Faculty of Women for, Arts, Science, and Education

Scientific Publishing Unit

Journal of Scientific Research in Science

Biological Sciences

Volume 41, Issue 2, 2024

ISSN 2356-8372 (Online) \ ISSN 2356-8364 (print)

Journal of Scientific Research in Science 2024, 41, (2),18:46

Contents lists available at EKB

Journal of Scientific Research in Science

Journal homepage: https://jsrs.journals.ekb.eg/

Improvement of inulin production in Jerusalem artichoke (*Helianthus tuberosus* L.) through foliar application of certain sugars

Dina E. Sakr[1, *], Mohamed Abdelsattar[2], Tahani A. Hathout[1], Samia M. El khallal[1], Sameh E. Hassanein[3] and Zinab A. Abdelgawad[1]

[1]Botany Department, Faculty of Women for Arts, Science and Education, Ain Shams University, Cairo, Egypt.
[2]Agricultural Genetic Engineering Research Institute (AGERI), Agricultural Research Center (ARC), Giza, Egypt.
[3]School of Biotechnology, Nile University, Giza, Egypt.

Abstract

A field experiment was conducted at the experimental farm of the Agricultural Genetic Engineering Research Institute (AGERI), Agriculture Research Centre (ARC) to investigate the response of Jerusalem artichoke plant to foliar application of Raffinose, Dextrin, and Sucrose at 10, 25, 50, and 100 mM in addition to control (tap water only) using Randomized complete block design with 3 replicates. Tested parameters were: plant fresh weight, dry weight, plant height, number of main branches/plants, number of lateral branches/plants, leaf area, photosynthetic pigment, total carbohydrate, inulin content, Fructan exohydrolase (FEH) activity, SDS-PAGE analysis of protein, soluble sugar content by HPLC, number of tubers produced, the yield per plant, and the overall yield per feddan (tons). Results indicated that spraying plants with Raffinose and Dextrin at 50 mM level enhanced all growth parameters, total carbohydrates, and inulin content. In addition, the dose of Raffinose 50 mM exhibits promising inhibition activity of FEH enzyme, increasing the production of inulin and tuber yield, followed by the same dose from Dextrin and Sucrose treatments. Based on this supposition, the results of this study suggested that the exogenous application of particular sugars, Raffinose and Dextrin, as an eco-friendly material, can improve the plant's overall growth, development, and yield of Jerusalem artichoke plant.

Keywords:

Jerusalem artichoke, Inulin, Fructan exohydrolase, Raffinose, Dextrin, Sucrose, Tubers.

***Corresponding author**: **Dina E. Sakr1**,Botany Department, Faculty of Women for Arts, Science and Education, Ain Shams University, Cairo, Egypt.
Email: dinaehab89@yahoo.com
(Received 26 Jan 2024, revised 10 Mar 2024, accepted 12 Mar 2024)
https://doi.org/ 10.21608/JSRS.2024.265530.1124

1. Introduction

Jerusalem artichoke, scientifically known as *Helianthus tuberosus* L., is a perennial plant classified under the family Compositae, which includes sunflower plants. The plant is grown specifically for its tubers, which are used as a vegetable. Additionally, it is cultivated for animal feed or silage purposes, serving as a fodder crop. Furthermore, it is grown to produce a large amount of biomass, making it suitable for use as a bioenergy crop [1]. It is a native plant to North America. It has been recognized recently as a promising biomass for bioeconomy development, with several great ecological restoration characteristics, including large biomass, fast growth, inexpensive management, minimal water and fertilizer needs, and strong adaptability, which make its production supportive of food security [2]. The Jerusalem artichoke's first successful cultivation took place in Egypt, where it was introduced as a new commercial crop through the Desert Research Station in Sheikh Zuweid as part of the country's initiative to cultivate novel, non-traditional crops on recently reclaimed land [3]. Additionally, this crop species holds economic significance due to its tubers, which contain a high amount of inulin, serving as a reserve carbohydrate that makes up 70-90% of their dry mass [4].

The inulin in the Jerusalem artichoke plant comprises fructofuranosyl units linked together in a linear β (2–>1) pattern. These units are connected to a glucose residue through a sucrose-type linkage. Inulin is a soluble dietary fiber and a functional food component. Inulin possesses numerous health-enhancing characteristics due to its resistance to digestion in the human stomach and small intestine, owing to fructose-linked molecules. However, bacteria in the large intestine can ferment them, producing short-chain fatty acids (SCFAs) [5]. As a result, it effectively controls blood sugar and lowers the risk of obesity [6]. Inulin, a prevalent prebiotic, reduces the risk of cancer by augmenting the probiotic activity of Bifidobacterium and lactobacilli and boosting the absorption of minerals in the human body [7]. Inulin can also be used to generate fructooligosaccharides and high-fructose syrup. Acetone, butanol, bioethanol, organic acids, and other essential industrial products can also be made from inulin. It also makes children's products and food items like meat and poultry [8].

In Jerusalem artichoke, the production of inulin is accomplished through the action of two enzymes. The first enzyme is sucrose 1-fructosyltransferase, also known as 1-SST (EC 2.4.1.99), which initiates sucrose synthesis. The second enzyme is fructan: fructan 1-

fructosyltransferase (also known as elongator enzyme) (1-FFT, EC 2.4.1.100) [9]. However, a major limitation of the J.A. plant as a source of inulin is the presence of 1-fructan exohydrolase (1-FEH, EC 3.2.1.80), an inulinase that degrades terminal fructosyl-fructose linkages of inulin [10], reducing the quality of inulin at the end of the growth period and during tubers storage.

Carbohydrates significantly influence every step of the plant life cycle since they serve as sources of energy and carbon, as well as perform regulatory tasks. In addition, they engage in interactions with other signalling molecules, such as phytohormones, to govern the growth and development of plants [11**,**12]. The regulation of sugar levels in plant cells, as well as their transportation, consumption, and storage, is closely monitored and greatly impacted by the physiological activity of the cells, various plant organs, external influences, circadian rhythms, and developmental stages [13].

Carbohydrates are receiving more attention because of their functions in plant immunity. Some trigger plant defences, while others function as signalling molecules, much like phytohormones, and are largely investigated nowadays [14]. In addition, several studies have demonstrated that sugars are essential for plants to respond defensively to various biotic and abiotic stressors [15]. It is well known that sugars are the primary substrates for respiration processes and the carbon skeleton for synthesizing defence compounds, including secondary metabolites like flavonoids, stilbenes, and lignins. These processes provide energy for cellular defence responses against pathogens [16]. In addition, saccharides such as Sucrose, glucose, fructose, and trehalose function as metabolic signalling molecules within host plant cells, stimulating the activation of many genes, including defence genes [17].

Raffinose is a soluble galactosyl-sucrose carbohydrate in the RFOs family, found in plant species like Raffinose, stachyose, and verbascose, and is transported through the phloem [18]. Dextrin is a carbohydrate produced when glycogen or starch is partially hydrolyzed, contains glucose and has the same basic formula as starch [19]. Sucrose regulates anthocyanin accumulation, sucrose transport, and carbohydrate metabolism [20].

According to Klinker *et al*. [21], using Sucrose, dextrose, or fructose, in addition to a topical application of urea, led to less leaf burn, enhanced several quality characteristics, and amplified tomato yield. Additionally, lettuce produced a larger yield and had better quality when urea and 2% sucrose were applied topically [22].

Zakinthinos *et al.* [23] found that applying a solution containing 3% sucrose, 2% glucose, 1% raffinose, and inositol to the leaves of pistachio cv. Aegina, one month before harvest, can enhance the crop's quality and quantity. This therapy significantly increased pistachio dehiscence by over 85%. Studies on different crops have demonstrated considerable impacts of Sucrose and other carbohydrates applied topically [24, 25]. According to Qiuxia. [26] applying exogenous raffinose increased the saturated fresh weight and dry weight of Arabidopsis thaliana leaves *in vitro*.

In wheat plants (*Triticum aestivum* L. cv. Giza 168), Ibrahim and Abdellatif. [27] found that applying maltose and trehalose treatments dramatically boosted total soluble sugars. Additionally, Sapindus mukorossi Gaertn entire trees were given 1.5% and 3% treatments of Sucrose, which considerably boosted the carbohydrate content, according to Gao *et al*. [28].

Sugars, including fructose, trehalose, Sucrose, and glucose, participate in plants' metabolic, regulatory, and signalling pathways. These routes include resistance to biotic stress; for example, it has been demonstrated that exogenous injection of trehalose improves wheat (*Triticum aestivum* L.) resistance to powdery mildew caused by *Blumeria graminis* [29].

Subsequently, most research has shown that applying carbohydrates to a plant's leaves can improve crops' quality and quantity. This study aimed to investigate the effects of eco-friendly and novel substances, such as Raffinose, Dextrin, and Sucrose, in relation to a number of morphological and biochemical traits of Jerusalem artichoke plants, including vegetative growth parameters, photosynthetic pigments, total carbohydrate, inulin content, Fructan exohydrolase (FEH) activity, SDS-PAGE analysis of protein, soluble sugar content by HPLC, and yield parameters. The outcomes of our investigation are expected to provide novel understandings regarding the physiological and biochemical pathways through which these sugars act as growth promoters or inducers.

2. Materials and methods

2.1. Materials:

The Jerusalem artichoke tubers cultivar Fuseau was acquired from the Strawberry and Non-Traditional Crops Improvement Centre at Ain Shams University in Cairo, Egypt. Raffinose, Dextrin, Sucrose, Fructose, Glucose, Methanol, Acetone were acquired from Sigma-Aldrich (São Paulo, Brazil).

