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EFFECT OF SHEEP MANURE, PHOSPHORUS LEVELS AND CHEMICAL FERTILIZERS AS WELL AS BIOFERTILIZERS ON GROWTH, YIELD, NITRATE ACCUMULATION AND CHEMICAL COMPONENTS OF BROCCOLI (*Brassica oleracea* var *italica*) PLANTS

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ABSTRACT

Two field experiments were carried out on broccoli (*Brassica oleracea* var *italica*) plants cv. Premium to study the effect of sheep manure at the rate 10 m^3 (4.28 tons/fed.), 3 levels of phosphate fertilizer i.e. 0, 10 or 20 kg P_2O_5/fed.or the recommended rate of chemical fertilizers (40 N + 30 P_2O_5 + 50 K_2O kg/fed.) with three kinds of biofertilizers i.e. yeast, *Pseudomonas*, *Bacillus* (bacteria dissolving phosphate) inoculated with seedling or uninoculation on vegetative growth characters at head initiation and at side head initiation as well as days from transplanting until head initiation, yield and chemical components in leaves and heads. Applying sheep manure plus 20 kg P_2O_5/fed. followed by sheep manure plus 10 kg P_2O_5/fed. gave higher values of plant length, plant, stem, leaves fresh weights, number of leaves per plant, head, side heads weights per plant and total yield/fed. as well as the percentage of N, P, K,dry matter, total sugar, soluble phenols and total free amino acids concentration in leaves and heads comparing with adding recommended chemical fertilizers. Days from transplanting until head initiation not affected by fertilization. The result indicated that using sheep manure plus 10 kg P_2O_5 gave better values than using chemical fertilizers. On the other hand, ascorbic acid of heads was higher by adding sheep manure plus10kgP_2O_5 than values of sheep manure plus 20 kg P_2O_5 or chemical fertilizers. Nitrate content of heads were the highest with application chemical fertilizer. The three biofertilizers treatments increased vegetative growth parameters, total yield and its components as well as chemical components in the leaves and heads as comparing with uninoculation treatment. *Pseudomonas aeruginosa* resulted in the highest yield of heads in the first season also side head weight and total yield per fed. as well as total sugars and amino acids in leaves in both seasons . Yeast caused the higher values of heads yield per fed. and total free amino acids in heads, in the second season. On the other hand, nitrate content of heads were the highest with inoculated plants with yeast, while *Bacillus* caused the lowest values. *Bacillus* results in the highest total amino acids in heads in the first season and ascorbic acid content in heads in both season.The highest total yields were obtained of plants received sheep manure plus 20 kg P_2O_5/fed and inoculated with *pseudomonas* or *Bacillus* in the first and second seasons, respectively.

INTRODUCTION

Broccoli (*Brassica oleracea* L. var *italica* Plenk) is an important nutrional cole vegetable. It is high in vitamin A, ascorbic acid and is a good source of calcium, niacin and riboflavin (Decoteau, 2000).

Broccoli is a cool-season crucifer. It has about the same climatic requirements as cauliflower. It is well adapted to all areas when grown during the coolest months of year, although it is not as sensitive to hot weather. It is

harvested over a longer period of time than cauliflower since lateral broccoli shoot develop marketable head after the center head is harvested. The upper stem and clusters of unopened flower buds (heads) are the edible parts of the plant (Johnson, 2000).

Broccoli product in combination of organic with inorganic fertilizer gave as good yields as those obtained with conventional inorganic fertilizer N. Biological activity in the soil tended to increase with the increase organo-mineral fertilizer rates (Belec *et al.*, 2001).

For increasing P uptake, plants can directly modify the rhizophere in order to gain access to unavailable soil P reserves through exuding chemical compounds into rhizoshere and through association of roots with mycorrhiza (Raghothama, 1999).

Organic and bio-fertilizers are very important sources for providing the plants with their nutritional requirements without having an undesirable impact on the environment. Many investigators compared the effect of different organic manures (Perin *et al.*, 2004 and Perez-Murcia *et al.*, 2006), biofertilizers (Manivannan and Singh, 2004 and Chaterjee *et al.*, 2005)and mineral fertilizer especially phosphorus , Kumar and Sharma (2001) reported that the maximum values for growth, yield and quality characteristics were obtained at the highest N, P and K levels (150, 90 and 60 kg/ha., respectively) as well as their interaction on broccoli growth, yield and contents of dry matter, N, P, K and chemical components in leaves and heads (Chaterjee *et al.*, 2005).

Nitrate concentrations in vegetables were positively correlated with N rates. (Karitonas, 1999 and Rydz, 2001). Nitrate may be formed from NO_3 after ingestion, causing methaemoglobinemia (Wright and Davison, 1964). Presence of NO_2 in blood might result also in the formation nitroseamines, which are carcinogenic (Craddock, 1983). Recent attention has been given to less pollution practices in modern agriculture. One of the ways to reduce soil pollution is the use of biofertilizers which have been recommended by several investigators to substitute chemical fertilizers.

Therefore, the objectives of this investigation were to find out the optimal phosphorus level and most efficient biofertilizers and organic fertilizers treatments and their combination to increase broccoli growth and increase yield as well as improve head quality.

MATERIALS AND METHODS

This investigation was conducted at The Experimental Station of the Faculty of Agriculture, Cairo University, Giza during the two successive winter seasons of 2002/2003 and 2003/2004 to study the effect of different levels of phosphorus, sheep manure or the biofertilizers treatments in single or in combined applications on broccoli growth characters, yield and nutritive value of heads. The physical and chemical characteristics of the experimental soil are presented in Table (1) as average in both seasons.

The chemical properties of soil were determined using the methods described by Jackson (1962). Seeds of broccoli hybrid prominence (Takii co.,

Japan) were sown in the two seasons on mid October in seedling trays. Transplanting was carried out four weeks later.

Table (1): Physical and chemical characteristic of experimental soil

Clay %	Silt %	Fine sand %	Coarse sand %	$CaCO_3$ %	pH	EC ds/m	Organic mater %	Total N %	P_2O_5 ppm	K_2O ppm
21.2	40.5	32.6	5.3	1.6	7.8	1.01	2.11	0.09	30.1	105.8

The experimental included 16 treatments which were the combinations of sheep manure with 3 levels of phosphorus fertilizers, i.e. 4.28 Tons/fed. (10 m^2/fed.) sheep manure + 0 kg P_2O_5 (P_1), sheep manure + 10 kg P_2O_5 (P_2), sheep manure + 20 Kg P_2O_5 as calcium superphosphate 15.5% P_2O_5 (P_3) or the recommended chemical fertilizers 40 kg N + 30 kg P_2O_5 + 50 kg K_2O/ fed.. All organic treatment received potassium sulphate until received 50 kg KO_2 / fed..

Broccoli seedling non inoculated or inoculated of with 3 biofertilizers treatments i.e. without biofertilizers (M_1), soil yeast *Candida tropicolis* (M_2), *Bacillus megatherium* (M_3), phosphate dissolving bacteria and *Pseudomonas aeruginosa* (M_4) at transplanting.

Sheep manure and calcium superphosphate were applied during soil preparation. On the other hand, N, P and K_2O applied as ammonium sulphate (20.5% N), calcium superphosphate 15.5% P_2O_5 and potassium sulphate (48% KO_2), respectively for chemical treatment. Application was in two equal parts at 3 and 6 weeks after transplanting.

Chemical analysis of sheep manure used is presented in Table (2) as average in both seasons. Various cultural practices were conducted as commonly followed in commercial cauliflower field.

Table (2): Chemical analysis of sheep manure used at experimental period.

Organic matter %	pH	EC ds/m	N %	P %	K %
65.7	8.01	2.3	1.9	0.4	1.2

Experimental design:

The experiment was conducted in three replicates in split plot design, where sheep manure plus levels of phosphorus presented as the main plots and biofertilizers treatments were presented as the sub plot.

The experimental plot was 14 m^2 and consisted of 5 rows, each 4.0 m long and 0.75 m wide. The distance between plants were 60 cm.

Data were recorded on the following characters:

Vegetative growth characters:

Ten plants taken from each plot were chosen randomly when top head initiated (55 days after planting) and when side head initiated (85 days after planting) for measuring the following characteristics:

Plant length, fresh weights of plant, stem and leaves as well as number of leaves per plant. Days from transplanting until head initiated were determined on 50% of plants.

Yield and its component: Average fresh weight of top head and side head per plant were determined from each plot. Yield of top head, yield of side head and total yield (top head + side head) were recorded in two rows and calculated as ton per feddan.

Chemical composition of leaves and heads:

Samples of leaves, at head initiation and samples of top heads at harvesting were taken and dry matter percentage was determined.

