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IMPROVEMENT OF MAIZE PRODUCTIVITY (ZEA MAYS L.) BY MYCORRHIZAL INOCULATION ON FERRUGINOUS SOIL IN CENTER OF BENIN

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ABSTRACT

The objective of our study was to evaluate the promoted effects of three arbuscular mycorrhizal fungi (AMF) on maize growth and yield on ferruginous soil in center of Benin. The maize variety used was EVDT 97 STR C1. The maize seeds were inoculated with each of three arbuscular mycorrhizal fungi (*Glomus intraradices, Funneliformis mosseae and Rhizophagus cubens*) combined with or not to mineral fertilizer dose recommended (50% of NPK). The experimental device was a completely randomized random block of nine treatments with four repetitions. The data collected were related to growth (height, diameter and leaf area), yield (biomass and grain yield), mycorrhization (frequency, intensity and number of spores) and NPK content. The maximum height, best seeds yield and larger leaf surface are obtained with maize plants treated with *R. intraradices* combined with 50% of NPK respectively surpassing of 17.44%, 38.14%, 45.99% the values obtained with the controls plants. *G. cubens* combined with 50% of NPK was induced an increase of diameter (6.50%), dry aerial biomass (32.86%) and dry ground biomass (94.73%) compared to the control. *G. cubens* only has led to the best frequency (41.25%), intensity of mycorrhization (6.07%) and a high number of spore (1.81 spores/g soil). These results show the potential of these arbuscular mycorrhizal fungi to improve the maize productivity on ferruginous soil in center of Benin.

Contribution/Originality: The paper's primary contribution is finding that mycorrhizal inoculation increase maize productivity by reducing the use of mineral fertilizer.

1. INTRODUCTION

The problem of food insecurity arises with acuity in Sub-Saharan Africa (Fao/Stat, 2013). This situation is maintained by the lower continual productivity of major crops including maize (*Zea mays* L.), due among other things to the soil impoverishment (Azontondé *et al*., 2009).

In Benin, maize occupies an important place in agricultural production systems in all agro-ecological areas of the country (Adjanohoun *et al*., 2012). Unfortunately, some producers continue to have lower seeds yields (0.5 t/ha) against potential yields in research station from 3 to 5 t/ha (Azontondé *et al*., 2010). Thus, to increase maize grain yield, beninese farmers use mineral fertilizers. These inputs are a problem for the farmers because of their relatively high costs and not always available, for the environment and the health of populations through the pollution of water courses and products of harvest by the heavy metals they contain. Thus, for efficient agriculture, sustainable and respectful of the environment, scientists advocate wholesome ecologically practices such as the use of bio-resources like mycorrhizal fungi.

Indeed, these mycorrhizal fungi are microorganisms that live in obligatory symbiosis with over 200,000 cultivated and uncultivated crops (Oehl and Sieverding, 2004; Smith and Read, 2008). These symbiotic fungi are considered as a microbial group 'keys' in the functioning of terrestrial ecosystems in particular for their capacity to promote the development of plants in degraded areas. Within this group of fungi are those referred to as Arbusculars Mycorrhizal Fungi (AMF). The plant provides to AMF the sugars (hexoses) (Read and Perez-Moreno, 2003) and in return the AMF are very vital for the absorption of phosphorus (P) by the plant, mainly in tropical areas where the P-assimilable is often limiting (Matos *et al*., 1999). The AMF facilitate feeding of the plant mineral elements (Fortin *et al*., 2008) and water (Grümberg *et al*., 2010). In addition, they are involved in the tolerance of plants to biotic (Whipps, 2004) and abiotic (Tao and Zhiwei, 2005) stresses. Some previous work such as Leye *et al.* (2009) in Senegal and Tchabi *et al.* (2008) in Benin showed a significant improvement in the growth of mycorrhizal plants.

Given the importance that are mycorrhizal fungi and in order to improve maize productivity while reducing the use of fertilizers, the present study aims to assess the effect of three arbuscular mycorrhizal fungi (*Glomus cubens*, *Rhizophagus intraradices*, *Funneliformis mosseae*) on the growth and yield of maize on ferruginous soil in center of Benin.

2. MATERIAL AND METHODS

The maize variety used was EVDT 97 STR C1. It is an early variety of 90 days. It has a good resistance to american rust, the stripe, and drought (Yallou *et al*., 2010). It was provided by National Institute of Agricultural Research of Benin (INRAB).

The three mycorrhizal fungi (*Rhizophagus intraradices*, *Glomus cubense* and *Funneliformis mosseae*) used in this study were obtained from partners of laboratory of Mycorrhizae of Instituto Nacional de Ciencias Agricolas (INCA) of Cuba (Latin America).

2.1. Geographical Location of Experimental Site

The trial has been installed on the experimental station of Agricultural Research Center of Save (CRA-Centre) of Beninese National Institute of agricultural research (INRAB). It is located in the commune of “Gobé” at an altitude of 105.1 m, longitude 8 ° 00' 149 "North, latitude 2 ° 25'646" East (Figure 1). This site is characterized by a subequatorial climate with two rainy seasons and two dry seasons. The average annual pluviometry is 1,100 mm (Capo-Chichi, 2006).

