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Effect of potassium humate spray on some biochemical characteristics in potato leaves *Solanum tuberosum* under water stress conditions

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Abstract

The research was carried out at Al-Hanadi Research Station - Lattakia Agricultural Scientific Research Center, during spring season 2020 .To experience the effect of different potassium humate levels 500,1000,1500 ppm as a foliar spray on potato leaves variety Spunta , at 40 and 80% of field capacity humidity . The research involved four treatments with 3 replicates at each irrigation level. A Completely Randomized design was used. Plants were sprayed with potassium humate HA three times, with an interval of ten days after 35 days of planting. The results showed that water stress caused an increase in total soluble sugars, proline content and catalase activity in Spunta potato leaves, but total chlorophyll content was decreased. Potassium humate treatments improved biochemical parameters at 40% of field capacity. Potassium humate 1000 ppm treatment increased significantly total chlorophyll content 0.142 mmol/mg, and total soluble sugars 1.67mg/g in Spunta leaves ,while 1500 ppm treatment increased significantly proline content 45.65 ppm and catalase activity 0.04 mg/g at the same level 40% . Also, at 80% of field capacity, potassium humate 1000 ppm increased the total chlorophyll content 0.140 mmol/mg and proline content in potato leaves 11.63 ppm. Thus, potassium humate can be applied at a concentration 1000 and 1500 ppm to enhance the efficiency of potato plant Spunta tolerance under water shortage conditions.

Keywords: biochemical characters, potato, potassium humate, spray, water stress.

Introduction

Potato crop occupies great importance in global food security[1], it is the fourth most important food after wheat, rice and maize [2]. It is considered an important food source in developing countries, due to its low cost of production, and a rich source of carbohydrates [3]. In addition to, the importance of the crop as food, it provides employment opportunities, and a source of income [4].

In Syria, water resources deficiency, differently weather and uneven rain, in addition to ground water level shortage effectes in plant growth and causes yield loss. The response of plants to low soil moisture can vary greatly between species [5]. Compared with other crops, the potato plant is sensitive to water stress [6], or very sensitive [7], due to the shallow root system 85% of the root is localized in the upper 0.3-

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0.4 m of the soil [8]. The tuber initiation is considered to be the most sensitive stage of moisture deficiency compared to the tuber growth stage[9]. The lack of water at this stage causes a delay in the emergence and swelling of the tubers and a decrease in the yield, size and quality of the resulting tubers [3].

The lack of water directly affected chlorophyll, transpiration, vegetative and root development, metabolism and plant growing hormones, and this is followed by an oxidative stress due to the accumulation of free radicals ROS [10]: O_2, H_2O_2 and OH, subsequent from partial reduction of oxygen[11]

These radicals causes lipid peroxidation and protein degradation and DNA damage [12]. Dehydration also effects injury to chlorophyll, carotenoids and plastids. plants tend to increase tolerant stress by modulating the osmotic pressure in cells through the accumulation organic substances [13] such as proline, and many kinds of sugars [14] or by increasing enzymatic defense which decreases the free radicals accumulation damage in water stress conditions [15].

The enzymes catalase, peroxidase, superoxide dismutase, and glutathione reductase are the first line of cell defense as enzymatic antioxidants [16], they scavenge and remove free radicals in mitochondria, cytoplasm and peroxisomes [17].

Humic acid improves plant resistance under water stress conditions[18]. It increases hormonal activity, releasing different types of auxins that regulate plant growth and response to environmental conditions [19]. Humic acid is a vital catalyst for plant growth, development and productivity by increasing the activity of hormones and enzymes [20]. Many studies have shown that humic substances increases yield and improves quality of potato tubers [21].

It was found that treatment with humic 15-30 liters/ha led to an increase in the total potato yield by 2250 kg/ha and an increase in marketed tubers by 2750 kg/ha [22]. Other results showed that humic acid 120 kg/ha enhanced total chlorophyll content, vitamin C, total nitrogen[23]. And tuber growth and productivity characters of Spunta plants in drought conditions [24]. Also, humic acid application increased potato plant growth, chlorophyll content, plastids and stomata conductance and tuber weight in glasshouse and water stress conditions [18].

