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Research Article

Evaluation of the Plant Growth Promotion Effect of *Bacillus* Species on Different Varieties of Tomato (*Solanum lycopersicum* L.) Seedlings

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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. The study was done to evaluate the effect of *Bacillus* species isolated from the sediment of Lake Tana on the early growth of different tomato (*Solanum lycopersicum* L.) varieties. All *Bacillus* isolates significantly ($P < 0.05$) increased the growth of all three tomato varieties in terms of shoot length, shoot fresh weight, shoot dry weight, root length, and fresh and dry weights of the root. The isolates' efficacy varied among tomato varieties. *Bacillus* isolate B2 was more effective in the Maya variety, with a potency of 85% and 71.2% shoot and root lengths, respectively. *Bacillus* isolate B3 was more efficient in the Melkesalsa variety, with a shoot and root length efficiency of 57% and 68%, respectively. *Bacillus* isolate B1 was more successful in the Kochero variety with 65 and 70% shoot and root length efficacy. Individual isolates' PGPR characteristics differed, resulting in a wide range of effectiveness among different varieties. More research studies are needed to fully know the mechanism of action and efficacy of these isolates in the field. The isolates must also be identified using molecular techniques.

1. Introduction

According to the data of [1], the world population is increasing at the rate of roughly 1.05% every single year due to the problems of food insecurity and famine aggravating throughout the world. The problem is severe in developing countries. Therefore, it is urgent to double the agricultural production to reduce the risk of malnutrition and poverty. Agriculture sectors try to develop different strategies to alleviate the above problems. From these strategies, applying agrochemicals to boost the production of crops is the major one in different parts of the world to minimize the risk of malnutrition and poverty. However, the overuse of these agrochemicals leads to various problems such as groundwater and crop products' contamination by heavy metals, interruption of the natural ecological cycle of nutrients, destruction of soil biological communities, and physical and chemical deterioration of agricultural soils which may cause environmental and public health problems [2, 3].

Tomato is the most widely eaten vegetable, ranking first as a processing vegetable [4] and second in terms of production after potato [5] in the world. Nutritionally, it serves as a source of vitamins A, B, C, and D and minerals such as calcium, phosphorus, and iron [6]. It is widely produced in Ethiopia in both small-scale farmers' and commercial producer's level. In Ethiopian households, it is consumed in fresh or processed forms. However, the national average yield of tomatoes in Ethiopia is low compared with the average production yield of other countries such as China (59.4 tons ha^{-1}), India (24.6 tons ha^{-1}), the USA (96.8 tons ha^{-1}), Turkey (68.8 tons ha^{-1}), and Egypt (40.9 tons ha^{-1}) [7]. The national average yield of tomatoes in Ethiopia is 5.3 tons ha^{-1} [8]. It is due to environmental constraints, i.e., biotic and abiotic.

To reduce the application of agrochemicals that caused several problems on human welfare in significant amounts, beneficial microbes have a vital role because they can promote the exchange of plant nutrients and can influence soil fertility [9–11]. Among these, plant growth-promoting rhizobacteria (PGPR) are the most promising one. PGPR are used to boost crop yield without increasing environmental contamination [12]. Previously, many pieces of research have been done on the effect of different PGPR strains in tomato production under different environments for plant growth promotion and controlling various tomato diseases [13–16].

Bacillus is one of the most important genera of beneficial bacteria that promotes plant growth via direct or indirect mechanisms [17]. However, the effect of *Bacillus* species on the early growth of different tomato (*Solanum lycopersicum L.*) variety seedlings in Ethiopia, specifically in the study area, is not reported previously. Therefore, this study aimed to evaluate the effect of *Bacillus* species on the early growth of different tomato (*Solanum lycopersicum L.*) varieties in Northwest Ethiopia.

2. Materials and Methods

2.1. Sampling and Sample Collection Methods. Sediment samples used to isolate *Bacillus* species were collected randomly from three different sites of Lake Tana, Northwest Ethiopia, with the help of sterilized pots, and transferred to sterilized polyethylene bags. The samples were labeled accordingly, transported to the University of Gondar, Department of Biology, Microbiology Laboratory, and deposited at 4°C for further use. The seeds of tomato varieties, namely, Kochero, Melkesalsa, and Maya, were collected from Adet Agricultural Research Center, Northwest Ethiopia.