2.2. Experimental Design

The experimental design was a block completely randomized of 9 treatments with 4 repetitions. Each basic plot (12.8 m^2) consists of 4 lines of 4 m long and the distance between the lines is 0.80 m. spacing of seedlings was 0.80 m x 0.40 m or a density of 31.250 plants/ha after the divorce (Hernandez *et al.*, 1995). The distances between basic plots and between rehearsals were respectively 1.8 m and 2 m. The useful parcel of 6.4 m^2 was represented by the 2 central lines on which the data were collected. The different treatments applied were: T1 = Control (without inoculation and mineral fertilizers); T2 = *Glomus cubens*; T3 = *Rhizophagus intraradices;* T4 = *Funneliformis mosseae;* T5 = 50% $N_{15}P_{15}K_{15}$ recommended; T6 = *Glomus cubens* + 50% $N_{15}P_{15}K_{15}$; T7 = *Rhizophagus intraradices* + 50% $N_{15}P_{15}K_{15}$; T8 = *Funneliformis mosseae* + 50% $N_{15}P_{15}K_{15}$; T9 = 100% $N_{15}P_{15}K_{15}$.

Mineral fertilizer dose recommended by INRAB (1995) is 200 kg/ha of NPK to sowing and 100 kg/ha of urea 45 days after sowing $N_{15}P_{15}K_{15}$ indicates the proportion of N, P and K in 100 kg of NPK fertilizer.

2.3. Determination of the Chemical Characteristics of Station Soil

Station soil samples (500 g) were taken at the beginning of experimentation at a depth of 0-20 cm and placed in a sterile plastic bag, labeled and transported to the laboratory for determination of their chemical properties. The soil pH was measured by electrometric method using a pH meter. The organic matter was determined by the Walkley and Black (1934). The exchangeable cations were determined by ammonium acetate method described by Thomas (1982) and assimilable phosphorus by color method of Metson (1956) at 660 nm The cationic exchange capacity was evaluated by the method described by Ramón *et al.* (2003).

2.4. AMF Inoculation of Maize Seeds

The inoculation of maize seeds with three fungi (*G. cubense*, *R. intraradices* and *F. mosseae)* was made on the eve of seedling according to the method described by Fernández *et al.* (2000). Indeed, every fungi spore has trapped in a sandy support to formulate the fertilizer product (inoculum) at a concentration of 20 spores/ gram of soil. The maize seeds were mixed with inoculum at ratio of 10:1 for each type of fungi. Each mixture was again mixed with 600 $ml.kg^{-1}$ of fertilizer. Thus, coated seeds have been air dried for 12 hours according to the recommendations published by Ruget and Chartier (1996).

2.5. Sowing and Maintenance of Plants

Two maize seeds inoculated or not have put in a seed hole about 5 cm of depth and the hole was immediately closed. Three Weeding were made, the first was coupled thinned to one plant per hole two weeks after sowing, the less vigorous plant was torn, the second and third times has been made respectively six weeks and eight weeks after sowing.

2.6. Collection of Growth Data

To assess the effect of different treatments on growth of maize plants, some growth parameters were measured. It's the height, diameter at the collar and leaf area of plants. The height and the diameter were then measured respectively through a ruler tape and the caliper 15^{th}, 30^{th}, 45^{th} and 60^{th} Days After Sowing (DAS). The leaf area of plants was estimated at 60^{th} JAS by the product of length and width of leaves affected by coefficient 0.75 (Valdés *et al.*, 2013).

2.7. Collection of Yield Data Biomass Produced (Fresh and Dry)

After grains harvest (95^{th} DAS), maize plants were cut at ground to constitute fresh biomass. The underground part (essentially the roots) represents the fresh biomass. Different biomass was weighed using a precision scale (Highland HCB 3001, max: 3000 g x 0,1 g). To determine the dry biomass, 1 kg of fresh biomass was steamed at

100 °C for 72 hours and weighed to determine the weight of dry biomass. Yields average fresh biomass and dry maize plants have been determined according to the formula:

$$R' = \frac{P' \times 10.000}{S' \times 1.000}$$ Where:

- R' = average yield in dry biomass of plants of maize in $t.ha^{-1}$
- P' = weight in dry biomass of maize plants in kg
- S = area of crop in m^2

2.8. Determination of Maize Grain Yield

After harvest, the maize cobs were shelled and weighed according each treatment using a precision scale. The moisture percentage of the grain was determined using a moisture meter (LDS - 1F). The values of average seeds yield were determined by following formula (Kjeldahl, 1883):

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

- R = is maize yield, expressed in t/ha ,
- P is the weight of maize seeds per field pot expressed in kg
- 10000 is the conversion of ha in m^2 ; 1000 is the conversion of t in kg
- S = is the field pot area expressed in m^2; % H = humidity of the grain in %.

2.9. Assessment of Nutritional Status of Maize Plants

The assessment of nutritional status of maize plants consisted in the determination of nitrogen (N), phosphorus (P) and potassium (K) contents. Indeed, after mineralization of plant material (whole maize plant) and their distillation by the Bray and Kurtz (1945) the nitrogen content was determined by titration, the phosphorus by Metson (1956) and potassium by atomic absorption spectrophotometer (Thomas, 1982).

