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# MORPHOPHYSIOLOGICAL AND ANATOMICAL STUDIES ON MORINGA (*MORINGA OLIEFERA* L.)

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## Abstract

Two pot experiments were carried at the Experimental farm of the Botany Department, Faculty of Agriculture, Benha University, Qlubia Governorate and Agricultural- Botany Department, Fac. of Agricultural, Assiut, AL- Azhar Univ, Egypt, during two successive seasons of 2015 and 2016. The aim of this work was to study the effect of foliar application with benzyladenine (BA) at (25, 50 and 100 $mgl^{-1}$), paclobutrazol ($pp_{33}$) at (5, 10 and 20 $mgl^{-1}$), algae extract at (5,10 and 20 $ml^{-1}$),mineral nutrients (Ca, Mg and Fe chelated) at 500 $mgl^{-1}$ and lithovit at 500 $mgl^{-1}$ on some morphological, physiological and anatomical characteristics of *Moringa oliefera* plant at 90 and 180 days after sowing.

The obtained results indicated that the foliar application of benzyl adenine (100 $mgl^{-1}$), lithovit (500 mgl-1), algae extract (20 $ml^{-1}$) and mineral elements (Ca, Mg and Fe) at 500 $mgl^{-1}$ significantly increased all the studied morphological parameters *i.e.*, stem length, leavesnumber and leaf area/$plant^{-1}$ as well as dry weight of roots, stems and leaves at different ages (90 and 180 days after sowing) during the two growth seasons. On the contrary, these measured morphological characteristics were significantly decreased with paclobutrazol treatment in both two seasons compared with the control treatment. Also, foliar application with all used treatments increased physiological characteristics of moringa leaves represented as photosynthetic pigments content (chlorophyll a, b and carotenoids), antioxidant activity, total phenolic and total flavonoids & flavonols concentration compared with the control plants. Results also showed that foliar spray treatments with benzyl adenine (100 mgl-1) followed by lethovit (500 $mgl^{-1}$), algae extract (20 $ml^{-1}$), paclobutrazol (20 mgl-1) respectively were the most effective in this respect.

Results revealed that different anatomical characteristics of moringa leaflet and stem were positively responded in the two stages of growth.

Add to the obtained data showed that the mosttrait sofmoringa leaflet anatomical characteristics were increased with different applied treatments, especially with paclobutrazol ($PP_{333}$) at 20$mgl^{-1}$, algae extractat 20$mll^{-1}$and lithovitat 500 $mgl^{-1}$ respectively compared with the control treatment in the two stages. The anatomical characteristics of the leaves were increased *i.e*, increasing thickness of palisade and spongy tissues as well as thickness of leaflet lamina. Also, the increasing of number and diameter of xylem vessels $arch^{-1}$ led to increase in the vascular bundle thickness. More over the increasing of number and larger of xylem vessels $arch^{-1}$and number of vascular bundles led for increasing the thicknesses of midrib at 90 or 180 days after sowing comparing to control treatment. The results showed that the increasing of stem diameter was a result for increasing the thickness of cuticle, epidermis, cortex, phloem and xylem tissues, cambial region, number of xylem vessels $arch^{-1}$, number of xylem arches vascular $bundle^{-1}$, number & diameter of the vascular bundles and pith in the stemat the two stages. Generally, in this study the morphological and physiological characteristics of *Moringa oleifera* were recorded the highest values with benzyladenine (100$mgl^{-1}$). At the same time, the best anatomical characteristics were recorded with the foliar application of paclobutrazol ($PP_{333}$) at 20$mgl^{-1}$at 90 or 180 days after sowing at the same field conditions.

***Key words :*** Moringa, benzyl adenine, paclobutrazol, algae extract, mineral nutrients, lithovit, anatomical structure, photosynthetic pigments, antioxidants activity, total flavonoids, flavonols and foliar application.

## Introduction

*Moringa oleifera* belongs to Kingdom: Plantae, Division: Magnoliphyta, Class: agnoliopsida, Order: Brassicales, Family: Moringaceae, Genus: Moringa, Species: *M. oleifera* Fahey (2005). 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. Paclobutrazol is a triazole compound widely used as retardant for controlling the vegetative growth of a wide range of angiosperm Terri and Millie (2000) and Ahamad Nazaradin *et al*. (2007). Paclobutrazol induce a variety of morphological, physiological and biochemical responses in plants, including are duction in stem elongation, stimulation of rooting, increased chlorophyll and carotenoids content, altered carbohydrate status, increased stress tolerance, delayed senescence, reducing gibberellin biosynthesis, increased cytokinins synthesis and alterations in secondary metabolite contents Abdul Jaleel *et al*. (2007), Gopi *et al*. (2009), Lolaei *et al*. (2013), Mansour (2014), Youssef and AbdEl-Aal (2013) and Ismail (2016). The main effect of paclobutrazol take splace throught healteration of hormonal balance. Since, it in hibits gibberellin biosynthesis hence, reducing cell division and cell elongation and retarding plant growth, where as it promotes treated plants to create morecytokinins Fletcher *et al*. (2000), Rademacher (2000) and Gopi *et al*. (2009). Several researchers have reported that paclobutrazol reduced plant growth of different plants Zhang *et al*.(2006), Hamza *et al*. (2007), Youssef and AbdEl-Aal (2013), Mausour (2014) and Ismail (2016). The positive effect of lithovit compound on plant growth and biochemical constituents was reportedby Abo-Sedera *et al*. (2016) found that foliar application of lithovitat 3gl$^{-1}$ and seaweed extracts at 2mll$^{-1}$ increased vegetative growth, total green podsyield and it compounds as well as pod quality of snapbean plants, Also, Abdel ghafar *et al*. (2016) showed that foliar application with lithovitat 0.5gl$^{-1}$ significant increased number of umbel, height of number scape,diameter of umbel, chlorophy ll and carotenoids, seed yield/plot and seed germination% of onion plants.

Youssef and Abd El-Aal (2013) found that most an atomicaltraits 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 $PP_{333}$ at150 and 100mgl$^{-1}$compared with the untreated plants. Youssef (2004) mentioned that treated Strelitzia reginae plants with $PP_{333}$ at 300 and 200 mgl$^{-1}$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 stemand leaf of *X. chrysanthus*. Palisade and spongymesophyll 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 Benzy ladenine, many authors reported that Benzyladenine is an important plant hormone that regulate various processes of plant growth and development including cell division and differentiation, organogenes is in developing plants and enhancement of leaf expansion and nutrientmobilization, improveplant growth and development Davies (1995). Itisalso 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 thic kness of mango plant. Youssef and AbdEl-Aal (2014) found that treatment sofkinet in increased histological characteristics of Hippeastrum leaf (*i.e.*, cuticle thickness, epidermis thickness, length & width of vascular bundle, phloem & xylemt issues 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 growthof plantt issue. This may increase the proportion of protoplasm to cell wall with the result of increased cell size Marschner (1995). Regarding to Algae extract, Blunden (1991), Crouch and Van Staden (1994) and Khan *et al*. (2009) reported that sea weed extract application for different crops was agreat importance due to contains high levels of or ganic matter, microelements (Fe, Cu, Zn, Co, Mo, Mn and Ni), vitamins and amino acids and also, rich in growth regulators such asauxins, cytokinin and gibberellins. Abd El-Aziz *et al*. (2011) on *Amaranthustricolor* plants, showed that foliar application of algae extracts significantly increased numberof leaves, dry weight of root, stem and leaves as well as photosynthetic pigments and carotenoids content. Salama and Yousef (2015) recorded that foliar applications of *Ocimumsanctum* L. plant with sea weed extractat 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 extractat1.5 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 founda pronounce effects on thickness of epidermis and cortex and number and diameterof vascular bundles o fstem with some mineral nutrients treatment. Mohammed (2005), who foundan increase in stemanatomical characteristics of treated Dill plant with some elements. Abbas (2013) reported that foliar fertilizer (Oligogreen HFcontains Fe, Zn, Mn, Cu and B) atrates of 0, 50, 100 and 150mgl$^{-1}$ on Dill

(*Anethumgraveolens* 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 stemanatomy due to the effect of nutrients mixture.

Therefore, the present investigation aimed to study the effect of foliar application with benzyladenine, paclobutrazol, algae extract, some mineral elements (Ca, Mg and Fe chelated) and lithovit on some morphological and physiological characters and 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 $mg^{-1}$, Paclobutrazol ($PP_{333}$) at 20 $mg^{-1}$, algae extract at 20 $ml^{-1}$, some mineral nutrients at 500 $mg^{-1}$ and lithovit at 500 $mg^{-1}$ compared with the control (the untreated one) treatment at 90 and 180 days after sowing. of *Moringa olifera* plant.

## Materials and Methods

The present study was carried out in pots at the Experimental Farm of Agricultural Botany Department, Faculty of Agriculture, Moshtohor, Qalubia Governorate, and Agric. Botany Department, Fac. of Agriculture, Assiut, AL- Azhar Univ., Egypt during the two growing successive seasons of 2015 and 2016.

The experiment was performed as a complete randomized design with 20 replicates. In both seasons the normal agricultural practices of growing *Moringa oleifera* plant including equal amounts of fertilizer and irrigation water/pot were applied.

The applied treatments were used as foliar application at different concentrations as follows: Control (distilled water) 0.0, benzyladenine (25, 50 and 100 $mgl^{-1}$), paclobutrazol at (5, 10 and 20 $mgl^{-1}$), algae extract at (5,10 and 20 $ml^{-1}$),mineral nutrient (Ca, Mg and Fe chelated) at 500 $mgl^{-1}$ and lithovit at 500 $mgl^{-1}$. Plants of each treatment were sprayed four times at 15, 30, 45 and 60 days after sowing, the first foliar spray was carried out after 15 days from sowing throughout the two successive seasons of 2015 and 2016.

