

## Assessment of Selenium Role in Promoting or Inhibiting Potato Plants under Water Stress

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**Abstract:** Two field experiments were conducted during two seasons of 2014 and 2015 to study the effect of foliar application of selenium (Se) as Na<sub>2</sub>SeO<sub>4</sub> at 0, 10 and 100 µM on potato plants grown under two different levels of irrigation (50 and 100% of irrigation requirements (IR)). Obtained results showed that there were dual effects of foliar applied Se on potato plants according to its concentration. The lower concentration (10 µM) revealed multiple positive effects; whereas, the high concentration (100 µM) showed adverse influences without observing any toxic features. Applied Se at 10 µM achieved significant ( $P \leq 0.05$ ) increases in the plant growth under well-watered condition; whereas, these increments were found to be mostly insignificant under water stress compared to the untreated plants. The biochemical constituents revealed that total chlorophyll was affected significantly by the treatment of Se at 10 µM under both levels of irrigation; but this response was only obvious in carotenoids under well irrigation level in the first season. Both proline and soluble sugars showed significant increases by the same treatment compared to the control plants under water deficit in both seasons. Moreover, modifying the accumulation rate of H<sub>2</sub>O<sub>2</sub> in leaf tissues and altering the activity of antioxidant enzymes including catalase (CAT), peroxidase (POD) and ascorbate peroxidase (APX) had been observed. Additionally, morphological studies of the stomatal parameters by scanning electron microscopy (SEM) were also examined. Finally, Tuber yield was increased significantly by the treatment of Se at 10 µM compared to the untreated plants under well irrigation conditions in both seasons and under drought stress in the second one. The parameters of quality including the average weight of individual tuber, starch and specific gravity were also investigated. Water use efficiency (WUE) for yield (kg.m<sup>-3</sup>) showed significant increases under water stress conditions.

**Key words:** Selenium • Potato • Water stress • Antioxidant enzymes and SEM

### INTRODUCTION

Potato (*Solanum tuberosum* L.) is considered an important part of the global food system. It is cultivated in more than 125 countries and consumed by more than a billion people around the world [1]. Potato tubers are rich in carbohydrates, proteins, vitamins, minerals, organic acids and antioxidants [2 -4]. Also, they contain a variety of secondary metabolites including phenolic compounds which act as valuable antioxidants [5] and contribute to protect against cancers [6].

In Egypt, potatoes have paid a considerable attention especially with increasing populations which have reached more than 90 million people. Besides, increasing the demand for potato day by day, it may also help in

raising the standard of living for farmers through its high market value and exporting to some European countries. One of the most problematic challenges to increase the cultivated area is water scarcity especially under arid and land with extreme climatic conditions which form more than 96% of total area in Egypt.

Drought stress is considered as one of the most important factor which limits production of potatoes. It can decrease the plant growth [7] and affect negatively on the number and size of the producing tubers [8]. Furthermore, the exposing to short period of water deficit during tuber bulking led to many defects and deformities such as dumbbell-shaped and knobby [9]. In general; there were several changes at the physiological, biochemical and molecular levels associated with drought

stress. Among these responses; decline the photosynthetic rate, stomatal conductance, chlorophyll concentration and modify the balance of water status, phytohormones, reactive oxygen species (ROS) and activities of antioxidants in plant tissues.

Selenium (Se) is a trace element which is essential for humans, animals and microorganisms [10]. It plays multiple vital roles in human body including thyroid hormone metabolism and anticancer through its antioxidant properties [11].

In the higher plants, its influence is often depends on its concentration. At low concentrations, Se 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 [12]. Moreover, it increased the starch accumulation in the chloroplasts [13] and had positive effect on the accumulation of carbohydrates in potato [14].

On the other hand, it is well known that Se at the higher concentrations could be toxic because it may be replaced with sulphur in the amino acids and consequently changing the three-dimensional structure of the proteins and corrupt the enzymatic functions [15].

Nowadays, most of the new varieties of potato are highly susceptible to drought stress than the wild ones and Andean species in the Americas [16]. Therefore, the purpose of this study was to evaluate the role of Se ( $\text{Na}_2\text{SeO}_4$ ) as foliar application at both low (10  $\mu\text{M}$ ) and high concentration (100  $\mu\text{M}$ ) for positive or negative regulation of potato growth and productivity under water deficit.

## MATERIALS AND METHODS

Two field experiments were conducted during the seasons of 2014 and 2015 in Taba farm, Sadat city, Menofia Governorate, Egypt, to investigate the effect of foliar application of sodium selenate ( $\text{Na}_2\text{SeO}_4$ ) as a source for Se at 0 (distilled water) as control, 10 and 100  $\mu\text{M}$  on potato plants grown under two different levels of irrigation (50 and 100% of water requirements). The texture of the experimental soil was loamy sand and its physical and chemical properties are shown in Table (1).

**The Experimental Layout:** Imported certified potato seed tubers of cv. Draga were purchased from Daltex Company, El-Tawfikia, Behira Governorate. The seed tubers were planted on January 28<sup>th</sup> in both seasons, the experiment was arranged in split plot design with three replicates.

Table 1: Physical and chemical properties of the experimental soil.

Physical properties					
Seasons	Clay (%)	Silt (%)	Sand (%)	Soil type	
2014	2.2	13.6	84.2	Loamy sand	
2015	3.1	14.2	82.7	Loamy sand	
Chemical properties					
Seasons	pH	N P K		CaCO <sub>3</sub> (%)	
		ppm			
2014	8.1	12.2	19.5	42.9	2.50
2015	7.95	12.9	20.0	43.2	2.50

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Drip irrigation with two levels (50 and 100 %) of water requirements were carried out in the main plots and the foliar applications of Se treatments were distributed in the sub-plots. The plot area was 15 m (length) x 3 m (width) with 70 cm between rows and 30 cm plant spacing. Three rows were left without irrigation as a border between both irrigation levels. Each plant in the rows had a single plastic irrigation dripper (4 L h<sup>-1</sup>). The efficiency of these drippers was multiple tested in many sites by collecting the drainage water in graduated cylinders. The average of drainage water per each dripper was about 3.25 L.h<sup>-1</sup> for all experiment.

