

Research Article

Effect of fertilizer Microdosing of and Microbial Inoculation on Pearl Millet (*Pennisetum glaucum* (L.) R. Br) Growth on Two Soils of the Peanut Basin of Senegal

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Abstract

Pearl millet (*Pennisetum glaucum* (L.) R. Br) is a very important cereal crop in the semi-arid regions of West Africa, serving as a primary food source for local populations. Therefore, its productivity remains lowered by soil degradation and low availability of inputs as fertilizers. In this context, biofertilizers and organic amendments offer sustainable and ecological alternatives for enhancing crop performance. This study aims to contribute to improve pearl millet production through the application of biofertilizers arbuscular mycorrhizal fungi (AMF) and plant growth-promoting rhizobacteria (PGPR) combined with microdose NPK. The research focuses on two soils of the peanut basin of Senegal (Touba Toul and Gossas). Thus, a greenhouse experiment was conducted using a completely randomized block design with five treatments (control, microdose, fungal inoculation (AMF), bacterial inoculation (PGPR) and dual inoculation (AMF+PGPR)) and five replicates, on each of the two soils. The parameters assessed included mycorrhization, collar diameter, number of leaves, chlorophyll content, shoot and root biomass, and ears length. Results revealed that, the microdose generated the best agronomic performance, including 46% increase in chlorophyll content and 30% increase in collar diameter compared to control. The AMF, PGPR and AMF+PGPR treatments showed more variable effects. While close to some parameters such as shoot biomass, improved significantly (up to 20% increase), but with no significant improvement in root biomass (1.02% increase). A notable site effect was observed: Touba Toul proved to be more favourable for millet growth, with an overall performance increase of around 60% compared to Gossas. These findings suggest that combining biofertilization with fertilizer microdosing could be a promising strategy for sustainable pearl millet production in sahelian regions.

Keywords

Pearl Millet (*Pennisetum glaucum*), Arbuscular Mycorrhizal Fungi (AMF), Plant Growth Promoting Rhizobacteria (PGPR), Fertilizer Microdose, Inoculation

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Received: 25 May 2026; Accepted: 8 June 2026; Published: 3 July 2026



1. Introduction

Pearl millet is staple food crops in the sahelian regions of West Africa, playing an essential role in food security and enhancing the resilience of agricultural systems [2, 38]. Its remarkable ability to tolerate harsh climatic conditions, such as low and erratic rainfall, high temperatures and nutrient-deficient soils, makes it a strategic crop for smallholder farmers [14, 21, 32]. Despite this hardness, pearl millet yields remain low, largely due to the progressive degradation of soil fertility and the inefficiency use of mineral fertilizer use [23]. Sahelian soils are generally characterized by low organic matter content, limited availability of essential nutrients and reduced biological activity [1]. These soil constraints severely limit nutrient uptake by plants and exacerbate nutrient losses through leaching, particularly nitrogen and phosphorus [8]. In this context, increasing mineral fertilizer does not guarantee sustainable yields improvement. On the contrary, excessive fertilizer application can contribute to further soil degradation and reduced nutrient use efficiency [33, 34]. Improving productivity through novel cropping-system approaches that integrate *in situ* soil fertility improvement techniques is, therefore attracting growing interest [10, 11, 16]. Soil microbes that enhance

nutrient availability and uptake efficiency are, therefore valuable technologies. Beneficial soil microorganisms, particularly arbuscular mycorrhizal fungi (AMF) and plant growth promoting rhizobacteria (PGPR), play a key role in improving hydromineral nutrition, plant growth [28, 36, 40, 42]. Microdosing, which involves the direct application of small amounts of mineral fertilizers combined with organic matter near roots, optimizes nutrient use, increases input efficiency and lowers production cost [15, 22, 24]. Several studies have highlighted the positive effects of integrated fertilization and microbial inoculation on cereal crop productivity in tropical conditions [14, 24, 29]. However, plant responses to these practices depends heavily on soil characteristics and local agro-ecological conditions. Furthermore, the interactions between mineral fertilization, organic amendments and soil microorganisms remain insufficiently documented for pearl millet, particularly regarding their combined effects on growth, mycorrhization, physiological parameters and yield. In this context, the present study aims to evaluate the effect of microbial inoculation and microdosing fertilizer practices on the growth of pearl millet.