**Sampling and collecting data**

Different vegetative growth characteristics of plant samples were taken at the following dates during the experimental period. Three samples of moringa plant for each treatment 90 and 180 days after sowing) were taken in 2015 and 2016 seasons. 3 plants from each treatment were randomly taken for different measurements. Then the plants were separated into their organs (roots, stems and leaves). The samples of these organs were dried in the oven at $70^0$C for 48 hours till constant weight. The dried samples of different organs were weighted for dry weight estimation then kept for chemical analysis determinations.

**I. Morphological characteristics**

The following vegetative growth characteristics were estimated :

Stem length / plant, Number of leaves/plant, Leaf area / plant, Dry weight of roots stems and leaves /plant

**II. Physiological characters**

Chemical analysis was carried out on the samples of leaves at 90 and 180 days during season 2016. Chlorophyll a, b and carotenoids were calorimetrically determined in the fresh leaves according to the method described by Wettestein (1957). The antioxidant activity was determined in dry leaves according to the method by Saint-Cricq De Gaulejae *et al.* (1999) modified by Lu, Zhao *et al*. (2007). Total phenolic compounds content was determined according to Folin-Ciocalteu spectrophotometric method Lu, Zhao *et al.* (2007). Total Flavonoids was determined according to the method of Mohdaly *et al.* (2012) and the total flavonols content was determined according to Kumaran and Karunakaran (2007).

**III. Anatomical characters**

The effect of foliar application with Benzyl Adenine (BA) at 100 $mg^{-1}$, Paclobutrazol ($PP_{333}$) at 20 $mg^{-1}$, Algae extract at 20 $ml^{-1}$, some mineral nutrients (Ca, Mg and Fe) at 500 $mg^{-1}$ and Lithovit at 500 $mg^{-1}$ treatments comparing with the control (tap water) on leaflet and stem anatomical characters of potted *Moringa olifera* plants at 90 and 180 days after sowing.

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 characteristics of the treated plants compared with the control based on transverse sections of the leaflets and stems were studied.

**IV. Statistical analysis**

Data of vegetative growth characteristics and chemical constituents were statistically analyzed and the means were compared using the Least Significant Difference test (L.S.D) at 5% level according to Snedecor and Cochran (1980).

## Results and Discussion

**I. Morphological characteristics**

1. Stem length, number of leaves, leaf area plant$^{-1}$, root dry weight(g) plant$^{-1}$, stem dry weight(g) plant and leaves dry weight(g)plant$^{-1}$.

Data recorded in table 1 show the effect of foliar spray with benzyl adenine, paclobutrazol, Algae extract, some mineral elements and lithovit at different tested concentrations on stem length, number of leaves, leaf area(cm$^2$) plant, root dry weight(g) plant$^{-1}$, stem dry weight(g)plant$^{-1}$ and leaves dry weight(g) plant$^{-1}$ at 90 and 180 days from seed sowing during both seasons of study. Such data indicate that foliar spray of moringa plants using benzyl adenine at 25, 50 and 100mgl$^{-1}$, paclobutrazol at 5, 10 and 20 mgl-1, Algae extract at 5, 10 and 20 ml$^{-1}$, mineral elements Ca, Mg and Fe chelated at 500mgl$^{-1}$ and lithovit at 500mgl$^{-1}$ significantly affected all measured morphological parameters of stem length, number of leaves plant$^{-1}$, leaf area, root dry weight g/plant, stem dry weight g/plant and leaves dry weight g/plant at 90 and 180 days at different stage of growth at 90 and 180 days from seed sowing compared with the control treatment in both growth seasons of study. In this respect, using the different tested concentration for benzyl adenine, Algae extract, mineral elements and lithovit significantly increased all aforementioned parameters, while using paclobutrazol at different studied concentrations significantly decrease such parameter compared with the control treatment In this connection using benzyladenine as growth promoter at 100mgl$^{-1}$. exhibited the highest values of stem length, leaves number and leaf area plant$^{-1}$ followed by using lithovit at 500mgl$^{-1}$, Algae extract at 20 ml$^{-1}$, Benzyl adenine at 50mgl$^{-1}$ and mineral elements at 500mgl$^{-1}$ and finally using benzyl adenine at 25mgl$^{-1}$ in descending order compared to the control. On contrast, such parameters were decreased with increasing the concentration of paclobutrazol up to the highest used levels (20mgl$^{-1}$) obtained results are true during both seasons of study.

These results of benzyl adenine are in agreement with those reported by Ramadan (1992), Ibrahim *et al*. (2010), Abdel-Dayem *et al*. (2012) and Saad (2015). In this regard such increment effect of each benzyl adenine, algae extract, mineral elements and lithovit may be due to the important role of BA and algae extract in the regulation meristematic cell division, cell enlargement, differentiation activity and organogenesis in developing plants, which in turn may stimulate plant growth rate. In this regard, Arigita *et al*. (2005) mentioned that cytokinins promote shoot development through increasing cell division, regulation of the cell cycle and the number of cycles which cells undergo in the meristems and organ primordial are the primary regulatory that targets cytokinins. Abdel-Dayem *et al*. (2012) had shown that the foliar application of benzyl adenine at 40 mgl$^{-1}$ significantly increased all vegetative growth characteristics of sesame plant *i.e.*, stem length, number and leaf area plant$^{-1}$.

Regarding to the stimulative effects of algae extract could be explained by Thirumaran *et al*. (2009) stated that seaweed liquid fertilizer (SLF) contained macro and micronutrients, organic substances like amino acids, vitamins and plant growth regulators such as, cytokinins, auxin and gibberellins.

The aforementioned results of algae extract are in conformity with those obtained by Craigie (2011), Abou El-Yazied *et al*. (2012), Tarraf *et al*. (2015), Abo-Sedera *et al*. (2016), Ismail (2016) and Boghdady *et al*. (2016) they showed that foliar application with algae extracts significantly increased different growth characteristics of several plants *i.e.*, root length, shoot length, stem diameter, number of leaves.

Also, Boghdady *et al*. (2016) found that foliar application with 0.75 and 1.00 ml seaweed extract l$^{-1}$ induced significant promoting effects on vegetative growth characteristics of chickpea plant.

Such results of some mineral nutrients have been previously recommended by Abbas (2013) reported that foliar fertilizer, (contains Fe, Zn, Mn, Cu and B), at rates of 0, 50, 100 and 150mg$^{-1}$ increased plant height, stem diameter and number of leaves of Dill (*Anethum graveolens* L.) plant.

Abbas *et al*. (2009) showed that application of minerals fertilizer significantly increased all growth parameters, *i.e.* Plant height, spike length and number of spikelets spike$^{-1}$ of wheat.

Atress and Mohamed (2014) concluded that spraying broccoli plants with Fe at 100mgl$^{-1}$ plus Mg at 0.5% or 1.0% significantly increased all the studied vegetative growth traits as compared with the control.

In the same respect, Lashkari *et al*. (2007) reported that leaf area of cauliflower were found significantly highest with combined foliar spray of zinc and iron at 0.5% concentration for each. Spraying pea plants with Fe at 100mgl$^{-1}$ gave the highest values of growth characters (Mansour *et al*., 2012) Spraying broad bean plants with Fe at 500mgl$^{-1}$ increased plant height El-Tantawy and Nawar (2013).

Kazemi (2014) reported that foliar application with iron, calcium increased leaf area, length of roots of strawberry.

Several studies have been dealing with the role of minerals on vegetable crops, Mg as a foliar application significantly enhanced plant height (Ahmed *et al*., 2011).

Also, the above mentioned results of lithovit are in harmony with those attained by Abo-Sedera *et al*. (2016) found that foliar application of lithovit. 3g$^{-1}$ increased vegetative growth characteristics of snap bean plant. Also, Abdel ghafar *et al*. (2016) showed that foliar application with lithovit at 0.5g$^{-1}$ significantly increased number of umbel, height of umbel scape, diameter of umbel of onion plants. Abou El-Nasr *et al*. (2015) reported that an increase in biomass parameters *i.e.* sapling height, stem diameter and leaf area. Previous studies showed that nanoparticles can have a beneficial effect on plants growth and development Zhu *et al*. (2008) and Roghayyeh *et al*. (2010). Nanoparticles improved the plant growth characteristics Esitken and Turan (2004) and Carbonell *et al*. (2011).

As for paclobutrazol the abovementioned results of paclobutrazol are in harmony with those attained by Zhang *et al*. (2006), Hamza *et al.* (2007), Youssef and Abd El-Aal (2013), Mausour (2014) and Ismail (2016) they concluded that treating plants with paclobutrazol resulted in reducing different plant growth characteristics. Cimen *et al.* (2004) reported that paclobutrazol retarded vegetative growth, reduced internode length, plant height, leaf area in maize. Amin (2007) deduced that spraying maize with paclobutrazol up to 60 mg-1 resulted in significant decrease in plant height and leaf area.

