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Research Article

Comparative growth analysis of cumin plants (*Cuminum cyminum* L.) treated with glycerol and talc based phosphate solubilizing bacterial consortia

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Abstract
Cumin (*Cuminum cyminum* L.) is a versatile annual herb cultivated in the Middle East, India, China, and Tunisia. The seeds of this plant are primarily used in cooking as common food additives and traditional medicines to treat hypolipidemia, cancer and diabetes. However, cumin plants have poor germination and weak establishment rate, particularly under drought stress. The present study aimed to examine the growth promotion potential of cumin plants treated with talc and glycerol-based phosphate solubilizing bacteria (PSB) consortia. Four efficient PSB were isolated from the rhizosphere of *Calotropis procera* and *Solanum lycopersicum* and identified through 16S rRNA sequencing as *Pseudomonas nitritireducens* MF351819*, Klebsiella pneumoniae* MF351845, *Erwinia* sp. MF351846 and *Pantoea dispersa* MF351847. In a nursery experiment on cumin (*Cuminum cyminum*) plants, 15 treatments of single, dual, triple and quadruple combinations of four PSB isolates were formulated on glycerol and talcum powder-based bacteriological carriers. The 100-day pot experiment was initiated during the winter of 2016 (the last week of October) containing loamy sand soil of Sardarkrushinagar, Gujarat. The formulations were applied to pot soil containing seven-day-old cumin seedlings. With glycerol-based PSB inoculations, the P14 treatment containing a consortium of *K. pneumoniae* MF351845, *Erwinia* sp. MF351846, and *P. dispersa* MF351847 provided the highest per plant seed yield of 0.19 g. With talc-based PSB inoculations, the P11 treatment containing a consortium of *K. pneumoniae* MF351845, *Erwinia* sp. MF351846, and *P. nitritireducens* MF351919 produced the highest per-plant cumin seed yield of 0.42 g. Overall, talc-based PSB consortial treatments improved height, dry weight, 100 seed weight and yield of the cumin plant.

Keywords: Cumin plants, Bacteriological carriers, Glycerol, PSB, Talc

INTRODUCTION

In nutrient-poor and semiarid regions of Gujarat, the growing crops are subjected to several environmental stresses (Zala *et al*., 2014). Such pressures more severely affect slow-growing crops like cumin, making it prone to crop weed competition. Although the Gujarat cumin (*Cuminum cyminum*) GC-4 variety is considered wilt resistant (Olsen, 1982), the physiological effect of drought on the plant cannot be undermined. Drought-induced stress in cumin plants results in higher levels of ethylene production, negatively impacting growth (Pishva *et al*., 2020). Thus a growth supplement in the form of inorganic fertilizers is often provided for the proper development of cumin plants (Patel *et al*., 2004). However, the concomitant increase in chemical fertilizer usage is only inching us nearer to complete soil fertility loss, which also impacts plant growth (Pahalvi *et al*., 2021). Under such circumstances, sustainable soil management strategies using "Plant Growth Promoting-Microbial Consortia" seem a viable solution for promoting cumin plant growth, without losing soil fertility. Such approaches often increase cumin growth, evident in the form of improved dry weight, chlorophyll content, fruit yield, etc. (Faravani *et al*., 2012; Jamir *et al*., 2021).

However, the rhizosphere-introduced Plant growth promoting rhizobacteria (PGPR) microflora often fails to survive due to the varied environmental conditions (Dutta and Podile, 2010; Yadav and Yadav, 2018). Nevertheless, when applied as consortia in the cumin plant rhizosphere, the PGPR successfully colonizes this region which becomes evident in the form of improved growth (Piri *et al*., 2019). The phosphate solubilizing bacteria (PSB) are a PGPR type that improves plant growth by solubilizing insoluble soil phosphate. Most commonly, PGPR like *Pseudomonas fluorescens*, *Bacillus subtilis* (Moradi and Piri, 2018) have been isolated from the cumin rhizosphere. Such bacteria improve plant growth through phytohormone secretion, N_2 fixation, P and Zn solubilization, mitigation of salinity stress, etc. (Moradi and Piri, 2018). Rhizobacteria having ACC deaminase (1-aminocyclopropane-1-carboxylate) activity facilitates plant growth by overcoming deleterious effects. The application PGPRs has been reported to enhance cumin seed and oil yield (Sedigh *et al*., 2014), comparable to chemical fertilizer application. The growth promotion due to PSB in various other plants is well documented in the scientific literature (Bashan *et al*., 2013; Khanna and Sharma, 2011; Qureshi *et al*., 2012; Shravanthi *et al*., 2019; Viruel *et al*., 2014; Yadav *et al*., 2016, 2017). Similarly, the role of PSB in cumin growth has been well studied (Jamir *et al*., 2021; Patel *et al*., 2013; Singh and Rao, 2011; Zaker Tavallaie and Khorramdel, 2012).

Apart from phosphate solubilization, the PSB also improves plant growth by mitigating salinity stress (Moradi and Piri, 2018). The application of PSB as a bioinoculant has been reported to enhance cumin seed and oil yield, comparable with chemical fertilizers (Mishra *et al*., 2019; Sedigh *et al*., 2014; Shivran *et al*., 2012). In previous studies, PSB like *P. fluorescens*, *Bacillus subtilis* (Moradi and Piri, 2018) and *Bacillus* sp. (Singh *et al*., 2016) were isolated from the cumin rhizosphere. The potential of *Pantoea dispersa* as a biocontrol agent against *Xanthomonas albilineans* and *Fusarium udum* on sugarcane and pigeon pea is well documented in the literature (Jiang *et al*., 2019; Zhang and Birch, 1996, 1997a; Zhang and Birch, 1997b). *P. dispersa* specific esterase attenuates sugarcane-specific pathogenicity of *X. albilineans* (Zhang and Birch, 1997b). A literature search documents the phosphate solubilizing potential of *P. dispersa* (Chen *et al*., 2014; Ghosh *et al*., 2021; Panwar *et al*., 2016). In addition to P solubilization, salt tolerance (Panwar *et al*., 2016) and arsenic resistance of *Pantoea dispersa* MF351847 are known (Ghosh *et al*., 2021). To our knowledge, the P solubilizing property of *P. nitritireducens* is not available in the literature. However, its nitrite-reducing potential is well documented (Wang *et al*., 2012). *Klebsiella* genus is known for P solubilizing ability (Bhardwaj *et al*., 2017; Li *et al*., 2011; Sadiq *et al*., 2013). Endophytic *K. pneumoniae* with biocontrol properties has also been recorded (Dey *et al*., 2019). The present study aimed to study a comparative growth analysis of cumin plants (*Cuminum cyminum* L.) treated with talc and glycerol based PSB consortia.

