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The Effect of Plant Growth Promoting Rhizobacteria (PGPR) on Biochemical Parameters of Coriander (*Coriandrum sativum* L.) Seedling

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A B S T R A C T

Many, microorganisms playing an important role in plant growth are used in agriculture system, especially that group of microorganisms called plant growth promoting rhizobacteria (PGPR), which can increase the growth of plant directly and indirectly; acting as biofertilizers, phytostimulators and biocontrol agent. A pot experiment was conducted to evaluate the effect of inoculation of three plant growth promoting rhizobacteira (*Azatobacer*, PSB, *Pseudomonas*) either singly or in combination on biochemical parameters of coriander seedling. There were four different seedling stages 5 DAG, 10 DAG, 15 DAG, and 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 given three PGPR had significantly increased biochemical parameters such as moisture content, total phenol, true protein, Indole-3-acetia acid (IAA), total soluble sugar, and reducing sugar in comparison to the individual and control treatment. Our study suggests that PGPR are environmental friendly and offer sustainable approach to increase production of crop and heath. So PGPR will restrict the use of chemical fertilizer in agriculture area.

Introduction

India is recognized as the "Home of spices" in all over the world. Whenever we think about spices it immediately strikes our mind about the hot, pungent, aromatic and spicy Indian dishes and cuisine, which are now becoming increasingly popular in the western countries. Coriander (*Coriandrum sativum* L.) is an important seed spice crop belonging to the family Apiaceae (previously classified under the family Umbelliferae) with a diploid chromosome number 2n=22. Coriander displays broad adaptation as a crop around the world, growing well under many different types of soil and weather conditions (Guenther 1952; Purseglove *et al.*, 1981 and Simon, 1990). The green herb has high vitamin C, vitamin A, and vitamin B_2 content (Girenko, 1982 and Prakash, 1990). Coriander is extensively used in 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. Among the essential nutrients, Nitrogen (N) and Phosphorus (P) are the primary nutrients in the soil which play crucial role in improving plant growth (Mohamed *et al.*, 2011). Phosphorus is

another most growth limiting nutrient for plant growth (Ezawa, 2002). Phosphorus is called "Key to life" because it is directly involved in most living process. Biological fertilizers like phosphate solubilizing microorganism (PSM) and plant growth promoting rhizobacteria (PGPR) are considered among the most important plant helper microorganism to supply nutrient at a favourable level and these fertilizers are absorbed on the basis of selection of beneficial soil microorganisms which has the highest efficiency to enhance plant growth by providing nutrients in a readily absorbable form. Surrounding plant roots there is an extremely important and active area for root activity and metabolism which is known as rhizosphere (Garcia *et al*., 2001). Bacteria inhabiting the rhizosphere and beneficial to plants are termed plant growth promoting rhizobacteria – PGPR (Kloepper *et al*., 1980). A rhizobacteria is qualified as PGPR when it is able to produce a positive effect on the plant upon inoculation (Barriuso *et al*., 2008). These bacteria significantly affect plant growth by: providing the host plant with fixed atmospheric nitrogen (Zhang *et al.,* 1996), solubilization of soil phosphorus compounds (De Freitas *et al*., 1997), producing biologically active substances such as auxins and other plant hormones (Khalid *et al*., 2004), suppressing pathogens by producing antibiotics and siderophores (Khan and Almas, 2002). So the present investigation was planned to evaluate effect of PGPR on biochemical parameters of coriander seedling.

Materials 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. These soil sterilized in autoclave dried properly and used for pot trial. There were 24 Pots, each with 40 cm deep and 45 cm wide, having capacity 40 kg soil/pot. Experimental soil was calcareous in texture and slightly alkaline in reaction having normal electrical conductivity.

PGPR culture

Three plant growth promoting rhizobacteria (*Azatobacter*, PSB, 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, India.

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 month. Sufficient water is 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).

Biochemical parameters

Moisture

Seedling moisture was measured by weighing randomly selected five seedlings and they placed in hot air oven for drying. Finally these samples was weighed and calculated the difference between fresh and oven dried seedlings, AOAC, (2005).

