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Effect of spraying with chitosan and whey on the content of nutrients and active compounds in leaves of *Duranta erecta* L.

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Abstract

The experiment was conducted in lathhouse of the Department of Horticulture and Landscape Engineering, College of Agriculture / University of Basra during the agricultural seasons (2022 and 2023) on Duranta plants, to know the effect of spraying different levels of chitosan at levels (0, 100, 200) mg L^{-1} and whey, which are (0, 75, 150) ml L^{-1}, on the content of nutrients and active compounds of Duranta plant leaves. The study followed the design of complete randomized sectors for factorial experiments with two factors of spraying chitosan and whey, with three concentrations for each, with three replicates for the treatment, so that the number of experimental units became 27 experimental units. The least significant difference (LSD) test was used at a significance level of 0.05) to compare the averages. The results show that the plants sprayed with chitosan at a concentration of 200 mg L^{-1} were significantly excelled and gave the highest nitrogen content in the leaves, which amounted to (3.070 and 3.483) %, phosphorus (0.260 and 0.286) % and potassium (2.486 and 2.742) %. The leaf content of active compounds increased when treated with chitosan at a concentration of 200 mg L^{-1}, as the phenols content in the leaves reached (215.359 and 378.491) mg g^{-1} and flavonoids reached (20.257 and 19.426) mg 100 g^{-1}, respectively, for both seasons of the study. The plants that were sprayed with whey protein at a concentration of 150 ml L^{-1} were significantly superior in giving the highest content of nutrients in their leaves, as the nitrogen content reached (3.439 and 3.769) %, phosphorus reached (0.269 and 0.300) % and potassium reached (2.596 and 2.906) %. The content of the leaves of the plant also increased. Duranta is one of the active compounds when sprayed with high concentration of whey, as the content of phenols reached (223.177 and 374.827) mg g^{-1} and flavonoids (21.232 and 20.271) mg 100 g^{-1}in the leaves respectively for both seasons of the study.

Keywords

chitosan; Duranta; flavonoids; phenols; whey

Introduction

Duranta plants are one of the most important branches of ornamental plants, which are beautiful and famous shrubs found all over the world and are usually used as plant hedges (1). Duranta has several names, the most important of which are the golden dewdrop, the flower of heaven, the whisper of angels, pigeon berries and the Brazilian flower of heaven (2). In addition to the importance of the Duranta plant as an ornamental plant, it is one of the most important medicinal plants, due to its content of active substances that have

been used in many medical fields. The Duranta plant has shown toxic effects on cancer cells, especially breast cancer cells, which paves the way for more research on its partial mechanism in treating cancer (3). The fruits and leaves have also been used in traditional folk medicine to treat malaria and worms. It also works as a diuretic, in addition to having anti-tumor and anti-bacterial activity (4). The Duranta plant has also shown many biological activities. It is antimicrobial, antioxidant and anti-cancerous. It protects the liver and nerves and relieves osteoarthritis due to its content of active compounds, estimated at 64 compounds, such as glycosides, flavonoids, phenols, steroids, terpenes and saponins (5, 6). It has many natural uses, as it is a promising agent in biological control against insects (7). The extract of the fruits of the Duranta plant showed great activity against malaria (8). Duranta essential oil also contains many biologically active compounds such as (β-sitosterol; naringenin; acteoside; lamiide; sucrose; raffinose) (9). The leaves of the Duranta plant also contain (limonene, β-caryophyllenel, pentadecanal, 1-octen-3-ol, α-humulene), which are among the most important active substances in the leaves (10). Therefore, it is necessary to pay attention to its cultivation and use modern methods in plant management and service and the most important of these methods is the use of stimulants, biofertilizers and growth regulators.

