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Combined Use of Microbial and Synthetic Amendments can Improve Radish (*Raphanus sativus*) Yield

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Abstract: The use of beneficial microbes, generally known as Plant Growth Promoting Rhizobacteria (PGPRs), decreases the dependency on the synthetic fertilizers in leguminous crops and some non-leguminous crops. This experiment was conducted at Institute of Horticultural Sciences, University of Agriculture, Faisalabad during 2014, to evaluate the effect of seed treatment with PGPRs viz., Azotobacter spp., Phosphorus solubilizing bacteria (PSB), Germinator (Ger; a synthetic germination and early growth enhancer) and PSB+Ger in combination with full (recommended dose of fertilizer; RDF), half dose of nitrogen, and half dose of phosphorus on yield of radish (*Raphanus sativus* L.) cv. 'Mino Early'. The results showed that Azotobacter spp. improved plants and yield related attributes, while germinator negatively affected them. Number of leaves per plant, root fresh weight, and marketable yield were significantly high owing to the combined application of PSB and RDF. While, leaf fresh weight, above ground plant biomass, biological yield, agronomic efficiency, and yield response was at the highest point in response to the application of Azotobacter spp. combined with a half dose of nitrogen as well as with a half dose of phosphorus. Moreover, root diameter increased when RDF was used along with PSB or Azotobacter spp., while plants treated with Azotobacter spp. along with a half dose of phosphorus had the longest roots. Correlation analysis revealed that marketable yield of radish was dependent on root fresh weight. Furthermore, agronomic efficiency and yield response to any treatment can be reliably estimated by root fresh weight of radish.

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1. Introduction

Sustainable crop production depends on good soil health. Soil health maintenance warrants optimum combination of organic and inorganic components of the soil. Nitrogen and phosphorus are essential plant nutrients and are widely used to increase the yield of crops (Reinhold-Hurek and Hurek, 2003). However, the use of elevated doses of nitrogenous fertilizers may cause negative and unpredictable effects on the environment, and contributes to the contamination of underground water and pose a serious threat to human and animal health. Phosphorus does not leach down like nitrogen, but it become insoluble and unavailable to plants (Antouna, 2012). Moreover, the cost of production can increase by excessive use of fertilizers.

In nature, there are a number of useful soil microorganisms such as bacteria, fungi, protozoa and algae, which coexist in the rhizosphere and are able to exert a beneficial effect on plant growth. Those microorganisms prevailing in the soil and benefiting the crop growth are termed as PGPRs. Among them, there are different strains of genera such as *Pseudomonas*, *Azospirillum*, *Azotobacter*, *Burkholderia*, *Bacillus*, *Enterobacter*, *Rhizobium*, *Erwinia*, *Serratia*, *Arthrobacter*, *Acinetobacter* and *Flavobacterium*. These are capable of promoting plant growth through different mechanisms, such as biological nitrogen fixation (BNF), phytohormone production (Naveed et al., 2014), phosphate solubilization (Chattopadhyay et al., 2007), and siderophore production (Karl et al., 2002). Their application has increased growth and yield (Shanthi et al., 2012) and also helped in preventing diseases by producing antibiotics and inducing the general resistance against diseases (do Vale Barreto Figueiredo et al., 2010). Strains of *Pseudomonas* and *Azotobacter* have increased root and shoot elongation in canola, lettuce, and tomato (Glick et al., 1997). Crop yield was increased by inoculation of the seed with Phosphorus solubilizing bacteria (PSB) before sowing (Chattopadhyay et al., 2007).

Radish (*Raphanus sativus* L.) is a winter vegetable crop that was cultivated on 10153 hectares with the production of 168257 tonnes during 2013-2014 (MNFSR, 2015). It is consumed as an appetizer and used as a salad. It has the fleshy root of different sizes, colour, and shape. It provides a considerable amount of nutrients (calcium, sodium, phosphorus and potassium), and is rich in carbohydrates and fiber. Both roots and leaves of radish are a good source of ascorbic acid. Farmers are usually not willing to spend much more money for fertilizer application in radish because of the uncertainty of the market value of their produce. Moreover, to make its cultivation profitable, it is necessary to decrease the cost of production.

