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Improvement of Maize (*Zea mays* L.) Production by the Use of Biofertilizers Based on Arbuscular Mycorrhizal Fungi and Tilemsi Natural Phosphate in the Field in Koumantou, Mali

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Abstract | **Original Research Article**

In Mali, maize cultivation is highly dependent on the input of chemical materials, including chemical fertilizers, which enrich the soil with nutrients necessary for plant growth. However, the high cost of these fertilizers and the risks to the environment increasingly require the use of biofertilizers such as mycorrhizal fungi and natural phosphates. Indeed, Arbuscular Mycorrhizal Fungi (AMF), when used as inoculum, improve plant growth through better uptake and transfer of nutrients, particularly phosphorus (P). AMF have proven to be very effective for improving plant growth, especially when coupled with Natural Phosphate (NP). The objective of this study is to improve maize growth through the use of biofertilizers based on Arbuscular Mycorrhizal Fungi and Tilemsi Natural Phosphate. For this purpose, a field experiment was conducted. At the time of sowing the maize seeds, the mycorrhizal inoculum and natural phosphate were applied. The design adopted was a randomized block. The experiment lasted 70 days. The results showed that under the conditions of our experiment, fertilization with AMF inoculum in the presence of phosphorus from TNP significantly stimulated maize yield compared to the Control, with. Fertilization with Arbuscular Mycorrhizal Fungi and Tilemsi Natural Phosphate improved maize growth and yield and could be an alternative to the use of chemical fertilizers in the prospect of sustainable agriculture and the long-term preservation of soil fertility and the environment.

Keywords : Arbuscular mycorrhizal fungi, Tilemsi natural phosphate, maize, Mali.

1. INTRODUCTION

In Sub-Saharan Africa, particularly Mali, the deficit of nutrients in the soil limits crop yield. Indeed, almost 80% of soils are poor, especially in phosphorus (P) and nitrogen (N) « Bâ *et al,* 2001 ; Bationo *et al,* 1998 ».

Cereals contribute significantly to food security. As such, maize, with 8.34% of calorie intake, is a valuable agricultural production in Mali « FAOSTAT, 2010 ». The cereal maize is primarily for human consumption in various culinary preparations such as *tô*, couscous, fresh or grilled cobs. However, it is increasingly used as poultry feed « Bazile et *al.,* 2004 ; Criado, 2002 ».

In Mali, phosphate and nitrogen fertilizers play a crucial role in the fertilization of cereal crops like maize « FAO, 2021 »). However, their use on crops is expensive and can lead to the proliferation of algae in rivers and lakes « Gruhn *et al,* 2000 ».

It is therefore necessary to seek reasonable, more effective, and more economical fertilization techniques to improve crop growth. Thus, the use of local fertilizing resources like natural phosphates (NP) is an alternative to the dependence on chemical fertilizers « Bationo *et al,* 1997 ; Pieri, 1989 ». Indeed, the phosphorus in NP is affordable for the farmer and could be produced as chemical fertilizers. Furthermore, to improve phosphorus solubility, inoculation with Arbuscular Mycorrhizal Fungi (AMF) is a possible recourse « Fernando et *al*, 2023 ; Prabawardan et *al*,

2023 ; Babana and Antoun, 2003 ». In fact, the presence of AMF could be an asset for maintaining the productivity of cultivated plants « Bâ *et al,* 2000 ; Al-Karaki *et al,* 2004 ; Schroeder and Janos, 2004 ». For this purpose, inoculation with AMF coupled with phosphate fertilization in the form of TNP increases the availability of phosphorus (P) « Fernando et *al*, 2023 ; Prabawardan et *al*, 2023 ».

The objective of this study is to improve maize (*Zea mays L.*) production by the use of biofertilizers based on Arbuscular Mycorrhizal Fungi and Tilemsi Natural Phosphate in the field in Koumantou, Bougouni region, Mali.

2. MATERIAL AND METHODS

2.1. Study Site

Our experiment was conducted in the main town of the rural commune of Koumantou, located in the Bougouni region of Mali and crossed by the national road (RN7).

2.2. Plant Material

Maize seeds belonging to the Tzee-y (Brico) variety were used. They were supplied by FASOKABA.

2.3. Fertilizers

For the fertilization of the maize crop, the inoculum of three species of Arbuscular Mycorrhizal Fungi: *Rhizophagus irregulare* (Ri), *Glomus aggregatum* (IR-27) and *Glomus mosseae* (Gm), from the collection of the Common Laboratory of Microbiology (LCM), IRD/ISRA/UCAD of Dakar, which have shown their effectiveness on crops, were used in this experiment. Tilemsi Natural Phosphate with a P_2O_5 content of approximately 30% and an interesting liming value, 45% CaO « Pieri, 1989 » as well as mineral fertilizer (DAP + Urea) were used.