**Calculations of Water Regimes:** Two levels of irrigation according to the water requirements of potato crop in the experimental site (50 and 100 %) were started applied 38 days after planting (DAP). Calculations of irrigation levels were done whereas the irrigation control was practiced via manual valves for each experimental plot. The total amount of irrigation water was calculated according to Food and Agricultural Organization (FAO) Penman- Monteith (PM) procedure, FAO 56 [17]. The potential evapotranspiration was calculated as follows:

$$ET_o = \frac{0.408\Delta(R_n - G) + \gamma \frac{900}{T + 273} u_2 (e_s - e_a)}{\Delta + \gamma(1 + 0.34u_2)} \quad (1)$$

where:

- ET<sub>o</sub> = Daily reference evapotranspiration [mm day<sup>-1</sup>].
- R<sub>n</sub> = Net radiation at the crop surface (MJ m<sup>-2</sup> day<sup>-1</sup>),
- G = Soil heat flux density (MJ m<sup>-2</sup> day<sup>-1</sup>),
- T = Mean daily air temperature at 2 m height (°C),
- U<sub>2</sub> = Wind speed at 2 m height (m s<sup>-1</sup>),
- e<sub>s</sub> = Saturation vapor pressure (kPa),
- e<sub>a</sub> = Actual vapor pressure (kPa),
- Δ = The slope of vapor pressure curve (kPa °C<sup>-1</sup>)
- γ = The psychrometric constant (kPa °C<sup>-1</sup>).

The second step was to obtain values of crop water consumptive use (ET<sub>crop</sub>) as described by Doorenbos and Pruitt [18], since the crop evapotranspiration (E<sub>crop</sub>) was calculated as follows:

$$E_{crop} = E_{To} \times K_c \dots \dots \text{mm / day} \quad (2)$$

where:

E<sub>to</sub> = The rate of evapotranspiration from an excessive surface of green cover of uniform height (8 to 15 cm), actively growing, completely shading the ground and did not suffer water shortage.

K<sub>c</sub> = Crop coefficient (between 0.6 to 1.2).

Water requirements (WR) for each treatment were calculated as following:

$$WR = E_{Tcrop} \times LR \% \dots \dots \text{mm / day} \quad (3)$$

where:

LR % = Leaching requirement percentage (22% of the water requirement based on the Leaching Fraction equation – according to equation 5).

Irrigation requirement (IR) was calculated as follows:

$$IR = WR \times R * 4200/1000 (\text{m}^3 / \text{feddan} / \text{day}) \quad (4)$$

where:

R = Reduction factor for drip irrigation that only covers a part of land and the rest dry leaves. It was recommend by Doorenbos and Pruitt [18] to use R value which ranges between 0.25 and 0.9 for drip irrigation system.

The total amount of irrigation water was measured by water flow-meter for each treatment. Table (2) shows the seasonal irrigation quantities for potato plants under both different irrigation treatments at the experimental site during the two seasons. Plants were irrigated by using drippers with 4 L.h<sup>-1</sup> capacity. The fertigation technique was used. Leaching requirements was calculated based on Allen *et al.* [17].

$$LF = EC_{iw} / EC_d \quad (5)$$

where:

LF = Leaching fraction

EC<sub>iw</sub> = Electrical conductivity of irrigation water (0.35 dS/m).

EC<sub>d</sub> = Electrical conductivity of drainage water salinity threshold [17].

**Amount of Used Water:** Total amount of the added water through the drip irrigation system was measured for each water regime treatment (Table, 2).

Water Use Efficiency (WUE): Water use efficiency was calculated for both different water regimes using the following equation of Srinivas *et al.*, [19].

$$WUE = \text{Total water consumption} (\text{m}^3 / \text{fed.}) / \text{Total yield} (\text{kg} / \text{fed.}).$$

**Foliar Application and Sampling:** Potato plants were subjected to the foliar application of Na<sub>2</sub>SeO<sub>4</sub> as a source of Se at 0 (distilled water), 10 and 100 μM two times: at 35 (after full emergence) and 55 days after planting (DAP) (initiation of tubers). The first time was done before the application of irrigation treatments by 3 days to help plants to assimilate Se in their tissues before exposing to water deficit. Tween 20 at 0.05 ml L<sup>-1</sup> was used as a wetting agent. Moreover, four samples were collected randomly from the inner rows: the first one at 60 DAP to estimate the plant growth after short term of water shortage; the second sample (75 DAP) in the middle period of water stress was taken to determine the biochemical constituents (the optimal time to determine the biochemical constituents for potato plants in several previous literatures); the third sample was taken at 90 DAP to record the plant growth after long term of water deficit and the final one (110 DAP) were done in the end of experiments after the maturity of the producing tubers to estimate the yield and some quality indicators.

**Fertilization and Cultural Management:** During the preparation of experimental soil, 30 m<sup>3</sup> compost, 30 kg N as ammonium sulfate (20.5% N) and 75 kg P as calcium superphosphate (15.5% P<sub>2</sub>O<sub>3</sub>) were dressed in the soil per fed. After that, 150 kg N as ammonium nitrate (33.5% N) and 96 kg K as potassium sulfate (48% K<sub>2</sub>O) were applied through 10 equal doses with irrigation water; this procedure was started after full emergence until 66 DAP. All the other cultural processes, disease and pest control programs were followed according to the recommendations of the Egyptian Ministry of Agriculture.

Table 2: Average amounts of applied water (m<sup>3</sup>.fed<sup>-1</sup>) in the seasons of 2014 and 2015

Date	Irrigation treatments (m <sup>3</sup> . fed <sup>-1</sup> )			
	50% of water requirements (Drought)	Average of daily requirements	100% of water requirements (Well- watered)	Average of daily requirements
Jan 28 – Feb 8 (12 days)	20.1	1.7	20.1	1.7
Feb 9-19 (11 days)	29	2.6	29	2.6
Feb 20- Mar 2 (10 days)	33.6	3.4	33.6	3.4
Mar 3-6 (4 days)	30	7.5	30	7.5
Starting date of both different irrigation treatments				
Mar 7-12 (6 days)	22.8	3.8	45	7.5
Mar13-23 (11 days)	64.4	5.9	128.8	11.7
Mar 24- April 4 (12 days)	83.4	7.0	166.7	14
April 5-15 (11 days)	96.6	8.8	193.2	17.6
April 16-26 (11 days)	181.7	18.2	363.3	36.4
April 27- May 8 (12 days)	180.1	15.6	360.2	31.4
May 9-17 (Stop watering)	0.0	0.0	0.0	0.0
Total (m <sup>3</sup> . fed <sup>-1</sup> )	741.7		1370	

### Studied Parameters

**Vegetative Growth:** Plant growth was estimated by recording the changes in shoot fresh and dry weights at 60 and 90 DAP respectively. 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 [20].