Total phenol

Suitable aliquot (0.1 ml) of was taken from methanol extract prepared for total free amino acids analysis and evaporated to dryness in water bath. One ml of millipore water in each test tube and 0.5 ml of Folin Ciocalteu's phenol reagent (1:1 with water) was added and kept for 3 min. After this 2 ml of 20% Sodium carbonate was added and mixed thoroughly.

The tubes were placed in boiling water for exactly one minute and cooled in ice water. The absorbance was read at 650 nm against a reagent blank (Bray and Thorpe, (1954). A standard graph was prepared using pyrocatachol ranging between 10- concentrations. The amount of phenols present in the sample was calculated as –

Phenol ($mg.g^{-1}$) = Sample O.D. x Standard O.D. x Dilution factor

True protein

The method of Folin-Lowry (Lowry *et al*., 1951) was used to estimate the protein content in the supernatant of enzyme extracts. Suitable aliquot (0.2 ml) was taken and total 3 ml volume was made with distilled water. To that, 5.0 ml of reagent C (Prepared by mixing 50 ml of reagent A with 1 ml of reagent B; A: 2 % Sodium Carbonate in 0.1 N Sodium Hydroxide. B: 0.5 % Copper sulphate in 1 % Sodium Potassium tartrate) was added and mixed properly. After 10 minutes, 0.5 ml of reagent D (D: Folin Ciocalteau reagent diluted with distilled water in 1:1 ratio) was added, thoroughly mixed and kept for 30 minutes at room temperature. The absorbance was measured at 660 nm. The protein content was calculated by using Bovine serum albumin as standard.

Protein content ($mg.g^{-1}$) = Sample O.D. x Graph factor x Dilution factor

Free amino acid

Free amino acid content was estimated as described by Lee and Takahashi (1966). Suitable aliquots were taken and volume made up to 1 ml by adding distilled water. To this, 5 ml ninhydrin reagent (1 % ninhydrin in 500mM citrate buffer, pure glycerol, and 500 mM citrate buffer pH 5.5 in the ratio of 5:12:2) was added, mixed thoroughly and then, tubes were kept in a boiling water bath for 12 minutes. After that, the tubes were transferred to an ice bath for immediate cooling.

The tubes were brought to room temperature and the absorbance was measured at 530 nm. The free amino acid content was calculated from reference curve prepared using glycine (10-100 µg) as standard and expressed as appropriate.

Total free amino acids:

($mg.ml^{-1}$ or $mg.g^{-1}$) = Sample O.D. x Standard O.D. x Dilution Factor

Indole-3-acetic acid

IAA content was determined as per the method given by Mazumdar *et al.,* (2007). 0.5 gm seedling sample was extracted in 10 ml of 80% methanol. The Tube was incubated overnight at room temperature. Aliquot of 0.2, 0.4 ml was taken in test tubes and volume made to 1ml with D/W. In that tube 2ml of $FeHClO_4$ Solution was added and after 25 minutes reading was taken at 530 nm.

Total soluble sugar

Seedlings (100 mg) were extracted with 5 ml of 80% ethanol and centrifuged at 3000 rpm for 10 minutes. Extraction was repeated 4 times with 80% ethanol and supernatants were collected into 25 ml volumetric flasks. Final volume of the extract was made to 25 ml with 80 % methanol. The extract (0.3 ml) was pipetted into separate test tubes and the tubes were placed in a boiling water bath to evaporate the methanol. One ml of millipore water and 1ml of 5% phenol was added in each test tube. Then 5 ml of sulphuric acid was added. The tubes were allowed to cool in ice-bath for 10-15 minutes. The intensity of colour was read at 490 nm on spectrophotometer. A standard curve was prepared using 10 mg glucose per 100 ml distilled water (Hedge and Hofreiter, (1962).

