



## Effect of the combination *Rhizophagus intraradices* and 50% of NPK on maize (*Zea mays* L.) growth and yield in central Benin

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Article published on January 30, 2020

**Key words:** CMA, *Rhizophagus intraradices*, NPK, Yield, Maize

### Abstract

The growth and grain yield of maize (*Zea mays*), variety 2000 SYN EE-W were assessed in response to combined inoculation of native arbuscular mycorrhizal fungus (AMF) *Rhizophagus intraradices* with NPK fertilizer in rhizosphere of ferruginous soil of Benin. The experimental design was a randomized complete block of three treatments (CTL = Peasant Practice (without AMF, with mineral fertilizers); Ri<sup>1/2</sup>NPK = *R. intraradices* + 50%N<sub>15</sub>P<sub>15</sub>K<sub>15</sub>; NPK = 100% N<sub>15</sub>P<sub>15</sub>K<sub>15</sub>) with three repetitions. Each elementary plot was 4m length and 3.2m width. After opening a seed hole, two maize seeds previously coated or not with *Rhizophagus intraradices* strains the day before were put into the hole. Seeding was done by keeping a distance of 0.80m x 0.40m and a density of 31.250plants/ha. The maximum heights, larger diameters and leaf area, the highest dry aerial and underground dry biomass of plants, including the best yield in maize seeds were recorded with *R. intraradices* treatment combined with 50% of NPK with respective increases of 36.80%, 82.89%, 88.73%, 91.74%, 92.22% and 38.14% compared to the values obtained with Peasant practices. *R. intraradices* produced 2 spores/g of soil and hold averages of 51.67% and 6.17% respectively for mycorrhization frequency and intensity. These results portend the use of this strain as an alternative solution to increase the productivity of maize in Benin.

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## Introduction

In the next decade, the major challenge for agriculture will be sustainable production of sufficient quantities of food crops to meet global increasing demand (Battini *et al.*, 2017). The current agricultural systems rely heavily on the continued application of chemical inputs including chemical fertilizers, mainly nitrogen (N), phosphorus (P) and potassium (K) that contribute to the decline of land-based biological fertility (Gruhn *et al.*, 2000; Plenchette *et al.*, 2005). This excessive dependence creates many problems in soil, plants and human health through adverse effects on food quality, soil health, including air and water systems (Yang *et al.*, 2004). Therefore, increasing attention is being today paid to the downside of high-input agricultural systems and much research aimed developing alternative ways to produce sufficient food in a sustainable and environmentally sound way (Thonar *et al.*, 2017). Several and different approaches have been investigated with the intention to reduce fertilizer inputs into agroecosystems, including breeding plant varieties with better Phosphore acquisition efficiency (Lynch et Brown, 2001), fertilizer placement strategies (Dunbabin *et al.*, 2009), or application of soil organisms and natural extracts with plant growth-promoting potential (Agbodjato *et al.*, 2016 ; Nkebiwe *et al.*, 2016). Among the abundant microbiota associated with the root zone of the plant, Arbuscular Mycorrhizal Fungi (AMF) occupy a preponderant place. The Arbuscular mycorrhizal fungi (AMF, *Glomeromycota*) are important beneficial soil microorganisms establishing mutualistic associations with most food crops (Berruti *et al.*, 2016) especially maize by improving soil characteristics, thereby promoting plant growth in normal and stressful environments (Navarro *et al.*, 2014, Alqarawi *et al.*, 2014). Similarly, they reinforce the efficiency of nutrient uptake, removal of soil pathogens, tolerance to drought stress, salinity and heavy metals, and ameliorate biomass by the production of plant hormones and root morphology changes (Filho *et al.*, 2017). Indeed, AMF are obligate biotrophs that colonize host roots to obtain sugars in exchange of mineral nutrients, absorbed and translocated through a fine network of extra radical mycelium spreading

from colonized roots into the soil (Smith & Read, 2008). Thus, AMF emerge as the appropriate solution for the reduction of chemical fertilizers, insecticides and herbicides (Berruti *et al.*, 2016) with the enhancement of food crop yield (Caruso, 2018).

In Benin, some studies (Assogba *et al.*, 2017; Aguegue *et al.*, 2017) had involved implementing different combinations of exogenous strains of AMF in particular *Glomus cubens*, *Funneliformis mossae* and *Rhizophagus intraradices* with chemical fertilizers on maize. The combination *Rhizophagus intraradices* with 50% NPK recorded the best yield despite the risks of inadequacy incurred by the AMF (Duponnois *et al.*, 2013). These results interested us because this strain had already been identified in soils of Benin by Tchabi *et al.* (2008). Formerly named *Glomus intraradices*, *Rhizophagus intraradices* is an AMF that colonize a wide range of host plants with high rate of root colonization and a rapid generation of a high number of spores (Lenoir *et al.*, 2016). Dias *et al.* (2018) revealed that species with the same characteristics as *Rhizophagus intraradices* are suitable for a large scale production programs of AMF-based biofertilizers for use in agriculture and in maize monocultures particularly. The present study is carried out in order to valorize endogenous soil microorganisms in the improvement of soil fertility. Thus, the work aimed to assess the effect of the combination *Rhizophagus intraradices* with 50% NPK on the growth and the grain yield of maize in peasant farms in Benin.

