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THE EFFECT OF EXCESS NUTRIENTS ON ANATOMICAL TRAITS IN THE STEM OF TWO SPECIES OF AQUATIC PLANTS

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Abstract

A competition experiment design had five treatment combinations to terminal shoots of three different treatment combinations of *Ceratophyllum demersum* and *Hydrilla verticillata* together *(T1, T2, T3)*, terminal shoots of *H. verticillata* only (T4) and *C. demersum* only (T5). Recorded significantly differences ($p<0.05$) in all characters between the maximum and minimum values, Therefore, recorded during measurement variations in the rate of the thickness of the stem and its component parts of *H. verticillata*, where; showed the highest value in vascular bundles 1.7µm at T3 in control and lowest value 0 at T2 in low concentration. and aerenchyma tissue recorded the highest value 2.5µm at T1 in high concentration. Recorded during measurement variations in the rate of the thickness of the *C. demersum* stem and its component parts, where; showed the highest value in vascular bundles 1.6µm at T3 in low concentration. and lowest value 0.5µm at T2 and T4 in high concentration, and aerenchyma tissue recorded the highest value 0.9µm at T1 in the control treatment. The *H. verticillata* plants in 30th day at high concentration showed the surface of the epidermis appears limp and a small size and reduction in the vascular bundle tissue appeared with disintegration in parenchyma, but during the 45th day at high concentration, where the degradation of photosynthesis pigments and deposition of materials in the parenchyma tissue was observed by increasing the pigmentation of the cells and increasing the shrivel epidermis. while in the *C. demersum* plant, shown in the 15th day, increase branches number with reducing aerenchyma size, and Plants in the 30th day, where the surface of epidermis appear shrivel with minimizing the cortex thickness and initiation of aerenchyma dissolution. while in the 45th day, where increased aerenchyma layer decay due to the dissolution of the layer, With the remain vascular bundle intact, But the leaves looked weak and detachable.

***Keyword*:** Anatomy, Ceratophyllum, Characters, Hydrilla, Nutrients.

Introduction

Aquatic plants are those species that complement their life cycle in lentic or lotic water bodies (Philbrick and Les, 1996). In comparison with a terrestrial habitat, water bodies such as spring, river, lake and stream, ponds, ditches, waterlogged localities etc. Supplying permanent and stable habitat is exhibited by its greater thermal and chemical stability (Tiffney, 1981). Compared with terrestrial plants the aquatic plants present anatomical specificities, such as the small amount of supporting tissues. In many species, are concentrated in the stem in an endodermis like structure rich in lignified cell walls and separating from the cortex is the central cylinder. The cortex of lacunar parenchyma (also called aerenchyma) develops around the central cylinder, and consists of a system of interconnected airspaces, allowing plant flotation and gas diffusion (Raven, 1996; Rascio, 2002). Have been shown to favors in the production of low-density tissues Nutrient enrichment and low oxygen concentrations often observed in nutrient-rich environments, (Puijalon *et al*., 2007), particularly through the development of aerenchyma tissue in stems and roots (Hussner *et al*., 2009; Jampeetong and Brix, 2009).

Generally characterized stems of dicot species that the essential tissue system is distinctive to the cortex and pith, either vascular histological system it's a vascular cylinder in which vascular bundles are arranged in a circular manner. However, aquatic species usually acquire anatomical and morphological characteristics suitable for the environment in which the plant lives (Al-Bayoumi *et al*., 1996). The difference in function and structure of epidermis in aquatic plants as compared with that of plants growing in aerial habitat is outstanding. The epidermis is not protective in aquatic plants but absorbs nutrients and gases directly from the water. The epidermis on all parts of water hyacinth consists of a single layer of rectangular cells which is characteristically a constant feature of this species thin cellulose walls of epidermal cells in a typical hydrophyte assist steady absorption from surrounding water (Mahmood *et al*., 2005).

Various anatomical and physiological attributes may be important in the competitive ability of aquatic plants, particularly invasive species. For instance, the ability of *Elodea canadensis* and *Myriophyllum spicatum* to exclude other submerged hydrophytes from shallow habitats is largely attributed to their superior ability to appropriate light and space, particularly in relatively fertile habitats (Nichols and Shaw 1986; Madsen et al. 1991). Free-floating *Azolla pinnata*, *Eichhornia crassipes*, and *Salvinia molesta* are similarly effective at appropriating surface and in casting shade in subtropical and tropical habitats (Scheffer 1997; Rea and Storrs 1999).

Hydrilla verticillata (L.f.)

In stem epidermal of H. verticillata consists of a monolayer thin-walled, this feature facilitates the direct absorption of gases and mineral salts dissolved in water, and the cortex is occupying the biggest part of the stem and become very wide. The outer layers of the cortex (Exocortex) are usually without aerobic spaces (intercellular) and thick, While the inner layers of the cortex (Endocortex) are has a large aerobic spaces arranged symmetrically and aerial where the air filled in these cavities helps float the plant, it also facilitates the exchange of gases during photosynthesis and respiration. The vascular tissues development is poor and doesn't show a remarkable differentiation between the xylem and phloem, the xylem representation of one tape is located

in the center of the central cylinder and there's no mechanical tissue exists in the stem of the submerged plant as the water column itself provides mechanical support to the plant (Ancibor; 1979).

