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 (PP333) 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 (PP333) 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 (PP333) 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 Moringaceae 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: Magnoliophyta, Class: angioiptherida, Order: Brassicales, Family: Moringaceae, Genus: Moringa, Species: M.oleifera (Fahney, 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 affluent 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); Ferretra 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 its 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 epidermal 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 PP333 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, organogogenesis 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 Youssef (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 Annals of Agric. Sci., Moshtohor, Vol. 55 (1) 2017.
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 characteristics of several plants. Agamy (2004) on sweet fennel plant and Xu et al., (2008) 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)** found an increase in stem anatomical characteristics of treated Dill plant with some elements. **Abbas (2013)** reported that foliar fertilizer (Oligo green HF contains 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 Elsa (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 (PP333) 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, Qubia governorate, Egypt during 2015 and 2016 seasons to evaluate the effect of foliar application with Benzyl Adenine (BA) at 100 mg/L, Paclobutrazol (PP333) 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 oleifera* plants at 90 and 180 days after sowing. Uniform *Moringa oleifera* seeds were obtained from Agriculture Research Centre, Giza, Egypt. Moringa seeds were sowed at 5th 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 (PP333) 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 (PP333) 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 (PP333) 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 (PP333) 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 (PP333) 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.

<table>
<thead>
<tr>
<th>Treatments</th>
<th>90 days after sowing</th>
<th>180 days after sowing</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Thickness of upper epidermis cuticle layer</td>
<td>Thickness of Lower epidermis cuticle layer</td>
</tr>
<tr>
<td>Control</td>
<td>10 9 13 10 23 4 37 102 233 69 62 4 20</td>
<td>13 10 19 12 34 6 44 132 231 101 75 7 25</td>
</tr>
<tr>
<td>Benzyl adenine at 100 mg/L</td>
<td>12 8 16 9 28 6 36 109 251 83 102 12 19</td>
<td>12 11 16 13 55 7 68 175 301 122 167 18 27</td>
</tr>
<tr>
<td>Paclobutrazol (PP333) at 20 mg/L</td>
<td>11 7 15 11 47 5 58 149 293 138 153 13 24</td>
<td>15 10 19 12 36 4 42 131 241 97 106 9 25</td>
</tr>
<tr>
<td>Algae extract at 20 ml/L</td>
<td>9 6 19 10 39 5 46 129 254 113 144 8 22</td>
<td>15 10 19 12 36 4 42 131 241 97 106 9 25</td>
</tr>
<tr>
<td>Mineral nutrients at 500 mg/L</td>
<td>10 8 15 12 24 5 31 100 237 76 92 11 21</td>
<td>15 10 19 12 36 4 42 131 241 97 106 9 25</td>
</tr>
<tr>
<td>Lithovit at 500 mg/L</td>
<td>12 7 14 10 36 4 42 131 241 97 106 9 25</td>
<td>15 10 19 12 36 4 42 131 241 97 106 9 25</td>
</tr>
</tbody>
</table>

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 (PP333) 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 (PP333) 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 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 (PP333) at 20 mg/L, algae extract at 20 ml/L, benzyadenine 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.


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 inducable 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., benzyadenine at 100 mg/L, Paclobutrazol (PP333) 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 (PP333) 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 (PP333) at 20 mg/L (4395µ), algae extract at 20 ml/L (4088µ) and benzyadenine 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 (PP333) at 20 mg/L (5642µ) followed by benzyadenine 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 (PP333) at 20 mg/L, lithovit at 500 mg/L, algae extract at 20 ml/L and some mineral nutrients at 500 mg/L.
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 (PP333) 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 (PP333) 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 (PP333) 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 (PP333) 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 and 180 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.