**Fresh and dry weights**

Data in table 1 revealed the effect of foliar spray of moringa plant with using benzyl adenine (25, 50 and 100mgl$^{-1}$), paclobutrazol (5, 10 and 20mgl$^{-1}$), algae extract (5, 10 and 20 ml$^{-1}$), some mineral elements (Ca, Mg and Fe) at 500mgl$^{-1}$ and lithovit at 500 mgl$^{-1}$ on fresh and dry weights of each of root, stems and leaves per/plant after 75, 90 and 180 days from seed sowing compared with the control during both season of study. Such data reveal that there were significant differences in fresh and dry weight of different plant parts among the all tested treatments compared with the control treatment in both seasons of study. In this regard using benzyl adenine, Algae extract, mineral elements and lithovit had positive increase in all measured parameters, while spraying the plants with paclobutrazol at different tested concentration had a negative effect on fresh and dry weight of different plant parts compared to the control treatment in the two seasons of study. In addition using benzyl adenine at 100mgl$^{-1}$ resulted in the highest value of root, stem and leaves fresh and dry weight followed by using lithovit at 500mgl$^{-1}$, algae extract at 20 mll$^{-1}$, benzyl adenine at 50 mgl$^{-1}$ and mineral elements at 500 mgl$^{-1}$ in descending order in the two seasons of study. Moreover, such morphological parameters were decreased with increasing the concentration of paclobutrazol, since the lowest growth values were connected with the highest used concentration (20mgl$^{-1}$). Such results may be due to the effect of used growth enhancement substances on cell division and enlargement in the meristems and organ primordial and increasing endogenous promoters substances which increased expansion.

These results are of great interest, because at this early stage of growth great stimulative effects existed with various applied treatments. Hence, that could be prolonged to the advanced growth stages including the final fruit yield as well as its quality. Besides, increasing of stem diameter accompanied with increasing of plant height means that applied treatments lead to vigorous growth and more healthy moringa plant.

In addition, increment of shoots (stems and leaves) fresh weight due to increases of leaves number and the total leaf area. Increment of leaf characteristics (number and area) as well as their content of bioconstituents *i.e.*, photosynthetic as mentioned later tables 1 and 2 could be a basic for increasing the photosynthetic efficiency.

Also, increment of leaf area is of great interest because that could be reflected upon the efficiency of photosynthesis by accumulating more assimilates and high rates of their translocation. Also, it could be noticed that increment of this area was preceded with high number of leaves.

Our results are in harmony with those reported by Arigita *et al.* (2005) mentioned that cytokinins promote shoot development through increasing cell division, regulation of the cell cycle and the number of cycles which cells undergo in the meristems and organ primordial

**Table 1 :** Morphological characters of the leaves and stem as affected by different applied treatments of moringa (*Moringa olifera* L.) at 90 and 180 days during 2015 and 2016 seasons.

| Treatments | Concentration | Stem length (cm)/plant$^{-1}$ | | leaves Number /plant$^{-1}$ | | Leaf area (cm$^2$) /plant$^{-1}$ | | Root dry weight (g)/plant$^{-1}$ | | Stem dry weight (g)/plant$^{-1}$ | | Leaves dry weight (g)/plant$^{-1}$ | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | 2015 | 2016 | 2015 | 2016 | 2015 | 2016 | 2015 | 2016 | 2015 | 2016 | 2015 | 2016 |
| | | **Morphological characters at 90 days after sowing** | | | | | | | | | | | |
| Control | 0.00 | 70.00 | 71.66 | 23.67 | 26.00 | 148.87 | 153.81 | 4.28 | 4.80 | 3.86 | 4.08 | 3.79 | 4.27 |
| Benzyl adenine | 25 mgl$^{-1}$ | 74.67 | 76.33 | 27.67 | 29.66 | 160.99 | 161.40 | 4.44 | 4.92 | 4.03 | 4.25 | 4.38 | 4.84 |
| | 50 mgl$^{-1}$ | 77.00 | 78.66 | 30.00 | 31.66 | 189.74 | 197.84 | 5.41 | 5.91 | 4.47 | 4.69 | 4.59 | 5.06 |
| | 100 mgl$^{-1}$ | 85.67 | 87.33 | 34.67 | 36.33 | 212.23 | 219.44 | 6.97 | 7.39 | 4.89 | 5.10 | 5.69 | 6.17 |
| Paclobutrazol | 5 mgl$^{-1}$ | 57.33 | 60.33 | 21.67 | 24.33 | 134.02 | 141.81 | 3.42 | 4.21 | 2.85 | 3.64 | 3.24 | 3.93 |
| | 10 mgl$^{-1}$ | 53.67 | 57.66 | 19.67 | 21.66 | 121.94 | 136.38 | 2.75 | 3.57 | 2.60 | 3.62 | 2.83 | 3.39 |
| | 20 mgl$^{-1}$ | 50.67 | 54.00 | 16.00 | 18.66 | 113.29 | 130.33 | 2.47 | 3.42 | 2.38 | 3.60 | 2.05 | 3.05 |
| Algae extract | 5 mll$^{-1}$ | 72.67 | 74.33 | 25.33 | 27.33 | 154.32 | 158.85 | 4.37 | 4.83 | 3.97 | 4.19 | 4.15 | 4.63 |
| | 10 mll$^{-1}$ | 74.67 | 76.33 | 28.67 | 30.33 | 174.87 | 177.48 | 4.95 | 5.43 | 4.31 | 4.55 | 4.42 | 4.88 |
| | 20 mll$^{-1}$ | 82.67 | 84.33 | 32.67 | 34.33 | 197.05 | 204.68 | 5.60 | 6.05 | 4.72 | 4.95 | 4.76 | 5.23 |
| Elements | 500 mgl$^{-1}$ | 76.67 | 78.33 | 29.00 | 31.00 | 181.03 | 187.93 | 5.33 | 5.80 | 4.40 | 4.66 | 4.57 | 5.03 |
| Lithovit | 500 mgl$^{-1}$ | 84.00 | 85.66 | 33.00 | 34.33 | 204.90 | 214.21 | 5.66 | 6.07 | 4.76 | 4.96 | 4.93 | 5.40 |
| LSD 5% | at 0.05 | 2.12 | 2.34 | 2.21 | 2.34 | 5.63 | 3.89 | 0.52 | 1.01 | 0.42 | 1.01 | 0.42 | 1.14 |
| | | **Morphological Characters at 180 days after sowing** | | | | | | | | | | | |
| Control | 0.00 | 98.67 | 102.00 | 32.67 | 34.33 | 153.55 | 158.68 | 8.14 | 8.23 | 8.73 | 8.80 | 5.79 | 6.26 |
| Benzyl adenine | 25 mgl$^{-1}$ | 106.33 | 111.00 | 36.67 | 38.00 | 169.42 | 172.98 | 9.06 | 9.12 | 9.04 | 9.14 | 6.37 | 6.85 |
| | 50 mgl$^{-1}$ | 110.67 | 114.67 | 41.00 | 42.33 | 196.77 | 199.22 | 9.47 | 9.58 | 9.25 | 9.41 | 7.20 | 7.58 |
| | 100 mgl$^{-1}$ | 119.33 | 122.67 | 46.33 | 47.67 | 215.08 | 222.31 | 10.38 | 10.53 | 9.97 | 10.05 | 7.57 | 8.00 |
| Paclobutrazol | 5 mgl$^{-1}$ | 88.33 | 92.00 | 30.00 | 31.33 | 146.63 | 151.06 | 7.30 | 7.42 | 7.21 | 7.35 | 3.61 | 5.75 |
| | 10 mgl$^{-1}$ | 82.33 | 86.33 | 27.33 | 28.33 | 141.26 | 143.53 | 6.80 | 6.87 | 6.77 | 6.91 | 3.01 | 5.29 |
| | 20 mgl$^{-1}$ | 76.00 | 80.00 | 24.67 | 25.67 | 134.15 | 138.00 | 6.34 | 6.35 | 6.56 | 6.62 | 2.80 | 4.71 |
| Algae extract | 5 mll$^{-1}$ | 102.67 | 106.33 | 34.67 | 35.67 | 163.09 | 168.38 | 8.57 | 8.61 | 9.00 | 9.05 | 5.97 | 6.37 |
| | 10 mll$^{-1}$ | 106.33 | 110.00 | 37.33 | 38.67 | 179.59 | 185.99 | 9.29 | 9.37 | 9.09 | 9.09 | 6.42 | 6.90 |
| | 20 mll$^{-1}$ | 113.33 | 117.33 | 43.33 | 44.33 | 202.96 | 205.73 | 9.69 | 9.84 | 9.57 | 9.73 | 7.33 | 7.79 |
| Elements | 500 mgl$^{-1}$ | 111.00 | 114.33 | 40.67 | 41.67 | 187.44 | 188.10 | 9.34 | 9.39 | 9.14 | 9.19 | 6.79 | 7.25 |
| Lithovit | 500 mgl$^{-1}$ | 116.67 | 120.33 | 44.33 | 45.33 | 210.00 | 216.86 | 9.81 | 9.95 | 9.75 | 9.94 | 7.41 | 7.87 |
| LSD 5% | at 0.05 | 3.65 | 3.67 | 1.51 | 1.61 | 2.71 | 3.12 | 1.03 | 1.06 | 1.03 | 1.04 | 0.92 | 1.11 |

are the primary regulatory that targets cytokinins. Abdel-Dayem *et al.* (2012) reported that the foliar application of benzyl adenine at 40 $mgl^{-1}$ significantly increased all vegetative growth characteristics of sesame plant *i.e.*, plant stems & leaves fresh and dry weights.

Ismail (2016) found that increases in fresh and dry weights of both shoots and roots of wheat plant in response to benzyladenine at 200 mgl-1 treatments.

As for the stimulative effects of algae extract could be explained by Abbas *et al*. (2009), Abo-Sedera *et al*. (2016), Ismail (2016) and Boghdady *et al*. (2016) they showed that foliar application with algae extracts significantly increased different growth characteristics of several plants.

Such results of some mineral nutrients have been previously recommended by Abbas (2013) showed that foliar fertilizer, (contains Fe, Zn, Mn, Cu and B), at rates of 0, 50, 100 and 150mg-1 increased shoot dry weight of dill plant.

