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Seed Priming with Zinc Oxide Nanoparticles and Plant Growth-promoting Rhizobacteria to Enhance the Seedling Development of Fenugreek

Jahal Dangar[1], Gunja Vasant[2], Shweta Bhatt[3], Ragini Raghav[4,]*

Abstract

Globally, agrochemicals are introduced to the soil as additional nutrients to increase soil fertility. A broad variety of situations in nature can be adapted by Plant Growth Promoting Rhizobacteria (PGPRs), thus making them a potential ecologically sustainable alternative. In addition, they are involved in a variety of ecologically important activities and interact with soil microorganisms both effectively and antagonistically. The growth of plants is known to be influenced by the presence of rhizobacteria in cultivated crops through a variety of direct or indirect mechanisms. In this paper, we report the isolation and characterization of rhizobacteria from soil samples of fenugreek (Trigonella foenum graecum L.) taken from different regions of Saurashtra and Gujarat, India. From the primary isolation, fifty-four bacterial isolates were obtained and their potential for encouraging plant growth was examined. Based on the test results of qualitative and quantitative physiological properties, of all the bacterial isolates, PGRJ3 showed the most advantageous traits as rhizobacteria that encourage plant growth, indicating that it can be useful for enhancing yield, growth, and sustainable agriculture. A chemical synthesis method was employed for the synthesis of ZnO nanoparticles, Synthesized particles were characterized by UV visible spectrophotometer and Transmission electron microscopy. Synergistic effect of ZnO NPs and potent rhizobacteria J3 were evaluated for seed priming of fenugreek. The obtained result suggests positive impact of nanoparticles and rhizobacteria for seed priming of fenugreek. Therefore, this study suggests that this potent rhizobacteria and nanoparticles could be used to formulate biofertilizers, which could be applied to sustainable cultivation to improve crop health.

Keywords: Plant Growth Promoting Rhizobacteria; PGP traits; Isolation; Screening

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INTRODUCTION

India is the world's biggest producer, consumer, and exporter of spices. Indian spices are highly valued for their amazing flavour, texture, aroma, and therapeutic qualities all over the world. The principal spices that are exported by India are pepper, chili, turmeric, ginger, cardamom, coriander, cumin, fennel, fenugreek, celery, nutmeg, mace, garlic, tamarind, and vanilla [1]. According to the Spices Board India, Ministry of Commerce & Industry, Government of India, total Indian spices exported in 2021-22 was about 3,032,432.44 lakhs whereas in 2019-20 year it was estimated about 2,206,279.91 lakhs indicating an 27.26% increase [2]. This significant increase in the export of spices is primarily due to excessive use of chemical fertilizers to enhance crop yield.

Trigonella foenum graecum L. (fenugreek) is an herb and one of the Indian spices which uses in medicinal purposes, in food industry and in cosmetic industry [3]. The states that produce the majority of fenugreek are Rajasthan, Gujarat and Madhya Pradesh. Gujarat contributes about 20% in total production of fenugreek in India. Furthermore, fenugreek growth and production are negatively impacted due to low soil quality and limited nutrient availability, particularly nitrogen [4]. The crop is sown twice a year, i.e. once in the Kharif season (June–July) and again in the Rabi season (September–December). Moreover, fenugreek can be grown in almost all type of soils having good drainage facilities, however it thrives on well drained loamy soils. The fenugreek yield has been impacted with various biotic and abiotic stresses which has frequently been compensated by the application of chemical fertilizers [5]. Recent studies suggest that the excessive use of chemical fertilizers has exponentially increased over the past 30 years to meet the demand for crop production. These disproportionate uses of chemical fertilizers have adverse effects on soil texture, soil salinity content, heavy metal accumulation, eutrophication, soil leaching, accumulation of nitrate, and other gases [6].

Rhizobacteria are soil bacteria that naturally adhere to plant roots and promote plant growth. These growth-promoting rhizobacteria plays a major role in producing growth hormones, mineral solubilizing activities, siderophore production, hydrogen cyanide production, and enzyme production. Modern methods of agriculture require the use of PGPR extensively while applying a reduced quantity of detrimental pesticides and fertilizers [7, 8]. Their potential to be employed as biofertilizers influences crop quality and yield while reducing the application of chemical fertilizer in sustainable production systems by promoting soil microbial activity and health.

Zinc is a vital element necessary for a greater range of plants' key processes such as enhancing water usage efficiency, the process of photosynthesis, controlling of reactive oxygen species, antioxidant activity, preservation of membranes credibility, growth control, and the expression of genes. The critical role that zinc oxide nanoparticles (ZnO NPs) play in crop growth and productivity has been extensively researched. This role includes nitrogen uptake, respiration, and photosynthesis, as well as the activation of other physiological processes like enzyme activation, protein synthesis, and glycogen and nucleic acid [9]. Zinc oxide nanoparticles differ from bulk materials in optical, mechanical, catalytic, and biological properties due to their small size (up to 100 nm) and high surface area to volume ratio. Nanoparticles (NPs) have drawn a lot of interest because of their special qualities and advantageous uses in agriculture, health, food, chemical, and cosmetic industries. Moreover, these nanoparticles have been studied for their potential to boost crop yields and alleviate environmental stress including salt and drought [10]. Several studies have claimed different effects of using nanoparticles on plant growth. Afrayeem et al., (2017) studied the effect of zinc oxide nanoparticles in seed germination and seed vigor in chili [11]. Similarly, Rai-Kalal et al., (2021) studied the seed priming with zinc oxide nanoparticles to improve germination and photosynthetic performance in wheat [12]. Adrees et al., (2021) studied the foliar exposure of zinc oxide nanoparticles to improve the growth of wheat and to decrease cadmium (Cd) concentration in grains under simultaneous Cd and water deficient stress [13].