## Material and methods

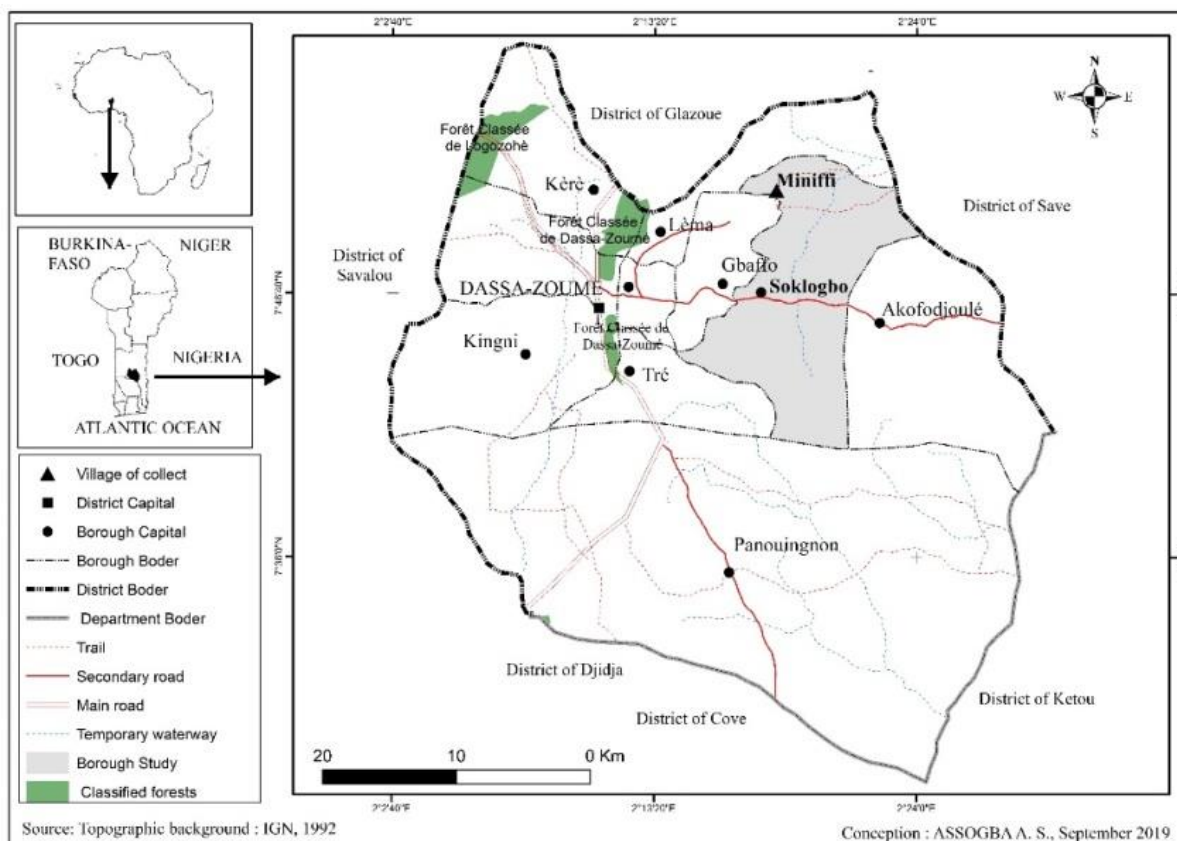
### Equipment

- The maize variety 2000 SYN EE-W was used during the experiment. It is an extra early variety, 75 days, developed by the International Institute of Tropical Agriculture (IITA) and the National Institute of Agricultural Research of Benin (INRAB). This is a variety with a good resistance to breaking, streak, american rust and tan spots. In addition, it tolerates drought and pests (MAEP, 2017).

- The mychorizal fungus *Rhizophagus intraradices* used was isolated and identified from the rhizosphere

of maize in the 6 agro-ecological zones in the departement of Collines in the centre Benin ( Dassa-Zoumé, Savalou, Savè, Glazoué, Bantè et Ouèssè) using the Davis- INVAM key. This mycorrhizal fungus was isolated and multiplied over a period of 12 weeks using *Sorghum bicolor* L. as host plant. The substrate used is clay and sterile soil at respective doses of 3/4 and 1/4 into a plastic pot (250 ml). The sorghum seeds were previously soaked for 2 min in a

sodium hypochlorite solution at 0.0024% and then rinsed five times in distilled water under vortex shaking (Gholami *et al.*, 2009). Then, 10 sorghum seeds were sowed in each pot containing different soil substrate dilution, in greenhouse at ambient temperature. Plants were watered daily with distilled water to the water capacity of substrate similar to that of the field for 6 weeks in the Laboratory of Biology and Molecular Typing in Microbiology of FAST / UAC.



**Fig. 1.** Geographical location of the experimentation site.

#### Location of experimentation site

This study was conducted in the village Miniffi belonging to Dassa-Zoumé municipality situated in the Centre of Benin. It is located between 7°50.4' north latitude and 2°10' east longitude. The city of Dassa-Zoumé is characterized by Guinean Sudan climate recording an annual rainfall patterns and large variations in temperature with an average around 28°C (Balogoun *et al.*, 2013).

