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The Effect of Seed Priming with Plant Growth Promoting Rhizobacteria (PGPR) on Growth of Coriander (*Coriandrum sativum* L.) Seedling

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A B S T R A C T

Plant growth promoting rhizobacteria (PGPR) are a wide range of root colonizing bacteria with the capacity to enhance plant growth by increasing seed emergence, producing lytic enzyme and bacteriocin. Soil or seed application of PGPR have been used to enhance growth of the several crops as well as to suppress the growth of the plant pathogens. The pot experiment was conducted during winter season to find out the effect of three plant growth promoting rhizobacteria (PGPR) either singly or in combination on vegetative growth parameters of coriander seedling. We observed four growth stages of *C.* sativum viz., 5 DAG, 10 DAG, 15 DAG, & 20 DAG. Seeds were inoculated with single and combined solution of 10^8 CFU/ml of rhizobacteria. Seeds were not inoculated for the control variant. The combinations of three PGPR i.e., *Azatobacter* + PSB + *Pseudomonas* significantly increased plant growth parameters such as shoot length, root length, shoot weight, root weight, total biomass and total chlorophyll contents in comparison to the individual and control treatment. The results of this study suggest that PGPR is a promising solution for sustainable, environmentally friendly agriculture, and its co-inoculation have the potential to increase the plant growth of *C. sativum* and it reduces the use of chemical fertilizer.

Introduction

The seed spice coriander (*Coriandrum sativum* L.) belonging to the family *Apiaceae*, having a diploid chromosome number 2n=22. Coriander leaves is extensively used in India as well as western countries in flavouring of processed foods, including breads, cakes, sauces, meat products, soup and confectionery. Coriander seeds are used in tonic, carminative, diuretic, stomachic and as an aphrodisiac. Oleoresin from coriander is used as a flavouring agent, as an ingredient in pharmaceutical formulations and in perfumery. A number of chemical constituents such as volatile constituents, flavonoids, isocoumarins, and coriandrones have been isolated from different parts of the plant (Taniguchi *et al.*, 1996). Due to the easy collection of the plant and being wide spread and also remarkable biological activities, this plant has become both food and medicine in many parts of the world, (Jinous and Nastaran, 2012). The term 'plant growth promotion' (PGP) is often used to describe increased plant growth followed by increased crop yield. Many microbes especially, rhizobacteria (bacteria from rhizosphere) are

known for their PGP properties. Plant growth promoting rhizobacteria (PGPR) are a wide range of root colonizing bacteria with the capacity to produce IAA like compounds (Kandoliya and Vakharia, 2013), enhance plant growth by increasing seed emergence (Herlache & Triplett, 2002), plant growth and crop yield (Kloepper, 1992). The use of PGPR offers an attractive way to replace chemical fertilizer, pesticides and supplements. Some PGPR have been produced commercially as inoculants for agriculture to improve plant growth through supply of plant nutrients and may help to sustain environmental health and soil productivity. So the present investigation was planned to evaluate effect of PGPR on vegetative growth parameters of coriander seedling.

Material and Methods

Experimental site

The present investigation was conducted in green house condition at Department of Biochemistry, College of Agriculture, Junagadh Agricultural University, Junagadh (Gujarat) during *Rabi* 2015-16.

Experimental soil

The soil was collected from Agronomy farm, Junagadh Agricultural University, Junagadh, sterilized in autoclave dried properly and used for pot trial. There were 24 Pots, each with 40cm deep and 45cm wide, having capacity 40kg soil/pot. Experimental soil was calcareous in texture and slightly alkaline in reaction having normal electrical conductivity.

PGPR culture

Three plant growth promoting rhizobacterial cultures (*Azatobacter*, Phosphate solubilizing bacteria, and *Pseudomonas*) were obtained from Microbial Cell, Department of Biotechnology, Junagadh Agricultural University, Junagadh.

Seed materials

The coriander seeds (cv. Gujarat Coriander-2) were obtained from Department of seed science and technology, Junagadh Agricultural University, Junagadh.

Seed treatment

Prior to treatments coriander seeds (Gujarat coriander-2) were sterilized with 70% ethanol and 0.1% mercuric chloride (Hg) and washed with distilled water for 4 times.

