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Synergistic Interaction Study of *Sinorhizobium meliloti* and *Pseudomonas aeruginosa* on Medicinal Legume *Trigonella foenum-graecum*

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Abstract—*The present study dealt with the synergistic effect of Sinorhizobium meliloti TN2 and Pseudomonas aeruginosa TO3 on the medicinally important Trigonella foenum-graecum plants. Bacteria were identified by biochemical experimentations as per Bergey's manual and 16S rDNA gene sequencing. The experiments were conducted to check the effect of selected bacterial isolates on the growth of Trigonella plants in in-vitro andIn-vivo conditions. The seeds were treated with Sinorhizobium meliloti TN2 as mono-inoculant and co-inoculant with Pseudomonas aeruginosa TO3. It was observed that Sinorhizobium meliloti TN2 – Pseudomonas aeruginosa co-inoculation in comparison to mono-inoculation of Sinorhizobium meliloti TN2 and Pseudomonas aeruginosa TO3 inoculation increased the nodulation, growth parameters and productivity in In-vitro andIn-vivo conditions. The interaction studies helped to understand the relationship among Sinorhizobium, Plant Growth Promoting Rhizobacteria (PGPR) and the plant host. More than this, co-inoculation with Sinorhizobium meliloti and Pseudomonas aeruginosa could be an effective biofertilization option for crop improvement along with productivity of medicinally important Trigonella foenum-graecum. The bioformulation formed from co-inoculation of Sinorhizobium meliloti and Pseudomonas aeruginosa proved to be the better option over chemical fertilizers to obtain higher productivity.*

INTRODUCTION

Soil constitutes a multifaceted, fundamental, irreplaceable, and dynamic biological system, harboring colonies of microbes in close proximity to plant roots. Microorganisms within the soil perform vital functions in nutrient cycles, sustaining soil fertility (Johns C., 2017). The term 'rhizobacteria,' introduced by Kloepper and Schroth (1978), characterizes soil bacteria that competitively inhabit plant roots, stimulating growth and reducing the occurrence of plant diseases. These beneficial microorganisms, known as Plant Growth Promoting Rhizobacteria (PGPR), successfully establish themselves in soil ecosystems due to their adaptability to diverse environments and rapid growth.

Numerous studies have highlighted the interactions between soil-borne microbes and plant roots, emphasizing the significance of these relationships in either enhancing or inhibiting the individual species' beneficial effects (Kannan et al., 2011). Scientific evidence demonstrates that PGPR present in legume root nodules is responsible for atmospheric nitrogen fixation (Bhat et al., 2023). Legumes, acting as essential colonizers in low-N environments, contribute significantly to biological nitrogen fixation in natural ecosystems, agriculture, and agro-forestry. Rhizobia, a well-known group of bacteria, serves as primary symbiotic nitrogen fixers, forming N_2-fixing nodules in collaboration with leguminous plants (Shimoda et al., 2020; Jaiswal et al., 2021).

Additionally, *Pseudomonas*, an important PGPR, exhibits advantages such as rapid multiplication and higher rhizosphere competence, making it effective in the biological control of various plant pathogens and suppressing plant diseases (Khare and Arora, 2011). *Pseudomonas sp.* influences plant growth directly through phytohormone production and indirectly through N2 fixation, producing biocontrol metabolites against soil-borne phytopathogens (Khare and Arora, 2010).

Co-inoculation of PGPR (*Sinorhizobium* and *Pseudomonas*) at optimal doses enhances nodule number, plant dry weight, and nitrogen fixation efficiency (Farssi et al., 2021). Therefore, exploring the interaction between the biological agent *P. aeruginosa* and the phyto-stimulator *Sinorhizobium* is crucial, especially in the context of legumes, for enhancing crop production. The use of mixed biocontrol agents offers adaptability to environmental changes, broad-spectrum pathogen protection, increased genetic diversity, and the utilization of diverse biocontrol mechanisms (Pierson & Weller, 1994; Duffy & Weller, 1995). This approach enhances the efficacy and reliability of control without resorting to genetic engineering (Janisiewicz, 1988).

