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Efficiency of Bio-Fertigation on Nutrients Uptake by Broccoli and Soil Microbial Biomass under Sandy Soil Conditions

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Abstract: A bio-fertigation trial was conducted at the private farm of El-Saff district, Egypt during winter season 2008 to evaluate the application of liquid formulation of N-fixer (*Azotobacter sp.*), P-solubilizer (*Bacillus megatherium*) and silicates decomposing (*Bacillus circulans*) along with N, P and K fertilizers through drip irrigation system compared with the conventional method of the same treatments under drip irrigation system on broccoli grown under arid condition. Split plot design with three replicates was done, the main plots were occupied with soil application and fertigation methods, while subplots were four rates of 75 and 100% recommended rates of NPK fertilizers and/or bio inoculants additives. Results summarized that better performance of the combined inoculations of *Azotobacter chroocccum, Bacillus megatherium* and *Bacillus circulans* through bio fertigation along with both 75 and 100% recommended rates of NPK fertilizers under drip irrigation system on plant growth, spear yield and its quality, nutrients uptake by broccoli and microbial indicators i.e. total account of bacteria, Azotobacter, total fungi and actinomycetes in the soil after harvesting was observed compared to the soil application of bio inoculants along the aforementioned rates of NPK fertilizers, respectively. Drip fertigation with 100 % recommended rate of NPK fertilizers plus bio fertigation significantly gave the highest values of all studied parameters as compared to the others. The regression analysis exerted that the most important variables, which influence the spear yield were, total N uptake ($r^2 = 0.644$), total P uptake ($r^2 = 0.717$), total bacteria account ($r^2 = 0.759$) and Azotobacter count ($r^2 = 0.781$) in the soil after harvesting.

Key words: Bio-fertigation · Microbial inoculants · Broccoli · Drip irrigation · Arid condition

INTRODUCTION

The production of horticultural crops has undergone enormous change in recent years due to development of innovative technologies including integrated nutrient management practices using biofertilizers [1]. The bio-fertigation can precisely deliver the bio-inoculants in the root zone [2]. It is an added advantage whereas microbial inoculants are supplied through bio-fertigation as it has more water use efficiency and fertilizer use efficiency, quality etc. Effective microorganisms can also be applied in the filed along with inorganic materials [3]. Nevertheless, this technology needs to be standardized, as the literature availability related to bio-fertigation is very scarce.

Beneficial bacteria such as *Azospirillum, Phosphobacteria* and *Methylotroph* colonizing in the rhizosphere region and has the ability to fix nitrogen, solubilize phosphorus and stimulate plant growth [4, 5]. Broccoli (*Brassica oleracea* var. *italica*), a member of the crucifer family of vegetables, is a rich source of sulphoraphane, which has been shown to display potent anti carcinogenic properties. However, over half of the national population fails to benefit from this because they lack a specific gene (GSTM1) that helps retain the compound in the body [6].

Some investigators reported that bio-fertilization increased yield of broccoli and cruciferous vegetables [7-9]. This study was undertaken to assess the efficiency of biofertigation on plant growth, yield and nutrient uptake by broccoli and soil microbial biomass under arid condition along with application of inorganic fertilizers.

MATERIALS AND METHODS

A field experiment was laid out at a private farm, El-Saff district, Egypt (altitude of 30°- 30" N and longitude of 30°-20'E), an arid climatic region during October to

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Table 1: Some physical and chemical properties of the studied soil.

Particle Size (%)								Macronutrients (mg kg^{-1} soil)			Micronutrients (mg kg^{-1} soil)		
Sand	Silt	Clay	Soil texture	EC(dSm^{-1})	pH	OM%	$CaCO_3$%	N	P	K	Fe	Zn	Mn
90.84	5.95	3.21	Sand	0.80	7.8	0.19	14.3	6.85	2.07	43.76	4.28	0.45	0.07

December of 2008 season in sandy soil (*Typic torripsamments*). Some physical and chemical properties of the investigated soil are listed in Table 1 as described by Hesse [10].