2.10. Assessment of Endomycorrhizal Infection of the Plants

The roots of maize plants were taken at 68th DAS. After coloring with blue trypan according to the method described by Phillips and Hayman (1970) arbuscular mycorrhizal fungi associated with roots of maize plants were observed through binocular microscope (XSP-BM-2CEA). The estimation of roots mycorrhizal infection was performed according to intersection method (Giovannetti and Mosse, 1980; Trouvelot *et al.*, 1986). The mycorrhization rates was estimated by two parameters of arbuscular mycorrhizal infections arbuscular namely:

-The frequency of mycorrhization (F) which reflects the infection degree of the root:

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

Where: N is the number of fragments observed and n_o the number of fragments no mycorrhization

- The intensity of mycorrhization: m expresses the portion of root colonized over the entire root system:

$$\frac{95n_5 + 70n_4 + 30n_3 + 5n_2 + n_1}{N - n_o}$$

In this formula, $\mathbf{n_5}$, $\mathbf{n_4}$, $\mathbf{n_3}$, $\mathbf{n_2}$ and $\mathbf{n_1}$ represent respectively the numbers of fragments scored in the five classes of infection, marking the importance of the mycorrhization namely: 5 = more than 95%, 4 = 1% of the cortex, 50 to 95%, 3 = 30 to 50%, 2 = 1 to 30%.

2.11. Statistical Analysis

The influence of treatments on growth, yield and mycorrhizal parameters, and nutritional status of plants compared to control was examined using analysis of variance model based to two factors (Block and treatment). Then the Dunnett test was used to assess the relative performance of each treatment compared to controls.

The determination of the best's treatment between them was made using an analysis of variance model based to two factors (Block and treatment). Then the Student Newman and Keuls test was used to assess the relative performance of treatment between them using the R

The influence of the AMF on the mycorrhization parameters was analyzed with a fixed pattern of analysis of variance followed by the test of Student Newman and Keuls for the structuring of the treatments.

A categorization and discrimination of treatment was made with hierarchical digital classification followed by Analysis in Principal Component (ACP) with the HPC function using the R software 3.3.2 with Facto Mine R package.

3. RESULTS

3.1. Chemical Characteristics of the Experimental Soil

The assessment of the chemical characteristics of experimental soil in the 0-20 cm horizon revealed that the rate of organic matter is low (1.16%), as well as nitrogen (0,076%). Soils are poor in phosphorus (5 ppm) and potassium (0.16 meq / 100g). The sum and the saturation of bases and the cationic exchange capacity are low, but the rate of the base saturation is average. The ground proved slightly acid (pH 6.5) (Table 1).

3.2. Influence of AMF on Maize Plants Growth

The results of mixed model variance based two factors (Block and treatments) revealed a highly significant difference ($p < 0.01$) between treatments for following parameters height, diameter and leaf area of maize plants.

The treatments explained at 69.62% the variability of heights, 46.80% the variability of diameter and 60.83% the variability of leaf area. The Dunnett test results showed that only the plants treated with combinations of *G. cubens* and 50% NPK; *R. intraradices* and 50% NPK; *F. mosseae* and 50% NPK and 100% NPK were different from the control with greater height (Table 2). It is the same for leaf area and those for plants treated with combinations of *R. intraradices* and 50% $N_{15}P_{15}K_{15}$; *F. mosseae* and 50% NPK and 100% NPK. A negative and significant difference was noted for the diameter and only for processing *F. mosseae.* The values of height, diameter and leaf area per maize plant obtained according to different treatments are presented in Table 3. It shows that the plants inoculated with the combination of *R. intraradices* with 50% NPK had the most important heights and leaf area (141.02 cm and 390.74 cm^2). They are followed by the plants inoculated by combination of *G. cubens* and 50% NPK (140.61 cm for the height) and 100% NPK (368.94 cm^2 for leaf area). For diameter, the best performance was observed with plants inoculated with the combination of *G. cubens* and 50% NPK (15.24 mm). The results of the variance analysis indicated overall a difference very highly significant ($p < 0.001$) between all treatments for all growth parameters (table 3).

3.3. Influence of AMF Treatments on Yield Parameters

The analysis of treatment on yields parameters revealed overall a difference very highly significant ($P < 0.001$) for biomass of plants treated compared to the control plant (Table 4). The result of Dunnett structuring test revealed that treatment *R. intraradices*, *F. mosseae* , 50% NPK and *F. mosseae* + 50% NPK are not significantly different compared to control for dry aerial biomass. As for dry underground biomass, *G. cubens*, *R. intraradices*, *F. mosseae* and 50% NPK are not too different compared to control and only plants treated with *R. intraradices* do not differ compared to control for fresh biomass underground ($P > 0.05$). A significant difference was noted for the combinations of *G. cubens* and 50% NPK and *R. intraradices* and 50% NPK for fresh area biomass and only for the treatment *R. intraradices* + 50% NPK for the performance. The variability induced by treatment are very high for all parameters of biomass yield ($R^2 > 50\%$) except for grain yield that is relatively average.

The biomasses of maize plants obtained varied significantly according to treatments. We noted a very highly significant difference ($P < 0.001$) between the treatments for all yield parameters (Table 5). Plants treated with 100% NPK had a higher fresh aerial biomass production (5.76 g) followed by plants inoculated by *R. intraradices* + 50% NPK (5.22 g) whereas control plants had average value of 3.57 g (Table 5). Similarly, 100% of NPK induced the highest fresh underground biomass production (0.58 g) followed by *G. cubens* + 50% NPK (0.49 g) compared to the control plants (0.26 g).