2.4. Determination of the Physicochemical Characteristics of the Cultivation Field in Koumantou

This work was carried out at the HEINZ IMHOF soil laboratory of IPR/IFRA in Katibougou (Mali). For this purpose, a composite sample of the cultivation field was used, and the analyses focused on in the Ph of water, in KCl pH, organic matter content, total nitrogen content, and assimilable potassium and phosphorus content.

The following methods were used :

- Potentiometry method for measuring the activity of hydrogen ions in soil/water and/or suspension for measurement. The soil/solution (water and/or KCl) ratio is.
- Anne method for organic matter determination.
- Kjeldahl method for total nitrogen assay.
- Water extraction method for phosphorus and potassium analyses.

2.5. Evaluation of the Effect of Arbuscular Mycorrhizal Fungi and Tilemsi Natural Phosphate on Maize Growth and Yield

2.5.1. Sowing, Inoculation, and Weeding

Maize seeds were sown at a rate of 2 seeds per planting hole. Mycorrhizal inoculum was applied at a dose of 20 g per planting hole, PNT at a dose of 30 g/m² (540 g/plot), and fertilizer (DAP + Urea) at 10 g + 5 g/m² (180 g + 90 g/plot).

Manual weeding was carried out to avoid transferring inoculated material from one plot to another. Weeding was performed twice at emergence and again 17 days later to maintain weed control in the experimental plot.

2.5.2. Description of the Experimental Design

The maize field constitutes an experimental plot which is subdivided into four blocks of five elementary plots, each representing a treatment, making a total of 20 elementary plots with an area of each. The completely randomized blocks are spaced one (1) meter apart. The elementary plots are also separated from each other by two-meter aisles. The arrangement of the elementary plots is random.

Thus, the experiment includes 5 treatments with 4 replicates of 2 factors (inoculation and fertilization):

- Mixed inoculum of arbuscular mycorrhizal fungi (M)
- Fertilizer (E) (DAP + Urea)
- Tilemsi Natural Phosphate (TNP)
- Mixed inoculum of AMF + Tilemsi natural phosphate (M + TNP)
- Control (T) (non-inoculated and non-fertilized)

In total, the experimental design includes :

- Four (04) elementary plots inoculated with the mixed inoculum of arbuscular mycorrhizal fungi
- Four (04) elementary plots fertilized with fertilizer (positive control)
- Four (04) elementary plots fertilized with TNP and non-inoculated
- Four (04) elementary plots inoculated with the mixed inoculum of arbuscular mycorrhizal fungi and fertilized with TNP
- Four (04) elementary plots non-inoculated and non-fertilized (absolute control).

The distance between the rows is 75 centimeters, and the distance between the hills on a row is 40 centimeters. Each elementary plot includes seven rows of eleven hills each, for a total of 77 hills. Plants from fifteen (15) hills per elementary plot (central hills) were measured during the experiment.