Although radish is a non-leguminous crop, yet the use of PSB has been reported that enhance root yield and productivity of radish (Chattopadhyay et al., 2007). Previously, we evaluated several PGPRs to enhance root yield of radish and found Azotobacter spp. and Phosphorus solubilizing bacteria (PSB) have a significant effect (Asghar, 2013). But, it was unclear from previous studies (Chattopadhyay et al., 2007; Asghar, 2013) that whether the use of Azotobacter spp. and PSB can substantially reduce the need of synthetic fertilizers or not. Therefore, this study was conducted to study the effect of seed inoculation with different PGPRs, in combination with a recommended or a half dose of nitrogen and phosphorus on growth and yield of radish crop.

2. Materials and Methods

2.1 Site and Materials

This study was conducted at the Vegetable Farm, Institute of Horticultural Sciences, University of Agriculture, Faisalabad, Pakistan (latitude 31°30 N, longitude 73°10 E and altitude 213 m). Soil analysis of the area showed that pH, EC, N, P, K, and organic matter, was 8.2, 0.32 dS/m, 0.03%, 3.01 ppm, 173 ppm and 1.19%, respectively. Seeds of radish cultivar ‘Mino Early’ were treated with Germinator (a synthetic germination enhancer; a product of NFC Institute, Faisalabad, Pakistan) and PGPRs (Azotobacter spp., Phosphorus solubilizing bacteria [PSB] obtained from Institute of Soil Chemistry and Environmental Sciences, AARI, Faisalabad, Pakistan) and a combination of PSB+Germinator. Azotobacter spp. and PSB were selected on the basis of our previous experiment in which several PGPRs were used for inoculation of two radish cultivars (Mino Early and 40 days) (Asghar, 2013).

2.2 Treatments and Experimental Layout

Slurry of compost containing the inoculum(s) was prepared using sugar solution in which seed were mixed and stirred gently so that seed get coated with the slurry. After coating, seeds were surface dried and then sown in the field. Treated and untreated seeds were sown during the first fortnight of October, 2014. The crop was fertilized with recommended dose of nitrogen and phosphorus (i.e. N & P @ 25 and 20 kg per acre, respectively), 50% of recommended dose of nitrogen+full recommended dose of phosphorus (12.5 and 20 kg per acre, respectively), or the full recommended dose of nitrogen and 50% of recommended dose of phosphorus (25 and 10 kg per acre, respectively). The levels of two factors along with their levels, which were tested in this study, are described below.

Factor I (Levels of Fertilizers): Recommended dose of fertilizer (N& P @ 25 and 20 kg per acre, respectively). ½ dose of nitrogen (N& P @ 12.5 and 20 kg per acre, respectively). ½ dose of phosphorus (N& P @ 25 and 10 kg per acre, respectively).

Factor II (Seed Treatments): Control, *Azotobacter spp.*, Phosphorus solubilizing bacteria (PSB), Germinator (Ger), PSB+Germinator (PSB+Ger).

The whole quantity of phosphorus and potassium (@ 25 kg per acre) while half of the nitrogen was applied at the time of land preparation while, remaining half of the nitrogen was applied at the after 20 to 25 days of sowing.

The experimental layout was according to the randomized complete block design under the factorial arrangement with three replications. All recommended cultural practices were carried out and the crop was harvested during the second fortnight of December 2014.

Data Collection and Statistical Analysis: Data was collected for number of leaves per plant, length of leaves and roots (edible), diameter of roots (edible), fresh weight of leaves and root, biological yield (kg ft^{-2}), marketable yield (kg ft^{-2}), agronomic efficiency (Jatav et al., 2012), and yield response (Jatav et al., 2012).

$$\text{Agronomic efficiency (kg/kg)} = \frac{\text{Root yield in fertilized plot} - \text{Root yield in unfertilized plot}}{\text{Quantity of total nutrient applied}}$$

$$\text{Yield Response (\%)} = \frac{\text{Root yield in fertilized plot} - \text{Root yield in unfertilized plot}}{\text{Root yield in unfertilized plot}} \times 100$$

Data was analyzed using ANOVA technique by statistical software (Statistix 8, version 8.1, USA) and treatment means were separated by Tukey's test ($P \leq 0.05$).

3. Results

3.1 Yield and related traits

Sustainability is a hot issue in agriculture and demands reduction in usage of synthetic products such as fertilizers, pesticides, and fungicides, etc. Importance of PGPRs is well established now. Therefore, the impact of local isolated strains of PGPRs were used for inoculation of radish seed and effect of these PGPRs on growth and productivity of radish was assessed using recommended dose of nitrogen and phosphorus or half dose of nitrogen or phosphorus. Analysis of variance table revealed significant changes for all individual and interactive effect of all studied parameters except the interactive effect of seed treatment and fertilizer application on leaf length (Table 1).