Pure culture of PGPR (10^8 CFU/ml) individually or in combination were treated with seeds. Seeds were not inoculated for control variant.

T_1-Control
T_2-*Azatobacter*
T_3-PSB (Phosphate solubilizing bacteria)
T_4-Pseudomonas
T_5-*Azatobacter* + PSB
T_6-*Azatobacter* + *Pseudomonas*
T_7-PSB + *Pseudomonas*
T_8-*Azatobacter* + PSB + *Pseudomonas*

Pot trial

Pot trials are conducted in green house of Biochemistry Department, College of Agriculture, J.A.U., Junagadh. After half an hour of seed treatment, they were sown in pots in three replications during December.

Sufficient water was supplied to pots till the last stage. The seedlings were analyzed in four stages viz., S_1 (5 DAG), S_2 (10 DAG), S_3 (15 DAG) and S_4 (20 DAG).

Physiological analysis of growth parameters:

Root Length (cm)

Root length of five randomly selected normal seedlings after 5 DAG, 10 DAG, 15 DAG, and 20 DAG was measured in three replications. Fresh plants from the pots were uprooted, washed with distilled water to remove soil and any dirt present, wiped with filter paper to remove extra moisture from the sample. After separating the root and shoot part with the sharp knife the length of root was measured in cm.

Shoot length (cm)

Shoot length of five randomly selected normal seedlings after 5 DAG, 10 DAG, 15 DAG, and 20 DAG was measured same as in root length.

Root fresh weight (mg)

Root fresh samples of five seedlings randomly selected for root length as described above were weighed on electronic balance and expressed as (mg. 5 plant^{-1}).

Shoot fresh weight (mg)

Shoot fresh samples of five seedlings selected for shoot length were weighed same as in root fresh weight.

Total biomass (mg)

Five seedlings selected for shoot/root length were weighed on electronic balance and expressed as (mg. 5 plant^{-1}).

Total chlorophyll content

Total chlorophyll in seedling was determined by DMSO method (Hiscox and Israelstam, 1979). Finely chopped 50 mg coriander seedlings were weighed in graduated test tube. 10 ml of DMSO was added in each test tube. The tubes were incubated at 65°C for 3 hours, after incubation the tubes were allowed to cool at room temperature and the OD of supernatant was recorded at 663 and 645 nm by taking DMSO as blank. The amount of chlorophyll present in the sample was calculated using standard formula:

Chlorophyll a (mg.g-^{1}) = 12.7 (A_{663}) – 2.69 (A_{645}) × V/1000 × W
Chlorophyll b (mg.g-^{1}) = 22.9 (A_{645}) – 4.68 (A_{663}) × V/1000 × W
Total chlorophyll (mg.g-^{1}) = [22.2 (OD at 645 nm) + 8.02 (OD at 663 nm)] × [V/ (1000 × W)]

Where, A= Absorbance at specific wavelength
V= Final volume of extract in DMSO (ml)
W=Fresh weight of tissue extracted (g)

Statistical design

Data obtained were analyzed statistically as per FCRD (1st factor- seedling stage, 2nd factor- treatments)

Result and Discussion

Root length and shoot length (cm)

Changes of root length (cm) and shoot length(cm), due to various treatment of plant growth promoting rhizobacteria (PGPR) during different growth stages viz., S_1 (5 DAG), S_2 (10 DAG), S_3 (15 DAG) and S_4 (20 DAG), are presented in Table.1. The data showed significant differences for growth stages, treatments as well as for interaction effect. There were increasing trend for the root length and shoot length from S_1 to S_4 stage (2.33-3.56 cm) and (5.38-12 cm) respectively. Irrespective of stages, mean

treatment effect concerned, the highest root length, and shoot length (3.46 cm) and (9.65 cm) respectively were found for the treatment T_8 (*Azatobacter* + PSB + *Pseudomonas*). S_4T_8 (14.13 cm) found significantly higher whereas S_1T_1 remain significantly lower (4.50 cm). Glick *et al.*, 1995 also reported that seed treatments of PGPR enhanced growth of the several crops. It was also reported that the PGPR decrease the application of chemical fertilizers (Adesemoye *et al.*, 2009), either by stimulating root growth or by directly stimulating plant nutrient uptake. Mahato *et al.*, (2009) found that PGPR had increased shoot length in tomato plant.

