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Determination of Mycorrhization Under Field-Fertilized Maize and Sorghum Crops in Koumantou, Bougouni Region, Southern Mali

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A B S T R A C T

Mali, facing drought and experiencing population growth, possesses significant agricultural potential. Cereals, particularly sorghum and maize, are produced and contribute to the country's food security. Soils are becoming increasingly infertile and are in dire need of amendments, including chemical fertilizers, which are costly and environmentally harmful. However, improved productivity is possible through the use of plant inoculation with high-performing strains of arbuscular mycorrhizal fungi (AMF). To this end, these AMF were applied to sorghum and maize crops at the time of sowing in the field in Koumantou. After 70 and 100 days of cultivation for maize and sorghum, respectively, mycorrhization parameters, namely frequency and intensity, were assessed. The results showed that the introduced fungi appeared to be effective in root colonization, with a mycorrhization frequency of 86.25% and an intensity of 24.21% in maize. In the field, the introduced mycorrhizal fungi showed low mycorrhization intensities in both crops, particularly in sorghum. Despite this low root colonization rate by arbuscular mycorrhizal fungi (AMF), the high mycorrhization frequencies induced some growth and, consequently, a noticeable yield compared to traditional farming practices using chemical fertilizers.

Introduction

Mali, a landlocked Sahelian country in West Africa, is experiencing rapid population growth of 3.6% (RGPH, 2019). Significant fluctuations in its cereal production, linked to the late start of the rainy season and its poor distribution, appear to be major issues (Traore *et al.,* 2000). Nowadays, declining soil fertility, and consequently reduced crop yields, has become a major constraint in all ecosystems.

In the fields surrounding the village, cultivated plots located in the immediate vicinity of dwellings, soil fertility is partially improved by the addition of organic fertilizers through the practice of raising cattle, sheep, goats, and poultry. These plots are improved with amendments of straw and other plant residues intended to enhance soil fertility. In addition, chemical fertilizers are used as a supplement, depending on their availability.

However, these phosphate fertilizers are not always affordable for farmers and are particularly harmful to the environment.

However, the use of appropriate technologies, such as inoculation with arbuscular mycorrhizal fungi (AMF), is an alternative to chemical fertilizers for improving crop productivity, including that of maize and sorghum. Inoculation involves introducing mycorrhizal propagules, namely AMF spores and mycorrhizal root fragments, to plants at the time of sowing (Fitter, 2012).

AMF are obligate symbionts that improve water and mineral nutrition and protect host plants against drought, pathogens, and salinity (Smith and Read, 2008).

In these fields, sorghum and maize are most commonly cultivated. They are major crops for ensuring the country's food needs (Bazile *et al.,* 2008). Total sorghum production reached 650,000 tonnes in 2004, representing 28% of total cereal production (FAO, 2005). Sorghum accounted for 35% of dry cereal production in 2003 (CILSS, 2002; Bazile *et al.,* 2008). It is consumed in the form of tô (a type of porridge), couscous, and also serves as animal feed, roofing material, and fuel for cooking (Bazile *et al.,* 2004).

Total maize production was 500,000 tonnes, representing 4.71% of total production between 2005 and 2009. Contributing 8.34% of the country's calories, maize is a crop with strong potential and has seen steadily increasing production levels in recent years (FAO, 2013).

While maize is primarily consumed by humans in various culinary preparations, such as tô, couscous, and fresh or roasted corn on the cob, it is increasingly used as poultry feed and as a biofuel energy source, an alternative to petroleum (Criado, 2002; Ekobo, 2006).

The objective of this study is to determine mycorrhization under fertilized sorghum and maize crops in the field in Koumantou.

Materials and Methods

Study Site

The commune of Koumantou, the main town of the rural commune of the same name, is located in the Bougouni region. It covers an area of 1,790 km² and is crossed by the national highway (RN7). The commune has a population of 41,168 inhabitants and a population density of 27 inhabitants per km² (CSA, 2007). The commune's terrain is very rugged.

The relatively well-developed hydrographic network is part of the Bagoé River watershed. Soil types are predominantly ferruginous tropical soils. In the lowlands and depressions, there are silty, sandy, or clayey soils. The fairly abundant natural vegetation consists mainly of shea (*Vitellaria paradoxa*), néré (*Parkia biglobosa*), baobab (*Adansonia digitata*), mahogany (*Khaya senegalensis*), and other trees. Resources are under considerable pressure. The main harvested products are néré (African locust bean), shea, and baobab.

