

## EFFECT OF *MORINGA OLEIFERA* LEAF EXTRACT ON GROWTH, METABOLITES AND ANTIOXIDANT SYSTEM OF BARLEY PLANTS

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### ABSTRACT

*Moringa oleifera* is an important and promising tree, growing in many arid and semi-arid areas. Moringa plant is effective and suitable source of nutrition and medication. The present work was carried out to study the response of two barley genotypes to a foliar application of moringa leaf extract (MLE) as a growth enhancer at the vegetative stage. The obtained result showed enhanced antioxidants activities (superoxide dismutase, peroxidase & catalase) in leaves of the barley genotypes under MLE application compared to the control. Similar results were detected in the case of plant growth, leaf photosynthetic pigments, building material and some minerals contents in both tested barley genotypes. In contrast, lower malonaldehyde (MDA) content was obtained under MLE treatment in the studied barley genotypes. The application of MLE resulted in good growth stimulation. Better enhancement was detected in Giza124 than Giza129 barley genotypes. In conclusion, foliar treatment with MLE as a bio-growth enhancer improves the antioxidant capacity and plant secondary metabolites of barley leaves. Thus, *Moringa oleifera* leaf extract is recommended to be applied widely as a bio-organic fertilizer for various crops due to its good productivity, highly nutritive and antioxidant system and low cost.

**Keywords:** *Moringa*, *Barley*, *Antioxidants*, *MDA*, *Chlorophyll*, *Biofertilizer*

### المخلص العربي

تعتبر شجرة المورينجا أوليفيرا شجرة هامة واعدة وهي تنمو في العديد من المناطق الجافة وشبه الجافة. نبات المورينجا هو مصدر فعال ومناسب في التغذية والأدوية. تهدف هذه الدراسة إلى دراسة استجابة صنفين من الشعير في المرحلة الخضريّة إلى المعاملة بمستخلص أوراق المورينجا (إم إل إي) باعتباره محسن للنمو. أظهرت النتائج تحسّين في نشاط مضادات الأكسدة (سوبرأوكسيد ديزميوتيز، البيروكسيديز والكتاليز) داخل أوراق نباتات الشعير المعاملة بمستخلص المورينجا مقارنة بنباتات الكنترول الغير معاملة بالمورينجا. تم رصد نتائج مشابهة محسنة أيضا لصنفى الشعير من خلال دراسة دالات النمو، أصباغ البناء الضوئي في الورقة، مواد البناء، بعض المعادن الأساسية. على النقيض تناقص محتوى المألونداي أدهيد نتيجة للمعاملة بمستخلص أوراق المورينجا في كلا صنفى نبات الشعير. أدت المعاملة بال (إم إل إي) الى تحسّين عام في النمو. تم تسجيل استجابة أفضل لصنف الشعير جيزة ١٢٤ عن صنف جيزة ١٢٩. وبالتالي فإن استخدام المعاملة الورقية بمستخلص المورينجا كمحسن نمو حيوي يمكنه تحسّين محتوى مضادات الأكسدة و مختلف المواد الأيضية الثانوية لأوراق نباتات الشعير. وبالتالي فإنه يوصى باستخدام مستخلص أوراق نبات المورينجا أوليفيرا على نطاق واسع كسماد عضوي حيوي لمختلف المحاصيل نظرا لأن هذا المستخلص يؤدي الى إنتاجية أفضل، قيمة غذائية ومضادات أكسدة أعلى بالإضافة إلى أنه ذات تكلفة أقل.

### INTRODUCTION

The use of inorganic fertilizers as an essential source of plant nutrients causing high cost and is also accompanied with environmental pollution. Thus, there is necessity to find alternative safe natural sources of plant biofertilizers. *Moringa oleifera* can be promoted among farmers as a possible substitute to the inorganic fertilizers (Phiri, 2010). Several searches have proved that *M. oleifera* is a valuable plant with multipurpose effects (Moyo *et al.*, 2011; Mishra *et al.*, 2011).

