Original Research

**Effect of Bio-Fertilizer and Foliar Spray of Selenium of Growth, Yield and Quality of Potato Plants**

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**Abstract**

Two field experiments were conducted during two seasons of 2017 and 2018 to evaluate the effect of both bio-fertilizer (inoculated or uninoculated) and selenium (Se) spray at 0.5, and 10 ppm on vegetative growth, pigments, yield and quality of potato plants. The results indicated that, vegetative growth characters (number of leaves, plant height, plant fresh weight, plant dry weight, leaf area and leaf pigments (chlorophyll a, chlorophyll b and chlorophyll a and b) were significantly increased with Bio-fertilizers (Halex-2, and High rate of Se (10 ppm), in both seasons. Plant yield of tuber, number of tubers/plant, average tuber weight and tuber yield/Fed and (tuber content of starch and protein), generally, seemed to be increased with the Bio-fertilizer (Halex-2) and high rate of Se, in both growing seasons. The interaction between Bio-fertilizer (Halex-2) and Se reflected positive effects on the all studied quantitative and qualitative characters of potato plants.

**Keywords:** Bio-fertilizer; Selenium; Potato; yield.

**1. Introduction**

Potato (*Solanum tuberosum* L.) is grown for food, animals feed and industry as well as seed tuber production [1]. Potato tubers are a good source of energy, and contain high quality proteins, vitamin c and minerals including calcium and magnesium [2]. The development of potato plants can be divided into five different growth stages, which include sprout development, vegetative growth, tuber initiation set, tuber bulking and tuber maturation [3]. It is recognized as one of the most important vegetable crops for local consumption and exportation and is known as the fourth most important world crop after rice, wheat and maize. It is considered one of the national income resources [4].

Bio-fertilizers improve soil fertility and help plant growth by increasing the number and biological activity of desired microorganisms in root environment [5]. Its enhance crop productivity through nitrogen fixation, phosphate solubilization, plant hormone production, ammonia excretion and controlling. Various plant diseases also biofertilizers and eco-friendly which at any rate cannot replace chemical fertilizers that are indispensable for getting maximum yield of crops [6].

Ghoneim and AbdEl-Razik [5] Indicated that, inoculation potato tuber seeds with the bio-fertilizer Halex-2 was responsible for statistically increases of plant height, leaf area, foliage fresh and dry weight Compared with the uninoculated (control). Also, Shibboob [7] found that, inoculation common bean with different bacterial bio-fertilizer types (Akadin+Halex-2), generally increased all vegetative growth characters expressed as root weight, plant height, shoot fresh weight, number of leaves and leaf area.

Higher plants are thought not to require Se and to have a low tolerance to it but there are increasing indications that Se may also have beneficial biological functions in higher plants. Selenium was shown to affect several physiological and biochemical processes in planet species [8-11]. In plants, Se may serve a role in antioxidative mechanisms and was indicated to be a component of glutathione peroxidase [12]. At low concentrations, it has many beneficial effects, it can increase the antioxidant capacity under different stresses conditions, delay senescence, promote the growth of seedlings and regulate the water status of plants under drought conditions [13]. Yassen, *et al.* [4], demonstrated that, Se application promoted plant growth. Tuber yield was increased significantly by the treatment of Se at 10 ppm compared with control (untreated plants), also parameter of potato quality i.e. average weight of individual tuber starch and specific gravity were significantly compared control [14].

More recently, it was revealed that, Se applied at low concentrations enhanced growth and antioxidative capacity of both mono and dicotyledonous plants. The growth promoting response to Se demonstrated in soybean (*Glycine max* L.) [15]. Pennamen, *et al.* [10], also observed that, Se induced starch accumulation in chloroplasts of young leaves. Addition of low concentrations alleviated in oxidative stress caused by UV-irradiation in lettuce and...
ryegrass [16, 17]. Therefore, the present investigation aimed to study the response of potato (Solanum tuberosum L.) to bio-fertilizer (Halex-2) and foliar spray of Se of the vegetative growth, tuber yield and chemical composition. The study aims to evaluate the role of fertilizer on uptake of Selenium (Se) by Potato plants in association with plant quality and yield.