For the production of dry biomass, plants treated with combination of *G. cubens* and 50% NPK had the highest dry aerial biomass (1.86 g) followed by those treated with combination of *R. intraradices* and 50% NPK (1.85 g). These plants showed increases in aerial and underground dry biomass respectively from 32.86% and 94.73% r compared to control plants. The lower values was observed with *F. mosseae* (1.39 g) followed by control treatment.

Similarly, the stronger aerial and underground dry biomass were obtained by plants inoculated by combinations of *G. cubens* and 50% NPK followed by plants inoculated by *R. intraradices* and 50% NPK**.** These treatments induced increases respectively around 32.86% and 32.14% compared to control for the dry aerial biomass and respectively from 94.73%, 63.16% for dry underground biomass.

For maize seeds yield, the highest values were obtained with plants treated with combination *A. intraradices* and 50% NPK (3.26 t/ha) with an increase of 38.14% compared to control plants. The weaker performance was observed with the plants treated with *F. mosseae* and the control plants.

3.4. Effect of CMA on Mycorrhization Parameters

The results of analysis of variance (Table 6) indicated that the differences in effect induced by the treatments on mycorrhization parameters is very highly significant ($p < 0.001$). Plants treated with *G. cubens* presents the best values of frequency (41.25%), intensity (6.07%) and the number of spores/g of soil (1.81). The highest intensities of infection were induced by all treatments constituted by only AMF It's *G. cubens* (6.05%), *R. intraradices* (4.75%) and *F. mosseae* (3.39%) Same observations was made for frequency of infection (41.25%. 27.50% and 26.25%). In addition, the highest number of spores has been registered in the soil treated with *G. cubens* (1.81) while the lowest number of spores was recorded with *F. mosseae* + 50% NPK (0.64/g).

3.5. Effects of Mycorrhizal Fungi on Nutritional Status of Maize Plant

The results of the mixed model variance based on two factors (block and treatments) revealed generally that no significant difference between N, P and K contents for all treatments ($p > 0.05$). The different values of these macronutrients (nitrogen, phosphorus and potassium) in maize plants are presented in table 7. Nitrogen (number) is weak and almost identical for all treatments. All inoculated plants have experienced improvements in their content of phosphorus and potassium. Indeed, plants which received only the contribution of *G. cubens*, *R. intraradices* and *F. mosseae* respectively present increases of 38%; 43% and 46% for phosphorus (P) and 46%; 44% and 51% for potassium (K) compared to the absolute control. Combined with 50% NPK, these same strains show respective increases of 11%; 10% and 37% for phosphorus (P) and 15%; 38% and 58% for potassium (K) compared to the absolute control. Thus the value the highest phosphorus was obtained with *F. mosseae* while that potassium was obtained with the combination of *F. mosseae* + 50% of NPK.

3.6. Categorization and Discrimination of Treatment by the Most Discriminating Characteristics

The categorization of various treatments according to growth, yield and nutritional parameters of the plants using the hierarchical classification followed by analysis in principal component (APC) has revealed four treatment groups. The first group (cluster 1) constituted of *F. mosseae* and *R. intraradices* treatment characterized only by high phosphorus content induction as opposed to cluster 4composed of *R. intraradices* + 50% NPK, 100% NPK and *G. cubens* + 50% NPK that induce higher growth with high yields and other production's parameters very high and a high content in nitrogen (N) (Figure 2). The Cluster 2 is constituted of control treatment and 50% NPK which produce high value of the nutritional status of plants in nitrogen and phosphorus with large diameter and opposes to the Group 3 composed of treatment *F. mosseae* + 50% NPK and *G. cubens* which gives very high plants, large leaf area with a very high nutritional status in potassium (K). It is also from the analysis of figure 1 that the elevation of nitrogen in plants treated with the treatment of Group 4 causes growth in diameter at the collar and in height very high which in turn ensures a good production of plants with high yields. Any elevation of phosphorus slows growth and led to lower production.