#### Preparation of the experimentation site

Plots were flat (no more than 2% slope) and not flooded. Clearing preceded ploughing which has been made to a depth of 20cm with a hoe.

Each elementary plot had an area of 12.8m<sup>2</sup> (4m x 3.2m). It included 4 lines of 4m long. Seeding was done at a distance of 0.80m x 0.40m with a density of 31,250plants / ha (Hernandez *et al.*, 1995). Data were collected on both centre lines of the useful plot of 6.4m<sup>2</sup>.

#### Determination of chemical soil parameters

Soil samples were taken at 0-20cm; 20-40cm and 40-60cm deep (Adjanohoun *et al.*, 2011). The samples consisted of the realization of 500g composite samples in each plot before installing the essay after ploughing and before the supply of some fertilizer amendment.

These samples were sent to the Laboratory of Soil, Water and Environment Sciences (LSSEE) of the National Institute of Agricultural Research of Benin (INRAB). These analyses consisted of determining the soil pH using Boudoudou *et al.* (2009) method, organic carbon by the method of Walkley and Black (1934), available phosphorus (Bray, 1945), total nitrogen (Kjeldahl, 1883), and the exchangeable bases by Metson (1956) method in ammonium acetate at a pH equal to 7.

*Evaluation of the effect of the combination of Rhizopagous intraradices with a half dose of NPK and Urea on the growth and yield of farm's maize*

*Experimental design*

The trial was installed in three producer's areas. At each producer's area, the experimental design was a completely randomized com block of three treatments with three repetitions. Each treatment therefore covered three elementary plots (plot 1 x 3 repeats) separated by aisles of 1m wide. The treatments formulated were T1-peasant's Practice (farmer crop's technology); T2-*Rhizopagus intraradices*+ 50% NPK and Urea and T3-100 percentage of NPK and Urea.

*Seeding and inoculation of corn seeds*

After the completion of about 5cm depth of seeds holes, two maize seeds previously coated with the bio-fertilizers were deposited in and the hole immediately closed. For the coating of the seeds, 1kg of bio-fertilizer is mixed with 600 ml of distilled water to obtain a paste in which 10kg of seeds were added for mixing. The thus treated seeds were dried at surrounding temperature for 12h (Fernandez *et al.*, 2000).

*Maintenance of experimental plots*

➤ Application of mineral fertilizers: the day of sowing, a basal fertilizer at the dose of 200kg/ha of fertilizers (NPK) was applied in accordance with the experimental protocol. The 40<sup>th</sup> Day after Sowing (DAS), a maintenance fertilizer consisting of urea (40% N) was applied at the dose of 100kg/ha according to the experimental protocol.

➤ Weeding: Weeding was done 15 DAS and 40 DAS.

*Evaluation of growth parameters*

The height of maize plants, the noose diameter of the seedlings were registered from 12 plants of the two central lines of each elementary plot, every 15 days from the 15<sup>th</sup> DAS until the 60<sup>th</sup> (DAS). The height of a maize plant (distance between the snare and the last ligule) was measured using a graduated tape while the noose diameter and the leaf diameter were taken using a caliper to calculate the leaf area.

*Evaluation of performance settings*

The produced biomass and grain yield of maize were evaluated at harvest (80 DAS). The biomass of 12 maize plants (roots, stem, leaves and grains) per basic plot was determined by weighing using a precision scale (GOLDEN-METTLER-USA, NT2000xE, Max: 20000g x 0,1g).

*Biomass*

After harvesting the grain (80 DAS), maize plants were cut flush with the ground to form fresh ground biomass. The underground part (mainly roots) was dug up to form the fresh-ground biomass. The different biomass (depending on treatment) were weighed using a precision balance (Highland HCB 3001. Max 3000g x 0.1g). To determine dry biomass yield, 1kg of fresh biomass was baked at 100°C for 72 hours and then weighed to determine the weight of the dry biomass. The average yield of fresh and dry biomass of maize plants were determined according to the formula:

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

Whereas *R* is average yield of dry biomass of maize plants  $t.ha^{-1}$ ; *P* the dry biomass weight of maize plants  $inkg$ ; 10000 the conversion of  $ha$  in  $m^2$ ; *S* the harvest area ( $m^2$ ) and 1000 represents the conversion of  $ton$  ( $t$ )  $inkg$

*Yield of corn seeds*

To obtain grain yield, maize cobs were harvested and shelled per plant and per elementary plot. The moisture content of the grains was determined using a moisture meter (LDS-1E). The average yield value in grains of maize plants were determined by the formula used by Valdes *et al.* (2013):

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

Whereas *R* is yield of maize kernels expressed in *t. ha<sup>-1</sup>*; *P* represents the grain weight (kg); 10000 represents the conversion of ha in m<sup>2</sup>; *S* is the harvest area (m<sup>2</sup>); 1000 represents the conversion of tone (t) in kg and % *H* the percentage of grain moisture .

