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PHYSIOLOGICAL AND BIOCHEMICAL INFLUENCES OF SOME SAFETY TREATMENTS ON ONION PLANTS

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ABSTRACT: *Effects of five different non-traditional treatments i.e. magnetic water, humic acid, seaweeds extracts, mycorrhzyia fungi and organic fertilizers in addition to the traditional mineral recommended fertilizer were determined in aspect to different growth characteristics within onion plants grown in two subsequent seasons under open field conditions in station of faculty of agricultural at minufiya university. The correlation between of the tested treatments and the accumulation of different biochemical active compounds within treated onion such as ploy phenols, and peroxidases substrates were investigated through using HPLC apparatus. The results showed that the highest recorded growth and vegetative criteria were observed on plants treated with mycorrhyza fungi followed by magnetized water. On contrary, the lowest growth and vegetative values were recorded by organic fertilizers and traditional recommend fertilizers. Biochemical analysis using HPLC revealed that cinamic acid substrate was detected only within treated plants by sea weeds extracts, humic acid and recommended mineral fertilizers. The highest accumulation of peroxidase enzyme was observed with mycorrhyza and sea weeds treatments. The results showed also that Mycorrhyza fungi, sea weeds extracts, organic fertilizers in addition to recommend mono fertilizers increased the accumulation of poly phenols substrates more than all other tested treatments.*

Key words: *Onion – Mycorrhyza- magnetized water- seaweds extracts – humic acid.*

INTRODUCTION

Onion (*Allium Cepa L*.) being among the most widely distributed Liliaceous plants in world. Besides, the onion is a species of the Alianceae family of great economic importance, and is the second most important vegetable crop in the world (FAO, 2006). However, making a significant contribution to the human diet, onions also have medicinal and functional properties (Lanzotti, 2006).

In Egypt onion is cultivated not only for local consumption, but also for export. Most of the area grown for export located in middle and upper Egypt. The early production of onion in the nile delta region gave the opportunity to increase bulbs yield for export. In Delta, Gizza 20 cv. Is a new cultivar well adapted for local conditions and show good uniformity in shape and color, this is may considered an exportable cultivar.

No doubt, the use of chemical fertilizers was boon for the past but ban for the present.

Where as organic fertilizers can be used in vegetable farming for safe food production and also sustain soil health. Using organic fertilizers the soil status can also be improved as it is rich in humus, different minerals, vitamins and growth substances that support excellent plant growth and production.

Humic acid is commercial product contains many elements which improve the soil fertility and increasing the availability of nutrient elements and consequently increased plant growth and yield. Moreover, humic acid particularly is used to ameliorate or reduce the negative effect of chemical fertilizers and some chemicals of soil. (Asmaa *et al.,* 2010).

Seaweeds and various seaweeds extracts have been utilized in agricultural

practices since long. The extracts of marine macroalgae viz. Brown , red and green algae are known to have positive effect on growth and yield of crops. The brown algae are the most commonly used seaweeds in agriculture. Seaweed extracts contain different phytohormones like auxins, gibberellins, cytokinines, absesic acid, ethylene, betaine and polyamines and other growth promoters along with trace elements, vitamins, amino acids, antibiotics and micronutrients which enhance the yield and yield attributes of crops, when applied exogenously (Panda et al., 2012).

Phosphorous (P) and sulfur (S) are two necessary nutrients that increase the growth and yield of plants. Defect of this nutrients in soil, as usually compensated by using of chemical fertilizers but this fertilizers have harmful effects on the environment and decrease the quality of agricultural products. Therefore, biological fertilizers are more interested for using in agriculture ecosystems.

Endomycorrhyzal fungi are an integral part of most plant species in nature. It is well documented that application of mycorrhyzal fungi caused increases in water and nutrients absorption and translocation special phosphorous to host plant cells and improve growth as well as photosynthesis which reduce more assemelation. Also, mycorrhyza has synergistic effects with most of other microorganisms that have increasing effect on yield and yield components of most crops (Lukiwalid and Simanungkalit, 2002, Marulanda *et al.,* 2003, Heggo and barakah, 2003).

