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Influence of Beneficial Bacterial Inoculation on Nitrogen Concentration and Tomato Seedling Growth Under Glasshouse Conditions

(Pengaruh Inokulasi Bakteria Bermanfaat terhadap Kepekatan Nitrogen dan Pertumbuhan Anak Benih Tomato di bawah Keadaan Rumah Kaca)

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ABSTRACT

Many types of soil bacteria through antagonistic activity, thrive in the rhizosphere of plants or surround the tissues of plants and encourage plant development and reduce the nematode population. Bacteria as such are commonly known as Plant Growth-Promoting Rhizobacteria (PGPR). The purpose of this research was to determine *Bacillus* spp. inoculations impact on tomato seedling development with varying rates of chemical nitrogen-fertilizer. To minimize the recommended quantity of N fertilizer for tomato seedling development, a small pot experiment with selected PGPB was undertaken with varying amount of N fertilizer. Plant growth-promoting bacteria (PGPB) labeled as UPMB10 and UPMRB9 (identified as *Bacillus subtilis* and *Bacillus tequilensis,* respectively) were utilized as microbial inoculants because they showed a significant improvement in seedling growth and N concentration in tomato plant tissues in a pot culture investigation. These microbial inoculants significantly improved the development of the plants, stem length, root length, leaves number, dry weight of shoots (stem, leaves), dry weight of roots, SPAD value, N concentration in tissues, and soil bacterial population. Bacteria-treated seedlings with 50% N fertilizer significantly increased stem length (69.07%), root length (78.51%), leaves number (68.58%), shoots (92.45%, 90.39%, stem and leaves, respectively), roots (73.33%), SPAD value (50.31%), and N concentration in plant tissues (63.79%) as compared to the uninoculated control. The findings also showed that inoculation of the *Bacillus* spp. tomato seedlings could save up to 50 percent of the recommended rate of chemical N fertilizer without affecting tomato seedling growth. The findings of this study suggest that the amount of nitrogen fertilizer given during tomato seedling development can be reduced by half, resulting in increased soil health and reduced environmental pollution.

Keywords: Inoculation; N levels; plant growth-promoting bacteria; tomato

ABSTRAK

Pelbagai jenis bakteria tanah melalui aktiviti antagonis, tumbuh subur dalam rizosfera tumbuhan atau mengelilingi tisu tumbuhan dan menggalakkan perkembangan tumbuhan dan mengurangkan populasi nematod. Bakteria seperti ini biasanya dikenali sebagai Rizobakteria Penggalak Pertumbuhan Tumbuhan (PGPR). Tujuan penyelidikan ini adalah untuk menentukan impak inokulasi *Bacillus* spp. kepada perkembangan anak benih tomato dengan kadar baja nitrogen kimia yang berbeza-beza. Untuk meminimumkan kuantiti baja N yang disyorkan untuk pembangunan anak

benih tomato, satu uji kaji pasu kecil dengan PGPB terpilih telah dijalankan dengan jumlah baja N yang berbeza-beza. Bakteria penggalak pertumbuhan tumbuhan (PGPB) yang dilabelkan sebagai UPMB10 dan UPMRB9 (masing-masing dikenal pasti sebagai *Bacillus subtilis* dan *Bacillus tequilensis*) telah digunakan sebagai inokulan mikrob kerana ia menunjukkan peningkatan yang ketara dalam pertumbuhan anak benih dan kepekatan N dalam tisu tumbuhan tomato kajian kultur pasu. Inokulan mikrob ini dengan ketara meningkatkan perkembangan tumbuhan, panjang batang, panjang akar, bilangan daun, berat kering pucuk (batang, daun), berat kering akar, nilai SPAD, kepekatan N dalam tisu dan populasi bakteria tanah. Anak benih yang dirawat dengan 50% N bakteria baja dengan ketara meningkatkan panjang batang (69.07%), panjang akar (78.51%), bilangan daun (68.58%), pucuk (masing-masing 92.45%, 90.39% untuk batang dan daun), akar (73.33%), nilai SPAD (50.31%) dan kepekatan N dalam tisu tumbuhan (63.79%) berbanding kawalan tanpa inokulasi. Hasil kajian juga menunjukkan bahawa inokulasi *Bacillus* spp. anak benih tomato boleh menjimatkan sehingga 50 peratus daripada kadar baja N kimia yang disyorkan tanpa menjejaskan pertumbuhan anak benih tomato. Hasil kajian ini juga mencadangkan bahawa jumlah baja nitrogen yang diberikan semasa pembangunan anak benih tomato dapat dikurangkan sebanyak separuh, menyebabkan kesihatan tanah meningkat dan pencemaran alam sekitar berkurangan.

Kata kunci: Bakteria penggalak pertumbuhan tumbuhan; inokulasi; tahap N; tomato

INTRODUCTION

Tomatoes (*Lycopersicum esculentum*) are the world's second most grown vegetable after potatoes, and according to the FAO, the yearly output of over 10^8 tons of fresh tomatoes in 3.7×10^6 ha globally, with China, the United States, and Turkey leading the way (FAO 2004; Ordookhani et al. 2010); In 2017, it ranked 1st in both production (18 million tons) and consumption of vegetables (FAO 2019; Yildizhan & Taki 2018). Tomato consumption has lately been proved to provide health benefits to people due to its rich level of phytochemicals and a variety of other essential minerals (Beutner et al. 2001; Ordookhani et al. 2010). Tomato is the most widely eaten vegetable and used as a good source of vitamins A, B, C, and D and minerals such as calcium, phosphorus, and iron (Mengistie & Awlachew 2022). This arrangement (Gahler, Otto & Böhm 2003) clarified the strong antioxidant capacity of both raw and cooked tomatoes, linking the fruit to decreased cancer and cardiovascular disease rates. Chemical fertilizers were introduced in the last century, which initially pleased farmers by increasing agricultural production. Chemical fertilizers, on the other hand, gradually began to show their negative effects, such as leaching, polluting water basins, harming microorganisms and beneficial insects, making the crop more prone to disease attacks, lowering the productivity of soil, and causing irreversible harm to the system (Pedraza 2016). The most critical success factors in increasing plant output is seed inoculation or priming with plant growth-promoting bacteria (PGPB) (Ashrafi & Seiedi 2011). PGPB is a consortium of beneficial bacteria that affects plant growth (Huang et al. 2016), productivity (Liu et al. 2016), and nutritional composition (Calvo et al. 2017), and were shown to be able to prevent plant disease, biologically (Xiang et al. 2017). In order to maximize the beneficial effects of these bacteria, it is essential to choose the right PGPB strain for each soil-plant-PGPB system and to optimize the inoculation method in both greenhouse and open-field experiments (Ruzzi & Aroca 2015). This beneficial microbe could enhance plant growth by invading the plant roots and interacting beneficially with the crop (Heidari, Mousavinik & Golpayegani 2011; Subba Rao 1999; Wu et al. 2005). Plant growth-promoting bacteria can promote the growth and development of plants through direct and indirect mechanisms, by production and secretion of chemical substances in the rhizosphere (Borges et al. 2019). According to Kloepper and Beauchamp (1992), *Azotobacter* and *Bacillus* inoculations enhanced cereal output by up to 30% and 43%, respectively. Development of seedlings is one of the most critical stages for vegetable crops to produce excellent yields. The efficient establishment of stands, as well as the uniform growth and production of plants, are all dependent on seedling quality. The PGPB boost plant growth characteristics by raising auxin and cytokinin levels while lowering ethylene and abscisic acid levels in the plants, which leads to improved cell division and elongation (Tinna et al. 2020). On the other hand, they improve the plant's ability to access nitrogen and phosphorus by fixing and solubilizing those elements, respectively. This leads to greater root and shoots development and an improvement in the plant's ability to absorb water and nutrients (Ruzzi & Aroca

