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Organic Fertilizer Based on *Rhizophagus intraradices*: Valorization in a Farming Environment for Maize in the South, Center and North of Benin

*Ricardos M. Aguégué[1], Sylvestre A. Assogba[1], Hafiz A. A. Salami[1], Abdel D. Koda[1], Nadège A. Agbodjato[1,2], Olaréwadjou Amogou[1], Haziz Sina[1], K. Valère Salako[3], Nestor R. Ahoyo Adjovi[4], Gustave Dagbénonbakin[4], Romain Glélé Kakai[3], Adolphe Adjanohoun[4] and Lamine Baba-Moussa[1]**

[1] Laboratory of Biology and Molecular Typing in Microbiology, Department of Biochemistry and Cell Biology, Faculty of Sciences and Techniques, University of Abomey-Calavi, Cotonou, Benin, [2] Food Security and Safety Niche, Faculty of Natural and Agricultural Sciences, North-West University, Mmabatho, South Africa, [3] Biomathematics and Forest Estimation Laboratory, Faculty of Agronomic Sciences, University of Abomey-Calavi, Cotonou, Benin, [4] Scientific Management Department of the Institute, National Institute of Agricultural Research of Benin, Cotonou, Benin

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***Correspondence:**
Lamine Baba-Moussa
laminesaid@Yahoo.fr

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Maize plays an important role in agricultural production systems in all agro-ecological zones of Benin. Despite its importance, its production faces many constraints including soil fertility. One of the ecological technologies aimed at improving agricultural production is the use of soil microorganisms including arbuscular mycorrhizal fungi (AMF). This study aims to evaluate the effectiveness of *Rhizophagus intraradices*, an indigenous strain, on maize productivity in farmers' areas in the Research and Development (RD) sites of the North (Ouénou), Center (Miniffi), and South (Zouzouvou). Three maize producers were selected at each RD site, for nine maize producers. The experimental design was a randomized complete block of three treatments with three replications. The different treatments were (i) Control–farmer's practice, (ii) *R. intraradices* + 50% of the recommended dose of NPK and urea, and (iii) 100% of the recommended dose of NPK and urea. Soil samples from the different RD sites were taken at a depth of 0–20 cm before sowing for chemical analysis. The different growth parameters (height, crown diameter, and leaf area), grain yield, and endomycorrhizal infection of maize plants were evaluated. The results showed that the soils were moderately acidic ($5.5 \leq$ pH waters ≤ 6.8) and low in organic matter ($0.95 \leq 33$ OM ≤ 1.17) regardless of the study area. The greater maize grain yield was recorded with application of 100% of the recommended dose of NPK and urea, and *R. intraradices* + 50% of the recommended dose of NPK and urea. In the RD sites at the South, Center, and North recorded with *R. intraradices* + 50% of recommended dose of NPK and urea, the grain yields of 1.9, 3.4, and 1.74 t/ha with an increase of 28, 38.21, and 13.21%, respectively, compared with farmer's practice. Mycorrhization frequencies in plants treated with Ri½ $N_{15}P_{15}K_{15}$ vary between 37.44 and 51.67% in the three zones. The results of the current study have proven the potential use of *R. intraradices* in sustainable intensification of maize production in Benin.

Keywords: ***Rhizophagus intraradices*, farming environment, ecological systems, yield, maize, Benin**

INTRODUCTION

The decadent productivity of agricultural speculation, in general, and that of maize, in particular, in Benin, a West African country, could be justified by certain constraints including climate change, declining soil fertility illustrated by nitrogen and phosphorus deficiencies (Benbrahim et al., 2004; Balogoun et al., 2014) caused by poor farming practices such as slash-and-burn agriculture. In tropical and subtropical countries, phosphorus is essential because of its natural unavailability in soils (Hailemariam et al., 2013), and nitrogen (N) is a limiting factor for cereal crops (Batamoussi et al., 2014). In this context, the satisfaction of food needs, in general, and maize demands, in particular, will have to be based on productivity improvement. Thus, producers resort to intensive use of inputs, especially mineral fertilizers, which are leached and easily diluted into rivers, lakes, and streams with adverse consequences on the environment and the health of humans and animals. According to Alalaoui (2007), the prolonged use of mineral fertilizers without organic inputs leads to the depletion of soil organic matter, which is more sensitive to wind and rain erosion.

For these reasons, it is imperative to have an understanding of the processes underlying the bioavailability of soil nutrients to plants, as well as the soil–root interactions of microorganisms (Eisenhauer, 2017; Bi et al., 2018). One of the oldest and most widespread mutualistic associations of microorganisms concern symbiosis in which a particular soil fungi called arbuscular mycorrhizal fungi (AMF) colonize the roots of most (74%) of the terrestrial plant families (van der Heijden et al., 2015). These fungi belong to the family of glomeromycetes, which includes at least 313 characterized species. AMF are key elements of soil fertility (Bedini et al., 2018). Several examples suggest the use of AMF for the promotion of plant performance (growth, survival, and tolerance) because they improve nutrition (water and minerals), photosynthesis, protection against biotic and abiotic stresses, regulation of development processes (flowering, fruit formation, rooting, etc.), (Bedini et al., 2018), and participate in soil structuring (Alqarawi et al., 2014). However, the wide use of mycorrhizal inocula in agriculture remains a challenge due to their cost, variability, quality, and effects on the plant such as their incompatibility with high levels of phosphorus (P) in the soil (Usharani et al., 2014; Berruti et al., 2016).