The water treaded by magnetic field or pass through a magnetic devise called magnetized water. The effect of magnetic fields on running water have been observed for years. Decreases of soil salinity, increases in mobile forms of fertilizers, increases in crop yield and earlier vegetation periods can be achieved by magnetized water treatment.

The objective of this investigation was to increases onion growth and productivity in addition to improve bulbs quality via using some safety treatments to growing plants.

MATERIALS AND METHODS

The investigation was conducted at the experimental farm, Faculty of Agriculture, Menoufia University during 2014 and 2015 seasons. The experiment was laid out in randomized block design with three replicates. Onion transplants of Giza 20 cv. were transplanted on December and in 2014 and 2015, respectively. Transplants received the normal cultural practices of onion cultivation.

Six rows (3.5m long and 50 cm width) with a total square area 10.5 m^2 were devoted for each experimental unit, the other two rows were considered as guard rows, two of witch were to study vegetative growth characteristics and chemical composition of plants, while the other two rows were left to yield investigation.

The treatments comprises of control where plants received mineral fertilizers according to the recommendation of Agriculture Ministry and five treatments which were seaweed extracts, mycorrhiza, magnetized water irrigation, organic fertilized and humic acid:

1- **Seaweed extracts:** Was added to the irrigation water 7cm/L twice after transplanting at 15 days intervals (Techno green group company, Cairo, Egypt, according to the recommendation of Agriculture Ministry).

2- **Mycorrhiza:** Was added at 3cm^3 for every transplant during transplanting, another 3cm^3 was added after 45 days from transplanting. Transplants roots were dipped in mycorrhiza solution before transplanting (Biological Unit of Ein Shams University) .

3- Magnetized irrigation water: Irrigation of the treated plants with magnetized water treated by a magnetic tube used for treating the water was manetron (model U.T.I 1 inch diameter, output 4-6 m^3/h and its strength 50mT), Fig. (1) produced by magnetic technologies L.C.C., Russia, branch United Arab Emirates, was executed during growth period, irrigation according to the recommendation of Agriculture Ministry.

4- Organic fertilizer: A compost of debris sugars factories with compound A (energy compounds + mineral substances) and compound B (vegetables extract from natural sources contains felavonoids + afrofelavonoids). The compost will mixed with a compound A and compound B as the organic complex was added to the soil before transplanting and repeat the addition after 30 days from transplanting, obtained from Prolina Company for Agriculture development, Dr. Mohamed Sherif Abdel-Maksoud .

NPK contents in the organic fertilizers were determined as data are presented in Table (1).

5- Humic acid: Humic acid was added to the soil at 2L/fed after transplanting for three times with 10 days intervals (according to the recommendation of Agriculture Ministry).

Thus, the experiment included five treatments in three replicates for each.

Data recorded:

During growth period, data were recorded for following items.

I- Vegetative growth:

Random 10 plants were dug out from each experimental unit 90 and 105 days from transplanting and subjected to the following measurements:

1.1. leaves fresh weight.
1.2. leaves dry weight.
1.3. Neck fresh weight.
1.4. Neck dry weight.
1.5. Bulb fresh weight.
1.6. Bulb dry weight.
1.7. Total plant fresh weight.
1.8. Total plant dry weight.