It is evident from results (Table 2) that seed treatment with PSB in combination with the recommended dose of fertilizers (RDF) significantly increased number of leaves per plant (19.8), root fresh weight (247.48 g), biological yield (1.245 kg ft^{-2}) and marketable yield (0.791 kg ft^{-2}) of radish cultivar 'Mino Early'. Seed inoculated with Azotobacter spp. and sown in plots with half of recommended dose of nitrogen (½ N) resulted in highest leaf fresh weight (161.05 g) and biological yield (1.273 g).

Maximum root length (40.6 cm) was recorded in plants raised from seed inoculated with Azotobacter spp. and sown in plots receiving half of the recommended dose of phosphorus (½ P), but were at par with root length in response to PSB treated seeds sown in plots receiving RDF (40.0 cm). However, root diameter was maximum (38.7 mm) in response to seed treatment with PSB+Germinator and Azotobacter spp. in combination with recommended dose of nitrogen and phosphorus (38.3 mm). Moreover, marketable yield of seed treated with PSB in combination with RDF (0.791 kg ft^{-2}) and seed inoculation with Azotobacter spp. in combination with ½ N (0.757 kg ft^{-2}) were statistically at par.

3.2 Agronomic efficiency and yield response

To have a more clear insight in the utilization of resources, we determined agronomic efficiency and yield response of different treatment combinations. It was visualized that agronomic efficiency (351.67) and yield response (92.1%) was highest for seed treatment with Azotobacter spp. in combination with ½ N followed by PSB treated seed in combination with RDF (266.02 and 53.1%, respectively) and Azotobacter spp. in combination with ½ P (250.05 and 50.4%, respectively) (Fig. 1 & 2). Moreover, it is also evident from results that agronomic efficiency was minimum for seeds treated with Germinator with ½ N (72.81) and ½ P (-191.78). Seeds treated with Germinator exhibited minimum yield response with 0.190 and -0.386 ½ N and ½ P, respectively.

3.3 Correlation patterns among various parameters

All the studied parameters showed significant correlation with each other except following: Agronomic efficiency showed non-significant relationship with leaf length (0.3054) and root diameter (0.3083); leaf length with number of leaves (0.0687); number of leaves with root diameter (0.1756); root diameter with root length (0.3066) (Table 3). Biological yield and marketable yield (1.00) showed strong correlation with root fresh weight (0.9656 and 1.00, respectively) as compared to root diameter (0.5647 and 0.4765, respectively) and root length (0.8003 and 0.7985, respectively).

Table 1. Mean square values of yield and yield related traits in radish cv. 'Mino Early'.

Source of Variation	df	Leaves $plant^{-1}$	Leaf length	Root length	Root diameter	Leaf fresh weight	Root fresh weight	Biological yield	Marketable yield	Agronomic efficiency	Yield response
Blocks	2	5.6	4.3	13.4	14.5	116.5	37.6	0.00	0.00	236.9	0.03
Fertilizer (F)	2	15.7	76.6*	54.9*	43.7*	3453.7*	5354.1*	0.17*	0.05*	57744.7*	42.35*
Seed Treatments (S)	4	38.1	55.2*	68.2*	19.9*	3648.2*	9565.8*	0.24*	0.09*	85355.4*	42.53*
F × S	8	11	30.1^{ns}	59.9*	35.8*	1342.7*	3170.1*	0.08*	0.032*	37115.0*	16.99*
Error	28	3.7	14.9	7.0	4.56	115.1	192.3	0.00	0.00	2044.9*	0.01*
Total	44										
CV		12.6	11.1	8.1	6.3	9.5	7.5	6.8	7.5	33.6	33.4

df=degree of freedom; ns= Non-significant , ***=** Significant at $P \leq 0.05$.

Table 2: Effect of various seed treatments and fertilizer doses on root yield and yield related parameters of radish cultivar "Mino Early"

