

MORPHOLOGICAL AND ANATOMICAL STUDIES ON POTATOES AS AFFECTED BY BIO-AND MINERAL FERTILIZERS

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ABSTRACT

Plant height, number of branches and leaves per plant, leaf area, fresh and dry weights of the shoot system, specific leaf weight (SLW), leaf weight ratio (LWR), specific leaf area (SLA) and leaf area ratio (LAR), estimated at the active growth period; after 90 days from planting, were decreased due to decreasing NPK dose less than the recommended one during the two growing seasons. Bio-fertilizers used, over all NPK doses, counteracted, in general the depressing effect of NPK stresses on most growth parameters studied. The most effective strain restoring most of the plant growth capacity was found with NFB when used individually or in combination with the others.

The anatomical structure, of the terminal leaflet blade, taken from the 3rd compound leaf from the plant tip, taken at the middle part indicated that NPK stress decreased thickness leaflet in the midrib region, mesophyll tissue, palisade tissue and spongy tissue. Midrib V.B. dimension (length and width), as well as thickness of xylem tissue and phloem tissue at the midrib bundle (external and internal) and metaxylem vessel dimension were also decreased. The most striking effects were found with respect to components of the vascular system.

The inoculation of bacterial strains used counteracted the depressing effects of NPK stresses on all these parameters. The most effective treatments were found with NFB+PDB+SB, NFB+PDB, NFB+SB, NFB alone in a descending order.

The stem structure at the 3rd internode of the main stem of potato plants as seen in T.S. indicated that stem diameter, cortex thickness, large vascular bundle dimension, external and internal phloem and xylem tissue thickness as well as pith tissue dimension were decreased due to NPK stress compared with the recommended one. All bacterial inoculation treatments counteracted the reduction in cell volume caused by NPK stress and increased stem diameter, cortex thickness, large vascular bundle dimension, external and internal phloem and xylem tissue thickness as well as pith tissue dimension. Metaxylem vessel dimension was also increased. Inoculation with NFB alone as well as inoculation with either NFB+PDB or mixed strains (NFB+PDB+SB) were generally the best treatment compared with plants grown without inoculation under any NPK doses.

Keywords: potatoes, NPK, SLW, LWR, SLA, LAR, Biofertilizers, NFB, PDB, SB.

INTRODUCTION

Potatoes (*Solanum tuberosum*, L) is one of the most important vegetable crops in Egypt for both local consumption and export, belong to the family Solanaceae which includes about 90 genera and 2000 species (Cobley, 1976), distributed in the tropical and temperature regions. It shows successive quick increase in both area and production.

There is a great need for further studies under Egypt condition to establish recommendations for reducing the amount of chemical nitrogen, phosphorus and potassium fertilizers to raising the quantity, improving the

quality and limiting the environment of pollution. It has focused the light on the use untraditional fertilizer especially the bio-fertilizer and slow release nitrogen fertilizer to reduce NO_2^- and NO_3^- accumulation.

Generally, bio-fertilizers are microbial preparations containing, primary, sufficient numbers of potent strains of microorganisms, having a definite beneficial role in furnishing a proper rhizosphere for plant growth (Abou-Hussein *et al.*, 2002). These strains bacteria not only caused the solubilization of minerals (Glick, 1995), but also stimulate plant growth by facilitating the uptake of minerals by plant (Kloepper *et al.*, 1989) and increased the amount of available N for plant uptake (Kapulnik *et al.*, 1985; Michiels *et al.*, 1989).

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

Certain morphological and anatomical structure of the stem and leaves were evaluated.

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).

Experimental design:

Farm yard manure has been added during soil preparation as organic fertilization at dose (40 m³/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 m²) 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 5×10^8 , 9×10^8 and 2.15×10^8 cells/ml of NFB, DPB and SB, respectively.

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°	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		

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% P₂O₅), as a source of phosphorus, at the dose of 75 kg P₂O₅ /fed., was added to the soil before planting and during soil preparation. Potassium sulphate (48 % K₂O) was used as a source of potassium at the dose of 96 kg K₂O/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. Each treatment was replicated 5 times. Three of them were kept till the end of the experimental period; 105 days from planting, and used for the morphological characters.

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.

Morphological characters:

Five plants were chosen randomly at the active growth period (75 days from planting) and carefully taken out of the soil with the aid of a water stream to insure minimal losses of the root system and the tubers if present. Plants were divided into their components organs. Each part was cleared with moistures cloth to remove surface contamination.

For the growth parameters determination, plant height (cm) of the aerial main stem, started from the soil surface to plant apex, numbers of branches and leaves per plant, leaf area per plant calculated as presented below, as well as fresh and dry weights of the shoots, tubers, if present, and the whole plant (g) were recorded.

Leaf area (cm)² per plant calculated based on area unit using disk method according to (Koller, 1972). Samples of ten disks were taken from the 3rd fresh leaf from plant tip and estimated their area. Leaf area per plant was calculated in square centimeters (cm)² using the following equation:

$$\text{Leaf area (cm)}^2 \text{ per plant} = \frac{\text{Disk area of 10 disks (cm}^2\text{) x fresh weight of the leaves}}{\text{Fresh weight of 10 disks}}$$

The following parameters were also estimated according to the formula outlined by Radford (1967) as follows:

- Specific leaf area (SLA) (dm²/g) = Leaf area per plant (dm²) / Leaf weight per plant
- Leaf area ratio (LAR)(cm²/g) = Leaf area per plant (cm²) / Total dry weight per plant (g)
- Specific leaf weight (SLW) (g/dm²) = Leaf dry weight per plant (g) / Leaf area per plant (dm²)
- Leaf weight ratio (LWR) = Leaf dry weight per plant (g) / Total dry weight per plant (g)

The remained plant material was subsequently oven dried at 70 °C for 48 hours in an electric oven to a constant weight and their crude dried weights were measured and the moisture percentages (leaf water potential; LWP) was calculated according to the formula of Peynado and Young (1968) as follows:

$$\text{-LWP} = \frac{\text{Fresh weight} - \text{dry weight (g)}}{\text{Fresh weight (g)}}$$

As for the anatomical studies samples were taken from the plants that grown only in the second season for the anatomical studies. Specimens (5 mm in length) were taken at the middle part from the terminal leaflet of the 3rd compound leaf and the middle part of the 3rd internode from the plant tip.

Samples were killed and fixed in Formalin- Alcohol-Acetic acid glacial mixture (FAA 17:2:1 v/v) for 72 hours, washed and dehydrated in alcohol series, cleared by xylene and embedded in paraffin wax (52- 54° cmp.). Cross sections 12-15 μ thick were prepared by a rotary Microtome, stained in Saffranin – light green combination, cleared with oil cloves and mounted in canado balsam (Gerlach, 1977).

The section were examined microscopically and the following characters were recorded quantitatively as described by Pazourek and Na'tr (1981).

Thickness (μ) of the leaflet, as well as mesophyll, palisade and spongy tissues; midrib vascular bundles dimension (length and width), xylem and phloem tissues thickness.

Stem diameter (μ), cortex thickness, number. of cortical cell layers. Large vascular bundles dimension (length and width), metaxylem vessels diameter as well as thickness of phloem, xylem and pith tissues.

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

I: Growth characters:

Tables, (2-4) show that all growth parameters studied as affected by the different treatments were similar in the two growing seasons. A significant decrease was noticed with decreasing NPK mineral fertilizers treatments dose less than the recommended one (control). The decrease was a concentration dependent. The lowest NPK dose (50% from recommended dose) recorded lowest values on all plant growth parameters compound with the control (100% from the recommended dose). The reduction in growth due to decreasing NPK dose may be related to difference in photosynthesis and respiration rates as well as the inhibition of both meristematic activity and elongation of cells under nutrients stress.

Regarding the effects of bio-fertilizers used, the data indicated, in general, that, all bacterial strains increased most of the plant growth parameters. Less increase in potato growth was detected in plants inoculated with PDB+SB, PDB and SB in a descending order.

The interaction treatments indicated that, the variation among numbers of branches and leaves as well as dry weight were less than that among leaf area and fresh weight. Wider variation were noted in all growth parameters studied at the low NPK dose whereas narrower ones were attained at 75% NPK from the recommended dose.