Agriculture is the commune's primary economic activity. It has significant potential due to the presence of plains. Crops grown are quite diverse: maize, sorghum, millet, rice, peanuts, cowpeas, yams, sweet potatoes, cassava, cotton, and sesame. The commune receives good rainfall (an average of 1130 mm per year). Nevertheless, farmers' incomes remain low due to the high cost of inputs.

Study material

Sorghum and maize roots constitute our study material. They come from maize and sorghum crop plots that have benefited from amendments based on a mixture of mycorrhizal fungi, natural phosphate and mineral fertilizer including diamond phosphate (DAP) and urea.

Methods

Physicochemical Characteristics of Cultivated Soils

The soil laboratory, HEINZ IMHOF, at the IPR/IFRA in Katibougou, served as the setting for this activity. The pH of water and KCl, the total nitrogen, available phosphorus, and potassium content, as well as the organic matter content, were analyzed using the following methods:

- Potentiometric analysis of hydrogen ion activity in suspended soil/water and/or KCl for pH measurement. The soil/solution (water and/or KCl) ratio was 1 g/2.5 ml;
- The Kjeldahl method for total nitrogen determination;
- Water extraction for phosphorus and potassium analysis;
- The Anne method for organic matter determination.

Previous Cropping Practices of the Fields

A reasoned survey was conducted with the owners of sorghum and maize fields to learn about their crop history. Its aim was to determine the crops grown in these fields over the past two years and the type of input used for their mineralization.

Root Sampling

In each sorghum and maize field, one plant was randomly selected per plot, for a total of four plants per treatment: inoculum M (mixture of arbuscular mycorrhizal fungi), Fertilizer E (DAP and urea), Phosphate Natural from Tilemsi (PNT), AMF and PNT inoculum, and the control T (uninoculated and unfertilized). Each plant was carefully dug up with a hoe to minimize root loss. In total, the roots of 20 plants from the different treatments (one plant per plot) were sampled at each sampling point during cultivation, at 20- and 40-days post-emergence, and at harvest. The roots were placed in tubes containing 70% alcohol and transported to the laboratory for mycorrhization assessment.

Staining and mounting of sorghum and maize roots

The roots were stained according to the method of Phillips and Hayman (1970). For this, twenty (20) thin root fragments, approximately one (1) cm long, were taken from different locations within the stained sample and then mounted between a slide and coverslip in glycerol. Three (3) slides were prepared for each of the eight (8) root replicates per treatment and per culture.

Determination of Mycorrhization Parameters in Cultures

The endomycorrhization rate, infection frequency (F%), the number of times the root is infected, and the mycorrhization intensity (I%), the degree of root infection by the fungus, are evaluated under a light microscope according to the mycorrhizal infection rating system (class 0 to 5) of Trouvelot *et al.,* 1986, and calculated using the following formulas:

F% = (number of mycorrhizal fragments / total number of fragments observed) x 100

I% = (95n5+70n4+30n3+5n2+n1) / total number of fragments observed.

Data Analysis

An analysis of variance (ANOVA) of all data was performed using R software (version R i386 3.2.2). Tukey's Contrasts test at a significance level of 5% was used to compare means.

Results and Discussion

Physico-chemical characteristics of sorghum and maize soils in Koumantou

The physico-chemical constituents in the sorghum and maize plots were determined, and the available phosphorus content was 2.6 for sorghum and 2.36 for maize.

Previous crops of the maize and sorghum plots.

The survey results showed that the fertilizer used in these fields near the village was manure. In the field previously planted with sorghum, maize and millet were grown for the past two years. Meanwhile, in the field where maize was cultivated for the past two years, cotton and sorghum were grown.

Determination of mycorrhization in maize and sorghum crops

Maize mycorrhization rate

20 days after emergence

On day 20 after emergence, the effect of inoculation with all treatments: Control (T), Fertilizer (E), Phosphate Natural from Tilemsi (PNT), Fungal Mixture (M) and (M+PNT) on the intensities and frequencies of maize mycorrhization was determined. No significant differences were observed between the treatments.

However, the highest mycorrhization rate, at 27.36% intensity, was obtained with the Control treatment (Figure 1).

40 days after emergence

On the 40th day after emergence, the previously observed trends (frequency and intensity of mycorrhization) appear to be confirmed, with the difference this time that the fungal mixture treatment in the presence of the PNT performs better (Figure 2).

Indeed, the intensity of root colonization by the applied endomycorrhizal fungi has significantly increased (38.43% versus 7.05%).