Atress and Mohamed (2014) concluded that spraying broccoli plants with Fe at 100mgl-1 plus Mg at 0.5% or 1.0% significantly increased all vegetative growth traits. In the same order, spraying pea plants with Fe at 100$mgl^{-1}$ gave the highest values of growth characters (Mansour *et al*., 2012). Kazemi (2014) found that foliar application with iron, calcium increased dry weight of strawberry.

Nutrients are essential for plant growth and plays an important role in many plant physiological processes (Mengel and Kirkby, 1987; Marschner, 1995 and Allison *et al*., 2001). Several studies have been dealing with the role of minerals on vegetable crops, Mg as a foliar application significantly enhanced fresh weight of cauliflower (Ahmed *et al*., 2011).

In the same order, the stimulative effects induced by lithovit application on growth characteristics could be explained by Abo-Sedera *et al*. (2016), Abdel Ghafar *et al*. (2016) and Abou El-Nasr *et al*. (2015) reported that foliar sprays with magnetite nano particaleas resulted in increasing plants dry weights.

Abou El-Nasr *et al.* (2015) reported that an increase in biomass parameters *i.e.*, dry weight of plant treated with nano particles.

Previous studies showed that nanoparticles can have a beneficial effect on plants growth and development (Zhu *et al*., 2008 and Roghayyeh *et al*., 2010). Nanoparticles improved the plant growth characteristics (Esitken and Turan, 2004 and Carbonell *et al*., 2011).

As for paclobutrazol the above mentioned results of paclobutrazol are in harmony with those attained by Zhang *et al*., 2006; Hamza *et al*., 2007; Youssef and Abd El-Aal, 2013; Mausour, 2014 and Ismail, 2016) they concluded that treating plants with paclobutrazol resulted in reducing different plant growth characteristics

Cimen *et al.* (2004) reported that paclobutrazol retarded vegetative growth but increased the dry matter percentage in maize. Amin (2007) deduced that spraying maize with paclobutrazol up to 60 $mg^{-1}$ resulted in significant decrease in dry weight (g)/plant.

## II. Physiological characters of leaves

### 1-photosynthetic pigments

Data recorded in table 2 show the effect of foliar application of benzyl adenine, paclobutrazol, Algae extract, some nutrient elements (Ca, Mg and Fe) and lithovit on photosynthetic pigments content of moringa plant leaves after 90 and 180 days from seeds sowing during 2016 growth season. Such data reveal that chlorophyll a , b and carotenoids content were enhanced as a result of all aforementioned growth enhancement treatments compared to the control treatment during the growth season after 90 and 180 days from seed sowing. In addition, the highest used concentrations of benzyl adenine (100 $mgl^{-1}$), paclobutrazol (20 $mgl^{-1}$) and algae extract (20 $mll^{-1}$) reflected the highest photosynthetic pigments content followed by using lithovit and nutrient elements in descending order during the growth season. Moreover, foliar application with different tested concentration of paclobutrazol (5, 10 and 20$mgl^{-1}$), exhibited the highest photosynthetic pigments content compared with other tested treatments and the control one. Furthermore, photosynthetic pigments content lavas tended to decrease with the age of growth and plant development. In this connection the newly formed leaves at 90 days from sowing had more chlorophyll and carotenoid stains than the older leaves after 180 days from seed sowing.

Same data also evidently confirmed the stimulatory and significantly effects of different applied treatments upon dry matter production and accumulation in leaves and branches In general, data in table 2 not only being a direct results for that vigorous growth obtained in table 2, but also could be considered an indicator for expectable high yield of moringa plant.

These results of benzyl adenine are in agreement with those reported by Ramadan (2005), Ibrahim *et al.* (2010), Abdel-Dayem *et al.* (2012) and Saad (2015).

Also, the obtained results of algae extract are in conformity with those obtained by Craigie (2011), Abou El-Yazied *et al.* (2012), Tarraf *et al*. (2015), Abo-Sedera *et al*. (2016), Ismail (2016), Boghdady *et al*. (2016) and Ismail (2016) found that treated plants with *Corallina*

**Table 2 :** Physiological characters as affected by different applied treatments of moringa (*Moringa olifera* L.) at 90 and 180 days during 2016 season.

| Treatments | Concentration | Physiological Characters of the leaves at 90 days after sowing | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | Chlorophyll (mg/g$^{-1}$ F.W.) | | | Antioxidant activity mgg$^{-1}$ D.W | Total Phenolic mgg$^{-1}$ D.W | Total Flavonoids mgg$^{-1}$ D.W | Total Flavonols mgg$^{-1}$ D.W |
| | | Chl.a | Chlb | Carot. | | | | |
| Control | 0.00 | 1.069 | 0.727 | 0.833 | 75.44 | 6.22 | 2.07 | 1.05 |
| Benzyl adenine | 25 mgl$^{-1}$ | 1.098 | 0.882 | 0.842 | 83.75 | 6.69 | 2.26 | 1.22 |
| | 50 mgl$^{-1}$ | 1.187 | 0.980 | 0.909 | 90.29 | 7.13 | 2.37 | 1.30 |
| | 100 mgl$^{-1}$ | 1.222 | 0.875 | 0.940 | 93.98 | 7.66 | 2.56 | 1.37 |
| Paclobutrazol | 5 mgl$^{-1}$ | 1.316 | 0.996 | 1.023 | 76.49 | 6.31 | 2.10 | 1.10 |
| | 10 mgl$^{-1}$ | 1.405 | 0.966 | 1.054 | 78.77 | 6.47 | 2.16 | 1.12 |
| | 20 mgl$^{-1}$ | 1.491 | 0.918 | 1.185 | 79.38 | 6.54 | 2.20 | 1.15 |
| Algae extract | 5 mll$^{-1}$ | 1.095 | 0.946 | 0.826 | 80.58 | 6.63 | 2.24 | 1.18 |
| | 10 mll$^{-1}$ | 1.144 | 0.912 | 0.869 | 84.35 | 6.82 | 2.32 | 1.27 |
| | 20 mll$^{-1}$ | 1.191 | 0.926 | 0.887 | 91.61 | 7.20 | 2.41 | 1.31 |
| Elements | 500 mgl$^{-1}$ | 1.150 | 0.970 | 0.852 | 84.59 | 6.90 | 2.35 | 1.28 |
| Lithovit | 500 mgl$^{-1}$ | 1.192 | 0.805 | 0.908 | 92.69 | 7.50 | 2.50 | 1.34 |
| LSD 5% | 0.05 | 0.004 | 0.006 | 0.007 | 2.21 | 0.12 | N.S | N.S |
| | | Physiological characters of the leaves at 180 days after sowing | | | | | | |
| Control | 0.00 | 0.818 | 0.360 | 0.538 | 80.46 | 8.14 | 2.63 | 1.38 |
| Benzyl adenine | 25 mgl$^{-1}$ | 0.954 | 0.562 | 0.849 | 86.96 | 9.20 | 3.12 | 1.67 |
| | 50 mgl$^{-1}$ | 1.009 | 0.730 | 0.871 | 92.49 | 9.87 | 3.36 | 1.73 |
| | 100 mgl$^{-1}$ | 1.028 | 0.842 | 0.872 | 95.64 | 10.54 | 3.52 | 1.81 |
| Paclobutrazol | 5 mgl$^{-1}$ | 1.033 | 0.707 | 0.918 | 81.66 | 8.49 | 2.72 | 1.43 |
| | 10 mgl$^{-1}$ | 1.161 | 0.857 | 0.902 | 82.26 | 8.91 | 2.83 | 1.53 |
| | 20 mgl$^{-1}$ | 1.661 | 0.593 | 0.557 | 82.87 | 9.03 | 2.96 | 1.59 |
| Algae extract | 5 mll$^{-1}$ | 0.949 | 0.580 | 0.781 | 84.40 | 9.10 | 3.06 | 1.63 |
| | 10 mll$^{-1}$ | 0.957 | 0.608 | 0.812 | 88.08 | 9.34 | 3.17 | 1.70 |
| | 20 mll$^{-1}$ | 1.021 | 0.562 | 0.790 | 93.50 | 10.05 | 3.40 | 1.75 |
| Elements | 500 mgl$^{-1}$ | 0.979 | 0.655 | 0.864 | 89.24 | 9.46 | 3.31 | 1.72 |
| Lithovit | 500 mgl$^{-1}$ | 1.021 | 0.672 | 0.825 | 94.58 | 10.30 | 3.43 | 1.78 |
| LSD 5% | 0.05 | 0.005 | 0.013 | 0.012 | 2.10 | 0.10 | 0.10 | N.S |

*elongate* increased photosynthetic pigments (chl. a, chl. b and chl. a+b) and carotenoids contents of wheat plants.

Such results of some mineral nutrients have been previously recommended by Abbas *et al.* (2009) and Abbas (2013).

For the used mineral elements, Iron (Fe) is a factor for approximately 140 enzymes that catalyze unique biochemical reactions Brittenham, (1994). Iron is critical for chlorophyll formation and photosynthesis and is important in the enzyme systems of plants Havlin *et al*., (1999). Foliar spraying of broad plants with Fe at 500 mgl$^{-1}$ significantly increased chlorophyll a, b and total chlorophyll El-Tantawy and Nawar (2013).

Nutrients are essential for plant growth and plays an important role in many plant physiological processes such as photosynthesis, sugar synthesis and starch translocation. It also works as an enzyme activator, a constituent of many enzymes in the plant Marschner, (1995) and Allison *et al*. (2001). Several studies have been dealing with the role of minerals on vegetable crops, Mg as a foliar application significantly enhanced chemical composition of cauliflower leaves (Ahmed *et al*., 2011).

