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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 due to poor agricultural practices, including excessive use of chemical fertilizers, there is an urgent need to seek solutions that integrate microorganisms of interest. The aim of the study was to assess the effect of combining three strains of indigenous arbuscular mycorrhizal fungi on maize production in northern Benin. The study involved 34 growers in Ouénou, Bagou, and Kokey. The experimental set-up consisted of three elementary plots with three treatments. Growth parameters were measured every 15 days, from the 15th to the 60th day after sowing, on ten plants per plot. Plant nutritional status, grain yield and mycorrhization were measured. Results showed that biostimulant + 50% NPK_Urea 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 corn; sustainable production; SDG2; combination

1. Introduction

Benin covers an area of 114,763 square kilometers, of which 32.8% is used for agriculture [1]. The agricultural sector in Benin is an important source of economic wealth as it contributes to 33% of the total gross domestic product, provides about 75% of operating revenues and 15% of government revenues [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, which benefits from special attention and its demand is constantly growing [5]. According to the [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 1,376 to 1,206 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 US$30 billion [7].

Land degradation, low investment in the agricultural sector, and changing climatic variables (high average temperature, scarce and irregular rainfall) that have characterized agricultural activities in the region [8,9] are believed to be the cause of this situation. A recent study conducted in Benin in the departments of Zou, Borgou, and Alibori indicates that 90% of the land has a low level of fertility [10].

The land is then subject to severe degradation due to poor farming practices that destroy the soil's flora, organic matter, fauna, and microfauna [11].

Soil degradation is, therefore, a serious threat to food production and rural livelihoods [12,13,14].

This continuous production coupled with inadequate use of mineral fertilizers [15] has resulted in increased rates of nutrient extraction from the soil; contributed to soil infertility [16] and contamination of groundwater and surface water [17].

As a result, the emphasis in recent years has been on reducing high-input farming systems [18]. The application of microbial biostimulants, which take advantage of symbiotic relationships, is a long-term strategy for improving plant productivity and performance [19]. 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 [20].

To reduce the effects of mineral fertilizers, several strategies have been researched, including the association of arbuscular mycorrhizal fungi (AMF) with plants. Arbuscular mycorrhizal fungi (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 [21]. AMFs are used as biofertilizers/biostimulants to improve soil nutrient availability and uptake [22] although they are neither nutrients nor pesticides. [23].

Indeed, AMF allows the plant to acquire mineral elements, in particular, elements that are not very mobile in the soil such as phosphorus, copper, and zinc [24]. They increase plant tolerance to environmental stress and induce plant resistance to pathogens [25], water stress [26], and salinity [27]. The value of using and preserving AMF, for use as a bio-fertilizer 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) [28].

In addition to its application as a biofertilizer, new 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 [29]. The plant symbiotic association involving these fungi is the subject of scientific debate. [30].

In the sub-region, many research works have been conducted by different researchers on AMF-based biofertilizers. Thus [31,32,33] studied the effect of mycorrhizal inoculation with strains of arbuscular mycorrhizal fungi on white fonio, mucuna, and sesame respectively.

In Benin, several studies have also been carried out, including those of [34] 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, Centre and North of Benin. The results obtained show an improvement in the growth of maize plants and an increase in yields of 28, 38.21 and 13.21% respectively in the South, Centre and North of Benin compared to the farmers' practice.

The synergistic effect of a co-inoculation of Arbuscular Mycorrhizal Fungi in comparison to mono-inoculation would be a possibility to improve corn production. It is in this perspective that the fungal inoculum used in the present study is composed of three species of the genus Glomus (*Glomus caledonius, Rhizophagus intraradices, and Funneliformis geosporum*), isolated from the soil of the maize rhizosphere in Benin by [35].

The objective of this study is to evaluate the combined effect of Glomus fungi on maize growth and yield in three Research and Development (R&D) sites in northern Benin.

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2. Results

2.1. Chemical characteristics of the study soils

The chemical characteristics of the soil at the RD sites are presented in Table 1. Soil water pH at Ouénou (pH=6.6), Bagou (6.4), and Kokey (6.7) are acidic. Organic matter varies from 1.2 to 1.5% while assimilable phosphorus has a value of 6.2 mg/kg in Ouénou, 7.7 mg/kg in Bagou, and 4.8 mg/kg in Kokey. Exchangeable bases vary between 5.6 and 7.1 meq/100 g soil. The cation exchange capacities at Ouénou, Bagou, and Kokey are 6.5, 10.3 and 7.8 respectively.