In the present work, we have isolated potential PGPR which exhibits various plant growth promoting traits such as indole acetic acid production, ammonia production, nitrogen fixation activity, phosphate solubilization, potassium solubilization, zinc solubilization, siderophore production, hydrogen cyanide production, gibberellic acid production, and chitinase activity. Furthermore, we have investigated the synergistic effect of ZnO NPs along with the potent PGP isolate as a potent biopriming agent for improving seed germination in fenugreek seeds.

MATERIALS AND METHOD

Collection of Soil Samples

Soil samples were collected from the rhizosphere of five different locations where fenugreek plants were cultivated. The different regions include Jalsika village situated in Wankaner (22.4846° N, 70.9822° E), Thikaryala village situated in Wankaner (22.4143° N,71.0878° E), Diu (20.7693° N, 73.6228 E), Talaja, Bhavnagar (21.3555° N, 72.0327° E) and Kherdi village situated in Rajkot (22.3039° N, 70.8021° E), Gujarat. The soil samples were collected into the sterilized autoclave bags and stored for further estimations.

Isolation of Microorganisms from Rhizospheric Soils

Isolation of microorganisms was done by spread plate method [14]. Briefly, 0.1 mL dilutions from the dilution tubes of 10^{-2} to 10^{-7} were spread onto the nutrient agar plate and incubated at 37 °C for 24 h. The colonies exhibiting distinct morphologies were streaked onto sterile plates of nutrient agar plates and incubated at 37 °C for 24 h. Gram's staining and Potassium hydroxide (KOH) method was performed to characterize the isolates microorganisms [15]. The purified microorganisms were stored at 4°C for further analysis.

Screening of Microorganisms for their Plant Growth-Promoting (PGP) Traits

Indole Acetic Acid Production

All the microorganisms were screened qualitatively and quantitatively for their capacity to generate indole acetic acid (IAA) by Gordon and Weber method [16]. Briefly, the bacterial cultures were inoculated in sterile nutrient broth supplemented with 0.1 g of L-tryptophan. The cultures were incubated at 30±2°C for 3-4 days. The cultures were centrifuged at 5000 rpm for 20 minutes, and 1 mL of supernatant was mixed with 2 mL of Salkowski's reagent (1 mL of 0.5 M ferric chloride in 50 mL of 35% perchloric acid). After that, the tubes were incubated for 20-30 minutes in the dark. The change in coloration to light pink was observed and absorbance was spectrophotometrically (Shimadzu UV-1800) recorded at 530 nm. The standard curve was recorded for IAA in 20-200 µg/mL range.

Ammonia Production

Each isolate was examined to determine their ability to produce ammonia in peptone water using the method established by Cappuccino and Sherman [17]. Briefly, the bacterial cultures were inoculated in 10 mL of peptone water (10 g bacteriological peptone and 5 g of sodium chloride) and incubated at 30±2°C for 2-3 days. Later, 1 mL of Nessler's reagent was added to each tube and color change was observed. The change in coloration from yellow to brown indicated production of ammonia and the absorbance was measured by UV-visible spectrophotometer at 450 nm. The concentration of ammonia was estimated based on a standard curve of ammonium sulphate ranging from 20-100 µg/mL.

Nitrogen Fixation Activity

The nitrogen fixation ability of rhizobacterial strains was examined by the methodology described by Jensen [18]. The isolates were streaked onto Jensen's agar media and cultured for 24-48 hrs. Colony growth demonstrated confirmation of nitrogen fixing activities.

Activity of Phosphate Solubilization

Evaluation of Phosphate Solubilization Qualitatively

The method described by Pikovskaya (1948) was used to determine the solubilization of inorganic phosphate. The spot inoculation was done for all the microorganisms onto Pikovskaya's agar media [19]. Later, the plates were incubated at 37°C for 6-7 days. The clear zone of phosphate solubilization was observed after 7 days of incubation. The phosphate solubilizing index was calculated using the following formula:

$$\text{Phosphate Solubilizing Index (PSI)} = ((\text{CD} + \text{ZD}))\ /\ \text{CD}$$

where, CD is Colony Diameter (in cm) and ZD is Zone Diameter (in cm).

Quantitative Estimation of Phosphate Solubilization

The phosphate solubilizing bacteria were examined to determine their quantitative amount of solubilizing phosphate. The amount of phosphate released was quantitatively estimated using the spectrophotometric chlorostannous reduced molybdophosphoric acid blue technique [20]. In brief, 1.0 mL of each of freshly grown culture was inoculated separately into 110 mL of sterile in Pikovskaya's broth in 250 mL Erlenmeyer flasks and incubated at 28±2°C at 120 rpm for 10 days along with uninoculated media as control. Later, 10 mL of each sample was collected in fresh tubes on 3rd, 5th, 7th, 9th, and 11th day and estimated for amount of soluble phosphorus and pH. After the culture broths were extracted by centrifuging the mixture for 15 minutes at 10,000 rpm, 100 µL of the supernatant was combined with 10 mL of the chloromolybdic reagent (15 g of ammonium molybdate in 400 mL of distilled water and 342 mL of concentrated hydrochloric acid (HCl)). The final volume was adjusted to 50 mL with distilled water after adding 5 drops of the chlorostannous acid reagent (10 g tin chloride in 25 mL concentrated HCl) and thoroughly stirring. Finally, the absorbance of the resulting blue color was determined using spectrophotometry at 660 nm. The concentration of phosphate solubilized was calculated from the standard curve of potassium dihydrogen phosphate (KH_2PO_4), and was expressed in µg/mL [21].