2015). Plant growth-promoting bacteria (PGPB) are utilized to stimulate the growth of plants, although the mechanism(s) behind it are largely unknown, including their influence on seedling formation (Yildirim et al. 2011). The uncontrolled chemicals used in agriculture have a detrimental effect on the ecosystem, resulting in a loss of biodiversity as hazardous substances accumulate in the soil, water, and water tables, polluting the soil, water, and water tables (Camelo, Vera & Bonilla 2011). There has been growing evidence that it can be expensive to use chemical fertilizers widely and cause significant environmental problems. Massive amounts of chemical fertilizers are utilized to replenish soil phosphorus and nitrogen, but they are expensive and pollute the environment. In chemical fertilizers, nitrogen, phosphorus and potassium nutrient uptake efficiency (NUE) is calculated to be roughly or lower than 50%, 10% and 40%, respectively (Baligar et al. 2001). Despite the negative environmental consequences, it is projected that the global use of inorganic fertilizers will rise to meet the growing global population's demand for food through intensive agriculture (Adesemoye, Torbert & Kloepper 2009). Efforts are currently being made to reduce the usage of N, a chemical fertilizer used by tomatoes, which has prompted studies into other methods of boosting soil fertility and crop output. Since the use of PGPBs to boost plant nutrient availability could be beneficial to agriculture (de Freitas et al.2007), it has become more widely employed as biological fertilizers in long-term agriculture around the world (Yildirim et al. 2011). The inoculated plant's biocontrol and disease resistance induction, biological N_2 fixation, phosphorus solubilization, and phytohormone synthesis are all examples of PGPB's growth-promoting activities in plants (Baset Mia, Shamsuddin & Maziah 2012). PGPB play an important role for increasing soil fertility, plant growth promotion, and suppression of phytopathogens for the development of eco-friendly and sustainable agriculture (Mittal et al. 2017). *Bacillus* strains like *Bacillus subtilis, Bacillus cereus, Bacillus licheniformis, Bacillus pumilus, Bacillus amyloliquefaciens, Bacillus polymyxa*, and *Bacillus megaterium* have successfully colonized the roots and rhizosphere of a variety of plants, including tomato, banana, canola, wheat, apple, red pepper, and arabidopsis, as a result, plant growth and output are raised, disease resistance is improved, and drought tolerance is improved (Chen et al. 2013). Via direct or indirect channels, the PGPB may promote the growth of plants. In the absence of other microorganisms, the direct effects can be shown (i.e., only the microbe being studied interacts with the plant), and the indirect processes are discovered when a microorganism interest and organism that causes phytopathology to interact with each other, hence reducing the plant's detrimental impacts (Acebo-Guerrero et al. 2015). PGPB directly impacts phytohormone production, siderophores creation, mineral soluble, and nitrogen fixation in the atmosphere (Estrada et al. 2013; Kaur & Reddy 2014; Reis, Divan Baldani & Baldani 2015). Furthermore, the impact of its vaccination on development, nutrition, and the importance of achievement is highlighted (*Solanum lycopersicum* L.) (Terry-Alfonso et al. 2005; Widnyana Ketut & Javandira 2016). Likewise, numerous *Bacillus* species have been tested for their capacity to boost tomato plant development. Regarding this, Gül, Kidoglu and Tüzel (2008) employed *Bacillus amyloliquefaciens* strain FZB24 and *Bacillus amyloliquefaciens* strain FZB42 to test their performance in the development of tomatoes in controlled and field environments with varying amounts of nutrients. According to Myresiotis, Vryzas and Papadopoulou-Mourkidou (2014), a mixture of *B. subtilis* GB03 and *B. pumilus* SE34 inoculated tomato plants improved absorption of nutrients and pathogen suppression. The potentiality for PGPB inoculation to improve plant production through processes that do not require biological nitrogen fixation should not be overlooked by researchers. Many rhizobacteria associated with soil and plants can produce phytohormones (Bastian et al. 1998). PGPB treatments boosted the biomass of plants while also improving watermelon and melon seedling's quality (Kokalis-Burelle et al. 2003).

From the foregoing explanation, it could be understood that most PGPB research has focused on proving that it directly influences plant development and yield, but little is known about how it affects tomato seedling growth. A good and established tomato seedling will lead to better plant and affects the yield and production, which is needed to meet the growing demand. The hypothesis of the study is that plant growth-promoting bacteria in combination with optimum nitrogen levels could lead to healthy seedling growth; while the objective was to optimize nitrogen fertilization levels with bacterial inoculations and to observe the effect on the early growth of tomato seedlings. Therefore, this study aimed to determine the influence of PGPB seed inoculation on tomato seedling growth under glasshouse conditions.

Methods and Materials

The location of the experiment, the experimental setup, and the treatments

The research was undertaken at the Soil Microbiology Laboratory and glasshouse of Land Management Department, Agriculture Faculty, UPM, Malaysia. The cultivar (cv) of tomato (*Lycopersicum esculentum*) used in this study was 303 hybrids. The seeds were collected from the Malaysian local market and kept at -20 °C to maintain their freshness and viability. Two contributing parameters are: PGPB treatments which had three levels (non-inoculation, *B. subtilis* inoculation, *B. tequilensis* inoculation), and N-fertilizer levels (0%, 25%, 50%, 75% and 100%). A total of 45 pots were used in a 3 × 5 factorial experiments, done in triplicates of randomized block designs.

