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Impact of the foliar spray with benzyl adenine, paclobutrazol, algae extract, some mineral nutrients and lithovit on anatomical features of moringa olifera plant

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Abstract

This study was carried out at The Experimental Station of Agricultural Botany Department, Faculty of Agriculture, Benha university, Qlubia governorate, Egypt during 2015 and 2016 seasons to evaluate the effect of foliar application with benzyl adenine (BA) at 100 mg/L, Paclobutrazol (PP_{333}) at 20 mg/L, algae extract at 20 ml/L, some mineral nutrients (i.e., Ca,Mg and Fe) at 500 mg/L and lithovit at 500 mg/L on leaflets and stems histological features of *Moringa olifera* plants at 90 and 180 days after sowing. Results revealed that different anatomical characteristics of moringa leaflets and stems were positively responded in the two stages of growth. Also, the obtained data showed that: the most traits of moringa leaflet anatomical features were increased with different applied treatments. In this respect, Paclobutrazol (PP_{333}) at 20 mg/L, algae extract at 20 ml/L and lithovit at 500 mg/L were the most pronounced treatments. Of these anatomical features were thickness of leaflet midvein, length & width of midvein vascular bundle, phloem & xylem tissues thickness, number of xylem vessels in midvein vascular bundle as well as the leaflet lamina thickness for leaflet. The cuticle thickness, epidermis thickness, cortex thickness, thickness of phloem tissue, thickness of cambial region, xylem tissue thickness, number of xylem vessels /xylem row, number of xylem rows /vascular cylinder and diameter of the xylem vessel in the stem. Generally, Paclobutrazol (PP_{333}) at 20 mg/L and algae extract at 20 ml/L appeared to be the most effective treatments on increasing counts and measurements of the studied histological features of *Moringa oleifera* leaflets and stems either at 90 or 180 days after sowing.

Key Words: Moringa, benzyladenine, paclobutrazol, algae extract, minerals, lithovit and anatomy.

Introduction

Moringa (*Moringa oleifera* L.) is the most widely cultivated species of Moringace family due to its easy propagation, fast growth and its numerous economic uses.

Moringa oleifera is shown in scientific division to become from Kingdom: Plantae, Division: Magnoliphyta, Class: agnoliopsida, Order: Brassicales, Family: Moringaceae, Genus: Moringa, Species: M.oleifera **(Fahey, 2005)**. *Moringa oleifera* is one of the most useful tropical trees. Its leaves are extremely valuable source of nutrition for people of all ages. Nutritional analysis indicates that Moringa leaves contain affluence of essential, disease preventing nutrients. They even contain all of the essential amino acids, which is unusual for a plant source. The young leaves are edible and are commonly cooked and eaten like spinach or used to make soups and salads. They are an exceptionally good source of antioxidant compounds such as flavonoids, ascorbic acid, carotenoids phenolics and some mineral nutrients (in particularly iron), and the sulphur-containing amino acids methionine and cystine. The composition of the amino acids in the leaf protein is well balanced; they contain high amounts of many of these nutrients and total phenols also a very low source of fat **Mishra *et al.*,(2012); Osman and Abohassan (2012)*;* Abdull Razis *et al.*,(2014) and Dao & Kabore (2015).** The leaves, fruit, flowers and immature pods of this tree are used as a highly nutritive vegetable in many countries, particularly in India, Pakistan, Philippines, Hawaii and many parts of Africa **Mughal *et al.*,(1999)**; **Ferreira *et al.*, (2008) and Mishra *et al.*,(2011)**.
Moringa is a fast growing deciduous shrub or small tree up to 12 m tall and 30 cm in diameter with an umbrella-shaped open crown. The bark is corky and gummy. Leaves are alternate, oddly bi- or tri-pinnate compound, triangular in outline and 20– 70 cm long. Each pinnae has 3–9 pairs of 1–2 cm long ovate leaflets, soft dark green above and whitish below. The white, fragrant flowers that are obliquely monosymmetric and papilionoid with five stamens, are in axillary pendulous panicles 1.5–2 cm long from leaf corners. The fruit pods, called “drumsticks” are 15–45 cm long, 9-ribbed capsules **Foidl *et al.*,(2001); National Research Council (2006) and Orwa *et al.*,(2009)**.

Nowadays, it has become necessary to search for untraditional growth substances as substitutes for increasing plant growth and productivity to particularly reduce the gap between production and consumption.

In the same order, the cultivated area of Moringa (*Moringa oleifera*) plant at ascend increasing in

Egypt. It gains worldwide attention due to it's nutritional quality, genetic variability, adaptability to adverse climate and soil conditions, and low production cost. It is acclaimed universally as a power house of plant products. Apart from this, Moringa a rich source of a wide range of mineral nutrients, vitamins **Fuglie (1999)** , large amounts of natural powerful antioxidants and medicinal purposes **Foidl *et al*., (1999) and Ghasi *et al*., (2000).**

Many investigators reported that improving the physiological, morphological and anatomical performances of plants could be achieved by application of different natural and chemical growth substances to enhance and maximizing its growth characteristics.

Regarding to paclobutrazol, it is a triazole compound widely used as retardant for the controlling the vegetative growth of a wide range of angiosperm (**Terri and Millie (2000) and Ahamed Nazaradin *et al.,* (2007)**. Paclobutrazol induce a variety of morphological and anatomical responses in plants. The main effect of paclobutrazol takes place through the alteration of hormonal balance. Since, it inhibits gibberellin biosynthesis, reducing cell division and cell elongation and retarding plant growth **Gopi et al.,(2009)** and **Youssef & Abd El-Aal (2013). Berova and Zlatev (2000)** reported that a reduced height and an increased stem thickness of tomato in response to paclobutrazol treatment. **Fletcher *et al.,* (2000)** had shown that Paclobutrazol inhibits gibberellin biosynthesis, hence, reducing cell elongation and retarding plant growth. In addition, **Bai *et al.,* (2004)** stated that the cambial growth of *Liquidambar styraciflua* and *Alnus glutinosa* was reduced following Paclobutrazol treatment. **Tekalign *et al.,* (2005)** concluded that the leaves of *Solanum tuberosum* treated with Paclobutrazol showed increase epicuticular wax layer, elongated and thicker epidermal as well as the palisade and spongy mesophyll cells. **Kishorekumar *et al.,* (2006)** found that the number of cells per unit area in the palisade spongy layers and chloroplast number per cells in the leaves of *Solenostemon rotundifolius* increased by Paclobutrazol treatment when compared to the control leaves. **Ahamed Nazaradin *et al.,* (2007)** concluded that Scanning electron micrographs of stems showed reduction in xylem thickness, while in the leaves, palisade and spongy parenchyma cells were more closely arranged in response to paclobutrazol. There was also an increase in palisade thickness of leaves of plants treated with 3.75 g l–1 paclobutrazol. Paclobutrazol was found to effectively inhibit plant height and leaf expansion and alter the stem and leaf anatomy of *S. campanulatum*. **Youssef and Abd El-Aal (2013)** found that most anatomical traits of Tabernaemontana plant leaf i.e., thickness of leaf midrib, length & width of vascular bundle, phloem & xylem tissues and number of xylem vessels in vascular bundle as well as the leaf blade thickness were increased with different applied treatments, especially PP333 at 150 and 100 ppm compared with the untreated plants. **Youssef (2004)** mentioned that treated *Strelitzia reginae* plants with PP_{333} at 300 and 200 ppm increased blade thickness, palisade thickness and phloem tissue thickness.