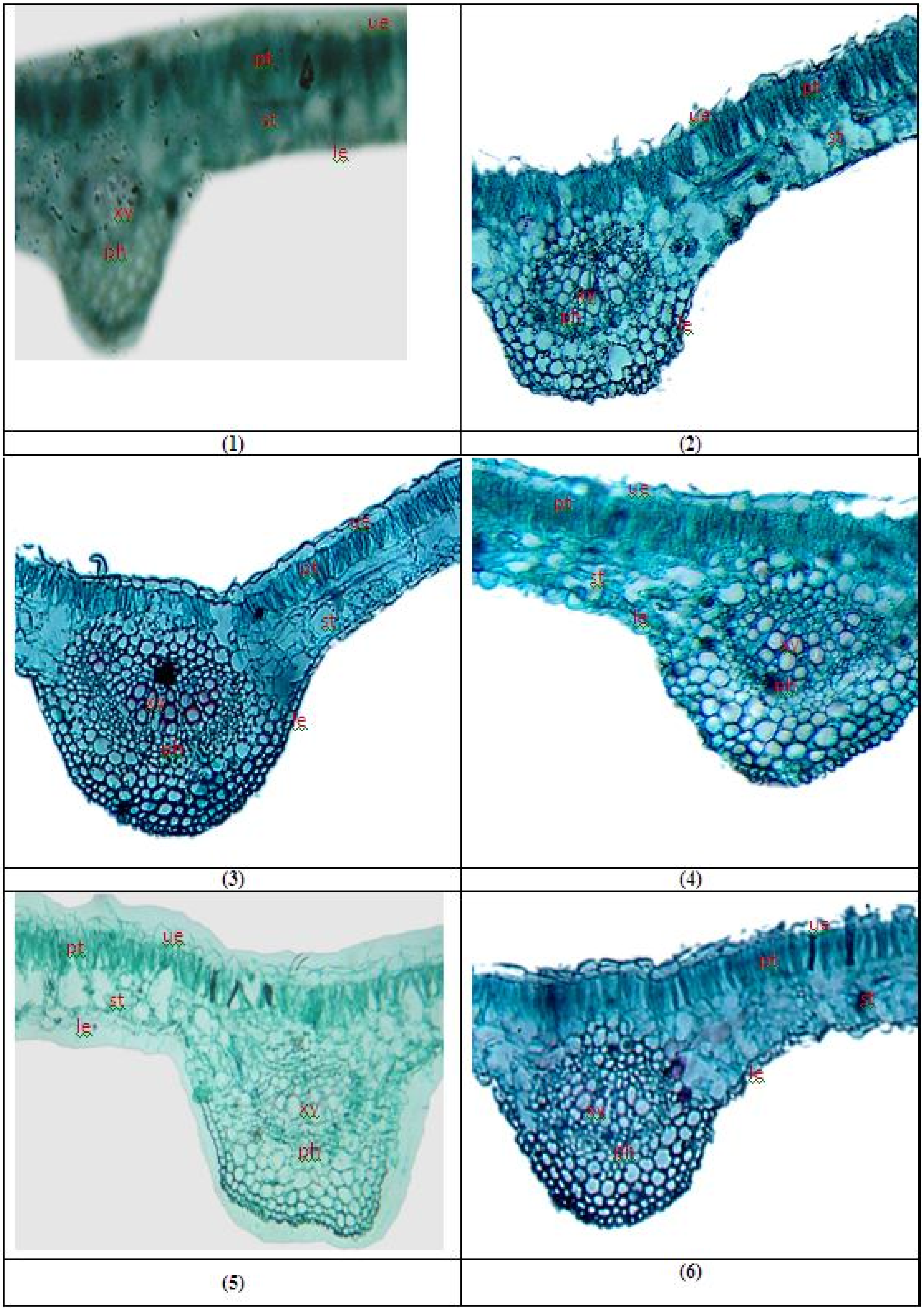


**Fig. 1 :** Transverse sections (X 40) of moringa (*Moringa oleifera*) leaflet at 90 days after sowing as affected by different applied treatments.

Where, (1): Control (2): Benzyl adenine at 100 $mgl^{-1}$ (3): Paclobutrazol (PP333) at 20 $mgl^{-1}$
(4): Algae extract at 20 $mll^{-1}$ (5): Mineral nutrients at 500 $mgl^{-1}$ (6): Lithovit at 500 $mgl^{-1}$
ue = Upper epidermis pt= Palisade tissue st= Spongy tissue le= Lower epidermis
ph = phloem tissue xy = Xylem tissue Ø V= Diameter of vessels

For the results of lithovit are in harmony with those attained by Abou El-Nasr *et al*. (2015) and Abo-Sedera *et al*. (2016).

Also, effects of lithovit application on photosynthetic pigments could be explained by Abou El-Nasr *et al.* (2015) reported that an increase in leaf bio-chemicals showed a tremendous increase in total and refraction of chlorophyll and carotenoids content using magnetite treatments compared to traditional iron chelate treatment. 250 mgl$^{-1}$ MNPs proved to be the highly effective than the same concentration of traditional iron chelate. Previous studies showed that nanoparticles can have a beneficial effect on plants chemical composition Radhakrishnan and Kumari (2012).

As for paclobutrazol the abovementioned results of paclobutrazol are in harmony with those attained by Zhang *et al.* (2006), Hamza *et al*. (2007), Youssef and Abd El-Aal (2013), Mausour (2014) and Ismail (2016) reported that treating wheat plants with paclobutrazol at 50 mgl$^{-1}$, significantly increased chlorophylls and carotenoids.

Dulizhao and Derrick (2000) showed that mepiquate chloride and chlormequate chloride increases photosynthetic rate by increasing leaf chlorophyll content and mesophyll cell size of cotton plant which is due to more rapid exchange of $CO_2$ into mesophyll cell by virtue of their large surface area.

**II Antioxidant activity:**

Regarding the effect of all tested foliar application treatments on antioxidant activity after 90 and 180 days from seed sowing the some data in table 2 show that the assayed antioxidant activity was increased as a result of using each of benzyl adenine, growth retardant (paclobutrazol), Algae extract, some nutrient elements (Ca, Mg and Fe) and lithovit at different used concentrations compared with the control treatment. In this connection, the highest used concentration of benzyl adenine (50 and 100 mgl$^{-1}$), algae extract 20 gl$^{-1}$ and lethovit at 500 mgl$^{-1}$ recorded the highest values of antioxidant activity compared with other tested treatments after 90 and 180 days from seed sowing. In addition using paclobutrazol at different concentrations gave the lowest values compared with other tested treatments in both sample of determinations.

These results of benzyl adenine are in harmony with those reported by Ramadan (1992), Ibrahim *et al.* (2010), Abdel-Dayem *et al.* (2012) and Saad (2015). In this regard such increment effect of each benzyl adenine, Algae extract, mineral elements and lithovit may be due to the important role of BA and algae extract in the regulation meristematic cell division, cell enlargement ,differentiation activity and organogenesis in developing plants, which in turn may stimulate plant growth rate.

Regarding to the stimulative effects of algae extract could be explained by Thirumaran *et al*. (2009) stated that seaweed liquid fertilizer (SLF) contained macro and micronutrients, organic substances like amino acids, vitamins and plant growth regulators such as, cytokinins, auxin and gibberellins.

Results of some mineral nutrients are in harmony with those reported by Abbas *et al.* (2009), Abbas (2013) and Atress and Mohamed (2014). Iron (Fe) is a factor for approximately 140 enzymes that catalyze unique biochemical reactions Brittenham (1994). Iron is important in the enzyme systems of plants Havlin *et al.*(1999).

Nutrients are essential for plant growth and plays an important role in many plant physiological processes. It also works as an enzyme activator, a constituent of many enzymes in the plant Mengel and Kirkby (1987), Marschner (1995) and Allison *et al.*(2001).

The effects induced by lithovit application on growth characteristics could by indicated by Abou El-Nasr *et al.* (2015) reported that an increase in leaf bio-chemicals showed a tremendous increasing using magnetite treatments compared to traditional iron chelate treatment. 250 mgl$^{-1}$ MNPs proved to be the highly effective than the same concentration of traditional iron chelate.

For paclobutrazol the abovementioned results of paclobutrazol are in harmony with those attained by Zhang *et al.*(2006), Hamza *et al.*(2007), Youssef and Abd El-Aal (2013), Mausour (2014) and Ismail (2016).

**1-Total phenolic:**

The same data in table 2 indicate that spraying moringa plants with benzyl adenine, paclobutrazol, Algae extract, nutrient elements (Ca, Mg and Fe) and lithovit at different tested concentrations tended to increase the content of total phenolic in leaves after 90 and 180 days from seed sowing compared with the control treatment. However, such increments in total phenolic compounds reach the level of significantly only in case of using lethovit in both sample of determination and using benzyl adenine at 100 mgl$^{-1}$ and Algae extract at 20 gl$^{-1}$ in the second sample of determination only.

Our results are go well with those reported by Brittenham (1994), Havlin *et al.* (1999), Lashkari *et al.* (2007), Thirumaran *et al*. (2009), Mansour *et al.* (2012), Radhakrishnan and Kumari (2012) and El-Tantawy and Nawar (2013).

