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EFFECT OF BIO-AND MINERAL FERTILIZERS ON PHOTOSYNTHETIC ACTIVITY OF POTATOES.

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ABSTRACT

Decreasing NPK doses, less than the recommended one, decreased chlorophylls a, b and their total as well as reducing sugars, non-reducing sugars and total carbohydrates, whereas carotenoieds and poly saccharides were increased during the two growing seasons. Chlorophyll a/b as well as total chlorophylls/ carotenoides ratios were also increased due to NPK stresses.

Application of bio-fertilizers, over all the NPK minerals doses, improved the accumulation of all photosynthetic pigments fraction as well as reducing sugars, non-reducing sugars, total sugars and total carbohydrates whereas, decreased that of polysaccharides compared to the plants grown without bio-fertilizers inoculation. Similarly, chlorophyll a/ chlorophyll b and chlorophylls /carotenoides ratios were increased with the same manner due to the inoculation of bio fertilizers used. The application of NFB was more effective than the other strains used followed by PDB and SB when used individually or incombinations compared with plants inoculated without bio fertilizers.

The interaction treatments between minerals and bio fertilizers, show that, bio fertilizers counteracted the depressing effects of decreasing NPK dose less than the recommended one on all photosynthetic pigments as well as reducing, non-reducing and total sugars as well as total carbohydrates whereas, decreased insoluble carbohydrates in the shoot system of potato plants during the two growing seasons. An additive effects were recorded in plants grown in 100% NPK and inoculated with bio-fertilizers mixture. Again, NFB strain was most effective than the other strains if inoculated individually or in combination with the others. Application of 75% NPK combined with bio fertilizers showed high values in this respect. The most effective treatment was found with NFB+PDB+SB followed by NFB+PDB and NFB+SB respectively.

Generally, it seems that all bio-fertilizers used, with the superiority of NFB strain, counteracted the depressing effect of NPK decreases on photosynthetic capacity up to 75% dose. At 75% NPK dose combined with bio-fertilizers attained nearly similar results with those recorded in the control plant. On the other, bio-fertilizers used failed to counteracted the harmful effects of NPK at 50% dose from the recommended dose. Bio-fertilizers in the presence of NPK at 50% dose from the recommended dose attained the minimum values in this respect.

Key words: potatoes, NPK, Biofertilizers, NFB, PDB, SB.

INTRODUCTION

Potato (*Solanum tuberosum, L;* Solanaceae) is considered one of the most important and popular vegetable crop in Egypt. Potato tubers are an excellent source of nutrients, protein, carbohydrates, mineral and ascorbic acid (Pondey and Chadha, 1996). It requires much more nutrients, particularly, N, P and K as compared with other vegetable crops.

Chemical fertilizers, particularly nitrogen salts are commonly used to improve potatoes growth and its productivity (Hussein and Radwan, 2002). Several investigators showed that mineral sources of N-fertilizers, especially

NO^{-}_{3} salts, accumulate more NO^{-}_{3} and NO^{-}_{2} ions within the plant tissues. From the nutritional point of view, NO^{-}_{3} and NO^{-}_{2} accumulations in the edible parts of the vegetable crops represented a serious problem for human health because their absorption into the blood. They may oxidize Fe^{++} of hemoglobin to Fe^{+++} and producing methemoglobin, which cannot transport oxygen (Swann, 1975).The toxicity of NO^{-}_{3} may be due to the formation of carcinogenic N- nitrous compounds by reaction with amino compounds. The toxic ions of nitrate and nitrite forming from nitrification are well known as an environmental pollutant (Alexander, 1977).

Great efforts have been directed to overcome the problems of chemical fertilizers which are generally represented in increasing costs as well as environmental pollution and its negative effects on human health. These efforts have been given to decrease the recommended chemical fertilizer doses by application of bio-fertilizers (Abd El-Naem *et al.,* 1999). Bio-fertilization is used in order to compensate a part of the mineral fertilizer doses, taking in consideration the complementary or synergistic effects of such combination between bio-and mineral fertilization. This could be of economic value from the applied point of view of minimizing the used doses of the mineral fertilizers and consequently reduce agricultural costs as well as soil pollution. In addition, the bio-fertilizers are increasingly used in modern agriculture due to the extensive knowledge in rhizosphere biology and the discovery of the promotive function of special groups of microorganisms such as *Azospirillum, Azotobacter, Acetobacter, Bacillus, Serratia* and *Pseudomonas* which known as plant growth promoting rhizobacteria (PGPR). Such beneficial effects of these promoting rhizobacteria may be attributed to the biological nitrogen fixation and production of phytohormones (gibberillin, cytokinin like substances and auxins) that promote root development and proliferation, resulting in efficient uptake of water and nutrients (Hartmann *et al*., 1983 and Haaktel *et al.*, 1998)

Application of bio-fertilizer is an important economically to reduce the cost of fertilizers and ecologically to reduce pollution of the environment (Verma, 1990).

The present investigation aimed to study to what extent the bio-fertilizers can replace some of the recommended NPK mineral fertilizers without affecting on growth. Photosynthetic activity.

MATERIALS AND METHODS

Two field experiments were carried out at the Agriculture Experimental Station, Faculty of Agriculture, Mansoura University, Egypt during the two growing seasons of 2001/2002 and 2002/2003. Different rates of the recommended NPK mineral fertilizers and three strains of non-symbiotic bacteria as a bio-fertilizers sources of N, P and K were used.

Potatoes tubers; Spunta cv (imported from Holland) were used in the present investigation and obtained from Agric. Res. Center (ARC),

Ministry of Agric., Egypt. Tubers were divided to pieces, averaging approximately 50 g weight.

Soil samples and analysis:

Twenty surface samples (0-20 cm depth) were taken at ten different locations before the experimental design, air dried, grounded, mixed and kept in plastic bags for the analyses. The mechanical and chemical analyses of the soil used were carried out in the two growing seasons as described by Jackson (1973) and Page *et al.,* (1982) and presented in Table (I).

Table (1): The physiochemical properties of the experimental soil used during the two growing seasons of 2001/2002 and 2002/2003.

