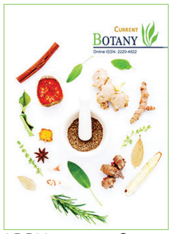
Current Botany 2024, 15: 81-86
doi: 10.25081/cb.2024.v15.8839
https://updatepublishing.com/journal/index.php/cb

Research Article



ISSN: 2220-4822

Effect of vermicompost enriched with bacterial endophytes (*Azospirillum* and *Rhizobium*) on growth and yield of tomato

S. Nithya, K. Sasikumar, O. S. Sethu Raman*

Department of Zoology, Kandaswami Kandar's College, Velur, Namakkal-638182, Tamil Nadu, India

ABSTRACT

The extensive use of chemical fertilizers has served as a response to the increasing need for crop production in recent decades. While it addresses the demand for food, it has resulted in a decline in crop productivity and a heightened negative environmental impact. In contrast, bacterial endophytes namely *Azospirillum* and *Rhizobium* and vermicompost offer a promising alternative to mitigate the negative consequences of chemical fertilizers. It can enhance nutrient availability, promote plant growth, and improve nutrient uptake efficiency, thereby reducing the reliance on chemical fertilizers. In this study, two bacterial endophytes *Azospirillum* and *Rhizobium*, combination with vermicompost and chemical fertilizer were used to investigate their potential role in the enhancement of growth yields of tomato. The inoculation of bacterial endophytes enhanced the root and shoot length, biomass and leaf chlorophyll contents. The fruit weight of the tomato (kg/plant) was also higher in the vermicompost and bacteria inoculated plants of tomato than in the chemical fertilizer. The mixed application of vermicompost with the combination of *Azospirillum* and *Rhizobium* showed the best performance compared to other treatments.

Received: September 21, 2023
Revised: June 16, 2024
Accepted: June 25, 2024
Published: July 05, 2024

***Corresponding author:**
O. S. Sethu Raman
E-mail: jayasamys@gmail.com

KEYWORDS: Vermicompost, Tomato, Endophytes, *Rhizobium*, *Azospirillum*, Chemical fetilizer

INTRODUCTION

Environmental degradation results from the excessive application of synthetic fertilizers, which have become more expensive as time passes. It is urgent to implement organic agricultural practices to increase crop yields while reducing environmental impact and costs. Additionally, the environmental pollution that is exacerbated during the production of synthetic fertilizers will be diminished through the use of organic agricultural methods. By employing vermitechnology, a biotechnological advancement, one of the primary instruments for valorization is transforming solid organic wastes into a useful compound through bioconversion; this holds great promise for the sustainable management of wastes under favourable ecological conditions (Hait & Tare, 2012). An acceptable biodegradation method (Bhat *et al.*, 2018), is defined by the severe molding of organic solid wastes generated by the combined activity of earthworms and microorganisms. Endophytes are microorganisms found within plants that include fungi, actinomycetes, bacteria, and other microbes. Throughout its life cycle, it appears to have no noticeable negative effect on the plant tissues it occupies. These bacteria, which normally inhabit intercellular gaps, have been isolated from a wide range of plant species and tissues, forming a huge reservoir of bacterial diversity (Ulrich *et al.*, 2008).

Endophytic bacteria, which infiltrate the internal tissues of their host plants and are capable of creating a range of partnerships such as symbiosis, mutualism, neutralism, and commensalism, have been found in almost every plant investigated. Endophytic bacteria include Acetobacter, Arthrobacter, *Bacillus*, *Burkholderia*, *Enterobacter*, *Herbaspirillum* and *Pseudomonas* (Lodewyckz *et al.*, 2002). There has recently been a surge in the number of newly discovered endophytic nitrogen-fixing bacteria. Among these are *Alcaligenes faecalis* (Zhou & You, 1988), *Herbaspirillum seropedicae* (Cavalcante & Dobereiner, 1998), and *Pseudomonas*, which is now recognized as a second species of *Herbaspirillum*, *Azoarcus spp*. (Reinhold-Hurek *et al.*, 1993).