Hydrogen Cyanide (HCN) Production

The potential of the bacterial isolates for producing hydrogen cyanide was investigated using the Lorck method [22]. All of the bacterial colonies were spread out on sterile nutritional agar medium enriched with 4.4 g/L glycine. After immersing the Whattman filter paper-1 in a sodium picrate solution containing 0.5% picric acid and 1% sodium carbonate, it was placed on the lid of a petri dish, sealed with parafilm, and kept at 30°C for two to five days. The formation of hydrogen cyanide caused the change in the color of the filter paper from yellow to brown or red.

Zinc Solubilizing Activity

All the bacterial cultures were spot inoculated onto sterile Pikovskaya's agar media containing 0.1% zinc insoluble compound such as zinc oxide [23]. The plates were incubated at 30±2°C for 4-5 days. The diameter of the halo zones surrounding the colonies was measured.

Potassium Solubilizing Activity

All isolates were tested for their capacity to solubilize potassium using the Aleksandrow agar medium with slight modification. The bacterial cultures were spot inoculated aseptically on Alexsandrow agar medium containing bromothymol blue as a pH indicator and incubated at 30±2°C for 48-72 hrs [24]. The diameter of the halo zones surrounding the colonies was measured as follows [25]

$$\text{Ratio} = D \,/\, d$$

where, D is diameter of zone of clearance (in cm) and d is diameter of growth (in cm).

Siderophore Production Assay

Qualitative Estimation of Siderophore

The CAS reagents were synthesized by Schwyn and Neilands' (1987) technique, with a slight modification [26]. The bacterial cultures were spot inoculated onto chrome azurol sulphonate (CAS) medium. The plates were incubated at 37 °C for 5-6 days. Positive siderophore production is indicated by the zone surrounding the colony changing from blue to deep yellow to orange color.

Quantitative estimation of siderophore

Siderophore synthesis is quantitatively assessed using liquid culture media and CAS reagent production siderophore units (psu). The bacterial cultures were inoculated in 1 mL of Luria Bertani (LB) broth and then incubated for 48 hours at 37°C. After incubation, the cultures underwent a 10-minute centrifugation at 10,000 rpm. Later, 0.5 mL of supernatant was mixed with 0.5 mL of CAS reagent. With the use of a UV-VIS spectrophotometer, the color shift was measured at optical density 630 nm [27].

$$\text{Production siderophore unit (psu)} = (((\text{Ar} - \text{As})) \,/\, ((\text{Ar}))) \times 100$$

where, Ar is absorbance of reference (CAS solution and uninoculated broth) and As is absorbance of sample (CAS solution and cell free supernatant of sample).

Gibberellic Acid Production

The estimation of the production of gibberellic acid was determined by the folin-ciocalteu reagent method. The 100 µL bacterial cultures were inoculated into 1 ml of Luria Bertani (LB) broth which was allowed to incubate for two days at room temperature. Later, the cultures were centrifuged at 10,000 rpm for 10 min. Subsequently, 1 mL of supernatant was mixed with 1 mL of HCl and 1 mL of Folin-Ciocalteu reagent (FCR). Later, the tubes were placed in boiling water bath for five minutes. The resulting bluish-green color was measured at 760 nm spectrophotometrically. The standard curve of gibberellic acid (GA_3) was recorded in the range 10-100 µg/mL.

Synthesis and Characterization of ZnO NPs

ZnO nanoparticle were synthesized using sol-gel method. Briefly, 0.02 M zinc acetate was dissolved in 100 mL distilled water and boiled with continuous stirring for 30–40 minutes. Later, aqueous 2 M sodium hydroxide as reducing agent and 0.5 M sodium citrate as capping agent were added at a constant flow rate until the coloration of the solution changed from colorless to white. The synthesized nanoparticles were allowed to cool for 30 minutes and settled in an ice bath for 2 h. A UV spectrophotometer was used to measure the solution's wavelength, which was within the range of 300–800 nm, following centrifugation of the solution at 8000 rpm for 10 minutes. The pellet was air dried for the entire night at 60°C in a hot air oven and stored for further characterization [28].

Seed Bio-priming

T. foenum-graecum L. seeds were surface sterilized in 0.5% sodium hypochlorite for five minutes, subsequently rinsed five times in sterile distilled water and finally one minute in 70% ethanol [5].

Further for seed priming, the seeds were exposed to separate (a) bacterial cultures and (b) nanoparticles and (c) a mixed solution of bacterial culture and ZnO NPs. An overnight-cultured bacterial suspension was added to nutritional broth to prepare the bacterial inoculum, which was then incubated at 28°C for 24 hours at 120 rpm in a shaking incubator.

Later, bacterial biomass was extracted from the culture and suspended in distilled water using a centrifuge. At 600 nm, the optical density of the culture was adjusted to 0.1, indicating about 10^7 CFU ml^{-1}. *T. foenum-graecum* seeds that had been surface-sterilized were in bacterial cultures for 30 minutes. Similarly, 400 ppm solution of ZnO NPs was used to submerge the seeds. Sterile distilled water was used to submerge seeds as a control.

Effect of Potent Rhizobacteria and ZnO NPs Seed Bio-priming on the Germination of Fenugreek Seeds

The germination of the seeds was recorded after two days of seedling growth. Calculations were performed for the vigor index and seed germination % [29]. The germination of seed was measured by the equation using following:

$$Seed\ germination\ (\%) = ((No.of\ germinated\ seeds)\ /\ (Total\ no.of\ sown\ seeds)) \times 100$$

Vigor index was estimated by using following equation:

$$Vigor\ index = \ seed\ germination\ (\%) \times total\ length\ of\ seed$$

RESULTS AND DISCUSSION

Fifty-four morphologically different colonies were isolated from the rhizospheric soils of fenugreek (*Trigonella foenum-graecum* L.) plant. The isolates were named according to sample location as J1-14 (Jalsika), T15-23, T30 (Thikariyala) and D24-29, D31-35 (Diu), TB57-68 (Talaja-Bhavnagar), and Kh69-75 (Kherdi). Traits that promote plant growth were evaluated for all of the fifty-four isolates.