4. DISCUSSION

On top of the light and CO_2, the plants need nutrients, oxygen and water for good growth. Nutrients can be found in the ground and in the air. A deficiency or an excess of nutrients can cause various problems of the plants. Unfortunately, the results of chemical analysis of experimental soil (Table 1) reveal that the soil had a low fertility and the maize plants cultivated in this soil had poor nitrogen, phosphorus and potassium content. the In addition, the highly acidity of experimental soil would justify the ameliorative effect of arbuscular mycorrhizal fungi on development of maize plants on ferruginous soil in center of Benin especially with *G. cubens* because this specie impacted positively the plants growth with acid pH (Wang *et al.*, 1993). This deficiency of phosphorus could be explained this significant effect of fungi on the leaf area and dry biomass of plants. The combination of *G. cubens* with 50% of NPK led to improve aerial (32.86%) and underground dry biomass (94.73%) production (Table 5). Indeed, the study of Gabriel and Cristina (Gabriel and Cristina, 2007) in Colombia revealed that inoculation of different arbuscular mycorrhizal fungi is effective on maize plants growth, particularly on the increase in dry matter and leaf area in soils lower in phosphorus. In our study, the AMF promoting effect is better when they combined with 50% of NPK resulting in increase of height (17.44%) and leaf area (45.99%) of maize plants compared to the control plants (Table 3). In addition, the diameter (15.24 mm) obtained with the plants treated with combination of *G. cubens* and 50% NPK surpassing of 6.50% the value of the control plants. Notice that the best parameters of growth have been achieved at the level of the plants inoculated and treated with a half dose of NPK in comparison to the non-inoculated maize plants. (Table 3). Jansa et *al*; Tchabi et *al*; Schoebitz Cid; Hamza and HAFSI (Jansa *et al.*, 2005; Schoebitz, 2010; Tchabi *et al.*, 2010; Hamza and Hafsi, 2014) attributed this beneficial effect of CMAs on plant growth to greater absorption of nutrients (N, P, K, Fe, Zn, Mn and Cu) by the plants. In addition to the plant acquisition systems of nutrients through epidermis and root hairs absorbent, CMAs are considered as 'additional organs' absorption of the nutrients in most vascular plants (Smith *et al.*, 2009). Indeed once associated with the host plant, fungi form a root extra Mycelial network which is an extension of root system and allows to uptake of nutrients in a volume of soil that explored by the only roots. The fungus also provides water to the plant. Note that the plants inoculated with AMFs are better ability to uptake water than the no inoculated plants, whether in water or no deficiency conditions (Bárzana *et al.*, 2012). The arbusculaires AMFs-plants symbiosis are reciprocal benefits to plants and AMFs, because the fungi get photosynthesis of carbon that the plants provide to fungi hexoses and which can constitute up to 20% of carbon fixed by the plants during photosynthesis (Smith and Read, 2008).

In our study, the best maize grain yield was obtained with plants treated by the combination of *R. intraradices* with 50% NPK. This treatment induced an increase of grains yield of 38.14% compared to control plants. Noticed that the increase of grain yield is 7.20% when *R. intraradices* is not combined with NPK. On one hand, this result shows that these AMFs have promoting effect on maize seeds yield, and their combination with 50% of NPK allows to perform better. These results are close to those reported by Hamza and Hafsi (2014) in Algeria who have shown that yield is higher when we combine the inoculum "symbivit" to 50% of synthesis fertilizer.

However, there was a very highly significant difference ($P < 0.000$) between the CMAs for parameters of mycorrhization. Thus, *G. cubens* have induced the best frequency and intensity of mycorrhization and a high number of spore per gram of soil (Table 6). On the contrary, *F. mosseae* + 50% NP have induced the lowest intensity of mycorrhization (1.85%) and the lowest number of spore per gram of soil (0.64/g). A reduction of values of three mycorrhizal variables measured was observed when AMFs are combined with NPK. The same trend has been observed in the work carried out by (Dafi and Nicolson, 1969; Mosse, 1973) which found that the contribution of phosphorus in high concentrations increases the roots resistance to mycorhization. The best value induced by *G. cubens* for growth and yield parameters is confirmed by the mycorrhization parameters. However, when combined with 50% of NPK, the best values are observed with *R. intraradices* followed of *G. cubens*. We deduce that *R. intraradices* resists more to the mineral fertilizer than G. *cubens*. Although soil chemical analysis has revealed that the experimental soil has a low fertility level, the values of mycorrhizal parameters are relatively small compared to those obtained by Pérez-Luna *et al.* (2012). In the previously study, the number of spores per gram of soil was 18 for *G intraradices* in Mexico. The results of our study are slightly higher than those of Chantelot (2003) who noted that the Most Probable Number (MPN) is considered acceptable around 1.5 and too low below 0.5 propagules per gram of soil. Moreover, the three weeding during our experiment in the field would have strongly affected the CMAs establishment on maize roots. Indeed, (Evans and Miller, 1990; McKonigle and Miller, 2000) showed that the work on the ground cause the destruction of the mycelium and are widely at origin of the reduction of maize roots colonization by fungi. The previous work conducted by Hayman (1983) also showed that the plants colonizationby CMAs may vary according to the CMA train used in the culture. Regarding the effectiveness of this strains of *G. cubens* is the most performant. Hetrick *et al.* (1995) have shown that mycorrhizal development is an expression of an interaction which varies strongly according to the varieties of plants and fungi strains.

The results of this study in general revealed that the use of mycorrhizal fungi has been beneficial. Indeed, without contribution of NPK, frequency, root mycorrhization rates and mycorhizogene power of soil are relatively high and allows a better growth and yield compared to control plants. However, all of results show that the use of mycorrhizal inoculum combined with 50% dose NPK increase the growth and yield parameters of maize. Despite the fact that the synthetic fertilizer has been a source of stress for the mushrooms, which slowed their mycorhizogene power and infecting, it led to outcomes compared to the absolute control. This lets consider that the recommendations of synthetic fertilizer application could possibly be reduced to less than half by combining the mycorrhizal inoculum. Hamza and Hafsi (2014) with a similar score claim that this reduction of synthesis fertilizer presents a very interesting economic and ecological benefit.

5. CONCLUSION

According to the results obtained in this study, we can say that arbuscular mycorrhizal fungi can provide to farmers an additional tool to reduce contributions of chemical fertilizers, improve soil fertility and increase their yields. These CMAs are most effective when they are combined with a half recommended dose of NPK. Let's Retain that it's *G. cubens* which proved to be more effective than other species. It is more adapted to the environmental conditions by giving overall, the best results for the growth parameters of, performance and mycorrhization, these results are more interesting when combined with 50% of NPK.