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

Parameters / Locality	C/N	M.O .%	pH_{eau} (1/2,5)	pH_{KCl} (1/2,5)	P ass (mg/Kg)	Ca éch .méq/100g	Mg méq/100g	K éch.méq/100g	Na éch.méq/100g	Sum of cations méq/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: cathionic exchange capacity; %V: volume.

2.2. Effect of CMA-based biostimulant on growth parameters

2.2.1. Height of maize plants

Table 2 shows the results of the maize plant height measurements. The plant heights obtained vary between 133.20 ± 3.42 cm and 212.72 ± 1.98 cm. In fact, regardless of the treatment applied to the plants, the vegetative development in height of these plants is lower in the Ouénou locality, whereas the best developments in height were observed in the Kokey locality. Moreover, regardless of the locality, the best performance in height 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 have a significant effect on the vegetative development in height of the plants (P-value=6.289e-06).

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 higher than the performances of the plants subjected to the control treatment (T0).

In the Bagou locality, the plants subjected to the effect of treatments T1 (CMA+ ½ NPK_Urea) and T2 (100% NPK_Urea) had relatively intermediate responses and 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 constitute the second-best performance obtained, namely 190.77 ± 2.14 cm for T1 and 188.62 ± 1.99 cm for T2.

The highest 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 are statistically similar for both treatments in this locality.

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

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

Area	Treatment	Height of the plants		Plant diameter		Leaf area of the plants	
		Mean	SE	Mean	SE	Mean	SE
Bagou	T0	156.01b	1.73	1.48c	0.04	1279.60	29.78
	T1	190.77d	2.14	1.80d	0.03	1659.88	32.87
	T2	188.62d	1.99	1.91de	0.04	1702.36	31.34
Kokey	T0	156.00b	3.30	1.44c	0.05	1339.36	29.64
	T1	203.19e	2.15	2.06ef	0.02	1652.78	28.19
	T2	212.72e	1.98	2.15f	0.03	1796.85	25.38
Ouénou	T0	133.20a	3.42	1.02a	0.03	1329.83	36.05
	T1	165.79bc	2.21	1.23b	0.03	1567.12	36.76
	T2	169.20c	2.62	1.36bc	0.04	1660.69	40.92
P. Value		6.289e-06 ***		7.706e-09 ***			

Values that are not followed by the same letters in the same column are significantly different according to Tukey test ($p < 0.05$). SE = Standrd Error; *** Très significatif.

2.2.2. Neck diameter of maize plants

Table 2 provides information on the results of the mean values of the diameter at the neck of the maize plants. The diameters of the plants subjected to the T1 treatment (CMA+ ½ NPK_Urea) increased from 20.58 to 43.05% compared to the control treatment (T0). Regardless of the treatment applied to the seedlings, the vegetative development in diameter at the crown of these seedlings is lower in Ouénou locality whereas the best developments in diameter at the crown were observed in Kokey locality (Table 2). Also, regardless of the locality, the best performance in diameter at the collar was observed in the plants subjected to the T2 treatments (100% NPK_Urea). The analysis of variance test showed that the experimental locality and the treatment have a significant effect on the vegetative development in diameter at the crown of the plants (p-value=7.706e-09).

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

Plants treated with T1 (CMA+ ½ NPK_Urea) and T2 (100% NPK_Urea) in Ouénou locality had equally low performances respectively (1.23 ± 0.03) and (1.36 ± 0.04) compared to plants subjected to the control treatment (T0) in the other localities.

In Bagou, the plants subjected to the effect of treatments T1 (CMA+ ½ NPK_Urea) and T2 (100% NPK_Urea) are statically different. The average diameters of plants subjected to T1 and T2 treatments in this locality represent the second-best performance obtained, namely 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 the plants subjected to the T2 treatments (2.15±0.03 cm). The plants subjected to treatment T1 (CMA+ ½ NPK_Urea) in this locality had a mean diameter development of 2.06±0.02 cm. These mean diameters were statistically different for the two treatments in this locality.

As with plant heights, plant diameter development was significantly influenced by the treatment applied but also by locality.

