Improving sorghum (*Sorghum bicolor* L.) production by using *Azospirillum* sp. AZ6 and *Trichoderma harzianum* T4-based biostimulants

ABSTRACT

Aims: The aim of this work 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 field in Mali, in rainy season.
Study design: The experimental design was a completely randomized block design with four replications, and three treatments randomly distributed. The blocking factor was the direction of rainwater runoff.
Place and Duration of Study: The trials were conducted in the rural commune of Oueléssebougou, located 80 km south of Bamako. The commune is located in the Sudanian climate zone with an annual rainfall of 600 mm to 1100 mm, but exceeds 900 mm.
Methodology: Sorghum seeds inoculated or not with *Azozpirillum* sp. AZ6, *Trichoderma harzianum* T4, were randomly sowed in single-cropped with no N application. For the control, 100% of the dose of Nitrogen was applied at sowing at a dose of 120 kg ha-1 N. Sorghum plant height, sorghum yield, number of panicles and the weight of 1000 grains weight were evaluated.
Results:
The results obtained showed that *Azospirillum* sp. *AZ6* and *Trichoderma harzianum* T4-based biostimulants increased significanly all parameters. Inoculation of AZ6 improved sorghum yield by 68,25%, while T4 by 8.73%, compared to the control.
Conclusion: The present work specify that, *Azospirillum* sp. *AZ6* and *Trichoderma harzianum* T4 were suitable for inoculating on sorghum (*Sorghum bicolore* L.). Azospirillum sp. Az6 produced the highest plant height, yield, number of panicles, and 1000 grain weight, when compared to *Trichoderma harzianum* T4 and the non-inoculated control. Therefore, *Azospirillum* sp. Az6 is suggested for large scale sorghum fields' application, which may reduce production cost, by increasing sorghum yield while reducing Nitrogen application.

1. INTRODUCTION

In Sahelian countries, such as Mali, Niger, Burkina Faso and Senegal, the basic diet of the populations is of cereal origin[1]. Sorghum, one of the main cereals consumed in the Sahel, is the fifth most cultivated cereal in the world 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 West Africa is 13,648,844 tonnes on 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 tonnes 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 microorganisms, mainly rhizosphere microorganisms capable of improving plant growth (MFCP) or PGPM (Plant Growth Promoting Microorganisms) in English. The results of the work of several researchers around the world have shown that the use of these rhizosphere microorganisms as biofertilizers constitutes a credible alternative to the use of chemical fertilizers. The *Azospirillum* genus is generally used to improve the mineral nutrition of plants [14], while the Trichoderma genus fungus is used to stimulate plant growth and protect them against a wide range of phytopathogens [15], [16], [17]. The use of PGPM-based bioinoculants can be considered as a means to minimize the adverse effects of biotic and abiotic constraints and move to a sustainable agricultural production system [18]. The present study therefore aims to (i) formulate biostimulants based on *Azospirillum* sp. Az6 and *Trichoderma harzianum* T4 and (ii) determine their effects on the growth and production of sorghum in fields.

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2. MATERIAL AND METHODS

2.1 Study site

The trials were conducted in the rural commune of Oueléssebougou. The commune is composed of 45 villages and is located 80 km south of Bamako. It covers an area of 1,117 km2. 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.

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2.2 Material
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 de dit Akar, researcher at 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 [19].

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 subcultured on a specific medium (Nutrient Agar) and incubated at 30°C for 48 hours; The Trichoderma harzianum strain was subcultured on PDA medium, then incubated at 25°C for seven (7) days

2.3.1.2 Production of *Trichoderma harzianum* T4 inoculum

The fungal inoculum (*Trichoderma harzianum* T4) was prepared by producing an 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 [21].

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 [22].

2.3.2 Experimental design

To determine the effect of the strains (treatments) on sorghum, we used a complete block design with 4 blocks and three treatments (T1: Control; T2: coated with *Azospirillum* sp. AZ6, and T3: coated with *Trichoderma harzianum* T4) randomly distributed 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 distance between the plots was 1 m and the distance between the pockets was 0.80 cm.

2.3.2.1 Sowing

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

2.3.2.2 Monitoring and collection of agronomic parameters

To evaluate the effect of biostimulants on sorghum, the following agronomic parameters were taken into account: 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), Th (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

Concerning the number of panicles per treatment (AZ6, T4, Control), it was counted after harvesting in the field and this at each of the four repetitions.

2.3.2.2.2 Yield of each treatment in g

To calculate the yield for each treatment (AZ6, T4, Control), the weight of the harvested seeds was determined by weighing using an electronic scale. Before determining the total weight and the weight of 1000 seeds by weighing, the moisture content was determined for each treatment using a moisture meter.