However, knowledge and understanding of the mechanisms that govern the functioning of these AMF communities, particularly in poor tropical agrosystems where sustainable management of generally low soil nutrient resources, must take into account the benefits of indigenous microorganisms. Fortunately, with the advent of molecular biology, considerable advances in AMF identification and nomenclature have been noted (Oehl et al., 2011).

Although the importance of intraspecific plant diversity and AMF for ecosystem functioning has often been highlighted (Wall et al., 2015), the interactive influences on their respective and reciprocal performance are still not well-understood (Sendek et al., 2019).

Although work on AMF diversity and use is not legion in Benin (Tchabi et al., 2008; Balogoun et al., 2014), it is, nonetheless, oriented toward speculation other than maize in small geographical study areas and has mainly concerned exotic strains of AMF. However, Benin has indigenous strains of AMF in regions characterized by different types of climate and soils from North to South through the center and which could each present significant specificity to be taken into account in the analyses. The objective of this study is to evaluate the effect of the fungus *Rhizophagus intraradices* on maize growth and yield at three Research and Development (RD) sites in Benin.

MATERIALS AND METHODS

Materials

The maize variety 2,000 SYNEE-W was used during the experimentation at the different sites where the trials were carried out. It is an extra-early variety, 75 days old, developed by the International Institute of Tropical Agriculture (IITA) and the Institut National des Recherches Agricoles du Bénin (INRAB). It is a variety that presents a good resistance to the rot of the stem, to the maize streak virus (MSV) of the *Mastrevirus* genus, to the American rust, to the helminthosporiose caused by the *Cochliobolus heterostrophus* fungus. In addition, it tolerates drought (MAEP, 2016).

The mycorrhizal inoculum of the *Rhizophagus intraradices* species used was isolated, identified, and characterized on the basis of morphological criteria (diameter, color, ornamentation, thickness of the spore wall) from the rhizosphere of maize soils in different agro-ecological zones of Benin (Aguégué et al., 2021). This mychorizial fungus was preserved at the Laboratory of Biology and Molecular Typing in Microbiology of Faculty of Sciences and Techniques of University of Abomey-Calavi (FAST/UAC). The inocula were produced and multiplied by associating spores of *Rhizophagus intraradices* with sorghum seedlings. Sorghum seeds were disinfected in a bleach solution (5%), then rinsed and soaked in sterile distilled water for 24 h. Sorghum plants were grown in glasshouses in pots containing sterilized substrate consisting of a mixture of clay and peat (2:1 v/v), for 4 months to ensure good sporulation of the strains. After 4 months of cultivation, the inoculum, consisting of spores and root fragment mixture, was collected (Rivera et al., 2003).

Study Area

The tests were installed on Research and Development sites respectively in the South at Zouzouvou (Commune of Djakotomey), in the Center at Miniffi (Commune of Dassa) and in the North at Ouénou (Commune of N'Dali), (**Figure 1**). In each of the zones, the trials were set up at three (3) different producers. The choice of sites was made taking into account the fact that they are the sites of Research and Development programs and that declining soil fertility is a priority constraint. The sites are flat with a maximum 2% slope and are not flooded.

Determination of Soil Chemical Parameters

Soil samples were taken at a depth of 0–20 cm (Adjanohoun et al., 2011) at various sites in the South (Zouzouvou), Center (Miniffi), and North (Ouénou). A 500-g composite sample was collected at each site prior to the installation of the experimental

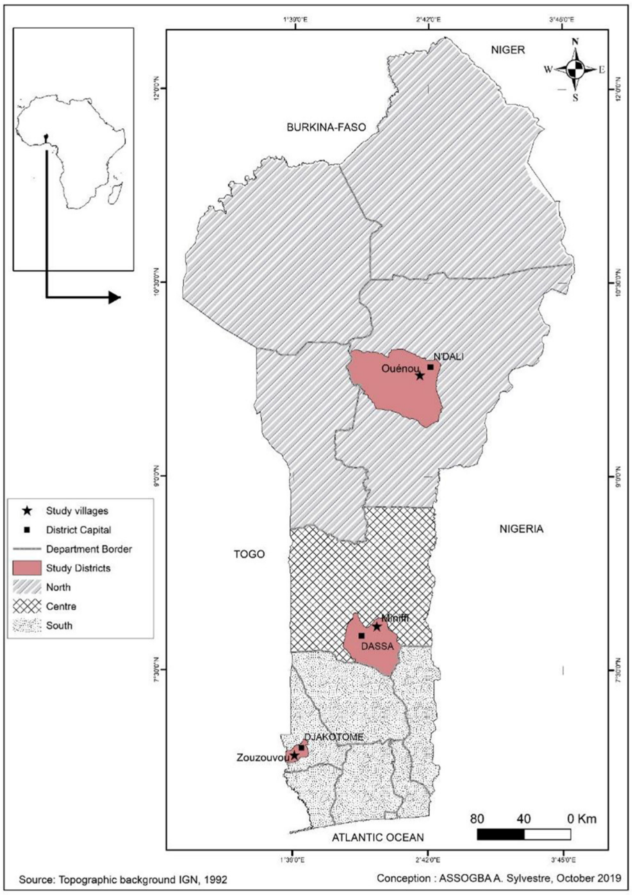

FIGURE 1 | Geographic location of experimental sites.