2. Enzyme activity in leaves:

Chromatogram analysis of onion leaves obtained from plants treated with five different biological and biochemical agents was performed on HPLC apparatus, (Agilent Technologies 1262 Infinity) system, using a preceded by a Eclipse plus ® C18 reversed phase guard column (4.6 by 10.0 mm, 3.5 pm). The HPLC system consisted of a 1260 Quat Pump unit, Agilent Technologies 1260 diode array detector, fluorescence array detector, and a 1260 auto sampler which were controlled by ChemoStation for LC 3D system. Before samples injection, the column had been equilibrated with 90% (v/v) water and 10% Acetonitril (solvent B). After injection, the samples were eluted at a flow rate of 1.0 ml min1 using an isocratic flow of 90% solvent A and 10% solvent B for 2 min, a linear gradient to 10% solvent A and 90% solvent B for 28 min, followed by an isocratic flow for 5 min with 90% solvent B. For conducting HPLC analysis, 4 g of fresh leaves collected from each treatment were freeze dried using liquid nitrogen. The Freeze-dried leaves were grinded using pestle and mortal before mixing thoroughly with 5 ml of ethyl acetate in plastic test tubes and gently hand shacked for 15 min. After shacking, the suspension was centrifuged at 12,000 rpm for 3 minutes. After separation, the suspension was filtrated into new tubes through 2 cotton layers and evaporated under vacuum to completion. The extracted compounds were then dissolved in 250 ul methanol and 50 pi of each chemical extraction analyzed by HPLC. Spectral analysis was conducted to compare the detected peaks with similar retention times in all extractions (Schouten *et al.,* 2004) modified by (Selim 2010).

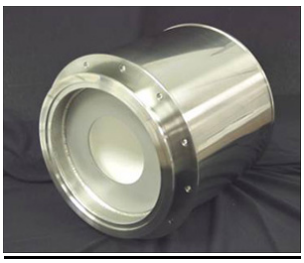

Fig. (1): Magnetic tube

Table (1) NPK contents in organic fertilizers used in 2014 and 2015 s4easons

2014			2015		
N %	P %	K%	N %	P %	K%
1.68	0.74	0.62	3.29	0.54	0.98

HPLC chemical standards of mixture of different chemical active compounds *i.e.* Acetone-DNPH, Acrolein-DNPH, 2, 5-Dimethylbezaldehyde -DNPH, Formaldehyde- DNPH, Isovaleraldehyde-DNPH, Propionaldehyde-DNPH and peroxides were used as references to compare the spectra pattern of obtained peaks within tested onion plants.

Results

- **Vegetative and growth criteria**

Different vegetative and growth criteria were recorded from two independent open field experiments conducted on two subsequent seasons (2013/2014 and 2014/2015).

The obtained results from first season experiment showed that either after 90 or 105 days, the highest leaves fresh and dry weight was observed on plants treated with mycorrhyza and magnetic water (Tables 2, 3, 4, and 5). In contrary, organic fertilizers followed by humic acid resulted in lowest leaves fresh and dry weight (Tables 2, 3, 4, and 5).

Ninety days after transplanting, the highest fresh and dry weight of neck plant was observed on plants treated with mycorrhyza followed by magnetic water and seaweeds extracts (table 6, 7, 8 and 9). Similar results were recorded again on treated plants with mycorrhyza and magnetic water after 105 days post transplanting (Tables 6, 7, 8 and 9).

Similar to the results observed with leaves and neck fresh and dry weight, the highest weight of both fresh and dry bulbs was recorded on plants treated by mycorrhyza and magnetic water, respectively either 90 or 105 days post transplanting. Moreover, the least bulbs fresh weight was recorded on control plants while the lowest bulb dry weight was observed on plants treated with organic fertilizers (Tables 10, 11, 12 and 13). In harmony with the previous mentioned results, significant increase of whole plant fresh weight was noticed on plants treated with magnetic water as well as mycorrhyza treatments either 90 or 105 day (Tables 14, 15, 16 and 17).

Remarkable, similar results were obtained again from the second season experiment. Conversely, seaweeds extract affected positively both total plant fresh and dry weight more than the all other tested treatments in the second season experiment.

Table (2): Leaves fresh weight as affected by some safety treatments during the winter season (2013/2014) at 90 and 105 days.

Treatments	90 days	105 days
Control	85.24	116.80
Organic	86.41	118.47
Mychorrhyza	104.4	130.6
Magnetic water	95.55	125.2
Humic acid	93.73	118.25
Sea weed extract	95.11	122.6
LSD value at alpha = 5%	3.339	4.314

Table (3): Leaves fresh weight as affected by some safety treatments during the winter season of 2014/2015 at 90 and 105 days.

