International Journal of Botany

ISSN: 1811-9700

OPEN ACCESS International Journal of Botany

ISSN 1811-9700
DOI: 10.3923/ijb.2024.129.134

Research Article
Assessment of Different *Rhizobium* spp. Strains as Bio-Fertilizer in Tomato Plants

[1]Gbenga Oluwayomi Agbowuro, [2]Olubunmi Olufunke Olajide and [2]Mose Jimoh Falade

[1]Department of Biosciences and Biotechnology, University of Medical Science, Ondo, Nigeria
[2]Department of Crop, Horticulture and Landscape Design, Ekiti State University, Ado Ekiti, Nigeria

Abstract

Background and Objective: Insufficient soil nutrients lead to poor crop yield, which warrants using fertilizers to boost yield. However, synthetic fertilizers at farmers' disposal are hazardous to humans and their environment. Thus, there is a need to find a sustainable approach to augmenting soil nutrients for better yield without hampering man and his environment. This research aimed at evaluating *Rhizobium* strains as bio-fertilizers on tomato plants and comparing them with NPK 15:15:15. **Materials and Methods:** Two *Rhizobium* strains (*Bradyrhizobium* spp., strain USDA 3384 and *B. japonicum* strain IRJ 2180A) were used to inoculate tomato plants and NPK 15:15:15 were applied on plants separately in a pot experiment. Data were obtained on plant height (cm), number of leaves per plant, number of fruit per plant, fresh fruit weight per plant (g), day to 50% flowering and fresh root weight (g) and were analyzed using IRRI STAR Software. **Results:** There were no significant differences in fresh fruits of tomato plants inoculated with *Rhizobium* strains compared with those fertilized with NPK 15:15:15. However, NPK 15:15:15 had the highest fresh fruit weight per plant (3.28) followed by *Bradyrhizobium* spp., strain USDA 3384 (3.16), *Bradyrhizobium japonicum* strain IRJ 2180A (3.04) and control (2.68), respectively. **Conclusion:** This study shows that *Bradyrhizobium* spp., strain USDA 3384 and *B. japonicum* strain IRJ 2180A increase fruit yield in tomato plants. They can be used in formulating biofertilizers.

Key words: Bio-fertilizers, *Rhizobium* strains, synthetic fertilizers, *Bradyrhizobium* spp., strain USDA 3384, *B. japonicum* strain IRJ 2180A yield

Citation: Agbowuro, G.O., O.O. Olajide and M.J. Falade, 2024. Assessment of different *Rhizobium* spp., strains as bio-fertilizer in tomato plants. Int. J. Bot., 20: 129-134.

Corresponding Author: Gbenga Oluwayomi Agbowuro, Department of Biosciences and Biotechnology, University of Medical Science, Ondo, Nigeria

Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

The tomato (*Solanum lycopersicum* L.) is an important fruit vegetable consumed and cultivated globally[1]. Its high demand is attributed to its excellent nutritional properties, health benefits and diverse products obtained from it domestically and industrially, as well as its ease of cultivation. Tomato fruits are eaten raw or cooked. It is processed into products such as juice, sauce ketchup, stew, etc[2]. According to the Centre for Overseas Development and Natural Resources Institute[3], tomato seeds contain 24% unsaturated fatty acid. The pressed cake residue is used for fertilizer production. It is a perfect dietary source of vitamins, minerals, carotenoids, antioxidant compounds and an appreciable quantity of protein[1,4]. It is a good source of income and employment for those who are involved in its cultivation, transporting, processing and marketing.

Despite the economic and nutritional importance of tomato, there is a wide gap between its production (supply) and its demand in Nigeria. The gap is attributed to low yield due to insufficient availability of soil nutrients, the use of crude implemented by poor resource farmers, the use of non-improved varieties that are susceptible to pests and diseases and low fruit quality and yield. However, post-harvest losses have been another source of wastage in tomato fruits in Nigeria that brings about its shortage in the market.

