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Response of Chive (*Allium Schoenoprasum* L.) Plant to Natural Fertilizers

Ahmed Mohamed Algharib[1], Ahmed Ebrahim El-Gohary[2], Saber Fayez Hendawy[2], Mohamed Salah Hussein[2]

[1] Environment and Bio-agriculture Department, Faculty of Agriculture, Al-Azhar University, 11884 Nasr City, Cairo, Egypt

[2] Medicinal and Aromatic Plants Department, National Research Centre, 12622 Dokki, Giza, Egypt

* Corresponding author's email: aelghareb@gmail.com

ABSTRACT

A field experiment was carried out at the Experimental Farm of SEKEM Company in Bilbes, Sharqia Governorate, Egypt during the 2019 season, in order to study the effect of adding compost tea and humic acid on the growth and active ingredient of the chive (*Allium schoenoprasum* L.) plant. Chive seeds were sown in nursery in August, two months later seedlings were transplanted to permanent soil. The chive plants received two organic fertilizers: the first one was compost tea (0, 100 and 200 ml/L) as main plot, and the second one was humic acid (0, 2 and 4 g/L) as sub plot. The two fertilizers were sprayed twice, 45 and 75 days after sowing. The results revealed that: the applied compost tea significantly increased the growth and yield characteristics as well as oil percentage and yield, compared with untreated plants. The plants treated with compost tea at 200 ml / L produced the maximum mean values of plant height, fresh and dry weights of herb, as well as essential oil yield. However, humic acid at 4 g /L recorded the highest mean values of plant height, fresh and dry weights of herb. On the other hand, humic acid at 2 g/L gave the highest values of essential oil yield. Moreover, the interaction treatments had a significant effect on all traits under study. Thirty compounds of Chive essential oil (EO) were identified by GC-MS analysis, representing from 65.07 to 93.29% of the total EO. The main compounds found were dipropyl disulfide (12.8–35.4%), dipropyl trisulfide (12.9–30.05%), methyl propyl trisulfide (3.80–9.03%) and 1-propenyl propyl disulfide (1.56–10%). The highest amounts of dipropyl disulfide and1-propenyl propyl disulfide were detected with humic acid at 4 g/L treatment. The treatment of compost tea at 200 ml/L + humic acid at 2 g/L caused the greatest accumulation of dipropyl trisulfide which recorded 30.05%, while the greatest values of methyl propyl trisulfide (9.03%) were recorded as a result of tea compost at 100 ml/L.

Keywords: Chive, organic fertilizers, humic acid, compost tea.

INTRODUCTION

Chives (*Allium schoenoprasum* L.) is a perennial plant [Singh et al., 2018], which was recently introduced to the Egyptian market. It belongs to the *Alliaceae* family and the *Allium* genus, which contains many important plants such as garlic, leeks, shallots, and onion [Yoldas et al., 2019]. Because it is has medicinal and edible functions [Jiang-xue et al., 2018], it is widely cultivated around the world. The plant is distinguished in taste and rich in sulfur compounds that can be used for its antimicrobial properties [Krishnan and Nair, 2016]. Ghasemian et al., [2018], found that; the *A. schoenoprasum* extracts exhibited higher activity against *S. aureus* and *B. cereus* strains. Moreover, numerous studies indicate that the plant has antioxidant properties [Lim, 2015]. In a study conducted in 2017, it was reported that; a chives plant could be used as an antioxidant [Bezmaternykh et al., 2017]. Another study, confirmed that; the *A. schoenoprasum* L. leaves can be considered as a potential antioxidant [Pesantes et al., 2019]. Moreover, chives are a nutrient-dense food, which contains thiamin, zinc and phosphorus; they are a very good source of fibers, vitamins, and minerals such as: Ca, Cu, Mn, Fe and K [Ghasemian et al., 2018]. The plant also, contains phenolic compounds, so there is a possibility of using the plant, especially flowers, as anti-proliferative and tumor arresting effects [Kucekova et al., 2011]. Parvu et al. [2014] reported that the *A. schoenoprasum* leaves extracts exert anti-inflammatory activities by inhibiting phagocytosis through the reduction of nitro-oxidative stress. Because of the side effects of pesticides and the extensive use of chemical fertilizers, many problems occur, such as environmental pollution, pest resistance development and food safety decline [Ye et al., 2020]. Therefore, there is an urgent need to use natural materials to control insects and fertilize plants. Organic fertilizers are one of these natural materials. The organic fertilizers improve soil fertility, physical properties viz. declining sodicity, reducing bulk density, water infiltration rate, increasing porosity and aeration, improving saline water leaching and chemical properties, that is, decreasing acidity [Singh et al., 2020]. Moreover, organic fertilizers contain many nutrients necessary to plants, in addition to raising the organic matter content of soil, thus improving its physical and chemical properties, which positively affects the yield and improves crop quality [Dongmei et al., 201]). Ramos-González et al. [2019]; found that chives grow better with vermi-compost fertilizer than with the other treatments. Therefore, the objective of this study was to evaluate the effect of organic fertilizers (compost tea and humic acid) on the yield and essential oil contents of chives (*Allium schoenoprasum* L.).

