

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

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ABSTRACT

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

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

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

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

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

INTRODUCTION

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

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

NO_3^- salts, accumulate more NO_3^- and NO_2^- ions within the plant tissues. From the nutritional point of view, NO_3^- and NO_2^- accumulations in the edible parts of the vegetable crops represented a serious problem for human health because their absorption into the blood. They may oxidize Fe^{++} of hemoglobin to Fe^{+++} and producing methemoglobin, which cannot transport oxygen (Swann, 1975). The toxicity of NO_3^- may be due to the formation of carcinogenic N- nitrous compounds by reaction with amino compounds. The toxic ions of nitrate and nitrite forming from nitrification are well known as an environmental pollutant (Alexander, 1977).

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

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

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

MATERIALS AND METHODS

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

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

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

Soil samples and analysis:

Twenty surface samples (0-20 cm depth) were taken at ten different locations before the experimental design, air dried, grounded, mixed and kept in plastic bags for the analyses. The mechanical and chemical analyses of the soil used were carried out in the two growing seasons as described by Jackson (1973) and Page *et al.*, (1982) and presented in Table (1).

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 | | | | |

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.

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.

Planting procedure:

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

Photosynthetic pigments:

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

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

$$\text{Chl (a)} = 9.784. E662 - 0.99. E644 = \text{mg/l}$$

$$\text{Chl (b)} = 21.426. E644 - 4.650. E662 = \text{mg/l}$$

$$\text{Carotenoides} = 4.695. E440.5 - 0.268 (\text{chl a} + \text{chl b}) = \text{mg/l}$$

Carbohydrate fractions:

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

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

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

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

Statistical analysis:

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

RESULTS AND DISCUSSION

Photosynthetic pigments:

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

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

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

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

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

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

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

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

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

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

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

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

| Treatments | | Chlorophyll a/Chlorophyll b ratio | | | Carotenoides Concentration (mg /g FWt) | | | Carotene content concentration (mg /plant) | | | Chlorophylls/ Carotenoides ratio | | |
|--------------------|------------------|-----------------------------------|-------|-------|--|-------|-------|--|------|------|----------------------------------|-------|-------|
| M-Mineral NPK | B-Bio-fertilizer | S1 | S2 | Mean | S1 | S2 | Mean | S1 | S2 | Mean | S1 | S2 | Mean |
| Control 100% | Without | 2.252 | 2.287 | 2.269 | 0.397 | 0.473 | 0.435 | 24.4 | 36.2 | 30.3 | 3.383 | 3.023 | 3.203 |
| | NFB | 2.155 | 2.054 | 2.104 | 0.416 | 0.479 | 0.447 | 30.4 | 37.8 | 34.1 | 3.526 | 3.157 | 3.341 |
| | PDB | 2.325 | 2.148 | 2.236 | 0.411 | 0.476 | 0.444 | 30.3 | 38.0 | 34.2 | 3.431 | 3.122 | 3.276 |
| | SB | 2.264 | 2.271 | 2.267 | 0.406 | 0.477 | 0.441 | 29.5 | 36.6 | 33.0 | 3.345 | 3.038 | 3.191 |
| | NFP+PDB | 2.030 | 2.060 | 2.045 | 0.450 | 0.496 | 0.473 | 33.2 | 40.2 | 36.7 | 3.333 | 2.883 | 3.108 |
| | NFB+SB | 2.138 | 2.050 | 2.094 | 0.423 | 0.487 | 0.458 | 31.0 | 38.7 | 34.8 | 3.487 | 3.131 | 3.309 |
| | PDB+SB | 2.106 | 2.099 | 2.102 | 0.431 | 0.485 | 0.455 | 31.7 | 38.5 | 35.1 | 3.401 | 3.105 | 3.255 |
| | NFB+PDB+SB | 2.014 | 2.045 | 2.029 | 0.456 | 0.499 | 0.457 | 33.9 | 41.3 | 37.6 | 3.305 | 3.082 | 3.193 |
| Mean | | 2.160 | 2.127 | 2.143 | 0.324 | 0.484 | 0.454 | 30.5 | 38.4 | 34.5 | 3.405 | 3.068 | 3.236 |
| 75% | Without | 2.505 | 2.484 | 2.494 | 0.412 | 0.479 | 0.446 | 29.8 | 38.3 | 34.0 | 3.063 | 2.902 | 2.982 |
| | NFB | 2.206 | 2.295 | 2.250 | 0.445 | 0.488 | 0.466 | 33.6 | 40.6 | 37.1 | 3.220 | 2.971 | 3.095 |
| | PDB | 2.407 | 2.370 | 2.388 | 0.433 | 0.486 | 0.459 | 32.8 | 40.7 | 36.7 | 3.210 | 2.921 | 3.065 |
| | SB | 2.558 | 2.452 | 2.505 | 0.413 | 0.485 | 0.449 | 30.6 | 38.6 | 34.5 | 3.136 | 2.911 | 3.023 |