2.2.3. Leaf area of maize plants

The mean values of the leaf area of maize plants are presented in Table 2. The leaf areas of the obtained plants varied from 1279.60±29.78 cm to 1796.85±25.38 cm. The lowest vegetative development in the leaf area was obtained in Bagou locality (1279.60±29.78 cm); while the best vegetative development in the leaf area was observed in Kokey (1796.85±25.38 cm). Regardless of the location, the best leaf area performance was observed in the T2 (100% NPK_Urea) treatments. The leaf area performance of the plants subjected to treatment T0 (control, not inoculated) was the lowest. However, statistical analysis showed that experimental location and treatment had no significant effect on the vegetative development in the leaf area of the plants.

2.2.4. Maize Yield Assessment

Table 3 shows the grain yields of the maize plants. The grain yields of the plants subjected to treatment T1 (CMA+ ½ NPK_Urea) increased by 32.40 to 43.70% compared to the control treatment (T0). Regardless of the treatment applied to the plants, grain yields were lower in the Kokey locality, while the best grain yields were recorded in Ouénou locality (Table 3). The best grain yields were observed in the plants subjected to the T1 treatments (CMA+ ½ NPK_Urea); while the lowest yields were recorded in the plants subjected to the control treatment (T0). In contrast, at Kokey, the grain yield for T2 (2.44±0.40 t/ha) was slightly higher than 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.

The interaction between zone and treatment did not significantly affect plant yield. However, the zone in isolation had an impact on plant yield and the treatments in isolation also had an impact on plant yield.

Table 3. Grain yields of maize plants.

ZONE	TREATMENT	Plant yield (t/ha)		
		Average	Standard error	IC-95%
Bagou	T0	1,13	0,4	[0.86 ; 1.40]
	T1	1,59	0,41	[1.32 ; 1.87]
	T2	1,55	0,26	[1.37 ; 1.72]
Kokey	T0	1,08	0,36	[0.85 ; 1.31]
	T1	1,43	0,33	[1.22 ; 1.64]
	T2	1,44	0,4	[1.19 ; 1.70]
Ouénou	T0	1,35	0,45	[1.04 ; 1.65]
	T1	1,94	0,35	[1.71 ; 2.18]
	T2	1,79	0,4	[1.53 ; 2.06]

95% CI= 95% confidence interval.

2.2.5. Nutritional status of the plants

Table 4 presents the nutritional status of maize plants. The results of the analysis of variance show that the Area-Treatment interaction has a significant effect (p-value = 0.005 **) on the Nitrogen concentration of the maize plants. Treatments T1 and T2 induced the best Nitrogen uptake regardless of the experimental area. However, the highest average Nitrogen values at Kokey (2 ± 0.05) and Bagou (1.98± 0.046) 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.

Phosphorus uptake was significantly influenced by the Area-Treatment interaction. At Bagou (2.3± 0.053), Kokey (2.01± 0.047), and Ouénou (1.98± 0.045), the best phosphorus uptake was obtained in plants that received the biostimulant + 50% NPK_Urea (T1). The biostimulant induced a phosphorus accumulation of the order of (21.08 to 27.77%) in the maize plants.

As for potassium, the highest levels (2.1±0.061 and 1.94d±0.025) in the 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.

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

Area	Treatment	Nitrogen (N) %		Phosphorus (P) %		Potassium (K) %	
		Mean	SE	Mean	SE	Mean	SE
Bagou	T0	1,63ab	0,023	1,8bc	0,026	1,71ab	0,026
	T1	1,98d	0,046	2,3e	0,053	2,1e	0,061
	T2	1,82c	0,033	1,92cd	0,038	1,88cd	0,047
Kokey	T0	1,72ac	0,021	1,66ab	0,024	1,7ab	0,021
	T1	2d	0,055	2,01d	0,047	1,94d	0,025
	T2	1,81c	0,032	2d	0,045	1,93d	0,044
Ouénou	T0	1,6a	0,03	1,61a	0,018	1,59a	0,017
	T1	1,74ac	0,014	1,98d	0,045	1,83bd	0,026
	T2	1,75bc	0,017	1,81bc	0,014	1,73abc	0,017
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 ($p < 0.05$). SE = Standard Error.