The experiment set up in split plot design with three replicates, the main plots were assigned to application methods of bio inoculants along with NPK fertilizers i.e. soil application and fertigation (bio-fertigation). While, the sub-plots were presented with combinations of NPK rates and/or bio inoculants as the following,

- 75% recommended rate of NPK fertilizer.
- 75% recommended rate of NPK + bio inoculants.
- 100% recommended rate of NPK fertilizer.
- 100% recommended rate of NPK + bio inoculants (bio-fertigation).

The area of each plot was 10m ×10m, hence the total area was 2400 m^2. Plants raw spacing was 0.5 m and the distance between plants was 0.5m. Cultures of N-fixer (*Azotobacter sp.*), P-solubilizer (*Bacillus megatherium*) and silicates decomposing (*Bacillus circulans*) at 500 ml (10^{10} cells ml^{-1}) was diluted in 200 liters of water acre^{-1} and added through drip irrigation system. The efficient strains of bacteria in peat growth media were obtained from general organization for Agriculture Equalization Fund (GDAEF), Ministry of Agriculture and Land Reclamation, Egypt. Bio inoculants solution was prepared in a container from which it was sucked by venture assembly and allowed through the irrigation system at an interval of 15 days commencing from 15 DAS up to 45 DAS (3 times). The drip irrigation lines were used GR with built-in drippers spaced 0.5m apart with a flow capacity of 4 liters hour^{-1} at 1.5 bar working pressure and the spacing between lateral lines was 0.5 m and irrigation water was from ground water source. Seeds of broccoli (var. calabrese, American) were sown in the nursery in foam trays filled with a mixture of peat moss and vermiculite (1:1 volume) on 5th September of 2008 season and these were transplanted in the open field on 10th October of 2008 season with the spacing of 0.7 m between the ridges and 0.50 m between the plants in a ridge. Mineral fertilizers were applied using the following methods, soil application by conventional method (Side dressing) and the other was fertigation method. Nitrogen was added in the form of ammonium nitrate (33.5% N), phosphorus as super phosphate (15% P_2O_5) and potassium as potassium sulfate (48% K_2O) were considered 75% and 100% of the total NPK requirements for broccoli grown under drip irrigation system. During soil preparation, farmyard manure and 2/3 of different P rates in the form of single super phosphate (15.5% P_2O_5) were added as basal dose. The rest of P fertilizer was applied also with the first irrigation. Nitrogen fertilizer was added at three equal doses with the first, second and third irrigations, respectively as ammonium sulphate (20.5% N), for the first dose and ammonium nitrate (33.5%N) for the second and third ones. Potassium sulphate (48% K_2O) rates were divided into two equal doses and applied with the second and the third additions of N.

With respect to fertigation method, the same N and K fertilizers with replacing of phosphoric acid instead of super phosphate were added weekly intervals through the in-line drippers. The rate of fertilizer adopted in the present study was 100 kg N acre^{-1}, 60 kg P_2O_5 acre^{-1} and 80 kg K_2O acre^{-1} according to the Ministry of Agriculture recommendations. After 90 days from transplanting, the spear yields of broccoli were harvested and data were recorded for the following, fresh and dry weights of vegetative growth, spear yield (ton acre^{-1}) and physical quality i.e., stalk number, head diameter (cm) and head height (cm). To calculate the macro- and micronutrients uptake by broccoli, samples were taken from each plot, dried at 70°C and grounded using stainless steel equipments. From each sample 0.2 g was digested using 5 cm^3 from the mixture of sulfuric (H_2SO_4) and perchloric ($HClO_4$) acids (1:1) to determine nutrients i.e. N, P, K, Fe, Mn, Zn and Cu concentrations as mentioned by Cottenie [11]. Rhizosphere soil samples were collected one day after bio fertigation to determine the total bacteria and Azotobacter counts, total fungi and actinomycetes. The statistical analysis was done according to the methods described by Gomez and Gomez [12].