Concerning the effects of NPK on plant growth of potatoes and other plant genera, many reports were recorded in literature. Nevertheless, a comparatively extensive work in this field covering several plant species has accomplished. The general pattern of response to the imposition of mineral nutrients stress is the suppression of growth which increases more or less in proportion to the stress of NPK, a trend that seems to agree with the results obtained in the present investigation. Among the studies available in this field of research are those of Rizk, Fatma, 2003 and Sarhan *et al.*, 2004.

It seems that one of the primary effects of mineral deficiencies or low NPK dose is to reduce considerably net photosynthesis (Awad, 2004) on the other hand, attributed this reduction to high respiration rates. Both reasons may be operative simultaneously.

The results also indicate that, all growth parameters studied of potatoes inoculated without any bio-fertilizers used decreased significantly with decreasing NPK dose less than the recommended dose. However, it can be safely stated that, with no exception, all bio-fertilizer treatments used counteracted, to one degree or another, the depressing effects of low dose of NPK up to 50% level on plant growth but showed an additive effects with 75%.

The specific effects of each treatments are viewed and discussed as follows:

I.1. Plant height, numbers of branches and leaves as well as leaf area:

Table (2) shows that the trend of all growth parameters studies under different treatments were similar in the two growing seasons. However, all values detected in the 2nd season were higher than that recorded the 1st one which may be due to the environmental condition.

The same table shows also that plant height, numbers of branches as well as leaves and leaf area of potato plants were decreased with decreasing the level of NPK less than the recommended dose (control). This inhibition rate was more pronounced under 50% NPK from the recommended dose. The role of N in protoplasm formation and all proteins, e.g. amino acids, nucleic acids and many enzymes as well as energy transfer materials; ADP and ATP was previously reported by Russel, (1973). Similarly, the role of P as a major nutrient element was reported by Yagodin (1982). He stated that P compounds are of absolute necessity for all living organisms, nucleoproteins constituting the essential substances of the cell and for cell division and development of meristematic tissues. K is an important element for plant growth and it is involved in very metabolic processes, including

carbohydrates metabolism, protein biosynthesis, assimilate translocation, conformation of enzymes and stomatal movement (Krauss, 2003). These effects of N, P and K reflected on vigorous vegetation growth such as plant height, number of branches, leaf area, fresh and dry weights.

Bacterial strains used, overall NPK level, increased these parameters during the two growing seasons. The most effective treatments were found with NFB strain when used individually or in combination with others. Therefore, the high values were recorded with NFB+PDB+SB, NFB+PDB and NFB+SB as well as NFB in a descending order. These results are true during the two growing seasons with the superiority of the 2nd one.

The interaction treatments showed that, all bacterial strains used showed an additive effect to the effects of 100% NPK on potatoes growth. Again, the most effective strain was found with NFB when used individually or in combination with the others in the two growing seasons. Moreover it was found that, all bacterial strains largely counteracted the mineral nutrients stress depressing effect on potatoes growth. Better counteraction effect was achieved at 75% NPK dose. The results also indicate that, the effects of bacterial strain of N (NFB) used individually or in combination with other strains used were more pronounced on restoring plant growth than the other strains used without N strain especially at 75% NPK. Less counteraction was achieved at 50% NPK with N bacterial strain. However, the other strains were not effective in this respect, and failed to replace mineral NPK fertilizers, since it rather reduced potatoes growth compared with the control.

The best treatment restoring most of the plant growth capacity under nutrients stress was the NFB+PDB+SB treatment. Its effect in counteracting stress was particularly evident under 75% NPK. These results are true during the two growing seasons with the superiority of the 2nd one. Similarly, plants inoculated with NFB+PDB showed higher values rather than NFB+SB or PDB+SB treatment. This may suggest that potato plants with NFB+PDB+SB acquired a good nutrients stress tolerance capacity. This effect was partly due to the N application and consequently its availability to the plant.

The synergistic effect of bio-fertilizer (NFB) on plant growth may be due to the role of N-microorganisms on supplying treated plants with their fixed nitrogen and improving plant growth (El-Agroudi *et al.*, 2003). The increasing effect of bio-fertilizers on plant growth may be attributed to its effects on synthesizing and producing several different plant hormones mainly, IAA, GA₃ and cytokinins, which play an important role in the formation of new cells and plant tissues resulting in stimulation of plant growth (Salisbury and Ross, 1992; Glick, 1995 and Kawthar *et al.*, 2002). Peng and Li, (1991) reported that the increase in plant height may be due to the role of bio-fertilization on stimulation of plant cell division and internodes elongation.

Arteca, (1996) noted that auxins and cytokinins increased cell division and cell enlargement. He added that exogenous application of cytokinins promote cell enlargement caused by an increase of water uptake as a result of an increase in the osmotic potential of the cells. Moreover, these strains bacteria not only caused the solubilization of minerals (Glick, 1995), but also stimulate plant growth by facilitating the uptake of minerals by

plant (Kloepper *et al.*, 1989) and increased the amount of available N for plant uptake (Kapulnik *et al.*, 1985; Michiels *et al.*, 1989).

Table (2): Effects of mineral and/or bio-fertilizers on plant height (cm), number of branches and leaves as well as leaf area (cm²) per plant of potato plants grown during the two growing seasons of 2001/2002 (S1) and 2002/2003 (S2).

Treatments		Plant height (cm)			Number of branches/plant			Number of leaves /plant			Leaf area cm ² /plant		
M-Mineral NPK	B-Bio-fertilizer	S1	S2	Mean	S1	S2	Mean	S1	S2	Mean	S1	S2	Mean
Control 100%	Without	30.30	34.50	31.40	3.11	3.30	3.20	27.9	28.6	28.25	2582	2708	2645
	NFB	36.30	38.60	36.45	3.93	4.11	4.00	31.4	31.0	30.82	2741	2866	2803.5
	PDB	35.10	38.00	35.55	3.60	3.90	3.75	30.6	32.5	31.95	2709	2832	2770.5
	SB	31.80	34.70	32.25	3.22	3.50	3.35	28.1	29.9	29.00	2602	2769	2685.5
	NFP+PDB	38.00	40.00	38.00	4.14	4.37	4.23	32.4	33.3	32.85	2849	2908	2878.5
	NFB+SB	37.00	39.50	37.25	4.06	4.30	4.30	32.3	32.4	32.35	2811	2900	2855.5
	PDB+SB	36.80	39.20	37.00	3.97	4.27	4.12	31.9	32.2	32.05	2768	2860	2814
	NFB+PDB+SB	38.60	41.00	38.80	4.20	4.60	4.40	32.6	33.6	33.10	2910	2981	2945.5
Mean		35.49	39.34	37.41	3.81	4.04	3.92	30.8	31.8	31.55	2756	2803	2799.5
75%	Without	27.50	30.90	29.20	2.50	2.89	2.70	24.0	24.5	24.25	2447	2467	2457
	NFB	33.30	36.20	34.75	3.63	3.90	3.75	28.9	29.1	29.00	2688	2693	2690.5
	PDB	32.90	34.80	33.85	3.37	3.71	3.53	28.2	28.6	28.40	2664	2623	2643.5
	SB	28.80	31.70	30.25	3.10	3.50	3.30	26.3	26.8	26.53	2582	2591	2586.5
	NFP+PDB	35.40	37.50	36.45	3.72	3.91	3.80	29.0	30.2	29.60	2722	2779	2750.5
	NFB+SB	34.10	37.23	35.67	3.80	4.07	3.93	28.9	30.0	29.45	2702	2740	2721
	PDB+SB	34.13	34.70	34.42	3.52	4.03	3.75	28.5	29.9	29.20	2700	2725	2712.5
	NFB+PDB+SB	35.40	40.20	37.75	3.83	4.02	3.90	30.1	31.6	30.80	2828	2790	2809
Mean		32.68	35.40	34.04	3.42	3.74	3.58	28.1	28.8	28.65	2666	2676	2671
50%	Without	24.80	25.00	24.90	2.13	2.22	2.15	18.3	20.7	19.50	2058	2177	2117.5
	NFB	27.60	29.10	28.35	2.62	3.01	2.80	22.8	23.7	23.25	2347	2453	2450
	PDB	27.40	29.00	28.20	2.70	2.80	2.75	22.2	23.1	22.65	2331	2444	2387.5
	SB	26.00	26.80	26.40	2.11	2.39	2.25	21.6	21.7	21.65	2376	2369	2372.5
	NFP+PDB	27.77	29.10	28.43	2.90	3.17	3.13	23.6	25.2	24.38	2391	2580	2485.5
	NFB+SB	27.30	29.70	28.50	2.73	3.01	2.87	23.5	25.0	24.25	2370	2559	2464.5
	PDB+SB	27.90	29.30	28.60	2.70	3.00	2.85	23.4	24.8	24.10	2354	2531	2442.5
	NFB+PDB+SB	29.70	32.70	31.20	3.17	3.13	3.15	24.2	25.4	24.82	2483	2603	2543
Mean		27.31	28.84	28.07	2.83	3.08	2.95	22.4	23.7	25.20	2338	2474	2406
Mean	Without	27.53	30.13	28.83	2.57	2.80	2.68	23.4	24.6	24.0	2362	2400	2381
	NFB	32.40	34.63	33.51	3.40	3.60	3.50	27.5	27.7	27.9	2692	2670	2681
	PDB	31.80	33.93	32.86	3.19	3.53	3.36	27.3	28.3	27.5	2668	2632	2650
	SB	28.86	31.06	29.96	2.80	3.13	2.96	25.3	26.1	25.7	2620	2576	2598
	NFP+PDB	33.72	38.60	36.16	3.57	3.81	3.71	28.2	29.7	28.9	2604	2755	2679.5
	NFB+SB	32.80	35.47	34.14	3.61	3.79	3.68	28.1	29.1	28.6	2622	2733	2677.5
	PDB+SB	32.94	34.47	33.67	3.39	3.77	3.58	28.1	29.0	28.5	2607	2705	2656
	NFB+PDB+SB	34.53	37.96	36.25	3.72	3.91	3.81	29.9	31.4	30.6	2740	3791	2765.5
LSD at 5% for: SxM		1.74			0.08			NS			2.9		
SxB		NS			0.04			0.36			1.8		
BxM		NS			0.11			0.45			3.6		
SxMxB		NS			0.14			0.63			5.0		

