



TOMATO SEEDLING PRODUCTION USING AN INOCULUM PREPARED WITH PLANT GROWTH-PROMOTING RHIZOBACTERIA (PGPR) ISOLATES

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SUMMARY

A study to attain healthy tomato (*Lycopersicon esculentum* Mill) seedlings through environment-friendly natural biological products took place from January to March 2022. Determining the effects of the inoculum prepared from five plant growth-promoting rhizobacteria (PGPR) isolates, i.e., *Pseudomonas fluorescens*, *Pseudomonas putida*, *Lysinibacillus fusiformis*, *Enterobacter cloacae*, and *Kineococcus radiotolerans* on the production of tomato seedlings, experiments proceeded at the Soil Microbiology Laboratory, Department of Soil Science and Water Resources, College of Agriculture, Tikrit University, Iraq. The isolates underwent screening for their efficiency as a biostimulant to dissolve insoluble phosphate compounds and produce indole acetic acid (IAA) and chelating compounds. The results showed the ability of all the isolates to produce IAA, chelating compounds, and solubility of phosphates. The *P. fluorescens* isolate showed superior in its phosphate solubilization and IAA production (41.30 mg p⁻¹, 13.00 mg ml⁻¹), followed by *P. putida*, *E. cloacae*, *L. fusiformis*, and *K. radiotolerans*, respectively, with the production of medium chelating compounds. The results also showed the superiority of the inoculated treatments over the non-inoculated treatments in the percentage and speed of germination, the length of tomato seedlings, the shoot dry weight, the number of leaves per plant, and root weight parts. The treatments with *P. fluorescens* displayed significant superiority in all studied traits, followed by *P. putida*, *E. cloacae*, *L. fusiformis*, and *K. radiotolerans*.

Keywords: Tomato seedlings, bacterial inoculation, *Enterobacter cloacae*, *Pseudomonas fluorescens*, *Kineococcus radiotolerans*, *Pseudomonas putida*

Key findings: The inoculum prepared from bacteria *P. fluorescens* proved superior over the rest of the bacterial species. Hence, highly recommended for adoption to produce healthy seedlings of tomato by relying on the biological inoculum.

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INTRODUCTION

The tomato (*Lycopersicon esculentum* Mill) is one of the important vegetable crops because

its fruit contains various essential elements necessary to build the human body and maintain health, such as calcium, phosphorous, iron, and carbohydrates (Mwangi *et al.*, 2011).

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Tomatoes are a good source of lycopene (carotenoids + flavonoids), which has antioxidant properties that help prevent cell damage and ward off daily toxins. It also prevents the adverse effects of lead in the blood constituents (Mallick, 2021). Tomatoes have many uses, such as, raw or cooked, in many dishes, sauces, salads, pickles, puree, paste, juice, sun-dried, and drink. These fruits contain 94% water, citric acid, malic acid, soluble sugars, vitamins A, B1, B2, and C, and many mineral salts.

Producing healthy and sturdy seedlings of crops is the basis for obtaining high production with early maturity. For crops reproduced by the seedling method, such as tomatoes, eggplants, and peppers, this method provides a beneficial opportunity and economy in the number of seeds, excluding diseased and weak seedlings, and obtaining higher production (Radhi and Hayder, 2011; Al-Khayri *et al.*, 2022; Javed *et al.*, 2022).

In tomato production, the main problem is its dependence on various chemicals (fertilizers, pesticides, and hormones) to encourage its growth for increased production and provide resistance to many pathogens. Therefore, the trend should be to use natural, organic, and environmentally-friendly products that stimulate seed germination, encourage plant growth, increase resistance to diseases, reduce the use of chemical products, and contribute to a clean and sustainable environment. Thus, healthy seedlings could be obtained, which can serve as a basis for earlier high production in vegetable crops (Moustaine *et al.*, 2017; Perez-Rodriguez *et al.*, 2020).