2. Materials and Methods

2.1. Soil Characterization

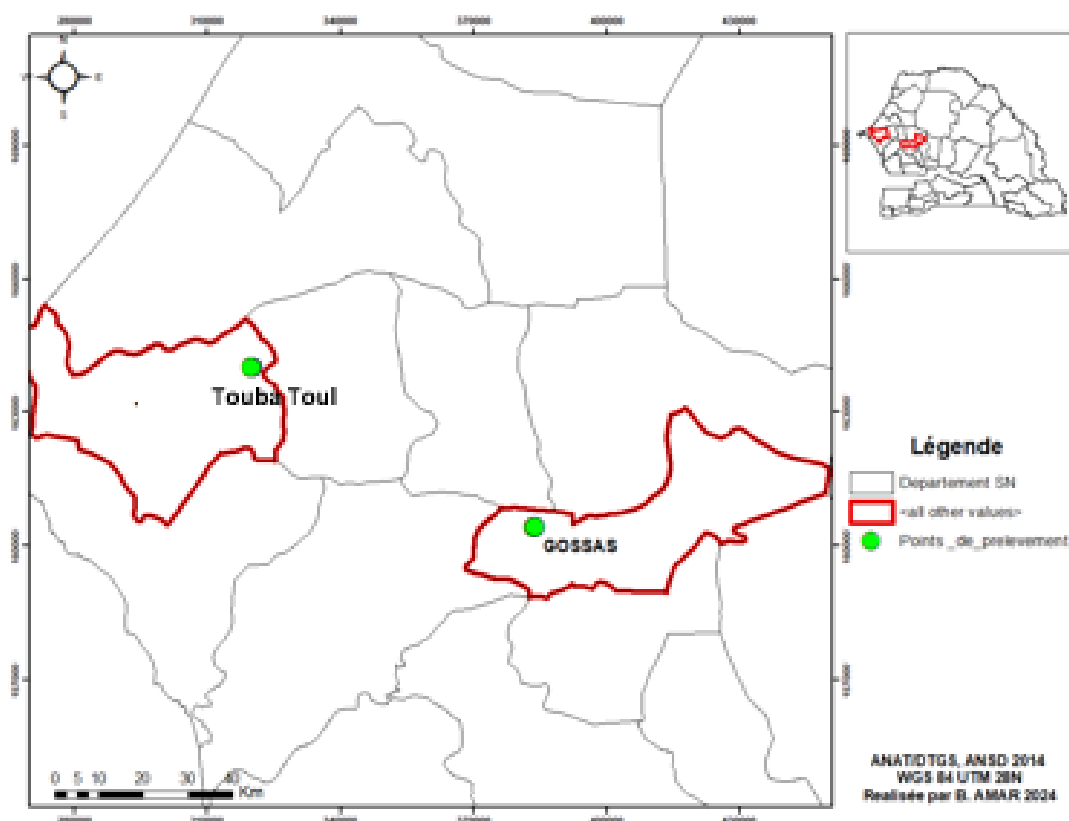


Figure 1. Geographical location of the soil sampling sites of Touba Toul and Gossas.

Table 1. Applied fertilizer treatments.

Treatments	Description
T0	Control (without any fertilization, or inoculation)
T1	Microdose = Compost (400g) + Urea (2g) + NPK (3g)
T2	AMF (<i>Rhizoglossus irregularis</i> + <i>Rhizoglossus aggregatum</i>) (20g)
T3	PGPR (LCM5016 + LCM 4370) (5mL)
T4	AMF (<i>Rhizoglossus irregularis</i> + <i>Rhizoglossus aggregatum</i>) (20g) + PGPR (LCM5016 + LCM 4370) (5mL)