Ahamed Nazaradin *et al.,* (2015) had concluded that Paclobutrazol (PBZ) altered the anatomical structure in the stem and leaf of *X. chrysanthus*. Palisade and spongy mesophyll cells in the leaf of the treated plants were tightly arranged as compared to that of the control plants. The palisade parenchyma thickness in the leaf was increased, while xylem thickness in the stem was reduced with PBZ application.

For Benzyl adenine, many authors reported that Benzyl adenine is an important plant hormone that regulate various processes of plant growth and development including cell division and differentiation, organogenesis in developing plants and enhancement of leaf expansion and nutrient mobilization, improve plant growth and development Davies**, (1995).** It is also implicated in the vascular development and synthesis of secondary metabolites like indols, alkaloids and anthocyanins. **Duszka et al., (2009). El-Badawy and Abd El-Aal (2013)** stated that all tested treatments of kinetin enhanced most of the studied leaf anatomical features i.e., midrib thickness, length and width of vascular bundle, phloem and xylem tissues and number of xylem vessels in vascular bundle as well as the leaf blade thickness of mango plant.

Youssef and Abd El-Aal (2014) found that treatments of kinetin increased histological characteristics of Hippeastrum leaf (i.e., cuticle thickness, epidermis thickness, length & width of vascular bundle, phloem & xylem tissues thickness and number of xylem vessels in vascular bundle as well as the lamina thickness). Also, **Devlin and Witham (1983)** concluded that cytokinins known and will recommended to increase the extension growth of plant tissue. This may increase the proportion of protoplasm to cell wall with the result of increased cell size **(Marschner, 1997)**

Regarding to Algae extract, **Blunden (1991) ; Crouch and Van Staden (1994) and Khan *et al.,* (2009)** reported that seaweed extract application for different crops was a great importance due to contains high levels of organic matter, micro elements (Fe, Cu, Zn, Co, Mo, Mn,and Ni), vitamins and amino acids and also, rich in growth regulators such as auxins, cytokinin and gibberellins .

Salama and Yousef (2015) recorded that foliar applications of *Ocimum sanctum* L. plant with seaweed extract at concentration of 1.5ml/L, increased the diameter of the main stem by 2.6% more than that of the control. The thickness of epidermis, cortex, phloem and xylem tissues were 13.3, 2.9, 21.0 and 47.6% respectively, more than those of the control, while a decrement of 4.6% in parenchymatous pith thickness was observed in plants treated with seaweed extract at 1.5 ml/L less than the control.

For Some mineral nutrients, several investigators studied the effect of nutrients on the anatomical characters of several plants. **Agamy(2004)** on sweet fennel plant and **Xu *et al.*, (2008)** they found a pronounce effects on thickness of epidermis and cortex and number and diameter of vascular bundles of stem with some mineral nutrients treatment. **Mohammed (2005)** who found an increase in stem anatomical characteristics of treated Dill plant with some elements. **Abbas (2013)** reported that foliar fertilizer (Oligo green HFcontains Fe, Zn, Mn, Cu and B) at rates of 0, 50, 100 and 150mg/L on Dill (*Anethum graveolens* L.) plant has positive effect on most anatomical characters. Foliar fertilizer caused significant increase in cortex thickness, number and thickness of vascular bundles and vascular units diameter, on the other hand Pith thickness decreased significantly. **El-Tantawy and Eisa (2009)** recorded favorable anatomical changes in leaf and stem anatomy due to the effect of nutrients mixture.

So, the aim of this study was conducted to follow up the internal structure of Moringa (*Moringa oleifera*) leaflets and stems which exhibit the most noticeable response to individually used growth substances i.e., benzyl adenine at 100 mg/L, Paclobutrazol (PP_{333}) at 20 mg/L, algae extract at 20 ml/L, some mineral nutrients at 500 mg/L and lithovit at 500 mg/L compared with the control (the untreated one) treatment at 90 and 180 days after sowing.

Material and Methods

This study was carried out at the Experimental Station of Agricultural Botany Department, Faculty of Agriculture, Benha university, Qlubia governorate, Egypt during 2015 and 2016 seasons to evaluate the effect of foliar application with Benzyl Adenine (BA) at 100 mg/L, Paclobutrazol (PP_{333}) at 20 mg/L, Algae extract at 20 ml/L, Some mineral nutrients (Ca,Mg and Fe) at 500 mg/L and Lithovit at 500 mg/L treatments comparing with the control (Tap water) on leaflet and stem histological features of potted *Moringa olifera* plants at 90 and 180 days after sowing. Uniform *Moringa olifera* seeds were obtained from Agriculture Research Centre, Giza, Egypt. Moringa seeds were sowed at 5 th May in 30 cm diameter plastic pots filled with a mixture of 1 clay: 2 sand (w:w). The pots were arranged in a complete randomized design with 10 replicates. All agricultural practices were done as usual. Foliar application with treatments was carried out four times, first at 15 days after sowing and repeated every 15 days as foliar spray to cover completely the plant foliage. It was intended to carry out a comparative anatomical study on leaflets and stems of treated Moringa plants.

Anatomical study:

Specimens of Moringa (*Moringa oleifera*) stems and terminal leaflets were taken from the 5th apical internode of the main stem and its corresponding leaf of treated plants and those of the control either at 90 and 180 days after sowing during season of 2016 only. These specimens were then killed and fixed in F.A.A. (10 ml formalin, 5 ml glacial acetic acid and 85 ml ethyl alcohol 70%), washed in 50% ethyl alcohol, dehydrated in a series of ethyl alcohols 70, 90, 95 and 100%, infiltrated in xylene embedded in paraffin wax with a melting point 60-63°C, sectioned 12 microns in thickness for stems and the leaflets **(Sass, 1951)**, stained with the double stain method (Fast green and safranin), cleared in xylene and mounted in Canada balsam **(Johanson, 1940).** Four sections treatment were microscopically inspected to detect histological manifestations of noticeable responses resulted from treatments. Counts and measurements (μ) were taken using a micrometer eye piece. Averages of readings from 4 slides / treatment were calculated. Anatomical features of the treated plants compared with the control based on transverse sections of the leaflets and stems were studied.