2.2.6. Evaluation of mycorrhization frequency and intensity

Figure 2 shows the frequency and intensity of mycorrhization of maize plants subjected to T1 treatment (CMA+ ½ NPK_Urea). The highest mycorrhization frequency (87%) was obtained in Kokey locality; while the lowest (12%) was recorded in Ouénou. Like the frequency, the highest intensity (50.59%) was recorded in Kokey, while the lowest (10%) was recorded in all three zones. Statistical analysis did not show any significant difference between treatments since treatments T0 (control, not inoculated) and T2 (100% NPK_Urea) were not mycorrhized.

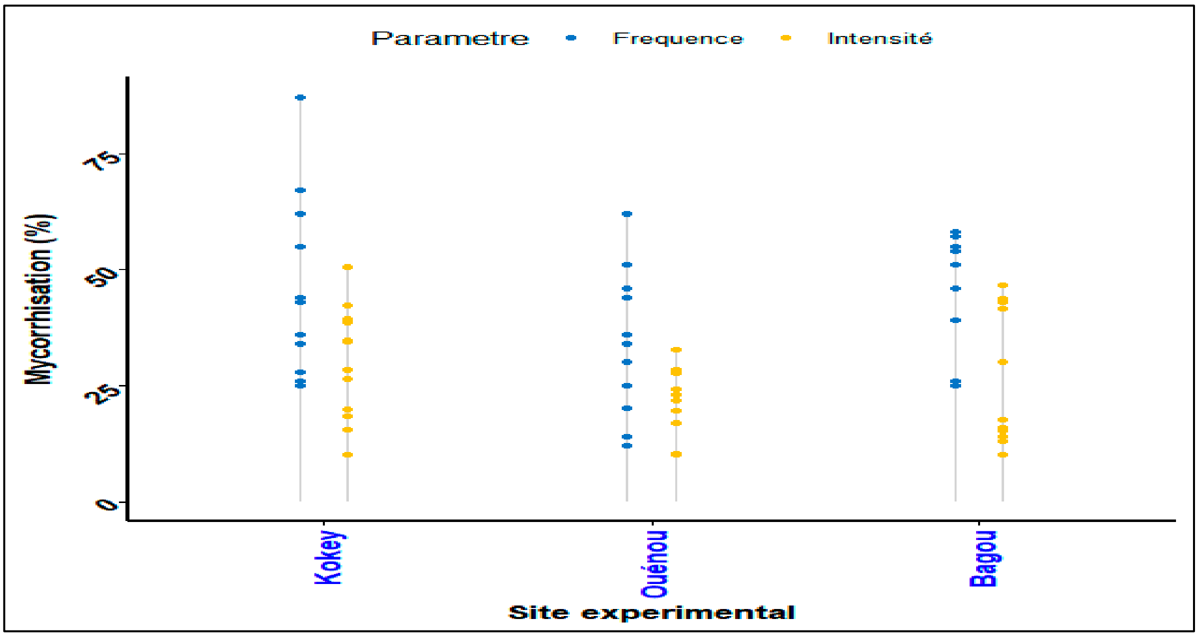

Figure 2. Frequency and intensity of mycorrhization of plants.

2.2.7. Participatory component analysis

A principal component analysis (PCA) was carried out to establish the relationship between the parameters of growth and nutritional status of maize plants (Fig.3). The analysis shows that the first two axes retain 77.8% of the cumulative variance and can thus be retained for the interpretation of the results. Indeed, both the parameters of plant nutritional status (Nitrogen, Phosphorus, and Potassium) and those of plant growth (Height, Diameter, and Leaf surface) are positively correlated with axis 1 (Dim 1). The variables such as Potassium, Height, and Diameter are the ones with the best representation quality and are strongly positively correlated with each other. Thus, the more the capacity of absorption by the plant of nutrients, especially potassium, is improved, the better the growth of the plants is, especially the growth in height of these plants. Also, a projection of the different treatments applied to the plants in the system of axes, indicates that the plants subjected to the T0 treatment do not have better performances in terms of absorption of the nutrients as well as the growth parameters whereas the plants subjected to the T1 and T2 treatments are those having the good performances in terms of absorption and growth. The plants subjected to treatment T1 (CMA+ ½ NPK_Urea) are those having known the strong absorption rates and by ricochet the best developments in height. As for the plants subjected to treatment T2 (100% NPK_Urea of the recommended dose), they are associated more with an important development in diameter.