Treatments	90 days	105 days
Control	89.16	121.2
Organic	99.46	128.8
Mycorrhiza	103.6	148.8
Magnetic water	106.8	143.4
Humic acid	99.72	136.1
Sea weed extract	102.9	142.8
LSD value at alpha = 5%	4.600	4.523

Table (4): leaves dry weight as affected by some safety treatments during the winter (2013/2014) at 90 and 105days.

Treatments	105 days	90 days
Control	6.613	6.813
Organic	6.825	6.950
Mychorrhiza	7.950	8.250
Magnetic water	7.713	7.962
Humic acid	7.115	7.825
Sea weed extract	7.162	7.950
LSD value at alpha = 5%	0.9147	1.289

Table (5): leaves dry weight as affected by some safety treatments during the winter season of 2014/2015 at 90 and 105 days.

Treatments	90 days	105 days
Control	4.625	5.100
Organic	5.375	6.463
Mycorrhiza	6.075	8.025
Magnetic water	5.963	7.963
Humic acid	5.600	7.225
Sea weed extract	5.563	7.512
LSD value at alpha = 5%	0.5703	0.7615

Table (6): Neck fresh weight as affected by some safety treatments during the winter season (2013/2014) at 90 and 105 days.

Treatments	90 days	105 days
Control	12.49	20.35
Organic	14.70	23.75
Mychorrhiza	19.35	26.65
Magnetic water	17.95	26.33
Humic acid	16.90	24.48
Sea weed extract	16.88	24.83
LSD value at alpha = 5%	1.262	2.666

Table (7): Neck fresh weight as affected by some safety treatments during the winter season of 2014/2015 at 90 and 105 days.

Treatments	90 days	105 days
Control	12.38	25.67
Organic	13.50	25.90
Mycorrhiza	15.30	30.13
Magnetic water	15.05	30.00
Humic acid	14.32	26.75
Sea weed extract	14.93	27.02
LSD value at alpha = 5%	1.747	2.521

Table (8): Neck dry weight as affected by some safety treatments during the winter season (2013/2014) at 90 and 105 days.

Treatments	90 days	105 days
Control	1.650	2.070
Organic	1.813	2.243
Mychorrhiza	2.263	2.855
Magnetic water	2.225	2.827
Humic acid	1.925	2.375
Sea weed extract	2.037	2.773
LSD value at alpha = 5%	0.3137	0.5777

Table (9): Neck dry weight as affected by some safety treatments during the winter season of 2014/2015 at 90 and 105 days.

Treatments	90 days	105 days
Control	1.600	2.662
Organic	1.663	2.938
Mycorrhiza	2.300	3.475
Magnetic water	2.175	3.275
Humic acid	1.688	3.100
Sea weed extract	1.900	3.300
LSD value at alpha = 5%	0.5512	0.3368

Table (10): Bulb fresh weight as affected by some safety treatments during the winter season (2013/2014) of at 90 and 105 days.

Treatments	105 days	90 days
Control	21.08	45.32
Organic	22.15	47.92
Mychorrhiza	27.77	60.33
Magnetic water	27.42	57.72
Humic acid	23.55	48.33
Sea weed extract	23.60	53.60
LSD value at alpha = 5%	1.964	2.357

Table (11): Bulb fresh weight as affected by some safety treatments during the winter season of 2014/2015 at 90 and 105 days.

Treatments	90 days	105 days
Control	19.50	40.42
Organic	18.80	42.63
Mycorrhiza	24.50	56.50
Magnetic water	23.02	52.45
Humic acid	22.65	45.20
Sea weed extract	22.58	48.34
LSD value at alpha = 5%	1.205	2.037

Table (12): Bulb dry weight as affected by some safety treatments during the winter season 1 of 2013/2014 at 90 and 105 days.

Treatments	105 days	90 days
Control	2.237	5.600
Organic	2.350	6.048
Mychorrhiza	3.150	6.988
Magnetic water	2.787	6.637
Humic acid	2.487	6.238
Sea weed extract	2.700	6.338
LSD value at alpha = 5%	0.5355	0.8789

Table (13): Bulb dry weight as affected by some safety treatments during the winter season of 2014/2015 at 90 and 105days.

