Response of chemically active compounds and some anatomical features of water hyacinth (*Eichhornia crassipes*) leaves grown in glass basins to the phosphate agent in Al-Diwaniyah Governorate

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Abstract

Eichhornia crassipes (Mart) Solms belongs to the family Pontederiaceae. The present study showed the response of chemical and anatomical characteristics to treatment with different concentrations of phosphate factor (0.19, 0.12, 0.05, 0.015) mg.L^{-1}. The plant was grown in 30 x 30 x 50 cm glass basins in March 2024, using Hoagland's solution as a source of macro and micronutrients. Secondary compounds were analyzed using GCMS technology, and 27 compounds were recorded across all treatments. The difference in concentrations led to the appearance of some compounds and the absence of others, such as the appearance of the fatty compound 1-Eicosanol when the concentration decreased to (0.05) mg.L^{-1} and its absence from all treatments including the control treatment. In addition to the participation and variation in concentrations of a single compound. The increase in phosphate concentrations led to an increase in most anatomical characteristics. Conversely, when it decreased to (0.05, 0.015) mg.L^{-1}, it caused a decrease in the thickness of the cuticle and epidermis layer, the density of the stomata and their distribution on the upper and lower surfaces, in addition to the appearance and disappearance of various hairs and crystals. The sharp decrease (0.015) mg.L^{-1} also led to the rupture of mesophyll cells and a decrease in their thickness.

Keywords: Phosphate factor, Chemical and Anatomical characteristics, *Eichhornia crassipes*

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Introduction

Eichhornia crassipes (Mart) Solms belongs to the family Pontederiaceae (Tham, 2012). This family includes nine genera and eight species (Onyango et al, 2015). It is a monocotyledonous plant and is considered an aquatic plant and floats on the surface of the water (Al-Hadeethi et al, 2017) . Its original habitat is the Amazon Basin and South America, reaching many regions of the world, such as tropical and subtropical regions, Japan, India, China, Brazil, and others (Dersseh et al, 2019).

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Al-Wagga (2012) pointed out that these plants spread in more than 70 countries, including Iraq in the mid-eighties, where they were introduced as ornamental plants, which led to their spread in various governorates of the country, especially in Karbala and Samarra. They are characterized by rapid growth and spread, reaching Wasit Governorate. According to what was indicated by (Washeeh,2024), this type of plant is characterized by perennial growth and can reach a height of 0.4 meters in fresh water and shallow ponds.It is characterized by its large leaves and symmetrical ray-shaped flowers, and its spike inflorescence contains six stamens and six petals (Aboul-Enein *et al*., 2011). Both (Elenwo & Akankali, 2016) and (Anuja et al., 2016) stated that it may cause environmental damage due to rapid growth in water, forming a floating mat on the water surface, especially when suitable conditions are provided. Despite its environmental damage, it has many benefits. (Balasubramanian *et. al*. 2012; Xie et al. 2016) mentioned the importance of the *Eichhornia crassipes* plant in the aquatic ecosystem through its ability to recycle nutrients and clean the water of various pollutants. Some studies have mentioned the importance of nutrients, especially phosphate. (Wieczorek *et al*., 2021) stated that it is one of the most important nutrients that affect the growth and reproduction of plants due to its effective role in controlling most physiological functions such as protein formation and others. It also plays an important role in various biochemical reactions, in addition to its importance in the formation of nucleic acids (DNA, RNA), sugar phosphates, phospholipids, and energy production in the form of adenosine triphosphate (ATP) (Dissanayaka *et al*, 2021). Several studies have indicated the importance of phosphate in enhancing chemical compounds in various plant tissues.(Liu *et al*. ,2021) reported that a deficiency in the concentration of this element enhances the accumulation of active substances, especially alkaloids and sugars. Peleja *et al*, (2022) also stated that variations in phosphate concentrations affect energy production, which may be reflected in carbohydrate formation in plants. (Vance *et al*. ,2003) indicated that these effects are not limited to physiological and morphological interactions, but extend to include anatomical traits in different plants. Loudari *et al*., (2022) also reported the effect of varying concentrations of phosphate and nitrate on anatomical traits in wheat roots.

Global studies on the effect of different phosphate concentrations on the chemical and anatomical characteristics of *Eichhornia crassipes* are very few. For example, Dubey *et al*. (2022) investigated the effect of increased nutrients, including phosphate, in tropical lakes in India on the anatomical characteristics of aquatic plant leaves. Chemical and anatomical studies have also been conducted, such as Ben Bakrim et al. (2022) on the properties of active chemical compounds in the Maghreb. In addition, researchers Bibi et al. (2023) conducted an anatomical and physiological study in Pakistan. Xie *et al*., (2004) indicated that high phosphate concentrations cause damage and decomposition of some aquatic plants, including *E. crassipes*.

Very few studies exist, particularly in Iraq, specifically in the Diwaniyah Governorate, including a study by Al-Hadeethi *et al* (2017) on the anatomical characteristics of water hyacinth (*E.crassipes*) leaves. Therefore, this research aims to highlight the effect of phosphates on the chemical and anatomical properties of this plant and to identify the changes that occur in this aquatic plant (*Eichhornia crassipes*).

Materials and methods :

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An experiment was conducted on water hyacinth (*Eichhornia crassipes*) plants grown hydroponically in 30 × 30 × 50 cm glass tanks in March 2024. The tanks and samples were thoroughly washed with distilled water to remove impurities and suspended microorganisms. The plants were cultured using chlorine-free water and Hoagland's solution as a source of macro and micronutrients and treated with different phosphate concentrations in descending order (0.19, 0.12, 0.05, 0.015) mg/L.The experimental results were compared with the control treatment (0.06 mg/L) for 20 days, where the control coefficient represents the level of phosphate in the environmental medium from which the plant samples were collected.Subsequently, the samples were collected, and the chemically active compounds were analyzed using gas chromatography-mass spectrometry (GC-MS). Anatomical characteristics were also studied at the end of the experiment.



Figure (1) Treatment of *E.crassipes* plants with different concentrations of phosphate.

A- Study of the properties of active secondary compounds.