MATERIALS AND METHODS:

This experiment was carried out at the Experimental Farm of SEKEM Company in Bilbes, Sharqia Governorate, Egypt, in 2019, in order to study the effect of adding different organic fertilizers on the growth and active ingredient of a chive plant. The seeds were purchased from the Pharmaceutical Company, Germany. The seeds were planted in the nursery

Table 1. The chemical and physical characteristics of study soil

A - Mechanical analysis			
Sand %	Silt %	Clay %	Texture
77.5	16.25	6.25	Loamy Sand

B - Soluble cations and anions (meq 100 gm^{-1} soil)							
Anions				Cations			
SO4	Cl	HCO_3	CO_3	K	Na	Mg	Ca
…	1	…	…..	0.24	0.34	0.72	2.06

C - Chemical properties*						
$CaCO_3$ %	C/N Ratio	N %	O.M %	O.C %	EC (dSm-1)**	pH (1: 2.5)
7.26	1.67	0.39	1.12	0.65	0.65	7.21

* Soil suspension; Soil: water (1: 2.5)
** dSm-1 (Siemens per meter)

Table 2. The physio-chemical properties and microbial population of organic compost tea

Bacterial Plate Count (CFU/ml)	7×10^7	EC ds / m	0.923
Bacterial Direct Count (Cell / ml)	6.4×10^8	PH	6.56
Spore Forming Bacteria (CFU / ml)	7×10^4	Mineral nitrogen ppm	249
Total Fungi (CFU / ml)	2.8×10^5	Available phosphorus ppm	7.3
		Available potassium ppm	201
		Ca (ppm)	88
		Mg (ppm)	115
		Fe (ppm)	66
		Zn (ppm)	7.33

Table 3. The physio-chemical properties humic acid

Chemical data	
Humic acid	80%
Potassium (K_2O)	10 – 12%
Zn, Fe, Mn, etc…	100 ppm
Physical data	
Appearance	Black powder
pH	9 – 10%
Water solubility	98%

in August 2019 and after two months of sowing the seedlings were transferred to the open field. The soil was analyzed and its chemical and physical characteristics are shown in Table 1. The experiment was statistically designed according to the split plot design with three replicates. The plot size was 2×2 m, containing 4 rows; the distance between hills was 25 and 50 cm apart. After 45 days of planting the seedlings, were thinned to 3 seedlings per hill.

The chive plants were fertilized with two types of organic fertilizers: the first type compost tea (0, 100 and 200 ml/L) as main plot, which was obtained from Fertility Lab. Sekem Academy for Science, and its physio-chemical properties and microbial population are shown in Table 2, while the second type of fertilizer was humic acid as sub plot at rates (0, 2 and 4 g/L), which was purchased from Leili Agrochemistry Co., LTD, China, and its properties are shown in Table 3. The two organic fertilizers were added by foliar spray to plants after 45 and 60 days of sowing.

Measurements

Vegetative growth characters

The following growth attributes were measured after harvesting, using ten random plants from each plot: plant height (the height from the base of the stem to the growth point, cm), Herb fresh weight (g), Herb dry weight (g), and yield of Herb (kg/ plot).