Results and Discussion

1. Anatomical alterations of Moringa *(Moringa oleifera*) leaflets in response to different applied treatments either at 90 and 180 days after sowing:

As shown in **Table 1** and **Figs. 1&2** data indicate the effect of different applied treatments i.e., benzyl adenine at 100 mg/L, Paclobutrazol (PP_{333}) at 20 mg/L, algae extract at 20 ml/L, some mineral nutrients at 500 mg/L and lithovit at 500 mg/L compared with the control treatment upon different anatomical features of Moringa (*Moringa oleifera)* leaflet. In this respect, most of the applied treatments have a positively impact on most studied histological characteristics of *Moringa oleifera* leaflets (cuticle thickness, epidermis thickness, midvein thickness, length & width of vascular bundle, phloem and xylem tissues thickness and number of xylem vessels in vascular bundle in addition to the lamina thickness) under different used treatments compared with the untreated one either at 90 and 180 days after sowing.

The obtained results clearly show that *Moringa oleifera* plants treated with Paclobutrazol (PP_{333}) at 20 mg/L, algae extract at 20 ml/L and Lithovit at 500 mg/L appeared to be the most effective treatments on increasing counts and measurements of certain histological features of *Moringa oleifera* leaflet compared with the other and the control treatments either at 90 or 180 days after sowing.

With regard to the leaflet lamina thickness it was increased to reach its maximum value (149 and 131μ) with Paclobutrazol (PP_{333}) at 20 mg/L and lithovit at 500 mg/L treatments respectively, followed by algae extract at 20 ml/L (129µ) and benzyl adenine at 100 mg/L (109µ) respectively, compared with the control treatment (102µ) at 90 days after sowing, while their values were (175,156,148,167 and 132µ) respectively, at 180 days after sowing.

For mesophyll tissue, thickness of both spongy and palisade tissues were recorded their maximum values with Paclobutrazol (PP_{333}) at 20 mg/L and algae extract at 20 ml/L treatments. Here, spongy tissue thickness was (23µ) with the control but increased to reach (47µ) and (39µ) with Paclobutrazol (PP_{333}) at 20 mg/L and algae extract at 20 ml/L treatments respectively, which were the most effective treatments. For palisade tissue thickness reached its maximum value (58 and 46 µ) with the same treatments respectively, but decreased to reach its minimum value (37µ) with the untreated (the control) treatment at 90 days after sowing.

Table 1. Effect of different applied treatments on the mean counts and measurements of certain histological features of Moringa (*Moringa oleifera*) leaflet at 90 and 180 days after sowing.

Histological Characteristics / Treatments	Thickness of upper epidermis cuticle layer	Thickness of Lower epidermis cuticle layer	Upper epidermis thickness	Lower epidermis thickness	Spongy tissue thickness	Number of spongy tissue layers	Palisade tissue thickness	Lamina thickness	Midvein thickness	Length of midvein vascular bundle	Width of midvein vascular bundle	Number of vessels i/ midvein vascular bundle	Vessel diameter
At 90 days after sowing													
Control	10	9	13	10	23	4	37	102	233	69	62	4	20
Benzyl adenine at 100 mg/L	12	8	16	9	28	6	36	109	251	83	102	12	19
Paclobutrazol (PP_{333}) at 20 mg/L	11	7	15	11	47	5	58	149	293	138	153	13	24
Algae extract at 20 ml/L	9	6	19	10	39	5	46	129	254	113	144	8	22
Mineral nutrients at 500 mg/L	10	8	15	12	24	5	31	100	237	76	92	11	21
Lithovit at 500 mg/L	12	7	14	10	36	4	42	131	241	97	106	9	25
At 180 days after sowing													
Control	13	10	19	12	34	6	44	132	231	101	75	7	25
Benzyl adenine at 100 mg/L	14	11	18	10	53	7	61	167	287	153	141	14	23
Paclobutrazol (PP_{333}) at 20 mg/L	12	11	16	13	55	7	68	175	301	122	167	18	27
Algae extract at 20 ml/L	11	10	18	11	45	6	53	148	276	107	164	12	28
Mineral nutrients at 500 mg/L	12	9	15	14	38	5	52	147	262	108	119	13	28
Lithovit at 500 mg/L	13	8	18	11	51	6	55	156	284	111	161	11	31

On the other hand for mesophyll tissue at 180 days after sowing, thickness of both spongy and palisade tissues were recorded their maximum values with Paclobutrazol (PP_{333}) at 20 mg/L and benzyl adenine at 100 mg/L treatments. Here, spongy tissue thickness was (34µ) with the control but increased to reach (55µ) and (53µ) with Paclobutrazol (PP_{333}) at 20 mg/L and benzyl adenine at 100 mg/L treatments respectively, which were the most effective treatments. For palisade tissue thickness reached its maximum value (68 and 61 µ) with the same treatments respectively, but decreased to reach its minimum value (44µ) with the untreated (the control) treatment.

This may explain the positive effect of used treatments in increasing cell division and elongation.

Regarding, vascular tissues at 90 days after sowing (i.e., xylem and phloem tissues) length and width of midvein vascular bundle were reached their maximum values with Paclobutrazol (PP333) at 20 mg/L, algae extract at 20 ml/L and lithovit at 500 mg/L treatments respectively. Here, length of midvein vascular bundle were 138,113 µ and 97 µ respectively, which were the more effective treatments in the same order. Also, width of midvein vascular bundle values were153,144 µ and 106 µ with the same treatments respectively

As for length and width of midvein vascular bundle at 180 days after sowing, behaved as the same as at 90 days after sowing.

With regard to midvein anatomical features, it could be noticed that there an increment in the midrib thickness especially, in case of Paclobutrazol (PP333) at 20 mg/L (293µ), algae extract at 20 ml/L, (254µ) and benzyl adenine at 100 mg/L (251µ) treatments compared with other treatments and the control (233µ) at 90 days after sowing, while their values were (301, 276 and 287 µ) compared with the control (231µ) respectively at 180 days after sowing. Increasing midrib thickness is attributed to the increase in many of its histological features such as thickness of both uppermost and lower most collenchyma tissues, lower most parenchyma tissue and dimensions of main vascular bundle as well as thickness of phloem tissue, xylem tissue and also number and diameter of xylem vessels in the vascular bundle.

