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MINERALS CONSTITUENTS, TOTAL FREE AMINO ACIDS AND NITRATE REDUCTASE ACTIVITY OF POTATOES (*Solanum tuberosum,* L.)AS AFFECTED BY BIO-AND MINERAL FERTILIZERS .

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ABSTRACT

During the two growing seasons, nitrogen, phosphorus and potassium concentrations and their content of potato shoots were negatively affected by decreasing NPK dose less than the recommended one (control). However, bio-fertilizers application, overall NPK dose, significantly increased N, P and P concentrations and their contents. The most effective strain was found with NFB when used individually or incombination with the other strains used. Therefore, NFB+PDB+SB recorded high values regarding N, P and K concentrations and their content.

The interaction treatments, indicate that, bio-fertilizers counteracted the depressing effects of NPK stress and showed an increase in N, P and K concentrations and their content in potato shoots. On the other hand, No_3^- concentration and nitrate reductase activity (NRA) were decreased whereas, free amino acids were increased. Inoculation with the three bacterial strains used recorded best results in this respect.

Keywords: Potato, biofertilizer, NPK fertilizer, NRA .

INTRODUCTION

Potatoes have a very exhausting effects on soil fertility. Its growth is responsible for a liberal dressing of readily available nutrients. Supplying the necessary nutrients in the correct proportion may be produced high growth and satisfactory tubers yield. Several investigators used different ways of planet nutrient analysis programs to evaluate the adequacy of soil fertility and nutrients sources on plant growth, chemical constituents as well as yield and its quality. Most of these ways are *i* : Critical or Sufficiency level (SL) approach by plant nutrient analysis programs (Russel, 1973). A critical value is the concentration of a nutrient in a specific plant part sampled at particular growth stage where yield is 90 to 95% of the established maximum (Salisbury and Ross, 1992). *ii* : Diagnosis and Recommendation Integrated alternative approach System (DRIS), which used nutrient ratio to interpret tissue nutrient data. Ideally DRIS identifies and quantifies the plant, soil and environmental factors affecting yield of express them numerically (Devlin and Witham, 1985). The interrelationships of these factors are expressed as "norms". After (norm) establishment from high yielding plants index for each nutrient approaches (Cooke, 1982 and Abd El-Aty, 1997), DRIS can be used for diagnosis making.

Several workers concluded that, the effects of minerals and/or biofertilizers depend on plant genera, type and minerals doses as well as bacterial strains (Abou-Hussein *et al.*, 2002 ;El-Shimi, Amal, 2003 and

Ramadan, 2007). The deleterious effects of No_3^- accumulation in potatoes was also reported (Abou-Hussein *et al.*, 2002 and Ramadan, 2007).

Therefore, the present investigation aimed to study the internal nutrients status of potatoes as affects by using biofertilization as a substitute of the mineral fertilizers. Nitrate - reductase activity (NRA) in relation to total free amino acid were also examined .

MATERIAL 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 2^{nd} and the 3^{rd} 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 N,P and K levels as well as nitrate-reductase activity and free amino acids.

Minerals constituents :

Determination of mineral nutrients concentrations were carried out on the ground dried kept materials and the concentrations were calculated on the dry weight basis.

The modified Micro-Kjeldahl apparatus of Parnars and Wagner as described by Jones *et al.,* (1991) was employed for total N determination. Nitrate-N was also estimated (Sandell, 1950).For total P and K determinations;0.4 g crude dried kept powder from each sample was wet digested with a mixture of concentrated sulphuric acid and perchloric acid, then heated until become clear solution (Peterburgski, 1968). Phosphorus was determined spectrophotometrically by Milton Roy Spectronic 1201 at wave length of 725 nm using stannous chloride reduced molybdophosphoric blue colur method in sulphoric acid system as described by Jackson (1973). Potassium was determined Flamephotometrically using Jenway Flamephotometer model CORNING 400 (Peterburgski, 1968).

In the fresh samples, nitrate-reductase activity; NAR (Jaworsky, 1971) and total free amino acids (Rosen, 1957) were also determined.