Determination of essential oil percentage

The essential oil% was determined according to Buitrago Díaz et al. [2011] by distilling fresh leaves (960 g) and roots (1050 g) from each replicate using a Clevenger-type apparatus for 4 hours. The oil obtained was dried over anhydrous sodium sulfate and stored at 4 °C.

Gas chromatography

The GC analysis was performed in the Central Laboratory, National Research Center, Cairo, Egypt, according to Buitrago Díaz et al. [2011]. The GC analysis of the oil was carried out using a Hewlett Packard Model 5985 apparatus equipped with flame ionization detector and a 60 m x 0.32 mm i.d. fused silica column coated with Carbowax. The oven temperature was programmed from 60 °C to 260 °C at a rate of 4 °C/min. Helium was used as a carrier gas at a flow rate of 1.0 ml./min. The sample was injected using a split ratio of 1:100. Retention indices were calculated relative to C8-C24 n-alkanes, and compared with the values reported in the literature.

Gas chromatography-Mass spectrometry

The GC/MS analysis was conducted on a Hewlett-Packard apparatus according to Buitrago Díaz et al. [2011]. Ionized voltage was 70ev and the ion source temperature was 200 °C. Other parameters were as under GC conditions. The components were identified using the Wiley MS data library (6th ed), followed by comparisons of MS data with published data.

Statistical analyses

The field data were statistically analyzed using the MSTAT program Version 2.1. Least significant differences (LSD) at the level of 5% were considered.

RESULTS AND DISCUSSION

Effect of compost tea:

Effect on growth and yield characters

The data tabulated in Table 4 and illustrated in Figure 1, clearly exhibit the effects of three compost tea levels (0, 100, 200 ml/l on

Table 4. Effect of compost tea on the growth and yield of a chive (*Allium schoenoprasum* L.) plant

Compost tea levels	Plant height (cm)	Herb fresh weight (g/plant)	Herb dry weight (g/plant)
Control (0 ml/L)	34.7[C]	36.9[b]	6.3[b]
100 ml/L	39.3[b]	41.3[a]	6.8[a]
200 ml/L	41.4[a]	41.4[a]	6.9[a]
LSD at 5%	1.05	1.0	0.5

the vegetative growth characteristics i.e. plant height, herb fresh and dry weight. All the applied compost tea levels significantly increased the plant height compared with untreated plants. The compost tea at 200 ml/l gave the tallest plant (41.4 cm) compared to 34.7 cm in the control plants. It is clear from the same table and figure that the unfertilized level (control) gave lower herb fresh and dry weights (36.9 and 6.3 g/plant) than other treatments.

The differences between control and compost tea levels were significant while there are no significant differences between two levels of compost tea. Compost tea at 200 ml/l gave the maximum mean values of herb fresh and dry weights, which recorded 41.4 and 6.9 g/plant, respectively. The promotion effect of compost tea on the growth and yield characteristics of chive plants may be due to the microbial function and chelated micronutrients content that provide mineral and biological nutrition. These results were in agreement with those obtained by Kim et al., [2015], who found that compost tea could be used as liquid nutrient fertilizer with active microorganisms for culture of variable crops under organic farming condition.

Effect on essential oil percentage and yield

All the applied compost tea levels increased essential oil% and essential oil yield (ml/fed. or ml/ha). Compost tea level at 200 ml/l caused a significant increment in essential oil percentage (0.05%) compared with control (0.03%). It is obvious from the same table that compost tea level at 200 ml/l gave the highest values of 109.8 ml/fed., and 274.4 ml/ha. Compost tea level at 100 ml/l came in the following rank, which gave 95.5 ml/fed., and 238.7 ml/ha. Similar increases were obtained on Salvia officinalis [El-Haddad et al., 2020]. The increment in oil yield may be due to either increase in vegetative growth or changes in the leaf oil gland population and monoterpenes biosynthesis.