According to the method of (Washeeh *et al*., 2024) , the plant leaves were taken at the end of the experiment and washed well with distilled water to remove impurities and microorganisms stuck to them. It was dried at laboratory temperature in a dark place to prevent secondary compounds from reacting with high light intensity. Then take 2 grams of each plant treatment and grind them finely separately in an electric grinder for (16-20) minutes depending on the thickness of the plant leaves to obtain a fine powder. One gram of the ground plant leaves was extracted from each treatment using 10 ml of 99% methanol. The samples were shaken in a shaker for approximately 15 minutes and stored in a dark place for 8 hours. Each treatment was then filtered through a 0.45-micrometer microfilter connected to a medical syringe. In addition, 1 ml of 99% hexane was added to the extract to concentrate it and remove water, then it was filtered in a centrifuge at 500 rpm. After that, the supernatant was drawn from the filtrate of each treatment and kept in other glass tubes for the determination of secondary active compounds using a GC-MS spectrometer .

B- Anatomical study

1- Preparation of Epidermis Samples.

At the end of the experiment, leaves of each treatment were collected for epidermal preparation. The upper and lower epidermis of each treatment were peeled off using a sharp blade and double-ended forceps. The epidermis was transferred to Petri dishes containing 2% NaOH to remove tissue stain. The leaves were then washed with distilled water and transferred to Petri dishes containing Safranin and then to other Petri dishes containing 70% ethyl alcohol for 4-6 minutes to remove excess stain. The epidermal cells were then transferred to glass slides and a drop of glycerin was added. The samples were then covered with a cover slide, and the samples were ready for examination.

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2- **A method of preparing slides for vertical sections in plants**.

Vertical sections of tissue from the leaves of each treatment were prepared separately using a rotating microtome at the Life Sciences Laboratory at the University of Baghdad. This was done after applying several different methods to prepare the samples (Washeeh *et al.*, 2024).

a- Fixation:

Each sample was placed in small glass bottles, 15 ml of Formalin Acetic Acid Alcohol (FAA) solution was added, and left at laboratory temperature for 24 hours.

b- :Washing and Dehyration

The samples were washed with 70% alcohol to remove traces of the fixative. The sections were then passed through an ascending series of alcohols (80, 90, 96)% for an hour, then in absolute ethyl alcohol for half an hour to remove water from the plant sections.

c- Embedding and Mounting :

Each specimen was placed in a mold and molten wax was poured into it. The base of the molds was then placed in cold water until the wax hardened sufficiently. The specimens were fixed in a position suitable for dissection and paraffin was poured over them until they hardened. After trimming the molds, excess wax was cut off from the sides.

d- Sectioning and Streching

The samples were cut into very fine slices using a Rotary Microtome and then the slices were spread on glass slides previously covered with glycerin.

e- Remove wax from the samples and stain them.

The slides containing the plant sections were passed through several solutions. Then the specimens were cleaned and dried and permanent mounting was prepared by placing drops of Distrene-Plasticzes-Xylene (P.D.X) adhesive on the sample sections and covered with a cover slide. The slides were transferred to a hot plate for 24 hours and the specimens were ready for examination under the light microscope. All measurements were taken using an ocular micrometer and the specimens were photographed with a camera (Panasonic, 1.3 pcs, Japan) installed on the same microscope.

Results and Discussion

The results of this study demonstrated the effect of phosphates on the chemically active compounds in plant leaves using gas chromatography-mass spectrometry. A number of compounds were observed in some treatments, while others disappeared when the plant was treated with different concentrations of phosphate. A total of twenty-seven compounds were recorded in all treatments of *E. crassipes* plants. These compounds included phenols, terpenes, amino compounds, alkanes, fatty acids, alkaloids, and steroid compounds, as shown in Table(1).

Reducing phosphate to (0.05 and 15.0) mg.L-1 resulted in the appearance of two phenolic compounds Isopropyl octadecyl ether and Methyl 4-bromo-7-(1,3-dioxan-2-yl)-3-methyl-6-phenylthio-4-octenoate, respectively, compared to the control treatment, Table (1). The difference in phosphate concentrations may have acted as a chemical stimulus to defend plant cells, which is often accompanied by an increase in compounds, especially phenolics, when exposed to different nutritional stresses. The study agreed with the study of (Stewart *et al*., 2001), who showed that the decrease in phosphate concentrations was the reason for the presence of phenolic compounds as a result of the plant being exposed to different nutritional stresses. On the other hand, the study also showed the appearance of four

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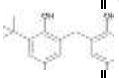
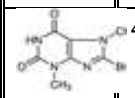
terpene compounds when phosphate increased and decreased. The decrease in concentrations caused the absence of the terpene compound Cyclohexasiloxane and dodecamethyl compared to the control treatment. It was also noted that some active compounds participated in all plant treatments with different phosphate concentrations and varying concentrations of each compound. This may be due to the decomposition of some terpene compounds and their conversion into other compounds, which may have led to their absence or variation in concentrations. The study was consistent with the study of (Bustamante *et al*., 2020), who mentioned in their study on the treatment of *Rosmarinus officinalis* plant to nutritional stresses with different concentrations that affect the formation of active compounds, including terpene compounds.

On the other hand, varying phosphate concentrations resulted in the absence of 1-Docosene in all treatments compared to the control treatment. Furthermore, a decrease in the concentration of 0.015 mg L^{-1} resulted in the disappearance of 1,1-bis(dodecyloxy)-Hexadecane. The lower concentrations above also resulted in the absence of the steroid Stigmasterin compared to the control treatment. (Song *et al*. , 2023) reported in their study on nitrogen deficiency and the stimulation of alkanes in cannabis plants that providing nutrients enhances photosynthesis and the production of secondary metabolites.

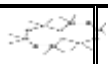
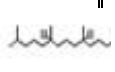
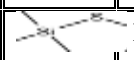
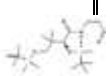
The current results showed the presence of equal numbers of amino acids and fatty acids, which were seven compounds for each, Table (1). The increase and decrease in concentrations led to the appearance and disappearance of some of them. The difference in concentrations led to the appearance of three amino acid compounds, such as Pantothenic acid tritbdms, and the absence of others. While the variation in phosphate concentrations led to the disappearance of some fatty acids, in addition to the appearance of Eicosanol-1, compared to the control treatment. The sharp decrease in concentration also caused the appearance of the carbohydrate complex L-(-)-Fucose, tetrakis (trifluoroacetate), benzyl alcohol (isomer 2) and its disappearance in the other treatments, while the decrease led to the absence of the Sucrose compound compared to the control treatment, Table (1). This may be due to differences in phosphate concentrations, which may affect energy production and various biological reactions, which may be reflected positively or negatively in the formation of secondary compounds in the plant. (Washeeh, 2018) stated that exposing plants to different nutritional environments affects most biological and biochemical reactions, leading to the conversion of some carbohydrate compounds into amino and fatty acids. This was confirmed by the current study, which observed a very small percentage of carbohydrate compounds and the absence of others compared to the number of amino and fatty acids.