It can be deduced that the treatments (CMA+ ½ NPK_Urea and 100% NPK_Urea of the recommended dose) improve the plant's capacity to absorb nutrients and ensure better vegetative development of the plants than the current farming practices in the experimental regions

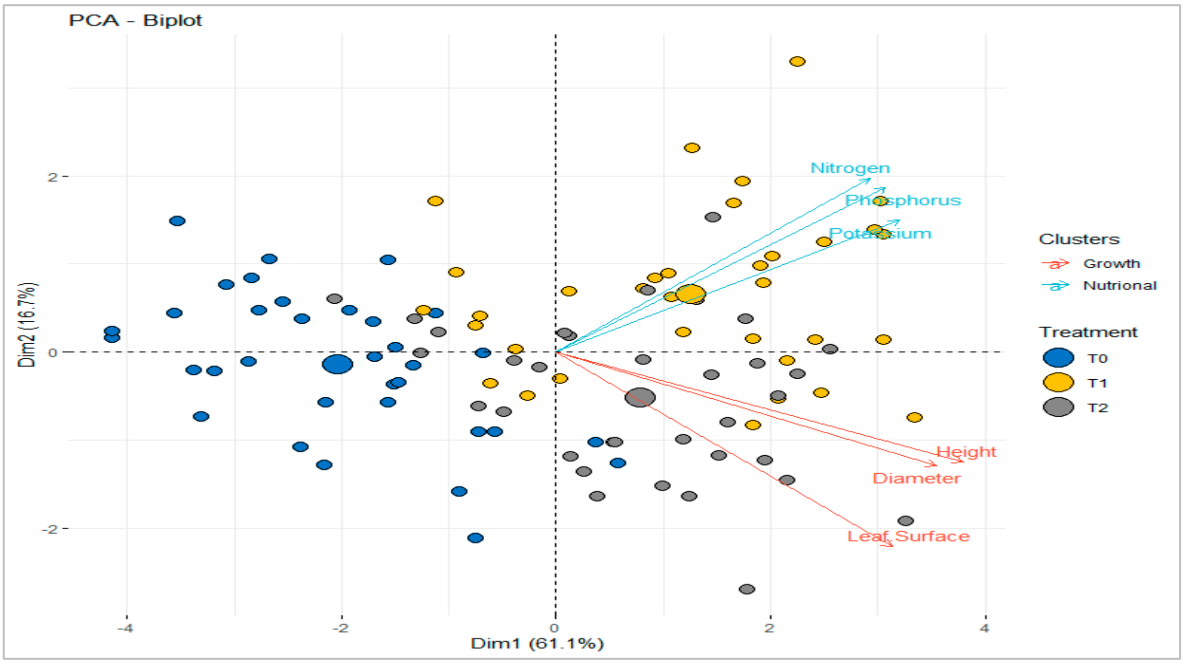

Figure 3. Principal Component Analysis (PCA) of the relationship between height, diameter, leaf area and nutritional status of maize plants. *T0: Absolute control; T1: biostimulant + 50% NPK_Urea and T2: 100% NPK_ Urea.*

3. Discussion

The chemical characteristics of the soils in our study show that organic matter varies 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 is 5.6 meq/100g and in Bagou is 7.1 meq/100g.

In addition, soil organic matter, assimilable phosphorus and exchangeable base contents are good for an experiment on the effects of NPK mineral fertilizers and are in agreement with those obtained by [34] in northern Benin. In addition, these soils had a low level of fertility characterized by high C/N ratios. The pHkcl shows lower values than the pHwater (6.6 at Ouénou; 6.4 at Bagou and 6.7 at Kokey). This shows that the soils in our study are acidic. This result confirms those of [34] who showed that the soils of northern Benin were moderately acidic and poor in organic matter. This pHwater promotes the growth of fungi, which thrive best in acidic environments [25]. According to [36], arbuscular mycorrhizal fungi are preponderant in acidic soils. Indeed, pH influences the activity of soil microorganisms that participate in the mineralization of organic matter as well as that of mycorrhizal fungi [37]. [38] 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 (CMA+ 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 are not directly accessible to the roots. Generally, mycorrhizal symbioses improve host plant development through improved plant nutrition [30].

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 [39]. These results are similar to those obtained by [40] who showed that inoculation with *F. mosseae* + ½ dose of NPK resulted in improved growth in length and thickness of maize plants. Also, [33] showed that inoculation of plants with *G. aggregatum* resulted in greater vegetative development of sesame.