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

Nitrogen:

It is clear from the results tabulated in Tables 2 and 3 that during the two growing seasons, nitrogen concentrations and its content of the shoots were negatively affected by decreasing NPK dose less than the recommended one (control). The high values were recorded in full recommended NPK dose and decreased with decreasing them. The increase in total N due to N fertilization may be , in part, attributed to the limited utilization of N substances and consequently their osculation is more rapid them their utilization for the formation of new cell and tissues (Esmail, 2005).

Bio-fertilizers application, overall NPK dose, significantly increased N concentrations and its content in the potato shoots. All inoculated plants showed high values more than those of uninoculated plants. The most effective strain was found with NFB when used individually or incombination with the other strain used. Therefore, NFB+PDB+SB recorded high values regarding N concentration and its content.

Regarding the interaction treatment, data in the same table 2 indicate that, bio-fertilizers counteracted the depressing effects of decreasing NPK doses and showed an increase in N concentration and its content in potato shoots. The increasing rate was decreased with decreasing NPK dose. Therefore, all bio- fertilizers used showed an additive effects to the recommended dose of NPK (control) on N concentration and its content. Inoculation with the three bacterial strains used recorded high values regarding N concentration and its content in the shoot system of potatoes. Comparing the effects of the three strains when used individually, data also indicate that inoculation with nitrogen-fixing bacteria (NFB) was more effective than that of phosphate-dissolving bacteria (PDB) or silicate bacteria (SB) in a descending order. Inoculation with NFB with either of PDB+SB or with PDB strains were the most effective treatments regarding N concentration and its content.

Comparing with the control (100% NPK from the recommended dose), data in the same tables show that plants received mixed bacterial strains used (NFB+PDB+SB) and grown under 75% NPK from the recommended doses showed high values regarding nitrogen concentration and its content. There is no significant differences between 75% and 50% from the recommended NPK doses and the control (100% recommended NPK).

The counteraction effects of bio-fertilizers on N may explain the N supplementary effect of the bio-fertilizers and the role of bacteria on increasing nitrogen absorption and increasing the production of auxins and gibberellin-like substances (Stizelezyk and Pokojski-Burdziej, 1984). Okon (1985) and Harari *et al.*, (1989) who found that, NFB or PDB increased nitrogen percentage as a results of their used inoculation on N2-fixation and plant growth stimulation. The role of PDB for activated the count of N2-fixing bacteria was recorded (El-Agroudi *et al.*, 2003).

Phosphorus:

As shown in tables (2 and 3), it was found that, application of NPK fertilizers at full recommended dose gave higher phosphorus concentrations and its content than the other NPK doses during the two growing seasons. The increase in phosphorus content due to phosphorus fertilization may be attributed to its availability and consequently the efficiency for roots absorbing (El-Shahawy, 2003). Neeru *et al.*, (2000) reported that, the high level of phosphorus in the soil was associated with an increase in nitrogen and phosphorus content in the plant tissues. Phosphorus uptake and its level depressed by decreasing its level in the plant media. It known to be taken as indicator for energy status, so its level directly associated with nutrients stress or phosphorus tolerability.

Bio-fertilizers application, over all NPK doses, increased phosphorus concentration and its content in potato shoots in comparison to plants inoculated without bacterial strains. The most effective treatments was found with PDB strain when used individually or in combination with the other strains used. The most effective treatments was found with NFB+PDB+SB followed by NFB+PDB, PDB+SB, PDB, NFB, SB in a descending order.

Data in the same tables show that, potato plant grown at any levels of NPK used and inoculated with all the used bacterial strains were higher in phosphorus concentration and its content values compared with the untreated ones. Inoculation with PDB was the most effective treatment at any NPK level in this respect. A synergistic effects were detected with all strains when used all together (NFB+PDB+SB) during the two growing seasons especially with the recommended dose (control).

Compared with the control (100% recommended NPK), data also indicate that, mixed strains of used bacteria (NFB+PDB+SB) 75% counteracted the depressing effect of decreasing NPK to 75% from the recommended dose. However, application of 50% NPK dose combined with bio-fertilizers used resulted in the lowest increases values in phosphorus concentration and its content. The most effective treatments on counteraction the phosphorus decreases was recorded with NFB+PDB+SB treatment. The favorable effect of bio-fertilizers on P content may be due to fact that non-symbiotic bacteria have the ability to supply the plants with N and thereby increase chemical contents in different plant tissues (Bashan and Holguim, 1997).