2.3.2.2.3 Grain size:
After destoning the weight of 1000 seeds for each treatment was weighed and repeated three times to take the average. In the process, 1000 seeds were first 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 analysis (ANOVA) for the different agronomic parameters measured was done for all data using the procedures of the General Linear Model (GLM) of the Statistical Analysis System (SAS). For all parameters whose F was found to be significant, the means were compared using the least significant difference (LSD) test protected by Fischer [23].

Ethical considerations
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 a cassava powder solution as an adhesive (Figure 1)

Figure 1. Coating of sorghum seeds with the formulated biostimulants: (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 richoderma-basedbiostimulants on sorghum growth and production

Inoculation of sorghum (*Sorghum bicolore* L.) seeds with biostimulants formulated with *Azospirillum* sp. Az6 and *Trichoderma harzianum* T4, all isolated in Mali, significantly enhanced plant height, yield, number of panicles and 1000 grain weight of sorghum (Table 1). However, the rate of enhancement varied with treatments. The *Azospirillum* sp. *AZ6* and *Trichoderma harzianum* T4 bioinocula significantly increased the height, the yield, the number of panicles, and the 1000 grain weight of the sorghum; over the non-treated control. The highest values of height, yield, number of panicles, and 1000 grains weight of sorghum cultivated in field; were recorded with *Azospirillum* sp Az6, followed by *Trichoderma harzianum* T4 (Table 2, Figure 2).

Table 1. Analyze of variance for plant height, yield, number of panicles and 1000 grains weight of sorghum (*Sorghum vulgarae*) cultivated in field.

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 bioinoculants 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 cultivated in field.

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. Photos of sorghum ears inoculated with *Azozpirillum* sp. Az6 and *Trichoderma harzianum* T4 compared to the control

3.2 Discussion

In this study, the results obtained showed that *Azospirillum* sp. *AZ6* and *Trichoderma harzianum* T4-based biostimulants increased significantly Sorghum plant height, number of panicles, yield and weight of 1000 grains. Inoculation of *Azospirillum* sp. AZ6, significantly increased Sorghum plant height and improved its yield by 68,25% compared to the control. Indeed, [26] showed that the *Azospirillum* sp. AZ6 strain, used in this study, improved significantly maize plant growth, yield and the weight of 1000 grains by producing Indole Acetic

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Acid (IAA), Siderophores and fixing atmospheric nitrogen which has a direct effect on plant growth. They obtained in field with *Azpspirillum* sp. AZ6 an increase of 20% in maize plant height compared to the non-inoculated control. [22] reported an improvement in maize plant height by 44% and yield by 21.3%, results which are lower than those obtained in this study. These differences may be due to the quality of cereal used, because tests was done in the same fields. In the same way, [27] showed 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, increases the specific surface area (SSA) of sorghum roots compared with non-inoculated plants grown only with nitrogen fertilization in soil contaminated with copper.

Earlier, [20] [28] [29] showed an improvement in plant productivity due to the growth-stimulating activity of *Trichoderma harzianum.* In dry climate conditions in the off-season, [30] obtained significative increases in yield, after inoculation of sorghum seeds cv. Ranchero with *Azospirillum brasilens.* In the same way, [31] showed that the *Trichoderma harzianum* T4 strain was capable of increasing the germination rate and growth of maize and rice, mainly by effectively controlling pathogenic fungi and producing growth substances. [32] showed that compared with the non-inoculated control, *Trichoderma* inoculation significantly increased sweet sorghum plant height, and promote its growth under saline conditions by regulating available nutrients and the bacterial community in the rhizosphere soil. Three *T. harzianum* strains, in field tests, showed outstanding capabilities to adapt to alkalinity stress. They also showed antagonistic activity against three phytopathogenic fungi. Additionally, they provided evidence of significant growth promotion in Sorghum bicolor [33] in Burkina Faso who obtained an improvement in plant height ranging from 19.75% to 33.33% on six varieties of maize inoculated with *Trichoderma harzianum*. The *Trichoderma harzianum* strain increased yield by 0.52%. This result is lower than that obtained by [33] with 15.69%. 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 inoculating on sorghum (*Sorghum bicolore* L.). Azospirillum sp. Az6 produced the highest plant height, yield, number of panicles, and 1000 grain weight, when compared to *Trichoderma harzianum* T4 and the non-inoculated control. Therefore, *Azospirillum* sp. Az6 is suggested for large scale sorghum fields' application, which may reduce production cost, by increasing sorghum yield while reducing Nitrogen application

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