MATERIALS AND METHODS

A study was initiated at Sardarkurshinagar Dantiwada Agricultural University, Sardarkrushinagar, Gujarat, in 2016 to screen PSB from rhizosphere soil of several plants and improve cumin GC-4 growth through their application as glycerol and talc based formulations in various combinations. The region falls under the semi-arid zone of Gujarat, India. The experimental soil was loamy sand with 0.21% organic and 4.65 % total C content.

PSB screening and identification

The rhizosphere soil of *Abelmoschus esculentus*, *Calotropis procera*, *Cynodon dactylon*, *Lantana camera*, *Senna tora*, *Solanum lycopersicum*, and *Solanum tuberosum* plants was screened for PSB isolation from Banaskantha, Gujarat (Table 1). One gram of rhizosphere soil from each sample was serially diluted and suspensions were transferred onto a Petri plate containing Pikovskaya's medium. The Petri plates were incubated at 30±1°C for three days. Well-developed and separated colonies with a clear zone of P solubilization on Pikovskaya agar were aseptically cultured through repetitive subculturing (Ranjan *et al*., 2013). The phosphate solubilizing index (PSI) of PSB was calculated by dividing solubilizing diameter by the growth diameter (Nguyen *et al*., 1992).

In this experiment, two morphological (capsule formation and motility) and 12 biochemical characterizations (catalase test, citrate test, gas production, gelatine hydrolysis, Gram staining, growth in KCN, H_2S production, indole production, methyl red test, nitrate reduction, urease test and Voges Proskauer test) of four PSB isolates were performed (Table 2). The PSB isolates were identified through 16S rRNA partial sequencing followed by BLAST of sequences from the NCBI database. The partial sequencing of the identified PSB isolates was submitted to NCBI GenBank®.

PSB consortia and carrier formulation

The four PSB isolates were mixed in glycerol and talc based formulations in all possible combinations (Table 3). The treatments comprised single (P1–4), dual (P5–10), triple (P11–14) and quadruplet (P15) PSB combinations. In vitro compatibility testing of all four PSBs, in all the possible combinations, was done before initiating the pot experiment. The control of glycerol and talc based treatment pots received sterilized 15 ml glycerol and 15 g talc, respectively. The 24 h old PSB inoculated 1.5 ml nutrient broth, with a population not less than 12 log cfu ml^{-1}, was diluted with sterile distilled water

Table 1. Phosphate solubilizing bacteria (PSB) isolated from various locations of Banaskantha District, Gujarat

No.	Soil sample collection location	Coordinates	Plant rhizosphere	Isolate code	Colony dia. (cm)	Dia. of clear zone (cm)	PSI*
1.	Agronomy Farm, S.K. Nagar	*24°19'22", 072°18'19*	*Calotropis procera*	A1	3.10	4.22	1.36
				A2	3.48	5.02	1.44
				A3	1.38	2.19	1.59
				A4	0.95	1.15	1.21
				A5	0.81	1.13	1.40
				A6	0.31	0.38	1.23
				A7	0.46	0.48	1.04
2.	College of Basic Science & Humanities, S.K. Nagar	*24°19'32", 072°18'34"*	*Solanum tuberosum*	B1	2.52	3.61	1.43
				B2	3.56	4.9	1.38
				B3	3.88	5.31	1.37
				B4	0.20	0.25	1.25
3.	Gowswami farmhouse, Deesa	*24°15'18", 072°12'41"*	*Lantana camera*	C1	1.31	1.54	1.18
				C2	3.34	4.33	1.30
				C3	3.34	4.75	1.42
				C4	1.73	1.68	0.97
				C5	2.56	3.64	1.42
4.	Dantiwada dam, Dantiwada	*24°20'34", 072°20'21*	*Cynodon dactylon*	D1	1.68	1.92	1.14
				D2	1.64	1.74	1.06
				D3	1.81	2.55	1.41
				D4	3.34	4.21	1.26
				D5	0.31	0.37	1.19
				D6	1.31	1.55	1.18
5.	Chaturbhuj farmhouse, Siddhpur	23°53'27", 072°22'15"	*Abelmoschus esculentus*	E1	1.98	2.39	1.21
				E2	1.58	1.88	1.19
				E3	2.43	2.86	1.18
				E4	1.32	1.71	1.30
6.	Shipu dam, Dantiwada	24°24'51", 072°18'24"	*Senna tora*	F1	0.92	1.26	1.37
				F2	0.84	1.26	1.50
				F3	0.85	1.16	1.36
7.	Kamaniya farm, Palanpur	24°10'37", 072°27'54	*Solanum lycopersicum*	G1	1.78	3.62	2.03
				G2	1.59	3.16	1.99
				G3	1.73	2.28	1.32
				G4	1.31	2.47	1.89

* PSI – phosphate solubilizing index

and glycerol to make a 15 ml of 20% glycerol solution and also mixed with 15 g talc powder. The glycerol or talc based inoculants were allowed to settle at room temperature for 24 h. The inoculants containing consortia of double, triple or quadruplet PSB isolate combinations were prepared by mixing 0.75 ml, 0.5 ml or 0.375 ml of 24 h old nutrient broth containing PSB population ≥12 log cfu ml^{-1}, respectively.