Strains of bacteria and their cultivation conditions

B. subtilis strain UPMB10 and *B. tequilensis* strain UPMRB9 were used in this investigation, and they were collected from the Soil Microbiology Lab's culture collection, Land Management Department, Agriculture Faculty, UPM, Malaysia. Each bacterial strain was streaked onto tryptic soy agar plates and transferred into broth and optical density at 600 nm was used to determine growth, and bacteria were harvested when the OD_{600} value reached 1.0. To determine the bacterial population, a series of 10-fold dilutions were used and the colony forming unit (CFU) were counted (Myresiotis, Vryzas & Papadopoulou-Mourkidou 2012). The broth contains 10^6-10^8 CFU mL^{-1} of the *Bacillus* spp. isolates were used for small pot trials.

Seed surface sterilization

Tomato seeds from the store were sterilized before being used in a 5% NaOCl solution for 60 s, then in 70% ethanol for 60 s, followed by sterile distilled water washes.

Inoculation of seeds with PGPB

360 seeds were inoculated with a 10^8 cells/mL microbes' solution in TSB. Different bacterial suspensions (*Bacillus subtilis, Bacillus tequilensis*) were used to treat seeds before sowing by soaking in each broth for 6 h. The seeds were then sewed immediately into the appropriate planting pot. In inoculated treatments, plants were given second inoculation with 2.0 mL of washed bacterial cells at 14 days after sowing.

Soil samples: collection and preparation

The soil sample (sandy clay) was collected from Field Kongsi, UPM, Malaysia. The top 15 cm of soil was dug out, two weeks of air drying, and sieving were performed on the soil (2 mm) (Ali-Tan et al. 2017) before being transferred into plastic pots (350 g soil each) having 8 cm diameter × 8 cm height and soil evenly placed into planting pots. The planting pots were properly labelled and hydrated in preparation for seeding.

Seed viability test

The viability of the seeds was verified by sowing 100 tomato seeds on a tray. 100 seeds were sown in 100 holes and covered lightly with soil. Every hole has single openings to drain out excess water. To increase humidity, the tray was wrapped in a plastic bag for two days. About 88 percent of seeds germinated within 5-6 days, indicating that the seeds were extremely viable.

Design of the treatment and inoculum description

Treatments	Inoculum	Urea as a N -fertilizer levels				
Uninoculated control	-	0% N	25% N	50% N	75% N	100% N
Inoculated	UPMB10	0% N	25% N	50% N	75% N	100% N
Inoculated	UPMRB9	0% N	25% N	50% N	75% N	100% N

UPMB10: *Bacillus subtilis*, UPMRB9: *Bacillus tequilensis*

GROWTH PARAMETERS OF TOMATO SEEDLINGS

Plant height (cm), stem diameter (mm), root length (cm), leaves number, leaves dry weight (g), shoot dry weight (g), root dry weight (g), number of secondary roots, and SPAD readings of the plant were taken every 10 days' intervals until 30 days at the end of the experiment.

DESIGN OF THE EXPERIMENT AND DATA ANALYSIS

The study used a randomized complete block design with two factors: Nitrogen levels and PGPB strains. The data was statistically examined using ANOVA analysis by Statistical analysis software (SAS 9.4) and at a 5% level of confidence, the means were compared using Duncan's Multiple Range Test (DMRT).

ANALYSIS OF THE FINDINGS

PLANT GROWTH PARAMETERS AND THEIR MEASUREMENT

The application of bacterial isolates with various N rates of the soil had a significant ($p<0.05$) impact on the growth parameters of the tomatoes (Figure 1(A)-1(C)). N levels in the soil influence plant development characteristics in a beneficial way. Parameters associated with plant growth were investigated at 0%, 25% 50%, 75%, and 100% N under inoculated (UPMB10, UPMRB9) and uninoculated plants at 10 days and 30 days after harvest. The stem, root and leaves dry weight increased significantly by 72.17% (0.0097 g), 80% (0.0050 g), and 72.50% (0.0120 g) at 10 days when soil N fertilization was increased to 100% N in a non-inoculated controlled environment (Figure 2(a)). After 30 days of inoculation, there was a significant increase in biomass indicators by 74.60% (0.0433 g), 63.64% (0.0110 g) and 70.99% (0.0517 g) from their respective control at 100% N (Figure 2(b)). Fertilization enhanced the growth of plant components, while the inoculated plants in treatment UPMB10N0, UPMB10N25, UPMB10N50, UPMB10N75, UPMB10N100, UPMRB9N0, UPMRB9N25, UPMRB9N50, UPMRB9N75, and UPMRB9N100 performed much better than their uninoculated counterparts (Figure 1(B) & 1(C)). The application of microbial isolates such as UPMB10, UPMRB9 with 0% N to 100% N plants exhibited an increase in stem, root and leaves dry weight compared to control plants (Figure 2). At the same application rate higher dry weight was recorded in UPMB10N50 and UPMRB9N50 treated plants compared to control plants. With N fertilization for dry biomass, the inoculated plants had higher values and greater enhancement percentages, indicating that the microbial inoculation had a good effect with N levels to the tomato plants. In uninoculated plants, other growth measures such as stem length, root length, and number of leaves showed a significant improvement in control (Figure 1(A)). The same pattern of enhancement was also detected

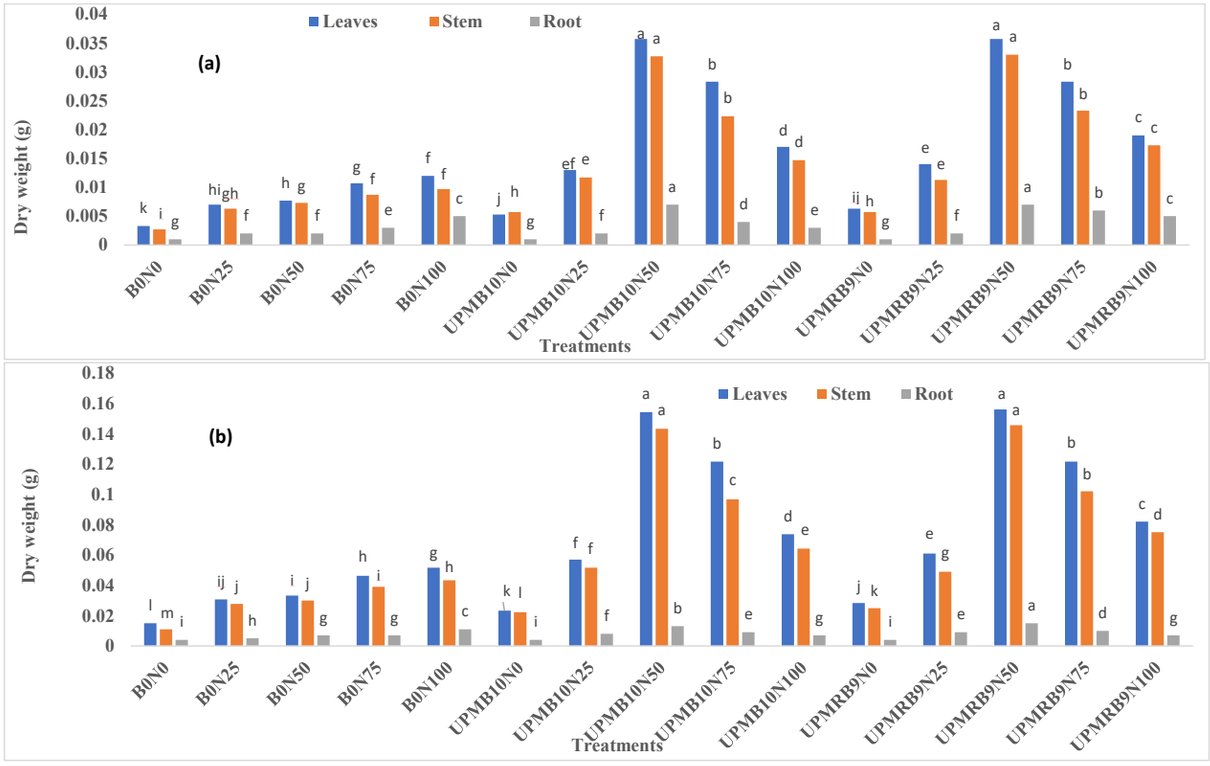
FIGURE 1(A). (1) Treatment B0N0- Uninoculated soil with a 0% N value was used to grow the plant. (2) Treatment-B0N25- Uninoculated soil with a 25% N value was used to grow the plant. (3) Treatment B0N50- Uninoculated soil with a 50% N value was used to grow the plant. (4) Treatment B0N75- Uninoculated soil with a 75% N value was used to grow the plant. (5) Treatment B0N100- Uninoculated soil with a 100% N value was used to grow the plant