Several studies revealed the ability of plant growth-promoting rhizobacteria (PGPR) species to stimulate the growth of different crops. These rhizobacteria PGPR species also enhance the crops' yield and resistance to diseases through various mechanisms, such as, nitrogen fixation, solubilization of insoluble phosphorous and potassium compounds, and the production of growth regulators, such as, IAA and chelating compounds (Abdulla and Alkurtany, 2017; Khalil and Alkurtany, 2018; Alkurtany and Alwandawi, 2020). Therefore, the conduct of this study aimed to a) use the bacterial inoculum to produce healthy tomato seedlings and b) evaluate the effects of the inoculum prepared from five local bacterial isolates in stimulating seed germination and encouraging the growth and production of tomato seedlings.

MATERIAL AND METHODS

Selection of bacterial isolates

Five isolates belonging to different bacterial genera received isolation from the gypsiferous soil surrounding the roots of different plants, obtained from January to March 2022 at the Microbiology Laboratory, Department of Soil Science and Water Resources, College of Agriculture, Tikrit University, Iraq. Two bacteria species belonging to *Pseudomonas* bacteria *P. fluorescens* and *P. putida* got diagnosed and confirmed by VITEK, Portland, Oregon, USA (Alkurtany and Alwandawi, 2020), while the National Center for Biotechnology Information (NCBI), Bethesda, Maryland, USA, molecularly identified and recorded the other three species, *Enterobacter cloacae*, *Lysinibacillus fusiformis*, and *Kineococcus radiotolerans* with No. MW979610, MW979607, and MW979598, respectively.

The efficiency of bacterial isolates

The efficiency of the bacterial isolates in dissolving phosphate testing used a Pikovskaya liquid medium, with the amount of dissolved phosphorus recorded according to the method described by Olsen and Watanabe (1965), as well as, their ability to produce indole acetic acid (Patten and Glick, 2002) and iron-chelating compounds (Payne, 1980).

Preparation of bacterial inoculum

Pure and 24-hour-old isolates of five young bacterial cultures (*P. fluorescens*, *P. putida*, *E. cloacae*, *L. fusiformis*, and *K. radiotolerans*) were grown in 500 ml flasks containing 300 ml of nutrient broth liquid media. The cultures, incubated in a shaking incubator at 28 °C at a speed of 100 cycles minute⁻¹ for 3–5 days, had the numbers of bacterial cells counted by dilution and plate counting to ensure that the bacterial number reached the threshold number of 10⁸ cfu.ml⁻¹ wherever received (Alkurtany *et al.*, 2018a, b). The seedling medium was sterilized, with the pH adjusted by adding 5% calcium carbonate. Filling seedling dishes with sterilized peat moss followed, with the tomato seeds sterilized by washing them with water several times to remove the fumigated substance sticking to their surfaces completely.

Afterward, soaking the tomato seeds in a mercury chloride HgCl₂ solution at a

concentration of 0.01% for 30 s eliminated the organisms on their surfaces, and then washed well with water 3–4 times to get rid of the remaining traces of the sterile material. Tomato variety 'Rogena' seeds, sown in seedling dishes by placing a seed in each hole, had the seedling dishes inoculated according to the treatments by injecting 3 ml of the inoculum into each hole around the seeds. Treatment of the experiment took place in the greenhouse in a complete randomized design (CRD) with three replications to study the inoculation effect with five local bacterial isolates on promoting the growth of tomato seedlings.

Traits measurement

Recording and evaluation among the various tomato seedlings occurred for the following traits: germination percentage, germination speed according to Kotowski (1962), fresh root and vegetative weight (g seedling⁻¹), dry root and vegetative weight (g seedling⁻¹), number of plant leaves (leaf. plant⁻¹), and seedling height.

Statistical analysis

The experiment, designed according to a completely randomized design (CRD), had the results statistically analyzed using SAS program V. 9, with the arithmetic means compared using the least significant difference (LSD_{0.05}) test to compare and separate the treatments.

