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THE IMPACT OF SOME BIOREGULATORS ON GROWTH, CHEMICAL CHARACTERS AND RADICAL SCAVENGING PROPERTIES OF ONION VOLATILE OIL

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The influence of different concentrations of some bioregulators by foliar application {methyl jasmonate (MJ) and Prohexadione- Ca (Pro-Ca)} on growth, pigment content and volatile oil composition and radical scavenging activity of onion plant were accomplished during two consecutive seasons. The obtained results indicated that foliar application of MJ (at 150mg/l) or Pro-Ca at (30 ppm), significantly promoted onion growth and quality as height, branches number and leaves weights for new and dry onion leaves, crisp and dry loads of branches and yield (g/plant). On the other hand, MJ application led to noteworthy decrease in the plant height at all the used treatments. A significant increase in mineral ions content of the produced onion seeds by foliar application of MJ and Pro-Ca at all the used levels. All the usages of bioregulators produced positive modifications in the photosynthetic pigments as well as yield and oil content of onion plants. Based on the finding, it is recommended the use of MJ and Pro-Ca twice, at the rate of 100 and 20 ppm, respectively for higher onion yield /acre (AC). Moreover, GC/MS analysis shown that the chief identified constituents of volatile oil of onion treated with MJ and Pro-Ca were 4-Mercaptononan-2-ol, 3-Mercaptononyl acetate, Diallyl thiosulfinate, 4-Mercaptononan-2-one, Diallyl trisulphide, 2-Heptyl thiophene, Methyl propyl trisulphide, 1,3-Propanedithiol, 3,5-Diethyl-1,2,4 trithiolane and 3,5-Methyl ethyl-1,2,4,trithiolane. MJ and Pro-Ca usages caused qualitative alterations in these components of onion volatile oil. In addition, the bioregulators used caused significant variation in the free radical scavenging effect measured by DPPH and B- carotene assays of onion volatile oil compared to TBHQ.
Keywords: Bioregulators, methyl jasmonate, prohexadione-Ca, *Allium cepa* L., DPPH

INTRODUCTION

Onion plants (*Allium cepa* L.) are one the utmost substantial harvests grown-up in Egypt and might be considered the principal cultured crops in the world owing to their extended storing time and convenience. Production of good quality onion is an important goal by the growers for local market and exports. Many biological activities of Alliums were valued to possess like phenolic and potent sulfur compounds, which provoke unlimited interest (Benkeblia, 2004, 2005; Packia *et al.*, 2015; Aslam *et al*., 2017). From inception, onion has been used as a food as well as medicine (Martinez *et al.,* 2007). Traditionally, onions were used to prevent infections as they contain many valuable compounds exerted anti-inflammatory, antihistamine effects, anti-parasitic, antifungal, antibacterial (including *H. pylori*) (Wani and Nisa, 2011; Kirilov *et al*., 2014; Lekshmi *et al*., 2015). According to FAO, tomato followed by onion in terms of yearly world production, improved varieties and crop production techniques project to raise economic yields of this crop. Application of bioregulators for onion cultivation is an easy and popular means for improving the yield, oil yield quality and quantity (Shafeek *et al*., 2015).

Current horticulture pursues substitutes to increase the plant quality palatability like biotechnology, crop controlling systems and the biological regulators. Growth regulators are considered the fundamental elements in growth, flowering, fruit setting and seed production in vegetative plants as well as onion and are well examined. Lokhande and Gaikwad (2014) stated that the foliar applications of GA, SA, 6BA, CCC, methionine and cysteine on the two onion varieties significantly increased the chlorophyll content, slightly altered the chlorophyll a/b ratio, decreased carotenoid and total carbohydrate contents, while total sugars were slightly elevated. Numerous classes of plant growth regulators disturb physiological activities of onion plants. Ebrahim and Abu-Grab (1997) found that CCC application increased potassium (K), nitrogen (N) and phosphorus (P) concentrations in onion bulbs. In addition, Amin *et al.* (2007) reported that application of different rates of salicylic acid and indole -3-butyric acid on onion improved growth, yield and chemical components.

Prohexadione calcium (Pro-Ca) inhibited gibberellins biosynthesis (A1, A4, and A7) by blocking the 3, β hydroxylation of GA_{20} to GA_1) and enhanced fruits quality in apple, pear and cherry trees under biotic and abiotic stress situations (Rademacher, 2000). Sharma *et al.* (2005) established that the application of prohexadione-Ca improved cucumber seedlings growth. Little is acknowledged upon the Pro-Ca influence on planting crop. It has been proposed that Pro-Ca may contribute in secondary metabolite pathways related to antioxidant status in palatable fruits, in addition to adapting the enzyme system activity (Ramirez *et al*., 2006). The second applied growth regulator is methyl-jasmonate (MJ) which influences on abundant developmental and physiological processes exaggerated by ABA water deficit stress, maturing, defense and may occupy as intermediates in signaling pathway of ABA (Wang *et al.*, 2003). Plant jasmonates were produced in reaction to various biotic and abiotic stresses, figuring up in the injured plant parts (Flecher, 2005). It appears to elaborate in countless plants morphogenic events like tuberization, tuberous root development, bulb establishment (onion and garlic). Also, it has been informed that MJ makes terpenoids in sweet basil (Deschamps and Simon, 2006) and metabolites like aliphatic alcohols as methyl chavicol (Deschamps *et al*., 2008). However, despite the powerful effect of MJ and Pro-Ca on initiation of numerous plant secondary metabolites; up to our knowledge, the effect of MJ and Pro-Ca on secondary metabolites as well as on the growth and productivity of onion plant has not been investigated.