### Biochemical Constituents and Antioxidant Enzymes

**Leaf Pigments:** Leaves sample (75 DAP) were collected to determine plant pigments according to the method of Lichtenthaler and Buschmann [21] with some modifications. 0.2 g of fresh leaves was homogenized with acetone 80% in dark at room temperature. Absorbance was measured in a UV/ VIS spectrophotometer. The concentrations were calculated using the following equations:

$$\text{Chl a } (\mu\text{g/ml}) = 12.25 A_{663.2} - 2.79 A_{646.8}$$

$$\text{Chl b } (\mu\text{g/ml}) = 21.50 A_{646.8} - 5.10 A_{663.2}$$

$$\text{Total Chl} = \text{Chl a} + \text{Chl b}$$

$$\text{Carotenoids } (\mu\text{g/ml}) = (1000 A_{470} - 1.82 \text{ Chl a} - 85.02 \text{ Chl b}) / 198$$

After that the calculations were done as mg. g<sup>-1</sup> f.wt

**Osmotic Solutes:** Proline concentration was determined by the method of ninhydrin reagent as described by Bates *et al.*, [22]. Total soluble sugars were estimated by the phenol sulphoric acid method as described by Chow and Landhauser [23].

**Hydrogen Peroxide (H<sub>2</sub>O<sub>2</sub>) Concentration:** The colorimetric determination of hydrogen peroxide

concentration at 390 nm in potato leaf tissues using potassium iodide was assayed as described by Loreto and Velikova [24]. A half gram of f.wt was homogenized in 3 mL of 1% (w/v) tri-chloroacetic acid (TCA). The homogenate was centrifuged at 10,000 rpm at 4°C for 10 min. Subsequently, 0.75 mL of the supernatant was added to 0.75 mL of 10 mM K-phosphate buffer (pH 7.0) and 1.5 mL of 1M KI. A Standard curve of H<sub>2</sub>O<sub>2</sub> was done to calculate its concentration (imol g<sup>-1</sup> f.wt) in plant tissues.

**Antioxidant Enzymes:** Antioxidant enzymes were extracted from potato plants by homogenizing 0.5 g f.wt Leaf tissue in 4 ml 0.1M sodium phosphate buffer (pH 7.0) containing 1% (w:v) polyvinylpyrrolidone (PVP) and 0.1mM EDTA. The mixture was centrifuged at 15000 xg for 15 min and supernatant was used as crude enzyme extract. All the preparation steps were carried out at 0–4°C. The activities of peroxidase, (POD, EC 1.11.1.7), ascorbate peroxidase (APX, EC 1.11.1.11) and catalase (CAT, EC 1.11.1.6) were assayed according to the methods of Dias and Costa [25], Nakano and Asada [26] and Cakmak *et al.*, [27] respectively. The activities of these enzymes were expressed as Δ A (absorbance). min<sup>-1</sup>.

**Morphological Studies by Scanning Electron Microscope (SEM):** Fresh materials from the terminal leaflet of the 4<sup>th</sup> leaf from the top of potato were fixed in 3% glutaraldehyde for 24 h at 4°C [28]. The specimens were dehydrated using ascending concentration of ethanol; critical point dried and finally coated with gold. The morphological examination was achieved through a Jeol Scanning Electron Microscope (JES-T330A) equipped with image recording and processing system (Sem Afor), Central Lab, Faculty of Agriculture, Ain shams University.

### **Yield Components and Estimation of Tuber Quality Indicators:**

Each experimental plot was harvested individually after 110 days from planting, number of tubers/plant, tuber yield / plant and feddan recorded, a random sample of three plants from each plot was chosen to estimate some of quality indicators including the weight, specific gravity and starch of the produced tubers. The specific gravity (SG) was estimated by the ratio of tuber density to water. 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 % was calculated according to the formula of Burton [29]: Starch (%) = 17.546+199.07 (SG – 1.0988).

**Statistical Analysis:** Data were analyzed using SAS Institute Inc. [30]. Standard deviations of the means were calculated and LSD's test ( $P \leq 0.05$ ) was used to determine significant differences between means.

## **RESULTS AND DISCUSSION**

**Changes in Growth Parameters:** Data presented in Figure (1) show that reducing the irrigation level inhibited significantly ( $P \leq 0.05$ ) plant growth as it evidenced by decreasing the shoot fresh and dry weights compared to the unstressed plants in both seasons. This effect was more conspicuous in the second sample at 90 DAP than the first one at 60 DAP. The importance of sufficient water supply for normal plant growth had been well documented in many previous studies. Water deficit can impair the stomatal conductance and limit CO<sub>2</sub> access leading to reduce photosynthesis and assimilates which play an important role in the plant growth and development [31]. In addition, plant growth at the cellular level depends on cell turgor and cell wall extensibility [32] where, both of them at mostly decrease dramatically under drought stress. In this study, the more clear negative effect of water stress on plant growth at 90 DAP than 60 DAP could be attributed to increase the period of water deficit simultaneously with onset of tuberization which need a lot of water to synthesis carbohydrates for tubers.

As for the effect of foliar application of Se, the general tendency was that Se-treated plants at low concentration (10 µM) revealed positive effects on shoot fresh and dry weights while, contrary results were achieved at the high concentration (100 µM) in comparison to the untreated plants under both examined levels of irrigation. In this respect, the highest significant ( $P \leq 0.05$ ) increases were obtained by Se-treated plants at

10 µM under well-watered conditions whereas; the lowest significant ( $P \leq 0.05$ ) decreases were recorded by the high concentration 100 µM under short water supply compared to the other treatments.

The influence of Se on plant growth at mostly depends on its concentration, at low concentrations; it can promote plant growth through enhancing the antioxidant contents by increasing the activity of superoxide dismutase (SOD) and preventing the decline of tocopherols [33]. Also, Se is capable of inhibiting lipid peroxidation [34] by reducing the level of reactive oxygen species (ROS) in plant tissues. On the other hand, Se at high concentrations may cause pro-oxidative stress [12] which can be reflected negatively on plant growth. Furthermore, Se at high levels may inhibit photosynthesis, impair nutrient uptake and transport [35,36]. Sometimes these effects reach the level of toxicity because excessive Se could lead to incorporation of Se instead of sulphur in amino acids and consequently altering the three-dimensional structure of protein and impairment the enzymatic functions [15]. The positive effect of Se application on plant growth has been documented previously in many plant species such as tobacco [37], lettuce [33], potato [14] and spinach [38].

### **Changes in the Biochemical Constituents**

**Leaf Pigments:** As shown in Figure (2), it is obvious that decreasing the level of irrigation caused significant reduction ( $P \leq 0.05$ ) in the concentration of photosynthesis pigments (chlorophylls and carotenoids) under all different foliar treatments. These findings could be attributed to increase the level of the producing ROS and occurrence the oxidative stress leading to slow synthesis or fast breakdown for leaf pigments under drought stress [39].

Concerning the effect of Se treatments, it can be observed that the low concentration of Se at 10 µM exhibited an improving in the concentrations of leaf pigments (chlorophylls and carotenoids) under both levels of irrigation in both seasons. These increases reached to the level of significance ( $P \leq 0.05$ ) compared to the untreated plants in total chlorophylls under both irrigation levels in the two seasons, whereas the only significant ( $P \leq 0.05$ ) increases in carotenoids were obtained under the high level of irrigation in the first season. Adversely, the high concentration of Se at 100 µM achieved significant ( $P \leq 0.05$ ) decreases in the total chlorophylls under well irrigation conditions in the two seasons. Moreover, a similar trend was observed in respect to carotenoids under both irrigation levels and the lowest one in both seasons respectively.