The simulative effect of mineral fertilizers on plant growth many be attributed to its effects on increasing both endogenous plant hormones and nutrients uptake (Helaly *et al.*, 1985; Ghallab and Salem, 2001; Hammad and El-Gamal, 2005). Arish and Bardisi, (1999) reported that, nitrogen is an

essential elements for building up protoplasm, amino acids and proteins which induce cell division and initiate meristematic activity. Phosphorus also is a part of molecular structure of nucleic acid (DNA and RNA), the energy transfer compounds and phosphoproteins. They added that, potassium elements are very important in overall metabolism of plant enzymes activity. Moreover, potassium has a beneficial effect on water consumption (Gardener *et al.*, 1985).

The increase in leaf number may be due to the positive effect of both mineral and/or bio-fertilizers which may be attributed to an increase in nutrients uptake (El-Gamal, 1999 a). Nitrogen caused an increase in the meristematic activity of the plant (Saleh *et al.*, 1982). Moreover, phosphorus play an important role in cell division and development of meristematic tissues (Ashour, 1998).

Omay *et al.*, (1993) attributed the increase in plant growth caused by bio-fertilizers probably to the effect of growth substances produced by the microorganisms i.e. IAA, GA₃ and cytokinins. These growth substances plays an important role in break plant apical dominance (El-Rewainy and Galal, 2004). Increment of branches number is of great importance, since it could be latter accompanied with an increase of formed and potato yield. Moreover, increasing the leaf number is of great interest because their reflection upon the final photosynthetic area, thereby the net assimilates that could be directed to the developing potato tubers. Moreover, the bio-fertilizers caused by inoculation with the three bacterial strains used facilitate the uptake of certain nutrients, which are necessary for plant growth. In addition, both mineral and/or bio-fertilizers caused an increase in the levels of the endogenous GA₃ and auxin contents (Helaly *et al.*, 1985; Noel *et al.*, 1996). GA₃ is known to cell be as an active material for elongation increase.

Concerning the increase in the leaf area under inoculation with *Pseudomonas fluorescens* (PDB) treatment, it may be due to its positive effect on P availability in the soil and its uptake by the plants (Hauka, *et al.*, 1996). 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). Abdel-Ati *et al.*, (1996) and Ashour, (1998) reported that, the reducing in soil- PH values due to P-dissolving bacteria inoculation may lead to an increase in the micronutrient contents and their availability, in addition to activity of N₂-fixing bacteria which increased cell division and cell elongation reflected an increase in plant height and dry matter accumulation (Hamed, 1998). Zaghoul *et al.*, (1996) found that, the highest count of *Azospirillum* was activated in soil inoculated with P-dissolving bacteria. The role of phosphorus in plant photosynthetic and respiration in addition to its role in cell division and development of meristematic tissues was reported (Ashour, 1998).

The beneficial effects of both mineral and/or bio-fertilizers on leaf number and their area may be attributed to an increase not only on mineral uptake (Hauka, 2000) but also on production of growth substances (Omay, *et al.*, 1993), which cause an increase in both cell division and cell elongation (Said and Keshta, 1999).

I.2. Fresh and dry weights as well as specific leaf weight (SLW) and leaf weight ratio (LWR):

Data indicate that, decreasing the NPK level rather than the recommended dose (control) resulted in a gradual decrease and even significant effects on the fresh and dry weights of potato shoots as well as LWR whereas increased SLW. These results are true during the two growing seasons.

Comparing the effects of different bio-fertilizers used on the fresh and dry weights, as well as SLW and LWR in both season (Tables 3), it is clear that, all bacterial strains used caused a favourable significant effects (especially when used all together; (NFB+PDB+SB) on all these parameters. Moreover, it is clear that, NFB strain was more effective than the others in this respect. Inoculation with nitrogen –fixing bacteria (NFB) was the most effective strain followed by phosphate-dissolving bacteria (PDB) on increasing fresh and dry weight of potato shoots as well as SLW and LWR during the two growing seasons. Bacterial strains used all together; (NFB+PDB+SB treatment) produced better effect than the other treatments of combined inoculation with strains used bacteria. Therefore, using NFB strain as an inoculation alone or in combination with the other strains used, with the superiority of PDB followed by SB, increased these parameters compared with the uninoculated plants. However, SB strain recorded less increases in this respect during the two growing seasons. These results may indicate that, these organisms, all together, exerts a growth stimulating and a synergistic effects as had been reported for other plant species (Raj *et al.*, 1981; Hauka *et al.*, 1996; El-Shahawy, 2003).

When the different doses of NPK fertilizers were associated with the inoculation treatments with the bio-fertilizers used (Tables VII and VIII), high positive responses and even highly significant effects on fresh and dry weights of potato shoots as well as SLW and LWR were recorded against control. Under each level of NPK fertilizers, such positive responses reached their maximum level when potato plants inoculated with the three strains used (NFB+PDB+SB) followed by NFB+PDB, NFB+SB and NFB alone respectively.

Comparing the values of both fresh and dry weights of the shoot system as well as SLW and LWR for the different treatments, it seen that all these parameters under 75% and 50% of the recommended quantities dose of NPK fertilizers plus mixed strains of used bacteria were high significant values compared with the control (100% recommended dose). It appears that, potato tubers which inoculated with mixed strains of bacteria plus 75% NPK (from the recommended dose) gave generally the best higher values of both fresh and dry weights of shoot systems as well as SLW than the other treatments. However, LWR showed lowest values in this respect.

The same tables indicated that, the superiority of combination between the recommended dose of NPK fertilizer and the dual bio-fertilizer treatments which recorded the highest significant increments over the all other treatments in both seasons.

Moreover, it was found that, although the positive effects of the single treatments with NFB, PDB or SB strains on the all studied growth

characters failed to counteracted the depressing effects of using 50% dose of the recommended quantitative of NPK fertilizer, the obtained data (Tables VII and VIII) clearly reviewed that, NFB strain was more effective than the other used strains under the all used level of NPK fertilizers.

The calculated assimilation rate (leaf area in cm² required for production one of dry matter) may be support the previously mentioned data about vigorous growth of potatoes as affected by the recommended NPK dose. Since, significant reduction in growth parameters was existed with decreasing the recommended dose of NPK up to 50%. That could be considered on evidence to decrease the efficiency of photosynthesis and also synthesize low assimilates per each unit of leaf area, hence, low rate of their translocation specially towards sink sites (potato tubers).

