Evaluation of the Mixed Effects of Some Indigenous Strains of Arbuscular Mycorrhizal Fungi on the Growth of Maize Seedlings Under Greenhouse Conditions

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Doi:10.19044/esj.2020.v16n3p275 URL:http://dx.doi.org/10.19044/esj.2020.v16n3p275

Abstract:

The objective of the study was to evaluate the joint effects of three groups of native arbuscular mycorrhizal fungi (AMF) (*Glomeraceae*, *Acaulosporaceae* and *Diversisporaceae*) on the growth of Maize Seedlings. The mycorrhizal fungi were isolated by the wet sieving method through decreasing sieve (300 µm, 125 µm, 63 µm and 38 µm) followed by centrifugation on a sucrose gradient. The growth tests were evaluated in greenhouse conditions for 40 days. After opening a planting hole, two maize (2000 SYNEE-W) seeds, one coated with AMF and the other not coated (Control) were introduced into the planting hole for each treatment. Data on different parameters were evaluated. The results of this study revealed that the maximum heights, the largest noose diameters and the largest numbers of leaves were obtained with treatment "*Acaulosporaceae* + 50% NPK-Urea"

having 20.55% and 17.04% respectively and 11.77% for that of the control. The produced biomass and the leaf area of the maize plants were improved by the treatment "*Glomeraceae*+ 50NPK-Urea" with a respective increase of 54.97% for fresh above biomass (FAB), 42.94% for fresh underground biomass (FUB) and 55.23% for the leaf area compared with the control. Also, very high frequency of mycorrhiza was recorded with treatment "Glomeraceae" while the largest numbers of mycorrhiza spores and intensity were recorded with treatment "Acaulosporaceae". These results augur the possibility of using these mixed AMF bio-products as organic fertilizers to improve maize productivity in Benin.

Keywords: Mixed Effect, Indigenous Arbuscular Mycorrhizal Fungi, Maize, Greenhouse, Benin

Introduction

Soil microorganisms such as Arbuscular Mycorrhizal Fungi (AMF) feed a growing interest as bio-stimulants (Caser et al., 2019). They may form mutual symbiosis with 80% of terrestrial plant species and several speculations (Berruti et al., 2016). Within the interface between the plant and the fungus, carbohydrates and mineral nutrients (N, P, Zn and B) are exchanged (Smith and Read, 1997). Thus, AMF alleviate slowdown of plant growth caused by the depletion of nutrient reserves and may improve tolerance to biotic and abiotic stresses. Inoculation of AMF increases water and nutrient uptake including phosphorus as this fungal symbiosis multiply the capacity of the host plant to explore a larger volume of soil compared to non-inoculated plants (Ruiz-Lozano and Azcón 1995; Joner et al., 2000; Smith and Read, 2008; Zhang et al., 2019).

In a context where agriculture of Benin is marked by the decline of soil fertility (Saïdou et al., 2012), it urges to find corrective solutions. Maize produced throughout in Benin involved into various economic transactions and thereby represents a significant source of income for producers and traders (Balogoun et al., 2013). Unfortunately, the national yields of maize estimated to 1.3 t/ha are still below those expected to meet the demand (DSA/MAEP, 2018; FAO, 2018). The AMF could be used to improve the productivity of maize. The role of AMF in growth and yield amelioration of maize plants (*Zea mays* L.) in Benin and elsewhere were mentioned in previous studies (Assogba et al., 2017, Aguégué et al., 2017, Koda et al., 2018, Liu et al., 2016; Polcyn et al., 2019).

