Improving sorghum (*Sorghum bicolor* L.) production through the use of *Azospirillum* sp. AZ6 and *Trichoderma harzianum* T4-based biostimulants

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ABSTRACT

Objectives: The objective(s) of the present experiment was to evaluate the effect of *Azospirillum* sp. AZ6 and *Trichoderma harzianum* T4-based biostimulants formulated in Mali on the growth and yield of Sorghum in Mali field during monsoon season.
Study design: The experiment was conducted in a completely randomized block design with three treatments.. The blocking factor was the direction of rainwater runoff.
Methodology: The study was carried out in the rural commune of Ouéléssebougou located in the Sudanian. The sorghum seeds inoculated or non-inoculated with *Azozpirillum* sp. AZ6, *Trichoderma harzianum* T4, were randomly sown in single-cropped with no nitrogen (N) application. For the control, 100% dose of N was applied at sowing time at 120 kg/ha. Sorghum plant height, sorghum yield, number of panicles and the weight of 1000 grains weight were evaluated.
Results:
The salient findings showed a significant increase of the parameters examined by *Azospirillum* sp. *AZ6* and *Trichoderma harzianum* T4-based biostimulants. Inoculation of AZ6 improved sorghum yield by 68,25%, while T4 by 8.73% in comparison with the control.
Conclusion: It is evident that *Azospirillum* sp. *AZ6* and *Trichoderma harzianum* T4 are suitable for inoculating sorghum (*Sorghum bicolore* L.). *Azospirillum* sp. *AZ6* produced the highest plant height, yield, number of panicles, and 1000 grain weight relative to *Trichoderma harzianum* T4 and the non-inoculated control. Therefore, this suggest that *Azospirillum* sp. *AZ6* is suitable for large scale application in sorghum fields to reduce the production costs and increase yield while reducing N fertilizer application rate

Keywords: Biostimulant, Microorganisms, Grain, Sowing, Genetic Diversity

1. INTRODUCTION

In Sahelian countries the basic diet of the populations is of cereal origin[1]. Sorghum is one of the main cereals consumed world including Sahel after maize, rice, wheat and barley [2], [3], [4]. In the poorest areas where food security is threatened, sorghum is a commodity sought after in human food, mainly for its pasty, sweet and fructose-rich grains [5] [6]. Sorghum fodder is also used in livestock feed [7]. World sorghum production is 58,705,915 tonnes on an area of approximately 40,251,818 ha with an average yield of 1,458.5 kg/ha [8]. Production in the Western Africa is 13,648,844 tons in an area of 14,025,233 ha, *i.e*. a yield of 973.2 kg/ha [8]. In Mali, sorghum production was 1,801,017 tons for an area of 1,831,825 ha with an average yield of 983 kg/ha [9]. Sorghum constitutes 35% of dry cereal production [10]. *Sorghum* cultivation is characterized by low production due to biotic and abiotic constraints including: insufficient and poorly distributed rainfall, low soil fertility, low production potential of so-called local varieties, the sensitivity of certain varieties to insect pests, weeds, a wide range of diseases and generally unfavorable socio-economic conditions [11], [12], [4].

The use of chemical fertilizers to increase production has led to contamination of groundwater and surface water, harming fish and wildlife. This has led to increased agricultural dependence on fossil fuel resources. It is therefore necessary to develop an effective alternative method to sustainably improve agricultural production while reducing the use of chemicals that have shown their limitations [13]. Research has been conducted in recent years on the use of soil microorganisms, mainly rhizosphere microbes capable of improving plant growth Plant Growth Promoting Microorganisms (PGPM). The several findings of other researchers around the globe have demonstrated the use of these rhizosphere microorganisms as bio-fertilizers, constituting a credible alternative to the use of chemical fertilizers [14] [15],. The *Azospirillum* genus is generally used to improve the mineral nutrition of plants [16], while the Trichoderma genus fungus is used to stimulate plant growth and protect them against a wide range of phytopathogens [17], [18], [19]. The use of PGPM-based bioinoculants can be considered a means to minimize the adverse effects of biotic and abiotic constraints and this aid to transition towards a sustainable agricultural production system [20]. Thus, the present investigation was implemented to (i) formulate biostimulants based on *Azospirillum* sp. AZ6 and *Trichoderma harzianum* T4 and (ii) determine the effects of biostimulants on the growth and production of sorghum in the fields.