The naturally occurring primary auxin, which is actively involved in plant development is called indole-3-acetic acid (IAA) [30]. Auxin, a vital hormone for plants, regulates a wide range of functions, including general root and shoot development, organ patterning, vascular development, and growth in tissue culture [31]. The biological processes that are linked to plant growth, includes seed germination, cell division, elongation, and differentiation, gene expression, photosynthesis initiation, and the growth of lateral and adventitious roots, are all regulated by IAA [32]. Each of the fifty-four isolates exhibited different capacities to generate indole acetic acid by changing the coloration from yellow to pink (Figure 5 a). Figure 1 shows that the highest IAA production was 49.9 µg/mL by TB58 and the lowest was16.6 µg/mL by TB61. Similarly, Bellotti et al., (2023) reported the IAA production within the range of 0.94 µg/mL by isolate TE105 to 22.14 µg/mL by isolate TE103. Additionally, the highest IAA was recorded by our isolate TB58, which was 55.63% greater than the 22.14 µg/mL concentration that isolate TE103 had recorded [33]. Sritongon et al., (2023) reported maximum IAA production of 13.53 µg/mL by isolate S81 identified as *Enterobacter cloacae* which is 18.49% lower compared our least IAA producer TB61 [34].

The process of ammonia synthesis by PGPR is essential for plant growth because it helps plants to accumulate nitrogen, which has a direct effect on plant growth [35]. Thirty-five isolates showed color change from yellow to brown which indicates ammonia production (Figure 5 d). Figure 2 shows the production of ammonia amongst 22.8 µg/mL to 97.6 µg/mL. The isolate J3 produced the highest concentration of ammonia, at 97.6 µg/mL. In a similar study, Joshi et al., (2023), reported *Serratia marcescens*, labelled as NJC21 isolate, produced the maximum amount of ammonia, 22.3 µg/mL. Compared to the stated NJC21 isolate, J3 generated 77.15% higher ammonia in the current study [36].

The biological nitrogen fixation by bacteria is one of the biggest benefits plants receive from their interactions with microorganisms. Plant growth depends significantly on PGPR's capacity to fix nitrogen into organic forms that plants can easily absorb. All the rhizobacteria were subjected to test their nitrogen fixing activity. Out of fifty-four isolates thirty-five isolates showed formation of colonies on Jensen's media (Figure 4 e).

Phosphate solubilizing bacteria (PSB) efficiently provide phosphate to plants in an environmentally friendly and sustainable manner by solubilizing Ca-bound compounds in soil [37]. We qualitatively estimated the phosphate solubilization for all the isolates, and found that twenty-five of them displayed a high to moderately clear zone of solubilization on Pikovskaya's agar media (Figure 4 a). Furthermore, the obtained twenty-five isolates were analyzed for the quantity of released phosphate.

A number of rhizobacterial strains have been specifically documented in the literature to be able to solubilize insoluble forms of phosphorus into soluble forms that are readily absorbed by plants. Phosphorus is available in soils in amount of 1 µmol/L while plants require 30 µmol/L for maximum growth and production [38]. It was showed that all twenty-five isolates demonstrated the solubility of phosphate in the range of 62.6 µg P/mL to 322.6 µg P/mL. After the incubation period, J3 and D28 isolates showed the highest solubilization of 322.6 µg P/mL and 194 µg P/mL, respectively, at the 7th day (Figure 3). TB64 showed least solubilization of 62.6 µg P/mL.

Numerous volatile bacterial compounds can help plants cultivate better and/or be more able to adapt to environmental stresses [39]. Hydrogen cyanide (HCN) producing bacteria can help plants in their defense against fungal pathogens [42]. HCN production was estimated by nutrient media having glycine. A total of twenty-one isolates produced hydrogen cyanide, as evidenced by the filter paper's color changing from yellow to light orange and reddish brown. (Figure 4 f). Similarly, Verma et al., (2020) observed hydrogen cyanide production by 5 rhizobacterial strains out of 10 rhizobacterial strains [40].

Rhizospheric bacteria that solubilize zinc are essential in mobilizing insoluble zinc from the soil, thereby increasing the availability of zinc that is accessible to plants [41].

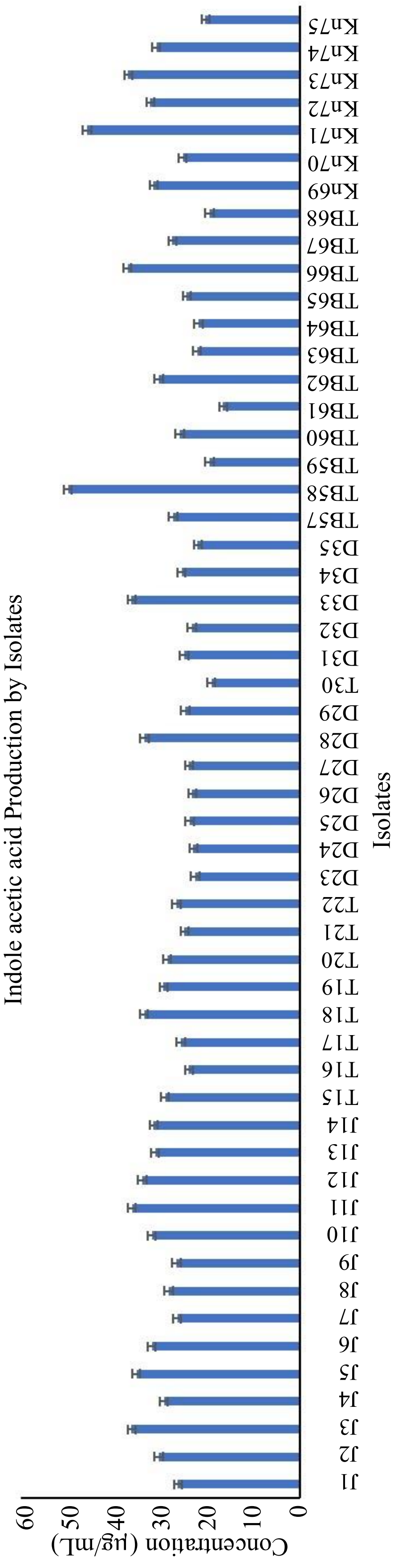

Figure 1. Quantitative assessment of isolates producing Indole acetic acid (IAA). The error bars represent Standard deviation.