Treatments	90 days	105 days
Control	1.975	4.125
Organic	2.125	4.737
Mycorrhiza	2.775	6.280
Magnetic water	2.300	6.113
Humic acid	2.138	5.901
Sea weed extract	2.287	5.987
LSD value at alpha = 5%	0.4189	0.689

Table (14): Total plant fresh weight (Bulb -leaves-neck) as affected by some safety treatments during the winter season of 2013/2014 at 90 days.

Treatments	90 days	105 days
Control	118.8	157.3
Organic	123.2	165.1
Mychorrhiza	151.4	191.5
Magnetic water	140.9	188.2
Humic acid	134.2	171.0
Sea weed extract	135.6	181.0
LSD value at alpha = 5%	4.837	7.840

Table (15): Total plant fresh weight (Bulb-leaves-neck) as affected by some safety treatments during the winter season of 2014/2015 at 90 and 105 days.

Treatments	90 days	105 days
Control	121.0	172.3
Organic	131.8	182.3
Mycorrhiza	143.3	215.4
Magnetic water	144.9	208.9
Humic acid	136.7	190.1
Sea weed extract	140.4	198.1
LSD value at alpha = 5%	5.486	6.012

Table (16): Total plant dry weight (Bulb-leaves-neck) as affected by some safety treatments during the winter season1 of 2013/2014 at 90 and 105 days.

Treatments	90 days	105 days
Control	11.49	14.48
Organic	11.56	14.75
Mychorrhiza	14.46	16.77
Magnetic water	13.39	16.11
Humic acid	13.16	15.05
Sea weed extract	13.28	15.57
LSD value at alpha = 5%	1.195	1.305

Table (17): Total plant dry weight (Bulb-leaves-neck) as affected by some safety treatments during the winter season of 2014/2015 at 90 and 105 days.

Treatments	90 days	105 days
Control	9.575	12.66
Organic	9.962	13.26
Mycorrhiza	12.34	15.46
Magnetic water	12.21	15.10
Humic acid	11.453	13.94
Sea weed extract	11.04	14.49
LSD value at alpha = 5%	1.107	1.352

- **Chromatogram analysis**

The results demonstated also that, all tested treatments induced the accumulation of peroxidase enzyme within individual treated plants. The highest accumlation levels was detected with seaweeds and mycorhyza treatemnts followed by organic fertiliyers and humic acid. In contary, lowest accumulation of peroxidase substance was recorded on plants treated with magnetic water (Fig. 2).

Chromatogram analysis showed also that seaweeds extract was superior comparing to the all other tested subtances in aspect to increasing levels of total phenol componds on treated onion plants while the lowest concentration of phenolic compounds was recorded by humic acid (Fig. 3).

Discussion

The obtained results showed that mycorrhyza and magnetic water were superior comparing to the all other treatments. Thus the most highest vegetative and growth criteria recorded with the mycorrhyza followed by the magnetic water. In contrary, the lowest fresh and dry weight of leaves, bulbls and neck organs were observed on plants treated with control, humic and organic fertilizers, respectively. Remarkable, in second season trail the results demonstrated that seaweed extracts resulted in the highest data values with treated plants in comparing to control as well as the rest of other treatments.

These results are in agreement with the study of Watts-Williams *et al.,* (2014) who demonstrated that mycorrhyza fungi play a significant role in increasing and improving the plant growth. Selim (2009, 2013) and Zayed (2010) reported also that magnetized water irrigation increased the accumulation of different important chemical ingredients as carbohydrates which lead to increasing the dry weight of treated plants.

Furthermore, in 2012, Dagro and Rekesh mentioned that seaweeds application enhanced dry matter accumulation on treated onion plants.

On the other hand, in the present study, the obtained results seaweeds and mycorhyza fungi recorded the highest accumulation of peroxidase and total phenols.