Moringa is a very useful tree can be called as the miracle tree. It has an obvious role in different purposes such as food, medication and also in industry (Moyo *et al.*, 2011). Moringa contains important minerals, proteins, carotene and various phenolics. It is rich in combination of zeatin with several flavonoid pigments which gave *M. oleifera* a good natural antioxidant system (Anwar *et al.*, 2007). Moringa leaf extract (MLE) stimulated growth of young plants, provide strength for adult plants and enhanced plant tolerance towards diseases.

Several recent studies were undertaken to stimulate the growth and yield of different plants by foliar treatment of MLE (Nouman *et al.*, 2011). There is only little scientific knowledge about the effect of MLE as a biofertilizer on the growth, metabolic and antioxidant enzymes of plants. Therefore, this work was carried out to study the effect of the aqueous extract of *M. oleifera* in improving the growth, metabolic and antioxidant activities of barley genotypes.

Barley (*Hordeum vulgare* L.) is one of the important crops and considered one of the first cultivated cereals. It considered as suitable crop for arid and semiarid regions, as it tolerates the abiotic conditions as compared to wheat (Karami *et al.* 2013).

Plant tolerance can be enhanced by the exogenous application of stimulators. Moringa oleifera leaf extract considered one of the natural plant growth stimulator which not only stimulates the plant growth, secondary metabolites, antioxidant system and dry matter production but also improves tolerance under the different stresses (Yasmeen *et al.*, 2012 a, b).

Reactive oxygen species (ROS) accumulations are greatly harmful for plants because of lipid peroxidation, protein deterioration and finally cell death (Karami *et al.* 2013). Malondialdehyde (MDA) is a suitable marker expressing the membrane lipid peroxidation content.

The foliar applied MLE produced maximum growth parameters, photosynthetic pigments and building material comparing with the control conditions. Foliar applied MLE increased the antioxidants such as peroxidase, catalase and superoxide dismutase and also leaf minerals contents such as  $K^+$ ,  $Ca^{+2}$ ,  $Mg^{+2}$  compared with the untreated barley plants.

The different effects of MLE application to enhance barley growth, metabolites and antioxidant system have not been well established yet. Therefore, this study was conducted to assess the effect of MLE as an organic plant growth stimulator in improving the growth, biomass production and antioxidant activity of barley plant.

## **MATERIAL AND METHODS**

### ***PLANT TREATMENTS***

Grains of barley genotypes (*Hordeum vulgare* L.); Giza124 and Giza129 were provided by Agricultural Research Centre, Giza, Egypt. Six grains were sown in each pot containing 5 kg of clay soil. All pots were irrigated to the soil water content with tap water. Pots were placed in an open air under natural conditions. Three replicates treatments were classified into two groups; the first group represented the control and the second group was twice foliar-sprayed with MLE 20 ml/plant at 30 and 45 days. The control plants were foliar-sprayed only with distilled water. Plants were harvested at 60 days to determine and clarify their physiological responses.

### ***PREPARATION OF MLE AQUEOUS EXTRACTS***

Fresh green leaves of *Moringa oleifera* were obtained from botanical garden, Sadat City, Egypt. The extract was prepared according to (Hanan and Salama, 2014) with

little modification. Fresh leaves of moringa were air-dried at room temperature ( $22 \pm 2$  °C) for 7 days then ground to keep in powder form. 100g of the air-dried plant leaves were soaked in one liter of distilled water at room temperature for 24 hours with occasional shaking. Then it was filtered through four layers of cheese cloth to remove the fiber, then through Whatman No.1 paper. MLE 30% concentration was prepared from the previous stock solution by dilution with distilled water.