Total soluble sugar ($mg.g^{-1}$) = Sample O.D. × Standard O.D. × Dilution factor

Reducing sugar

The dinitrosalicylic acid (DNSA) method was used to estimate the glucose and galacturonic acid released by cellulase, polygalacturonase and β-1,3 glucanase enzymes (Miller, 1972). A known volume of aliquot was taken in test tube and final volume of 1.0 ml adjusted with distilled water. To this 0.5 ml DNSA reagent (1g DNSA + 200 mg crystalline phenol + 50 mg sodium sulphite in 100 ml of 1% sodium hydroxide) was added and mixed properly. The content was heated in a boiling water bath for 5 min. When the contents of the tubes were still warm, 1.0 ml of 40% sodium potassium tartrate (Rochelle salt) solution was added. Cool it and final volume was made 5.0 ml with distilled water. After that the tubes were read at 540 nm using spectrophotometer. Reagent blank was also performed by addition of 1.0 ml of distilled water in place of enzyme aliquot. A known concentration of standard (0.5-2.5 µM) of glucose or galacturonic acid was carried out and was calibrated and expresses as appropriate.

Glucose/ galacturonic acid = Sample O.D. x Standard O.D. x Dilution Factor ($\mu M.mg^{-1}$ protein) $mg.ml^{-1}$ or $mg.g^{-1}$ protein

Results and Discussion

Moisture content

Changes of moisture content (%) due to various treatment of plant growth promoting rhizobacteria (PGPR) during different growth stages were presented in Table.1. The data showed significant differences for growth stages and treatments. For interaction effect it was non-significant. The value for the moisture content at different stages in a coriander seedling was varied from 89.27 % to 90.67 %. The data at stage S_1 (90.67 %) was found significantly highest. Stage S_4 (89.27 %) showed significantly lowest value indicates gains in dry matter with the growth of seedlings. So for as treatments and combinations, no clear cut trend was found for the moisture content. PGPR treatment increased moisture content in cabbage

seedling compared to control one (Metin *et al*., 2014).

Total phenol content, true protein, and free amino acids

Total phenol content (mg.g^{-1}%), true protein (%), and free amino acids (%) data varied due to various treatment of plant growth promoting rhizobacteria (PGPR) during different growth stages were presented in Table.2. The data showed significant differences for growth stages, treatments, and interaction effect. The total phenol content in a coriander seedling was varied from 0.254 to 0.295 mg.g-1%. The value for total phenol content was found for stage S_4 (0.295 mg.g^{-1}%) was significantly highest. Also reported that the plants growth promoting rhizobacteria (PGPR) induced the synthesis of specific phenolic acids, salicylic acid (SA) with varied amounts at different growth stages (Singh *et al*., 2003; Kandoliya and Vakharia, 2013). T_8 (*Azatobacter* + PSB + *Pseudomonas*) (0.299 mg.g^{-1}%) found significantly higher phenol content. In case of combination highest value was found for S_4T_8 (0.324 mg.g^{-1}%). Alireza Pazoki (2015) reported that the, PGPR (*Azospirillium*, *Azotobacter* and *Pseudomonas*) diminished flavonoids (22%) and increased phenols (17.9%). Marcela *et al*., (2014) also reported that the combination of PGPR increased the total phenol content.

The value for true protein varies from 3.74 to 4.41 %. Highest true protein content in a coriander seedling was recorded for stage S_4 (4.41 %). Stage S_1 recorded significantly lowest value for the true protein content. This indicates gain in protein content with the advancement of the growth stages. Irrespective of stages treatment T_8 (5.03 %) found significantly higher. Aishwath *et al*., (2012) observed that at 60 DAS the protein content was enhanced with individual and combine use of inoculants in coriander straw.

Table.1 Effect of Plant growth promoting rhizobacteria (PGPR) on moisture content of Coriander (*C. sativum* L.) seedling

Treatments	Stages				Mean T
	S_1 (5 DAG)	**S_2 (10 DAG)**	**S_3 (15 DAG)**	**S_4 (20 DAG)**	
T_1	90.13	89.48	89.25	88.92	**89.44**
T_2	90.49	89.71	89.54	89.11	**89.71**
T_3	90.58	89.85	89.70	89.23	**89.84**
T_4	90.28	89.59	89.41	89.01	**89.57**
T_5	91.01	90.31	90.02	89.55	**90.22**
T_6	90.75	89.98	89.80	89.34	**89.97**
T_7	90.92	90.13	89.87	89.43	**90.08**
T_8	91.17	90.49	90.15	89.58	**90.35**
Mean (S)	**90.67**	**89.94**	**89.72**	**89.27**	
	S	**T**	**S x T**		
S.Em.±	0.06	0.07	0.16		
C.D. at5%	0.16	0.20	N.S.		
C.V. %	0.38				