as mentioned for dry biomass. There was a noticeable difference enhancement in stem length, root length, the number of leaves, the number of secondary roots, and stem diameter by 91.45% (1.17 cm), 69.09% (1.65 cm), 55.50% (6.00), 59.97% (6.67), and 41.10% (0.73 mm), respectively, at 100% N levels after 10 days (Figure 1(A)). After application of UPMB10 to the plants grown at 0% to 100% N levels, the stem length, root length, leaves number, number of secondary roots, and stem diameter increased by 44.44% (2.07 cm), 69.23% (1.95 cm), and 61.72% (8.70), 49.93% (7.33), and 37.18% (0.78 mm) at 100% N from their inoculated control that means UPMB10N0 counterparts, respectively, after 10 days. There was a slight improvement in value at 100% N after 20 and 30 days of inoculation compared to inoculated control (Figure 1(B)). Numerically different from UPMB10 but statistically similar results were found when inoculated with UPMRB9 (Figure 1(C) & Table 1). The stem length (UPMB10N50, 13.67 cm), root length (UPMB10N50, 11.50 cm), leaves number (UPMB10N50,

FIGURE 1(B). (1) Treatment B0N0- Uninoculated soil with a 0% N value was used to grow the plant. (2) Treatment UPMB10N0- plant cultivated on *Bacillus subtilis*-inoculated soil with a 0% N value. (3) Treatment UPMB10N25- plant cultivated on *Bacillus subtilis*-inoculated soil with a 25% N value. (4) Treatment UPMB10N50- plant cultivated on *Bacillus subtilis*-inoculated soil with a 50% N value. (5) Treatment UPMB10N75- plant cultivated on *Bacillus subtilis*-inoculated soil with a 75% N value. (6) Treatment UPMB10N100- plant cultivated on *Bacillus subtilis*-inoculated soil with a 100% N value

FIGURE 1(C). (1) Treatment B0N0- plant grown uninoculated soil having a N value of 0%. (2) Treatment UPMRB9N0- plant grown inoculated with *Bacillus tequilensis* soil having a N value 0% (3) Treatment-UPMRB9N25- plant cultivated on *Bacillus tequilensis* inoculated soil with a 25% N value (4) Treatment UPMRB9N50-plant cultivated on *Bacillus tequilensis* inoculated soil with 50% N value. (5) Treatment UPMRB9N75- plant grown inoculated with *Bacillus tequilensis* soil having a N value of 75%. (6) Treatment UPMRB9N100- plant grown inoculated with *Bacillus tequilensis* soil having a N value 100%

38.33), number of secondary roots (UPMB10N50, 50), and stem diameter (UPMB10N50, 4.03 mm) were detected to be considerably greater at 50% N with UPMB10; thus, it implies that optimum N level with bacteria both have a beneficial effect on plant growth. Statistically similar results were shown with UPMRB9 at 30 days after sowing (Table 1). However, the SPAD value also showed to be significantly higher improvement at 50% N with each inoculant (UPMB10, UPMRB9) which showed the positive effect of optimum N on photosynthesis, and when compared to the uninoculated plants, the inoculated plants increased chlorophyll content. The values in Table 1 reflect the effects of bacteria with N levels.



The results are the averages of three replicates. Duncan's multiple range test shows that values in a column that start with the same letter are not statistically different at the 5% level of probability

FIGURE 2. Dry biomass in response to treatments on tomato plant (a) at 10 days' inoculation (b) at 30 days' inoculation

MEASUREMENT OF N CONCENTRATION IN PLANT TISSUES

Plants using 75% and full N rates had significantly higher N concentrations in their roots, stems, and leaves than those receiving 25% of the suggested N level and without fertilizer control, moreover, when compared to those without fertilizer control, the half N level treatments dramatically raised N concentrations in plant tissues (Figure 3). Figure 3 shows plant tissue N concentrations compared to without fertilizer control rate. These findings indicated that under correctly managed glasshouse conditions that prevented nutrient loss through leaching, 50% of the required N level might satisfy plant N requirements during seedling stages, potentially masking the benefits of inoculated PGPB strains. However, PGPB inoculation with N levels could produce high N concentrations in plant tissues compared to unfertilized control. Leaves (UPMB10N50, 26.15 mg), stem (UPMB10N50, 11.64 mg), and root (UPMB10N50, 18.26 mg) had detected to be significantly greater N concentrations at 50% N with PGPB, thus suggests that optimum N level and microbial isolates both have a beneficial effect on plant N concentrations.