device. At each site, a mixture of soil samples was collected using an auger at a depth of 0–20 cm. Five (05) sampling points were randomly selected. Four (04) of the five (5) sampling points are each located on the four cardinal points (North–South–West–East). The fifth sampling point is located approximately at the junction of the four (04) preceding points. These samples were sent to the Laboratoire des Sciences du Sol, Eau et Environment (LSSEE) of the Institut National des Recherches Agricoles du Bénin (INRAB). These analyses consisted of the determination of pH (water), (by glass electrode in a soil/water ratio of 1/2.5), organic matter and carbon (Walkley and Black, 1934), assimilable phosphorus (Bray and Kurtz, 1945), total nitrogen (Kjedahl, 1883), and exchangeable bases by the method of Metson (1957) with ammonium acetate at a pH equal to 7.

Experimental Device

The plowing was done at a depth of 20 cm using a hoe on each site. Each 12.8 m² (4 × 3.2 m) elementary plot had four lines of 4-m long. The trials were installed at three growers on each site. At each grower, the experimental design used was a completely randomized block of three treatments with three replicates. Each treatment had, thus, covered three elementary plots (1 plot × 3 replicates) separated by alleys 1-m wide. The treatments were defined as follows:

- T1: Farmer's practice (control);
- T2: Ri½ $N_{15}P_{15}K_{15}$_Urea (*R. intraradices* + 50% of the recommended dose of NPK and urea);
- T3: $N_{15}P_{15}K_{15}$_Urea (100% of the recommended dose of NPK and urea).

Farmer's practice technique in the present study is characterized by the application of the recommended dose of mineral fertilizer $N_{15}P_{15}K_{15}$ on the 15th day after sowing and urea on the 45th day after sowing. However, for treatments T2 and T3, the mineral fertilizer $N_{15}P_{15}K_{15}$ was applied on the day of sowing, and urea was applied on the 45th day after sowing. The recommended rate of mineral fertilizer for the maize crop used in this study is 200 kg ha^{-1} of $N_{15}P_{15}K_{15}$ and 100 kg ha^{-1} of urea (46% N).

Sowing and Inoculation of Maize Seeds

Before sowing, the maize seeds were coated with the inoculum of *Rhizuphagus intraradices*. Seed coating was done according to the methodology described by Fernandez et al. (2000). A 1 kg of the inoculum was mixed with 600 ml of distilled water to obtain a paste to which 10 kg of seeds were added for mixing. The coated seeds were dried at room temperature for 12 h. Sowing was done in plots about 5-cm deep at a spacing of 0.80 × 0.40 m, i.e., a density of 31,250 plants/ha (Hernandez et al., 1995). On the day of sowing, a bottom manuring at the rate of 200 kg/ha of fertilizer ($N_{15}P_{15}K_{15}$) was applied in accordance with the experimental protocol. Then, on the 40th day after sowing (DAS), maintenance manure consisting of urea (40% N) was applied in accordance with the experimental design. Data for the various growth, yield, and mycorrhization parameters were collected on the two central lines of the 6.4-m² working plot at each site.

Evaluation of Growth Parameters

The height of the maize plants and the diameter at the collar of the plants were collected on 12 plants from the two central lines of each elementary plot, every 15 days from DAS until the 60th DAS on the different RD sites. The height of a maize plant was measured with a graduated ruler, the diameter was measured with a caliper at the plant collar, and the leaf area was estimated at the 60th DAS by the product of the length and width of the leaves multiplied by 0.75 (Ruget et al., 1996).

Evaluation of Grain Yield

Corn grain yield was assessed at harvest (80th DAS). Corn cobs were harvested, dispatched, and shelled per plant and per elementary plot. Grain moisture percentage was determined using a moisture meter (LDS-1F). The average grain yield values of the maize plants were determined by Equation 1 used by Ferro Valdés et al. (2013):

$$\mathbf{R} = \frac{\mathbf{P} \times \mathbf{10.000}}{\mathbf{S} \times \mathbf{1.000}} \times \frac{\mathbf{14}}{\mathbf{\% \, H}} \tag{1}$$

Where,

R = the average maize grain yield in t ha^{-1};

TABLE 1 | Chemical characteristics of the study soil.

Characteristics	pH (water)	OM (%)	*P*-ass (ppm)	C/N	Sum of exchangeable bases (meq/100 g)	CEC (meq/100 g)
Miniffi	6.20	1.16	11.75	13.33	7.84	8
Ouénou	5.54	0.95	6.91	6.88	1.82	4.64
Zouzouvou	5.6	1.17	2	10.75	2.47	6.5

$pH_{(water)}$; OM, organic matter; P-ass, assimilable phosphorus; C, carbon; N, Nitrogen; CEC, cation exchange capacity.