Determination of N, P and K were carried out on the ground dry materials of plants which were digested using sulfuric acid, salicylic acid and hydrogen peroxide according to Linder (1944). Nitrogen was determined using the micro-kejeldahl apparotus of Parnos–Wagner as described by Van-Schouwenburg and Walinga (1978). Phosphorus was estimated colorometically by using chlorostannous reduced molybdophosphoric blue color method according to Chapman and Parker (1961). Potassium was determined using the flame photometer . NO_3 – N was determined in distilled water extracts of dried tissue by the procedure of Cataldo *et al.*, (1975) by using salicylic acid and then calculated as mg/100 gram fresh weight. Ethanol extracts of fresh materials were used for the determination of total sugars, total free amino acids and total soluble phenols. Total sugar were determined by using the phenol-sulphuric acid method (Dubois *et al.*, 1956). Total free amino acids were determined by using ninhydrin reagent according to (Moore and Stein, 1954). Total soluble phenols were estimated using the Folin-ciocalteau colorimetric method (Swain and Hillis, 1959). Vitamin C content of heads determined by the methods of A. O. A. C. (1980) .

Statistical analysis:

Yield and yield components as well as chemical composition of leaves and heads were analysis as split plot design, whereas the vegetative characters of plant samples were analyzed as split – split plot in time, plant samples were considered as sub-sub plots. Data were subjected to the proper statistical analysis of variance according to Snedecor and Cochran (1980) using the LSD at 5% level.

RESULTS AND DISCUSSION

Vegetative Growth:

Results presented in Tables (3,4and5) indicate that the most studied vegetative growth parameters, i.e., plant length, fresh weights of plant, stem and leaves as well as number of leaves per plant were significantly influenced by different levels of phosphorus and biofertilizers treatments at the two different samples (55 and 85 days after transplanting).

Regarding to the effect of phosphorus levels, significant differences were detected on vegetative parameters, the highest values were obtained by adding mixture of sheep manure + 20 kg P_2O_5 (P_3) followed by mixture of sheep manure + 10 kg P_2O_5. (P_2)

On the other hand, applying chemical fertilizers (NPK) alone or sheep manure alone (P_1) gave the lowest values, in both seasons. While, the number of leaves per plant, were not significantly affected by the different levels of P_2O_5 fertilization in the second season.

Table (3): Effect of sheep manure, phosphorus levels, chemical fertilizers, biofertilizers treatment, plant age and their interaction on length and fresh weight of plant.

Fertili-zers	Biofertilizers	Plant length (cm.)						Plant weight (g)					
		2002-2003			2003-2004			2002-2003			2003-2004		
		C_1	C_2	X	C_1	C_2	X	C_1	C_2	X	C_1	C_2	X
P_1	M_1	58.0	70.0	64.0	49.6	66.5	58.1	460.0	1178.3	819.2	378.8	891.7	625.2
	M_2	60.3	70.7	65.5	58.0	68.0	63.0	489.2	1176.7	832.9	455.0	1390.0	922.5
	M_3	58.1	69.0	63.5	60.8	72.0	66.4	469.6	1166.7	818.1	456.3	1091.7	774.0
	M_4	58.7	73.3	66.0	60.0	77.0	68.5	511.3	1227.5	869.4	507.5	920.0	713.8
	X	58.8	70.8	64.8	57.1	70.9	63.98	482.5	1187.0	834.9	449.4	1073.0	761.4
P_2	M_1	55.5	65.3	60.4	54.0	66.7	60.3	462.5	1175.0	818.8	440.0	990.0	715.0
	M_2	57.1	70.7	63.9	64.0	76.3	70.2	532.5	1133.3	832.9	637.3	1346.7	992.0
	M_3	59.8	71.7	65.8	53.5	71.7	62.7	508.8	1220.0	864.4	492.5	1306.7	899.6
	M_4	61.6	78.5	69.8	61.8	72.3	67.1	520.0	1271.7	895.8	568.8	1150.0	859.4
	X	58.4	71.5	64.96	58.3	71.8	65.1	505.0	1200.0	853.0	534.8	1198.0	866.5
P_3	M_1	57.9	74.0	65.97	53.0	67.3	60.2	506.3	1246.7	876.5	497.5	983.3	740.4
	M_2	60.6	74.7	67.6	59.0	74.7	66.8	525.0	1268.3	896.7	503.8	1281.7	892.7
	M_3	62.5	76.0	69.3	61.5	74.7	68.1	577.5	1360.0	968.8	608.8	1146.7	877.7
	M_4	61.7	74.3	68.9	60.3	74.3	67.3	605.0	1391.7	998.3	626.3	1390.0	1008.0
	X	60.7	74.8	67.7	58.4	72.8	65.6	553.4	1317.0	935.1	559.1	1200.0	879.7
NPK	M_1	56.7	67.7	62.2	52.5	64.7	58.6	422.5	920.0	671.3	451.3	1008.3	729.8
	M_2	61.6	67.5	64.5	54.7	67.3	61.0	580.0	1038.3	809.2	494.2	1013.3	753.8
	M_3	56.8	67.5	62.2	53.0	70.7	61.8	453.8	1100.0	776.9	461.3	1006.7	734.0
	M_4	55.3	69.8	62.6	57.3	75.7	66.5	458.8	1185.0	821.9	493.8	1220.0	856.9
	X	57.6	68.1	62.9	54.4	69.6	62.0	478.8	1061.1	769.8	475.1	1062.0	768.6
The interaction between biofertilizers treatments and plant age on length and fresh weight of plant.													
	M_1	57.0	69.3	63.2	52.3	66.3	59.3	462.8	1130.0	796.4	441.9	968.3	705.1
	M_2	59.9	70.9	65.4	58.9	71.6	65.3	531.7	1154.0	842.9	522.5	1258.0	890.2
	M_3	59.3	71.0	65.2	57.2	72.3	64.7	502.4	1212.0	857.0	504.7	1138.0	821.3
	M_4	59.2	74.0	66.6	59.8	74.8	67.3	523.8	1269.0	896.4	549.1	1170.0	859.5
	X	58.9	71.3		57.1	71.3		505.2	1191.0		504.6	1134.0	
LSD 0.05	P: 2.99 M: 2.42 C: 1.34				P: 1.19 M: 2.28 C: 1.52			P: 4.89 M: 74.7 C: 64.72			P: 100.5 M: 85.6 C: 54.04		
	P x M: 4.84				P x M: 4.92			P x M: 149.3			P x M: 171.2		
	P x C : 2.69				P x C : 3.03			P x C : 105.7			P x C : 108.1		
	M x C: 2.69				M x C: 3.03			M x C: 105.7			M x C: 108.1		
	P x M x C: 5.37				P x M x C: 6.06			A x M x C: 211.4			P x M x C: 216.1		

P_1 : Sheep manure+0 kg P_2O_5/fed P_2:Sheep manure + 10 kg P_2O_5 fed. P3:Sheep manure + 20 kg P_2O_5/fed.
NPK: 40 kg. N+ 30 kg P_2O_5+ 50 kg K_2O/fed.
M_1: Without inoculation M_2:*Candida tropiclis* M_3 : *Bacillus megatherium* M_4: *Pseudomonas aeruginosa*
C_1: 55 days after transplanting C_2 : 85 days after transplanting

Concerning the effect of the three different types of biofertilizers, data presented in Tables (3,4 and 5) reveal that plant length was the tallest by applying *Pseudomonas aeruginosa* (M_4) followed by *Candida tropicalis* (M_2) and *Bacillus megatherium* (M_3) while without using biofertilizers plant length was the lowest, in both seasons.

Plant fresh weight was the heaviest by applying M_4, while there were no significant differences between M_2 and M_3 in the first season. On the other, hand M_2 resulted in the highest values, while there were no significant differences between M_2, M_3 and M_4 in the second season. Without using biofertilizers (M_1), plants had the lowest values in both seasons.

Stem fresh weight was the heaviest by using *Pseudomonas aeruginosa* (M_4) or without using biofertilizers, while *Candida tropicalis* (M_2)gave the lowest values in the first season. *Candida tropicalis* (M_2) followed by *Pseudomonas aeruginosa* (M_4) gave the heaviest values while without using biofertilizers (M_1) gave the lowest values in the second season.

Leaves fresh weight per plant were the highest by using M_4 and M_2 in the first and second seasons, respectively. While, leaves fresh weight were the lowest without using biofertilizers in both seasons.

Number of leaves per plant were the highest by applying *Pseudomonas aeruginosa* (M_4)or *Bacillus megatherium* (M_3) without significant differences between them, in the first season. *Candida tropicalis* (M_2) followed by *Pseudomonas aeruginosa* (M_4) gave the highest values in the second season. The lowest values were obtained without using biofertilizers in both seasons.