The soils of this study were sampled in two locations in the peanut basin of Senegal: Touba Toul and Gossas (Figure 1). Three composite samples of 50 kg of soil were taken diagonally at depths of 5 - 20 cm from farmers' plots where peanuts had been grown previously at each site. Analysis of Touba-Toul soil properties (Table 1) shows it is a sandy, hence light-textured and well-drained, but has low water holding capacity. It has low salinity and a favourable pH (6.8). It contains a low organic matter content of less than 2%. It is poor in available nitrogen and phosphorus, indicating that the soil requires amendments to improve fertility and would be favourable to mycorrhization. The properties of the Gossas soil (Table 1) show that it is predominantly sandy with a light texture, well-drained and with low water retention. It has low salinity, a slightly acidic pH (5.6) and low organic matter content. This soil is poor in assimilable phosphorus and total nitrogen, reflecting limited chemical fertility and a need for organic or mineral inputs to improve its productivity.

2.2. Plant Material

The seeds of pearl millet pre-base (*Pennisetum glaucum*) Souna 3 variety were supplied by the Seed Production Unit (UPSEM) of the Senegalese Agricultural Research Institute (ISRA). It is a short-cycle variety with a maturation period of 75-90 days, making it particularly suitable for regions with low and irregular rainfall. This widely cultivated variety is adapted to Sahelian agroecological conditions and is preferred by small-holder farmers in Senegal. Maize seeds (*Zea mays*) supplied by the Senegalese Company for the Production and Distribution of Tropical Seeds (TROPICASEM) were also used.

2.3. Fertilizers

The fungal inoculum is a mixture of two strains, *Rhizoglossus irregularis* and *Rhizoglossus aggregatum*, from the Laboratory of Fungal Biotechnology (LBC) of the Department of Plant Biology, Cheikh Anta Diop University, Senegal. Mycorrhizal inoculant for each endophyte consisted of mixed soil, spores, mycelium and infected root fragments from pot culture of *Zea mays*. *Rhizoglossus* isolate preparations with similar characteristics (an average of 40 spores per gram & 85% of

infected roots) were used as inoculant. The bacterial inoculum consists of a liquid culture of strains LCM5016 and LCM4370 isolated from the rhizosphere of wheat and belonging to the collection of the Commun Microbiology Laboratory (LCM).

The fertilizers used are compost produced in this study, NPK mineral fertilizer (15 10 10) and urea (46%).

2.4. Assessment of Effect of Biofertilizers Inoculation and Fertilizer Microdosing on Millet Plants

Experimental trial implementation

The experiment was conducted in a greenhouse using a completely randomized design, with five treatments (T0: Control, T1: Microdose, T2: AMF, T3: PGPR, T4: AMF + PGPR) and five replicates (Table 2). Two types of unsterilized soils were used, with 25 pots per site. Each pot was filled with 3 kg of soil. Before sowing, 400 g of compost was incorporated into the soil, as well as 20 g of a mixed fungal inoculum composed of *Rhizoglossus irregularis* and *Rhizoglossus aggregatum*, depending on the treatment. Five seeds of pearl millet variety Souna 3 were then sown per pot. After germination, the seedlings were thinned to keep one plant per pot. Plant growth-promoting bacteria (PGPR) inoculum was applied at a rate of 5 ml per pot according to the treatments. Mineral fertilization was carried out by applying per pot 3 g of NPK (15-10-10) ten days after seedling emergence, followed by 2 g of urea (46%) at the tillering stage. The pots were then arranged randomly and redistributed weekly. Watering was carried out every two days during three months.

Table 2. Physico-chemical characteristics of the soils of Touba-Toul and Gossas.

Component elements (content per 100g of soil)	Touba Toul	Gossas
Clay	3.6%	3.4%
Silt	1.6%	1.7%
Fine silt	2.9%	Nd

Component elements (content per 100g of soil)	Touba Toul	Gossas
Fine sand	51%	4.3%
Medium sand	Nd	69.9%
Coarse sand	40.9%	20.8%
Conductivity	65 $\mu\text{s}/\text{cm}$	2 $\mu\text{s}/\text{cm}$
C	1.57%	2.23%
Organic matter	1.24%	1.41%
N	0.11%	0.21%
C / N	11%	11.6%
Total P	47 ppm	39 ppm
Assimilable phosphorus	3.1 ppm	2.1 ppm
pH (soil/water ratio 1: 2)	6.8	5.6

Nd = not determined

2.5. Parameters Assessed

Following parameters were measured initially: plant height and stem diameters at 2 cm from the base, number of leaves and tillers, chlorophyll content, Shoot and root biomass, and ear length. Chlorophyll content was estimated using a SPAD chlorophyll meter. After harvest, dry biomass was obtained after oven drying the samples at 65°C during 5 days.