2.2. Isolation, Characterization, and Identification of Bacillus Species. Physical pretreatment methods were applied to the sediment samples through air and heat drying to facilitate the isolation of *Bacillus* species. *Bacillus* species were isolated through serial dilution followed by spread plates and streak plates for purification from physically pretreated sediments. The purified colonies were characterized morphologically (colony color, form, margin, and elevation); microscopically (endospore formation and shape); and biochemically and physiologically (Gram reaction, indole test, catalase test, MR-VP test, TSI test, citrate utilization, and hydrolysis of starch, gelatin, casein, and urea, growing at various NaCl concentrations and temperatures) [18]. Bergey's manual of determinative bacteriology was used as a guide to identify the isolates.

2.3. Inoculum Preparation and Pot Experiments. The experiment was performed in 2017 from March to June at the University of Gondar, Biology Department. To prepare the inoculum, a purified single *Bacillus* colony was transferred to a 100 ml flask containing 25 ml of nutrient broth and grown aerobically at 30°C for 24 hours. Then, bacterial suspension was diluted in sterile distilled water before being inoculated into the seedling.

Healthy seeds of tomato were sown on the prepared soil and were watered regularly for up to 45 days. After 45 days of growth, the tomato seedlings having equal size and looking healthy were selected. The selected seedlings were dipped three times into sterile water to remove the attached soil and dipped into bacterial spore suspensions or distilled water (control) for 30 minutes immediately before transferring to the prepared pot, and each pot received three seedlings [19]. This experiment was carried out at the University of Gondar in 2017 in sterilized pots (20 cm diam.) containing sterilized soil in a CRD manner. Pot and soil sterilization was performed by 5% formalin solution. Three replications were used for each treatment. Pots were observed regularly and watered up to 45 days with enough sterilized water per day on each pot. After 45 days, the seedlings were uprooted, and shoot and root fresh and dry weights and root and shoot lengths were measured and recorded. Efficacy test was applied to determine the effectiveness of each isolate from each variety on each growth parameter by the following formula: efficacy = treated − control/treated × 100%.

2.4. Data Analysis. The collected data were statistically analyzed by using SPSS 20 software version and one-way ANOVA. The effect of isolated *Bacillus* strains on growth promotion effect was compared using the least significant difference (LSD) at a 5% probability level ($P \leq 0.05$).

3. Results

3.1. Isolation of Bacillus Species. Five *Bacillus* colonies with different growth characteristics were successfully isolated from sediment samples obtained from Lake Tana and represented as B1, B2, B3, B4, and, B5 where "B" stands for *Bacillus*.

3.1.1. Colony Characteristics of Bacillus Isolates. The colony characteristics of the isolated *Bacillus* species exist in Table 1.

3.1.2. Physiological and Biochemical Characteristics of Bacillus Isolates. The result indicates that all isolates produced catalase (catalase positive). *Bacillus* isolates B1, B2, and B5 had the capacity to produce H_2S gas and acid, and *Bacillus* isolates B2, B3, and B4 utilized citrate as a carbon source (Table 2). About hydrolytic enzyme production, the isolated species except B5 were able to hydrolyze casein, B1 and B4 were able to hydrolyze starch, while gelatin hydrolysis was observed in all species except B2 (Table 2).

The result (Table 2) revealed that *Bacillus* isolates were able to survive at different temperatures (20°C, 30°C, 37°C, 45°C, and 55°C) and concentrations of sodium chloride. The isolates except B3 were able to grow at 5% concentration of sodium chloride, whereas only B3 was resistant to 10% sodium chloride.

3.2. Effect of Bacillus on the Growth of Tomato Seedlings. All five *Bacillus* isolates (B1, B2, B3, B4, and B5) showed positive effects on the seedling growth of all three tomato

Table 1: The morphological and microscopic results of the isolated *Bacillus* colony.

Colony character	*Bacillus* species				
	B1	B2	B3	B4	B5
Color	White	White	White	White	White
Form	Circular	Circular	Circular	Irregular	Circular
Elevation	Convex	Convex	Flat	Flat	Flat
Margin	Entire	Entire	Entire	Lobate	Entire
Endospores	+	+	+	+	+
Shape	Rod	Rod	Rod	Rod	Rod

Table 2: Physiological and biochemical characteristics of *Bacillus* isolates.