Understanding the physiological and biochemical structures of plant tolerance serves as the basis for crops development under water stress conditions [25]. Because of the importance of humic acid, and its effective role in growth and development of potato crop, therefore, the research aimed to increase the potato plants growth under drought by treating them with potassium humate.

Materials and Methods

The research was carried out at Al-Hanadi Research Station, Agricultural Scientific Research Center, Lattakia. Using Spunta potato variety. It is a medium-ripened varieties 100-150 days, spunta tubers are oblong and attractive. The variety is distinguished by a good size of the vegetative group, high yield in spring and good in autumn[26]. Spunta tubers were bought from the Lattakia Seed Multiplication General Organization. Tubers were planted in sandy loamy soil with good content of organic matter, a high phosphorous content and medium potassium content .Table 1.

Table 1. Physical and chemical properties of agricultural soil

Clay %	silt %	sand %	Available potassium mg kg-1 soil	Available phosphorous	mineral nitrogen	Total calcium carbonate mg g-1	Organic matter mg g-1	EC ds/m	pH	Depth cm
18	8.5	73.5	228.75	22.5	6.5	340	13.3	0.44	7.61	30-0

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Source: Soil, Plant and Fertilizer Analysis Methodologies Book - General Authority for Scientific and Agricultural Research.

The soil was prepared and plowed three times at the end of January by cultivator at a depth of 40 cm. field soil land was planned before sowing potato tubers in the last week of January. The tubers were manually planted in the field at a10 cm depth and 30*70cm distance between plants and lines. After planting, service practices included the irrigation soil and fertilizing with 369.5 kg ha^{-1} urea fertilizer and 240 kg ha^{-1} potassium sulfate fertilizer [27].

Next 35 days of planting, potato plants irrigated at two levels: 40% and 80% of field capacity humidity. the field capacity was calculated based on the soil volumetric moisture content and by using tensiometer Each plot included an irrigation level involved all separate treatments in completely randomized blocks with three replicates for each treatment. Humate potassium was sprayed

three times every week, the first spray after 35days of planting. As in the table below:

Level 40% :	Level 80 % :
• T_1-C_{40} : using water .	• T_5-C_{80}: using water.
T_2-HA_1 : using potassium humate 500mg/l	T_6- HA_1 : using potassium humate 500mg/l
T_3-HA_2 :using potassium humate 1000mg/l	T_7HA_2 : using potassium humate 1000 mg/l
T_4-HA_3: using potassium humate 1500mg/l	T_8-HA_3 using potassium humate 1500mg/l

Measurements:

It was identified 15 plants before applying humate spray taking into account the homogeneity of the selected plants in each treatment, and the following indicators were taken after the third spray:

Chlorophyll content: According to Mackiney[28] 1941, weight 100 mg of fresh leaves, soaked them in a mixture of **:**75% acetone + 25% alcohol**.** Read the absorbance of the samples on a Spectrophotometer 200 Analytikjena at wave length 645 and 663 nm The concentration of chlorophyll a,b was calculated from the equation:

Chlorophyll a = ((12.3 x absorbance at wavelength 663) - (0.86 x absorbance at wavelength 645)) /100

Chlorophyll b = ((9.3 x absorbance at wavelength 645) - (3.6 x absorbance at wavelength 663))/100

Total chlorophyll mmol/mg = chlorophyll a + chlorophyll b

Total soluble Sugars content mgg^{-1}**:** collect 100 mg of fresh leaves (fourth leaf on stem), marinade them in 3 ml of alcohol 80%. Add 20 m of distilled water, take 1 ml of sample in plots, 1 ml phenol 5% and 5 ml of sulfuric acid 96%. Read the absorption D at 490 nm wavelength in spectrophotometer[29].

Total soluble Sugars content mg g^{-1} = D x 1.657.