Season	1. Mechanical Analysis				Organic Matter	Calcium carbonate	PH (1:2.5 soil: water suspension)	Soil texture
	Soil Fraction %							
	Coarse sand	Fine sand	Silt	Clay				
2001/2002	2.43	21.43	27.66	48.29	0.99	2.09	7.80	Clayey
2002/2003	2.58	22.50	25.92	49.00	1.10	2.12	7.65	

	2. Chemical Analysis								
	EC dsm^{-1} soil paste extract at 25 C^0	CATIONS (meq/L)				ANIONS (meq/L)			
		Ca^{++}	Mg^{++}	Na^{+}	K^{+}	HCO_3^-	$CO_3^=$	$SO_4^=$	Cl^-
2001/2002	1.31	5.33	4.22	10.40	0.39	2.44	-	7.68	10.63
2002/2003	1.45	5.21	4.11	10.99	0.37	2.07	-	7.80	11.00

	3. Nutrients Analysis		
	mg/100 g soil		
	N	P	K
2001/2002	25.00	8.30	268..91
2002/2003	33.00	8.50	335.10

Experimental design:

Farm yard manure has been added during soil preparation as organic fertilization at dose (40 m^3/fed.). The experiments comprised of 24 treatments included three different rates of the recommended NPK mineral fertilizers used individually or in combinations with three strains of non-symbiotic bacteria as a bio-fertilizer sources for N, P and K. The experiments design used was a two factor randomized complete block system distributed as a split plot combined with five replications. Each plot was (14 m^2) included four ridges, each five meters long and 70 cm apart; the distance between hills was 25 cm apart.

Bio-fertilizer treatments:

Three strains of non-symbiotic bacteria were used in the present investigation as bio-fertilizers sources; "*Azospirillum brasilense*" , nitrogen-fixing bacteria (NFB) , "*Pseudomonas fluorescens*" , phosphate-dissolving bacteria (PDB) and "*Bacillus circulans*" , silicate bacteria (SB) which able to release K from clay minerals (Monib *et al.*, 1984). The two former strains were obtained from Microbiol. Res.Dept., Soil, Water and Environ. Res. Inst., ARC. Giza, Egypt, whereas the third organism was obtained from Microbiol.

Dept., Fac. of Agric., Mansoura Univ. Egypt. All bacterial strains were multiplied in nutrient liquid broth and centrifuged then prepared again in suspension. Liquid broth cultures contains $5x10^8$, $9x10^8$and $2.15x10^8$ cells/ml of NFB, DPB and SB, respectively.

Microbial inoculum treatments:

As recommended by the Pathology Dept. Ministry of Agric. Egypt,potato tubers pieces were sterilized with Vitavax Kapetan 1% at the rate of 1.25 kg/ton. and then inoculated with bacteria suspension, individually or in combinations directly before planting to form the following treatments:

1- Without bio-fertilizers.
2- Inoculation with *Azospirillum brasilense* (NFB).
3- Inoculation with *Pseudomonas fluorescens* (PDB).
4- Inoculation with *Bacillus circulans* (SB).
5- Inoculation with (NFB + PDB).
6- Inoculation with (NFB + SB).
7- Inoculation with (PDB + SB).
8- Inoculation with (NFB + PDB + SB).

Mineral fertilizer treatments:

As recommended by the Agric. Res. Center, Egypt, nitrogen fertilizer in the form of ammonium nitrate (33.3% N) was used at the dose of 180 kg N/fed. at three equal doses. The first was used after emergence (18-21 days from planting), whereas the second and third doses were applied before the 2nd and the 3rd irrigations respectively (31 and 46 days from planting). Calcium superphosphate (15.5% P_2O_5), as a source of phosphorus, at the dose of 75 kg P_2O_5 /fed., was added to the soil before planting and during soil preparation. Potassium sulphate (48 % K_2O) was used as a source of potassium at the dose of 96 kg K_2O/fed. at two times, the first half was added with the first addition of N-fertilizer, and the second with the third doses of N-fertilizer.

The mineral fertilizer treatments were used at the three following different rates:

1- 100% NPK from the recommended dose (control).
2- 75% NPK.
3- 50% NPK.

These treatments were used with or without the bio-fertilizer treatments.

Planting procedure:

The treated potato pieces were planted in the ridges at 12-15 cm depth (25 cm apart) on 12nd October, 2001 and 15th October, 2002 growing season, respectively. Irrigation was done immediately. All usual cultural practices of potatoes cultivation were carried out according to the procedures that recommended by the Ministry of Agric. Egypt. at the active growth period (75 days from planting) samples were taken to determinations of photosynthetic pigments, carbohydrate fractions and minerals concentrations

Photosynthetic pigments:

The blade of the 3rd upper compound leaf was chosen from the apex of the main stem to determine photosynthetic pigments (chlorophylls a, b and

their total as well as carotenoides) concentrations. The optical density of the filtrate was determined spectrophotometry by Milton Roy Spectronic 1201. The concentrations of chl (a) and chl (b) as well as carotenoides were calculated according to Wettstein's formula (Wettestein, 1957) as follows:

Chl (a) = 9.784. E662 – 0.99. E644 = mg/l

Chl (b) = 21.426. E644 – 4.650. E662 = mg/l

Carotenoides = 4.695. E440.5 – 0.268 (chl a + chl b) = mg/l

Carbohydrate fractions:

Total soluble carbohydrate (sugars) were extracted from 5 g crude dried material of the shoots from each treatment by ethanol 70%, and kept overnight at room temperature (Kayani *et al.,* 1990) before being filtered. Protein was precipitated by using trichloroacetic acid (TCA).

Reducing sugars (R.S) was determined using (modified Nelson, s solutions;(Naguib 1964) and the intensity of the colour was measured by Milton Roy Spectronic 1201 spectrophotometer at 620 nm against a blank containing only distilled water and modified Nolson's reagent.

For estimation non-reducing sugars (NRS), 10 ml of the cleared extract previously mentioned, were mixed with 5 ml of 1.5 N hydrochloric acid and the mixture was kept in a water bath at 60 ºC for 30 minutes (Naguib, 1964) and the reducing values were determined as described before. The differences between the value obtained by this methods and that of reducing sugars is an estimate of non-reducing sugars content as sucrose.

Total carbohydrates were determined as described by Amberger (1954). Hydrolsis was carried out in the homogenates of 5 g crude dried materials by boiling it for 3 hours with 5 ml 25% hydrochloric acid.

Statistical analysis:

The experiment of the present investigation was laid out as a factorial complete randomized block design system with split plot combined over locations. Data were statistically analyzed according to the technique of analysis of variance (ANOVA). Least Significant Difference test (L.S.D.) method was used to test the differences between treatments means at 5% [in case of significant difference (*)] and 1% [in case of highly significant difference (**)] levels of probability, as published by Gomez and Gomez (1984).