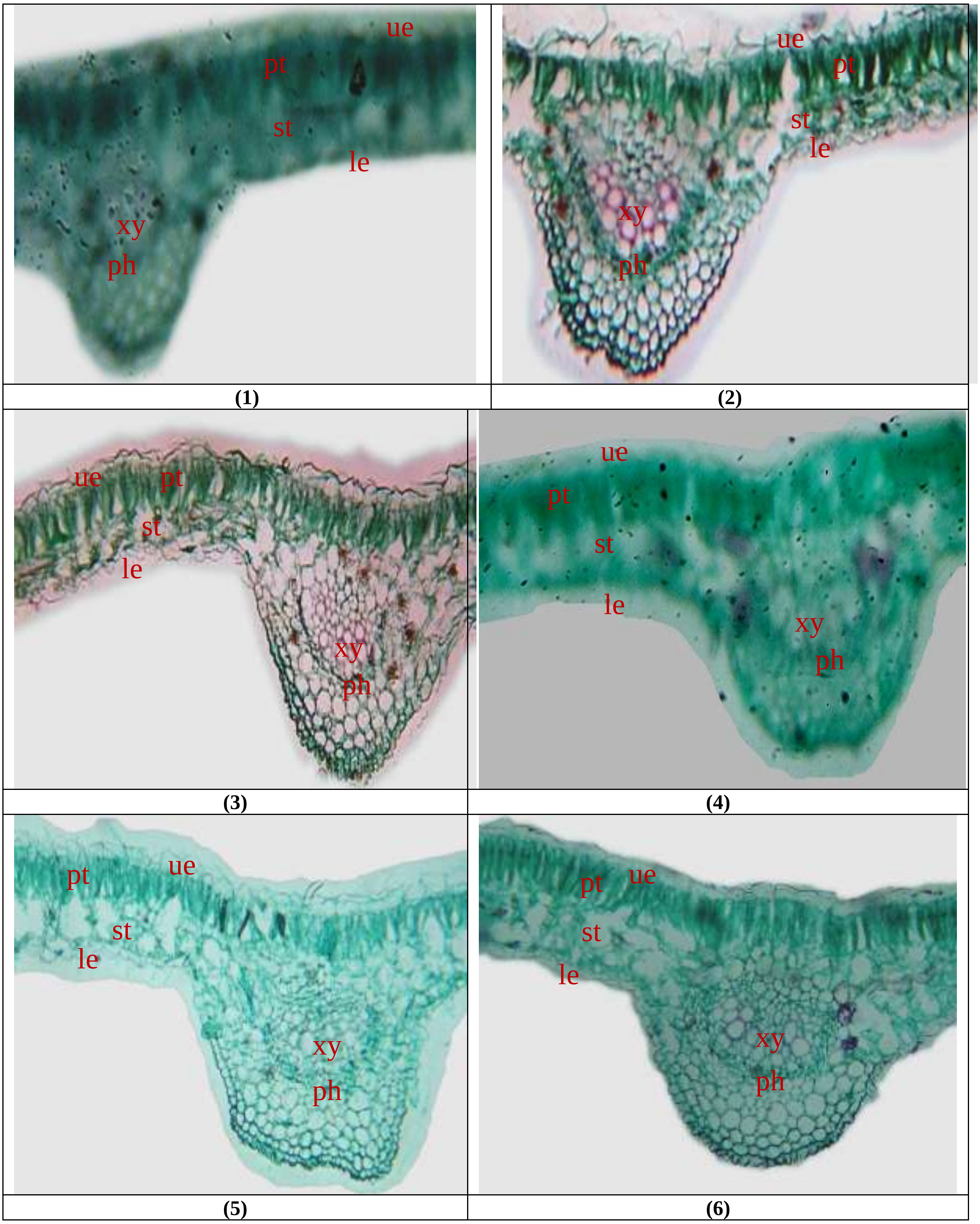


Fig. 1: Transverse sections (X 40) of Moringa (*Moringa oleifera*) leaflet at 90 days after sowing as affected by different applied treatments.

Where: (1): Control (2): Benzyl adenine at 100 mg/L (3): Paclobutrazol (PP333) at 20 mg/L (4): Algae extract at 20 ml/L (5): Mineral nutrients at 500 mg/L (6): Lithovit at 500 mg/L

ue= Upper epidermis pt= Palisade tissue st= Spongy tissue le= Lower epidermis

ph= phloem tissue xy= Xylem tissue

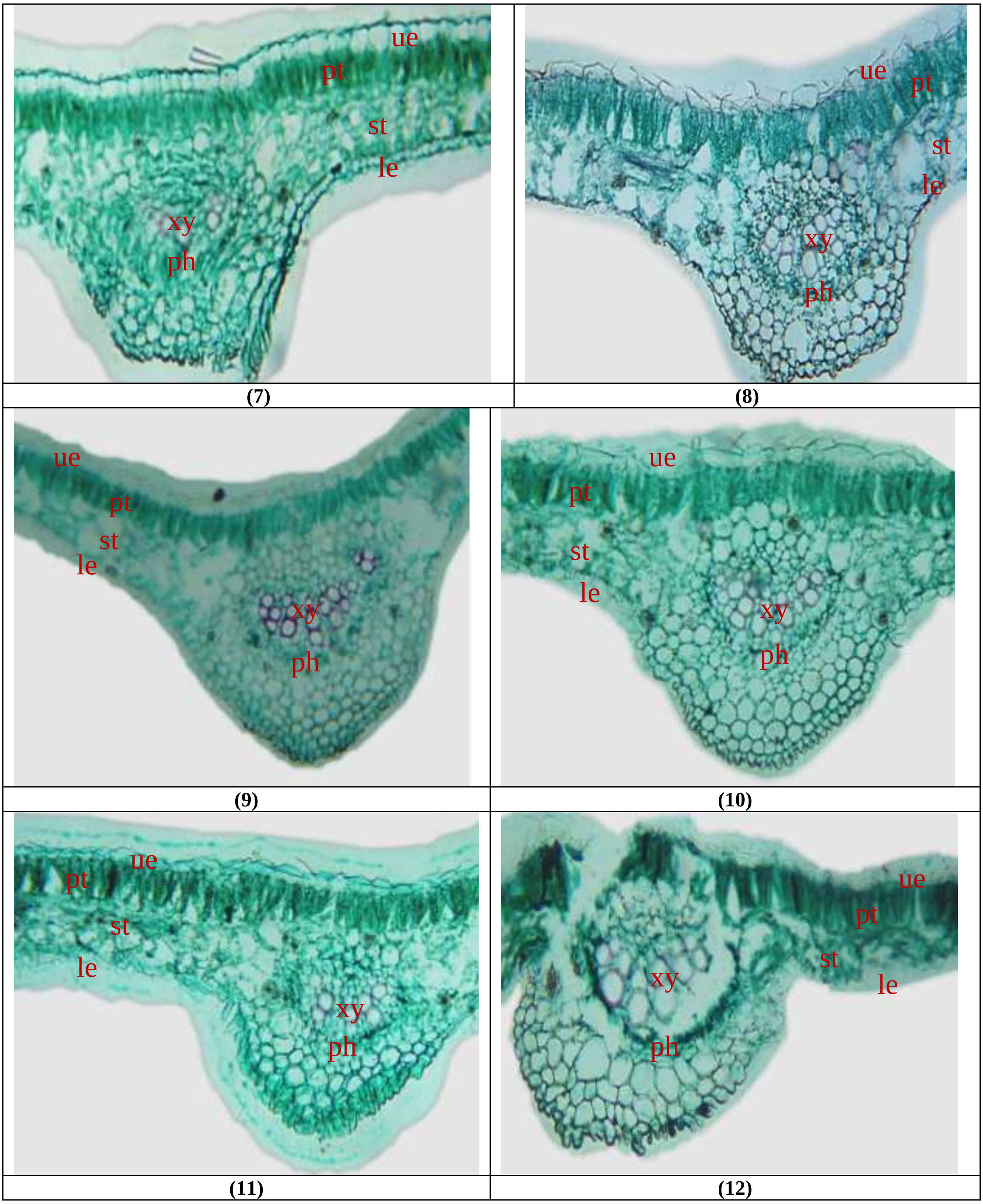


Fig. 2 : Transverse sections (X 40) *of* Moringa (*Moringa oleifera*) leaflet at 180 days after sowing as affected by different applied treatments.