### **GROWTH CRITERIA**

Root length and shoot length was determined by direct measurement. Leaf area was determined according to (McKee, 1964; Bonhomme *et al.*, 1974) and recently was reviewed by (Norman and Campbell, 1994). Leaf area = k (leaf length x leaf maximum width)  $\text{Cm}^2 \text{ plant}^{-1}$

### **FRESH AND DRY WEIGHT**

The shoot fresh and dry weights were expressed as g/plant. Fresh organs were dried in an aerated oven (Hotbox Oven, Gallenkamp, England) at 80°C.

### **PHOTOSYNTHETIC PIGMENTS**

Contents of chlorophyll a, chlorophyll b, and carotenoids were estimated by the spectrophotometric method recommended by (Lichtenthaler, 1987) and expressed as mg/g FW.

### **CARBOHYDRATES:**

Soluble and total carbohydrates were estimated by the MDA reagent method which was carried out by (Fales, 1951; Schlegel, 1956) and adopted by (Badour, 1959).

### **PROTEINS**

Soluble protein and total protein content of root, stem and leaves were determined according to (Lowry *et al.*, 1951) and expressed as mg/g DW.

### **MINERALS**

- 1-  $\text{K}^+$  content was estimated by (Williams and Twine, 1960) using Carl Zeiss 2-flame photometer as mg/g DW.
- 2- The versene-titration method by (Schwarzenbach and Biedermann, 1948) was employed for calcium and magnesium determination. The data of  $\text{Ca}^{++}$  and  $\text{Mg}^{++}$  expressed as mg/g DW.

### **DETERMINATION OF MEMBRANE DAMAGE**

Lipid peroxidation was measured by determining malonaldehyde (MDA) levels. MDA content was determined as an indicator of leaf lipid peroxidation according to (Hernández and Almansa, 2002). Fresh leaf samples (500 mg) were homogenized in 5 mL of 0.1% trichloroacetic acid (TCA). The homogenate was centrifuged at 15,000g for 20 min at 4°C. One mL aliquot of the supernatant was mixed with 3 mL of 0.5% thiobarbituric acid (TBA) prepared in 20% TCA and incubated at 90°C for 20 min. After stopping the reaction in an ice bath, samples were centrifuged at 10,000g for 5 min. The supernatant absorbance at 532 nm was then measured. After subtracting the non-specific absorbance at 600 nm, MDA concentration was determined using the extinction coefficient  $155 \text{ mM}^{-1} \text{ cm}^{-1}$

## **ANTIOXIDANT DEFENSE SYSTEM**

Antioxidant enzymes in the fresh shoots were extracted according to (Mukherjee and Choudhuri, 1983). Superoxide dismutase (SOD, EC 1.12.1.1.) was measured according to (Noctor and Foyer, 1998), Peroxidase (POX, EC 1.11.1.7) activity was detected by (Kong *et al.*, 1999) and catalase (CAT, EC 1.11.1.6) activity was assayed according to (Chen *et al.*, 2000).

## **STATISTICAL ANALYSIS**

Significance between means of the control and MLE-treated plants and the standard errors were analyzed using SPSS at 5% (Gomez and Gomez, 1984).

## **RESULTS**

### **GROWTH PARAMETERS**

As shown in figure (1), shoot length of Giza124 was more than in Giza129 barley genotype while root length was higher in Giza129 than Giza124 barley genotype. Shoot fresh weight, shoot dry weight and root dry matter recorded higher measures in Giza124 than Giza129 barley genotype. Root/shoot dry weight ratio was higher in Giza129 than Giza124. MLE stimulated the root length, shoot length and leaf area of the two barley genotypes. Shoot fresh weight, shoot dry weight, root dry weight and root/shoot dry weight ratio enhanced markedly by MLE application. The highest stimulation in shoot fresh weight was detected in Giza129 genotype while Giza124 gave the best stimulation in shoot dry weight as indicated in figure (2).