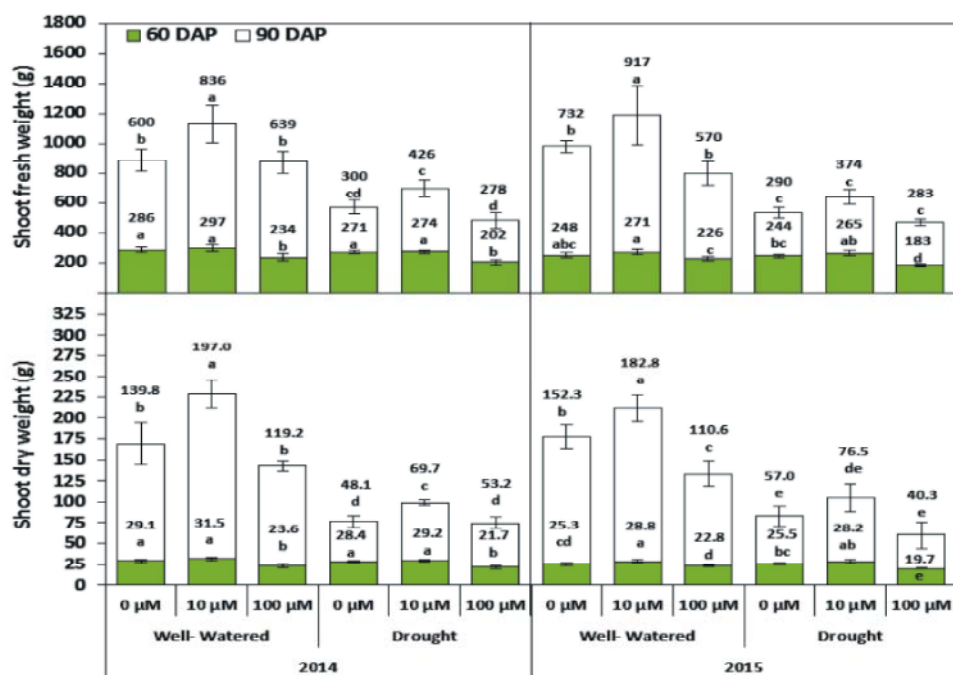


Fig. 1: Effect of foliar application of  $\text{Na}_2\text{SeO}_4$  at 0 (distilled water) as control, 10 and 100  $\mu\text{M}$  on shoot fresh and dry weights of potato plants at 60 and 90 DAP under two different levels of irrigation in the seasons of 2014 and 2015

Increasing the concentration of leaf pigments in the Se-treated plants in comparison to the untreated ones had been reported in many previous studies [38, 40-41]. The optimal concentration of Se may play an important role in enhancing leaf pigments by increasing the capacity of antioxidants and delaying the senescence [12] of leaf tissues. It can also, stimulate the biosynthesis of polyamines especially putrescine in potato leaves [42]. Polyamines were proved to implicate in many physiological events including cell division, embryogenesis [43], delaying the plant senescence and reducing the decline of leaf pigments under stress conditions [44]. In addition, Se can inhibit lipid peroxidation [34] which may reflect positively on the integrity of the cellular and sub cellular membranes of some organelles such as the chloroplast. These membranes are considered to be responsible for leaf pigment synthesis and localization. Conversely, the high concentrations of applied Se may induce pro-oxidative reactions and numerous of harmful effects in plant tissues [12] leading to increase the level of ROS and suppression the contents of leaf pigments.

**Osmolytes:** As can be seen in Figure (3), there was a considerable accumulation in the osmotic solutes (proline and soluble sugars) in the potato leaves

associated with reducing the irrigation level in comparison to the other one. These results had been early confirmed in many plant species including green bean [45], sunflower [46] and potato [47-49]. These solutes under drought stress act as osmoregulators; they prevent water loss from cells and keeping their turgidity by stabilizing the protein of the biological membranes [50]. Moreover, they regulate the osmotic potential of the cell sap leading to increase the absorption of water under the circumstances of water deficiency [51].

Regarding the influence of foliar application of Se, it is obvious that both examined concentrations of Se enhanced the amounts of proline compared to the controls under both levels of irrigation. The highest significant ( $P < 0.05$ ) increases were obtained by the lower concentration of Se under drought stress compared to the untreated plants in both seasons. In addition, except under drought in the first season, no significant differences were observed between the both investigated concentrations of Se under the same level of irrigation. Increasing the concentration of proline in the Se-treated plants could be attributed to alter the activity of some enzymes which involved in the biosynthesis of proline. For instance, Khan *et al.* [52] showed that applied Se has caused increasing in the activity of glutamyl kinase (GK) and decreasing the activity of proline oxidase (PROX).

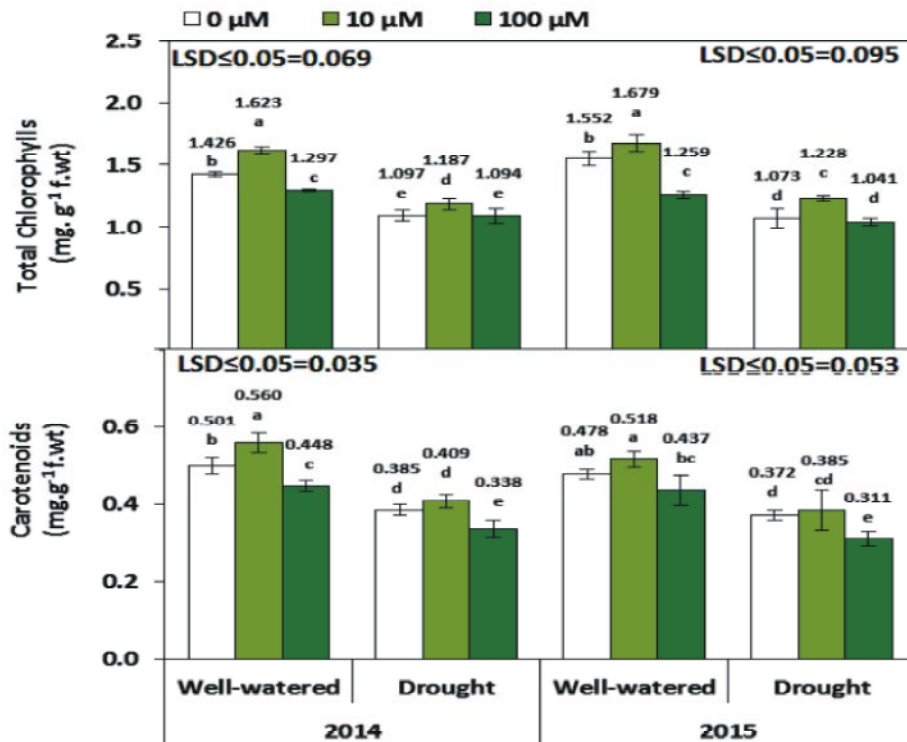


Fig. 2: Effect of foliar application of Na<sub>2</sub>SeO<sub>4</sub> at 0 (distilled water) as control, 10 and 100 μM on leaf pigments of potato plants under two different levels of irrigation in the seasons of 2014 and 2015