70 days after emergence; at harvest

At harvest, on day 70 after emergence, the analysis of variance (ANOVA) showed a highly significant difference between the treatments. Treatment T resulted in the highest mycorrhization intensity (35.15%). However, the mixture of endomycorrhizal fungi appeared to colonize the roots better, with 86.25%, the highest mycorrhization frequency, and 24.21% intensity at the end of cultivation (Table 1).

Sorghum Mycorrhization Rates

On Day 20 After Emergence

Twenty (20) days after emergence, the effect of inoculation with the treatments (T, E, PNT, M, M+PNT) on the intensities and frequencies of sorghum mycorrhization was evaluated. No significant difference was observed between the different treatments for frequencies ($P = 0.814$). Analysis of variance (ANOVA) showed a significant difference ($p \leq 0.05$) between the different treatments for mycorrhization intensities. The control treatment had a much higher mycorrhization intensity than the other treatments (Figure 3).

On day 40 after emergence

Forty (40) days after emergence, mycorrhization frequencies were significant ($p \leq 0.01$) and intensities showed a tendency ($p \leq 0.0643$) for the applied fungi to colonize the roots. The highest root colonization rate was observed with the fungal mixture alone or with the application of PNT. However, the chemical fertilizer treatment had a depressive effect on mycorrhization intensity, decreasing from 3.37% to 0.83% (Figure 4).

At 100 days after emergence, harvest

One hundred (100) days after emergence, no significant difference was noted at harvest between the treatments for mycorrhization rate following the analysis of variance. The control treatment showed the best mycorrhization rate at this time with an intensity of 14.83%. The mixture of endomycorrhizal fungi applied with the PNT appears to colonize less at the end of the growing season (Figure 5).

During our work to improve the productivity of sorghum and maize through biological fertilization, the mycorrhizal fungal fertilizer applied was able to induce increased productivity in cereals, sorghum and maize.

The results showed that the introduced (allogeneic) fungi appeared to be effective in colonizing roots, with a mycorrhization frequency of 86.25% and an intensity of 24.21% in maize. Our results are consistent with those of Smith and Read (2008); Kung'u *et al.,* (2008); Graham and Abbott (2000); Li (2005); and Li *et al.,* (2008), who found a beneficial effect of endomycorrhizal inoculation on the infection of roots in agroforestry species.

Soils cultivated under sorghum and maize were very poor in available phosphorus, at 2.36 ppm and 2.6 ppm for maize and sorghum, respectively (IPR/IFRA, 2016). Phosphorus levels of 5 to 14 ppm P were observed in soils in Mali by Traoré (2003). At the same time, Duponnois *et al.,* (2001) found that very poor soils ($P < 10$ ppm) had a low mycorrhization intensity ($I \leq 39.5$). The low intensities ($\leq 35.15\%$) observed in our work reinforce those of Duponnois *et al.,* (2001). These results are also consistent with those of Diouf *et al.,* (2008), who obtained mycorrhization intensities of less than 40% in the roots of *Gliricidia sepium* inoculated with *Glomus aggregatum* in Senegal.

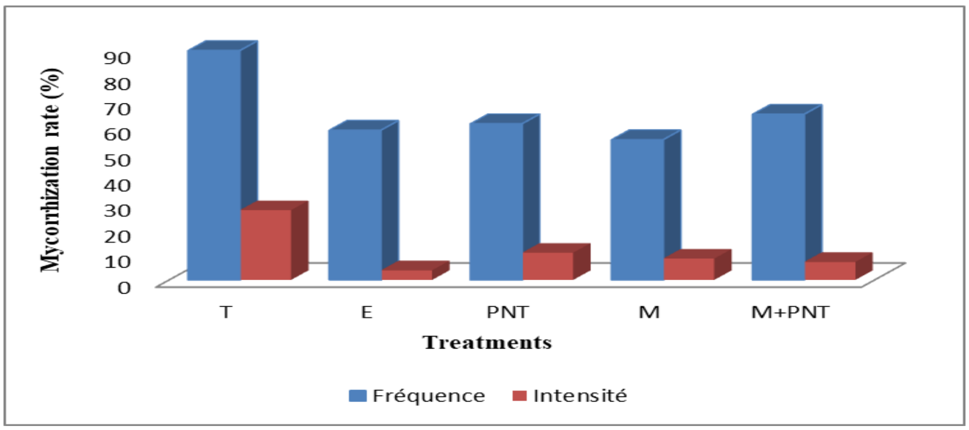
Our results show no positive correlation between the low level of available phosphorus in the soils of our sorghum and maize crops and the low mycorrhization intensities of the plants of these crops. These results contradict those of Bâ *et al.,* (1997, 1998, 2001), Sacko (1997), and Smith and Read (2008), who found that arbuscular mycorrhizal fungi are more efficient and thrive in soils low in available phosphorus.