Chitosan is a biopolymer with the chemical formula ($C_6H_{11}O_4N$) n and its chemical name is Poly (1,4)-2-amino-2-deoxy-B-d-glucose (11). Chitosan is also known as a polysaccharide, which can be practically obtained by removing acetyl from chitin, a long-chain polymer of N-acetyl -glucosamine that is easily extracted from fungal cells and the exoskeleton of oysters, shrimp and other shellfish. These shell and crustacean materials are ground together and then subjected to a chemical stripping process to extract a part of the composition of these materials called acetyl, so that the chemical composition of chitosan becomes ready and carries a positive charge of ammonium that works to attract molecules of other materials that carry a negative charge (12). The positive charge of chitosan gives the polymer many unique physiological and biological properties. As a result of separating the acetyl group from chitin and its content of a free amine group provides it with a high ability to bind with the negative charge in fats, oils, cholesterol, negative metal ions, proteins and other large molecules that distinguish it from chitin to become ß-1,4- β-1,4-Poly-D-glucosamine. To gain its chemical properties (13-15) it was found that spraying chitosan at concentrations of 100, 200 and 300 mg L^{-1} on Duranta versicolor plants caused a significant increase in the leaf content of elements, as the plants are sprayed at a concentration of 300 mg L^{-1} gave the highest nitrogen content in the leaves, reaching 2.89 and 3.03 % for both growing seasons 2017 and 2018, respectively, while the lowest nitrogen content in the leaves was 1.42 and 1.49 % for both seasons, respectively, in control treatment. The same treatment also gave the highest phosphorus content in the leaves, reaching 0.644 and 0.679 % compared to the control treatment, which gave 0.351 and 0.370 % for both study seasons, respectively and gave the highest. The potassium content in the leaves reached 2.64 and 2.76 % compared to the control treatment that gave 1.64 and 1.70 % for both study seasons, respectively. It was showed that spraying spinach plants (*Spinacia oleracea*) with five concentrations of chitosan, which are (0, 100, 150, 200, 250, 300) parts per million, led to a significant increase in the phosphorus content of the leaves at concentrations of 200 and 250 ppm and potassium increased at concentrations of 150 and 250 parts per million compared to control treatment (16).

Many studies show the effect of chitosan on improving the content of active compounds in leaves. Previous research concluded that spraying sage plants (*Salvia officinalis* L.) at a concentration of 0.50 g L^{-1} led to an increase in the content of total phenols and flavonoids and the amount of oil in the leaves compared to the control treatment (17). Spraying Cordyline plants with five concentrations of chitosan 0, 25, 50, 75, 100 mg L^{-1} exceeded the 100 mg L^{-1} treatment and resulted in the highest total phenols content in the leaves, with an increase rate of 35.8 % compared to the control treatment (18). Spraying *Tecoma stans* (L.) Juss. ex Kunth seedlings with normal or nano chitosan at concentrations of 0, 150 and 300 mg L^{-1} increased the leaf content of the nutrients N, P and K. The 300 mg L^{-1} treatment resulted in the highest nutrient content, reaching 2.232 % for N, 1.223 % for K and 0.311 % for P, compared to the control treatment, which had the lowest values at 1.591 %, 1.142 % and 0.254 %, respectively (19).

Whey is a by-product of the dairy industry and one of the most important nutrients due to its content of protein, lactose, phosphorus, potassium, calcium, magnesium and manganese (20). Whey is added as a supplement to the fertilization process to reduce the number of added fertilizers that cause many environmental problems. Whey contains many important elements for the plant, as one ton contains 1.36 kg of nitrogenous compounds, 0.314 kg of phosphorus compounds, 1.59 kg of potassium salts and small amounts of magnesium and calcium (21, 22). Previous research showed that adding four concentrations of whey, namely 0, 8, 12, 24 litres ha^{-1} to Chinese cabbage plants, led to improving the content of nutrients in plant leaves (23), the concentration exceeded 24 litres ha^{-1} and gave the highest content of nitrogen, phosphorus and potassium, reaching 2.547, 0.316 and 1.403 % respectively, compared to control treatment, which gave the lowest content of elements, reaching 2.163, 0.276 and 1.301 % respectively.

Therefore, the study aims to know the extent of the effect of spraying different concentrations of chitosan and whey in improving the content of nutrients and active compounds in the leaves of Duranta plants under environmental conditions in Basra Governorate.

Materials and Methods

The experiment was conducted in the canopy of the Department of Horticulture and Landscape Engineering, College of Agriculture / University of Basra during the two seasons 2022 and 2023 on Duranta plants, to know the effect of spraying different levels of benzyl adenine 0, 75, 150 mg L^{-1} and chitosan at levels 0, 100, 200 mg L^{-1} in growth traits. Some chemical traits of the leaves of the Duranta plant. Seedlings were brought from one of the private nurseries in Baghdad at

one year old and transferred to plastic anvils with a diameter of 25 cm, filled with a medium consisting of 2 mol + 1 peat moss and several random samples were taken. of the soil before planting and some chemical and physical traits of the potting soil were estimated in the central laboratory of the College of Agriculture / University of Basra, Table 1.