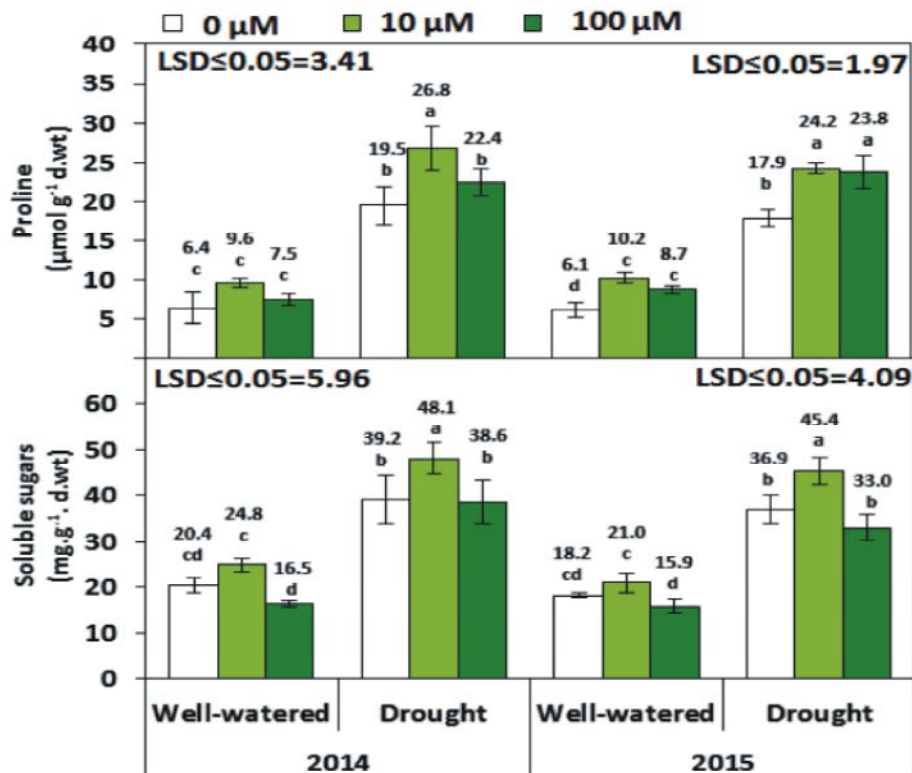


Fig. 3: Effect of foliar application of Na<sub>2</sub>SeO<sub>4</sub> at 0 (distilled water) as control, 10 and 100 μM on osmolytes concentration of potato plants at under two different levels of irrigation in the seasons of 2014 and 2015

As for the soluble sugars, it can be observed that Se treatment at low concentration improved the content of soluble sugars. These increases reached the level of significance ( $P \leq 0.05$ ) compared to the controls under drought stress in both seasons. The second concentration of Se lowered the soluble sugars but these decreases were insignificant ( $P \leq 0.05$ ) compared to the untreated plants under both levels of irrigation in the two seasons. Increasing the soluble sugars by the application of Se at low concentrations ( $5-15 \mu\text{mol L}^{-1}$ ) had been observed earlier in alfalfa plants, this response was related to increase the activity of fructose 1, 6-bisphosphatase (F1, 6-BPase), a key enzyme in carbohydrate metabolism [53].

**H<sub>2</sub>O<sub>2</sub> Concentration and Activity of Antioxidant Enzymes:** In several previous studies, applied Se led to reduce the oxidative damage in a lot of biological organisms. This influence may be attributed to its ability in stimulating the activity of some antioxidant enzymes and preventing the accumulation of hydrogen peroxide in tissues [54-55].

In the current study, the foliar treatments of Se had a dual effect on the accumulation of H<sub>2</sub>O<sub>2</sub> (Figure, 4) in leaf tissues according to its concentration. The high concentration (100  $\mu\text{M}$ ) resulted in the highest significant ( $P \leq 0.05$ ) increases in H<sub>2</sub>O<sub>2</sub> compared to the other treatments under both levels of irrigations. Meanwhile, the lowest one (10  $\mu\text{M}$ ) revealed dramatic decreases reached the level of significance ( $P \leq 0.05$ ) compared to the untreated plants under drought stress in both seasons. These results indicated that applied Se at low concentration (10  $\mu\text{M}$ ) may play an important role in scavenging of ROS from plant tissues especially H<sub>2</sub>O<sub>2</sub> molecules under water stress. Adversely, the high concentration (100  $\mu\text{M}$ ) triggered considerable amounts of H<sub>2</sub>O<sub>2</sub> and caused several oxidative damages to the leaf tissues. These findings may explain the promoting and inhibiting effects of both examined Se concentrations on plant growth (Figure, 1) under the circumstances of this study.

Concerning the activity of antioxidant enzymes (CAT, APX and POD), these enzymes are considered to be the most important tools for the decomposition of H<sub>2</sub>O<sub>2</sub> in the biological systems. The general trend was that decreasing the level of irrigation exhibited significant ( $P \leq 0.05$ ) increases in the activity of these enzymes compared to the unstressed one. Many of the previous investigations reported that usually there was a substantially progressive in the activity of many

antioxidant enzymes under water deficit [45, 56-57]. This behavior leads to keep the level of the producing ROS under control in plant tissues and improves plant growth and its performance under these conditions.

As for the effect of Se treatments, the general tendency was that applied Se at 10  $\mu\text{M}$  under water stress achieved the highest activities for all investigated enzymes. These increases were significant ( $P \leq 0.05$ ) with regard to CAT and POD whereas; APX did not show any significant changes in comparison to the untreated plants under the same level of irrigation in both seasons. On contrary, except under drought stress in the second season, a significant ( $P \leq 0.05$ ) suppression was noticed in the activity of CAT by the treatment of Se at 100  $\mu\text{M}$  under all studied conditions. A similar trend was observed in respect to APX but the only significant ( $P \leq 0.05$ ) reduction was obtained under drought stress in the second season in comparison to the untreated plants. On the other hand, POD with exception under well-watered conditions in the first season was decreased insignificantly ( $P \leq 0.05$ ) by the treatment of Se at 100  $\mu\text{M}$  compared to the untreated plants.