Soil treatment of PSB consortia in a pot experiment

A pot experiment was initiated during the winters of 2016 containing loamy sand soil at Sardarkrushinagar Gujarat to determine the effect of glycerol and talc based PSB formulations on cumin plant growth. The experiment consisted of four treatment levels with untreated control. Table 4 describes the treatment layout of the cumin plants. The cumin seeds were aseptically germinated on water agar Petri plates at 30°C for a week. Meanwhile, the 32 cemented pots in sets of glycerol and talc based treatments were filled with 15 kg solarized garden soil and 15 one-week-old seedlings growing on Petri plates were transplanted per pot. The glycerol or talc based inoculants were mixed in the pot soil containing transplanted seedlings so that each gram of soil received not less than 8 log cfu of PSB. The pots were kept in the shade overnight for bioinoculants to overcome the transfer shock. Later the pots were kept under direct sunlight in a completely random design (CRD) and watered regularly. Each pot received approximately 35 watering during the study. No additional fertilizer or pesticide was added to the treatments. No weeding was done, and no pest attack or rainfall was reported during the study period.

After 100 days of growth, the plants were harvested and measured for growth parameters. The cumin plants were cut into roots and shoots. The shoots were washed with distilled water and roots with tap water to remove adhering soil particles and air-dried before re-

Table 2. Morphological and biochemical characterization of PSB isolates

Characteristics	**Isolate code**			
	A3	**G1**	**G2**	**G4**
	***Pseudomonas nitritireducens* MF351819**	***Klebsiella pneumoniae* MF351845**	***Erwinia* sp. MF351846**	***Pantoea dispersa* MF351847**
Capsule formation	-	+	-	-
Motility	+	-	+	+
Catalase test	+	+	+	+
Citrate test	+	+	+	+
Gas production	-	-	-	-
Gelatin Hydrolysis	-	-	-	+
Gram Staining	-	-	-	-
Growth in KCN	+	+	+	-
H_2S production	-	-	+	-
Indole	-	-	-	-
MR (Methyl Red)	-	-	+	+
Nitrate Reduction	+	+	+	-
Urease	-	+	-	-
VP (Voges Proskauer)	-	+	+	+

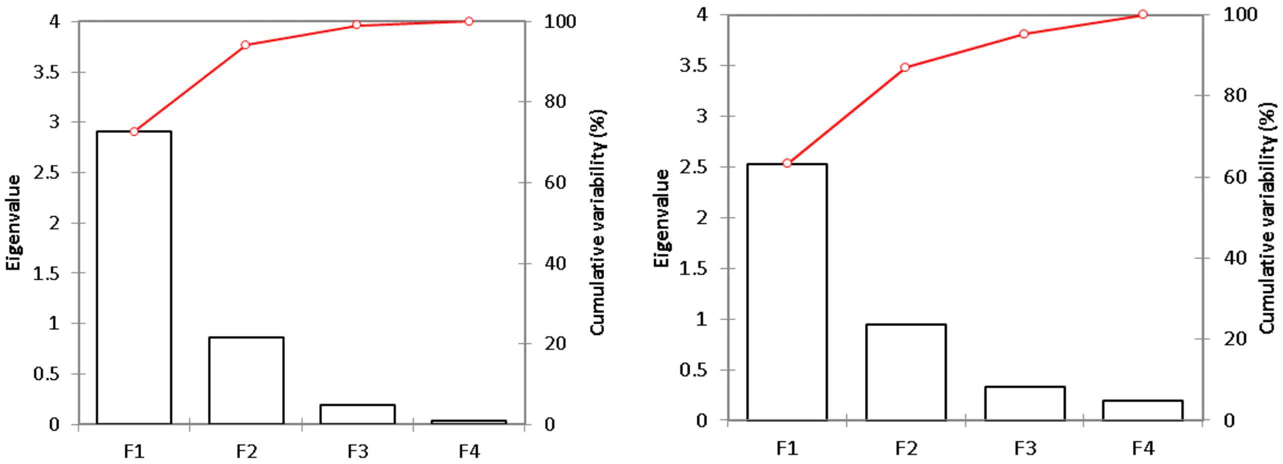
Table 3. PSB consortial treatments used in the glycerol and talc based formulations

Treatment code	**Treatment level**	**PSB treatment/treatment combination**
P0	Nil	Control
P1	Single	*Pseudomonas nitritireducens* MF351819
P2		*Klebsiella pneumoniae* MF351845
P3		*Erwinia* sp. MF351846
P4		*Pantoea dispersa* MF351847
P5	Dual	*K. pneumoniae* MF351845 and *P. nitritireducens* MF351819
P6		*Erwinia* sp. MF351846 and *P. nitritireducens* MF351819
P7		*P. nitritireducens* MF351819 and *P. dispersa* MF351847
P8		*K. pneumoniae* MF351845 and *Erwinia* sp. *MF351846*
P9		*P. dispersa* MF351847 and *K. pneumoniae* MF351845
P10		*Erwinia* sp. and *P. dispersa* MF351847
P11	Triple	*K. pneumoniae* MF351845, *Erwinia* sp. MF351846 and *P. nitritireducens* MF351819
P12		*K. pneumoniae* MF351845, *P. nitritireducens* MF351819 and *P. dispersa* MF351847
P13		*Erwinia* sp. MF351846, *P. nitritireducens* MF351819 and *P. dispersa* MF351847
P14		*K. pneumoniae* MF351845, *Erwinia* sp. MF351846 and *P. dispersa* MF351847
P15	Quadruplet	*K. pneumoniae* MF351845, *P. nitritireducens* MF351819, *Erwinia* sp. MF351846 and *P. dispersa* MF351847

Experiment design setup = CRD; Number of replicates =15

A. Glycerol formulation showing growth of cumin plants *B. Talc formulation showing better growth of cumin plants*

Fig. 1. *Cumin pots treated with glycerol and talc based phosphate solubilizing bacterial (PSB) consortia of Pseudomonas nitritireducens MF351819, Klebsiella pneumoniae MF351845, Erwinia sp. MF351846 and Pantoea dispersa MF351847*



A. Glycerol formulation *B. Talc formulation*

Fig. 2. *Principal component analysis (PCA) scree plot for low-coverage sequencing data for analyzed glycerol and talc based PSB consortia treated cumin plant growth variables*

cording growth measurements. The plant height (root and shoot length), dry weight, 100 seeds weight and yields were observed. Plant dry weights were observed after overnight drying the root and shoot systems in a hot air oven at 80°C.