As shown in Tables (3, 4 and 5), there were significant increases in plant length, fresh weight of plant, stem and leaves as well as number of leaves per plant with the progress of plant age.

It is clear from Tables (3,4 and 5) that the interaction between phosphorus fertilization and biofertilizers treatments were significant in both seasons. The tallest plants were obtained when using sheep manure + 10 kg P_2O_5 (P_2) and *Pseudomonas aeruginosa*(M_4) in the first season while the interaction between $P_2 \times M_2$ (*Candida tropicalis*) resulted in the tallest one in the second season.

The heaviest fresh weight of plants were obtained as a result of interaction between $P_3 \times M_4$ (P_3 : sheep manure + 20 kg P_2O_5, M_4 : *Pseudomonas aeruginosa*) in
both seasons.

Without using biofertilizers treatments (M_1), broccoli plants were the lower in the length and fresh weight at all levels of phosphorus fertilizer. $P_1 \times M_1$ gave the lowest values.

The highest fresh weight of stem, leaves and number of leaves per plant were obtained by the interaction between $P_3 \times M_4$ in the first season , but the interaction between $P_2 \times M_2$ gave the highest values in the second season.The lowest values of stem fresh weight were obtained with the interaction NPK$\times M_4$ and NPK$\times M_2$ in the first and second seasons, respectively. The lowest leaves fresh weight and number of leaves per plant were obtained from the interaction NPK $\times M_1$ and $P_1 \times M_1$ in the first and second seasons, respectively.

Table (4): Effect of sheep manure, phosphorus levels, chemical fertilizers, biofertilizers treatment, plant age and their interactions on stem and leaves fresh weights per plant

Fertili-zers	Biofertillizers	Stem weight /plant(g)						Leaves weight/plant (g)					
		2002-2003			2003-2004			2002-2003			2003-2004		
		C_1	C_2	X	C_1	C_2	X	C_1	C_2	X	C_1	C_2	X
P_1	M_1	87.5	356.7	222.1	76.3	251.7	164.0	372.5	805.0	588.8	302.5	591.7	447.1
	M_2	95.8	380.0	237.9	82.5	366.7	224.6	393.4	796.7	595.0	372.5	906.7	639.6
	M_3	80.0	434.3	211.7	88.8	285.0	186.9	389.6	823.3	606.5	367.5	677.7	567.1
	M_4	121.3	322.5	221.9	80.0	285.0	182.5	390.0	905.0	647.5	427.5	617.5	522.5
	X	96.1	350.6	223.4	81.9	297.1	189.5	386.4	832.5	609.4	367.5	720.6	544.1
P_2	M_1	91.2	407.5	249.4	110.0	316.7	213.3	351.3	725.0	538.1	330.0	630.0	480.0
	M_2	91.2	323.3	207.3	160.4	350.0	255.2	441.3	843.3	642.3	479.2	946.7	712.9
	M_3	97.5	311.7	204.6	101.2	333.3	217.3	411.3	873.3	642.3	391.3	826.7	609.0
	M_4	95.4	425.0	260.2	88.8	266.7	177.7	424.6	888.3	656.5	480.0	805.0	642.5
	X	93.8	366.9	230.4	115.1	316.7	215.9	407.1	832.5	619.8	420.1	802.1	611.1
P_3	M_1	132.5	383.3	257.9	85.0	283.3	184.2	392.5	863.3	627.9	412.5	656.7	534.6
	M_2	90.0	380.0	235.0	82.5	373.3	227.9	416.3	888.3	652.3	421.3	866.7	644.0
	M_3	105.0	400.0	252.5	67.5	340.0	203.8	472.5	923.3	697.9	541.3	743.3	642.3
	M_4	108.7	466.7	287.7	127.5	361.7	244.6	496.3	966.7	731.5	498.8	916.7	707.7
	X	109.1	407.5	258.3	90.6	339.6	215.1	444.4	910.4	677.4	468.5	795.8	632.1
NPK	M_1	111.2	350.0	230.6	67.5	283.3	175.4	311.3	570.0	440.6	383.8	661.7	522.7
	M_2	113.7	313.3	213.5	41.7	233.3	137.5	466.3	725.0	595.6	452.5	700.0	576.3
	M_3	118.8	390.0	254.4	46.3	251.7	149.0	335.0	710.0	522.5	415.0	700.0	557.5
	M_4	97.5	270.0	183.8	105.0	308.3	206.7	361.2	915.0	638.1	388.8	840.0	614.4
	X	110.3	330.8	220.6	65.1	269.2	167.1	368.4	730.0	549.2	410.0	725.4	567.7
The interaction between biofertilizers treatments and plant age on stem and leaves fresh weight per plant.													
	M_1	105.6	374.3	240.0	84.7	283.8	184.2	356.9	740.0	548.9	357.2	635.0	496.1
	M_2	97.7	349.2	223.4	91.8	330.8	211.4	429.3	813.3	621.3	431.4	855.0	643.2
	M_3	100.3	361.3	230.8	75.9	302.5	189.2	402.1	832.5	617.3	428.8	759.2	594.0
	M_4	105.7	371.0	238.4	100.3	305.4	202.9	418.0	918.8	668.4	448.8	794.8	621.8
	X	102.3	364.0		88.2	305.6		401.6	826.4		416.6	671.0	
LSD 0.05	P: 15.85 M: 12.92 C: 8.47				P: 13.71 M: 15.91 C: 9.82			P: 46.4 M: 53.3 C: 37.8			P: 74.9 M: 61.9 C: 39.5		
	P x M: 25.83				P x M: 31.8			P x M: 106.5			P x M: 123.9		
	P x C : 16.95				P x C : 19.6			P x C : 75.6			P x C : 78.9		
	M x C: 16.95				M x C: 19.6			M x C: 75.6			M x C: 78.9		
	P x M x C: 33.89				P x M x C: 39.3			A x M x C: 151.2			P x M x C:157.9		

P_1: Sheep manure+0 kg P_2O_5/fed. P_2: Sheep manure+10 kg P_2O_5/fed.
P_3: Sheep manure + 20 kg P_2O_5/fed. NPK: 40 kg. N+ 30 kg P_2O_5+ 50 kg K_2O/fed.
M_1:Without inoculation M_2:*Candida tropiclis*
M_3 : *Bacillus megatherium* M_4: *Pseudomonas aerugionsa*
C_1: 55 days after transplanting C_2 : 85 days after transplanting

In general, without applying biofertilizers treatments the vegetative growth values were lower comparing with using biofertilizers treatments. The interaction between levels of phosphorus fertilization and the age of the plant were significant in both seasons .The highest values of vegetative growth were obtained by using sheep manure + 20 kg P_2O_5 (P_3)at the second stage of growth C_2 (at side heads initiation) in both seasons.

Results presented in Tables (3,4 and 5) indicated that there were significant differences in all studied vegetative growth characters due to the effect of interaction of biofertilizers treatments and the age of the plant. The greatest values of these parameters were recorded in the plant treated with *Pseudomonas aeruginosa* (M_4) and *Candida tropicallis* (M_2) in the first and

second seasons respectively at the second stage of plant growth. Whereas , the lowest values were recorded in plants untreated with biofertilizers treatment at the first stage of growth in both seasons.

The effect of the interaction between levels of phosphorus fertilization, biofertilizers treatments and the age of the plant were significant. The greatest values of the most vegetative growth characters were obtained with using sheep manure + 20 kg P_2O_5 (P_3) accompanied with the application of *Pseudomonas* (M_4) at the second stage of the growth ($P_3 \times M_4 \times C_2$).

Table (5): Effect of sheep manure, phosphorus levels, chemical fertilizers, biofertilizers treatment, plant age and their interactions on number of leaves per plant.