**2-Total Flavonoids and flavonols:**

With regard to the effect of spray treatments on total Flavonoids and flavonols contents of moringa plant leaves

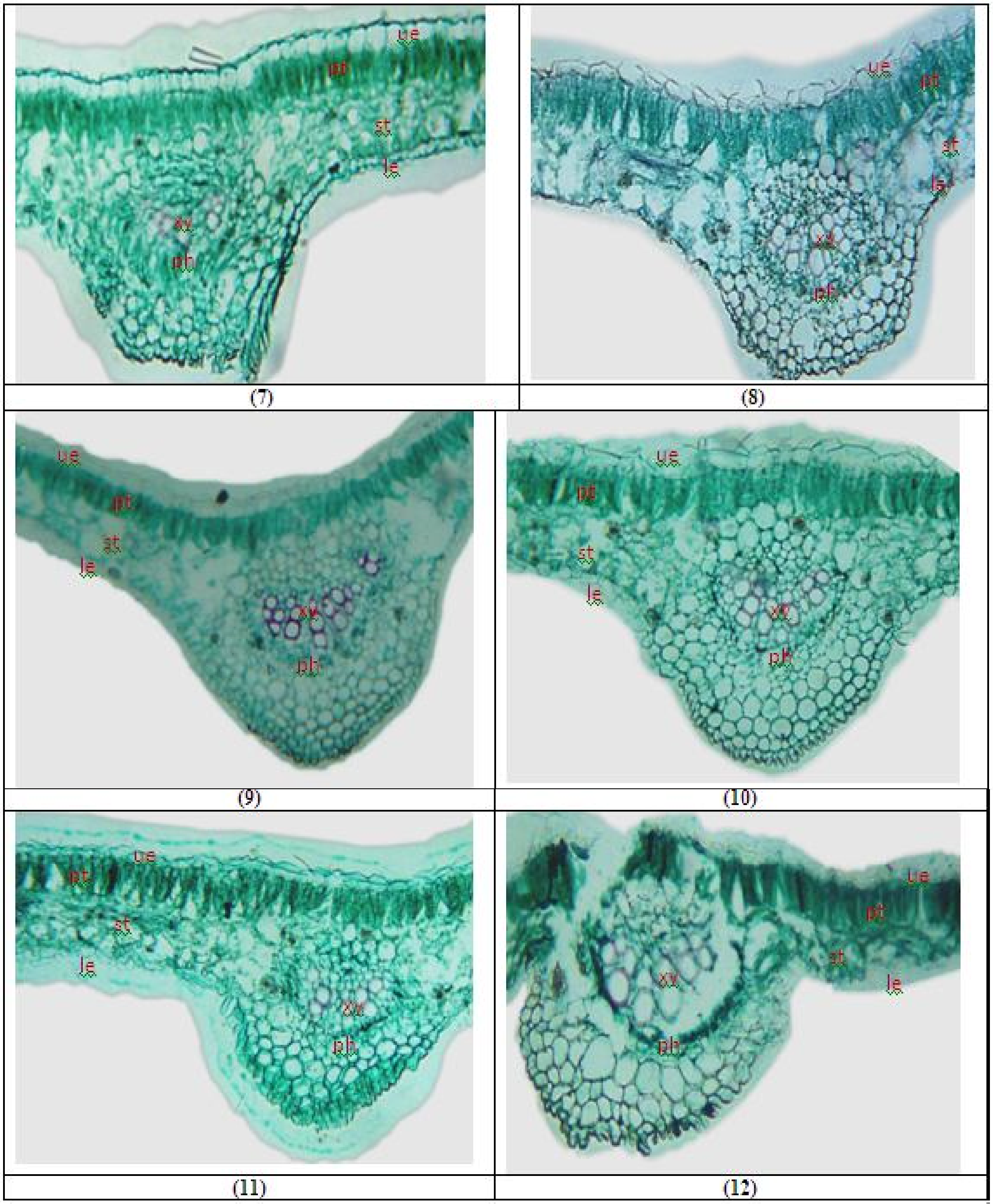


**Fig. 2 :** Transverse sections (X 40) of moringa (*Moringa oleifera*) leaflet at 180 days after sowing as affected by different applied treatments.

Where, (7): Control (8 ): Benzyl adenine at 100 $mgl^{-1}$ (9): Paclobutrazol ($PP_{333}$) at 20 $mgl^{-1}$
(10): Algae extract at 20 $mll^{-1}$ (11): Mineral nutrients at 500 $mgl^{-1}$ (12): Lithovit at 500 $mgl^{-1}$
ue= Upper epidermis pt= Palisade tissue st= Spongy tissue le= Lower epidermis
ph= phloem tissue xy= Xylem tissue Ø V= Diameter of vessels

after 90 and 180 days from seed sowing, the same data in table 2 indicate that there no significantly differences in both total flavonoids and flavonols contents among all tested treatments and the control treatment in the two samples of determinations, However, the highest concentration was found in case of spraying the plants with the highest concentration of benzyl adenine (100 $mgl^{-1}$) and lithovit treatment at 500 $mgl^{-1}$ compared with other studied treatments in both flavonoids and flavonols compounds.

The previously mentioned results are in conformity with those reported by Ramadan (1992), Ibrahim *et al.*

(2010), Abdel-Dayem *et al*. (2012) and saad (2015). In this regard such increment effect of each benzyl adenine, Algae extract, mineral elements and lithovit may be due to the important role of BA and algae extract in the regulation meristematic cell division, cell enlargement ,differentiation activity and organogenesis in developing plants, which in turn may stimulate plant growth rate. In this regard, Arigita *et al.* (2005) mentioned that cytokinins promote shoot development through increasing cell division, regulation of the cell cycle and the number of cycles which cells undergo in the meristems and organ primordial are the primary regulatory that targets cytokinins.

Regarding to the activation effects of algae extract could be explained by Thirumaran *et al*. (2009) stated that seaweed liquid fertilizer (SLF) contained macro and micronutrients, organic substances like amino acids, vitamins and plant growth regulators such as, cytokinins, auxin and gibberellins.

The aforementioned results of algae extract have been previously recommended by Craigie (2011), Abou El-Yazied *et al.* (2012), Atress and Mohamed (2014), Tarraf *et al*. (2015), Abo-Sedera *et al*. (2016), Boghddy *et al*. (2016) and Ismail (2016).

On the other side, it was reported that Iron (Fe) is a factor for approximately 140 enzymes that catalyze unique biochemical reactions Brittenham (1994). Iron is important in the enzyme systems of plants Havlin *et al.* (1999). Also, nutrients are essential for plant growth and plays an important role in many plant physiological processes such as photosynthesis, sugar synthesis and starch translocation, It also works as an enzyme activator, a constituent of many enzymes in the plant Mengel and Kirkby (1987), Marschner (1995) and Allison *et al.* (2001). Several studies have been dealing with the role of minerals on vegetable crops, Mg as a foliar application significantly enhanced chemical composition of cauliflower leaves and curds Ahmed *et al.* (2011).

In the same order, The abovementioned results of lithovit are in harmony with those attained by Abou El-Nasr *et al.* (2015) who reported that an increase in leaf bio-chemicals showed a tremendous increasing using magnetite treatments compared to traditional iron chelate treatment. 250 $mgl^{-1}$ MNPs proved to be the highly effective than the same concentration of traditional iron chelate. Using Nano fertilzers to plants is one of the critical importance due to its unique properties and activities Xia *et al.* (2009). Previous studies showed that nanoparticles can have a beneficial effect on plants Zhu *et al.* (2008), Roghayyeh *et al.* (2010), Esitken and Turan (2004) and Carbonell *et al.* (2011) Nanoparticles improved the chemical composition of plants Radhakrishnan and Kumari (2012).

As for paclobutrazol the abovementioned results of paclobutrazol are in harmony with those attained by Zhang *et al.* (2006), Hamza *et al.* (2007), Youssef and Abd El-Aal (2013), Mausour (2014) and Ismail (2016).

## III. Anatomical characters

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

As shown in table 3 and figs. 1 & 2 data indicate the effect of different applied treatments *i.e.*, benzyl adenine at 100 $mgl^{-1}$, Paclobutrazol ($PP_{333}$) at 20 $mgl^{-1}$, algae extract at 20 $mll^{-1}$, some mineral nutrients at 500 $mg^{-1}$ and lithovit at 500 $mgl^{-1}$ 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, midrib 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. extract at 20 $mll^{-1}$ (129µ) and benzyl adenine at 100 $mgl^{-1}$ (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 $mgl^{-1}$ and algae extract at 20 $mll^{-1}$ treatments.

The obtained results clearly show that *Moringa oleifera* plants treated with Paclobutrazol ($PP_{333}$) at 20 $mg^{-1}$, algae extract at 20 $mll^{-1}$ and Lithovit at 500 $mg^{-1}$ appeared to be the most effective treatments on increasing counts and measurements of certain anatomical characters 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 $mgl^{-1}$ and lithovit at 500 $mg^{-1}$ treatments respectively, followed by algae tissue thickness was (23µ) with the control but increased to reach (47µ) and (39µ) with Paclobutrazol ($PP_{333}$) at 20 $mgl^{-1}$ and algae extract at 20 $mll^{-1}$ treatments respectively, which were the most effective treatments. For palisade