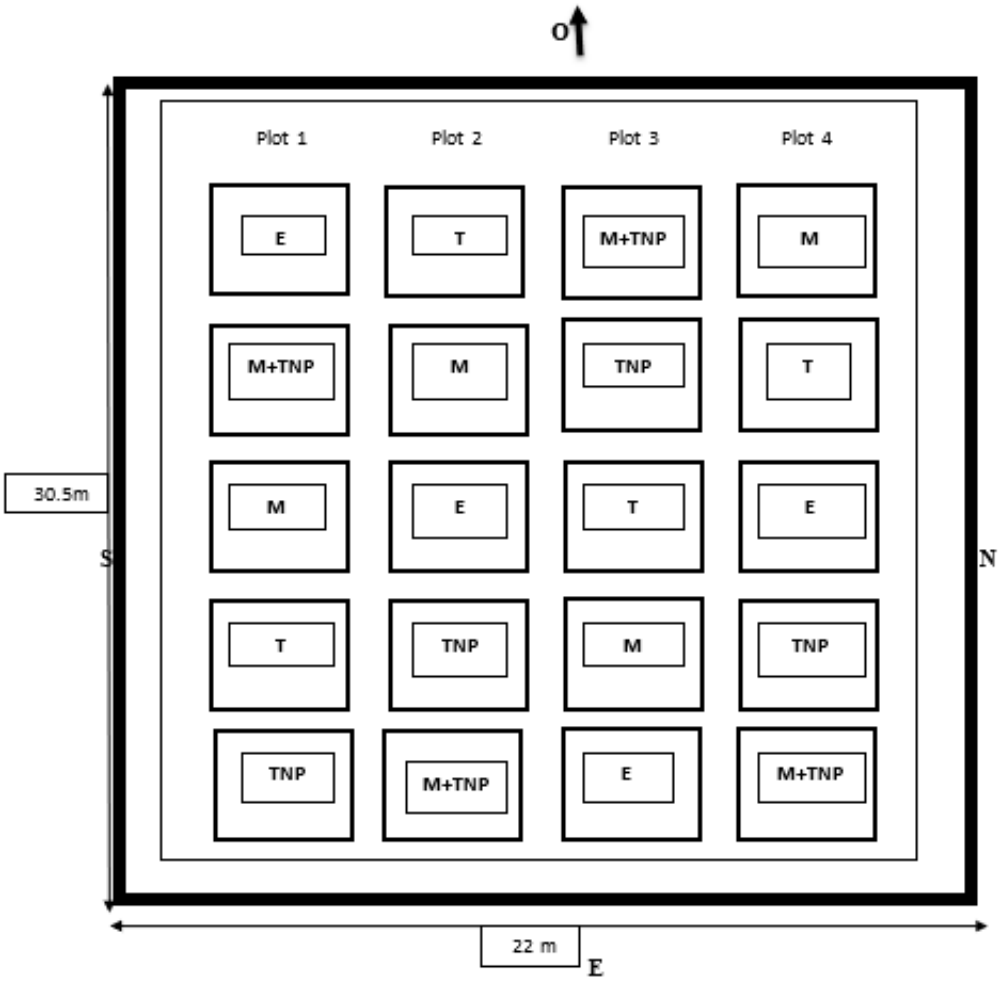

Fig. 1. Field experimental design for maize cultivation

Légende : *M : AMF inoculum ; TNP : Tilemsi Natural Phosphate ; E : Fertilizer (DAP : Di ammonium phosphate + Urea) ; T : Control.*

2.5.3. Parameters Measured and Crop Harvest

For each elementary plot, measurements focused on the 15 central plants. Plant height and diameter were measured every 20 days starting from emergence : at 20 days after emergence (DAE), 40 DAE, and 60 DAE. After 70 days of cultivation, the maize plants were harvested and dried in an oven at for two days, then weighed. The harvest concerned the 15 plants that were measured in each elementary plot, i.e., 60 plants per treatment. A total of 300 plants were harvested per cultivation plot. Panicle length, panicle weight, and above-ground dry biomass (stem) were determined. The number of cobs was also counted. The obtained grains were weighed to estimate the crop yield and the weight of one thousand (1000) grains per treatment.

2.6. Data Analysis

Analysis of variance (ANOVA) of the data was performed using the R software (Version R i386 3.2.2), and the Tukey Contrast test at the significance level « Bertrand, 2011 » was used to compare the means.

3. RESULTS

3.1. Physicochemical Characteristics of the Maize Cultivation Field in Koumantou

The determination of physicochemical constituents in the maize plot showed an acidic of water (4,20 to 4,30) and KCl (4,08 to 4,09), a very low proportion of organic matter (0,6 to 0,7) and nitrogen (0,01 to 0,03), and also a very low quantity of potassium (2,36 to 2,6) and assimilable phosphorus (3,85 to 4,49) (Table 1).

Table 1: Physicochemical Characterization of Cultivation Soils at the Koumantou Site

Constituents (content per 100g of soil)	water pH	KCL pH	Organic Matter %	Nitrogen %	Assimilable Phosphorus (P) ppm	Potassium (K) ppm
Maize plot	4.30	4.09	0.6	0.01	2.36	3.85

3.2. Effect of Arbuscular Mycorrhizal Fungi and Tilemsi Natural Phosphate on Maize Growth and Yield in the Field in Koumantou

3.2.1. Maize Height and Diameter at 20 and 40 Days of Growth

During development, the height and diameter of the maize plants were measured on the 20th and 40th day after emergence. Analysis of variance (ANOVA) showed a highly significant difference (p≤0,001) between the plants of the different treatments. All fertilizer-based treatments stimulated growth better than the Control treatment. However, the mycorrhizal fungi inoculum (M) in the presence of TNP had a greater effect on growth (height and diameter) than the 2 fertilizers applied separately and the chemical fertilizer (Table 2).