*Evaluation Endomycorrhizal Infection of Plant Roots*

Samples of maize roots were collected at 68<sup>th</sup> DAS using a holland auger. After coloring to Trypan blue according to the method described by Phillips and Haymain (1970), the AMF associated with the roots of maize plants were observed in the binocular (XSP-BM-2CEA. 2013). The estimation of mycorrhizal root infection was performed according to the method of intersection (Giovannetti and Mosse, 1980; Trouvelot *et al.*, 1986). The mycorrhizal infection rate was estimated by two parameters of arbuscular mycorrhizal infection which are:

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

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

Where *N* is the number of fragments observed and *n<sub>0</sub>* the number of fragments without a trace of mycorrhiza.

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

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

In this formula, *n<sub>5</sub>*, *n<sub>4</sub>*, *n<sub>3</sub>*, *n<sub>2</sub>* and *n<sub>1</sub>* are numbers of respectively noted fragments 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.

*Statistical analysis*

All analyses were performed with the software R (3.5.3) (R Core Team 2018). Repeated measurement ANOVA was performed on the height, diameter and

the values of nlme sheet (Pinheiro *et al.*, 2019). For aerial fresh biomass, underground fresh biomass and leaf area, we conducted multiple analyses of variance.

**Results**

*Chemical Characteristics of the Soil of the Experimental Site*

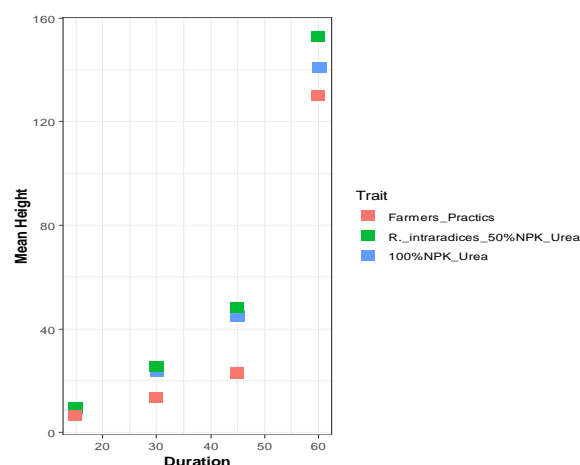
The chemical properties of soil before installation of fertilization essays (Table 1) showed in general that soil of Miniffi in central Benin is slightly basic (pH = 7.8) at the first two horizons and acid at the third horizon. The soil recorded an intermediate fertility level and are richer in Ca<sup>2+</sup> than potassium ions K<sup>+</sup>. The nitrogen to carbon ratio C / N is relatively low. The phosphorus content in topsoil (47,5mg/kg soil) is lower than the horizon 2 (55.02mg/kg of soil).

**Table 1.** Chemical characteristics of the soil of the experimental site.

| Site  | Depth (Cm) | pH (H <sub>2</sub> O) | C-org (G /kg) | N-total (G /kg) | P-Bray1 (Mg /kg) | Exchangeable bases (Cmol /kg) |                  |                |
|-------|------------|-----------------------|---------------|-----------------|------------------|-------------------------------|------------------|----------------|
|       |            |                       |               |                 |                  | Ca <sup>2+</sup>              | Mg <sup>2+</sup> | K <sup>+</sup> |
| Dassa | 0-20       | 7.80                  | 8.0           | 0.60            | 47.50            | 3.32                          | 2.31             | 2.21           |
|       | 20-40      | 7.50                  | 7.2           | 0.40            | 55.02            | 3.89                          | 2.72             | 1.48           |
|       | 40-60      | 6.90                  | 7.2           | 0.20            | 36,80            | 4.20                          | 3.15             | 2.48           |

*Effect of treatments on the growth of maize plants Height*

The fig. 2 shows the average plant height among the three producers. The results on height growth of seedlings showed that the type of treatment and the duration significantly influence growth in plant height (P <0.001).



**Fig. 2.** Average heights in function of time and the type of treatment in the three producer's sites.

We note that from the 45<sup>th</sup> to the 60<sup>th</sup> DAS, the plants have given strong growth in height regardless of the type of treatment. In addition, plants treated with the combination of *R. intraradices* + 50% Urea NPK-gave the greatest heights, followed by plants receiving 100% NPK-Urea. Cultural practices of producers also increase the height with time, but this growth is very low compared to the two others.

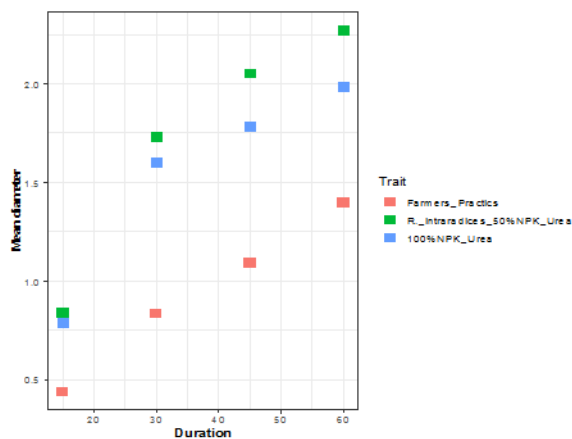
*Stem diameter*

The analysis of the results shows that the type of treatment and duration have a highly significant effect on diameter growth of plants ( $P < 0.001$ ) (Table 2).