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 total phenol, true protein, and free amino acids of Coriander (*C. sativum* L.) seedling

Treatments	Total phenol (mg.g^{-1}%)					True protein (%)					Free amino acids (%)				
	DAG					DAG					DAG				
	5	10	15	20	Mean (T)	5	10	15	20	Mean (T)	5	10	15	20	Mean (T)
T_1	0.231	0.241	0.259	0.269	**0.250**	3.18	3.39	3.66	0.269	**3.52**	0.031	0.026	0.019	0.010	**0.022**
T_2	0.243	0.251	0.272	0.282	**0.262**	3.61	3.73	3.87	0.282	**3.82**	0.034	0.029	0.022	0.014	**0.025**
T_3	0.249	0.256	0.276	0.287	**0.267**	3.64	3.78	3.96	0.287	**3.90**	0.036	0.030	0.023	0.015	**0.026**
T_4	0.241	0.246	0.265	0.277	**0.257**	3.41	3.56	3.75	0.277	**3.66**	0.033	0.028	0.021	0.012	**0.024**
T_5	0.270	0.279	0.301	0.315	**0.291**	3.90	4.32	4.51	0.315	**4.38**	0.040	0.035	0.027	0.020	**0.031**
T_6	0.260	0.267	0.289	0.297	**0.278**	3.71	3.87	4.11	0.297	**4.01**	0.038	0.032	0.026	0.017	**0.028**
T_7	0.264	0.273	0.295	0.305	**0.284**	3.84	4.12	4.38	0.305	**4.26**	0.039	0.034	0.027	0.019	**0.030**
T_8	0.276	0.284	0.311	0.324	**0.299**	4.63	4.85	5.25	0.324	**5.03**	0.031	0.035	0.028	0.019	**0.031**
Mean (S)	**0.254**	**0.262**	**0.284**	**0.295**		**3.74**	**3.95**	**0.284**	**4.41**		**0.036**	**0.031**	**0.024**	**0.016**	
	S	**T**	**S × T**			**S**	**T**	**S × T**			**S**	**T**	**S × T**		
S.Em. ±	0.002	0.002	0.004			0.05	0.07	0.15			0.003	0.0003	0.001		
C.D. at 5 %	0.004	0.005	0.012			0.15	0.18	0.42			0.0007	0.0009	0.002		
C.V. %	3.17					5.00					5.30				

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 Indole-3- acetic acid, Total soluble sugar, and Reducing sugar of Coriander (*C. sativum* L.) seedling

Treatments	Indole acetic acid (µM.g^{-1})					Total soluble sugar (%)					Reducing sugar (%)				
	DAG					DAG					DAG				
	5	10	15	20	Mean (T)	5	10	15	20	Mean (T)	5	10	15	20	Mean (T)
T_1	4.5	6.0	7.0	9.5	**6.8**	0.32	0.33	0.35	0.37	**0.34**	0.06	0.06	0.08	0.09	**0.07**
T_2	8.5	10.5	11.5	14.1	**11.2**	0.35	0.36	0.37	0.40	**0.37**	0.07	0.07	0.09	0.12	**0.09**
T_3	10.5	12.5	13.5	16.1	**13.1**	0.36	0.38	0.39	0.41	**0.39**	0.07	0.07	0.09	0.14	**0.09**
T_4	7.0	8.5	9.0	12.4	**9.2**	0.34	0.36	0.37	0.40	**0.37**	0.06	0.07	0.09	0.10	**0.08**
T_5	16.5	18.5	21.6	22.5	**19.8**	0.40	0.43	0.46	0.48	**0.44**	0.08	0.08	0.12	0.18	**0.12**
T_6	13.0	14.5	15.5	33.1	**19.0**	0.38	0.40	0.41	0.44	**0.41**	0.07	0.08	0.10	0.16	**0.10**
T_7	15.0	16.5	18.1	22.3	**18.0**	0.39	0.41	0.43	0.47	**0.43**	0.08	0.08	0.11	0.17	**0.11**
T_8	18.0	20.0	22.5	36.5	**24.2**	0.49	0.44	0.47	0.60	**0.50**	0.08	0.10	0.13	0.19	**0.13**
Mean (S)	**11.6**	**13.4**	**14.8**	**20.8**		**0.38**	**0.39**	**0.41**	**0.44**		**0.07**	**0.08**	**0.10**	**0.14**	
	S	**T**	**S × T**			**S**	**T**	**S × T**			**S**	**T**	**S × T**		
S.Em. ±	0.2	0.2	0.5			0.012	0.015	0.034			0.002	0.002	0.005		
C.D. at 5 %	0.6	0.6	1.4			0.034	0.043	0.096			0.005	0.006	0.013		
C.V. %	6.41					9.01					7.43				