The simulative effects of both mineral and/or bio fertilizers on plant growth may be attributed to the effects of minerals on increasing both endogenous plant hormones and nutrients uptake (Kawthar *et al.*, 2002). Arisha and Bardisi (1999) added that, nitrogen is an essential element for building up protoplasm, amino acids and proteins which induce cell division and initiate meristematic activity. Phosphorus also is a part of molecular structure of nucleic acid (DNA and RNA), the energy transfer compounds and phosphoproteins. Potassium element is very important nutrient in overall metabolism of plant enzymes activity (Mengel and Kirkby, 1978). Moreover, potassium has a beneficial effect on water consumption (Gardener, 1985).

1.3. Leaf water potential (LWP), leaf relative turgidity (LRT) , specific leaf area (SLA)and leaf area ratio (LAR):

Regarding the effects of different NPK doses data in Table (4) indicate that all these parameters, in both seasons, were decreased significantly with an increase in NPK stress compared with the complete recommended dose (100% NPK ; control).

The negative effects of decreasing NPK fertilizers doses less than the recommended one on LWP, LRT, SLW and LAR of potatoes may be due to the stress effects of these elements on plant growth. The importance of N, P and K for normal growth was previously mentioned (Yagodin, 1982). Munson (1972) added that K is an important element for plant growth and it is involved in very metabolic processes including carbohydrates metabolism, protein biosynthesis, assimilate translocation, conformation of enzymes and stomatal movement.

Increment of branches number is of great importance, since it could be latter accompanied with an increase of formed and potato yield. Moreover, increasing the leaf number is of great interest because their reflection upon the final photosynthetic area, thereby the net assimilates that could be directed to the developing potato tubers.

The calculated assimilation rate (leaf area in cm² required for production one gramm of dry matter) may be support the previously mentioned data about vigorous growth of potatoes as affected by the recommended NPK dose. Since, significant reduction in growth parameters was existed with decreasing the recommended dose of NPK up to 50%. That could be considered on evidence to decrease the efficiency of photosynthesis and also synthesize low assimilates per each unit of leaf

area, hence, low rate of their translocation specially towards sink sites (potato tubers).

Table (3): Effects of mineral and/or bio-fertilizers on fresh and dry weight of the shoot system (g) per plant as well as specific leaf weight (SLW) and leaf weight ratio (LWR) of potato plants grown during the two growing seasons of 2001/2002 (S1) and 2002/2003 (S2).

Treatments		Fresh weight g/plant			Dry weight g/plant			Specific leaf weight (SLW)			Leaf weight ratio (LWR)		
M-Mineral NPK	B-Bio-fertilizer	S1	S2	Mean	S1	S2	Mean	S1	S2	Mean	S1	S2	Mean
Control 100%	Without	97.97	120.33	109.15	10.22	13.65	11.93	0.323	0.331	0.327	0.769	0.624	0.697
	NFB	109.40	139.86	124.63	11.61	14.99	13.30	0.330	0.336	0.333	0.786	0.648	0.717
	PDB	109.12	138.28	123.70	11.59	14.28	12.93	0.335	0.338	0.337	0.788	0.647	0.718
	SB	100.00	129.22	114.61	10.79	14.38	12.58	0.324	0.333	0.329	0.781	0.641	0.710
	NFP+PDB	113.16	142.35	127.75	12.09	15.61	13.85	0.340	0.341	0.341	0.802	0.656	0.729
	NFB+SB	110.28	142.02	126.15	11.80	15.44	13.62	0.334	0.337	0.336	0.795	0.651	0.723
	PDB+SB	110.28	138.03	124.15	11.69	15.33	13.51	0.331	0.336	0.334	0.805	0.642	0.724
	NFB+PDB+SB	121.81	150.01	135.91	12.79	16.15	14.47	0.341	0.344	0.343	0.809	0.657	0.733
Mean		109.31	130.27	120.28	11.07	14.97	13.27	0.331	0.330	0.331	0.791	0.646	0.725
75%	Without	90.27	110.13	100.20	9.77	12.75	11.26	0.325	0.349	0.337	0.742	0.642	0.692
	NFB	106.74	132.00	119.37	11.55	14.79	13.17	0.328	0.367	0.348	0.749	0.652	0.701
	PDB	105.71	131.70	118.70	11.26	14.70	12.98	0.328	0.360	0.344	0.746	0.645	0.695
	SB	96.97	120.51	108.74	10.33	13.67	12.00	0.327	0.359	0.343	0.745	0.643	0.694
	NFP+PDB	108.98	140.27	124.62	11.75	15.46	13.61	0.336	0.371	0.354	0.758	0.658	0.708
	NFB+SB	107.82	136.47	122.15	11.62	15.22	13.42	0.335	0.370	0.353	0.750	0.656	0.703
	PDB+SB	107.57	135.37	121.47	11.56	15.01	13.28	0.335	0.367	0.351	0.749	0.652	0.701
	NFB+PDB+SB	118.43	149.13	133.78	12.80	16.30	14.55	0.338	0.371	0.355	0.766	0.662	0.714
Mean		105.31	121.94	117.08	11.33	14.73	13.03	0.331	0.350	0.341	0.751	0.646	0.700
50%	Without	67.53	95.47	81.50	8.49	10.67	9.58	0.341	0.352	0.347	0.733	0.661	0.697
	NFB	94.49	114.92	104.70	10.02	12.99	11.50	0.349	0.355	0.352	0.743	0.671	0.707
	PDB	93.77	114.71	104.24	9.92	12.97	11.44	0.348	0.356	0.352	0.743	0.671	0.707
	SB	90.57	104.89	97.73	9.46	12.18	10.82	0.346	0.353	0.350	0.738	0.667	0.702
	NFP+PDB	101.47	127.21	114.34	10.30	13.58	11.94	0.351	0.358	0.355	0.754	0.681	0.718
	NFB+SB	98.29	125.07	111.68	10.00	13.58	11.79	0.349	0.356	0.353	0.751	0.677	0.714
	PDB+SB	97.12	123.51	110.31	9.90	13.50	11.70	0.351	0.357	0.354	0.749	0.676	0.713
	NFB+PDB+SB	106.27	130.67	118.47	10.94	14.45	12.70	0.354	0.362	0.358	0.757	0.685	0.721
Mean		93.68	113.93	104.31	9.87	12.99	11.43	0.341	0.350	0.346	0.746	0.667	0.708
Mean	Without	80.20	106.97	93.07	9.49	12.30	11.25	0.341	0.330	0.336	0.748	0.642	0.697
	NFB	103.04	127.71	115.37	11.06	14.20	12.66	0.340	0.349	0.345	0.759	0.657	0.708
	PDB	102.86	120.06	111.50	10.92	13.98	12.60	0.340	0.351	0.346	0.757	0.654	0.706
	SB	90.84	118.20	104.52	10.19	13.41	11.88	0.340	0.342	0.341	0.750	0.650	0.702
	NFP+PDB	107.87	134.71	118.19	11.38	14.88	13.05	0.340	0.352	0.346	0.750	0.660	0.709
	NFB+SB	100.46	124.18	112.27	11.14	14.74	12.97	0.346	0.351	0.349	0.750	0.661	0.707
	PDB+SB	104.99	131.30	116.81	11.00	14.71	12.91	0.344	0.351	0.348	0.748	0.657	0.705
	NFB+PDB+SB	110.00	139.96	124.98	12.17	15.73	13.90	0.348	0.353	0.351	0.757	0.657	0.709
LSD at 5% for: SxM			0.28		0.14			0.001			0.001		
SxB			0.46		0.31			0.002			0.001		
MxB			0.57		0.38			0.002			0.001		
SxMxB			0.80		0.54			0.003			0.002		

Regarding the effects of different NPK doses data in the tables indicate that all these parameters in both seasons, were decreased significantly with an increase in NPK stress compared with the complete recommended dose (100% NPK; control). The negative effects of decreasing NPK fertilizer doses, less than the recommended one, on LWP, LRT, SLA and LAR of potatoes may be due to the stress effects of these elements on

plant growth. The importance of N, P and K for normal growth was previously mentioned (Yagodin, 1982). Munson (1972) added that K is an element important for plant growth and it is involved in very metabolic processes including carbohydrates metabolism, protein biosynthesis, assimilate translocation, conformation of enzymes and stomatal movement.