Tropical soils are inherently low in soil organic matter and not fertile[5,6]. To advert poor fruit yield in tomatoes due to insufficient soil nutrients, the use of fertilizers has been adopted by the farmers. The fertilizer could be organic, inorganic or a combination of the two. However, the accessibility of organic fertilizer in the large quantity required at the right time and its slow release of nutrients forced more farmers to opt for inorganic fertilizer. Inappropriate usage of these chemical fertilizers by most farmers due to their level of illiteracy or being curious to get more yield made them apply fertilizer more than the required quantity leading to environmental degradation which is detrimental to man, livestock and the environment.

The excessive use of chemical agro-inputs such as fertilizer and pesticides on a large scale has resulted in the outcry of environmentalists, concerned government and non-governmental organizations and International bodies for environmental protection and its sustainability[7]. Therefore, the use of bio-fertilizers become necessary for a sustainable environment and the ecosystem at large. Bio-fertilizers not only promote plant growth and enhance soil fertility but also reduce environmental pollution[8]. Plant probiotic bacteria (PPB) are a good component in formulating bio-fertilizers. The PPB are microorganisms that are capable of colonizing the plant root system, promoting plant growth, development and health and eventually increasing its productivity. The PPB can achieve these by interacting with the host plant through various mechanisms such as nitrogen fixation from the atmosphere, production of enzymes, phyto-hormones and secondary metabolites[9]. The use of PPB as s bio-fertilizer will reduce the use of chemical fertilizers and promote sustainable agricultural practices[10]. However, PPBs that are safe for consumption should be used in the formulation of bio-fertilizers to prevent sanitary problems or disease outbreaks as some of the final products from some plants may be consumed raw or half-cooked[11]. Some of the commonly safe PPBs used as bio-fertilizers belong to *Bacillus, Pseudomonas* and *Rhizobium* genera[1]. The genus *Rhizobium* belongs to a diverse group of symbiotic nitrogen-fixing rhizospheric bacteria that are phylogenetically in nature. They can survive without the host plant for some time. *Rhizobium* are harmless to man, animals and plant's health. They have the attributes of Plant Growth-Promoting Bacteria (PGPB) and also act as plant fertilizers[11]. The high capability of *Rhizobium* to establish a symbiotic relationship with a host plant is considered to be an excellent plant probiotic bacteria[12].

This study aimed to assess the potential of two *Rhizobium* strains as Plant Growth-Promoting Bacteria (PGPB) in enhancing tomato vegetative growth and fruit yield and to compare its fruit yield to a commonly used synthetic fertilizer by tomato farmers (NPK 15:15:15).

MATERIALS AND METHODS

Description of the experimental sites: The multi-locational pot experiment was conducted at the screen house of Teaching and Research Farm, Ekiti State University, Ado-Ekiti Nigeria, The Biological Garden, University of Medical Sciences, Ondo-City, Nigeria and Oke-Ako/Irele Farm Settlement, Oke-Ako-Ekiti, Nigeria at the early cropping season of 2023 within March, to August. Ondo lies in the rainforest agroecological zone while Oke-Ako-Ekiti falls in the derived guinea savanna of Nigeria. Ado-Ekiti lies between the rainforest and the derived guinea Savanna Agroecological Zone of Nigeria. The 12 soil samples from the soil used for the pot experiment were collected with the use of a sterilized soil auger at a depth of 0-30 cm at each of the environments for analysis. The soil samples were properly packed in a labeled envelope for physiochemical analysis. The soil analysis was carried out at the Laboratory of the Department of Environmental and Toxicology Management of Elizade University, Ilara-Mokin and Nigeria.

Experimental materials: The two tomato varieties and NPK 15:15:15 fertilizer used for the research were obtained at the Ekiti State Government Ministry of Agriculture and Food Security, Ado-Ekiti, Ekiti State, Nigeria. The 5 g of the two tomato seeds were used for the experiment. The two tomato varieties used are Roma VF and Platinum F_1, five of the seeds were produced by Premier Seed Nigeria Limited, Kaduna and East-West Seed Company, Philippines, respectively. The NPK 15:15:15 fertilizer used was produced by Wacot Limited, Ogba Lagos. The two *Rhizobium* spp., used for the research were obtained from the Soil Microbiology Laboratory of the International Institute of Tropical Agriculture, Ibadan. The two *Rhizobium* spp., are *Bradyrhizobium* spp., strain USDA 3384 and *B. japonicum* strain IRJ 2180A. The two *Rhizobium* spp., were sub-culture at the laboratory of the Department of Crop, Horticulture and Landscape Design, Ekiti State University, Ado-Ekiti, Nigeria.