Bio-fertilizers play a fundamental role in converting fixed P form to available for plant nutrition making the uptake of nutrients by plants more easy (Abou-Hussein *et al.*, 2002). The observed increase was definitely due to the synergistic effect between the examined bacterial groups in plant rhizosphere (El-Agroudi *et al.*, 2003).

Quastel (1965) reported that, soil microorganisms known as phosphate solubilizing bacteria (PSB) play a fundamental role in converting P fixed form to be soluble and available for plant nutrition. He added that, the microbial breakdown of soil organic matter is associated with an increase CO_2 which possibly increases the solubility of soil phosphate.

In addition to the plant phosphorus status may also be improved by applying certain bacteria that release fixed phosphorus, such as *Bacillus polymyxa, Bucillus megaterium*, or *Pseudomoas fluorescens*. phosphorus occurs in soil both in the organic and inorganic form (phosphate), but only a small fraction of the total soil phosphate is directly available for plants, and cycling of phosphorus by bacteria may increase its availability. However, the conditions required for bacterial phosphorus cycling to provide a substantial contribution to the P nutrition of plants are not known (Brown, 1974).

Potassium:

It is noted from the data tabulated in Tables 2 and 3 that, decreasing NPK minerals fertilizers dose decreased significantly potassium concentration and its content of potato shoots during the two growing seasons. The rate of decrease was increased significantly with decreasing NPK dose up to 50% of the recommended dose.

Table (2): Effects of mineral and/or bio-fertilizers on nitrogen, phosphore and potassium concentrations (mg/g D.Wt.) in the shoot system of potato plant grown during the two growing seasons of 2001/2002 (S1) and 2002/2003 (S2).

Treatments		N concentration (mg/g D.Wt.)			P concentration (mg/g D.Wt.)			K concentration (mg/g D.Wt.)		
M-Mineral NPK	B-Bio-fertilizer	S1	S2	Mean	S1	S2	Mean	S1	S2	Mean
Control 100%	Without	26.730	26.960	26.845	4.963	5.511	5.237	56.720	79.540	68.130
	NFB	27.250	27.600	27.425	5.281	5.999	5.540	57.220	79.690	68.455
	PDB	26.860	27.070	26.965	5.652	6.003	5.827	57.460	79.900	68.680
	SB	26.770	27.007	26.888	5.215	5.733	5.474	69.910	86.660	78.285
	NFP+PDB	27.370	27.790	27.580	5.696	6.133	5.914	57.560	80.140	68.850
	NFB+SB	27.270	27.730	27.500	5.400	5.833	5.617	71.460	86.820	79.140
	PDB+SB	26.950	27.140	27.045	5.822	6.096	5.909	76.120	85.390	80.755
	NFB+PDB+SB	27.420	27.913	27.667	5.763	6.252	6.007	77.150	85.220	81.185
	Mean	26.777	27.401	27.089	5.461	5.920	5.695	65.700	83.170	74.435
75%	Without	25.560	26.030	25.795	4.644	4.933	4.788	47.110	67.030	57.070
	NFB	26.380	26.830	26.605	4.722	5.022	4.872	49.350	67.530	58.440
	PDB	25.700	26.140	25.920	5.203	5.552	5.377	51.120	67.630	59.375
	SB	25.640	26.080	25.860	4.703	5.001	4.852	70.390	74.610	72.500
	NFP+PDB	26.570	26.950	26.760	5.252	5.488	5.370	52.200	68.860	60.530
	NFB+SB	26.320	26.910	26.615	4.822	5.033	4.928	70.920	75.140	73.030
	PDB+SB	25.770	26.210	25.990	5.222	5.411	5.317	73.760	75.720	74.740
	NFB+PDB+SB	26.610	27.080	26.845	5.302	5.564	5.433	74.850	76.570	75.710
	Mean	26.069	26.529	26.299	4.873	5.160	5.016	63.213	73.886	68.549
50%	Without	24.830	25.250	25.040	4.322	4.611	4.466	43.520	61.550	52.535
	NFB	25.460	25.660	25.560	4.374	4.652	4.513	45.160	63.370	54.265
	PDB	24.970	25.370	25.170	4.744	5.122	4.933	46.840	63.660	55.250
	SB	24.850	25.300	25.075	4.344	4.674	4.509	64.130	65.440	64.785
	NFP+PDB	25.500	25.800	25.650	4.922	5.263	5.092	47.050	64.130	55.590
	NFB+SB	25.380	25.740	25.560	4.444	4.707	4.575	66.260	66.640	66.450
	PDB+SB	25.040	25.420	25.230	4.874	5.222	5.048	67.200	66.850	67.025
	NFB+PDB+SB	25.630	25.860	25.745	4.963	5.311	5.137	67.330	67.710	67.520
	Mean	25.208	25.550	25.379	4.623	4.945	4.811	56.186	65.169	60.677
Mean	Without	25.473	25.847	25.747	4.510	4.885	4.993	48.117	68.373	58.245
	NFB	26.130	26.463	26.364	4.592	4.958	5.018	49.577	69.197	59.387
	PDB	25.610	25.960	25.852	5.000	5.329	5.134	50.807	69.397	60.102
	SB	25.520	25.896	25.775	4.554	4.936	4.811	67.143	75.903	71.523
	NFP+PDB	26.247	26.613	26.497	5.090	5.428	5.325	51.270	70.430	60.657
	NFB+SB	26.090	26.560	26.392	4.689	4.991	4.906	68.547	74.533	71.873
	PDB+SB	25.687	26.023	25.922	5.073	5.376	5.290	74.693	78.320	73.173
	NFB+PDB+SB	26.320	26.718	26.753	5.143	5.509	5.458	76.777	80.167	73.805
LSD at 5% for: SxM		NS			0.154			0.949		
SxB		NS			0.179			1.363		
MxB		NS			0.219			1.671		
SxMxB		NS			0.311			2.446		