2. MATERIAL AND METHODS

2.1 Site Description

The study was conducted in the rural commune of Oueléssebougou. The commune is composed of 45 villages and is at 80 km south of Bamako. It covers an area of 1,117 km^2. It is bordered to the north by the commune of Dialakoroba, to the south by the commune of Kéléya to the east by the commune of Sanakoro-djitoumou and to the west by the commune of Faraba. The climate of this commune is semi-arid, characterized by heat, with two major seasons: a dry season that runs from November to April and a rainy season that extends from May to October. The commune is located in the Sudanian climate zone with an annual rainfall of 600 mm to 1100 mm, but exceeds 900 mm. The relief is monotonous overall with altitudes between 90 m and 100 m. The vegetation consists of shrub savannah.

2.2 Material(s)

2.2.1 Biological material

2.2.1.1 Plant material

The seeds of the SOUBA TIMI variety, used during the trial, were kindly donated by Dr. Aboubacar Touré dit Akar, researcher at International Crops Research Institute for the Semi-Arid Tropics (ICRISAT). The variety has a cycle of 120 days with a potential yield of 3t/ha and a weight of 1000 seeds of 23.7 g. It is a dual-use variety: the grain is mainly for human consumption and fodder for livestock feed. It is possible to produce this variety in the off-season. It is also a variety called stay green, a variety useful for drought resistance [21].

2.2.1.2 Microorganisms

Two strains from the LaboREM-Biotech collection were used during the trial. These are: *Trichodermas harzianum* T4 (fungus) and *Azospirillum* sp. Az6 (Bacteria).

2.3 Methods

2.3.1 Production of biostimulants

2.3.1.1 Culture of *Azospirillum* sp. Az6 and *Trichoderma harzianum* T4

Azospirillum sp. was sub-cultured on a specific medium (Nutrient Agar) and incubated at 30°C for 48 hours; The *Trichoderma harzianum* strain was also sub-cultured on potato dextrose agar (PDA) medium followed by, incubation in BOD at 25°C for seven (7) days.

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2.3.1.2 Production of *Trichoderma harzianum* T4 inoculum

The fungal inoculum (*Trichoderma harzianum* T4) was prepared by producing a homogeneous mixture of mycelial and spores of the fungus in 9 mL of sterile distilled water. One hundred (100) µL of this solution were spread on PDA medium, and incubated for 7 days at 25°C. After incubation, the spores were recovered and transferred to a bottle containing 500 ml of sterile distilled water [20]. Before use in the fields, the sorghum seeds were coated with the spores for 24 hours. After the mixture of charcoal and gum arabic was added [22].

2.3.1.3 Production of *Azospirillum* sp. inoculum. AZ6

The bacterial inoculum (*Azospirillum* sp. AZ6), it was taken to inoculate the nutrient broth and stirred for 72 hours at 200 rpm. After incubation the optical density of the bacterial suspension was adjusted to 108 cfu / ml. Before sowing, the sorghum seeds were sanitized using 2% chloramine T and soaked in the bacterial solution for 60 minutes [23].

2.3.2 Experimental design

To determine the effect of the strains (treatments) on sorghum, a completely randomized block design with 4 blocks and three treatments (T_1: Control; T_2: coated with *Azospirillum* sp. AZ6, and T_3: coated with *Trichoderma harzianum* T4) was used, distributed randomly between the blocks. The blocking factor was the direction of rainwater runoff. The experimental unit was an elementary plot of 4.8 m x 4.8 m or 23.4 m^2. The distance between the blocks was 1 m, the plots was 1 m and the pockets was 0.80 cm.

2.3.2.1 Sowing

Sowing was achieved i on the 17th July, 2022 using the hand at a rate of 5 to 10 seeds per pocket on the 12 elementary plots for the treatments. Before planting the crops, the seedbed was prepared by shallow plowing (30 cm deep). The 15 days after sowing (DAS), thinning was done at the rate of 2 plants per pocket, followed by first weeding using the daba. A second weeding was carried out 30 DAS of sorghum.

2.3.2.2 Monitoring and collection of agronomic parameters

To evaluate the effect of biostimulants on sorghum, the following agronomic parameters wereconsidered the plant size, number of panicles, yield and weight of 1000 grains. Plant heights in cm. All the measurements of the different parameters were carried out on a sample of five (5) plants taken at random for the first time per treatment and repetition: Az6 (Five plants), T_4 (Five plants), Control (Five plants). The five plants of each elementary plot were identified and referenced for the following measurements.