2.2. The experimental condition and cultivation:

The field investigations took place in the experimental farm belonging to the Agricultural Genetic Engineering Research Institute (AGERI), a part of the Agriculture Research Centre (ARC)., located in Giza, Egypt. The soil under investigation exhibited a clay texture and pH level of 9, indicating alkalinity.

Whole tubers were used within a range of 20 to 25 grams and sown during the summer season of 2022 on April 1st. The experimental design comprised thirteen treatments involving Raffinose, Dextrin, and Sucrose, each at four concentration levels for foliar application. Details of these treatments are presented in Table 1.

Table 1: Treatments of foliar application.

T1:	**Control (sprayed with tap water) and 0.1% Tween 20**		
T2:	10 mM Raffinose	**T8:**	50 mM Dextrin
T3:	25 mM Raffinose	**T9:**	100 mM Dextrin
T4:	50 mM Raffinose	**T10:**	10 mM Sucrose
T5:	100 mM Raffinose	**T11:**	25 mM Sucrose
T6:	10 mM Dextrin	**T12:**	50 mM Sucrose
T7:	25 mM Dextrin	**T13:**	100 mM Sucrose

The treatments were performed in a Complete Randomized Design with five replications. The area of the experimental plot was 39 m^2, consisting of thirteen ridges that were three meters long and one meter in width. Tubers were planted on one side of the ridge at 50 cm hill spacing and 5 cm depth with one tuber per hill to avoid plant overlapping. All treatments were applied as foliar spray thrice at 40 (Photo 1: A1), 60 (Photo 1: A2), and 80

(Photo 1: A3) days after sowing. Tween 20 (0.1%) was added as a wetting agent and surface spreader for each treatment, including the control [30]**.**

stages at 40, 60, and 80. A4 and A5 represent the Flowering stage at 180 DAS and harvesting of tubers at 240 DAS respectively.

2.3. Vegetative growth parameters:

At 82 days after sowing (DAS), five random plants were picked up from all treatments to determine plant fresh weight (g), dry weight (g), plant height (m), number of main branches/plants, number of lateral branches/plant and leaf area (cm^2) [30].

2.4. Determination of photosynthetic pigment contents:

Leaf samples (0.2 g) harvested at 82 days after sowing (DAS) from control and treated plants were homogenized in acetone 80% (v/v). The extract was centrifuged at 5,000 rpm for 15 minutes, and absorbance was recorded at 646 and 663 nm for chlorophyll (a and b) estimation and at 470 nm for carotenoids. The calculation of pigment content was performed using the equations described by Lichtenthaler and Buschmann. [31]:

Chlorophyll a = 12.25 A663 – 2.79 A646
Chlorophyll b= 21.21 A646 – 5.1 A663
Carotenoids = (1000 A470 – 1.8 Chl a – 85.02 Chl b) / 198

2.5. Determination of total carbohydrate content:

Extraction and determination of total carbohydrates were estimated following Hedge and Hofreiter. [32] method in which the carbohydrate content is determined by hydrolyzing the polysaccharides first into simple sugars by acid hydrolysis and then estimating the resulting monosaccharides. An amount of 0.5 g dried plant material (leaves or tubers) was placed into a boiling tube containing 5 ml of 2.5 N HCL, then the solution was boiled in the water bath for 2 hours and cooled to room temperature. Neutralize it with solid sodium carbonate until the effervescence ceases, make up the volume to 100 mL, and centrifuge. Take 0.5 mL from the supernatant for analysis by mixing with 4 ml of anthrone reagent and heat for eight minutes in a boiling water bath. Finally, cool rapidly and read the green to a dark green color product at 625 nm using a spectrophotometer (UV/Vis Double Beam Spectrophotometer T80+). The standard curve was drawn using different concentrations of glucose (100 µg/ml) and a glucose-free solution as blank. Values were expressed as mg glucose /g D.W.

2.6. Determination of inulin content:

Extraction and determination of inulin were determined according to Gibson *et al*. [33]. About 200 mg of dried plant material (leaves or tubers) was extracted in 10 ml of warm water. To 1 ml of extract, an equal volume of conc. HCl and 100 µl resorcinol (1 mg/ml) were added,

and the mixture was made up to 10 ml with distilled water. The mixture was warmed in a water bath for 10 minutes, and the absorbance was read at 490 nm. Inulin content was calculated as mg fructose/g DW from the calibration curve established with fructose concentrations in the 0.5: 20 µg/ mL range.

2.7. Determination of Fructan exohydrolase (FEH) activity:

The study prepared the crude extract of the FEH enzyme from Jerusalem artichoke leaves using Krivorotova and Sereikaite's [34] method. Frozen leaf tissue was suspended in a buffer, and the reaction mixture was heated. The enzyme activity was assessed using Zhang *et al*.'s [35] spectrophotometric method, using a 3.5-dinitrosalicylic acid colorimetry reagent. The enzyme activity is determined by the amount of fructose freed per hour under reaction conditions.

2.8. SDS-PAGE analysis of protein:

Protein profiles of Raffinose, Dextrin, and Sucrose treatments and control samples were determined using Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) in 12% acrylamide slab gels, colored, photographed, and stained with Coomassie Brilliant Blue following Laemmli's [36] technique. Gel Doc VILBER LOURMAT system (Kapelan Bio-Imaging, Germany) was used to photograph, scan, and analyze gels.

2.9. Determination of soluble sugar content by high-performance liquid chromatography (HPLC):

The study extracted and quantified soluble sugars from dried leaf powder using the Romani *et al*. [37] method, determining their free sugar profile using HPLC-IR and identifying their relative retention times. The Eurospher 100-5 NH2 column (4.6 x 250 mm, 5 mm, Knauer) and Knauer Smartline 2300 RI detector were both included in the HPLC system. The mobile phase was acetonitrile/deionized water (7:3) at a 1 ml/min flow rate. The results are expressed in g/100 g of dried weight, calculated by internal normalization of the chromatographic peak area. Sugar identification was made by comparing the relative retention times of sample peaks of standards (Ribose, Glucose, Fructose, Sucrose).

2.10. Tuber yield parameters:

Five plants were randomly selected at harvest to count the number of tubers on each, the yield per plant in kilograms, and the overall yield per feddan in tons [30].

2.11. Statistical analysis

All data analysis was performed using SPSS software version 23.0 (SPSS, Chicago, IL, USA). Values were expressed as means of three replicates (n = 3) along with standard error for biochemical analysis and five replicates (n = 5) for vegetative parameters. The statistical analysis was performed by one-way analysis of variance (ANOVA). Duncan's multiple range tests compared significant differences between means. The differences were considered statistically significant when $p \leq 0.05$.

3. Results and Discussion

3.1. Vegetative growth parameters:

The effect of Raffinose, Dextrin, and Sucrose on plant height, the number of main branches/plants, the number of lateral branches/plants, and leaf area (cm^2) (Table 2) were significantly increased compared to the control treatment. Raffinose treatment 50 mM appeared superior in all tested morphological parameters (120 cm plant height, 5 main branches, 58 lateral branches, 66.5 m^2 leaf area) followed by a dose of 25 mM Raffinose (4 main branches, 42 lateral branches, 61.5 m^2 leaf area).

In this context, Ibrahim and Abdellatif. [27] proved that maltose and trehalose as types of carbohydrates foliar application to wheat plants (*Triticum aestivum* L. cv. Giza 168) caused a significant increase in all plant growth parameters as plant fresh, dry weight and leaf area and indicated that maltose had a higher significant effect in no. of spikes/plant and no. of spikelet/plant when compared with control and better than trehalose treatment.

Results in Table 2 revealed the behaviour of all Raffinose, Dextrin, and Sucrose treatments on the fresh and dry weight of Jerusalem artichoke plants taken 82 days after sowing. As regards plant fresh weight, the highest values were detected for spraying with Raffinose at 50 and 100 mM (228.54 g and 223.49 g, respectively), followed by foliar application of Dextrin at 50 and 100 mM (212.19 g and 205.63 g, respectively). These

increments were significant as evaluated by the control. The data listed in Table 2 for plant dry weight indicated that the treatments of Raffinose at 50 and 100 mM doses were significantly increased (77.42 g and 70.05 g).

This result was in harmony with Qiuxia. [26] who found that applying exogenous Raffinose increased the saturated fresh weight and dry weight of *Arabidopsis thaliana* leaves *in vitro*. Furthermore, Zakinthinos *et al.* [23] discovered that applying a combination of carbohydrates, specifically 3% sucrose, 2% glucose, 1% raffinose, and inositol, to the leaves of pistachio cv. Aegina one month prior to harvesting, can enhance both the quality and productivity of the crop. Previous studies on different crops, such as cotton and lettuce, have demonstrated notable impacts of applying Sucrose and other carbs directly to the leaves [24, 25].

A significant decrement in plant fresh weight was exhibited for low doses of Raffinose and Dextrin treatments of 10 and 25 mM (Table 2) in addition to all doses of Sucrose treatments compared to the control. For plant dry weight, the low doses of Raffinose treatments 10 and 25 mM (42.99 g and 49.05 g**)** and all doses tested for Dextrin and Sucrose treatments were not significantly different compared to the control (Table 2). Arzani *et al*. [38] deduced that the dry and fresh weight of pistachio was not affected by carbohydrate application because of the low concentrations of carbohydrates they used.

Table 2: Effect of foliar application of Raffinose, Dextrin, and Sucrose on vegetative growth parameters of Jerusalem artichoke plants.