RESULTS

In the isolates' efficiency in dissolving phosphate and producing indole acetic acid (IAA) and chelating compounds, notably, all the isolates could dissolve insoluble phosphate compounds and produce IAA and chelating compounds (Table 1). The superiority of isolate *P. fluorescens* in its ability to dissolve insoluble phosphate compounds appeared as it gave the highest values for the solubility of phosphate (41.30 mg p L⁻¹) compared with the rest of the isolates. The solubility values of insoluble phosphate compounds for *P. putida*, *E. cloacae*, *L. fusiformis*, and *K. radiotolerans* amounted to 38.05, 37.30, 27.40, and 25.20 mg p L⁻¹, respectively. The results also revealed that all the isolates could produce IAA.

The superiority of *P. fluorescens* was also confirmed by producing the highest value for IAA (13.00 mg p l⁻¹), followed by isolates *P.*

putida (11.00 mg p L⁻¹), *E. cloacae* (10.20 mg p L⁻¹), *L. fusiformis* (8.80 mg p L⁻¹), with the lowest IAA value recorded in isolate *K. radiotolerans* (7.21 mg p L⁻¹) (Table 1). The results also showed the ability of all isolates to produce chelating compounds, with the two isolates, *P. fluorescens* and *P. putida*, exhibiting higher production of chelating compounds. However, the three Isolates, i.e., *E. cloacae*, *L. fusiformis*, and *K. radiotolerans*, only enunciated average production of iron chelating compounds.

For tomato seed germination, Table 2 shows the effect of bacterial inoculation on the rate and speed of germination in tomato seedlings. Results revealed that inoculating the medium with the prepared inoculum had a significant effect on the percentage and speed of germination and seedling growth. The average percentage and speed of germination for inoculated treatments were 82.80% and 11.86 days, respectively, to 63% and 13.30 days for non-inoculated treatment, respectively. The treatment inoculated with the *P. fluorescens* isolates outperformed the others by showing full germination percentage (100%) and speed of germination with the least period (11.00 days) over the rest of the inoculated treatments and the control. The treatment inoculated with *K. radiotolerans* recorded a germination percentage of 70% and took 12.18 days to reach the final germination percentage (Table 2).

The studied effects of inoculation from the prepared inoculum from various bacterial isolates on the plant height and the number of leaves per plant in the tomato seedlings appear in Table 3. The effect of inoculation with bacterial isolates showed significance in plant height and the number of leaves, with the impact of inoculation most pronounced in plant height, followed by the number of leaves. The treatment inoculated with isolate *P. fluorescens* displayed superior to all other treatments in plant height and the number of leaves, which reached 11.87 cm and 5.00 leaves per plant⁻¹, respectively. These values of plant height and the number of leaves indicated significant superiority to the non-inoculated treatment (8.02 cm, 9.00 leaves per plant⁻¹) and the treatment inoculated with isolate *K. radiotolerans* (4.25 cm, 4.25 leaves per plant⁻¹).

The inoculation of various bacterial isolates inoculum had a significant effect on the fresh and dry weight of tomato seedlings' vegetative and root parts (Table 4). Results revealed that the treatments inoculated with the bacterial isolates, *P. fluorescens*, *P. putida*,

Table 1. The efficiency of bacterial isolates in phosphate solubilizing and production of indole acetic acid and chelating compound used in tomato seedlings.

Isolates	Chelating Compound	Phosphate (mg p l ⁻¹)	Solubilizing	Indole Acetic Acid (IAA) µg.ml ⁻¹
<i>Pseudomonas fluorescens</i>	+++	41.30		13.00
<i>Pseudomonas putida</i>	+++	38.05		11.00
<i>Enterobacter cloacae</i>	++	37.30		10.20
<i>Lysinibacillus fusiformis</i>	++	27.40		8.80
<i>Kineococcus radiotolerans</i>	++	25.20		7.21

Table 2. Effect of inoculation with bacterial inoculum on the percentage and speed of germination of tomato seedlings.

Treatments	Germination (%)	Germination speed (days)
Control	63	13.30
<i>P. fluorescens</i>	100	11.25
<i>P. putida</i>	94	11.93
<i>E. cloacae</i>	85	11.95
<i>L. fusiformis</i>	75	12.00
<i>K. radiotolerans</i>	70	12.18
LSD _{0.05}	2.4	0.02
Average germination (%) for inoculated treatments	82.80	
Average germination speed for inoculated treatments	11.86 days	

Table 3. Effect of inoculation with bacterial inoculum on the seedling height and the number of leaves per plant in tomato seedlings.