Factor	Levels	No. of leaves plant^{-1}	Root length (cm)	Root diameter (mm)	Leaf fresh weight (g)	Root fresh weight (g)	Biological yield (kg. ft^{-2})	Marketable yield (kg. ft^{-2})
RDF	**Control**	14.4 a-e	32.2 a-e	30.0 cd	94.7 d-f	171.7 de	0.91 c-f	0.55 de
	Azotobacter spp.	17.2a-c	32.1 b-f	38.3 a	129.6 a-c	191.7 cd	1.03 b-d	0.61 cd
	PSB	**19.8a**	40.0 ab	33.6 a-d	141.6 ab	**247.6 a**	**1.25 a**	**0.79 a**
	Germinator (Ger)	16.0 a-e	33.3 a-e	36.1 a-c	139.7 ab	200.6 b-d	1.09 a-c	0.64 b-d
	PSB+Ger	13.5 b-e	33.4 a-e	**38.7 a**	124.5 b-d	216.0 a-c	1.09 a-c	0.69 a-c
N_{50}	**Control**	11.2 de	26.9 ef	29.2 d	73.0 f	123.3 fg	0.63 gh	0.39 fg
	Azotobacter spp.	18.9 ab	36.4 a-c	35.8 a-c	**161.0 a**	236.8 ab	1.27 a	0.76 ab
	PSB	16.1 a-e	31.2 c-f	34.2 a-d	99.4 c-f	171.2 de	0.87 d-f	0.55 de
	Germinator (Ger)	16.7 a-e	27.7 d-f	32.3 a-d	89.9 ef	146.8 ef	0.76 fg	0.47 ef
	PSB+Ger	14.8 a-e	34.1 a-e	30.9 cd	109.2 b-e	177.7 c-e	0.92 c-f	0.57 c-e
P_{50}	**Control**	12.3 c-e	35.0 a-d	37.4 ab	113.6 b-e	160.1 d-f	0.82 e-f	0.51 d-f
	Azotobacter spp.	17.0 a-d	**40.6 a**	34.2 a-d	140.2 ab	240.8 ab	1.22 ab	0.77 ab
	PSB	15.2 a-e	28.9 c-f	31.6 b-d	122.9 b-d	187.4 c-e	0.99 c-e	0.60 c-e
	Germinator (Ger)	11.1 e	25.0 f	30.5 cd	73.1 f	98.1 g	0.55 h	0.31 g
	PSB+Ger	15.3 a-e	29.7 c-f	29.2 d	75.51 f	185.7 c-e	0.84 d-f	0.59 c-e

Values sharing same letters in a column are statistically non-significant at P ≤ 0.05. RDF= Recommended dose of fertilizers, N_{50}= 50% of recommended dose of nitrogen, P_{50}= 50% of recommended dose of phosphorus.

We also found that yield response can be assessed from marketable yield and root fresh weight and thus can be used in experiments involving PGPRs and fertilizers. Moreover, the agronomic efficiency of radish can be reliably estimated from marketable yield, root fresh weight and, whole plant fresh weight.

4. Discussion

Due to continuous cropping, the productivity of most of the agricultural lands is declining. A large number of synthetic fertilizers are used to supply macro-nutrients. Depending on management practices, soil and environmental conditions; and, nutrient losses in terms of leaching, volatilization aggravate the cost of production without increasing the production. Moreover, depending on soil reaction, even macronutrient got fixed in the soil. To avoid these losses and fixation as well as to increase nitrogen level in the soil, soil microbes can play an important role. Plant growth promoting rhizobacteria are free living heterogeneous group of bacteria which colonize the roots and improve growth, yield and quality of various crops by enhancing production of plant growth regulators, increasing solubility of phosphate, and fixation of atmospheric nitrogen (Singh, 2013), or by suppressing the activity of phytopathogenic microorganisms (Cuppels, 1999; Zahir et al., 2004).

In this study, it was observed that seeds treated with PSB showed better results (growth and yield) only when they were used with a RDF. Chattopadhyay et al. (2007) also reported that root yield of radish enhanced when PSB inoculated seeds were used in combination with a full dose of NPK fertilizers. This increase in yield owing to PSB treatment can be attributed to the increased (10 to 15 kg per ha) availability of phosphorus (Gaur, 2006) and/or owing to enhanced production of IAA (Wani et al., 2007).