Biochemical tests	*Bacillus* species				
	B1	B2	B3	B4	B5
Gram RXN	+	+	+	+	+
Catalase test	+	+	+	+	+
Casein hydrolysis	+	+	+	+	−
Citrate utilization	−	+	+	+	−
VP test	−	−	+	−	−
MR test	+	−	−	+	+
Indole test	+	+	−	−	+
H_2S production	+	+	−	−	+
Glucose (acid)	+	+	−	+	+
Glucose (gas)	+	−	−	−	+
Urea hydrolysis	+	−	+	+	−
Starch hydrolysis	+	−	−	+	−
Gelatin hydrolysis	+	−	+	+	+
Resistance to 5% NaCl	+	+	−	+	+
Resistance to 7% NaCl	+	+	−	+	−
Resistance to 10% NaCl	+	−	−	−	−
Growth at 20°C	−	−	+	−	−
Growth at 30°C	+	+	+	+	+
Growth at 37°C	+	+	+	+	+
Growth at 45°C	+	−	−	−	−
Growth at 55°C	+	−	_	−	−

+: positive; −: negative.

varieties (Melkesalsa, Kochero, and Maya) in all measured growth parameters (shoot and root fresh and dry weights, root length, and stem length); however, different isolates showed different effects on different varieties at different growth parameters (Tables 3–5). As presented in Table 3, tomato variety Melkesalsa seedlings treated with B5 showed the highest shoot length (40.3 ± 1.85 cm), with statistical significance at $P < 0.01$ level of confidence, B1 showed the highest shoot fresh weight (15.31 ± 0.33 g) and shoot dry weights (12.5 ± 0.60 g), and B3 showed the highest root length (12.9 ± 1.8 cm), root fresh weight (3.11 ± 0.18 g), and root dry weight (1.4 ± 0.23 g) compared to the control.

As presented in Table 4, tomato variety Kochero seedlings treated with B1 showed the highest shoot length (34.86 ± 3.1 cm), shoot fresh weight (10.86 ± 0.4 g), shoot dry weight (8.33 ± 0.76 g), root length (12.0 ± 0.0 cm), root fresh weight (3.0 ± 0.06), and root dry weight (1.2 ± 0.08). As the data shown in Table 5, tomato variety Maya seedlings treated with B2 showed the highest shoot length (23.0 ± 4.16 cm), shoot fresh weight (5.66 ± 0.33 g), shoot dry weight (2.8 ± 0.08 g), root length (8.0 ± 0.57 cm), shoot fresh weight (2.70 ± 0.05 g), and root dry weight (1.71 ± 0.00).

Efficacy of *Bacillus* isolates on the growth promotion of tomato seedlings was different among each variety and each growth parameter. The variety Melkesalsa treated with *Bacillus* isolate B5 showed the highest growth promotion efficacy in shoot length (62%), variety Kochero treated with *Bacillus* isolate B1 showed the highest growth promotion efficacy in shoot length (65%), and variety Maya treated with *Bacillus* isolate B2 showed the highest growth promotion efficacy in shoot length (85%). The variety Melkesalsa treated with *Bacillus* isolate B3 showed the highest growth promotion efficacy in root length (68.9%), variety Kochero treated with *Bacillus* isolate B1 showed the highest growth promotion efficacy in root length (70%), and variety Maya treated with *Bacillus* isolate B2 showed the highest growth promotion efficacy in root length (71.2%).

4. Discussion

In the present study, five *Bacillus* species which showed the characteristics such as Gram-positive, endospore-forming, catalase-positive, and rod-shaped were isolated from the sediment of Lake Tana. The result of the current study agreed with the characteristics of *Bacillus* described in Bergey's Manual of Systematic Bacteriology.

The isolated Bacillus species were evaluated for their effect on the early growth of different tomato varieties. The results revealed that the *Bacillus* spp. improved the growth of tomato seedlings in all varieties with different efficacies in all growth parameters. As presented in Tables 3–6, the growth promotion effect of each *Bacillus* species varies among variety and growth parameters. Tomato variety Melkesalsa seedlings treated with B5 showed the highest shoot length (40.3 ± 1.85 cm), variety Kochero seedlings treated with B1 showed the highest shoot length (34.86 ± 3.1 cm), and variety Maya seedlings treated with B2 showed the highest shoot length (23.0 ± 4.16 cm) with statistical significance at $P < 0.01$ compared to the control.

The efficacy of *Bacillus* isolates on the growth promotion of tomato seedlings was different among each variety and each growth parameter. The variety Melkesalsa treated with *Bacillus* isolate B5 showed the highest growth promotion efficacy in shoot length (62%), variety Kochero treated with *Bacillus* isolate B1 showed the highest growth promotion efficacy in shoot length (65%), and variety Maya treated with *Bacillus* isolate B2 showed the highest growth promotion efficacy in shoot length (85%). The variety Melkesalsa treated with *Bacillus* isolate B3 showed the highest growth promotion efficacy in root length (68.9%), variety Kochero treated with *Bacillus* isolate B1 showed the highest growth promotion efficacy in root length (70%), and variety Maya treated with *Bacillus* isolate B2 showed the highest growth promotion efficacy in root length (71.2%).