RESULTS AND DISCUSSION

Photosynthetic pigments:

The highest chlorophylls values were resulted from the control plants which received full recommended dose of NPK during the two growing seasons (Tables 2 and 3). Decreasing NPK doses less than the recommended one decreased the concentrations of chlorophylls a, b and their total. Total chlorophylls content was also decreased and the decrease was a concentration dependent.

On the other hand, the data clearly show that carotenoides concentration and their content were increased under NPK stresses. The rate

of decrease was noticed to be greater in chlorophyll b than in chlorophyll a. Therefore, ratios of chlorophyll a/b as well as total chlorophylls/ carotenoides were increased as a result of decreasing NPK doses.

Each of the bio-fertilizers used had a stimulative effect on all photosynthetic pigments fraction concentrations as well as their content during the two growing seasons. Therefore, inoculation of potato tubers with either of NFB, PDB and /or SB increased significantly chlorophylls a, b and their total as well as carotenoid concentration in the 3rd upper compound leaf of potato when compared with uninoculation one. The application of NFB was more effective than the other strains used followed by PDB and SB compared with plants inoculated without bio fertilizers. An additive effects were shown with NFB if used incombination with the other strains used. The synergistic effects of PDB on increasing photosynthetic pigments were more pronounced than that with SB. Therefore, the photosynthetic pigments concentrations as well as its content were higher in NFB+PDB+SB followed by NFB+PDB, NFB+SB, NFB, PDB+SB, SB in a descending order. These results are true during the two growing seasons overall NPK level. Similarly, chlorophyll a/ chlorophyll b and chlorophylls /carotenoides ratios were increased, with the same manner, due to the inoculation of bio fertilizers used. These results indicate that the rate of increase in chlorophylls especially chlorophyll a was more affected positively than chlorophyll b and carotenoides.

The stimulating effect of *Azospirillum brasilens* (NFB), *Pseudomonas fluorescens* (PDB) and *Bacillus circulans* (SB) on photosynthetic pigments might be due to their action as antioxidants, in which protect chloroplasts against the formation of toxic free radicals, thereby prevent degradation of pigments and inhibit the photooxidation of pigments that arise under stressful conditions (Abo-Aly and Gomaa, 2002).

The increases of leaf area and photosynthetic pigments as well as increament of the dry matter accumulation of the leaves indicate the stimulatory effects of *Azospirillum brasilens* (NFB), *Pseudomonas fluorescens* (PDB) and *Bacillus circulans* (SB) upon the efficiency of photosynthesis processes, hence more photosynthates being created as well as enhancement of minerals translocation from roots to leaves and, in turn, the sufficient assimilates supply.

All of these advantages, in addition to higher potato yield, with good quality achieved make the applied of *Azospirillum brasilens* (NFB), *Pseudomonas fluorescens* (PDB) and *Bacillus circulans* (SB) may be recommended as effective and safe agricultural practice in cultivation of potatoes under nutrients stress condition.

Regarding the interaction treatments between minerals and bio fertilizers, data in the same tables show that, bio fertilizers counteracted the depressing effects of mineral deficiency (decreasing NPK dose less than the recommended one) on all photosynthetic pigments concentration as well as their content during the two growing seasons. An additive effects were recorded in plants grown in 100% NPK and inoculated with bio-fertilizers mixture. Again, NFB strain was the most effective than the other strains if

inoculated individually or in combination with the others. Application of 75% NPK combined with bio fertilizers showed high values in this respect.

Table (2): Effects of mineral and/or bio- fertilizers on chlorophyll a , b and total chlorophylls (a+b) concentrations (mg/g F.Wt.) and their content (mg/plant) in the 3rd upper compound leaf of potato plants grown in the two growing seasons of 2001/2002 (S1) and 2002/2003.(S2)

Treatments		Chlorophyll a Concentration (mg/g F.Wt.)			Chlorophyll b Concentration (mg/g F.Wt.)			Chlorophylls (a+b) Concentration (mg/g F.Wt.)			Chlorophylls (a+b) content (mg/plant)		
M- Mineral NPK	B- Bio-fertilizer	S1	S2	Mean	S1	S2	Mean	S1	S2	Mean	S1	S2	Mean
Control 100%	Without	0.930	0.995	0.961	0.413	0.435	0.424	1.343	1.430	1.386	98.0	117.7	107.9
	NFB	1.002	1.017	1.008	0.465	0.495	0.480	1.467	1.512	1.489	111.7	125.9	118.8
	PDB	0.986	1.014	1.000	0.424	0.472	0.448	1.410	1.486	1.448	107.7	124.7	116.2
	SB	0.942	1.006	0.974	0.416	0.443	0.430	1.358	1.449	1.403	101.8	119.1	110.5
	NFP+PDB	1.005	1.030	1.017	0.495	0.500	0.497	1.500	1.430	1.515	117.0	114.4	115.7
	NFB+SB	1.005	1.025	1.015	0.470	0.500	0.485	1.475	1.525	1.500	113.9	128.1	121.1
	PDB+SB	0.994	1.020	1.007	0.472	0.486	0.479	1.466	1.506	1.486	114.3	128.0	121.2
	NFB+PDB+SB	1.007	1.033	1.020	0.500	0.505	0.502	1.507	1.538	1.522	118.8	132.4	125.6
Mean		0.985	1.020	1.002	0.456	0.474	0.465	1.441	1.497	1.457	110.4	123.8	117.1
75%	Without	0.902	0.991	0.946	0.360	0.399	0.379	1.262	1.390	1.326	91.1	111.2	101.1
	NFB	0.986	1.010	0.998	0.447	0.440	0.443	1.433	1.450	1.441	108.0	120.6	114.3
	PDB	0.982	0.998	0.990	0.408	0.421	0.414	1.390	1.419	1.404	105.2	118.8	112.0
	SB	0.931	1.003	0.967	0.364	0.409	0.386	1.295	1.412	1.353	95.8	112.2	104.0
	NFP+PDB	0.997	1.019	1.008	0.478	0.472	0.475	1.475	1.491	1.483	115.0	127.1	121.1
	NFB+SB	0.997	1.016	1.006	0.456	0.459	0.457	1.453	1.475	1.464	110.8	124.4	117.6
	PDB+SB	0.981	1.017	0.999	0.421	0.441	0.431	1.402	1.427	1.414	107.5	120.5	114.0
	NFB+PDB+SB	1.002	1.018	1.010	0.492	0.485	0.488	1.494	1.503	1.498	116.5	128.5	122.5
Mean		0.976	1.011	0.993	0.420	0.446	0.433	1.397	1.452	1.426	106.3	120.4	113.3
50%	Without	0.892	0.966	0.929	0.303	0.323	0.313	1.195	1.279	1.237	73.4	97.9	85.7
	NFB	0.915	0.988	0.951	0.349	0.351	0.350	1.264	1.339	1.301	92.5	105.8	99.1
	PDB	0.904	0.985	0.945	0.318	0.347	0.333	1.222	1.332	1.277	90.3	106.4	98.4
	SB	0.900	0.980	0.940	0.315	0.325	0.320	1.215	1.305	1.260	88.2	101.7	94.9
	NFP+PDB	0.939	0.993	0.966	0.367	0.382	0.374	1.306	1.375	1.340	96.4	111.5	103.9
	NFB+SB	0.934	0.993	0.963	0.370	0.365	0.367	1.304	1.358	1.331	95.6	107.9	101.8
	PDB+SB	0.905	0.990	0.947	0.351	0.359	0.355	1.256	1.349	1.302	92.4	106.9	99.7
	NFB+PDB+SB	0.943	1.000	0.971	0.367	0.391	0.379	1.310	1.391	1.350	97.3	115.0	106.2
Mean		0.924	0.996	0.960	0.342	0.367	0.349	1.134	1.342	1.309	90.8	106.7	98.7
Mean	Without	0.908	0.991	0.949	0.359	0.386	0.372	1.267	1.366	1.321	87.5	108.9	98.2
	NFB	0.971	1.005	0.985	0.420	0.439	0.429	1.391	1.444	1.414	104.1	117.4	110.7
	PDB	0.960	0.999	0.983	0.383	0.430	0.406	1.344	1.429	1.389	101.1	116.6	108.8
	SB	0.924	0.996	0.961	0.364	0.392	0.378	1.289	1.389	1.339	95.3	111.0	103.1
	NFP+PDB	0.980	1.014	0.996	0.447	0.451	0.449	1.427	1.465	1.446	109.5	117.7	113.6
	NFB+SB	0.978	1.011	0.993	0.422	0.441	0.431	1.401	1.443	1.424	106.8	120.2	113.5
	PDB+SB	0.960	1.009	0.985	0.408	0.399	0.403	1.368	1.416	1.388	104.8	118.5	111.6
	NFB+PDB+SB	0.984	1.021	0.999	0.453	0.460	0.456	1.437	1.477	1.455	110.9	125.3	118.1
LSD at 5% for: SxB		0.003			0.003			NS			0.2		
SxM		0.001			0.002			0.019			0.4		
BxM		0.004			0.004			NS			0.5		
SxBxM		0.005			0.006			NS			0.7		

