

Original Research Article

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Improving Tomato (*Solanum lycopersicum* L.) Production in Greenhouses Using PGPR Bacteria, Actinomycetes and *Pseudomonas* sp.

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ABSTRACT

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The tomato (*Solanum lycopersicum* L.) is a highly important cash crop for market gardeners. It is the most widely consumed culinary ingredient globally and contributes to a healthy, balanced diet. Tomatoes have high water and fertilizer requirements. While organic fertilization is possible, chemical fertilization is the predominant method used. However, the use of chemical fertilizers is becoming increasingly problematic due to high costs and various environmental issues. Consequently, there is a need for alternative fertilization methods that are less expensive, environmentally friendly, and capable of boosting crop yields. This study investigated the use of agriculturally beneficial bacteria specifically Actinomycetes and *Pseudomonas* to enhance greenhouse tomato production. Soil samples were collected, and a tomato fertilization trial was established using sterilized soil. Liquid cultures of isolated Actinomycetes and *Pseudomonas* strains were prepared. A single-block experimental design without replication was employed, focusing on a single factor: fertilization. Data collection covered seed germination rates, the number of branches, the number of fruits, and fruit weight. After a four-month growing period, analysis of variance revealed no significant differences in seed germination rates among the treatments. However, highly significant differences were observed between treatment groups regarding the number of branches, the number of fruits, and fruit weight. Treatments involving these bacteria successfully stimulated tomato production.

Introduction

The tomato (*Solanum lycopersicum* L.) is one of the most important and widely grown cash crops in the world

(Shankara *et al.*, 2005). It ranks second globally after the potato, with approximately 182.3 million tonnes produced annually (FAOSTAT, 2019). The tomato is a very important crop for both smallholders and

commercial farmers (Shankara *et al.*, 2005). Production is concentrated primarily in Asia (61%), while Europe, the Americas, and Africa account for 14%, 13%, and 12% of global production, respectively. China is the world's largest consumer of tomatoes, followed by India, Africa, including North Africa, the Middle East, the United States, and Brazil (FAOSTAT, 2019).

The tomato is the most widely consumed culinary ingredient in the world (Martínez-Damián *et al.*, 2019). Tomato consumption contributes to a healthy, balanced diet (Shankara *et al.*, 2005). Tomatoes are rich in minerals, vitamins, essential amino acids, sugars, and dietary fiber all bioactive compounds that are beneficial to health (Martínez-Damián *et al.*, 2019; Wakchaure *et al.*, 2020). They can be consumed fresh in salads or cooked in sauces, soups, and meat or fish dishes (Krauss *et al.*, 2006). They can also be processed into purée, juice, and ketchup (Krauss *et al.*, 2006; Shankara *et al.*, 2005). Tomatoes can be dried or canned, resulting in processed products of economic importance (Shankara *et al.*, 2005).

Tomatoes are an ingredient in many traditional African dishes (Courchinoux, 2008). They are the most widely cultivated vegetable species in Africa (Courchinoux, 2008). Tomatoes contain a variety of specialized metabolites beneficial to health, including vitamins, carotenoids, phenolic compounds, and glycoalkaloids (Liu *et al.*, 2016; Raiola *et al.*, 2014). These metabolites are bioactive compounds with a wide range of therapeutic properties: anti-inflammatory, anti-allergic, antimicrobial, vasodilatory, antithrombotic, cardioprotective, and antioxidant effects (Raiola *et al.*, 2014).

These molecules are also key determinants of the fruit's taste, color, and texture (Quinet *et al.*, 2019; Martí *et al.*, 2016; Raiola *et al.*, 2014). Tomatoes are thus the primary source of lycopene in the human diet (Viuda-Martos *et al.*, 2014). In addition to carotenoids, tomatoes contain over 500 forms of flavonoids (Quinet *et al.*, 2019; Bovy *et al.*, 2010), more than 100 glycoalkaloids the most abundant being tomatine (Alseekh *et al.*, 2015) and over 400 volatile compounds (Quinet *et al.*, 2019).