However, a decisive step in the implementation of AMF technology is the appropriate selection of effective fungal isolates to inoculate plants (Songachan and Kayang, 2018). Therefore, identification of AMF is considered as a prerequisite for mycorrhizal inoculation programs since the

effectiveness of their application on plant growth depends on AMF isolation (Duponnois et al., 2001). Thus, it is crucial to choose the appropriate AMF to target host plant to enable it to make the maximum profit (Bagyaraj and Varma, 1995). Even if AMF appear more effective in natural conditions, it is crucial to collect isolates which must be easily reproducible and multipliable in one hand and can sporulate under laboratory conditions in the other hand, to produce a very suitable inoculum for practical applications (Songachan and Kayang, 2018). The development of techniques to improve crop production with a production of a high number of propagules CMA (Gaur and Adholeya, 2002) appears as a soil fertility and nutrition management technology of plants requiring low inputs. It is the reason why studying the endomycorrhizae characteristics associated with this plant and assessment of their application in a controlled environment becomes important. Moreover, ecological studies on the diversity of AMF, the morphological characterization of spores, molecular biology techniques or assessment of endomycorrhizae inoculants generally rely on exogenous strains with little investigations on native stains (Leal et al., 2009; Symanczik, 2016; Aguégué et al., 2017). Based on these observations, the main objective of this study was to determine the status of AMF associated to *Zea mays* cultivated in the Centre of Benin. It aimed to characterize the native AMF, to isolate them and to assess their effects on the growth of maize seedlings in greenhouse conditions.

Materials And Methods

Collection of Test Materials

Vegetable materials

The maize variety *2000*SYN EE-W was used during the experiment. It is an early variety of 75 days, developed by the International Institute of Tropical Agriculture (IITA) and the National Institute of Agricultural Research of Benin (INRAB) (MAEP, 2017).

Isolation of AMF

The mycorrhizal fungal groups Glomeraceae (*Funeliformis mosseae*, *Funeliformis geosporum*, *Glomus Caledonius*, *Glomus ambiosporum*, *Rhizophagus intraradices* and *Septoglomus contrictum*) Acaulosporaceae (*Acaulospora capsicula*, *Acaulospora denticulata*) and Diversisporaceae (*Diversispora globifera*) were isolated and identified from the maize rhizosphere of six (6) different municipalities (Dassa-Zoumé, Savalou, Savè, Glazoué, Bantè and Ouèssè) of Collines department by using of Davis – INVAM's key. These different groups of fungi were isolated and multiplied over a period of twelve weeks using sorghum (Sorghum bicolour) as a trap plant because of its important mycorrhizal potential and the substrate used is

a mixture of clay and sterile soil at respective doses of 3/4 and 1/4 in a pot of 250 millilitres (ml) of capacity.

Capture and Inoculum Preparation

Sorghum seeds were soaked for two (2 min) minutes in a sodium hypochlorite solution at 0.024% and then rinsed five (5) times with distilled water under vortex shaking (Gholami et al., 2009). Then, ten (10) grains of sorghum (*Sorghum bicolor* L.) were sown in each pot containing soil substrate of different dilution levels. The pots were then placed in a greenhouse at room temperature. The plants were watered daily with distilled water to maintain the capacity of the soil similar to the field one for six (6) weeks. The whole root biomass and substrate were thus crushed to obtain inocula of all strains.

Experimental design

The experimental design was a Randomized Complete Block Design (RCBD) of nine (9) treatments with three (3) repetitions. The treatments applied were: T1 = control (no inoculation or mineral fertilizers); T2 = Glomeraceae; T3 = Acaulosporaceae; T4 =*Diversisporaceae;* T5 = 50% + NPK Urea recommended; T6 = *Glomeraceae* + 50% N15P15K15; T7 = *Acaulosporaceae* + 50% NPK; T8 =*Diversisporaceae* + 50% NPK; T9 = 100% NPK.

The dose of mineral fertilizers recommended by INRAB (1995) is 200 kg / ha of NPK.

Seed coating

The inoculum thus obtained was used to coat the seeds in the ratio 10: 1 (10 kg of Seed per 1 kg of bio-product) for each fungi group. Each mixture was kneaded with an amount of water equivalent to 600 $ml.kg^{-1}$ of fertilizers. The coated seeds were dried at ambient air for 12 hours in accordance with the recommendations of Fernández et al. (2000).