2. Material and Methods

2.1. Experimental Design

Two field experiments were carried out during the two growing seasons of 2017/2018 and 2017/2018 at South Tahrir, newly reclaimed sandy soils under drip irrigation system at the experimental station, El-Beihira Governorate, Egypt. The texture of the experimental soil was sandy and its physical and chemical properties are shown in Table (1): imported certified potato seed tubers of CV. Sponta were purchased from Daltex Company, El-Tawfikia, El-Beihira Governorate. The seed tubers were planted on January 26th in both seasons potato tuber seed took place in row 0.15 m width and 0.25 m inter row spacing.

The experimental layout was split-plot system in a randomized complete blocks design with three replications-bio-fertilizer (treated or untreated with Halex-2) were arranged as the main plots. Selenium rates were considered as the sub plot. Each sub-plot was two rows of 9 m long and 0.80 m width (2 rows), having an area of 14.40 m². Hales-2 as a bio-fertilizer contacted a mixture of non-symbiotic. Fixing non-bacteria of the genera Azotobacter, Azospirillium and Kelbsiella, was supplied by Bio-fertilization Unit, Plant Pathology Department, Faculty of Agriculture, Alexandria University.

Two bio-fertilizer treatments were used in this study, i.e. uninoculated (control) and inoculated with Halex-2. The bio-fertilizer was used at rate of 400 g/Feddan during planting. After two weeks, the inoculation process was again repeated by side dressing the inoculation suspension beside the plant, by fertigation system.

Potato plants were subjected to the foliar application of Na₂CO₃ as a source of Se at concentrations; 5 and 10 ppm for two times 40 day after full emergence and 50 day after planting (DAP) (initiation of tubers). During the preparation of experimental soil, 40 m³ compost, 40 kg N as ammonium sulphate (20.5% N) and 45 kg P as calcium superphosphate (15.5% P₂O₅) were dressed in the soil/Feddan. After that, 160 kg N as ammonium Nitrate (33% N), 145 kg K as potassium sulphate (48% K₂O) and 45 15 kg P₂O₅ as phosphoric acid (80% P₂O₅) were applied through 20 equal doses with irrigation water (fertigation), this procedure was started after full emergence until 70 DAP. The all other culture processes, disease and pest control programs were followed according to the recommendations of the Egyptian Ministry of Agriculture.

2.2. Vegetative Parameters

Vegetative growth characters: plant growth was estimated by recording the change in shoot fresh, number of branches, plant height and dry weight at 95 DAP. Samples were randomly taken from the inner rows for each sub-plot and immediately their fresh weight was recorded. The dry weight was estimated by drying the samples in air-forced ventilated oven at 70 °C until a constant weight [18].

2.3. Plant Pigments

Leaves sample (80 DAP) were collected to determine plant pigments according to the method of Lichtenhaler and Buschmaan [19] with some modifications. Two hundred mg of fresh leaves were homogenized with acetone 80% in a dark bottle at room temperature. The absorbance was measured in a UV/VIS spectrophotometer, and the concentrations were calculated using the following equations:

\[ \text{Chl } a \text{ (ng/ml)} = 12.25 \times \frac{A_{663.2}}{A_{646.8}} - 2.79 \times \frac{A_{646.8}}{A_{663.2}} \]
\[ \text{Chl } b \text{ (ng/ml)} = 21.50 \times \frac{A_{663.9}}{A_{663.2}} - 5.10 \times \frac{A_{663.2}}{A_{663.9}} \]
\[ \text{Total Chl} = \text{Chl } a + \text{Chl } b \]

Carotenoids (ng/ml) = \( 100A_{470} - 1.82 \times \text{Chl } a - 85.02 \times \text{Chl } b/198. \)

After that, the calculations were done as mg/g fresh w yield components and estimation of tuber quality indicators: each experimental plot was harvested individually after 110 days from planting. Number of tuber/plant, tuber yield/plant, average tuber weight, and tuber yield/Feddan were estimated in a random sample of three plants from each plot. Some of quality indicators including specific gravity, protein and starch of produced tubers were estimated. The specific gravity (SG) was estimated by the ratio of tuber density to water.
2.4. Tuber Characters

Tuber density was estimated by divide the weight of tuber on its volume, the volume was determined by the
volume of water which displaced by the tuber in a graduated cylinder. The starch and protein percent were calculated
according to the formula of Burton [20].

