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Anatomical structure of *Antirrhinum majus* plant stem and leaf as affected by diatomite, putrescine and alpha- tocopherol treatments

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Abstract: Two pot experiments were conducted to study the effect of diatomite at the rate of 10, 20 and 30%, Putrescine at the rate of 100 ,200 and 300 ppm and alpha-tocopherol (Vitamine E) at the rate of400,800 and 1200 ppm on anatomical structure of stem and leaf of *Antirrhinum majus* L. plants. Data indicated that, all concentrations of diatomite, putrescine and alpha tocopherol increased stem anatomical structure expressed;stem diameter,phloem thickness,xylem thickness and pith thickness.The best results were found at the concentration of 30%diatomite,200ppm putrescine and 800 ppm alpha tocopherol. On the other hand the thickness of epidermis did not show any difference with the control,while all treatments decreased thicknessof cortex than control plants.All concentrations of diatomite,putrescine and alpha tocopherol increased leaf anatomical structure than control plant expressed; palisade thickness except 300 ppm putrescine, midrib thickness,spongy except 10 and20%diatomite,while the upper epidermis did not show any difference with control and the lower epidermis has similar structure with that shown in control and other concentrations.
Key words: *Antirrhinum majus* L.,diatomite, putrescine, alpha-tocopherol, anatomical structure.

Introduction

Antirrhinum majus L. plant (Snapdragon) family Scrophulariaceae, native to the Mediterranean Region. Recently, 36 species and 20,000 cultivars ofAntirrhinum have been renowned[1]. These cultivars were classified on the basis of their growth and flowering response to day length and temperatures[2] .For landscape dwarf snapdragon cultivars are excellent to be planted at border edges or raised beds and rock gardens. The tall varieties are used as background or as the main feature in a mixed bed as well as cut flowers, while the dwarf and trailing cultivars are great in containers. Growing flower crops compared with traditional crops provide more marketing at both small and large scale for growers and it becoming more selective choice nowadays. Globally, horticultural crops are cultivated in more than 140 countries[3].

Diatomite has neutral pH and stable and will not contribute to change in pH[4] .

Putrescine concentration increases in plants in response to a number of stress factors including water stress, acid treatment, mineral deficiency, and osmotic shock or CO_2 treatment[5].

Alpha tocopherol (Vitamin E) is a powerful biological antioxidant, fertilizers and amendments also induced positive effects on ameliorate the bad effects of salinity stress[6,7] emphasized the beneficial effects of antioxidants on growth characters, leaf chemical composition, yield and yield components of different horticultural crops.

The aim of this study was to investigate the effects of diatomite,Putrescine and Alpha-tocopherol on anatomical structure of *Antirrhinum majus* L.plant.

Materials and Methods

Two pot experiments were carried out during (2011-2012) and (2012-2013) at the nursery of Ornamental Horticultural Department, Faculty of Agriculture, Cairo University, Giza, Egypt and laboratories of Egypt Green farm, Giza, Egypt. The aim was to study the effect of using of diatomite before transplanting and foliar spraying with different levels of Putrescine and alpha-tocopherol application on anatomical structure of *Antirrhinum majus* L. plant.

Seedlings of *Antirrhinum majus* L. dwarf bicolor were obtained uniformly (5cm) in length and carry (2 pairs) of leaves from Egypt Green farm, Giza, Egypt cultured individually in(25 cm diameter) clay pots in the open field on the 15thof November 2011 and 2012. Filled with 4 Kg growing media (clay and sand at the ratio of 1:1 (v/v). After a week from transplanting in both seasons; all seedlings received equal dose (4g/pot) of N.P.K fertilizer (19:19:19) from ammonium nitrate (33%N) calcium super phosphate (15.5%P_2O_5) and potassium sulphate (48%K_2O), respectively and irrigated regularly with tap water. Diatomite application had been added a week before transplanting at the concentrations of (10%, 20% and 30%),i.e. (40, 80 and 120 g/kg) of diatomite having SiO_2 (86-89%) in a soluble form and neutral pH beneficial to plants after three weeks from 1st fertilization on 15 th December in both seasons, the plants were sprayed for three times after a month from transplanting at fortnight intervals i.e (15thDecember, 1st January and 15th January) in both seasons. till run off point with two growth regulators Putrescine (Put) at the concentrations of (100, 200 and 300 ppm) and Alpha-tocopherol (vitamin E) at the concentrations of (400, 800 and 1200 ppm), while the control plants were sprayed with distilled water. Each treatment had three replicates and each replicate contained five plants. The experiment was arranged in completely randomized design, the anatomical studied characteristics in stem were (stem diameter, thickness of epidermis , thickness of cortex, thickness of phloem, thickness of xylem and thickness of pith) and in leaf were (upper epidermis, lower epidermis, thickness of palisade, thickness of spongy, thickness of midrib, thickness of xylem and thickness of phloem).