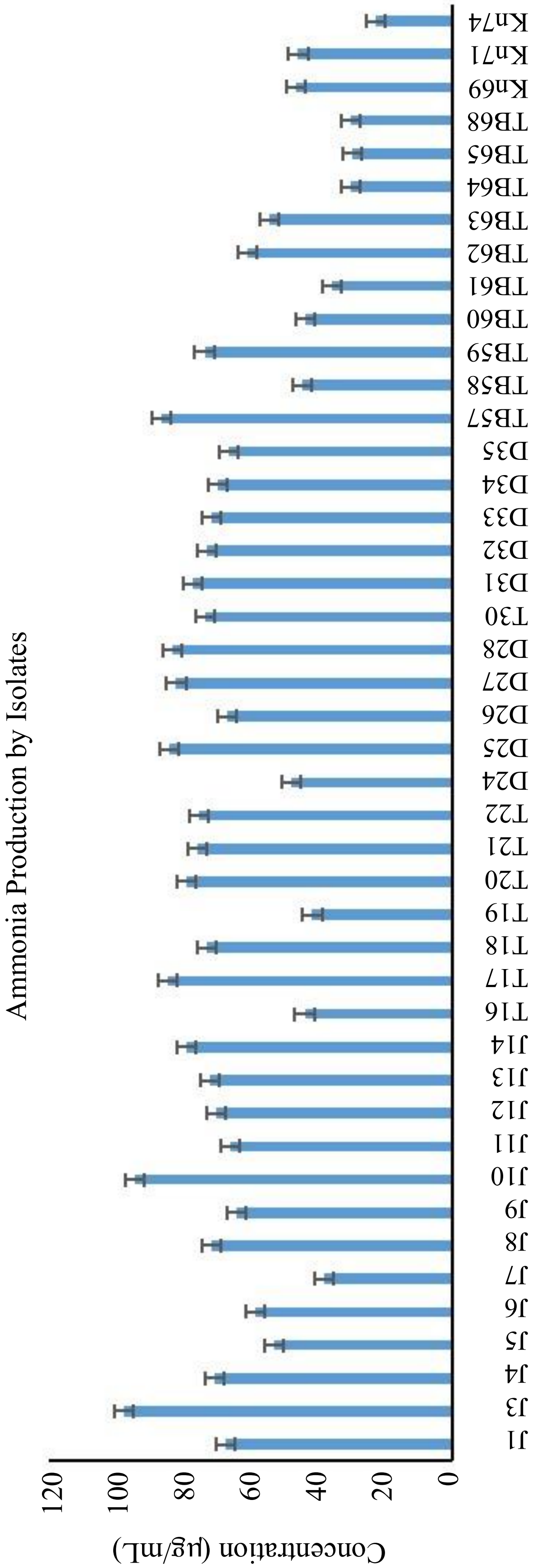

Figure 2. Quantitative assessment of isolates producing Ammonia. The error bars represent standard deviation.

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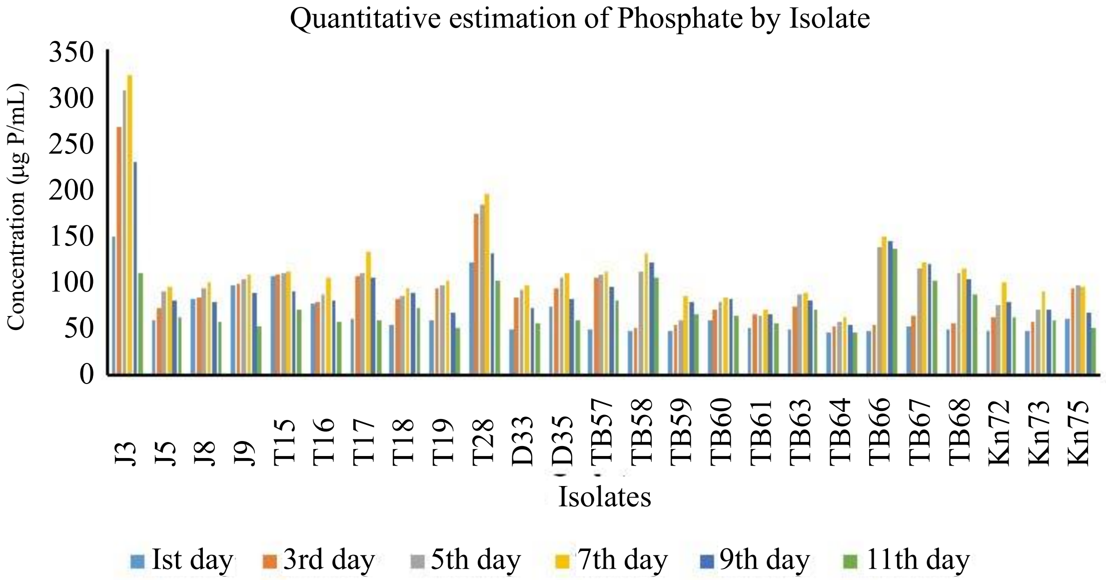

Figure 3. Quantitative assessment of isolates exhibiting phosphate solubilization ability.