2.3.2.2.1 The number of panicles per treatment

With regard to the number of panicles per treatment (AZ6, T4, Control) was counted after harvesting in each of the four repetitions.

2.3.2.2.2 Yield of each treatment in grams

To calculate the yield for each treatment (AZ6, T4, Control), the weight of the harvested seeds was determined by weighing in anelectronic balance. Before obtaining the total weight and weight of 1000 seeds, the moisture content was estimated for each treatment using a moisture meter.

2.3.2.2.3 Grain size:

The weighing of grain was done after destoning the weight of 1000 seeds for each treatment and this was repeated three times to take the average. In the process, 1000 seeds were first

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counted using a counting device (CONTADOR Type: PFEUFFER). An electronic balance was then used to determine the weight of the 1000 seeds.

2.3.2.3 Statistical analysis of data collected in the field

The homogeneity of variance was verified for all data collected. Non-homogeneous data were transformed before analysis. The ANOVA analysis for different agronomic parameters examined was done for all the data using the General Linear Model (GLM) procedures of the Statistical Analysis System (SAS). For all parameters whose F-calculated value was found to be significant, the means were compared using the least significant difference (LSD) as given by Fischer [24].

The strains *Trichoderma harzianum* T4 and *Azospirillum* sp. AZ6, do not constitute dangers for human health, even less for the environment [24], [25].

3. RESULTS AND DISCUSSION

3.1 Biostimulants formulated with *Trichoderma harzanum* T4 and *Azospirillum* sp. AZ6

The biosinoculants based on *Azospirillium* sp. AZ6 and *Trichoderma harzianum* were produced and used to inoculate sorghum seeds using charcoal powder as a carrier and cassava powder solution as an adhesive (Figure 1 A, B, C)

Figure 1. Coating of sorghum seeds with the formulated bio-stimulants: (A) Inoculum of *Azospirillum* sp. AZ6, (B) sorghum seeds coated with *Azospirillum* sp. AZ6, and (C) sorghum seeds coated with *Trichoderma harzanum* T4.

3.2 Effect of the *Azospirillum* and *Trichoderma*-based biostimulants on sorghum growth and production

The inoculation of sorghum (*Sorghum bicolore* L.) seeds with bio-stimulants formulated with *Azospirillum* sp. AZ6 and *Trichoderma harzianum* T4 isolated in Mali has significantly enhanced plant height, yield, number of panicles and 1000 grain weight of sorghum (Table 1). However, the rate of enhancement varied significantly with the treatments. The *Azospirillum* sp. *AZ6* and *Trichoderma harzianum* T4 bio-inocula were observed to have increased the height, the yield, the number of panicles, and the 1000 grain weight of the sorghum significantly over (control) non-treated. The significantly highest values of height, yield, number of panicles, and 1000 grains weight of sorghum were recorded under *Azospirillum* sp AZ6, followed by *Trichoderma harzianum* T4 (Table 2 and Figure 2).

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Table 1: Analysis of variance for plant height, yield, number of panicles and 1000 grains weight of sorghum (*Sorghum vulgarae*) under cultivated field conditions cond.

Sources of variation	DF	Mean square			
		Plant height (cm)	Yield (T/ha)	Panicles number	1000 seeds weight
Treatments	2	967,80***	0,12***	180,10***	17,54***

DF: Degree of freedom, *Significant, **Very significant, ***Highly significant

Table 2: Effect of bio-inoculants formulated with *Azospirillum* sp. AZ6 and *Trichoderma harzianum* T4 on the height, the yield, the number of panicle, and the 1000 grain weight of sorghum **(*Sorghum vulgarae*) under cultivated field conditions**.

Treatments	Height (Cm)	Yield (T/ha)	Number of panicles	1000 grains weight (G)
Azospirillum sp. AZ6	159.26	2.12	67.17	23.98
Trichoderma harzianum T4	155.62	1.37	55.12	22.08
Control	138.65	1.26	46.02	20.15