In this study, onion was cultivated as one of the supreme promising aromatic and medicinal plants in Egypt and the effects of foliar application of some bioregulators (MJ and Pro- Ca) were evaluated on growth, productivity, mineral ions content and volatile oil composition. This study can provide useful information for improvement of cultivated conditions of onion bulb plants and investigation on the special effects of MJ as well Pro-Ca on the volatile oil yield and composition under certain land conditions in Egypt.

MATERIALS AND METHODS

This trial was conducted in El-Gharbia Governate, Middle Delta, Egypt (Mahalla El-Kobra region) for two winter seasons(2010/2011and2011/2012) in clay loamy soil. Onion seeds (cv. Giza red) were cultivated in the garden centeron1 November in both seasons. Sprouts were transplanted to the trial field at 15cm spacing using a randomized complete block design (RCBD) with four replications. After 30 and 45 days from transplanting, plants were sown in plots (3.5 × 3.0 m area) with five rows 60 cm apart and sprayed twice with freshly prepared MJ and Pro-Ca bioregulators solutions. Other recommended agronomic practices were applied (Agricultural Research Center, Ministry of Agriculture, Giza, Egypt).

***Bioregulators*:** Methyl jasmonate (MJ) was used in plant defense ($C_{13}H_{20}O_3$) with MW224.3g/mol). Growth retardant Prohexadione-Ca (Sigma-Aldrich) was used on plants. Pro-Ca wet table powder with 82.5% activity has chemical assembly of calcium salt [3,5 dioxo- 4 propionylcyclohexan-carboxylic acid], ($C_{10}H_{10}CaO_5$) (250.26 g/mol), water solubility 96.60% at 20°C. Based on the active ingredient, different amounts of MJ and Prohexadione-Ca were calculated and applied (0, 50,100 and 150 ppm and 0, 10, 20 and 30 ppm, respectively).

***Sampling*:** After 15 days from the second application of MJ and Prohexadione–Ca, onion plants were collected and grouped as follows:

***Set 1: For vegetative growth parameters*:** These included plant height (cm), sum of leaves/plant, fresh and dry weight/plant (g), bulb length (cm), bulb diameter (cm) and bulb weight(g). Photosynthetic pigments were also calculated using young leaves of the plants.

***Set 2: For minerals content*:** Leaves content of N, P, K and S was recorded.

***Set 3: For onion volatile oil*:** The fresh onion plants were weighed and subjected to hydro distillation.

***Chemical analysis photosynthetic pigments*:** Determination of photosynthetic pigments (Chl. 'a', Chl. 'b' and carotenoids) was made in homogenized fresh young onion leaves (0.5 g) in acetone (85%) (Metzziner *et al.*, 1965). Samples were red at 663, 664 and 452.5 nm, respectively.

***Mineral ions content*:** Determination of mineral ions contents of N, P, K and S in onion dry leaves powder (0.5 g) was made according to Page (1982).

***Extraction of onion volatile oil*:** Volatile onion oil extraction was carried out by the liquid-liquid continuous steam distillation (Lee and Shibamoto, 2001). Fresh onion (300 g/L deionized water) was steam-distilled for 4 h. Liquid-liquid continuous extraction of onion volatile oil was made by dichloromethane extraction for 6 h and then dried over anhydrous sodium sulfate. The solvent was removed by a rotary flash evaporator (BUCHI 461 Switzerland) followed by nitrogen streaming (reduced to 0.5 mL).

***Investigation of volatile oils*:** The identity of volatile compounds in onion oils were examined by association with the KI, mass spectral, NIST AMDIS (version 2.1 software) and published KI data (16- 20). Gas chromatograph (Hewlett-Packard 6890) had a DB- 5 capillary column (30 m × 0.25 mm i.d. with df= 0.25 um) (Agilent, CA) and flame ionization detector. The injector and detector temperatures (200 and 250°C, respectively), the oven temperature (35 to 220°C at 3°C/min and held for 40 min) and the helium carrier gas (29 cm/s injections in the split- less mode) were the other conditions. GC/MS (HP 6890) interfaced to an HP 5791 was used for identification of GC components mass spectral (MS ionization voltage of 70 eV. A 30m × 0.25 mm i.d. (df= 0.25 um) and fortified with a DB wax bonded- phase fused- silica capillary column (Agilent, Folsom, CA). The helium carrier gas linear velocity was 30 cm/s, injector and the detector(250°C) and the oven temperature (35 to 180 at 3°C/ min and held for 40 min).

***Antioxidant activity*:**

***1,2- diphenyl picrylhydrazyl (DPPH) radical scavenging assay*:** The onion volatile oils antioxidant activity was inspected by quantifying the bleaching of DPPH purple-colored ethanol solution. The effect of onion volatile oil extracted from onion plants treated by foliar application of different bioregulators (MJ and Pro-Ca) on the DPPH radical were valued according to Hatano *et al.* (1988). Different concentrations of onion volatile oils (2mL) was mixed with one mL from a 0.5 mM DPPH radical DPPH ethanol solution. Then, added 2 ml of sodium acetate (0.1 M) buffer (pH 5.5). The mixtures were well shaken and kept at room temperature for 30 min (dark). The absorbance was measured at 517 nm. Ethanol and tertiary butyl hydroxyl quinone (TBHQ) were used (negative and positive controls, respectively). The reduction in DPPH radicals (I%) results are the average of triplicate analyses (El-Massry *et al.*, 2002).

***-Carotene bleaching assay*:** The onion volatile oil antioxidant activity was examined by assessing the volatile compound reticence's and the conjugated diene hydroperoxides originate from linoleic acid oxidation using-carotene bleaching assay (Siddhuraju and Becker, 2003) with slight alterations.