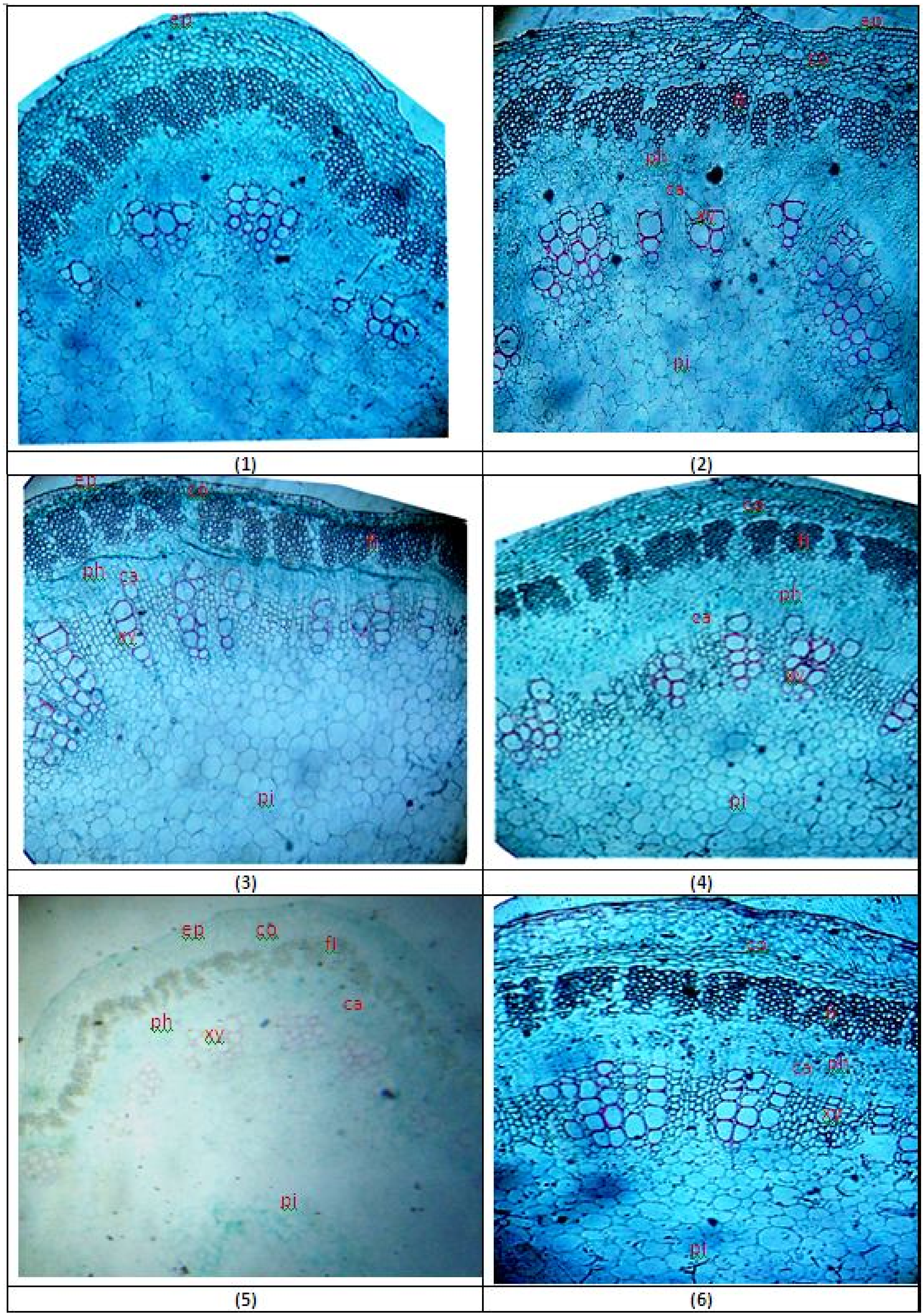


**Fig. 3 :** Transverse sections (X 40) of moringa (*Moringa oleifera*) stem at 90 days after sowing as affected by different applied treatments.

Where, (1): Control (2): Benzyl adenine at 100 $mgl^{-1}$ (3): Paclobutrazol ($PP_{333}$) at 20 $mgl^{-1}$

(4): Algae extract at 20 $mll^{-1}$ (5): Mineral nutrients at 500 $mgl^{-1}$ (6): Lithovit at 500 $mgl^{-1}$

ep = epidermis co = cortex tissue ph = phloem tissue ca = cambium tissue xy = Xylem tissue

pi = pith tissue fi = fibers Ø V = Diameter of vessels

**Table 3 :** Anatomical structure of the leaves as affected by different applied treatments of moringa (*Moringa olifera* L. ) at 90 and 180 days during 2016 season.

| Treatments | Anatomical Characters (μ) of the leaves at 90 days after sowing | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | Lamina Thcikness | Upper epidermis | Lower epidermis | Spongy tissue | Palisade tissue | Midvein Thickness | Vascular bundle length | Diameter of vessels | No. of vesseles |
| Control | 102 d | 13 d | 10 bc | 23 e | 37 d | 233 e | 65 f | 20 cd | 4 d |
| Benzyl | 109 c | 16 b | 9 c | 28 d | 36 d | 251 b | 92 d | 19 d | 12 ab |
| Paclo batro 20 mgl$^{-1}$ | 149 a | 15 bc | 11 ab | 47 a | 58 a | 293 a | 145 a | 24 a | 13 a |
| Algae extract 20 mll$^{-1}$ | 129 b | 19 a | 10 bc | 39 b | 46 b | 254 b | 128 b | 22 b | 8 c |
| Elements 500 mgl$^{-1}$ | 100 e | 15 bc | 12 a | 24 e | 31 e | 237 d | 84 e | 21 bc | 11b |
| Lithovit 500 mgl$^{-1}$ | 31 f | 14 cd | 10 bc | 36 c | 42 c | 241 c | 101 c | 25 a | 9 c |
| Mean | 120.00 | 15.33 | 10.33 | 32.83 | 41.66 | 251.50 | 102.80 | 21.83 | 9.50 |
| F test | ** | ** | ** | ** | ** | ** | ** | ** | ** |
| | Anatomical Characters (μ) of the leaves at 180 days after sowing | | | | | | | | |
| Control | 132 e | 19 a | 12 bc | 34 f | 44 e | 231 e | 88 e | 25 cd | 7 e |
| Benzyl adenine | 167 b | 18 a | 10 d | 53 b | 61 b | 287 b | 147 b | 23 e | 14 b |
| Paclo batro 20 mgl$^{-1}$ | 175 a | 16 b | 13 ab | 55 a | 68 a | 301 a | 154 a | 27 b | 18 a |
| Algae extract 20 mll$^{-1}$ | 148 d | 18 a | 11 cd | 45 d | 53 d | 276 c | 135 c | 28 b | 12 cd |
| Elements 500 mgl$^{-1}$ | 147 d | 15 b | 14 a | 38 e | 52 d | 262 d | 113 d | 28 b | 13 bc |
| Lithovit 500 mgl$^{-1}$ | 156 c | 18 a | 11 cd | 51 c | 55 c | 284 b | 134 c | 31 a | 11 d |
| Mean | 154.16 | 17.33 | 11.83 | 46.00 | 55.50 | 273.50 | 128.87 | 27.00 | 12.50 |
| F test | ** | ** | ** | * | ** | ** | ** | ** | ** |

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.

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 mgl$^{-1}$ and benzyl adenine at 100 mgl$^{-1}$ treatments. Here, spongy tissue thickness was (34μ) with the control but increased to reach (55μ) and (53μ) with Paclobutrazol ($PP_{333}$) at 20 mgl$^{-1}$ and benzyl adenine at 100 mgl$^{-1}$ 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 ($PP_{333}$) at 20 mgl$^{-1}$, algae extract at 20 mll$^{-1}$ and lithovit at 500 mgl$^{-1}$ 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 were 153, 144 μ and 106 μ with the same treatments, respectively.

With regard to midvein anatomical characters, it could be noticed that there an increment in the midrib thickness especially, in case of Paclobutrazol (PP333) at 20 mgl$^{-1}$ (293μ), algae extract at 20 ml-1, (254μ) and benzyl adenine at 100 mgl$^{-1}$ (251μ) treatments compared with other treatments and the control (233μ) at 90 days after sowing, while their values were (301, 276 and 287μ) compared with the control (231μ), respectively at 180 days after sowing. Increasing midrib thickness is attributed to the increase in many of its anatomical characters 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 The above mentioned results specially increment of the conductive tissues.

Regarding to the vascular tissues *i.e.*, 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 ($PP_{333}$) at 20 mgl$^{-1}$, algae extract at 20 mll$^{-1}$ and lithovit at 500 mgl$^{-1}$ treatments respectively compared with other treatments and the untreated plants.