Leaf Proline content ppm**:** according to Bates [4], weight 100 mg of fresh tubers, put it in 10 ml salphosalicylic acid 3%, take 2 ml in a test tube with 2 ml acetic acid and 2 ml nenhydrin, add 5ml toluene. read absorption at 520 nm wavelength on spectrophotometer.

Catalase activity mg/g : The enzyme activity was estimated by Belchkov method[30], by calculate the enzymatic activity as the number of milligrams of H_2O_2 dissociated in the incubation time calculated for 1 g of the studied plant material according to the following equation: $P =((A-B)\times 0.17)) / (T\times H)$

P: Catalase activity, A: volume of potassium permanganate 0.01 N consumed to calibrate the control experiment, B: volume of potassium permanganate 0.01 regular used to calibrate the sample experiment, 0.17: milligrams of H_2O_2 equivalent to 1 mL of KMnO4: N 0.01, H: weight of the plant sample taken for enzyme study, T: incubation time.

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Statistical analysis:

Trial was designed as a factorial experiment in a completely randomized design in split plots. The resulting data were analyzed using Costat and treatments differences were evaluated using Duncan s test[31] at least significant differences LSD 0.05.

Results

Total chlorophyll (mmol/mg): Results showed a significant decrease in total chlorophyll content in potatoes leaves stressed and untreated at level 40% of the field capacity, compared to the level 80%. Treatment with potassium humate 500, 1000 and 1500 ppm increased significantly total chlorophyll in potato leaves 13.01and 24.64% compared to the control at level 40% of field capacity. Also, at the level 80%, total chlorophyll increased significantly in potato leaves treated with potassium humate 1000 and 1500ppm 21.42 and 19.11%, compared to the control. Potassium humate 1000 ppm increased total chlorophyll significantly in stressed and irrigated potato leaves Fig. 1.

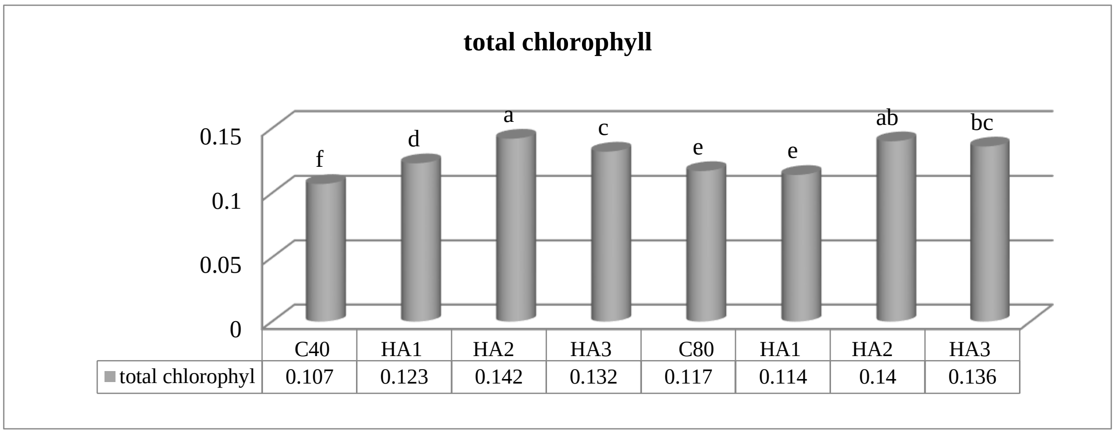

Figure 1. Total chlorophyll content in spunta leaves treated with potassium humate at 40 and 80% of field capacity .lsd0.05=0.005. For each parameter, treatments followed by the same letter are not significantly different at p < _ 0.05.

Sugars content: Soil moisture decreasing into 40% of field capacity level led to a significant soluble sugars accumulation increasing in potato leaves stressed and untreated with potassium humate 1.28 mg/g compared to the level 80% 0.15 mg/g. Fig. 2. At level 40%, soluble sugars accumulation increased significantly in potato leaves treated with potassium humate 1000 ppm 1.67 mg/g, while potassium humate 500 and 1500 ppm did not affect in soluble sugars accumulation, compared to the control 1.28 mg/g. As for the level 80% , potassium humate treatment 1500ppm led to a significant increase in soluble sugars in irrigated potato leaves 0.545mg/g.