***Statistical analysis*:** Statistical analysis was accomplished by following Steel *et al*. (1997) and Snedecor and Cochran (1994). Each year was separately analyzed and then combined analysis across the two years was performed if homogeneity according to Bartlet test was insignificant. Results are presented as mean ±SE. ANOVA is followed by Tukey multiple comparisons using IBM SPSS Statistics for Windows (version 22) (Armonk, NY: IBM Corp). Differences were deliberated to be statistically significant when $p < 0.05$.

RESULTS

Results presented in Table 1 show that an apparent association seems to exist between bioregulators and onion traits. Plant tallness, sum of leaves plant^{-1}, fresh and dry weights plant^{-1}, bulb length, diameter and weight, and onion yield / AC were noteworthy affected by MJ and Pro-Ca. In addition, onion yield and all its traits were significantly affected by concentration of bioregulators.

The decrease in plant height was observed as concentration of MJ and Pro-Ca increased up to 150 and 30 ppm, respectively. On the other side, the results revealed that foliar application of MJ 100 and 150 ppm, as well as, Pro-Ca 20 and 30 ppm concentrations directed to substantial increase in the sum of leaves plant^{-1}, fresh and dry weight plant^{-1}, bulb length, diameter and weight. The maximum values were attained from applying MJ150 or Pro-Ca30 ppm as compared with that achieved from other treatments in all traits except, bulb dimension and bulb diameter.

Chlorophyll "a" and "b" and total carotenoids as affected by the used bioregulators are presented in Table 2. Mineral ions contents of N, P, K and S were used as indicator for onion plant nutritional status. The results show that the application of methyl jasmonate or prohexadione-Ca at the rate of 100 and 150 ppm or 20 and 30 ppm, respectively, increased the chlorophyll "a" and total carotenoids contents of onion green leaves as associated with that has gotten from the untreated plants.

Table 1. Effect of different concentrations of Methyl Jasmonate (MJ) and Prohexadione-Ca (Pro-Ca) on onion yield and vegetative growth traits (combined data of the two seasons 2010/2011 and 2011/2012).

Treatment	Trait							
	Plant height (cm)	**No. of leaves Plant^{-1}**	**Fresh wt. plant^{-1} (g)**	**Dry wt. plant^{-1} (g)**	**Bulb length (cm)**	**Bulb diameter (cm)**	**Bulb wt. (g)**	**Yield (ton/acre)**
Control	61.00	7.50	130.50	22.10	4.10	3.0	80.67	10.10
MJ_{50}	60.50	8.90	144.00	24.40	4.32	3.69	86.50	10.50
MJ_{100}	56.70	10.00	156.50	26.48	5.11	3.83	93.00	12.60
MJ_{150}	54.60	12.10	160.00	27.09	5.15	3.85	99.70	12.70
$LSD_{0.05}$	**4.62**	**0.96**	**14.77**	**2.75**	**0.59**	**0.43**	**5.40**	**1.04**
Control	63.20	8.00	132.40	22.80	3.92	3.50	82.10	9.85
$Pro\text{-}Ca_{10}$	60.00	8.10	145.10	24.88	4.99	3.80	88.00	10.00
$Pro\text{-}Ca_{20}$	52.00	9.90	152.00	26.11	5.11	4.10	94.50	12.25
$Pro\text{-}Ca_{30}$	47.80	10.00	157.45	27.27	5.00	4.00	100.05	12.91
$LSD_{0.05}$	**4.11**	**0.92**	**13.25**	**2.55**	**0.48**	**0.27**	**5.97**	**1.02**

Control: no bioregulators; MJ_{50}: Methyl jasmonate at 50 ppm; MJ_{100}: Methyl jasmonate at 100 ppm; MJ_{150}: Methyl jasmonate at 150 ppm; Pro-Ca $_{10}$: Prohexadione-Ca at 10 ppm; Pro-Ca $_{20}$: Prohexadione-Ca at 20 ppm; $Pro\text{-}Ca_{30}$: Prohexadione-Ca at 30 ppm.

Table 2. Photosynthetic pigments and mineral ions content of green leaves of onion plants as influenced by different concentrations of Methyl Jasmonate (MJ) and Prohexadione-ca (Pro-Ca) (combined data of the two seasons 2010/2011 and 2011/2012).

Treatment	Trait							
	Chl. a (mg/gf. wt.)	Chl. b (mg/gf. wt.)	Total chl. a+b (mg/gf. wt.)	Carotenoids (mg/g)	Nitrogen (mg/g)	Phosphorus (mg/g)	Potassium (mg/g)	Sulphur (mg/g)
Control	0.80	0.24	1.04	0.46	28.5	2.63	16.31	1.20
MJ_{50}	0.87	0.30	1.17	0.55	30.5	2.71	17.19	1.28
MJ_{100}	0.90	0.33	1.23	0.58	32.60	2.83	18.86	1.35
MJ_{150}	0.97	0.36	1.33	0.53	32.89	3.01	19.50	1.39
$LSD_{0.05}$	**0.09**	**ns**	**ns**	**0.05**	**3.11**	**0.28**	**1.96**	**0.14**
Control	0.73	0.21	0.94	0.41	3.92	3.5	82.10	1.32
$Pro\text{-}Ca_{10}$	0.79	0.27	1.06	0.46	4.99	3.8	88.00	1.47
$Pro\text{-}Ca_{20}$	0.88	0.31	1.19	0.53	5.11	4.1	94.50	1.60
$Pro\text{-}Ca_{30}$	0.95	0.32	1.27	0.57	5.00	4.0	100.0	1.65
$LSD_{0.05}$	**0.1**	**ns**	**ns**	**0.06**	**0.48**	**0.27**	**5.97**	**0.15**

Control: no bioregulators; MJ_{50}: Methyl jasmonate at 50 ppm; MJ_{100}: Methyl jasmonate at 100 ppm; MJ_{150}: Methyl jasmonate at 150 ppm; Pro-Ca $_{10}$: Prohexadione-Ca at 10 ppm; Pro-Ca $_{20}$: Prohexadione-Ca at 20 ppm; $Pro\text{-}Ca_{30}$: Prohexadione-Ca at 30 ppm; Chl.:Chlorophyll.