### **PHOTOSYNTHETIC PIGMENTS**

The different photosynthetic pigments fractions (Chl.a, Chl.a.b, carotenoids) along with the total pigments and Chl a/b ratio gave much more content in Giza124 than that in Giza129 barley genotype as expressed in figure (3). MLE foliar application enhanced Chl.a, carotenoid and total pigments content in Giza124 genotype while slightly reduced Chl.b content leading to higher Chl.a/b ratio. In Giza129 genotype, MLE enhanced all studied photosynthetic fractions. Giza124 showed mostly higher stimulation than Giza129 barley genotype.

### **CARBOHYDRATE AND PROTEIN CONTENTS**

Soluble and total carbohydrate contents in root and shoot recorded higher values in Giza124 than in Giza129 barley genotype as found in table 1. MLE enhanced the different carbohydrate fractions in roots and shoots of both studied barley genotypes. Roots showed more stimulation than shoots especially in Giza129 genotype.

Total protein contents in root and shoot were much more in Giza124 than Giza129 barley genotype, while soluble proteins were lesser in roots and shoots of Giza124 comparing with Giza129 of roots and shoots. MLE enhanced the soluble and total protein accumulations in root and in shoot of both studied barley genotypes.

### **DIFFERENT MINERALS CONTENTS ( $K^+$ , $CA^{+2}$ AND $MG^{+2}$ )**

Table 2 indicated that  $K^+$  determination gave highest content in roots and shoots of Giza124 than Giza129 barley genotype. MLE enhanced the  $K^+$  content in root and in shoot of both studied barley genotypes.

Ca<sup>2+</sup> and Mg<sup>2+</sup> content were higher in Giza124 than Giza129 barley genotype. MLE stimulated the Ca<sup>2+</sup> content of roots and shoots of the studied barley genotypes. The highest stimulation was recorded in Giza124 genotype and in roots than shoots. MLE foliar application resulted in little enhancement in Mg<sup>2+</sup> content in the two studied barley genotypes.

#### **MDA Content**

MDA content was much more in Giza129 than Giza124 genotype as shown in figure (4). MLE reduced MDA content in both genotypes, the higher response recorded in Giza124 genotype.

#### **ANTIOXIDANT ENZYMES**

CAT, POD and SOD enzyme activities was much higher in Giza124 than in Giza129 barley genotype. MLE stimulated the different antioxidant enzymes in both barley genotypes, CAT and SOD gave better stimulation in Giza124 genotype however, POD resulted in better response in Giza129 barley genotype figure (5).

#### **DISCUSSION**

Different growth stimulants often improve plant growth and various developmental processes. *Moringa oleifera* leaf extract is rich in zeatin, carotenoids, phenols, antioxidants and some main nutrients so it has the ability to regulate plant growth and often used as exogenous plant growth stimulant (Fuglie 1999; Foidl et al. 2001).

Under control conditions the shoot length, leaf area, shoot fresh weight, shoot and root dry weights were higher in Giza124 than Giza129 barley genotype. This indicates better growth and better yield of Giza124 genotype than Giza129 barley genotype. While root length and root/shoot dry weight ratio was higher in Giza129 than Giza124 and this may indicate the strategy of Giza129 plants in improving the root status to enable their survival.

MLE markedly enhanced the different studied growth parameters. Commonly the highest stimulation was recorded in Giza124 than Giza129 barley genotype. The photosynthetic pigments results confirmed the previous data of growth. The applied moringa extract may enhance earlier formation of cytokinin thus reducing the premature leaf senescence leading to increased leaf area and more photosynthetic pigments (Ali et al., 2011). So it could be concluded that the increase in chlorophylls and different growth characteristics of barley genotypes might have been correlated with high cytokinin levels in MLE (Rady et al., 2013).

Soluble and total carbohydrate contents in root and shoot recorded higher values in Giza124 than in Giza129 barley genotype this is mainly resulted due to higher photosynthetic pigments in Giza124 barley genotype and confirming better dry matter yield in Giza124 barley genotype. Total protein contents in root and shoot were also much higher in Giza124 than Giza129 barley genotype. Moringa extract significantly stimulated the soluble and total carbohydrate contents and relatively the protein contents in the different organs of both studied barley genotypes. MLE helps in the osmotic adjustment and it can regulate the expression of genes of metabolic processes and defense (Hebers and Sonnewald, 1998).