Filling Jars

The substrate used is a ferruginous soil collected between 0 and 20 cm deep with a shovel spade. The substrate was sterilized twice at 120 ° C for 20 minutes to 24 hours; Two kilograms of the substrate was then weighed in a jar of 15 cm diameter (Gholami et al., 2009). Each pot was dampened at 2 / 9th of the Maximum Water Retention Capacity (WRC) of the substrate corresponding to 224 ml of sterile distilled water 24 hours before sowing (Eteka, 2005).

Sowing and pots maintaining

Two maize seeds, one coated with AMF and the other not coated (Control) have been introduced in a central seed hole approximately 5 cm deep per pot. The pots were watered with 1/9 of the Maximum Water Retention Capacity (112 ml) daily. Thinning to one plant per hole was made the 7th Day After Sowing (DAS) The different parameters to evaluate were collected from the 7th to 40th DAS.

Evaluation of Growth Parameters

The evaluation of the effects of different treatments on the growth of maize plants was made by taking the measurement of the variables: height, noose diameter, leaf number and leaf area. The height of maize plant (*Zea mays* L.) is the distance between the noose of the last ligule. It was measured by using a measuring tape while; the noose diameter was measured using a caliper and leaves were counted every 96 h from the thinning of plants namely the 7th, 11th, 15th,19th, 23rd, 27th, 31st, 35th and 39th Day After Sowing (DAS). The surface of the last two leaves with ligule of each plant was estimated by the product of the length (the top of the sheath at the tip of the blade) and; the leaf width (measured in the middle of the blade) multiplied by the coefficient 0.75 (Ruget et al., 1996).

Evaluation of growth Parameters

The precision balance (Highland HCB 3001. Max 3000g x 0.1g) was used to weigh the different biomass (depending on treatment). The determination of yield of above and underground biomass was determined according to the formula:

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

- R '= average yield of dry biomass of maize plants t.ha^{-1}
- P = weight in dry biomass of corn plants in kg
- 10,000 is the hectare conversion in m²; 1000 converting tonne (t) into kg
- S = crop area cultivated in m²

Evaluation Endomycorrhizal Infection of Plant Roots

The samples of maize root were collected on the 40th DAS. After staining with trypan blue according to the method described by Phillips and Haymain (1970), fragments of maize roots were observed in the binocular (XSP-BM-2CEA. 2013). The estimation of mycorrhizal root infection was performed using intersection method (Giovannetti and Mosse, 1980;

Trouvelot et al., 1986). Two parameters of arbuscular mycorrhizal infection were used for the calculation of mycorrhizal rate:

- The frequency of Mycorhization (F), which reflects the degree of infection of the root system:

$$\mathbf{F\ (\%)} = \frac{(\mathbf{N} - \mathbf{n_o})}{\boldsymbol{N}}$$

Where N is the number of fragments observed and No the number of fragments without a trace of mycorrhiza.

- The intensity of mycorrhiza: m (absolute mycorhization intensity) that expresses the portion of the cortex colonized according to the entire root system:

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

In this formula, $\mathbf{n_5}$, $\mathbf{n_4}$, $\mathbf{n_3}$, $\mathbf{n_2}$ and n_1 are a number of fragments respectively noted in five classes of infection; marking the importance of mycorrhiza as follows: 5 = more than 95%, 4 = 50 to 95%, 3 = 30 to 50%, 2 = 1 to 30%, 1 = 1% of the cortex.

Data analysis

All analyses were performed with the software R (3.5.3) (R Core Team 2018). Repeated measure analysis of variance was performed on the height, diameter and the values of the sheet with the package Nonlinear Mixed Effects (Pinheiro et al., 2019). For above fresh biomass, the leaf area and underground fresh biomass, we conducted multiple analyses of variance.

Results

Effects of AMF on height, noose diameter and the number of leaves of the maize plants

The results of Analysis of Variance revealed a significant difference in the height and number of leaves, while the diameters were not significantly different and varied over time. Acaul_50NPKUrea generated the greatest heights (Figure 1), diameter (Figure 2) and number of leaves (Figure 3) over time.