RESULTS AND DISCUSSION

Plant Growth Parameters: Combination of bio inoculants along with inorganic fertilizers injected through drip irrigation system had a significant effect on plant growth parameters of broccoli crop as compared to the conventional method of bio inoculants application along with inorganic fertilizers as presented in Table 2.

Data revealed that increasing rates of NPK fertilizers under drip irrigation system favorably affected fresh and dry leaves, stems, spears and total weights of broccoli crop. Drip fertigation with 100 % recommended rate of NPK fertilizers plus bio-fertigation significantly recorded the highest growth parameters, respectively. While, the lowest values of the same traits obtained from soil addition with 75% NPK fertilizers alone. This may be due to the higher frequency of biofertigation with bio inoculants and the availability of soil moisture, which leads to the effective absorption of nutrients and better proliferation of roots as reported by Gomathy [2].

Yield and physical quality: As shown in Table 3, yield and physical quality of spear was significantly influenced by the bio- fertigation and soil application of bio inoculants along with application of chemical NPK fertilizers under drip irrigation system. Increasing rates from 75 to 100% recommended rate of NPK fertilizer and/or bio inoculants markedly influenced spear yield and its quality under drip irrigation system. Better performance of the combined inoculations of *Azotobacter chroocccum, Bacillus megatherium* and *Bacillus circulans* on broccoli crop was observed through bio fertigation along with fertigation of NPK fertilizers compared to the soil application of these inoculants under drip irrigation system.

When comparing all the treatments, maximum yield was 1.673 ton acre^{-1} recorded in drip fertigation with 100% recommended rate of NPK and bio fertigation. On the other hand, the minimum yield was 0.951ton acre^{-1} obtained from soil application with 75% recommended rate of NPK fertilizers solely. Meanwhile, the same Table revealed that the maximum stalk number per plant and head diameter were 14.88 and 12.83 cm occurred with drip fertigation with 75% rate of NPK + bio fertigation followed by drip fertigation with 100% recommended NPK fertilizer along with bio fertigation. On the contrary, drip fertigation with 75% recommended rate of NPK alone produced 16.60 cm head height followed by drip fertigation with 100% NPK + bio fertigation (16.50 cm). Higher spear yield, spear quality may be attributed to the cumulative effect of nutrient transformation and plant growth promotion. Moreover, application of *Azotobacter chroocccum, Bacillus megaterium* and *Bacillus circulans* giving maximum benefit in terms of broccoli yield and spear quality [9]. Significantly higher values of yield attributes were determined due to drip fertigation than conventional method of fertilizer application [13].

Table 2: Effect of application methods of bio inoculants and NPK fertilizer rates on plant growth of broccoli crop under drip irrigation system

		Fresh weight (g plant^{-1})				Dry weight (g 100 g^{-1} fresh weight)			
Treatments		Leaves	Stems	Spears	Total	Leaves	Stems	Spears	Total
Application methods									
Soil application		471.32	161.16	146.87	779.35	12.43	8.20	12.58	33.20
Fertigation		607.23	214.08	183.88	1005.19	13.75	11.09	15.32	40.16
F Test		*	--	*	**	--	*	*	*
Bio inoculants and NPK fertilizer rates									
75% NPK		456.23b	171.63b	147.96b	775.81c	12.18b	8.97	12.83	33.98
75% NPK + Bio.		526.75ab	178.08b	149.44b	854.27b	12.80b	9.09	14.14	36.03
100% NPK		485.98b	202.43a	174.52ab	862.92b	13.48a	9.75b	13.95	37.18
100% NPK + Bio.		688.14a	198.35a	189.59a	1076.08a	13.89a	10.7	14.90	39.54
Application methods x bio inoculants and NPK fertilizer rates									
Soil application	75% NPK	138.96e	138.96e	113.26e	618.82e	11.58d	7.80c	11.28d	30.66d
	75% NPK + Bio.	165.43d	165.43d	121.51d	744.58d	12.66cd	7.90c	12.66cd	32.22d
	100% NPK	181.75cd	181.75c	167.26cd	725.42d	12.91c	8.26c	12.54cd	33.71c
	100% NPK + Bio.	158.51d	158.51d	185.46ab	1028.59ab	12.55c	8.82c	13.85bc	35.22bc
Fertigation	75% NPK	545.86c	204.29b	182.65b	932.80c	12.78c	10.14b	14.37ab	37.29b
	75% NPK + Bio.	595.86ab	190.73bc	177.37c	963.96c	12.94c	10.28b	15.61a	38.83ab
	100% NPK	595.54b	223.11ab	181.77b	1000.42b	14.05b	11.23ab	15.36ab	40.64a
	100% NPK + Bio.	691.66a	238.19a	193.72a	1123.57a	15.22a	12.69a	15.95a	43.86a