Table 3: Correlation matrix among various yield related traits in radish

	AE	BY	LFW	LL	MY	NL	RD	RFW	RL	YR	FWWP
Agronomic efficiency (AE)	1										
Biological yield (BY)	**0.8271**	1									
Leaf Fresh Weight (LFW)	**0.7091**	**0.9229**	1								
Leaf Length (LL)	**0.3054** N.S	**0.5328**	**0.5343**	1							
Marketable Yield (MY)	**0.8353**	**0.9656**	**0.7911**	**0.7911**	1						
Number of leaves per plant (NL)	**0.6310**	**0.5751**	**0.4737**	**0.0687** ns	**0.5939**	1					
Root Diameter (RD)	**0.3083** N.S	**0.5647**	**0.6231**	**0.5829**	**0.4765**	**0.1756** N.S	1				
Root Fresh Weight (RFW)	**0.8353**	**0.9656**	**0.7911**	**0.4858**	**1.0000**	**0.5939**	**0.4765**	1			
Root Length (RL)	**0.6956**	**0.8003**	**0.7004**	**0.3954**	**0.7985**	**0.5079**	**0.3066** N.S	**0.7985**	1		
Yield Response (YR)	**0.9823**	**0.8907**	**0.7429**	**0.3465**	**0.9136**	**0.6498**	**0.3544**	**0.9136**	**0.7550**	1	
Whole Plant Fresh Weight (FWWP)	**0.8271**	**1.0000**	**0.9229**	**0.5328**	**0.9656**	**0.5751**	**0.5647**	**0.9656**	**0.8003**	**0.8907**	1

ns= Non-significant at P ≤ 0.05; all other correlation values are statistically significant at P ≤ 0.05.

It was also observed that when nitrogen or phosphorus was applied at half rate, seed inoculated with Azotobacter spp. exhibited superiority, in terms of biological and marketable yield, over other seed treated with the same level of nitrogen and phosphorus. Moreover, Azotobacter treated seeds in combination with a half dose of nitrogen (½ N) and phosphorus (½ P) produced yield at par with PSB treated seeds at RDF. Further, it is also evident that Azotobacter spp. with ½ phosphorus and PSB treated seeds with RDF exhibited agronomic efficiency and yield response statistically at par with each other, which might be due to phosphate solubilization by *Azotobacter* spp. (Wani et al., 2007).

Azotobacter chroococcum treated plants grown in phosphate soil with (50 %) less phosphorus showed more growth than untreated plants of tomato (Puertas and Gonzales, 1999) and onion (Mandhare et al., 1998). Kalita et al. (2015) developed bioformulations from Bacillus that increased biomass production in several vegetable crops. Our results provide evidence that PSB treated seed can give good results when applied with RDF.

The PSB treated seeds did not improve yield under these fertilizers (½ N or ½ P) levels. Furthermore, maximum percent increase (92%) in marketable yield, highest agronomic efficiency and yield was achieved with *Azotobacter* inoculation and a ½ dose of nitrogen. This observation was in accordance with the findings of Bhardwaj et al. (2007) who concluded that 75% of recommended dose of nitrogen in combination with *Azotobacter* seed treatment gave the same yield for broccoli as the recommended dose of fertilizer without *Azotobacter*. Kamalkannan and Manivannam (2003) recorded maximum radish root yield from Azospirillum treated seed sown in the soil fertilized with 75% nitrogen and phosphorus. Similarly, Bashyal (2011) recorded the non-significant difference in yield of cauliflower fertilized with 120 or 60 kg nitrogen along with *Azotobacter*, thus reducing the cost of production by saving 50% nitrogen as observed in our study.

Other seed treatments did not improve growth and productivity of radish to the same extent. Rather, germinator treated seeds showed a decline in yield as compared to untreated seeds at 50% phosphorus level. The increase in yield and agronomic efficiency of *Azotobacter* treated radish seeds can be attributed to the nitrogen fixing (15 to 20 kg ha^{-1} $crop^{-1}$) capability (Singh and Singh, 2007) and synthesis of growth hormones (auxins, cytokinins and GA-like substances) by *Azotobacter* that promote growth and productivity (Wani et al., 2013).

5. Conclusion

It can be concluded that the PSB treated seeds enhanced marketable yield only at RDF, but not at lower level nitrogen (½ N) or phosphorus (½ P). However, seeds inoculated with *Azotobacter* spp. produced more marketable yield by increasing agronomic efficiency and yield response with a half dose of nitrogen (½ N) and phosphorus (½ P) and thus can help in reducing the cost of production besides decreasing the pollution due to nitrogen leaching. Seed inoculation with *Azotobacter* spp. can be a promise for root of crops such as radish with limited root system because nitrogen is fixed in the vicinity of the root system of inoculated plants. Moreover, agronomic efficiency and yield response must be evaluated while performing fertilizer trials to have a clear response of various treatments. It is recommended that farmers should try *Azotobacter* spp. to inoculate radish seed that can reduce their cost of nitrogenous fertilizers for this crop. While in soils having phosphorus non-available form, farmers must use PSB to release the phosphorus and thus they can reduce the cost incurred on phosphatic fertilizers.

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Competing Interests

The authors declare that there is no potential conflict of interest.

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