Treatment, field experimental design and cultivation condition: The experiment was laid out in a Complete Randomized Design (CRD) with three replicates across the three locations via University Teaching and Research Farm, Ekiti State University, Ado-Ekiti, Nigeria, The Biological Garden, University of Medical Sciences, Ondo-City, Nigeria and Oke-Ako/Irele Farm Settlement, Oke-Ako, Ekiti. The $2\times4\times3$ factorial involves two tomato varieties and four levels of treatments including control in three environments. The soil used for the experiment was obtained at each of the environments. The soil was sterilized to get rid of pathogens. The 5 kg of sterilized soil was packed into 10 L of perforated pots and labeled accordingly. The seeds were surface sterilized with the use of hydrogen peroxide solution, nursed on sterilized cocopeat for 21 days and transplanted. Transplanting was done at 18 hrs of the day. The 2 weeks after planting, the suspension of each of the two *Rhizobium* (1×10^3 spores/mL of distilled water) was used to inoculate the plant by injecting it into the root zone of the plants. The 9.6 g of NPK 15:15:15 was applied to some of the plants according to the design using the ring method[13]. The sites were kept weed-free throughout the experiment. Split bamboo poles were used as stakes for the tomato plant. At 3 week intervals, insect pests were controlled with the use of 30 and 70 mL of Lambda cyhalothrin and Cypermethrin plus Dimethoate, respectively in 16 L of Knapsack sprayer.

Data collection and statistical analysis: Data were collected on plant height (cm), number of leaves per plant, number of fruit per plant, fresh fruit weight per plant (g), days to 50% flowering and fresh root weight (g) at days to 50% flowering. Data were collected by adopting Robertson and Labate[14]. Fresh root weight was determined using the destructive method of uprooting the plant. Data were subjected to analysis of variance using Statistical Tools for Agricultural Research software. Means were separated using the Duncan's Multiple Range Test at $p\leq0.05$ level of significance.

RESULTS AND DISCUSSION

Table 1 presented the physical and chemical properties of the soil used for the pot experiment at the three experimental sites. The pH values for the soil were 5.92, 5.11 and 5.63 at Ado-Ekiti, Ondo-City and Oke-Ako, respectively. The textural class across the three locations was sandy loam. Total carbon (%) across the three environment ranges from 0.83-0.90. The available organic matter, total nitrogen values and nitrogen were 1.69, 0.10 and 0.10 (%) for Ado-Ekiti, respectively while that of Ondo-City were 1.54, 0.13 and 7.82 for available organic matter, total nitrogen values and nitrogen, respectively. The values for available organic matter, total nitrogen values and nitrogen at Oke-Ako were 1.60, 0.19 and 9.36%, respectively. Phosphorus was measured in mg/kg while Ca^{2+} and Mg^{2+} were measured in mol/kg.

The soil pH values in the three environments show that the soil is slightly acidic which is still within an acceptable range[15]. The pH values across the environments will still make soil nutrients readily available for plant root uptake[16]. According to the soil nitrogen critical value by Bao *et al.*[17], the soil at the experimental sites was deficient in soil nitrogen. This result conformed to the findings of Shiyam and Binangi[5] that tropical soils are inherently low in soil nitrogen. The total available phosphorous across the three environments falls below the soil phosphorus critical level of 10-15 g/kg[18]. Texture is a vital soil property that determines the water and nutrient holding capacity of the soil which invariably enhances plant growth and development[19]. The textural class of the soil across the three environments has the potential to hold water with high humus content.