According to the (FAO, 2011), 30% to 50% of global food production is estimated to be lost between harvest and consumption. However, drying and packaging tomatoes reduce post-harvest losses, ensure food security, and increase producers' incomes.

The tomato is a crop with high water and fertilizer requirements. Fertilization can be achieved using organic fertilizers such as farmyard manure, poultry manure, and compost or chemical fertilizers (Shankara *et al.*, 2005).

In Mali, tomato crops in the main production zones Ségou, Koulikoro, and Sikasso are primarily fertilized using mineral fertilizers.

However, the use of these chemical fertilizers is becoming increasingly problematic due to their high cost and the various environmental issues they pose, such as the pollution of agricultural soils; the soil balance specifically the Carbon/Nitrogen (C/N) ratio could be disrupted by the presence of heavy metal traces.

Against this backdrop, it is essential to utilize alternative fertilization methods that are less costly, environmentally friendly, and capable of boosting tomato production.

To this end, certain agriculturally beneficial bacteria specifically Plant Growth-Promoting Rhizobacteria (PGPR), such as those belonging to the *Pseudomonas* genus and the Actinomycetes group, which are naturally present in the soil could be harnessed.

This study aims to improve tomato production through the use of Actinomycetes and *Pseudomonas* strains.

Materials and Methods

Study site

The work was conducted using sterilized soil in a greenhouse at the Soil Microbiology Unit of the Laboratory for Research in Microbiology and Microbial Biotechnology (LaboREM-Biotech), within the Faculty of Science and Technology (FST) at the University of Sciences, Techniques and Technologies of Bamako (USTT-B).

Materials

Plant material used

Seeds of the tomato variety Solo were used.

Bacterial strains used

Actinomycete and *Pseudomonas* isolates were used.

Culture media used

Actinomycete Isolation Agar and King B media were used for the isolation and cultivation of Actinomycetes and *Pseudomonas sp.*

Methods

Soil sampling

Two sets of soil samples were collected from a tomato field in the village of Torodo, located in the rural commune of Kambila, Kati Cercle, Mali. The samples in the first set consisted of rhizosphere soil, soil in direct contact with the tomato plant roots. To obtain these, tomato plants were dug up using a hoe, and the soil adhering directly to the roots was collected by scraping. These soil samples were pooled to form a composite sample and placed in a sterile sampling bag. The samples in the second set were collected from various points within the field away from the tomato plants at a depth of 30 cm, with 5 kg collected at each point. These samples were mixed, distributed into sterile sampling bags, labeled, and transported to the laboratory; the samples from the first set were transported in a cooler.

Isolation of Actinomycetes and *Pseudomonas sp.* strains from tomato field soil

A stock solution was prepared using 10 g of soil from plot 1 and 90 mL of sterile distilled water. Serial dilutions, 10^{-1} , 10^{-2} , 10^{-3} , up to 10^{-6} were then prepared from this stock solution in 20 mL test tubes numbered 1 to 6, each containing 9 mL of physiological saline, 8 g NaCl per 1000 mL sterile distilled water. The final two dilutions, 10^{-5} and 10^{-6} were used to separately inoculate AIA and King B media in Petri dishes, using 1 mL of dilution per dish. Three dishes were used for each dilution and medium. The dishes were then incubated at 37°C for 48 hours.

Following incubation, the resulting isolates were subcultured onto the corresponding media and preserved.

Evaluation of the effects of isolated Actinomycetes and *Pseudomonas sp.* strains on the growth of greenhouse grown tomatoes

A tomato fertilization trial using liquid cultures of Actinomycetes and *Pseudomonas* bacteria was conducted

in a greenhouse, using pots filled with the sterilized study soil. Small holes were made in the bottom of each pot.

Preparation of liquid bacterial inocula

A colony from each isolate was picked using a sterile platinum loop and used to inoculate the corresponding liquid medium (without agar) in a flask. After inoculation, the flasks were securely sealed with aluminum foil. All these operations were performed next to a lit Bunsen burner inside a thoroughly cleaned hood. The flasks were then agitated on an orbital shaker.