Table 3. Chemical compositions of the volatile oils of Egyptian onions treated by different concentration of bioregulators identified by GC-MS (combined across seasons).

Compound Name	kI	MJ 50	MJ 100	MJ 150	Pro-Ca 10	Pro-Ca 20	Pro-Ca 30	Control
THIOLS								
1-Propanethiol	722	4.76	nd	2.79	2.67	8.92	0.4	0.52
1,3-Propanedithiol	859	7.32	7.52	8.73	2.49	6.32	25.7	4.21
1-(Propylthio)1-propanethiol	1083	1.40	nd	0.95	0.45	2.18	2.45	1.60
THIOLANES								
3,5-Diethyl-1,2,4 trithiolane	1283	1.11	1.91	1.17	0.64	1.74	1.45	2.90
3,5-Methyl ethyl-1,2,4-trithiolane	1239	1.05	1.24	nd	0.53	1.14	1.31	0.50
SULPHIDES								
Dimethyl disulfide	747	0.84	nd	nd	Nd	nd	3.67	0.56
Diallyl sulfide	907	0.69	nd	nd	Nd	0.33	nd	nd
Methyl propyl disulfide	912	1.82	5.34	1.68	1.57	2.82	1.30	0.80
Propyl-propenyl trisulphide, cis	1299	0.37	nd	nd	Nd	nd	0.76	nd
1-Propenyl propyl trisulfide (E/Z)	1343	0.80	1.82	0.99	Nd	0.99	nd	1.40
Diallyl trisulfide	1371	8.98	2.74	8.97	3.36	1.72	1.29	7.28
Methyl propyl trisulphide	1114	2.88	1.65	0.72	7.66	0.79	4.03	5.39
Methyl-3,4-dimethyl-2-thienyldisulfide	1515	0.93	1.90	0.76	1.21	0.86	1.20	1.28
Dipropyl tetrasulphide	1526	1.72	nd	1.27	0.55	0.46	0.51	0.54
THIO DERIVATIVES								
Propyl methane thiosulfonate	1157	1.02	nd	1.28	1.43	1.31	5.84	1.87
Diallyl thiosulfinate	1326	1.17	11.76	4.43	10.7	9.2	3.14	9.39
THIA DERIVATIVES								
2,4,5-Trimethyl thiazole	993	0.29	0.40	nd	Nd	0.60	2.55	0.89
2,4,6-Triethyl-1,3,5-trithiane	1563	1.30	1.78	nd	1.64	1.45	1.12	1.70
4-Ethyl-2,3,5,6-tetrathianone	1597	3.04	11.2	7.91	3.69	6.38	7.16	2.35
6-Ethyl-4,5,7-thiadecane	1537	nd	0.47	nd	0.55	0.31	nd	nd
6-Ethyl-4,5,7-trithiaoctane	1384	nd	1.25	0.27	0.66	0.31	nd	nd
THIAZINES								
5,6-Dihydro-2,4,6-triethyl-4H-1,3,5-dithiazine (isome)	1448	4.53	8.01	nd	1.76	1.00	2.99	nd
5,6-Dihydro-2,4,6-triethyl-4H-1,3,5-dithiazine	1474	0.66	nd	nd	0.45	0.16	nd	0.43
THIOPHENE &THIIN								
2- Heptyl thiophene	1671	2.51	6.55	5.96	2.94	5.02	4.15	5.42
3,4-Dihydro-3-vinyl-1,2-dithiin	1190	0.85	nd	0.45	1.87	1.09	1.00	0.75
Mercaptans								
1-Mercaptopentan-3-ol	1722	2.43	1.40	1.88	2.14	1.33	Nd	2.54
4-Mercaptononan-2-one	1800	6.61	4.44	8.51	7.36	3.97	7.00	8.02

Compound Name	kI	MJ 50	MJ 100	MJ 150	Pro-Ca 10	Pro-Ca 20	Pro-Ca 30	Control
3-Mercaptoheptan-1-ol	1959	2.68	1.13	4.53	1.06	nd	nd	2.46
4-Mercaptononan-2-ol	1978	15.99	nd	15.2	16.4	17.4	14.1	16.04
3-Mercaptononyl acetate	1998	8.9	17.66	15.9	10.9	5.79	nd	12.93
MICELLANEOUS								
2-Methyl-2-pentanal	806	nd	1.31	0.7	Nd	1.32	4.53	nd
4-Methoxy benzoic acid	1490	1.82	nd	nd	Nd	0.22	nd	nd
Decanal	1498	nd	1.56	nd	Nd	0.8	nd	0.46
Tetradecan-1-ol	1676	4.68	nd	1.23	5.46	nd	nd	nd
(Z)-Tetradecanal	1604	3.7	nd	1.88	5.87	1.96	nd	5.77
Dodecyl acetate	1607	1.08	1.62	0.53	2.06	0.69	1.04	nd
Tetradecanal	1616	nd	0.57	0.54	0.67	0.24	nd	0.37
Propyl dodecanoate	1628	0.84	1.85	1.29	0.7	2.48	1.46	1.51
Chemical Classes								
Total thiols		**13.48**	7.52	12.47	5.61	17.42	**28.55**	6.33
Total sulphides		19.03	13.45	14.39	14.35	9.41	12.76	17.25
Total thio derivatives		2.19	11.76	5.71	12.13	10.51	8.98	11.26
Total thia derivatives		4.63	15.11	8.18	6.54	9.05	10.83	4.94
Total thiazines		**5.19**	**8.01**	0.00	2.21	1.16	2.99	**0.43**
Total thiophenes &thins		3.36	6.55	6.41	4.81	6.11	5.15	6.17
Total miscellaneous		12.12	5.6	5.47	**14.76**	6.39	2.5	**8.11**
Total mercaptans		36.61	24.63	**46.02**	37.86	28.49	21.1	**41.99**
Total thiolanes		2.16	3.15	1.17	1.17	2.88	2.76	3.4