Treatments	Plant height (cm)	No. of main branches /plant	No. of lateral branches /plant	Leaf area (cm^2)	Plant fresh weight (g)	Plant dry weight (g)
Control	76 ± 15.6 c	2 ± 1 bc	18 ± 4 f	30.2 ± 4.9 f	203.45 ± 0.25 d	41.13 ± 0.31 h
R 10 mM	82 ± 14.7 bc	3 ± 1.7 abc	32 ± 2.6 bcde	58 ± 10 abc	178.59 ± 0.29 g	42.99 ± 0.13 g
R 25 mM	82 ± 10.5 bc	4 ± 1 ab	42 ± 5.5 b	61.6 ± 7.2 ab	197.34 ± 0.33 e	49.05 ± 0.04 e
R 50 mM	120 ± 9.5 a	5 ± 1 a	58 ± 9.1 a	66.5 ± 1.5 a	228.54 ± 0.57 a	77.42 ± 0.31 a
R 100 mM	98 ± 8.7 b	4 ± 1 ab	38 ± 3.6 bcd	56.2 ± 9.1 abcd	223.49 ± 0.43 b	70.05 ± 0.34 b
D 10 mM	77 ± 7.2 c	3 ± 0 abc	24 ± 7.2 ef	53.2 ± 12.9 abcd	162.36 ± 0.34 i	37.83 ± 0.24 j
D 25 mM	82 ± 8.7 bc	3 ± 1 abc	29 ± 6.1 cde	55.1 ± 11.7 abcd	173.49 ± 0.22 h	42.75 ± 0.41 g
D 50 mM	82 ± 3.4 bc	3 ± 0 abc	32 ± 3 bcde	43.6 ± 10.6 cdef	212.19 ± 0.57 c	39.63 ± 0.30 i
D 100 mM	81 ± 8.٢ bc	3 ± 1.7 abc	25 ± 2 ef	43.1 ± 2.4 cdef	205.63 ± 0.88 d	50.92 ± 0.14 d
S 10 mM	91 ± 4.5 bc	3 ± 0 abc	27 ± 7.5 ef	34.2 ± 2.8 ef	154.83 ± 0.30 l	40.34 ± 0.33 h
S 25 mM	92 ± 11.5 bc	3 ± 0 abc	30 ± 3 cde	44.7 ± 4.3 cdef	157.32 ± 0.31 k	35.67 ± 0.28 k
S 50 mM	92 ± 9.1 bc	3 ± 2 abc	39 ± 5.2 bc	49.6 ± 11 bcde	160.68 ± 0.39 j	45.51 ± 0.63 f
S 100 mM	87 ± 11.5 bc	3 ± 0 abc	28 ± 7.2 def	40.5 ± 9.8 def	184.83 ± 0.30 f	51.78 ± 0.65 c
F value	4.05**	3.96**	9.94***	4.79***	3497.1***	1176.2***

Means having the same letters in a column were not significantly different according to Duncan's multiple range test Where One star (*) means p-value<0.05, two stars (**) mean p-value<0.01, and three stars (***) mean p-value<0.001, R: Raffinose, D: Dextrin, S: Sucrose. Data represents as means ± Standard error.

3. 2. Photosynthetic pigment contents:

Results in Figure 1 indicate that foliar application of tested Sugars exhibited significant and positive effects on photosynthetic pigments, namely chlorophyll a, b, and carotenoids. The highest significant value of chlorophyll a, b, and carotenoids content was recorded when foliar treatments were applied with 50 mM Raffinose followed by 100 mM Raffinose, 100 mM Dextrin, and 25 mM Sucrose, respectively. On the other hand, the foliar treatments of lower doses of Raffinose and Dextrin (10 and 25 mM), in addition to the dose of Sucrose (10 mM), had the lower significant value from all tested treatments.

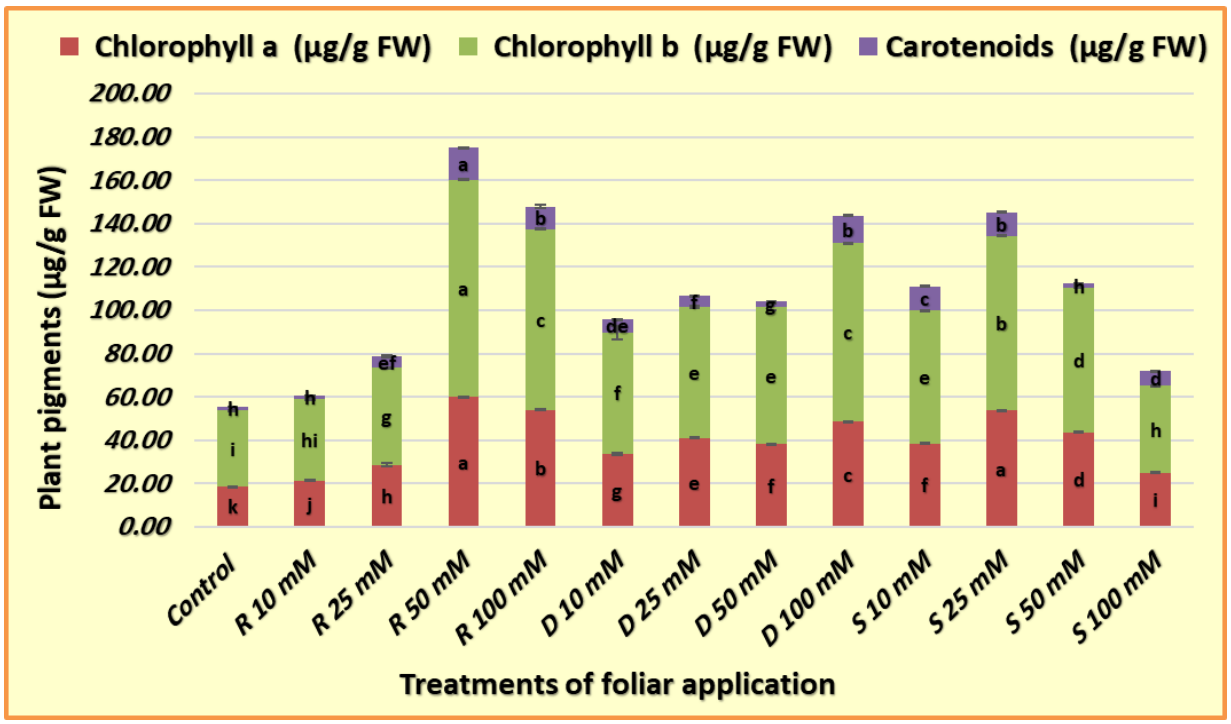

Fig.1: Effect of foliar application of Raffinose, Dextrin, and Sucrose on the content of photosynthetic pigments of Jerusalem artichoke plants. Where R: Raffinose, D: Dextrin, S:

These presented results are in accordance with those obtained by Khater *et al*. [39] with a type of sugar trehalose, which has a stimulatory effect on photosynthetic pigments, explaining that might be due to the role of trehalose in stabilizing macromolecules (membrane lipids and proteins) and biological structures thereby helping to maintain photosynthesis under stress [40].

Trehalose may influence the regulation of carbon metabolism and photosynthesis, according to Lunn *et al*. [41]. Furthermore, Sadak [42] asserted that the utilization of trehalose as an osmoprotectant substance enhanced the photosynthetic pigments of the fenugreek plant when subjected to drought conditions. This improvement was achieved by increasing the activity of the photosynthetic process and water relation attributes and enhancing the antioxidant defence mechanism.

Raffinose is a rare osmoprotectant sugar regularly observed in plants, according to Bougouffa *et al*. [43]. From this perspective, as it is likewise thought of as an osmoprotectant sugar, we expected it to exhibit the same behaviour as trehalose in improving photosynthetic pigment content.

3. 3. Total carbohydrate content:

Figure 2 displays the impact of the sugars Raffinose, Dextrin, and Sucrose on the total carbohydrate content recovered from leaf samples collected 82 days after planting. As shown, the highest significant value was recorded from 50 mM of Raffinose (46.7 mg glucose/g DW) followed by 100 mM of Raffinose (37.32 mg glucose/g DW), 50 mM of Sucrose (29.23 mg glucose/g DW), and 50 mM of Dextrin (23.8 mg glucose/g DW). Unlike the foliar treatments with lower doses of Raffinose, Dextrin, and Sucrose (10 mM) were not significantly different compared to the control.

These results were supported in other types of carbohydrates by Ibrahim and Abdellatif [27]**,** who documented that applying trehalose and maltose treatments significantly increased total soluble sugars in wheat plants (*Triticum aestivum* L. cv. Giza 168). In addition, Gao *et al*. [28] stated that the 1.5% and 3% treatments of Sucrose significantly increased the carbohydrate content when applied exogenously to whole trees of *Sapindus mukorossi* Gaertn. From those observations, Raffinose may have a trehalose effect on plants, affecting plant growth and development by controlling carbon metabolism [44].