TABLE 1. Effects of bacterial inoculation on the growth-related parameters of tomato at different levels of N

Treatment	Stem length(cm)		Root length (cm)		Leaves number		No. of Secondary roots		Stem diameter (mm)		SPAD value	
	10 DAS	30 DAS	10 DAS	30 DAS	10DAS	30 DAS	10DAS	30DAS	10DAS	30DAS	10DAS	30DAS
B0N0	0.10^{f}	4.33^{f}	0.57^{h}	2.60^{o}	2.67^{j}	12.67^{m}	2.67^{h}	9.00^{l}	0.43^{i}	1.96^{l}	5.74^{l}	25.05^{m}
B0N25	1.03^{ef}	4.50^{f}	1.15^{f}	4.80^{l}	4.00^{ih}	16.67^{k}	4.67^{ef}	20.00^{j}	0.48^{hi}	2.03^{k}	6.71^{j}	29.22^{k}
B0N50	1.11^{ef}	4.83^{ef}	1.22^{e}	5.05^{j}	4.67^{gh}	19.67^{i}	5.00^{def}	22.00^{hi}	0.58^{gh}	2.40^{j}	7.65^{h}	33.10^{i}
B0N75	1.16^{ef}	5.03^{ef}	1.05^{fg}	5.27^{h}	5.33^{gf}	22.67^{h}	5.67^{d}	25.00^{g}	0.67^{ef}	2.82^{h}	8.72^{g}	37.96^{h}
B0N100	1.17^{ef}	5.07^{ef}	1.77^{c}	8.90^{d}	6.00^{ef}	26.67^{g}	6.67^{c}	30.00^{f}	0.73^{de}	3.19^{g}	9.40^{f}	40.65^{g}
UPMB10N0	1.15^{ef}	5.00^{ef}	2.16^{b}	2.70^{n}	3.33^{ij}	14.67^{l}	3.67^{g}	15.00^{k}	0.49^{hi}	1.97^{l}	5.99^{k}	25.79^{l}
UPMB10N25	1.32^{e}	5.73^{e}	0.90^{g}	5.00^{k}	3.67^{ij}	17.67^{jk}	5.00^{def}	21.00^{ij}	0.62^{fg}	2.42^{j}	6.88^{i}	29.91^{j}
UPMB10N50	3.14^{a}	13.67^{a}	2.13^{b}	11.50^{b}	9.67^{a}	38.33^{b}	11.33^{a}	50.00^{b}	0.94^{a}	4.03^{b}	11.41^{b}	49.07^{b}
UPMB10N75	2.42^{bc}	10.50^{bc}	1.73^{c}	8.85^{e}	7.67^{c}	34.67^{d}	9.33^{b}	39.67^{d}	0.84^{bc}	3.69^{d}	10.60^{c}	45.58^{d}
UPMB10N100	2.07^{d}	9.00^{d}	1.42^{de}	8.70^{g}	6.67^{ed}	29.67^{f}	7.33^{c}	32.00^{e}	0.78^{cd}	3.25^{f}	9.90^{e}	42.76^{f}
UPMRB9N0	1.21^{ef}	5.27^{ef}	0.67^{h}	2.75^{m}	3.00^{ij}	13.67^{lm}	4.33^{fg}	16.00^{k}	0.56^{gh}	1.98^{l}	6.00^{k}	25.81^{l}
UPMRB9N25	1.33^{e}	5.77^{e}	1.05^{fg}	5.15^{i}	3.67^{ij}	18.00^{j}	5.33^{de}	23.00^{h}	0.63^{fg}	2.46^{i}	6.91^{i}	30.04^{j}
UPMRB9N50	3.14^{a}	14.00^{a}	3.15^{a}	12.10^{a}	10.00^{a}	40.33^{a}	12.00^{a}	52.00^{a}	0.97^{a}	4.08^{a}	11.57^{a}	50.41^{a}
UPMRB9N75	2.53^{b}	11.00^{b}	1.80^{c}	8.98^{c}	8.67^{b}	36.67^{c}	10.00^{b}	42.00^{c}	0.88^{ab}	3.72^{c}	10.65^{c}	46.22^{c}
UPMRB9N100	2.19^{dc}	9.50^{dc}	1.50^{d}	8.82^{f}	7.00^{cd}	31.00^{e}	6.67^{c}	30.00^{f}	0.82^{bcd}	3.31^{e}	9.99^{d}	43.17^{e}
B	**	**	**	**	**	**	**	**	**	**	**	**
N	**	**	**	**	**	**	**	**	**	**	**	**
B×N	**	**	**	**	**	**	**	**	**	**	**	**

The results are the averages of three replicates. Duncan's multiple range test shows that values in a column that start with the same letter are not statistically different at the 5% level of probability. ** Significant at the 5% level

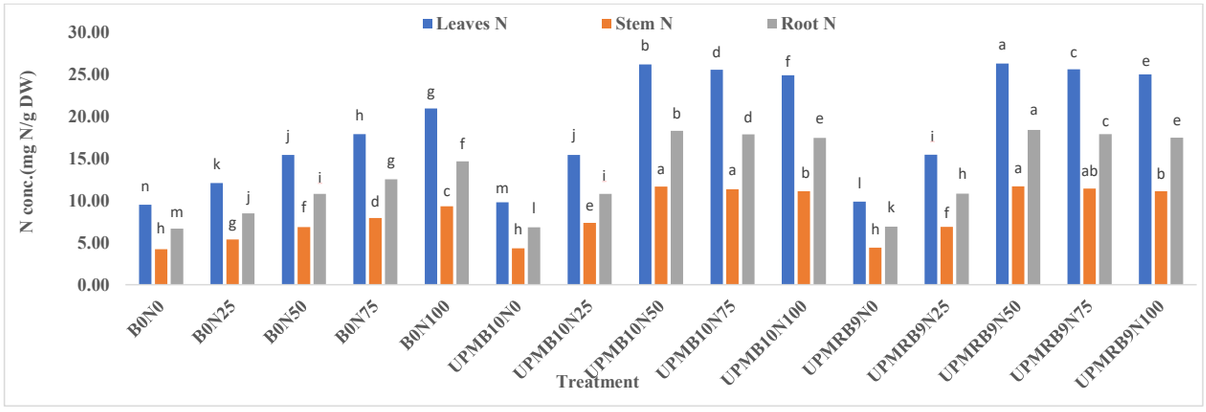
N REMAINING IN THE SOIL AT HARVEST

After harvest, Figure 4 shows the total remaining N (percentage) in the soil. When the N fertilizer application rate was higher, the remaining total N in the soil was higher. The amount of nitrogen left in the soil was related to how much urea was applied. In the uninoculated treatments, the soil applied with 100 percent N had the highest remaining N, while the control had the lowest (unfertilized). The amount of residual N in the soil rose with the increased rate of N levels. According to our findings, the total residual N in soil was higher in inoculated soil with N levels than in uninoculated soil with N levels (Figure 4).