Table 1. Some primary traits of the soil used in the study

Particle size			Texture	PH	EC	O.M	N	P	K
Clay (%)	Silt (%)	Sand (%)			dsm^{-1}	%	mg L^{-1}		
10.0	8.0	82.0	Loamy Sand	7.34	1.0	26	53	26.3	66.4

Studied traits

Leaf content of macro elements: According to the method mentioned by Cresser and Parsons (24), the leaf content of major elements was estimated according to the following steps:

1. 0.2 of the dry ground sample was taken for each experimental unit and placed in the digestion tube.
2. 5 ml of concentrated sulfuric acid was added and left for 24 hr (overnight).
3. The digestion device was heated until it reached a temperature of 400 °C.
4. The samples were placed in the device for half an hour until boiling.

3 ml of the acid mixture (4 mL of concentrated perchloric acid + 96 mL of concentrated sulfuric acid) was added and then heated until the solution became clear. The solution was left to cool and the volume was completed to 50 mL with distilled water.

Nitrogen percentage in leaves %: Total nitrogen was measured in the digested samples using a Microkjeldahl steam distillation device according to the method of Page et al (25).

Phosphorus percentage in leaves %: Phosphorus percentage in leaves was estimated using a Spectrophotometer with a wavelength of 470 nm according to the method of Murphy and Riley (26).

Potassium percentage in leaves %: Potassium was estimated in the leaves using a Flame photometer type PEP 73 JEN WA 73 according to the method of (25). The results are expressed according to the standard curve in which potassium chloride is used.

Active ingredients in the leaves

Preparation of extract: The plant extract was prepared by taking 1 g of the dry plant sample and adding 10 mL of the prepared solution consisting of 70 % ethanol and 100 µL of hydrochloric acid HCl, then leaving it for three days and then filtering it and evaporating it with a rotary evaporator at a temperature of 40 °C

Total phenols content in the leaves: (mg 100 g^{-1}) 450 µL of Follen's reagent were taken and 450 µL of distilled water were added to it, the mixture was mixed and 50 µL of the prepared extract were added to it, then left for 5 min at room temperature and then 500 µL of 15 % sodium carbonate were added to it, then mixed and left for 60 min, then the absorbance was measured at a wavelength of 765 nm using a spectrophotometer and the phenols were estimated using the curve using calcidic acid (27).

Total flavonoids content in leaves: The total flavonoids in the leaves were estimated using the method described by Borrás-Linares I et al (27) and based on the standard curve of quercetin, 0.5 mL of the filtrate was taken and 1.5 mL of methanol was added to it and mixed, then 0.1 of 10 % aluminium chloride was added to it and 1 M of potassium acetate was prepared and 2.8 mL distilled water was added to it and left for 30 min, then measured by a spectrophotometer at a wavelength of 415 nm and flavonoids were estimated using quercetin as a standard substance.

Statistical analysis

The experiment was conducted according to a Randomized Complete Block Design (R.C.B.D) and in a factorial experiment with two factors, the first representing spraying chitosan at three levels 0, 100, 200 mg L^{-1} and the second factor representing spraying whey at three levels 0, 75, 150 ml L^{-1}. The number of experimental treatments was 9 factorial treatments and the number of replicates for each treatment was 3 replicates. The total number of experimental units was 27 experimental units. The statistical analysis of the experiment data was done using the Genestat statistical program and the Least Significant Differences (L.S.D) test was used at a probability level of 0.05 to compare the averages of the treatments.

Results

Nutrient content of Duranta leaves

The results in Table 2 shows the significant effect of spraying with chitosan on the content of Duranta leaves of nitrogen, phosphorus and potassium, as the plants treated with a concentration of 200 mg L^{-1} recorded the highest nitrogen content in their leaves for both study seasons, reaching 3.070 and 3.483 %, respectively, compared to control plants, whose average nitrogen content of leaves reached 2.310 and 2.723 %, respectively, for both seasons, respectively. It also gave the highest phosphorus content in the leaves, reaching 0.260 and 0.286 %, respectively, compared to control plants, whose average phosphorus content reached 0.212 and 0.238 %, respectively, for both study seasons, while the potassium content of the leaves reached 2.486 and 2.742 %, respectively, compared to control plants, whose average Its potassium content was 1.591 and 1.848 % respectively for both seasons. As for the effect of spraying with whey, the results of Table 2 showed the moral superiority of plants sprayed with whey at a concentration of 150 mL L^{-1} on plants sprayed with other concentrations for both seasons, as the highest nitrogen content in their leaves reached 3.439 and 3.769 % compared to control plants whose nitrogen content in their leaves reached 1.841 and 2.341 %, respectively. The plants sprayed with the same concentration also surpassed morally in the phosphorus content of their leaves, as it reached 0.269 and 0.300 % compared to control plants whose phosphorus content reached 0.189 and 0.210 % for both seasons,