Measurement of inorganic phosphate and carbon contents of the pot soil after plant harvesting

After cumin plant harvesting, the 20 g pot soil was collected in plastic vials from each treatment and measured for inorganic phosphate (Pi), total C and organic C through the methods described below.

The inorganic phosphate in soil was estimated through Olsen (1982) method. The organic soil C content was measured by adding 0.5 g soil to 4 ml $K_2Cr_2O_7$ and 10 ml H_2SO_4 solution, followed by mixing and incubation for 30 minutes. The samples were centrifuged at 5000 rpm for 10 minutes, and the collected supernatant's optical density (OD) was measured at 660 nm (Schollenberger, 1927).

Total C in the soil was measured by combusting 5 g soil in a resistance furnace in the presence of pure oxygen at a temperature exceeding 500°C for six hours. Later the oven temperature was decreased and samples were collected and weighed to find the weight loss (Winters Jr and Smith, 1929).

RESULTS AND DISCUSSION

PSB screening and identification

The 33 PSB with various degrees of P solubilization were isolated from seven locations in north Gujarat (Table 1). The four most potent PSB with PSI range between 1.59–2.03 were characterized through biochemical tests (Table 2) and identified as *Pseudomonas nitritireducens* MF351819, *Klebsiella pneumoniae* MF351845, *Erwinia* sp. MF351846 and *Pantoea dispersa* MF351847 through 16S rRNA sequencing. The *in*

Table 4. Experimental details of the pot study

Location and agroclimatic zone: S.K. Nagar, Gujarat, India; semiarid
Experimental details
Bacterial treatment: Four PSB in various combinations
Carrier application: Glycerol and talc powder
Design: CRD
Crop and variety: *Cumin cyminum* GC4
Seeding rate: Fifteen per pot
Soil contained in pot: 15 kg
Manuring and fertilizers: Nil
Cultural details
Date of sowing: 27/10/2016
Date of harvesting: 04/02/2017
Experimental duration: 100 days
Number. of waterings: 35
Number of weedings: Nil
General conditions
Plant Stand: Direct sunlight
Season: Winter
Rainfall (mm) & rainy Days: Nil

vitro compatibility testing of the isolates before the pot experiment found none to be antagonistic or synergistic to one another. However, PSB interaction in the rhizosphere remains unpredictable due to the broader range of metabolic pathway expressions, which may have a delirious or synergistic effect on the microbial community.

Pot experiment of PSB inoculation on cumin plant

Before the pot study, the four PSB isolates were mixed in 15 treatment combinations to make glycerol and talc based bioformulations (Table 3). The pot experiment was initiated in the winter of 2016 (last week of October); the details are available in Table 4.

Effect of glycerol-based inoculants on cumin growth

The highest plant length of 19.22 cm and dry weight of 0.22 g were observed with P3 treatment of *Erwinia* sp. MF351846 (Table 5 and Fig. 1). The maximum 100 seed weight of 0.47 g was obtained with P11 treatment containing a consortium of *K. pneumoniae* MF351845, *Erwinia* sp. MF351846 and *P. nitritireducens*

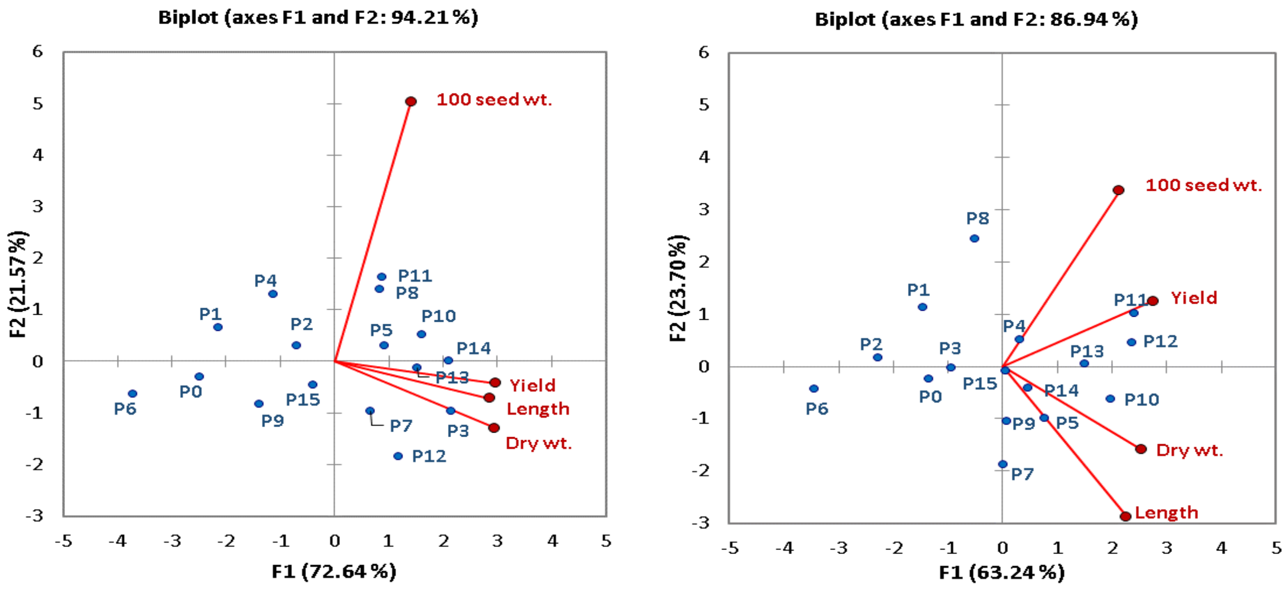
Table 5. Growth of cumin plants treated with glycerol and talc based PSB consortia*