Table (3): Effects of mineral and/or bio-fertilizers on chlorophyll a/chlorophyll b ratio, carotenoides concentration (mg /g FWt) and their content (mg/plant) and chlorophylls/carotenoides ratio in the 3rd upper compound leaf of potato plants grown in the two growing seasons of 2001/2002 (S1) and 2002/2003 (S2).

Treatments		Chlorophyll a/Chlorophyll b ratio			Carotenoides Concentration (mg /g FWt)			Carotene content concentration (mg /plant)			Chlorophylls/ Carotenoides ratio		
M-Mineral NPK	B-Bio-fertilizer	S1	S2	Mean	S1	S2	Mean	S1	S2	Mean	S1	S2	Mean
	Without	2.252	2.287	2.269	0.397	0.473	0.435	24.4	36.2	30.3	3.383	3.023	3.203
	NFB	2.155	2.054	2.104	0.416	0.479	0.447	30.4	37.8	34.1	3.526	3.157	3.341
	PDB	2.325	2.148	2.236	0.411	0.476	0.444	30.3	38.0	34.2	3.431	3.122	3.276
Control	SB	2.264	2.271	2.267	0.406	0.477	0.441	29.5	36.6	33.0	3.345	3.038	3.191
100%	NFP+PDB	2.030	2.060	2.045	0.450	0.496	0.473	33.2	40.2	36.7	3.333	2.883	3.108
	NFB+SB	2.138	2.050	2.094	0.423	0.487	0.458	31.0	38.7	34.8	3.487	3.131	3.309
	PDB+SB	2.106	2.099	2.102	0.431	0.485	0.455	31.7	38.5	35.1	3.401	3.105	3.255
	NFB+PDB+SB	2.014	2.045	2.029	0.456	0.499	0.457	33.9	41.3	37. 6	3.305	3.082	3.193
Mean		2.160	2.127	2.143	0.324	0.484	0.454	30.5	38.4	34.5	٣,٤٠٥	٣,٠٦٨	٣,٢٣٦
	Without	2.505	2.484	2.494	0.412	0.479	0.446	29.8	38.3	34.0	3.063	2.902	2.982
	NFB	2.206	2.295	2.250	0.445	0.488	0.466	33.6	40.6	37.1	3.220	2.971	3.095
	PDB	2.407	2.370	2.388	0.433	0.486	0.459	32.8	40.7	36.7	3.210	2.921	3.065
75%	SB	2.558	2.452	2.505	0.413	0.485	0.449	30.6	38.6	34.5	3.136	2.911	3.023
	NFP+PDB	2.086	2.159	2.122	0.477	0.511	0.494	36.6	43.6	40.1	3.092	2.918	3.005
	NFB+SB	2.186	2.213	2.199	0.455	0.491	0.472	34.3	41.4	37. 9	3.193	3.004	3.096
	PDB+SB	2.330	2.306	2.318	0.450	0.498	0.474	34.5	421	38.3	3.115	2.865	2.990
	NFB+PDB+SB	2.037	2.099	2.068	0.485	0.515	0.500	37.8	44.0	40.9	3.080	2.918	2.999
Mean		2.289	2.297	2.293	0.446	0.491	0.469	33.8	41.2	37.4	٢,٩٤٩	٢,٩٢٦	٣,٠٢٩
	Without	2.944	2.991	2.857	0.485	0.520	0.503	35.4	42.8	39.1	2.464	2.461	2.462
	NFB	2.622	2.815	2.916	0.518	0.563	0.541	39.4	46.9	43.2	2.440	2.378	2.409
	PDB	2.843	2.839	2.885	0.508	0.553	0.530	38.9	44.5	41.8	2.405	2.409	2.407
50%	SB	2.857	3.015	2.864	0.493	0.538	0.515	36.9	44.2	40.6	2.464	2.426	2.445
	NFP+PDB	2.559	2.599	2.837	0.585	0.661	0.623	45.6	52.9	49.2	2.232	2.080	2.156
	NFB+SB	2.524	2.720	2.935	0.533	0.588	0.560	41.2	49.4	45.3	2.446	2.309	2.377
	PDB+SB	2.578	2.758	2.847	0.528	0.580	0.554	41.2	49.3	45.2	2.379	2.326	2.352
	NFB+PDB+SB	2.297	2.557	2.830	0.593	0.678	0.635	46.7	58.4	52.5	2.209	2.052	2.130
Mean		2.653	2.787	2.720	0.530	0.585	0.558	40.7	48.5	44.6	٢,٣٨٠	٢,٣٠٥	٢,٣٤٣
	Without	2.567	2.587	2.577	0.431	0.491	0.538	29.8	39.1	34.5	٢,٩٧٠	٢,٧٩٥	٢,٨٨٣
	NFB	2.328	2.388	2.358	0.461	0.510	0.495	34.5	41.8	38.1	٣,٠٦٢	٢,٨٣٥	٢,٩٤٩
	PDB	2.525	2.452	2.488	0.451	0.505	0.491	34.0	41.1	37.6	٣,٠١٥	٢,٨١٧	٢,٩١٦
Mean	SB	2.561	2.579	2.570	0.437	0.500	0.530	32.4	39.8	36.1	٢,٩٩٢	٢,٧٩٢	٢,٨٩٢
	NFP+PDB	2.225	2.273	2.249	0.504	0.556	0.469	38.5	45.5	42.0	٢,٨٨٦	٢,٦٢٧	٢,٧٥٦
	NFB+SB	2.283	2.328	2.305	0.470	0.522	0.485	35.5	43.2	39.3	٣,٠٤١	٢,٨١٥	٢,٩٢٨
	PDB+SB	2.338	2.388	2.363	0.471	0.521	0.478	35.8	43.3	39.5	٢,٩٦٥	٢,٧٦٥	٢,٨٦٥
	NFB+PDB+SB	2.116	2.234	2.175	0.511	0.564	0.461	39.5	47.9	43.7	٢,٨٤٩	٢,٦٨٤	٢,٧٦٦
LSD at 5% for: SxM			0.008			0.003			2.3			0.007	
SxB			0.036			0.005			2.6			0.011	
MxB			0.044			0.006			3.0			0.014	
SxMxB			0.063			0.008			3.6			0.019	