Table 2. Effect of spraying chitosan and whey on the content of nutrients in the leaves of Duranta plants

whey	Chitosan	Nitrogen content in leaves (%)		Phosphorus content in leaves (%)		Potassium content in leaves (%)	
		2022	2023	2022	2023	2022	2023
0	0	1.490	1.990	0.168	0.189	1.124	1.334
	100	1.900	2.400	0.186	0.207	1.475	1.685
	200	2.133	2.633	0.214	0.235	1.894	2.104
75	0	2.460	2.870	0.222	0.247	1.629	1.879
	100	2.887	3.297	0.258	0.283	1.970	2.220
	200	3.170	3.580	0.277	0.302	2.370	2.620
150	0	2.980	3.310	0.246	0.277	2.020	2.330
	100	3.430	3.760	0.272	0.303	2.575	2.885
	200	3.907	4.237	0.290	0.321	3.193	3.503
	LSD	0.1371	0.1360	0.0071	0.0069	N.S.	0.0877
whey	0	1.841	2.341	0.189	0.210	1.498	1.708
	75	2.839	3.249	0.252	0.277	1.990	2.240
	150	3.439	3.769	0.269	0.300	2.596	2.906
	LSD	0.0792	0.0780	0.0041	0.0039	0.1252	0.0506
Chitosan	0	2.310	2.723	0.212	0.238	1.591	1.848
	100	2.739	3.152	0.239	0.264	2.007	2.263
	200	3.070	3.483	0.260	0.286	2.486	2.742
	LSD	0.0792	0.0780	0.0041	0.0039	0.1252	0.0506

respectively. The highest potassium content reached 2.596 and 2.906 % compared to control plants whose potassium content reached 1.498 and 1.708 % for both seasons of the study. In sequence. As the results in Table 2 shows the significant effect of the interaction between chitosan and whey protein on the nitrogen and phosphorus content of leaves for both seasons of the study, the interaction between 200 mg L^{-1} chitosan + 150 mL L^{-1} whey protein) was significantly superior to the rest of the treatments and gave the highest nitrogen content in its leaves, reaching 3.907 and 4.237 % compared to control plants, whose average nitrogen content was 1.490 and 1.990 % for both seasons of the study, respectively. This treatment also gave the highest phosphorus content in its leaves, reaching 0.290 and 0.321 % compared to control plants, whose average phosphorus content was 0.168 and 0.189 %, respectively, for both seasons. As for the interaction effect on the potassium content, it was significant in the second season only. The interaction treatment between 200 mg L^{-1} chitosan and 150 mL L^{-1} whey) the highest content reached 3.503 % compared to control treatment which gave the lowest potassium content reached 1.334 %.

Active compounds in the leaves of Duranta plants

The results of Table 3 shows the significant effect of spraying with chitosan on the content of active compounds in the leaves of Duranta plants, as when comparing the averages of the treatments, the plants treated with chitosan showed significant differences between them, as the plants treated with 200 mg L^{-1} of chitosan recorded a significant superiority in the content of their leaves of total phenols, which reached 27.116 and 29.559 mg g^{-1} and the highest content of total flavonoids in the leaves reached 20.257 and 19.426 mg 100 g^{-1} for both seasons of the study, in succession, compared to control treatment, which gave the lowest content of total phenols in its leaves, which reached 24.779 and 27.213 mg g^{-1} and the lowest content of total flavonoids, which reached 15.313 and 14.864 mg 100 g^{-1} For both study seasons, respectively. As for the effect of spraying with milk whey on the content of active compounds in the leaves, it was significant, as the results of Table 3 showed that the plants sprayed with milk whey at a concentration of 150 mL L^{-1} were superior in the content of their leaves of total phenols, which reached 36.387 and 36.838 mg g^{-1} and the highest content of total flavonoids reached 21.232 and 20.271 mg 100 g^{-1} for