All the above mentioned findings indicated that Se at 10  $\mu\text{M}$  was more potent in increasing the activity of CAT and POD than APX whereas; Se at 100  $\mu\text{M}$  was more effective on inhibition the activity of CAT than both APX and POD. In another meaning CAT was more sensitive to the high foliar applied concentration of Se than APX and POD. Changing the metabolism of antioxidant systems by Se application under water deficit had been proved in many previous studies, for example Wang *et al.*, [58] reported that applied Se altered the metabolism of ascorbate-glutathione (ASC-GSH) system in *Trifolium repens*. Moreover, it can increase the activity of CAT and glutathione peroxidase (GSH-Px) in barley [59] and tomato [60] in comparison to the untreated plants.

**Morphological Studies by SEM:** Concerning the morphological changes of stomatal apparatus (Figure 5; Table 3), it can be observed that generally, water stress decreased significantly ( $P \leq 0.05$ ) the diameter, length and the percent of open stomata, whereas the number of stomata per leaf area unit tended to increase insignificantly ( $P \leq 0.05$ ) compared to the well-watered control. Under drought stress, plants resort to reduce the transpiration rate and regulate the amount of water lost. This behavior requires inducing some physiological and morphological changes including altering the stomatal pore dimensions, density and the percent of the open stomata in many plant species [61-62].



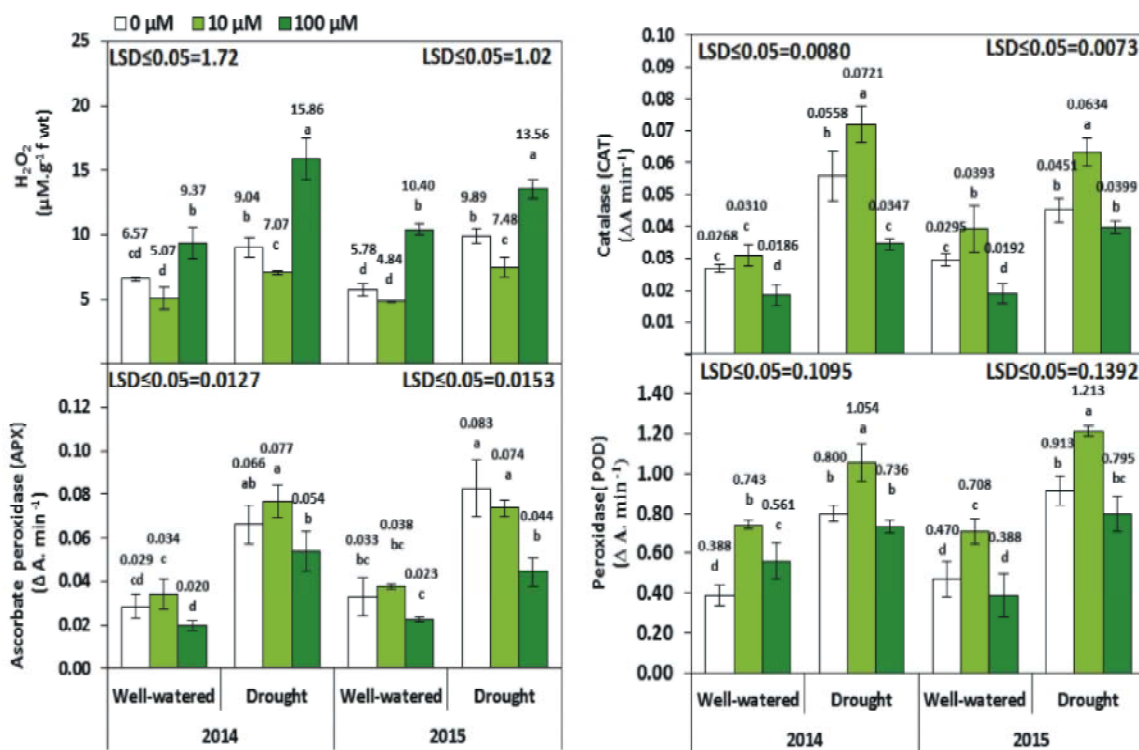


Fig. 4: Effect of foliar application of Na<sub>2</sub>SeO<sub>4</sub> at 0 (distilled water) as control, 10 and 100 µM on H<sub>2</sub>O<sub>2</sub> concentration and antioxidant enzymes of potato plants under two different levels of irrigation in the seasons of 2014 and 2015.

Table 3: Effect of foliar application of sodium selenate (Na<sub>2</sub>SeO<sub>4</sub>) at 0, 10 and 100 µM on some stomatal characteristics of the lower surface (abaxial side) of the terminal leaflet of the 4<sup>th</sup> leaf from the top of potato plants at 75 DAP under two different irrigation levels

Irrigation level	Na <sub>2</sub> SeO <sub>4</sub> concentration	Stomatal pore dimensions (µm)		Stomatal density (No./mm <sup>2</sup> )	Opened/closed stomatal (%)
		Width	Length		
Well-watered					
100% of irrigation requirements	0 µM	1.96±0.12 a	8.66±0.69 a	780±113 b	77.3 ±4.9a
	10 µM	1.30±0.33 b	8.30 ±0.62a	879±87 ab	73.7 ±5.5a
	100 µM	0.88 ±0.05c	7.39 ±1.26ab	850 ±124ab	71.8 ±3.0a
Drought					
50% of irrigation requirements	0 µM	0.68 ±0.02cd	6.85±0.19 bc	876 ±47ab	40.1 ±6.1b
	10 µM	0.58±0.04 d	6.06±0.42 c	947±76 a	43.7 ±2.9b
	100 µM	0.45±0.03 d	5.78±0.80 c	912 ±40 ab	21.7±2.9 c
LSD=0.05		0.26	1.32	154.5	7.81

The foliar treatments of Se caused substantially decreases in the stomatal pore dimensions compared to the untreated plants under both levels of irrigation. In this regard, the high concentration of Se at 100 µM achieved the lowest values under water shortage compared to the other treatments. On the other hand, remarkable increments in the number of stomata per area unit were obtained by the foliar treatments of Se. The highest significant ( $P \leq 0.05$ ) increases in this trait were resulted in the treatment of Se at 10 µM under drought stress. Moreover, open stomata ratio has not been changed

significantly ( $P \leq 0.05$ ) in both examined Se-treated plants in comparison to the untreated ones under well-watered conditions. Adversely under water deficit conditions, this trait reduced significantly ( $P \leq 0.05$ ) in Se-treated plants at 100 µM compared to the control.

