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Consortium of plant growth promoting rhizobacteria improves growth, yield and nutrient uptake in spinach (*Spinacia oleracea*)

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Abstract
A field experiment was conducted at the experimental farm of Department of Plant Pathology and Agricultural Microbiology, College of Agriculture, Pune (Maharashtra, India), during the *kharif* season of year 2020, to study the effect of consortium of plant growth promoting rhizobacteria (PGPR) (*Azotobacter chroococcum, Pseudomonas fluorescens, Bacillus subtilis, Bacillus megaterium, Frateuria aurantia*) on growth and yield of spinach (*Spinacia oleracea*). Results of the investigation explicitly unveiled that, the seed inoculation with consortium of PGPR consisting of *A. chroococcum, P. fluorescens, B. subtilis, B. megaterium* and *F. aurantia* in conjunction with application of 75% of recommended dose of chemical fertilizers (RDF) was the most effective in improving growth, yield and nutrient uptake in spinach. This treatment had significantly the highest plant height (21.50 cm) and number of leaves (10) at 15 days after sowing. Observations recorded at 30 days after sowing as well indicated that, this treatment had the highest plant height (27.67 cm), number of leaves (18.67), fresh plant weight (99.50 g/plant), fresh shoot and root weight (67.80 and 31.10 g/plant). The treatment also recorded minimum days for crop maturity (28.33 days) and yielded the highest yield (95.89 q/ha). In regard to nutrient uptake, this treatment was found most effective in improving N, P and K uptake (62.67, 22.67 and 37.23 kg/ha, respectively) by plants. However, this treatment did not differ significantly from the treatment consisting of seed inoculation with consortium of *A. chroococcum, P. fluorescens, B. subtilis, B. megaterium, F. aurantia* in conjunction with application of 100% RDF and, the treatment of application of RDF only, in augmenting growth and yield of spinach. These three treatments, thus, were found to be significantly superior over rest of the treatments in improving growth parameters and yield attributes of the crop. The results explicitly substantiate that, the treatment comprising of seed inoculation with consortium of *A. chroococcum, P. fluorescens, B. subtilis, B. megaterium, F. aurantia* in conjunction with application of 75% of recommended dose of fertilizers (RDF) saves 25% of recommended dose of nitrogen, phosphorus and potassium in the cultivation of spinach.

Keywords: Consortium, *Azotobacter chroococcum, Pseudomonas fluorescens, Bacillus subtilis, Bacillus megaterium, Frateuria aurantia*, spinach

Introduction
The spinach (*Spinacia oleracea*) is a very popular leafy vegetable and is largely grown and consumed in India. This crop is commonly known as *palak*, in India, which is mainly cultivated for its fresh leaves. The leaves are bright green, fleshy, lustrous and are accepted by the large population in India and other parts of the world as leafy vegetable. In the nutritional context, 100 g edible spinach contains energy 97 kJ (23 kcal), carbohydrates 3.6 g, sugars 0.4 g, dietary fiber 2.2 g, fat 0.4 g, protein 2.9 g, water 91.4 g, vitamin A equiv. 469 µg (59%), vitamin A 9377 IU, Beta-carotene 5626 µg (52%), Lutein and zeaxanthin 12198 µg, thiamine (vit. B1) 0.078 mg (7%), riboflavin (vit. B2) 0.189 mg (16%), niacin (vit. B3) 0.724 mg (5%), vitamin B6 0.195 mg (15%), folate (vit. B9) 194 µg (49%), vitamin C 28 mg (34%), vitamin E 2 mg (13%), vitamin K 483 µg (460%), calcium 99 mg (10%), iron 2.71 mg (21%), magnesium 79 mg (22%), manganese 0.897 mg (43%), phosphorus 49 mg (7%), potassium 558 mg (12%), sodium 79 mg (5%) and zinc 0.53 mg (6%).
Nowadays the continuous use of chemical fertilizers creates an imbalance among the beneficial activities of microorganisms and thereby indirectly affects the biological properties of soil leading to deterioration of soil quality. Moreover, due to increased cost of chemical fertilizers, marginal farmers cannot afford to use these fertilizers. Currently, it is urgent to maintain high productivity impacting the environment as little as possible (Montano *et al*., 2014) [9]. In this context, plant growth promoting rhizobacteria (PGPR) is an excellent

alternative to farmers to face the new challenges of modern agriculture, as serious environmental and social problems emerged as a consequence of industrialization of agriculture provoked by necessity to increase a great amount of food to the general population. PGPR represent a wide variety of soil bacteria, which when grown in association with a host plant, result in stimulation of host growth. PGPR modes include fixing atmospheric N_2, increasing availability of nutrients in the rhizosphere, positively influencing root growth and morphology and, promoting other beneficial plant–microbe symbioses. Use of PGPR is one of the potential ways to decrease negative environmental impacts, resulting from continued use of chemical fertilizers, pesticides and herbicides. The term PGPR was first defined by Kloepper and Schroth (1978) [6] to describe soil bacteria that colonize the rhizosphere of plants, growing in, on or around plant tissues that stimulate plant growth by several mechanisms. Since that time, research activities aimed at understanding how these bacteria perform their positive (or negative) effect have steadily increased and many studies have been published on these microorganisms (Montano *et al*., 2014) [9].