Table 1. Analysis of variance in height, noose diameter and number of leaves

	Height		**Diameter**		**Leaf**	
Factors	chisq	Pr(>chisq)	chisq	Pr(>chisq)	chisq	Pr(>chisq)
Treatment	38942	<0.001	13739	0088	17.4113	0026
Time	1653.75	<0.001	1198.615	<0.001	860.0237	<0.001
Treatment: time	12,501	0.13	4.345	0825	1.9046	0984

Height

The figure 1 shows the evolution of plant height over time. There is a gradual evolution of plants over time. The results of analysis of variance showed that there is a significant difference depending on the type of treatment and time especially late in the cycle (P <0.001). Maximum heights are obtained with treatments that have received *Acaulosporaceae* + 50% NPK-urea with an increase of 20.55% compared to the control followed by those received only the Acaulosporaceae Glomeraceae treatment and combined with 50% NPK-Urea.

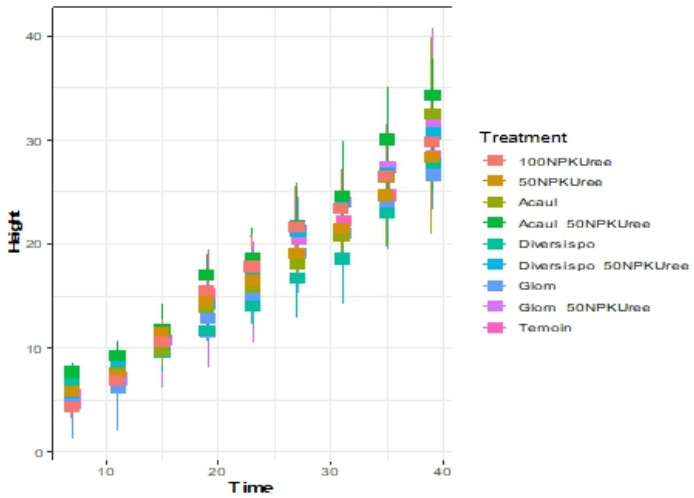

Figure 1. Variation in height depending on the type of treatment and time.

Diameter

Noose diameters of the seedlings grew progressively (Figure 2) from the first to the 40th day after sowing (DAS). Diameters were similar with a significant difference over time (P <0.001). The largest diameters were recorded with the same treatments (*Acaulosporaceae* + 50% NPK-Urea) observed at the maximum heights with an increase of 17.04% compared to the control.

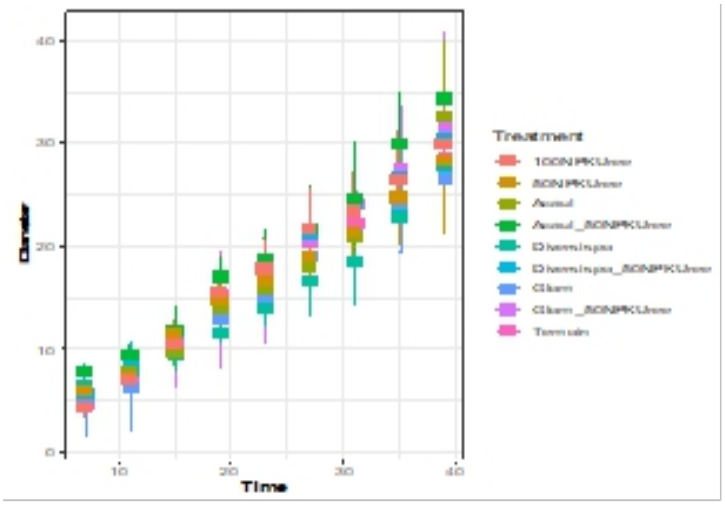

Figure 2. Changes in noose diameter depending on the type of treatment and time.

Number of leaves

Figure 5 shows the gradual progress of the number of leaves depending on the type of treatment and time. The variance of analysis of the results showed a highly significant difference (P <0.001). A slight demarcation between all other treatments and the control was thus observed from the 30th DAS. The highest numbers of leaves were also recorded with *Acaulosporaceae* + 50% NPK-Urea with an increase of 11.77%.