2.5. Selenium Quantification

The collected tubers for the treatments and control were cute for small pieces and dried at 70 °C until ash. Then,
1 g of each was digested in HNO₃ plus 5 drops of H₂O₂ for enough time to receive clear and constant solution. The
solution was employed for cooling, dilution with deionized water and filtrated to a remarkable volume [21]. The
examined metal was quantified on Inductive Coupled Plasma Optical Emission Spectroscopy (ICP-OES) instrument
(Optima 7000 Perkin Elmer, USA). The samples were injected into the cyclonic spray chamber with mass flow-
controlled laser nebulizer with gas flow at rate 0.65 L/min. The instrument was operated in a fast-sequential mode
and featured to cooled CCD detector. Background and spectral interferences could be simply corrected and
accurately using Agilent's MP Expert software. The output data were estimated for metal concentration and expressed as mg/kg dry w.

2.6. Statistical Analysis

Data were analyzed using SHS Institute Inc. SAS institute, 1988 standard divisions of the means were calculated
and LSD test (P=0.05) was used to determine significant differences between means.

3. Results and Discussion

3.1. Vegetative Growth Characters

Data presented in Table 2 and 3 indicated that, the vegetative growth characters of Potato plants, i.e number of
leaves, branches plant height, plant fresh weight, plant dry weight and leaf area were significantly affected by the
bio-fertilizer treatments, compared with the control, in both seasons. So, a positive effect of bio-fertilizer could be
explained on the ground that bio-fertilizer plays an important role in enhancing effects of the non-symbiotic N₂-
fixing bacteria on morphology and/or physiology of the root system which, consequently, promoted the vegetative
growth to go forward. In this respect, Martin, et al. [22] and Janow, et al. [23] indicated that, the Azotobacter
and Asosprillum strains produced adequate amounts of indol acetic acid and cytokinen which increased the surface area
per unit root length and enhanced root hair branching with an eventual increase in the uptake of nutrients from the soil.

Data in Table 2 show that, increasing the foliar spray of Se from 0 to 5 or 10 ppm, resulted in corresponding
and significant increases in the all studied vegetative growth characters, i.e. number of leaves, plant height, plant
fresh weight, plant dry weight and leaf area, during both seasons.

Table 2. Vegetative growth characters of Potato as affected by bio-fertilizer and Selenium levels during the seasons 2017/2018 and 2018/2019

| Characters       | No. of Leaf/plant | No. of branch/plant | Plant height (cm) | Plant fresh weight (g) | Plant dry weight (g) | Leaf area (cm²) |
|------------------|-------------------|---------------------|-------------------|------------------------|---------------------|-----------------|
| Season Treatment | S₁  | S₂  | S₁  | S₂  | S₁  | S₂  | S₁  | S₂  | S₁  | S₂  |
| Bio-fertilizer   |     |     |     |     |     |     |     |     |     |     |
| Non-BF          | 43.61 | 50.35 | 4.21 | 4.19 | 77.3 | 55.9 | 370.6 | 413.9 | 44.9 | 62.8 |
| BF              | 53.57 | 59.39 | 4.25 | 4.26 | 85.2 | 67.6 | 515.5 | 511.0 | 65.8 | 68.6 |
| LSD             | 1.04  | 1.12  | 0.11 | 0.13 | 1.83 | 2.12 | 5.09  | 4.35  | 1.2  | 3.41 |
| Se              |     |     |     |     |     |     |     |     |     |     |
| 0 ppm           | 43.83 | 49.96 | 4.22 | 4.18 | 52.7 | 51.1 | 366.6 | 369.7 | 44.1 | 51.3 |
| 5 ppm           | 48.89 | 55.73 | 4.24 | 4.23 | 69.1 | 65.1 | 499.0 | 500.4 | 60.0 | 61.8 |
| 10 ppm          | 53.05 | 58.91 | 4.33 | 4.25 | 68.3 | 68.9 | 463.6 | 517.1 | 62.1 | 84.1 |
| LSD             | 0.85  | 0.95  | 0.12 | 0.10 | 1.8  | 1.7  | 4.16  | 3.5  | 0.9  | 2.7  |