K<sup>+</sup>, Ca<sup>2+</sup> and Mg<sup>2+</sup> contents were higher in Giza124 than Giza129 barley genotype. MLE stimulated the K<sup>+</sup>, Ca<sup>2+</sup> with little stimulation in Mg<sup>2+</sup> content of roots and shoots of barley genotypes and this agreed with (Yasmeen et al. 2013) who found enhancement in minerals (K, Ca, Mg) contents by MLE application to improve the

tolerance in wheat plant under saline conditions. MLE is rich in zeatin, calcium, potassium, magnesium and other growth components enabling it to act as a perfect growth stimulant for crops cultivation (Yasmeen *et al.*, 2012a; Nouman *et al.*, 2012a). Both barley genotypes under the MLE treatment exhibited direct increase in the shoot  $K^+$  content which ultimately stimulated the uptake of  $K^+$  during stomatal conductance (Cakmak, 2005). According to (Sivakumar and Ponnusami, 2011), the uptake and accumulation of some minerals as K, Na, Ca, Mg as a result of the organic fertilization was investigated. Leaves of moringa are rich source of protein,  $\beta$ -carotene, vitamin C, calcium and potassium and considered as a source of natural antioxidants (Siddhuraju and Becker, 2003).

In this study the exogenous applications of MLE improved the antioxidant activities. MLE exogenous application gave maximum activities of the studied antioxidant enzymes (SOD, CAT and POD) comparing with control plants. MLE is a natural biofertilizer inducing positive release of antioxidants and this may be due to involving zeatin in it and also due to leaf chlorophyll contents that has the main role in photosynthesis process (Foidl *et al.*, 2001).

MDA is a good index for membrane lipid peroxidation where the reduction in membrane stability indicates the degree of lipid peroxidation caused by ROS (Anjum *et al.*, 2011). The lower level of lipid peroxidation in leaves of Giza124 barley genotype suggests that this genotype is better protected from oxidative damage than Giza129 barley genotype. MLE treatment decreased MDA contents in leaves of both genotypes and this is agreed with (Hung *et al.*, 2006).

Enhanced antioxidant system under MLE application is related to higher mineral contents found in moringa leaves which make it perfect plant growth stimulant (Yasmeen *et al.*, 2013). Its growth enhancing behavior was studied on different plants (Nouman *et al.*, 2012a,b). In this study the recorded enhancement caused by treatment with MLE in barley genotypes could be attributed to hormonal effect especially rich zeatin contents of moringa. Zeatin improves the antioxidant properties of many enzymes and retards the cell aging effects of ROS (Zhang and Ervin, 2004).

In conclusion, MLE is to be used as a promising plant growth stimulant or a biofertilizer which can induce cultivation of barley. It improved the biomass production of barley plants, stimulated the photosynthetic pigments, soluble and total sugars, soluble and total proteins and (potassium, calcium, magnesium) as essential minerals. This study supported the potent antioxidant capacity of *Moringa* extracts as MLE improved the antioxidant activities of SOD, CAT and POD whereas decreased the lipid peroxidation levels in barley plants. The enhancement gained under MLE treatment was more pronounced in the Giza124 barley genotype plant comparing to Giza129 barley genotype and this difference may be due to their genetic variations.