* Mean values followed by the same letter within the treatments are not significantly different ($P < 0.05$).

Table 3: Effect of application methods of bio inoculants and NPK fertilizer rates on spear yield (Ton acre^{-1}) and physical quality of broccoli crop under drip irrigation system

		Physical quality		
Treatments	Spear yield (Ton acre^{-1})	Stalk No.	Head diameter (cm)	Head height (cm)
Application methods				
Soil application	1.21	12.42	10.48	10.49
Fertigation	1.56	13.42	11.42	16.27
F Test	*	*	--	*
Bio inoculants and NPK fertilizer rates				
75% NPK	1.22b	12.61b	10.32	13.00b
75% NPK + Bio.	1.47ab	12.77b	11.88	11.95c
100% NPK	1.29b	13.06a	10.11	14.58a
100% NPK + Bio.	1.58a	13.23a	11.50	14.00ab
Application methods x Bio inoculants and NPK fertilizer rates				
Soil application	75% NPK	0.951b	12.33b	10.08bc	9.40c
	75% NPK + Bio.	1.405ab	10.66c	11.90ab	8.40c
	100% NPK	1.021ab	13.00ab	9.77c	12.67b
	100% NPK + Bio.	1.477ab	13.67ab	10.17bc	11.50b
Fertigation	75% NPK	1.489ab	12.89ab	10.55bc	16.60a
	75% NPK + Bio.	1.534ab	14.88a	11.85bc	15.50a
	100% NPK	1.558ab	13.11ab	10.45bc	16.48a
	100% NPK + Bio.	1.673a	12.78ab	12.83a	16.50a

•Mean values followed by the same letter within the treatments are not significantly different ($P < 0.05$).

Nutrients uptake by broccoli crop: With the exception of Mn, Zn and Cu nutrients, Data in Table 4 revealed that Fe, N, P and K nutrients absorbed by shoot tissues of broccoli crop were increased significantly with increasing N, P and K fertilizer rates.

Application of varying rates of N, P and K fertilizers as fertigation in combination with bio fertigation positively influenced the nutrients taken up by broccoli shoot as compared with the conventional application of N, P and K fertilizers along with bio inoculants. Drip fertigation with 100 % recommended rate of NPK and liquid formulation of *Azotobacter chroocccum, Bacillus megatherium* and *Bacillus circulans* injected through drip irrigation system recorded the highest values of macro and micronutrients absorbed by shoot of broccoli tissues. In this study as the water supply is continuous through drip, the plants tend to take more nutrients in the soil resulted in higher yield under higher available supply of fertilizer. Similar finding showed the enrichment of bio fertigation using effective microorganisms' increased 60% availability of major nutrients [14]. Similarly, Table 5 indicates that the variation in macro and micronutrients absorbed by spear tissues of broccoli crop among the biofertigation and soil application treatments followed the similar trend as that of shoots (Table 4).