The current study focuses on isolating rhizobia (from root nodules) and *Pseudomonas* (from the rhizospheric region) from the medicinally important leguminous plant *Trigonella foenum-graecum*. Additionally, rhizobial isolates undergo scrutiny for *in-vitro* interactions with fungal phytopathogens.

MATERIALS AND METHODS

Rhizobial isolates were obtained from the root nodules of various wild but medicinally significant leguminous plants thriving in the Kanpur and adjoining regions (26.46°N, 80.33°E), characterized by an annual rainfall ranging from 70 to 100cm and temperatures spanning from 3°C to 45°C. The selection and identification of isolates followed protocols outlined in Bergey's Manual of Systematic Bacteriology (Garrity et al., 2005).

Two rhizobial isolates, TN2 and TN5, along with *Pseudomonas* sp. TO3, were chosen based on their plant growth-promoting (PGP) qualities and biocontrol potential. Identification of these isolates relied on the analysis of 1.5kb 16S rRNA sequences. Similarities in the 16S gene sequences were determined using BLAST (Altschul et al., 1990) and the Ribosomal Database Project (RDP) (Khare and Arora, 2010).

Phytopathogenic strains of *Macrophomina phaseolina* ARIFCC257 and Fusarium solani ARIFCC852 were acquired from the Mycology and Plant Pathology Group, Division of Plant Sciences, Agharkar Research Institute, Pune. These strains were cultured and maintained on Potato Dextrose Agar (PDA) (Hi Media, Mumbai) at temperatures of 28°C and 4°C, respectively

IN-VITRO INTERACTION STUDY

Interaction with common fungal phytopathogens

The rhizobial isolates and two different phytopathogens *M. phaseolina* ARIFCC257, *F. solani* ARIFCC852 were interacted *in-vitro* in plate by dual culture method (Kumar et al., 2005). These plates were incubated at 28°C for 5 days to measure the inhibition of radial fungal growth as a clear zone between bacterial colonies and fungal isolates.

INTERACTION WITH FLUORESCENT *PSEUDOMONAS*

Rhizobial isolates selected on the basis of PGP characteristics were checked for their interaction with selected *Pseudomonas aeruginosa* TO3. An amoxycilin-resistant strain of TO3 was isolated on King's B medium containing 100mgl^{-1} of amoxycilin (*P. aeruginosa* TO3^{amoxy+}). TO3^{amoxy+} was engineered for ciphalexin resistance (100mgl^{-1}) (designated TO3 $^{amoxy+ciphalexin+}$) as described by Kumar et al. (2003). Similarly, nalidixic acid resistant rhizobial isolate TN2 was isolated on YEMA medium containing 100mgl^{-1} of nalidixic acid (*S. meliloti* TN2$^{nalidixic\ acid+}$). TN2$^{nalidixic\ acid+}$ was developed for ciproflexin resistance (100mgl-1) was denoted as TN2nalidixic acid+ ciproflexin+ according to Kumar et al., (2003). Antibiotic resistant marker strain of another selected rhizobial isolate S. meliloti TN5 was also developed and denoted as TN5nalidixic acid+ ciproflexin+. YEMA media containing amoxycilin and ciphalexin (100mgl-1 each) was used to enumerate the bacterial population. The in-vitro interaction studies of rhizobial isolates TN2 and TN5 with Pseudomonas aeruginosa TO3 as mono-inoculants as well as co-inoculants were conducted by Colony forming units (Cfu) counting method and Spectrophotometric method.

CFU COUNTING METHOD

Selected rhizobial isolates and *P. aeruginosa* TO3 were cultured on YEM agar and KB agar plates respectively at 28°C for 24h. Cell suspension of rhizobial isolates and strain TO3 was prepared by scraping colonies from agar plates and mixing in phosphate buffer. The OD-610 (optical density at 610nm) of cell suspensions was adjusted to 0.1. These cell suspensions (10µl) were inoculated in YEM broth (5ml) as mono-inoculants as well as co-inoculants as (a) only rhizobial isolate, (b) only *P. aeruginosa* TO3, and (c) rhizobial isolate + *P. aeruginosa* TO3 in equal amount and incubated for 48h. Then dilution of each inoculant was done up to 10^8 and 100µl of each.