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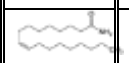
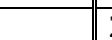
Table (1): Analysis of the active compounds in the leaves of the *E. crassipes* plant under the influence of different concentrations of phosphates using GC-MS technology.

plant type \ *E.crassipes*										Name of compound	Formula Chemical	chemical composition	sequencing	compound type
Compound concentration =(area %) \ R.Time														
C4=0.015 mg.L^{-1}		C3=0.05 mg.L^{-1}		C2=0.12 mg.L^{-1}		C1=0.19 mg.L^{-1}		C0						
R. Time	concentration	R. Time	concentration	R. Time	concentration	R. Time	concentration	R. Time	concentration					
5.51	1.73	5.51	1.39	5.51	1.61	5.51	1.85	5.52	1.70	Pinostrobin, TMS	C19H22O4Si		1	Phenolic compound
15.93	1.55	15.93	4.06	15.93	3.23	15.93	2.71	15.93	5.29	Phenol, 2,2'-methylenebis[6-(1,1-dimethylethyl)-4-methyl	C23H32O2		2	
-	-	6.96	2.56	-	-	-	-	-	-	Isopropyl octadecyl ether	C21H44O		3	
13.36	4.20	-	-	-	-	-	-	-	-	Methyl 4-bromo-7-(1,3-dioxan-2-yl)-3-methyl-6-phenylthio-4-octenoate	C19H25BrO4S		4	
						8.		8.		Cyclohexasiloxa	C12H36O6S		5	Terp

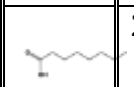
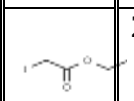
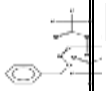
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-	-	-	-	-	-	50	1.27	50	1.50	ne, dodeca methyl-	i6			
11.53	7.14	11.12	12.77	11.12	16.66	11.12	17.11	11.12	15.72	Neophytadiene	C20H38		6	
11.35	3.13	11.35	3.14	11.53	6.47	11.53	6.96	11.53	4.87	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	C20H40O		7	
13.61	19.86	13.61	12.49	13.61	15.44	13.61	9.98	13.61	7.54	Phytol	C20H40O		8	
-	-	-	-	-	-	-	-	18.28	3.16	1-Docosene	C22H44		9	alkanes
-	-	19.80	2.60	16.88	1.13	18.28	2.45	16.75	3.62	Hexadecane, 1,1-bis (dodecyloxy)-	C40H82O2		10	
6.86	1.28	-	-	-	-	6.86	1.89	6.86	2.08	Disilathiane, hexamethyl	C6H18SSi2		11	Amino acid
10.20	1.36	10.20	1.52	10.21	2.25	-	-	-	-	Pantothenic acid tritbdms	C27H59NO5Si3		12	
13.70	1.29	13.70	2.80	13.70	1.54	-	-	13.70	1.60	Methyl stearate	C19H38O2		13	

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19.80	4.78	-	-	19.80	4.49	19.80	4.13	-	-	Tetrapentacontane, 1,54-dibromo-	C54H108Br2		14	
-	-	8.50	1.60	8.50	1.38	10.20	1.91	-	-	N-(4-Nitrobenzyl) propan-1-amine, TMS	C13H22N2O2Si		15	
13.50	2.20	13.36	4.45	13.49	2.33	-	-	13.49	2.80	9Octadecenamide, (Z)-	C18H35NO		16	
-	-	-	-	18.28	3.90	-	-	-	-	Cycloeicosane	C20H40		17	
11.81	7.91	11.82	10.02	11.82	9.14	11.82	9.17	11.82	9.32	Hexadecanoic acid, methyl ester	C17H34O2		18	Fatty acid
-	-	-	-	13.36	4.80	13.36	5.26	13.36	5.19	Octadecanoic acid, 9,10-dihydroxy-, methyl ester, bis(trifluoroacetate	C23H36F6O6		19	
16.83	5.98	16.83	10.41	16.83	6.87	16.83	10.21	16.83	8.48	13-Docosenoic acid, methyl ester, (Z)-	C23H44O2		20	
13.5	2.20	13.4	3.55	13.4	2.33	-	-	13.4	2.80	Oleic acid	$C_{19}H_{36}O_2$		21	

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0		9		9				9					
12.13	3.20	-	-	-	-	12.12	2.09	12.31	2.43	n-Hexadecanoic acid	$C_{16}H_{32}O_2$		22
-	-	-	-	-	-	-	-	6.77	1.66	E-2-Heptenoic acid, 3-iodo-	C7H11IO2		23
-	-	19.44	2.04	-	-	-	-	-		1-Eicosanol	C20H42O		24
-	-	19.67	4.40	19.67	4.44	19.67	4.51	19.68	7.03	Stigmasterin	C29H48O		25
-	-	-	-	13.53	2.39	13.53	2.41	13.53	2.40	Sucrose	C12H22O11		26
3.18	0.81	-	-	-	-	-	-	-	-	L-(-)-Fucose, tetrakis(trifluoroacetate), benzyloxime (isomer 2)	C21H15F12NO9		27