These results indicated that, *Azospirillum brasilens* (NFB) had a synergistic action effects when used inoculation with either of *Pseudomonas fluorescens* (PDB) and/or *Basillus circulans* (SB) on photosynthetic pigments concentrations and their content. Therefore, plants inoculated with

NFB+PDB showed high values in their chlorophylls and carotenoides concentrations as well as their content followed by that inoculated with NFB+SB and with PDB+SB. Inoculated with NFB+PDB+SB was the most effective in this respect.

The addition of mineral fertilizer showed a synergistic effect to that of the bacterial strains used on increasing all photosynthetic pigments concentrations and their content.Compared with the control (100% recommended NPK), data also show that, the plants which received mixed strains of used bacteria (NFB+PDB+SB) and grown under 75% NPK (from recommended dose) showed higher values of chlorophyll a, b and their total as well as carotenoides than the plants treated with mixed bacterial strains and grown under 50% NPK (from recommended dose). On the other hand, plants treated with NFB+PDB+SB plus 75% NPK (from recommended dose) showed higher values in this respect. Ratios of chlorophyll a/ chlorophyll b and chlorophylls / carotenoides showed similar trend during the two growingseasons. The reduction in chlorophylls was accompanied with an irregular fluctuation values regarding chlorophyll a/ chlorophyll b ratio , whereas chlorophylls / carotenoides ratio decreased due to the increase in carotenoides values and the decrease in chlorophylls concentrations.

The decrease in chlorophylls under stress may be due to the inhibiting effects of nutrients deficiency on the activity of Fe-containing enzymes; cytochrom oxidase (Maximova and Matychen, 1965), The disruption in chloroplast structure (Helaly 1984) which in turn may decrease the rate of chlorophylls biosynthesis and their accumulation. Prisco and O'Leary (1972) interpreted the enhancement of chlorophyll decay in the leaves of stressed plants to the disrupted hormonal balance in the leaves. One possibility of how this could occur would be due to less synthesis of cytokinins in the roots and as a consequence less hormone delivering to the shoots. Another possibility would be that absicic acid activity increased in stressed plants and this compound is known to accelerate leaf senescence (Hatung, 2004).

The behavior of carotenoides under stress condition may reflect its well known role in plant tissues as a protective compound against the unfavorable conditions (Helaly, 1977 and Jeffrey, 1987).

The enhancing effects of bio fertilizers on chlorophylls concentration and their content may be attributed to their effects on increasing not only mineral uptake (Hauka, 2000) but also the production of growth substances especially cytokinins (Omay *et al.,* 1993). Cytokinins are known to stimulate chlorophyll synthesis and delay chlorophyll destruction and senescence (Dalzeil and Lawrence, 1984).

Jagnow *et al.*, (1991) and Gabr *et al.,* (2001) reported that, the role of non-symbiotic N2-fixing bacteria on the availability of nutrients and the modification of root growth morphology and physiology would be through hormonal exudates of bio-fertilizer bacteria which led to more efficient absorption of available nutrients which are main components of photosynthetic pigments.

Subba rao, (1993) added that, the beneficial effects of bacterization on chlorophylls may be attributed to N2-fixation process, and/or to the

production of growth promoting substances like gibberellins and other compounds of auxin type which gave a positive effect of plant growth, chlorophyll content nutrient uptake (Frankenberger and Arshad, 1995; Bashan and Holguim, 1997).

The increase of chlorophylls and carotenoides due to PDB treatment reflects may be attributed to the effects of phosphate-dissolving bacteria on decreasing soil PH, increasing the availability of some nutrients such as Fe, Zn, Mn and Cu to plant uptake (Gaur and Ostwal, 1972; Alexander, 1982; El-Dahtory *et al.,* 1989; Hauka *et al.,* 1990), potassium content (El-Shahawy, 2003), stimulating and surviving nitrogen fixing bacteria such as *Azospirillum* (Algawady and Gaur, 1988).In addition, the phosphate-dissolving bacteria may play a desirable role as a source for certain nutrients for supplying the plants by their nutrient requirements (Saber *et al.,* 1983). Moreover, Sobh *et al.,* (2000) reported that, inoculation with phosphorus bio-fertilizers increased phosphatase activity, available P and producing growth regulating hormones. Moreover, Bender *et al.,* (1986) revealed that, available P increased the photosynthetic Co_2 fixation and assimilates translocation.