**Table 1. Effect of foliar application of moringa leaf extract on soluble carbohydrates (S.Car), total carbohydrates (T. Car), soluble proteins (S.P) and total proteins (T.P) of roots and shoots of two barley genotypes.**

Building material	Parameter	Giza124		Giza129	
		Control	MLE spray	Control	MLE spray
Carbohydrate	R.S.Car	29.31±0.58	35.20**±0.29	22.30±0.17	30.20**±0.11
	R.T.Car	48.33±0.86	55.50**±0.28	45.33±0.19	66.27±0.57
	Sh.S.Car	30.87±0.29	33.57**±0.28	30.80±0.40	37.40**±0.23
	Sh.T.Car	60.80±0.23	73.00**±0.57	57.87±0.28	60.40*±0.58
Protein	R.S.P	30.00±0.57	28.50*±0.28	33.50±0.29	36.00*±0.57
	R.T.P	131.00±0.86	147.50**±0.29	117.50±1.15	122.66*±0.57
	Sh.S.P	65.50±0.86	88.00**±0.57	89.33±0.19	86.50*±0.28
	Sh.T.P	139.00±1.15	168.31**±1.15	136.00±1.15	167.00**±1.70

Significance between means of the control and MLE-treated plants expressed as \* and highly significance \*\* and ± standard errors were calculated for each treatment at 5%.

**Table 2. Effect of foliar application of moringa leaf extract on K<sup>+</sup>, Ca<sup>2+</sup> and Mg<sup>2+</sup> contents of roots and shoots of two barley genotypes.**

Minerals	Organ	Giza124		Giza129	
		Control	MLE spray	Control	MLE spray
K <sup>+</sup>	Root	5.49±0.14	3.36*±0.20	4.56±0.10	3.93**±0.15
	Shoot	15.60±0.29	7.50**±0.23	12.90±0.27	4.79**±0.17
Ca <sup>++</sup>	Root	4.85±0.11	0.00±0.35	4.50±0.05	0.75**±0.04
	Shoot	4.50±0.04	0.25**±0.04	3.25±0.03	0.75**±0.02
Mg <sup>++</sup>	Root	5.85±0.02	0.90±0.14	5.40±0.04	0.50±0.04
	Shoot	3.15±0.02	0.40**±0.01	3.15±0.01	0.27*±0.02

Significance between means of the control and MLE-treated plants expressed as \* and highly significance \*\* and ± standard errors were calculated for each treatment at 5%.

Figures

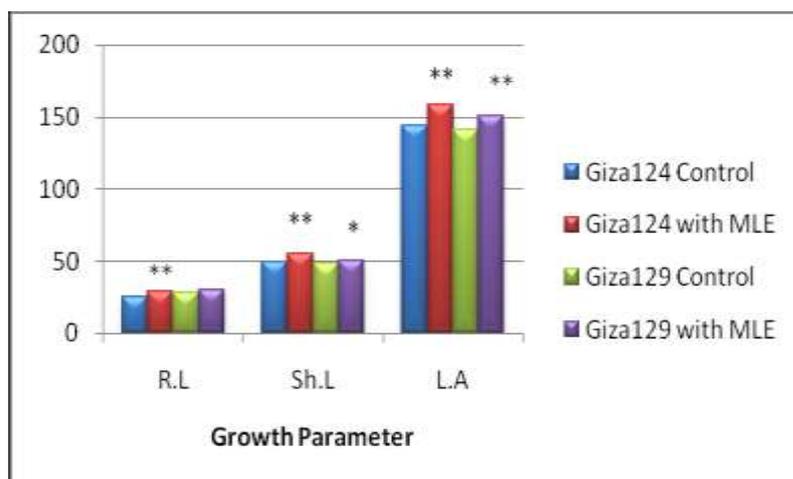


Fig. (1): Effect of foliar application of moringa leaf extract on root length (R.L), shoot length (Sh.L) and leaf area (L.A) of two barley genotypes.

Significance between means of the control and MLE-treated plants expressed as \* and highly significance \*\* at 5%.