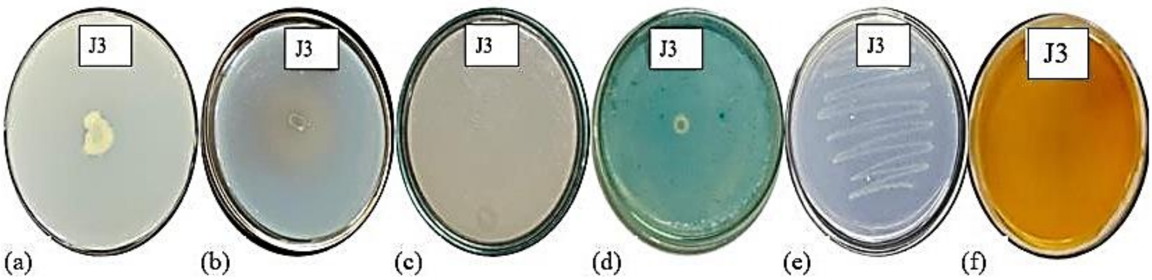

Figure 4. (a) Phosphate solubilization; (b) Potassium solubilization; (c) Zinc solubilization; (d) Siderophore production; (e) Growth in Jensen media indicating Nitrogen-fixing ability; (f) Hydrogen cyanide production

The diameter of the halo zone that formed on a growth medium treated with zinc oxide compound was used to calculate the area of solubilization. Twenty isolates displayed zone of solubilization for zinc in different range (Figure 4 c). The maximum solubilization index was observed by T19 that was about 4.16, whereas the least solubilization index was identified by TB68 about 2.07. Mumtaz et al., (2017) reported solubilization index for zinc was within the limits of 0.72 to 2.2. The highest solubilization index were displayed by isolate ZM20 (*Bacillus* sp.) and S10 (*Bacillus aryabhattai*) [42].

Potassium is one of the necessary mineral for the growth and development of plants. The majority of potassium in soil is found in a variety of insoluble minerals, rocks, and sedimentary materials. Figure 4 b shows the measurement of the greatest potassium solubilization ratio was 1.83 for TB58 and TB64 on Aleksandrow agar supplemented with bromothymol blue. Bopin et al., (2023) reported the highest potassium solubilization index by isolate KSA16 was about 1.162 which was identified as *Streptomyces atacamensis* [43]. Zhang et al., (2014) reported the solubilizing zones of different isolates from 0.65 cm to 1.50 cm while the results illustrate the solubilizing zones of isolates in the range of 0.5 to 2.3 cm in our study [44].

The usage of siderophores in agriculture has drawn a lot of interest to siderophore production in recent decades. Each of the fifty-four bacterial isolates recovered from the fenugreek rhizosphere was examined for the formation of siderophores on CAS agar plates. Figure 4 d shows the development of an orange-colored halo zone surrounding the bacterial colonies indicating the production of siderophores. Further, twenty-five bacterial isolates produced siderophores based on the extent of the halo formation on CAS agar. Twenty-five bacterial isolates generated siderophore units in the range of 6.66% – 70% on quantitative estimation of siderophore by changing green to yellow color of the solution (Figure 5b). In similar studies, Sarwar et al., 2020 reported siderophore production by MGS14 isolate (73%) [45]. The maximal siderophore production seen in J6 (70%). Moreover, the least siderophore producers were Kh71 and Kh72 about 6.66%.

Gibberellin increases the capacity for embryonic development, enhances fruit development, stimulates seed germination, and causes root and stem elongation [46]. Only fifteen isolates showed gibberellic acid synthesis by changing the coloration of the solution to bluish green (Figure 5c) in the range of 15.1 µg/mL to 86.2 µg/mL (Figure 6). The isolate Kh69 produced the least gibberellic acid recorded about 15.1 µg/mL, while J1 estimated the maximum production at about 86.2 µg/mL. Ashry et al., (2022) reported the highest amount of GA3 was about 49.95 µg/mL by isolate DS9, which was 42.05 % lower than the gibberellic acid produced by our isolate [47].

Furthermore, ZnO NPs were produced using the sol-gel method. The change in coloration of colorless solution to white-colored sol indicated the formation of nanomaterials which were characterized using UV visible spectrophotometer. Figure 7 a shows the absorption maxima of the synthesized nanoparticles was obtained at 367 nm which is characteristic for zinc oxide nanoparticles [48]. A transmission electron microscope (TEM) was used to characterize synthesized nanoparticles. The micrograph generated by TEM displayed that the average diameter of spherical nanoparticles was 51–57 nm.

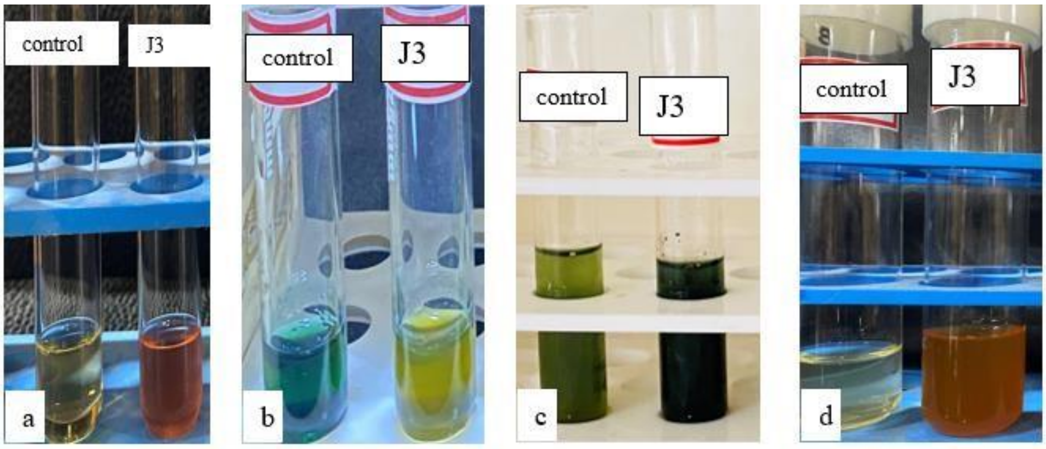

Figure 5. (a) Quantification of IAA production; (b) Quantification of siderophore production; (c) Quantification of gibberellic acid production; (d) Quantification of ammonia production.