Considering the facts mentioned in the foregoing paragraphs, it was thought worthwhile to appraise the effect of consortium of PGPR, in conjunction with different doses of chemical fertilizers, in augmenting growth, yield and nutrient uptake in spinach (*Spinacia oleracea*).

Materials and Methods

A field experiment was conducted at the experimental farm of Department of Plant Pathology and Agricultural Microbiology, College of Agriculture, Pune (Maharashtra, India), during the *kharif* season of year 2020, to study the effect of consortium of plant growth promoting rhizobacteria (PGPR) (*Azotobacter chroococcum, Pseudomonas fluorescens, Bacillus subtilis, Bacillus megaterium, Frateuria aurantia*) in improving growth and yield of spinach (*Spinacia oleracea*). The experiment was laid out in RBD (Randomized Block Design) with eleven treatments and three replications. The treatments were: T_1: seed treatment (ST) with consortium of PGPR, T_2: ST with consortium of PGPR + 100% NPK, T_3: ST with consortium of PGPR + 100% N +75% P&K, T_4: ST with consortium of PGPR + 100% N +50% P&K, T_5: ST with consortium of PGPR + 75% N +100% P&K, T_6: ST with consortium of PGPR + 75% NPK, T_7: ST with consortium of PGPR + 75% N +50% P&K, T_8: ST with consortium of PGPR + 50% N +100% P&K, T_9: ST with consortium of PGPR + 50% N +75% P&K, T_{10}: ST with consortium of PGPR + 50% NPK, T_{11}: recommended dose of chemical fertilizers (RDF). The lignite based powder formulation of PGPR consortium consisted of *A. chroococcum, P. fluorescens, B. subtilis, B. megaterium* and *F. aurantia.* The seeds of spinach were treated with the consortium at the rate of 25 g/kg seed. The spinach cv. All Green was used as a test crop. The crop was sown by maintaining 15 cm distance between rows. The chemical fertilizers were applied, as per the treatments, at the rate of 40:40:40 NPK kg/ha. The cultural operations like irrigation, weeding were uniformly carried out to all the treatments. The observations were recorded, at 15 and 30 days after sowing, on plant height (cm), number of leaves, fresh weight per plant (g/plant), fresh weight of shoot (g/plant), days required for maturity, yield per plot (kg/plot), yield per hectare (q/ha). For recording observations, five plants per plot were randomly selected. The statistical analysis was carried out by using OPSTAT statistical analysis tool (*www.hau.ernet.in*) [13].

Results and Discussion

Results of the present investigation pertaining to various growth and yield attributes of spinach (*Spinacia oleracea*) as influenced by inoculation with consortium of *Azotobacter chroococcum, Pseudomonas fluorescens, Bacillus subtilis, Bacillus* megaterium and *Frateuria aurantia* in conjunction with graded levels of recommended dose of chemical fertilizers are discussed below under different sub-headings.

Plant height

The results pertaining to plant height of spinach at 15 and 30 days after sowing (DAS), as influenced by inoculation with consortium of *A. chroococcum, P. fluorescens, B.subtilis, B. megaterium* and *F. aurantia,* are presented in Table 1. Results of the investigation explicitly revealed that, among the different treatments, seed inoculation with consortium of PGPR + 75% of recommended dose of fertilizers (RDF) was the most effective, as it recorded significantly the highest plant height (21.50 cm at 15 DAS, 27.67 cm at 30 DAS) in comparison to rest of the treatments. However, plant height recorded with treatment was statistically at par with those of the treatments comprising of seed inoculation with consortium of PGPR + 100% RDF (21.00 cm at 15 DAS, 27.17 cm at 30 DAS) and, application of RDF (20.67 cm at 15 DAS, 26.83 cm at 30 DAS). The lowest plant height at 15 DAS (11.67 cm) and 30 DAS (16.33 cm) of the crop was noticed in the treatment of consortium alone. The results obtained in the present investigation are in close confirmation with findings of Paithankar and Gore (2019) [10], Yadav *et al.* (2019) [14], Ali *et al.* (2013) [1] who reported increased plant height in Indian spinach, whereas Sahu *et al.* (2013) [12] found similar results in coriander.