***Ceratophyllum demersum* (L.)**

In stem cross section of *C. demersum* consists from the outer-layer of thin cells representing the epidermis followed by the cortex that can distinguished into three regions (Khazraji *et al*., 1990; Schneider & Carlquist 1996). The first consists of numerous layers of the cells characterize the external cortex followed by the middle part of the cortex which is rich in air-conditioned rooms that play a role in storing some oxygen produced by the process of photosynthesis to be used in the process of respiration (Gonzalez-Meler *et al.*, 2004), Finally the internal cortex area is a single row of cells called endodermis, Either the vascular cylinder it's a central location and this characteristic give plant characteristic of resistance against water flows (Al-Arousi *et al*., 1977), Also characterized with air channel central location surrounded by parenchyma tissue, the vascular cylinder also contains cells are relatively wide believed to be vessels it has been mentioned by (Schweingrube *et al*., 2011) these cells do not have any distinctive structures in their walls, Which makes it hard to determine their own identity, And this prompted Schneider & Carlquist 1996 to believe that xylem is not found in *C. demersum* (Schweingruber *et al*., 2011). The present study aims to effect of nutrients on stem anatomical traits to inter-specific competition between *Hydrilla verticillata* and *Ceratophyllum demersum*.

Materiel and Methods

Experimental condition

Terminal shoots of both species were cut into 10 cm length fragments while existing branches, roots and flower buds were removed. They were planted in plastic containers all were equal dimensions (40cm length x 25cm width x 25cm height) filled with 15 liters of water per container and the water level of each container was maintained at a constant level throughout the experiment. Our experiment was designed in 63 containers (plastic tank). divided into five treatments, everyone has four combinations, each combination has 12 containers. In each combination, three different concentrations (three containers for each concentration), A fourth combinations were only plant without nutrients (control), Remain 3 containers were water control (only water) Table 1.

The experimental were arranged in Completely Randomized Design and plant anatomy was recorded after (15, 30, and 45) day of planting after acclimatized of the plant for 14 days in tap water; after acclimation, plant exposed to chosen concentrations of the nutrients; nitrogen (2, 6 and 10) ppm and phosphorus (0.1, 0.5 and 1) ppm, with constant light irrationally (400 Lux.); photoperiod 12/12 light/dark (h./h.) and temperature 30 ºC.

Table 1: The Number and ratio of terminal shoots of each species used per container in each treatment.

Treatment	Combination	Number and Ratio of Plants / Container			
		Low con.	Medium con.	High con.	Control
T1	*H. verticillata : C.demersm*	10:30	10:30	10:30	10:30
T2	*H. verticillata : C.demersm*	20:20	20:20	20:20	20:20
T3	*H. verticillata : C.demersm*	30:10	30:10	30:10	30:10
T4	*H. verticillata* only	40:00	40:00	40:00	40:00
T5	*C.demersm* only	00:40	00:40	00:40	00:40

- Low concentration = (P 0.1 mg/l, NO_3 2 mg/l)
- Moderate concentration = (P 0.5 mg/l, NO_3 6 mg/l)
- High concentration = (P 1 mg/l, NO_3 10 mg/l)

Forty terminal shoots were planted per container and the method of planting (arrangement of plants in the container). The containers were laid out in a Completely Randomized Design (CRD) representing three replicates per treatment. Hence altogether 2400 terminal shoots 1200 each from *Hydrilla verticillata* and *Ceratophyllum demersum* were used in the experiment.

Preparation of tissue sections

Stem of each studied plants anatomical procedures can be divided into a series of steps. For the paraffin double stain method with modification (Brown, 1975) (these steps are as follows:

Tissue removal by sharp blade into pieces of 10*10mm then Installation in FAA (Formaline -Acetic Acid- Ethanol 15: 15: 70) for one hour followed by Dehydration in a gradual series of ethanol. (70%) for 6 hours to remove the water from the tissue and then the process of Infiltration with a mixture of paraffin wax and zillion for two hours and then the process of embedding in pure paraffin at 58 °C. (Bearing in plastic molds). Sectioning with microtome (8 mm thick). At last Mounting on microscope slides. After that Clearing and Staining with Safranin solution for 3 hours and washing with ethanol 70% then fast green solution for 30 mint. Set up durable mounts. Studies were performed on prosthesis and other anatomical characteristics. Measurements and photographs were taken with the help of a camera microscope (AMCAP 104, China) using an optical micrometer, calibrated by a phase micrometer.

Statically analysis:

The experimental plots were arranged in a Random Design Completely; data were analyzed by using SPSS statistical software (version 16)

Results and Discussion

The present study showed clear variation on anatomical level (Stem) for understudy plants when examined by light microscopy.

***Hydrilla verticillata* stem**

The results showed during measurement of rate of thickness in *H. verticillata* stem a variation in the rate of the thickness of the stem and its component parts, where; showed highest value in vascular bundles 1.7µm at T3 in control and lowest value 0 at T2 in low concentration and aerenchyma recorded highest value 2.5µm at T1 in high concentration and lowest value 0.3µm at T2 in control, While in parenchyma appeared highest reading 1µm at T4 in control and lowest reading 0.1µm at T1 in low and moderate concentration, whereas recorded in epidermis highest value 0.6µm at T4 in moderate concentration and lowest value 0.1µm at T2 in low concentration, finally in the total stem was maximum value 8.3µm at T2 in low concentration and minimum value 3.7µm at T1 in high concentration these clear in the table (2).

Statically analysis under probability ($p < 0.05$) showed there are significant differences between all interactions.

The stem showed a clear change in treatments T2, T3 and T4 under low concentration in a 15th day decrease in vascular bundle tissues and smaller in size, then in 30th day begin the decay of the parenchyma tissue and the connection point between stem and leave. So the stems float on the surface of the water and at the end of the experiment (45th day) shown dissolution in the vascular bundle and parenchyma tissue and increase epidermis shrivel. And this is consistent with (AL-Zurfi *et al*., 2018) where, stress exposure leads to a decrease in the thickness of parenchyma cells. Either, under moderate conc. to same treatments in the 15th day, where appeared shrivel in stem surface and increase cell walls thickness. While in the 30th day showed smaller in vascular bundle diameter and increased in epidermis shrivel and start disintegration aerenchyma were increased in size and decreased in number. Then in the 45th day become yellowing in the plant, low of thickness parenchyma layer, Increase the aerenchyma layer, Dissolving and decreasing the thickness of vascular bundles concurrent with increased period of exposing to nutrients stress. Whereas, Exposure to high conc. after the 15th day in the same treatments leads to increases thickness of cell walls and decrease the diameter of the vascular bundle and its central pore. While after 30th day showed a decrease in size of the aerenchyma tissues but increased in number, and increase cell thickness. In the 45th day viewed decay in the parenchyma tissue with remain of the vascular bundle intact and increase in the shivering of the epidermis and decrease in general in the thickness of cells and tissues. In addition to all the above-mentioned variations, it emerged in treatment (T1) degradation of photosynthesis pigments and deposition of materials in the parenchyma tissue was observed by increasing the pigmentation of the cells and increasing the shrivel epidermis.