The results from the combined analysis of variance across the three environments of the study were presented in Table 2. The mean squares due to varieties were significant in all the traits studied except for fresh root weight. The significance level within the varieties shows the extent of genetic variability among these cultivars[20]. This finding conformed to the results of some authors that genetic variability exists among crop genotypes[21,22]. Agbowuro *et al.*[23] reported some levels of genetic variability in African yam beans. The treatment has a significant effect on all the traits studied except for the number of leaves per plant

Table1: Physiochemical properties of the experimental sites

	Environments		
Properties	Ado-Ekiti	Ondo City	Oke-Ako
Sand (%)	59.6	64.0	60.1
Clay (%)	19.4	18.3	21.7
Silt (%)	21.0	18.7	18.1
Texture	Sandy loam	Sandy loam	Sandy loam
pH (H_2O)	5.92	5.11	5.63
Total carbon (%)	0.86	0.90	0.83
Organic matter (%)	1.69	1.54	1.60
Nitrogen (%)	0.10	0.13	0.19
Phosphorus (mg/kg)	9.63	7.82	9.36
Ca^{2+} (mol/kg)	1.47	1.86	1.70
Mg^{2+} (mol/kg)	0.73	0.71	0.70

Table 2: Combined analysis of variance across the three environments for tomato traits

SV	DF	PH (cm)	NLP^{-1}	NFP^{-1}	$FFWP^{-1}$ (g)	DT50% F	FRW (g)
Rep.	2	486.058	10.01	51.12	0.006	261.43	9.46
Var.	1	1653.12**	6179.01**	2688.88**	1.084**	938.88*	23.57
Trt.	3	273.53**	104.71*	311.81**	1.176**	47.75**	16.46**
Var×Trt	3	88.23*	31.82*	17.59**	0.213**	15.11**	11.61*
Env.	2	25.68	0.05*	84.50	0.144	5.010	10.56
Var.×Env.	2	0.12	0.05*	234.72	0.517	0.667	12.45
Trt.×Env.	6	4.13	17.20**	21.42	0.027	0.385	7.958
Var.×Trt.×Env.	6	6.68*	12.75*	8.75**	0.036**	0.123*	5.181**
Error	71						

Significant at 5 and 1% level, respectively SV: Source of variation, DF: Degree of freedom, PH: Plant height (cm), NLP^{-1}: Number of leaves per plant, NFP^{-1}: Number of fruit per plant, $FFWP^{-1}$: Fresh fruit weight per plant, DT50% F: Day to 50% flowering and FRW: Fresh root weight

Table 3: Mean performance for some tomato traits studied across the three environments

	Traits					
Treatment	PH (cm)	NLP^{-1}	NFP^{-1}	$FFWP^{-1}$ (g)	DT50% F	FRW (g)
Control	90.06^{b}	51.54^{c}	25.16^{a}	2.68^{b}	71.0^{b}	65.74^{c}
NPK 15:15:15	97.89^{a}	69.42^{a}	31.24^{a}	3.28^{a}	63.93^{a}	70.35^{b}
Bradyrhizobium spp., strain USDA 3384	98.67^{a}	63.13^{b}	32.16^{a}	3.16^{a}	68.6^{b}	89.94^{a}
B. japonicum strain IRJ 2180A	95.00^{a}	60.98^{b}	31.16^{a}	3.04^{a}	69.3^{b}	86.47^{a}

Means with the same letter (s) in each column are not significantly different ($p<0.05$) according to Duncan's Multiple Range Test (DMRT), PH: Plant height, NLP^{-1}: Number of leaves per plant, NFP^{-1}: Number of fresh fruits per plant, $FFWP^{-1}$: Fresh fruit weight per weight, DT50% F: Days to 50% flowering and FRW: Fresh root weight

which was significant at ($p<0.01$). This shows that the treatments have effects on all measured traits. Variety×treatment interaction influences all the studied traits.