Application of different rates of N, P and K fertilizers as fertigation along with bio fertigation had a pronounced effect on the absorbed nutrients by broccoli spear exterior to the other treatments. Drip fertigation with 100% recommended rate of NPK and bio fertigation produced the highest values of N, 26.48, P, 10.40, K, 22.65, Fe, 84.00, Zn: 2.49 mg plant^{-1} except for Mn and Cu nutrients, respectively. Meanwhile, soil application with 75% recommended rate of NPK produced the lowest values of N, 5.41, P, 3.95, K, 7.67, Fe, 71.85, Mn: 0.56 and Cu, 0.45 mg plant^{-1} respectively. These results were in accordance with Zaki [9], who reported that plants treated with bio inoculants absorbed N, P and K nutrients by tissues of broccoli spear higher than untreated plants.

Microbial indicators: Data in Table 6 illustrated that microbial indicators i.e., total account of bacteria, Azotobacter, total fungi and actinomycetes in the soil after harvesting.

Application of varying rates of N, P and K fertilizers as fertigation with biofertigation had a marked influence on microbial indicators as compared to the conventional method of N, P and K fertilizers application in conjunction with bio inoculants. Drip fertigation with 100 % recommended dose of N, P and K fertilizers and liquid

Table 4: Effect of application methods of bio inoculants and NPK fertilizer rates on nutrients uptake (mg plant^{-1}) by broccoli shoot under drip irrigation system

		Macronutrients			Micronutrients			
Treatments		N	P	K	Fe	Mn	Zn	Cu
Application methods								
Soil application		21.03	3.89	40.00	72.45	3.40	2.58	2.25
Fertigation		37.65	6.04	73.72	73.11	4.40	2.21	2.26
F Test		**	*	*	*	--	--	--
Bio inoculants and NPK fertilizer rates								
75% Mineral		21.23c	3.94b	43.36c	68.78	2.79	2.33	2.31
75% M + Bio.		28.02b	4.59ab	51.69c	70.96	4.44	2.43	2.56
100% Mineral		32.76b	5.38ab	61.53b	70.28	4.02	2.31	1.89
100% M + Bio.		35.36a	5.95a	70.87a	81.10	4.36	2.52	2.28
Application methods X bio inoculants and NPK fertilizer rates								
Soil application	75% NPK	12.21e	3.18b	29.65c	68.60d	2.33	1.93	2.33
	75% NPK + Bio.	22.14d	3.82b	31.00c	70.82c	4.23	2.32	2.42
	100% NPK	24.56c	4.26ab	47.21c	69.18bc	3.18	3.16	1.27
	100% NPK + Bio.	25.22c	4.30ab	52.14b	81.21a	3.85	2.90	2.99
Fertigation	75% NPK	30.25b	4.70ab	57.07ab	68.96d	4.88	2.72	2.29
	75% NPK + Bio.	33.90ab	5.36ab	72.37ab	71.10b	4.64	2.53	2.70
	100% NPK	40.95ab	6.50a	75.84a	71.38b	4.85	1.46	2.50
	100% NPK + Bio.	45.49a	7.59a	89.59a	80.98b	4.86	2.13	1.56

•Mean values followed by the same letter within the treatments are not significantly different ($P < 0.05$).

Table 5: Effect of application methods of bio inoculants and NPK fertilizer rates on nutrients uptake (mg plant^{-1}) by broccoli spear under drip irrigation system