steroid

carbohydrates

The scientist (-) points to the absence of bioactive compounds

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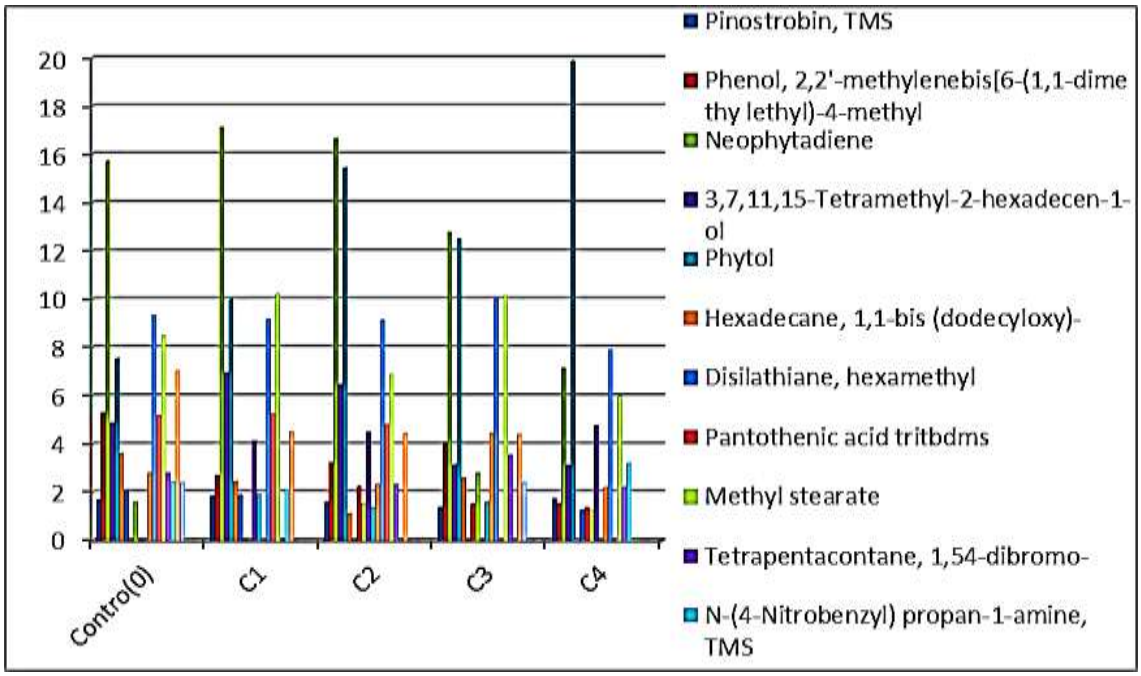


Figure (2): Analysis of the active compounds in the *E. crassips* plant under the influence of different concentrations of phosphate using GC-MS technology .

The effect of different concentrations of phosphate on anatomical characteristics :

A - rdinary Epidermal Cell

The present results showed that the difference in phosphate factor concentrations had an effect on most of the anatomical characteristics in *E. crassipes*. It also had no effect on the shape of the regular cells and their walls on the upper and lower surfaces, as they were polygonal or hexagonal, while their walls were straight and straight-curved Figure (3). The study agreed with the study of(DeSousa *et al*., 2016) that the shape of the cells was polygonal or hexagonal in the studied plant. While increasing the concentration (0.12 and 0.19) mg.L^{-1} caused an increase in the thickness of the walls compared to the control treatment, which was thinner. While the opposite happens when the concentration decreases to (0.015 and 0.05) mg . L^{-1}, it causes a decrease in the wall thickness, Figure (3). The increase and decrease of phosphate also affected the dimensions of plant epidermal cells, the highest rate of the upper surface ranged between (35.67 . 22.08) micrometers when the concentration increased to (0.19) mg . L^{-1}, while the lowest rate was (15.58 * 11.25) micrometers when the concentration decreased to (0.015) mg . L^{-1}. As for the lower surface, the highest rate of epidermal cell dimensions ranged between (24.83 * 19.58) micrometers when the concentration increased to (0.19) mg . L^{-1}, while the lowest rate of epidermal cell dimensions was (13.33 * 9.42) micrometers at the low concentration (0.015) mg . L^{-1}, Table (2). On the other hand, high concentrations of phosphate affected the stomatal complexes in *E. crassipes*. The increase in phosphate led to an increase in the dimensions of guard cells and stomatal dimensions, in addition to its effect on the spread of stomatal cells on both surfaces.

While the stomatal index decreased with low phosphate on both the upper and lower surfaces compared to the control treatment,

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Figure (3). This may be due to the effect of plant growth as a result of exposure to different levels of phosphate. This increase may have led to an increase in the activity of enzymes and growth regulators, and consequently the activity of meristematic tissues and the efficiency of photosynthesis, in contrast to the decrease in phosphate concentrations. The study is consistent with what Liu & Wu (2017) indicated regarding the physiological importance of phosphate and its effective role in building and forming cells as well as in the efficiency of photosynthesis by releasing the energy needed for the various vital processes that occur within the plant. This is reflected in most of the anatomical characteristics. This study is consistent with the study by (Sarker et al., 2010) regarding the decrease in stomatal dimensions and spread when phosphate concentrations are low. The study also agreed with what was indicated by (Soliman *et al*., 2015) that different nutritional stresses affect some anatomical characteristics, especially the thickness of the vertical walls and cells and their dimensions in their study on the Moringa plant. On the other hand, the difference in phosphate concentrations affected the surface coating of *E. crassipes*, Figure (4). Conical unicellular hairs were found on the upper surface of all plant treatments, including the control treatment. While the increase in phosphate led to the appearance of dense cylindrical unicellular hairs on both the upper and lower surfaces.

In addition, high levels (0.12 and 0.19) mg.L-1 of phosphate led to the appearance of sand crystals on both surfaces, while increasing the concentration to 0.19 mg.L-1 led to the appearance of prismatic crystals on the lower surface compared to other treatments with low concentrations, as well as the control treatment, in which no such crystals were observed. The appearance and disappearance of these types of hairs and crystals in the studied plant may be attributed to a method of reducing the effects of excess phosphate by converting it to calcium oxalate in the form of prismatic or sand crystals. The study agreed with (Washeeh et al., 2024), who showed in their anatomical study of *Azolla pinnata* and its treatment with different light levels, the effect of the surface coating on the plant's exposure to environmental factors. The current study confirmed this through the appearance and disappearance of some different hairs and crystals. The same source also mentioned that some anatomical characteristics are affected by various environmental stresses, leading to the emergence of new characteristics and the disappearance of others as a result of the plants' resistance to these factors and the protection of their cells from excessive influences.

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Table (2): Variations in the quantitative characteristics of both the upper and lower surfaces of leaves and stomata of *E.crassipes* treated with different concentrations of phosphate (measured in micrometers).

