

INHANCEMENT OF SOME CITRUS TREES TOLERANCE TO GASEOUS POLLUTANTS AND HEAVY METALS BY USING DIFFERENT ANTIOXIDANTS AND EFFECTIVE MICROORGANISMS

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ABSTRACT

The pattern of changes in reducing the total amount of both Pb and Cd in the leaves and fruits of Valencia orange and Balady mandarin were apparent .Also, the combined treatments V.C,V.E. and EM produced a synergistic influence on the reduction of Pb and Cd inside the leaves tissue and fruits . It is interesting to mention that the combination of the treatments used induced a marked decrease in the toxic elements in the leaves and fruits peel and their pulp ,below the values maintained under single treatment through the both seasons of the study .A decrease in both Cd and Pb in leaves and fruits ,attribute to the effect of different treatments applied. Also ,significant increase in the concentrations of chlorophyll a,b and total chlorophyll were detected in both cultivars. Moreover , stimulatory effects of the treatments on total number of fruits ,fruit weight and yield per tree of Valencia orange and Balady mandarin were recorded .

INTRODUCTION

In recent past ,air pollutants are ,responsible for vegetation injury and crop yield losses (Joshi and swami ,2007) .Therefore, air pollution is one of the most significant environmental concerns in both developed and un developing countries. The urban air quality is continuously affected by emissions from both stationary and mobile combustion sources. Mobile sources also contribute to the emission of major urban air pollutants including: carbon monoxide (COx),nitrogen oxides (NOx), sulphur oxides (SOx), particulate matter (PM), lead (Pb), photochemical oxidants such as ozone (O₃) and ozone precursors like hydrocarbons and volatile organic compounds(Costa, 2001). Various physical, chemical and dynamic processes may generate air pollutants including particulates and gaseous contaminants that may cause adverse health effects on human or animals, affect plant life and impact the global environment by changing the atmosphere of the earth (Raabe, 1999; Bakand *et al.*, 2005; Hayes *et al.*, 2007). While plants can improve the air quality in some extent, air pollution may adversely influence the plant life. Air pollutants such as ozone may inter into plant tissues via stomata and elevate the level of reactive oxygen species (ROS) causing serious damage to the DNA, proteins and lipids (Sharma and Davis, 1997; Hippeli and Elstner, 1996). Also, It is well known that plant cells have several antioxidative defence mechanisms such as tocopherol, carotenoids, glutathione and glutathione reductase enzymes, superoxide dismutase, catalase, ascorbate peroxidase, polyphenol oxidase and particularly peroxidase to protect plants against these oxidative stresses (Kangasjarvi et

al., 1994; Pell *et al.*, 1997; Noctor and Foyer, 1998; Sanderman *et al.*, 1998).

However, the activation of antioxidative defence mechanisms requires a high consumption of energy which may consequently inhibit the plant growth, heavy metals as lead, cadmium, manganese and copper are important elements for plants but when increase over the critical levels become environmental pollutants and toxicants, particularly in areas with high anthropogenic pressure (critical levels :Pb10-300 ppm ,Cd 5 -30ppm ,Mn300-1500 ppm ,Cu 20-60 ppm in plant and soil (Kapata *et al.*,1992,Alloway,1995and Zengin *et al.*,2005). Antioxidants is safe to human and environmental and use successfully to control some plant diseases , (Prusky,1988). The antioxidants play an important role in protecting the cell from senescence as well as enhancing the formation of organic foods .Also, they prevent producing free radicals during plant metabolism from oxidation of lipids, the components of plasma membrane which is an accompanied with the loss of membrane permeability and the death of cell, (Raskin,1992) . The following study was carried out to alleviate the adverse impact of environmental pollution by using antioxidants and other effective microorganisms.

MATERIALS AND METHODS

This study was carried out during the two successive seasons of 2011 /2012 and 2012/ 2013 on 20-year-old of citrus trees; Valencia orange and Balady mandarin on sour orange rootstock . Trees, subjected to the citrus orchard management as recommended by the Ministry of Agriculture. Trees were arranged in a randomized complete block design with 3 replicates. The data were statistically analyzed according to Snedecor and Cochran, (1980).