Fertilizers	Biofertilizers	Leaves numbers/plant					
		2002-2003			2003-2004		
		C_1	C_2	X	C_1	C_2	X
P_1	M_1	16.43	16.66	16.55	13.93	14.33	14.13
	M_2	16.70	17.30	17.02	16.10	20.33	18.22
	M_3	18.30	17.33	17.82	15.27	17.00	16.13
	M_4	17.33	19.33	18.33	15.83	16.00	15.92
	X	17.19	17.67	17.43	15.28	16.92	16.10
P_2	M_1	15.66	16.00	15.83	15.00	15.67	15.33
	M_2	15.90	18.33	17.12	16.27	19.33	17.80
	M_3	18.50	19.50	19.00	16.83	17.67	17.25
	M_4	16.33	21.00	18.67	16.67	17.67	17.17
	X	16.60	18.71	17.65	16.19	17.58	16.89
P_3	M_1	15.90	17.60	16.75	15.00	16.67	15.83
	M_2	17.43	18.33	17.88	15.77	18.67	17.22
	M_3	17.93	22.00	19.97	15.83	17.00	16.42
	M_4	18.90	23.0	20.95	15.83	18.67	17.25
	X	17.54	20.33	18.89	15.61	17.75	16.68
NPK	M_1	14.67	15.00	14.83	14.53	17.00	15.77
	M_2	15.43	15.50	15.47	15.83	17.00	16.42
	M_3	15.60	18.30	16.97	15.17	17.00	16.08
	M_4	15.30	21.00	18.17	15.00	18.30	16.67
	X	15.26	17.46	16.36	15.13	17.33	16.23
The interaction between biofertilizers treatments and plant age on number of leaves per plant.							
	M_1	15.67	16.32	15.90	14.62	15.92	15.27
	M_2	16.37	17.38	16.87	15.99	18.83	17.41
	M_3	17.58	19.29	18.44	15.77	17.17	16.47
	M_4	16.98	21.08	19.03	15.83	17.67	16.75
	X	16.65	18.52		15.55	17.40	
LSD 0.05	P: 0.64 M: 0.89 C: 0.51				P: ns M: 0.77 C: 0.67		
	P x M: 1.78				P x M: 1.53		
	P x C : 1.02				P x C : 1.33		
	M x C: 1.02				M x C: 1.33		
	P x M x C: 2.03				P x M x C: 2.66		

P_1 : Sheep manure+0 kg P_2O_5/fed
P_2: Sheep manure + 10 kg P_2O_5/fed.
P_3: Sheep manure + 20 kg P_2O_5/fed.
NPK: 40 kg. N+ 30 kg P_2O_5+ 50 kg K_2O/fed.
M_1:Without inoculation
M_2:*Candida tropiclis*
M_3: *Bacillus megatherium*
M_4: *Pseudomonas aerugionsa*
C_1: 55 days after transplanting
C_2 : 85 days after transplanting

Days from transplanting to head initiation (DTH):

Concerning the effect of phosphorus levels and biofertilizers treatments and their interactions, data presented in Table (6) revealed that there were no significant differences between phosphorus levels on days from transplanting to head initiation (DTH) in both seasons. On the other hand, there were significant differences among biofertilizers treatment for DTH. Applying biofertilizers resulted in lower days from transplanting to head initiation compared to without using biofertilizers. There were no significant differences between biofertilizers treatments in the first season, while *Bacillus* and *Pseudomonas* resulted in lower days compared to *Candida*, in the second season.

With respect to the interaction between phosphorus fertilization and biofertilizers treatments, data in Table (6) show significant differences. The lowest days from transplanting to head initiation were obtained from plants fertilized with P_2 and treated with M_3 (*Bacillus*) or M_2 (*Candida*) as well as from plant fertilized with P_3 and treated with M_3 (*Bacillus*) in the first season. The same trend was obtained by M_4 (*Pseudomonas*) or M_3 (Bacillus) treatments which gave the lowest values when combined with all different kinds of fertilizers in the second season.

In general, applying biofertilizers treatments with all kinds of fertilizers resulted in the lowest days from transplanting to head initiation compared to without biofertilizers treatments.

Yield and its components:

Data presented in Table (6) revealed significant differences in the average top head and side head weight per plant as well as top heads, side head yields/fed. and total yield/fed. due to the effect of phosphorus levels. The highest value of these characters were recorded by the plants fertilized with sheep manure + 20 kg P_2O_5 (P_3), meanwhile the lowest values were obtained when applying sheep manure (P_1)or (chemical fertilization) NPK in the first and second seasons, respectively.

Concerning the effect biofertilizers treatments, data presented in Table (6) indicated that the highest values of average head weight and yield of heads/fed. were obtained by the plant supplied with *Pseudomonas* (M_4) followed by *Bacillus* (M_3)in the first season. Meanwhile, (*Candida*) M_2 followed by (*Pseudomonas*) M_4 gave the highest values in the second season. On the other hand, average side head weight per plant and yield of side heads/fed. were the greatest when applying *Pseudomonas* (M_4) followed by *Candida* (M_2)in the first season. In the second season, *Pseudomonas* (M_4) followed *Bacillus* (M_3) resulted in the highest values .While , total yield/fed. was the greatest when applying (*Pseudomonas*) M_4 in both seasons. On the other hand, the lowest values of these characters were obtained by the plants untreated with any of biofertilizers treatments in both seasons.

With respect to the effect of interaction between levels of phosphorus fertilization and biofertilizers treatments on yield and its components , data in Table (6) indicated significant differences. The highest average head weight, yield of heads/fed. and total yield /fed. were obtained by the plants fertilized with sheep manure+20 kg P_2O_5(P_3) and treated with *Pseudomonas*(M_4)in the first season. While, in the second season the interaction between P_3 and M_3

(*Bacillus*) gave the highest values. Meanwhile, the highest values of side heads weight / plant and yield of side heads /fed. were noticed in plants fertilized with sheep manure+10 kgP_2O_5 (P_2) and treated with *Pseudomonas* (M_4) in the first season. On the other hand , $P_3 \times M_3$ gave the highest values in the second season. However, the lowest values of average side heads/plant, yield of side heads/fed. and total yield/fed. were obtained with interaction $P_2 \times M_1$ in the first season. Meanwhile, average head weight and yield of heads/fed., in both seasons , average side heads/plant, yield of side heads / fed. and total yield were the lowest with using the interaction $P_1 \times M_1$ in the second season.

The results indicated that combined of sheep manure, phosphorus fertilizer and biofertilizers treatments affected in the productivity of broccoli plants.

From these results, it can be concluded that most of the studied vegetative growth parameters as well as yield and its components were increased with increasing phosphorus levels. Similar results and suggestion were reported by many workers on broccoli plants.

The effect of N, P and K fertilizers were in the same trend of studies were obtained by Dufault (1988), Kumar and Sharma (2001), Brahma *et al.*, (2002), Sharma *et al.*, (2002) and El-Magd *et al.*, (2005) who reported that increasing any of N, P or K ratio improved vegetative growth expressed as plant height and number of leaves per plant as well as total yield and head quality.

The vegetative growth and yield were affected by phosphorus levels. Wang *et al.*, (1997) Cited that application of N+ P gave 110% higher yields than N alone.

In this respect, this enhancement may be explained that phosphorus is a part of molecular structural of several vitally important compounds notable nucleic acids (DNA, the two forms of RNA). In addition, phosphorus plays role in the enzyme system necessary for energy transformation in photosynthesis and respiration. It is also a constituent of cell nucleus and essential for division for the development of merstimatic tissue (Edmont *et al.*,1981) .

Furthermore, from the results it can be suggest that, vegetative growth parameters and yield of plants received combined of sheep manure and mineral fertilizer were higher than those supplied with mineral fertilizers alone or organic manure. These results are in agreement with those obtained by Subhan (1989) on cabbage, Sharma (2000) on broccoli plants and Bhardwai *et al.*,(2000) on cauliflower and tomato. The yield of broccoli harvested one week earlier and higher with combination of organic and mineral fertilizer. This was related to improved P nutrition (Brown *et al.*, 1994). In manured plots available P_2O_5 content increased. Cabbage yield were highest when chemical fertilizer were applied with FYM (Kamaiyama *et al.*, 1995). For every 1 ton compost applied, fertilizer N application could be reduced by about 10 kg (Inoune *et al.*, 1996).

Regarding the effect of biofertilizers treatments, results indicated that broccoli plants treated with biofertilizers, i.e. *Candida tropicalis, Bacillus megatherium* (Phosphate dissolving) or *Pseudomonas* had higher vegetative growth parameters, yield and its components compared with non biofertilizers

treatment. *B. megatherium* or *P. aeruginosa* were more superior than *C. tropicalis* in most characters. These results were confirmed by Subba Rao (1993) who reported that biofertilizers stimulate plant growth by increasing the number and biological activity of desired micro-organisms in root environment. Some of these micro-organisms fix-atmospheric nitrogen or convert of bond phosphorus or bond potassium to easily stimulated form. Broccoli plant received combination of organic, mineral fertilizer and biofertilizers had higher vegetative parameter and yield comparing with using organic or chemical alone. Inoculation plants or soil with different micro-organic has become recently new methods to reduce the amount of inorganic complete fertilizers.