Anatomical studies

Samples were taken from fifth brunches on the plant after 55 days from transplanting; Transported to the laboratory, then cleaned with tap water cutted into suitable parts, killed and fixed in FAA. Solution (10ml formalin,5 ml acetic acid and 85 ml ethyl alcohol 70%),dehydrated in different concentrations of ethyl alcohol, clearing in different concentrations of ethyl alcohol+Xylene, infiltrated and embedded in pure paraffin wax (M.P.58-60 c^{o})[8]. Sectioning at thickness of 20 µ was performed by using arotary microtome. Paraffin ribbons were mounted on slides and sections stained with safranine and fast green[9]. Sections were mounted in Canda balsam then examined microscopically and microphotography.

Results and Discussion

1-Effect of diatomite, putrescine and alpha-tocopherol treatments on stem anatomical structure (µ) of *Antirrhinum majus* L. Plant.

A-Stem diameter

Data in Table (1)and Fig.(1) stated that, spraying of *Antirrhinum majus* L. plants with diatomite at the concentrations of (10, 20 and 30%) increased stem diameter as compared with control plants. In case of diatomite it was found that the maximum increase in stem diameter (2212.50µ) had been measured for the plants treated with 30% as compared with the untreated plants (2175.00 µ).

Treating the plants with putrescine at the concentrations of (100,200 and 300 ppm) increased stem diameter as compared with control plants. The highest value (2487.50µ) was measured for the plants treated with 200 ppm putrescine as compared with control plants.

Alpha-tocopherol treatments at the concentrations of (400,800 and 1200ppm) increased stem diameter as compared with control plants. The highest value for stem diameter (2200.00µ) was recorded for plants sprayed with 800 ppm alpha-tocopherol as compared with the untreated plants.

B-Thickness of epidermis

The thickness of epidermis did not show any difference to the control treatment.All samples had the same structure thickness (25.00µ)**.**

C-Thickness of cortex

All treatments decreased the thickness of cortex than that of the control plants.

D-Thickness of phloem

Results in Table (1) and Fig (1) showed that, application of diatomite at the concentrations of (10, 20 and 30%) showed simulative in thickness of phloem;except the concentration of 10% diatomite, which recorded the lowest values (50.00µ) compared with control and other treatments, whereas application of 20,30% diatomite increased thickness of phloem searching (75.00 µ) as compared with control plants (62.50µ).

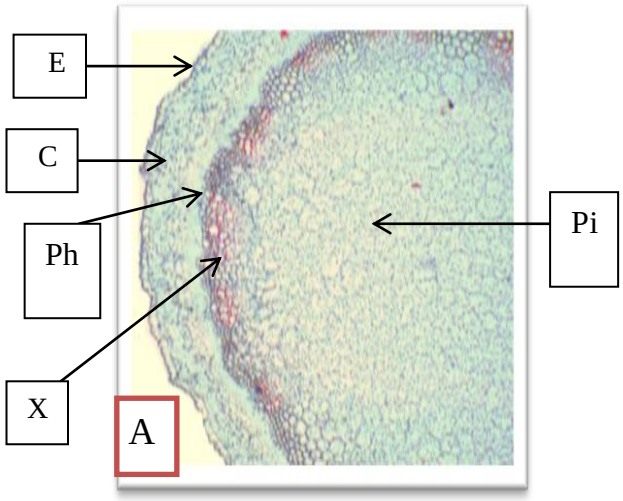
Treating the plants with putrescine at the concentrations of 100 and 300ppm decreased thickness of phloem, while using the concentration of 200 ppm increased thickness of phloem to (68.75 µ) as compared with control plants.