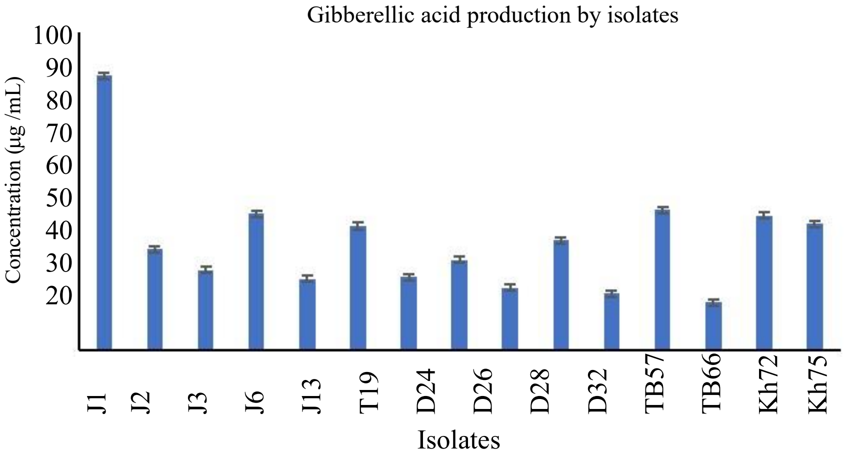

Figure 6. Quantitative estimation of Gibberellic acid. The error bars represent Standard deviation.

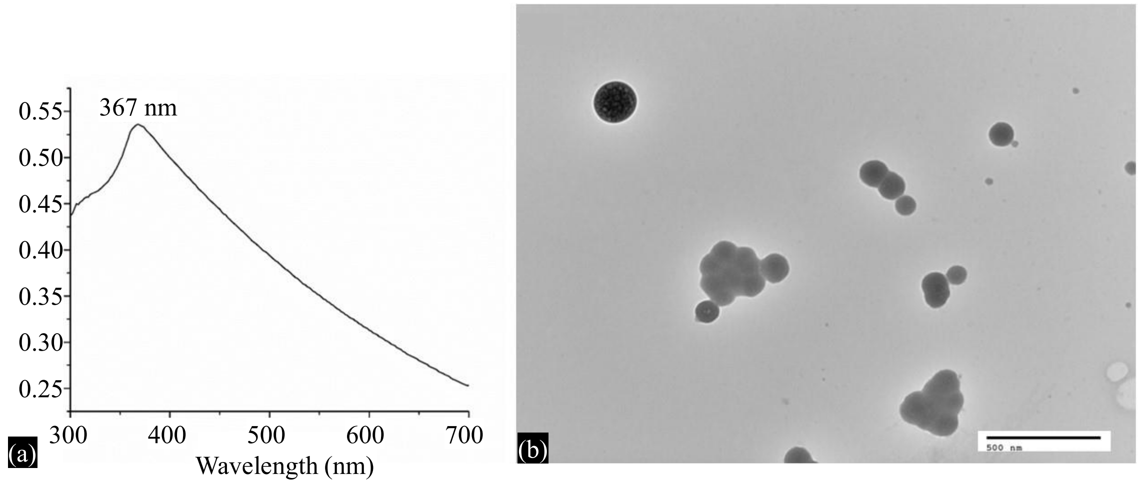

Figure 7. (a) UV–visible absorption spectrum of the ZnO nanoparticles (b) TEM micrograph of zinc oxide synthesized nanoparticles.

The emergence and growth of fenugreek seeds was substantially improved by seed bio-priming using bacterial culture and nanoparticles. The treatments of seeds with potent rhizobacteria and ZnO-NPs cumulatively and individually showed the variation in seed germination and vigor index parameters. In comparison to control and individual treatments of ZnO-NPs and J3 potent bacterial culture, seeds combinedly treated with ZnO-NPs and potent bacterial culture J3 showed notable increase in germination (%) and vigor index. ZnO-NPs and J3 potent bacterial culture significantly increased the germination and vigor index of the seeds by around 72% and 88.4%, respectively, in comparison to the control group (Figure 8).