PSB treatment code	Glycerol formulation				Talc formulation			
	Length (cm)	Dry wt. (g)	100 seed weight (g)	Yield per plant (g)	Length (cm)	Dry wt. (g)	100 seed weight (g)	Yield per plant (g)
P0	14.64^{ef}	0.05^{fg}	0.34^{def}	0.03^{e}	19.69^{ab}	0.19^{b}	0.49^{de}	0.31^{abc}
P1	14.29^{fg}	0.07^{efg}	0.39^{bcd}	0.03^{e}	18.47^{ab}	0.21^{ab}	0.59^{abcd}	0.27^{abc}
P2	16.73^{bcde}	0.09^{cdefg}	0.39^{bcd}	0.07^{de}	18.51^{ab}	0.22^{ab}	0.53^{cde}	0.21^{bc}
P3	19.22^{a}	0.22^{a}	0.37^{cde}	0.15^{ab}	18.88^{ab}	0.26^{ab}	0.53^{abcde}	0.28^{abc}
P4	15.35^{cdef}	0.08^{defg}	0.43^{ab}	0.06^{de}	19.36^{ab}	0.27^{ab}	0.6^{abcd}	0.33^{abc}
P5	17.83^{ab}	0.15^{abcd}	0.41^{bc}	0.12^{bcd}	19.93^{ab}	0.33^{a}	0.53^{bcde}	0.32^{abc}
P6	12.33^{g}	0.03^{g}	0.31^{f}	0.02^{e}	18.05^{b}	0.2^{ab}	0.43^{e}	0.20^{c}
P7	17.82^{ab}	0.16^{abc}	0.35^{cdef}	0.12^{bcd}	20.91^{a}	0.29^{ab}	0.49^{de}	0.27^{abc}
P8	17.05^{bcd}	0.15^{abcde}	0.46^{a}	0.11^{bcd}	18.07^{b}	0.22^{ab}	0.69^{a}	0.31^{abc}
P9	15.02^{def}	0.10^{cdefg}	0.33^{ef}	0.08^{cde}	20.83^{a}	0.27^{ab}	0.56^{abcde}	0.26^{abc}
P10	16.75^{bcde}	0.20^{ab}	0.43^{ab}	0.16^{ab}	20.77^{a}	0.33^{ab}	0.6^{abcd}	0.36^{abc}
P11	16.92^{bcd}	0.14^{bcde}	0.47^{a}	0.12^{bcd}	20.12^{ab}	0.3^{ab}	0.68^{ab}	0.42^{a}
P12	17.84^{ab}	0.22^{a}	0.32^{ef}	0.13^{abc}	20.56^{ab}	0.31^{ab}	0.67^{abc}	0.39^{ab}
P13	17.33^{abc}	0.20^{ab}	0.4^{bc}	0.15^{ab}	20.56^{ab}	0.29^{ab}	0.63^{abcd}	0.35^{abc}
P14	17.54^{ab}	0.20^{ab}	0.41^{bc}	0.19^{a}	20.92^{a}	0.24^{ab}	0.58^{abcde}	0.32^{abc}
P15	16.93^{bcd}	0.12^{cdef}	0.36^{cdef}	0.08^{cde}	19.92^{ab}	0.24^{ab}	0.54^{abcde}	0.35^{abc}
CV	3.47	3.47	3.47	3.47	4.90	4.90	4.90	4.90
R^2	0.51	0.49	0.55	0.52	0.20	0.15	0.26	0.14
F	15.47	14.27	18.24	16.06	3.72	2.54	5.35	2.51
Pr > F	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	0.001671	< 0.0001	0.00187
LSD	1.221	0.046	0.031	0.034	1.502	0.079	0.089	0.107

* Each value is a mean of 15 replicates

Table 6. Cumin plant growth parameter correlation matrix (Pearson (n)) treated with glycerol and talc based bioinoculants

Variables	Length	Dry wt.	100 seed wt.	Yield
Glycerol formulation				
Length	1	0.868	0.312	0.821
Dry wt.	0.868	1	0.239	0.945
100 seed wt.	0.312	0.239	1	0.371
Yield	0.821	0.945	0.371	1
Talc formulation				
Length	1	0.658	0.162	0.525
Dry wt.	0.658	1	0.371	0.588
100 seed wt.	0.162	0.371	1	0.710
Yield	0.525	0.588	0.710	1



A. Glycerol formulation *B. Talc formulation*

Fig. 3. *Factor loadings of the F1 and F2 projection from PCA of glycerol and talc based PSB consortia treated cumin plant growth parameters.*

MF351819. In contrast, the highest per plant yield of 0.19 g was observed with P14 treatment containing consortium of *K. pneumoniae* MF351845, *Erwinia* sp. MF351846 and *P. dispersa* MF351847. It can be summarized that maximum observed parameters yielded higher values with consortia of three PSB isolates (P11 - P14). The lowest growth parameter values were observed with P6 treatment containing a consortium of *Erwinia* sp. MF351846 and *P. nitritireducens* MF351819.

The glycerol application in bioformulation improves the shelf life of bioinoculants and is a readily biodegradable non-polar solvent (Sehrawat *et al.*, 2017). Glycerol readily distributes in water when released in the environment, with negligible factions in air, soil or sediment (Wernke, 2014). Once the glycerol-based bioinoculant reaches the soil, the glycerol provides no existential benefit to the bacteria and plays no role in soil texture improvement.