The experimental procedurs:-

This experiment included five treatments as follows :- -

- | | |
|--|-------------------------|
| 1- control treatments (spray only with tap water) | 2- Vitamin C (1000 ppm) |
| 3- Vitamin E (1000 ppm) | 4- EM (10ml/l) |
| 5- Mixture of (V.C.,V.E.,EM) | |

Solutions was sprayed on trees five times on the season,(At blooming stage ,At fruit set stage ,At pre maturity stage ,At ripening stage ,At pre harvest)

- Monitoring of air pollutants:-

Monitoring of ozone (O₃), sulphur dioxide (SO₂) and nitrogen dioxide concentrations were also determined in the air at different spots during the whole period of the study at the site of experimentation. Also, total suspended particulate matter (TSP) were also determined in the air for 24 hrs every week. Mobile laboratory (Environmental S.A) was used to determine NO₂ using Ac 31M–LCD (R) (Chemiluminescent nitrogen oxide Analyzer) to monitor nitrogen dioxide NO₂ in low concentration of ambient air. The principle of operation based on emit light at the presence highly oxidizing ozone. The measurement was indicated by a liquid crystals displays on the front panel. SO₂was determined by using AF 21 M – LCD (R) (UV fluorescent

sulfur dioxide Analyzer) used for monitoring low concentration of SO₂ at ambient air

High volume Model (HVC – 1000 N) was used for analysis of TSP (20 – 50 um in diameter) depending on wind speed and direction sampler flow rate ; the type of filter used is glass filter

Calculation of T.S.P concentration

The T.S.P is calculated from the following equation :

$$\text{T.S.P} = \frac{(\text{wf} - \text{wi}) \times 10^6}{V}$$

Where:-

TSP = mass con of total suspended particulate matter

Wi = initial weight of clean filter, per g.

Wf = final weight of exposed filter, per g

10⁶ = conversion factor of g to mg.

The data were also calculated as µg/ m³ of the air for 24 hrs.

-Preparation and determination of heavy metals in leaves, peels and pulps of fruits:-

Samples were carried out as previously mentioned in the published work of EL-Baz and Aboryia,2008, mature leaves of non-fruited branches of Valencia orange and Balady mandarin located at the middle of the branches. were collected in November; washed several time with redistilled water to remove dust, then dried at 65-70 °c for 3 days till reaching constant dry weight, finely ground and 0.2 gram of dry material of each sample was wet digested in digestion flask by conc. nitric acid and perchloric acid and heated on hot plate to obtain a gentle refluxing action (Fick *et al.*, 1979) lead and cadmium were determined using the atomic absorption spectrophotometer, (Perkin Elmer 5000). Results were expressed as milligram per 100 gram of dry weigh of leaves. Fruits of both Valencia orange and Balady mandarin as peel and pulp were treated with the same procedure, Shallari *et al.*, 1998 and Shendeh, 1992).

- Determination of chlorophyll a , b and total chlorophyll contents :- (mg/100g) (FW)

Freeze-dried samples were added to 5 ml DMF (dimethylformamide) . The suspension was sonicated for 15 min at 4 °C and then stored at 4 °C for 16 hours to allow the DMF (dimethylformamide) to leach the pigments from the sample. Finally, 1 ml of the supernatant was centrifuged for 5 min at 16000 rpm and 4C to remove any suspended material and the clarified supernatant was then analyzed by spectrophotometer on 662 nm (E 662) and on 650 nm (E 650). E 662:- The spectrophotometer's result at 662 nm (nanometer) when the sample treats with DMF (dimethylformamide) .E 650:- The spectrophotometer's result at 650 nm (nanometer) when the sample treats with DMF . (Porra., 2002) .

- Fruit yield and fruit quality :

At harvest time when the fruits attained their maturation (At December for Balady mandarin, At April for Valencia orange) the mature fruits were counted as a number per tree, and then a representative sample was weighted to determine the average fruit weight and the yield per tree.

RESULTS AND DISCUSSION

1- Monitoring of O₃, SO₂ and NO₂ in the ambient air :

In recent years, it has become evident that pollution in rural areas of Egypt are dominated by pollutants such as O₃, SO₂ and NO₂ which are emitted in excessive levels, especially at some areas adjacent to the main roads and industrial areas. The increase in the ambient O₃ level in urban and even in rural areas has been primarily due to the presence of " photochemical smog " produced through the increased industrial activities or the emission of volatile organic compounds (VOCs) from citrus trees. The orchard of the Faculty of Agriculture, Mansoura University represents polluted area near to Mansoura – Cairo highway from the western direction of the orchard.