KI: confirmed by comparison with Kovat's index on DB5 column (Adams, 1995); MJ_{50}: Methyl jasmonate at 50 ppm; MJ_{100}: Methyl jasmonate at 100 ppm; MJ_{150}: Methyl jasmonate at 150 ppm; Pro-Ca_{10}: Prohexadione-Ca at 10 ppm; Pro-Ca_{20}: Prohexadione-Ca at 20 ppm; Pro-Ca_{30}: Prohexadione-Ca at 30 ppm; Control: no bioregulators.

Table 4. Mean squares analysis of variance for main chemical classes of the volatile oil of Egyptian onion treated by different concentrations of bioregulators identified by GC-MS.

ANOVA*						
Volatile oils main groups	**Source of variation**	**Sum of Squares**	**df**	**Mean Square**	**F**	**Sig.**
Total thiols	Between Groups	1172.883	6	195.480	522.216	0.000
	Within Groups	5.241	14	0.374		
	Total	1178.124	20			
Total sulphides	Between Groups	174.152	6	29.025	38.194	0.000
	Within Groups	10.639	14	0.760		
	Total	184.791	20			
Total thio derivatives	Between Groups	245.918	6	40.986	110.513	0.000
	Within Groups	5.192	14	0.371		
	Total	251.110	20			
Total thia derivatives	Between Groups	244.231	6	40.705	481.067	0.000
	Within Groups	1.185	14	0.085		
	Total	245.416	20			
Total thiophenes & thiins	Between Groups	148.096	6	24.683	7447.332	0.000
	Within Groups	.046	14	0.003		
	Total	148.142	20			
Total thiazines	Between Groups	23.788	6	3.965	20.642	0.000
	Within Groups	2.689	14	0.192		
	Total	26.477	20			
Total miscellaneous	Between Groups	322.589	6	53.765	324.975	0.000
	Within Groups	2.316	14	0.165		
	Total	324.905	20			
Total mercaptans	Between Groups	1543.073	6	257.179	312.083	0.000
	Within Groups	11.537	14	0.824		
	Total	1554.610	20			
Total thiolanes	Between Groups	15.013	6	2.502	40.319	0.000
	Within Groups	.869	14	0.062		
	Total	15.881	20			

*****One way analysis of variance (ANOVA) was conducted and included the main effects of treatment.

Table 5. Chemical compositions of the volatile oils main groups in Egyptian onions treated by different concentration of bioregulators identified by GC-MS (combined across seasons).

Compound Name	Thiols	Sulphides	Thio derivatives	Thia derivatives	Thiazines	Thiophenes & thins	Miscellaneous	Mercaptans	Thiolanes
Control	6.33±0.26e	17.25±0.15a	11.26±0.48a	4.94±0.04f	0.43±0.02f	6.17±0.14ab	8.11±0.8c	41.99±0.61b	3.40±0.23a
MJ_{50}	13.48±0.29c	19.03±0.31a	2.19±0.06d	4.63±0.14f	5.19±0.03b	3.36±0.17d	12.12±0.44b	36.61±0.36c	2.16±0.05b
MJ_{100}	7.52±0.54d	13.45±0.58b	11.76±0.69a	15.14±0.16a	8.01±0.01a	6.55±0.27a	5.60±0.23d	24.63±0.70e	3.15±0.29a
MJ_{150}	12.47±0.40c	14.39±0.11b	5.71±0.87c	8.18±0.08d	0.00±0.00g	6.41±0.08a	5.47±0.28d	46.02±0.09a	1.17±0.02c
$Pro\text{-}Ca_{10}$	5.61±0.11e	14.35±0.50b	12.13±0.32a	6.54±0.31e	2.21±0.07d	4.81±0.34c	14.76±0.14a	37.86±0.57c	1.17±0.01c
$Pro\text{-}Ca_{20}$	17.42±0.33b	9.41±0.54c	10.51±0.21ab	9.05±0.03c	1.16±0.03e	6.11±0.44ab	6.39±0.16d	28.49±0.78d	2.88±0.02a
$Pro\text{-}Ca_{30}$	28.55±0.38a	12.76±0.88b	8.98±0.1b	10.83±0.23b	2.99±0.03c	5.15±0.9bc	2.50±0.12e	21.10±0.06f	2.76±0.07ab

Control: No bioregulators; MJ 50 : Methyl jasmonate (50 ppm); MJ 100 : Methyl jasmonate (100 ppm); MJ 150 : Methyl jasmonate (150 ppm); Pro-Ca 10 : Prohexadione-Ca (10 ppm); Pro-Ca 20 : Prohexadione-Ca (20 ppm); Pro-Ca 30 : Prohexadione-Ca (30 ppm). Each value with the same column followed by the same letters is not significantly different at level of 0.01

Table 6. Antioxidant activity using DPPH (1,2- diphenyl picrylhydrazyl) assay of green leaves of onion plants as influenced by spraying different concentration of Methyl Jasmonate (MJ) and Prohexadione-Ca (Pro-Ca) combined across seasons.