Table 6 describes the Pearson correlation matrix of four plant growth parameters: length, dry weight, 100 seed weight and yield. Strong correlations between dry weight and yield (0.945), length and dry weight (0.868) and between length and yield (0.821) were observed. Fig. 2A describes the principal component analysis (PCA) scree plot of low-coverage sequencing data for glycerol based treated cumin plant growth variables. Four growth variables driving F1 and F2 had the most significant relative contribution in principal component analysis (PCA) of Fig. 3A. The F1 component was influenced by yield, plant dry weigh and length with 72.64% loadings, while 100 seeds weight influenced F2 component with 21.57% loadings. The treatments P3, P14, P10, P13 and P12 were grouped to plant yield, dry

Table 7. Two-Factor ANOVA of cumin plant growth parameters for glycerol and talc based PSB treatments

Glycerol formulation						
Summary	**Total plant length**	**Total plant dry wt.**	**100 seed wt. (g)**	**Yield per plant (g) (A*B)**	**Total**	
Count	240.00	240.00	240.00	240.00	960.00	
Sum	3953.70	32.91	92.41	24.15	4103.17	
Average	16.47	0.14	0.39	0.10	4.27	
Variance	5.49	0.01	0.00	0.00	51.05	
Talc formulation						
Count	240	240	240	240	960	
Sum	4733.40	62.65	136.92	74.40	5007.37	
Average	19.72	0.26	0.57	0.31	5.22	
Variance	5.10	0.01	0.02	0.02	71.52	
Total						
Count	480	480	480	480		
Sum	8687.10	95.56	229.33	98.55		
Average	18.10	0.20	0.48	0.21		
Variance	7.93	0.01	0.02	0.03		
ANOVA						
Source of Variation	***SS***	***df***	***MS***	***F***	***P-value***	***F crit***
Sample	425.83	1.00	425.83	319.38	<0.01	3.85
Columns	114138.87	3.00	38046.29	28535.53	<0.01	2.61
Interaction	851.93	3.00	283.98	212.99	<0.01	2.61
Within	2549.26	1912.00	1.33			
Total	117965.89	1919.00				

weight and length with strong loadings on F1 component, implying higher growth values. In contrast, P4, P8 and P11 treatments were grouped with F2 component as per their factor scores. While P0, P1, P4, P6 and P9 had weaker factor loading on F1 component, implying lower growth.

Effect of talc-based inoculants on cumin growth

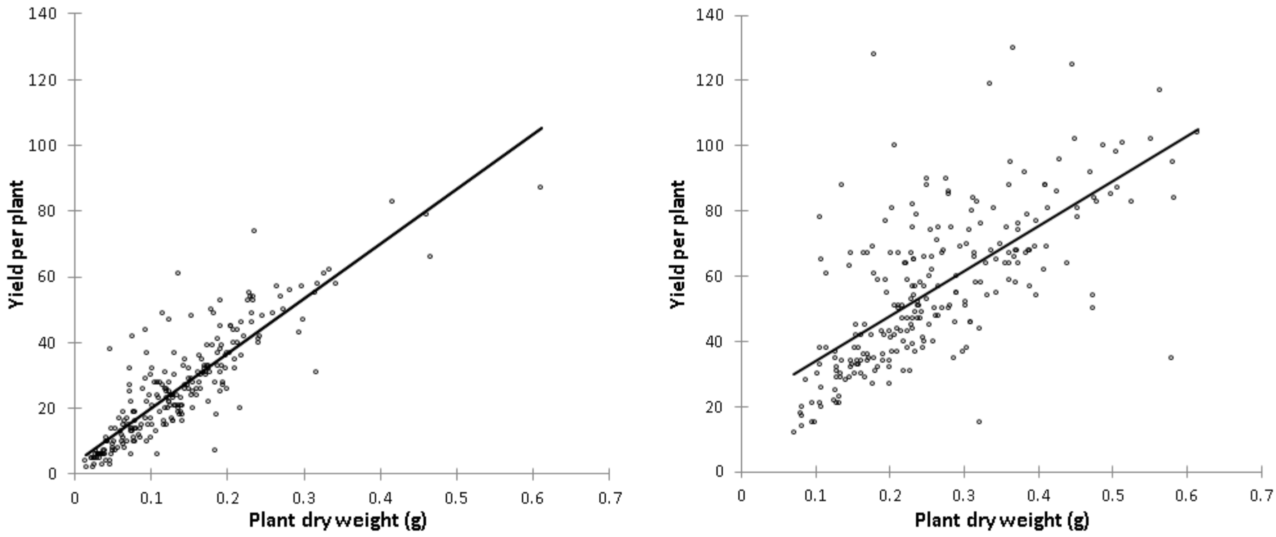
The present study observed a maximum plant length of 20.92 cm with P14 treatment containing the consortium of *K. pneumoniae* MF351845*, Erwinia* sp. MF351846 and *P. dispersa* MF351847. The maximum dry weight of 0.33 g was found with P5 treatment with a consortium of *K. pneumoniae* MF351845 and *P. nitritireducens* MF351819 and with P10 treatment, a consortium of *Erwinia* sp. and *P. dispersa* MF351847 (Table 5). A maximum 100 seed weight of 0.69 g was obtained with P8 treatment of *K. pneumoniae* MF351845 with *Erwinia* sp., while the highest cumin yield of 0.42 g was obtained with P11 treatment having a consortium of *K. pneumoniae* MF351845*, Erwinia* sp. MF351846 and *P. nitritireducens* MF351819. The P6 treatment had values lower than the control in three of the four growth parameters measured.

Talc is a known anti-caking and dispersing agent that improves plant nourishment from the soil. The role of talc-based formulations in bioinoculation survival is well documented (Arpitha and Brahmaprakash, 2016; Mishra *et al*., 2019; Shivran *et al*., 2012). The talc-based bioinoculants have been reported to enhance the soil's organic carbon, nitrogen, phosphorous, and potassium (Pahari *et al*., 2017). Growth promotion of cumn through talc-based formulation containing consortia of *P. pituda* and *Microbacterium taraoxidens* (Shivran *et al*., 2012), and *Bacillus subtilis*, *B. megaterium* and *Bacillus* sp. (Mishra *et al*., 2019), have been reported. The talc based bioinoculants enhanced the quality and quantity of soil microflora, perhaps by improving the soil texture. The same can be verified in Table 5 by comparing the dry weights of controls for glycerol and talc-based treatments (0.05 g and 0.19 g, respectively). In a similar study, the pot and field experiment using talc-based formulations improved rice plant dry weight (Tamreihao *et al*., 2016).

Fig. 2B describes the PCA scree plot of low-coverage sequencing data for talc based PSB treated cumin plant growth variables. The PCA study of four growth parameters of cumin shows that variables driving F1 and F2 had the most significant relative contribution to the variation between analyzed variables. The yield and dry weight contributed the most to the F1 component with 63.24% loadings, while 100 seed weight and yield influenced F2 component with 23.7% loadings as indicated in Fig. 3B. The treatments P0, P1, P2, P3, P6 and P8 had weaker factor loadings on the F1 component, implying lower values of all four parameters.