SPECTROPHOTOMETRIC METHOD

For the spectrophotometric method of interaction study 100µl cell suspension of *S. meliloti* TN2 and cell free culture supernatant of *P. aeruginosa* TO3 (100µl) was added in 50ml YEM broth. Cell suspension of rhizobial isolate TN2 (100µl) inoculated in YEM broth was taken as control. Similar sets were also maintained for another selected rhizobial isolate *S. meliloti* TN5 for interaction study. The flasks were shaken continuously in an orbital shaker for 48h at 28°C. The OD at 610nm of rhizobial isolate (set A and B) was assessed by spectrophotometer (GENESYSTM 6, Model, 335908-02) at an interval of 2 h up to 48h. The experiments were done in triplicate.

IN-VIVO INTERACTION STUDY WITH *P. AERUGINOSA* TO3

Selected rhizobial isolates *S. meliloti* TN2 and *S. meliloti* TN5 based on *in-vitro* interaction studies were further taken for *in-vivo* studies. It caused nodulation on the host plant (*T. foenum-graecum*) and was taken for further pot and field study.

Pot Study

The effect of pseudomonal strain *P. aeruginosa* TO3 on plant growth, nodule number and nodule fresh weight as mono-inoculation as well as co-inoculation with *S. meliloti* TN2 was examined on fenugreek or methi (*T. foenum-graecum*) in pots. Pre-sterilized plastic pots (20*15) (capacity to hold 2kg of soil) were filled with garden soil and sterilized two times for 20min at 120°C with a 24h interval. Sterilized soil without any inoculum was taken as control. Seed bacterization was done according to Arora et al., (2001). Cells of *S. meliloti* TN2, *P. aeruginosa* TO3 and combination of *S. meliloti* TN2 and *P. aeruginosa* TO3 were grown under continuous shaking conditions (at 150 rpm) on yeast extract mannitol broth at 28˚C for 48h. Cultures were centrifuged separately at 7000rpm

for 15min at 4˚C. The culture supernatant was discarded and pellets were collected and washed with sterile distilled water and resuspended in SDW (sterile distal water) to obtain a population density of 10^8Cfu/ml. Cell suspension was mixed with 1%CMC solution and seeds were coated with the slurry and allowed to air dry in aseptic conditions. The seeds coated with 1%CMC slurry without any bacterial strain served as control. The treatment combinations were: (1) Uninoculated control; (2) *S. meliloti* TN2 mono-inoculation; (3) *S. meliloti* TN5 mono-inoculation; (4) *P. aeruginosa* TO3 mono-inoculation; (5) *S. meliloti* TN2 + *P. aeruginosa* TO3; (6) *S. meliloti* TN5 + *P. aeruginosa* TO3 co-inoculated. Seedlings were provided with broth cultures of *S. meliloti* TN2, *S. meliloti* TN5 *P. aeruginosa* TO3 and combination of *S. meliloti* TN2 + *P. aeruginosa* TO3 and *S. meliloti* TN5 + *P. aeruginosa* TO3 to their respective pots for 2-4times/15 days after sowing as booster dose and no artificial fertilization were used. The water was provided to the plants after the same interval in equal amounts. The plants were uprooted carefully 60 days after sowing (DAS) and studied for their increased nodulation, vigor index, and enhanced plant biomass. Fresh weight was determined and dry weight was calculated by drying plants in an oven at 75°C until the weight became constant.

Field Study

Field experiments were conducted under irrigated conditions with three replications in a randomized block design at the Medicinal Garden, CSJM University, Kanpur (20°38'E and 80°21'N; temperature maximum 48°C and minimum 3°C), Uttar Pradesh (northern India) Kanpur. *Trigonella foenum-graecum* (methi) seeds were surface sterilized with 0.2% $HgCl_2$ for nodulation and interaction studies in field. The treatments were provided in following manner (1) Untreated control, (2) *S. meliloti* TN2 mono-inoculation, (3) *S. meliloti* TN5 mono-inoculation, (4) *P. aeruginosa* TO3 mono-inoculation, (5) *S. meliloti* TN2 + *P. aeruginosa* TO3 co-inoculated, and (6) *S. meliloti* TN5 + *P. aeruginosa* TO3 co-inoculated. Seeds were treated with *S. meliloti* TN2, *S. meliloti* TN5 and *P. aeruginosa* TO3 as mono-inoculants as well as co-inoculants. Uncoated seeds were served as control and sown just as they were procured after washing. No chemical fertilizer was applied to the field. Root nodulation, plant growth (shoot and root length), vigor index, seedling biomass and total yield were assessed. Ten plants were selected randomly from each plot 90 DAS.