The number of fruits per plant, fruit weight per plant and days to 50% flowering were significant ($p<0.05$) while other studied traits were significant ($p<0.01$). For the environment, variety×environment interaction and treatment×environment interaction, the traits studied were not significantly different except for the number of leaves per plant which was significantly different ($p<0.01$) and significantly different ($p<0.05$) for environments, variety×environment interaction, respectively. The result shows that the environment does not have much influence on the traits studied except for the number of leaves per plant ($p<0.01$). The interaction of the environment with the varieties and the treatments shows some levels of consistency of the varieties and the treatments across the environment. However, the interaction of the varieties, treatments and environments was significantly different thought at different levels. The mean performance of the studied traits for the two tomato varieties across the three environments was presented in Table 3. *Bradyrhizobium* spp., strain USDA 3384 had the highest mean value for plant height (98.67 cm) followed by NPK 15:15:15 (97.89), *B. japonicum* strain IRJ 2180A (95.00) and control: The plants that received no treatment (90.06).

Though there were no significant differences among the three treatments applied (*Bradyrhizobium* spp., strain USDA 3384, *B. japonicum* strain IRJ 2180A and NPK 15:15:15). However, the values differ. For the number of leaves per plant, there were significant differences among the traits studied across the three environments. The NPK 15:15:15 (69.42) recorded the highest mean value while control (51.54) recorded the least mean value. For the number of fruits per plant and fruit weight per plant, the two traits follow the same trend. The three treatments were not significantly different from each other though their mean values were different. *Bradyrhizobium* spp., strain USDA 3384 recorded the highest mean value for the number of fruits per plant (32.16) while NPK 15:15:15 recorded the highest mean value for fresh fruit weight per plant. *Bradyrhizobium* spp., strain USDA 3384 (89.94) recorded the highest fresh root weight followed by *B. japonicum* strain IRJ 2180A (86.47), NPK 15:15:15 (70.35) while control (15.74) had the least value for root length.

The plant treated with the *Rhizobium* spp., has a massive root system. The results recorded from the research show the efficiency of the *Rhizobium* strains used as a PGPR. The tomato plants inoculated with the *Bradyrhizobium* spp., strain USDA 3384 and *B. japonicum* strain IRJ 2180A produced reasonably as that of plants fertilized with NPK 15:15:15 fertilizer and gave better results compared to the control. This finding was in agreement with the work of Cabrera[24] who reported that tomato plants that were treated with R. etliCE-3 and R.ISCR strains produced reasonably more than the plants that were not inoculated. The better yield obtained in *Rhizobium* inoculated plants could be attributed to their ability to fix nitrogen symbiotically. They also can dissolve phosphates. The nitrogen fixed and the phosphates dissolved will favour tomato plant nutrition[25]. Moreover, *Rhizobium* spp., has direct action b y producing phyto-hormones naturally[24]. These phytohormones include auxins, ethylene, gibberellins, cytokines and abscisic acid. These hormones perform different functions on the plants ranging from cell division, cell elongation, flowering, fruiting, increase in fruit size, ripening of the fruits, etc. which invariably enhance growth and development[26]. The massive growth in the root system of the plants treated with *Rhizobium* spp., shows that the phytohormones produced by the *Rhizobium* spp., help in developing water and nutrient absorption capacity of the plant through its root system.

CONCLUSION

The findings from the research work showed the positive effect of *Rhizobium* spp., in the plants inoculated with two *Rhizobium* strains concerning control, plants without inoculation and plants fertilized with NPK 15:15:15. With the use of *Bradyrhizobium* spp., strain USDA 3384, *B. japonicum* strain IRJ 2180A, higher yield is guarantee. Hence, the use of bio-fertilizers formulated from these *Rhizobium* strains will ensure a sustainable agriculture approach that will ensure higher yield, cost-effectiveness and eco-friendliness.

SIGNIFICANCE STATEMENT

Using expensive chemical fertilizers is the main strategy for supplementing soil nutrients to enhance crop growth and yield. However, inappropriate use of chemical fertilizer causes soil degradation and negatively affects the environment. The use of *Rhizobium* spp., as a bio-fertilizer is a promising option for increasing vegetative and fruit yield in tomato plants without hampering the environment.

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