It's important to think about how plant-beneficial bacteria can be exploited in agriculture as biofertilizers, insecticides, and phytoremediation approaches (Weyens *et al.*, 2009). According to Gamo and Ahn (1991), *Azospirillum* sp. wasisolated from a range of non-graminous cereals and successfully colonized and enhanced acetylene reduction. Steenhoudt and Vanderleyden (2000) described *Azospirillum*, a bacterium that is closely related to grasses and fixes nitrogen. Nitrogen fixation by bacteria in non-leguminous field crops enabled a favorable connection between *Azospirillum* and wheat. Studying the mechanisms that stimulate plant development, as well as understanding each stage of PGPB plant colonization, is critical for improving the reliability and efficacy of inoculant strains. Over the last decade, numerous investigations have shown that a wide variety of endophytes, predominantly from the rhizosphere, establish themselves inside plants (Hardoim *et al.*, 2008). Certain endophytes have also been shown to benefit plant health and growth.

To conduct this study, endophytic bacteria that fix nitrogen were obtained from tomatoes. Vegetables are the most strategically important food commodity in India, particularly in Tamil Nadu, in terms of both cultivation and consumption. Vegetables are now incorporated into everyday routines in a variety of ways, including as snacks and components of main meals. Identification and isolation of effective nitrogen-fixing bacteria is critical for increasing productivity and growth while reducing the usage of dangerous nitrogen fertilizers. This is due to the fact that nitrogen is the key element necessary for its formation. The primary focus of the study has been on the properties of these isolates as agronomic inoculants; nevertheless, the investigation of the bacterial community that pervades plants has received very little attention.

Tomatoes (*Solanumlycopersicum*) are a well-known vegetable all over the world for theirnutritional, economic, and social value. Its antioxidant capabilities play an important function in human nutrition (Stoleru *et al.*, 2020). According to FAOSTAT (2020), tomatoes have been grown on more than 5 million hectares globally with an average yield of 35.9 t/ha, with an annual production of approximately 180 million tons. Tomatoes can be eaten in a variety of ways, both fresh and processed, making them an important global crop (Ray *et al.*, 2011). Tomato consumption is currently prioritized in rational nutrition views; owing to the vast array of nutritious and beneficial components it contains (Felföldi *et al.*, 2021). So, the present study was aimed to investigate the effects of a nutrient-dense diet and endophytic nitrogen-fixing bacterial species on tomato yield and growth.

MATERIALS AND METHODS

Seed Collection Andbacterial Endophytes Treatment

The tomato seeds were obtained from KrishiVigyan Kendra (KVK), Namakkal, Tamilnadu.Seed inoculationwas done with viable bacterial isolates of *Azospirillum* and *Rhizobium*and cultivated. Two grams of tomato seeds were treated with one-half milliliter each of *Azospirillum* and *Rhizobium* in their individual and dual forms, as described in the following procedure. As a control, the untreated seedlings were maintained. Following shade drying, the treated seeds were promptly sown in plates at a rate of three seeds per cup.

Preparation of Inoculum

After preparing liquid media devoid of nitrogen for *Azospirillum* and liquid medium containing yeast extract mannitol for *Rhizobium*, the cultures were inoculated and kept at 32 °C in a rotating agitator for 48 hours. The population test was carried out as a seed inoculation using a normal procedure. The density of the culture was calculated using turbidity after shaking.

Vermicompost Preparation

The vermicompost was prepared using circular plastic pots. Each pot contained a 1:1:1 mixture of paper mill sludge (PMS), sugar mill press mud (SPM) and cow dung. Each batch included fifteen adult worms. A 90-day-old vermicompost sample was harvested and used for the experiment.

Treatments and Field Trial

The research study was conducted in a cultivable land area between July and October 2022 using a randomized block design. It comprised three replications and eight treatments (T_1-Chemical fertilizer; T_2-Vermicompost; T_3-Chemical fertilizer+*Azospirillum*; T_4-Chemical fertilizer+*Rhizobium*; T_5-Vermicompst+*Azospirillum*; T_6-Vermicompost+*Rhizobium*; T_7-Chemical fertilizer+*Azospirillum*+*Rhizobium*; T_8-Vermico mpost+*Azospirillum*+*Rhizobium*). Seedlings were grown on plates for 30 days and submerged for 60 seconds in a solution containing cultures of *Azospirillum* and *Rhizobium*. Finally, the tomato seedlings were transplanted into the field at a rate of two seedlings per pit with 75 cm spacing between rows and plants.