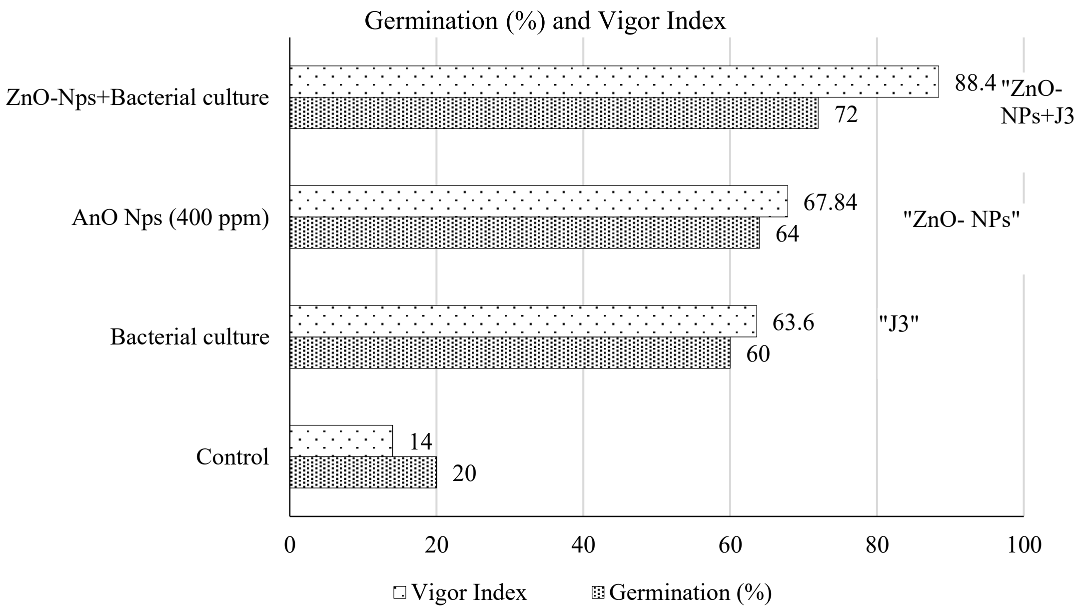

Figure 8. Germination (%) and Vigor Index of Fenugreek seeds treated with potent rhizobacteria (J3) and ZnO-NPs.

CONCLUSION

In the present study, PGPR from fenugreek crops were isolated and screened. Each isolated colony was examined for characteristics that would encourage plant growth. Thirty-five isolates were able to produce nitrogen, twenty-five isolates were able to solubilize phosphate, thirty-three isolates were able to solubilize potassium, twenty isolates were able to solubilize zinc, twenty-one isolates were able to produce hydrogen cyanide, fifteen isolates were able to produce the phytohormone gibberellic acid, and twenty-five isolates were able to produce siderophore. Further, all fifty-four were all capable of producing indole acetic acid (Table 1). However, only one isolate J3 out of fifty-four isolates showed positive results for all the plant growth promoting traits and was selected for further experiments.

Table 1. Screening of isolates exhibiting plant growth promoting traits.

Isolate	IAA	Ammonia production	PSB	KSB	ZSB	HCN	N_2 fixation activity	Siderophore production	Gibberellic acid production
J1	+	+	-	-	-	-	-	+	+
J2	+	-	-	-	-	-	+	+	+
J3	+	+	+	+	+	+	+	+	+
J4	+	+	-	-	+	-	+	-	-
J5	+	+	+	+	-	-	+	+	+
J6	+	+	-	-	-	-	-	+	+
J7	+	+	-	-	+	-	+	-	+
J8	+	+	+	+	-	+	-	-	+
J9	+	+	+	+	-	-	-	+	+
J10	+	+	-	-	-	+	+	-	-
J11	+	-	-	-	-	+	+	-	+
J12	+	+	-	-	-	-	-	+	+
J13	+	+	-	-	-	-	+	-	+
J14	+	+	-	-	-	+	-	-	-
T15	+	-	+	+	-	-	-	+	+
T16	+	+	+	+	+	-	+	+	+
T17	+	+	+	+	-	-	-	-	+
T18	+	+	+	+	-	-	+	+	-
T19	+	+	+	+	+	-	+	+	+
T20	+	+	-	+	-	-	+	-	+
T21	+	+	-	+	+	-	+	-	-
T22	+	+	-	+	-	-	-	-	+
T23	+	+	-	+	-	-	+	-	-
D24	+	+	-	+	-	+	-	-	+
D25	+	+	-	+	-	-	+	-	-
D26	+	+	-	+	+	-	+	-	+
D27	+	+	-	+	-	-	+	-	-
D28	+	+	+	+	-	+	+	+	+
D29	+	+	-	+	-	+	+	-	-
T30	+	+	-	+	+	-	+	-	-
D31	+	+	-	+	-	-	+	-	-
D32	+	+	-	+	-	+	-	-	+
D33	+	+	+	+	+	+	-	-	-
D34	+	+	+	+	-	+	-	-	-
D35	+	+	+	+	-	+	+	-	-
TB57	+	+	+	-	+	-	+	+	+
TB58	+	+	+	+	-	-	+	-	-
TB59	+	+	+	+	-	+	+	+	-
TB60	+	-	+	-	+	+	+	-	-
TB61	+	+	+	+	-	+	-	+	-

TB62	+	+	-	-	+	+	+	-	-
TB63	+	+	+	+	+	-	-	+	-
TB64	+	+	+	-	+	-	+	+	-
TB65	+	+	-	+	-	-	+	+	-
TB66	+	-	+	+	+	+	+	+	+
TB67	+	-	+	-	-	-	+	+	-
TB68	+	+	+	-	+	-	+	-	-
Kh69	+	+	-	+	+	+	+	+	+
Kh70	+	-	-	-	-	-	-	+	-
Kh71	+	+	-	-	+	+	-	+	-
Kh72	+	+	+	-	-	-	-	+	+
Kh73	+	-	+	+	-	+	-	+	-
Kh74	+	-	-	-	+	-	+	+	-
Kh75	+	+	+	-	+	-	+	-	+

For synthesizing zinc oxide nanoparticles, the sol-gel method was employed. ZnO NPs (400 ppm) with potent rhizobacterial culture (J3) were applied to seeds resulting in the enhancement of germination and an increased seedling vigor index. The synergistic effect ZnO and J3 PGPR was observed, which has an impact on biological processes involved in growth and development of the fenugreek seeds. Furthermore, this potent isolate will be analyzed for molecular identification and the co-application of J3 isolate and ZnO NPs will be studied on plant growth and development by evaluating various physicochemical parameter in pot experiments.

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