**Table 2.** Repeated measurements analysis of variance (ANOVA) of the diameter depending on treatment and duration.

| Source of variation  | Degree of freedom | Chi-square | Pr (> Chi-square) |
|----------------------|-------------------|------------|-------------------|
| Treatments           | 2                 | 152.14     | <0.001            |
| Duration             | 1                 | 253.95     | <0.001            |
| Treatments *duration | 2                 | 6.14       | 0047              |

In opposition, the interaction and treatment duration significantly influence the diameter growth of plants. The fig. 3 shows a strong rapid diameter growth between the 1<sup>st</sup> and the 30<sup>th</sup> DAS. The larger diameters were recorded with plants treated with *R intraradices*- 50% NPK- Urea followed by those treated with 100% NPK-Urea. Cultural practices of producers also increase the diameter over time, but this growth is very low compared to the other two treatments.



**Fig. 3.** Average diameters over time and the type of treatment of three producer's sites.

*Leaf area*

The analysis of the results revealed (table 3) that only the type of treatment induced a highly significant variation on the leaf surface ( $P < 0.001$ ).

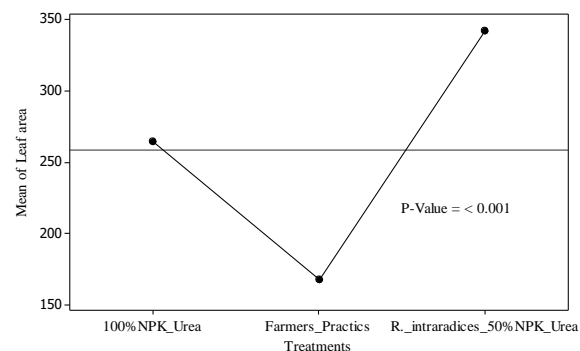
The fig. 4 shows that for 100% of NPK-urea, leaf area pass the average and when this treatment consists of *R. intraradices*- 50% NPK-Urea, a better average of the leaf area is achieved. The lower leaf area is recorded with the use of peasant farming practices.

**Table 3.** Analyses of mixed effect variance of leaf area based on treatments and producers.

| Source                 | Degree of freedom | F value | P-value |
|------------------------|-------------------|---------|---------|
| Producers              | 2                 | 2.70    | 0181    |
| Treatments             | 2                 | 133.99  | <0.001  |
| Treatments * Producers | 4                 | 0.56    | 0695    |

*Fresh aerial biomass*

The results of the mixed effect ANOVA of fresh aerial biomass showed a highly significant difference ( $P = 0.006 < 0.05$ ) between the three treatments applied (Table 3). Also, a significant difference ( $P = 0.031 < 0.05$ ) was observed between the various production sites. The Tukey test of average structuration showed that 50% *R\_intraradices*-NPK\_Urea is not different from 100% Urea NPK-but both are different from the farming practices ( $P = 0.005$  and  $0.034$  respectively). The analysis of fig. 5 shows that the largest fresh aerial biomass were recorded with *R. intraradices*-50% NPK-Urea.



**Fig. 4.** Average leaf area based on treatments.

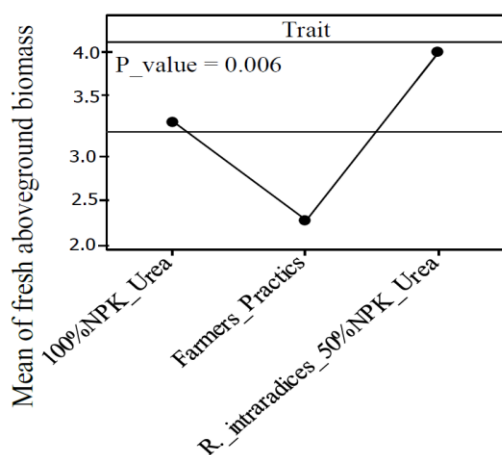
*Dry aerial biomass*

The analysis of the table 4 shows that the experimentation site of the three different producers and the type of treatment affect very significantly the fresh aerial biomass of plants ( $P < 0.001$ ).

According to the fig. 6, the producer treatment interaction does not influence the fresh aerial biomass. When the treatment given to plants consists of 100% of NPK-Urea, dry aerial biomass exceeds the average and when this treatment consists of *R. intraradices*- 50% NPK-Urea, the average of dry aerial biomass reached 4g. The lowest dry aerial biomass was obtained with plants of peasant practice treatment (Fig. 6).