Table (6): Effect of sheep manure, phosphorus levels, chemical fertilizers, biofertilizers treatment, and their interactions on day to head initiation, head weight, side head weight, head yield, side head yield and total yield

Fertilizers	Biofertilizers	Day to head initiation		Head weight (gm.)		Side head weight (gm/plant)		Head yield (tons/fed.)		Side head yield (tons/fed.)		Total yield (Tons/fed.)	
		2002-2003	2003-2004	2002-2003	2003-2004	2002-2003	2003-2004	2002-2003	2003-2004	2002-2003	2003-2004	2002-2003	2003-2004
P_1		57.2	58.7	504.0	557.0	290.0	268.8	4.480	4.951	2.578	2.389	7.059	7.341
P_2		56.2	58.8	523.7	576.0	311.1	271.6	4.655	5.119	2.766	2.415	7.421	7.534
P_3		56.9	59.0	589.1	606.9	332.9	298.6	5.237	5.394	2.959	2.654	8.192	8.049
NPK		56.6	58.4	539.2	548.0	291.0	243.8	4.793	4.871	2.588	2.167	7.380	7.039
LSD 0.05		NS	NS	50.12	25.66	40.42	5.69	0.445	0.230	0.360	0.055	0.551	0.209
	M_1	57.8	60.6	520.9	502.3	263.7	181.9	4.631	4.464	2.344	1.617	6.975	6.082
	M_2	56.1	58.7	521.5	625.7	311.3	243.7	4.635	5.562	2.768	2.167	7.404	7.728
	M_3	56.4	57.8	556.3	546.5	301.6	319.9	4.945	4.858	2.681	2.844	7.626	7.702
	M_4	56.7	57.9	557.2	613.5	348.5	337.3	4.953	5.453	3.098	2.998	8.051	8.451
LSD 0.05		0.59	0.75	32.38	31.68	35.1	20.66	0.288	0.276	0.312	0.183	0.476	0.332
P_1	M_1	59.1	60.6	470.9	413.2	289.5	143.7	4.186	3.673	2.573	1.277	6.759	4.950
	M_2	56.0	59.4	474.4	626.1	293.2	256.7	4.217	5.563	2.607	2.282	6.824	7.847
	M_3	57.2	57.6	522.5	537.2	289.3	352.9	4.644	4.774	2.572	3.129	7.217	7.903
	M_4	56.7	57.5	547.9	651.8	287.9	322.9	4.870	5.794	2.559	2.870	7.429	8.664
P_2	M_1	56.5	60.4	515.1	536.2	220	219.5	4.579	4.767	1.926	1.951	6.535	6.718
	M_2	55.8	58.5	495.0	603.2	302.9	271.1	4.400	5.362	2.692	2.410	7.092	7.772
	M_3	55.8	57.9	573.9	536.2	300.8	236.3	5.102	4.764	2.673	2.100	7.775	6.864
	M_4	56.8	58.3	510.2	628.5	420.8	359.7	4.540	5.586	3.741	3.197	8.281	8.783
P_3	M_1	59.3	61.8	590.2	509.5	296.0	186.8	5.246	4.528	2.631	1.661	7.877	6.189
	M_2	56.2	59.1	506.6	658.0	373.0	264.6	4.503	5.849	3.316	2.352	7.819	8.201
	M_3	55.8	57.3	594.8	666.2	290.1	409.6	5.287	5.922	2.579	3.641	7.866	9.563
	M_4	56.3	57.9	664.9	593.9	372.6	333.4	5.911	5.279	3.312	2.963	9.223	8.242
NPK	M_1	56.3	59.4	507.5	550.2	249.2	177.8	4.511	4.890	2.215	1.581	6.726	6.471
	M_2	56.4	58.0	609.9	615.4	276.0	182.6	5.422	5.471	2.458	1.623	7.880	7.094
	M_3	56.7	58.3	534.2	446.6	326.2	281.7	4.748	3.970	2.900	2.504	6.648	6.474
	M_4	57.0	57.8	505.1	579.8	312.5	333.1	4.49	5.154	2.778	2.961	7.268	8.150
LSD 0.05		1.19	1.50	64.77	63.35	70.20	41.32	0.576	0.551	0.624	0.365	0.953	0.666

P_1:Sheep manure+0 kgP_2O_5/fed P_2:Sheep manure+10kgP_2O_5/fed.
P_3:Sheep manure+ 20 kg P_2O_5/fed. NPK: 40 kg. N+ 30 kg P_2O_5+ 50 kg K_2O/fed.
M_1: Without inoculation M_2: *Candida tropiclis*
M_3 : *Bacillus megatherium* M_4: *Pseudomonas aerugionsa*

Many investigators studied the effect of microorganism on the growth and yield. Hsieh and Hsu (1995) studied the effect of addition of microorganism mixture EM (containing nitrogen-fixing, phosphate-dissolving and photosynthetic microorganisms, vesicular – arbuscular mycorrhizas, *Bacillus, Lactobacillus*, yeast and *actionmycetes*). The increases in cabbage yield in the organic plots compared with chemical plots were greater, especially when the microorganism mixture were included in the manures. Cabbage performed best with chicken manure compost combined with microorganisms. Bahadur *et al.*, (2005) evaluated the effect of organic manures and biofertilizers on growth and yield of cabbage. Biofertilizers were Azospirillum, vesicular abrbuscular mycorrhiza (VAM) and phosphate solubilizers microorganisms (PSM). Pressmud + VAM+ the recommended dose of NPK (120:60:80 kg/ha) recorded the highest values for all parameters of growth, yield and average head weight. Chaterjee *et al.* (2005) studied the effect of different organic amendments and biofertilizers (Azotobacter, phosphate solubilizer and potash mobilizer) on broccoli. Mustard oil cake+ biofertilizer resulted in highest yield while recommended NPK rate, 150:60:80 kg/ha gave the lowest value.

Concerning the day from transplanting until head initiation, Gauss and Taylor (1996) reported on broccoli that at 8 weeks the apices reached its maximum width and 83% of the apices were initiation first order floral stalks. At 9 weeks, when the plants had an averages of 17 leaves, all apices were in the same stage of flowering and 52% of the plants sampled had inflorescence heads or button greater than 5.0 mm in diameter. Fortunato and Damato (2000) found that broccoli plants sown at October, apex transition occurred 55 days and ended 30 days afterwards.

Inoculation plants with microbial mixture and supplemented with organic and chemical fertilizer led to significantly decreased days from transplanting until flowering comparing with applying chemical fertilization (Darwesh, 2002 on tomato and Alian, 2005 on artishok).

Regarding the relationship between the growth of broccoli plants and plant age, results indicated that the highest values of vegetative growth parameter were recorded when side heads initiated after 83 days of transplanting. These results agreement with results obtained by Rincon *et al.*, (1999) who reported that the highest growth rate occurred between 73 and 87 days after transplanting.

Chemical composition in leaves and heads:

Data in Table (7) indicated clearly significant differences between phosphorus levels on the percentage of (N, P and K. The maximum values of N, P and K in leaves and heads were obtained as a result of fertilization with the highest phosphorus level 20 kg P_2O_5 + sheep manure (P_3). While, application of sheep manure only (P_1) gave the lowest values of N and K in leaves as well as N, P and K in heads. However, the lowest values of P in leaves were obtained with applying chemical fertilization (NPK), in both seasons.

Moreover, it is clear from data in Table (7) that application of M_4 (*Pseudomonas*) resulted in the highest values of N, P and K in the first season in the leaves. While M_2 (*Candida*) and M_3 (*Bacillus*) resulted in the

highest values of P and K, respectively in the second season. As regard to heads, (*Pseudomonas*) M_4 increased significantly N% in the first season. Meanwhile, (*Candida*) M_2 resulted in the highest values of N in the second season. The percentage of P and K% in head increased significantly with applying (*Bacillus*) M_3 in both seasons. Without using biofertilizers treatments (M_1), N, P and K % in the leaves and heads were the lowest in both seasons.

Table (7): Effect of sheep manure, phosphorus levels, chemical fertilizers, biofertilizers treatment, and their interactions on N% , P% and K% in leaves and heads.