| | NFP+PDB | 2.086 | 2.159 | 2.122 | 0.477 | 0.511 | 0.494 | 36.6 | 43.6 | 40.1 | 3.092 | 2.918 | 3.005 |
| | NFB+SB | 2.186 | 2.213 | 2.199 | 0.455 | 0.491 | 0.472 | 34.3 | 41.4 | 37.9 | 3.193 | 3.004 | 3.096 |
| | PDB+SB | 2.330 | 2.306 | 2.318 | 0.450 | 0.498 | 0.474 | 34.5 | 42.1 | 38.3 | 3.115 | 2.865 | 2.990 |
| | NFB+PDB+SB | 2.037 | 2.099 | 2.068 | 0.485 | 0.515 | 0.500 | 37.8 | 44.0 | 40.9 | 3.080 | 2.918 | 2.999 |
| Mean | | 2.289 | 2.297 | 2.293 | 0.446 | 0.491 | 0.469 | 33.8 | 41.2 | 37.4 | 2.949 | 2.926 | 3.029 |
| 50% | Without | 2.944 | 2.991 | 2.857 | 0.485 | 0.520 | 0.503 | 35.4 | 42.8 | 39.1 | 2.464 | 2.461 | 2.462 |
| | NFB | 2.622 | 2.815 | 2.916 | 0.518 | 0.563 | 0.541 | 39.4 | 46.9 | 43.2 | 2.440 | 2.378 | 2.409 |
| | PDB | 2.843 | 2.839 | 2.885 | 0.508 | 0.553 | 0.530 | 38.9 | 44.5 | 41.8 | 2.405 | 2.409 | 2.407 |
| | SB | 2.857 | 3.015 | 2.864 | 0.493 | 0.538 | 0.515 | 36.9 | 44.2 | 40.6 | 2.464 | 2.426 | 2.445 |
| | NFP+PDB | 2.559 | 2.599 | 2.837 | 0.585 | 0.661 | 0.623 | 45.6 | 52.9 | 49.2 | 2.232 | 2.080 | 2.156 |
| | NFB+SB | 2.524 | 2.720 | 2.935 | 0.533 | 0.588 | 0.560 | 41.2 | 49.4 | 45.3 | 2.446 | 2.309 | 2.377 |
| | PDB+SB | 2.578 | 2.758 | 2.847 | 0.528 | 0.580 | 0.554 | 41.2 | 49.3 | 45.2 | 2.379 | 2.326 | 2.352 |
| | NFB+PDB+SB | 2.297 | 2.557 | 2.830 | 0.593 | 0.678 | 0.635 | 46.7 | 58.4 | 52.5 | 2.209 | 2.052 | 2.130 |
| Mean | | 2.653 | 2.787 | 2.720 | 0.530 | 0.585 | 0.558 | 40.7 | 48.5 | 44.6 | 2.380 | 2.305 | 2.343 |
| Mean | Without | 2.567 | 2.587 | 2.577 | 0.431 | 0.491 | 0.538 | 29.8 | 39.1 | 34.5 | 2.970 | 2.795 | 2.883 |
| | NFB | 2.328 | 2.388 | 2.358 | 0.461 | 0.510 | 0.495 | 34.5 | 41.8 | 38.1 | 3.062 | 2.835 | 2.949 |
| | PDB | 2.525 | 2.452 | 2.488 | 0.451 | 0.505 | 0.491 | 34.0 | 41.1 | 37.6 | 3.015 | 2.817 | 2.916 |
| | SB | 2.561 | 2.579 | 2.570 | 0.437 | 0.500 | 0.530 | 32.4 | 39.8 | 36.1 | 2.992 | 2.792 | 2.892 |
| | NFP+PDB | 2.225 | 2.273 | 2.249 | 0.504 | 0.556 | 0.469 | 38.5 | 45.5 | 42.0 | 2.886 | 2.627 | 2.756 |
| | NFB+SB | 2.283 | 2.328 | 2.305 | 0.470 | 0.522 | 0.485 | 35.5 | 43.2 | 39.3 | 3.041 | 2.815 | 2.928 |
| | PDB+SB | 2.338 | 2.388 | 2.363 | 0.471 | 0.521 | 0.478 | 35.8 | 43.3 | 39.5 | 2.965 | 2.765 | 2.865 |
| | NFB+PDB+SB | 2.116 | 2.234 | 2.175 | 0.511 | 0.564 | 0.461 | 39.5 | 47.9 | 43.7 | 2.849 | 2.684 | 2.766 |
| LSD at 5% for: SxM | | 0.008 | | | 0.003 | | | 2.3 | | | 0.007 | | |
| SxB | | 0.036 | | | 0.005 | | | 2.6 | | | 0.011 | | |
| MxB | | 0.044 | | | 0.006 | | | 3.0 | | | 0.014 | | |
| SxMxB | | 0.063 | | | 0.008 | | | 3.6 | | | 0.019 | | |