These results showed the benefits of biotechnology based on CMAs in agriculture to improve the growth and yield of maize in Benin while protecting the environment for sustainable development.

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ILLUSTRATION

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Table-1. Chemical characteristics of soil ofexperimental site (0-20 cm depth).

Sample	pH (water)	P a (ppm)	Nitrogen (%)	MO (%)	K - ech (meq / 100g)	Exchangeable bases (meq/100 g)	CEC (meq/100)
Tropical ferruginous soil depleted	6.5	5	0.076	1.16	0.16	3.66	8.00

MO: organic matter; P a (ppm): assimilable phosphorus; K - ech: exchangeable Potassium; CEC: Ability to Exchange Cationique, ppm: percentage per thousand; meq: milliequivalent.

Table-2. Average value of growth parameters of plants inoculated compared to control plants: results of the Dunnett test.

Treatments	Height (cm)	Diameter at the collar (cm)	Leaf area (cm^2)
G. cub	5.168	-0.575	30.4
A. int	3.52	-1.065	-16.04
F. mos	-1.415	-1.695*	18.25
50% NPK	-0.892	-0.23	-11.24
***G.cub* + 50% NPK**	20.523***	0.932	46.42
***R.int* + 50% NPK**	20.938***	0.1	123.09**
***F. mos* + 50% NPK**	19.628***	-0.05	97.23*
100% NPK	13.083*	0.737	101.29**
R^2 (%)	69.62	46.80	60.83

*: significant 0.0; *: significant at 0.01; *: significant at 0,001(P<0,001). *G. cub*: *Glomus cubens; A. int*: *Rhizophagus intraradices; F. mos*: *Funneliformis mosseae; G.cub*+ 50% NPK: *Glomus cubens* + 50% NPK; *R.int*+ 50% NPK: *Rhizophagus intraradices* + 50% NPK, *F. mos* + 50% NPK: *Funneliformis mosseae*, 100% NPK: 100% NPK

Table-3. Influence of the CMAs on height, diameter and leaf area of maize plants at 60th DAS

Treatments	Height (cm)		Diameter at the neck (mm)		Leaf area (cm²)	
	M	CV (%)	M	CV (%)	m	CV (%)
Witness	120.08^{c}	2.61	14.31^{ab}	6.65	267.65^{c}	10.88
G. cub	125.25^{bc}	1.17	13.74^{abc}	6.44	298.05^{bc}	4.36
A. int	123.60^{bc}	7.97	13.25^{bc}	8.17	251.60^{c}	4.22
F. mos	118.67^{c}	4.32	12.62^{c}	6.05	285.89^{c}	19.84
50% NPK	119.19^{c}	4.80	14.08^{abc}	6.21	256.40^{bc}	8.19
G.cub + 50% NPK	140.61^{a}	6.99	15.24^{a}	2.04	314.07^{abc}	16.50
R.int + 50% NPK	141.02^{a}	4.13	14.41^{ab}	6.71	390.74^{a}	20.11
F. mos + 50% NPK	139.71^{a}	4.77	14.26^{ab}	3.60	364.88^{ab}	5.69
100% NPK	133.17^{ab}	3.99	15.05^{a}	2.25	368.94^{ab}	12.88
Probability	0.000		0.001		0.000	
Meaning	***		***		***	

* = p < 0.001 (very highly significant.) , followed the same letter averages differ not significantly at the 5% threshold; m: average; cv: EMA of variation; *G.cub: Glomus cubens; A. int: Rhizophagus intraradices ; NPK: Nitrogen-Phosphorus-Potassium; F. mos: Funneliformis mosseae; G. cub + 50% NPK: Glomus cubens +* 50% NPK ; *R. int + 50% NPK: Rhizophagus intraradices +* 50% NPK; *F. mos + 50% NPK: Funneliformis mosseae +* 50% NPK.

Table-4. Average value difference of the production parameters of treatment compared to controls T1: Dunnett test results

Treatments	DAB	DUB	CUB	BAF	Yield
G. cub	0.33**	0.06	0.07***	0.31	0.52
A. int	0.13	0.02	0.01	0.30	0.17
F. mos	-0.01	0.04	0.08***	-0.22	-0.52
50% NPK	0.14	0.03	0.05**	0.22	0.30
G.cub + 50% NPK	0.46 ***	0.19***	0.24***	1.45***	0.70
R.int + 50% NPK	0.45***	0.12***	0.13***	1.65***	0.89*
F. mos + 50% NPK	0.02	0.12***	0.16***	0.10	0.20
100% NPK	0.35**	0.24***	0.33***	2.19***	0.72
R^2 (%)	64.21	85.40	97.06	87.44	49.62

*: significant 0.0; *: significant at 0.01; *: significant at 0,001(P<0,001). R^2 (%): coefficient of determination that explains the overall variability generated by all of the factors below: DAB: dry aerial biomass, DUB: dry underground biomass, CUB: cool underground biomass, BAF: fresh aerial biomass; Yield: performance; *G.cub: Glomus cubens; A. int: Rhizophagus intraradices; NPK: Nitrogen-Phosphorus-Potassium; F. mos: Funneliformis mosseae; G. cub + 50% NPK: Glomus cubens +* 50% NPK ; *R. int + 50% NPK: Rhizophagus intraradices +* 50% NPK. *F. mos + 50% NPK: Funneliformis mosseae +* 50% NPK.