Table 3. Effect of spraying chitosan and whey on some active compounds in the leaves of Duranta plants

whey	Chitosan	Total phenolics content (mg g^{-1})		Total flavonoids content (mg/100g^{-1})	
		2022	2023	2022	2023
0	0	15.635	18.755	11.273	11.253
	100	16.591	20.262	13.523	13.270
	200	17.601	21.646	16.900	15.243
75	0	23.972	27.313	15.683	15.097
	100	25.182	27.984	19.067	19.203
	200	25.864	28.846	20.357	21.163
150	0	34.728	35.571	18.983	18.243
	100	36.549	36.756	21.200	20.700
	200	37.884	38.186	23.513	21.870
	LSD	0.5501	0.2493	N.S.	1.1769
whey	0	16.609	20.221	13.899	13.256
	75	25.006	28.048	18.369	18.488
	150	36.387	36.838	21.232	20.271
	LSD	0.3176	0.1439	0.8571	0.6795
Chitosan	0	24.779	27.213	15.313	14.864
	100	26.107	28.334	17.930	17.724
	200	27.116	29.559	20.257	19.426
	LSD	0.3176	0.1439	0.8571	0.6795

both study seasons, respectively, compared to control treatment, which gave the lowest content of total phenols in its leaves, which reached 16.609 and 20.221 mg g^{-1} and the lowest content of total flavonoids in the leaves, which reached 13.899 and 13.256 mg 100 g^{-1} for both study seasons, respectively. As for the effect of the interaction between the two study factors on the content of active compounds in the leaves of Duranta plants, Table 3 shows that there are significant effects of the interaction in this trait, as the plants that were sprayed (200 mg L^{-1} chitosan with 150 mL L^{-1} whey) significantly outperformed the rest of the treatments in the content of their leaves of total phenols, as they gave the highest content of total phenols in the first and second seasons, reaching 37.884 and 38.186 mg g^{-1} compared to control treatment, which gave the lowest content of total phenols, reaching 15.635 and 18.755 mg g^{-1}. They also significantly outperformed in the content of their leaves of total flavonoids in the second season, reaching 21.870 mg 100 g^{-1} compared to control treatment, which gave the lowest content of total flavonoids in its leaves, reaching 11.253 mg 100 gm^{-1}.

Discussion

We noted from Table 2 that high concentrations of chitosan spraying are superior in improving the leaf content of nutrients and active compounds. This may be because chitosan treatment plays a role in increasing the biomass of roots and shoots and stimulates the pathways of building auxin and cytokinin and stimulates the activity of enzymes (28). Or it may be because chitosan stimulates the series of primary and secondary metabolic pathways for building carbon and N. It may stimulate the levels of CO_2, N and P fixation, which leads to increased production of sucrose, which provides carbon structures for the synthesis of phospholipids and antioxidants (29). Or it may be due to the fact that chitosan works to increase the absorption of nutrients and increase the net rate of photosynthesis, build phenols and flavonoids and works to increase the activity of enzymes (30, 31). As there was a superiority of high concentrations of whey protein and the reason for improving the content of nutrients and active compounds of the leaves of the Duranta plants is attributed to the role of whey protein, which contains many nutrients on the one hand and increases their readiness on the other hand, in addition to containing amino acids, organic acids and proteins. This led to an increase in the percentage of nitrogen in the leaves due to the presence of a sufficient amount of nitrogen in the whey protein (32). Also, whey protein is a nutrient because it contains several elements, including phosphorus and thus increases its absorption by the green group of plants, which led to an increase in the percentage of phosphorus in the leaves of the Duranta plants with an increase in the concentration of whey protein.

Conclusion

Considering the field experiment, spraying chitosan led to an increase in the content of the nutrients important for plant growth and active compounds in the leaves. Spraying Duranta plants with whey protein also led to positive results in improving the active compounds through the positive relationship between the two factors, which led to improving the cultivation of Duranta plants and their tolerance to climatic conditions in Basra Governorate.

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Authors' contributions

All authors made equal contributions in conducting the research and drafting the manuscript.

Compliance with ethical standards

Conflict of interest: The writers admitted no conflict of interest.