Table 1: Effect of consortium of PGPR on growth parameters and days required for maturity of spinach

Treatment	Plant height (cm)		No. of leaves/plant		Days required for crop maturity
	15 DAS	30 DAS	15 DAS	30 DAS	
T_1 - Consortium	11.67	16.33	5.67	9.33	34.00
T_2 - Consortium + 100% NPK	21.00	27.17	9.67	18.33	28.67
T_3 - Consortium + 100% N + 75% PK	17.33	22.50	8.00	16.67	29.00
T_4 - Consortium + 100% N + 50% PK	16.83	21.83	8.00	16.33	29.33
T_5 - Consortium + 75%N + 100% PK	16.33	21.50	7.67	15.67	29.67
T_6 - Consortium + 75% NPK	21.50	27.67	10.00	18.67	28.33
T_7 - Consortium + 75% N + 50% P&K	15.83	21.00	7.67	14.33	30.33
T_8 - Consortium + 50% N + 100% P&K	15.67	20.00	7.33	14.00	30.33
T_9 - Consortium + 50% N + 75% P&K	15.17	19.00	7.00	12.67	31.67
T_{10} - Consortium + 50% NPK	14.00	18.17	7.00	12.00	32.67
T_{11} - Recommended dose of fertilizer	20.67	26.83	9.33	18.00	29.00
S.E(m)±	0.51	0.37	0.40	0.37	0.42
CD at 5%	1.51	1.09	1.18	1.08	1.24

Number of leaves

The results pertaining to number of leaves of spinach recorded at 15 DAS and 30 DAS as influenced by inoculation with consortium of *A. chroococcum*, *P. fluorescens*, *B. subtilis*, *B. megaterium* and *F. aurantia* are presented in Table 1. Results of the investigation clearly indicated that, among the different inoculation treatments, the treatment comprising of seed inoculation with consortium of PGPR + 75% RDF was the most effective, as it recorded significantly maximum number of leaves at 15 DAS (10.00) and 30 DAS (18.67) of the crop in comparison to rest of the treatments. However, it was statistically at par with those recoerded in the treatments comprising of seed inoculation with consortium of PGPR + 100% RDF (9.67 at 15 DAS, 18.33 at 30 DAS) and, the treatment consisting of application of RDF (9.33 at 15 DAS, 18.00 at 30 DAS). Least number of leaves, at 15 DAS (5.67) and 30 DAS (9.33) of the crop, was noticed in the treatment wherein the seeds were treated with consortium of PGPR. Results obtained in the present experimentation are in agreement with the findings of Jat *et al.* (2006) [3] and, Mehta *et al.* (2010) [8] on fenugreek; Kalidasu *et al.* (2008) [4] and, Patidar *et al.* (2016) [11] on coriander and, Paithankar and Gore (2019) [10] in Indian spinach.

Days required for maturity of spinach

Data pertaining to days required for maturity of spinach, as influenced by inoculation with consortium of *A. chroococcum*, *P. fluorescens*, *B. subtilis*, *B. megaterium* and *F. aurantia* are presented in Table 1. Data of the investigation explicitly indicated that, among the different treatments, a treatment comprising of seed inoculation with consortium of PGPR + 75% of RDF was the most effective, since it recorded significantly least days (28.33) for maturity of the crop, in comparison to rest of the treatments. However, this treatment was statistically at par with the treatments comprising of seed inoculation with consortium of PGPR + 100% RDF (28.67 days), seed inoculation with consortium of PGPR + 100% N and 75% P&K (29.00 days), RDF (29.00 days) and, seed inoculation with consortium of PGPR + 100%N and 50% P&K (29.33 days). The maximum days (34.00 days) were required for maturity of the crop in the treatment comprising of seed inoculation with consortium only. The results obtained in the present experiementation are in close confirmation with findings of Paithankar and Gore (2019) [10], Yadav *et al.* (2019) [14], Ali *et al.* (2013) [1], who reported similar observations in Indian spinach, whereas Meena *et al.* (2015) [7] reported similar observations in fenugreek.