**Table 4.** Mixed effect ANOVA of the fresh aerial biomass based on treatments and producers.

| Source                 | Degree of freedom | F value | P-value |
|------------------------|-------------------|---------|---------|
| Treatments             | 2                 | 12.14   | <0.001  |
| Producers              | 2                 | 31.61   | <0.001  |
| Treatments * Producers | 4                 | 1.30    | 0308    |



**Fig. 5.** Average of fresh aerial biomass based on treatments.

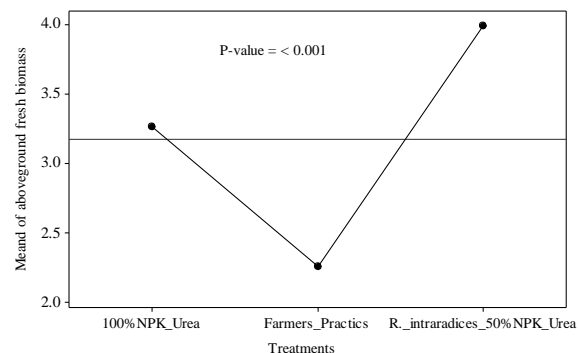
*Fresh ground biomass*

The analysis of the table 5 shows that only the type of treatment has a significant effect on the fresh ground biomass ( $P < 0.001$ ).

When the treatment that is given to plants is consisted of 100% of NPK-Urea, the average fresh-ground biomass is 0.255g and when this treatment consists of *R. intraradices*- NPK- 50% Urea, an important fresh ground biomass is noticed up to 0.45g (Fig. 7).

**Table 5.** Mixed effect ANOVA of the fresh ground biomass based treatments and producers.

| Source                 | Degree of freedom | F value | P-value |
|------------------------|-------------------|---------|---------|
| Producers              | 2                 | 1.48    | 0.330   |
| Treatments             | 2                 | 22.37   | 0007    |
| Treatments * Producers | 4                 | 0.80    | 0541    |



**Fig. 6.** Average of aboveground dry biomass based on treatments.

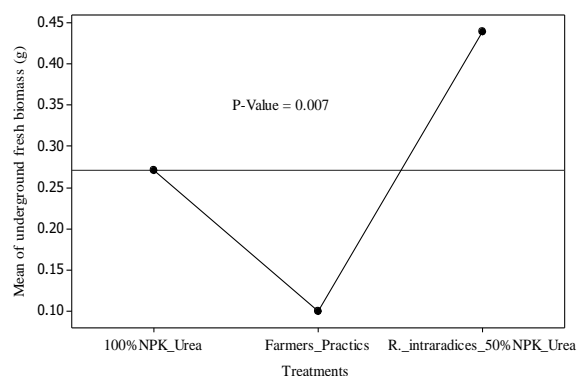
*Dry ground biomass*

Analysis of the results of the dry-ground biomass shows that the treatment did not induce a significant change at 5% of the fresh ground biomass of plants (Table 6).

The experimentation sites have led to a slight variation of this biomass beyond 5% (Fig. 8).

**Table 6.** Mixed effect analysis of variance of the dry ground biomass based on treatments and producers.

| Source                 | Degree of freedom | F value | P-value |
|------------------------|-------------------|---------|---------|
| Producers              | 2                 | 5.54    | 0070    |
| Treatments             | 2                 | 3.76    | 0120    |
| Treatments * Producers | 4                 | 1.31    | 0305    |



**Fig. 7.** Average fresh ground biomass based on treatments.

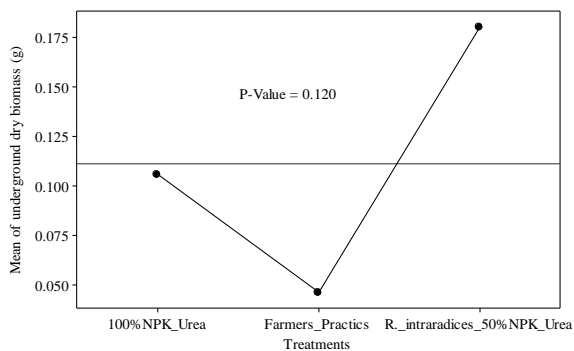
*Yield*

The analysis of the results (Table 7) shows that the producers of experimentation sites and the interaction treatment and producer experimentation sites does not affect the yield per hectare of production. Contrarily, the nature of the treatment has a significant effect on yield ( $P < 0.001$ ).

We remark in the fig. 9 that for 100% of NPK-Urea, the average production yield is 2.5t/ha and *R. intraradices*-50% NPK-Urea gives a yield around 3,4t/ha. The lowest production yield is achieved with the use of Peasant practices, about 1.5t/ha. Consequently, the best treatment *R. intraradices*-50% NPK-Urea which permits to obtain a yield close to 3.5t/ha.

**Table 7.** Mixed effect ANOVA based on treatments and producers.

| Source                 | Degree of freedom | F value | P-value |
|------------------------|-------------------|---------|---------|
| Producers              | 2                 | 3.50    | 0052    |
| Treatments             | 2                 | 23.77   | <0.001  |
| Treatments * Producers | 4                 | 0.48    | 0748    |



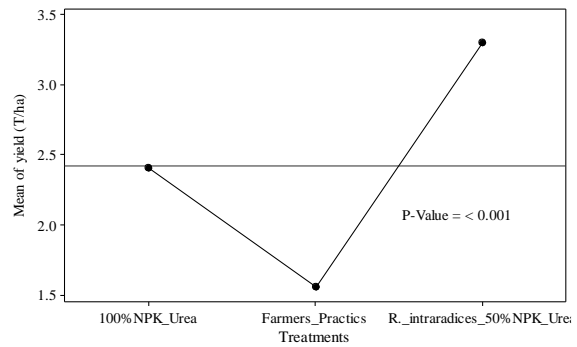
**Fig. 8.** Underground dry biomass average based on treatments.