Treatments	Plant height (cm)	No. of leaves plant ⁻¹
Control	8.02	4.25
<i>P. fluorescens</i>	11.87	5.00
<i>P. putida</i>	11.00	4.75
<i>E. cloacae</i>	10.27	4.75
<i>L. fusiformis</i>	10.22	4.50
<i>K. radiotolerans</i>	9.00	4.25
LSD _{0.05}	1.49	0.70

Table 4. Effect of inoculation with bacterial inoculum on the fresh and dry weight of vegetative and root parts in tomato seedlings.

Treatments	Vegetative fresh weight (g plant ⁻¹)	Vegetative dry weight (g plant ⁻¹)	Root fresh weight (g plant ⁻¹)	Root dry weight (g plant ⁻¹)
Control	0.61	0.08	0.11	0.05
<i>P. fluorescens</i>	1.46	0.16	0.37	0.14
<i>P. putida</i>	1.18	0.11	0.25	0.09
<i>E. cloacae</i>	1.08	0.11	0.19	0.08
<i>L. fusiformis</i>	1.05	0.10	0.12	0.06
<i>K. radiotolerans</i>	1.01	0.10	0.11	0.06
LSD _{0.05}	0.24	0.06	0.13	0.04

and *E. cloacae*, showed significant superiority in the fresh and dry weights of the vegetative and root parts of tomato seedlings compared with the non-inoculated treatment. The results also showed that the highest values of these traits emerged in inoculations made with the bacterial isolate *P. fluorescens*, followed by *P. putida*, *E. cloacae*, *L. fusiformis*, *K.*

radiotolerans, and control. The fresh and dry weights of the vegetative and root parts for isolate *P. fluorescens* were 1.46, 0.16, 0.37, and 0.14 g seedling⁻¹, respectively, while the same trait values for the control treatments were 0.61, 0.08, 0.11, and 0.05 g seedling⁻¹, respectively.

DISCUSSION

More evidence show that the PGPR bacteria can fix nitrogen, dissolve insoluble phosphate compounds, and produce plant hormones, such as, indole acetic acid and iron chelating compounds. Phosphorus is the second necessary element after nitrogen, based on the importance and quantity needed by crop plants. This element mostly appears in insoluble organic forms and mineral compounds unavailable for plants. However, the bacterial isolates can dissolve insoluble phosphate compounds to produce organic acids (Satyaprakash *et al.*, 2017; Alkurtany *et al.*, 2022) and the phosphatase enzyme, which plays a major role in dissolving phosphorus (Alori *et al.*, 2017), and the production of the phytase enzyme (Maougal *et al.*, 2014). It leads to the acidification of cells and the surrounding environment, which then boosts the dissolution of unready phosphate compounds and the release of available phosphorus for crop plants. Results also revealed that the treatments inoculated with gram-negative bacteria exhibited superior to the gram-positive treatments in phosphate solubilizing, with the same previously confirmed by Abdelrahman *et al.* (2021) and Kalayu (2019). These results also align with the past findings in tomatoes (Alkurtany and Alwandawi, 2020), maize (Khalid, 2019), and other crop plants (Khalil and Alkurtany, 2018).

Indole acetic acid (IAA) is one of the most vital plant hormones. It directly affects the growth, cell division, and root formation, as well as, encourages the length and density of the root system, which leads to an enhancement in the surface area of the root absorption zone, enabling the root to reach the nutrients in the medium in which it grows (Hayat *et al.*, 2012). The variation in the amount of IAA produced by different bacterial isolates could be attributed to their genetic variation, as they belong to varied species, reflected in their biological properties, including their secretions in the growth medium. These results are consistent with the past findings in maize (Abdulla and Alkurtany, 2017) and cowpea (Alsamarrai, 2021). The superiority of the bacteria *P. fluorescens* could confirm as attributable to its higher IAA production, supported by previous findings in tomatoes (Alkurtany and Alwandawi, 2020).