Fresh plant weight

The results with regard to fresh weight of whole plant of spinach as influenced by inoculation with consortium of *A. chroococcum*, *P. fluorescens*, *B. subtilis*, *B. megaterium*, *F. aurantia* are presented in Table 2. Among different inoculation treatments appraised, inoculation with consortium + 75% RDF was found to be the most effective, as it recorded significantly maximum fresh weight of whole plant (99.50 g) over rest of the treatments. However, fresh plant weight recorded with this treatment did not differ significantly from those recorded with the treatments comprising of inoculation with consortium + 100% RDF (98.67 g) and, RDF alone (98.50 g). Minimum fresh weight of whole plant was noticed in the treatment of consortium alone (71.60 g). The results obtained are in close confirmation with findings of Paithankar and Gore (2019) [10], Yadav *et al.* (2019) [14], Ali *et al.* (2013) [1] who reported increased fresh weight of whole plant in spinach whereas, Sahu *et al.* (2013) [12] reported similar results in coriander.

Fresh shoot weight

Results pertaining to fresh shoot weight per plant as influenced by inoculation of consortium of *A. chroococcum*, *P. fluorescens*, *B. subtilis*, *B. megaterium*, *F. aurantia* are presented in Table 2. Among different inoculation treatments tried in the investigation, inoculation with consortium + 75% RDF was found to be most effective as it recorded significantly maximum fresh shoot weight (67.80 g/plant) over rest of the treatments. However, fresh shoot weight recorded with this treatment was statistically at par with those of the treatments consisting of consortium + 100% RDF (67.70 g/plant), consortium + 100% N + 75% P and K (66.60 g/plant) and, RDF (67.50 g/plant). Minimum fresh shoot weight was noticed in the treatment of consortium alone (42.90 g/plant).

Table 2: Effect of consortium of PGPR on fresh shoot and root weight of spinach

Treatment	Fresh plant weight (g/plant)	Fresh shoot weight (g/plant)	Fresh root weight (g/plant)
T_1 - Consortium	71.60	42.90	28.10
T_2 - Consortium + 100% NPK	98.67	67.70	30.97
T_3 - Consortium + 100% N + 75%PK	97.50	66.60	30.90
T_4 - Consortium + 100% N + 50% PK	96.60	65.70	30.83
T_5 - Consortium + 75%N + 100% PK	94.77	64.76	30.00
T_6 - Consortium + 75% NPK	99.50	67.80	31.10
T_7 - Consortium + 75% N + 50% P&K	90.63	60.95	29.65
T_8 - Consortium + 50% N + 100% P&K	86.17	56.07	30.00
T_9 - Consortium + 50% N + 75% P&K	80.00	51.20	28.73
T_{10} - Consortium + 50% NPK	77.47	49.37	28.7
T_{11} - Recommended dose of fertilizer	98.50	67.50	31.00
S.E(m)±	0.67	0.65	0.27
CD at 5%	1.96	1.92	0.79

Fresh root weight

Results in regard to fresh root weight per plant as influenced by inoculation with consortium of *A. chroococcum, P. fluorescens, B. subtilis, B. megaterium, F. aurantia* are presented in Table 2. Among the different inoculation treatments evaluated in the present investigation, inoculation with consortium + 75% RDF recorded significantly highest fresh root weight (31.10 g/plant) over rest of the treatments. Nevertheless, this treatment was statistically at par with the treatments comprising inoculation with consortium + 100% RDF (30.97 g/plant), RDF (31.00 g/plant), consortium + 100% N + 75% P and K (30.90 g/plant) and, consortium + 100% N + 50% P and K (30.83 g/plant). The lowest fresh root weight was noticed in the treatment of consortium only (28.10 g/plant).

Yield

Results pertaining to yield of spinach as influenced by inoculation with consortium of *A. chroococcum, P. fluorescens, B. subtilis, B. megaterium, F. aurantia* are presented in Table 3. Among the different inoculation treatments evaluated in the investigation, the treatment comprising of seed inoculation with consortium + 75% RDF was found to be most effective as it recorded significantly the highest yield (95.89 q/ha) over rest of the treatments. However, the yield obtained with this treatment was statistically at par with those of treatments consisting of seed inoculation with consortium + 100% RDF (94.46 q/ha) and, RDF alone (93.90 q/ha). The least yield of the crop was obtained in the treatment of consortium only (75.01 q/ha). The results of the present investigation are in conformity with those of Assiouty and Abo-sedera (2005) [2] in spinach, Kavitha *et al.* (2013) [5] in amaranthus, Sahu *et al.* (2013) [12] in coriander, Paithankar and Gore (2019) [10], Yadav *et al.* (2019) [14], Ali *et al.* (2013) [1] in spinach and, Meena *et al.* (2015) [7] in fenugreek.