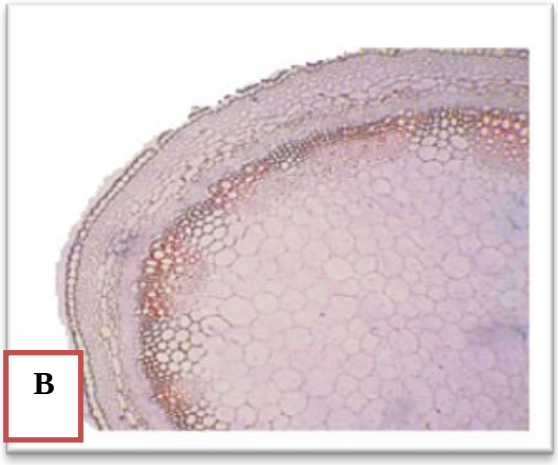
Spraying the plants with alpha-tocopherol at the concentrations of (400 and 1200 ppm) decreased thickness of phloem, whereas application of with800ppm increased thickness of phloem to (75.00µ) as compared with the control plants.

Table (1) Effect of Diatomite, Putrescine and Alpha tocopherol on stem anatomical structure on *Antirrhinum majus* L. plant

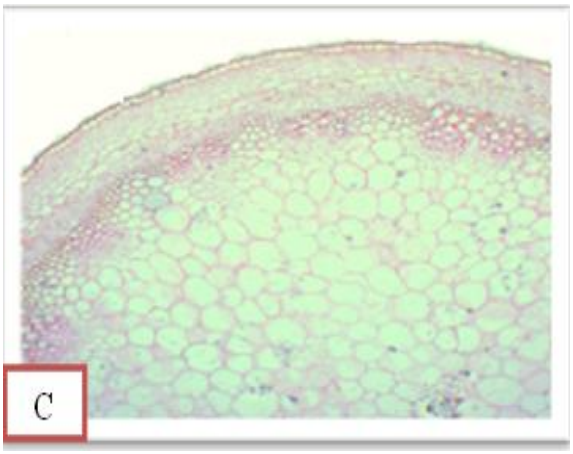
Treatments	Stem diameter	Thickness of epidermis	Thickness of cortex	Thickness of Phloem	Thickness of xylem	Thickness of pith
Control	2175.00	25.00	162.50	62.50	112.50	1437.50
DM.10 %	1987.50	25.00	125.00	50.00	150.00	1137.50
DM.20 %	2200.00	25.00	125.00	75.00	162.50	1137.50
DM.30 %	2212.50	25.00	125.00	75.00	175.00	1137.50
Put.100 ppm	2362.50	25.00	150.00	50.00	175.00	1475.00
Put.200 ppm	2487.50	25.00	150.00	68.75	200.00	1625.00
Put.300 ppm	2087.50	25.00	125.00	50.00	137.50	1312.50
Toco.400 ppm	1950.00	25.00	150.00	50.00	162.50	1137.50
Toco.800 ppm	2200.00	25.00	131.25	75.00	175.00	1337.50
Toco.1200 ppm	1787.50	25.00	150.00	50.00	162.50	1050.00

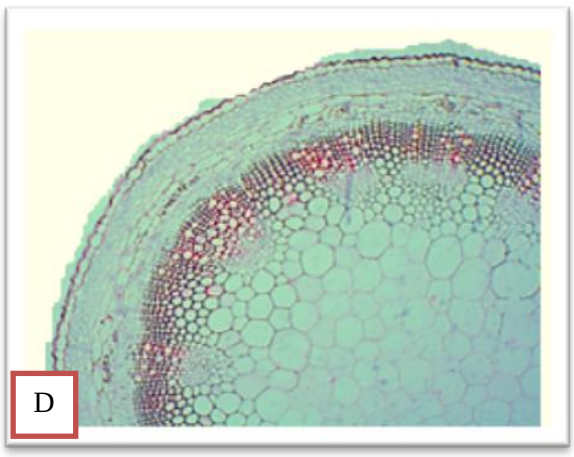
Diatomite: DM , Putrescine: put. , Alpha-tocopherol: Toco