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

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

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

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

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

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

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

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

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

The increase of chlorophylls and carotenoides due to PDB treatment reflects may be attributed to the effects of phosphate-dissolving bacteria on decreasing soil PH, increasing the availability of some nutrients such as Fe, Zn, Mn and Cu to plant uptake (Gaur and Ostwal, 1972; Alexander, 1982; El-Dahtory *et al.*, 1989; Hauka *et al.*, 1990), potassium content (El-Shahawy, 2003), stimulating and surviving nitrogen fixing bacteria such as *Azospirillum* (Algawady and Gaur, 1988). In addition, the phosphate-dissolving bacteria may play a desirable role as a source for certain nutrients for supplying the plants by their nutrient requirements (Saber *et al.*, 1983). Moreover, Sobh *et al.*, (2000) reported that, inoculation with phosphorus bio-fertilizers increased phosphatase activity, available P and producing growth regulating hormones. Moreover, Bender *et al.*, (1986) revealed that, available P increased the photosynthetic CO_2 fixation and assimilates translocation.

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

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

Carbohydrate fractions:

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

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

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

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

| Treatments | | Reducing sugars | | | Non-reducing sugars | | | Total sugars | | | Polysaccharides | | | Total carbohydrates | | |
|-----------------|--------------------|-----------------|-------|-------|---------------------|------|------|--------------|-------|-------|-----------------|--------|--------|---------------------|--------|--------|
| | | S1 | S2 | Mean | S1 | S2 | Mean | S1 | S2 | Mean | S1 | S2 | Mean | S1 | S2 | Mean |
| Control 100% | Without | 20.98 | 19.17 | 20.07 | 2.91 | 2.59 | 2.75 | 22.08 | 23.57 | 22.82 | 100.08 | 102.11 | 101.09 | 122.88 | 125.68 | 124.28 |
| | NFB | 23.17 | 21.48 | 22.32 | 2.65 | 3.72 | 3.18 | 24.13 | 28.89 | 25.51 | 98.88 | 99.27 | 99.08 | 123.01 | 126.16 | 124.58 |
| | PDB | 21.75 | 21.66 | 21.20 | 1.87 | 2.54 | 2.20 | 23.53 | 24.49 | 23.51 | 99.43 | 101.23 | 100.33 | 122.96 | 125.72 | 124.34 |
| | SB | 20.62 | 21.52 | 19.82 | 1.90 | 2.27 | 2.08 | 22.42 | 24.89 | 23.65 | 99.98 | 100.08 | 100.03 | 122.99 | 124.97 | 124.34 |
| | NFP+PDB | 30.62 | 26.96 | 30.29 | 3.66 | 4.19 | 3.92 | 33.62 | 31.81 | 32.62 | 98.60 | 98.27 | 98.43 | 132.22 | 130.08 | 131.15 |
| | NFB+SB | 21.21 | 24.82 | 25.41 | 3.35 | 3.42 | 3.38 | 27.97 | 29.63 | 28.80 | 98.33 | 98.55 | 98.44 | 126.30 | 128.18 | 127.24 |
| | PDB+SB | 23.04 | 25.54 | 25.41 | 3.25 | 3.65 | 3.45 | 25.79 | 29.69 | 30.30 | 98.28 | 98.58 | 98.43 | 124.00 | 128.57 | 126.28 |
| | NFB+PDB+SB | 35.44 | 32.93 | 34.18 | 4.47 | 4.96 | 4.71 | 41.40 | 40.40 | 40.90 | 95.68 | 96.07 | 96.43 | 137.08 | 136.47 | 136.77 |
| | Mean | | 23.86 | 25.16 | 24.51 | 3.01 | 3.21 | 27.24 | 28.61 | 27.92 | | 99.27 | 99.21 | 126.43 | 128.22 | 127.58 |
| 75% | Without | 18.44 | 16.16 | 17.30 | 2.07 | 3.72 | 4.71 | 18.23 | 22.16 | 20.19 | 99.54 | 101.41 | 102.46 | 120.71 | 124.60 | 122.65 |
