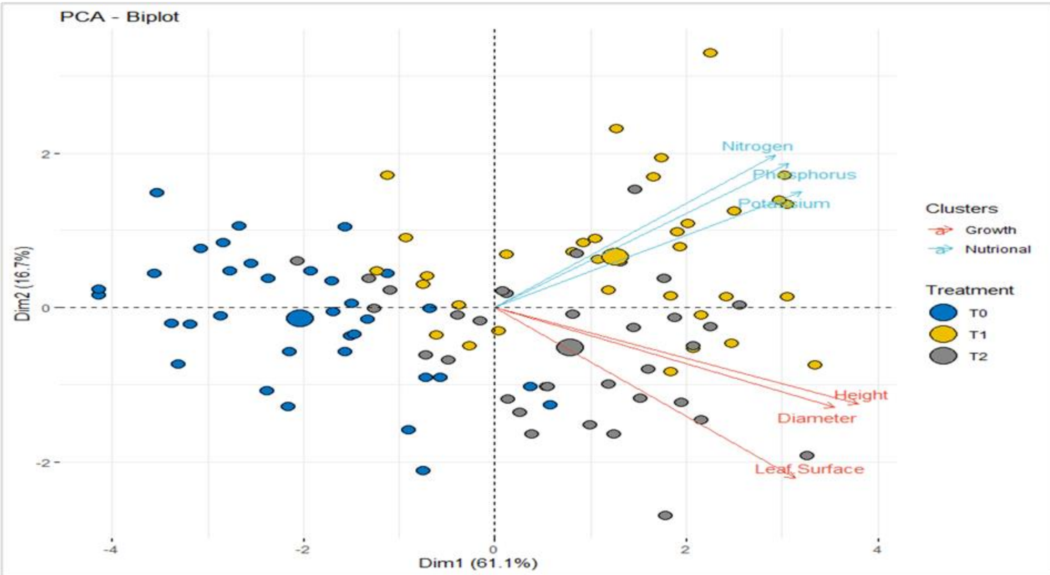

Figure 3. Principal component analysis (PCA) of the relationship between height, diameter, leaf area and nutritional status of maize plants. T0: Absolute control; T1: biostimulant + 50% NPK_Urea and T2: 100% NPK_ Urea.

We can then deduce that the treatments (AMF+ ½ NPK_Urea and 100% NPK_Urea of the recommended dose) improved the plants' capacity to absorb nutrients and ensured better vegetative development of the plants than the current farming practices in the experimental regions.

4. Discussion

The chemical characteristics of the soils in our study show that organic matter varied between 1.2% and 1.5%, while assimilable phosphorus had a value of 4.8 mg/kg in Kokey, 6.2 mg/kg in Ouénou and 7.7 mg/kg in Bagou. The exchangeable base in Ouénou and Kokey was 5.6 meq/100g and in Bagou 7.1 meq/100g.

In addition, soil organic matter, assimilable phosphorus and exchangeable base contents were good for the experiment on the effects of NPK mineral fertilizers and agreed with those obtained by Aguégué et al. [32] in northern Benin. Furthermore, these soils had a low level of fertility characterized by high C/N ratios. The pH-KCl showed lower values than the pH of water (6.6 at Ouénou; 6.4 at Bagou and 6.7 at Kokey). This shows that the soils in our study were acidic. This result confirms those of Aguégué et al. [32], who showed that the soils of northern Benin were moderately acidic and poor in organic matter. This water pH promotes the growth of fungi, which thrive best in acidic environments [23]. According to Zhu et al. [53], AMF proliferation is pH-dependent, with a preference for slightly acidic conditions. Indeed, pH influences the activity of soil microorganisms that participate in the mineralization of organic matter as well as that of mycorrhizal fungi [54]. Coughlan et al. [55] stated that mycorrhizal colonization is high at pH levels between 5 and 7, but low at pH levels around 4.

Results of agronomic parameters showed that inoculation improved vegetative development in the height and crown diameter of maize plants throughout the growing season (Table 2). Inoculated maize plants had better vegetative development compared to non-inoculated control treatments. In contrast, treatments T1 (AMF + ½ NPK_Urea) and T2 (100% NPK_Urea of the recommended rate) showed almost similar values. However, statistical analyses of plant height and crown diameter showed significant differences between the different treatments and locations (Table 2). This is explained by the fact that the necessary nutrients were not directly accessible to the roots. Generally, mycorrhizal symbioses improve host plant development through improved plant nutrition [28].

Thus, the hyphae would colonize a large volume of soil and penetrate it to depths inaccessible by the roots to provide hydromineral nutrition to the roots. The beneficial role of AMF on plant growth is attributed to improved uptake, transport and absorption of mineral elements, primarily phosphorus, by plant tissues [56]. These results are similar to those obtained by Koda et al. [57], who showed that inoculation with *F. mosseae* + ½ dose of NPK resulted in improved growth in length and thickness of maize plants. Zoungrana et al. [31] showed that inoculation of plants with *G. aggregatum* resulted in greater vegetative development of sesame.