Where: (7): Control (8): Benzyl adenine at 100 mg/L (9): Paclobutrazol (PP333) at 20 mg/L (10): Algae extract at 20 ml/L (11): Mineral nutrients at 500 mg/L (12): Lithovit at 500 mg/L

ue= Upper epidermis pt= Palisade tissue st= Spongy tissue le= Lower epidermis

ph= phloem tissue xy= Xylem tissue

The above mentioned results specially increment of the conductive tissues.

(xylem & phloem) are also of great importance because they could be also involved in the interpretation about why vigorous growth was existed with different applied treatments especially with Paclobutrazol (PP333) at 20 mg/L , algae extract at 20 ml/L and lithovit at 500 mg/L treatments respectively compared with other treatments and the untreated plants.

In general, the stimulatory effects of applied treatments upon the anatomy features of treated plants could mainly attributed to the increase of endogenous hormones level especially cytokinins and auxins, **(Sotiropoulos *et al.*, (2002) and (Youssef and Abd El-Aal 2013)**.

Of interest to note that these positive responses of different anatomical aspects to treatments especially, in case of Paclobutrazol (PP_{333}) at 20 mg/L, algae extract at 20 ml/L,benzyl adenine at 100 mg/L and lithovit at 500 mg/L treatments followed by some mineral nutrients at 500 mg/L compared with the control were completely reversed upon enhancing vegetative growth of treated plants. So, present study revealed those increases of xylem tissue, i.e., the route of some mineral nutrients and water translocation from roots to leaves and the phloem tissue i.e., the pathway of different assimilates from leaves to other plant sinks. Thereby, improvement of translocation events directly could be considered a direct reason for increment of the expected vegetative growth.

The aforementioned results of Paclobutrazol (PP_{333}) are in conformity with those obtained by **Berova and Zlatev (2000)** on *Lycopersicon esculentum*, **Fletcher *et al.*, (2000) , Youssef (2004)** on *Strelitzia reginae* **,Tekalign *et al.*, (2005)** on *Solanum tuberosum* **, Kishorekumar *et al.*, (2006)** on *Solenostemon rotundifolius* **Gopi *et al.*,(2009)** on *Ocimum sanctum* **and Youssef & Abd El-Aal (2013)** on *Tabernaemontana coronaria*. The abovementioned results of benzyl adenine are in harmony with those attained by **Davies, (1995), El-Badawy and Abd El-Aal (2013)** on *Mangifera indica* plant **and Youssef and Abd El-Aal (2014)** on *Hippeastrum vittatum*. also, These results of algae extract go on line with that obtained by **Crouch and Van Staden (1994) and Khan *et al.*, (2009) and Salama and Yousef (2015)** on *Ocimum sanctum*. Such results of some mineral nutrients have been previously recommended by **Agamy(2004)** on *Foeniculum vulgare*, **Xu *et al.*, (2008), Mohammed (2005)** on *Anethum graveolens*, **Abbas (2013)** on *Anethum graveolens* **and El-Tantawy & Eisa (2009)** on table beet plants.

Also, the previously mentioned and discussed results of *Moringa oleifera* leaflet anatomy of treated plants, reveal that increasing of leaf anatomy features compared with the control confirmed by vigorous growth of *Moringa oleifera* may be positively correlated with photosynthesis pigments, carbohydrates and total sugars content. This confirmed the previously discussed results of anatomy and growth, proved that the best morphological behavior of plants as affected by the applied treatments was mainly due to their induceable best morphological and anatomical performances.

2. Anatomical alterations of Moringa *(Moringa oleifera*) stems in response to different applied treatments at 90 and 180 days after sowing:

Data in Table 2 and Figs. 3 and 4 indicate the effect of different applied treatments (i.e., benzyl adenine at 100 mg/L, Paclobutrazol (PP_{333}) at 20 mg/L, algae extract at 20 ml/L, some mineral nutrients at 500 mg/L and lithovit at 500 mg/L compared with the control (the untreated one) treatment at 90 and 180 days after sowing on different anatomical characteristics of Moringa (*Moringa oleifera*). In this respect, most of the applied treatments have a positively impact on most studied histological characteristics of Moringa stem (cuticle thickness, epidermis thickness, cortex, thickness of phloem tissue, thickness of cambial region, xylem tissue thickness, number of xylem vessels /xylem row, number of xylem rows /vascular cylinder and diameter of the xylem vessel) under different used treatments compared with the control.

In general, Paclobutrazol (PP_{333}) at 20 mg/L, algae extract at 20 ml/L and lithovit at 500 mg/L treatments recorded the best results as exceeded that of other used and the control treatments in terms of the most studied anatomical features either at 90 or 180 days after sowing.

With regard to the stem diameter, could be noticed that increment in the stem diameter especially in case of Paclobutrazol (PP_{333}) at 20 mg/L (4395µ), algae extract at 20 ml/L (4088µ) and benzyl adenine at 100 mg/L (3912µ) treatments compared with other treatments and the control at 90 days after sowing , meanwhile in case of 180 days after sowing, stem diameter was reached its maximum values with Paclobutrazol (PP_{333}) at 20 mg/L (5642µ) followed by benzyl adenine at 100 mg/L (5535µ) and algae extract at 20 ml/L (5417µ) respectively which recorded the best results as exceeded that of other used and the control treatments.

Regarding, vascular tissues of Moringa stem i.e., xylem and phloem tissues thickness of both xylem and phloem tissues were reached their maximum values with Paclobutrazol (PP333) at 20 mg/L, lithovit at 500 mg/L and algae extract at 20 ml/L treatments, respectively. Here, xylem tissue thickness was 292, 278µ and 274µ which were the more effective treatments in the same order. Also, phloem tissue thickness behaved as the same as the xylem tissue thickness, it was 283,194µ and 189µ with Paclobutrazol (PP_{333}) at 20 mg/L, lithovit at 500 mg/L and algae extract at 20 ml/L treatments respectively, while their values with the control treatment were 207 and 159 µ for xylem and phloem tissues respectively, at 90 days after sowing.

As for vascular tissues of Moringa stem at 180 days after sowing, thickness of both xylem and phloem tissues were reached their maximum values with Paclobutrazol (PP_{333}) at 20 mg/L (451µ) , algae extract at 20 ml/L (447µ) followed by benzyl adenine at 100 mg/L (366µ) appeared to be the most effective treatments for xylem tissue, respectively compared with the control (217µ). Here, phloem tissue thickness behaved as the same as vascular xylem tissue since, its values were 329,309 and 253 µ with the same treatments, respectively and the control (196µ) .

The increase in size of vascular bundles due to the used treatments may be as a result of the enhancement of the activity of cambium to form and differentiate new vascular bundles **Agamy (2004) and Mohammed (2005)**.