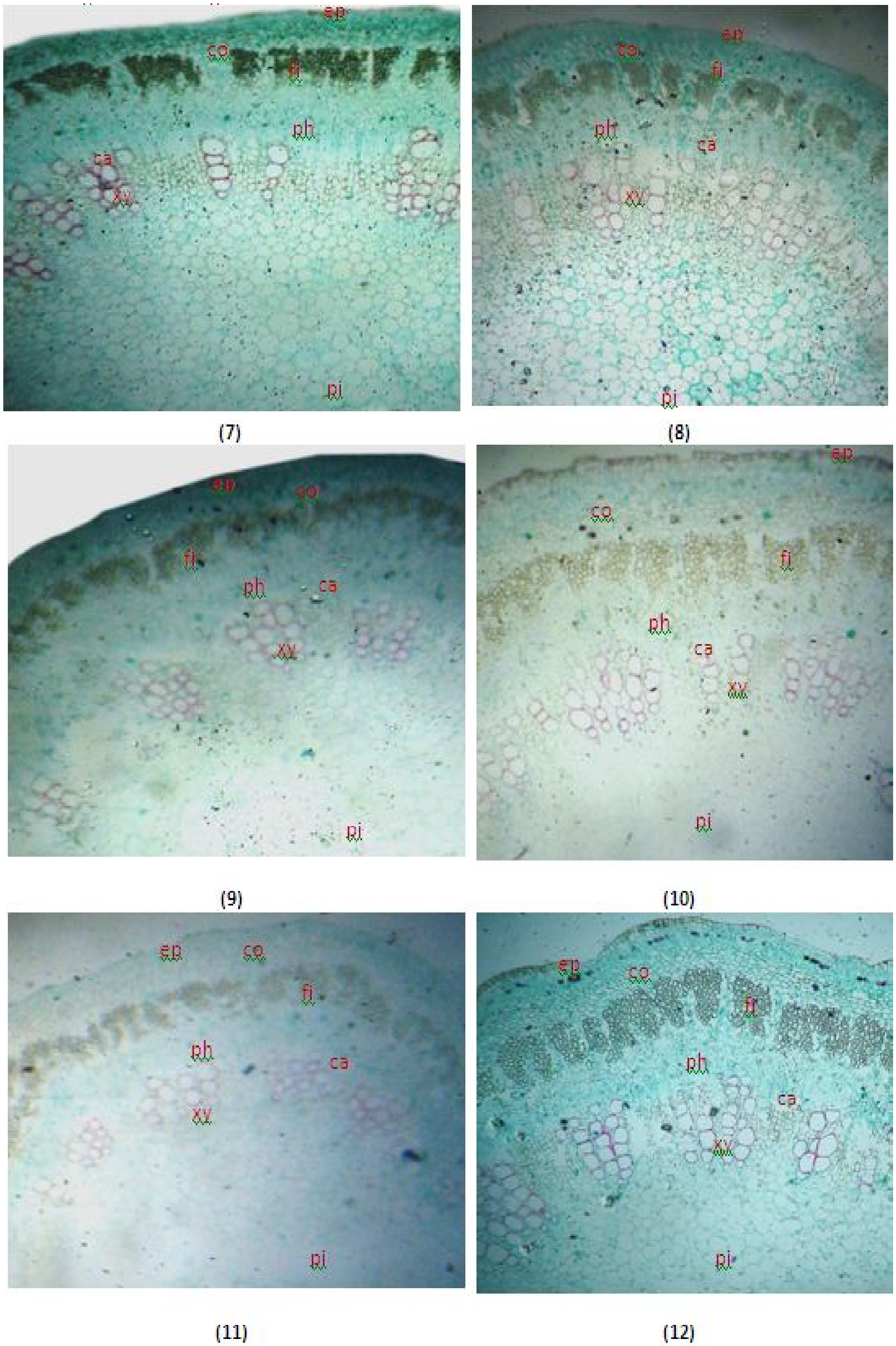


**Fig. 4 :** Transverse sections (X 40) of *moringa* (*Moringa oleifera*) stem at 180 days after sowing as affected by different applied treatments.

Where, (7): Control (8): Benzyl adenine at 100 $mgl^{-1}$ (9): Paclobutrazol ($PP_{333}$) at 20 $mgl^{-1}$

(10): Algae extract at 20 $mll^{-1}$ (11): Mineral nutrients at 500 $mgl^{-1}$ (12): Lithovit at 500 $mgl^{-1}$

ep=epidermis co=cortex tissue ph=phloem tissue ca=cambium tissue xy=Xylem tissue pi=pith tissue fi=fibers ØV=Diameter of vessels

**Table 4 :** Anotomical structure of the stems as affected by different applied treatments of moringa (*Moringa olifera* L.) at 90 and 180 days during 2016 season.

| Treatments | Stem diameter | Cuticle thickness | Epidermis thickness | Cortex thickness | Xylem thickness | Phloem thickness | Cambium thickness | Pith diameter | Vesseles diameter | No. of xyllem /arch$^{-1}$ | No. of vessels/ arch$^{-1}$ |
|---|---|---|---|---|---|---|---|---|---|---|---|
| **Anatomical characters (µ) of the stem at 90 days after sowing** | | | | | | | | | | | |
| Control | 3577 d | 11 ab | 12 c | 275 c | 207 b | 159 c | 21 d | 2107 c | 62 f | 64 d | 3a |
| Benzyl adenine 100 mgl$^{-1}$ | 3912 c | 10 bc | 14 b | 369 a | 216 b | 187 b | 31 a | 2149 bc | 69 e | 74 c | 4a |
| Paclobutrazol 20 mgl$^{-1}$ | 4395c | 12 a | 12 c | 374 a | 292 a | 283 a | 27 b | 2292 a | 76 b | 91 b | 4a |
| Algae extract 20 mll$^{-1}$ | 4088a | 7 d | 15 ab | 363 a | 274 a | 189 b | 24 c | 2243 ab | 72 d | 112 a | 4a |
| Elements 500 mgl$^{-1}$ | 3946 b | 9 c | 12 c | 325 b | 221 b | 166 c | 24 c | 2325 a | 74 c | 106 a | 3a |
| Lithovit 500 mgl$^{-1}$ | 3863 c | 11 ab | 16 a | 358 a | 278 a | 194 b | 25 c | 1986 d | 78 a | 83 bc | 3a |
| Mean | 3963.33 | 10.00 | 13.50 | 344.00 | 247.78 | 196.333 | 25.33 | 2181.94 | 71.83 | 88.33 | 3.50 |
| F test | ** | ** | ** | ** | ** | ** | ** | ** | ** | ** | ** |
| **Anatomical Characters of the stem at 180 days after sowing** | | | | | | | | | | | |
| Control | 4334 d | 14 ab | 15 ab | 458 e | 217 e | 196 e | 32 c | 2368 c | 72 e | 87 c | 5 ab |
| Benzyl adenine 100 mgl$^{-1}$ | 5535 b | 14 ab | 16 a | 662 a | 366 b | 253 c | 34 ab | 2754 a | 81 c | 94 c | 6 a |
| Paclobutrazol 20 mgl$^{-1}$ | 5642 a | 15 a | 15 ab | 674 a | 451 a | 329 a | 33 bc | 2488 b | 89 a | 126 a | 4 b |
| Algae extract 20 mll$^{-1}$ | 5417 c | 11 c | 16 a | 592 b | 447 a | 309 b | 29 d | 2504 b | 88 a | 96 c | 5ab |
| Elements 500 mgl$^{-1}$ | 4177 f | 13 b | 14 b | 495 d | 254 | 211 d | 28 d | 2347 cd | 78 d | 114 b | 4 b |
| Lithovit 500 mgl$^{-1}$ | 4682 d | 13 b | 16 a | 553 c | 297 cd | 244 c | 35 a | 2264 d | 86 b | 132 a | 5 ab |
| Mean | 5134.61 | 13.33 | 15.333 | 572.33 | 338.67 | 257.00 | 31.83 | 2453.83 | 82.333 | 108.17 | 4.83 |
| F test | ** | ** | ** | ** | ** | ** | ** | ** | ** | ** | ** |

In general, the stimulatory effects of applied treatments upon the anatomy characters of treated plants could mainly attributed to the increase of endogenous hormones level especially cytokinins and auxins, (Sotiropoulos *et al*., 2002; 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 mgl$^{-1}$, algae extract at 20 mll$^{-1}$, benzyl adenine at 100 mgl$^{-1}$ and lithovit at 500 mgl$^{-1}$ treatments followed by some mineral nutrients at 500 mgl$^{-1}$ 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 and Abd El-Aal (2013) on *Tabernaemontana coronaria*. The above mentioned 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 characters 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 characters of *Moringa* (*Moringa oleifera*) stems in response to different applied treatments at 90 and 180 days after sowing:**

Data in table 4 and figs. 3 and 4 indicate the effect of different applied treatments (*i.e.*, benzyl adenine at 100 mgl$^{-1}$, paclobutrazol ($PP_{333}$) at 20 mgl$^{-1}$, algae extract at 20 mll$^{-1}$, some mineral nutrients at 500 mg$^{-1}$ and lithovit at 500 mgl$^{-1}$ 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$^{-1}$, number of xylem rows vascular cylinder$^{-1}$ and diameter of the xylem vessel) under different used treatments compared with the control.

In general, paclobutrazol ($PP_{333}$) at 20 mgl$^{-1}$, algae extract at 20 mll$^{-1}$ and lithovit at 500 mgl$^{-1}$ treatments recorded the best results as exceeded that of other used and the control treatments in terms of the most studied anatomical characters 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 mgl$^{-1}$ (4395µ), algae extract at 20 mll$^{-1}$ (4088µ) and benzyl adenine at 100 mgl$^{-1}$ (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 mgl$^{-1}$ (5642µ) followed by benzyl adenine at 100 mgl$^{-1}$ (5535µ) and algae extract at 20 mll$^{-1}$ (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 ($PP_{333}$) at 20 mgl$^{-1}$, mgl$^{-1}$ and algae extract at 20 mll$^{-1}$ 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 mgl$^{-1}$ (451µ), algae extract at 20 mll$^{-1}$ (447µ) followed by benzyl adenine at 100 mgl$^{-1}$ (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-1 followed by paclobutrazol ($PP_{333}$) at 20 mgl$^{-1}$ and lithovit at 500 mgl$^{-1}$ 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 lithovit at 500 mgl$^{-1}$ and algae extract at 20 mll$^{-1}$ 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 mgl$^{-1}$, lithovit at 500 case of 180 days after sowing its maximum value (35µ) was recorded with lithovit at 500 mg-1 treatment followed by benzyl adenine at 100 mgl$^{-1}$ (34µ) and paclobutrazol ($PP_{333}$) at 20 mgl$^{-1}$ 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 mgl$^{-1}$, benzyl adenine at 100 mgl$^{-1}$ and algae extract at 20 $mll^{-1}$ 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 4.

In general, the stimulatory effects of applied treatments upon the anatomy characters 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 4 and figs. 3 and 4 the treatments of paclobutrazol ($PP_{333}$) at 20 $mgl^{-1}$, benzyl adenine at 100 $mgl^{-1}$ and algae extract at 20 $mll^{-1}$ recorded highly values of the stem studied anatomical characteristics especially, in number of xylem rows vascular $cylinder^{-1}$, number of xylem vessels xylem $row^{-1}$ 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 $mgl^{-1}$, algae extract at 20 $mll^{-1}$ followed by benzyl adenine at 100 $mgl^{-1}$ and lithovit at 500 $mgl^{-1}$ 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 characters counts from one side and moringa age (*i.e.*, either at 90 or 180 days after sowing) and 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 $mgl^{-1}$ or Algae extract at 20 $mll^{-1}$ four times to enhance the internal anatomical status towards maximizing its growth and productivity.

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