As for the effects of bio-fertilizers, overall NPK doses, data in the same tables indicate that, inoculation with each of the bacterial strains used increased LWP and LRT in potato shoots whereas decreased that of SLA and LAR compared with potatoes without bio-fertilizers treatment. Again, the most effective strain was found with NFB followed by PDB and SB in a descending order. A synergistic effects on these parameters were found with mixture strains. Therefore, the most effective treatments on increasing LWR and LRT and decreasing SLA and LAR was found with NFB+PDB+SB followed with NFB+PDB, NFB+SB, PDB+SB, NFB, PDB and SB respectively.

The interaction treatments indicate that, all bio-fertilizers strains used not only nullified the depressing effects of stress at 75% NPK on LWR and LRT but increased these parameters over the control (100% NPK) without inoculation bio-fertilizers. Similarly all bio- fertilizers strains counteracted, to some extent, the depending effects of stress at 50% NPK on LWR and LRT during the two growing seasons. However, SLA and LAR were decreased in the plants inoculated with any of the bacterial strains used and grown in the different NPK doses. However, plants inoculated with any strains used showed an additive effects to the effects of NPK stress on SLA and LAR.

Under each level of NPK fertilizer, the responses on these parameters reached their maximum level when the plants inoculated with all strains when used together (NFB+PDB+SB). It is reported the superiority of combination between 75% from the recommended doses of NPK fertilization and the dual bio fertilizers treatments which recorded the highest significant effects on LWP and LRT over the all other treatments in both seasons. On the other hand, all combination treatments had a negative effects on SLA and LAR. Moreover, the obtained data clearly indicated that, nitrogen bacteria strain was more effective than PDB or SB strains under the all used doses of NPK fertilizers.

The positive effect of mineral fertilizers on growth characters of potatoes may be attributed to the role of N in protoplasm formation and all proteins, e.g. amino acids, nucleic acids and many enzymes as well as energy transfer materials; ADP and ATP (Russel, 1973).The role of P as a major nutrient elements, where P compounds are of absolute necessity for all living organisms, nucleoproteins consisting the essential substances of the cell and for cell division and development of meristematic tissues (Yagodin (1982). un Vao day nghe bai nay di ban [http:// nhatquanglan1.0catch.com](http://nhatquanglan1.0catch.com) K is important for plant growth and is involved in every metabolic processes including carbohydrates metabolism, protein biosynthesis, assimilate translocation, conformation of enzymes and stomatal movement (Munson, 1972). Potassium has regulating and catalytic roles on plant metabolism and is involved in numerous functions in the plant such as enzyme activation, cation-anion balance, stomatal movement, phloem loading, assimilate translocation and turgor regulation to name only few

(Glass, 1989 and Krauss, 2001). At the biochemical level, K plays some irreplaceable and versatile roles that catalyze the metabolic activities at the cellular and organ levels which be translated at the whole plant into promotion in energy conversion, carbohydrate assimilation and translocation and nutrient uptake, transfer and metabolism (Johanston, 1997 and Krauss, 2003) . These effects were reflected on vigorous vegetation growth such as plant height, number of branches , leaf area and consequently fresh and dry weights of the shoots per plant. Thus adequate K supply is important for driving up the plant morphology, anatomy and physiology towards greater crop yield with better quality, more resistance to biotic stresses; pests and pathogens, and higher tolerance to abiotic stress; drought, salinity, improper temperature and high light intensity (Krauss, 2001 and Krauss, 2003).

The vigour of potato plants growth reflected with LWP, LRT, SLA and LAR supplied with 100% NPK as chemical fertilizers over than that obtained bio fertilizers might be due to the more solubility and availability of NPK in the chemical fertilizers form compared to the microorganisms activity. It mean that bio fertilizers sources needed a longer time to change from organic to mineral form which directly absorbed by the root system.

The increasing of LWP, LRT, SLA and LAR values due to the treatment of NFB+PDB+SB may be attributed to the increasing of the nutritional elements in the rooting zone, and consequently the absorption of more nutrients. The beneficial effects of microorganisms on rhizosphere intensification, N₂-fixing potential, plant growth substances production and antimicrobial substances production that could be useful against pathogenic microorganisms were previously mentioned (Bhattarai and Hess (1998). Zhang *et al.*, (1996) suggested that *Serratia* stimulate soybeans growth through the production of plant growth regulating compound, which stimulate nitrogen fixation by stimulating over-all plant vigor and growth, resulting in a subsequent increase in nitrogen fixation. Bhattarai and Hess (1998) reported that *Azospirillum* + Vesicular-arbuscular mycorrhizas (VAM) inoculation enhanced the growth at early stage as compared with NPK alone, which induced an increase of shoots and spike dry weight of wheat plant.

El-Borollosy *et al.*, (2000) reported that the stimulatory effect of *Azotobacter chroococum* on increasing growth and yield can be attributed not only to N₂ fixation activity, but also to the production of growth promoters (indol acetic acid , gibberellin and cytokinine) substances which help in greater absorption of nutrients from the soil. Similarly, Valssak and Reynders (1980) found that *Azospirillum* produces IAA, indol lactic acid, gibberellins and cytokinins like substances.

Bashan *et al.*, (1990) concluded that, the significant favorable effects of the combination between NPK fertilizer doses and *Azospirillum* as well as *Pseudomonas* bio-fertilizers (especially between the recommended dose and bio-fertilizer) on LWP, LRT, SLA and LAR may be explained on the basis of the beneficial effects of bacteria on the nutrient availability, vital enzymes, hormonal stimulating effects on plant growth or the increasing evidence for this view.

Bashan and Levanony (1990) proposed several possible modes of action due to *Azospirillum* on plant growth: N₂ fixation, which contributes N to

the plant, hormonal effects, which alter plant metabolism and growth, general improvement in the growth of the entire root system, resulting in enhanced mineral and water uptake.

Table (4): Effects of mineral and/or bio -fertilizers on leaf water potential (LWP), leaf relative turgidity (LRT) as well as specific leaf area (SLA) and leaf area ratio (LAR) of potato plants grown during the two growing seasons of 2001/2002 (S1) and 2002/2003 (S2).