Data in table (1) and illustrated in Fig. (1) showed that O₃ in ambient air, also SO₂ and NO₂ concentrations ranged from 290-682, 400-695 and 320-670 µg / m³ during the period of monitoring on Sep. 2012-2013 respectively .The concentration of pollutants increased in the summer months, and that may be due to the emission of high density traffic or the emission of VOCs from the trees and the burning of rice straw.

Generally, the concentrations of gases at experiment location appear to be higher than the maximum permissible limits of the environmental protection standards law (4 / 1994) that modified by the law (9/ 2009); 120 for O₃, 125 for SO₂ and 150 µg / m³ for NO₂ at exposure duration for 24 hrs.

Consequently, it can be observed that the rate of increase of gaseous pollutants were apparently higher as compared to the earlier study of the same authors at the same site (EL-Baz and Aboryia., 2008)

Table (1): Monitoring of O₃, SO₂ and NO₂ in ambient air for experiment site during 2011/2012– 2012/2013 seasons.

Month	O ₃ (µg/m ³)	SO ₂ (µg/m ³)	NO ₂ (µg/m ³)
Sep	680.2	690.2	670.8
Oct	690.0	691.0	667.7
Nov	584.0	586.5	485.0
Dec	493.2	432.3	300.3
Jan	292.7	400.0	390.0
Feb	298.8	425.3	320.5
Mar	290.1	487.3	332.6
April	399.2	423.2	415.7
May	506.7	511.5	460.0
Jun	550.2	518.0	665.7
July	580.0	682.8	667.3
Aug	695.0	694.5	650.0
Sep	682.9	695.3	575.7
Mean	518.6	506.7	507.7

2- Monitoring of (TSP) total suspended matter, heavy metals Pb and Cd in the air :

From the data presented in table (2) it can be observed that TSP levels at the site of experiment , were ranged from 260, to 403 $\mu\text{g} / \text{m}^3$ air. TSP in the air at the site of experiment was also increased during the summer months. In connection with the present data, it was reported that average day of TSP matter approximately 25 $\mu\text{g} / \text{m}^3$ is considered to be a good air quality, but 69 $\mu\text{g}/\text{m}^3$ is considered to be moderate air quality and 255 $\mu\text{g}/\text{m}^3$ is unhealthful air quality. Also, heavy loads of particles can also result in reducing light transmission to the chloroplast and the occlusion of stomata, decreasing the efficiency of gaseous exchange (Beckett *et al.*, 1998). There is already extensive evidence on the health effects of air pollution , i.e. exposure to particulate matter can contribute to cancer (Bernard *et al.*, 2001). It can be also observed that the rate of increase of TSP were doubled at the same site if compared to the data of previous study of the same authors at the same site (EL-Baz and Aboryia., 2008) .The data tabulated in table (2) also showed an increase of Pb and Cd concentrations in the dust collected in the filter of Pb ranged at the site of experiment from 1.60 to 7.48 $\mu\text{g} / \text{m}^3$ air,. Pb concentration also tend to increase at the summer months. The data of Pb and Cd concentrations in the air tend to be proportional with the increase of total suspended matter recorded. Hosie *et al.* (1978) found that Cd was significantly affected the growing crops especially adjacent to high ways. It can be observed that continues increase in heavy metals estimated at the site of experiment to Pb and Cd in the ambient air from 2004 to 2012 (EL-Baz and Aboryia., 2008).

Table (2) : Monitoring of dust and heavy metals in ambient air at experiment site during 2012/2013-2012/2013 seasons.