Table 2: Mean thickness of vascular bundle, Aerenchyma, Parenchyma and epidermis, of *H. verticillata* stem during the experiment.

Number of plant	Conc.	Vascular bundle (µm)	Aerenchyma thickness (µm)	Parenchyma thickness (µm)	Epidermis thickness (µm)	Total stem diameter (µm)
40 (100%) T4	Low	0.2	1.3	0.5	0.3	8.1
	Moderate	0.5	1.5	0.1	0.6	6.1
	High	0.8	2	0.5	0.4	5.5
	Control	1.3	0.4	1	0.3	6.7
30 (75%) T3	Low	0.2	2	0.3	0.3	7.9
	Moderate	1.3	2.3	0.5	0.5	5.8
	High	1	2.5	0.3	0.2	4.5
	Control	1.7	0.4	0.9	0.2	6.5
20 (50%) T2	Low	0	2.3	0.2	0.1	8.3
	Moderate	0.5	1.2	0.7	0.5	5
	High	0.2	1.7	0.2	0.2	4.3
	Control	1.2	0.3	0.8	0.2	6.5
10 (25%) T1	Low	0.5	1.5	0.1	0.2	7.2
	Moderate	0.3	0.7	0.1	0.5	5.7
	High	1.2	2.5	0.3	0.2	3.7
	Control	1.3	0.5	0.9	0.2	6.7

***Ceratophyllum demersum* Stem**

The recorded rate of the stem thickness of *C. demersum* during measurement variations and its component parts, Where; showed the highest value in vascular bundles 1.6µm at T3 in low conc. and lowest value 0.5µm at T2,T5 in high concentration and aerenchyma tissue recorded the highest value 0.9µm at T1 in control and lowest value 0.3µm at T5 in high concentration , While in parenchyma appeared highest reading 1.5µm at T1 in control and lowest reading 0.1µm at T5 in high concentration ,whereas recorded in epidermis highest value 0.5µm at T2,T3 in high concentration and lowest value 0.1µm at T1,T2 and T5 in low concentration, finally in total stem was maximum value 6.1µm at T1 in low concentration and minimum value 3.1µm at T3 in high concentration. Statically analysis under probability ($p < 0.05$) showed there are significant differences between all interactions.

The *C. demersum* stem recorded a marked weakness at T5 in all its layers, reversing what appeared in its leaves. This may give an indication of the different response method of nutrients between the stem and leaf, While the thickness of the stem increased in the competition of aquariums and this may explain that the stem needs to stress conditions in order to stimulate its construction, While the lowest thickness of all layers was recorded in the high concentrations except the epidermis layer, where the highest value recorded in high concentration 5.1µm. This may explain that the epidermis of *C. demersum* stem is a good conductor of nutrients. these clear in the table (3).

Table 3: Mean thickness of vascular bundle, Aerenchyma, Parenchyma and epidermis, of *C. demersum* stem during the experiment.

Number of plant	Conc.	Vascular bundle (µm)	Aerenchyma Thickness (µm)	Parenchyma Thickness (µm)	Epidermis Thickness (µm)	Total Stem diameter (µm)
40 (100%) T5	Low	1.2	0.6	1.3	0.1	5.5
	Moderate	0.9	0.5	0.8	0.2	4.6
	High	0.5	0.3	0.1	0.3	3.2
	Control	0.9	0.7	1.2	0.2	3.5
30 (75%) T1	Low	1.5	0.7	1.3	0.1	6.1
	Moderate	1.3	0.5	0.9	0.2	5.6
	High	1	0.6	0.2	0.4	3.3
	Control	0.9	0.9	1.5	0.2	3.7
20 (50%) T2	Low	1.2	0.8	1.2	0.1	5.9
	Moderate	0.8	0.5	0.8	0.4	5.8
	High	0.5	0.6	0.5	0.5	2.8
	Control	1	0.8	1.5	0.2	3.9
10 (25%) T3	Low	1.6	0.8	1.3	0.2	5.7
	Moderate	1.4	0.6	1	0.3	5.5
	High	0.8	0.5	0.6	0.5	3.1
	Control	0.8	0.7	1	0.2	3.5

Histological variations of the study plants

Histological variations of *H. verticillata*

Showed the plate (2) cross-section of *Hydrilla verticillata* stem that exposing to the low concentration of nutrients were showed decrease in vascular bundle tissues and smaller in size in low concentration during the 15th day, while during the 30th day were, begin the decay of the parenchyma tissue and the connection point between stem and leaves. So the stems float on the surface of the water (plate 2-D) and during the 45th day of the experiment, where dissolution the vascular bundle and parenchyma tissue and increase epidermis shrivel (plate 2-E).

In plate (3) showed the cross-section of *H. verticillata* stem that exposing to moderate concentration of nutrients during the 15th day of the experiment, were appeared shrivel in stem surface and increase cell walls thickness (Plate 3-C), while showed during the 30th day appeared smaller in vascular bundle diameter and increased in epidermis shrivel and start disintegration aerenchyma were increased in size and decreased in number(plate 3-D), as for during the 45th day of the experiment showed yellowing the plant, decrease in thickness parenchyma layer, Increase the aerenchyma layer, Dissolving and decreasing the thickness of vascular bundles concurrent with increased period of exposing nutrients stress (plate 3-E).