TABLE 2 | ANOVA results of height and collar diameter.

Sources of variation	Height		Collar diameter	
	F-value	*p*-value	*F*-value	*p*-value
(Intercept)	334.5508	<0.0001	1,148.9774	<0.0001
Time	**140.5530**	**<0.0001**	**128.8457**	**<0.0001**
Treatment	0.1180	0.8888	**74.4053**	**<0.0001**
Areas	**56.2697**	**<0.0001**	**4.3320**	**0.0161**
Time:treatment	1.3592	0.2622	0.2867	0.7514
Time:areas	**22.5223**	**<0.0001**	**4.0523**	**0.0207**
Treatment:areas	1.5527	0.1941	**20.1609**	**<0.0001**
Time:treatment:areas	0.5470	0.7017	0.7745	0.5447

The bold values show the significance level of the different variables under study.

P = the maize grain weight in kilograms (kg);
S = the harvest area in m², and
% H = the grain moisture content in %.

Evaluation of Endo Mycorrhizal Infection of Maize Plant Roots

Corn root samples were collected at the 80th DAS. After staining with trypan blue according to the method described by Phillips and Hayman (1970), arbuscular mycorrhizal fungi associated with maize plant roots were observed with binoculars (XSP-BM-2CEA. 2013). Estimation of mycorrhizal infection of roots was carried out according to the intersection method described by Giovanetti and Mosse (1980). The rate of mycorrhization was estimated by two parameters of arbuscular mycorrhizal infection described by Trouvelot et al. (1986), namely: (i) mycorrhization frequency (F), which reflects the degree of infection of the root system, and (ii) mycorrhization intensity or absolute mycorrhization intensity (m), which expresses the portion of the colonized cortex in relation to the entire root system, calculated according to Equations 2, 3, respectively.

$$F(\%) = \frac{(\mathbf{N}-\mathbf{n_o})}{\mathbf{N}} \quad (2)$$

where

N is the number of fragments observed and
n_o is the number of fragments with no trace of mycorhization;

$$\mathbf{m}(\%) = \frac{\mathbf{95n_5 + 70n_4 + 30n_3 + 5n_2 + n_1}}{\mathbf{N - n_o}} \quad (3)$$

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

Statistical Analysis

Data per site for all parameters assessed were collected. Mixed-effect linear models on longitudinal data were fitted to evaluate the effect of treatments and area on plant growth parameters (height and crown diameter). In each model, treatments and zones were considered fixed factors, and time was considered random. Adjusted averages were also calculated to represent trends in each growth parameter at the treatment and zone level. These analyses were performed using the nlme (for model fitting), and it means (for the calculation of adjusted averages) packages. Descriptive statistics were calculated for each growth parameter measured.

In order to assess the effect of treatments and zone on yield and leaf area, it was evaluated using a two-criteria analysis of variance (treatment and zone). The Shapiro-Wilk and Levene tests (Glèlè Kakaï et al., 2006) were performed to verify the conditions of normality and homoscedasticity of the data required for ANOVA. As the experimental design was balanced, the ANOVA type II test was adopted. Once the ANOVA test was significant, a pairwise comparison *post-hoc* test using the Tuckey's *post-hoc* test (Douglas and Fligner, 1991) was performed to assess the statistical differences in the means. In addition, descriptive statistics were calculated for each measured parameter. These analyses required the use of the dplyr and DescTools packages for the calculation of descriptive statistics, the ggplot2, and ggpur packages for the mustache boxes, the stats package for the Shapiro test and Levene test, the car package for the ANOVA, and the multcomp package for the pairwise comparison *post-hoc* test.

The significance threshold retained is 5%, and all the different tests were performed in R 4.0.2 software (R Core Team, 2020).

RESULTS

Chemical Characteristics of the Soil

The soil chemistry characteristics of the R&D Sites are presented in **Table 1**. The soil water pH in Zouzouvou (pH = 5.6), Ouénou (pH = 5.5), and Minifi (pH = 6.2) is acidic. Organic matter varies between 0.95 and 1.17%, while assimilable phosphorus has a respective value of 2 ppm at Zouzouvou, 11.7 ppm at Miniffi, and 6.9 ppm at Ouénou. Exchangeable bases vary between 1.82 and 7.84 meq/100 g of soil.

Effect of the Mycorrhizal Fungus *Rhizophagus intradices* on the Growth of Maize Seedlings

The results of the analysis of variance show that the height variations observed at the plant level depend only on the area (p-value < 0.0001) and over time (**Table 2**).

The evolution of these average plant heights over time and by treatment are presented graphically by zone (**Figure 2**). From the figure, it appears that whatever the zone, the growth in height of the plants reaches its maximum around 60 days after sowing. The highest maize plant heights were observed in the south regardless of treatment. However, in the Center and North, the best heights were observed, respectively, at the level of plants treated with Ri½ $N_{15}P_{15}K_{15}$_Urea (T2) and $N_{15}P_{15}K_{15}$_Urea (T3), (**Figure 2**).