Table 3. Vegetative growth characters of Potato as affected by the interaction between bio-fertilizer and Se levels (ppm) during the seasons 2017/2018 and 2018/2019

| Characters       | No. of Leaves | No. of branches | Plant height (cm) | Plant fresh weight (g) | Plant dry weight (g) | Leaf area (cm²) |
|------------------|---------------|-----------------|-------------------|------------------------|---------------------|-----------------|
| Season Treatment | S₁  | S₂  | S₁  | S₂  | S₁  | S₂  | S₁  | S₂  | S₁  | S₂  |
| Se               |     |     |     |     |     |     |     |     |     |     |
| Non-BF          | 38.78 | 46.36 | 4.16 | 4.19 | 70.17 | 69.15 | 319.3 | 321.2 | 35.67 | 42.83 |
| BF              | 54.49 | 60.74 | 4.19 | 4.34 | 87.05 | 88.93 | 564.9 | 534.4 | 71.33 | 61.60 |
| LSD             | 0.9  | 1.05 | 0.21 | 0.188 | 2.59 | 2.60 | 7.20 | 6.15 | 1.70 | 4.83 |

The enhancing effects of Se application on plant growth may be attributed to its beneficial effects, it can
increase the antioxidant capacity under different stresses conditions, delay senescence, promote the growth of
seedlings and regulate the water status of plants under drought condition [8, 13]. Similar results were obtained by Ibrahim and Ibrahim [14]. Concerning the interaction effect between bio-fertilizer treatment and Se spray on the studied vegetative growth characters of potato plants, the obtained results in Table 3 reflect significant differences for the all vegetative growth characters during both seasons. The foliar spray of 10 ppm Se combined with potato plants inoculated by bio-fertilizer, can be considered the best choice as it attained the best results for most vegetative growth characters during both seasons. Similar results were obtained by Shiboob [7], Moussa, et al. [8] and Ibrahim and Ibrahim [14].

3.2. Leaf Pigments Characters
As for the effects of bio-fertilizer treatments on pigment trails i.e. chlorophyll a, chlorophyll b and carotenoids of Potato plants, the results presented in Table 4, clearly, indicate that, inoculation Potato plants with the bio-fertilizer Holex-2 was superior to the uninoculated plants in terms of the all pigments, traits during both seasons. Bio-fertilizer can convert nutritionally important elements from unavailable to available form through biological processes leading to improving growth, yield and quality [24]. Many researchers studied the role of bio-fertilizer as stimulating for plant growth and yield of vegetables [25, 26].

The effect of Se rates on the leaf pigmentation traits were found significant during both seasons (Table 4). The addition of Se at rate 10 ppm reflected more significant than the control. The optimal concentrated (10 kg) of Se may play an important role in enhancing leaf pigments by increasing the capacity of antioxidants and delaying the senescence [13]. Similar results were recorded by Moldovan, et al. [27], Duma, et al. [28] and Saffaryezadi, et al. [29].

Data in Table 5 show that, the interaction effect between bio-fertilizer treatments and Se rates on leaf pigmentation traits, were significant during both seasons. The treatment combination of 10 ppm Se and bio-fertilizer (Halex-2) proved the highest mean values for these traits. Similar results were obtained by Ibrahim and Ibrahim [14], Hegde, et al. [24], Manjurul, et al. [26].