Lower epidermis							Upper epidermis							
Stomatal Index	Stomata dimensions		Guard cell dimensions		Ordinary epidermal cell dimensions		Stomatal Index	Stomata dimensions		Guard cell dimensions		Ordinary epidermal cell dimensions		Species of plants	
	Width	length	Width	length	Width	length		Width	length	Width	length	Width	length	*E.crassipes*	Phosphate factor ($mg.L^{-1}$)
10.13	1.5-2 (1.92)	4-5.5 (5.08)	2.5-3 (2.58)	10-12 (10.67)	5.5-10 (8.92)	10-22 (13.5)	15.16	1.5-2.5 (1.92)	5-10 (6.5)	2-4 (3.17)	10 - 15 (12.08)	5 - 10 (7.17)	20 - 13.5 (17.67)	Control	
11.12	2.5-4 (2.92)	10-15 (11.17)	2.5-4 (3.42)	15-20 (17.08)	12.5-28 (19.58)	14-48 (24.83)	16.5	1.5-3.5 (2.5)	8-18.5 (14.08)	3-8 (5.5)	18.5 - 25 (22.08)	12.5 - 30 (22.08)	18.5-38 (35.67)	C1=0.19	
11.11	2.1-1.8	5-10 (8.33	2.5-3 (2.58)	12-19 (15.67)	8-23 (16.67)	18-28 (21.	16.2	1.5-3.5	8-16 (13.17	3.5-5 (4.23)	18.5-23.5	18 - 28 (19.33	18-38 (30.92)	C2=0.12	

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	(2.33)	)				5)		(2.37)	)		(21.92)	)		
10.96	1.5-2 (1.67)	5-8.5 (7.33)	4-5 (4.92)	11.5-13.5 (12.33)	5-10 (8.17)	10-25 (17.83)	13.51	1.5-2.5 (1.92)	5-11 (7.75)	3.5 - 4.5 (4.17)	15-17.5 (15.75)	7.5-30 (18.58)	12.5 - 33.5 (23.08)	C3=0.05
10.81	1.5-2 (1.7)	2.5-3 (2.58)	2.5-5 (3.67)	12-15 (14.5)	7.5-14 (9.42)	10-20 (13.33)	13.16	1.5 - 2.0 (1.58)	3.5-7 (5.67)	2.5 - 4.5 (2.92)	10-15 (13.25)	7.5 - 20 (11.25)	9.5 - 20.5 (15.58)	C4=0.015

.The numbers outside the parentheses represent the upper and lower limits. *The numbers inside the parentheses represent the average.

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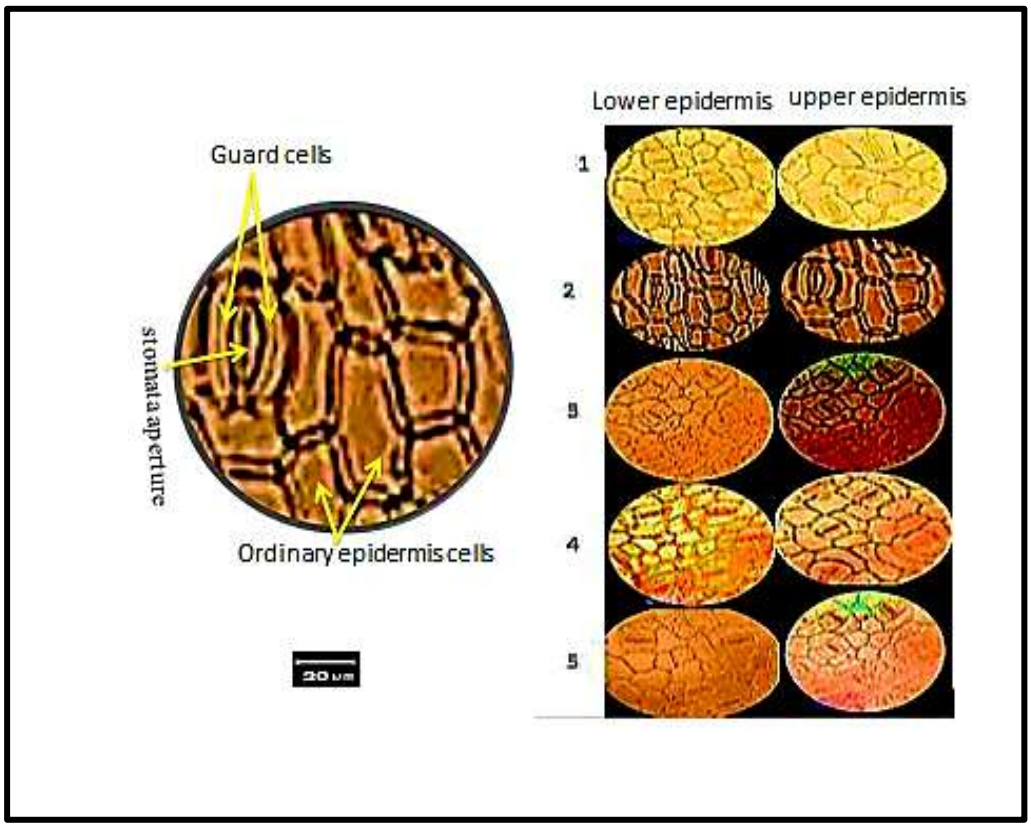
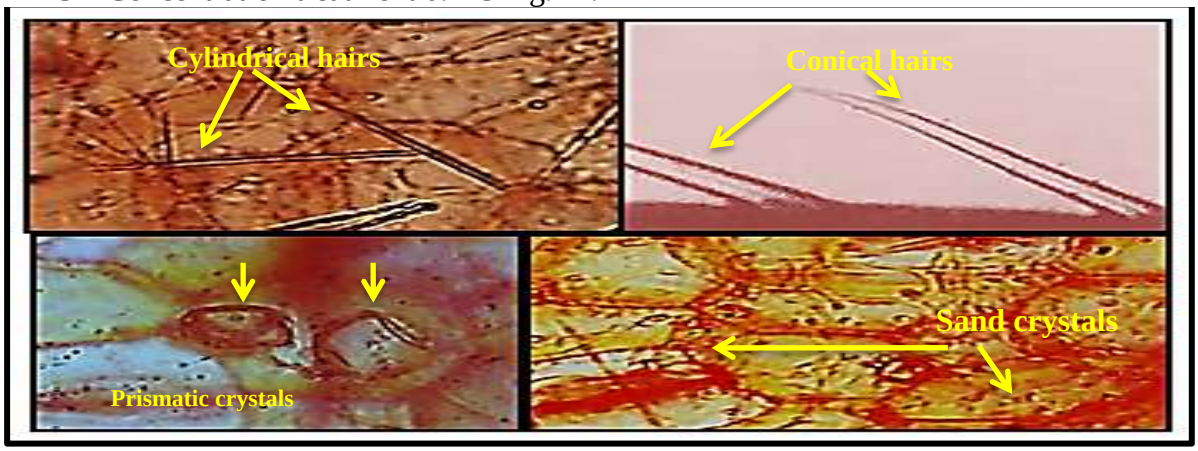


Fig (3): Variations in the epidermis of *E. crassipes* leaves treated with different concentrations of phosphate factor.