Percentage of mycorrhizal root infection was estimated by microscope observation of fungal colonization after clearing washed roots in 10% KOH and staining with 0.05% trypan blue in lactophenol (v/v), according to [26]. Mycorrhizal colonization was calculated according the method of [31] and the

statistical tables of [6]. The frequency of mycorrhization were estimated according to [35], to quantify the proportion of colonized roots and the degree of colonization of the root cortex, respectively.

2.6. Statistical Analysis

Statistical analyses were performed using RStudio (version 2023.12.1-402). Data normality was assessed using the Shapiro–Wilk test, and analyses of variance were conducted accordingly. Data following a normal distribution were analyzed with ANOVA, while non-normal data were analyzed using the Kruskal–Wallis test. Data visualization (boxplots) and multivariate analyses also performed in RStudio. Correlations between inoculated and non-inoculated parameters were assessed using Spearman’s test, and results from the two sites were compared using PCA.

3. Results

3.1. Effects of Biofertilizers and Microdosing on Millet Growth and Mycorrhization on Gossas Soil

3.1.1. Effect on Mycorrhization

Results on the effect on fertilization on millet mycorrhization in Gossas soil is showed in Figure 2. The microdose treatment (T1) stands out with the lowest frequency (3%), showing no significant difference from the control treatment T0, which has an intermediate frequency (47.8%). Conversely, the AMF treatment (T2) (66.6%), PGPR (T3) (75.4%) and AMF+PGPR (T4) (65.2%) treatments exhibit the highest frequencies and not significantly different from one another.

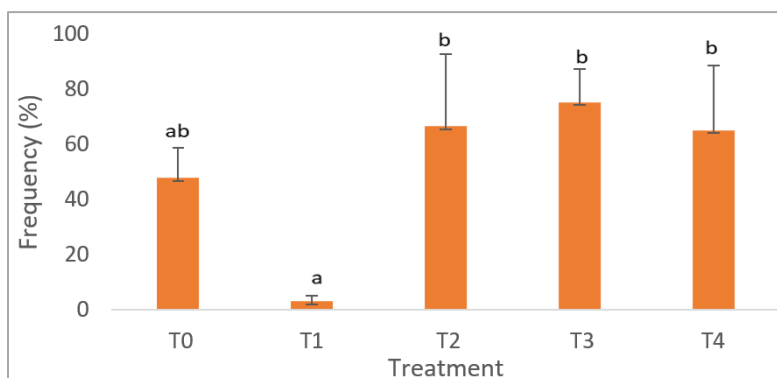


Figure 2. Frequency of mycorrhization of millet cultivated on Gossas soil under different inoculated and fertilized treatments.

3.1.2. Effect on Growth Parameters

No significant differences in plant height ($p = 0.201$), although the minimum height was observed in the T0 control

(87.4 \pm 16.56 cm) and the maximum in the T1 microdose (125.2 \pm 33.38 cm) (Table 3).

Chlorophyll content did not differ significantly between treatments T1, T2, T3 and T4, with the highest value under T1

(38 SPAD). The control T0 had the lowest value (19 SPAD), comparable to T4.

In contrast, collar diameter varied significantly ($p = 0.016$). The AMF treatment (T2) showed the lowest value (3.57 ± 1.93 mm), with no significant difference from T0, T3 and T4, while the microdose (T1) recorded the highest value (16.65 ± 5.02 mm), comparable to T3 and T0.

The number of leaves differed significantly between treatments ($p = 0.016$), with a maximum under microdose T1 (27.0 ± 16.01), significantly comparable to T2 and T3. The lowest values were observed with double inoculation T4 (7.6 ± 2.3),

with no significant difference from T0.