Regarding the effects of bio-fertilizers, overall NPK doses, data in the same tables show that, potassium concentration and its content of potato shoots inoculated with mixture of bio-fertilizers containing nitrogen fixers and phosphate dissolving bacteria and silicate bacteria had more K concentration and content than those obtained from uninoculated one.

Table (3): Effects of mineral and/or bio-fertilizers on nitrogen, phosphore and potassium contents (mg/plant) in the shoot system of potato plant grown during the two growing seasons of 2001/2002 (S1) and 2002/2003 (S2).

Treatments		N content (mg/plant.)			P content (mg/plant.)			K content (mg/plant.)		
M-Mineral NPK	B-Bio-fertilizer	S1	S2	Mean	S1	S2	Mean	S1	S2	Mean
Control 100%	Without	281.201	384.180	332.690	49.055	74.257	61.656	565.13	1090.69	806.63
	NFB	314.465	408.756	361.105	55.173	78.478	66.825	625.71	1135.78	862.30
	PDB	311.845	403.072	357.458	59.815	81.941	70.878	632.28	1145.04	870.26
	SB	290.454	388.281	339.367	51.158	75.235	63.196	733.24	1202.78	953.79
	NFP+PDB	330.849	433.468	382.159	63.963	89.114	76.538	720.87	1062.15	883.98
	NFB+SB	318.786	425.101	371.943	57.281	81.755	69.518	800.31	1284.96	1028.65
	PDB+SB	318.010	419.042	368.526	61.621	86.402	74.011	886.42	1302.98	1086.33
	NFB+PDB+SB	336.882	436.727	386.804	66.998	92.551	79.774	994.85	1387.28	1184.36
Mean		312.811	412.328	362.506	58.132	82.467	75.981	741.82	1201.31	989.613
75%	Without	255.600	341.514	298.557	45.440	63.409	54.424	471.10	879.43	675.57
	NFB	299.677	394.401	347.039	52.506	72.353	62.429	560.62	992.69	773 .72
	PDB	293.931	384.519	339.225	58.363	80.199	69.281	584.66	994.84	776.14
	SB	263.836	354.949	309.392	47.365	66.703	57.034	724.31	1069.88	890.27
	NFP+PDB	304.359	413.898	359.128	60.161	83.945	72.053	609.54	1072.84	824.90
	NFB+SB	308.470	404.188	356.329	55.342	74.094	64.718	831.18	1098.56	963.04
	PDB+SB	297.901	397.606	347.753	59.210	80.568	69.889	945.15	1270.03	1105.82
	NFB+PDB+SB	319.772	420.742	370.257	64.492	89.570	77.031	1023.21	1326.26	1172.82
Mean		292.943	388.977	342.176	55.361	76.355	64.862	714.21	1085.97	886.818
50%	Without	229.901	254.343	242.122	40.017	46.447	43.232	402.95	619.99	507.85
	NFB	260.227	332.810	296.519	44.707	60.336	52.215	461.53	821.91	629.21
	PDB	257.790	329.556	293.673	48.977	66.535	57.756	483.58	826.94	640.49
	SB	247.481	308.154	277.817	43.262	56.929	50.095	638.67	797.06	717.16
	NFP+PDB	261.145	350.312	305.729	51.499	72.629	62.064	492.28	884.99	673.62
	NFB+SB	267.108	343.886	305.497	45.955	62.885	54.420	685. 19	890.31	787.43
	PDB+SB	259.665	342.153	300.910	50.543	70.288	60.415	696.86	899.80	798 .54
	NFB+PDB+SB	262.374	360.669	311.522	52.459	75.841	64.150	838.52	1138.26	969.41
Mean		255.711	327.735	292.848	47.177	63.986	55.177	581.33	856.09	695.904