Table 4. Pigments content in Potato plants treated with bio-fertilizer and Selenium during the seasons 2017/2018 and 2018/2019

| Characters       | Chlorophyll a | Chlorophyll b | Chlorophyll (a x b) | Carotenoid |
|------------------|---------------|---------------|---------------------|------------|
| Season Treatment | S1            | S2            | S1                  | S2         |
| Bio              |               |               |                     |            |
| 0                | 1.042         | 0.921         | 0.263               | 0.314      |
| BF               | 1.132         | 1.091         | 0.403               | 0.356      |
| LSD              | 0.039         | 0.39          | 0.038               | 0.038      |
| Se               |               |               |                     |            |
| 0                | 1.043         | 0.976         | 0.320               | 0.315      |
| 5 ppm            | 1.073         | 1.012         | 0.331               | 0.336      |
| 10 ppm           | 1.145         | 1.030         | 0.348               | 0.355      |
| LSD              | 0.031         | 0.031         | 0.031               | 0.031      |

Table 5. Pigment contents (ng/ml) in Potato plants as affected by the interaction between bio-fertilizer and Se levels during seasons 2017/2018 and 2018/2019

| Characters       | Chlorophyll a | Chlorophyll b | Chlorophyll (a x b) | Carotenoid |
|------------------|---------------|---------------|---------------------|------------|
| Season Treatment | S1            | S2            | S1                  | S2         |
| 0                | Non-BF        | 0.990         | 0.880               | 0.260      |
| BF               | 1.100         |               | 0.403               | 0.357      |
| 5                | Non-BF        | 1.097         | 1.073               | 0.380      |
| BF               | 1.090         | 0.960         | 0.270               | 0.330      |
| 10               | Non-BF        | 1.047         | 0.923               | 0.260      |
| BF               | 1.200         | 1.100         | 0.426               | 0.380      |
| LSD              | 0.054         | 0.054         | 0.055               | 0.054      |

3.3. Potato yield and Quality
Data presented in Table 6 showed that, yield/plant, number of tubers/plant, average tuber weight, yield/Fed, tuber content of starch, and tuber content of protein, were significantly affected by bio-fertilizer treatments with Hales-2, during both seasons. Similar results were recorded by Hegde, et al. [24] and Eldad, et al. [25]. They showed that bio-fertilizer was stimulator for plant yield and quality of some vegetable crops. Regarding the effect of Se addition, data shown in Table 6 indicate that, addition of Se at rate of 10 ppm significantly increased most Potato yield and quality as compared with the control, during both seasons. Selenium (Se) supplementation to plants enhances the production and quality of edible plant products, by increasing anti-oxidant activity of the plant [4, 14, 30].

According to the results in Table 7, some significant differences were observed due to the interaction effects between the bio-fertilizer with Halix-2 and Se rate on yield a quality of Potato plants, during both seasons. The addition of bio-fertilizer (Halex-2) coupled by 10 ppm of Se gave the highest mean values for yield and quality,
except number of tubers/plants which showed no significant interaction during both seasons. Similar results also were recorded by Manjurul, et al. [26], Ibrahim and Ibrahim [14], and Nossier, et al. [30].

The all treatments exhibited Se accumulation in the collected tubers greater than the control (without treatment). So, the obtained data showed Se levels during 2017 greater than those during 2018 for both treatments (Figure 1 and 2). In case of bio-fertilizer, the levels were 1.407, 2.066 ppm and 1.168, 1.850 ppm during 2017 and 2018, respectively. Without bio-fertilizer treatment exhibited the values; 1.528, 2.255 ppm and 1.36, 2.07 ppm during the described seasons. The levels of Se in the control samples did not exceed 0.604 ppm. From these findings, it is obtained that, biosafety procedures and further toxicological studies may be considered, before any decision concern Se treatments on potato plants.