Ethical issues: None

References

1. Said RM. Response of sky flower (*Duranta erecta* L. var. *Variegata*) transplants as pot-plant to growing media and water amounts. Middle East Journal of Agriculture Research 2016;5(2):201-7.
2. Butle S, Wagh A, Jadhav P. Plant profile, phytochemical and pharmacological properties of *Duranta erecta* (golden dew drop): a review. Asian Journal of Pharmacognosy. 2020;4(2):42-9.
3. Puri AV. *Duranta Repens* Linn. (Verbenaceae): A comprehensive review of pharmacognostic, ethnomedicinal, pharmacological and phytochemical aspects. Asian Journal of Pharmaceutical and Clinical Research 2018;11(11):91-6. https://doi.org/10.22159/ajpcr.2018.v11i11.28509
4. Bhar K, Kanthal LK, Manna S, Nagalaxmi P, Eswar K, Satya C. *In-vitro* cytotoxic activity and anthelmintic activity of chloroform extract of *Duranta erecta L.* ripe fruits. Research Journal of Pharmaceutical, Biological and Chemical Sciences. 2016;7(6):860-5.
5. Srivastava M, Shanker K. *Duranta erecta* Linn: A critical review on phytochemistry, traditional uses, pharmacology and toxicity from phytopharmaceutical perspective. Journal of Ethnopharmacology. 2022;293:115274. https://doi.org/10.1016/j.jep.2022.115274
6. Sahu A, Jain D. A review of the extent of explored potential for phytochemical and pharmacological activity of *Duranta erecta* leaves. International Journal of Pharmaceutical Sciences and Research. 2023;14(3):1071-75.
7. Julia AE, Gregory OE, Felicia E, Michael U, Godwin N, Chinelo E. Bioefficacy of *Duranta erecta* leaf extract on yellow fever and dengue vector, *Aedes aegypti* Linn. in Nigeria. Journal of Medicinal Plants Research. 2018;12(11):124-32. https://doi.org/10.5897/JMPR2016.6277
8. Castro O, Barrios M, Chinchilla M, Guerrero O. Chemical and biological evaluation of the effect of plant extracts against *Plasmodium berghei.* Revista de Biología Tropical / International Journal of Tropical Biology and Conservation. 1996;44(2A):361-7.
9. Abou-Setta LM, Nazif NM, Shahat AA. Phytochemical investigation and antiviral activity of *Duranta repens.* Journal of Applied Sciences Research. 2007;3(11):1426-33.