AFTER PLANT HARVEST, THE MICROBIAL POPULATION IN THE SOIL

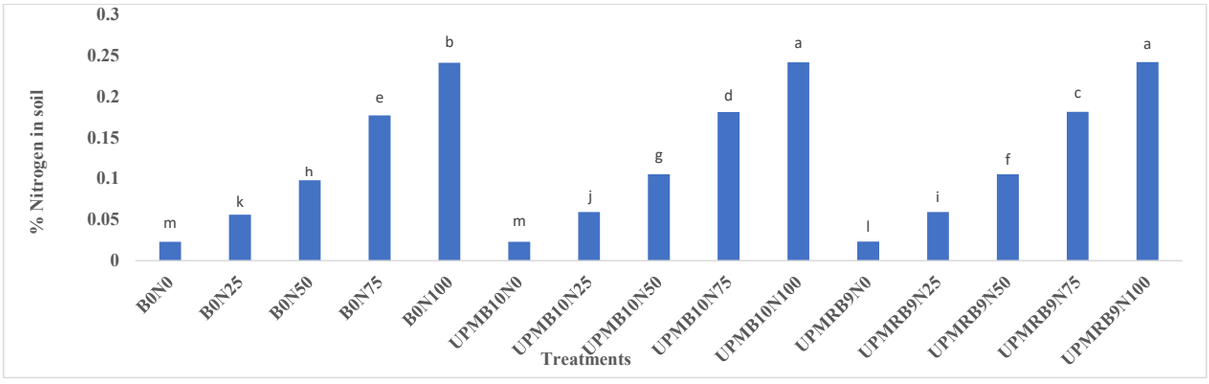
Plant growth-promoting bacteria inoculation with the N fertilizer enhanced the rhizobacterial population in the

harvested soil, compared to uninoculated soil (Figure 5). UPMB10 and UPMRB9 both significantly gave higher the rhizobacterial population under 50% N level compared to other treatments, our study showed that after 50% N levels if N rates increased, the bacterial population gradually decreases such as 75% N and 100% N levels gave lower population than 50% N level, which means at a certain level, bacteria grow faster before eventually becoming slower, and the highest bacterial population was found at 50% N level (Figure 5). UPMB10N50 (98×10^5 CFU/g Fresh soil) and UPMRB9N50 (110×10^4 CFU/g fresh soil) gave a higher population than other treatments, which are significantly higher among treatments.



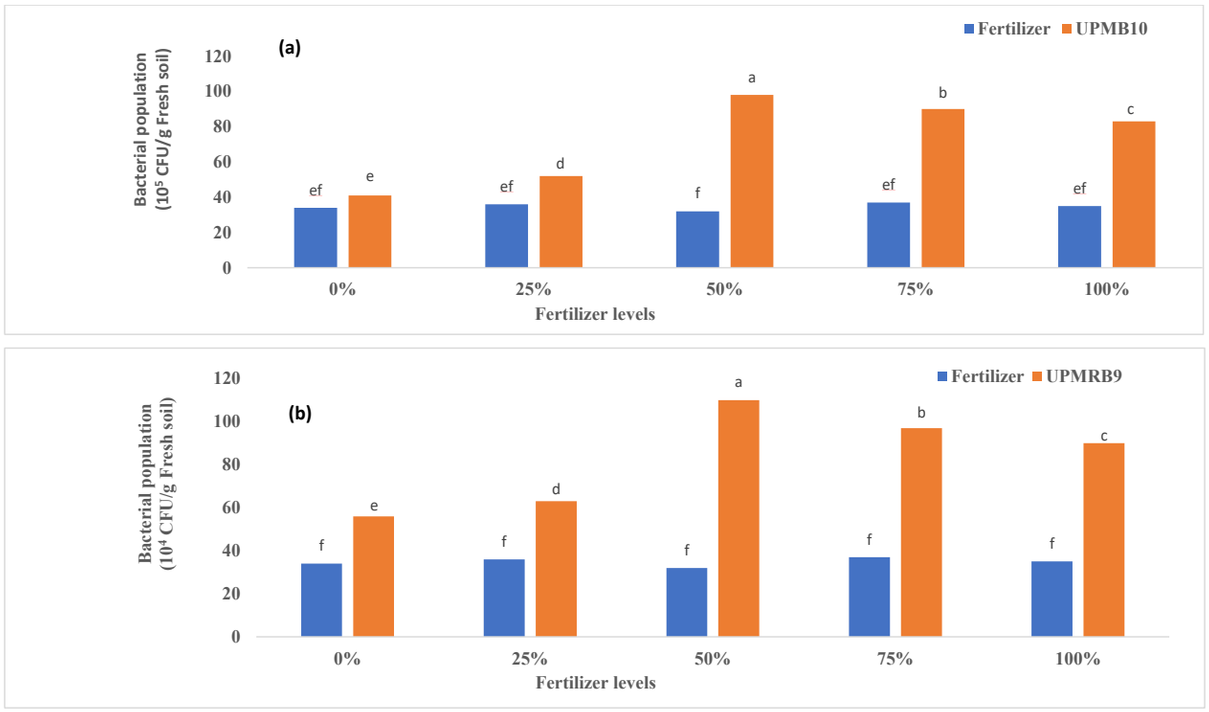
The results are the averages of three replicates. Duncan's multiple range test shows that values in a column that start with the same letter are not statistically different at the 5% level of probability

FIGURE 3. N concentration in leaves, stem, and root in response to treatments on tomato plant



The results are the averages of three replicates. Duncan's multiple range test shows that values in a column that start with the same letter are not statistically different at the 5% level of probability

FIGURE 4. Total remaining N (%) in harvest soil with or without microbial inoculants



The results are the averages of three replicates. Duncan's multiple range test shows that values in a column that start with the same letter are not statistically different at the 5% level of probability

FIGURE 5. Effect of rhizobacterial population numbers in soil after inoculation with N fertilizer (a) Bacterial population in UPMB10 inoculated soil (b) Bacterial population in UPMRB9 inoculated soil

CORRELATION AMONG PARAMETERS

The correlation coefficient (Table 2) showed a significant positive correlation between most parameters studied. This indicated that the performance of the parameters was interdependent. Stem length was positively, strongly correlated with root length (r=0.882), leaves number (r=0.928), SPAD value (r=0.878), number of secondary roots (r=0.934), stem diameter (r=0.910), stem dry weight (r=0.977), leaves dry weight (r=0.972), and bacterial population (r=0.953). It also indicated a weak relationship with soil total N (r=0.362). Number of secondary roots was also strongly, positively correlated with stem diameter (r=0.969), stem dry weight (r=0.968), leaves dry weight (r=0.970), leaves N concentration (r=0.915), stem N concentration (r=0.912), root N concentration (r=0.914), and bacterial population (r=0.845). It also indicated a weak relationship with soil N concentration (r=0.497). Bacterial population strongly correlated with leaves dry weight (r=0.913) and N plant tissues (stem, leaves, root), indicating that these parameters were more efficient with adequate amounts of N with PGPB.