A) - Untreated plants (control) **(B) - Plants treated with 30% diatomit**





(C)- Palnts treated with 200 ppm Putrescine **(D) - plants treated with 800 ppm Alpha tocopherol (V. E).**

Photo (1) Transverse section through the fifth branch of the developed on the main stem of *Antirrhinum majus*L.plant.

pi: epidermis cor: cortex ph: phloem xy: xylm pi: pith

E- Thickness of xylem

The data showed that using diatomite at the concentrations of 10, 20 and 30% increased thickness of xylem giving the highest value was(175.00µ) at 30% as compared with the untreated plants (112.50µ).

Using putrescine at the concentrations of 100,200 and 300 ppm affected positively on xylem thickness compared with the untreated plants. The highest value (200.00µ) was obtained for the application of putrescine of 200 ppm at the concentration as compared with control (112.50µ) this effect might be related to its positive effect on lignifiation.

Spraying the plants with alpha-tocopherol at the concentrations of 400,800 and 1200 ppm increased thickness of xylem as compared with the control plants. The highest value (175.00µ) was obtained from plants treated with alpha-tocopherol at the concentration of 800 ppm as compared with the untreated plants (112.50µ).

F-Thickness of pith

The data in Table (1) and Fig (1) showed that using diatomite at the concentrations of (10, 20 and 30%) resulted in (1137.50µ), which in thinner than that of the control treatments (1437.50µ)**.**These results are in

agreement with those obtained by[10] on rice plants,they found that under silicon application bending moment and culm thickness was increased. [11] on herbaceous peony (*Paeonialaeti flora Pall*) found that, using silicon at 500 µg/mL increased the lapped number of thickened sclerenchyma cells and the thickness of cell walls increased. Moreover, silicon application increased also lignin content.

Spraying putrescine at the concentrations of 100 and 200 ppm increased thickness of pith to (1475.00 and 1625.00 µ), while,raising the concentration to 300 ppm decreased this character to (1312.50µ) as compared with the control, Our results are in agreement with those obtained by [12], they concluded that polyamine metabolism has been related to the formation of the reactive oxygen species (ROS) that play a key role in cell wall expansion and growth, vascular differentiation, and lignin polymerization during vascular differentiation, lignin is formed with the concurrence of H_2O_2 and cell wall bound peroxidases.

In case of alpha tocopherol it was found that all concentrations decreased in pith thickness than control plants. Our results are in agreement with those obtained by [13]on papaya plants and [14]on snap bean plants.The effectiveness of any given antioxidant in the plant depends on which free radical is involved, and where the target of damage is. Thus, while in one particular system an antioxidant may protect against free radicals, in other system it could have no effect at all, or in certain circumstances, an antioxidant even act a "pro-oxidant" that generates toxic oxygen species[15].

2-Effect of diatomite, putrescine and alpha-tocopherol treatments on leaf anatomical structure of *Antirrhinum majus* L. Plant.

A-Upper epidermis

The upper epidermis did not show any difference between the treated plants and the control, giving is similar structure to each other.

B-Lower epidermis

The lower epidermis showed similar structure for all plants either treated or not treated plants.

C-Thickness of palisade

The data presented in Table (2) and Fig. (2) stated that, spraying *Antirrhinum majus* L. plant with diatomite at the concentrations of 10, 20 and 30% increased in thickness of palisade as compared with the control. The thickness of palisade increased steadily as the diatomite concentration was raised, giving the highest value (160.00µ) showed a highest increase than both other concentrations and control by (100.00µ).

Concerning the effect of putrescine treatments, the highest value for palisade thickness (170.00µ) was measured for that treated with 200 ppm as compared with the other concentrations and the control plants.

All concentrations of alpha-tocopherol increased the palisade thickness as compared with the control plants, giving its highest value (170.00 µ) for the plants treated with (800 ppm),as compared with the other concentrations and the control treatments.

D-Thickness of the spongy tissue

The data in Table(2) and Fig (2) revealed that, application of diatomite at the concentration of 30 % resulted in the highest value (135.00 µ) for thickness of spongy as compared with the two other concentrations and untreated plants (120.00µ).