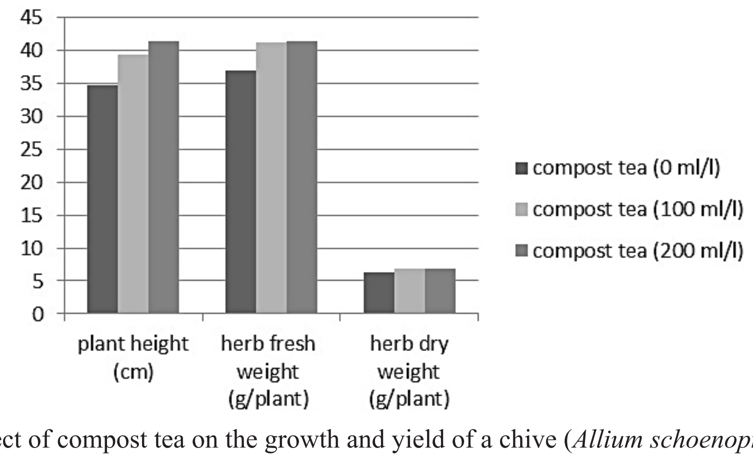

Figure 1. Effect of compost tea on the growth and yield of a chive (*Allium schoenoprasum* L.) plant

Table 5. Effect of compost tea levels on the essential oil yield of a chive (*Allium schoenoprasum* L.) plant

Compost tea levels	EO %	EO (ml / feddan)	EO (ml / hectare)
Control (0 ml/L)	0.03[b]	54.9[c]	137.3[c]
100 ml/L	0.04[ab]	95.5[b]	238.7 [b]
200 ml/L	0.05[a]	109.8[a]	274.4[a]
LSD at 5%	0.01	1.2	3.5

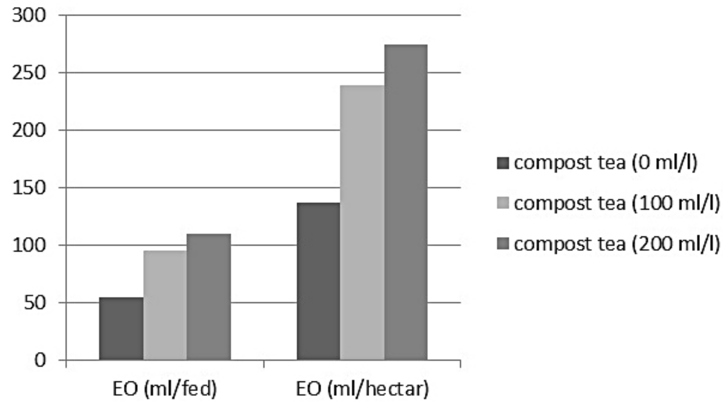

Figure 2. Effect of compost tea on the essential oil yield of a chive (*Allium schoenoprasum* L.) plant

Effect of Humic Acid

Effect on growth and yield characters

The results presented in Table 6 and Figure 3 show that all applied humic acid levels significantly increased the growth and yield of chive plant. Humic acid at 4 g/l gave the highest mean values of plant height, herb fresh weight as well as herb dry weight which recorded 43 cm, 42.9 g/ plant and 7.4 g/plant, respectively. In this respect, Yousef et al. [2011] concluded that humic acid had positive effects on olive seedlings. Another study conducted in 2011 on potato plants showed that humic acid had positive effects on plant growth and on all yield quantitative characters [Sarhan, 2011]. The positive effects of humic acid may be due to increasing the cation exchange capacity of the soil, reducing soil pH, enhancing the root development, increasing the root/shoot ratio, and production of root hairs which increase the active uptake for most of the nutrients in the soil [Abd El-Razek et al., 2020].

Effect on essential oil percentage and yield

The results of the effect of foliar spraying with humic acid on the essential oil percentage and yield of the chive (*Allium schoenoprasum* L.) plant were presented in Table 7 and Figure 4. It is clear that the humic acid levels had no significant

Table 6. Effect of humic acid levels on the growth and yield of a chive (*Allium schoenoprasum* L.) plant

Humic acid levels	Plant height (cm)	Herb fresh weight (g/plant)	Herb dry weight (g / plant)
Control (0 g/L)	33.5[c]	36.0[c]	5.6[c]
2 g / L	38.7[b]	40.6 [b]	6.9[b]
4 g / L	43.0[a]	42.9[a]	7.4[a]
LSD at 5%	1.1	1.0	0.5