DISCUSSION

A new investigation has discovered that optimum N fertilizer combined with PGPB results in a positive effect on the soil microbial population, N concentration in tissues, reduced loss of applied N and overall seedling growth. Furthermore, using bacterial inoculants to minimize the negative impact of inorganic fertilizers. Improvements in tomato development mediated by PGPB could be attributed to a variety of pathways, including the production of ACC deaminase (Afridi et al. 2019), Phosphate solubilizer (Kadmiri et al. 2018), IAA production (Myo et al. 2019; Zahir et al. 2010), and siderophore synthesis (Kumar et al. 2018).

GROWTH ATTRIBUTES

In this research, only under N fertilization conditions

could *Bacillus* spp. inoculation promotes tomato growth, demonstrating the importance of N to all living organisms, including PGPB (UPMB10, UPMRB9). *Bacillus* spp. can initiate BNF using N as a source of energy. *Bacillus* spp. capacity to fix N_2 as well as increase the development of plants. PGPB-induced increases in dry matter and yield were seen in strawberry plants (Karlidag et al. 2010), wheat (Turan et al. 2014, 2012) and cabbage and broccoli (Yildirim et al. 2011). As an example, Ratti et al. (2001); Sundara, Natarajan and Hari (2002); Woodard and Bly (2000) and Zahir et al. (2012)

TABLE 2. Correlation coefficient between parameters

	SL30	RL30	LN30	SPAD30	NSR30	SD30	SDW30	RDW30	LDW30	LNC	SNC	RNC	SoilN	BaP
SL30	1	.882**	.928**	.878**	.934**	.910**	.977**	.764**	.972**	.855**	.853**	.854**	.362	.953**
RL30		1	.967**	.969**	.958**	.969**	.909**	.881**	.907**	.954**	.952**	.954**	.643**	.798**
LN30			1	.989**	.973**	.991**	.933**	.823**	.943**	.968**	.963**	.967**	.635*	.857**
SPAD30				1	.954**	.988**	.891**	.816**	.901**	.979**	.974**	.979**	.712**	.798**
NSR30					1	.969**	.968**	.894**	.970**	.915**	.912**	.914**	.497	.845**
SD30						1	.928**	.857**	.941**	.971**	.969**	.971**	.649**	.836**
SDW30							1	.865**	.994**	.859**	.861**	.859**	.347	.906**
RDW30								1	.859**	.773**	.777**	.773**	.366	.650**
LDW30									1	.877**	.878**	.877**	.380	.913**
LNC										1	.999**	1.000**	.769**	.820**
SNC											1	.999**	.763**	.820**
RNC												1	.769**	.819**
SoilN													1	.371
BaP														1

SL= Stem length; RL= Root length; LN= Leaves number; SPAD value; NSR= Number of secondary roots; SD= Stem diameter; SDW=Stem dry weight; RDW= Root dry weight; LDW= Leaves dry weight; LNC= Leaves N concentration; SNC= Stem N concentration; RNC= Root N concentration; SoilN= Soil N; BaP= Bacterial population, **, ns are significant and non-significant

showed that when PGPB inoculation is paired with chemical fertilizers, plant growth is boosted. Multiple applications of *Bacillus velezensis* have been reported to benefit radish biomass (Meng, Jiang & Hao 2016). *Bacillus subtilis* inoculation has a positive influence on the development of tomato plants, contributing to a significant increase in the mass and length of its stem and root (Cabra Cendales et al. 2017). To satisfy the food security concerns posed by a rapidly rising population, worldwide demand for nitrogen fertilizer is expected to rise (de Bruijn 2015). Considering outlook, Nitrogen-fixing microorganisms likewise PGPB utilization in agricultural systems is critical (Raymond et al. 2004). Furthermore, the application of PGPB as a partial alternative for chemical fertilizers in agricultural systems has been suggested (Wu et al. 2005). The results showed that if inoculants were not used, 100% fertilizer resulted in stronger plant growth than all other lower levels (Table 1 & Figure 1(A)). This finding is consistent with Biswas, Ladha and Dazzo (2000), who claimed that fertilizer N inputs and inoculants are interdependent for maximum production of rice gains. Compared to 100%

fertilizer, poorer growth of tomato seedlings was seen when 50% N fertilizer was treated at greater or lower levels with PGPB. According to the findings of our research, the result suggesting that nitrogen fertilization could be reduced by up to 50%, with the application of PGPB. This result is similar to Wong et al. (2014), who observed that supplementation with approximately 4.0×10^6 CFU g^{-1} of soil with a beneficial inoculant with half of the amount of fertilizer, consistently produced the same plant growth potential as 100% fertilization, whilst also increasing the nitrogen use efficiency of the applied fertilizer. Compared to control plants, the bacterial inoculation plants that were given 50% NPK had significantly higher rates of N, P, and K (Masciandaro, Ceccanti & García 1994). Chaichi, Shabani and Noori (2015) found that plant development and dry weight increased when berseem clover was fed with biofertilizer in combination with reduced chemical fertilizer. When inoculant or fertilizer alone was used, Canbolat et al. (2006) and Elkoca, Kantar and Sahin (2007) observed no significant change in plant biomass of barley, or seed yield and plant biomass of chickpea. On the basis of these findings, it had been proposed that inoculants might be used as a chickpea fertilizer alternative Elkoca, Kantar and Sahin (2007). Inoculants may allow an optimal fertilizer rate for tomatoes, but they will not replace fertilizer, according to the current findings. These findings matched those of Hernández and Chailloux (2004), who found that the dry mass of tomato plants cultivated in the controlled environment with 75% fertilizer with two co-inoculated PGPR was significantly higher than that of tomato plants cultivated in the greenhouse with full fertilizer rate but no PGPR. This research examines, in terms of soil N, there were no notable differences between inoculated control (UPMB10N0, UPMRB9N0) and uninoculated control (B0N0) treatments, whereas soil N concentration was much higher in 50% N with PGPB inoculation. We hypothesized that in the current investigation, the lack of accessible N may have hampered the establishment of bacterial populations in the absence of N fertilization. BNF and root biomass enhancement did not occur under these N-limited conditions because plant growth-promoting bacteria did not colonize the rhizosphere, and plant growth did not improve. Our findings contradict prior studies, which found that PGPB inoculation boosted cabbage (Turan et al. 2014) and banana (Baset Mia et al. 2010) growth in both N-free and N-limited conditions. These variances could be attributable to changes in experimental circumstances, plant species, and PGPB strains employed in the investigations. Turan et al. (2014) investigated the effects of *B. megaterium* TV-91C, *Pantoea agglomerans* RK-92, and *B. subtilis* TV-17C on cabbage cultivation using peat as a substrate. It is impossible to say whether the plant growth improvement reported in their study was attributable only to the PGPB inoculation because peat already contains high quantities of nutrients (including nitrogen). Plant growth-promoting bacteria significantly enhanced the height of rice seedlings (Tan et al. 2014). *Bacillus* sp. at the population of 4×10^5 CFU and 8×10^5 CFU showed significantly better plant height, leaf number, root length and number, and weight of tomato fruits (Widnyana Ketut 2018). When Baset Mia et al. (2010) studied the impact of PGPB inoculation on banana seedlings for 45 days, they employed nutritional solutions. Other nutrients, such as Mo, may be used by PGPB to begin BNF under these circumstances. Additionally, trace levels of N contained in the nutritional parts of a solution may have been sufficient for those PGPB strains to continue biological nitrogen fixation. According to current research (Israr et al. 2016; Khan et al. 2016), the inoculated control had a basal amount of N fertilizer, and they noticed an increase in plant growth. All these findings suggested that N is required for PGPB species to boost plant development and BNF levels. Our findings that PGPB did not trigger N fixing in the absence of nitrogen fertilizer back up this hypothesis. Inoculating legumes with PGPB has been shown to promote N_2-fixation, resulting in increased plant growth. PGPB supplementation has been shown to improve N_2-fixation in soybean (Dashti et al. 1998), chickpea (Valverde et al. 2006), and common bean (Figueiredo et al. 2008) under greenhouse and field conditions. *Bacillus* spp. tends to promote faster seedlings growth compared to the *Pseudomonas* spp. isolates (Widnyana Ketut & Javandira 2016). PGPB application also had a positive effect in tomato, as *B. subtilis* and *B. tequilensis* inoculations in the current study, greater N concentrations in plant parts (shoots, roots) were seen in N conditions.