The results demonstrate that differences in the PGPR properties of the individual isolates made their effectiveness wide ranging in different varieties. This result was in line

Table 3: The effect of *Bacillus* isolates on the growth of tomato variety Melkesalsa seedlings.

Treatment	Upper ground growth parameters			Underground growth parameters		
	Shoot length in cm	Shoot fresh weights in g	Shoot dry weights in g	Root length in cm	Root fresh weights in g	Root dry weights in g
B1	33.23 ± 2.3b*	15.3 ± 0.33a**	12.5 ± 0.6a**	12 ± 0.57a*	2.9 ± 0.03a*	1.06 ± 0.07b*
B2	27.76 ± 3.6b*	7.66 ± 0.33b*	6.0 ± 0.17b*	10.4 ± 0.29a*	2.6 ± 0.17b*	0.93 ± 0.03b*
B3	35.0 ± 5.68a*	11.33 ± 2.6a*	8.66 ± 2.3a*	12.9 ± 1.8a**	3.1 ± 0.18a*	1.40 ± 0.23a*
B4	25.6 ± 3.75b*	7.33 ± 0.33b*	5.20 ± 0.6b*	8.0 ± 0.57b*	2.2 ± 0.03b*	0.76 ± 0.08b*
B5	40.3 ± 1.85a**	12.1 ± 1.46a*	8.33 ± 0.6b*	10.6 ± 0.88a*	2.8 ± 0.05b*	0.98 ± 0.0b*
Control	15.00 ± 1.00c	1.80 ± 0.25c	1.00 ± 0.1c	4.0 ± 0.57c	0.93 ± 0.03c	0.59 ± 0.03c
LSD at 0.05	2.7	2.92	2.0	3.1	0.88	0.92

Values are mean ± standard error of three replications; *statistically significant at $P < 0.05$ (significant difference with the control); **statistically significant at $P < 0.01$ (highly significant difference to the control); ns indicates not statistically significant; means in each column followed by the same letter are not significantly different at $P < 0.05$ according to Fisher's LSD.

Table 4: The effect of *Bacillus* isolates on the growth of tomato variety Kochero seedlings.

Treatment	Upper ground growth parameters			Underground growth parameters		
	Shoot length in cm	Shoot fresh weights in g	Shoot dry weights in g	Root length in cm	Root fresh weights in g	Root dry weights in g
B1	34.86 ± 3.1a**	10.86 ± 0.4a*	8.33 ± 0.76a**	12.0 ± 0.0a**	3.0 ± 0.06a*	1.2 ± 0.08a*
B2	19.53 ± 2.02c*	7.20 ± 0.2b*	5.50 ± 0.25b*	7.3 ± 0.88b*	2.6 ± 0.03b*	1.03 ± 0.03b*
B3	16.53 ± 1.00ns	6.93 ± 0.17b*	5.03 ± 0.53b*	6.8 ± 0.27b*	2.2 ± 0.05b*	1.08 ± 0.04b*
B4	33.60 ± 2.0a*	10.06 ± 0.28a*	8.03 ± 0.54a*	8.0 ± 0.57b*	2.2 ± 0.09b*	1.14 ± 0.04a*
B5	30.30 ± 0.17b*	6.63 ± 0.27b*	5.03 ± 0.43b*	6.7 ± 0.14b*	2.0 ± 0.03b*	1.02 ± 0.03b*
Control	12.20 ± 0.1d	1.73 ± 0.08c	0.88 ± 0.00c	3.6 ± 0.33c	1.06 ± 0.07c	0.66 ± 0.14c
LSD at 0.05	2.5	2.06	2.45	2.0	1.67	1.6

Values are mean ± standard error of three replications; *statistically significant at $P < 0.05$ (significant difference with the control); **statistically significant at $P < 0.01$ (highly significant difference to the control); ns indicates not statistically significant; means in each column followed by the same letter are not significantly different at $P < 0.05$ according to Fisher's LSD.

Table 5: The effect of *Bacillus* isolates on the growth of tomato variety Maya seedlings.