Table.1 Effect of Plant growth promoting rhizobacteria (PGPR) on root and shoot length (cm) of coriander (*C. sativum* L.) seedling

Treatments	Root length (cm)					Shoot length (cm)				
	DAG					DAG				
	5	10	15	20	Mean (T)	5	10	15	20	Mean (T)
T_1	2.02	2.12	2.77	3.03	**2.48**	4.50	5.68	8.40	10.25	**7.21**
T_2	2.13	2.27	2.92	3.22	**2.63**	4.78	6.07	8.84	10.57	**7.56**
T_3	2.30	2.42	3.12	3.45	**2.82**	5.21	6.37	9.07	11.49	**8.04**
T_4	2.10	2.23	2.66	3.13	**2.53**	4.61	5.99	8.57	10.38	**7.39**
T_5	2.57	2.91	3.74	3.93	**3.29**	6.11	7.63	10.03	13.88	**9.41**
T_6	2.37	2.58	3.28	3.64	**2.97**	5.67	6.71	9.41	12.18	**8.49**
T_7	2.48	2.83	3.52	3.83	**3.17**	5.80	7.08	9.69	13.15	**8.93**
T_8	2.65	3.09	3.88	4.24	**3.46**	6.33	7.88	10.26	14.13	**9.65**
Mean (S)	**2.33**	**2.56**	**3.23**	**3.56**		**5.38**	**6.68**	**9.28**	**12.00**	
	S	**T**	**S x T**			**S**	**T**	**S x T**		
S.Em. ±	0.02	0.03	0.07			0.10	0.14	0.28		
C.D. at 5 %	0.07	0.10	0.20			0.28	0.39	0.79		
C.V. %	4.15					5.80				

The values are mean of three replications

Where, T_1- (Control), T_2- (*Azatobacter*), T_3- (PSB), T_4- (*Pseudomonas*), T_5-(*Azatobacter*+ PSB), T_6- (*Azatobacter*+ *Pseudomonas*), T_7- (PSB + *Pseudomonas*), T_8- (*Azatobacter* + PSB + *Pseudomonas*), C.D.-Critical Difference, C.V.-Coefficient of Variance, S.Em.-Standard Error of Mean.

Table.2 Effect of Plant growth promoting rhizobacteria (PGPR) on root weight, shoot weight, and total biomass (mg) of coriander (*C. sativum* L.) seedling

Treatments	Root wt. (mg. 5 plant^{-1})					Shoot wt. (mg. 5 plant^{-1})					Total Biomass (mg. 5 plant^{-1})				
	DAG					DAG					DAG				
	5	10	15	20	Mean (T)	5	10	15	20	Mean (T)	5	10	15	20	Mean (T)
T_1	4.49	6.84	8.22	10.89	**7.61**	24.75	49.02	140.45	182.31	**99.13**	29.25	55.87	148.67	193.20	**106.75**
T_2	7.10	7.65	10.41	13.39	**9.64**	47.91	79.29	186.30	312.84	**156.58**	55.32	87.46	197.15	328.31	**167.06**
T_3	7.57	8.53	10.96	17.58	**11.16**	48.60	80.99	186.67	331.74	**162.00**	55.85	89.00	197.19	347.25	**172.32**
T_4	6.29	7.46	9.88	11.57	**8.80**	42.74	76.95	148.38	229.84	**124.48**	49.02	84.40	158.26	241.41	**133.28**
T_5	9.71	10.82	12.11	23.54	**14.04**	53.53	155.70	208.96	552.88	**2.42.77**	63.08	166.52	221.07	576.42	**256.77**
T_6	8.15	9.57	10.99	18.00	**11.68**	53.37	81.11	188.32	351.76	**168.64**	59.67	90.68	199.31	369.76	**179.85**
T_7	8.95	9.76	11.42	19.99	**12.53**	51.52	151.59	192.55	483.40	**219.77**	62.48	161.35	203.97	503.39	**232.80**
T_8	10.13	11.51	12.44	26.01	**15.02**	55.16	155.98	216.54	575.12	**250.70**	65.29	167.49	228.97	601.13	**265.72**
Mean (S)	**7.80**	**9.02**	**10.80**	**17.62**		**47.20**	**103.83**	**183.52**	**377.48**		**55.00**	**112.85**	**194.32**	**395.11**	
	S	**T**	**S × T**			**S**	**T**	**S × T**			**S**	**T**	**S × T**		
S.Em. ±	0.22	0.32	0.63			1.61	2.28	4.55			1.63	2.30	4.61		
C.D. at 5 %	0.63	0.90	1.79			4.55	6.43	12.87			4.60	6.51	13.02		
C.V. %	9.72					4.43					4.21				