| | NFB | 22.29 | 20.65 | 21.47 | 2.47 | 2.72 | 2.59 | 23.12 | 25.01 | 24.06 | 99.01 | 100.63 | 101.26 | 123.80 | 125.86 | 124.83 |
| | PDB | 21.96 | 19.69 | 19.82 | 1.15 | 2.36 | 1.75 | 20.84 | 24.32 | 22.58 | 99.37 | 100.80 | 101.26 | 121.71 | 124.98 | 123.34 |
| | SB | 19.19 | 19.89 | 19.04 | 1.27 | 2.01 | 1.64 | 20.16 | 22.20 | 21.18 | 99.50 | 101.22 | 101.71 | 121.71 | 125.08 | 123.39 |
| | NFP+PDB | 30.83 | 27.51 | 29.17 | 3.45 | 3.69 | 3.57 | 30.96 | 34.52 | 32.74 | 98.55 | 99.88 | 99.13 | 130.00 | 133.74 | 131.87 |
| | NFB+SB | 25.98 | 23.63 | 24.80 | 1.72 | 2.34 | 2.03 | 25.35 | 28.32 | 26.83 | 98.76 | 99.66 | 100.10 | 125.44 | 128.43 | 126.93 |
| | PDB+SB | 22.95 | 21.54 | 22.24 | 1.09 | 2.33 | 1.71 | 22.63 | 25.28 | 23.95 | 98.81 | 99.29 | 100.25 | 122.81 | 125.81 | 124.21 |
| | NFB+PDB+SB | 35.49 | 31.85 | 33.67 | 4.10 | 4.51 | 4.30 | 35.95 | 40.00 | 37.97 | 95.10 | 96.54 | 95.65 | 135.93 | 135.33 | 135.63 |
| | Mean | | 22.36 | 24.52 | 23.44 | 2.16 | 2.96 | 2.56 | 24.53 | 27.48 | 26.00 | 98.58 | 99.93 | 99.50 | 125.63 | 128.32 |
| 50% | Without | 15.33 | 14.17 | 14.75 | 1.46 | 1.46 | 1.46 | 15.63 | 18.82 | 16.22 | 102.48 | 102.44 | 100.47 | 115.17 | 118.23 | 138.63 |
| | NFB | 17.88 | 16.95 | 17.58 | 1.33 | 2.48 | 1.90 | 18.21 | 20.39 | 19.30 | 101.68 | 100.85 | 99.82 | 117.22 | 121.02 | 119.12 |
| | PDB | 17.43 | 16.83 | 17.13 | 1.12 | 2.24 | 1.68 | 17.95 | 19.69 | 19.81 | 100.87 | 101.66 | 100.08 | 117.32 | 120.47 | 118.89 |
| | SB | 16.41 | 16.58 | 15.99 | 1.62 | 1.49 | 1.65 | 18.20 | 18.90 | 18.55 | 101.55 | 101.88 | 100.36 | 117.70 | 119.12 | 118.89 |
| | NFP+PDB | 23.42 | 20.06 | 21.74 | 2.27 | 2.21 | 2.24 | 18.33 | 25.73 | 24.03 | 99.04 | 99.22 | 99.22 | 120.88 | 125.61 | 123.24 |
| | NFB+SB | 19.27 | 18.49 | 18.88 | 1.84 | 2.76 | 2.30 | 20.33 | 22.03 | 21.18 | 100.09 | 100.11 | 99.21 | 119.09 | 121.69 | 120.39 |
| | PDB+SB | 19.09 | 18.21 | 18.65 | 1.54 | 1.85 | 1.69 | 19.75 | 20.04 | 19.89 | 100.18 | 100.33 | 99.04 | 118.58 | 119.33 | 118.94 |
| | NFB+PDB+SB | 29.48 | 27.43 | 28.45 | 3.06 | 3.55 | 3.30 | 29.49 | 33.03 | 31.26 | 95.98 | 95.33 | 95.82 | 124.59 | 129.57 | 127.08 |
| | Mean | | 18.46 | 19.83 | 19.15 | 2.16 | 2.96 | 2.02 | 20.24 | 21.95 | 21.09 | 100.23 | 100.23 | 100.60 | 119.06 | 122.13 |
| Mean | Without | 16.50 | 18.25 | 17.37 | 2.15 | 2.59 | 2.36 | 18.65 | 20.85 | 19.75 | 100.70 | 101.98 | 101.34 | 119.58 | 122.83 | 121.21 |
| | NFB | 19.69 | 21.22 | 17.36 | 2.15 | 2.97 | 2.56 | 21.82 | 24.09 | 22.96 | 99.85 | 100.25 | 100.05 | 119.58 | 124.34 | 121.21 |
| | PDB | 18.73 | 20.05 | 19.39 | 1.38 | 2.38 | 1.88 | 20.11 | 22.49 | 21.30 | 99.85 | 100.25 | 100.56 | 120.66 | 123.72 | 122.19 |
| | SB | 17.99 | 18.57 | 18.28 | 1.59 | 1.92 | 1.88 | 19.59 | 20.50 | 20.04 | 100.34 | 101.06 | 100.70 | 120.80 | 123.05 | 121.92 |
| | NFP+PDB | 25.84 | 28.29 | 27.07 | 3.13 | 3.36 | 3.24 | 28.97 | 31.68 | 30.33 | 98.73 | 99.12 | 98.93 | 127.70 | 129.81 | 128.75 |
| | NFB+SB | 22.25 | 23.82 | 23.03 | 2.30 | 2.84 | 2.24 | 24.55 | 26.66 | 25.60 | 99.06 | 99.44 | 99.25 | 123.61 | 126.10 | 124.85 |
| | PDB+SB | 20.76 | 21.69 | 21.23 | 1.96 | 2.84 | 2.28 | 24.55 | 24.00 | 23.36 | 99.09 | 99.40 | 99.24 | 121.79 | 124.50 | 123.14 |
| | NFB+PDB+SB | 30.74 | 33.47 | 32.10 | 3.88 | 4.34 | 4.11 | 35.61 | 37.81 | 36.71 | 95.58 | 95.98 | 95.78 | 134.86 | 136.12 | 135.49 |
| | LSD at 5% for: SxM | | | 0.05 | | | 0.01 | | | 0.02 | | | | | | 0.08 |
| SxB | | | 0.08 | | | 0.01 | | | 0.01 | | | | | | 0.14 | |
| MxB | | | 0.11 | | | 0.01 | | | 0.02 | | | | | | 0.17 | |
| SxMxB | | | 0.14 | | | 0.01 | | | 0.03 | | | | | | 0.23 | |