*Mycorrhization traits*

Treatments have induced here an average of 2 spores per gram of soil (Table 8). The mycorrhization frequency displayed a mean of 51.67% whereas the mycorrhization intensity recorded a mean of 6,19%

**Table 8.** Spore number and mycorrhization frequency and intensity.

| Spore (g/sol) |           | Frequency (%) |           | Intensity (%) |           |
|---------------|-----------|---------------|-----------|---------------|-----------|
| Mean          | St. Error | Mean          | St. Error | Mean          | St. Error |
| 2.00          | 0.58      | 51.67         | 3.93      | 6.19          | 1.02      |



**Fig. 9.** Average yield based on treatments.

**Discussion**

For optimal growth of the plant, nutrients must be available in sufficient and balanced quantities (Yayeh and Melkamu, 2017). From initial analyses of the chemical parameters of the experimental soils, it appears that the site soil is alkaline with a low C / N ratio at the topsoil. This soil also has a medium level of available phosphorus. Igue *et al.* (2016) abounded in the same direction, stating that the available phosphorus is moderately high in the tropical ferruginous soils with concretions than hydromorphic ferruginous tropical soils. Moreover, the sum of exchangeable bases is low and reflects their low fertility in accordance with the work of Adjanooun *et al.* (2011). It is apparent that potassium took the lowest values compared to all other chemical elements measured (Table 1). The availability of nutrients and slow release by microorganisms of the small proportion that is accessible to them (Mahmoud *et al.*, 2009) require a supply of chemical or organic fertilizers (Yayeh and Melkamu, 2017) in an integrated approach to their wisely use (Bünemann *et al.*, 2006, Diagne *et al.*, 2016). It follows that the amount of nutrients that these soils contain is insufficient to meet the needs of maize plants (Yallou *et al.*, 2010). Thus, effects of fertilizer combination on maize involving *R. intraradices* were evaluated.

The heights gradually increased from the first to the 30<sup>th</sup> DAS, but we noticed a significant increase ( $p < 0.001$ ) for this parameter 45 DAS whatever the type of treatment used. In addition, fast growth of the noose diameter was observed from the first to 30th DAS with a significant difference ( $p < 0.001$ ) between the type of



treatment and duration. For the leaf area, it is noted that only the type of treatment induced a very significant difference ( $p < 0.001$ ). A demarcation of all the growth variables with the contribution of *R. intraradices* was observed with increases of 36.80%, 82.89% and 88.73%. It appears that the AMF have contributed to the improved results practically at the end of the cycle. These results are supported by those of Pharudi (2010) that found that more time was needed to see the effect of inoculated cm A on growing speculation like maize. Chukwukwa *et al.* (2017) have for their part, discovered that the combination AMF + NPK (30g AMF and 60g NPK) significantly improved plant height ( $133 \pm 8$ ,  $145 \pm 10$  and  $149 \pm 13$ cm respectively), the width of the leaves, the circumference of the stem and leaf chlorophyll content ( $0.1825 \pm 0.0007$ ml/g) unlike the six other treatments implemented during their studies on yam. It is not exaggerated to deduce that the deficiencies of nitrogen and phosphorous in soil are filled with NPK and urea inputs during the period preceding the 60 DAS (Figs. 1 and 2). Ilunga *et al.* (2018) have proved here that early application of NPK on maize led to a better development of vegetative parts of plants. They attributed this to the presence of nitrogen in the basal fertilizer. From the moment the development of plants at the beginning depends heavily on soil nutrients, early root colonization by the AMF could be a serious advantage (Bini *et al.*, 2018) particularly in Benin, where the poor nitrogen and phosphorus ferruginous soils with concretions dominate (Igue *et al.*, 2016).

Beyond making available nutrients for plants, the AMF are known to give plants a better ability to acquire water compared to non-inoculated plants (Bárzana *et al.*, 2012; Malonda *et al.*, 2019), causing thereby better growth of inoculated plants (Gnamkoulamba *et al.*, 2018a). Therefore, microorganisms could induce growth of more roots (Fig. 7). These roots may in turn produce more enzymes as regard to higher P availability in the second horizon (Table 1). Similarly, AMF in synergy with the bacteria induce the growth of plants, on the one hand, by stimulating the production of hormones such as auxins or cytokinins (McNear, 2013; Hart *et al.*, 2014) or hormone-like compounds (Sharma *et al.*,

2013) and on the other hand by phosphatase production. Indeed, the bacteria stimulate the AMF by the mechanism 'mycorrhiza helper' (Garbaye, 1994), thereby enhancing their ability to make available the Phosphore (Hussain *et al.*, 2013; Thonar *et al.*, 2017) from soil particles (Cassan *et al.*, 2013) and phosphoric fertilizers synthesis (Jastrzębska *et al.*, 2016).