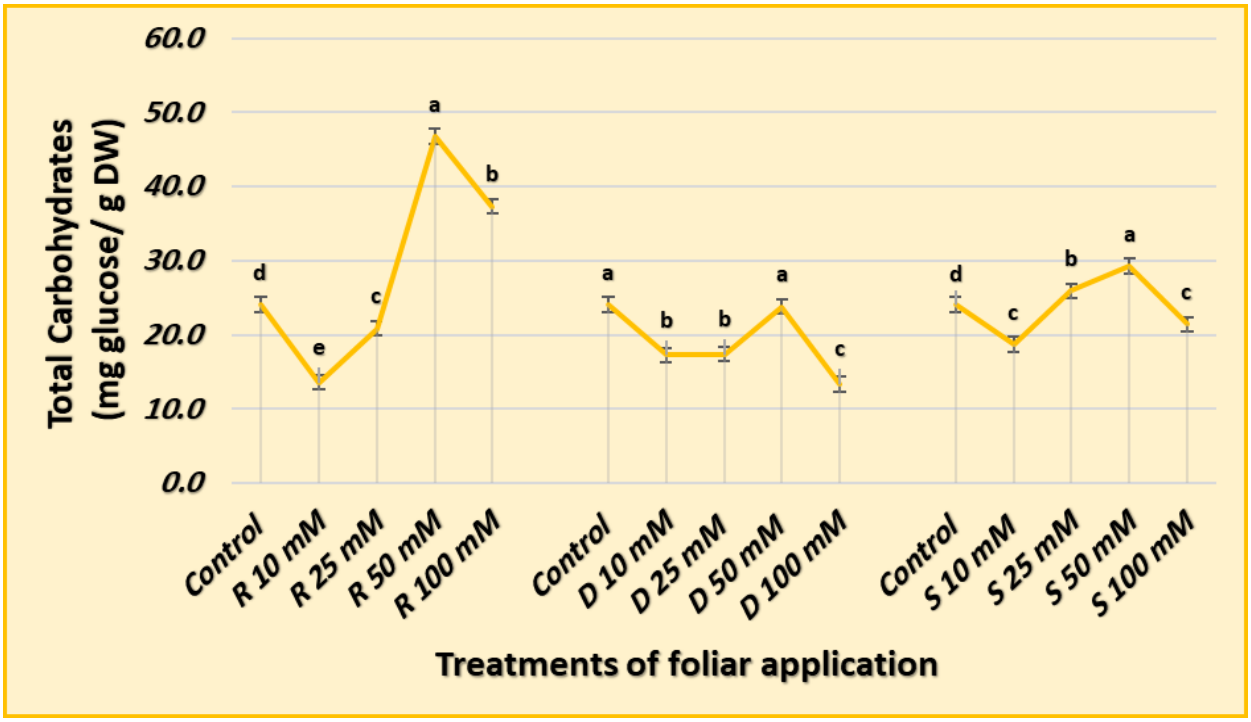

Fig.2: Effect of foliar application of Raffinose, Dextrin, and Sucrose on the total carbohydrate content of Jerusalem artichoke plants. Where R: Raffinose, D: Dextrin, S: Sucrose.

3.4. Inulin content:

Data presented in Figure ٣ demonstrates the effect of foliar application of Raffinose, Dextrin, and Sucrose on inulin content extracted from leaf samples harvested 82 days after sowing (DAS). The highest significant value was that recorded from 50 mM of Raffinose (3.16 mg fructose/g DW), which is more productive in enhancing inulin levels than other treatments, and then 25 mM of Raffinose (2.63 mg fructose/g DW) sequentially. Additionally, doses of Dextrin (25, 50, and 100 mM) and Sucrose (25 and 50 mM) positively affect inulin levels compared to control. The lower doses of Dextrin and Sucrose (10 mM; 1.75 mg fructose/g DW) similarly retarded the inulin content and were not significantly different compared to the control.

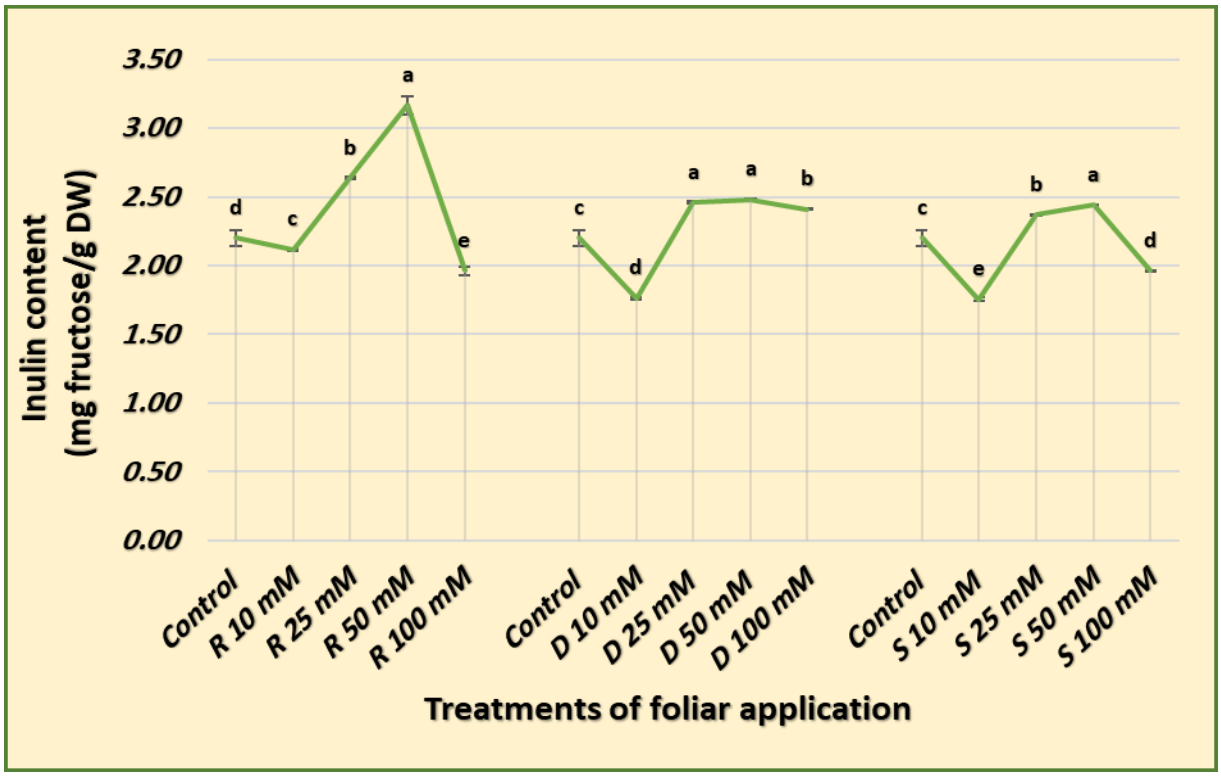

Fig. 3: Effect of foliar application of Raffinose, Dextrin, and Sucrose on inulin content of Jerusalem artichoke plants. Where R: Raffinose, D: Dextrin, S: Sucrose.**Photo 1:** Foliar times of Jerusalem artichoke (*Helianthus tuberosus* L.) plant, A1 to A3 represent vegetative

Tropical and subtropical grasses mostly store carbohydrates like Sucrose and starch, whereas calm and cool zone grasses primarily store fructose polymers known as Fructans [45]. Inulin is one of the five types of Fructans identified and characterized by glycosidic bonds and the Jerusalem artichoke plant's preserved variety. When photosynthesis is more than the demand, supposedly when the concentration of Sucrose in sink organs reaches an acceptable level, inulin synthesis begins [10].

According to Ueno *et al*. [9], the vacuole is where inulin production starts, using Sucrose as a donor and substrate. Sucrose-sucrose fructosyltransferase (1-SST), the first transferase enzyme, converts two sucrose molecules into 1-kestose, which releases a glucose molecule. Inulin, a type of fructan, is formed through the action of an enzyme called fructan-fructan fructosyltransferase (1-FFT). This enzyme transfers a fructose residue from one molecule to another, specifically to the identical carbon position on the receiving molecule. Thus, to sum up, the more Sucrose that is readily available, the more inulin is produced, and

this might be a result of the exogenous application of utilized sugars investigated in this work (Raffinose, Dextrin, and Sucrose) causing an increase in naturally occurring soluble sugars like Sucrose that result in higher inulin content formation.

Another explanation that caused the increments of inulin in Raffinose treatments was that it may have more inhibition activity to the Fructan exohydrolase (inulin degrading enzyme) than Dextrin and Sucrose. So, these doses had the highest inulin contents as the FEH enzyme did not decompose them.

3.5. Fructan exohydrolase (FEH) activity:

Figure 4 displays the impact of the sugars Raffinose, Dextrin, and Sucrose on the activity of fructan exohydrolase (FEH) recovered from leaf samples collected 82 days after planting. Notably, all treatments of Raffinose, Dextrin, and Sucrose positively affected the inhibition of Fructan exohydrolase (FEH) compared to the control (Figure 4). The potent and highest positive significant inhibition was Raffinose at a 50 mM dose (24.8 U/ g F.W.) compared with all tested sugars. The Dextrin, at a 50 mM dose (25.9 U/ g F.W.), responded positively, inhibiting the target enzyme after Raffinose. They were the best, more than Sucrose treatments.