Soil sterilization

Soil samples from batch 2 were autoclaved twice at 120°C for 20 minutes. After sterilization, the soil was stored in new, sterile bags.

Experimental setup

Filling and placement of pots

After sterilization, the soil was distributed into 10 kg pots. Each pot was filled to three-quarters of its capacity, i.e., 7.5 kg. Once filled, the pots were arranged in rows of ten (10) on a metal cultivation table inside the greenhouse. The spacing between the rows of pots was 30 cm.

Sowing, seed inoculation, and seedling thinning

The seeds were divided into four batches. Seeds from the first, second, and third batches were treated with the liquid culture of the two bacterial isolates, while those in the fourth batch served as controls.

Sowing was performed by hand in pots at a depth of 2 cm, with five seeds per pot. At the time of sowing, 10 mL of the isolate suspension was applied to each corresponding planting hole. Fifteen days after sowing, the seedlings were thinned to three per pot.

Experimental setup

A single-block design without replication was adopted, and a single factor was investigated: fertilization, comprising four (04) treatments: fertilization with an Actinomycete isolate, fertilization with a *Pseudomonas* isolate, fertilization with an Actinomycete isolate plus a

Pseudomonas isolate, and a control. Each treatment consisted of ten (10) pots, Figure 1.

Data collection

The experiment lasted four (4) months. Data collection focused on seed germination rate, number of branches, number of fruits, and fruit weight. The seed germination rate was assessed on the 21st day after sowing by calculating the percentage of germinated seeds. The number of branches was determined by counting in the third month after sowing. The number and weight of fruits were assessed at the time of harvest. These measurements were taken on one plant per pot.

Data analysis

Analysis of variance, ANOVA was performed using R software (version 4.2.1), and Tukey's contrast test at the 5% significance level (Bertrand and Maumy, 2011) was used to compare the means.

Results and Discussion

Actinomycete and *Pseudomonas* sp. isolates obtained

Figure 2 shows an image of the isolates obtained for the two groups of bacteria.

These results show that the experimental soil contains strains of Actinomycetes and *Pseudomonas*.

Effects of Actinomycetes and *Pseudomonas* sp. strains used to improve greenhouse tomato production

Analysis of variance showed no difference among the various treatments regarding seed germination rates. However, it revealed a highly significant difference among plants from the different treatments regarding the number of branches, number of fruits, and fruit weight (Table 1).

Indeed, the number of branches is higher in fertilized plants than in control plants. The number and weight of fruits are higher in plants fertilized with the Actinomycetes + *Pseudomonas* mixture than in plants from the other treatments and, above all, the controls (Table 2).

These results indicate that, with the exception of the seed germination rate, the values obtained for the other variables were higher in plants treated with the biofertilizers than in the control plants. Overall, fertilization using the Actinomycetes + *Pseudomonas* mixture proved more effective than fertilization with either type of bacterium used individually.

Analysis of the data showed that plants inoculated with the Actinomycetes + *Pseudomonas* combination yielded the best results, averaging 18 fruits per plant, followed by those inoculated with *Pseudomonas* and Actinomycetes alone, with averages of 16 and 14 fruits per plant, respectively. Significant differences were observed regarding fruit weight; the highest values were obtained with the Actinomycetes + *Pseudomonas*, Actinomycetes, and *Pseudomonas* treatments, which exceeded the non-inoculated control by 237.70 g, 206.85 g, and 179.34 g per plant, respectively.

Figure 3 shows tomato plants at the fruit-ripening stage, either fertilized or unfertilized with fertilizers based on Actinomycetes and *Pseudomonas*.

The present study reveals that seed germination rates were high across all plants, whether fertilized or not. Furthermore, fertilization using a mixture of the two rhizobacteria Actinomycetes and *Pseudomonas* proved more effective than using either bacterium alone. Our findings highlight the impact of Actinomycetes and *Pseudomonas* sp. strains on tomato production. These results corroborate earlier studies regarding the positive role these strains play in the growth and yield of vegetable crops, including tomatoes.