NUTRIENT CONTENT IN PLANTS

Our study observed that both with and without PGPB inoculation, the level of dispersed N in shoots (leaves, stem) was approximately 50% greater than in roots. Most bacteria, including PGPB, can express the nitrogenase enzyme that catalyzes the conversion of atmospheric N_2 to NH_3, according to Xiao et al. (2010). In addition, in our investigation, PGPB-initiated N uptake by tomatoes was linked to an enhancement in soil N concentrations.

This data is consistent with Figueiredo et al. (2008), who found that PGPB-induced BNF increased soil N levels. Accordingly, Malik et al. (1997) discovered that PGPB-associated with kallar grass (*Leptochloa fusca* (L.) Kunth) fixed optimal levels of nitrogen in the soil. PGPB may stimulate plant development by increasing the region of the roots or stimulating expansion of the roots, in addition to increasing BNF (Yildirim et al. 2011), both things help plants absorb more water (Mayak, Tirosh & Glick 2004). This hypothesis proved our results that PGPB inoculation plus N fertilizer boosted root weight more than only N fertilization. Growing proof suggests that PGPB-influenced changes in the structure of the root increase region of the roots and stimulate plant development (Bhattacharyya & Jha 2012); this is due to the fact that plant development is governed by internal systems that have been influenced by the availability of soil nutrients to the roots. Exudates from the roots released by plants, whether directly or indirectly improve nutrient uptake because in soil, plant roots are the principal locations of nutrient and microbial interactions (Bottini, Cassán & Piccoli 2004; Turan et al. 2012). *Bacillus pumilus* increases photosynthesis and leaf transpiration when N is available, according to Masood, Zhao and Shen (2020), and the rise in transpiration of leaves is suggestive of higher plant water intake, which also aids N transport, notably NO_3- into plants. Dey et al. (2004) also found that PGPB increased peanut plant's water and nutrient absorption. Our PGPB boosts BNF, which increases leaf transpiration and dissolved N transfer to aboveground plant tissues. Both with and without PGPB inoculation, the rate of dispersed N was around 50% greater in shoots (stem and leaves) than in roots, our finding supported a previous research study (Masood, Zhao & Shen 2020). Our findings back up this hypothesis, as shoot N concentrations were higher than root N concentrations. Due to photosynthesis and N_2 fixation are inextricably related (Ryle 1988), increased N uptake by plants adds to the creation of chlorophyll, hence enhancing plant photosynthetic potential (Baset Mia et al. 2010). In this case, PGPB can aid in providing nutrients to meet crop nutrition needs, hence enhancing plant development. *Bacillus* spp. inoculation did not improve plant development in our study when there was no N fertilizer input; this is likely due to the soil's low N fertility condition, which made *Bacillus* spp. survival is difficult. In this study, the available N in soil was 3381 ppm, and 156.8 ppm as urea was given to the N fertilization treatment as a 100% N dose. As a result, the available N in soil without and with N fertilization might be 3381 ppm and 3537.8 ppm, respectively. The findings back up the theory that PGPB in combination with optimum rate of fertilizer could improve soil nitrogen levels and plant growth. If the percentages of suggested fertilizer were decreased and inoculants were used, stem length, root length, shoot (stem, leaves) dry biomass, root dry biomass and the entire rate of fertilizer without inoculants resulted in higher N concentrations (Table 1 & Figure 2). After experimenting with various N fertilizer levels, it was discovered that 50% fertilizer was the secured optimum to which fertilizer may be reduced if applied with PGPB to generate higher growth than full fertilizer rates without PGPB under these experimental conditions.

RHIZOSPHERE POPULATION

According to the findings of our research, the result supports 50% N fertilizer reduced if PGPB was used as a result optimum where the outcomes were consistent. The soil microbial population has expanded because of the inoculation and the addition of 50% nitrogen, which has enhanced dehydrogenase activity. When comparing the MC + 50% NPK treatment to the 100% NPK treatment, the bacterial population in the rhizosphere soil was found to be considerably greater. Ali-Tan et al. (2017) noted that a higher amount of microbial population in the rhizosphere was observed when fertilizer-N levels were low. Previous research has shown that Chemical fertilizers reduce the number of bacteria in the rhizosphere soil (Marschner, Crowley & Yang 2004). Our result also observed that bacterial application significantly enhanced the rhizobacterial number under Nitrogen fertilization, but not without Nitrogen fertilization (Figure 5). Masood, Zhao and Shen (2020) back up our findings.

CONCLUSIONS

Due to the improved N uptake, *Bacillus* spp. inoculations improve tomato seedlings' growth under optimal N conditions. Increased nitrogen uptake by tomato plants is aided by *Bacillus* spp.-assisted augmentation of biological nitrogen fixation. Findings from this study has shown the potential of PGPB to enhance tomato seedling's growth, which will lead to better crop yield. This was achieved with the savings of up to 50% of the nitrogen fertilization, therefore, could reduce the input cost and environmental issue associated with excessive

use of chemicals in agriculture. This will certainly aid the government and relevant sectors in suggesting for alternative methods to increase the tomato production, to meet the increasing demand of the market, using sustainable approach.

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