Using putrescine at the concentrations of 100, 200 and 300ppm increased thickness of spongy as compared with the untreated plants. The highest value (160.00µ) was measured for the plants treated with 200 ppm putrescine compared with the control plants (120.00µ).

On the other hand spraying the plants with alpha-tocopherol at the concentrations of 400,800 and 1200 ppm increased thickness of spongy as compared with the untreated plants. The highest value (150.00µ) was measured when the plants were sprayed with 800 ppm alpha tocopherol as compared with the control (120.00µ) and other concentrations (130.00µ).

E-Thickness of midrib

The data presented in Table (2) and illustrated in Fig (2) showed that all the applied concentrations of diatomite increased the midrib thickness steadily as the concentration was raised giving the value(37.0 µ) for the plants treated with 30%diatomite compared with the untreated plants.

Spraying the plants with putrescine at the concentrations of 100, 200 and 300 ppm increased thickness of midrib giving the highest value (410.00µ) when the plants were treated with 200 ppm putrescine as compared with the untreated plants.

Concerning the application of alpha-tocopherol, all concentrations affected positively on the thickness of midrib giving the highest value (400.00µ) for the plants treated with 800 ppm as compared with the untreated plants and other treatments.

F-Thickness of xylem

The data in Table (2) and Fig (2) showed that, application of diatomite at the concentrations of 10, 20 and 30% increased thickness of xylem as compared with the control giving same value (50.00 µ) for all concentrations. This means that the concentrations did not have any variable effect on the thickness of xylem.

Treated plants with putrescine at the concentrations of 100,200 and 300 ppm affected positively on thickness of xylem resulting in its highest value (70.00µ) when the plants were treated with100 and 200 ppm putrescine as compared control plants (30.00µ).Higher concentration 300 ppm decreased the value to 50.0µ .This might explained that low or medium concentrations were more effective than the higher one.

In relation to alpha- tocopherol application of 400,800 and 1200ppm increased thickness of xylem as compared with the untreated plants resulting in its highest value (50.00µ) when the plants were treated with 400 and 800 ppm alpha tocopherol,while higher concentration (1200 ppm) decreased this value to(40.00µ) as compared with control plants(30.00µ).

G-Thickness of phloem

The data revealed that application of diatomite treatments at 10, 20 and 30% affected only on this character at 30% resulting in its highest value (30.00 µ) as compared with the other treatments and the untreated plants (20.00µ).These results are in agreement which with that obtained by [16] on orchid plant, they indicated that the Silicon may have contributed to the structural stability of the leaves. The silicon may have become incorporated in the cell walls and may have helped to maintain the leaf structure. The leaf structure is essential for the correct development of these plants because of its support on the chlorenchyma which is responsible for photosynthesis.

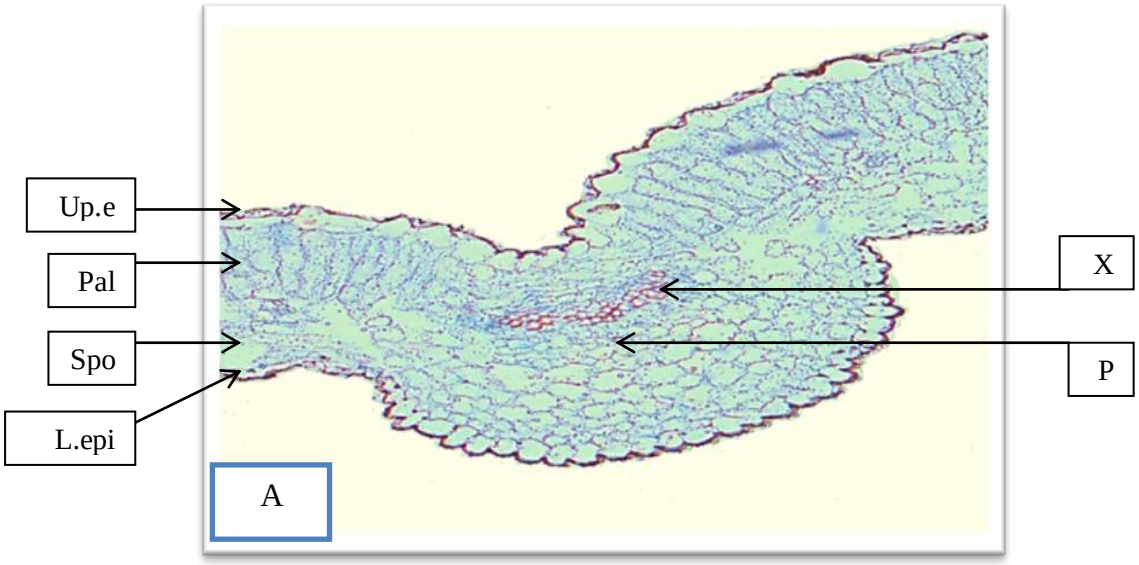
The application of putrescine at 200 ppm gave the highest value (30.00µ),while the other concentrations (100 and 300 ppm) resulted in the same values (20.00µ). These results are in hormony with that of the polyamine metabolism which related to the formation of the reactive oxygen species (ROS) that play a key role in cell wall expansion and growth, vascular differentiation, and lignin polymerization, so polyamines metabolism play an important role direct or indirect for increasing thickenss by increasing metabolism[12].