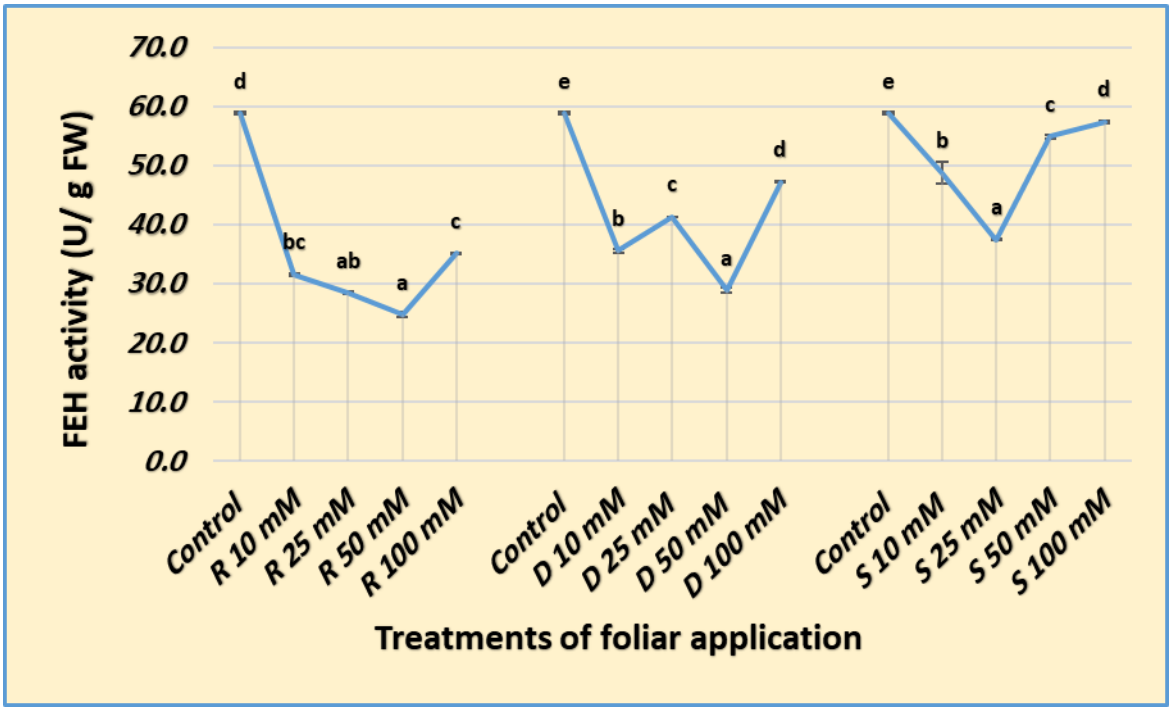

Fig. 4: Effect of foliar application of Raffinose, Dextrin, and Sucrose on Fructan exohydrolase (FEH) activity of Jerusalem artichoke plants. Where R: Raffinose, D: Dextrin, S: Sucrose.

According to studies conducted on *Helianthus tuberosus* [26], *Triticum aestivum* [47], *Chrysolaena intybus* [48], *Chrysolaena obovata* [49], and *Lolium perenne* [50], Sucrose suppresses the activity of fructan exohydrolases. Also, the presence of 1-fructan exohydrolase (1-FEH, EC 3.2.1.153), an inulinase that breaks down terminal fructosyl-fructose linkages of inulin, significantly reduces the quality of inulin at the end of the growth phase and during tubers storage which converts it to monosaccharides [10].

3.6. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE):

Photo 2 displays the SDS-PAGE electrophoretic pattern of soluble proteins obtained from leaf samples collected 82 days after sowing. The number and relative molecular weights are recorded in Table 3. The results showed 12 protein bands of molecular weights ranging between 82 and 8 KDa. Treatments of 10, 25, and 50 mM Raffinose, 50 mM Dextrin, and 50 mM Sucrose had the appearance of protein bands (12) in leaves as control, while the lowest number of protein bands (10) appeared in treatments of 100 mM Raffinose, 10, 25 mM Dextrin and 10, 25 mM Sucrose. In addition to 100 mM, Sucrose treatments had 11 protein bands. The protein patterns of Jerusalem artichoke plants comprise ten major bands (common bands) having molecular weights of 70, 66, 38, 36, 32, 30, 18, 12, and 8 KDa, recorded in all treatments. Two protein bands with 82 and 73 KDa molecular weights disappeared in Jerusalem artichoke leaves and were recorded as non-unique bands.

El Gengaihi *et al*. [51] stated that SDS-PAGE protein patterns of the Jerusalem artichoke plant were composed of 16 amplicons (bands), and its molecular weights ranged from 201. to 17.66 KDa. It was reported that the Fructan exohydrolase enzyme had comparable molecular weights between 68 and 70 KDa [46 , 47] In this regard, the protein band in our analysis, which had a molecular weight of 70 KDa, may be the FEH enzyme in all treatments. Foliar treatments may have no impact on enzyme biosynthesis but merely result in a decrease in enzyme activity.

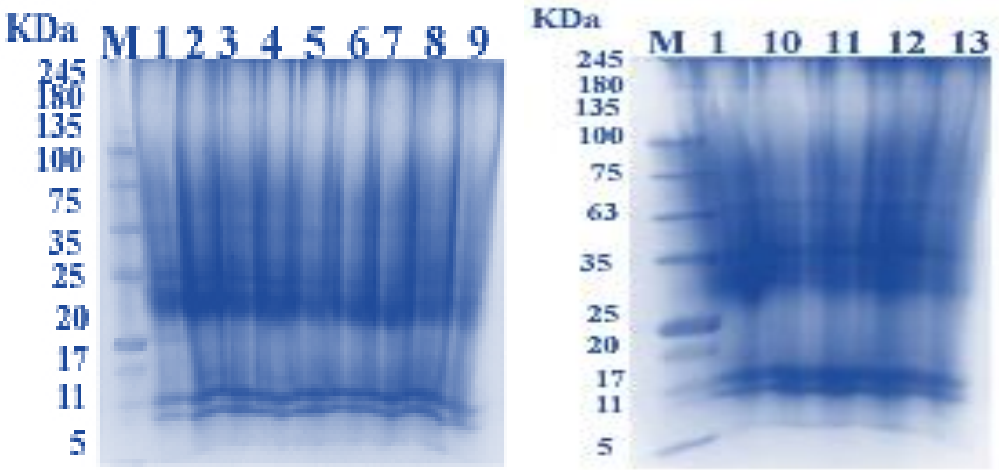

Photo 2: Electrophoretic banding patterns of Jerusalem artichoke leaves in response to foliar application of Raffinose, Dextrin, and Sucrose, while, M: Standard molecular weight Marker proteins, 1: Control, 2: 10 mM Raffinose, 3: 25 mM Raffinose, 4:50 mM Raffinose, 5: 100 mM Raffinose, 6: 10 mM Dextrin, 7: 25 mM Dextrin, 8: 50 mM Dextrin, 9: 100 mM Dextrin, 10: 10 mM Sucrose, 11: 25 mM Sucrose, 12: 50 mM Sucrose, 13: 100 mM Sucrose.

Table 3: Effect of foliar application of Raffinose, Dextrin, and Sucrose on the protein patterns separated by SDS-PAGE of Jerusalem artichoke leaves expressed as 1 (presence) and 0 as (absent).

Band No	M.W KDa	Control	R 10 mg	R 25 mg	R 50 mg	R 100	D 10 mg	D 25 mg	D 50 mg	D 100	S 10 mg	S 25 mg	S 50 mg	S 100
1	**82**	1	1	1	1	0	0	0	1	1	0	0	1	0
2	**73**	1	1	1	1	0	0	0	1	1	0	0	1	1
3	**70**	1	1	1	1	1	1	1	1	1	1	1	1	1
4	**66**	1	1	1	1	1	1	1	1	1	1	1	1	1
5	**38**	1	1	1	1	1	1	1	1	1	1	1	1	1
6	**36**	1	1	1	1	1	1	1	1	1	1	1	1	1
7	**33**	1	1	1	1	1	1	1	1	1	1	1	1	1
8	**32**	1	1	1	1	1	1	1	1	1	1	1	1	1
9	**30**	1	1	1	1	1	1	1	1	1	1	1	1	1
10	**18**	1	1	1	1	1	1	1	1	1	1	1	1	1
11	**12**	1	1	1	1	1	1	1	1	1	1	1	1	1
12	**8**	1	1	1	1	1	1	1	1	1	1	1	1	1
Total		**12**	**12**	**12**	**12**	**10**	**10**	**10**	**12**	**12**	**10**	**10**	**12**	**11**

3.7. Free soluble sugar content by (HPLC):

The results have shown that the tested Sugars had varying effects on the endogenous free soluble sugar concentration retrieved from leaf samples harvested at 82 days after sowing, compared to the control treatment. (Figure 5). Notably, the free soluble sugars (Ribose, Fructose, Glucose, and Sucrose) were significantly decreased in Raffinose and Dextrin, especially in 50 mM Raffinose and 100 mM Dextrin. Sucrose doses were not significantly different compared to the control.

The study results agreed with Shao *et al*. [52]**,** who suggest that during the initial phase of tuberization, the photosynthetic assimilation products of Jerusalem artichoke are transiently stored in the stem. Subsequently, the tuberization process redirects the storage of photosynthetic products from the stems to the tubers.

Reports have indicated that the products of assimilation resulting from photosynthesis in Jerusalem artichoke leaves are mostly carried to the underground parts of the plant as Sucrose but are retained in the tubers as fructan (polyfructosylsucrose) [53]. Sucrose served as the initial substance for producing Fructans; however, synthesizing and storing Fructans, specifically inulin, primarily occurred in the tubers of Jerusalem artichoke [54]. The obtained result in the behavior of tested Sugars in decreasing the free sugars may be due to the positive enhancement effects of their translocation from leaves to sink organs (tubers).