RESULTS

All the rhizobial strains (isolated from different medicinal and wild leguminous plants), were Gram-negative, non-spore-forming rods (bacilli) or oval (coccobacilli) but some formed white-colored, semi-translucent, rounded, mucoid colonies with 2-6mm diameter after 48h of incubation on YEMA were fast growers with an average mean generation time of 2.9-4.9, while formed white, rounded, opaque, convex, and granular colonies with diameter less than 2mm after 5-7days of incubation on YEMA were slow growers with an average generation time of 7.8 to 8.0h. All rhizobial isolates showed a negative response to gelatin and starch hydrolysis and a positive to nitrate reductase. Most of the rhizobial isolates did not show growth in GPA. All isolates were able to grow on HAB, 8% KNO_3 and able to tolerate 2% NaCl. None isolate was able to utilize citrate and all were negative for starch hydrolysis. The majority of isolates were able to utilize hexoses (glucose, galactose, mannitol, dextrose, and sorbitol), pentoses (arabinose, xylose), trioses (glycerol), disaccharides (sucrose, lactose, maltose and trehalose) and polysaccharide (starch). The majority of the isolates were able to utilize different amino acids except glycine and methionine. Fast growers were not able to utilize valine, and threonine also while slow growers did not utilize cystine. Based on complete 16SrRNA gene sequence analysis (1478bp), selected isolates TN2 and TN5 were identified as *Sinorhizobium meliloti*. Rhizobial isolates TN2 and TN5 showed maximum similarity to *S. meliloti* strain DASA12028 'AF417566'.

In culture tubes as well as in pot study rhizobial isolates TN2 and TN5 (from *Trigonella*) showed nodulation in methi plants. Most of the rhizobial isolates were positive to acetylene reduction assay. Rhizobial isolate TN2 was able to fix nitrogen up to $8.953*10^{-9}$ (mol C_2H_2/Cfu/hour) in microaerophilic conditions and $4.157*10^{-9}$(mol C_2H_2/Cfu/hour) under aerobic conditions. Nitrogen up to $8.226*10^{-9}$(mol C_2H_2/Cfu/hour) in microaerophilic conditions and $3.195*10^{-9}$(mol C_2H_2/Cfu/hour) under aerobic conditions was fixed by rhizobial isolate. TN5, while isolate CT3 fixed only $7.018*10^{-9}$(mol C_2H_2/Cfu/hour) in microaerophilic conditions and $2.972*10^{-9}$(mol C_2H_2/Cfu/hour) under aerobic conditions. Rhizobial isolates TN2 and TN5 showed maximum solubilization of inorganic phosphate on Pikovskya's agar showed the formation of a clear halo around their spot inoculation as well as the production of siderophore.

***IN-VITRO* INTERACTION STUDY**

Rhizobial isolate TN2 showed maximum antagonism against both fungal pathogens as proved by inhibition of the radial growth of the fungus on YEM agar plates (Figure: 1(a) and 1(b)). TN2 showed 77.78% inhibition of *M. phaseolina*, in comparison to control. On the other hand, maximum inhibition of *F. solani* was 75.55% (by the rhizobial isolate TN2). Rhizobial isolate TN5 showed 73.33% inhibition against *M. phaseolina* and 62.22% inhibition against *F. solani*. Rhizobial isolate TN2 was found more effective among all strains against all fungal phytopathogens.