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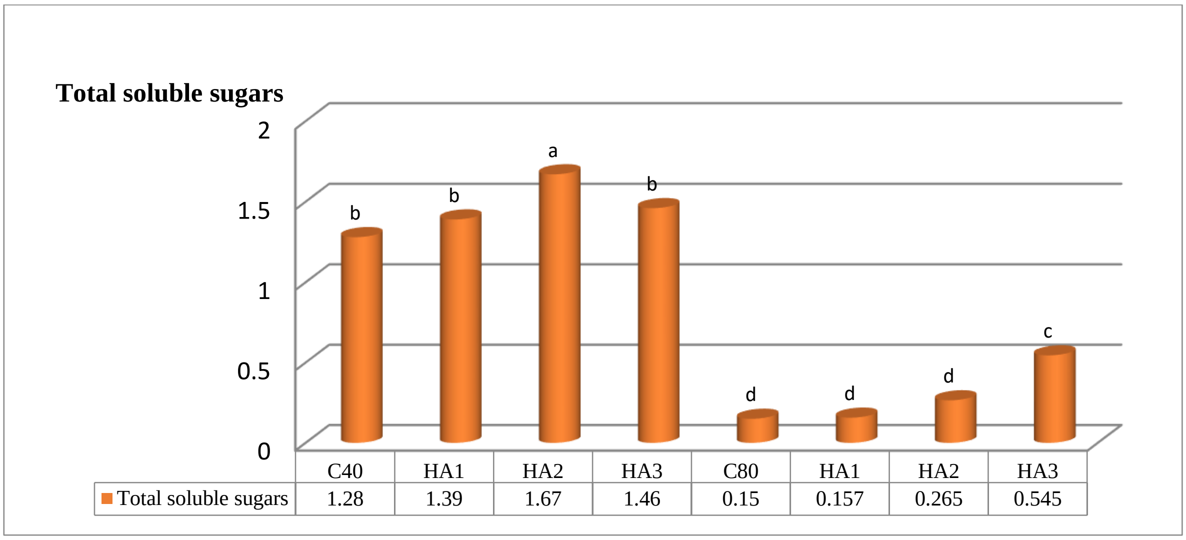

Figure 2. Total soluble sugars content in potato leaves treated with potassium humate at 40 and 80% of field capacity, LSD 0.05 =0.2.

Proline content: The results showed a significant increase in proline content in potato leaves stressed and untreated with potassium humate 55.33% ,compared to its content at the level of 80% . At the level 40% of field capacity, potassium humate treatment significantly increased proline associated with an increase in applied humate concentration 31.65, 41.83 and 45.65ppm, compared with control 17.26 ppm. Also, potassium humate treatment 1000 and 1500ppm increased proline content significantly of irrigated potato leaves 11.63 and 8.94ppm compared with the irrigated control 7.71ppm. Fig. 3.