Treatments		Leaf water potential (LWP)			Leaf relative turgidity (LRT)			Specific leaf area (SLA)			Leaf area ratio (LAR)		
M- Mineral NPK	B- Bio-fertilizer	S1	S2	Mean	S1	S2	Mean	S1	S2	Mean	S1	S2	Mean
Control 100%	Without	88.64	88.66	88.65	81.40	85.61	83.50	3.077	3.149	3.113	252.6	198.4	225.5
	NFB	88.98	88.97	88.97	83.62	86.42	85.02	3.002	2.973	2.987	236.1	191.2	213.6
	PDB	88.87	89.04	88.95	83.60	85.88	84.74	2.987	2.956	2.971	233.7	198.3	216.0
	SB	88.75	88.87	88.81	82.44	85.22	83.83	3.090	3.003	3.046	241.1	192.5	216.8
	NFP+PDB	89.02	89.03	89.02	85.63	87.14	86.38	2.934	2.934	2.934	235.6	186.3	210.9
	NFB+SB	88.94	88.89	88.91	84.71	86.82	85.76	2.994	2.968	2.981	238.2	187.8	213.0
	PDB+SB	88.89	88.89	88.89	83.85	86.01	84.93	3.101	2.973	3.037	236.8	186.6	211.7
	NFB+PDB+SB	89.05	89.02	89.03	87.92	90.33	89.12	2.936	2.957	2.946	227.5	184.6	206.0
Mean	88.89	88.76	88.91	84.14	86.28	85.15	3.010	2.989	2.999	237.7	190.7	214.2	
75%	Without	88.27	88.42	88.34	80.52	82.52	81.52	3.203	2.983	3.093	250.5	193.5	222.0
	NFB	88.76	88.79	88.77	83.33	86.01	84.77	3.136	2.808	2.972	232.7	182.1	207.4
	PDB	88.82	88.84	88.83	83.20	85.89	84.54	3.141	2.793	2.967	236.6	178.4	207.5
	SB	88.84	88.66	88.60	81.94	84.83	83.38	3.207	2.876	3.042	249.9	189.5	219.7
	NFP+PDB	89.22	88.98	89.10	85.72	88.00	86.86	3.091	2.807	2.949	231.7	179.7	205.7
	NFB+SB	89.01	88.85	88.93	84.78	87.72	86.25	3.113	2.782	2.947	232.5	180.0	206.2
	PDB+SB	88.94	88.91	88.92	83.93	87.11	85.52	3.072	2.806	2.939	233.6	181.5	207.5
	NFB+PDB+SB	89.19	89.07	89.13	89.50	91.13	90.31	3.071	2.784	2.928	220.9	171.2	196.0
Mean	88.84	88.81	88.83	84.11	86.70	85.38	3.129	2.820	2.974	237.0	181.9	209.0	
50%	Without	88.13	88.21	88.17	72.91	78.21	75.56	3.161	2.978	3.070	242.4	204.0	223.2
	NFB	88.76	88.71	88.72	80.83	83.49	82.16	3.061	2.813	2.937	234.2	188.8	211.5
	PDB	88.62	88.69	88.65	80.80	83.72	82.26	3.063	2.806	2.934	235.0	188.4	211.7
	SB	88.40	88.39	88.39	78.83	80.73	79.78	3.097	2.892	2.995	240.5	194.5	217.5
	NFP+PDB	89.10	88.81	88.95	82.74	84.90	83.82	3.065	2.792	2.928	232.1	190.5	211.1
	NFB+SB	89.05	88.78	88.91	81.49	84.22	82.85	3.082	2.791	2.936	237.0	188.4	211.1
	PDB+SB	89.01	88.81	88.91	80.54	82.87	81.70	3.081	2.775	2.928	237.8	187.5	212.1
	NFB+PDB+SB	89.09	88.94	89.01	84.11	88.13	86.12	3.143	2.766	2.928	227.1	180.1	203.6
Mean	88.76	88.67	88.72	80.28	83.28	81.78	3.094	2.827	2.960	230.8	190.2	213.0	
Mean	Without	88.35	88.43	88.39	78.27	82.11	80.19	3.147	2.973	3.060	244.0	198.7	221.3
	NFB	88.82	88.82	88.82	82.09	85.20	83.90	3.076	2.810	2.943	234.2	188.8	211.5
	PDB	88.77	88.86	88.81	82.03	85.16	83.84	3.074	2.802	2.938	235.0	188.4	211.7
	SB	88.57	88.64	88.60	81.07	83.09	82.13	3.131	2.924	3.027	243.8	192.1	218.0
	NFP+PDB	89.11	88.94	89.03	84.79	87.78	86.28	3.030	2.844	2.937	233.1	180.3	206.2
	NFB+SB	88.99	88.84	88.92	83.76	86.20	84.98	3.073	2.847	2.960	230.9	180.4	205.6
	PDB+SB	88.95	88.87	88.91	82.77	85.23	84.00	3.080	2.801	2.941	237.4	180.2	205.8
	NFB+PDB+SB	89.11	89.01	89.06	87.17	91.86	89.52	3.000	2.827	2.913	220.1	178.7	201.9
LSD at 5% for: SxM		0.01			0.02			0.002			0.1		
SxB		0.02			0.01			0.001			0.1		
MxB		0.03			0.03			0.002			0.1		
SxMxB		0.04			0.04			0.003			0.2		

II: Anatomical structure:

II.1. leaf internal structure :

The anatomical structure of the terminal leaflet blade taken from the 3rd compound leaf from the plant tip taken at the middle part as affected by mineral and/or bio-fertilizers of the potato plants grown only during the second season (2002/2003) are presented in Table (5) and illustrated in Figs (1 and 2).

Generally, it was found the leaflet blade internal structure of potato plants as seen in transfer section consists of lower and upper epidermis , mesophyll tissue differentiated into palisade and spongy parenchyma, the palisade parenchyma tissue cells are elongated and compactly arranged parenchymatous cells having few narrow intercellular spaces between them. The spongy parenchymatous cells are loosely arranged with numerous large intercellular spaces. Venation is reticulate pinnate with frequent secondary veins. The vascular bundle of the midvein is an open collateral having a narrow cambial zone (Fig.1 A).

Table (5) and Figs (2 and 3) give the details of the anatomical structure of the terminal leaflet blade of the 3rd compound leaf of *Solanum tuberosum* L. treated with bacterial strains used and grown under different doses of mineral NPK fertilizers.

Data indicate that, plants received NPK at the full (100%) recommended dose (control) were higher in all anatomical parameters studied than that received 75% NPK and the decrease was a concentration dependent overall the presence of bio-fertilizers. Reduced cell volume in various plant tissues due to stress condition was reported previously (Chaudhry and Qurat-w-Ain, 2003; Sridhar *et al.*, 2005;Esmail, 2005 and Abd El-Salam, Heba, 2006). The correlation between leaf thickness and area of cross section in possible showing that the changes of the leaf cross section area results in changes of dry matter corresponding to the surface area unit (Kallis and Tooming, 1974) and internal surface of the leaf (Nobel, 1977) and replaced the addition of 100 % NPK recommended dose .The enhancing effect of mineral fertilizers on the internal structure may be attributed to the essential role of nitrogen for building up the protoplasm and enhancing cell division and initiate of meristematic activity. Moreover, phosphorus enhanced the vascular tissues formation as well as is essential substance for cell division and development of meristematic tissues (Yagodin, 1982).

On the other hand, it was found that inoculation of bacterial strains used increased, overall NPK doses, all anatomical parameters studied of the leaf. The most effective treatments were found with NFB+PDB+SB, NFB+PDB, NFB+SB, NFB alone in a descending order. Less increases were recorded with the other strains.

The interaction treatments between mineral NPK and bio-fertilizers indicated that, all bacterial strains counteracted the depression effects of minimizing NPK up to 75% from the recommended dose and decreased afterwards. Leaflet thickness at the midrib was increased as a result of increasing palisade and spongy mesophyll tissues thickness. Midrib; vascular bundle as dimensions (length & width) were also increased as indicated by its dimensions well as xylem and external and internal phloem tissues

thickness. Metxylem vessels dimension was also increased when compared with non – inoculated plants (Fig. 1 A). Inoculation with *Azospirillum brasilense* (NFB) alone (Fig. 1 B) as well .as inoculation with either PDB (Fig. 1 C) or with mixed strains of used bacteria (NFP+ PDB+SB) (Fig. 1 D) were the best treatment in this respect compared with untreated plants.

Comparing, with the control (100% recommended dose) (Fig. 2 A), data in the same table indicate that, plants received mixed strains of used bacteria either NFB (Fig. 2 C) or (NFB+PDB+SB) plus 75% NPK from recommended dose (Figs. C and D) showed high values in this respect. Data in Table (8) and illustrated in fig. (2 D) revealed also that plant inoculated with mixed three strains of used bacteria plus 75 % NPK from recommended dose recorded higher values from all measured anatomical characters than other specially with respect to components of the vascular system. It recorded highest dimensions of the mid-rib vascular bundle and its constituents from phloem and xylem. The recorded increase in xylem tissues dimensions was accompanied with higher number of vessels. The modification of the leaf anatomy due to the bio-fertilizer application may be attributed to their effects on the environmental factors which required to a longer period of time.

The least values of the studied leaf anatomical structure were recorded with plants received with 50% NPK from the recommended dose as treated with no bio-fertilizers. The most striking effects were recorded with respect to components of the vascular system. Moreover, it was found that, the plants received mixed strains bacteria failed to replace mineral NPK at 50% from the recommended dose fertilizers. The reduction in leaf thickness with no bio-fertilizers and less dose of NPK may be due to the decrease in cell division as a results of nuclear degradation of meristimatic cells under stress condition (Katsuhara and Kawasaki, 1996).

Reduced cell volume in various plant tissues was reported in response to stress condition. The absence of bio-fertilizer under 50% NPK induced reduction in cell volume was recorded for epidermal and mesophyll cells as well as various cell types. Similar results were reported under heavy metals stress (Chaudhry and Qurat-w-Ain, 2003 and Abd El-Salam, Heba, 2006).

The increase of leaflet internal structure as responses to bio-fertilizers treatments may be attributed to its effects on increasing different plant hormones mainly, IAA, GA3 and cytokinins which enhanced cell division and cell enlargement as well as formation of new cells and plant tissues (Salisbury and Ross, 1992; Kawther *et al.*, 2002). Arteca (1996) reported that, auxins and cytokinins increased cell division and cell enlargement which caused by an increase of water uptake as a result of an increase in the osmotic potential of the cells.