For cambial region thickness, its maximum value (31µ) was recorded with benzyl adenine at 100 mg/L followed by Paclobutrazol (PP_{333}) at 20 mg/L and lithovit at 500 mg/L treatments (27 and 25µ) while its value was (21µ) with the control in case of 90 days after sowing. As for cambial region thickness in case of 180 days after sowing its maximum value (35µ) was recorded with lithovit at 500 mg/L treatment followed by benzyl adenine at 100 mg/L (34µ) and Paclobutrazol (PP_{333}) at 20 mg/L treatments (33µ) while its value was (32µ) with the control treatment.

For stem pith and cortex region thickness, they nearly behaved as the same as the above discussed stem vascular tissues.

Also, it could be noticed that increase of the stem diameter were reversed upon different tissues comprising the whole section. Since, thickness of each cuticle layer, epidermis, cortex (collenchyma and parenchyma tissues) and pith parenchyma layers, as well as the dimensions of vascular bundles. Moreover, thickness of phloem tissue, cambial region and xylem tissue, number of xylem vessels /vascular bundle and diameter of the widest xylem vessel were reached their maximum values with Paclobutrazol (PP_{333}) at 20 mg/L,benzyl adenine at 100 mg/L and algae extract at 20 ml/L treatments compared with other treatments and the control respectively, at 90 and180 days after sowing which recorded the best results as exceeded that of other used and the control treatments were the most pronounced in this respect as shown in **Table 2**.

Table 2. Effect of different applied treatments on the mean counts and measurements of certain histological features of Moringa (*Moringa oleifera*) stem at 90 and 180 days after sowing.

Treatments \ Histological Characteristics	Cuticle layer thickness	Epidermis thickness	Stem diameter	Cortex thickness	phloem tissue thickness	Cambial region thickness	xylem tissue thickness	Number of xylem rows/Vascular cylinder	No. of xylem vessels / row	Vessel diameter	pith diameter
At 90 days after sowing											
Control	11	12	3577	275	159	21	207	64	3	62	2107
Benzyl adenine at 100 mg/L	10	14	3912	369	187	31	216	74	4	69	2149
Paclobutrazol (PP333) at 20 mg/L	12	12	4395	374	283	27	292	91	4	76	2292
Algae extract at 20 ml/L	7	15	4088	363	189	24	274	112	4	72	2234
Mineral nutrients at 500 mg/L	9	12	3946	325	166	24	221	106	3	74	2325
Lithovit at 500 mg/L	11	16	3863	358	194	25	278	83	3	78	1986
At 180 days after sowing											
Control	14	15	4334	458	196	32	217	87	5	72	2368
Benzyl adenine at 100 mg/L	14	16	5535	662	253	34	366	94	6	81	2754
Paclobutrazol (PP333) at 20 mg/L	15	15	5642	674	329	33	451	126	4	89	2488
Algae extract at 20 ml/L	11	16	5417	592	309	29	447	96	5	88	2504
Mineral nutrients at 500 mg/L	13	14	4177	495	211	28	254	114	4	78	2347
Lithovit at 500 mg/L	13	16	4682	553	244	35	297	132	5	86	2264

In general, the stimulatory effects of applied treatments upon the anatomy features of stem for treated plants could be attributed to the effect of tested treatments upon cambium activity. Increment of cambium activity could mainly attributed to the increase of endogenous hormones level especially cytokinins and auxins, **Sotiropoulos *et al.*, (2002) ; Ismaeil (2005); Youssef and Abd El-Aal, (2014).**

In other words, a positive correlation was found between different applied treatments and studied Moringa leaflet & stem anatomical characteristics compared with the control.

Also, of interest to note that as shown in Table 2 and Fig. 4 the treatments of Paclobutrazol (PP333) at 20 mg/L., benzyl adenine at 100 mg/L and algae extract at 20 ml/L recorded highly values of the stem studied anatomical characteristics especially, in number of xylem Rows /Vascular cylinder, number of xylem vessels /xylem row and diameter of the xylem vessel compared with the control treatment either at 90 or 180 days after sowing.

Of interest to note that these positive responses of different anatomical aspects to treatments especially, in case of Paclobutrazol (PP_{333}) at 20 mg/L, algae extract at 20 ml/L followed by benzyl adenine at 100 mg/L and lithovit at 500 mg/L treatments compared with the control were completely reversed upon enhancing vegetative growth of treated plants. So, present study revealed those increases of xylem tissue, i.e., the route of some mineral nutrients and water translocation from roots to leaves and the phloem tissue i.e., the pathway of different assimilates from leaves to plant sinks. Thereby, improvement of translocation events directly could be considered a direct reason for increment the vegetative growth characteristics of Moringa plant. Our results are in harmony with those reported by **Youssef and Abd El-Aal (2013)** they reported that increasing of stem diameter may be accompanied with basic anatomical modification in different stem tissues especially phloem and xylem. **Devlin and Witham (1983)** ; **Crouch and Van Staden (1994)**;**Davies (1995)**;**Tekalign *et al.*,(2005)**; **Agamy(2004)**; **Youssef (2004)** ; **Xu *et al.*, (2008)**; **Khan *et al.*,(2009)**;**Abbas (2013)**;**Youssef and Abd El-Aal, (2013)** ;**Ahmed Nazarudin *et al.*, (2015)**;**Salama and Yousef (2015)**;**Salama *et al.*, (2016)**.

Herein, it was clear that there is a positive correlation between moringa anatomical features counts from one side and Moringa age (i.e., either at 90 or 180 days after sowing) & the applied treatments form the other side. Since, data revealed that the increment of histological features measurements at 180 days after sowing nearly reached to or even more than two times of 90 days after sowing values with the applied treatments.

Conclusion: Finally, the results of this study indicate that it could be spraying *Moringa oleifera* seedlings with Paclobutrazol (PP_{333}) at 20 mg/L or Algae extract at 20 ml/L four times to enhance the internal anatomical status towards maximizing its growth and productivity.