Treatment	Sample			
	50 □l	100 □l	200 □l	400 □l
MJ_{50}	39± 2.2	46±2.3	68±2.2	78±3.1
MJ_{100}	32±1.1	60±3.2	71±1.7	74±2.8
MJ_{150}	36±2.3	50±1.4	65±1.9	77±1.6
$Pro\text{-}Ca_{10}$	35±1.7	47±1.6	66±2.2	75±1.4
$Pro\text{-}Ca_{20}$	37±1.2	50±2.2	67±3.1	72±2.2
$Pro\text{-}Ca_{30}$	40±2.3	48±3.5	66±1.5	71±1.7
Control	37±2.5	52±1.8	66±2.1	73±2.3
TBHQ	-	-	86±1.2	-

Control: No bioregulators; MJ 50 : Methyl jasmonate (50 ppm); MJ 100 : Methyl jasmonate (100 ppm); MJ 150 : Methyl jasmonate (150 ppm); Pro-Ca 10 : Prohexadione-Ca (10 ppm); Pro-Ca 20 : Prohexadione-Ca (20 ppm); Pro-Ca 30 : Prohexadione-Ca (30 ppm) ; tert-Butylhydroquinone [TBHQ].Results are presented as mean ±SE.

Table 7. Antioxidant activity using □-Carotene assay content of green leaves of onion plants as influenced by spraying different concentration of Methyl Jasmonate (MJ) and Prohexadione-Ca (Pro-Ca) combined across seasons.

Treatment	Sample			
	50 □l	100□□l	200□□l	400□□l
MJ_{50}	33.2±1.2	49.2±1.3	62.4±3.6	74.35±4.1
MJ_{100}	38.7±1.8	56.2±2.2	69.2±2.8	78.20±3.4
MJ_{150}	40.1±3.1	45.3±1.9	63.2±2.5	74.90±2.1
$Pro\text{-}Ca_{10}$	39.6±2.2	48.5±2.5	67.2±1.6	77.40±1.5
$Pro\text{-}Ca_{20}$	34.3±0.6	53.2±3.3	63.3±2.4	74.24±2.4
$Pro\text{-}Ca_{30}$	40.9±1.3	51.3±3.1	68.4±2.2	77.21±1.6
Control	43.62±0.8	56.9±2.4	64.0±3.6	79.35±0.8
TBHQ	-	-	82.0±1.4	-

Control: No bioregulators; MJ 50 : Methyl jasmonate (50 ppm); MJ 100 : Methyl jasmonate (100 ppm); MJ 150 : Methyl jasmonate (150 ppm); Pro-Ca 10 : Prohexadione-Ca (10 ppm); Pro-Ca 20 : Prohexadione-Ca (20 ppm); Pro-Ca 30 : Prohexadione-Ca (30 ppm) ; tert-Butylhydroquinone [TBHQ].Results are presented as mean ±SE.

The average yield of onion volatile oil (0.66 ± 0.033 gm/ 100 gm fresh weight) varied. GC-MS analyses of the volatile oils obtained from Egyptian onion plant treated by different concentrations of MJ and Pro-Ca directed to the credentials of 39 compounds, demonstrating 96.88% of the whole oil. Onion volatile oil was mainly composed of mercaptans (44.99%), sulphides (18.28%), thio derivatives (11.26%), thiophines and thins (6.17%), thiols (6.33%), thia derivatives (4.94%), thiolanes (3.4%), thiazines (0.43%) and miscellaneous (8.11%) (Table 3, 4, 5).

The major constituents of onion volatile oils which treated by different concentration of bioregulators (MJ and Pro-Ca) were, 4-Mercaptononan-2-ol, 3-Mercaptononyl acetate, Diallyl thiosulfinate, 4-Mercaptononan-2-one, Diallyl trisulphide, Methyl propyl disulphide, Methyl propyl trisulphide, Dipropyl tetrasulphide (Table 1). MJ and Pro-Ca handlings caused significant qualitative variations in these constituents of chemical classes of onion volatile oils (Table 3, 5).

The DPPH radical scavenging results of the Egyptian onion treated by MJ and Pro-Ca volatile oils and compared with TBHQ as control are presented in Table 6. It was found the MJ100 had the highest antioxidant activity.

Antioxidant activity of onion volatile oils using β-carotene assay showed a moderate antioxidant activity (ranged from 33.2 to 69.2% at 200 µl), compared to TBHQ (82% at 200 µl) (Table 7).

DISCUSSION

***Onion yield and vegetative growth traits*:** An apparent association seems to exist between bioregulators and onion behaviors. Plant tallness, sum of leaves plant^{-1}, fresh and dry weights plant^{-1}, bulb length, diameter, and weight and onion yield / AC were noteworthy affected by MJ and Pro-Ca. In addition, onion yield and all its traits were significantly exaggerated by concentration of bioregulators.

A gradual decrease in plant height was recorded as concentration of MJ and Pro-Ca increased up to 150 and 30 ppm, respectively. Such decrease in plant height may be attributed to the anti-gibberellin effect of MJ and Pro-Ca, which inhibited the stem elongation. On the other hand, the results revealed that foliar application of MJ 100 and 150 ppm, as well as, Pro-Ca 20 and 30 ppm concentrations directed to substantial increase in vegetative traits like sum of leaves plant^{-1}, fresh and dry weight plant^{-1}, bulb length, diameter and weight. The maximum values were attained by applying MJ150 or Pro-Ca 30 ppm as compared with that achieved from other treatments in all traits except, bulb dimension and bulb diameter.