Indeed, our results demonstrated that tomato plants inoculated with Actinomycetes and *Pseudomonas* sp. Isolates applied separately showed a marked improvement in growth and fruiting compared to the control. These findings were confirmed by Nantoumé *et al.*, (2018), who showed that tomato seeds inoculated with rhizospheric bacterial isolates and grown in a greenhouse produced plants with improved growth.

The present study results also align with those of Giovanardi and Stefani (2018), who observed improved growth and yield in tomato plants inoculated with Actinomycete strains. Actinomycetes influence plant growth due to their ability to produce phytohormones and other important molecules Lima (2008) an effect reflected in our results regarding the number of branches on the tomato plants.

Figure.1 Young plants fertilized or not with Actinomycetes and Pseudomonas strains in a greenhouse



Figure.2 Image of Actinomycetes (A) and *Pseudomonas* sp. (B) isolates obtained from the study soil.

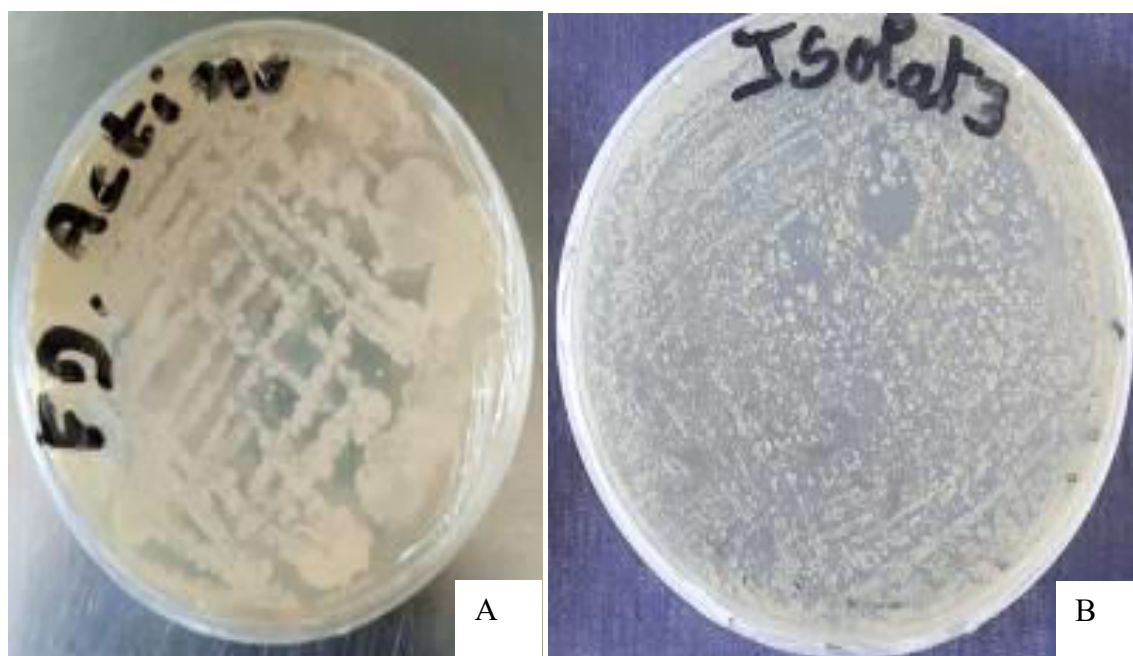


Figure.3 Image of greenhouse-grown tomato plants at the fruit-ripening stage, either fertilized or not with fertilizers based on Actinomycetes and *Pseudomonas*



Table.1 Analysis of variance of seed germination rate, number of branches, number of fruits, and fruit weight of fertilized and unfertilized tomato plants.

Source of variation	DDL	Mean of squares			
		Measured variables			
		Germination rate	Number of branches	Number of fruits	Fruit weight
Treatments	9	1.8***	1.6***	351.3***	75394***

*** Highly significant ; DDL: Degree of Freedom.

Table.2 Mean values for seed germination rate, number of branches, number of fruits, and fruit weight of fertilized and unfertilized tomato plants.