Month	TSP ($\mu\text{g}/\text{m}^3$)	Pb ($\mu\text{g}/\text{m}^3$)	Cd ($\mu\text{g}/\text{m}^3$)
Sep	403.9	7.32	0.092
Oct	400.0	6.953	0.083
Nov	295.0	3.667	0.044
Dec	296.0	2.456	0.034
Jan	290.0	1.603	0.030
Feb	260.0	1.76	0.033
Mar	262.5	1.236	0.039
April	273.2	3.587	0.052
May	383.2	5.411	0.050
Jun	390.0	6.344	0.065
July	400.0	7.225	0.066
Aug	410.1	7.482	0.098
Sep	402.8	7.681	0.097
Mean	343.0	4.825	0.060

3- Reducing Pb and Cd concentrations as a toxic elements in the leaves and fruits of both Valencia orange and Balady mandarin by using different antioxidants and EM:

Data presented in tables (3,4 and5) showed the effects of vitamin C (V.C) ,vitamin E (V.E) and Effective microorganisms (EM) foliar sprays on the pattern of changes in reducing the concentration of both Pb and Cd in the leaves and fruits of both Valencia orange and Balady mandarin were apparent .Also, the combined treatment produced a significant influence on the reduction of both Pb and Cd concentrations .In connection to other studies, Zengin and Munzuroglu (2005) found significant increases of many antioxidant in their concentration ;proplin ,retinal , α -tocopherol and ascorbic acid were detected after ten -days exposure to heavy metal (Pb,Cd). Demirevska - Kepora *et al.*(2006) reported that the content of oxidized ascorbate increased during Cd exposure in *Hordeum vulgare* plants .The data showed a clear decreasing in both Pb and Cd content in the leaves and fruits.. Zengin and Munzuroglu (2005) found an increase of some heavy metals concentrations such as Pb and Cd which may be increased and induced some antioxidants as ascorbic acid , α -tocopherol contents in the primary leaves of bean seedlings .Also ,they mentioned that total chlorophyll content declined progressively with increasing concentrations of heavy metal .Detailed studies indicate that heavy metals have effects on chlorophyll content in plants .Heavy metals are known to interfere with chlorophyll synthesis either through direct inhibition of an enzymatic step or by inducing deficiency of an essential nutrient needed the absences of some metals. (Joshi and Swami,2009; Agbaire,P.O., 2009; Honour 2009 and Iglesias *et al* .,2006 Joshi and Swami,2007;)

Table (3): Effect of treatments on reducing Concentrations of Pb and Cd in the leaves of Valencia orange and Balady mandarin (on dry weight basis).

Site	Species	Treatments	Pb (ppm)		Cd (ppm)	
			2012	2013	2012	2013
ELGamaa	Valencia orange	Tap water	13.282	13.859	8.405	8.759
		EM(10ml/l)	7.448	6.887	4.830	3.705
		V.C(1000ppm)	3.356	2.858	1.615	1.081
		V.E(1000ppm)	4.893	5.662	2.965	3.011
		EM,V.C,V.E 10ml/l 1000ppm,1000ppm	3.053	2.826	1.026	1.013
	Balady mandarin	Tap water	13.916	13.774	9.143	9.248
		EM(10ml/l)	8.863	7.822	3.568	3.480
		V.C(1000ppm)	3.696	2.722	1.132	1.218
		V.E(1000ppm)	5.697	6.334	2.877	3.851
		EM,V.C,V.E 10ml/l 1000ppm,1000ppm	3.773	2.754	0.808	0.944
		L.S.D at 5% CULTIVER:	0.4078	0.9903	0.5245	0.5126
		L.S.D at 5% TREATMENT:	0.9420	1.4480	0.7383	0.7889
L.S.D at 5% INTERACTION:	1.0922	2.1118	0.9674	1.1195		

Table (4) : Effect of treatments on reducing concentrations of Pb and Cd in the peel of Valencia orange and Balady mandarin fruit (on dry weight basis).

Site	Species	Treatments	Pb (ppm)		Cd (ppm)		
			2012	2013	2012	2013	
ELGamaa	Valencia orange	Tap water	10.175	10.305	5.665	6.125	
		EM(10ml/l)	7.843	7.601	3.775	3.093	
		V.C(1000ppm)	4.726	4.979	1.101	0.861	
		V.E(1000ppm)	5.729	5.606	4.132	3.001	
		EM,V.C,V.E 10ml/l 1000ppm,1000ppm	1.890	1.882	0.913	0.229	
	Balady mandarin	Tap water	10.381	10.598	6.156	6.012	
		EM(10ml/l)	7.602	7.293	3.545	3.398	
		V.C(1000ppm)	2.358	2.192	0.496	0.610	
		V.E(1000ppm)	5.733	5.096	2.750	2.827	
		EM,V.C,V.E 10ml/l 1000ppm,1000ppm	2.158	2.223	0.369	0.300	
	L.S.D at 5% CULTIVER:			0.4844	0.4468	0.5098	0.2528
	L.S.D at 5% TREATMENT:			0.6906	0.5041	0.5408	0.3553
L.S.D at 5% INTERACTION:			1.0572	0.8675	0.8471	0.5281	