However, results reflected irrelevant effect between the concentrations of MJ100 and 150 ppm of the following traits; fresh and dry weight plant^{-1}, bulb length and diameter, and yield / AC. Similarly, between Pro-Ca 20 and 30 ppm for number of leaves plant^{-1}, fresh and dry weight plant^{-1}, bulb length and diameter, and yield / AC. From economic point of view, medium concentration of MJ 100 and Pro-Ca 20 ppm led to not only save cost of foliar application but also gave the highest onion yield and its traits.

Regarding influence of methyl jasmonate or prohexadione-Ca on the onion yield / AC, application of MJ at the level of 100 or 150 ppm and prohexdione-Ca at the level of 20 or 30 ppm led to significant increase in the total yield/ AC as compared with control plants or the lowest concentration. Considerable increase in onion yield / AC may be due to increase in sum of leaves plant^{-1}, fresh and dry weight plant^{-1}, bulb length, bulb diameter and bulb weight.

In the current work, the outcomes obtained from applying MJ are in settlement with those obtained by Talaat and Lieberei (2001) and Wasternack (2007) who used MJ on different onion plants. While the results obtained from the application of growth retardant prohexadione-Ca "antigibberellin" on onion plant are in covenant with that obtained by several investigators (Gent, 1997; Bekheta *et al.*, 2006; Asin *et al.*, 2007; Abd El-Samad *et al*., 2011) who used diverse antigibberellins on different plants.

***Photosynthetic pigments*:** The results show that foliar application of methyl jasmonate or prohexadione-Ca at the rate of 100 and 150 ppm or 20 and 30 ppm, respectively, initiated significant increase in the chlorophyll "a" and total carotenoids contents of onion green leaves as compared to untreated plants. Also, the application of MJ or Pro-Ca led to insignificant increase in the amounts of chlorophyll type (b) and total chlorophyll as matched to the untreated plants.

The outcomes are in covenant with other investigators who found that application of growth regulators on onion plants led to increase in the photosynthetic pigments (Sharinath *et al*., 2007; El Tohamy *et al*., 2014). In addition, Bekheta *et al.* (2006) stated that antigibberellin (uniconazole) growth retardant caused some alterations in beans as for example a surge in chlorophyll intensities and distended chloroplasts. Similarly, Amin *et al.* (2007) testified that application of indole-3-acetic acid and salicylic acid (100 ppm) on onions led to significant increase in the photosynthetic pigments.

***Mineral ions content*:** N, P, K and S contents in onion were used as indicator for onion plant nutritional status (Table 2). Results clearly revealed that spraying onion plants with methyl jasmonate at the rate of 100 or 150 ppm or prohexadione-Ca at the rate of 20 or 30 ppm led to significant increase in the mineral ions content (N, P, K, and S) as compared to untreated plants. The highest values of all minerals were obtained from the application of MJ or Pro-Ca at the level of 100 or 30 ppm, respectively, as compared with other treatments. Use of bioregulators may encourage the role of macronutrients in activation of physiological processes (El-Awadi and Abd El Wahed, 2012). These out comes are in accordance with that recorded by Armengaud et al. (2004) who reported that jasmonic acid has a unique part in nutrient signaling and stress management of variable physiological processes such as nutrient storage, reutilizing and rearrangement. They also proposed that applicant genes elaborate K+ perception and signaling, as well as, a network of molecular processes underlying plant revision to K+ deficit.

***Onion volatile oil*:** The yield of onion treated by different concentrations of MJ and Pro-Ca and its volatile oils analyzed by GC/MS. Thirty nine compounds, demonstrating 96.88% of the whole oil were found (Table 3). Onion volatile oil was mainly composed of mercaptans (44.99%), sulphides (18.28%), thio derivatives (11.26%, thiophines and thins (6.17%), thiols (6.33%), thia derivatives (4.94%), thiolanes (3.4%), thiazines (0.43%) and miscellaneous (8.11%) (Table

4). The relative concentrations of many constituents of onion volatile oil were amplified, diminished or missing in onion plants treated with growth regulators as matched with untreated control plants (El-Shafie and El-Gamaily, 2002).

4-Mercaptononan-2-ol, 3-Mercaptononyl acetate, Diallyl thiosulfinate, 4-Mercaptononan-2-one, Diallyl trisulphide, Methyl propyl disulphide, Methyl propyl trisulphide, Dipropyl tetrasulphide, 2-Heptyl thiophene, 1-Propanethiol, 1,3-Propanedithiol, 3,5-Diethyl-1, 2,4 trithiolane, 3,5-Methyl ethyl-1, 2,4,trithiolane, Diallyl thiosulfinate, 4-Ethyl-2, 3,5,6-tetrathianone, 6-Ethyl-4, 5,7-trithiaoctane and 5,6-Dihydro-2,4,6-triethyl-4H-1,3,5-dithiazine (isomer) were the major constituents of onion volatile oil (table 3). MJ and Pro-Ca handlings caused significant qualitative variations in these constituents of chemical classes of onion volatile oils (Table 4, 5). These results are alike to El- Tohamy *et al.* (2009) and Iqbal *et al*. (2016) who established that the key components of volatile oil in onion plants treated by some micronutrient's are different Sulphur containing compound as 3,4-Dimethylthiophene; Propyl 1-propenydisulphide, cis; Propyl 1-propenyl disulphide, trans; Methylpropyl trisulphide; 3,5-Diethyl- 1,2,4-trithionale; diazines and thiane derivatives.