		Macronutrients			Micro nutrients			
Treatments		N	P	K	Fe	Mn	Zn	Cu
Application methods								
Soil application		11.17	5.50	11.62	81.27	1.67	1.84	0.90
Fertigation		23.48	9.06	19.09	81.08	1.79	1.71	0.91
F Test		*	*	*	--	--	--	--
Bio inoculants and NPK fertilizer rates								
75% NPK		12.34c	5.95	12.03c	77.43b	1.00	1.67	0.51
75% NPK + Bio.		15.91b	6.87	14.06b	83.10a	1.88	1.96	0.82
100% NPK		19.49a	7.91	16.19b	79.28b	2.05	1.64	0.99
100% NPK + Bio.		21.55a	8.41	19.15a	84.90a	1.99	1.85	1.30
Application methods X bio inoculants and NPK fertilizer rates								
Soil application	75% NPK	5.41e	3.95c	7.67c	71.85b	0.56e	2.13	0.45
	75% NPK + Bio.	7.47e	5.70bc	10.00b	82.29ab	1.90c	1.99	1.01
	100% NPK	15.17d	5.95bc	13.17ab	85.15a	2.14bc	2.05	0.75
	100% NPK + Bio.	16.62cd	6.41b	15.65ab	85.80a	2.08bc	1.20	1.39
Fertigation	75% NPK	19.26c	7.94ab	16.38ab	83.00a	1.44d	1.20	0.57
	75% NPK + Bio.	24.35ab	8.04ab	18.11a	83.90a	1.85b	1.93	0.62
	100% NPK	23.81b	9.86a	19.20a	73.40b	1.95a	1.23	1.22
	100% NPK + Bio.	26.48a	10.40a	22.65a	84.00a	1.90a	2.49	1.21

*Mean values followed by the same letter within the treatments are not significantly different ($P < 0.05$).

Table 6: Effect of application methods of bio inoculants and NPK fertilizer rates on microbial indicators in soil under drip irrigation system

Treatments		Total account (10^6)	Azotobacter(10^5)	Total fungi(10^4)	Actinomycetes
Application methods					
Soil application		117.95 x10^6	14.49 x10^5	16.81x10^4	23.91
Fertigation		198.89 x10^6	27.99 x10^5	26.98 x10^4	27.49
Bio inoculants and NPK fertilizer rates					
75% NPK		108.33x10^6	13.90 x10^5	12.50 x10^4	18.25
75% NPK + Bio.		154.13 x10^6	20.50 x10^5	20.48 x10^4	25.38
100% NPK		140.88 x10^6	23.35 x10^5	25.38 x10^4	27.18
100% NPK + Bio.		230.35 x10^6	27.20 x10^5	29.21 x10^4	32.00
Application methods X bio inoculants and NPK fertilizer rates					
Soil application	75% NPK	88.25 x 10^6	9.30 x 10^5	9.85 x 10^4	14.75
	75% NPK + Bio.	108.05 x 10^6	14.30 x 10^5	14.85 x 10^4	23.00
	100% NPK	102.55 x 10^6	14.70 x 10^5	17.35 x 10^4	26.25
	100% NPK + Bio.	172.95 x 10^6	19.65 x 10^5	25.17 x 10^4	31.65
Fertigation	75% NPK	128.4 x 10^6	18.50 x 10^5	15.15 x 10^4	21.75
	75% NPK + Bio.	200.20 x 10^6	26.70 x 10^5	26.10 x 10^4	27.75
	100% NPK	179.20 x 10^6	32.00 x 10^5	33.40 x 10^4	28.10
	100% NPK + Bio.	287.75 x 10^6	34.75 x 10^5	33.25 x 10^4	32.35