Treatment	Upper ground growth parameters			Underground growth parameters		
	Shoot length in cm	Shoot fresh weights in g	Shoot dry weights in g	Root length in cm	Root fresh weights in g	Root dry weights in g
B1	14.6 ± 2.7a*	3.83 ± 0.6b*	2.50 ± 0.2a*	6.00 ± 0.57b*	2.30 ± 0.05b*	1.60 ± 0.05b*
B2	23.0 ± 4.16a**	5.66 ± 0.33a*	2.8 ± 0.08a*	8.0 ± 0.57a*	2.70 ± 0.05a*	1.71 ± 0.00a*
B3	6.33 ± 0.3b*	2.2 ± 0.08b*	1.70 ± 0.1b*	4.03 ± 0.03b*	2.06 ± 0.03b*	1.57 ± 0.05b*
B4	6.33 ± 0.33b*	2.20 ± 0.05b*	1.46 ± 0.1c*	4.03 ± 0.03b*	2.10 ± 0.05b*	1.58 ± 0.06b*
B5	13.66 ± 1.2a*	3.33 ± 0.33b*	2.2 ± 0.05b*	6.00 ± 0.57b*	2.26 ± 0.03b*	1.65 ± 0.00b*
Control	3.30 ± 0.35c	1.17 ± 0.06c	0.76 ± 0.08c	2.30 ± 0.05c	1.06 ± 0.07c	0.48 ± 0.06c
LSD at 0.05	1.78	1.0	0.98	1.4	0.99	0.90

Values are mean ± standard error of three replications; *statistically significant at $P < 0.05$ (significant difference with the control); **statistically significant at $P < 0.01$ (highly significant difference to the control); ns indicates not statistically significant; means in each column followed by the same letter are not significantly different at $P < 0.05$ according to Fisher's LSD.

Table 6: Efficacy of *Bacillus* on the growth promotion of tomato seedlings.

Treatment	Melkesalsa variety		Kochero variety		Maya variety	
	Shoot length	Root length	Shoot length	Root length	Shoot length	Root length
B1	54%	66.6%	65%	70%	77%	61.6%
B2	46.23%	61.5%	37%	48.68%	85%	71.2%
B3	57%	68.9%	26%	43.8%	47.8%	42.9%
B4	41%	50%	61%	55%	47.8%	42.9%
B5	62%	62.2%	59%	46.2%	75.84%	61.6%
Control	—	—	—	—	—	—

with the report of [20, 21]. They suggested that different effects of different isolates were due to the variation of plant growth-enhancing mechanisms.

Bacillus species benefits plants through various mechanisms such as biofilm production [22], converting the complex form of essential nutrients (P and N) [23, 24], liberating ammonia from nitrogenous organic matter [25], fixing atmospheric N_2 [26], siderophore production [27], phytohormone production [28–30], and exudation of ACC deaminase [31, 32], controls pathogenic microbial growth, and boosts pest defense systems [33]. In the present study, we found that all five *Bacillus* species improved both upper and underground growth parameters of all three tomato varieties. The growth improvement of our isolates may be through one or more of the above mechanisms.

The results revealed that the *Bacillus* species improved the growth of tomato seedlings in all varieties with different efficacies in all growth parameters. The results demonstrated that differences in the PGPR properties of the individual isolates made their effectiveness wide ranging in different varieties. This result was supported by the result of [20, 21]. They suggested that different effects of different isolates were due to the variation of plant growth-enhancing mechanisms.

The result clearly indicates that the growth promotion effect of each *Bacillus* species varies in each variety of tomato. The composition and activity of bacterial communities associated with plants were regulated by plants [34]. Plant growth promotion by beneficial bacteria was strongly affected by the plant genotype [35].

In the present study, all isolated *Bacillus* species improved the growth parameters of tomato plants (increase in root length or fresh weight of the root and increase in shoot length or fresh weight of the shoot) over the control (untreated). This result agreed with the previous study done in [36–38] which reported that the positive correlations between the production of IAA by *Bacillus* species and growth parameters of tomato plants (increase in root length or fresh weight of the root) indicated the effect of IAA on plant growth. IAA primarily stimulates plant growth by expanding the root system to enter into the larger volume of soil for increased water and nutrient uptake [24]. In this regard, our *Bacillus* isolates may improve plant growth may be by producing IAA.

5. Conclusion

Indigenous *Bacillus* species as PGPR for tomato different varieties' seedling growth is quite promising under the conditions of Northwest Ethiopia. The tested *Bacillus* isolates improve tomato different varieties' seedling growth under the pot experiment. Further research should be directed towards ascertaining in detail the mode of action of these effective isolates. Field studies should be undertaken to confirm the effectiveness of the isolates under field conditions to use as biofertilizers.

Data Availability

The data used to support the findings of this study are available from the corresponding author upon request.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

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