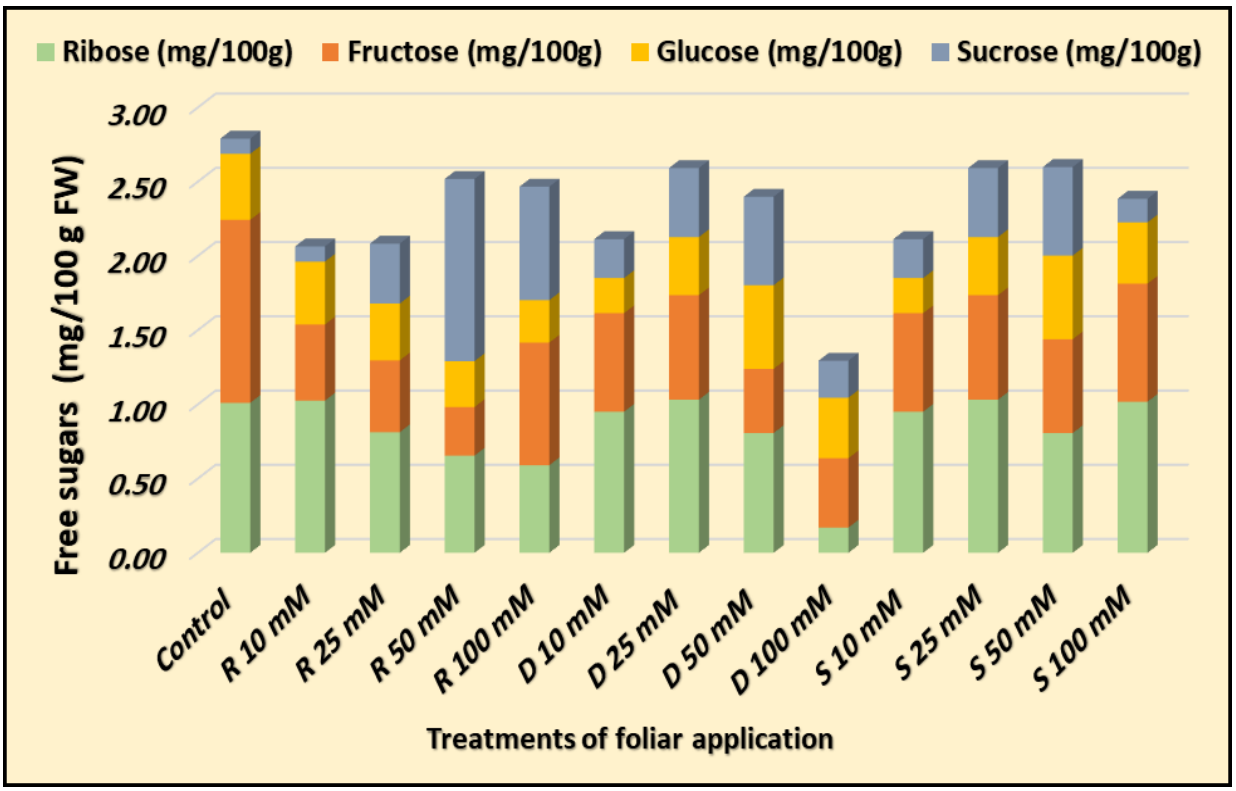

Fig.5: Effect of foliar application of Raffinose, Dextrin, and Sucrose on the total soluble sugar content of Jerusalem artichoke plants. Where R: Raffinose, D: Dextrin, S: Sucrose.

3.8. Tuber yield parameters:

The information in Table 4 illustrates how Raffinose, Dextrin, and Sucrose foliar treatments affected the features of tuber yields at harvest time. According to the data, the most significant number of tubers per plant (133 and 112 tubers), yield per plant (4.82 and 4.24 kg), and total yield per feddan (40.67 and 35.33 tons) were obtained when spraying Jerusalem artichoke plants with 50 mM of Raffinose and then 50 mM of Dextrin respectively. On the other hand, when compared to the control, the sucrose doses of 50 and 100 mM showed the lowest significant number of tubers per plant, yield per plant, and overall yield per feddan.

In line with our findings, foliar application of different types of carbohydrates Trehalose was sprayed on plants to improve their development, yield, and quality. Examples of these plants include those studied by Alam *et al*. [55] for Brassica species, Shafiq *et al*. [56] and Akram *et al*. [57] for radish, Khater *et al*. [39] for cowpea, and Wang *et al*. [12] for sweet potato. In addition, El Metwaly *et al*. [58] suggested applying trehalose topically to produce

high productivity and the best tuber root quality of sweet potato plants, from this point, may have the same behavior as Trehalose to plants. As shown, the previously tested parameters indicated that a significant improvement in plant growth was attributed to the Raffinose treatments, especially the 50 mM dose.

Table 4: Effect of foliar application of Raffinose, Dextrin, and Sucrose on tuber yield parameters of Jerusalem artichoke plants at harvesting.

Treatments	No. of tubers/plant	Yield/ plant (kg)	Total yield/ Feddan (ton)
Control	**76 ± 7.8 bc**	**2.09 ± 5.1 b**	**17.67 ± 4.3 b**
R 10 mM	**82 ± 20.9 bc**	**2.14 ± 4.5 b**	**18.00 ± 3.6 b**
R 25 mM	**85 ± 15.7 bc**	**3.22 ± 4.2 ab**	**27.00 ± 3.5 ab**
R 50 mM	**133 ± 21.1 a**	**4.82 ± 10.8 a**	**40.67 ± 3.1 a**
R 100 mM	**67 ± 9.2 bc**	**1.96 ± 4.6 b**	**16.33 ± 4.1 b**
D 10 mM	**78 ± 13.8 bc**	**2.45 ± 5.6 ab**	**20.67 ± 4.8 ab**
D 25 mM	**84 ± 15 bc**	**2.14 ± 3.5 b**	**18.00 ± 3 b**
D 50 mM	**112 ± 8.3 ab**	**4.24 ± 14.1 ab**	**35.33 ± 7.9 ab**
D 100 mM	**79 ± 9.9 bc**	**2.53 ± 11.7 ab**	**21.33 ± 9.6 ab**
S 10 mM	**83 ± 4.1 bc**	**2.60 ± 2.9 ab**	**21.67 ± 2.4 ab**
S 25 mM	**81 ± 14.9 bc**	**2.11 ± 8.5 b**	**15.33 ± 6.4 b**
S 50 mM	**55 ± 4.8 c**	**1.84 ± 7.3 b**	**18.00 ± 7.2 b**
S 100 mM	**53 ± 23.9 c**	**1.77 ± 8.7 b**	**15.00 ± 7.5 b**
F value	**2.135***	**1.458***	**1.435***

Means having the same letters in a column were not significantly different according to Duncan's multiple range test where One star (*) means p-value<0.05, two stars (**) mean p-value<0.01, and three stars (***) mean p-value<0.001, data represents as means ± Standard error.

4. Conclusion

The present study accomplished its major objective. Among all tested Sugars Raffinose treatments, especially the dose of 50 mM, has the most potent and positive effect of all tested morphological, physiological, and biochemical analyses, which explains the good behavior not only in enhancing the assimilation of photosynthesis and increasing the photosynthetic products but also redirecting the allocation of photosynthetic resources from the stems to the tubers which explain the high productivity and the quality of produced tubers—furthermore, controlling the Fructan exohydrolase activity and the enhancement of inulin production.

This research revealed that supplying plants with certain sugars, particularly Raffinose and Dextrin, as a sustainable substance might improve their overall growth and development, raise their total output, and enhance their ability to overcome environmental challenges. These findings will be proven and strengthened by future studies.

5. Funding:

This research received no external funding.

6. Institutional Review Board Statement:

Not applicable.

7. Informed Consent Statement:

Not applicable.

8. Data Availability Statement:

All data are within the paper.

9. Declarations:

All authors declare that there is no conflict of interest in submitting this manuscript.

References

[1] Qiu, Y., Lei, P., Zhang, Y., Sha, Y., Zhan, Y., Xu, Z., Li, S., Xu, H. & Ouyang, P. (2018). Recent advances in bio-based multi-products of agricultural Jerusalem artichoke resources. *Biotechnology for Biofuels*, 11(1). https://doi.org/10.1186/s13068-018-1152-6

[2] Kaszás, L., Alshaal, T., Kovács, Z., Koroknai, J., Elhawat, N., Nagy, É., & Domokos-Szabolcsy, É. (2020). Refining high-quality leaf protein and valuable co-products from green biomass of Jerusalem artichoke (*Helianthus tuberosus* L.) for sustainable protein supply. *Biomass Conversion and Biorefinery*, 1-16. https://doi.org/10.1007/s13399-020-00696-z

[3] Mohamed, H. E. (2020). Effect of Tuber Cutting and Planting Date on Yield and Quality of Jerusalem artichoke in New Reclaimed Soil under Upper Egypt Conditions. *Middle East* J, 9(4), 943-947. https://doi.org/10.36632/mejar/2020.9.4.72

[4] Yang, S., Wang, L., Zhong, Q., Zhang, G., Zhang, D., & Sun, X. (2020). Transcriptome changes induced by drought stress in Jerusalem artichoke (*Helianthus tuberosus* L.) and weighted gene co-expression network analysis (WGCNA). https://doi.org/10.21203/rs.3.rs-25678/v1

[5] Yu, Q., Zhao, J., Xu, Z., Chen, Y., Shao, T., Long, X., & Zhou, J. (2018). Inulin from Jerusalem artichoke tubers alleviates hyperlipidemia and increases abundance of bifidobacteria in the intestines of hyperlipidemic mice. *Journal of Functional Foods*, 40, 187-196. https://doi.org/10.1016/j.jff.2017.11.010