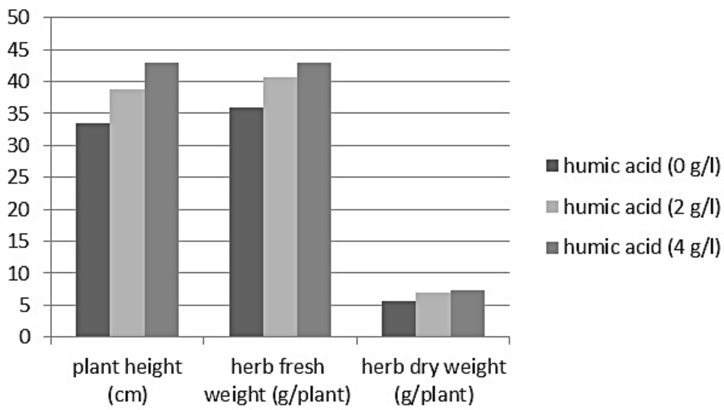

Figure 3. Effect of humic acid levels on the growth and yield of a chive (*Allium schoenoprasum* L.) plant

Table 7. Effect of humic acid levels on the essential oil of a chive (*Allium schoenoprasum* L.) plant

Humic acid levels	EO %	EO (ml / feddan)	EO (ml / hectare)
Control (0 g/L)	0.04[a]	68.16[b]	170.4[b]
2 g / L	0.04[a]	96.5[a]	241.3[a]
4 g / L	0.04[a]	95.5[a]	238.7[a]
LSD at 5%	NS	1.2	3.5

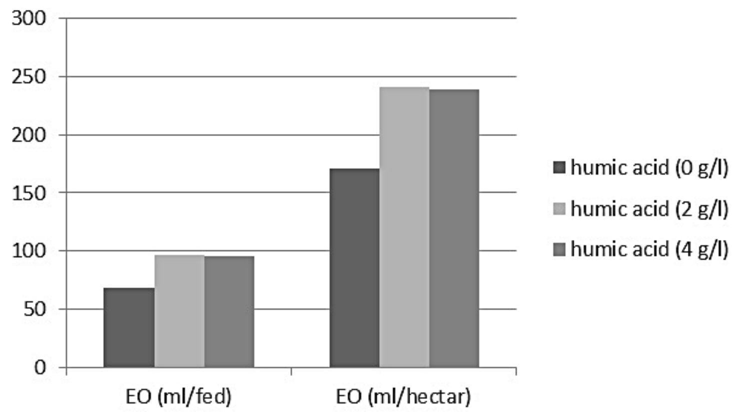

Figure 4. Effect of humic acid levels on the essential oil yield of a chive (*Allium schoenoprasum* L.) plant

Table 8. Effect of combination treatments between compost tea and humic acid on the growth and yield of a chive (*Allium schoenoprasum* L.) plant

Compost tea levels	Humic acid levels	Plant height (cm)	Herb fresh weight (g/plant)	Herb dry weight (g/plant)
0 ml / L	0	29.3[e]	29.3[c]	4.9[d]
	2 g / L	34.5[d]	38.9[b]	6.7 [abc]
	4 g / L	40.2[c]	42.5[a]	7.2[ab]
100 ml / L	0	34.6[d]	39.2[b]	5.9[c]
	2 g / L	39.2[c]	41.1[ab]	7.1[abc]
	4 g / L	44.0[ab]	43.5[a]	7.5[a]
200 ml / L	0	36.7[d]	39.4[b]	6.1[bc]
	2 g / L	42.5[b]	41.9[a]	7.0[abc]
	4 g / L	44.9[a]	42.8[a]	7.6[a]
LSD at 5 %		1.8	1.7	0.5

Table 9. Effect of combination treatments between compost tea and humic acid on the essential oil percentage and oil yield of a chive (*Allium schoenoprasum* L.) plant

Compost tea levels	Humic acid levels	Essential oil %	Essential oil yield	
			ml/ Fed.	ml/ ha.
0 ml / L	0	0.02[c]	31.36[h]	78.4[h]
	2 g / L	0.03[c]	64.32[g]	160.8[g]
	4 g / L	0.03[bc]	69.12[f]	172.8[f]
100 ml / L	0	0.04[abc]	75.52[e]	188.8[e]
	2 g / L	0.04[abc]	90.88[d]	227.2[d]
	4 g / L	0.05[ab]	120.0[b]	300.0[b]
200 ml / L	0	0.05[ab]	97.6[c]	244.0[c]
	2 g / L	0.06[a]	134.4[a]	336.0[a]
	4 g / L	0.04[abc]	97.28[c]	243.2[c]
LSD at 5 %		0.01	2.10	6.0