Table 2 : Effect of Inoculation on Maize Growth

Treatment	Growth Parameters and Day After Emergence				
	Heights (in cm)			Diameters (in cm)	
	20 DAE	40 DAE	70 DAE	20 DAE	40 DAE
T	44.06 a	80.83 a	136.16 a	0.11 a	0.51 a
E	50.90 ab	113.56 bc	158.23 bc	0.68 bc	1.28 bc
TNP	50.51 ab	104.06 b	147.71 ab	0.46 b	1.13 b
M	55.83 b	106.16 bc	147.40 ab	0.61 b	1.11 b
M+TNP	57.08 b	117.81 c	161.76 c	0.88 c	1.48 c
Probability	0.001	0.001	0.001	0.001	0.001
Significance	HS	HS	HS	HS	HS
C.V	0.30%	0.25%	0.16%	0.98%	0.53%

Légende : *Each value represents the mean for 300 plants, DAE = Days After Emergence ; T : Control ; E : Fertilizer ; TNP : Tilemsi Natural Phosphate ; M : AMF Inoculum ; HS : Highly Significant ; CV : Coefficient of Variation. In the same column, values followed by the same letter are not statistically different at the level for the Tukey Contrasts test.*

3.2.2. Diameter and Above-Ground Biomass of Maize at Harvest

After 70 days of cultivation, the results of the analysis of variance (ANOVA) of the parameters measured at harvest showed a highly significant difference (p≤0,001) between the treatments. The treatment with mineral fertilizer (E) resulted in significantly greater panicle lengths than the other treatments. The treatment with Arbuscular Mycorrhizal Fungi inoculum in the presence of TNP stimulated more during development (45.82%), while Fertilizer (E) had more effect on the parameters determined at harvest (panicle length, panicle weight, above-ground dry biomass). Compared to the control, the different treatments did not have significant effects on the number of panicles (Table 3).

Table 3: Effect of Inoculation at Maize Harvest

Treatment	Growth Parameters			
	Diameters (in cm)	Panicle Length (in cm)	Panicle Weight (in g)	Above-Ground Dry Biomass (in g)
T	1.03 a	17.45 a	31.50 a	23.01 a
E	1.75 cd	21.20 b	66.93 c	46.31 c
TNP	1.50 b	18.40 a	46.17 b	38.84 bc
M	1.60 bc	17.95 a	42.44 ab	35.07 b
M+TNP	1.82 d	18.89 a	49.51 b	45.82 c
Probability	0.001	0.001	0.001	0.001
Significance	HS	HS	HS	HS
C.V	0.31%	0.19%	0.60%	0.57%

Légende : *Each value represents the mean for 300 plants. T : Control ; E : Fertilizer ; TNP : Tilemsi Natural Phosphate ; M : AMF Inoculum ; HS : Highly Significant ; CV : Coefficient of Variation. In the same column, values followed by the same letter are not statistically different at the level for the Tukey Contrasts test.*

3.2.3. Effect on Maize Grain Yields

The effect of inoculation on maize grain yields in the field was evaluated. The analysis of variance shows a significant effect (p≤0,05) of fertilization on grain yields. Fertilization with chemical fertilizer, followed by treatments with Arbuscular Mycorrhizal Fungi inoculum in the presence of TNP and TNP alone, significantly increased maize yield compared to the Control, with, and respectively (Table 4).

Table 4 : Effect of Inoculation on Maize Grain Yields

Treatments	Yield per Hectare
T	0.62533 a
E	1.1924725 b
TNP	0.83375 ab
M	0.63267 a
M+TNP	0.8204875 ab
Probability	0.05
Significance	S

Légende : *Each value represents the mean for 300 plants. T : Control ; E : Fertilizer ; TNP : Tilemsi Natural Phosphate ; M : AMF Inoculum ; S : Significant. In the same column, values followed by the same letter are not statistically different at the level for the Tukey Contrasts test.*

3.2.4. Weight of 1000 Maize Grains

No treatment had a significant effect on the weight of 1000 grains compared to the control. However, the weight of 1000 grains is highest with treatments E and M+TNP. Treatment M shows the lowest value.