[6] Wan, X., Guo, H., Liang, Y., Zhou, C., Liu, Z., Li, K. & Wang, L. (2020). The physiological functions and pharmaceutical applications of inulin: A review. *Carbohydrate polymers*, 246, 116589. https://doi.org/10.1016/j.carbpol.2020.116589

[7] Singh, R. S., Chauhan, K., & Kennedy, J. F. (2017). A panorama of bacterial inulinases: production, purification, characterization, and industrial applications. *International journal of biological macromolecules*, 96, 312-322. https://doi.org/10.1016/j.ijbiomac.2016.12.004

[8] Singh, R. S., Singh, T., & Larroche, C. (2019). Biotechnological applications of inulin-rich feedstocks. *Bioresource technology*, 273, 641-653. https://doi.org/10.1016/j.biortech.2018.11.031

[9] Ueno, K., Sonoda, T., Yoshida, M., Kawakami, A., Shiomi, N., & Onodera, S. (2020). Decreased expression of fructosyltransferase genes in asparagus roots may contribute to efficient fructan degradation during *asparagus spear* harvesting. *Plant Physiology and Biochemistry*, 156, 192-200. https://doi.org/10.1016/j.plaphy.2020.09.007

[10] Wang, M., & Cheong, K. L. (2023). Preparation, structural characterization, and bioactivities of fructans: A review. *Molecules*, 28(4), 1613. https://doi.org/10.3390/molecules28041613

[11] Ciereszko, I. (2018). Regulatory roles of sugars in plant growth and development. *Acta Societatis Botanicorum Poloniae*, 87(2). https://doi.org/10.5586/asbp.3583

[12] Wang, W., Yu, H., Kim, H. S., Yang, Y., Qiu, X., & Kwak, S. S. (2019). Molecular characterization of a sweet potato stress tolerance-associated trehalose-6-phosphate synthase 1 gene (IbTPS1) in response to abiotic stress. *Plant Biotechnology Reports*, 13, 235-243. https://doi.org/10.1007/s11816-019-00532-5

[13] Lemoine, R., Camera, S. L., Atanassova, R., Dédaldéchamp, F., Allario, T., Pourtau, N. & Durand, M. (2013). Source-to-sink transport of sugar and regulation by environmental factors. *Frontiers in plant science*, 4, 272. https://doi.org/10.3389/fpls.2013.00272

[14] Trouvelot, S., Héloir, M. C., Poinssot, B., Gauthier, A., Paris, F., Guillier, C. & Adrian, M. (2014). Carbohydrates in plant immunity and plant protection: roles and potential application as foliar sprays. *Frontiers in plant science*, 5, 592. https://doi.org/10.3389/fpls.2014.00592

[15] Keunen, E. L. S., Peshev, D., Vangronsveld, J., Van Den Ende, W. I. M., & Cuypers, A. N. N. (2013). Plant sugars are crucial players in the oxidative challenge during abiotic stress: extending the traditional concept. *Plant, cell & environment*, 36(7), 1242-1255. https://doi.org/10.1111/pce.12061

[16] Jeandet, P., Vannozzi, A., Sobarzo-Sánchez, E., Uddin, M. S., Bru, R., Martínez-Márquez, A. & Nabavi, S. M. (2021). Phytostilbenes as agrochemicals: biosynthesis, bioactivity, metabolic engineering and biotechnology. *Natural product reports*, 38(7), 1282-1329. https://doi.org/10.1039/D0NP00030B

[17] Morkunas, I., Narożna, D., Nowak, W., Samardakiewicz, S., & Remlein-Starosta, D. (2011). Cross-talk interactions of Sucrose and *Fusarium oxysporum* in the phenylpropanoid pathway and the accumulation and localization of flavonoids in embryo axes of yellow lupine. *Journal of Plant Physiology*, 168(5), 424-433. https://doi.org/10.1016/j.jplph.2010.08.017

[18] Elango, D., Rajendran, K., Van der Laan, L., Sebastiar, S., Raigne, J., Thaiparambil, N. A. & Singh, A. K. (2022). Raffinose family oligosaccharides: friend or foe for human and plant health?. *Frontiers in Plant Science*, 13,829118. https://doi.org/10.3389/fpls.2022.829118

[19] Alvani, K., Qi, X., & Tester, R. F. (2011). Use of carbohydrates, including dextrins, for oral delivery. *Starch-Stärke*, 63(7), 424-431. https://doi.org/10.1002/star.201000110

[20] Yoon, J., Cho, L. H., Tun, W., Jeon, J. S., & An, G. (2021). Sucrose signaling in higher plants. *Plant Science*, 302, 110703. https://doi.org/10.1016/j.plantsci.2020.110703

[21] Klinker, J. E., & Emmert, E. M. (1953). Effect of foliar applications of urea, Sucrose, and dextrose on tomato yields and quality. https://books.google.com.eg/books?id=3kMnAQAAMAAJ

[22] Bassiouny, I., Mazrouh, A. Y., & Hassan, N. A. (1993). The Effect of foliar fertilizers, Sucrose and GA3 on growth and yield of lettuce plants (*Lactuca sativa L*.). *Journal*, 645-653.

[23] Zakinthinos, G. & Rouskas, D. (1995). Shell dehiscence improvement and weight increase in Aegina pistachio nuts with carbohydrate application. *Acta Horticulture* 419:143-48. https://doi.org/١٠,١٧٦٦٠/ActaHortic.1995.419.23

[24] Hoshino, J. Y., Nonaka, M. & Oda. M. 1987. Physiological and ecological study on cold hardiness of lettuce, 3; effect of cryoprotectant sprays on cold hardiness of lettuce. *Research Reports of the Rural Development Administration-Horticulture (Korea R.)*.

[25] Bhatt, J.G. & Renganayagi, R. (1987). Ascorbic acid turnover in cotton boll as affected by foliar application of Sucrose. *Annals of botany* 60:395-97. https://doi.org/10.1093/oxfordjournals.aob.a087459

[26] Qiuxia, W. (2007). Influence of Applying Exogenous Raffinose on the Water Loss Rate of *Arabidopsis thaliana* Leaf in Vitro. *JOURNAL OF ANHUI AGRICULTURAL SCIENCES*, *35*(33), 10607.https://api.semanticscholar.org/CorpusID:88450538

[27] Ibrahim, H. A., & Abdellatif, Y. M. (2016). Effect of maltose and trehalose on growth, yield and some biochemical components of wheat plant under water stress. *Annals of Agricultural Sciences*, *61*(2), 267-274. https://doi.org/10.1016/j.aoas.2016.05.002

[28] Gao, S., Gao, Y., Yang, Y., Jia, L., & Weng, X. (2022). Exogenous Sucrose Supply Regulates the Physiological Index Levels Before the Flower Blooming and Fruit Abscission Stages in the Biodiesel Tree *Sapindus mukorossi* Gaertn. *Hort Science*, *57*(11), 1397-1408. https://doi.org/10.21273/HORTSCI16626-22

[29] Tayeh, C., Randoux, B., Vincent, D., Bourdon, N., & Reignault, P. (2014). Exogenous trehalose induces defenses in wheat before and during a biotic stress caused by powdery mildew. *Phytopathology*, *104*(3), 293-305. https://doi.org/10.1094/PHYTO-07-13-0191-R

[30] Abo El-Fadel, N. I., & Shama, M. A. (2020). Foliar Application of Glycine Betaine and Potassium Silicate and Its Effect on Growth Performance of Jerusalem Artichoke Grown on Calcareous Soil Under Water Stress Conditions. *Journal of the Advances in Agricultural Researches*, *25*(1), 14-37. https://doi.org/10.21608/JALEXU.2020.163749

[31] Lichtenthaler, H. K., & Buschmann, C. (2001). Chlorophylls and carotenoids: Measurement and characterization by UV-VIS spectroscopy. *Current protocols in food analytical chemistry*, 1(1), F4-3. https://doi.org/10.1002/0471142913.faf0403s01

[32] Hedge, J.E. and Hofreiter, B.T. (1962). In: Carbohydrate Chemistry, 17 (Eds. Whistler R.L. and Be Miller, J.N.), *Academic Press, New York interactions. Ind. J. Plant Physiol*, *21*(4), 477-488.