10. Thomas PS, Essien EE, Ascrizzi R, Flamini G. Composition of volatile oils from *Duranta repens* leaves and fruits. Chemistry of Natural Compounds. 2019;55(2):359-60. https://doi.org/10.1007/s10600-019-02690-y
11. Pandey P, Verma MK, De N. Chitosan in agricultural context: A review. Bulletin of Environment, Pharmacology and Life Sciences. 2018;7(4):87-96.
12. Orzali L, Corsi B, Forni C, Riccioni L. Chitosan in agriculture: A new challenge for managing plant disease. In: Shalaby EA, editor. *Biological Activities and Application of Marine Polysaccharides.* INTECH; 2017. p. 17-36. https://doi.org/10.5772/66840
13. Suk YO. Interaction of breed-by-chitosan supplementation on growth and feed efficiency at different supplementing ages in broiler chickens. Asian-Australasian Journal of Animal Sciences. 2004;17(12):1705-11. https://doi.org/10.5713/ajas.2004.1705
14. Younes I, Rinaudo M. Chitin and chitosan preparation from marine sources: Structure, properties and applications. Marine Drugs. 2015;13(3):1133-74. https://doi.org/10.3390/md13031133
15. Nofal EMS, Menesy FA, El-Hady A, Shehab El-Deen EG. Improving vegetative growth, root traits and some chemical constituents of *Duranta erecta* Var. *Variegata* by using humic acid, salicylic acid and chitosan. International Journal of Plant Production. 2020;11(10):945-9. https://doi.org/10.21608/jpp.2020.124287
16. Nipa NA, Das P, Nasrin H, Zakir HM, Rahman A, Quadir QF. Growth, yield and biochemical qualities of spinach (*Spinacia oleracea*) being influenced by the foliar application of chitosan. Journal of Experimental Agriculture International. 2023;45(8):30-40. https://doi.org/10.9734/jeai/2023/v45i82152
17. Vosoughi N, Gomarian M, Pirbalouti AG, Khaghani S, Malekpoor F. Essential oil composition and total phenolic, flavonoid contents and antioxidant activity of sage (*Salvia officinalis* L.) extract under chitosan application and irrigation frequencies. Industrial Crops and Products. 2018;117:366-74. https://doi.org/10.1016/j.indcrop.2018.03.021
18. El-Serafy RS. Phenotypic plasticity, biomass allocation and biochemical analysis of cordyline seedlings in response to oligo-chitosan foliar spray. Journal of Plant Nutrition and Soil Science. 2020;20(3):1503-14. https://doi.org/10.1007/s42729-020-00229-7
19. Al-Sharifi AHH. Biostimulation with regular and nano chitosan in some growth characteristics of seedlings *Tecoma stans (L.) Juss. Ex Kunth.* [Master's thesis]. Karbala: University of Karbala, College of Agriculture; 2023.
20. Demir S, Şensoy S, Ocak E, Tüfenkçi Ş, Durak ED, Erdinc C, et al. Effects of arbuscular mycorrhizal fungus, humic acid and whey on wilt disease caused by *Verticillium dahliae* Kleb. in three solanaceous crops. Turkish Journal of Agriculture and Forestry. 2015;39(2):300-9. https://doi.org/10.3906/tar-1403-39
21. Brink AW. The faces of whey. Allergy Causes Nutr Remedies. 2002 Jan;48-3.
22. Bahaa AJA, Aati ASA, Abdul-Razzaq SA. The role of whey and organic matter in some chemical and physical properties of soil. Diyala The Journal of Agricultural Science. 2006;2(1).
23. Aleyashe HH, Obaid MH. The Effect of Adding Whey and Spraying with Silicone on the Chemical of Chinese Cabbage Plant *Brassica campestris var. Pekinensis.* IOP Conference: Series Earth and Environmental Science. 2023;1259(1):012051. https://doi.org/10.1088/1755-1315/1259/1/012051
24. Cresser MS, Parsons JW. Sulphuric-perchloric acid of digestion of plant material for determination of nitrogen, phosphorus, potassium, calcium and magnesium. Analytica Chimica Acta. 1979;109(2):431-6. https://doi.org/10.1016/S0003-2670(01)84273-2
25. Page AL, Miller RH, Keney DR. Methods of soil analysis. Part 2. 2nd ed. Madison, WI: Son, Wisconsin, USA; 1982. p. 1159.
26. Murphy J, Riley JP**.** A modified single solution method for determination of phosphate in natural waters. *Analytica Chimica Acta.* 1962;27:31-6. https://doi.org/10.1016/S0003-2670(00)88444-5
27. Borrás-Linares I, Fernández-Arroyo S, Arráez-Roman D, Palmeros-Suárez PA, Del Val-Díaz R, Andrade-Gonzáles I, et al. Characterization of phenolic compounds, anthocyanidin, antioxidant and antimicrobial activity of 25 varieties of Mexican Roselle (*Hibiscus sabdariffa*). Industrial Crops and Products. 2015;69:385-94. https://doi.org/10.1016/j.indcrop.2015.02.053
28. Colman SL, Salcedo MF, Mansilla AY, Iglesias MJ, Fiol DF, Martín-Saldaña S, et al. Chitosan microparticles improve tomato seedling biomass and modulate hormonal, redox and defense pathways. Plant Physiology and Biochemistry. 2019;14:203-11. https://doi.org/10.1016/j.plaphy.2019.09.002
29. El Amerany F, Rhazi M, Balcke G, Wahbi S, Meddich A, Taourirte M, et al. The effect of chitosan on plant physiology, wound response and fruit quality of tomato. Polymers. 2022;14(22):5006. https://doi.org/10.3390/polym14225006
30. Pichyangkura R, Chadchawan S. Biostimulant activity of chitosan in horticulture. Scientia Horticulturae. 2015;196:49-65. https://doi.org/10.1016/j.scienta.2015.09.031
31. Van SN, Minh HD, Anh DN. Study on chitosan nanoparticles on biophysical characteristics and growth of Robusta coffee in greenhouse. Biocatalysis and Agricultural Biotechnology. 2013;2(4):289-94. https://doi.org/10.1016/j.bcab.2013.06.001
32. Abdul Hamid BA. The role of whey in the growth of yellow corn and the microbial community in the soil. Anbar Journal of Agricultural Sciences. 2009;7(1):47-56. https://doi.org/10.32649/ajas.2009.14772