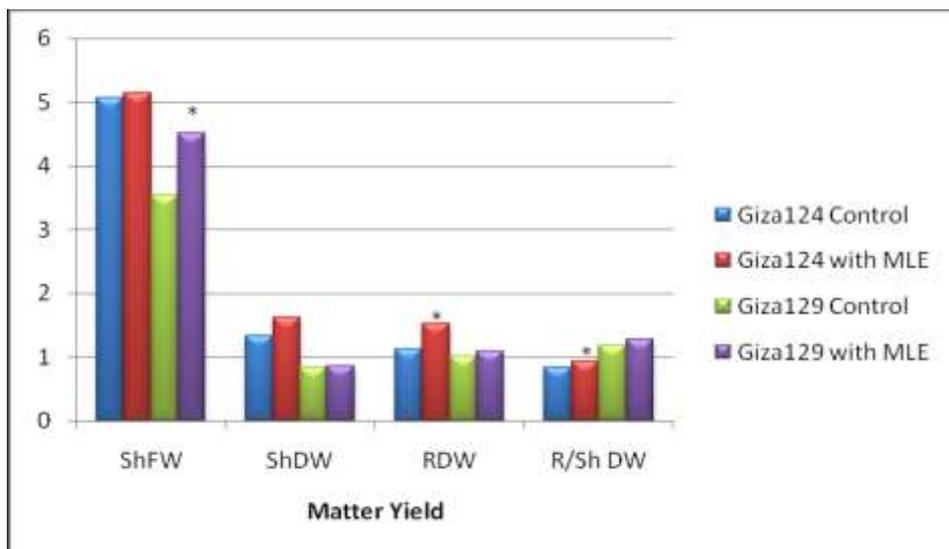


Fig. (2): Effect of foliar application of moringa leaf extract on shoot fresh weight (ShFW), shoot dry weight (ShDW) and root/shoot dry weight ratio (R/Sh DW) of two barley genotypes.

Significance between means of the control and MLE-treated plants expressed as \* and highly significance \*\* at 5%.

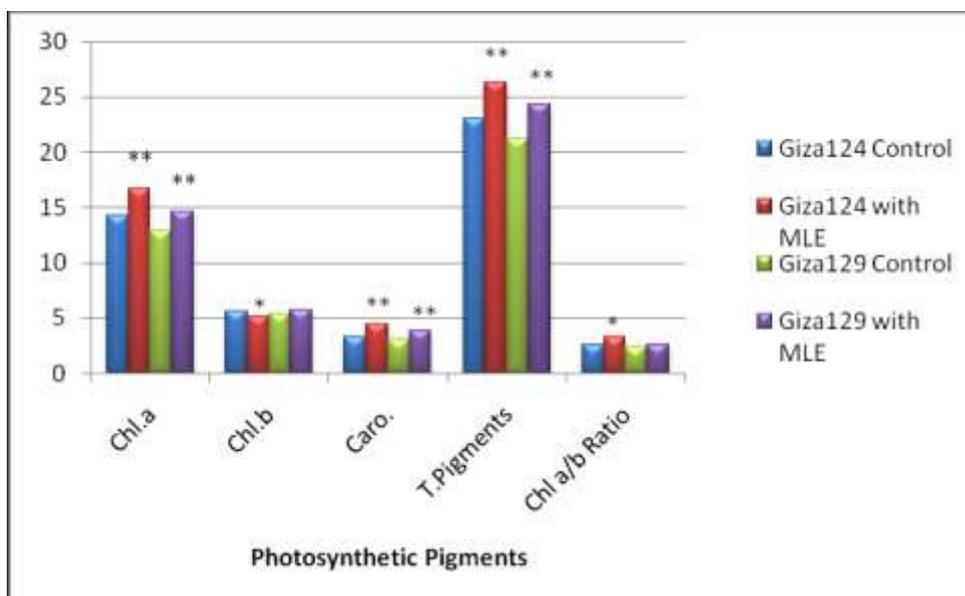


Fig. (3): Effect of foliar application of moringa leaf extract on Chl.a, Chl.b, Carotenoids, total pigments and Chl a/b ratio of two barley genotypes.

Significance between means of the control and MLE-treated plants expressed as \* and highly significance \*\* at 5%.