C

D

Fig (1): Cross sections of the terminal leaflet blade of the 3rd compound leaf from the potato plant tip as affected by some biofertilizers (Obj. x10. Oc. X 15)

**Pal= palisade tissue SP= spongy tissue X= xylem Ph= phlo
Mi=midvein vascular bundle**

Fig (2): Cross sections of the terminal leaflet blade of the 3rd compound leaf from the potato plant tip as affected by different doses of mineral fertilizers and their interactions with biofertilizers (Obj. x10. Oc. X 15)

A:100%NPK B:75% NPK C:75% NPK+NFB

D: 75% NPK +(NFB+PDB+SB)

Pal= palisade tissue SP= spongy tissue X= xylem Ph= phloem

Mi=midvein vascular bundle.

Table (5): Effects of mineral and/or bio-fertilizers on some anatomical characters (μm) of the terminal leaflet of the 3rd compound leaf from the potato plants tip during the second season of 2002/2003.

Treatments		Anatomical characters									
		Leaflet thickness in the midrib region μm	Mesophyll tissue thickness μm	Palisade tissue thickness μm	Spongy tissue thickness μm	Midrib V.B. dimension		Xylem tissue thickness of midrib bundle μm	Phloem tissue thickness of ss of midrib		Metaxylem vessel dimension μm
						Length	Width		External	Internal	
Mineral NPK (M)	Bio-fertilizer (B)										
100%	Control	1750	538	280	258	645	470	310	170	153	56
	NFB	1862	572	295	277	680	410	330	177	167	60
	PDB	1810	562	292	270	665	395	318	175	158	58
	SB	1785	554	290	264	648	375	315	172	154	55
	NFP+PDB	1935	580	310	290	700	475	340	180	166	62
	NFB+SB	1910	575	320	285	690	472	334	178	163	60
	PDB+SB	1880	564	324	270	680	450	338	172	154	64
	NFB+PDB+SB	2030	645	335	310	734	512	355	190	176	70
Mean		1870	508	305	278	680	444	330	156	161	60
75%	Control	1610	465	235	230	535	320	230	148	144	50
	NFB	1844	500	250	246	560	387	245	157	157	58
	PDB	1820	496	248	242	557	370	240	153	154	55
	SB	1815	490	245	242	510	335	190	155	152	48
	NFP+PDB	1865	562	284	255	605	354	265	164	162	60
	NFB+SB	1860	556	278	278	590	343	253	162	158	58
	PDB+SB	1852	531	275	256	580	346	247	160	150	60
	NFB+PDB+SB	2491	775	395	380	807	465	376	268	265	88
Mean		1894	546	276	266	593	365	255	170	167	59
50%	Control	1040	305	160	145	263	250	142	64	57	35
	NFB	1130	445	225	220	318	290	160	78	55	44
	PDB	1090	435	220	215	290	275	154	78	48	40
	SB	1075	395	205	190	270	270	147	70	42	36
	NFP+PDB	1145	430	230	225	310	278	163	77	63	48
	NFB+SB	1140	424	227	222	320	310	154	80	60	45
	PDB+SB	1135	421	224	220	332	302	177	82	58	46
	NFB+PDB+SB	1193	474	244	230	345	335	185	85	68	52
Mean		1118	416	216	208	306	288	160	76	56	43
Control		1466	436	225	211	481	346	227	127	118	47
NFB		1612	505	256	247	519	362	245	137	126	54
PDB		1573	497	253	242	504	346	237	135	120	51
SB		1558	479	246	232	476	326	217	132	116	46
NFP+PDB		1648	524	274	256	445	369	256	140	130	56
NFB+SB		1636	518	275	261	533	375	247	140	127	54
PDB+SB		1622	505	274	248	530	368	254	138	120	56
NFB+PDB+SB		1904	631	324	306	628	437	305	181	169	70
LSD at 5% for: M		1.3	1.2	1.4	1.3	1.9	0.7	0.3	0.1	0.2	1.0
B		2.2	2.0	2.2	2.1	3.2	1.1	0.4	0.2	0.4	1.6
MxB		3.7	3.5	3.9	3.6	5.5	1.9	0.8	0.3	0.7	2.8

II.2. Stem structure :

The stem structure of potato plants (Table 6) as seen in transfer section (Fig 3 A) consists of the epidermis, ground tissue, which differentiated into cortex and pith. The outer most layers constitutes the epidermis, followed by the cortex tissue, its outermost region consists of 4-5 cell layers of angular chlorenchymatous cells, and the middle region consists of 4-6 cell layers of chlorenchymatous cells.

The vascular bicollateral bundles arranged in complete cylinder. The vascular bundles are bicollateral. Two types of collateral bundles are presents i.e. large and small bundles. The large bundles were alternated with small one (Fig.3 A).

The vascular cambium first developed from the procambium between the primary xylem and phloem in the vascular bundles, and later continuous cylinder of vascular tissues are formed by the development of the interfascicular cambium.

The effects of bacterial inoculation with *Azospirillum brasilense* (NFB), *pseudomonas fluorescens* (PSB) and/or *Bacillus circulans* (SB) and mineral fertilizers on the 3rd internode the main stem of potato plants taken from the plant tip are presented in Table (XII) and illustrated in Figs. 3 and 4. Data show that, the anatomical parameters studied were decreased with decreasing NPK fertilizer doses. Plants treated with 100% recommended dose of NPK resulted high values than that treated with 75% and the decrease was a concentration dependent, overall the presence of bio-fertilizers. The increase in stem diameter under full recommended dose of mineral fertilization may be attributed to the effects of nutrients on increasing meristematic activity as well as cell division and its elongation through auxin production (Said and keshta ,1999 ; Salem, 2000). El-Rewainy and Gala, (2004) reported that, nitrogen not only increased the growth substances but also increase their translocation in the plant. In addition, phosphorus is a component of RNA and DNA (Marschner,1995) therefore it play an important role for cell division activity. EL- Shaarawy *et al.*, (2004) suggested that the increased stem diameter due to application of phosphorein at the dose of 200g/fed. may be attributed to the promotion of cambial activity which produced higher amount of secondary xylem. The physiological data recorded in the present investigation led to the suggestion that mineral deficiency induced reduction in cell volume (Chaudhry and Qurat-w-Ain, 2003). Pb decreased No. of xylem vessels and metaxylem vessel diameter. Functional abnormalities in the treachery elements in response to condition stress were also reported (Vollonweider *et al.*, 2005 and Abd El-Salam, Heha, 2006).

Data indicate also that all bacterial inoculation treatments increased stem diameter, cortex thickness, large vascular bundle dimensions, external and internal phloem and xylem tissues thickness as well as pith tissues dimension. Metaxylem vessel dimension was also increased. The increase in vascular tissue components may be due to the increase in vascular tissue differentiation compared with those non- inoculated plants. Inoculation with *Azospirillum brasilense* (NFB) alone (Fig. 3 B) as well as inoculation with either NFB + PDB (Fig. 3 C) or mixed strains of used bacteria (NFB + PDB+ SB) (Fig. 3 D) were generally the best treatments in this respect compared with those grown without inoculation (Fig. 3 A).

The increase in stem diameter due to the inoculation with mixed three strains of used bacteria may be attributed to their ability to release plant growth substances, mainly IAA, GA3 and cytokinines (Omay *et al.*, 1993). Auxins and cytokinins increased cell division and cell enlargement (Arteca, 1996). He added that the exogenous application of cytokinins

promote cell enlargement caused by an increase of water uptake as a result of an increase in the osmotic potential of the cells. The hormonal balance may be detected (Salama and Helaly, 1981). Moreover, the increase in vascular bundle dimensions may be due to a stimulation in cell division in the procambium and extension growth (Abd El-Salam, Heba, 2006).

Table (6): Effects of mineral and/or bio-fertilizers on some anatomical characters (μn) of the main stem at the 3rd internode of potato plants during the second season of 2002/2003.