Mean	Without	252.845	322.596	288.558	44.766	60.957	55.202	477.64	853.33	652.577
	NFB	288.475	374.716	332.405	50.696	70.205	62.395	547.36	979.87	748.276
	PDB	284.886	368.554	327.501	55.620	75.656	64.161	565.21	985.27	760.891
	SB	264.540	346.721	306.424	47.207	66.088	56.361	698.36	1013.55	849.550
	NFP+PDB	298.784	399.226	351.921	58.448	81.784	69.076	604.01	1005.12	791.756
	NFB+SB	293.512	386.979	340.919	52.751	72.719	62.484	771.19	1085.90	927.880
	PDB+SB	284.302	382.278	336.325	57.036	78.973	67.741	839.74	1150.52	948.834
	NFB+PDB+SB	306.343	406.046	356.790	61.185	85.835	72.033	940.06	1281.91	979.983
LSD at 5% for: SxM		2.612			1.918			4.306		
SxB		1.023			2.560			1.908		
MxB		3.084			2.976			5.056		
SxMxB		4.355			3.608			6.248		

Comparing the effects of the three strains used, it was found that, SB strain was most effective in this respect.

The interactions between the used level of mineral and bio-fertilizers proved significantly effect in both studied seasons. Data indicate also that, potassium concentration and its content were increased in the shoot system of potato plants as a result of inoculation with either of used bacterial strains under different doses of NPK compared with the control. It is realized that, increasing levels of the used mineral fertilizers from NPK or using a mixture of bio-fertilizers containing N fixers and phosphate dissolving bacteria as well as silicate bacteria induced significantly the increases in potassium concentration and its content of the potato shoots in both studied seasons. The maximum potassium concentration and content of potato shoots was detected with the treatment of 100% mineral fertilizers NPK combined with bio-fertilizers mixture (NFB+PDB+SB).

Comparing the three bacterial strains, when used individually or in combination, the data indicate that, inoculation with SB strain was more effective than PDB on increasing potassium whereas, NFB treatment recorded least values. The combined treatments showed that, SB as well as PDB strains caused an additive effect to that of NFB on increasing potassium concentration and its content. Therefore, all bacterial strains used together recorded high values in this respect. These results are true during the two growing seasons.

Regarding the interaction between bio-and mineral fertilizers at 75% NPK (from the recommended dose), the same data show that, plants received mixed strains of the three bacterial strains used recorded highest values compared with the control treatment (100% recommended NPK). At 50% NPK, all strains counteracted, to some extent, the depressing effect of decreasing NPK dose on potassium concentration and its content in the two growing seasons.