Table 8. Pearson correlation matrix between inorganic phosphate (mg Kg^{-1}), total and organic C from the soil of glycerol and talc based bioinoculant treated pots after cumin plant harvesting

a. Glycerol formulation			
Variables	**Pi**	**Total C**	**Organic C**
Pi	**1**	0.066	0.441
Total C	0.066	**1**	-0.078
Organic C	0.441	-0.078	**1**
b. Talc formulation			
Variables	**Pi**	**Total C**	**Organic C**
Pi	**1**	0.427	0.445
Total C	0.427	**1**	-0.116
Organic C	0.445	-0.116	**1**



Glycerol formulation (R^2=0.7528) *Talc formulation (R^2=0.4633)*

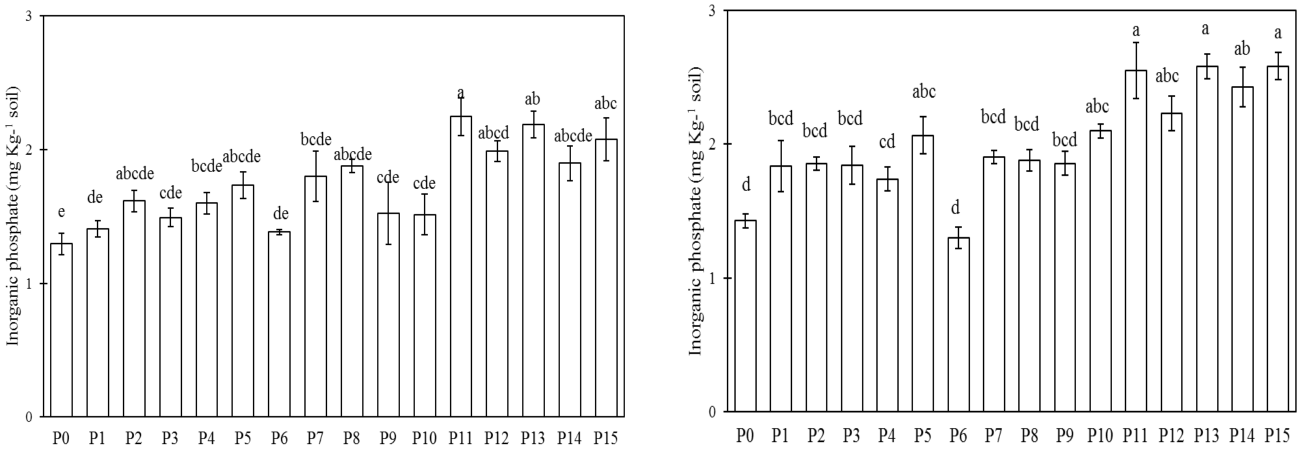
Fig. 4. *Regression of per plant cumin yield by plant dry weight treated with glycerol and talc based PSB consortia*

Treatments P10, P11, P12 and P13 had higher factor scores on F1 component, while P1, P8 and P11 were grouped with F2 component having lower growth values but higher 100 seed weight, implying that plant dry weight does not directly correlate with 100 seed weight and yield, which can be explained from Table 6 of the Pearson correlation matrix. Also, the correlations were not as strong as with glycerol treatments, signifying the contribution of a few additional factors in plant growth. A correlation of 0.710 was found between cumin yield and 100 seed weight, plant length and dry weight (0.658), and between dry weight and yield (0.588).

Comparing cumin plant growth with glycerol and talc-based inoculation treatments

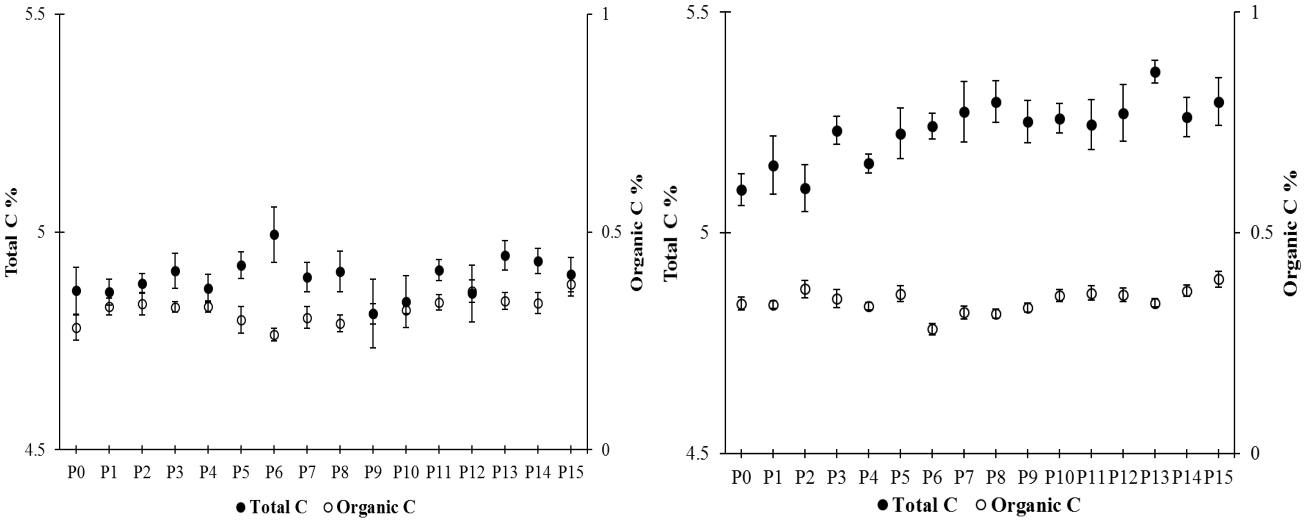
The cumin plants in the talc-based control had a comparatively higher dry weight of 0.19 g than glycerol based control (0.05 g). Similarly, plant length, 100 seed weight and talc based control had higher values than glycerol based control. Almost in every treatment, the growth parameter values in talc based treatment were higher than talc. It is hypothesized that supplementation of talc improved the texture of loamy sand soil, deficient in fine particles. The talc's fine particles might help form soil aggregates to hold more nutrients in micro pockets to sustain more bacteria in the rhizosphere. Talc based inoculants might also have enabled the PSB consortia to sustain and colonize the plant rhizosphere for better plant growth.