The values are mean of three replications

Where, T_1- (Control), T_2- (*Azatobacter*), T_3- (PSB), T_4- (*Pseudomonas*), T_5-(*Azatobacter*+ PSB), T_6- (*Azatobacter*+ *Pseudomonas*), T_7- (PSB + *Pseudomonas*), T_8- (*Azatobacter* + PSB + *Pseudomonas*), C.D.-Critical Difference, C.V.-Coefficient of Variance, S.Em.-Standard Error of Mean.

Table.3 Effect of plant growth promoting rhizobacteria (PGPR) on Chlorophyll A, B and total chlorophyll (mg.gm-1fr.wt.) of coriander (*C. sativum* L.) seedling

Treatments	Chlorophyll A (mg.gm^{-1}fr.wt.)					Chlorophyll B (mg.gm^{-1}fr.wt.)					Total Chlorophyll (mg.gm^{-1}fr.wt.)				
	DAG					DAG					DAG				
	5	10	15	20	Mean (T)	5	10	15	20	Mean (T)	5	10	15	20	Mean (T)
T_1	0.42	0.44	0.48	0.49	**0.46**	0.23	0.23	0.24	0.25	**0.24**	0.65	0.68	0.72	0.74	**0.69**
T_2	0.45	0.48	0.50	0.53	**0.49**	0.25	0.26	0.27	0.28	**0.26**	0.70	0.74	0.77	0.81	**0.75**
T_3	0.47	0.49	0.52	0.55	**0.51**	0.27	0.28	0.28	0.29	**0.28**	0.73	0.76	0.81	0.84	**0.79**
T_4	0.43	0.46	0.49	0.51	**0.47**	0.24	0.25	0.25	0.26	**0.25**	0.67	0.71	0.74	0.77	**0.72**
T_5	0.50	0.54	0.57	0.60	**0.55**	0.31	0.32	0.33	0.35	**0.33**	0.80	0.86	0.90	0.94	**0.88**
T_6	0.49	0.49	0.55	0.58	**0.53**	0.28	0.29	0.30	0.32	**0.30**	0.78	0.72	0.86	0.90	**0.82**
T_7	0.49	0.52	0.55	0.58	**0.54**	0.29	0.30	0.32	0.33	**0.31**	0.77	0.82	0.86	0.91	**0.84**
T_8	0.52	0.57	0.59	0.62	**0.58**	0.32	0.34	0.35	0.36	**0.34**	0.85	0.91	0.94	0.99	**0.92**
Mean (S)	**0.47**	**0.50**	**0.53**	**0.56**		**0.27**	**0.28**	**0.29**	**0.30**		**0.74**	**0.78**	**0.82**	**0.86**	
	S	**T**	**S × T**			**S**	**T**	**S × T**			**S**	**T**	**S × T**		
S.Em. ±	0.003	0.004	0.008			0.002	0.002	0.004			0.006	0.009	0.018		
C.D. at 5 %	0.008	0.011	N.S.			0.004	0.006	N.S.			0.018	0.025	N.S.		
C.V. %	2.51					2.57					3.82				

The values are mean of three replications

Where, T_1- (Control), T_2- (*Azatobacter*), T_3- (PSB), T_4- (*Pseudomonas*), T_5-(*Azatobacter*+ PSB), T_6- (*Azatobacter*+ *Pseudomonas*), T_7- (PSB + *Pseudomonas*), T_8- (*Azatobacter* + PSB + *Pseudomonas*), C.D.-Critical Difference, C.V.-Coefficient of Variance, S.Em.-Standard Error of Mean.