Growth Parameters

Shoot length

The shoot length of treated plants was measured at 30^{th}, 50^{th}, and 70^{th} day after transplanting (DAT).The branch length in centimeters from the base of the plant to its terminal growing point was measured on the selected plants.

Root length

Root length was determined from the base of the plant to the apex of the longest root on the 30^{th}, 50^{th} and 70^{th} day after transplanting (DAT).

Leaf Chlorophylls Content

The completely grown leaves were taken for chlorophyll content analysis of the treated plants. Fresh leaf extract (0.03 g) from each compost treatment was extracted using 5 mL of N dimethyl formamide and chlorophyll a, b, and total chlorophyll content were determined calorimetrically at wave lengths of 663.8 and 646.8 nm, respectively.

Determination of Number of Leaves and Quantity of Flowers

After the 30th, 50th, and 70th DAT, the number of leaves was tallied. At 30, 50 and 70 days after planting, the plants were counted to determine how many blooms had opened in each treatment.

Characteristics of Yield

The total number of fruits gathered at the 70th day and the mean number of fruits per plant was determined. The fruit weight was measured independently on the seventieth day. For fruit measurement, Vernier calipers were used to measure each fruit's length in centimeters from the base of the calyx to the tip, and the average was computed on a regular basis. Also, the girth of the fruit was measured and the average was then calculated and expressed in centimeters.

Characteristics of Fruit Quality

Ten tomato fruits were used to assess fruit quality. Fruits were cut into tiny pieces and a juice extractor was used to extract an exact volume (mL) of tomato juice from each of the ten fruits. Clear juice was used to conduct quality tests. After the extraction of the pulp, peels and seeds, the juice volume was measured in a graduated cylinder. The pH of tomato fruit juice was determined in a 50 mL filtrate juice generated from 10 g of fruit flesh tissues combined with 100 mL of distilled water (Polat *et al.*, 2010).

Determination of Titratable Acidity (TA) and Ascorbic Acid (AA)

Titratable acidity of the tomato was measured after diluting 10 g of a homogenized tomato juice sample with 50 mL of distilled water and 0.1% NaOH solution at a pH of 8.17, the result was expressed as g/L (Thakur *et al.*, 1996).

The ascorbic acid concentration of tomatoes was measured using a metod described by Tareen *et al.* (2021). 5 g of homogenized fruit pulp with 5 mL of 0.1% HCl (w/v) was taken and the mixture was then centrifuged at 10,000 rpm for 10 minutes to extract the supernatant. A spectrophotometer was employed at 243 nm to test the supernatant solution's absorbance.

Statistical Analysis

All of the data were analyzed (SPSS V15). A one-way analysis of variance (ANOVA) with a significance level of $p<0.05$ was employed to compare the experimental parameters. The data were reported as means ± the standard deviation (SD).

RESULTS AND DISCUSSION

The present study revealed that a significant statistical variations between the various types of vermicompost, biofertilizers and chemical fertilizer combinations. Non-sterile soil demonstrated a more prominent stimulatory effect of bacterial treatments on plant growth and development thansterile soil. The administration of superior endophytic nitrogen fixing bacterial strains to seeds resulted in significant improvements in tomato seed germination, growth, and yield. The results of this investigation showed that seed inoculation with T_8 vermicompost including *Azospirillum* and *Rhizobium* was superior to other combinations.

Growth Parameters

The shoot length, root length, leaf chlorophyll, leaf count, and flower count were all measured and recorded at 30, 50, and 70 days. Tables 1 to 3 shows that vermicompost coupled with *Azospirillum* and *Rhizobium* (T_8) grew faster on all testing days.