Shoot biomass varied significantly ($p = 0.005$), with a minimum value under T0 (1.44 ± 0.88 g), comparable to T2, T3 and T4, and a maximum value under microdose T1 (15.48 ± 1.32 g), significantly comparable to T3 and T4. This increase in biomass for T1 is 975% compared to T0. Similarly, root biomass shows highly significant differences ($p = 0.005$). The lowest value was observed with double inoculation T4 (0.68 ± 0.11 g), comparable to T0, T2 and T3, while microdose T1 had the highest value (4.34 ± 1.3 g), comparable to T0 and T2.

Table 3. Variation in growth and biomass parameters of millet plants inoculated and cultivated on Gossas soil.

Treatment	Plant height (cm)	Chlorophyll content (SPAD)	Neck diameter (mm)	Number of leaves	Shoot biomass (g)	Root biomass (g)
T0	87.4 ± 16.56^a	18.89 ± 0.43^a	5.9 ± 3.38^{ab}	8.2 ± 3.27^a	1.44 ± 0.88^a	1.04 ± 0.7^{ab}
T1	125.2 ± 33.38^a	38.65 ± 5.02^b	16.65 ± 5.02^b	27.0 ± 16.01^b	15.48 ± 1.32^b	4.34 ± 1.3^b
T2	92.8 ± 17.9^a	35.43 ± 0.38^b	3.57 ± 1.93^a	9.2 ± 1.3^{ab}	1.62 ± 1.00^a	1.36 ± 0.76^{ab}
T3	94.2 ± 13.36^a	32.31 ± 3.8^b	4.00 ± 0.43^{ab}	8.8 ± 0.83^{ab}	1.8 ± 0.94^{ab}	0.8 ± 0.15^a
T4	93.8 ± 15.27^a	19.06 ± 3.59^{ab}	3.96 ± 1.27^a	7.6 ± 2.3^a	1.92 ± 0.67^{ab}	0.68 ± 0.11^a
Average \pm SD	98.88 ± 23.26	28.86 ± 9.5	6.82 ± 5.73	12.16 ± 10.17	4.46 ± 5.95	1.64 ± 1.55
RESUME (%)	24	32.9	84	84	133	95
p-value	0.201	0.016*	0.016*	0.016*	0.005**	0.005*

Codes: 0 "****" 0.001 "***" 0.01 "**" 0.05 "." 0.1 " " 1 (significant difference according to Dunn's test); Mean \pm SD (total mean plus or minus standard deviation); CV (coefficient of variation). Values represent the mean of 5 replicates. Means followed by the same letter in the same column are not significantly different at the 5% level according to Dunn's test. The highest statistically significant values are assigned the letter "b".

3.1.3. Effect on Ears Length

No significant differences on Ears length were found

among the treatments (Figure 3). However, the microdose treatment (T1) showed the highest value of ear lengths (12.8 cm). The shortest ear lengths were recorded under bacterial treatment (T3) with an average length of 4.8 cm.

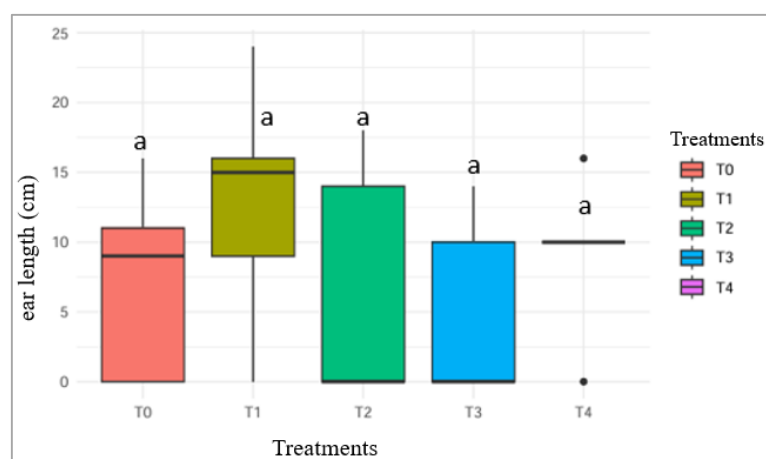


Figure 3. Effect of biofertilizers inoculation and microdose on ear length of millet cultivated on Gossas soil.