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

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

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

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

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

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

Zayed (1998) proved that, phosphorus dissolving bacteria is known by its ability to dissolve the precipitation form phosphorus: $\text{Ca}_3(\text{PO}_4)_2$ depending on its ability to produce inorganic, organic acids and/or CO_2 . Bender *et al.*, (1986) revealed that, phosphorus increased photosynthetic CO_2 fixation and assimilates translocation in carrot plants. Rabinoveich *et al.*, (1999) mentioned that, increased doses of bio-fertilizers for potato raised high concentration of denitrificating microorganisms.

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

REFERENCES

- Abd El-Naem, G.F.; H.A Ismail.; A.M. Zaki, and E.A.El-Morsi, (1999). Effect of fertilization on chemical constituents nitrates, nitrites, ascorbic acid and some antinutritional factors levels in potato tubers. Egypt J. Agric. Sci., Mansoura Univ., 24(2):873-889.
- Abou-Aly, H.E. and A.D. Gomaa, (2002). Influence of combined inoculation with diazotrophs and phosphate solubilizers on growth, yield and volatile oil content of Coriander (*Corianderum sativum* L.) plants. Egypt Bull. Fac. Agric., Cairo Univ., 53: 93-114.
- Abou-Hussein, S.D.; I. El-Oksh.; T.El-Shorbagy, and A.M.Gomaa, (2002). Effect of cattle manure, bio-fertilizers and reducing mineral fertilizer on nutrient content and yield of potato plant. Egypt J. Hort. 29 (1):99-115.
- Agamy, R.A., (2004). Effect of mineral and/or bio-fertilizers on morphological and anatomical characters, chemical constituents and yield of sweet fennel (*Foeniculum vulgare* P. Mill. cv. Dulc) plants in calcareous soil. Egypt. J. Appl. Sci., 19(3): 55-75.
- Alexander, M. (1977). Introduction to Soil Microbiology. 2nd Ed., John Wiley and Sons, Inc. New York. 397 pp.
- Alexander, M. (1982). Introduction to Soil Microbiology. 2nd Ed. John Wiely and Sons Inc., New York, 467 pp.
- Amberger, A. (1954). Einflub von Kalium und Stickstoff auf Ferment and Kolenhydrathaus halt von Grunland pflanzen. Z. pflanzenernahr. Dung Bodenkunde, 66(11), 3: 211-222.
- Arisha, H. M. and A. Bardisi (1999). Effect of mineral and organic fertilizers on growth, yield and tuber quality of potato under sandy soil conditions. Egypt Zagazig J. Agric. Res., 26(2): 391-409.
- Bender, L.; B. Steibeling and K.H. Neumann, (1986). Investigations on photosynthetic and assimilates translocation on *Daucus carota* L. as influenced by a varied phosphorus supply and changes in the endogenous hormonal system following GA₃ treatments. Zeitschrift für Pflanzenernahrung und Bodenkunde, 149(5): 533-540.
- Daiziel, J. and D.K.Lawrence (1984). Biochemical and biological effects of Kaureneoxidase inhibitors, such as paclobutrazol. In R. Menhenett, D.K.Lawrence, eds. Biochemical aspects of synthetic and naturally occurring . Plant Growth Regulators. Monograph.
- Davis, L.A. and F.T. Addicott, (1972). Abscic acid : Correlation with abscission and with development in the cotton fruit. Plant Physiolo. 49:644-646. dissolving bacterium on plant growth and ³²P-uptake. Soil Biology & Biochimestry, 13(2): 105-108.
- Effect of cattle manure, bio-fertilizers and reducing mineral fertilizer on nutrient content and yield of potato plant. Egypt J. Hort. 29 (1):99-115.
- El-Dahtory, Th.; M.Abdel-Nasser; A.R.AbdAllah and M.A.El-Mohandes (1989). Studies on phosphate solublizing bacteria under different soil amendments. Egypt Minia J. Agric. Res. & Dev., 11(2): 935-950.
- El-Shahawy, A.M.B. (2003.) Effect of phosphate dissolving bacteria on yield and nutrient uptake of faba bean and wheat plants. Ph. D. Thesis, Fac. of Agric., Mansoura Univ., Egypt.