When plants treated with vermicompost containing *Azospirillum* and *Rhizobium* were compared to the control group, plant growth parameters such as shoot length, root length, leaf count, leaf chlorophyll content, and blossom count performed better. The results presented here are consistent with previous publications (Joshi & Vig, 2010). Vermicompost enhances soil texture, aeration, and compaction, allowing plants to absorb more water and nutrients from the surrounding root zone. This is a significant contribution to soil health and plant development. The current findings are consistent with those of previous research conducted by Sangwan *et al.* (2010).

In the present study, vermicompost on tomato plants greatly increased growth parameters. Vermicompost contains hormone-like characteristics that promote increased biomass and root initiation improving the plant growth. Table 3 shows that the effects of varying vermicompost rates on the amount of chlorophyll in leaf tissue were statistically significant. The study's findings suggest that the amounts of chlorophyll a ranged from 0.258 to 0.176 mg/100 mL, while chlorophyll b ranged from 0.296 to 0.092 mg/100 mL. T_8 plants had the highest leaf concentrations of total chlorophyll, chlorophyll b, and chlorophyll a, whereas, T_1 plants had the lowest amounts. The amount of chlorophyll did not vary significantly, although T_5 and T_4 plants had more than T_1 plants. Because T8 had more nitrogen than the other kinds, it had the largest impact on chlorophyll levels (Table 2).

The soil's nitrogen content most certainly increased after adding vermicompost. However, there is frequently a link between chlorophyll concentration in a growing media and the amount of nitrogen available for plant development (FAOSTAT, 2020). The observed improvement in growth characteristics may be explained by the fact that *Azospirillum*-inoculated plants had faster nutritional absorption of dry matter, N, P and K in their stems and leaves than normal plants. *Azospirillum* sp. increases plant water interaction, mineral absorption, and root development to promote plant growth and productivity. In addition to its ability to fix nitrogen, *Azospirillum* produces growth-promoting chemicals such as GA and IAA. These hormones considerably aid crop development. Pollen germination and tube growth were increased, resulting in an

Table 1: Effect of efficient isolates of *Azospirillum* and *Rhizobium* on growth parameters of tomato on 30th day in field experiment

Treatments	Shoot length (cm)	Root length (cm)	Number of leaves	Number of flowers	chlorophyll 'a' (mg/g FW)	chlorophyll 'b'(mg/g FW)	Total chlorophyll (mg/g FW)
T1	9.35±2.04	4.18±1.05	9.27±2.03	0	0.117±0.05	0.132±0.11	0.249±0.27
T2	10.03±1.92	4.60±1.09	10.51±1.85	0	0.129±0.08	0.125±0.07	0.254±0.23
T3	10.39±2.38	5.29±0.92	9.96±1.02	0	0.131±0.11	0.142±0.16	0.273±0.35
T4	10.18±2.03	5.02±1.51	12.48±1.27	0	0.125±0.13	0.158±0.12	0.283±0.29
T5	10.5±1.91	5.48±1.30	10.26±2.15	0	0.115±0.09	0.163±0.18	0.278±0.21
T6	10.22±2.58	5.15±1.08	9.93±1.28	0	0.139±0.1	0.157±0.13	0.296±0.19
T7	10.98±3.01	5.62±1.63	11.42±1.92	0	0.128±0.08	0.146±0.21	0.274±0.34
T8	11.69±2.38	6.02±1.51	10.35±1.26	0	0.147±0.12	0.172±0.14	0.319±0.39

Values are mean±SD of three replicates

Table 2: Effect of efficient isolates of *Azospirillum* and *Rhizobium* on growth parameters of tomato on 50th day in field experiment