Overall, lower growth of cumin plants was observed with glycerol-based PSB consortial treatments than talc -based inoculants (Table 5). Interestingly, the treatments with higher growth values among the four measured parameters contained *P. dispersa* MF351847 (P4, P7, P9, P10, P12, P13, P14, and P15) in the consortium. With glycerol-based treatment, the highest cumin plant length and dry weights were obtained with P3 treatment of *Erwinia* sp. However, the talc-based P3 treatment fetched comparatively lower cumin seedling length and dry weight. Perhaps the pre-existing rhizospheric microbial interaction forbade the single inoculant of P3 treatment from colonizing. Overall dual and



A. Glycerol formulation *B. Talc formulation*

Fig. 5. *Inorganic phosphate in glycerol and talc based PSB consortia treated pot soil after cumin plant harvesting (* Vertical bars represent ± standard error)*



A. Glycerol formulation *B. Talc formulation*

Fig. 6. *Total and organic C in glycerol and talc based PSB consortia treated pot soil after cumin plant harvesting*α (* Vertical bars represent ± standard error;α The soil used in the experiment had initial 0.21% organic and 4.65 % total C content)*

triple PSB consortial treatments recorded maximum dry weights and yield per plant.

With glycerol and talc-based applications the P6 treatment containing consortium of *Erwinia* sp. MF351846 and *P. nitritireducens* MF351819 resulted in lowest plant growth suggesting negative interaction with each other or other rhizosphere bacteria. With P11 and P13 PSB consortial treatments, moderately higher plant growth was observed, perhaps due to dilution of negative interaction between *Erwinia* sp. and *P. nitritireducens*. As a result, with P16 treatment, relatively lower growth than P15 treatment was observed since it included *Erwinia* sp. and *P. nitritireducens* in a consortium. However, *in vitro* inoculant compatibility testing between PSB isolates found no interaction.

Table 7 describes the two-factor ANOVA of four cumin plant growth parameters viz. plant length, dry weight, 100 seed weight, and yield per plant) between glycerol and talc-based treatments. The two-way ANOVA compares 15+1(control) treatments, four observed growth parameters, and glycerol and talc-based treatment sets at P-value <0.01. Fig. 4 describes the regression of yield by plant dry weight, treated with glycerol and talc and glycerol based PSB consortia. The plant's dry weight in glycerol based formulation fairly correlated with yield (R^2=0.7528). However, with talc based formulation the lower correlation of 0.4633 was observed.

Inorganic phosphate and carbon content of glycerol and talc treated pot soil

The glycerol and talc based PSB consortial treatments considerably improved Pi content in the soil (Fig. 5). With glycerol based formulation the highest pot soil Pi of 2.25 mg Kg^{-1} was observed with P11 treatment. With talc based formulation the maximum Pi of 2.58 mg Kg^{-1} was observed with P15 treatment. Table 8 describes the Pearson correlation between Pi (mg Kg^{-1}), total and organic C from the soil of treatment pots after cumin plant harvesting. A moderate correlation of 0.441 was observed between organic C and Pi, signifying the indirect role of organic C in P solubilization.

The pot soil had initial 0.21% organic and 4.65% total carbon content. With glycerol-based PSB application, the total carbon content of soil remained almost similar in all the treatments, while organic C increased with inoculation treatment level and reached the maximum of 0.38 % in P15 treatment (Fig. 6). Similarly, the total C content of talc-based treatment was also constant in all treatments. However, the organic C increased with the treatment level reaching a maximum of 0.4% with P15 treatment, implying the importance of PSB consortia in improving the soil's organic C content. Overall, higher organic C contents were registered with talc-based inoculants. Perhaps the addition of talc in the soil is responsible for higher biological activity leading to higher organic C. The talc-based PSB consortia could improve the soil's biological activity to increase organic C content. A recent study shows a correlation between soil organic C content and P availability (Kumar and Rai, 2020). However, a similar study reported increased soil organic carbon levels in PSB inoculated and rock phosphate-fertilized soil, improving maize and wheat crop growth (Kaur and Reddy, 2014a, b). The poor organic C in semiarid soil reduces the rhizosphere PSB population and hampers P solubilization, impeding plant growth. In a previous pot study done by the authors, the biovars of *B. subtilis* and *P. fluorescens* introduced in the cumin rhizosphere at the rate of 6, 7 and 8 log cfu ml^{-1} reached below the detection limit after three to four weeks of study due to scarcity of carbon in the soil (Yadav and Yadav, 2016).

Conclusion

The study obtained four efficient PSB, viz. *P. nitritireducens* MF351819*, Klebsiella pneumoniae* MF351845, *Erwinia* sp. MF351846 and *Pantoea dispersa* MF351847 from the rhizosphere of *Calotropis procera* and *Solanum lycopersicum*. In the pot study, the consortia of these bacteria in different combinations improved cumin plant growth. Comparatively lower plant length and dry weight, 100 seeds weight and yield were obtained with glycerol-based treatments than with talc-based inoculation. Although liquid bioinoculants are in great demand due to prolonged bioinoculant survivability, the importance of talcum powder extends beyond its role as a carrier. The present results showed that talcum powder-based bioinoculants efficiently improved soil organic C levels in cumin-grown pots. Talcum powder indirectly enhances the organic C level in soil by improving soil texture, leading to higher biological activity near the rhizosphere. Thus talc is best suited as a carrier for bioinoculants in semiarid and nutrient-poor soils. However, more study is required to understand PSB survivability, population dynamics and interactions in semiarid rhizosphere soils to formulate better consortia with a longer shelf life that could also protect bioinoculants post field application.

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Conflict of interest

The authors declare that they have no conflict of interest.

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