The increase in potassium content in the shoot system under bio-fertilizer reflects an enhanced growth which might be possibly due to the role of ; *Bacillus circulans* strain in increasing K-uptake (El-Shahawy, 2003). Abou-Hussein *et al.,* (2002) found that, applying bio-fertilizer to potato plant with reducing mineral fertilizers increased the percentages of N, P and K in potato leaves.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. Terhan *et al.,* (1991) found that, P, P+K increased available soil K that increased K uptake. However, the fact tolerant for three strains of used bacteria treatment produced a better effect than the other treatments may indicate that three organisms all together exerts a growth stimulating and a synergistic effect as had been reported for other plant species (Raj *et al.*, 1981; Hauka *et al.,* 1996; El-Shahawy, 2003).

The forgoing results in the present investigation indicate that, both bio-and mineral fertilizers caused a positive effect on concentrations of N,P and K in the shoots of potato plants which may be attributed to the role of bacteria in the production of auxins that promoted root growth absorbed more nutrients from the soil (Helaly *et al.*, 1985; Kawther *et al.,* 2002).

Glick, (1995) noted that, plant growth promoting rhizobacteria (PGPR) can affect plant growth in two different ways, indirectly or directly. The indirect promotion of plant growth occurs when PGPR reduce or prevent the deleterious effects of one or more phytopathogenic organisms as well as enhancement of root nodule number or mass (Yahalom *et al.,* 1987). The direct promotion effects of PGPR include an increase in the mobilization of insoluble nutrients followed by facilitating the uptake of nutrient through their ability of mobilizing the unavailable forms of nutrients elements to available (Lifshits *et al.,*1987; Ishac, 1989). Abd-El-Moez *et al.,*(1999) added that, the beneficial effects of bio or organic fertilizers may be attributed to the increase in root surface per unit of soil volume. Improving not only soil physical but also chemical characteristics resulting in more the available nutrient elements to be absorbed by plant roots.

The increases of nitrogen, phosphorus and potassium due to increasing mineral fertilizers may be attributed to the beneficial effects of these elements for plant growth. N increases the capacity of plants to absorb nutrients by increasing the size of plant root surface / unit of soil volume as well as the high capacity of the plants supplied with N in building chlorophyll and other metabolites which in turn contributes much to the increase of nutrient uptake Marschner, 1995). The importance of phosphorus and potassium for plant growth were previously mentioned (Russel, 1973; Yagodin, 1982 and Munson, 1972) These results are in agreement with those of Arisha and Bardisi, (1999) ; Hedg *et al.,* (1999) and Abd El-Malck, (2004).

The beneficial effect of bio-fertilizers on increasing nitrogen, phosphorus and potassium accumulation may be due to their effects on the macro and microelements availability which increases at the root rhizosphere in addition to the increase in the root absorption surface. In this context,Gharib,(2001)reported that, using *Bacillus megatherium+Azotobacter +Azospirillum* led to increasing chlorophyll content and nutrients uptake in the leaves of cucumber plants. The increase of chlorophylls and nutrients uptake due to bio-fertilizer treatment may be attributed to the affects of phosphate – dissolving bacteria on decreasing soil PH, increasing the availability of some nutrients such as Fe, Zn, Mn and Cu to plant uptake (Alexander, 1982; El-Dahtory *et al.,* 1989; Hauka *et al.,* 1990).

Nitrate-Reductase Activity (NRA) as well as concentration of nitrate ion (NO_3^-) and total free amino acids:

Data in the two growing seasons tablested in Table (4) indicate that NRA and NO_3^- concentration were decreased in potato leaves with decreasing NPK dose less than the recommended one (control). However, total free amino acid concentrations were increased significantly. Maximum NRA and NO_3^- level were proportional to the NPK doses which recorded in the control (100% NPK from the recommended dose). The increase of NO_3^- level and NRA at the 100% NPK dose may be due to the effective of N absorption. It was found that nitrate reductase activity in the shoots depends on various factors including the level of nitrate supply, plant species and its age as well as carbon economy (Marschner, 1995). The same auther added that the increase in NRA was a companied with an increase in NO_3^- concentration.