The superiority of mineral NPK fertilizers interacted with inoculation of bacteria strains used on chlorophylls as well as carotenoides concentrations and their content of potato leaves may be attributed, mainly, to the effect of the three strains on increasing the efficiency of added mineral fertilizers (NPK) and consequently increased the absorbed nitrogen and other elements uptake by potatoes leading to an increase in chlorophyll pigments biosynthesis (Marschner, 1995; Hedge *et al.,* 1999).

Arisha and Bardisi (1999) reported that, nitrogen is a constituents on chlorophyll molecule. Moreover, nitrogen is the main constituent of all the amino acids and hence of proteins as well as lipids as galactolipids, acting as a structural components of the chloroplast. Correspondingly, an enhancement of protein synthesis and chloroplasts formation leads to an increase in chlorophyll biosynthesis (Marschner, 1995). The latter authors added that, P is a component of assimilatory ATP and $NADPH+H^+$ as well as other compounds that play a vital role in biosynthesis of chlorophylls and other pigments.

Carbohydrate fractions:

Results in the Tables (4) were paralled with those obtained above with respect to photosynthetic pigments. Mineral fertilizers at full recommended dose (control) attained the highest reducing sugars, non-reducing sugars, total sugars and total carbohydrates concentrations in the shoots of potato plants were decreased with decreasing NPK fertilizers doses less than the control. However, polysaccharides were increased as a result of NPK dose decrease and the lowest values were recorded in the control. Sugars and total carbohydrates link to the case of stress *via* their roles as cellular cryoprotective or osmoregulator agent (Hockaka and Somera, 1973), they protected proteins and enzymes against denaturation induced by nutrients stresses as well as basic substrate for ATP synthesis.

Application of bio-fertilizers, over all the NPK minerals doses, improved the accumulation of reducing sugars, non-reducing sugars, total sugars and total carbohydrates whereas, decreased that of polysaccharides in comparison to

the plants grown without bio-fertilizers inoculation. The most effective treatment was found with NFB+PDB+SB followed by NFB+PDB and NFB+SB respectively. Moreover, the data indicated that, NFB strain was most effective treatment followed by PDB and SB respectively.

Table (4): Effects of mineral and/or bio- fertilizers on reducing sugars, non-reducing sugars, total sugars, polysaccharides and total carbohydrates concentration (mg/g D.Wt) in the shoot system of potato plants grown during the two growing seasons of 2001/2002 (S1) and 2002/2003 (S2).