Spraying the plants with alpha tocopherol at the concentrations of 400,800 and 1200 ppmdid not effect on phloem thickness by giving equal to that of the control plants (20.0µ). These results are in agreement with those obtained by [14] on snap bean plants,they reported that ascorbic acid (antioxidant) plays important roles in the plant, such as protective role against reactive oxygen species that are formed from photosynthetic and respiratory processes. Moreover AsA is linked to cell growth, being involved in the cell cycle and other mechanisms of plant cell growth and division, as well as acting as a co-factor for many enzymes.

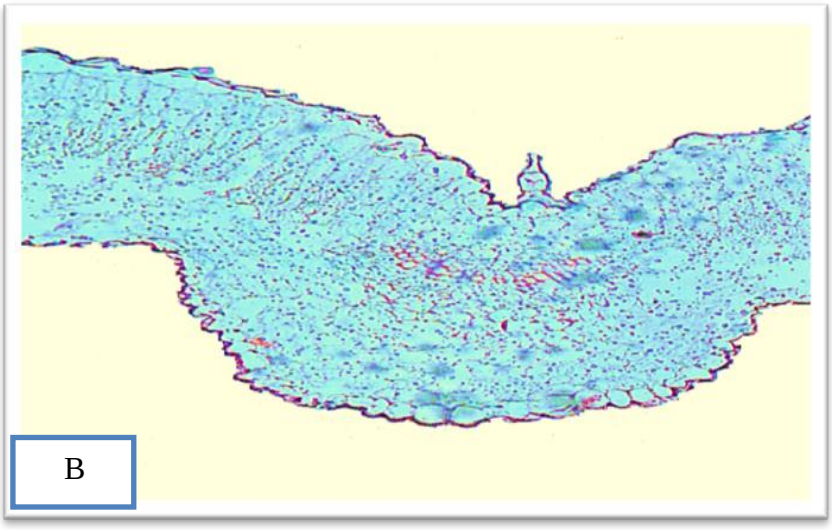
Table (2) Effect of Diatomite, Putrescine and Alpha tocopherol on leaf anatomical structure of*Antirrhinummajus* L. Plant.

Treatment	Upper epidermis	Lower epidermis	Thickness of Palisade	Thickness of spongy	Thickness of midrib	Thickness of xylem	Thickness of phloem
Control	30.00	20.00	100.00	120.00	330.00	30.00	20.00
DM.10 %	25.00	20.00	110.00	120.00	340.00	50.00	20.00
DM.20 %	30.00	20.00	140.00	120.00	360.00	50.00	20.00
DM.30 %	35.00	25.00	160.00	135.00	370.00	50.00	30.00
Put.100 ppm	30.00	20.00	150.00	140.00	400.00	70.00	20.00
Put.200 ppm	30.00	20.00	170.00	160.00	410.00	70.00	30.00
Put.300 ppm	30.00	20.00	100.00	100.00	350.00	50.00	20.00
Toco. 400 ppm	30.00	20.00	150.00	130.00	370.00	50.00	20.00
Toco. 800 ppm	30.00	20.00	170.00	150.00	400.00	50.00	20.00
Toco.1200 ppm	30.00	20.00	150.00	130.00	360.00	40.00	20.00