1- Control treatment
2- Concentration treatment 0.19 $mg.L^{-1}$
3- Concentration treatment 0.12 $mg.L^{-1}$
4- Concentration treatment 0.05 $mg.L^{-1}$
5- Concentration treatment 0.475 $mg.L^{-1}$.



Fig(4):Unicellular cylindrical hairs and sand crystals on both surfaces of *E. crassipes* plant when the phosphate concentration increased (0.19 and 0.12) $mg.L^{-1}$, and prismatic crystals on the lower surface at the concentration of 0.19 $mg.L^{-1}$, and unicellular conical hairs in the upper epidermis at all plant treatments (Scale bar 6um).

B - The effect of varying phosphate concentrations on the cross-sections of *E. crassipes* leaves:

Clear changes were observed between the cross sections after treating *E. crassipes* with different concentrations of phosphate. A variation was observed in the thickness and dimensions of the cuticle layer, the upper and lower epidermis, as well as an increase in the thickness of the mesophyll tissue when the concentration increased to 0.19 $mg.L^{-1}$, as the highest rate (3.3) micrometers in cuticle thickness was recorded, while on the other hand the lowest rate (2.12) micrometers was recorded when the concentration decreased to 0.015 $mg.L^{-1}$, Table (3) and Figure (5). Also, increasing the concentration by 0.12 $mg.L^{-1}$ led to an increase in the thickness of the upper epidermis layer (22.1) micrometers, while decreasing the concentration to 0.015 $mg.L^{-1}$ caused a decrease in the thickness rate (14.4) micrometers, Table (4). This may be due to the increased activity of some enzymes and internal activities when the phosphate factor increases, which may stimulate the plant to add materials to the surface layers (cuticle) and the upper and lower epidermal cell walls when the phosphate factor concentrations increase. The study agreed with(Barros *et al*., 2015) and (Balk *et al.*, 2023) that there are some internal factors such as physiological activities such as plant growth and development that stimulate the lignification process, which is the addition of materials to the plant cell wall that contributes to the plant's adaptation to the surrounding environmental factors, causing it to thicken. The second source indicated that lignin is, from a chemical point of view, a polyphenolic compound as it is produced under unfavorable conditions. The study did not agree with (Liu HouCheng *et al*., 2004) in their study on the effect of phosphate deficiency on the anatomical characteristics of Vigna unguiculata, as they showed that an increase in thickness occurs in the presence of phosphate deficiency. As for the mesophyll, the sharp decrease in phosphate factor caused more obvious tissue cell rupture, especially at a concentration of 0.015 $mg.L^{-1}$, Figure (5). The highest tissue thickness (43.3) micrometers) was recorded when the concentration increased to 0.19$mg.L^{-1}$, while the lowest (31.5) micrometers) was recorded when the low concentration was 0.015 $mg.L^{-1}$. This can be attributed to the role of phosphate in cell building, energy storage, and growth stimulation in the plant, as well as its impact on the anatomical aspect. It was also observed that the air spaces in the mesophyll tissue expanded as the concentrations decreased. This may be due to increased air storage to enable the plant to float on the water surface as a result of increased internal vital reactions, such as increased gas exchange between the internal and external environments. This led to damage and rupture of the mesophyll tissue cells. The study agreed with (AL-Hadeethi *et al*., 2017) who mentioned in their anatomical study of *E.crassipes* leaves that the expansion of the air chambers in the plant is a result of air storage and exchange with the thin walls surrounding the chloroplast cells from the inside, which helps the plant to carry out internal reactions such as completing the photosynthesis process and increasing respiration under different conditions.

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Table(3):Quantitative characteristics in cross sections of *E.crassipes* leaves treated with different concentrations of phosphate factor (measured in micrometers).

Mesophyll tissues	Epidermis thickness		cuticle thickness	phosphate factor $mg.l^{-1}$	Species	N
	Lower epidermis	Upper epidermis				
30.1- 36.5 (34.3)	-25.2 19.6 (24.2)	18.3- 14.2 (16.4)	1.8- 3.5 (2.7)	Control	*E.crassipes*	1
39.5- 45.5 (43.3)	-34.8 29.5 (31.5)	-23.5 17.5 (20.08)	2.7- 4.3 (3.3)	0.19		2
38.8-42.9 (40.87)	-35.5 29.5 (34.1)	-24.5 18.4 (22.1)	1.9- 3.7 (3.2)	0.12		3
36.1-39.5 (37.5)	-18.1 14.6 (17.5)	-15.3 10.7 (14.5)	1.9 -3.4 (2.6)	0.05		4
33.1-36.5 (31.5)	-16.1 12.6 (14.5)	-14.3 10.1 (14. 4)	1.7 -3.3 (2.12)	0.015		5

*The numbers outside the parentheses represent the upper and lower limits. *The numbers inside the parentheses represent the average.