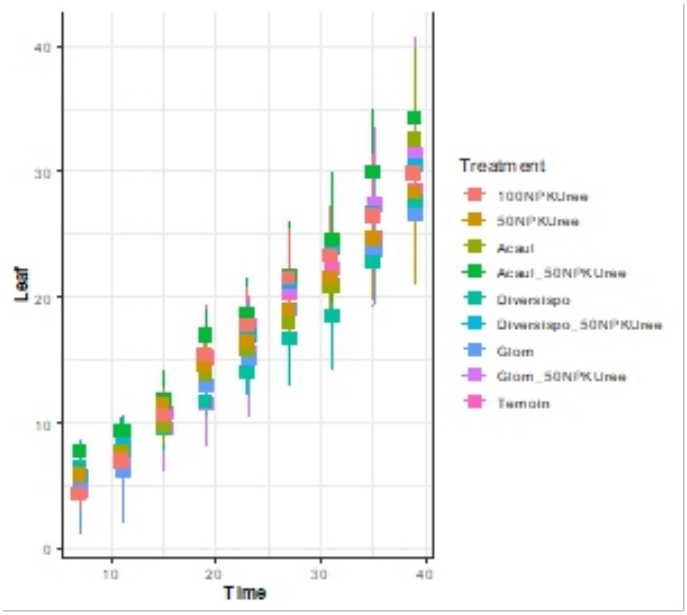

Figure 3. Number of leaves depending on the type of treatment and time

Effects of AMF on biomass (above and underground) and the leaf area of corn plants

The results of multiple analysis of variance of the three parameters indicate that; treatments have induced similar effects on aboveground biomass (P-value> 0.05), whereas they lead to significant changes on underground biomass and leaf area of maize plants (P-value< 0.05) (Table 2). The Student Newman and Keuls test results revealed that *Glomeraceae*+ 50NPK-Urea generated an important Underground Fresh Biomass (UFB) and a large leaf area (LA) exceeding 42.94% and 55.23% respectively in comparison to the control. The best fresh above biomass (FAB) was recorded with Glomeraceae + 50NP-KUrea with an increase of 54.97% compared to the control.

Table 2: Influence of AMF on fresh above biomass (FAB), fresh underground biomass FUB and leaf area (LA) of maize plants

	FAB		**FUB**		**LA**	
	Average	**CV**	**Average**	**CV**	**Average**	**CV**
100NPKUrea	0.35	16.09	133.07b	22.48	0.28a	5.39
50NPKUrea	0.26	45.78	122.10b	40.66	0.30A	5.04
Acaul	0.59	50.60	118.56b	27.57	0.36A	10.02
Acaul_50NPKUrea	0.50	19.90	151.55b	18.16	0.17b	25.64
Diversispo	0.27	31.64	130.34b	10.33	0.34a	6.18
Diversispo_50NPKUrea	0.40	36.74	145.36b	7.22	0.17b	17.63
glom	0.54	58.79	154.19b	20.74	0.33A	1.77
Glom_50NPKUrea	0.61	17.89	234.18a	48.01	0.17b	9.17
Witness	0.28	27.13	104.83c	17.12	0.19b	37.63
Probability	0097	-	0012	-	<0.001	-

Means followed by the same letter are not significantly different at the 5% level; cv = coefficient of variation

Mycorhization Settings

The figure 4 shows the variation in the intensity, frequency of mycorrhiza, number of spores according to the different strains. The greater intensity of mycorrhiza (Figure 4a) was recorded with Acaulosporaceae monitoring Diversisporaceae + 50% NPK + Urea. The greater frequency of mycorrhiza (Figure 4b) was recorded with Glomeraceae followed Acaulosporaceae + 50% NPK + Urea and Diversisporaceae while the largest number of spores (Figure 4c) was obtained with monitoring Acalulosporaceae Glomeraceae.