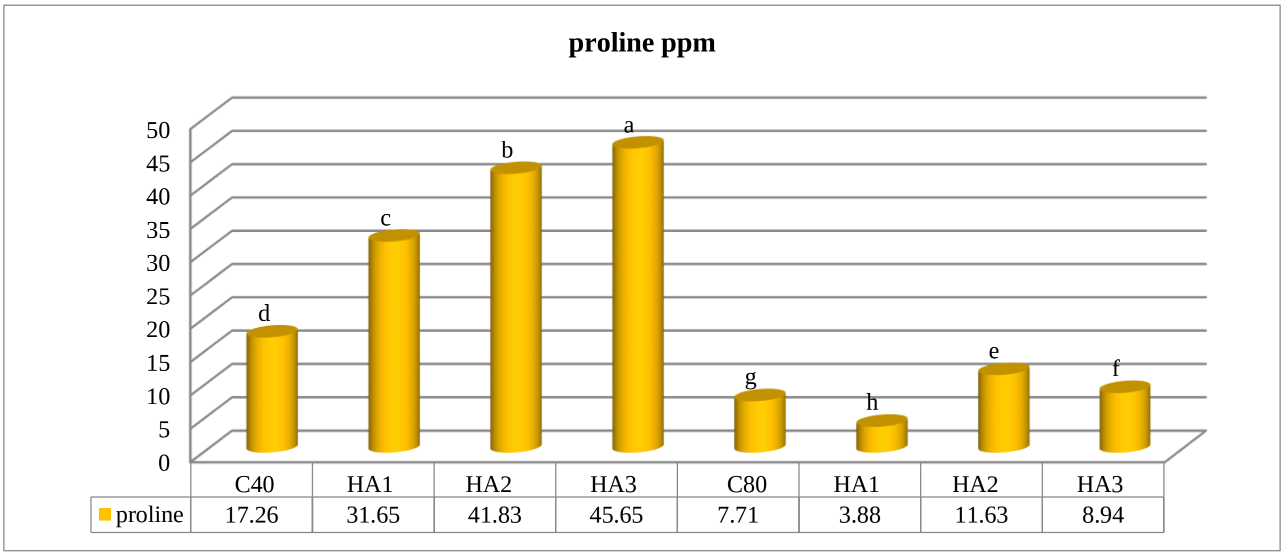

Figure 3. proline content in potato leaves treated with potassium humate at 40 and 80% of field capacity LSD 0.05=0.92.

Catalase activity: The results shown in Fig. 4, indicates a significant increase in catalase activity 0.037mg/g in stressed potato leaves at level 40% of the field capacity, compared to their content at

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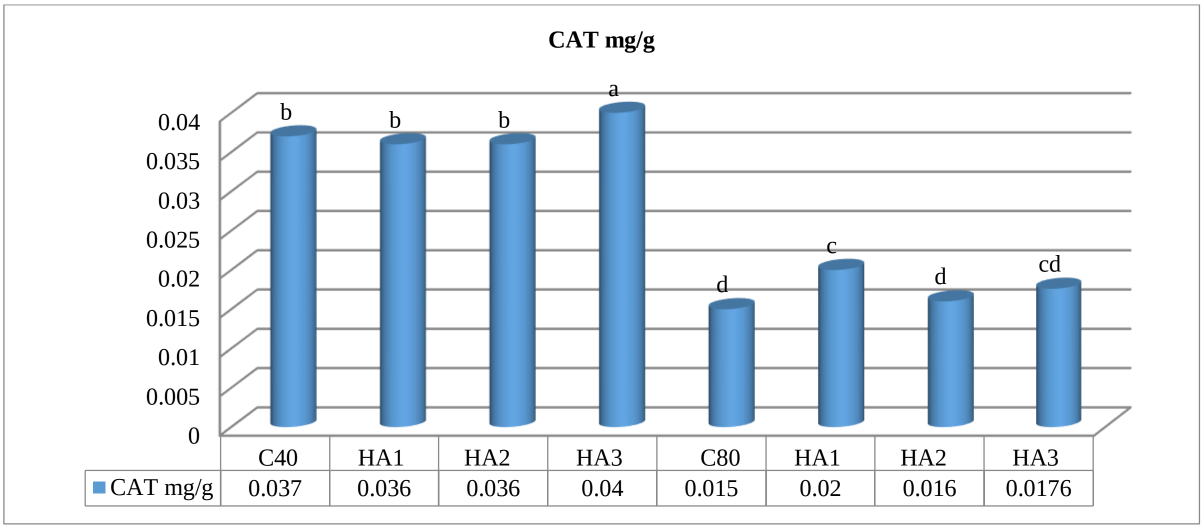

Figure 4. Catalase activity in potato leaves treated with potassium humate at 40 and 80% of field capacity . LSD0.05=0.002.

the level 80% 0.015mg/g, there was a significant increase in catalase activity at the irrigation level 40%, in potassium humate treatment 1500,while there wasn't actual effect of 500 and 1000 ppm concentration on catalase activity at the level 40% . Also, at 80% level, potassium humate 500 ppm increased catalase activity significantly in potato leaves 0.02mg/g compared to the control 0.015mg/g, and the other concentrations 0.016 and 0.017mg/g.