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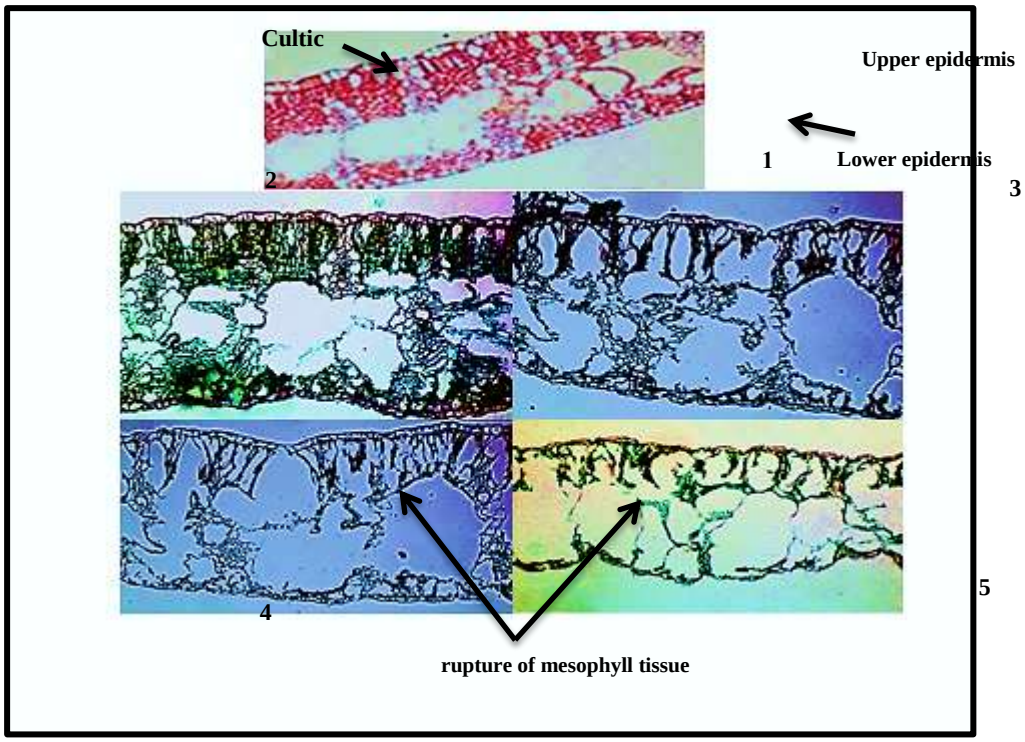


Fig (5): Cross sections of *E.crassipes* leaves treated with different concentrations of phosphate (Scale bar 20 µm).

Conclusions:

It can be concluded from the study that varying phosphate concentrations led to the appearance and disappearance of some chemical compounds in *E. crassipes* leaves. For example, decreasing the concentration to 0.05 mg. L^{-1} resulted in the appearance of the phenolic compound Isopropyl octadecyl ether compared to other treatments including the control treatment. The varying concentrations also led to variations in the concentration of each compound, in addition to its interaction with other treatments such as the terpene compound Phytol at different concentrations. On the other hand, varying the concentrations of the phosphate factor affected most of the anatomical characteristics. Increasing the concentrations (0.12 and 0.19) $mg.L^{-1}$ led to an increase in the thickness of the cuticle and epidermal layers, an increase in the dimensions of guard cells and their spread on the upper and lower surfaces, in addition to an increase in the thickness of the mesophyll. While the decrease in concentrations (0.015 and 0.05) $mg.L^{-1}$ led to the rupture of mesophyll cells and a decrease in the anatomical characteristics mentioned above.

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Researchers' contribution

The lead researcher ,Zainab Allawi Washeeh , conducted the experiment, managed the project, dissected the plants, extracted the active secondary compounds from their leaves, and then analyzed the data. Meanwhile, researchers Azhar

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Abdulameer SOsa and Raid Kadhim Abed AL-ASADY contributed by reading, organizing, and formatting the data and verifying its accuracy.

References:-

1. De Sousa, D. J. L., Scatena, V. L., Giulietti, A. M., and Oriani, A.(2016). Morphological and anatomical patterns in Pontederiaceae (Commelinales) and their evolutionary implications. Aquatic Botany,pp129, 19-30.
2. Aboul-Enein, A., Shanab, S., Al-Abd, A., Shalaby, E.,&El-Shemy, H. (2011). Biologically active compounds of Eichhornia crassipes extract manifest potent anticancer and antioxidant activities. Plant Signaling & Behavior, 6, 834-836.
3. Al-Hadeethi, M. A., Al-Obaidi, B. M., Khalaf, F. K., & Saleh, B. H. (2017). Anatomical features of (Eichhornia crassipes (Mart.) Solms) growing in Iraq. In 8th international conference on agricultural, environment, biology and medical sciences (AEBMS-2017) (pp. 155-161).
4. AL-SAADI., Z.A., Sosa A.A., AL-Sady R.K.A. (2024). Study of some anatomical and chemical characteristics of Eichhiornia Crassipes Plants Growing in ALDiwaniyah Province and treated with different lighting intensities. Minar International Journal of Applied Sciences and Technology, 6(2): 12-29.
5. Al-Wagga AHA, (2012). Studying of Growth, Reproduction and Control of Eichhornia crassipes (Mart) in Nenawa Province. Ph.D. Dissertation, Coll. of Agric. and Forest.Univ. of Mosul. pp. 181.
6. Anuja, S., Aggarwal, N. K., Anita, S., & Anita, Y. (2016). Beyond biocontrol: water hyacinth-opportunities and challenges. Journal of Environmental Science and Technology, 9(1), 26-48.
7. Balasubramanian D., Arunachalam K., Das A. and Arunachalam A. (2012) Decomposition and nutrient release of Eichhornia crassipes (Mart.) Solms. under different trophic conditions in wetlands of eastern Himalayan foothills. Ecological Engineering, 44, 111-122.
8. Balk, M., Sofia, P., Neffe, A. T., and Tirelli, N. (2023). Lignin, the lignification process, and advanced, lignin-based materials. International Journal of Molecular Sciences, 24(14), 11668.
9. Barros, J., Serk, H., Granlund, I., and Pesquet, E. (2015). The cell biology of lignification in higher plants. Annals of botany, 115(7), 1053-1074.
10. Ben Bakrim, W., Ezzariai, A., Karouach, F., Sobeh, M., Kibret, M., Hafidi, M., & Yasri, A. (2022). Eichhornia crassipes (Mart.) Solms: a comprehensive review of its chemical composition, traditional use, and value-added products . Frontiers in pharmacology, 13, 842511.
11. Bibi, M., Asad, F., Shahid, S., & Kiramat, H. (2023). Anatomical, histochemical and phytochemical screening of the vegetative parts of Eichhornia crassipes. Journal of Pure and Applied Agriculture, 8(3), 42-52.
12. Bustamante, M. Á., Michelozzi, M., Barra Caracciolo, A., Grenni, P., Verbokkem, J., Geerdink, P., ... and Nogues, I. (2020). Effects of soil fertilization on terpenoids and other carbon-based secondary metabolites in Rosmarinus officinalis plants: a comparative study. Plants, 9(7), 830.