Certain mutualistic endophytic isolates belonging to specific species of *Trichoderma, Gliocladium, Fusarium, Mucor, Penicillium, Aspergillus, Stachybotrys,* and *mycorrhzya* genera have the potential for application in agricultural systems besides the traditional chemical pesticides. Some can be beneficial with regard to tolerating biotic and a biotic stress caused by drought,

salinity, minerals deficiency and pathogen invasion through modulating an array of physiological, biochemical and morphological processes (Oelmüller, *et al.,* 2009; Sherameti *et al.,* 2005., Lukiwatid and simamyngkalit., 2002) resulting in the accumulation and translocation of assimilates, osmotic adjustment, maintenance of cell wall elasticity and the increase of the water use efficiency by the host plant. Others can support the plant in coping with biotic stress conditions.

Moreover, many of seaweeds (marine algae) attracted the interest of scientists as natural sources of different bio-active compounds. Therefore they are candidate to be promising bio-stimulator agents (Michael *et al*., 2005; El-gamal, 2010). Many bioactive compounds have been identified in seaweeds*;* antioxidants are often, reducing agents such as thiol*,* ascorbic acid or poly phenols (Hu*,* 2008; Li *et al*., 2011). Of the beneficial antioxidant compounds, phenolic compounds widely exist in plants and have been considered to have high antioxidant capacity and free radical scavenging capacity (Kahkonen *et al*., 2001).

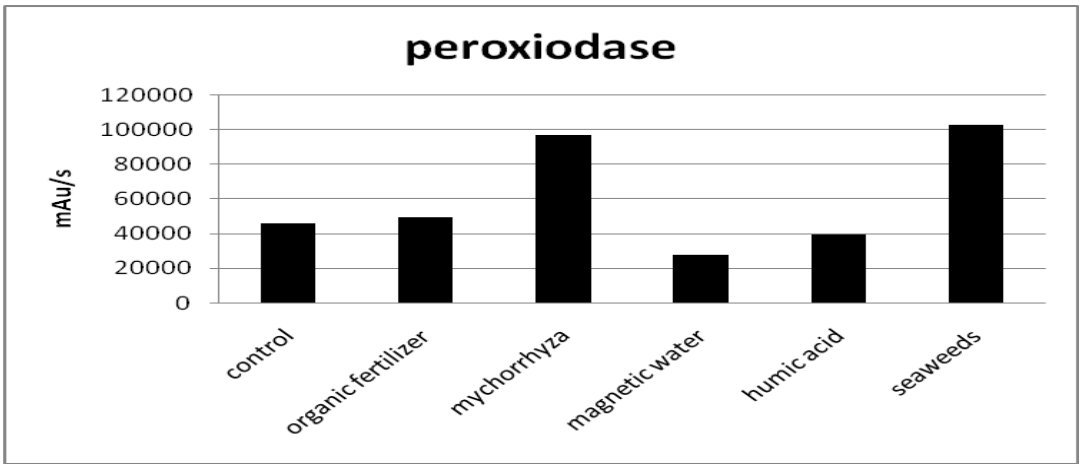

Figure (2): Accumulation of peroxidase enzyme within individual treated onion plants.