Table 3: Effect of consortium of PGPR on yield of spinach and available N, P&K in soil after harvest

Treatment	Yield (q/ha)	Available NPK (kg/ha) in soil after harvest		
		N	P	K
T_1 - Consortium	75.01	173.08	13.17	270.17
T_2 - Consortium + 100% NPK	94.46	201.75	27.17	329.39
T_3 - Consortium + 100% N + 75%PK	91.85	198.00	24.33	325.39
T_4 - Consortium + 100% N + 50% PK	90.18	197.92	22.00	320.33
T_5 - Consortium + 75%N + 100% PK	89.62	197.33	23.00	325.90
T_6 - Consortium + 75% NPK	95.89	199.75	26.00	328.67
T_7 - Consortium + 75% N + 50% P&K	88.34	197.20	22.33	319.50
T_8 - Consortium + 50% N + 100% P&K	86.67	190.00	23.00	325.00
T_9 - Consortium + 50% N + 75% P&K	85.84	188.00	24.67	325.83
T_{10} - Consortium + 50% NPK	83.34	187.17	21.33	314.33
T_{11} - Recommended dose of fertilizer	93.90	198.42	26.17	329.17
SE (m)±	0.89	1.74	1.46	2.55
CD at 5%	2.62	5.12	4.31	7.51

Available NPK in soil after harvest

After harvest of the spinach, soil was analyzed for available soil nutrients and the results regarding available NPK in soil as influenced by inoculation with consortium of *A. chroococcum, P. fluorescens, B. subtilis, B. megaterium, F. aurantia* are presented in Table 3. Among the different inoculation treatments, inoculation of spinach with consortium + 100% RDF recorded significantly highest available NPK (201.75, 27.17 and 329.39 kg/ha, respectively) in soil over rest of the treatments. However, in respect of N content in soil, this treatment was statistically at par with the treatments comprising of inoculation with consortium + 75% RDF, consortium + 100% N +75% P&K, consortium + 100% N + 50% P&K, consortium + 75%N + 100% P&K, consortium + 75%N + 50% P&K and, RDF alone. Whereas, in regard to P and K it was statistically at par with the treatments of consortium + 75% RDF, consortium + 100% N + 75% P&K, consortium + 75%N + 100% P&K, consortium + 50% N + 100% P&K, consortium + 50%N + 75% P&K and, RDF alone. The lowest available NPK in soil after the crop harvest was noticed in the treatment of consortium alone (173.08, 13.17 and 270.17 kg/ ha, respectively).

Nutrient uptake by crop

Results in respect of nutrient uptake by spinach plant as influenced by inoculation with consortium of *A. chroococcum, P. fluorescens, B. subtilis, B. megaterium, F. aurantia* are presented in Table 4. Among the different inoculation treatments tried in the investigation, inoculation with consortium + 75% RDF was found to be most effective as it recorded significantly the highest NPK uptake (62.67, 22.67 and 37.23 kg/ha, respectively) over rest of the treatments. However, it was statistically at par with those recorded with the treatments of inoculation with consortium + 100% RDF, consortium + 100% N +75% P&K, consortium + 100%N + 50% P&K, consortium + 75%N + 100% P&K and, RDF alone. The lowest NPK uptake by the spinach plants was noticed in the treatment consisting of inoculation with consortium alone (42.07, 14.67 and 24.67 kg /ha, respectively).

Table 4: Effect of consortium of PGPR on nutrient uptake by spinach

Treatment	Nutrient uptake (kg/ha) by plants		
	N	P	K
T_1 - Consortium	42.07	14.67	24.67
T_2 - Consortium + 100% NPK	61.33	22.33	37.00
T_3 - Consortium + 100% N + 75%PK	59.67	19.27	35.50
T_4 - Consortium + 100% N + 50% PK	58.00	18.17	34.33
T_5 - Consortium + 75%N + 100% PK	57.17	19.00	33.00
T_6 - Consortium + 75% NPK	62.67	22.67	37.23
T_7 - Consortium + 75% N + 50% P&K	55.33	17.67	32.33
T_8 - Consortium + 50% N + 100% P&K	54.00	18.33	29.90
T_9 - Consortium + 50% N + 75% P&K	52.83	18.00	29.33
T_{10} - Consortium + 50% NPK	50.67	16.67	28.67
T_{11} - Recommended dose of fertilizer	61.00	22.00	36.67
SE (m)±	2.11	0.66	1.67
CD at 5%	6.22	1.94	4.93

Conclusion

From the present investigation it can be concluded that the inoculation of spinach seeds with consortium of *A. chroococcum, P. fluorescens, B. subtilis, B. megaterium, F. aurantia* in conjunction with application of 75% RDF is effective for getting higher yield with 25% saving in nitrogenous, phosphatic and potassic chemical fertilizers.

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