- Fales, H.M. and T.M. Jaouni, (1973). Simple device for preparing ethereal diazomethane without resorting to co-distillation. *Nal Chem.*, 45: 2302-2303.
- Frankenberger, Jr.W.T. and Arshad, M. (1995). *Phytohormones on soils Microbiol production and function*. Marcei Dekker, Inc., New York, pp.503.
- Gabr, S.M.; I.M. Ghoneim and H.M.F.Hassan, (2001). Effect of bio and nitrogen fertilization on growth, flowering , chemical contents, yield and quality of sweet pepper. *J. Adv. Agric. Res.*, 6(4): 939-955.
- Gaskin, P.; J.MacMillon and J.A.D. Zeevaart, (1973). Identification of gibberellins A₂₀ abscisic acid and phaseic acid from flavoring *Bryophyllum daigrementation* by combined gas chromatography-mass spectrometry. *Planta*, 111:347-352.
- Gaur, A.C. and K.P. Ostwal, (1972). Influence of phosphate dissolving bacilli on yield and phosphate uptake of wheat crop. *Indian J. Exptl. Biol.*, 10: 393- 398. genobiotics conditions. *Can. J. Microbiol.*, 33: 390-395.
- Gharib, M.G.A., (2001). response of two cucumber cultivars to bio-fertilization under plastic house condition. M.Sc. Thesis, Fac. Agric., Cairo Univ., Egypt.
- Glick, B.R. (1995). The enhancement of plant growth by free-living bacteria. *Can. J. Microbiol.* 41: 109-117.
- Gomez, K. A. and A. A.Gomez (1984). *Statistical Procedure for Agricultural Research*. Jhn Ziley and Sons. Inc., New York, p. 680. Group Lid., London 642 pp.
- Hartmann, A.; A. Singh and W. Klingmuller, (1983). Isolation and characterization of *Azospirillum* mutants exccrting high amount of indole acetic acid. *Can. J. Microbiol.* 29:919-923.
- Hatung, W. , (2004). Plant response to stress: Abscisic acid fluxes. Marcel Dekker Inc., New York. Pp. 540-690.
- Hauka. F. I. A.; M.M.A. El-Sawah, and Kh. H. Hamdi, (1990). Effect of phosphate-solubilizing bacteria on growth and P-uptake by barley and tomatoes plants in soil amended with rock or tricalcium-phosphate. *Egypt J. Agric. Sci., Mansoura Univ.*, 15 (3): 450-459.
- Hauka. F. I. A. (2000). Effect of using single and composite inoculation with *Azospirillum brasilense*, *Bacillus megaterium* var. phosphaticum and *Glomus macocarpus* for improving growth of *Zea mays*. *Egypt J. Agric. Sci., Mansoura Univ.*, 25(4)2327-2338.
- Helaly, M.N. (1977). Some physiological studies in relation to salt tolerance of Egyption henbane; *Hyoscyamus muticas*, L. Ph. D. Thesis, Faculty of Agric, Cairo Univ., Egypt.
- Helaly, M.N. (1984). Effects of salinity on the chloroplast ultrastructure and photosynthetic activity in horse been plants. *Egypt. J. Agric. Sci., Mansoura Univ.*, 9: 241-250.
- Hockaka, P.W. and G.N. Somero, (1973). Increased chilling tolerance by using some minerals nutrients for cucumber seedling. *Egypt. J. Hort.*, 20(2): 243-256.
- Hussein, H.F. and S.M.A.Radwan (2002). Influnce of compined application of organic and inorganic fertilizations rates with multi bio-fertilizers on potato under integrated weed managements. *Egypt J. Agric. Sci., Mansoura Univ.*, 27(5):3035- 3055.

- Ismail, H.F. (2005). Effect of some trace-elements on *Zea mays*, L plants under saline condition. Ph.D. Thesis, Fac. of Agric. Mansoura Univ., Egypt.
- Jachson, M. L. (1973). Soil chemical analysis. Prentice-Hall of India. Private New Delhi, PP. 144-197.
- Jagnow, G.; G. Hoflich and K.H. Hoffman, (1991). Inoculation of non-symbiotic rhizosphere bacteria: Possibilities of increasing and stabilizing yield. *Angew Botanik*, 65: 97-126. (c.f. Computer Research; www.ovid.com).
- Kayani, S.A.; H.H. Naqvai and I.P. Ting, (1990). Salinity effects on germination and mobilization of reserves in Jojoba seed. *Crop Sci.*, 30: 704-708.
- Marschner, H. (1995). Mineral nutrition of higher plants. 2nd Ed. Academic press, Harcourt Brace and Company. Publishers. London, San Diego,
- Maximova, B.V. and G.G. Matychen, (1965). Effect of soil salinity on the intensity of respiration and activity of terminal oxidase in the leaves of oats plants. *Sov. Plant Physiol.*, 12: 540-542.
- Mohamed, Faten, F.E., (2007). The effect of growth regulators and partial replacement of mineral fertilizers by bio-fertilizers on botanical characters of caraway (*Carum carvi* L.) and anise (*Pimpinella anisum* L.) plants. Ph.D. Thesis, Fac. Agric. Fayoum Univ., Egypt.
- Monib, M.; M. K. Zahra; S. I. Abdel-Al And A. Hegazy, (1984). Role of silicate bacteria in releasing K and Si from biotite and orthoclase. *Soil biology and conservation of the biosphere*. 2: 733-743.
- Naguib, M.I., (1964). Modified Nelson Solution. Effect of serin on the carbohydrate and nitrogen metabolism during germination cotton seeds. *Indian J. Exptl. Biol.*, 2: 149.
- Omay, S.H.; W.A. Schmidt and P. Martin, (1993). Indole acetic acid productivity phosphate dissolving bacteria for increasing P-uptake and yield of *Vicia faba*, L., cultivated in a calcareous soil. *Egypt. J. Microbiol. Special Issue*, pp. 41-46.
- Page, A.I. ; R.H. Miller and T.R. Keeney, (1982). Methods of soil analysis part 2. *Amer. Soc. Agric. Inc. Madison Wig*: 595.
- Pondey S.N. and A. Chadha, (1996). *Economic Botany*, New Dalhi, p. 57-58.
- Prisco, J.T. and J.W. O'Leary, (1972). Enhancement of intact bean leaf senescence by NaCl salinity. *Physiol. Plant*, 27: 95-100.
- Rabinovich, G.Yu; N. Gkovalev and G.M. Goryachkin, (1999). Status of soil microorganisms at simultaneous application of mineral fertilizers and the products of aerobic bio-fermentation. *Set skokhzyaistvennaya-Biologia*, 3, 82.
- Saber, M.S.M ; H.K. Abd El-Maksoud and M.A. Khalafallah, (1983). The use of phosphate dissolving bacteria for increasing P-uptake and yield of *Vicia faba*, L., cultivated in a calcareous soil. *Egypt. J. Microbiol. Special Issue*, pp. 41-46.
- Sobh, M.M.; S. Genaidy and M., Hegazy, (2000). Comparative studies on mineral and biofertilization for some main field crops in northern delta soils. *Egypt Zagazig, J. Agric. Res.*, 27(1): 171-179.
- sources of nitrogen on growth, biochemical aspects and yield of tomatoes. *Egypt 2th Conf. Agric. Botany Sci.*, 21-24 Sept., 1985.