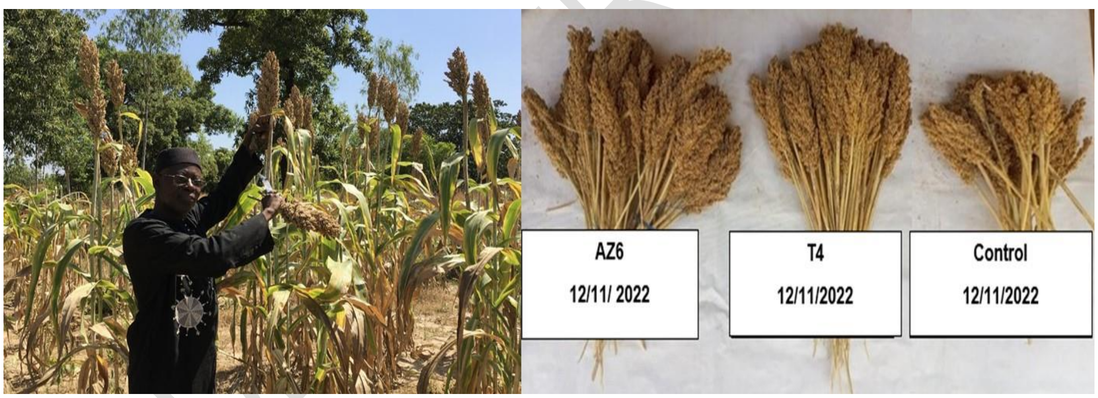

Figure 2. Sorghum in field (A) and a comparison of sorghum ears inoculated with *Azozpirillum* and *Trichoderma harzianum* T4 and with the control (B)

4. Discussions

The inoculation of *Azospirillum* sp. AZ6, significantly increased Sorghum plant height and improved its yield by 68,25% relative to the control. The use of *Azospirillum* sp. AZ6 strain applied in this study was found to have significantly improved maize plant growth, yield and the weight of 1000 grains by producing Indole Acetic Acid (IAA), siderophores and fixed atmospheric nitrogen which is well-known to have a direct effect on plant growth [26]. It was further observed that a significant increase of 20% in maize plant height was obtained while employing *Azpspirillum* sp. AZ6 in comparison with the control (non-inoculated maize seeds) An improvement in maize plant height by 44% and yield by 21.3% was also reported [22], even though the results were lower than those of the present study. These differences may be due to the quality of cereal used as the tests were performed under similar fields' conditions.

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In like manner, Lynch *et al*. [27] demonstrated that the inoculation of *Azospirillum brasilense* allows a higher index of chlorophyll a and b, and the development of the aerial part of the sorghum,thus increasing the specific surface area (SSA) of sorghum roots compared to non-inoculated plants grown only with nitrogen in the soil contaminated with copper.

Earlier findings showed an improvement in plant productivity due to the growth-stimulating activity of *Trichoderma harzianum* [20; 28-29]. In dry climatic conditions in the off-season as in the current study, Wei *et al*. [30] obtained a significant increase in yield after the inoculation of sorghum seeds cv. Ranchero with *Azospirillum brasilens.* Similarly, Cabral-Miramontes *et al*. [31] announced that the *Trichoderma harzianum* T4 strain was capable of increasing the germination rate and advancing maize and rice, mainly by effective control of pathogenic fungi and production of growth promoting substances. Dabire *et al*. [32] reported a significant increase on sweet sorghum plant height, and its growth under saline conditions promoted by *Trichoderma* inoculation through regulation of available nutrients and the bacterial community in the rhizosphere soil. The three *Trichoderma harzianum* strains under field tests demonstrated an outstanding capabilities to adapt to alkalinity stress as evidenced by the present study. These have also shown antagonistic activity against three phytopathogenic fungi. Additionally, the strains provided evidence of significant growth promotion in *Sorghum bicolor* who obtained and an improvement in plant height ranging from 19.75% to 33.33% on six varieties of maize inoculated with *Trichoderma harzianum* in Burkina Faso [33]. The *Trichoderma harzianum* strains significantly increased sorghum yield by 0.52% [33] and this result is lower (15.69%) than the one obtained in maize yield. These differences can be explained by the non-use of the same crop and the same agro-ecological conditions of the study areas.

4. CONCLUSION

The present studies specify that, *Azospirillum* sp. *AZ6* and *Trichoderma harzianum* T4 were suitable for inoculation on sorghum (*Sorghum bicolore* L.). *Azospirillum* sp. AZ6 produced the highest plant height, yield, number of panicles, and 1000 grain weight compared to *Trichoderma harzianum* T4 and the control (non-inoculated). Therefore, *Azospirillum* sp. AZ6 can be suggested for large scale application in the sorghum fields as to minimize the production costs, increase the yield of sorghum and ultimately reduce nitrogen application. The future activities will be the formulation of a bioinoculant and its and large-scale production.

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