According to Sampath et al. [58], AMF effectively enhance plants' nutritional capacity, especially phosphorus and water uptake, through the development of a telluric mycelial network, thereby increasing the surface areas and uptake volumes of mycorrhized roots. This results in significant improvement in height growth and total biomass of cowpea plants with the genus Glomus [59]. In addition, Agbodjato et al. [16] showed that inoculation of maize plants in a farming environment with the two endogenous strains of arbuscular mycorrhizal fungi (*Gloméracées and Acaulosporaceae*) revealed that inoculated plants were better developed than those not inoculated.

In contrast to the vegetative development in height and diameter at the collar of the plants, statistical analysis of leaf area showed that both the experimental locality and treatment had no significant effect on the vegetative development of the maize plants.

Regarding the action of AMF on maize grain yield, our results show that inoculation of maize plants with AMF increased the grain yield of inoculated plants in all locations compared to uninoculated plants (control treatment and treatment with 100% NPK_Urea of the recommended dose). This is explained by the success of mycorrhizal infection, due to the ability of AMF to develop hyphae and mobilize soluble phosphorus from the soil [60]. Similar results were obtained by Aguégué et al. [32], who showed that mycorrhizal inoculation of maize with *R. intraradices* combined with 50% of the recommended dose of NPK improved the yield of seed production of this plant.

The same observations were made by Haro et al. [61], who proved that the best maize grain yield was obtained with the treatment *Glomeracea* + 25% NPK-Urea.

Furthermore, our results also reveal that the interaction between the zone and the treatment did not have a significant effect on plant yield. These results confirm those of Gnamkoulamba et al. [62], who showed that inoculation with microorganisms indigenous to Burkina Faso improved aboveground biomass production and cowpea yield on par with fertilization with chemical fertilizers (NPK) at the rate of 100 kg/ha. On the other hand, our results are contrary to those of Coughlan et al. [55], who reported in a farmer setting that inoculated rice plants had a significantly higher increase in yield variables (number of tillers produced, number of fertile panicles per plant and number of grain per panicle) compared to non-mycorrhized plants. These studies prove that increased nutrient uptake in plants colonized by AMF can lead to a significant reduction in the rate of fertilizer and pesticide application while giving equal or even higher yields [63]. The results also showed a correlation between agronomic parameters and mycorrhization parameters. For example, mycorrhization results showed that maize roots were mycorrhized by the mycorrhizal strains used to formulate the inoculum (Figure 2). Thus, inoculated maize plants were more susceptible to the effect of the inoculum. The absence of mycorrhizal infection on the roots of the control plants and the plants that received the T2 treatment (100% NPK_Urea) shows that these treatments were free of any mycorrhizal colonization and that there was no competition effect between the strains native to its soils and those provided by the fungal inoculum.

Thus, root mycorrhization rates were greater than 50% regardless of location. This may be because maize roots were less abundant, stubby and lacking absorptive hairs, and therefore particularly dependent on AMF [64]. The maize root system is characterized by the presence of adventitious roots that only absorb nutrients in the surface layer of the soil. These results confirm those obtained by Houngnandan et al. [65] and Tawaraya [66], who observed a mycorrhization rate between 50% and 70%. Additionally, the best values of mycorrhization intensity (50.6%, 46.5% and 32.7%) observed respectively in Kokey, Bagou and Ouénou confirm the results obtained by Hijri et al. [67] and Breuillin et al. [68], who showed that the level of soil fertility, especially the high level of phosphorus, inhibited the plant–AMF symbiosis and in some cases eliminated the effect of mycorrhizal fungi.

5. Conclusions

The recent awareness of the limits of natural resources and the pollution of soil, air and water, reflects the need for sustainable agriculture. The latter aims at limiting the use fertilizers and pesticides by favoring biological processes. The objective of this study was to evaluate the combined effect of three strains of glomus fungi on the growth and yield of maize in North Benin.

These results showed that fungal inoculum composed of species isolated from the rhizosphere soils of maize in Benin plus a ½ dose of recommended NPK_Urea fertilizer improved the vegetative development of maize plants. This also resulted in improved maize grain yield. Finally, the use of arbuscular mycorrhizal fungi plus a ½ recommended fertilizer dose of NPK_Urea for maize cultivation was found to be more effective than the recommended dose translated by the use of 100% NPK_Urea. Its application by growers would therefore be ecologically profitable.

Author Contributions: This work was carried out in collaboration with all authors. L.T.A., A.D.K., R.M.A., C.G.O., S.A.A., M.Y.A., C.A. and O.A. carried out the trial setup, data collection and harvesting. L.T.A. wrote the first draft of the manuscript, managed the literature search, and performed the statistical analysis. S.M.I.H., N.A.A. (Nadège Adoukè Agbodjato), N.A.A. (Nestor Ahoyo Adjovi), A.A., L.B.-M. and O.O.B. wrote the protocol, managed the study analyses, and supervised the various activities. All authors have read and agreed to the published version of the manuscript.

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