According to [41], AMF effectively is plant 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 [42]. In addition, [18] showed that inoculation of maize plants in a farming environment with the two endogenous strains of arbuscular mycorrhizal fungi (*Glomeracees* et *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 water and soluble phosphorus from the soil [43]. Similar results were found by [34] 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 [44] who proved that the best maize grain yield was obtained with the treatment *Glomeracea* + 25% NPK-Urea.

However, our results also reveal that the interaction between the zone and the treatment did not give a significant effect on plant yield. These results confirm those of [45] who showed that inoculation with microorganisms indigenous to Burkina Faso improves 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 [38] 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 [46].

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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 are free of any mycorrhizal colonization and that there is 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 due to the fact that maize roots are less abundant, stubby, and lacking absorptive hairs, and therefore particularly dependent on AMFs [47]. 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 [48,49] who observed a mycorrhization rate between 50% and 70%. Also, 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 [50,51] 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.

4. Materials and Methods

4.1. Experimental site

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, on the Research and Development (RD) 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 1km apart.

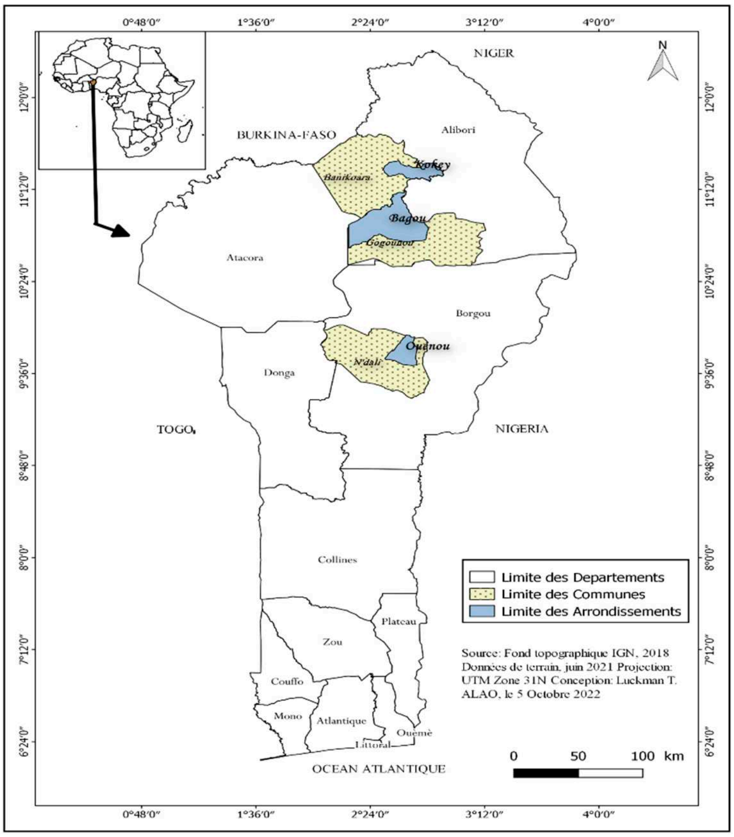

Figure 1. Map showing study areas.

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4.2. Materials

The maize variety QPM FAABA with an intermediate cycle of 105 days was used. It is supplied by the International Institute of Tropical Agriculture (IITA) and the National Institute of Agricultural Research of Benin (INRAB). It is 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 [52].

Fungal inoculum composed of three species of the genus Glomus (*Glomus caledonius, Rhizophagus intraradices and Funneliformis geosporum*), isolated from maize rhizosphere soils in Benin by [35].

4.3. Methods

4.3.1. Preparation of the inoculum

Maize seed inoculation was performed as described by [53]. The amount of inoculum applied was 10% of the weight of the maize seed with a quantity of distilled water equivalent to 600 ml.kg-1 of inoculum. The seeds were coated and then left to dry in the air for 12 hours before sowing.

4.3.2. Experimental set-up

The experimental set-up at each producer was made up of three elementary plots with three treatments. The area allocated to each elementary plot was 500 m² (25 m*20 m). The different treatments were: T0 (Control without CMA inoculum, representing the farmer's practice); T1 (CMA+ ½ NPK_Urea), and T2 (100% NPK_Urea of the recommended rate). The seeding spacing was 0.80 m x 0.40 m. The recommended dose of mineral fertilizer was 200 Kg/ha and that of Urea is 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.