Root fresh weight, shoot fresh weight, and total biomass (mg)

Changes of root weight, shoot weight, and total biomass (mg) due to various treatment of plant growth promoting rhizobacteria (PGPR) during different growth stages are presented in Table.2. The data showed significant differences for growth stages, treatments, and interaction effect. The mean value for root weight, shoot weight, and total biomass at stage S_4 (17.62 mg), (377.48 mg) and (395.11 mg) respectively were found significantly highest. Irrespective of stages, mean treatment effect concerned, the significantly highest root weight, shoot weight, and total biomass were found for the treatment T_8 (15.02 mg), (250.70 mg) and (265.72mg) respectively. Mathivanan *et al.,* (2014) reported that the combination of plant growth promoting rhizobacteria enhanced the root weight. Jha and subramanian, (2013) also reported that the plants inoculated with PGPR showed higher dry weight. So far as interaction effect for the root weight, shoot weight, and total biomass were concerned, the combination of treatment, S_4T_8 (26.01 mg), (575.12 mg), and (601.13 mg) respectively recorded significantly highest value. The result of present experiment was in agreement with Zahid *et al.,* (2013).

They reported that integrated effect of PGPR and PSB along with chemical fertilizers has great significance for the improvement root weight at vegetative stage. It was also reported that the plant growth promoting rhizobacteria (PGPR) had a capacity to enhance plant growth by increasing seed emergence, plant growth and crop yield (Kloepper, 1992). The application of plant growth promoting rhizobacteria (PGPR) increases the shoot weight might be bacterial synthesis of plant hormones including indole-3-acetic acid, cytokinin and gibberellins as well as by increased mineral and nitrogen availability in the soil reported by several researchers (Rodriguez and Fraga, 1999; Sturz and Nowak, 2000; Sudhakar *et al.,* 2000; Karlidag *et al.,* 2007). PGPR applied in combination have the potential to increase the plant growth of *C. forskohlii* **(**Damam *et al.,* 2013). Integrated effect of PGPR and PSB along with chemical fertilizers has great significance for the improvement of soil fertility as well as to increase the plant growth and its biomass (Zahid *et al.,* 2013). It was also reported that the, PGPR can also increase plant growth, by associative N_2 fixation (Hong *et al.,* 1991), solubilizing nutrients such as P (Whitelaw, 2000), regulating ethylene production in roots (Glick, 1995) as well as by the releasing phytohormones (Arshad and Frankenberger, 1993).

Chlorophyll A, B and total Chlorophyll (mg.gm^{-1}fr.wt.)

Changes of chlorophyll A, B and total chlorophyll (mg.gm^{-1}fr.wt.) due to various treatment of plant growth promoting rhizobacteria (PGPR) during different growth stages were presented in Table 3. The data showed significant differences for growth stages and treatments. For interaction effect it was non-significant. The value for chlorophyll A, B and total chlorophyll for the stage S_4 were significantly highest (0.56 mg.gm-1fr.wt.), (0.30 mg.gm^{-1}fr.wt.), and (0.86 mg.gm^{-1}fr.wt.) respectively. In case of different treatments of PGPR irrespective of stages were concerned, the PGPR combination of (*Azatobacter* + PSB + *Pseudomonas*) T_8 recorded significantly highest value (0.58 mg.gm^{-1}fr.wt.), (0.34 mg.gm^{-1}fr.wt.), and (0.92 mg.gm^{-1}fr.wt.) respectively. PGPR strains alone or in combination increases the leaves chlorophyll content (Marius, *et al.,* 2013). The results of present experiments were in agreement with their studies.

In conclusion the use of chemical fertilizer having adverse effect on soil fertility, also they are expensive to buy compared to biofertilizer. In contrast to chemical fertilizer the use of plant growth promoting rhizobacteria (PGPR) as a biofertilizer having no side effect and it increases the crop yield individually or in combination. Among the studied eight treatments and four stages, treatment T_8 (*Azatobacter* + PSB + *Pseudomonas*) & stage 4 (20 DAG) are most effective that increased the vegetative growth parameter either in combination or alone compared to control treatment.

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