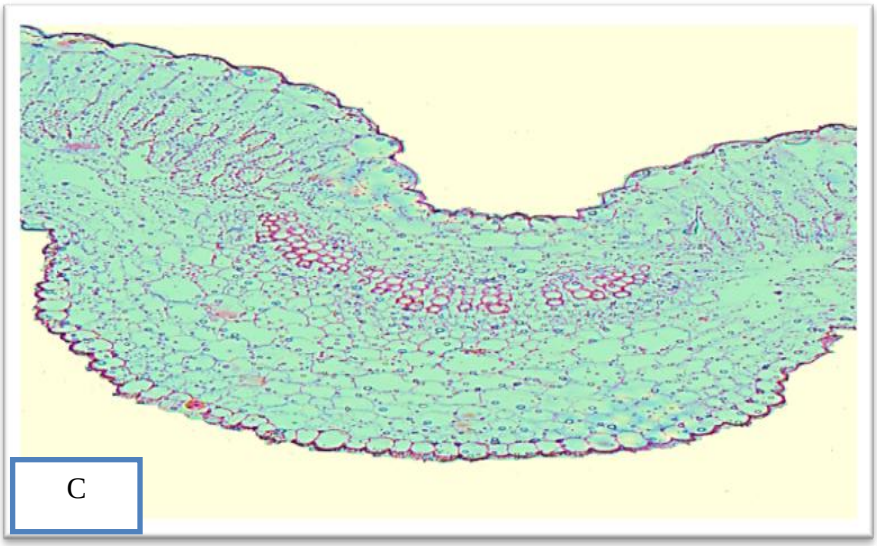
Diatomite: DM , Putrescine: put. , Alpha-tocopherol: Toco



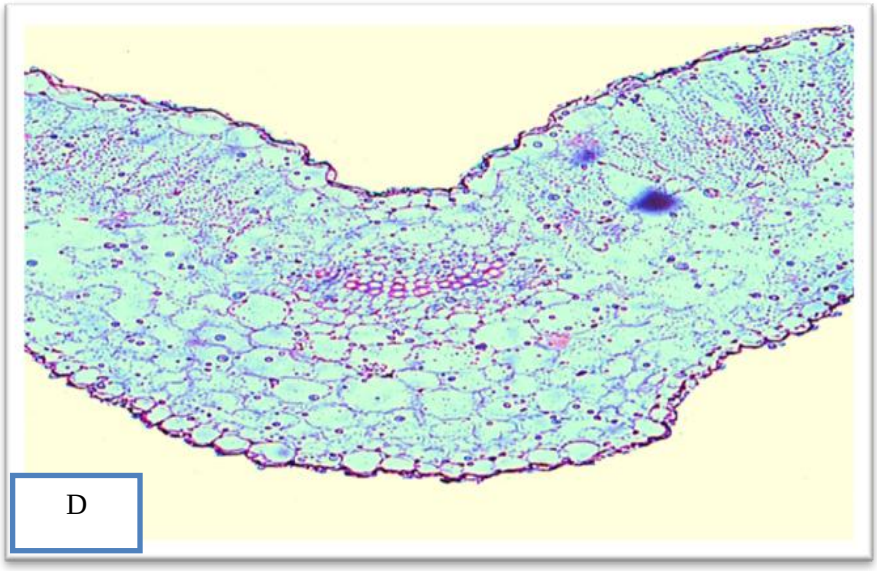
(A)- Untreated plants (control) Up.epi: upper epidermis Pal: palisade Spo: spongy l.ep: lower epidermis Xy: xylem Ph: phloem



(B)- Plants treated with 30% diatomite



(C)- Plants treated with 200 ppm Putrescine



(D) - Plants treated with 800 ppm Alpha tocopherol (vitamin E).

Photo. (2) Transverse section through the blade of the eighth leaf on the fifth branch developed on the main stem of *Antirrhinum majus*L.plant.

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