ISSN 2072-3857

13. Dersseh M. G., Melesse A. M., Tilahun S. A., Abate M., and Dagnew D. C.,(2019). Water hyacinth: review of its impacts on hydrology and ecosystem services-lessons for management of Lake Tana, Extreme Hydrology and Climate Variability. 237–251, https://doi.org/10.1016/b978-0-12-815998-9.00019-1.
14. Dissanayaka, D.M.S.B.; Ghahremani, M.; Siebers, M.; Wasaki, J.; Plaxton, W.C.(2021). Recent insights into the metabolic adaptations of phosphorus-deprived plants. J. Exp. Bot. , 72, 199–223. [Google Scholar] [CrossRef] [PubMed] .
15. Dubey, D., Kumar, S., & Dutta, V. (2022). In-situ anatomical and elemental response of aquatic macrophytes against nutrient enrichment in freshwater tropical lakes. Applied Water Science, 12(7), 163.
16. Elenwo, E., & Akankali, J. (2016). The estimation of potential yield of water hyacinth: a tool for environmental management and an economic resource for the Niger Delta region. *Journal of Sustainable Development Studies*, *9*(2), 115-137.
17. Liu HouCheng, L. H., Chen GuoJu, C. G., Chen RiYuan, C. R., Kuang YanHua, K. Y., & Wu XiaoYing, W. X. (2004). Anatomical structures of seedlings of Vigna unguiculata ssp. sesquipedalis cultivars under phosphorus-deficiency stress.
18. Liu, C. and Wu, Q.(2017). Responses of Plant Growth, Root Morphology, Chlorophyll and Indoleacetic Acid to Phosphorus Stress in Trifoliate Orange. Biotechnology, 16: 40-44.
19. Liu, L., Xiang, H., Shen, H., Dong, Y., Sun, X., Cai, Y., & Fan, H. (2021). Effects of low phosphorus stress on the main active ingredients and antioxidant activities of Dendrobium officinale. *Industrial Crops and Products*, *173*, 114095.
20. Loudari, A., Mayane, A., Naciri, R., Zeroual, Y., Colinet, G., & Oukarroum, A. (2022). Root morphological and anatomical responses to increasing phosphorus concentration of wheat plants grown under salinity. Plant Stress, 6, 100121.
21. Onyango J. P. and Ondeng M. A.,(2015). The contribution of the multiple usage of water hyacinth on the economic development of reparian communities in dunga and kichinjio of kisumu central sub county, Kenya, *American Journal of Renewable and Sustainable Energy*. 1, no. 3, 128–132.
22. Peleja, V. L., Peleja, P. L., Lara, T. S., Tribuzy, E. S., & de Moura, J. M. S. (2022). Seasonality and phosphate fertilization in carbohydrates storage: Carapa guianensis Aubl. seedlings responses. *Plants*, *11*(15), 1956.
23. Pereira, F. J., CASTRO, E. M. D., OLIVEIRA, C. D., Pires, M. F., Pereira, M. P., Ramos, S. J., & Faquin, V. (2014). Lead tolerance of water hyacinth (*Eichhornia crassipes* Mart.-Pontederiaceae) as defined by anatomical and physiological traits. Anais da Academia Brasileira de Ciências, 86(3), 1423-1433
24. Sarker, B. C., Karmoker, J. L.,&Rashid, P. (2010). Effects of phosphorus deficiency on anatomical structures in maize (*Zea mays* L.). *Bangladesh journal of botany*, *39*(1), 57-60.
25. Soliman, A. S.; El-feky, S. A .and Darwish, E. (2015). Alleviation of salt stress on Moringa peregrina

using foliar application of nanofertilizers. J. Hortic. For., 7 (2):36-47.

26. Song, C., Saloner, A., Fait, A., and Bernstein, N. (2023). Nitrogen deficiency stimulates cannabinoid biosynthesis in medical cannabis plants by inducing a metabolic shift towards production of low-N metabolites. Industrial Crops and Products, 202, 116969.
27. Stewart AJ, Chapman W, Jenkins GI, Graham I, Martin T and Crozier A: (2001). The effect of nitrogen and phosphorus deficiency on flavonol accumulation in plant tissues. Plant Cell Environment 24(11), 1189-1197.
28. Tham, H. T. (2012). Water Hyacinth (*Eichhornia crassipes*): Biomass production, ensilability and feeding value to growing cattle. Department of Animal Nutrition and Management, Swedish Universityof Agricultural Sciences.
29. Vance C.P., C. Uhde-Stone and D.L. Allan. (2003). Phosphorus acquisition and use: critical adaptations by plants for securing a nonrenewable resource. New Phytol. 157: 423-447.
30. Washeeh, Z. A., Sosa, A. A.,&Al-Asady, R. K. A. (2024). The effect of different light intensities on the anatomical and chemical characteristics of the aquatic plant of Azolla pinnata. *International Journal of Aquatic Biology*, *12*(3), 275-284.
31. Washeeh, Zainab. Allawi .,(2018) .Anatomical and chemical study of Two genera of Salicaceae Spread in Diwaniyah Province and Palynology. Master's thesis in the College of Education / University of Al-Qadisiyah in Biology Sciences – *Botany*, pp113.
32. Wieczorek, D., Żyszka-Haberecht, B., Kafka, A., & Lipok, J. (2021). Phosphonates as unique components of plant seeds—a promising approach to use phosphorus profiles in plant chemotaxonomy. *International Journal of Molecular Sciences*, *22*(21), 11501.
33. Xie Y., Xie Y., Hu C., Chen X. and Li F. (2016) Interaction between litter quality and simulated water depth on decomposition of two emergent macrophytes. Journal of limnology, 75(1).
34. Xie, Y., Yu, D., & Ren, B. (2004). Effects of nitrogen and phosphorus availability on the decomposition of aquatic plants. *Aquatic Botany*, *80*(1), 29-37.

ISSN 2072-3857