Discussion

In this study, Chlorophyll was decreased in leaves of Spunta potato stressed , and it may be due to the effect of water stress in plants, as it reduces chlorophyll-a, chlorophyll-b and carotenoids, proteolysis and photochemical reactions prevalent in most plants [32].In stress plants, cells loses the signals to stimulate pore closure [33], inhibit rubisco enzyme activity and disrupt biochemical and physiological processes [34].

The decrease in stomata conductivity under the influence of dehydration also leads to a change in electron transport as a result of the inhibitory damage to photosynthetic organisms, and energy dissipation in chloroplasts[35,36]. This leads to a decrease in the chlorophyll content and free radicals accumulation, that oxidize lipids and impair cells vital functions [37]. These results are consistent with Kiani [38] who showed a decrease in total chlorophyll content in the leaves of *sunflower* and *Helianthus annuus* L. under water stress conditions [39]. Other results showed that water stress dose not effect on chlorophyll content in potato leaves 40. The increase in proline and sugars in stressed and not treated leaves can be explained by the fact that the proline accumulation is an adaptive mechanism that contributes to increased plant tolerance to drought stress, as it adjusts osmotic to maintain filling pressure and preventing damage to cell parts [41].

The accumulation of Sugars increases cell membrane stability and maintenance of filling pressure through its role in modulating the osmotic pressure in the cells [14], increasing stress tolerance. These results are confirmed by studies found that water stress increases proline and soluble osmolites [41]. Increased soluble sugars in plants in response to water stress have been documented by researchers [35].

These results agree with Teixeira study[42], which indicated that proline increased in different parts of potato plants in response to water stress conditions, and was higher in roots and tubers than in leaves. other results showed an increase in the accumulation of sugars in the leaves of some plant varieties when exposed to water stress[35].

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The increase in catalase activity under stress conditions is due to the response of plants when soil moisture decreases, to reduce free radical damage through an increase in enzymatic antioxidants such as peroxidase and catalase [15]. This results is confirmed by other finding that increased activity of POD, SOD and CAT in potato plants as a defense mechanism is to mitigate the oxidative stress damage caused by free radicals accumulation [43]. Our study results are consistent with other findings that indicated an increase in the activity of CAT, proline, and soluble sugars in potato leaves at a moisture level as low as to 40% of the field capacity compared to higher irrigation levels [38] .Also, the results of Batool[44] found increased in enzymatic antioxidants activity CAT, PPO, SOD, sugars, proline, and proteins under water stress. And El-Yazied [45] found that the content of total chlorophyll, proline and soluble sugars content increased under drought conditions.

The increase in chlorophyll in potato leaves treated with potassium humate, especially at concentration 1000 ppm, maybe due to its effect in increasing photosynthesis by increasing the content of total chlorophyll and carotenoids in treated plants compared to control plants and stimulating the activity of enzymes that participate in the photosynthesis process, the uptake of Manganese and iron, which are necessary for metabolism of chlorophyll [46].

These results are consistent with studies that showed a high content of chlorophyll and mineral elements in potato leaves, as a result of treatment with potassium humate under water stress conditions [23]. The increase in sugars and proline Figs. 2,3**.** may be due to the positive effect of potassium humat on cell division, photosynthesis, the formation of proteins synthesis and the increased enzymes activity [47].

In addition to, potassium humate is a rich sources of mineral elements, especially potassium, which increases plant stress tolerance [48]. In addition to its important role in nurturing photosynthesis efficiency, regulating stomata conductance, decreasing transpiration rate and increasing intracellular carbon dioxide concentration between cells in such conditions [18], and increasing the chlorophyll content Fig. 1, which leads to an increase in metabolic products.