Table 10. Effect of combination treatments between compost tea and humic acid on the essential oil constituents of a chive (*Allium schoenoprasum* L.) plant

Compost tea Levels (ml/L)	0			100			200			Mean
Humic acid levels (g / L)	0	2	4	0	2	4	0	2	4	
Propanal diethyl acetal	0.10	0.09	1.10	1.02	0.08	0.98	1.11	0.89	1.01	0.71
1,3-Propanedithiol	0.10	0.12	0.13	0.12	0.00	0.00	0.11	0.10	0.20	0.10
Dimethyl thiophene	0.15	0.14	0.17	0.12	0.16	0.00	0.09	0.12	0.00	0.11
Dimethyl thiophene	0.26	0.30	0.28	0.22	0.32	0.29	0.21	0.23	0.23	0.26
Methyl propyl disulfide	3.55	1.59	4.1	2.30	3.10	2.55	3.66	2.55	2.97	2.78
Methyl 1-propenyl disulfide	0.95	0.89	0.90	0.78	0.81	0.89	0.83	0.67	0.59	0.81
Dimethyl trisulfide	0.65	0.71	0.63	0.75	0.67	0.81	0.68	0.71	0.69	0.70
2-Pentylfuran	0.14	0.09	0.18	0.08	0.05	0.15	0.13	0.00	0.00	0.09
Allyl propyl disulfide	0.34	0.41	0.39	0.43	0.51	0.33	0.43	0.42	0.31	0.40
Dipropyl disulfide	17.49	12.8	35.4	29.01	31.08	26.99	30.22	25.55	31.46	26.67
1-Propenyl propyl disulfide	4.84	9.05	10.0	8.02	7.90	5.32	1.56	7.44	5.66	6.64
Allyl methyl trisulfide	0.27	0.31	0.24	0.27	0.31	0.19	0.48	0.32	0.51	0.32
Methyl propyl trisulfide	5.47	3.8	5.50	9.03	8.55	9.00	6.99	6.89	5.83	6.78
Methyl 1-propenyl trisulfide	0.36	0.23	0.51	0.33	0.61	0.43	0.37	0.39	0.41	0.40
Methyl-1-(methylthio)ethyl-disulfide	0.51	0.42	0.31	0.52	0.43	0.45	0.49	0.51	0.49	0.46
Borneol	0.85	0.83	0.42	0.54	0.79	0.65	0.81	0.74	0.76	0.71
Dimethyl tetrasulfide	0.39	0.42	0.38	0.53	0.31	0.00	0.21	0.34	0.23	0.31
Methyl 1-(methylthiopropyl) Disulfide	0.20	0.00	0.00	0.19	0.31	0.24	0.00	0.00	0.00	0.10
3-Methoxyoctane	1.24	2.31	2.01	1.80	3.44	2.05	1.99	2.08	1.09	2.00
Dipropyl trisulfide	15.21	23.4	24.5	16.07	12.90	21.23	24.01	30.05	25.90	21.47
Allyl propyl trisulfide	1.52	0.90	1.30	1.55	0.75	0.86	1.09	1.62	1.91	1.28
6,10-Dimethyl 2-undecanone	0.32	0.22	0.43	0.12	0.26	0.24	0.41	0.45	0.39	0.32
β-Selinene	0.25	0.19	0.21	0.32	0.25	0.17	0.38	0.27	0.23	0.25
(*E*)-β-Farnesene	1.30	2.01	2.03	1.83	1.95	0.90	1.32	2.07	1.99	1.71
β-Ionone	0.30	0.52	0.32	0.34	0.27	0.51	0.50	0.00	0.41	0.35
α-Farnesene	2.46	3.01	1.99	2.56	2.34	1.89	2.07	2.03	1.97	2.26
2-Methyl-3,4-dithiaheptane	3.10	2.73	2.89	3.01	2.34	2.56	1.98	3.45	3.24	2.81
Dipropyl tetrasulfide	0.35	0.52	0.31	0.00	0.34	0.29	0.31	0.16	0.27	0.28
Propyl-1-(propylthio) ethyl trisulfide	1.10	0.87	1. 03	2.05	1.87	2.51	1.45	1.95	2.09	1.74
6,10,14-Trimethyl-2-pentadecanone	1.30	0.98	0.76	1.90	2.01	1.34	0.76	0.69	1.07	1.20
Total Identified Compounds	65.07	69.86	93.29	85.81	84.71	83.82	84.65	92.69	91.91	83.53