Table 6. Potato yield and quality as affected by bio-fertilizer and Selenium levels during seasons 2017/2018 and 2018/2019

| Characters | Yield/plant | No. of tuber/plant | N.F weight | Seed yield | Starch content | Protein content |
|------------|-------------|--------------------|------------|------------|----------------|----------------|
|            | S1    | S2    | S1    | S2    | S1    | S2    | S1    | S2    | S1    | S2    | S1    | S2    | S1    | S2    |
| Bio-fertilizer | 0.729 | 0.685 | 6.549 | 6.222 | 113.9 | 109.1 | 12.82 | 12.09 | 1.08 | 1.07 | 19.49 | 18.69 |
|             | BF    | 0.918 | 0.880 | 6.704 | 6.597 | 135.4 | 137.1 | 15.85 | 15.78 | 1.08 | 1.08 | 22.81 | 22.19 |
|             | LSD   | 0.038 | 0.039 | 0.32  | 0.491 | 6.32  | 7.43  | 0.41  | 0.22  | 0.04 | 0.04 | 0.16  | 0.18  |
| Se         | 0     | 0.770 | 0.737 | 6.480 | 6.333 | 118.1 | 117.4 | 13.38 | 13.01 | 1.08 | 1.07 | 20.46 | 19.81 |
|            | 5 ppm | 0.823 | 0.783 | 6.648 | 6.438 | 124.9 | 133.6 | 14.90 | 13.95 | 1.08 | 1.08 | 21.15 | 20.40 |
|            | 10 ppm| 0.876 | 0.843 | 6.887 | 6.592 | 130.9 | 128.3 | 15.33 | 14.84 | 1.09 | 1.08 | 21.84 | 21.12 |
| LSD        | 0.031 | 0.031 | 0.26  | 0.40  | 5.16  | 6.06  | 0.33  | 0.18  | 0.03 | 0.03 | 0.13  | 0.14  |

Table 7. Potato yield and Potato quality as affected by the interaction between bio-fertilizer and Selenium levels during the seasons 2017/2018 and 2018/2019

| Characters | Yield/plant | No. of tuber/plant | N.F weight | Seed yield | Starch content | Protein content |
|------------|-------------|--------------------|------------|------------|----------------|----------------|
|            | S1    | S2    | S1    | S2    | S1    | S2    | S1    | S2    | S1    | S2    | S1    | S2    | S1    | S2    |
| Bio-fertilizer | 0.68 | 0.64 | 6.32 | 6.20 | 108.3 | 101.9 | 12.00 | 11.34 | 1.07 | 1.06 | 11.97 | 12.20 |
|             | BF    | 0.91 | 0.87 | 6.85 | 6.55 | 135.0 | 137.4 | 15.69 | 15.72 | 1.09 | 1.08 | 21.79 | 22.07 |
|             | LSD   | 0.05 | 0.06 | 0.40 | 0.32 | 6.06 | 4.94 | 0.39 | 0.32 | 0.04 | 0.04 | 0.23 | 0.25 |
| Se         | 0     | 0.66 | 0.63 | 6.63 | 6.46 | 127.9 | 130.9 | 14.75 | 14.69 | 1.08 | 1.08 | 23.94 | 21.42 |
|            | 5 ppm | 0.77 | 0.72 | 6.87 | 6.44 | 118.3 | 111.5 | 13.54 | 12.75 | 1.08 | 1.07 | 18.88 | 19.15 |
|            | 10 ppm| 0.73 | 0.69 | 6.44 | 6.32 | 114.9 | 109.8 | 12.92 | 12.18 | 1.07 | 1.07 | 19.61 | 18.72 |
| LSD        | 0.054 | 0.054 | 0.46 | 0.69 | 8.94 | 10.51 | 0.58 | 0.31 | 0.054 | 0.054 | 0.23 | 0.25 |         |

Figure 1. The level of Se (ppm) in collected Potato tubers after bio-fertilizer treatment
Figure 2. Levels of Se (ppm) in Potato tubers of treatments without bio-fertilizer

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