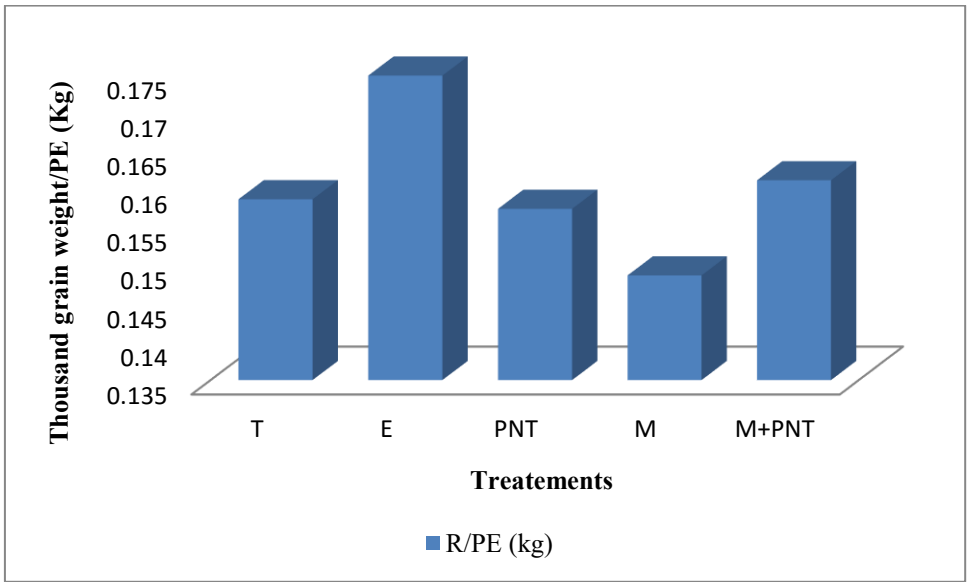

Fig. 2. Mean weight of 1000 maize grains as a function of treatments

Légende: *DAE = Days After Emergence ; T = Control ; E = Fertilizer ; TNP = Tilemsi Natural Phosphate ; M = AMF Inoculum ; R = Yield ; PE = elementary plot.*

4. DISCUSSION

The results of our work show that inoculation with AMF strains improved the growth and yield of maize in the presence of phosphorus (P) from Tilemsi Natural Phosphate (TNP). Work on improving the productivity of cereals in general, and maize in particular, through biological fertilization has been the subject of little investigation so far in Mali and elsewhere in the sub-region.

Maize Response to Mycorrhizal Inoculation and Phosphate Fertilization

The physicochemical characterization of the maize cultivation soils showed soils very poor in assimilable phosphorus : 2,36 ppm P. Indeed, the soils of Mali contain only to 5 to 14 ppm P « Traoré, 2003 ». However, it has been observed that AM fungi are more effective and express themselves better in soils poor in assimilable phosphorus (P < 10 ppm), « Bâ *et al,* 2001 ».

In our study, field fertilization of the maize crop with Arbuscular Mycorrhizal Fungi (AMF) inoculum in the presence of Tilemsi Natural Phosphate (TNP) had a significant positive effect on the height to 161,76% and above-ground biomass (stem and panicle) to 45,82% of the plants. Indeed, an improvement in height of and above-ground dry biomass of of the maize plants was noted. Our results are comparable to those of « Prabawardan *et al.,*2023 », who observed a positive effect of endomycorrhizal inoculation on the growth of plants cultivated on a P-poor substrate. Our results are also comparable to those of « Diouf *et al.,*2005; Bâ *et al.,*2000 » who noted significant growth of *A. mangium* and fruit trees inoculated with *G. fasciculatum* and *G. verriculosum* in Senegal.

In our work, in terms of growth, the application of TNP stimulated the effect of arbuscular mycorrhizal fungi. Our results differ from those of « Inkleby *et al.,*2001 », who noted that phosphorus application is not

the sole determinant of plant growth. These authors asserted that inoculation with high-performing AMF strains has a greater effect on plant growth than the application of natural phosphates. Indeed, Tilemsi natural phosphate (TNP), due to its low solubilization, is released slowly, and its effect on growth is delayed « Strullu, 1991 ».

The observed biomass production in our results could be positively correlated with yield. Indeed, with yields of 0,8204875 kg/ha for maize compared to the absolute control, an improvement in production was observed. Our results are confirmed by those of « Jansa *et al.,*2005 », who concluded that inoculation of leeks with a mixture of mycorrhizal fungi, namely *Rhizophagus irregularis* and *Glomus claroideum*, allowed better productivity consecutive to inoculation with *R. irregularis* or *G. claroideum* taken separately. These results are also similar to those of « Koide, 2000 », who found that the positive effects of arbuscular mycorrhizal fungal inoculum are due to the functional complementarity of the strains inoculated simultaneously. Our results are also comparable to those of « Diouf *et al.,*2008 », who observed a positive effect on maize yield with dual Rhizobium-mycorrhizal fungus inoculation in the presence of natural phosphate.

5. CONCLUSION

By improving the growth and yield of the maize crop in the field, the use of biofertilizers constitutes an ecological and economic alternative to chemical fertilization.

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