Table (5) : Effect of treatments on reducing Concentration of Pb and Cd in the pulp of Valencia orange and Balady mandarin fruits (on dry weight basis).

Site	Species	Treatments	Pb (ppm)		Cd (ppm)		
			2012	2013	2012	2013	
ELGamaa	Valencia orange	Tap water	6.221	6.665	3.576	3.374	
		EM(10ml/l)	4.623	4.554	2.321	2.310	
		V.C(1000ppm)	2.481	2.153	0.504	0.545	
		V.E(1000ppm)	3.768	3.744	1.959	1.965	
		EM,V.C,V.E 10ml/l 1000ppm,1000ppm	2.067	2.025	0.561	0.180	
	Balady mandarin	Tap water	6.927	6.950	4.328	3.976	
		EM(10ml/l)	3.737	3.711	2.207	2.438	
		V.C(1000ppm)	2.043	1.275	0.113	0.264	
		V.E(1000ppm)	3.072	3.161	1.584	1.458	
		EM,V.C,V.E 10ml/l 1000ppm,1000ppm	2.051	1.431	0.045	0.291	
	L.S.D at 5% CULTIVER:			0.3722	0.3325	0.3462	0.3516
	L.S.D at 5% TREATMENT:			0.5728	0.4845	0.3890	0.3345
L.S.D at 5% INTERACTION:			0.6301	0.6151	0.5477	0.5908	

4- Effect of treatments (EM, V.C, V.E) on chlorophyll (a) , chlorophyll (b) and total chlorophyll content mg/g fresh weight of Valencia orange and Balady mandarin leaves.

Data presented in table (6) showed that a significant increase in the concentrations of chlorophyll a ,b and total chlorophyll in both seasons and cultivars studied (Valencia orange and Balady mandarin) under the effect of applied treatments (EM,V.C,V.E) . The increase of antioxidant activity due to the treatments applied which may be increased stress tolerance of trees of Valencia orange and Balady mandarin to pollutant occurred and also protect plant cell from oxidative damage via scavenging of ROS (Gill and Tuteja,2010). The obtained results can be also supported by the findings of (Joshi and Swami,2007; Joshi and Swami,2009; Agbaire,P.O., 2009; Honour2009 and Iglesias *et al* .,2006) .

Zengin and Munzuroglu (2005) reported that in their study the increase of some heavy metals concentrations such as Pb and Cd may be increase and induced some antioxidant ; ascorbic acid , α -tocopherol content in primary leaves of bean seedling .Also ,they mentioned that chlorophyll a,b and total chlorophyll content declined progressively with increasing concentration of heavy metals .

Table (6) : Effect of treatments on chlorophyll (a), chlorophyll (b)and total chlorophyll mg/g fresh weight of Valencia orange and Balady mandarin.

Site	species	Treatments	Chlorophyll a mg/g		Chlorophyll b mg/g		Total chlorophyll mg/g	
			2012	2013	2012	2013	2012	2013
ELGamaa	Valencia orange	Tap water	18.83	18.70	31.76	31.87	50.60	50.58
		EM(10ml/l)	20.15	20.16	32.71	32.65	52.87	52.97
		V.C(1000ppm)	19.30	19.46	32.67	32.80	51.97	52.27
		V.E(1000ppm)	19.34	19.80	32.66	32.34	52.00	52.14
		EM,V.C,V.E 10ml/l 1000ppm,1000ppm	20.21	20.31	33.34	33.69	53.56	53.85
	Balady mandarin	Tap water	19.37	19.31	31.67	31.82	51.05	51.13
		EM(10ml/l)	19.68	19.79	33.62	33.64	53.30	53.44
		V.C(1000ppm)	20.14	20.22	33.83	34.06	53.97	54.28
		V.E(1000ppm)	20.06	19.88	33.86	33.80	53.92	53.69
		EM,V.C,V.E 10ml/l 1000ppm,1000ppm	20.43	20.55	33.90	33.85	54.33	54.41
L.S.D at 5% CULTIVER:			0.280	0.302	0.390	0.478	0.484	0.488
L.S.D at 5% TREATMENT:			0.200	0.195	0.514	0.504	0.602	0.557
L.S.D at 5% INTERACTION:			0.232	0.335	0.698	0.765	0.749	0.793