Treatments		Reducing sugars			Non-reducing sugars			Total sugars			Polysaccharides			Total carbohydrates		
M- Mineral NPK	B- Bio-fertilizer	S1	S2	Mean	S1	S2	Mean	S1	S2	Mean	S1	S2	Mean	S1	S2	Mean
Control 100%	Without	20.98	19.17	٢٠,٠٧	2.91	2.59	٢,٧٥	22.08	23.57	٢٢,٨٢	100.08	102.11	١٠١,٠٩	122.88	125.68	١٢٤,٢٨
	NFB	23.17	21.48	٢٢,٣٢	2.65	3.72	٣,١٨	24.13	28.89	٢٥,٥١	98.88	99.27	٩٩,٠٨	123.01	126.16	١٢٤,٥٨
	PDB	21.75	21.66	٢١,٢٠	1.87	2.54	٢,٢٠	23.53	24.49	٢٣,٥١	99.43	101.23	١٠٠,٣٣	122.96	125.72	١٢٤,٣٤
	SB	20.62	21.52	١٩,٨٢	1.90	2.27	٢,٠٨	22.42	24.89	23.65	99.98	100.08	١٠٠,٠٣	122.99	124.97	١٢٤,٣٤
	NFP+PDB	30.62	26.96	٣٠,٢٩	3.66	4.19	٣,٩٢	33.62	31.81	32.62	98.60	98.27	٩٨,٤٣	132.22	130.08	١٣١,١٥
	NFB+SB	21.21	24.62	٢٥,٤١	3.35	3.42	٣,٣٨	27.97	29.63	٢٨,٨٠	98.33	98.55	٩٨,٤٤	126.30	128.18	١٢٧,٢٤
	PDB+SB	23.04	25.54	٢٥,٤١	3.25	3.65	٣,٤٥	25.79	29.69	30.30	98.28	98.58	٩٨,٤٣	124.00	128.57	١٢٦,٢٨
	NFB+PDB+SB	35.44	32.93	٣٤,١٨	4.47	4.96	٤,٧١	41.40	40.40	٤٠,٩٠	95.68	96.07	٩6٤٣.	137.08	136.47	١٣٦,٧٧
	Mean	٢٣,٨٦	٢٥,١٦	٢٤,٥١	٣,٠١	٣,١٢	٣,٢١	٢٧,٢٤	٢٨,٦١	٢٧,٩٢		99,27	٩٩,٢١	١٢٦,٤٣	١٢٨,٢٢	١٢٧,٥٨
75%	Without	18.44	16.16	١٧,٣٠	2.07	3.72	٤,٧١	18.23	22.16	٢٠,١٩	99.54	101.41	١٠٢,٤٦	120.71	124.60	١٢٢,٦٥
	NFB	22.29	20.65	٢١,٤٧	2.47	2.72	٢,٥٩	23.12	25.01	٢٤,٠٦	99.01	100.63	١٠١,٢٦	123.80	125.86	١٢٤,٨٣
	PDB	21.96	19.69	١٩,٨٢	1.15	2.36	١,٧٥	20.84	24.32	٢2٥٨.	99.37	100.80	١٠١,٢٦	121.71	124.98	١٢٣,٣٤
	SB	19.19	19.89	١٩,٠٤	1.27	2.01	١,٦٤	20.16	22.20	٢1.1٨	99.50	101.22	١٠١,٧١	121.71	125.08	١٢٣,٣٩
	NFP+PDB	30.83	27.51	٢٩,١٧	3.45	3.69	٣,٥٧	30.96	34.52	٣٢,٧٤	98.55	99.88	٩٩,١٣	130.00	133.74	١٣١,٨٧
	NFB+SB	25.98	23.63	٢٤,٨٠	1.72	2.34	٢,٠٣	25.35	28.32	٢٦,٨٣	98.76	99.66	١٠٠,١٠	125.44	128.43	١٢٦,٩٣
	PDB+SB	22.95	21.54	٢٢,٢٤	1.09	2.33	١,٧١	22.63	25.28	٢٣,٩٥	98.81	99.29	١٠٠,٢٥	122.81	125.61	١٢٤,٢١
	NFB+PDB+SB	35.49	31.85	٣٣,٦٧	4.10	4.51	٤,٣٠	35.95	40.00	٣٧,٩٧	95.10	96.54	٩٥,٦٥	135.93	135.33	١٣٥,٦٣
	Mean	٢٢,٣٦	٢٤,٥٢	٢٣,٤٤	٢,١٦	٢,٩٦	٢,٥٦	٢٤,٥٣	٢٧,٤٨	٢٦,٠٠	٩٨.58	99.93	٩٩,٥٠	١٢٥,٦٣	١٢٨,32	١٢٧,٥٨
50%	Without	15.33	14.17	١٤,٧٥	1.46	1.46	١,٤٦	15.63	18.82	١٦,٢٢	102.48	102.44	١٠٠,٤٧	115.17	118.23	١٣٨,٦٣
	NFB	17.88	16.95	١٧,٥٨	1.33	2.48	١,٩٠	18.21	20.39	١٩,٣٥	101.68	100.85	٩٩,٨٢	117.22	121.02	١١٩,١٢
	PDB	17.43	16.83	١٧,١٣	1.12	2.24	١,٦٨	17.95	19.69	١٩,٨١	100.87	101.66	١٠٠,٠٨	117.32	120.47	١١٨,٨٩
	SB	16.41	16.58	١٥,٩٩	1.62	1.49	١,٥٥	18.20	18.90	١٨,5٥	101.55	101.88	١٠٠,٣٦	117.70	119.12	١١٨,٨٩
	NFP+PDB	23.42	20.06	٢١,٧٤	2.27	2.21	٢,٢٤	18.33	25.73	٢٤,٠٣	99.04	99.22	٩٩,٢٢	120.88	125.61	١٢٣,٢٤
	NFB+SB	19.27	18.49	١٨,٨٨	1.84	2.76	٢,٣٠	20.33	22.03	٢١,١٨	100.09	100.11	٩٩,٢١	119.09	121.69	١٢٠,٣٩
	PDB+SB	19.09	18.21	١٨,١٥	1.54	1.85	١,٦٩	19.75	20.04	١٩,٨٩	100.18	100.33	٩٩,٠٤	118.56	119.33	١١٨,٩٤
	NFB+PDB+SB	29.48	27.43	٢٨,٤٥	3.06	3.55	٣,٣٠	29.49	33.03	٣١,٢٦	95.98	95.33	٩٥,٨٢	124.59	129.57	١٢٧,٠٨
	Mean	١٨,٤٦	١٩,٨٣	١٩,١5	٢,١٦	٢,٩٦	٢,٠٢	٢٠,٢٤	٢١,٩٥	٢١,٠٩	١٠٠,23	١٠٠,23	١٠٠,٦٠	١١٩,٠٦	١٢٢,١٣	١٢٠,٥٩
Mean	Without	١٦,٥٠	١٨,٢٥	١٧,٣٧	٢,١٥	٢,٥٩	٢,٣٦	١٨,٦٥	٢٠,٨٥	١٩,٧٥	١٠٠,٧٠	١٠١,٩٨	١٠١,٣٤	١١٩,٥٨	١٢٢,٨٣	١٢١,٢١
	NFB	١٩,٦٩	٢١,٢٢	١٧,٣٦	٢,١٥	٢,٩٧	٢,٥٦	٢١,٨٢	٢٤,٠٩	٢٢,٩٦	٩٩,٨٥	١٠٠,٢٥	١٠٠,٠٥	١١٩,٥٨	١٢٤,٣٤	١٢١,٢١
	PDB	١٨,٧٣	٢٠,٠٥	١٩,٣٩	١,٣٨	٢,٣٨	١,٨٨	٢٠,١١	٢٢,٤٩	٢١,٣٠	٩٩,٨٥	١٠٠,٢٥	١٠٠,٥٦	١٢٠,٦٦	١٢٣,٧٢	١٢٢,١٩
	SB	١٧,٩٩	١٨,٥٧	١٨,٢٨	١,٥٩	١,٩٢	١,٨٨	١٩,٥٩	٢٠,٥٠	٢٠,٠٤	١٠٠,34	١٠١,٠٦	١٠٠,٧٠	١٢٠,٨٠	١٢٣,٠٥	١٢١,٩٢
	NFP+PDB	٢٥,٨٤	٢٨,٢٩	٢٧,٠٧	٣,١٣	٣,٣٦	٣,٢٤	٢٨,٩٧	٣١,٦٨	٣٠,٣٣	٩٨,٧٣	٩٩,١٢	٩٨,٩٣	١٢٧,٧٠	١٢٩,٨١	١٢٨,٧٥
	NFB+SB	٢٢,٢٥	٢٣,٨٢	٢٣,٠٣	٢,٣٠	٢,٨٤	٣,٢٤	٢٤,٥٥	٢٦,٦٦	٢٥,٦٠	٩٩,٠٦	٩٩,٤٤	٩٩,٢٥	١٢٣,٦١	١٢٦,١٠	١٢٤,٨٥
	PDB+SB	٢٠,٧٦	٢١,٦٩	٢١,٢٣	١,٩٦	٢,٨٤	٢,٢٨	٢٤,٥٥	٢٤,٠٠	٢٣,٣٦	٩٩,٠٩	٩٩,٤٠	٩٩,٢٤	١٢١,٧٩	١٢٤,٥٠	١٢٣,١٤
	NFB+PDB+SB	٣٠,٧٤	٣٣,٤٧	٣٢,١0	٣,٨٨	٤,٣٤	٤,١١	٣٥,٦١	٣٧,٨١	٣٦,٧١	95.58	95.98	95.78	١٣٤,٨٦	١٣٦,١٢	١٣٥,٤٩
LSD at 5% for: SxM			0.05			0.01			0.02			0.01			0.08	
SxB			0.08			0.01			0.01			0.02			0.14	
MxB			0.11			0.01			0.02			0.02			0.17	
SxMxB			0.14			0.01			0.03			0.03			0.23	

Regarding the interaction treatments, data in Table (4) clearly show that, inoculation with all used bacteria strains and their interactions with NPK doses increased significantly the concentrations of reducing, non-reducing and total sugars as well as total carbohydrates whereas, decreased insoluble carbohydrates in the shoot system of potato plants. These results are true in the two growing seasons.