Treatments	Germination rate	Number of branches	Number of fruits	Fruit weight (g)
Witness	100 a	1,20 a	5,00 a	46,77 a
Actinomycètes	100 a	2,00 b	14,00 b	206,85 c
<i>Pseudomonas</i>	100 a	2,00 b	16,00 c	179,34 b
Actinomycètes + <i>Pseudomonas</i>	100 a	2,00 b	18,00 d	237,70 d

Each value represents the mean of fifty (50) seeds for germination rate and of ten (10) plants for the number of branches, number of fruits, and fruit weight. Values followed by the same letter are not significantly different according to the Tukey contrasts test at the 5% level.

Patil *et al.*, (2011), as well as Elshafie and Camele (2022), demonstrated the growth-stimulating effect of Actinomycete isolates on tomato plants. The findings of

these authors corroborate our own regarding the beneficial impact of these microorganisms on tomato production.

Yogesh *et al.*, (2021) observed 100% seed germination and increased shoot and root lengths in vegetable crops including tomatoes following the in vitro antagonistic screening of actinobacteria strains. This result aligns with our findings regarding seed germination rates and, in terms of growth, the number of branches on fertilized plants compared to the controls.

Fernandez *et al.*, (2015) have already demonstrated that *Pseudomonas* bacteria are capable of growing in agricultural soils like the one used in our experiment. Sandhya *et al.*, (2010) observed enhanced stimulation of growth parameters in tomato plants attributed to *Pseudomonas sp.* These results confirm the findings of our own work on tomato plant growth.

Aminisarteshnizi (2022) inoculated greenhouse eggplant seedlings with *P. fluorescens* isolates, significantly improving plant growth parameters and reducing nematode reproduction associated with the crop. This finding supports our own results regarding the positive impact of these rhizobacteria on crops. Bossis *et al.*, (2000) reported that fluorescent *Pseudomonas* species are of potential environmental interest. Some enhance plant growth and health and help reduce the use of synthetic agricultural inputs a point we aim to highlight in our study. Similarly, studies by García-Villaraco *et al.*, (2021); Boubguel and Kacel (2016) demonstrated that certain *Pseudomonas fluorescens* strains increase tomato plant weight. These results, which align with our own, are supported by Lemanceau (1992), who reported that the use of fluorescent *Pseudomonas* strains capable of persisting and proliferating on the root system yields significant benefits for the inoculated plants.

Furthermore, the combined action of the two strains *Pseudomonas sp.* and actinomycetes stimulated tomato plant development, as demonstrated in the study by Gravel *et al.*, (2007), they noted that both fruit number and weight were higher in plants treated with the *Pseudomonas* and actinomycete mixture than in those treated with the strains individually or in the control group. These results regarding tomato growth and fruit weight confirm the positive effect of the synergy between *Pseudomonas* and Actinomycetes on production; they align with findings by Yattara *et al.*, (2008); Glick *et al.*, (2007) and Egamberdieva (2007), all of whom demonstrated that interactions between plants and rhizospheric microorganisms benefit plant production as is the case here with the tomato crop.

This study was conducted to investigate the use of two bacteria of agricultural interest Actinomycetes and *Pseudomonas* for enhancing greenhouse tomato production. The experiment demonstrated that treatments with these rhizobacteria stimulated tomato production.

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Author Contributions

Souleymane KONE: Investigation, formal analysis, writing—original draft. Fassely DIARRA: Validation, methodology, writing—reviewing. Mariam DIOP:—Formal analysis, writing—review and editing. Souleymane Bathié KONE: Investigation, writing—reviewing. Rokiatou FANE: Resources, investigation writing—reviewing. Mahomed Sanogo: Validation, formal analysis, writing—reviewing. Bakary CISSE: Conceptualization, methodology, data curation, supervision, writing—reviewing the final version of the manuscript.

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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