The results of the analysis of variance show that the variations in collar diameter observed at the plant level depend on the treatments (p-value < 0.001) and on the zone (p-value $= 0.016$). However, these variations over time do not depend on the treatments (p-value $= 0.751$) but rather on the zone (p-value < 0.001), (**Table 2**).

The curves of the evolution of the diameter at the collar of the plants over time and by treatment show these variations by zone (**Figure 3**). Thus, in general, and whatever the zone, the growth in diameter of the plants reaches its maximum around 60 days after sowing. The treatment Ri½ $N_{15}P_{15}K_{15}$_Urea (T2) followed by $N_{15}P_{15}K_{15}$_Urea (T3) induced the largest collar diameters (**Figure 3**) in the Center, while the largest collar diameter values were recorded with treatments Farmer's practice (T1) and $N_{15}P_{15}K_{15}$_Urea (T3) in the South and North, respectively.

From the analysis in **Figure 4**, it appears that the plants in the southern zone perform best for most treatments. The results of the analysis of variance show a significant difference in the effects of the interaction between treatment and zone (Df $= 8$, p-value $= <0.001$) on the leaf area of the plants. It can be deduced that the variation in plant leaf area per treatment depends on the experimental area. The Tuckey's test performed (**Figure 4**) shows the difference in performance between the treatments according to each zone. Thus, the treatment $N_{15}P_{15}K_{15}$_Urea (T3) in the South zone gives the best performance. Next comes the treatment Ri½ $N_{15}P_{15}K_{15}$_Urea (T2) and treatment Farmer's practice (T1) in the southern zone, followed by the treatment $N_{15}P_{15}K_{15}$_Urea (T3) in the northern zone. Treatments Ri½ $N_{15}P_{15}K_{15}$_Urea (T2), and Farmer's practice (T1) in the northern zone have similar performances as the treatment $N_{15}P_{15}K_{15}$_Urea (T3) in the central zone. The treatment Farmer's practice (T1) in the central zone, gives a low performance.

Effect of the Mycorrhizal Fungus *Rhizophagus intradices* on Maize Grain Yield

The analysis in **Figure 5** shows that the plants in the Southern and Central zone perform best for most treatments. The results of the analysis of variance show a significant difference in the effects of the interaction between treatment and zone (Df $= 8$, p-value $= <0.001$) on plant grain yield. This suggests that the variation in plant grain yield per treatment depends on the experimental area. The Tuckey's test performed (**Figure 5**) shows the difference in performance between the treatments according to each zone. Thus, the treatment $N_{15}P_{15}K_{15}$_Urea (T3) in the South and Central zone gives the best performance (3.3 t ha^{-1}). Next comes the treatment Ri½ $N_{15}P_{15}K_{15}$_Urea (T2), (2.8 t ha^{-1}) and the treatment Farmer's practice (T1) in the South and Central zones. The treatment Ri½ $N_{15}P_{15}K_{15}$_Urea (T2) in the North zone has similar performances as the treatment $N_{15}P_{15}K_{15}$_Urea (T3). Of all the zones, the treatment Farmer's practice (T1) in the North zone gives the lowest performance (1.6 t ha^{-1}).

Effect of the Mycorrhizal Fungus *Rhizophagus intradices* on the Mycorrhization of Maize Plant Roots

Mycorhization frequencies in plants treated with the treatment Ri½ $N_{15}P_{15}K_{15}$_Urea (T2) vary between 37.44 and 51.67% in the three zones (**Figure 6**). Mycorhization intensity varies between 6.19 and 27.02%. It should be noted, however, that we did not observe mycorhization at the root level of the plants treated with the treatment Farmer's practice (T1) and the plants treated with the treatment $N_{15}P_{15}K_{15}$_Urea (T3). The results of the analysis of variance of mycorhization intensity and frequency in the three study areas revealed a significant difference ($p < 0.001$) in mycorhization intensity between the study areas, while the frequency of mycorhization was similar in the different study areas. The contact of the line with a given arc represents the mean value, while the horizontal line indicates the median. Thus, the high intensity of mycorhization is observed in the South of Benin. The intensities of mycorhization in the Center and in the North are similar.

DISCUSSION

The chemical characteristics of our study soils show that organic matter varies between 0.95 and 1.17%, while assimilable phosphorus had a respective value of 2 ppm in the South, 47.5 ppm in the Center, and 6.7 ppm in the North. Exchangeable bases varied between 1.82 and 7.84 meq/100 g of soil. The chemical characterizations show that the experimental soils in the South, Center, and North were acidic and moderately poor (Sys et al., 1993). Moreover, soil contents in organic matter, phosphorus, and exchangeable bases are good for an expression of the effects of NPK mineral fertilizers (Igué et al., 2015). The water pH of the study soils varies between 5.5 and 6.20, which shows that our study soils are acidic. According to Davet (1996), AMF are preponderant in acidic soils. The pH influences the activity of soil microorganisms that participate in the mineralization of organic matter as well as that of mycorrhizal fungi (Parent and Gagné, 2010). Coughlan et al. (2000) demonstrated a positive correlation of pH with the quality and quantity of intra-root colonization. They stated that mycorrhizal colonization is high at pH levels between 5 and 7, but low at pH levels around 4,