All the bacterial isolates displayed the capability of producing chelating compounds because of their ability to grow on an iron-chelating medium. The two isolates, i.e., *P. fluorescens* and *P. putida*, showed the highest

capability to iron chelation, giving intense growth on the medium. However, the rest of the isolates, i.e., *E. cloacae*, *L. fusiformis*, and *K. radiotolerans*, provided medium growth, and thus have a medium ability to chelate iron. The difference in isolates' production of chelating compounds was due to the variation in their genetic composition, which caused differences in their growth on the iron-chelating medium (Khalil and Alkurtany, 2018). The bacteria producing these compounds can withdraw ferric ions from the 2, 2-dipyridyl compound, bind to it, and transport it into the bacterial cell to benefit from it in metabolic activity (Leoni *et al.*, 1996).

The superiority of treatments inoculated with bacterial isolates over non-inoculated ones in the percentage and speed of germination in tomatoes proved as attributable to the encouraging mechanisms for seed germination possessed by the bacterial isolates, especially the production of the hormone IAA. The role of IAA in stimulating the germination of seeds, cell division, and elongation and an increase in the rate and speed of germination cannot be ignored (Kamble and Galero, 2015). As far as the superiority of the treatment of inoculation with bacteria *P. fluorescens* over the rest of the treatments is concerned, it may result from its higher production of IAA, whereas a decrease in these traits occurred with bacteria *K. radiotolerans* due to less production of IAA.

The superiority of the treatments inoculated with bacterial isolates over non-inoculated ones in seedling height, number of leaves, and the fresh and dry weight of the vegetative and root parts of tomato seedlings could come from the isolates possessing different mechanisms to encourage plant growth, such as, dissolving insoluble phosphate compounds and producing IAA and iron-chelating compounds. Phosphorus also plays a key role in many vital processes, such as, photosynthesis, respiration, energy transfer, and the formation of DNA and RNA. The phosphorus role is essential in the growth, development, and straightening of roots, which helps distribute their spread and increase the absorption of nutrients (Curtin and Sysers, 2011). The increase in seedling height, number of leaves, and fresh and dry weight of vegetative and root parts of tomato seedlings inoculated by the prepared inoculum from bacterial isolates may be due to the direct and indirect mechanisms possessed by these isolates to encourage plant growth. These processes include the solubilizing of phosphate compounds, the liberation of plant-ready

phosphorus, and the secretion of stimulants to the medium as IAA promotes the growth of running hairs and the formation of a dense root system, which influences the process of absorbing nutrients from the growth medium, resulting in improved plant growth and increasing vegetative and root weight (Verma *et al.*, 2012).

The enhancement and improvement in the studied growth characteristics of tomato seedlings may refer to the release of chelating compounds called siderophores that contribute to the protection of nutrients, especially iron, and make them available for crop plants (Hayat *et al.*, 2012). It has a chief role in increasing the readiness of iron and its absorption by the plant, as these compounds work to convert the iron oxides present in the root ocean into a soluble form easily absorbed by the plant. As for the ethical superiority of the treatment inoculated with bacteria *P. fluorescens* over the rest of the inoculation treatments concerning the number of leaves, plant height, and fresh and dry weight of the vegetative and root parts of tomato seedlings, it could come from its superiority in the promising criteria for plant growth represented in the dissolution of phosphate and the production of IAA and iron-chelating compounds, with these results showing consistent with the past findings in tomatoes (Almaghabi *et al.*, 2013; Perez-Rodriguez *et al.*, 2020).

CONCLUSIONS

The efficiency of the prepared inoculum in encouraging the growth and production of tomato seedlings attained evaluation. The superiority of the inoculum prepared from the bacteria *P. fluorescens* over the rest of the bacterial species underwent observation. Thus, adopting this inoculum to produce healthy seedlings of tomato proved highly recommended. It will also help reduce dependency on chemical compounds with high costs and damage to the environment and human health.

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