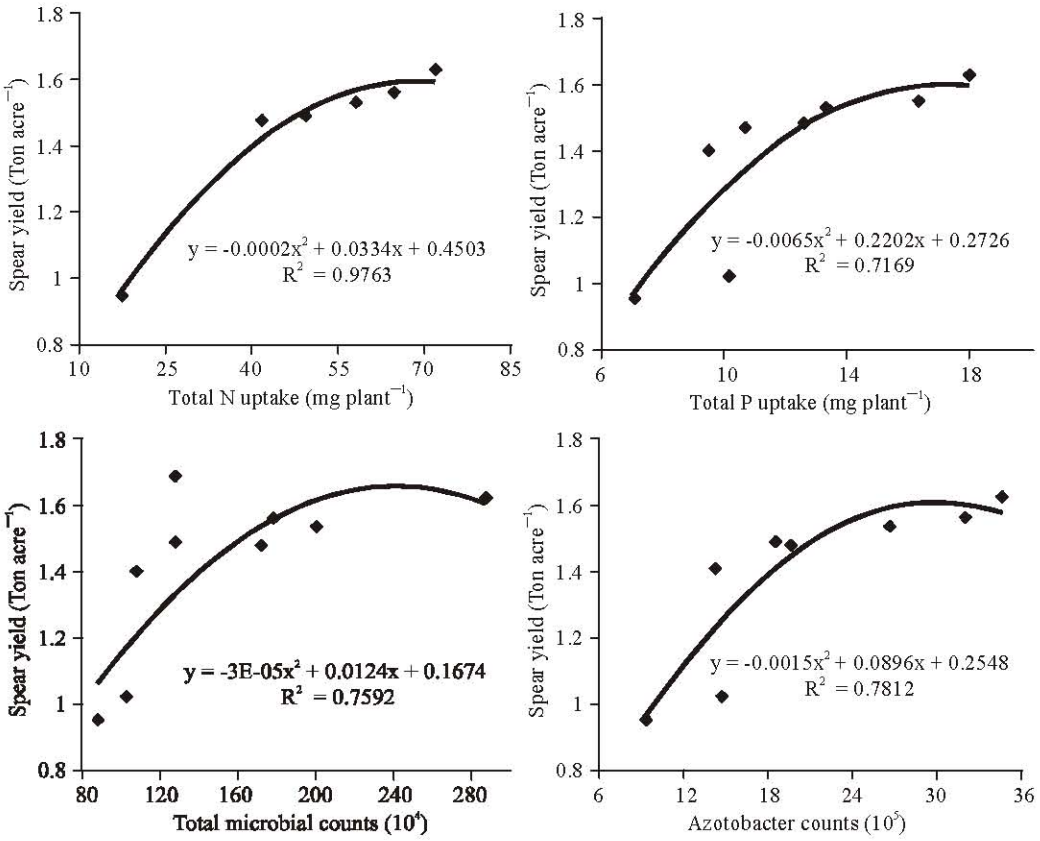

Fig. 1: Coefficient regression (r^2) between total N and P nutrients uptake (mg plant^{-1}), total microbial count and Azotobacter count and spear yield (Ton acre^{-1})

formulation of *Azotobacter chroocccum, Bacillus megatherium* and *Bacillus cirulans* injected through drip irrigation system produced the highest values of all studied attributes. It was found that the total bacteria accounts were 287.75 x 10^6, 34.75 x 10^5 for Azotobacter count, 33.25 x 10^4 for total fungi and 33.35 x 10^5 for

actinomycetes in the treatment which received 100% recommended rate of N, P and K fertilizers along with bio fertigation. It can be noticed that plants received bio inoculants combined with N, P and K fertilizers higher than that N, P and K fertilizers solely. Accepted, removed variables and their relative contribution in spear yield (Ton acre^{-1}) for broccoli crop and the technique stepwise regression analysis exerted that the most important variables, which influence the spear yield (ton acre^{-1}) were, total N uptake (r^2= 0.644), total P uptake (r^2 = 0.717), total account of bacteria (r^2 =0.759) and Azotobacter count (r^2 = 0.781) in soil after harvesting as illustrated in Fig 1.

CONCLUSION

In conclusion, better performance of the liquid formulation of *Azotobacter sp., Bacillus megaterium* and *Bacillus circulans* through bio fertigation along with fertigation of inorganic fertilizers on spear yield, quality, macro and micronutrient contents of broccoli crop and biomass soil after harvesting was observed compared to the same treatment under the conventional method. Drip fertigation with 100 % recommended rate of NPK fertilizers plus bio fertigation significantly gave the highest values of all studied parameters as compared to the others.

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