Treatments	Shoot length (cm)	Root length (cm)	Number of leaves	Number of flowers	chlorophyll a (mg/g FW)	chlorophyll b (mg/g FW)	Total chlorophyll (mg/g FW)
T1	25.42±1.32	10.23±0.25	24.00±1.10	5.42±0.05	0.152±0.03	0.193±0.04	0.345±0.18
T2	30.12±161	12. 25±0.32	19.93±3.11	6.10±0.08	0.161±0.04	0.198±0.05	0.359±0.13
T3	32.15±2.04	12.87±0.20	23.38±1.05	6.38±0.03	0.173±0.02	0.245±0.05	0.418±0.15
T4	29.15±1.42	11.97±1.29	22.43±1.08	6.98±0.04	0.142±0.02	0.176±0.03	0.318±0.12
T5	31.15±1.82	14.01±2.08	25.13±2.05	7.35±0.05	0.155±0.03	0.201±0.06	0.356±0.18
T6	30.05±1.03	11.31±1.02	22.73±1.28	8.12±0.07	0.147±0.05	0.194±0.04	0.341±0.12
T7	30.98±1.24	10.23±0.25	21.49±2.08	8.74±0.03	0.182±0.04	0.212±0.05	0.394±0.17
T8	33.17±1.08	12.45±0.23	20.87±1.15	9.69±0.08	0.199±0.05	0.243±0.06	0.442±0.20

Values are mean±S.D of three replicates

Table 3: Effect of efficient isolates of *Azospirillum* and *Rhizobium* on growth Parameters of tomato on 70th day in field experiment

Treatments	Shoot length (cm)	Root length (cm)	Number of leaves	Number of flowers	chlorophyll a (mg/g FW)	chlorophyll b (mg/g FW)	Total chlorophyll (mg/g FW)
T1	50.25±1.63	14.03±1.05	34.12±1.08	12.31±0.52	0.172±0.02	0.237±0.03	0.409±0.19
T2	50.98±2.16	15. 52±1.02	37.23±2.01	13.19±0.76	0.179±0.05	0.213±0.04	0.392±0.21
T3	51.39±1.08	15.07±0.70	33.58±1.75	14.20±0.71	0.183±0.03	0.285±0.05	0.468±0.09
T4	55.42±1.52	13.97±1.57	35.13±1.48	14.18±0.47	0.153±0.02	0.198±0.02	0.351±0.11
T5	53.12±1.81	15.71±1.52	32.51±1.08	15.72±0.60	0.165±0.04	0.217±0.04	0.382±0.15
T6	51.15±2.34	14.83±1.25	38.89±2.18	14.65±0.73	0.158±0.03	0.205±0.03	0.363±0.12
T7	53.87±2.08	15.68±1.13	35.17±1.05	14.26±1.03	0.197±0.01	0.245±0.04	0.442±0.18
T8	59.15±1.20	16.07±1.08	38.91±1.31	16.10±1.47	0.216±0.05	0.273±0.05	0.489±0.15

Values are mean±S.D of three replicates

increase in fruit set. This could be related to the increased levels of endogenous hormones in plant tissue caused by *Azospirillum* derived IAA.

Yield Parameters

The application of the optimal dose of vermicompost, which includes *Azospirillum* and *Rhizobium* (T_8) resulted in a significant increase in yield (Figure 1).

The results of the present study is comparable with the previous report published by Kashem *et al.* (2015) who studied the effects of bovine manure vermicompost and inorganic fertilizers on the vegetative growth and fruit production of tomato. The results showed that all parameters were increased significantly in plants treated with vermicompost compared to the plants treated with inorganic fertilizer (Table 4). Microorganisms improve plant nutrient availability by hastening the decomposition of organic manures. Vermicompost's establishment and productivity help a wide range of agricultural crops, vegetables, flowers, and fruits. Cantanazaro *et al.* (1998) highlighted the importance of synchronizing nutrient release with plant absorption, as well as how delayed release fertilizers can increase plant yields while reducing nutrient leaching. When utilized widely in horticulture, vermicompost can aid with macrophyte

Figure 1: Effect of vermicompost enriched *Azospirillum* and *Rhizobium* on yield of tomato

Table 4: Effect of efficient isolates of *Azospirillum* and *Rhizobium* on yield parameters of tomato