The negative effect of Se on the stomatal dimensions (diameter and length) was confirmed previously in some literatures [63-64]. The reason for this response is still unclear but several previous studies found that some genes which involved in the pathways of some phytohormones (polyamines, ethylene, jasmonic acids,

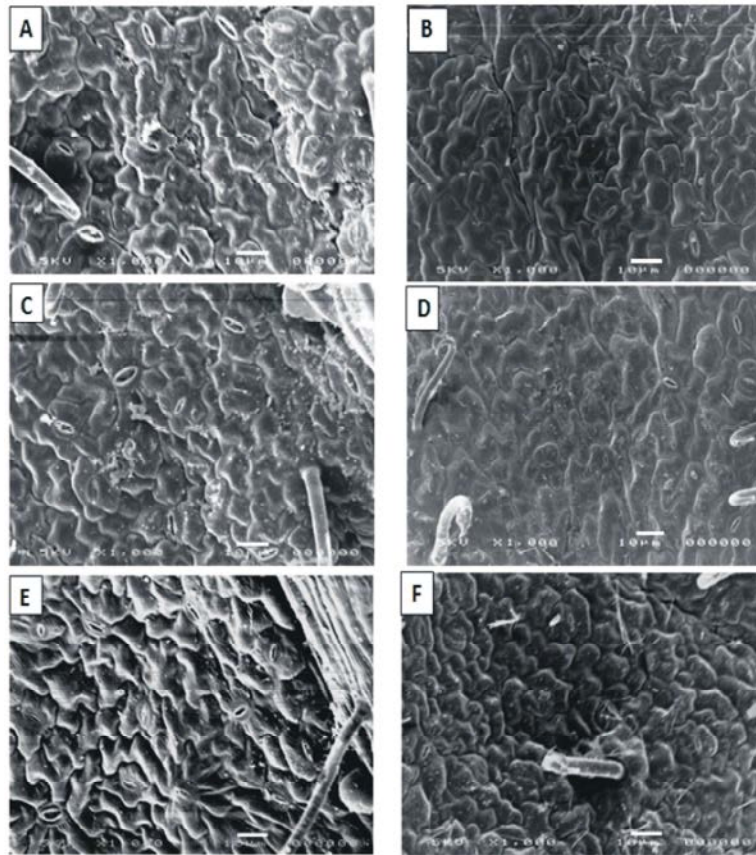


Fig. 5: SEM photographs showing the effect of foliar application of Se ( $\text{Na}_2\text{SeO}_4$ ) at 0 (distilled water) as control (A, B), 10 (C, D) and 100 (E, F)  $\mu\text{M}$  on the morphological structure of the epidermal cells of the lower surface (abaxial side) of the terminal leaflet of the 4<sup>th</sup> leaf from the top of potato plants at 75 DAP under two different levels of irrigation requirements (100% on the left and 50% on the right respectively).

salicylic acid etc.) were upregulated by Se application [65]. These hormones may be related indirectly to the differentiation and development of stomata. Moreover, Se is considered to be able to regulate the water status of plants under drought conditions [12]. It can increase the cytosolic calcium and ROS leading to enhance the production of stress hormones such as ethylene, salicylic acid and jasmonic acid [65]. This effect could explain the reduction of open stomata ratio with Se treatments especially at high concentration (100  $\mu\text{M}$ ) under drought stress compared to the control. Adversely, increasing the total number of stomata by the treatments of Se particularly under water deficit could be essentially to allow for continuous  $\text{CO}_2$  access to keep photosynthesis and survival under these circumstances; in another meaning, applied Se in this study may achieve the homeostasis between the control of water lost and maintaining the photosynthesis in the safe limit under water deficit conditions.

**Yield and Quality Indicators:** Respective the yield and its components (Figure 6), it was obvious that in general, water stress was more effective on reducing the weight of tubers than their number plant<sup>-1</sup>. This response reflected a significant ( $P \leq 0.05$ ) decline in the total yield of tubers fed<sup>-1</sup> compared to the well-watered level in both seasons. These results could be attributed to decrease the allocation of assimilates from leaves to the producing tubers under water deficiency, as well as reducing the rate of plant growth (Figure, 1) under these circumstances. As for the effects of Se applications, it can be observed that the number of tubers plant<sup>-1</sup> showed slight decreases in the first season as a result of both Se applications compared to the untreated plants. Meanwhile, these decreases reached the level of significance ( $P \leq 0.05$ ) as affected by Se at 100  $\mu\text{M}$  under well irrigation conditions compared to the control in the second season. On the other hand, Se treatment at 10  $\mu\text{M}$  revealed at mostly the highest significant ( $P \leq 0.05$ ) increases in the weight of