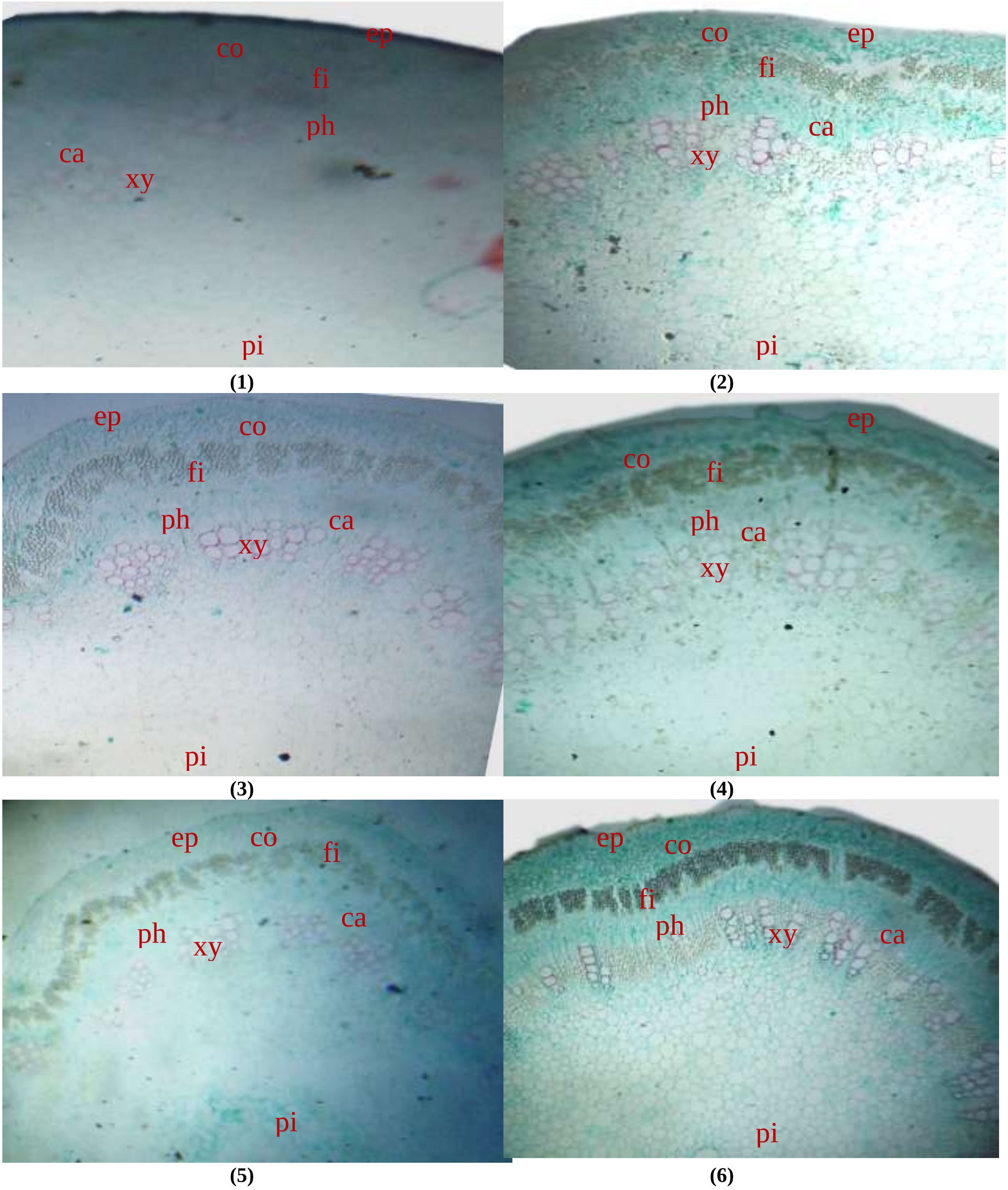
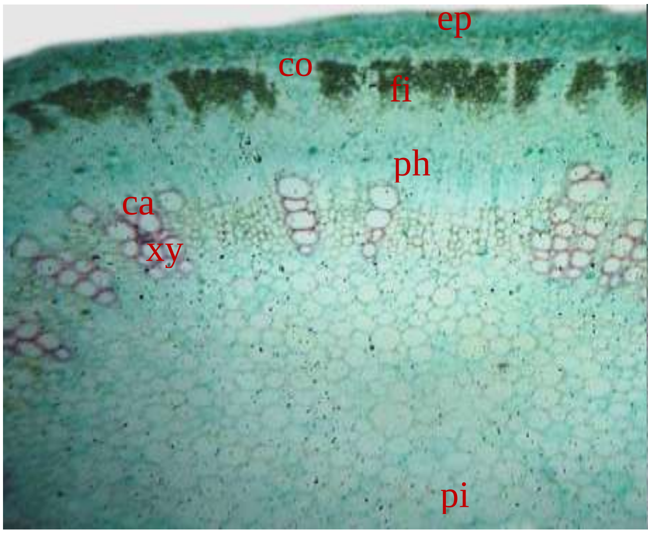
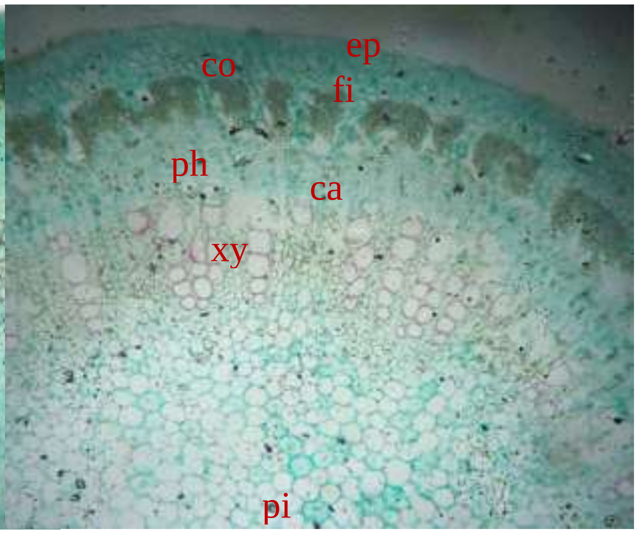


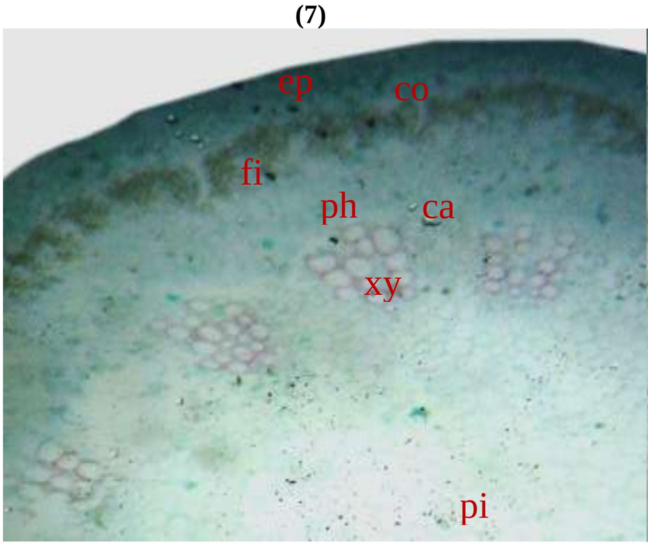
Fig. 3 : Transverse sections (X 40) *of* Moringa *(Moringa oleifera*) stem at 90 days after sowing as affected by different applied treatments.