5 - Effect of treatments(EM,V.C,V.E) on fruit yield and their quality of Valencia orange and Balady mandarin :

From the data presented in table (8) it is evident that both Valencia trees and mandarin trees which sprayed with the applied treatments increased their yield per tree during both seasons of the study , where applications significantly increased the total numbers of fruits per tree at harvest time . The highest yield were recorded from the sprayed trees with combined treatment of (EM,V.C,V.E).This reflect the different antioxidant effect on productivity of citrus trees .Generally (Thompson and Taylor, 1969 ; Olszyk *et al.*, 1990). who mentioned that ozone or sulfur dioxide affect fruit yield and quality and tree growth, where yield 31 % lower with half–ambient oxidants and 29 % lower with sulfur dioxide compared to filtered air. Also, they showed that number of fruit per tree was reduced by ambient oxidants and SO₂. Individual fruit weight was reduced by ambient oxidants. Reduced yield and fruit quality caused by O₃ was first observed in different parts, data of citrus trees have shown that ambient oxidants reduced yield 17 % to 27 % (Thompson and Taylor, 1969, 1967). The obtained data were in agreement with those reported by Paschoal *et al.* ,(1995), Paschoal *et al.* ,(1996)and Adilson *et al.* , (1996) on the effect of EM on the yield of orange trees also Samra *et al.* ,2012 on the effect of V.C on Balady mandarin .

Table (7) : Effect of treatments on number of fruits/ tree, fruit weight and yield per tree of Valencia orange and Balady mandarin .

Sites	Species	Treatments	No. of fruits / tree		Fruit weight (g)		Yield (kg / tree)		
			2012	2013	2012	2013	2012	2013	
ELGamaa	Valencia orange	Tap water	193.7	183.7	130.4	130.1	25.25	23.88	
		EM(10ml/l)	366.3	367.3	160.2	160.2	58.67	58.82	
		V.C(1000ppm)	335.7	331.7	169.6	171.4	56.92	56.82	
		V.E(1000ppm)	308.3	309.3	170.3	170.3	52.50	52.67	
		EM,V.C,V.E 10ml/l,1000ppm,1000ppm	376.3	377.3	157.9	157.0	59.43	59.25	
	Balady mandarin	Tap water	404.3	251.7	112.6	125.7	45.27	31.52	
		EM(10ml/l)	634.3	371.3	106.2	121.9	67.34	45.28	
		V.C(1000ppm)	565.3	346.7	108.5	128.2	61.35	44.42	
		V.E(1000ppm)	592.7	349.3	104.9	130.4	62.19	45.55	
		EM,V.C,V.E 10ml/l,1000ppm,1000ppm	670.3	366.7	108.3	131.7	72.61	48.28	
	L.S.D at 5% CULTIVER:			25.89	21.95	9.73	8.31	3.423	4.808
	L.S.D at 5% TREATMENT:			30.43	32.10	3.13	2.07	2.476	3.760
	L.S.D at 5% INTERACTION:			43.75	40.68	3.81	3.32	3.813	4.825

CONCLUSION

It could be concluded that ,reduction of the concentrations of heavy metals ,which can be toxic to citrus trees and the mankind have been achieved through foliar sprays of different antioxidants ,non - enzymatic such as vitamin C and E and use of EM sprays at different time . An increase

in physiological parameters and stimulatory action can be occur . Consequently ,increase in both yield of fruits and their quality attributes have been occurred.