4.3.3. Collection of soil samples

Soil samples were collected in diagonal order. Sampling was done prior to planting at a depth of 0-20 cm at each catch at each producer. A 200g composite soil sample was taken from the different Development Research sites. The method of [54] was used to determine soil pH. The method of [55] was used for the determination of assimilable phosphorus and that of [56] for the determination of exchangeable cations (Ca, Mg, K, and Na). In addition, according to the method of [57], organic matter and organic carbon were determined. The cation exchange capacity (CEC) was determined by the method of [58]. Finally, total nitrogen was determined by the method of [59].

4.3.4. Evaluation of growth parameters

At each elementary plot, the height and diameter at the neck of maize plants were collected 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 at the 60th DAS. The height of a maize plant was measured with a tape measure. Plant diameter was measured with a caliper at the collars of the plant and leaf area was estimated by the product of leaf length and width with a coefficient of 0.75 [60].

4.3.5. 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 x 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 [61].

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

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Where, R = average seed yield of maize plants, t/ha; P = seed mass of maize plants, kg; S = harvest area, m^2; H = percent grain moisture, %.

4.3.6. Assessment of the nutritional status of corn plants

The evaluation of the nutritional status of the maize plants consisted of the determination of the nitrogen (N), phosphorus (P), and potassium (K) contents. Indeed, after mineralization of the plant material (whole maize plant) and their distillation by the method of [55], the nitrogen content was determined by titration, phosphorus by the method of [58], and potassium by atomic absorption spectrophotometer [56].

4.3.7. 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 [62]. The observation was made by electron microscopy. The roots were first cut into 1cm long pieces, and 0.2g of these roots were introduced into test tubes. After the addition of 10% KOH, the contents were put in an oven at 90°C for 1h. Then, KOH was poured out and the roots were rinsed thoroughly with tap water. This operation emptied the cells of their cytoplasmic contents. The 0.05% trypan blue solution was added to the roots and the whole was put back in the oven at 70°C for 15 min. This allows staining of the fungal structures. A light rinse with water removed the excess dye.

After staining the root samples, preparations were made and mounted between the slide and coverslip for observation. The fragments were observed under an electron microscope at magnification (Gx100). The method described by [63] allowed us to determine the frequency and intensity of mycorrhization:

The mycorrhization frequency (F), which reflects the degree of infection of the root system, and the mycorrhization intensity or absolute mycorrhization intensity (I), which expresses the portion of the colonized cortex in relation to the whole root system, are calculated according to the following equations.

$$\mathbf{F}(\%) = ((\mathbf{N} - \mathbf{n_o}) / \mathbf{N}) \times \mathbf{100}$$

With (F) reflecting the degree of infection of the root system, N is the number of fragments observed and n0 is the number of fragments with no trace of mycorrhization.

$$I(\%) = (95n_5 + 70\ n_4 + 30\ n_3 + 5\ n_2 + n_1) / N - n_0$$

With: I: The intensity of mycorrhization, N: number of observed fragments; no: number of fragments without a trace of mycorrhization; n5, n4, n3, n2, and n1 are respectively the five classes of infection 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%.

4.4. 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. To capture the statistical differences in means when the ANOVA test is significant, a post hoc test of pairwise comparisons using the Tuckey post hoc test [64] was performed. The different tests were carried out in the R 4. 1. 3 software [65] and required the use of the dplyr and DescTools packages for the calculation of descriptive statistics, the ggplot2 and ggpur packages for the realization of whisker boxes, the "car" package for the ANOVA and the multcomp package for the realization of the post hoc test of comparison by pairs. The threshold of significance is 5%. Moreover, by means of the ggpubr package, a dotchart was realized in order to appreciate the frequency and intensity of mycorrhization of the plants at each experimental site.

5. Conclusions

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

From these results, it was found 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. TLA, ADK, RMA, SAA, OA, and CA carried out the trial set-up, data collection, and harvesting. TLA wrote the first draft of the manuscript, managed the literature search, and performed the statistical analysis. NAA, NA-A, AA, LB-M, and OOB wrote the protocol, managed the study analyses, and supervised the various activities. All authors read and approved the final manuscript.

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