Fertilize-rs	Biofertil-izers	Leaves						Heads					
		N%		P%		K%		N%		P%		K%	
		2002-2003	2003-2004	2002-2003	2003-2004	2002-2003	2003-2004	2002-2003	2003-2004	2002-2003	2003-2004	2002-2003	2003-2004
P_1		2.39	2.59	0.234	0.289	1.69	1.76	2.05	2.23	0.246	0.199	1.86	1.97
P_2		2.73	2.74	0.260	0.303	1.87	2.11	2.46	2.44	0.286	0.338	2.43	2.15
P_3		2.73	2.91	0.294	0.391	2.38	2.20	2.66	2.51	0.322	0.361	2.44	2.32
NPK		2.62	2.70	0.218	0.225	1.86	1.86	2.41	2.21	0.249	0.237	2.17	2.21
LSD 0.05		0.223	0.14	0.032	0.009	0.17	0.17	0.099	0.245	0.032	0.032	0.141	0.105
	M_1	2.01	2.52	0.155	0.222	1.93	1.71	2.09	2.03	0.218	0.214	1.97	2.01
	M_2	2.83	2.75	0.210	0.352	1.88	1.83	2.35	2.57	0.297	0.322	2.34	2.15
	M_3	2.69	2.85	0.308	0.333	1.77	2.41	2.37	2.42	0.379	0.353	2.38	2.33
	M_4	2.93	2.82	0.333	0.305	2.22	1.97	2.77	2.36	0.210	0.246	2.21	2.17
LSD 0.05		0.190	0.21	0.008	0.027	0.107	0.08	0.169	0.146	0.027	0.027	0.146	0.148
P_1	M_1	1.85	2.62	0.143	0.163	1.38	1.54	1.750	1.91	0.213	0.160	1.80	1.75
	M_2	2.44	2.59	0.133	0.410	2.08	1.54	1.93	2.54	0.253	0.208	1.83	1.94
	M_3	2.41	2.53	0.394	0.375	1.56	2.18	2.00	2.47	0.330	0.279	1.93	2.16
	M_4	2.88	2.66	0.265	0.208	1.73	1.76	2.53	1.99	0.188	0.149	1.86	2.04
P_2	M_1	2.61	2.16	0.181	0.214	1.50	2.00	2.35	2.07	0.203	0.213	2.04	1.96
	M_2	2.89	2.72	0.174	0.423	1.56	2.04	2.36	2.85	0.381	0.438	2.73	2.16
	M_3	2.62	3.01	0.321	0.264	2.20	2.19	2.54	2.63	0.325	0.353	2.53	2.30
	M_4	2.79	3.05	0.365	0.311	2.21	2.20	2.60	2.22	0.235	0.348	2.43	2.16
P_3	M_1	1.99	2.69	0.166	0.285	2.40	1.68	2.06	2.09	0.231	0.326	1.95	2.16
	M_2	2.86	2.75	0.298	0.419	2.40	1.98	2.68	2.68	0.328	0.419	2.53	2.28
	M_3	3.0	3.12	0.304	0.421	2.06	3.34	2.69	2.44	0.500	0.406	2.74	2.54
	M_4	3.06	3.07	0.409	0.440	2.66	1.78	3.21	2.81	0.230	0.291	2.54	2.30
NPK	M_1	1.61	2.59	0.130	0.226	2.43	1.64	2.23	2.05	0.224	0.156	2.07	2.16
	M_2	3.15	2.94	0.236	0.156	1.46	1.74	2.43	2.22	0.226	0.221	2.28	2.23
	M_3	2.76	2.75	0.211	0.258	1.26	1.93	2.25	2.14	0.361	0.375	2.33	2.30
	M_4	2.94	2.51	0.293	0.260	2.28	2.13	2.72	2.41	0.188	0.194	2.00	2.16
LSD 0.05		0.380	0.413	0.017	0.053	0.185	0.17	0.337	0.292	0.053	0.053	0.292	0.297

P_1: Sheep manure+0 kg P_2O_5 — P_2:Sheep manure+10 kg P_2O_5/fed.
P_3:Sheep manure + 20 kg P_2O_5/fed. — NPK: 40 kg. N+ 30 kg P_2O_5+ 50 kg K_2O/fed.
M_1: Without inoculation — M_2:*Candida tropiclis*
M_3 : *Bacillus megatherium* — M_4: *Pseudomonas aeruginosa*

Concerning the interaction between phosphorus level and biofertilizers treatments, there were significant differences. The interaction of $P_3 \times M_3$ or $P_3 \times M_4$ resulted in the highest percentage of N, P and K in both leaves and head in both seasons.

Dry matter percentage, total sugars, total soluble phenols, total free amino acids concentration in the leaves and heads (Tables 8 and 9) were significantly influenced by phosphorus levels. Sheep manures + 20 kg P_2O_5 (P_3) resulted in the greatest values followed by sheep manure + 10 kg P_2O_5

(P_2). Meanwhile, the lowest values were detected in leaves and heads of the plants fertilized with sheep manure only (P_1) or NPK chemical fertilizers .These results were obtained in two seasons.

Regarding the effect of biofertilizers treatments, data in Tables (8and 9)indicated that plants received *Bacillus* (M_3)or *Pseudomonas* (M_4)had the highest values of dry matter percentage, total sugars, total soluble phenols and total free amino acids concentration in the leaves in both seasons. Meanwhile, inoculated with (*Candida*) M_2 resulting in the greatest phenols and total free amino acids concentration of the head in the first and second seasons, respectively. On the other hand, plants untreated with biofertilizers gave the lowest values of chemical components in leaves and heads.

Table (8): Effect of sheep manure, phosphorus levels, chemical fertilizers, biofertilizers treatment, and their interactions on dry matter %, total sugars, total soluble phenols and total free amino acids concentration (mg/100g f . w) in leaves

Fertilize-rs	Biofertilizers	Dry matter %		Total sugars		Total soluble phenols		Total free amino acids	
		2002-2003	2003-2004	2002-2003	2003-2004	2002-2003	2003-2004	2002-2003	2003-2004
P_1		13.15	12.81	60.9	70.8	43.3	33.3	246.3	294.7
P_2		13.21	12.76	86.3	103.8	48.6	43.2	335.9	369.0
P_3		13.30	12.83	87.9	111.4	50.2	47.8	366.9	541.3
NPK		12.22	12.02	61.3	78.8	34.3	33.7	321.0	362.5
LSD 0.05		0.926	ns	3.66	3.35	2.86	4.92	11.83	8.97
	M_1	12.72	12.00	43.7	57.6	33.0	28.4	280.3	359.7
	M_2	12.85	12.71	78.1	98.5	36.5	39.6	317.8	384.2
	M_3	13.41	12.86	77.5	101.9	57.9	43.0	296.9	398.1
	M_4	12.88	12.85	96.3	106.7	48.9	46.9	375.0	425.6
LSD 0.05		0.601	0.521	4.23	5.22	4.17	3.83	7.14	4.410
P_1	M_1	12.69	12.60	34.2	38.1	30.9	24.9	181.1	274.2
	M_2	12.94	12.67	61.2	75.8	27.4	36.2	251.1	273.9
	M_3	13.85	12.72	72.8	83.7	64.2	30.6	206.1	307.8
	M_4	13.10	13.25	75.7	85.4	50.8	41.3	346.7	322.8
P_2	M_1	13.44	12.18	46.8	77.9	33.9	24.1	319.5	336.7
	M_2	13.25	13.10	106.9	142.2	40.7	40.2	366.1	398.9
	M_3	12.81	12.62	90.90	107.8	69.4	27.8	313.9	352.7
	M_4	13.35	13.14	100.5	87.3	50.3	80.5	343.9	387.8
P_3	M_1	12.97	12.18	53.2	69.7	40.8	27.5	305.6	501.2
	M_2	13.46	12.62	69.6	93.9	29.2	38.8	306.1	527.3
	M_3	13.94	13.0	92.6	135.1	64.6	86.9	362.6	543.9
	M_4	12.83	13.51	136.5	147.1	66.1	37.9	493.4	592.8
NPK	M_1	11.79	11.02	40.6	44.6	26.4	37.2	315.0	326.7
	M_2	11.74	12.47	78.6	82.2	48.8	43.2	347.0	336.7
	M_3	13.05	13.11	53.5	81.2	33.3	26.8	305.0	387.8
	M_4	12.32	11.47	72.6	107.0	28.7	27.7	316.1	398.9
LSD 0.05		1.201	1.043	8.56	9.04	8.34	7.65	14.28	8.82

P_1:Sheep manure+ 0kg kg P_2O_5/fed P_2:Sheep manure+10kg P_2O_5/fed.
P_3:Sheep manure + 20 kg P_2O_5/fed. NPK: 40 kg. N+ 30 kg P_2O_5+ 50 kg K_2O/fed.
M_1: Without inoculation M_2: *Candida tropiclis*
M_3 : *Bacillus megatherium* M_4: *Pseudomonas aerugionsa*

Data presented in Tables (8 and 9) show that dry matter percentage, total sugars, total soluble phenols and total free amino acids concentration in leaves and heads were significantly affected due to the interaction between phosphorus levels and biofertilizers treatments. The highest values of these chemical composition in leaves and heads were detected when plants fertilized with sheep manure + 20 kg P_2O_5 (P_3) and treated with (*Bacillus*) M_3 or (*Pseudomonas*) M_4 in both seasons.