3.2. Effect of Biofertilizers and Microdosing on the Growth and Mycorrhization of Millet on Touba Toul Soil

3.2.1. Effect of Mycorrhization

Results on the effect on fertilization on millet mycorrhization in Touba toul soil are showed in Figure 4. Treatment T1

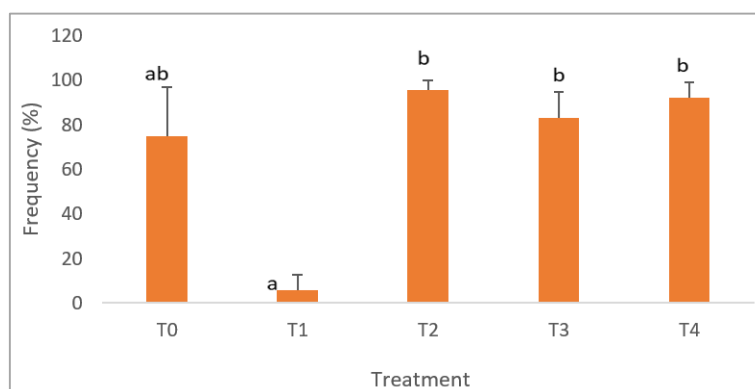


Figure 4. Frequency of mycorrhization of millet cultivated in Touba Toul soil, under different inoculated and fertilized treatments.

3.2.2. Effect on Growth Parameters of Millet Plants

Analysis of the results shows (Table 4) that the greatest heights were obtained with treatments T1 (142 cm) and T2 (138.4 cm), which are significantly comparable to each other and to treatments T0 (128 cm) and T3 (125 cm) while the lowest height was significantly noted with treatment T4 (112.4 cm). The chlorophyll content varied significantly ($p=0.001$). The lowest value was noted with the PGPR treatment (T3) (4.74 ± 0.9 SPAD value), showing no significant difference from treatments T0, T2 and T4. The highest value was observed with the microdose treatment (T1) (37.42 ± 3.31 SPAD value), which showed no significant difference from treatments T2 and T0. Significant effects were observed for collar diameter ($p = 0.023$) with the PGPR treatment (T3) (6.02 ± 0.53 mm), recording the lowest value and showing no significant difference from treatments T0, T2, T3 and T4. The microdose treatment (T1) showed the highest value (15.42 ± 5.07 mm) and did not differ significantly difference from treatment T4.

The number of leaves varied significantly, treatments T3

stands out clearly with the lowest frequency (5.6%), which is significantly different to that of treatment T0 (75%). In contrast, treatments T2 (95.4%), T3 (83%) and T4 (92.1%) show very high frequencies that are not significantly different from each other.

and T4 (6.8 ± 0.83 and 6.8 ± 1.3) respectively showed the lowest values, and were significantly comparable to each other and to treatments T0 and T2. Treatment T1 showed the highest leaf count (15.4 ± 7.09) and was not significantly different from treatments T0 and T4.

No significant difference was noted among treatments for the number of shoots ($p=0.213$). However, treatment T1 had the highest value (1.8), while treatments T0, T3 and T4, had the lowest values.

Shoot biomass varied significantly. Treatment T0 had the lowest value (7.18 ± 1.53 g), not significantly different from treatments T0, T2, T3 and T4. Treatment T1 has the highest value (23.22 ± 6.63 g), not significantly different from treatments T2 and T3, representing an increase of more than 200% compared to the control.

A significant difference was noted for root biomass. The double inoculation treatment T4 showed the lowest value (1.38 ± 0.67 g) and was not significantly different from treatments T0, T2 and T3. The microdose treatment T1 showed the highest value (4.04 ± 1.32 g) and was no significantly different from treatments T2 and T3.

Table 4. Variation in growth and biomass parameters of millet plants cultivated on the soil of Touba Toul.