Fresh underground and aboveground biomass also knew an increasing evolution during the crop cycle with a clear demarcation of the combination *R. intraradices*-50% NPK-Urea from 100% of NPK-Urea in one hand and farmer practices in a other hand. In 2015, Yagoob had evaluated the effect of mycorrhizal fungi on the phosphorus rate set on cucumber roots and observed that the fresh and dry weights had respective increases about 53.2 and 44.6% and also improved the phosphorus content in leaves when the soil was inoculated with *Glomus mosseae* and *Glomus intraradices*. At Ngaoundere in Cameroon, Megueni *et al.* (2011) had observed that the high AMF density in the soil tended to increase the water content and biomass of cowpea leaves. They noticed contrarily a decrease in mineral elements concentration in the leaves and they attributed it to the increase of the water balance (Bárzana *et al.*, 2012; Gnamkoulamba *et al.*, 2018a). Elsewhere, Malonda *et al.* (2019) found a decrease in dry matter content of mycorrhizal roots of cassava plants (26.1% in Kindisa and 30.76% in Liyayi) that appears to be specific to this speculation. Contrarily, Gnamkoulamba *et al.* (2018a, 2018b) found a rising trend for the measurements of the total dry biomass of rice and indicated a growing trend based on the inoculation dose.

Regarding maize yield in our study, analysis of fig. 8 shows that for 100% of NPK-Urea, the average production yield of 2.5t/ha and *R. intraradices*-50% NPK-Urea gives a yield of about 3,4t/ha (Fig. 8). These results are similar to those of Assogba *et al.* (2017) who obtained the best kernel yield with plants treated with the combination of *R. intraradices* - 50% NPK. This treatment induced an increase in grain yield of 38.14% compared to control. This may be due firstly to the synchronization between the release of

nutrients from fertilizers and their uptake by the plant because the decomposition of NPK is slow (Ilunga *et al.*, 2008) and the application of NPK had been done at sowing stage. Later, AMF established wide net of hyphae for their hosts (Nadeem *et al.*, 2014. Zhang *et al.*, 2017), making them benefit from increased access to nutrients with improved growth and yield (Hart *et al.*, 2014). Moreover, release of nitrogen from mineral fertilizers (Nyembo *et al.*, 2012 Cozzolino *et al.*, 2013) reinforced by the mobilization of certain trace elements and improved water balance of plants by the AMF allowed yield and biomass increases (Chen *et al.*, 2017,.. Mitra *et al.*, 2019) as nitrogen is the main limiting factor of the yield of cereal crops (Saïdou *et al.*, 2012; Batamoussi *et al.*, 2014). Ceballos *et al.* (2013) and Aboubacar *et al.* (2013) who observed increases respectively 20% and 51% for cassava and cowpeas through the predominance of phosphorus in the soil after AMF inoculation support these findings. Bakonyi *et al.* (2018) also found similar trends with wheat yields both with mineral fertilization (7.38- 8.31t ha<sup>-1</sup>) and the inoculation of the AMF (7.52 to 8.17t ha<sup>-1</sup>). This highlights all the benefit of the AMF characterized by the mycorrhization frequency and intensity, essential parameters for their activity evaluation.

An adequate balanced fertilization required adequate growing conditions of the plant for the hyphae initiation and conservation in both greenhouse crops (Balzergue *et al.*, 2013; Bonneau *et al.*, 2013) and agricultural practices on-farm (Verbruggen *et al.*, 2013.). In this study, the frequencies of mycorrhization of *Rhizophagus intraradices* in the maize-growing rhizosphere in Benin displayed an average of 51.67% very close to that found by Hounngandan *et al.* (2009) (53.58% and 63%) and higher than that obtained in Cameroon (1.66-20.5%) by Tobolbai *et al.* (2018). We can attribute the variations of these frequencies and intensities of mycorrhization not only to the type and origin of soil (Tobolbai *et al.*, 2018) but also to the AMF specie. Moreover, Thonar *et al.* (2011) and Ma *et al.* (2018) found a significant increase in *R. intraradices* whatever the experimental combinations in presence of the high fertilizer concentrations. This reflects the

resilience and tolerance of *R. intraradices* whatever are the soil conditions. The spore number, the percentage of root colonization and distribution of AMF are also affected by seasonal fluctuations in humidity, temperature, pH, and soil nutrients including N, P, K, Zn, Fe, etc. (Patale and Shinde, 2010a; Patale and Shinde, 2010b; Patale, 2016).

### Conclusion

This study revealed that the maize [*Zea mays* (L.)] was receptive to AMF. Among the three treatments evaluated, it appears that the combination that of *R. intraradices*-50% NPK-Urea gave the best results compared to other combinations. This result is a chance for agriculture in Benin that has one's ambition to be in a sustainable and environmentally sound way. The whole growth and yield parameters were significantly improved with *R. intraradices*. However, it is urgent to make this bio-fertilizer from the strains of *R. intraradices* that would show better ability to adapt to the soil and climate of Benin in order to improve grain yields in a sustainable farming approach and climate change. Clarification of mechanisms with new experiments including more diverse soils and combination with other bioeffectors like PGPR for fertilizer efficiency will give further insights about the window of conditions leading to successful use of cmA in agriculture.

### Acknowledgements

We would like to thank the project of West Africa Agricultural Productivity Program (WAAPP) 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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