Table-5. Average value of production parameters depending on the treatments: results of analysis of variance followed by Student Newman and Keuls test

Treatments	DAB		DUB		CUB		FAB		Yield	
	m	CV	m	CV	m	CV	m	CV	m	CV
Witness	1.4^{c}	2.54	0.18^{d}	14.47	0.26^{e}	5.09	3.57^{c}	7.36	2.36^{ab}	13.03
G. cub	1.73^{ab}	9.81	0.25^{d}	21.98	0.33^{d}	1.53	3.88^{c}	11.23	2.89^{a}	12.73
A. int	1.53^{bc}	8.73	0.20^{d}	12.91	0.27^{e}	0.00	3.87^{c}	9.89	2.53^{ab}	20.13
F. mos	1.39^{c}	16.37	0.23^{d}	7.7	0.34^{d}	4.8	3.35^{c}	9.08	1.84^{b}	13.74
50% NPK	1.54^{bc}	4	0.21^{d}	5.92	0.31^{d}	2.63	3.79^{c}	7.80	2.66^{ab}	19.52
G.cub + 50% NPK	1.86^{a}	3.58	0.37^{b}	15.45	0.49^{b}	7.48	5.02^{b}	3.02	3.06^{a}	18.37
R.int + 50% NPK	1.85^{a}	9.64	0.31^{c}	3.11	0.39^{c}	2.47	5.22^{b}	5.46	3.26^{a}	10.08
F. mos + 50% NPK	1.42^{c}	4.74	0.30^{c}	7.2	0.41^{c}	5	3.67^{c}	3.68	2.56^{ab}	11.24
100% NPK	1.75^{ab}	4.78	0.42^{a}	1.94	0.58^{a}	4.93	5.76^{a}	4.40	3.08^{a}	14.94
Probability	0.000		0.000		0.000		0.000		0.000	
Meaning	***		***		***		***		***	

* = p < 0.001 (very highly significant). DAB: Dry Aerial Biomass, DUB: Dry Underground Biomass, **CUB**: cool underground biomass, FAB: Fresh Aerial Biomass; Yield: performance m: average; cv: coefficient of variation; *G.cub: Glomus cubens; R. int: Rhizophagus intraradices; NPK: Nitrogen-Phosphorus-Potassium; F. mos: Funneliformis mosseae; G. cub + 50% NPK: Glomus cubens +* 50% NPK ; *R. int + 50% NPK: Rhizophagus intraradices +* 50% NPK. *F. mos + 50% NPK: Funneliformis mosseae +* 50% NPK**.**

Table-6. Intensity of mycorrhization, mycorrhization frequency and number of spores per gram of soil: results of analysis of variance.

Treatments	Intensity		Frequency		Number of spore	
	m	CV (%)	m	CV (%)	m	CV (%)
G. cub	6.07 ^{a}	20.93	41.25^{a}	14.86	1.81 ^{a}	13.18
A. int	4.75^{ab}	30.01	27.50^{b}	11.31	1.00^{b}	6.53
F. mos	3.39^{bc}	30.95	26.25^{b}	17.42	0.68^{c}	8.32
***G.cub* + 50% NPK**	1.96^{c}	9.01	20.75^{b}	30.57	0.80^{bc}	9.13
***R.int* + 50% NPK**	2.01^{c}	40.10	24.00^{b}	17.01	0.96^{b}	5.89
***F. mos* + 50% NPK**	1.85^{c}	29.93	22.75^{b}	16.59	0.64^{c}	11.41
Probability	0.000		0.000		0.000	
Meaning	***		***		***	

* = p < 0.001 (very highly significant). In the same column, with averages of different letters are significantly different at the 5% threshold according to the Student-Newman-Keuls test. m: average; cv: coefficient of variation; *G.cub: Glomus cubens; A. int: Rhizophagus intraradices; NPK: Nitrogen-Phosphorus-Potassium; F. mos: Funneliformis mosseae; G. cub + 50% NPK: Glomus cubens* + 50% NPK ; *R. int + 50% NPK: Rhizophagus intraradices* + 50% NPK; *F. mos + 50% NPK: Funneliformis mosseae* + 50% NPK.

Table-7. Nitrogen, phosphorus and potassium contents of the maize plants

Treatments	Nitrogen (N) (%)		Phosphorus (P) (mg/g)		Potassium (K) (mg/g)	
	m	CV	m	CV	M	CV
Witness	1.87 ^{a}	10.45	2.41 ^{a}	118.52	1.99 ^{a}	26.84
G. cub	1.80 ^{a}	5.98	1.47 ^{a}	59.82	3.87 ^{a}	93.16
A. int	1.76 ^{a}	6.77	1.60 ^{a}	44.93	3.57 ^{a}	54.93
F. mos	1.84 ^{a}	11.98	1,678^{a}	42.59	4.04 ^{a}	60.47
50% NPK	2.08 ^{a}	17.94	1.46 ^{a}	75.01	2.92 ^{a}	53.05
***G.cub* + 50% NPK**	1.95 ^{a}	16.86	1.02 ^{a}	25.05	2.32 ^{a}	47.87
***R.int* + 50% NPK**	1.97 ^{a}	13.55	1.01 ^{a}	10.65	3.22 ^{a}	23.26
***F. mos* + 50% NPK**	1.77 ^{a}	16.52	1.46 ^{a}	43.18	4.79 ^{a}	62.36
100% NPK	1.94 ^{a}	13.08	1.26 ^{a}	22.28	2.69 ^{a}	41.59
Probability	0.124		0.199		0.005	
Meaning	NS		NS		NS	