Treatments	Number of Fruits/Plant	Fruit weight (g)	Fruit diameter (cm)	Average Fruit Yield/ Plot
T1	8.42±1.37	60.13±0.72	2.61±0.18	4.23±1.27
T2	10.16±0.95	68.27±0.38	2.82±0.27	4.48±1.56
T3	9.87±0.53	71.15±0.59	3.79±0.59	5.97±1.26
T4	10.43±0.39	69.48±0.47	3.88±0.36	6.41±2.14
T5	10.91±0.41	68.17±0.82	3.74±0.27	7.41±2.08
T6	12.07±0.90	73.30±0.54	3.69±0.25	7.43±1.28
T7	13.85±1.03	73.49±0.39	3.98±0.20	8.61±1.17
T8	16.18±1.47	75.16±0.54	4.14±0.57	8.92±1.54

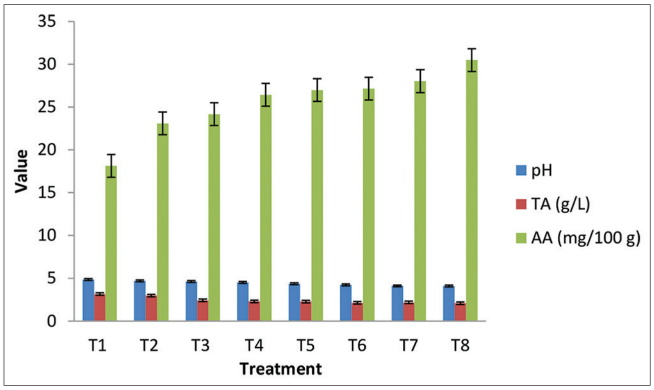


Figure 2: Effect of vermicompost enriched with bacterial endophyteson physico-biochemical parameters of tomato fruit

management and disposal, as well as nutrient depletion and a lack of organic matter in these soils (Najar & Khan, 2013).

The *Azospirillum* treatment increased the nitrogen content and availability in plants. The addition of *Azospirillum* boosts the nutrient uptake such as potassium and phosphate, and encourages the creation of phytohormone-like compounds. The biological activity of the microorganisms would have enhanced the soil condition, resulting in an area ideal for giving critical nutrients to the plant roots. It is possible that the macronutrients in vermicompost promote increased chlorophyll production, enzyme system improvement, normal fruit weight enhancement and plant development acceleration. Furthermore, earthworms produce humus through organic processes that boost plant metabolic rate and nutrient absorption. Vermicompost enhances plant development by promoting the transformation of rhizosphere microorganisms and the eradication of plant diseases.

Characteristics of Fruit Quality

Vermicompost with *Rhizobium* and *Azospirillum* produced the highest levels of fruit biochemical constituents, such as pH, TA, and vitamin 'C' (ascorbic acid). The observed growth features may have resulted from the increased rates of nutrient absorption displayed by *Azospirillum* inoculated plants compared to those that were not. N, P and K concentrations in the stems and leaves of the plant increased as a consequence of this. In comparison to the other treatments, the T_8 treatment produced the highest concentration of notable AA in tomato fruit per hundred grams, there were 21 to 35 milligrams of vitamin C. Better plant growth was observed in T_8 (Vermicom post+*Azospirillum*+*Rhizobium*) might be responsible for the higher quality fruit yield observed (Figure 2).

pH and TA values were much higher in T_1 than those of the other vermicomposts that were supplemented with varying amounts of PMS and SPM. The appropriate pH for tomatoes is required for fresh eating; a low pH brings out the flavor and aroma of the fruit. The pH of each compost variety ranged from 4.11 to 4.86. The measured values are consistent with those reported by other researchers in other investigations (Aurora *et al.*, 2019). TA levels in this experiment ranged from 3.03 to 4.15 (g/L). The T_1 compost had the highest TA value of 4.15 g/L, while the T_8 compost had the lowest TA value of 2.03 g/L. The findings of Zoltán *et al.* (2022) showed that the nutritional quality parameters of raw red tomato kinds grown organically are similar to our findings. However, it is necessary to have a more comprehensive understanding of the process of bacterial colonization of plants to make a more precise evaluation of the likelihood of bacterial establishment in the plant environment after the application of field biofertilizer, as well as the interactions that occur between bacteria and plants. Both the quantity and quality of nutrients found in vermicompost are improved by bacterial endophytes, which also stimulate the growth and production of crop plants.

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