In the interaction study by CFU method, on co-inoculation, the growth of *P. aeruginosa* TO3 was enhanced in the presence of both rhizobial isolates *S. meliloti* TN2 and *S. meliloti* TN5. The best result for growth enhancement of both rhizobial isolate (18.88%) and *P. aeruginosa* TO3 (14.21%) in co-inoculant was observed when *S. meliloti* TN2 was taken in the study (Table: 1). Similar results were found in *in-vitro* interaction study by spectrophotometric method. There was an enhancement in the growth rate of rhizobial isolates (TN2 and

TN5) in co-inoculant during the initial 12h over mono-inoculant (Figure: 2).

Table 1. Colony count and log CFU/ml of different rhizobial isolates on mono-inoculation as well as co-inoculation

S/N	Inoculants	Isolates	log CFU/ml	% enhancement in CFU over respective mono-inoculants
1.	Mono-inoculant	TO3	7.25±0.02	-
2.	Mono-inoculant	CT3	7.40±0.02	-
	Co-inoculant	CT3	8.43±0.04	13.92
		TO3	8.17±0.02	12.69
3.	Mono-inoculant	TN2	7.31±0.03	-
	Co-inoculant	TN2	8.69±0.02	18.88
		TO3	8.28±0.02	14.21
4.	Mono-inoculant	TN5	7.28±0.03	-
	Co-inoculant	TN5	8.26±0.02	13.46
		TO3	8.12±0.02	12.00

Values of CFU are the mean of 03 replicates ± SD

Figure 1(a): Dual culture showing interaction of rhizobial isolates (*S. meliloti* TN2 and TN5) with *M. phaseolina*

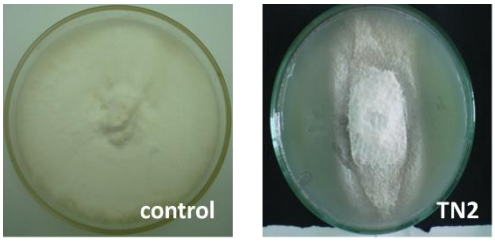
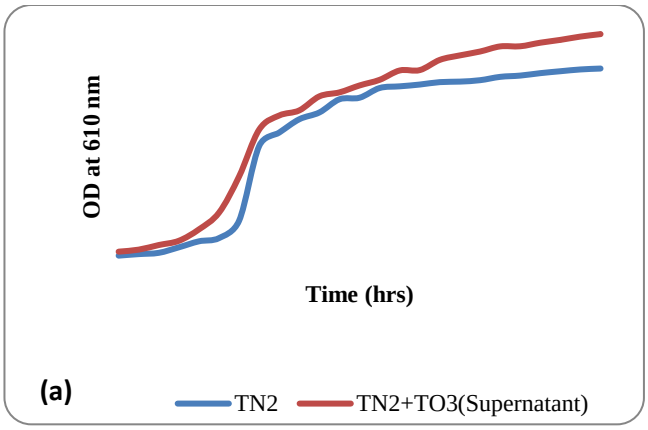


Figure 1(b): Dual culture showing the interaction of rhizobial isolate *S. meliloti* TN2 with *F. solani*



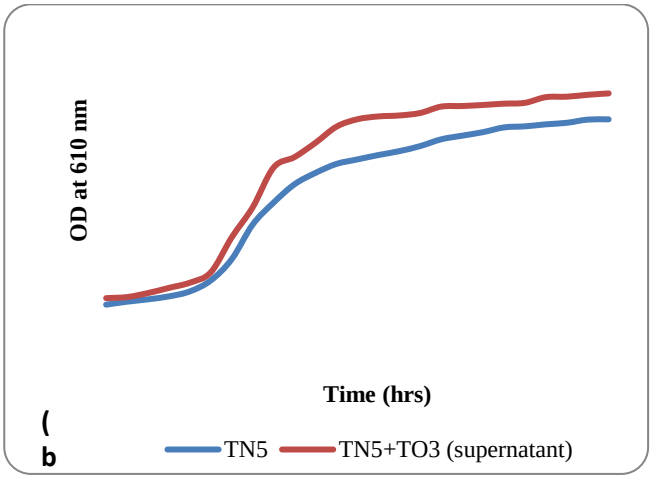


Figure 2: Spectrophotometric method: Effect of cell-free culture supernatant of *P. aeruginosa* TO3 on growth of rhizobial isolates (a) *S. meliloti* TN2 and (b) *S. meliloti* TN5