Treatment	Plant height (cm)	Chlorophyll content (SPAD value)	Neck diameter (mm)	Number of leaves	Shoot biomass (g)	Root biomass (g)
T0	124.2 ± 20.60 ab	14.56 ± 7.57 ab	6.63 ± 1.33 a	8.4 ± 2.07 ab	7.18 ± 1.53 a	1.98 ± 0.88 ab

Treatment	Plant height (cm)	Chlorophyll content (SPAD value)	Neck diameter (mm)	Number of leaves	Shoot biomass (g)	Root biomass (g)
T1	139.2 ± 14.11 ^b	37.42 ± 3.31 ^b	15.42 ± 5.07 ^b	15.4 ± 7.09 ^b	23.22 ± 6.63 ^b	4.04 ± 1.32 ^b
T2	138.4 ± 13.22 ^b	8.4 ± 3.0 ^{ab}	6.12 ± 0.96 ^a	8.0 ± 3.0 ^{ab}	9.88 ± 2.06 ^{ab}	1.94 ± 1.00 ^{ab}
T3	128.6 ± 8.64 ^{ab}	4.74 ± 0.9 ^a	6.02 ± 0.53 ^a	6.8 ± 0.83 ^a	9.14 ± 0.9 ^{ab}	1.54 ± 0.94 ^{ab}
T4	112.4 ± 6.64 ^a	4.96 ± 1.86 ^a	7.89 ± 1.84 ^{ab}	6.8 ± 1.3 ^a	8.64 ± 0.94 ^a	1.38 ± 0.67 ^a
Average ± SD	128.56 ± 15.97	14.01 ± 13.01	7.93 ± 4.48	9.08 ± 4.67	11.61 ± 6.68	2.17 ± 1.33
RESUME (%)	12.42	93	56	51	58	61
p-value	0.043*	0.001**	0.023*	0.019*	0.002**	0.04*

Codes: 0 "****" 0.001 "***" 0.01 "**" 0.05 "." 0.1 " " 1 (significant difference according to Dunn's test); Mean ± SD (total mean plus or minus standard deviation); CV (coefficient of variation).

Values represent the mean of 5 replicates. Means followed by the same letter in the same column are not significantly different at the 5% level according to Dunn's test.

The highest statistically significant values are assigned the letter "b".

3.2.3. Effect on the Ear Length of Millet Plants

Ears length showed no significant differences between

treatments (Figure 5) indicating treatments applied had no effect on millet ear length. However, treatment T3 showed the highest value (23.2 cm) and treatment T0 the lowest value (20 cm).

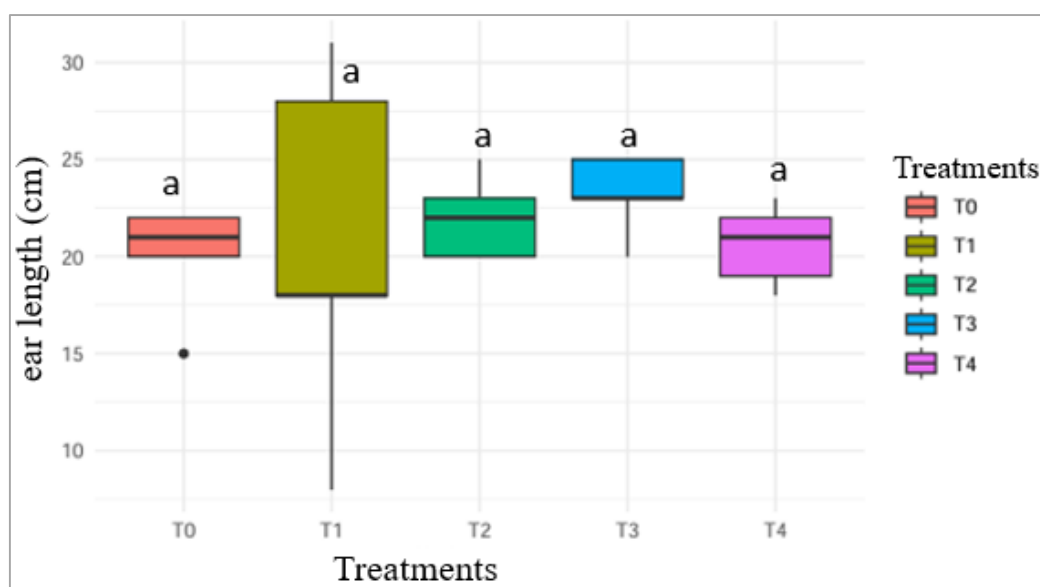


Figure 5. Effect of biofertilizers inoculation and microdose on ear length of millet cultivated on Touba Toul soil.