Showed the plate 4 showed cross-section of *H. verticillata* stem that exposing to high concentration of nutrients during the 15th day of the experiment, where the thickness of cell walls increased and decrease the diameter of the vascular bundle and its central pore (Plate 4-C), while during the 30th day appeared aerenchyma tissues decreased in size and increased in number, and increase cell thickness. (plate 4-D), as for during the 45th-day plant decay in the parenchyma tissue with remain of the vascular bundle intact and increase in the shivering of the epidermis and decrease in general in the thickness of cells and tissues (plate 4-E).

Clear the plate 5 cross-section of *H. verticillata* stem that exposing to low ,moderate and high concentration of nutrients in T1 during 15th day in moderate concentrations showed tortuosity in the epidermis surface with a narrow diameter central pore of vascular bundle and increase the thickness of cell walls (plate 5-C) ,while during the 30th day at high concentration showed the epidermis surface limp and a small size and reduction in the vascular bundle tissue appeared with disintegration in parenchyma (plate 5-D), during the 45th day at high concentration, where the degradation of photosynthesis pigments and deposition of materials in the parenchyma tissue was observed by increasing the pigmentation of the cells and increasing the shrivel epidermis (plate 5-E).

Anatomical characteristics were studied for *H. verticillata* after exposing it, To different concentrations of nutrients (P, N). In 45th day, competing with the other plant understudy (*C. demersum)* where many water adaptations for the studied plants, So the difference in the composition and function of the epidermis in hydrophytes compared with terrestrial plants is obvious. The epidermis in aquatic plants has no protective role but absorb gases and nutrients directly from water (Mahmood et al. 2015). And through the results of the *H. verticillata* plant, We observe that the stem is affected by nutrients. During the experiment for (15, 30 and 45) day.

The plant showed clear changes to the stem in treatments T2, T3 and T4 under low concentration in the 15th day decrease in vascular bundle tissues and smaller in size, then in 30th day begin the decay of the parenchyma tissue and the connection point between stem and leave. So the stems float on the surface of the water and at the end of the experiment (45th day) shown dissolution in the vascular bundle and parenchyma tissue and increase epidermis shrivel.

Either, under moderate conc. to same treatments in the 15th day, where appeared shrivel in stem surface and increase cell walls thickness. While in the 30th day showed smaller in vascular bundle diameter and increased in epidermis shrivel and start disintegration aerenchyma were increased in size and decreased in number. Then in the 45th day becomes yellowing in the plant, low of thickness parenchyma layer, Increase the aerenchyma layer, Dissolving and decreasing the thickness of vascular bundles concurrent with increased period of exposing to nutrients stress.

Whereas, Exposure to high conc. after the 15th day in the same treatments leads to increases thickness of cell walls and decrease the diameter of the vascular bundle and its central pore. While after 30th day showed a decrease in size of the aerenchyma tissues but increased in number, and increase cell thickness. In the 45th day viewed decay in the parenchyma tissue with remain of the vascular bundle intact and increase in the shivering of the epidermis and decrease in general in the thickness of cells and tissues. In addition to all the above-mentioned variations, it emerged in treatment (T1) degradation of photosynthesis pigments and deposition of materials in the parenchyma tissue was observed by increasing the pigmentation of the cells and increasing the shrivel epidermis.