The highest values of total phenols were found in leaves of plants fertilized with sheep manure + 10 kg P_2O_5 (P_2) and treated with *Bacillus* (M_3)in the first season, while the interaction between 20 kg P_2O_5+sheep fertilizer (P_3) and *Candida* (M_2) resulted in the highest values of dry matter percentage and total soluble phenols concentration in the heads in the first season. On the other hand, the lowest values of most chemical components were obtained with interaction NPK × M_1 or P_1 × M_1 in leaves and heads in both seasons.

As regarding ascorbic acid (VC) concentration in broccoli heads, data in Table 9 revealed that,VC values increased significantly with increasing phosphorus level up to 10 P_2O_5 + sheep manure (P_2)in both seasons. Ascorbic acid in heads of plants received chemical fertilizers alone (NPK) had lower values compared to P_2 in both seasons. The lowest values recorded with application sheep manure only (P_1). Biofertilizers treatments resulted in significant increasing in ascorbic acid concentration in heads comparing with unionculated ones. Plants were treated with *Bacillus* followed by *Pseudomonas* had the highest values of VC when compared with those inoculated with *Candida*. The interaction between phosphorus and biofertilizers treatments were significant. The highest values were obtained from plants received P_2× M_3 followed by NPK × M_4 in the first season , while the highest values were recorded by the plants treated with NPK × M_3 and P_2×M_3 in the second season. However, P_1×M_1 treatment gave the lowest values in both seasons.

Concerning the effect of phosphorus level on nitrate concentration, data in Table (9) revealed that nitrate concentration in broccoli heads significantly decreased with increasing P level. P_3 (sheep manure + 20 kg P_2O_5) treatment had the lowest values. Chemical fertilizer alone (NPK) gave the highest values in both seasons.

Moreover, the results indicated that biofertilizers treatments increased significantly nitrate concentration in head compared to uninoculated plants. Plants inoculated with *candida* (M_2) had the highest values while, inoculation with *Bacillus* (M_3) decreased nitrate in heads in both seasons.

The interaction between phosphorus fertilization and biofertilizers treatments were significant in both seasons. Plants received P_3×M_1 and P_3×M_3 gave the lowest values in the first and second seasons, respectively. Moreover, the results indicated that nitrate concentration in heads decreased with increasing phosphorus addition by adding phosphate to the soil or as a result of using active dissolving phosphate bacteria (*Bacillus megatherium*.) such values were still in the acceptable ranges for human health (Wang and Li , 2004)

Table (9): Effect of sheep manure, phosphorus levels, chemical fertilizers, biofertilizers teatment, and their interactions on dry matter%, total sugars, total soluble phenols,total free amino acids, ascorbic acid and nitrate concentration (mg/100g f. w) in heads.

Fertili-zers	Biofertil-izers	Dry matter %		Total sugars		Total phenols		Total mino acids		Ascorbic acid		Nitrate	
		2002-2003	2003-2004	2002-2003	2003-2004	2002-2003	2003-2004	2002-2003	2003-2004	2002-2003	2003-2004	2002-2003	2003-2004
P_1		11.67	11.28	125.8	120.3	36.5	40.4	484.0	576.1	108.1	76.3	243.2	228.6
P_2		12.45	11.62	166.0	124.8	44.9	42.5	517.8	613.6	146.2	147.5	231.2	220.3
P_3		13.60	13.19	179.4	180.1	50.2	51.6	605.0	636.1	126.3	146.3	210.6	192.0
NPK		11.73	11.10	157.9	106.5	38.0	39.5	466.0	540.7	135.6	126.9	245.3	235.7
LSD 0.05		1.235	0.649	0.510	3.13	3.89	2.49	12.51	10.17	4.31	5.93	6.44	7.90
	M_1	11.67	11.06	133.9	114.7	38.8	37.9	460.1	573.0	90.6	91.9	223.8	210.9
	M_2	12.26	11.87	152.7	139.3	52.5	42.3	505.4	616.0	133.8	119.4	254.9	236.1
	M_3	12.68	12.27	185.5	134.2	36.2	46.4	567.3	569.5	151.1	146.9	218	208.2
	M_4	12.85	11.99	157.1	143.3	41.9	47.4	539.9	607.7	140.6	138.8	233.6	221.5
LSD 0.05		0.674	0.636	4.05	2.55	3.41	4.21	9.60	7.33	4.50	4.34	5.36	4.71
P_1	M_1	11.1	10.63	121.9	106.6	36.2	36.8	419.4	537.5	45.0	50.0	236.4	225.9
	M_2	11.7	11.57	144.3	111.6	53.2	40.9	448.2	595.9	140.0	62.5	267.3	236.2
	M_3	11.66	11.61	157.1	107.5	31.6	42.1	491.7	551.4	135.0	92.5	220.8	211.6
	M_4	12.22	11.32	79.7	155.5	24.8	41.7	576.8	619.5	112.5	100.0	248.1	240.7
P_2	M_1	12.0	11.26	161.3	109.7	30.6	41.0	506.5	602.2	112.5	130	221.5	210.1
	M_2	12.16	11.52	130.6	129.9	46.5	42.3	430.5	622.3	132.5	132.5	247.7	228.2
	M_3	12.39	12.38	214.1	126.7	46.2	42.9	627.1	601.5	202.3	177.5	225.6	219.4
	M_4	13.27	11.32	158.1	133.1	56.1	43.8	507.6	628.5	137.5	150.0	230.1	223.6
P_3	M_1	12.79	12.23	132.5	140.5	52.1	44.7	547.2	612.6	100.0	125.0	191.6	175.4
	M_2	13.08	13.65	156.0	194.6	79.0	49.9	600.7	633.7	112.5	155.0	230.8	220.3
	M_3	14.41	13.28	201.1	219.5	33.4	59.6	613.0	678.5	155.0	137.5	201.3	170.5
	M_4	14.13	13.59	228.0	165.8	36.2	52.3	659.0	619.5	137.5	167.5	218.5	201.6
NPK	M_1	10.79	10.13	119.8	102.1	36.3	29.0	367.3	539.6	105.0	62.5	245.5	231.9
	M_2	12.11	10.75	180.0	120.9	31.3	35.9	542.4	613.4	150.0	127.5	273.7	259.7
	M_3	12.27	11.78	169.6	83.3	33.7	41.0	537.5	446.7	112.5	180.0	224.3	231.2
	M_4	11.76	11.73	162.4	119.7	50.8	52.0	416.7	563.2	175.0	137.0	237.8	220.0
LSD 0.05		1.347	1.272	8.11	4.42	5.91	7.29	16.63	12.69	8.75	8.18	10.57	9.43

P_1 : Sheep manure +0kg P_2O_5/fed **P_2:Sheep manure + 10 kg P_2O_5/fed.**
P_3: Sheep manure + 20 kg P_2O_5/fed. **NPK: 40 kg. N+ 30 kg P_2O_5+ 50 kg K_2O/fed.**
M_1: without inoculation **M_2: *Candida tropiclis***
M_3 : *Bacillus megatherium* **M_4: *Pseudomonas aerugionsa.***

Results indicated that the percentage of dry matter, N, P and K of leaves and heads as well as chemical component of leaves and heads, i.e. total sugar, soluble phenols, free amino acids, ascorbic acid and nitrate increased with increasing P level. Combination of phosphorus sheep manure + 10 kg P_2O_5 (P_2) was superior than chemical phosphorus fertilizer alone at 30 kg P_2O_5 / fed. Broccoli plants inoculated with biofertilizers had higher content of dry matter, N, P and K as well as chemical components of leaves and heads comparing with un inoculated plants. These results agree with those obtained by Prabhakar *et al.*, (1986) they reported that cabbage yield and P uptake increased with P up to 75 kg/ha. Lu *et al.*, (1997) mentioned that peak nutrient absorption occurred during the head formation period. The

descending order of dry weight proportions was leaves, head, stem and roots.

Bowen *et al.*, (1999) and Rincon *et al.*, (1999) found that the highest concentration of N, P and K were accumulation in leaves, head and stem respectively occurred between 73 and 87 day after transplanting. Nkoa *et al.*, (2001) reported that the greatest dry weight of shoot were obtained at inflorescence initiation but subsequently decreased until harvest. Brahma *et al.*, (2002) pointed out that the highest percentage of N, P and K in leaves were 3.9%, 0.44% and 2.75%, respectively and ascorbic acid content of the head were 128.05 mg/100g .

Vagen (2003) mentioned that rapid N uptake in broccoli approximately starts when the small heads just visible to the eye. Choudhary and Choudhary (2005) reported that ascorbic acid content of heads were not much influenced by the N and P treatments, but dry weight was influenced significantly by both levels of N and P.