Table.1 Effect of inoculation on the mycorrhization rate of maize at harvest (after 70 days of sowing)

Treatments	Mycorrhization parameters	
	Mycorrhization frequency (%)	Intensity of mycorrhization (%)
T	85 ab	**35,15 b**
E	57,5 a	9,45 a
PNT	73,75 ab	17,96 a
M	**86,25 b**	**24,21 ab**
M+PNT	77,5 ab	16,4 a
Probability	0,05	0.01
Significance	S	TS

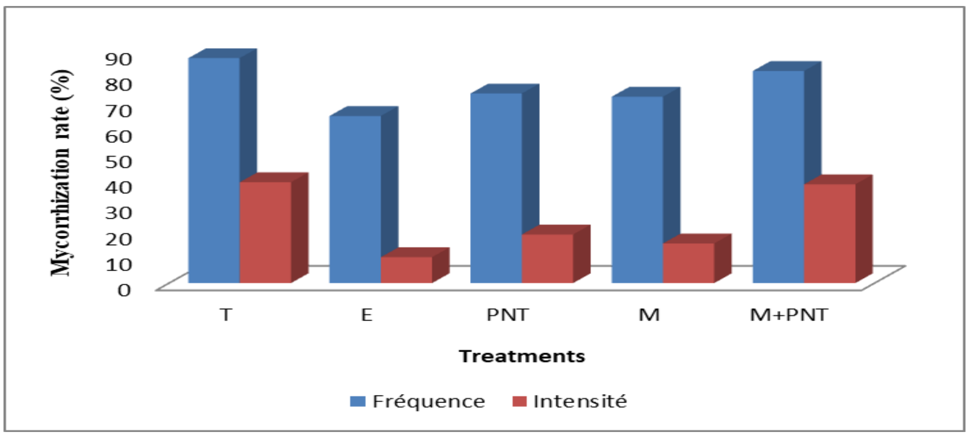
Legend: Each value represents the average for 20 plants. In the same column, values followed by the same letter are not statistically different from each other at the 5% level for the Tuckey Contrasts test. T: Control; E: Fertilizer; PNT: Phosphate Natural from Tilemsi; M: Mushroom Mixture; S: Significant; TS: Highly Significant.

Figure.1 Effect of inoculation on the frequencies and intensities of mycorrhization of maize on day 20 after emergence



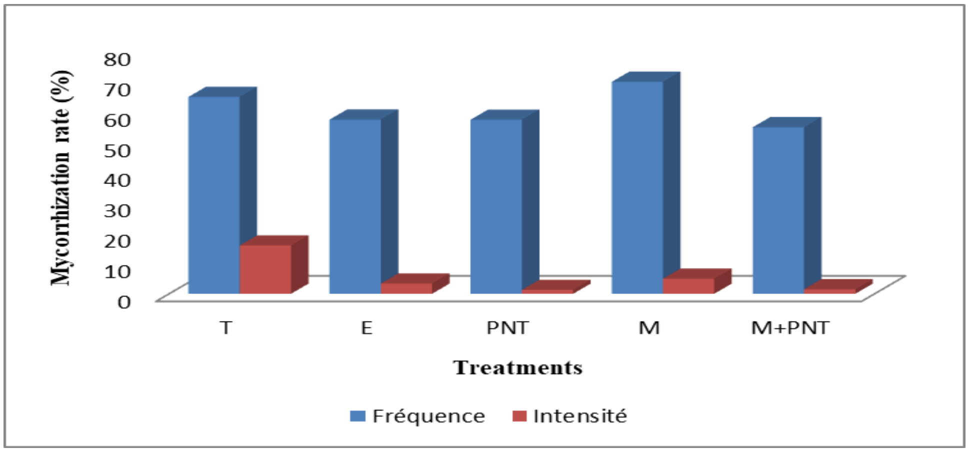
Key: T = Control; E = Fertilizer; PNT = Phosphate Natural from Tilemsi; M = Mushroom Mixture

Figure.2 Effect of inoculation on the frequencies and intensities of mycorrhization of maize 40 days after emergence