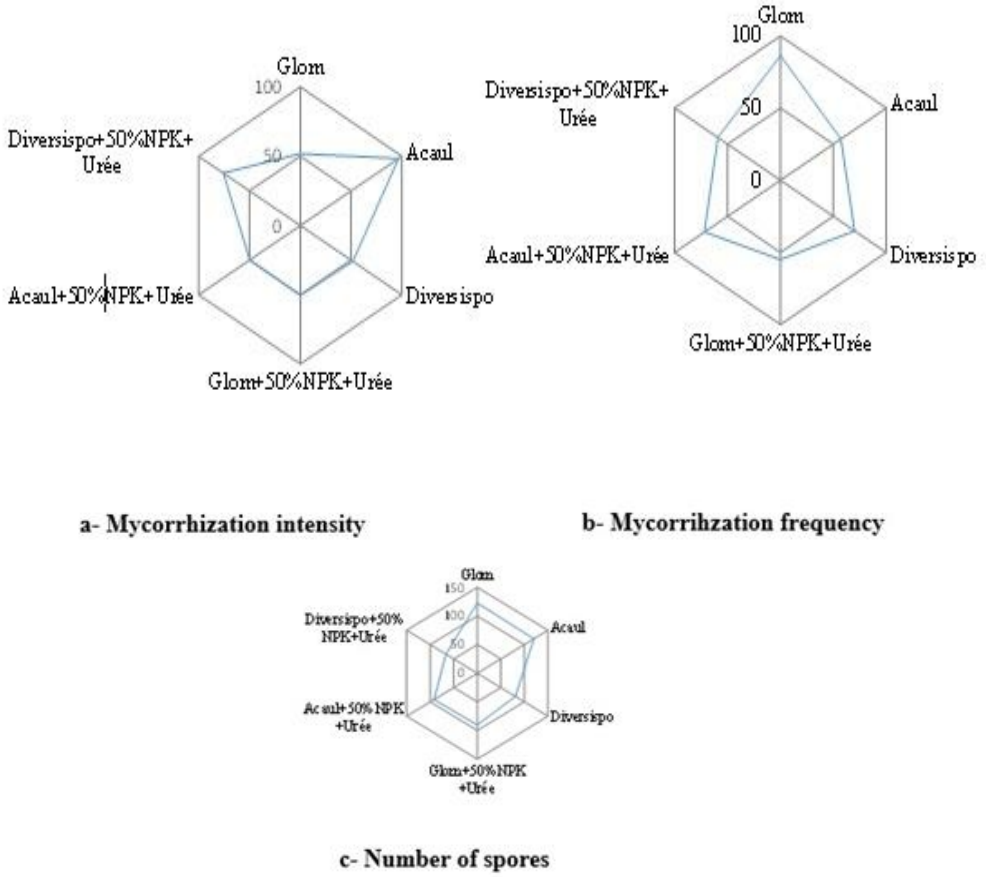

Figure 4: Variation in mycorrhization intensity (a) frequency (b) and the number of spores (c) according to the different AMF strains

Discussion

Plant growth parameters such as plant height, stem diameter and biomass are the external indicators of internal plant metabolism (Chen et al., 2017). In this study, the maize seeds inoculated with AMF induced improvement of growth parameters depending on the time and treatment. A significant difference ($P < 0.001$) was observed in height and number of leaves

while the noose diameters were similar and vary over time (P <0.001). The greatest heights, largest diameters and number of leaves were recorded with the plants that received the treatment Acaulosporaceae + 50% NPK-urea with respective increases of 20.55%, 11.77%, 17.04% compared to the control. These results could be attributed to the fact that experimental conditions might have been favourable to Acaulosporacea family since Walder and van der Heijden (2015) argued that several factors, such as environmental conditions and functional diversity, can affect nutrient exchange between the fungi and host plants. Similar results have been noted by Sery et al. (2016) who observed that *Acaulosporaceae colombiana* has significantly improved growth traits such as the leaf area, height of plants and biomass in greenhouse conditions.