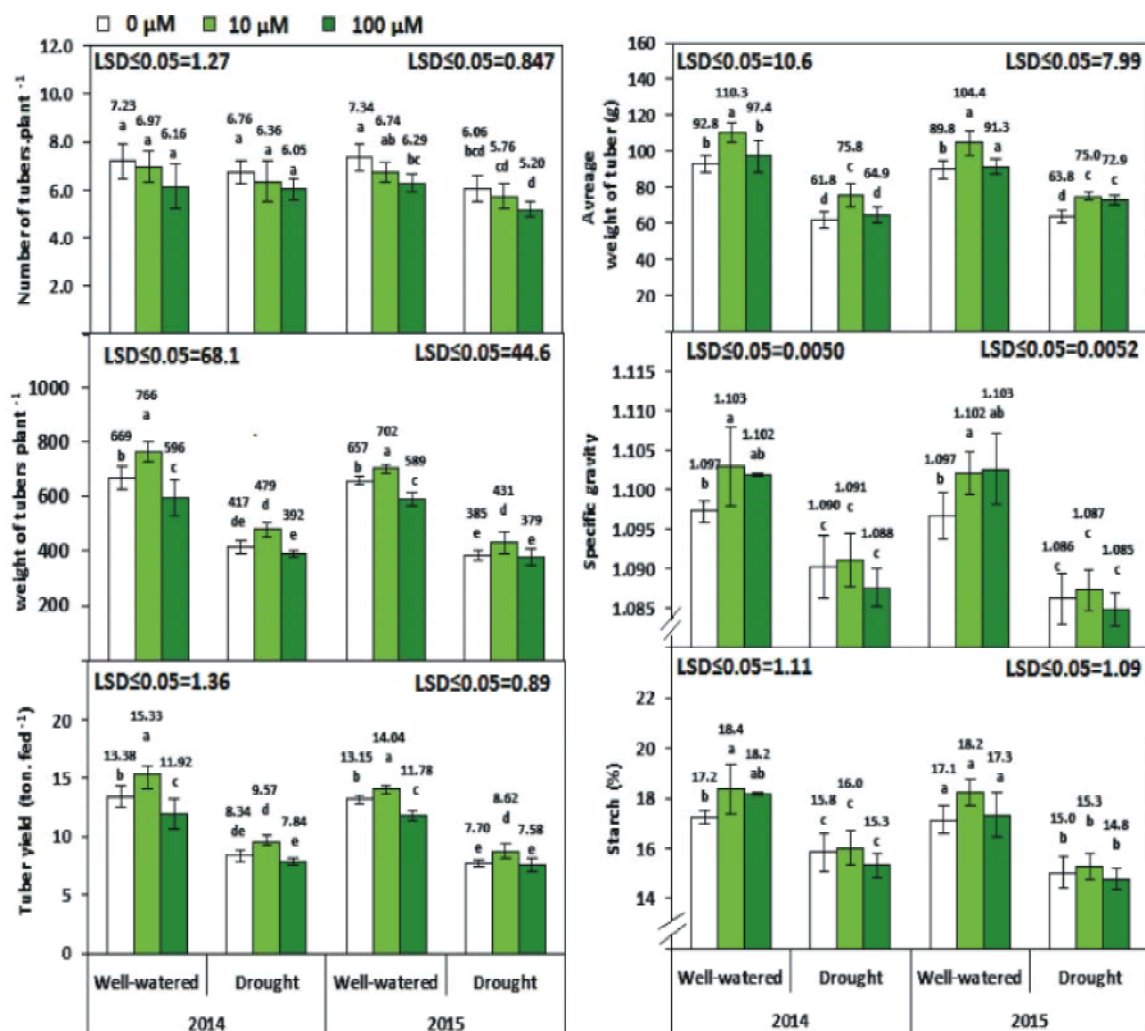


Fig. 6: Effect of foliar application of  $\text{Na}_2\text{SeO}_4$  at 0 (distilled water) as control, 10 and 100  $\mu\text{M}$  on the yield and some quality indicators of potato plants under two different levels of irrigation in the seasons of 2014 and 2015

tubers plant<sup>-1</sup> and total yield fed<sup>-1</sup>. On contrary, applied Se at 100  $\mu\text{M}$  reduced significantly ( $P \leq 0.05$ ) these traits compared to the untreated plants. These effects were more clarified under well irrigation than water shortage. These results might be explained by that plants under water stress could be more resistance to the exposing of the high concentrations of Se than the unstressed ones. In this study, this response was supported by the insignificant ( $P \leq 0.05$ ) differences in the growth rate (Figure, 1) between the shoot dry weights of high Se subjected plants and the controls under drought stress in both seasons.

Meanwhile, increasing the level of  $\text{H}_2\text{O}_2$  (Figure, 4) under drought stress + Se at 100  $\mu\text{M}$  might be essential to induce resistance of potato plants to the high concentrations of Se under the circumstances of this

study. Sometimes triggering ROS in plant tissues under stresses conditions may have beneficial effects in inducing resistance mechanisms, signaling and controlling the growth and development of plants under biotic and abiotic stresses [66-67].

Concerning the effects on the quality of the producing tubers, it can be noticed that all studied parameters including the average weight of individual tuber, specific gravity and starch% had been decreased significantly ( $P \leq 0.05$ ) by reducing the irrigation level in both seasons. These findings could be attributed to the negative effect of water stress on the partitioning of assimilates as well as inhibiting the activities of one or more of the enzymes contributed to the pathways of starch synthesis [68].

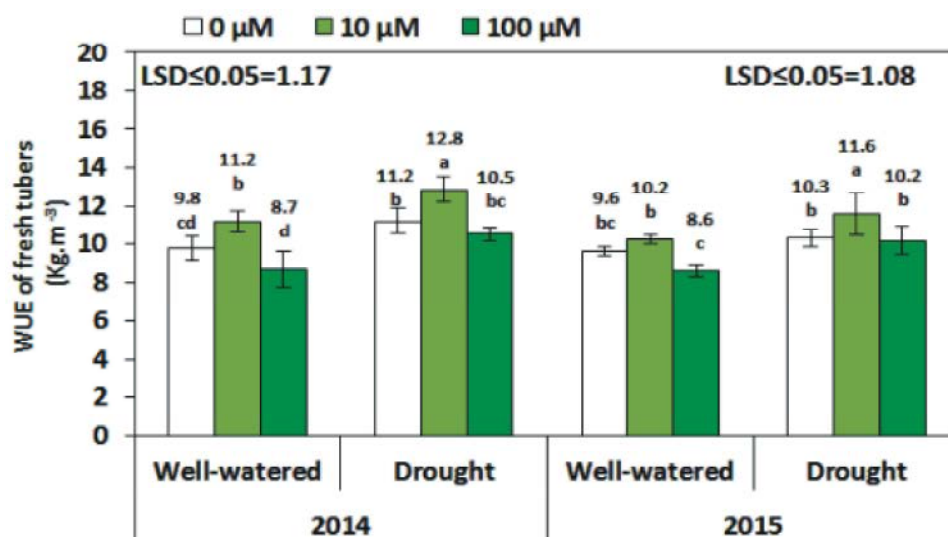


Fig. 7: Effect of foliar application of Na<sub>2</sub>SeO<sub>4</sub> at 0 (distilled water) as control, 10 and 100 μM on water use efficiency (WUE) of potato plants under two different levels of irrigation in the seasons of 2014 and 2015

The foliar application of Se at 10 μM enhanced significantly ( $P \leq 0.05$ ) the average weight of individual tuber compared to the untreated plants under both levels of irrigation in the two seasons, whereas; the only significant ( $P \leq 0.05$ ) increases of specific gravity were achieved under well-irrigation conditions by the same treatment (10 μM). On the other hand, except under the well irrigation level in the first season, no significant ( $P \leq 0.05$ ) differences were detected in the starch % between the different foliar treatments. In this study, all the above mentioned positive results of the foliar application of Se at 10 μM on plant growth, biochemical constituents and the activities of antioxidant enzymes may be related to enhance the quality indicators of the producing tubers.

**Water Use Efficiency (WUE):** Data presented in Figure (7) show that in general, WUE for tuber yield was increased significantly ( $P \leq 0.05$ ) as a result of reducing the irrigation levels under different foliar treatments. Moreover, the highest significant ( $P \leq 0.05$ ) values were obtained by the foliar application of Se at 10 μM compared to the other treatments under both levels of irrigation in the two seasons. In general, increasing plant production per unit of water used under water deficit conditions refers to increase crop drought resistance. These findings could be attributed to alter several physiological and biochemical responses which confirmed earlier in this study. Also, Se at 10 μM was confirmed to be an optimal foliar application to enhance plant performance under the circumstances of this study.

## CONCLUSION

The results of this study revealed that the foliar application of Se at 10 μM gave the best results on the growth, biochemical constituents and productivity of potato plants under both levels of irrigation. In addition, it increased the water use efficiency for yield under drought stress. Meanwhile, in general, the treatment of Se at 100 μM led to several negative effects on plants but without appearing any toxic features.

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