<table>
<thead>
<tr>
<th>Treatments</th>
<th>At 90 days after sowing</th>
<th>At 180 days after sowing</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Cuticle layer thickness</td>
<td>Epidermis thickness</td>
</tr>
<tr>
<td>Control</td>
<td>11</td>
<td>12</td>
</tr>
<tr>
<td>Benzyl adenine at 100 mg/L</td>
<td>10</td>
<td>14</td>
</tr>
<tr>
<td>Paclobutrazol (PP333) at 20 mg/L</td>
<td>12</td>
<td>12</td>
</tr>
<tr>
<td>Algae extract at 20 ml/L</td>
<td>7</td>
<td>15</td>
</tr>
<tr>
<td>Mineral nutrients at 500 mg/L</td>
<td>9</td>
<td>12</td>
</tr>
<tr>
<td>Lithovit at 500 mg/L</td>
<td>11</td>
<td>16</td>
</tr>
<tr>
<td>Control</td>
<td>14</td>
<td>15</td>
</tr>
<tr>
<td>Benzyl adenine at 100 mg/L</td>
<td>14</td>
<td>16</td>
</tr>
<tr>
<td>Paclobutrazol (PP333) at 20 mg/L</td>
<td>15</td>
<td>15</td>
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<tr>
<td>Algae extract at 20 ml/L</td>
<td>11</td>
<td>16</td>
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<tr>
<td>Mineral nutrients at 500 mg/L</td>
<td>13</td>
<td>14</td>
</tr>
<tr>
<td>Lithovit at 500 mg/L</td>
<td>13</td>
<td>16</td>
</tr>
</tbody>
</table>

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); Ismaiel (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 (PP333) 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 Youssef (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 (PP333) 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
Impact of the foliar spray with benzyl adenine, paclobutrazol, algae extract, some mineral nutrients, and Lithovit on the growth and yield of Moringa oleifera. Transverse sections (X 40) of 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
References


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

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**أجريت تجربتي أاصص بمحطة البحوث التابعة لقسم النبات الزراعي - كلية الزراعة - جامعة بنها - محافظة القليوبية - مصر خلال موسم 2015 - 2016 لدراسة تأثير الرش الورقي بمنظمات النمو البنزيل أدنين بتركيز 100 ملجم / لتر و الباكلوبترازول بتركيز 20 ملجم / لتر و مستخلص الطحالب بتركيز 20 مل/ لتر وبعض العناصر الغذائية (كالسيوم وماغنسيوم وحديد) بتركيز 50 ملجم / لتر والليثوفيت بتركيز 500 ملجم / لتر مقارنة بالزنك (ماء الصنبور) على الصفات التشريحية لنبات المورينجا النامي في الأصيص عند عمر 90 و 180 يوم من الزراعة. برزت النتائج المتبعة أوصى أن النمو الفموي للنباتات المورينجا نتيجة المعاملات المختلفة المستخدمة كان مصحوباً بتغيرات واضحة في العديد من الصفات التشريحية للأوراق والسوق فقد:

- زادت جميع المعاملات المستخدمة إلى تخسيس معظم قراءات التركيب التشريحي للورقة من أهمها (سمك العرق الومطي - طول وعرض الحزم الوعائية وسمك أنسجه الخشب والنسج - عدد صفوف الخشب في الحزم الوعائية - قطر أوعية الخشب - سمك الأنسجة الببتيدية بورقة متصلة في النسيج العمادى والأنسجي). وكذلك سمح للورقة بخفض عمودها والذي عبر عن عمر 90 و 180 يوم من الزراعة.

- كما أوضح النتائج أن المعاملات المستخدمة إلى زيادة سمك الورقة والأسسية الكاشفة له مثل سمح نسيج البشرة و نسيج القشرة وسمك الأنسجة الوعائية (نسيج الأوعية الوعائية) عدد صفوف الخشب في الأسطوانة الوعائية - أنساع旺盛 الخشب وسمك نسيج النذاع هذا بالإضافة إلى زيادة سماك نسيج الكامبيوم والذي يصبح زادة في كشف الأسسة الوعائية (نسيج الخشب واللحم) مقارنة بالزنك وذلك عند عمر الدراسة.

- وهذا يؤكد أهمية السماكة المتقلبة لنسيج الخشب واللحم (الأسسية القاطعة) والذي يحافظ تخليل ونقل كمية أكبر من نواتج التمثيل الضمي ويتسبب في تنبؤ معاملة الرش الورقي بالباكلوبترازول بتركيز 20 مل / لتر ومستخلص الطحالب بتركيز 20 مل / لتر مقارنة مع المعاملات الأخرى المستخدمة بالزوتي والكتربول.**