In-vivo interaction study

Pot Study

In-vivo studies of selected rhizobial isolates TN2 and TN5 with *Pseudomonas* as mono-inoculants and co-inoculants were performed on methi (*Trigonella foenum graecum*). In methi, dry weight was enhanced by 56.41% on co-inoculation in comparison to control while mono-inoculation of *S. meliloti* TN2 and *P. aeruginosa* TO3 resulted in 25.64% and 20.51% enhancement respectively, in comparison to control. Nodulation was also enhanced by co-inoculation of TN2 and TO3 in comparison to mono-inoculation. There was an 86.67% increase in nodulation due to co-inoculation in comparison to control. On the other hand, nodulation was enhanced by co-inoculation of TN5 with TO3 resulting in 54.43 % in comparison to mono-inoculation of TN5 over control. There was a 48.72% enhancement in dry weight on co-inoculation than that of mono-inoculation of TN5 (23.08%) and TO3 (20.51%) over control (Table: 2).

Table 2. Effect on the growth of methi on mono-inoculation and co-inoculation of *S. meliloti* TN2, *S. meliloti* TN5, and *P. aeruginosa* TO3 in pots

***In-vivo* interaction and nodulation study (in pots)**									
Crop	**Treatment**	**Germination %**	**Shoot length**	**Root length**	**No. of pods/ plant**	**No. of seeds per pod**	**No. of nodules/ plant**	**Plant fresh weight (gm)**	**Plant dry weight (gm)**
Methi	Control	83.33±0.04^{a}	12.9±0.02^{a}	5.7±0.02^{a}	1±0.03^{a}	4±0.03^{a}	-	0.86±0.02^{a}	0.39±0.03^{a}
	TO3	86.67±0.03^{b}	17.8±0.05^{b}	6.5±0.03^{b}	1±0.02^{a}	5±0.02^{b}	-	1.09±0.04^{b}	0.47±0.02^{b}
	TN2	88.57±0.02^{c}	19.7±0.02^{c}	6.9±0.02^{b}	2±0.05^{b}	7±0.05^{d}	9.0±0.02^{a}	1.13±0.03^{c}	0.49±0.02^{b}
	TN5	88.57±0.02^{c}	17.6±0.02^{b}	6.5±0.03^{b}	1±0.03^{a}	6±0.02^{c}	7.9±0.05^{a}	1.07±0.02^{b}	0.48±0.04^{ab}
	TN2+TO3	93.33±0.05^{d}	21.5±0.04^{d}	8.0±0.03^{c}	3±0.02^{c}	9±0.02^{e}	16.8±0.05^{c}	1.26±0.05^{d}	0.61±0.05^{c}
	TN5+TO3	92.10±0.02^{d}	20.1±0.04^{d}	7.4±0.02^{c}	2±0.05^{b}	7±0.03^{d}	12.2±0.04^{b}	1.19±0.02^{c}	0.58±0.02^{c}

Results are the mean of 03 replicates ±SD. Means in the column followed by the same superscript letters indicate no significant difference (P = 0.05) by Duncan's multiple range test.

1. Field Study

The maximum enhancement in plant growth parameters in the field study was also caused by co-inoculants, followed by mono-inoculants of rhizobial isolates (TN2 and TN5) and *Pseudomonas aeruginosa* TO3. Dual inoculation of *S. meliloti* TN2 + *P. aeruginosa* TO3 produced more nodule number, more nodule fresh weight, and more nodule dry weight in comparison to *S. meliloti* TN2 alone inoculation. Similarly, co-inoculation of *S. meliloti* TN5 + *P. aeruginosa* TO3 enhanced the plant biomass, nodule number, and plant vigor than that of mono-inoculation (Table: 4 and Figure: 3).