Similarly, Gonzalez *et al.*, (1990) found that increasing N fertilization increased NO_3^- content and NAR of wheat plant.

Table (4): Effects of mineral and/or bio-fertilizers on nitrate reductase activity (NAR) as well as concentration of nitrate ion (NO_3^-) and total free amino acids in the shoot system of potato plant grown during the two growing seasons of 2001/2002 (S1) and 2002/2003 (S2).

Treatments		NRA (µgNo2/ g Fwt.)			NO_3^- (mg/g Dwt.)			AA (mg/g Fwt.)		
M-Mineral NPK	B-Bio-fertilizer	S1	S2	Mean	S1	S2	Mean	S1	S2	Mean
Control 100%	Without	0.507	0.567	0.537	2.849	2.825	2.837	4.90	5.10	5.00-
	NFB	0.392	0.408	0.400	2.600	2.612	2.606	6.30	6.44	6.37
	PDB	0.430	0.390	0.420	2.710	.2.670	2.690	5.60	5.70	5.65
	SB	0.460	0.494	0.482	2.750	2.784	2.772	5.10	5.18	5.14
	NFP+PDB	0.397	0.357	0.377	2.570	2.574	2.572	6.74	6.70	6.72
	NFB+SB	0.400	0.370	0.385	2.605	2.575	2.590	6.44	6.30	6.37
	PDB+SB	0.411	0.409	0.410	2.670	2.692	2.681	5.73	5.77	5.75
	NFB+PDB+SB	0.326	0.306	0.316	2.330	2.344	2.337	6.93	6.94	6.93
Mean		0.415	0.413	0.414	2.636	1.780	2.610	5.966	6.016	5.991
75%	Without	0.520	0.516	0.518	2.037	2.117	2.077	5.04	5.00	5.02-
	NFB	0.320	0.332	0.326	2.090	1.910	2.000	6.99	6.99	6.99
	PDB	0.340	0.380	0.360	2.035	2.051	2.043	5.90	5.70	5.80
	SB	0.486	0.486	0.486	2.070	2.062	2.066	5.60	5.50	5.53
	NFP+PDB	0.276	0.270	0.273	1.900	1.916	1.908	7.64	7.00	7.32
	NFB+SB	0.280	0.300	0.290	1.971	1.910	1.941	7.10	7.24	7.17
	PDB+SB	0.386	0.378	0.382	2.071	2.039	2.050	5.88	5.96	5.92
	NFB+PDB+SB	0.250	0.234	0.242	1.500	1.508	1.504	7.97	7.85	7.91
Mean		0.357	0.361	0.359	1.915	1.951	1.933	6.265	6.405	6.335
.50%	Without	0.455	0.457	0.456	2.000	2.048	2.024	4.96	4.82	4.89-
	NFB	0.200	0.222	0.211	1.851	1.810	1.831	7.90	7.92	7.91
	PDB	0.285	0.275	0.280	1.850	1.870	1.860	7.03	6.85	6.94
	SB	0.348	0.358	0.353	1.870	1.874	1.872	5.44	5.00	5.22
	NFP+PDB	0.196	0.190	0.193	1.600	1.608	1.604	8.20	8.64	8.44
	NFB+SB	0.210	0.190	0.200	1.700	1.744	1.722	8.00	8.12	8.06
	PDB+SB	0.312	0.300	0.306	1.812	1.800	1.806	7.37	7.43	7.40
	NFB+PDB+SB	0.150	0.162	0.156	1.558	1.530	1.544	8.82	8.70	8.86
Mean		0.269	0.269	0.269	1.780	1.785	1.783	7.215	7.185	7.200
Mean	Without	0.494	0.513	0.504	2.295	2.330	2.313	4.300	4.973	4.637
	NFB	0.304	0.321	0.312	2.061	2.141	2.101	7.063	7.117	7.090
	PDB	0.352	0.348	0.350	2.198	2.197	2.198	6.177	6.083	6.130
	SB	0.431	0.444	0.438	2.230	2.240	2.235	5.380	5.227	5.303
	NFP+PDB	0.290	0.272	0.281	2.023	2.033	2.028	7.527	7.447	7.487
	NFB+SB	0.297	0.287	0.292	2.092	2.076	2.084	7.180	7.220	7.200
	PDB+SB	0.370	0.362	0.366	2.184	2.044	2.114	6.327	6.387	6.357
	NFB+PDB+SB	0.242	0.234	0.238	1.796	1.794	1.795	7.903	7.830	7.867
LSD at 5% for: SxM			0.003			0.042			0.025	
SxB			0.004			0.068			0.041	
MxB			0.005			0.084			0.051	
SxMxB			0.007			0.119			0.070	