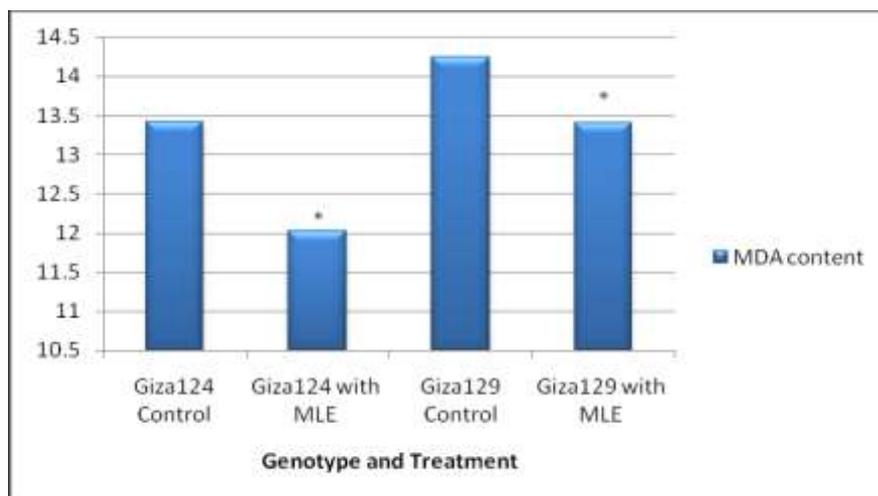


Fig. (4): Effect of foliar application of moringa leaf extract on Malondialdehyde (MDA) content of two barley genotypes.

Significance between means of the control and MLE-treated plants expressed as \* and highly significance \*\* at 5%.

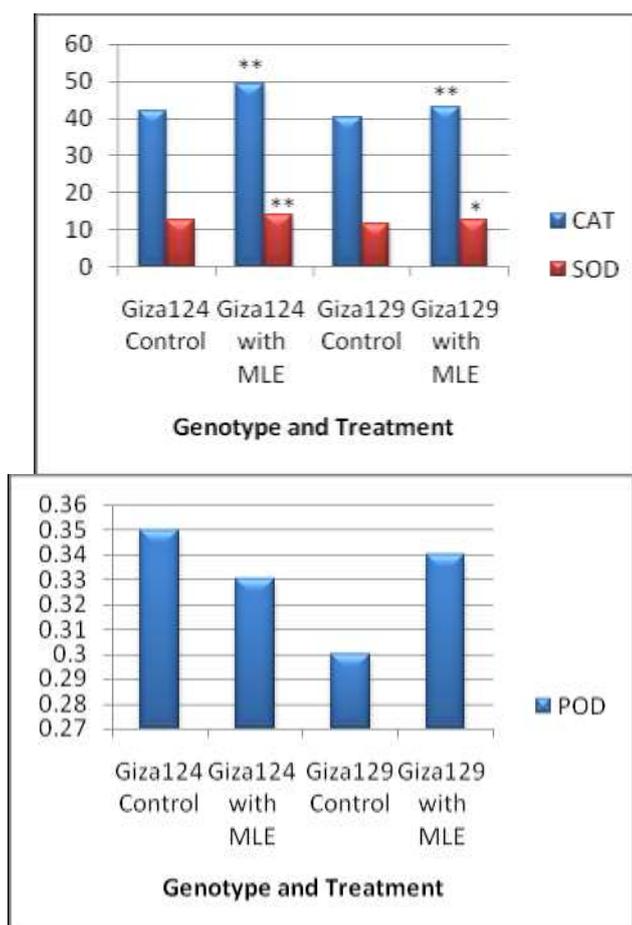


Fig. (5): Effect of foliar application of moringa leaf extract on malondialdehyde (MDA) content of two barley genotypes.

Significance between means of the control and MLE-treated plants expressed as \* and highly significance \*\* at 5%.

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