The AMF used without addition of NPK in this study involved in the improvement of all growth parameters whereas maize plants that received Glomeraceae generated high values in height exceeding 1.17% those that grew under Acaulosporaceae treatment and 11.21% those produced with Diversisporaceae. Moreover, these same strains induced high values in noose diameters, leaf numbers and leaf areas exceeding respectively 9.20%, 2.30%, and 23.11% for Acaulosporaceae, with increases of 2.30 %, 2.27% and 15.46% for Diversisporaceae. These results show the beneficial effect of AMF on the various growth variables and are supported by those of several authors namely El yazeid et al. (2007), Laminou et al. (2009), Leye et al. (2015) who reported the beneficial effect of the symbiosis between AMF and plants in their development, growth and production. Cardenas (2010) reported in their studies that the plants inoculated with the AMF would be more effective in the use of soil nutrients, by involvement of AMF in improvement of plants growth (El-yazeid et al., 2007).

The plants treated with the combination Glomeraceae + 50% NPK + Urea induced similar effects on fresh above biomass in comparison to those of other treatments in one hand and a production of underground biomass significantly different of the same treatments (P <0.001) with a respectively increase of 54.97% and 42.94 compared to the control in the other hand. According to fresh underground biomass, it is clear that treatments mainly composed of AMF have generated similar values corresponding to an average of 136.45 with the exception of Glom_50NPKUrea and control that diverge by displaying respectively the maximum and minimum values of 234.18 and 104.83. This could be explained by the fact that AMF improved the production of photosynthetates leading both to accumulation of underground and above biomass (Chen et al., 2017). These results are higher than those obtained by Hamza (2014) which showed the positive effect of AMF on the biomass produced. Moreover, leaf is the main vegetative part of the plant supplying carbons' assimilates to developing organs and a measure of its productivity. The assimilates vary depending on leaf area that is an indicator of

photosynthetic capacity in chlorophyll site (Ogoke et al., 2003) and their translocation (Law-Ogbomo and Remison, 2007). In this study, one noted that treatments, only composed of AMF (Acaul., Glom. and others.), competed with mineral treatments (100NPKUrea and 50NPKUree)by producing a mean leaf area of 0.32 in comparison to the control and Acaul respectively recording a leaf area average of 0.19 and the highest leaf area average of 0.36. These results should be attributed to AMF that develops symbiosis with roots to obtain essential nutrients from the host plant and consequently provide mineral nutrients in return, for example, N, P, K, Ca, Zn, and S (Begum et al., 2019). For example, Garcés-Ruiz (2017) remarked that pi uptake rate was markedly improved in the AMF-colonized maize plants. Laminou et al. (2009) in Niger have also shown that AMF (especially *Glomus intraradices*) inoculation of plants positively impact total biomass performance.

In addition to growth parameters, we recorded during this study: high mycorrhizal intensity, and frequency with a large number of spores. These results are slightly higher than those obtained by Wang et al. (2015) and Jeong et al. (2015) that reported the mycorrhizal rate of 50% for rice seedlings inoculated with AMF in nurseries. This could also be an expression of interaction variations depending on the host plant and fungal strains (Lumini et al., 2011). Nevertheless, these results indicated that the level of AM-root colonization remains a weak indicator of plant growth benefits (Nunes et al., 2008) because it was not always consistent with the impact AM symbiosis has on plant growth yields (Guissou et al., 2016).

Conclusion

This study confirms the ameliorative effect of the combination of different AMF strains on different variables measured in function of time. Among the AMF combinations assessed, the combination of Glomeraceae without NPK was the most effective treatment followed by Acaulosporaceae. We recorded with Glomeraceae the best performance without adding NPK for most parameters evaluated. These results were more interesting when the strains were combined with NPK + 50% Urea, and the best combination was *Acaulosporaceae* + 50% NPK + urea. These results demonstrate the possibility of using these native AMF of maize rhizosphere in Benin as organic fertilizers to improve maize productivity.

Acknowledgements

We would like to thank the project of West Africa Agricultural Productivity Program (WAAPP) and the National Fund of the Scientific Research and the Innovation (FNRSIT) for their financial supports of this work.

Conflicts of Interest

The authors declare that there is no conflict of interests regarding the publication of this paper.

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