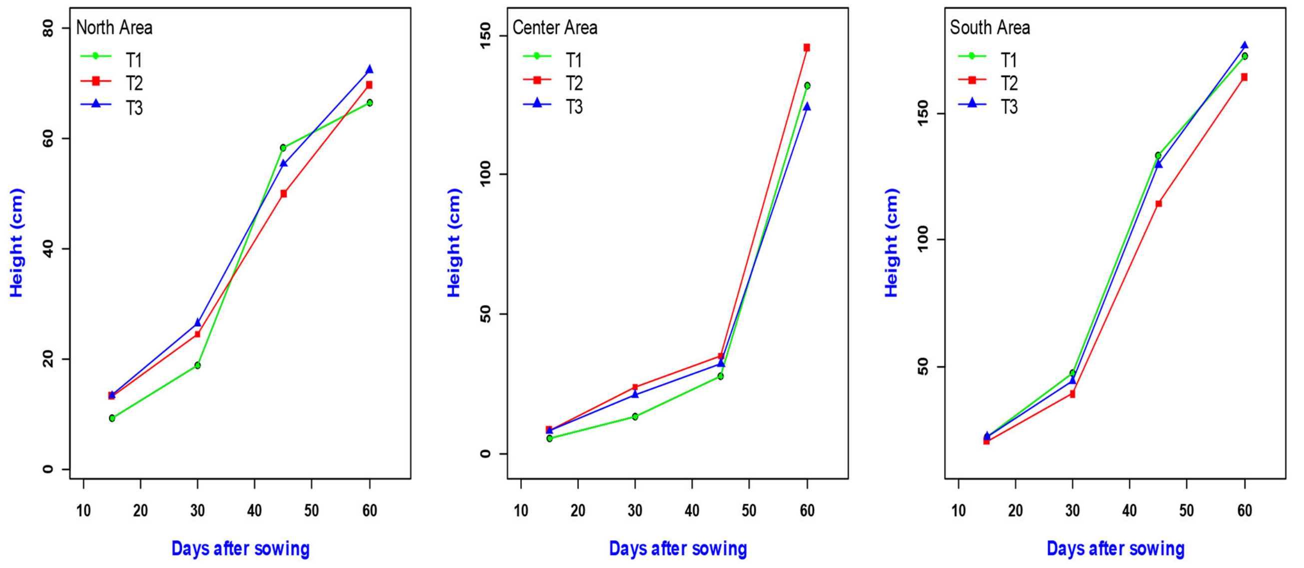

FIGURE 2 | Height variation by treatment and study area.

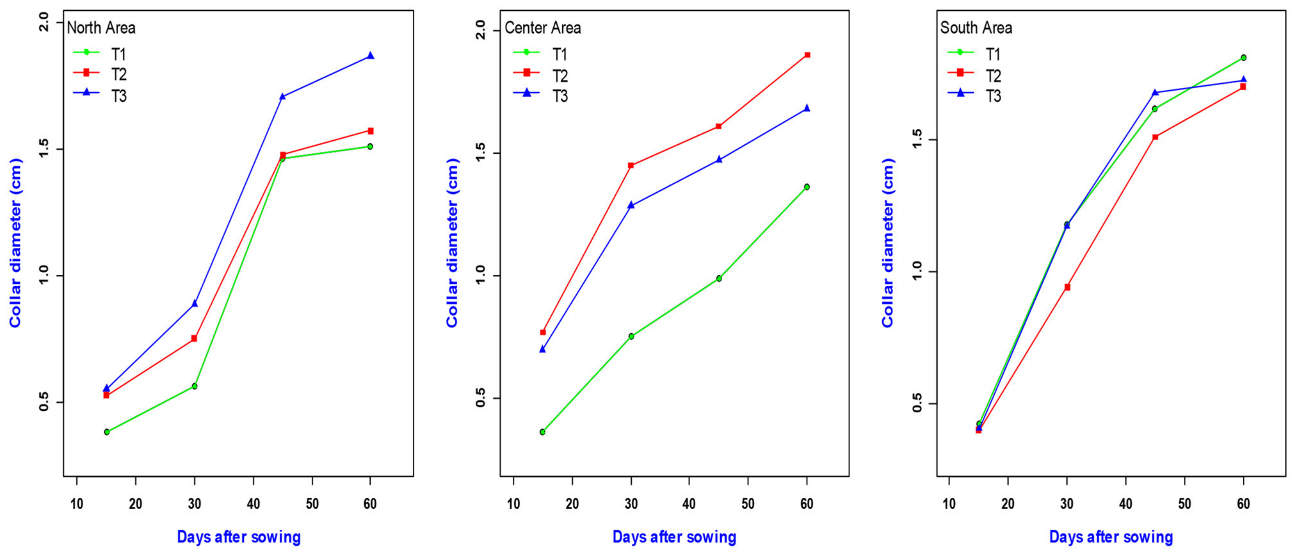

FIGURE 3 | Variation in collar diameter as a function of different treatments and study areas.

as the adaptation of fungi to different pH levels varies between species. In soils with pH between 7 and 7.6 *R. intraradices* colonizes more the roots of plants to stimulate their growth.