A significant increase in the content of thiols, others (miscellaneous) and thia derivatives regardless the variation in their contents in onion plants due to the foliar application by MJ and Pro-Ca (6.33% in control for thiols reaching 13.5% and 28.55% for MJ_{50} and $Pro\text{-}Ca_{30}$, respectively; 8.11% for miscellaneous in control and becomes 12.12% and 14.76% for MJ_{50} and $Pro\text{-}Ca_{10}$, respectively and 4.94% for thia compounds in control and reach 8.18% and 10.83% for MJ and Pro-Ca, respectively (Table 3,5). Conversely, a significant decline in mercapatans and thio derivatives was observed (41.99% in control reaching 24.63 and 21.10% for MJ_{100} and $Pro\text{-}Ca_{30}$, respectively and 11.26% in thio derivatives control decreased to 2.19 and 8.98% for MJ_{50} and $Pro\text{-}Ca_{30}$, respectively). The effect of diverse managements on onion volatile oil and its components may be ascribed to its enzymatic activity and metabolic influence of volatile oil assembly (Burbott and Loomis, 1969). MJ and Pro-Ca have a part in governing genes expression that are regulated by these plant regulators in response to biotic and abiotic stresses. Hence, these compounds alter the secondary metabolites and their paths affecting plastid, chlorophyll amount and tolerated ailment stress.

Origin of onion oil components**:** Onion volatile oil comprises a combination of predominately sulphur composites as alk(en)yldisulphides and higher sulphides (Brodnitz *et al.,* 1969). Onions are eaten for their distinctive taste and the imaginary health giving possessions of their flavor compounds containing sulphur; S-alk(en)yl-l-cysteine sulphoxides (ACSOs). Total ACSO is certainly associated with enzymatically formed pyruvate, which is sequentially positively allied with pungency (Chope *et al.* (2006).

Aroma of the *Allium cepa* is owing to alkyl thiosulphonates which are released on freshly cut bulb, while propyl and propenyl sulfides are responsible for cooked onion. Dimethylthiophenes is found in fried onion bulb. Precursors on the *Allium cepa* are S-methyl and S-propyl-L-cysteine sulfoxide which are biosynthesized from valine and cysteine (Michael, 2006). Recent studies have shown that diallyl disulfide and dipropyl disulfide lower the blood glucose and lipid levels in humans and animals (Corozmatine and Villamiel, 2007; Dima *et al*., 2014). S- allyl cysteine sulfoxide (odorless) is converted by allinase (unstable) into diallyl thiosulfinate and finally decomposed to thiosulfonate and a disulfide (decomposition products). Thus, explicating the origins of both allyl propyl disulfide as well as allylpropyltrisulfide (Farkas *et al.*,1992; Rose *et al*., 2005).

Volatile sulphides as methyl di- and tri-sulfides, propyl di- and tri- sulfides and methyl propyl di- and tri- sulfides were identified in volatile extract and in dehydrated powder of onion (Carson and Wong, 1961; Bernhard, 1968). S-methyl cysteine sulfsxide and S-propyl cysteine were splitted by allinase with subsequent formation of saturated di and tri-sulfides which were recognized in onion (Kupiecki and Virtanen, 1960).

Special organoleptic characteristics and lacrimation were observed in onion tissue originating from the enzymes action on *trans* (+)-s-1 -propenyl-L-cysteine-S-oxide (Schwimmer, 1968). The propenyl propyl disulfides are substantial components, while allyl disulfides are only trivial constituents' extract of commercial onion oil (Brodnitz and Pollock, 1968). The derivation of the allyl derivatives is not very clear. Probably, allyl and propenyl sulfides endure revocable isomerization. The absence of mercaptans, alcohols, and carbonyls in the onion oil designates that the profit making procedure engaged in onion oil manufacturing selectively recovers the onion di- and trisulfides. Meaningfully, Carson (1967) and Bernhard (1968) pointed out that the alignment of the di- and trisulfides is definitely the defining element in the Allium family aroma.

Onion aroma compounds biosynthesis undoubtedly involves enough level of sulphur. Generally, in commercially onion products, pungency is influenced by sulphur content; a greater sulphur availability, followed by larger flavor strength (Jones *et al.,* 2004).

Antioxidant activity of onion plants essential oil treated with bioregulators**:** Natural antioxidants in medicinal plants and foods can scavenge free radicals by preventing the radical chain oxidation that destroys cell membranes (Mangas *et al*., 2006).

The stable DPPH free radical has usually established as a mean for valuing the antioxidant activity (Nagai *et al*., 2003). In that assay, the antioxidants were capable to lessen the DPPH radicals into yellow- colored diphenyl pricryhydrazine comprehending that activity to their hydrogen contributing ability (Chen *et al*., 2008). The DPPH radical scavenging results of the Egyptian onion treated by MJ and Pro-Ca volatile oils and compared with TBHQ as control are shown in Table 6. It can be realized that the DPPH radical scavenging activity of onion volatile oils is dose dependent and increased from 32 to 71% (200□l), compare to TBHQ (86% at 200□l). The results pointed out that the onion volatile oil displayed a prospective antioxidant activity.

The onion volatile oil antioxidant activity using β-carotene/linoleic acid method was studied. Results showed moderate antioxidant activity (ranged from 33.2 to 69.2% at 200□l), compared to TBHQ (82% at 200□l) (Table 7). Our results suggested that the volatile oils of onion treated with MJ and Pro-Ca revealed moderate antioxidant activity with a dose dependent manner in the two assays. Data are reliable with aforementioned reports (Stajner *et al*., 2008; Takahashi and Shibamoto, 2008). It was found that the results obtained by DPPH as well as □-carotene assays were dependable and confirm the potent antioxidant activity of onion volatile oils.

Conclusion**:** The present research explored that two bioregulators with different concentrations had a major influence on the onion development, yield, quality and quantity, mineral ions content, volatile oil and its antioxidant activity. It was concluded that bioregulators could be successfully employed for enhancement of onion bulb directly or indirectly, through its components and their antioxidant activity. Based on the findings, the use of MJ and Pro-Ca twice, at the rate of 100 and 20 ppm, respectively, for higher onion yield /AC is recommended.

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