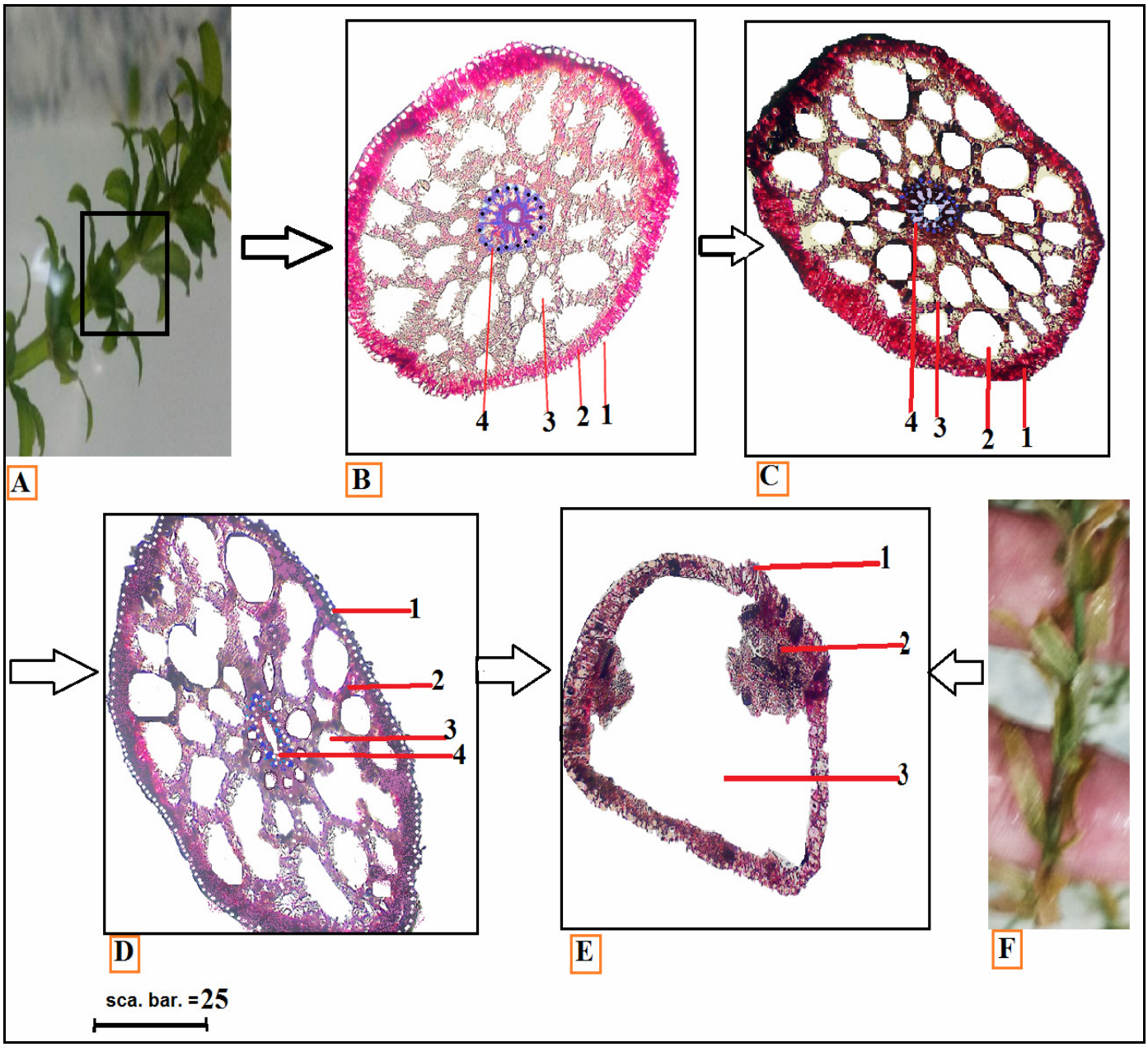

Plate 2 : Cross section of *Hydrilla verticillata* stem. shown in X 10 magnification (X)* with scale bars =25 µm----------1. Showing the effect of adding nutrients with low concentrations to T4, T3 and T2*

where:1=Epidermis cells, 2=Parenchyma tissue, 3= Aerenchyma tissue, 4=Vascular bundles. (X) * = power enlarge under microscope

T* = number of plant; T4 mean (only *H.verticillata*), T3 mean (30 *H.verticillata* :10 *Ceratophyllum*), T2 mean (20 *H.verticillata* : 20 *Ceratophyllum*), T1 mean (10 *H.verticillata* : 30 *Ceratophyllum*)

A= *H. verticillata* plant ,B= Control and low concentration plant in first day , C= Plants in low concentration during 15th day, D= Plants in 30th day E= Plants in 45th day, F= *H.verticillata* at the end of the experiment.

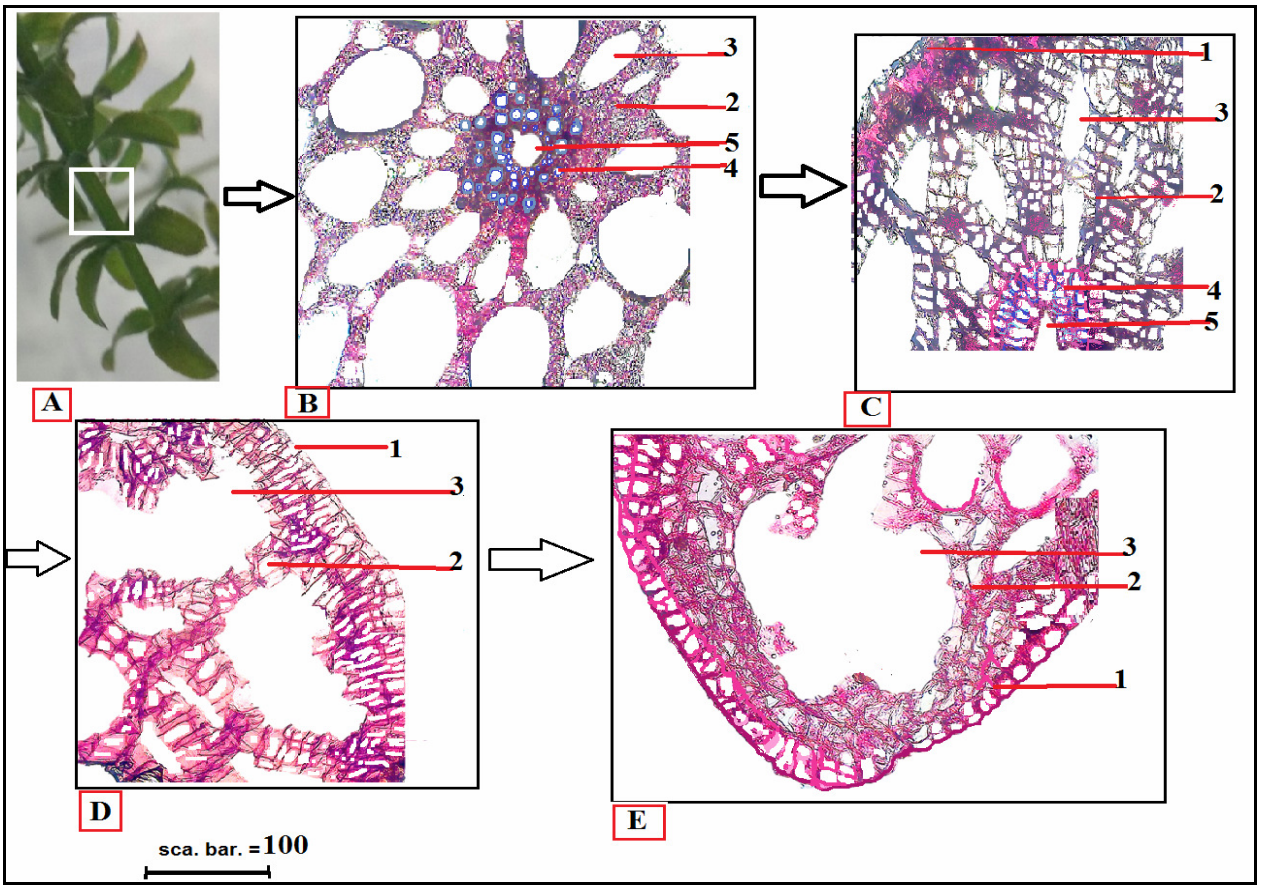

Plate 3 : Stem cross sections of *Hydrilla verticillata*. shown in X25 magnification with scale bars =100 µm----------1. Showing the effect of adding nutrients with moderate concentrations to T4, T3 and T2*