The contribution of arbuscular mycorrhizal fungi had a significant effect on maize plant growth. Indeed, the application of Ri½ $N_{15}P_{15}K_{15}$_Urea induced better growth of maize plants compared with the Farmer's practice and those receiving $N_{15}P_{15}K_{15}$_Urea in Southern Benin, while in Central and Northern Benin, it was the Ri½ $N_{15}P_{15}K_{15}$_Urea and $N_{15}P_{15}K_{15}$_Urea treatments that generated the greatest growth variables. These results are in agreement with those obtained by Ndoye et al. (2016) in Senegal who revealed that inoculation with arbuscular mycorrhizal fungi (*Glomus manihotis*) significantly improved the growth (+28.5%) of fonio (*Digitaria exilis* Stapf) under semi-controlled conditions. Also, (Sánchez-Roque et al., 2016) also observed a positive effect of AMF inoculation on three pepper varieties.

As for corn grain yield, the results of the analysis of the means revealed a significant difference. Ri½ $N_{15}P_{15}K_{15}$_Urea had generated the highest yields in the Center, while $N_{15}P_{15}K_{15}$_Urea had induced the highest yields in the South. However, there was no significant difference between the three treatments in the North with low performance. Indeed, grain yields

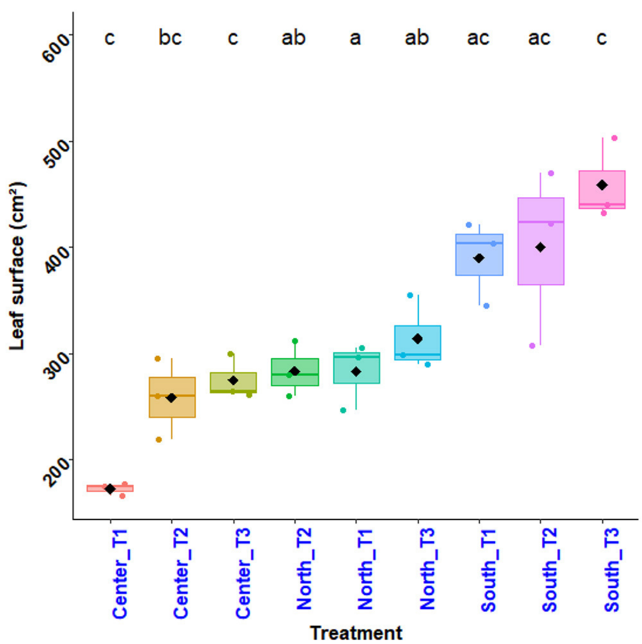

FIGURE 4 | Variation in maize plant leaf area as a function of treatments.

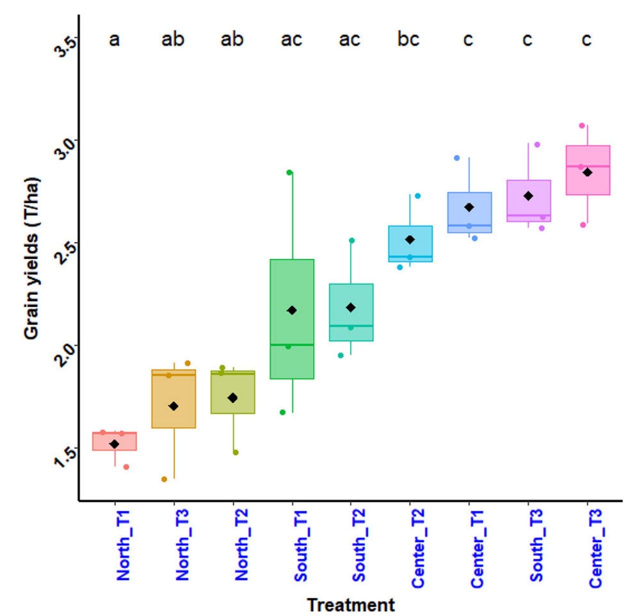

FIGURE 5 | Corn grain yield variation by treatment.

obtained in the South was 1.95 t ha^{-1} with the contribution of Ri½ $N_{15}P_{15}K_{15}$_Urea. These grain yield values are 28% higher than the grain yield obtained from the plants treated with Farmers' practices (without AMF, with $N_{15}P_{15}K_{15}$_Urea). Plants that received $N_{15}P_{15}K_{15}$_Urea in Central Benin had an average yield of 2.5 t ha^{-1}, and those that received Ri½ $N_{15}P_{15}K_{15}$_Urea yielded around 3.4 t ha^{-1}. In North Benin, there was no significant difference between the three treatments. These differences in yield are due to several factors such as the functional diversity of AMF and environmental conditions as so well-noted by (Walder and Van Der Heijden, 2015).