[33] Gibson, G., Willis, C. L., & Van Loo, J. (1994). Non digestible oligosaccharides and bifidobacteria: Implications for health. *International Sugar Journal*, (96), 381-387. https://api.semanticscholar.org/CorpusID:83087379

[34] Krivorotova, T., & Sereikaite, J. (2014). Determination of fructan exohydrolase activity in the crude extracts of plants. *Electronic Journal of Biotechnology*, *17*(6), 329-333. https://doi.org/10.1016/j.ejbt.2014.09.005

[35] Zhang, L., Liu, W., Ji, J., Deng, L., Feng, Q., Shi, W., & Gao, J. (2021). Inactivation of Inulinase and Marination of High-Quality Jerusalem Artichoke (*Helianthus tuberosus* L.) Pickles with Screened Dominant Strains. *Frontiers in bioengineering and biotechnology*, *8*, 626861.https://doi.org/10.3389/fbioe.2020.626861

[36] Laemmli, U. K. (1970). Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *nature*, *227*(5259), 680-685. https://doi.org/10.1038/227680a0

[37] Romani, A., Baldi, A., Tattini, M., & Vincieri, F. F. (1994). Extraction, purification procedures and HPLC-RI analysis of carbohydrates in olive (*Olea europaea* L.) plants. *Chromatographia*, *39*, 35-39. https://doi.org/10.1007/BF02320455

[38] Arzani, K., Hokmabadi, H., & Dehghani-Shuraki, Y. (2001). Effects of foliar application of some carbohydrates on qualitative and quantitative traits of pistachio nuts cv. Kalleh-Ghoochi. In *International Symposium on Foliar Nutrition of Perennial Fruit Plants 594* (pp. 291-295). https://doi.org/10.17660/ActaHortic.2002.594.34

[39] Khater, M. A., Dawood, M. G., Sadak, M. S., Shalaby, M. A., El-Awadi, M. E., & El-Din, K. G. (2018). Enhancement the performance of cowpea plants grown under drought conditions via trehalose application. *Middle East J*, *7*(3), 782-800. https://api.semanticscholar.org/CorpusID:212428980

[40] Crowe, J.H., Hoekstra, F.A. & Crowe, L.M. (1992). Anhydrobiosis. Annu. Rev. Physiol. 54: 570– 599. https://doi.org/10.1146/annurev.ph.54.030192.003051

[41] Lunn, J. E., Delorge, I., Figueroa, C. M., Van Dijck, P., & Stitt, M. (2014). Trehalose metabolism in plants. *The Plant Journal*, *79*(4), 544-567.https://doi.org/10.1111/tpj.12509

[42] Sadak, M. S. (2016). Mitigation of drought stress on fenugreek plant by foliar application of trehalose. *International Journal of Chemistry Technology Research*, *9*, 147-155. https://api.semanticscholar.org/CorpusID:212499581

[43] Bougouffa, S., Radovanovic, A., Essack, M., & Bajic, V. B. (2014). DEOP: a database on osmoprotectants and associated pathways. *Database*, 2014, bau100. https://doi.org/10.1093/database/bau100

[44] Lin, Y., Zhang, J., Gao, W., Chen, Y., Li, H., Lawlor, D. W. & Pan, W. (2017). Exogenous trehalose improves growth under limiting nitrogen through upregulation of nitrogen metabolism. *BMC plant biology*, *17*, 1-13. https://doi.org/10.1186/s12870-017-1207-z

[45] Aldrete-Herrera, P. I., López, M. G., Medina-Torres, L., Ragazzo-Sánchez, J. A., Calderón-Santoyo, M., González-Ávila, M., & Ortiz-Basurto, R. I. (2019). Physicochemical composition and apparent degree of polymerization of fructans in five wild agave varieties: potential industrial use. *Foods*, *8*(9), 404. https://doi.org/10.3390/foods8090404

[46] Marx, S. P., NÖSBERGER, J., & Frehner, M. (1997). Seasonal variation of fructan-β-fructosidase (FEH) activity and characterization of a β-(2-1)-linkage specific FEH from tubers of Jerusalem artichoke (*Helianthus tuberosus*). *The New Phytologist*, *135*(2), 267-277. https://doi.org/10.1046/j.1469-8137.1997.00641.x

[47] Van den Ende, W., Clerens, S., Vergauwen, R., Van Riet, L., Van Laere, A., Yoshida, M., & Kawakami, A. (2003). Fructan 1-exohydrolases. β-(2, 1)-trimmers during graminan biosynthesis in stems of wheat? Purification, characterization, mass mapping, and cloning of two fructan 1-exohydrolase isoforms. *Plant Physiology*, *131*(2), 621-631. https://doi.org/10.1104/pp.015305

[48] Verhaest, M., Lammens, W., Le Roy, K., De Ranter, C. J., Van Laere, A., Rabijns, A., & Van den Ende, W. (2007). Insights into the fine architecture of the active site of chicory fructan 1-exohydrolase: 1-kestose as substrate vs Sucrose as inhibitor. *New Phytologist*, *174*(1), 90-100. https://doi.org/10.1111/j.1469-8137.2007.01988.x

[49] Asega, A. F., do Nascimento, J. R. O., Schroeven, L., Van den Ende, W., & Carvalho, M. A. M. (2008). Cloning, characterization and functional analysis of a 1-FEH cDNA from *Vernonia herbacea* (Vell.) Rusby. *Plant and cell physiology*, *49*(8), 1185-1195. https://doi.org/10.1093/pcp/pcn094

[50] Lothier, J., Van Laere, A., Prud'homme, M. P., Van den Ende, W., & Morvan-Bertrand, A. (2014). Cloning and characterization of a novel fructan 6-exohydrolase strongly inhibited by Sucrose in *Lolium perenne*. *Planta*, *240*, 629-643. https://doi.org/10.1007/s00425-014-2110-6

[51] El Gengaihi, S. A., Enein, A. A., Abou Elalla, F. M., & Abou Baker, D. H. (2009). Molecular characterizations and antimicrobial activities of chicory and Jerusalem artichoke plants. *Int. J. Acad. Res, 1*(2), 66-71.

[52] Shao, T., Chen, Y., Gao, X., Zhou, Z., Long, X., & Rengel, Z. (2023). Salt stress affects the biomass of industrial crop Jerusalem artichoke by affecting sugar transport and metabolism. *Heliyon*, *9*(3). https://doi.org/10.1016/j.heliyon.2023.e14107

[53] Lv, S., Wang, R., Xiao, Y., Li, F., Mu, Y., Lu, Y., & Zhao, C. (2019). Growth, yield formation, and inulin performance of a non-food energy crop, Jerusalem artichoke (*Helianthus tuberosus* L.), in a semi-arid area of China. *Industrial crops and products*, *134*, 71-79. https://doi.org/10.1016/j. indcrop.2019.03.064

[54] Luo, R., Song, X., Li, Z., Zhang, A., Yan, X., & Pang, Q. (2018). Effect of soil salinity on fructan content and polymerization degree in the sprouting tubers of Jerusalem artichoke (*Helianthus tuberosus* L.). *Plant Physiology and Biochemistry*, *125*, 27-34. https://doi.org/10.1016/j.plaphy.2018.01.025

[55] Alam, M. M., Nahar, K., Hasanuzzaman, M., & Fujita, M. (2014). Trehalose-induced drought stress tolerance: A comparative study among different Brassica species. *Plant Omics*, *7*(4), 271-283.https://search.informit.org/doi/10.3316/informit.580352339413763

[56] Shafiq, S., Akram, N. A., & Ashraf, M. (2015). Does exogenously applied trehalose alter oxidative defense system in the edible part of radish (*Raphanus sativus* L.) under water-deficit conditions? *Scientia Horticulturae*, *185*, 68-75. https://doi.org/10.1016/j.scienta.2015.01.010

[57] Akram, N. A., Waseem, M., Ameen, R., & Ashraf, M. (2016). Trehalose pretreatment induces drought tolerance in radish (*Raphanus sativus* L.) plants: some key physio-biochemical traits. *Acta physiologiae plantarum*, *38*, 1-10. https://doi.org/10.1007/s11738-015-2018-1

[58] El-Metwaly, H. (2021). Response of yield and quality of sweet potato plants to irrigation intervals and some soil and foliar application treatments. *Journal of Productivity and Development*, *26*(1), 61-95.

الملخص العربي

تحسين إنتاجية مادة الإنيولين في نبات الطرطوفة من خلال الرش الورقي لبعض السكريات

دينا صقر*[١] ، محمد عبد الستار[٢]، تهاني حتحوت[١]، سامية الخلال[١]، سامح حسنين[٣]، زينب عبد الجواد[١]

[١]قسم النبات ،كلية البنات للآداب والعلوم والتربية، القاهرة، مصر.
[٢]معهد الهندسة الوراثية، مركز البحوث الزراعية، الجيزة، مصر.
[٣]كلية التكنولوجيا الحيوية، جامعة النيل، الجيزة، مصر.

الملخص العربي

أجريت تجربة حقليه في المزرعة التابعة لمعهد بحوث الهندسة الوراثية الزراعية (AGERI)، مركز البحوث الزراعية (ARC)، لدراسة استجابة نبات الطرطوفه لرش الاوراق بالرافينوز، والديكسترين، والسكروز بتركيزات ١٠، ٢٥، ٥٠، و١٠٠ ميلليمولر، بالإضافة إلى الكنترول (ماء الصنبور فقط) مع ٣ متكررات. القياسات التي تم اختبارها هي الوزن الرطب للنبات و الوزن الجاف و ارتفاع النبات و عدد الفروع الرئيسية و الجانبية لكل نبات و مساحة الورقة كما تم تقدير الاصباغ النباتيه و محتوي الكربوهيدرات و محتوي الانيولين و تقدير نشاط انزيم فركتان إكسوهيدرولاز (FEH) و تحليل التفريد الكهربي للبروتين SDS-PAGE و تقديرمحتوى السكريات الذائبة بواسطة HPLC و عدد الدرنات الناتجة لكل نبات و إنتاجية النبات و إنتاجية الفدان الإجمالية . وأشارت النتائج إلى أن رش النباتات بالرافينوز والدكسترين بمستوى ٥٠ ملم إلى تعزيز النمو وارتفاع خصائص إنتاجية الكربوهيدرات الكلية والأنولين. بالإضافة إلى ذلك، أظهرت جرعة رافينوز ٥٠ ملم نشاط تثبيطى واعد لإنزيم فركتان إكسوهيدرولاز (FEH) مما يزيد من إنتاج الأنيولين ومحصول الدرنات، تليها نفس الجرعة من الدكسترين والسكروز. بناءً على هذا الافتراض تشير نتائج هذه الدراسة إلى أن الإستخدام الخارجي لسكريات معينة مثل الرافينوز والدكسترين، كمادة صديقة للبيئة يمكن ان يحسن النمو الإجمالي وتطور وإنتاجية نبات الطرطوفة.