Biofertilizer application, overall NPK doses, increased significantly total free amino acids and decreased NRA and No_3^- concentration in potato leaves. All inoculated plants showed less NRA and NO_3^- concentration than those of uninoculated. The most effective treatments on increasing total free amino acids and on decreasing NRA and NO_3^- concentrations was found with (NFB+PDB+SB) treatment. In this context, Abo- El- Gheit (1993) mentioned that, the total free amino acids were increased in *Strelizia reginae Ait* plant as a result of an increase in N dose with its different forms.

The interaction treatments show that biofertilizers with any of the three bacterial strains used inhibited the accumulation of NO_3^- ion and NRA within the potatoes tissues and increased the concentrations of total amino acids (Table 4). Moreover, it was found that, inoculation with nitrogen fixing bacteria (NFB) was more effective than that of phosphrus –dissolving bacteria (PDB) or silicate bacteria (SB) in descending order . These results were true at any of the NPK doses used . Potato plants received mixture of bacterial strains used (NFB+PDB+SB) and grown under 75% NPK from the recommended doses showed high values of total free amino acids as well as lower values from NRA and NO_3^- concentrations. The inoculation with the three bacterial strains used was recommended to decease No_3^- toxicity.

The beneficial effects of nitrogen fixing bacteria (NFB) , phosphrus – dissolving bacteria (PDB) and silicate bacteria (SB) which used are known as a plant growth promoting rhizobacteria (PGPR) may be attributed to the biological nitrogen fixation and production of phytohormones ; gibberellins, cytokinin like substances and auxins (Ramadan, 2007 and Helaly, *et al.*, 2009) that promote root development and proliferation (Hartmann, *et al.*, 1998) and resulting in efficient uptake of water and nutrients (Hauka, 2000) .

It could be concluded that, using biofertilizers for potato plants as a substitute for the chemical fertilizers may be recommended to reduce nitrate accumulation as well as improve total free amino acids.

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تأثير التسميد الحيوى والمعدنى على المحتوى العنصرى والأحماض الأمينية الحرة الكلية ونشاط انزيم إختزال النترات فى البطاطس.

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نقصت تركيزات ومحتوي المجموع الخضري لنبات البطاطس من النتروجين و الفسفور و البوتاسيوم بزيادة النقص في نسبة التسميد المعدني المستخدم عن الجرعة الموصي بها خلال موسمى النمو. وعلى العكس من ذلك فقد أدي التسميد الحيوي ، بصرف النظر عن نسبة التسميد المعدنى المستخدم ، إلى زيادة معنوية في تركيز ومحتوي المجموع الخضري لنبات البطاطس من النتروجين والفوسفور والبوتاسيوم .و أعطى التلقيح بالبكتيريا المثبتة للنتروجين ، عند إستخدامها منفردة أو مختلطة ، مع السلالات الأخرى ، أعلي القيم فى هذا الشأن.

ولقد عوضت معاملات التفاعل بين التسميد المعدنى ، والحيوى النقص الناتج فى المحتوى العنصرى المتسبب عن نقص نسبة التسميد المعدنى حيث سببت زيادة فى تركيز ومحتوى النيتروجين الكلى والفوسفور والبوتاسيوم فى المجموع الخضرى للنبات.كما أدت الى زيادة الأحماض الأمينية الكلية الحرة ونقص فى تركيز أيون النترات ونشاط انزيم النيتريت ريدكتيز.