In methi (*T. foenum-graecum*), there was a 128.46% enhancement in nodule number per plant on co-inoculation in comparison to mono-inoculation of TN2(38.72%), and TO3(23.78%) concerning control. Maximum enhancement in dry weight (49.26 %) was caused by co-inoculants in comparison to mono-inoculants of *S. meliloti* TN2 (37.46 %) and *P. aeruginosa* TO3 (25.22 %) over uninoculated control. The vigor index increased by 57.91% on combined inoculation than mono-inoculation of *S. meliloti* TN2 (23.19 %) and *P. aeruginosa* TO3 (11.85%). Maximum percentage yield was gained on co-inoculation (25.84%) than mono-inoculation of *S. meliloti* TN2 (15.59%) and *P. aeruginosa* TO3 (9.88%) over control.

In the case of S. meliloti TN5 also maximum enhancement in dry weight was caused by co-inoculants (5.72%), followed by mono-inoculants of S. meliloti TN5 (32.01%) and then P. aeruginosa TO3 (25.22%) over control. Co-inoculant caused 104.04% enhancement in nodulation in comparison to mono-inoculation of S. meliloti TN5 (32.76%) and P. aeruginosa TO3 (23.78%) for control. The vigor index showed a 39.39% enhancement in co-inoculation in comparison to mono-inoculants TN5(13.25%) and TO3(11.85%) over control. There was a significant increase in yield by 22.38% than mono-inoculation of S. meliloti TN5(11.73 %) and P. aeruginosa TO3 (9.88%) (Table: 4).

Figure 3: Effect of mono-inoculation and co-inoculation of *S. meliloti* TN2 and *P. aeruginosa* TO3 on growth of *T. foenum-graecum* in field trial (1) control (2) TO3 (3) TN2 (4) TN2 + TO3 (5) TN5 (6) TN5 + TO3

Table 3. Effect of mono-inoculation and co-inoculation of *S. meliloti* TN2 and *P. aeruginosa* TO3 on growth of *T. foenum-graecum* in field trial

Treatment	Germination %	Shoot length / Plant (cm)	Root length / Plant (cm)	No. of pods / Plant	No. of nodule / plant	Nodule fresh wt. / plant (gm)	Nodule dry wt. / plant (gm)	Fresh wt. / Plant (gm)	Dry wt. / Plant (gm)	Total yield (kg/hectare)
Control	89.30±0.05^{a}	45.16±0.02^{a}	12.25±0.05^{a}	14.91±0.03^{a}	15.60±0.03^{a}	1.64±0.04^{a}	0.32±0.02^{a}	20.67±0.04^{a}	6.78±0.05^{a}	1277.54^{a}
TO3	93.33±0.05^{b}	48.32±0.05^{b}	13.12±0.05^{b}	24.08±0.03^{b}	19.31±0.05^{b}	1.81±0.05^{b}	0.61±0.05^{b}	26.81±0.05^{b}	8.49±0.03^{b}	1403.77^{b}
TN2	95.73±0.04^{c}	52.02±0.02^{d}	13.95±0.05^{c}	32.82±0.03^{d}	21.64±0.03^{d}	2.12±0.03^{c}	0.73±0.05^{c}	31.70±0.04^{d}	9.32±0.05^{d}	1476.69^{d}
TN5	93.33±0.04^{b}	49.14±0.04^{c}	13.07±0.04^{b}	28.28±0.04^{c}	20.71±0.04^{c}	1.82±0.03^{b}	0.63±0.03^{b}	29.52±0.04^{c}	8.95±0.03^{c}	1427.37^{c}
TN2+TO3	99.43±0.02^{d}	63.25±0.04^{f}	18.17±0.05^{e}	50.09±0.03^{f}	35.64±0.03^{f}	2.49±0.05^{e}	0.89±0.03^{e}	42.46±0.03^{f}	10.12±0.02^{f}	1607.62^{f}
TN5+TO3	95.33±0.03^{c}	58.17±0.04^{e}	16.79±0.04^{d}	46.21±0.04^{e}	31.83±0.04^{e}	2.28±0.03^{d}	0.86±0.03^{d}	38.18±0.03^{e}	9.88±0.04^{e}	1563.51^{e}

Results are the mean of 10 replicates ±SD. Means in the column followed by the same superscript letters indicate no significant difference (P=0.05) by Duncan's multiple range test.