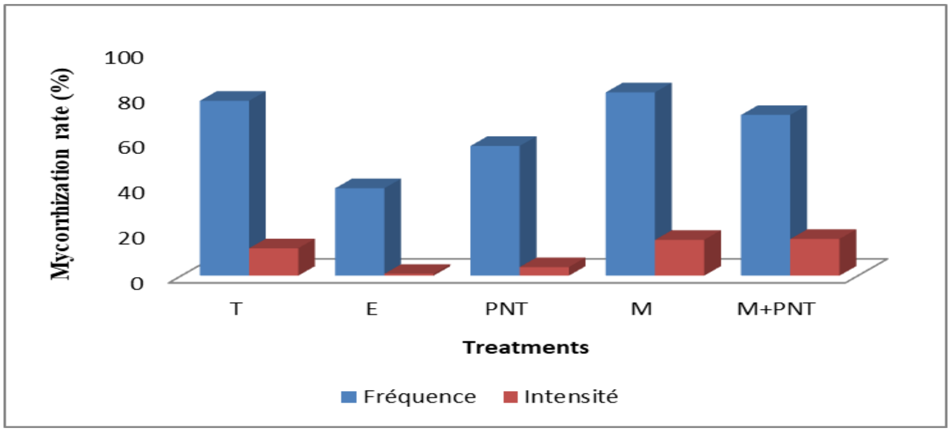
Key: T = Control; E = Fertilizer; PNT = Phosphate Natural from Tilemsi; M = Mushroom Mixture

Figure.3 Effect of inoculation on sorghum mycorrhization parameters at day 20



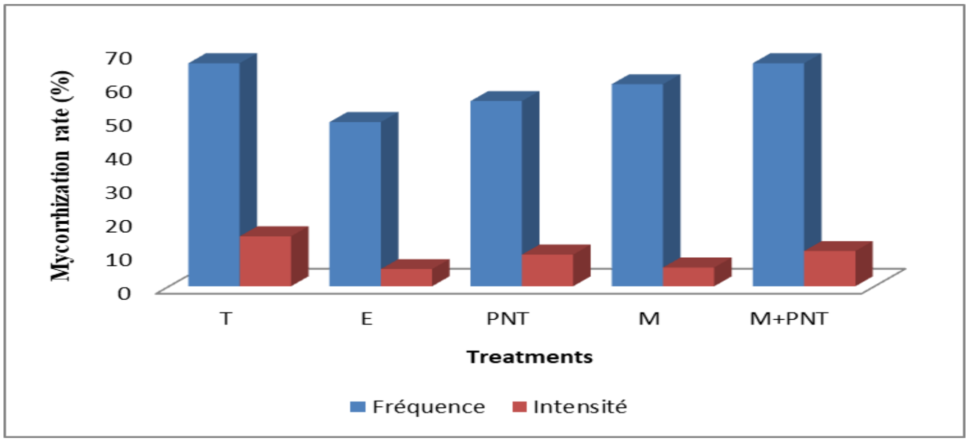
Legend: T = Control; E = Fertilizer; PNT = Phosphate Natural from Tilemsi; M = Mushroom Mixture

Figure.4 Effect of inoculation on the mycorrhization rate of sorghum at day 40



Key: T = Control; E = Fertilizer; PNT = Phosphate Natural from Tilemsi; M = Mushroom Mixture

Figure.5 Effect of inoculation on the mycorrhization rate of sorghum at harvest



Key: T = Control; E = Fertilizer; PNT = Phosphate Natural from Tilemsi; M = Mushroom Mixture

Our results, regarding the low values of mycorrhization intensity, can also be explained by the previous crop. Indeed, our crop trials (sorghum and maize) followed millet and sorghum crops, respectively. These results are corroborated by those of Asimi (2009), according to whom the frequency of root infection in cereal plants under continuous cultivation is significantly higher than in rotational crops such as the present ones, sorghum after millet and maize after sorghum.

In the field, allogeneic mycorrhizal fungi showed low mycorrhization intensities with both crops, particularly in sorghum. Despite this weak root colonization by arbuscular mycorrhizal fungi (AMF), the high mycorrhization frequencies induced some growth and consequently a significant yield compared to traditional farming practices using chemical fertilizers.

AMF-based biofertilization could be an alternative to the use of phosphate fertilizers.

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Author Contributions

Souleymane KONE: Investigation, formal analysis, writing—original draft. Souleymane Bathié KONE: Validation, methodology, writing—reviewing. Rokiatou FANE:—Formal analysis, writing—review and editing. Vital TRAORE: Investigation, writing—reviewing.

Data Availability

The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

Declarations

Ethical Approval Not applicable.

Consent to Participate Not applicable.

Consent to Publish Not applicable.

Conflict of Interest The authors declare no competing interests.

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