Combination of chemical, organic or biofertilizers fertilizers supplied to broccoli plants resulted higher chemical components in leaves and heads comparing with application of organic or chemical fertilizers alone. These results were confirmed by Machendran and Kumar (1997) they reported that the highest ascorbic acid content of cabbage were produced by applied 75% of the recommended rate of NPK combined with ver- micompost and digested organic supplement. Suchorska – Orowska, (1998), Morselli *et al.*, (1999) and Perin *et al.*, (2004) found that the dry matter, sugar content of heads and uptake of N, P and K of cabbage, cauliflower or broccoli were significantly increased by organomineral fertilizers applied compared to chemical fertilizer.

The enhancing effect of biofertilizers, i.e. yeast, *Pesudomonas* and *Bacillus* (phosphate solubilizing bacteria), on organic chemical component may be attributed to increase phosphatase activity, P availability or/and producing growth regulating hormones. The use of biofertilizers may have an additional benefits such as nitrogen fixation, mobilizing phosphate and micronutrients through the production of organic acids and lowering soil pH (Saber, 1993). In this respect, Hewedy (1999) stated that, inoculation of tomato plants with mixture of some biofertilizers (phosphorien, rhizobecterien and microbien) increased the availability of some nutrients which could be reflected on plants uptake and its content from these nutrients. Nanjian *et al.*, (2001) mentioned that increases in the level of amino acids and phenols were observed by inoculation with VAM (Vescicular arbuscular mycorrhiza) in tomato plants. The levels of peroxidase, phenol oxidase and phenylalanine ammonia – lyase increased with treated plants with VAM. Leaves and roots of treated plants with VAM had higher amount of cytokinins. In addition, microorganisms can secrete growth promoting substances, e.g., gibberellins, cytokinins and auxins (Brown, 1972). Abou-Hussein *et al.*, (2002) found that applying compost with chicken manure and biofertilizer, increased the percentage of nutrients in potato leaves (N, P, K and Ca) and total carbohydrates. On the other hand, using the chicken manure with biofertilizer increased nitrate content in potato tubers. Hanafy Ahmed *et al.*, (2002) working on lettuce, suggested that the use of rhizobactrein, nitrobein,

microbein and biogein may enhance the use of nitrate transformation with the available carbon into plant growth compounds which increased plant growth by increasing the organic components (total sugars, free amino acids and soluble phenols). Moreover, Chaterjee *et al.*, (2005) evaluated the effects of different organic amendments and biofertilizer (Azotobacter, vesicular arbuscular mycorrhiza and potash mobilizer. Poultry manure + biofertilizer produced broccoli heads with the highest ascorbic acid and reducing sugar contents.

Nitrate accumulation in plants occurs as a result of nitrate accumulation in the soil due to the activity of soil nitrifying organisms. This could be mainly due to the intensive application of nitrogen fertilizers which results in imbolancing nutritional status of the plants and consequently high nitrate accumulation as well as soil pollution. Nitrate content in broccoli heads varied significantly with the N and P rates (Karitonas, 1999 and Rydz, 2001). Nitrate concentrations in vegetables were positively correlated with N rates. As a result of addition N fertilizer to soil was the major cause for vegetables increasing their nitrate contents. With the addition of P fertilizer, yields of green cabbage and rape were increased, while those of spinach had no significant changes. The nitrate concentration was significantly decreased in green cabbage by P treatment. Different organs had different amount of nitrate accumulation, and it was found that nitrite concentration were much higher in roots, stems and petioles than in blades at any N rate. The head of broccoli had the lowest nitrate content (Dolanska, 2002 and Wang and Li, 2004).

It is important here to mention that, in the present work, there was a pronounce negative relationship between nitrate accumulation and organic compounds (total sugars, soluble phenols, free amino acids and ascorbic acid) which induce due to increasing phosphorus supply, either alone or combined with any of the different biofertilizers. In this respect, Hanafy Ahmed (1996) and Hanafy Ahmed *et al.*, (2002) suggested that simple organic molecules such as sugars, free amino acids, total soluble phenole and organic acids may act as an osmoticum for the regulation of plant osmosis. These simple organic molecules or solutes can replace nitrate in the cell vacuoles. Blom – Zandstra *et al.*, (1988) suggested that the availability of sugars might affect the need for nitrate as an osmoticum. The same authors working on lettuce, pointing out that NO_3 accumulation was inversely related to the accumulation of sugars and organic acids. Furthermore, Mulier and Touraine (1992) and Imsande and Touraine (1994) mentioned that increasing the concentration of certain amino acids in the phloem sap causes an inhibition of nitrates uptake.

Biofertilizers are prepared nowadays from different microorganisms having a define role in the transformations of nutrients from the soil the plant roots. The effect of plant inoculation with different types of soil microorgamisms has become recently new methods to reduce the amount of inorganic complete fertilizers.

Finally it could be concluded that total yield increased due to increasing of top head and side heads of plant, which was a reflection for optimum plant growth. The best yield and quality were obtained in the present

study with applying 10 m^3 sheep manure plus 20 Kg P_2O_5/fed. and inoculated plants with *Bacillus megatherium* or *Pseudomonas aerugionsa*.

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تاثيرالتسميد بمخلفات الغنم ومستويات الفوسفات والسماد الكيماوي والحيوي علي النمو والمحصول وتراكم النترات والمحتوي الكيماوي للبروكولي.

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اجريت تجربتين حقليتين على نباتات البروكلي صنف برميم لدراسة تأثير سماد الغنم بمعدل ١٠ م^3 للفدان (٤,٢٨ طن) وثلاث مستويات من السماد الفوسفاتى (صفر، ١٠، ٢٠ كجم فوأ / فدان) والسماد كيماوى الموصى به (٤٠ ن، ٣٠ فوأ، ٥٠ بوأ كجم / فدان) .الشتلات لقحت بالمخصبات الحيوية وهى الخميرة والباسيلس وبسيدموناس وكذلك عدم التلقيح وتاثرها على صفات النمو الخضرى عند بداية تكوين الرؤوس والرؤوس الجانبية وايضاعدد الأيام حتى بداية ظهور الأقراص، كمية المحصول ومكوناته والمحتوى الكيماوى للأوراق والأقراص .أظهرت النتائج ان استعمال سماد الغنم + ٢٠ كجم فوأ يليه سماد الغنم + ١٠ كجم فوأ أعطى اكبر القيم لصفات طول النبات ، وزن النبات، والساق والأوراق وعدد الأوراق للنبات مقارنة بإستخدام السماد الكيماوى الموصى به، كذلك متوسط وزن القرص ووزن الأقراص الجانبية للنبات والمحصول الكلى وأيضا النسبة المئوية النتروجين والفوسفور والبوتاسيوم والمادة الجافة و وتركيز السكريات الكلية والفينولات والأحماض الامينية الحرةالكلية فى الأوراق والأقراص.لم يتاثر عدد الأيام من الشتل حتى بداية ظهور القرص الرئيسى بأنواع أومستويات التسميد. استعمال سماد الغنم + ١٠ كجم فوأ / فدان أعطى نتائج أفضل من استعمال السماد الكيماوى الموصى به لمعظم الصفات. محتوى الأقراص من حمض الاسكوربيك كان أعلى عند استعمال سماد الغنم + ١٠ كجم فوأ / فدان .كان محتوى الأقراص من النترات أعلى باستعمال السماد الكيماوى.استعمال المخصبات الحيوية تسببت فى زيادة لصفات النمو الخضرى والمحصول ومكوناته وكذلك المكونات الكيماوية للأوراق والرؤوس مقارنة بعدم استعمال المخصبات الحيوية. بسيدموناس تسبب فى اكبر قيم محصول الأقراص للفدان فى الموسم الأول، أيضا محصول الأقراص الجانبية والمحصول الكلى للفدان والسكريات الكلية والأحماض الامينية الكلية فى الأوراق فى كلا الموسمين. الخميرة أعطت أعلى القيم لمحصول الاقراص للفدان والاحماض الامينية الكلية فى الاقراص فى الموسم الثانى. كان أعلى تراكم للنترات فى الاقراص عند تخصيب النباتات بالخميرة بينما استعمال الباسيلس تسبب فى أقل مقدار. بكتريا الباسيلس تسببت فى أعلى قيم الاحماض الامينية الحرة فى الاقراص فى الموسم الاول، وحمض الأسكوربيك فى الاقراص من الموسمين. أعلى محصول كلى تم الحصول عليه بتسميد النباتات بسماد الغنم + ٢٠ كجم فوأ وتلقيحها بالبسيدموناس فى الموسم الاول والباسيلس فى الموسم الثانى.