DISCUSSION

In this study, root nodulating bacteria were isolated from wild and medicinal legumes, encompassing both fast and slow growers. The isolates exhibited negative responses to citrate utilization, gelatin, and starch hydrolysis, yet were positive for nitrate reductase and HAB. All rhizobial isolates demonstrated tolerance to 8% KNO3 and 2% NaCl, while none could grow on GPA. Specifically, rhizobial isolates (TN2 and TN5) from the medicinally important leguminous plant T. foenum-graecum were identified as *Sinorhizobium meliloti* through morphological, biochemical, physiological characterization (Garrity et al., 2005), and 16S rRNA sequencing.

Effective nodulation was observed on the host plant *T. foenum-graecum* (methi) and *C. arietinum* (chickpea) by rhizobial isolates *S. meliloti* TN2 and *S. meliloti* TN5. Positive responses to acetylene reduction assay, siderophore production, and phosphate solubilization were noted, while HCN production was absent. The production of biologically fixed nitrogen, siderophores, and indole-3-acetic acid (IAA) was consistent with previous reports. Fast-growing rhizobia strains exhibited exopolysaccharide (EPS) production, a crucial signal for host specificity during early root hair infection.

S. meliloti TN2 and *S. meliloti* TN5 displayed significant inhibition of phytopathogens (*M. phaseolina* and *F. solani*), a rare but documented capability among rhizobia. *P. aeruginosa* TO3, isolated from the rhizosphere region of *Trigonella sp.*, exhibited antagonistic properties against various phytopathogens, producing antibiotics, HCN, siderophore, and IAA.

Interaction studies demonstrated that co-inoculation of *Pseudomonas* and rhizobia enhanced the growth of rhizobial isolates, with *P. aeruginosa* TO3 proving effective in *in-vitro* studies. *In-vivo* pot interaction studies further showed enhanced plant growth and yield upon co-inoculation, especially in methi and chickpeas. The co-inoculation of *Rhizobium* and *Pseudomonas* not only increased nodulation but also improved plant growth parameters, emphasizing the potential for sustainable agricultural practices.

Field interaction studies confirmed the positive impact of co-inoculation on plant growth and yield, with the best results observed in methi co-inoculated with *P. aeruginosa* TO3 and *S. meliloti* TN2. The application of pyocyanin-producing pseudomonads alongside rhizobia demonstrated increased biofilm development, contributing to enhanced nodulation ability and sustained growth and productivity.

The study highlighted the symbiotic effectiveness of co-inoculation with suitable non-rhizobial plant growth-promoting bacteria (PGPB) to improve rhizobial inoculants. Specifically, the interaction between *S. meliloti* TN2 and *P. aeruginosa* TO3 emerged as a favorable option for methi growth. This co-inoculation strategy demonstrated synergistic effects, combining the plant growth-promoting properties of *P. aeruginosa* TO3 with the nitrogen-fixing and nodulation capabilities of *S. meliloti* TN2.

The findings underscored the potential for developing bio-formulations through the combined inoculation of *S. meliloti* and *P. aeruginosa* for field applications, contributing to sustainable and enhanced crop production. Studies on the synergistic interaction between biocontrol and plant growth-promoting agents, such as *P. aeruginosa* and *S. meliloti*, are crucial, especially in the context of legumes. Overall, the co-inoculation of *S. meliloti* and *P. aeruginosa* demonstrated superior plant growth-promoting activity, suppression of fungal growth, and potential for bioformulation development.

CONCLUSIONS

In conclusion, our findings suggest that microbial colonization by two distinct bacterial isolates has the potential to enhance the growth of medicinally important *Trigonella sp.* crops under field conditions. The practice of applying a mixture of beneficial micro-organisms to boost crop production is currently in use in agriculture. This study underscores the development and formulation of Plant Growth Promoting Rhizobacteria (PGPRs) for the biological enhancement of various plant growth characteristics. The creation of stable formulations of antagonistic PGPRs in sustainable agricultural systems presents a promising approach, replacing the reliance on chemical fertilizers. Moreover, PGPRs contribute to the protection of natural environments. Nevertheless, cautiously conducted field trials with crop plants inoculated alongside rhizobacteria are essential for the optimal commercial utilization of PGPR strains.

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