The additive effects of bio fertilizers was more pronounced at the control (100% NPK). As NPK dose decreased, it seems that all bio-fertilizers used, with the superiority of NFB strain, counteracted the depressing effect of

NPK decreases up to 75% dose. At 75% NPK dose combined with bio-fertilizers attained nearly similar results with those recorded in the control plant with slight differences between them. Again, the most effective strains was found with NFB followed with PDB and SB respectively. However, using these strains, all together, recorded highest counteraction effect. On the other, bio-fertilizers used failed to counteracted the harmful effects of NPK at 50% dose from the recommended dose. Bio-fertilizer in the presence of NPK at 50% dose from the recommended dose attained the minimum values in this respect.

The obtained data did confirm the previous growth data recorded in regards to the gradual increases in sugars of shoots as the NPK dose increased. Carbohydrates, especially sugars, link to the case of stress *via* their roles as cellular cryoprotective or osmoregulator agent (Hockaka and Somera, 1973) they protected proteins and enzymes against denaturation induced by nutrients stress as well as basic substrate for ATP synthesis.

The increase of total sugars and total carbohydrates concentrations due to the bio-fertilizers as shown in the present study was supported by Agamy (2004) and Mohamed, Faten (2007). They showed that, bio fertilizers significantly increased both mineral and leaf chlorophylls and carotenoides concentrations than those of unfertilized plants. These results are good explanation to the obtained results regarding the favorable role of bio fertilizers on growth characters. The availability of N and P for plant growth due to diazotrophs and phosphate solubilizers inoculated to the large increase in the rate of photosynthesis by the plants which are sufficient to plant growth. The enhancing effect of bio fertilizers on growth and photosynthetic pigments with the same treatment may explain the increase of total carbohydrates concentration.

The stimulating effects of both bio-and mineral fertilizers on sugar concentration may be related to their effects on enhancing photosynthetic pigments in the leaves and different plant hormones as shown in the present investigation .

Zayed (1998) proved that, phosphorus dissolving bacteria is known by its ability to dissolve the precipitation form phosphorus: Ca_3 $(PO4)_2$ depending on its ability to produce inorganic, organic acids and/or Co_2. Bender *et al*., (1986) revealed that, phosphorus increased photosynthetic Co_2 fixation and assimilates translocation in carrot plants. Rabinoveich *et al.,* (1999) mentioned that, increased doses of bio-fertilizers for potato raised high concentration of denitrificating microorganisms.

Abou-Hussein *et al.,* (2002) found that, adding bio-fertilizers to potatoes increased dry matter and total carbohydrates of produced potato plants . This effect may be due to that bio-fertilizers play a fundamental role in converting P or K fixed form to be soluble ready for plant nutrition making the uptake of nutrition by plant more easy.

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تأثير التسميد الحيوى والمعدنى على نشاط التخليق الضوئى فى البطاطس.

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أدي نقص جرعات التسميد المعدني ، عن الجرعة الموصي بها ، إلي نقص في تركيزات كلوروفيل أ ، ب و مجموعهما والسكريات المختزلة والغير مختزلة ومحتوى الكربوهيدرات الكلية خلال موسمي النمو. وعلى العكس من ذلك ، فقد زادت تركيزات الكاروتنيدات والسكريات العديدة ومحتواهما مع نقص نسبة التسميد المعدني عن التركيز الموصى به. وكان النقص في الكلوروفيلات أكبر منه فى الكاروتنيدات مما أدى إلى خفض النسبة بين كلوروفيل أ وكلوروفيل ب وكذا بين الكلوروفيلات و الكاروتنيدات .

ولقد أدي التسميد الحيوي المستخدم إلي زيادة تركيز صبغات البناء الضوئي المقدرة و محتواها خلال موسمي النمو كما أدى الى زيادة السكريات المختزلة وغير المختزلة ومجموعهما والكربوهيدرات الكلية بينما نقصت السكريات العديدة . وبدرجة متشابهة فقد زادت النسبة بين بين كلوروفيل أ الى كلوروفيل ب وبين الكلوروفيلات و الكاروتنيدات كنتيجة لإستخدام التسميد الحيوى بصرف النظر عن وجود أو عدم وجود التسميد المعدنى. وكانت المعاملة بالبكتيريا المثبتة للنتروجين ، هي الأكثر تأثيرا فى هذا الشأن ، مقارنة بالسلالات البكتيرية الأخرى المستخدمة ، سواء كان استخدامها فرديا أو مختلطا.

كما أدي التفاعل بين التسميد الحيوي و التسميد المعدني ، إلي تعويض النقص المتسبب عن خفض نسبة التسميد المعدني عن الجرعة الموصي بها ، فى تركيزات صبغات البناء الضوئى ومحتواها فى الورقة خلال موسمي النمو وكذلك الى نقص السكريات المختزلة والسكريات الغير مختزلة ومجموعهما ومحتوى الكربوهيدرات الكلى بينما نقص الكربوهيدرات الغير زائبة فى المجموع الخضرى للبطاطس خلال موسمى النمو . وقد أدي التلقيح بالبكتيريا ، وخاصة المثبتة للنتروجين ، أعلي القيم في هذا الشأن .

ولقد أدت المعاملة بالتسميد الحيوي ، بصرف النظر عن النسب المستخدمة من التسميد المعدني ، إلي تاثيرات اضافية فى النباتات النامية تحت التركيز الموصى به من التسميد المعدنى والملقحة بخليط السلالات البكتيرية الثلاثة معا ، وكان التلقيح بالبكتريا المثبتة للنتروجين أفضل من التلقيح بغيرها من السلالات .

وبوجه عام فقد عوض التسميد الحيوي المستخدم ، وخاصة مع البكتيريا المثبتة للنتروجين التأثير الضار الناتج عن نقص نسبة التسميد المعدني ، إلى ٧٥% من الجرعة الموصي بها, فقد أدي التفاعل بين هذه النسبة والتسميد الحيوي إلي الحصول علي أفضل نتائج وكانت قريبة مع قيم نباتات المقارنة . وقد فشل إستخدام التسميد الحيوي بمفرده في تعويض التأثير الضار الناتج عن نقص نسبة التسميد المعدني إلى ٥٠% من الجرعة الموصي بها ، حيث أعطت أقل القيم في هذا الشأن .