NS = p > 0.05 (not significant), m: average; cv: coefficient of variation; *G.cub: Glomus cubens; A. int: Rhizophagus intraradices ; NPK: Nitrogen-Phosphorus-Potassium; F. mos: Funneliformis mosseae; G. cub + 50% NPK: Glomus cubens* + 50% NPK ; *R. int + 50% NPK: Rhizophagus intraradices* + 50% NPK; *F. mos + 50% NPK: Funneliformis mosseae* + 50% NPK

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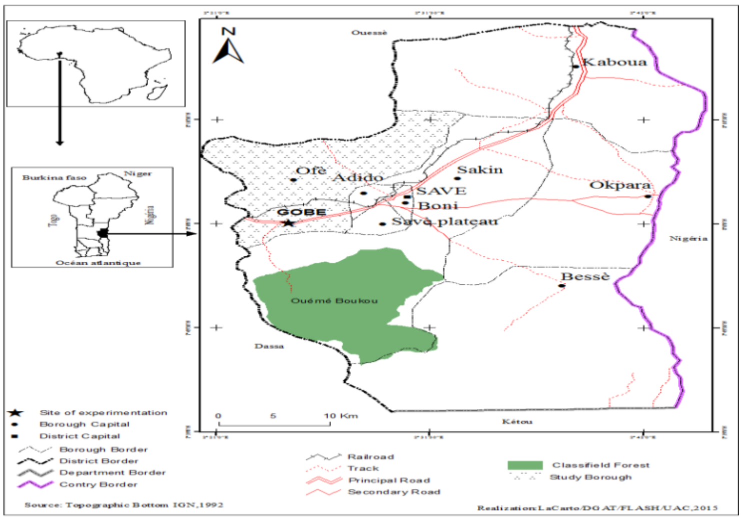

Figure-1. Geographical localization of the experimentation site
Source: Fond topographique IGN, 1992, Réalisation: LaCarto/DGAT/FLASH/UAC, 2015.

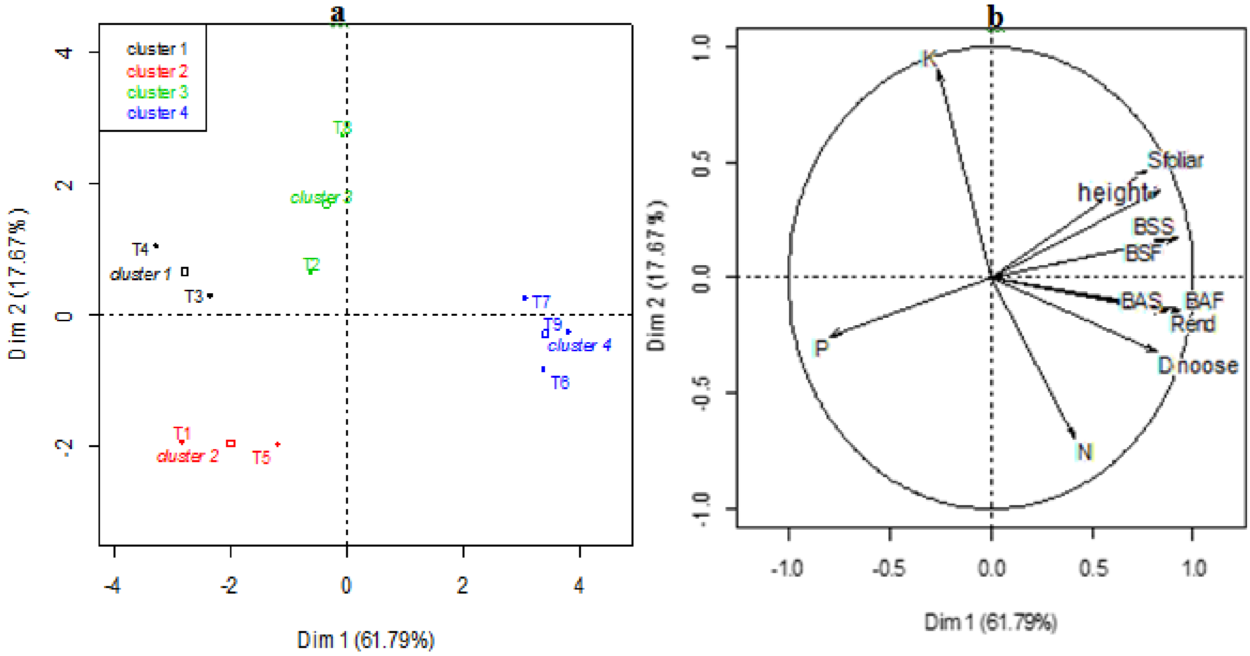

Figure-2. (a) categorization of treatments and their (b) discrimination in the factorial axis plan (1,2).