Where: 1=Epidermis cells, 2=Parenchyma tissue, 3= Aerenchyma tissue, 4=Vascular bundles, 5= The central pore of the vascular bundle. A= *H. verticillata* plant, B= Control in moderate concentration during first day, C= Plants during 15th day D= The plants during 30th day E= Plants during 45th day.

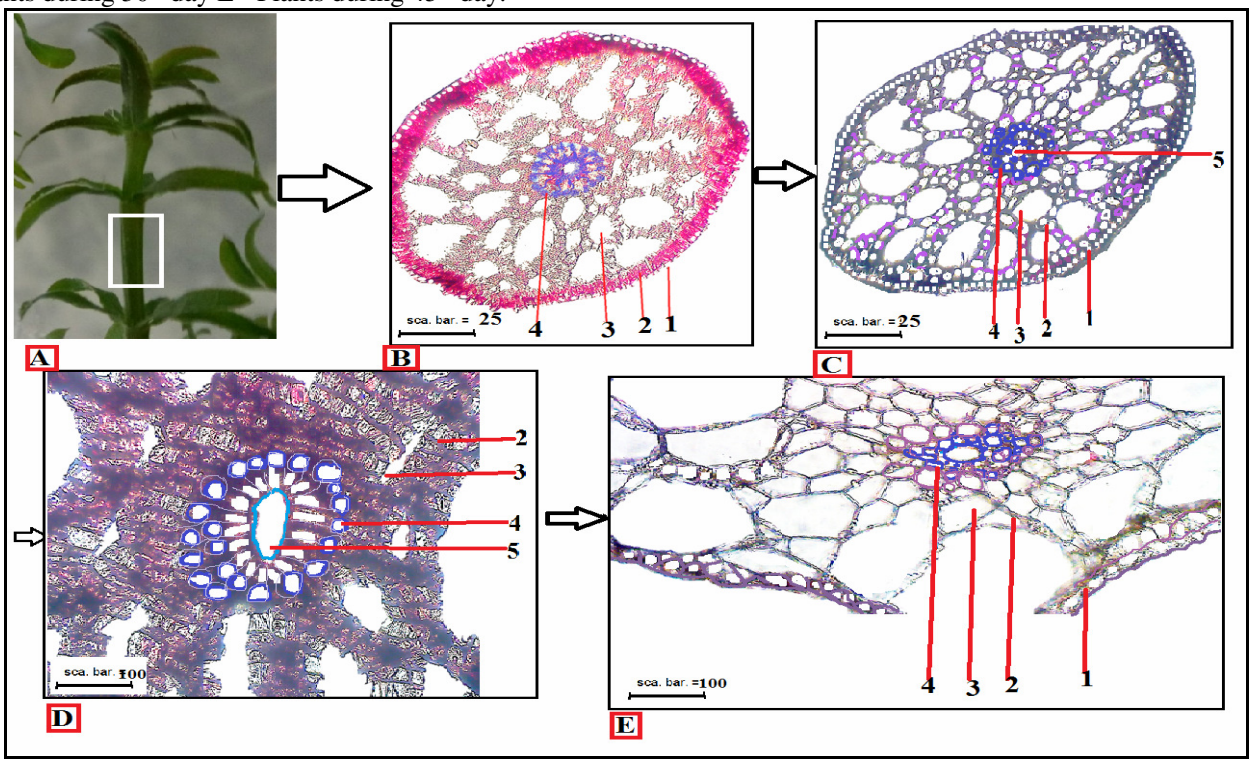

Plate 4 : Stem cross sections of *Hydrilla verticillata* shown in X10 magnification with scale bars =25 µm----------1 in (B and C) plates and X25 magnification with scale bars =100 µm----------1 in (D and E) plates . Showing the effect of adding nutrients with high concentrations to T4,T3 and T2*

Where:

1=Epidermis cells, 2=Parenchyma tissue, 3= Aerenchyma tissue, 4=Vascular bundles, 5= The central pore of vascular bundle. A= *H.verticillata* plants ,B= Plants in Control and first day in high concentration, C= Plants during the 15th day, D= The plants during 30th day E= Plants during 45th day.