Grain yield was higher on ferruginous soil in the Center compared with yields in the North and South. This is explained by a good level of assimilable phosphorus (11.75 ppm), which was higher than in the ferruginous soils of the North (6.91 ppm) and ferralitic soils of the South (2 ppm) in this study. In addition, the soils of Central Benin have an organic matter rate of 1.16% and a pH in water (6.2) allowing a good expression of *R. intraradices*, which shows a good colonization of plant roots in soils with a pH between 7 and 7.6. Rivera et al. (2003) in Cuba, Assogba et al. (2017), and Aguégué et al. (2021) in Benin also observed a 35–50% increase in maize yields compared with the control (without AMF or mineral fertilizers) following the application of $N_{15}P_{15}K_{15}$_Urea, which created a commercial strain. It should be noted that other factors such as temperature and pest attacks may explain the differences in yield observed from one area to another. According to the work of Hasanuzzaman et al. (2013), high temperatures in the northern zone of the country affected plant growth and development through mechanisms described by delayed growth rate, drop in biomass production, leaf and reproductive organ burning, leaf abscission and senescence, fruit damage and, in turn, yield reduction and cell death. Through symbiosis, AMF established mycorrhiza with their hosts (Nadeem et al., 2014; Zhang et al., 2017) and reduced drought-related consequences (Yooyongwech et al., 2016; Moradtalab et al., 2019). Thus, hosts benefited more often from increased access to nutrients with improved growth and yield (Hart et al., 2014; Liu et al., 2016; Chen et al., 2017). Also, the release of nitrogen from mineral fertilizers increased yield and its components (Torbert et al., 2001; Nyembo et al., 2012). Bakonyi and Csitári (2018) made the same observations, showing that AMF inoculation increased wheat grain yield from 7.52 to 8.17 t ha^{-1} in the same way as mineral fertilization (7.38 to 8.31 t ha^{-1}).

The frequency of mycorhization of maize roots in this study was low. Root colonization of maize plants ranged from 6.19 to 27.02%. These values are low in comparison with the work of Rivera et al. (2003) and Tian et al. (2013), which showed a 76–80% colonization of maize roots as a result of the combination of arbuscular mycorrhizal fungi with the recommended half-dose of mineral fertilizer during and after moderate states of hydric stress. Ndoye et al. (2016) observed the highest mycorhization frequencies and intensities of fonio root mycorhization with *G. aggregatum* and *R. irregularis*. On the other hand, Incesu et al. (2015) observed higher rates of root colonization of *Diospyros virginiana* with *R. irregularis* and *G. caledonium* compared with other AMF species (*G. etunicatum*, *Funneliformis mosseae*, and *G. clarium*). However, it is important to note that above 12% mycorhization intensity, the benefits derived by the plant symbiote are not negligible (Diagne et al., 2013).

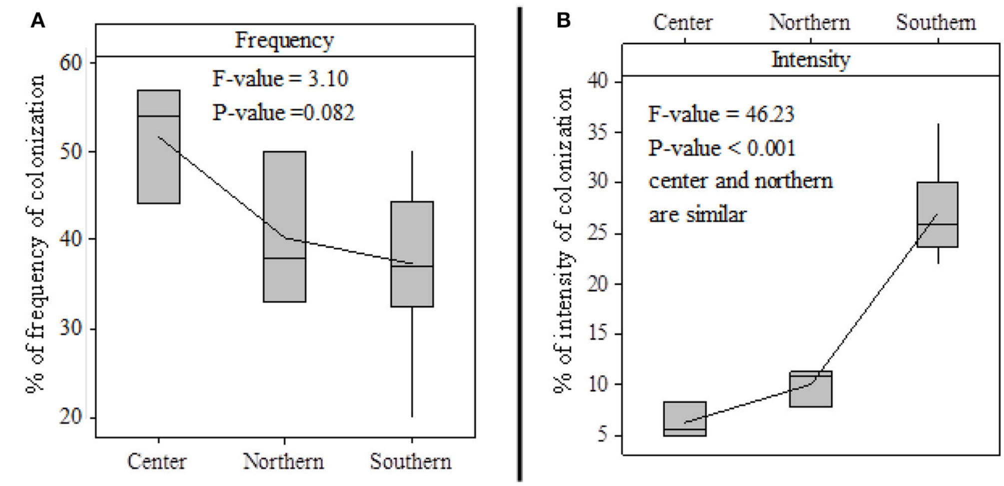

FIGURE 6 | Boxplot of mycorrhization across study areas: frequency of colonization **(A)**; intensity of colonization **(B)**.

CONCLUSION

The results of this study showed that Ri½ $N_{15}P_{15}K_{15}$_Urea had a positive impact on all the variables of grain growth and yield while reducing by half the use of mineral fertilizers at the different Research and Development Sites of Ouénou, Miniffi, and Zouzouvou. These results show that mycorhization of maize could play an important role in improving the growth and yield of maize plants and, thus, contribute to the development of methods that are environmentally friendly and guarantee sustainable agriculture in Benin. Further work is needed to better understand the behavior of AMF on maize growth and productivity, and soil fertility management in a large number of producers in order to make recommendations on the use of these fungus-based biofertilizers.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

AUTHOR CONTRIBUTIONS

This work was carried out in collaboration with all authors. RA, SA, HSa, AK, NAg, and OA conducted the trial set-up, data collection, and harvesting. RA wrote the first draft of the manuscript and managed the bibliographical research. KS with RGK performed the statistical analysis. NAh, HSi, GD, AA, and LB-M wrote the protocol, managed the study analyses, and supervised the various activities. All authors read and approved the final manuscript.

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