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زيادة مقاومة بعض اشجار الموالح للملوثات الغازية والعناصر الثقيلة باستخدام
مضادات الأكسدة المختلفة وبعض الكائنات الحية الدقيقة
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أجريت هذه الدراسة بمزرعة كلية الزراعة جامعة المنصورة خلال موسمين متتاليين لعامي ٢٠١١-٢٠١٢ و ٢٠١٢-٢٠١٣ على أشجار موالح عمرها حوالي ٢٠ سنة لصنفى البرتقال الفالانشيا واليوسفى البلدى مطعومين على نارنج وتخضع هذه الأشجار للمعاملات الموصى بها للموالم وتتضمن كل معاملة ثلاث مكررات تبعا للتصميم القطاعات الكامل العشوائية . وحيث ان أشجار برتقال الفالانشيا واليوسفى البلدى تتعرض باستمرار إلى ملوثات الهواء مثل ثاني أكسيد الكبريت وثاني أكسيد النيتروجين والأوزون بالإضافة إلى المعادن الثقيلة مثل الرصاص والكاديوم والأترية العالقة والتي تنبعث من احتراق وقود السيارات المارة على كوبرى المنصورة العلوى وطريق القاهرة دمياط السريع، وذلك بالإضافة إلى محطة كهرباء ظلخا ومصانع الطوب المقابلة لهذا البستان . لذلك تم اجراء هذه الدراسة لتقليل الأثر الضار لملوثات الهواء مثل اكاسيد الكبريت واكاسيد النيتروجين والأوزون على اشجار كل من البرتقال الفالانشيا واليوسفى البلدى وذلك باستخدام بعض مضادات الاكسدة مثل فيتامين C وفيتامين E والكائنات الحية الدقيقة (EM) كما يهدف البحث إلى تقليل تركيز العناصر الثقيلة مثل الرصاص والكاديوم داخل كل من أوراق وثمار اشجار الموالم وزيادة الإنتاج .

تم رش اشجار البرتقال الفالانشيا واليوسفى البلدى باستخدام مضادات الأكسدة فيتامين C وفيتامين E بتركيز ١٠٠٠ جزء فى المليون (مجم / لتر) والكائنات الحية الدقيقة (EM) بتركيز ١٠ملى /لتر كما تم الرش بمخاليط بنفس التركيزات وتم الرش خمسة مرات فى كل موسم (مرحلة التزهير - مرحلة العقد - مرحلة اكتمال النمو - مرحلة النضج- مرحلة قبل الجمع) .

- اعطت جميع المعاملات تقوفا معنوياً فى خفض تركيز العناصر الثقيلة (الرصاص - الكاديوم) فى اوراق وثمار كل من البرتقال الفالانشيا واليوسفى البلدى بالمقارنة بالكنترول ، ولم تسجل اى فروق معنوية بين المعاملات الفردية من فيتامين C وفيتامين E والكائنات الحية الدقيقة (EM) ولكن المعاملة باستخدام فيتامين C وفيتامين E والكائنات الحية الدقيقة (EM) كخليط اعطت تقوفا معنوياً فى خفض تركيزات العناصر الثقيلة عن المقارنة .

- اعطت جميع المعاملات تقوفا معنوياً فى زيادة محتوى الأوراق من كلوروفيل a وكلوروفيل b والكلوروفيل الكلى ولم تسجل اى فروق معنوية بين المعاملات الفردية من فيتامين C وفيتامين E والكائنات الحية الدقيقة (EM) ولكن المعاملة باستخدام فيتامين C وفيتامين E والكائنات الحية الدقيقة (EM) كخليط اعطت تقوفا معنوياً فى زيادة محتوى الكلوروفيل بالأوراق .

- اعطت جميع المعاملات تقوفا معنوياً فى زيادة المحصول فى كل من البرتقال الفالانشيا واليوسفى البلدى . وخلصت الدراسة إلى ان رش البرتقال الفالانشيا واليوسفى البلدى باستخدام مضادات الأكسدة فيتامين C وفيتامين E وايضا استخدام الكائنات الحية الدقيقة (EM) كان لة تأثيراً جوهرياً فى خفض الأثر الضار لملوثات الهواء و تركيزات العناصر الثقيلة داخل انسجة النبات وايضا تحسين الصفات المورفولوجية والفسيوولوجية تحت ظروف التلوث حيث ان لمضادات الأكسدة دور فعال فى تخلص النبات من الشقوق أو الروابط الحرة المتكونة داخل خلايا النبات نتيجة للتلوث .