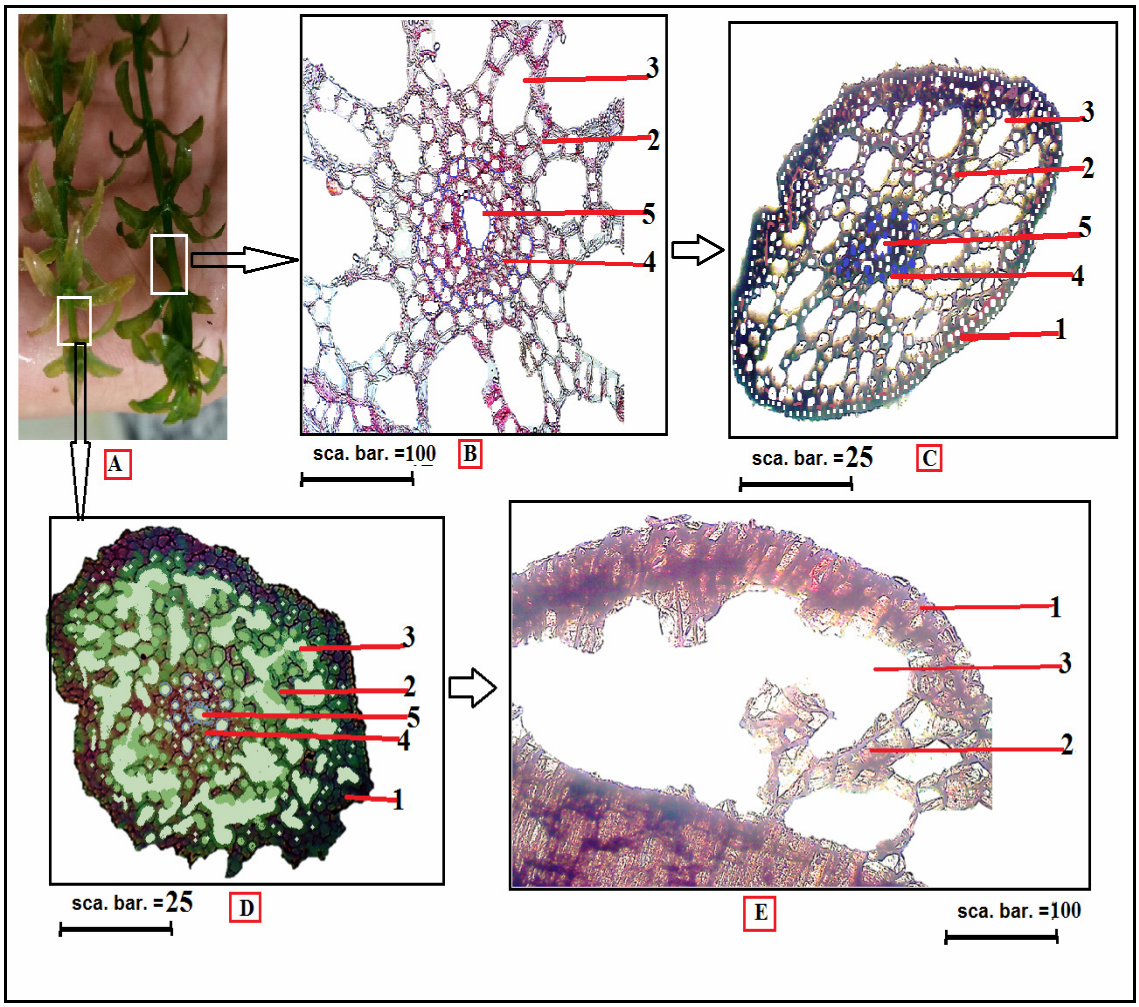

Plate 5: Stem cross sections of *H. verticillata* shown in X10 magnification with scale bars =25 µm----------1 in (C and D) plates and X25 magnification with scale bars =100 µm----------1 in (B and E) plates showing the effect of adding nutrients with low, moderate and high concentrations to T1 . Where: 1=Epidermis cells, 2=Parenchyma tissue, 3= Aerenchyma tissue, 4=Vascular bundles, 5= The central pore of the vascular bundle.
A= *H. verticillata* plants ,B= Plants in Control and first day in low concentration in, C= Plants during 15th day, D= The plants during 30th day E= Plants during 45th day at high concentration.

3.3.5.2. Histological variations of *C. demersum*

The results that observed in (plate 6-C) the cross sections of *C. demersum* stem, that exposing to all concentrations to all treatments during plants during the 15th day, showed shrivel in the surface of the epidermis and decrease the thickness of the cortex layer and increase the thickness of cell walls. Where plants during the 30th day, where shrivel epidermis surface increased. With thinner in cortex thickness and the dissolution of their cells and reduction in the aerenchyma tissue (plate 6–D), while during the 45th day, where the decay in most of the cortex layer resulting from the dissolution of the layer and increased the volume of aerenchyma spaces and fewer in number (plate 6-E). Where the plant at the end of the experiment, all these apparent have been repeated in all concentrations and in all treatments (Plate 6-F).

Anatomical characteristics were studied for *C. demersum* after exposing it, To different concentrations of nutrients (P,N). In the 45th day, Competing with the other plant understudy (*H. verticillata*). Where observed that the stem and leaves were affected by nutrients Throughout the experimental, As the plant showed clear changes in the stem for treatments (T1,T2 and T3) under low conc. where recorded in the 15th day increase branches number with reducing aerenchyma size. While in the 30th day the surface of epidermis appears to shrivel with minimizing the cortex thickness and initiation of aerenchyma dissolution. And in the 45th day where increased aerenchyma layer decay due to the dissolution of the layer, With the remain vascular bundle intact, But the leaves looked weak and detachable. This leads to deterioration cells and plant tissues to makes the plants enter in ageing phase. As the elements enter the leaves through the stomata and accumulate gradually until they reach the wood vessels (Günthardt and Vollenweider, 2007).

But when the plant is exposed to the moderate conc. of the same treatments show increase branches number with reducing aerenchyma size during the 15th day, while during 30th day obtained an increase in the epidermis thickness and sinuosity it, with stiffness in the new branches. In the 45th day were increased the aerenchyma layer deterioration with a minimize of leaves vascular bundle thickness especially a contact points between leaf and stem, in many cases the leaves were separated and floated on the surface of the water.

High conc. of the same treatments in the 15th day showed a decrease in size and the number of branches, at 30th day Where the shrivel in the surface of epidermis appear with a minimize in cortex thickness and initiation of aerenchyma dissolution. Then in the 45th day where plant stem at the end of the experiment becomes completely disintegrates and

remains a thin layer of the epidermis catcher for leaves and plant were floating.

Whereas, treatment (T5) in all concentrations during 15th and 30th day noted early dissolution of the aerenchyma layer appeared and smaller thickness of the stem and leaves in general. While in 45th day seen that a diameter of the vascular bundle become smaller with the disappearance of cortex and aerenchyma layers, finally stems and leaves were decay.