These results were confirmed by Abdelrasheed [49], who showed an increase in relative water content chlorophyll content, carotenoids, stomata conductance, proline, and soluble sugars under drought conditions after being treated with potassium humate. Similar results have been reported by Alharbi [50]. Many studies confirmed an increase in the relative water content, total soluble sugar, total acidic acid, proline, and mineral elements N, K, P and Ca**,** in faba bean plants treated with humate under water stress conditions [51]. Increased catalase in potato leaves treated with potassium humate may be due to the role of potassium humate in increasing the osmotic pressure-regulating antioxidants enzymes and protect cells from oxidative stress and improving electron transport, which enhance stress tolerance [52].

Conclusion

Cultivation of potato plants at different levels of humidity led to variation in biochemical properties of leaves, and that potassium humate spray on potato plants increased potato's tolerance to water deficit. Potassium humate positively affected in increasing potato leaves content of chlorophyll, soluble sugars and proline, and increasing the activity of catalase under stress conditions, especially at concentrations1000 and 1500 ppm.

Authors' Declaration

- Conflicts of Interest: None.
- We hereby confirm that all the Figures and Tables in the manuscript are ours. Furthermore, any Figures and images, that are not ours, have been included with the necessary permission for re-publication, which is attached to the manuscript.

- Ethical Clearance: The project was approved by the local ethical committee in University of Tishreen.

Authors' Contribution Statement

M. D. and S. S. conceived of the presented idea. H. H., developed the theory and performed the computations. M. D. and H. H., verified the analytical methods. S. S. and M. D. encouraged and supervised the findings of this work. All authors discussed the results and contributed to the final manuscript

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تأثير الرش بهيومات البوتاسيوم في بعض الصفات البيوكيميائية لأوراق البطاطا *Solanum tuberosum* المعرضة للإجهاد المائي

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الخلاصة

نفذت الدراسة في محطة بحوث الهنادي التابعة لمركز البحوث العلمية الزراعية في اللاذقية خلال العروة الربيعية لموسم 2020 وذلك لدراسة تأثير الرش الورقي بتراكيز متباينة من هيومات البوتاسيوم ppm 1500,1000,500 على نباتات البطاطا صنف سبونتا عند مستويي ري 40 و 80 % من السعة الحقلية . استخدم في تصميم التجربة نظام العشوائية الكاملة ضمن القطع المنشقة. شملت التجربة 4 معاملات و3 مكررات لكل معاملة عند كل مستوى رطوبة. تم رش النباتات بالهيوماتHA بعد 35 يوم من الزراعة لثلاثة مرات، بفاصل عشرة أيام بين الرشة والأخرى.

أظهرت النتائج زيادة محتوى السكريات الذائبة الكلية ومحتوى البرولين وفعالية الكاتالاز في أوراق البطاطا المجهدة في حين انخفض محتواها من الكلوروفيل .كما حسنت المعاملة بهيومات البوتاسيوم الصفات المدروسة عند المستوى 40 % ، وأعطت معاملة الهيومات بتركيز1000 ppm أعلى محتوى من الكلوروفيل الكلي 0.142 مليمول / مغ ، والسكريات الذائبة الكلية1.67ملغ/غ . بينما أعطت معاملة الهيومات بتركيز 1500 ppm أعلى محتوى للبرولين 45.65 ppm و زادت فعالية الكاتالاز 0.04مغ/غ عند المستوى 40 %. و عند المستوى 80 % تميزت معاملة الهيومات ppm1000 بأعلى محتوى للكلوروفيل الكلي في أوراق البطاطا 0.140 مليمول /مغ وأعلى محتوى من البرولين ppm11.63 . وبالتالي يمكن تطبيق هيومات البوتاسيوم بالتركيز 1000و 1500 ppm لرفع كفاءة تحمل نبات البطاطا صنف سبونتا تحت ظروف نقص الماء.

الكلمات المفتاحية: الصفات البيوكيميائية، البطاطا، هيومات البوتاسيوم ، الرش الورقي، الإجهاد المائي.