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

So far as mean free amino acid value for growth stages was concerned, it varied between 0.016 to 0.036 %. At stage S_1, in a coriander seedling the highest mean free amino acid content was recorded 0.036 % which gradually decreased to 0.016 % at the 20 DAG i.e., S_4 stage. For treatments mean free amino acid value varies between 0.016 to 0.036 %. At stage S_1, in a coriander seedling the highest mean free amino acid content was recorded 0.036 % which gradually decreased to 0.016 % at the 20 DAG i.e., S_4 stage. Irrespective of stages and treatments the highest value was recorded in a combination of S_1T_8 (0.040 %). The data was in agreement with Ahmed *et al.,* (2014). They reported that the effects of PGPR as seed inoculants, and white willow **(***Salix alba*) extract as foliar and seed treatment in faba bean plants against (BYMV) increased the free proline content in comparison with control plants.

Indole acetic acid, total soluble sugar, and reducing sugar

The changes in indole acetic acid content ($\mu M.g^{-1}$), Total soluble sugar (%), and reducing sugar (%) due to various treatment of plant growth promoting rhizobacteria (PGPR) during different growth stages in coriander seedlings were presented in Table.3. The data showed significant differences for growth stages, treatments, and interaction effect. The mean IAA value for growth stages, treatments, and their combinations varied from 11.6 to 20.8, 6.8 to 24.2, and 4.5 to 36.5 ($\mu M.g^{-1}$) respectively. Mean highest IAA for growth stages, treatments, and their combination found in stage S_4 (20.8 $\mu M.g^{-1}$), Treatment T_8 (24.2 $\mu M.g^{-1}$), and S_4T_8 (36.5 μM.g-1) respectively. IAA is the most quantitatively important phytohormone produced by PGPR (Vessey, 2003). The mean Total soluble sugar value for growth stages, treatments, and their combinations varied from 0.380 to 0.445, 0.344 to 0.501, and 0.318 to 0.602 (%) respectively. The mean highest Total soluble sugar for growth stages, treatments, and their combination found in stage S_4 (0.445 %), Treatment T_8 (0.501 %), and S_4T_8 (0.602 %) respectively. Hafsa *et al.,* (2014) reported that under drought stress PGPR application in maize increased the total soluble sugar.

So far as mean reducing sugar value for growth stages was concerned, it varied between 0.071 to 0.145 %. At stage S_1, in a coriander seedling the lowest mean reducing sugar content was recorded 0.071 % which gradually increased to 0.145 % at the 20 DAG i.e., S_4 stage. In case of mean value of reducing sugar for the treatments irrespective of stage was concerned, the highest value recorded from the treatment having combination of 3 PGPR, i.e., T_8 (0.126 %). The highest value was recorded in a combination of S_4T_8 (0.192 %). Marius *et al.,* (2013) reported that the PGPR strains improve the nutritive value of the harvested runner bean grains by enhancing the total reducing carbohydrates content up to 49.28%.

In conclusion 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. Author studied eight treatments and four growth stages among these treatment T_8 (*Azatobacter* + PSB + *Pseudomonas*) & stage 4 (i.e. 20 DAG) are most effective that increased the biochemical parameters in coriander seedling either singly or in combination compared to control treatment.

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