Through the foregoing in the above, the present study suggests that nutrients used (P, N). It was behind the deterioration of the internal structure (leaf and stem) of the two plants understudy (*H.verticillata and C. demersum*) at all concentrations used (High, moderate and low). In the results of our study, we were able to infer that there is a susceptibility to the two plants understudy on the absorption of nutrients and stored within the tissues in the form of crystals after taking the necessary quantity to meet the need it. With the continued absorption increases the accumulation of nutrients within the tissue, then behave nutrients like the behavior of heavy metals or pesticides with the harmful effect on the plant. and because they are collected inside they tend to destroy tissues from inside to the outside. Therefore, in most experimental treatments for all concentrations, the internal parts (central vascular bundle) after 15 days of nutrient supplementation were observed to be degraded and deformed. After 30 days the damage extends to the decomposition of the parenchyma tissue surrounding the vascular bundle and at the end of the experiment (45 days later). Then, symptoms of deformity are visible on the outer epidermis. This leads to the decomposition of the stem and leaves together. Due to lack of nutrient access due to decomposition of vascular bundles and parenchyma tissue, this may be consistent with some studies (Schulze *et al.*, 2002) which indicating that nutrients transport is via the xylem into the storage parenchyma of the stem or into the leaf where nitrate is initially stored together with other nutrient elements.

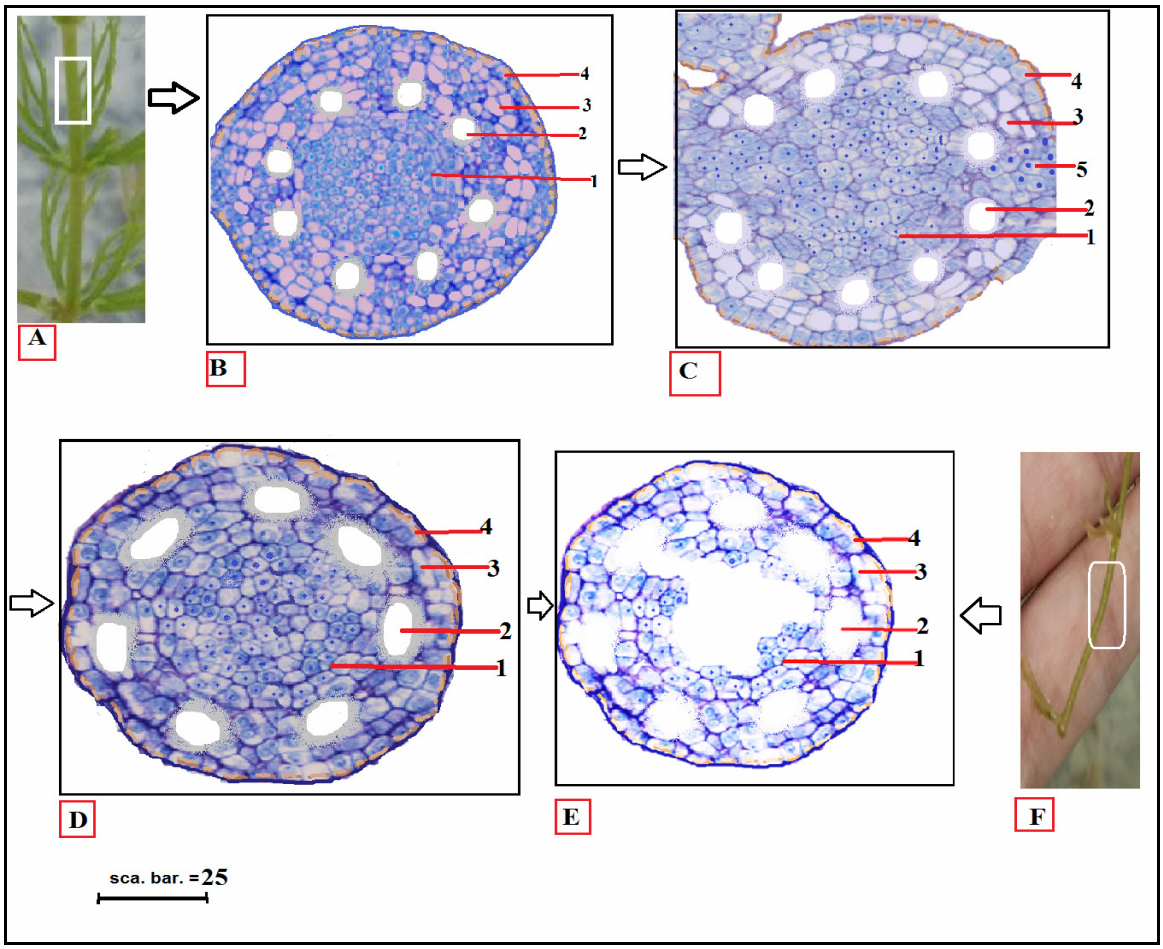

Plate 6 : Cross sections of *C. demersum* stem, shown in X10 magnification with scale bars =25 µm----------1 showing the effect of adding nutrients with all concentrations to all treatments.
where: 1=Vascular bundles, 2= Aerenchyma tissue, 3=Cortex tissue, 4=Epidermis cells.
A= *C. demersum* plant , B= plants in control and first day, C= plants during 15th day, D= plants during 30th day, E= plants during 45th day, F= Plant at the end of the experiment.

Conclusion

Concluded of our results the nutrients, it was behind the deterioration of the internal structure stem of the two plants understudy (*H. verticillata* and *C.demersum*) at all concentrations, where this deterioration began from the inner parts and then expanded outward. And these nutrients (especially in high and moderate concentrations) had a negative role on the structure of the plant stem and led to the degradation of the plants at the end of the experiment.

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