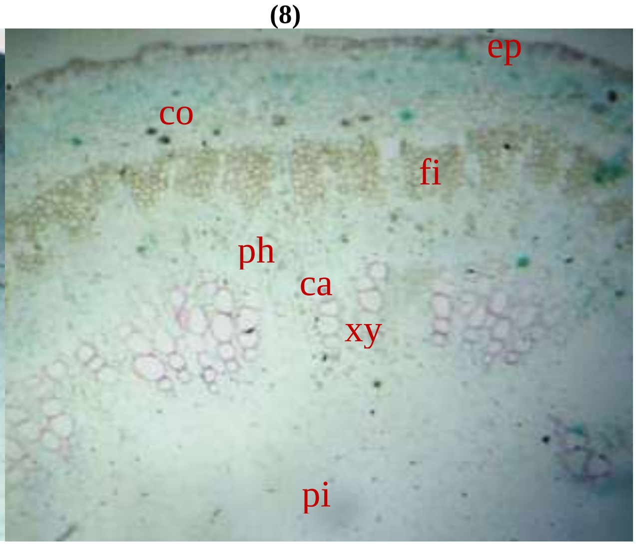
Where: (1): Control (2): Benzyl adenine at 100 mg/L (3): Paclobutrazol (PP333) at 20 mg/L
(4): Algae extract at 20 ml/L (5): Mineral nutrients at 500 mg/L (6): Lithovit at 500 mg/L
ep =epidermis co= cortex tissue ph= phloem tissue ca= cambium tissue xy= Xylem tissue
pi= pith tissue fi= fibers



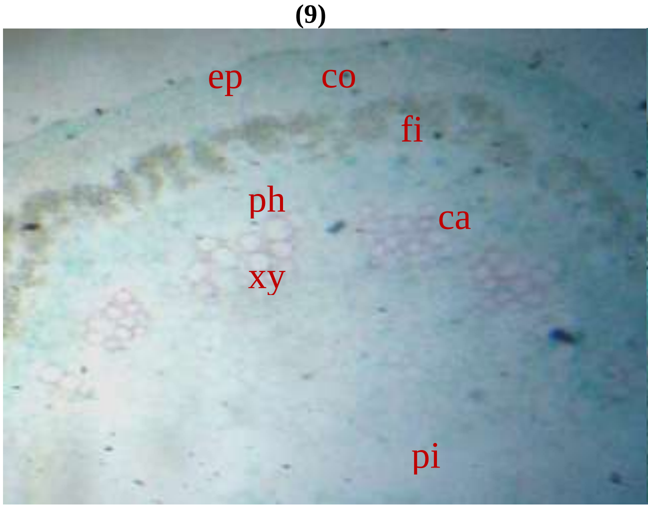


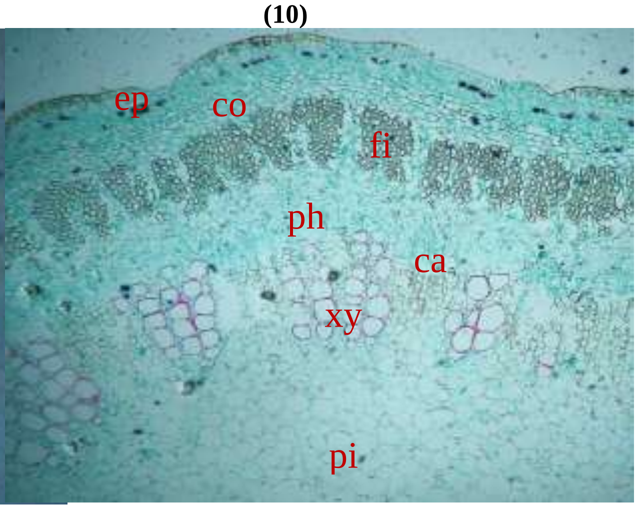
(7) (8)





(9) (10)





(11) (12)

Fig. 4 : Transverse sections (X 40) *of* Moringa (*Moringa oleifera*) stem at 180 days after sowing as affected by different applied treatments.

Where: (7): Control (8): Benzyl adenine at 100 mg/L (9): Paclobutrazol (PP333) at 20 mg/L
(10): Algae extract at 20 ml/L (11): Mineral nutrients at 500 mg/L (12): Lithovit at 500 mg/L
ep =epidermis co= cortex tissue ph= phloem tissue ca= cambium tissue xy= Xylem tissue
pi= pith tissue fi= fibers

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تأثير الرش الورقى بالبنزيل أدنين، الباكلوبترازول، مستخلص الطحالب، بعض العناصر الغذائية والليثوفيت على الصفات التشريحية لنبات المورينجا.

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أجريت تجربتى أصص بمحطة البحوث التابعة لقسم النبات الزراعى – كلية الزراعة – جامعة بنها – محافظة القليوبية – مصر خلال موسمى 2015 ، 2016 لدراسه تأثير الرش الورقى بمنظمات النمو البنزيل أدنين بتركيز100 ملجم / لتر و الباكلوبترازول بتركيز20 ملجم / لتر ومستخلص الطحالب بتركيز20 مل / لتر وبعض العناصر الغذائية (كالسيوم وماغنسيوم وحديد) بتركيز500 ملجم / لتر والليثوفيت بتركيز500 ملجم / لتر مقارنة بالكنترول (ماء الصنبور) على الصفات التشريحية لنبات المورينجا النامية في الأصيص عند عمر 90 و 180 يوم من الزراعة خلال موسم النمو 2016 تم رش النباتات بالمعاملات السابقة وكرر الرش كل 15 يوم.

أظهرت النتائج المتحصل عليها أن النمو القوي لنباتات المورينجا نتيجة المعاملات المختلفة المستخدمة كان مصحوباً بتغيرات واضحة في العديد من الصفات التشريحية للأوراق والسوق فقد:

أدت جميع المعاملات المستخدمة إلى تحسين معظم قراءات التركيب التشريحي للورقة من أهمها (سمك العرق الوسطي – طول وعرض الحزم الوعائية وسمك أنسجة اللحاء والخشب – عدد صفوف الخشب في الحزم الوعائية – قطر أوعية الخشب – سمك الأنسجة التمثيلية بالورقة متمثلة فى النسيج العمادى والأسفنجى وكذلك سمك نصل الورقة) مقارنة بالكنترول وذلك عند عمر90 و 180 يوم من الزراعة.

كما أوضحت النتائج أن المعاملات المستخدمة إلي زيادة سمك الساق والأنسجة المكونة له مثل سمك نسيج البشرة و نسيج القشرة وسمك الأنسجة الوعائية (نسيج الخشب و اللحاء) عدد صفوف الخشب في الأسطوانة الوعائية – أتساع الوعاء الخشبى وسمك نسيج النخاع هذا بالإضافة زيادة سمك نسيج الكامبيوم والذى يصحبه زيادة فى تكشف الأنسجة الوعائية (نسيج الخشب و اللحاء) مقارنة بالكنترول وذلك عند عمرى الدراسة.

وهذا يؤكد أهمية المساحة المقطعية لنسيجي الخشب و اللحاء (الأنسجة الناقلة) والذي يصاحبه تخليق ونقل كمية أكبر من نواتج التمثيل الضوئى وإمتصاص أكثر للعناصر المعدنية والذى يترتب عليه تحسين النمو والإنتاجية للنباتات المعاملة. وكانت أفضل المعاملات فى هذا الصدد معاملة الرش الورقى بالباكلوبترازول بتركيز20 ملجم / لتر ومستخلص الطحالب بتركيز20 مل / لتر مقارنة بالمعاملات الأخرى المستخدمة والكنترول.