4. Discussion

Microdose treatment (T1) produced the best results in terms of vegetative growth (collar diameter, leaf number), biomass and yield. The combined supply of nitrogen, phosphorus and potassium promotes chlorophyll synthesis, energy metabolism (ATP) and photosynthetic efficiency, respectively [13, 20].

Combining with compost enhances these effects by improving the biological and chemical properties of the soil [3, 9]. These results are consistent with studies carried out in the Sahel showing the effectiveness of fertilizer microdosing in improving millet and sorghum productivity [12, 19, 25]. However, inoculations with biofertilizers alone did not significantly improve plant millet growth compared to plant control, despite a good mycorrhization. This lack of effect could be explained

by the severe nutrient limitation in the studied soils, which restricts the functional effectiveness of AMF and PGPR [4, 7, 37]. Because biofertilizers needed minimum nutrients for its metabolism, and if not these biofertilizer organisms eventually starve to death, go into dormancy or die before they have had a chance to establish themselves in the rhizosphere [17]. Moreover, in non-sterilized, native strains which are better adapted to the soil, could be in competition with introduced biofertilizer strains and out pass them. Furthermore, the AMF+PGPR combination did not generate any notable synergy compared to microdose, suggesting that the test conditions or interactions between native microorganisms did not allow the optimal expression of their complementary effects [30, 41].

Finally, pearl millet performance was generally better in Touba Toul soil, with marked increases in shoot biomass and yield. This difference in inoculation response between the two soils could be explained mainly by the soil texture, which offers better water and nutrient retention, as well as a pH more favorable to phosphorus absorption [18, 39]. These results confirm that crop response to inoculation and fertilization are highly dependent on local soil conditions [5, 27].

5. Conclusion

Overall, inoculation with biofertilizers (AMF, PGPR and AMF+PGPR) improved root colonization, confirming their ability to stimulate mycorrhizal symbiosis. However, these treatments did not allow very significant gains in growth and yield compared to the control, likely due to the poor nutrient content of the soils. Conversely, organo-mineral fertilization (microdose) produced the best agronomic performance (growth, biomass, yield), confirming the effectiveness of direct inputs of assimilable nutrients. A combined strategy, that integrates biofertilization (AMF and PGPR) with microdosing, appears to be a promising approach to reconcile agricultural productivity, with reduced fertilizer costs and environmental sustainability.

Abbreviations

AMF	Arbuscular Mycorrhizal Fungi
PGPR	Plant Growth-promoting Rhizobacteria
NPK	Mineral Fertilizer, Nitrogen Phosphorus Potassium
LBC	Laboratory of Fungal Biotechnology (LBC)
LCM	Laboratory of Microbiology IRD/ISRA/UCAD
ANOVA	Analysis of Variance

Acknowledgments

The authors would like to thank Cheikh NDIAYE for their technical assistance.

Author Contributions

Khady Diaw: Data curation, Investigation, Methodology, Writing – original draft

Ramatoulaye Thiaba Samba: Conceptualization, Methodology, Supervision, Writing – original draft, Writing – review & editing

Malick Ndiaye: Conceptualization, Resources, Supervision, Writing – original draft, Writing – review & editing

Mame Arame Fall Ndiaye: Conceptualization, Supervision, Writing – review & editing

Funding

This study was supported by the consortium Research Center for International Development (CRDI) and the Ministry of Higher Education, Research and Innovation (MESRI) from Senegal (SGCI2 Program).

Conflicts of Interest

The authors declare any conflict of interest for this research.

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