- Subba Rao, N.S. (1993). Bio-fertilizers in agricultures and forestry. 3rd Ed. Oxford and IBH Publishing Co. Pvt. Ltd., New Delhi, Bomba, Calcutta, 219 pp.
- Swain, T. and W.F. Hillis, (1959). The quantitative analysis of phenolic constituent. J. Sc., Food Agric., 10: 63-69.
- Swann, P. F. (1975). The toxicology of nitrate, nitrite and N-nitroso compounds, J. Sci. FD.Agric., 26, 1761.
- Verma, L.N. 1990. Role of biotechnology in supplying plant nutrients in the nineties. Fertilizer-News, 35:87-97.
- Vogel, A.L. (1975). A Text Book of Practical Organic Chemistry. Publish by English Language Book Society and Longman Group Limited, 3rd Ed., pp. 197-569.
- Wettstein, D. (1957). Chlorophyll lethal under submikroskopische formmechanischer plastiden. Exp. Cell Res., 12: 427-433.
- Zayed, G. (1998). Can the encapsulation system protect the useful bacteria against their bacteriophages. Plant and Soil 197 : 1.

تأثير التسميد الحيوي والمعدني على نشاط التخليق الضوئي في البطاطس.
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أدى نقص جرعات التسميد المعدني ، عن الجرعة الموصى بها ، إلى نقص في تركيزات كلوروفيل أ ، ب و مجموعهما والسكريات المختزلة والغير مختزلة ومحتوى الكربوهيدرات الكلية خلال موسمي النمو. وعلى العكس من ذلك ، فقد زادت تركيزات الكاروتينيدات والسكريات العديدة ومحتواهما مع نقص نسبة التسميد المعدني عن التركيز الموصى به. وكان النقص في الكلوروفيلات أكبر منه في الكاروتينيدات مما أدى إلى خفض النسبة بين كلوروفيل أ وكلوروفيل ب وكذا بين الكلوروفيلات و الكاروتينيدات .

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

كما أدى التفاعل بين التسميد الحيوي و التسميد المعدني ، إلى تعويض النقص المتسبب عن خفض نسبة التسميد المعدني عن الجرعة الموصى بها ، فى تركيزات صبغات البناء الضوئي ومحتواها فى الورقة خلال موسمي النمو وكذلك إلى نقص السكريات المختزلة والسكريات الغير مختزلة ومجموعهما ومحتوى الكربوهيدرات الكلى بينما نقص الكربوهيدرات الغير زائبة فى المجموع الخضرى للبطاطس خلال موسمي النمو . وقد أدى التلقيح بالبكتيريا ، وخاصة المثبتة للنتروجين ، أعلى القيم فى هذا الشأن .

ولقد أدت المعاملة بالتسميد الحيوي ، بصرف النظر عن النسب المستخدمة من التسميد المعدني ، إلى تأثيرات اضافية فى النباتات النامية تحت اليركيز الموصى به من التسميد المعدني والملقحة بخليط السلالات البكتيرية الثلاثة معا ، وكان التلقيح بالبكتيريا المثبتة للنتروجين أفضل من التلقيح بغيرها من السلالات .

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