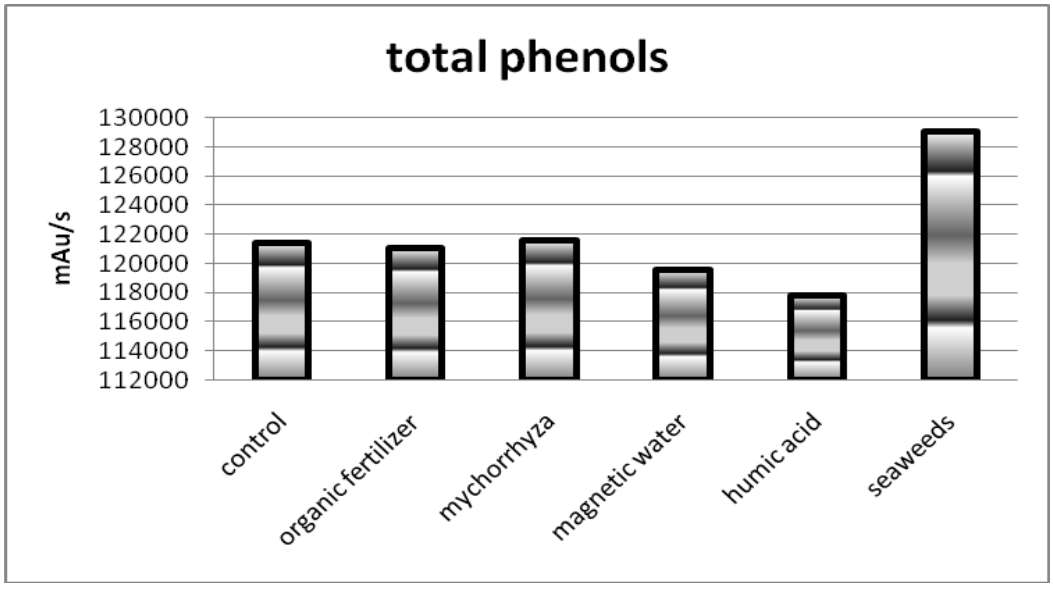

Figure (3): Accumulation of total phenols compounds within individual treated onion plants.

Recently, Macartain *et al*. (2007) proved that seaweeds generally have high concentrations of C, K, Mg, Na, Cu, Fe, I, and Zn. The presence of these value nutrient elements within seaweeds (marine Algae) make them excellent organic fertilizers and could explain the positive influence of the tested seaweeds on onion fresh and dry weights. Additionally, seaweeds algae, as bio/stimulators agents may increase RWC, antioxidant enzymes *i.e.* peroxidase and total phenols as well as increasing cinamic acid concentrations. Moreover increases in onion leaves, neck, bulb and total plants fresh and dry weight was demonstrated in present study as well (Bhattacharya *et. al*., 2013).

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التأثيرات الفسيولوجية والبيوكيميائية لبعض المعاملات الآمنة علي نباتات البصل

عبد الرازق ميدان ، محمد عبد الفتاح فتح الله ، سالي عبد الرازق ميدان ، آلاء كمال النمر

قسم البساتين – كلية الزراعة – جامعة المنوفية

الملخص العربى

تم دراسة تأثير خمس أنواع مختلفة من المعاملات الغير تقليدية وهى (الماء الممغنط - حمض الهيوميك – مستخلص الأعشاب البحرية – فطريات الميكروهيزا – السماد العضوى) بالإضافة إلى الكميات الموصى بها من الأسمدة التقليدية الموصى بها من وزارة الزراعة (كنترول) على الصفات الخضرية والإنتاجية المختلفة لنباتات البصل فى موسمين متتاليين فى مزرعة كلية الزراعة جامعة المنوفية. كما تم دراسة تأثيرات هذه المعاملات المختلفة المستخدمة على إنتاج بعض المركبات الكيمائية الحيوية داخل النبات مثل الفينولالت المرتبطة, انزيم بيرواكسيداز , ومادة السينامك حيث تعرف أهمية ودور هذه المركبات المختلفة وارتباطها بالعديد من الوظائف الحيوية المختلفة للنبات وتأثيرها على زيادة الإنتاجية. وقد أظهرت النتائج ان أفضل المعاملات التى ادت الى زيادة القياسات الخضرية والثمرية المستخدمة كانت معاملة الميكروهيزا يليها الماء الممغنط بينما كان اقل المعاملات تاثيرا هو السماد العضوى يليه السماد التقليدى. كما أظهرت نتائج التحليل الكميائى أن معاملة النباتات بفطريات الميكروهيزا او بمستخلصات الطحالب البحرية أدت الى إنتاج كميات اكبر من انزيم البيروكسيداز مقارنة بالمعاملات الأخرى. أظهرت معاملات الميكروهيزا والطحالب البحرية تفوقا على المعاملات الأخرى من حيث كمية وإنتاج المركبات الفينولية تحت ظروف الدراسة.