Treatments		Anatomical characters								
		Stem diameter μn	Cortex thickness μn	Large vascular dimension μn		Phloem tissue thickness μn		Xylem tissue thickness μn	Metaxylem vessel dimension μn	Pith dimension μn
				Length	Width	External	Internal			
100%	Control	2165	530	435	470	105	84	240	72	1200
	NFB	2490	550	490	495	130	88	264	80	1450
	PDB	2305	545	460	485	124	82	248	78	1300
	SB	2278	540	448	580	118	82	240	74	1290
	NFP+PDB	2680	580	560	530	178	96	280	88	1540
	NFB+SB	2626	582	544	515	168	92	278	82	1500
	PDB+SB	2602	578	534	500	164	90	270	80	1490
	NFB+PDB+SB	2640	528	570	500	170	96	310	100	1530
Mean		2473	554	505	453	144	88	239	81	1412
75%	Control	1850	450	400	310	100	78	210	70	1000
	NFB	2230	480	470	380	120	86	254	76	1280
	PDB	2112	464	448	364	115	84	238	74	1200
	SB	2036	456	430	348	110	80	230	72	1150
	NFP+PDB	2378	498	490	450	124	90	266	86	1390
	NFB+SB	2278	488	480	420	122	90	256	82	1310
	PDB+SB	2282	482	500	410	150	92	250	84	1300
	NFB+PDB+SB	2580	650	600	580	180	98	315	110	1500
Mean		2218	496	477	357	127	87	223	81	1266
50%	Control	1362	300	262	200	80	32	140	32	800
	NFB	1595	380	325	240	90	44	180	48	890
	PDB	1484	344	300	232	88	36	164	40	840
	SB	1425	330	275	224	84	36	148	34	820
	NFP+PDB	1638	394	334	248	92	48	195	64	910
	NFB+SB	1618	388	330	236	90	44	188	60	900
	PDB+SB	1614	384	325	230	88	42	186	52	905
	NFB+PDB+SB	1705	410	340	255	98	60	200	68	955
Mean		1555	366	311	233	88	42	175	49	877
Control		1792	426	365	326	95	64	196	58	1000
NFB		2105	470	428	371	113	72	232	68	1206
PDB		1967	451	402	360	109	67	216	64	1113
SB		1913	442	387	384	104	66	206	60	1086
NFP+PDB		2232	490	461	409	131	78	247	79	1280
NFB+SB		2174	486	451	390	126	75	240	74	1236
PDB+SB		2166	481	453	380	134	74	235	72	1231
NFB+PDB+SB		2308	529	503	445	149	84	275	92	1328
LSD at 5% for: M		1.1	1.8	1.8	1.9	1.2	0.9	1.9	1.0	1.5
B		1.7	3.1	3.0	3.2	2.0	1.4	3.1	1.6	2.4
MxB		3.0	5.1	5.2	5.5	3.5	2.5	5.1	2.7	4.2

Fig (3): Cross Sections of the 3rd internode from the potato plant tip as affected by some biofertilizers (Obj. x 10. Oc. X15)
Co= cortex EN. Ph= External phloem

Fig (4): Cross sections of the 3rd internode from the potato plant tip as affected by different doses of mineral fertilizers and their interactions with biofertilizers (Obj.x10.Oc.X 15)

A: unterated B: 100% recommended NPK
C: 75% NPK +(NFB+PDB+SB)
Col= colenchyma Co= cortex Exph= External phloem
Ph= Pholem Enph= Enternal phloem Pi= pith

Comparing, with the control (100% recommended dose without bio-fertilizers, data in the same table and illustrated in fig. (4 A) clearly show that plant inoculated with mixed three strains of used bacteria either (NFB+PDB) or (NFB+PDB+SB) plus 75 % NPK (from recommended dose) (Figs. 4 C and D) gave high values in this respect. Although, the plants received mixed strains bacteria failed to replace mineral NPK fertilizers since it recorded less values. Data in the same table and illustrated in fig.(4 D) clearly show that, plants inoculated with mixed three strains of used bacteria plus 75% NPK (from recommended dose) was the most effective in this respect.

The reduction in cell volume may be due to a reduction in cell turgid and cell wall elasticity (Baredo *et al.*, 1988). Decrease cell wall extensibility may arise from cross -linking the pectin carboxyl groups in walls (Baredo and Poschenrieder, 1990) or to H₂O₂-mediated cross linking between aromatic cell wall compounds (Schutzendubel *et al.*, 2002).

The most effects of minerals deficiencies are their effects on vascular elements, where a general suppression of their development was recorded. Similarly, Setia and Boula (1994) found that, No. of vascular bundle and their cell size were decreased in response to heavy metals stresses. According to (Vollonweider *et al.*, 2005 and Abd-Elsalam, Heba, 2006).

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دراسات مورفولوجية و تشريحية علي البطاطس تحت تأثير التسميد الحيوي والمعدني

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أجريت تجربتين حقليتين بمحطة البحوث الزراعية بكلية الزراعة جامعة المنصورة خلال موسمي النمو ٢٠٠١/٢٠٠٢ و ٢٠٠٢/٢٠٠٣ بهدف دراسة مدى إمكانية إحلال التسميد الحيوي كبديل عن بعض من جرعات التسميد المعدني الموصى بها لتسميد نباتات البطاطس صنف "سيونتا دون التأثير على النمو. لتقليل الأثر الضار لتراكم النترات والنيتريت في الدرنه حفاظا على صحة المستهلك مع تقليل تلوث البيئة. وأستخدم لهذا الغرض ثلاث مستويات مختلفة من التسميد المعدني أحدهما التركيز الموصى به كعامله مقارنة بالإضافة الى تقليل المستوى الي ٧٥%، ٥٠% من الجرعة الموصى بها ، كما أستخدم ثلاث سلالات بكتيرية كمصدر للتسميد الحيوي ؛ وهى سلالة أوسبريليم براسيلينز المثبتة للنيتروجين ، سلالة سيدوموناس فلوريسنس المذيبة للفوسفات وسلالة الباسلس سيركيولنس الميسرة لإطلاق البوتاسيوم المرتبط بمعادن التربة ، وقد أختبر أثر تلك المعاملات وتداخلاتها على بعض الصفات المورفولوجية والتشريحية خلال فترة النمو النشط ، وحلت النتائج إحصائيا خلال موسمي النمو.

و تتلخص أهم النتائج المتحصل عليها على النحو التالي:
أولاً: الدراسات المورفولوجية :

- ١- لوحظ إنخفاض معنوي في طول النبات ، عدد الأفرع والأوراق وكذلك الوزن الغصن والجاف للمجموع الخضرى للنبات كما إنخفض وزن الورقة النسبي ومساحتها والجهد المائي ودرجة الإمتلاء بها مع نقص جرعة التسميد المعدني عن التركيز الموصى به خلال موسمي النمو .
- ٢- عوضت السلالات البكتيرية المستخدمة كسماد حيوي النقص الناتج عن تخفيض معدلات التسميد المعدني على معظم قياسات النمو المدروسة، وكانت أفضل السلالات لهذا الغرض هى البكتيريات المثبتة للنيتروجين إذا أستخدمت بمفردها أو مرتبطة مع أى من السلالات الأخرى المستخدمة.

ثانياً: الدراسات التشريحية :

- ١- أوضحت الدراسات التشريحية عند فحص القطاع العرضي المأخوذ فى الوريقة الطرفية لنصل الورقة الثالثة المركبة من قمة النبات عند العرق الوسطى أن نقص التسميد المعدني عن الحد الموصى به قلل من سمك الوريقة عند العرق الوسطى و سمك النسيج الوسطى و النسيج العمادي و الإسفنجي وطول وعرض الحزمة الوعائية الوسطية وسمك نسيج الخشب و اللحاء فيها ولوحظ أن التأثير على الجهاز الوعاني كان أكبر منه على الأنسجة الأخرى..
- ٢- كان لإستخدام السلالات البكتيرية تأثير إيجابي على معظم الصفات التشريحية التى درست تحت جميع معاملات التسميد المعدني، وكانت أفضل المعاملات للسلالات الثلاثة معا : البكتريا المثبتة للنيتروجين مع البكتريا المذيبة للفوسفات و بكتيريا السيليكات. كما اوضحت معاملات التداخل أن جميع السلالات البكتيرية الثلاثة عوضت النقص المتسبب عن نقص مستوى التسميد المعدني فى جميع الصفات .
- ٣- أوضح القطاع العرضي فى السلامة الثالثة للساق الرئيسى للنبات إنخفاض قطر الساق و سمك القشرة و أبعاد الحزمة الوعائية الرئيسية وسمك نسيج الخشب واللحاء الخارجى والداخلى والنخاع مع نقص تركيز التسميد المعدني المستخدم عن التركيز الموصى به. بينما أدت جميع السلالات البكتيرية المستخدمة إلى زيادة فى هذه الصفات. كما أدت إلى تعويض النقص المتسبب عن إنخفاض تركيز السماد المعدني، وكانت أفضل السلالات للبكتريا المثبتة للنيتروجين .