or pronounced effect on the essential oil percentage. On the other hand, the humic acid levels significantly increased the essential oil yield comparing with untreated plants, where humic acid at 2 g/l gave the highest values of essential oil yield which recorded 96.5 ml/fed. and 241.3 ml / ha. The increment of essential oil yield may be due to increment of herb yield. Some studies have pointed to the positive effects of humic acid on the essential oil content, and yield of *Hisbiscus sabdariffa* L. [Heidari and Khalili, 2014], *Foeniculum vulgare* var. Dulce [Khalid et al., 2015], and *Hypericum perforatum* L. [Kaboli Farshchi et al., 2016].

Effect of combination treatments

Effect on growth and yield characters

The results of the interaction effect between compost tea and humic acid on chive plants were presented in Table 8. The results of the study indicate that the lowest plant height, herb fresh and dry weights resulted by untreated plants which recorded 29.3 cm, 29.3 g/plant and 4.9 g/plant, respectively. The highest mean values of plant height (44.9 cm) and herb dry weight (7.6 g/plant) were obtained from the interaction treatment between compost tea at 200 ml/l and humic acid at 4 g/l. However the highest mean value of herb fresh weight (43.5 g/plant) was obtained from the interaction treatment between compost tea at 100 ml/l and humic acid at 4 g/l.

Effect on essential oil percentage and yield

The results of the interaction between the two factors of the study in Table 9 indicated that the interaction treatments had a significant effect on essential oil% and yield. The interaction between compost tea at 200 ml/l and Humic acid 2 g/l gave the highest mean value of essential oil% which recorded 0.06% followed by compost tea at 200 ml/l without humic acid as well as the combination treatment compost tea at 100 ml/l + humic acid at 4 g/l which gave 0.05%. The essential oil yield reached maximum values (134.4 ml/fed. and 336.0 ml/ha.) as a result of the interaction treatment compost tea at 200 ml/l + humic acid at 2 g/l, then came compost tea at 100 ml/l + humic acid at 4 g/l which gave 120.0 ml/fed and 300 ml/ha. The increment in oil yield may be due to either increase in vegetative growth or changes in leaf oil gland population and monoterpenes biosynthesis. These results are in agreement with Buitrago Diaz et al. [2011] who found that the oils extracted from *Allium schoenoprasum* by hydrodistillation yielded 0.02–0.03%.

Chemical composition of the essential oil

The GC-MS analysis of chive EO identified 30 components, representing from 65.07 to 93.29% of the total EO. The main compounds found in chive EO were dipropyl disulfide (12.8–35.4%), dipropyl trisulfide (12.90–30.05%), methyl propyl trisulfide (3.80–9.03%) and 1-propenyl propyl disulfide (1.56–10%). The highest amounts of dipropyl disulfide and 1-propenyl propyl disulfide were detected with humic acid at 4 g/l treatment with the values of 35.4 and 10%, respectively. Treatment of compost tea at 200 ml/l + humic acid at 2 g/l caused the greatest accumulation of dipropyl trisulfide which recorded 30.05%, while the greatest values of methyl propyl trisulfide (9.03%) were recorded as a result of compost tea at 100 ml/l. The same components were discovered in the *Allium schoenoprasum* L. plant in previous studies [Mnayer et al., 2014], which found that the main compounds in chive EO were dipropyl disulfide (19.49%), dipropyl trisulfide (15.21%), methyl propyl trisulfide (8.47%) and 1-propenyl propyl disulfide (5.84%). The variations in the essential oil content and composition could be due to several factors, such as climatic, geographic conditions and growth stage of collected plants [Zouari et al., 2012]. The presented results agree with those obtained by other authors i.e. oregano essential oil production increased significantly with K-humate application [Said-Al Ahl and Hussein, 2010].

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