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Research Article
Selenium to Improve Growth Characters, Photosynthetic Pigments and Essential Oil Composition of Chives Varieties

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Abstract

Background and Objective: Chives (*Allium schoenoprasum*) has various medical uses and biological activities such as antioxidant and antimicrobial. Selenium (Se) plays an important role in some physiological process in higher plants. The aim of this study was to evaluate vegetative growth characters (VGC) {plant height {PH} (cm/plant), fresh weight (FW) and dry weight (DW) as g/plant}, photosynthetic pigments (PHP) {chlorophyll (Chl) a, Chl b and total carotenoids (TC)} and essential oil (EO) composition of two chives varieties under Se treatments. **Materials and Methods:** Two varieties of chives {*A. schoenoprasum* Moskurl 2 Petra (M2P) and *A. schoenoprasum* Moskurl 2 KRA USA (M2K)} were exposed to different doses of Se (control, 3, 6, 9 and 12 mg L^{-1}) as foliar spray. Randomized Complete block design (RCBD) was used and the averages of data were statistically analyzed using two-way ANOVA. **Results:** Six mg L^{-1} (Se) x M2K variety resulted in the greatest values (41.0, 55.6 and 9.6) of VGC. The highest accumulations of Chl a and TC (1.9 and 0.6) were obtained from M2P and M2K varieties treated with 12 mg L^{-1} (Se) while M2K plants x 6, 9 and 12 mg L^{-1} (Se) produced the highest amounts (0.3) of Chl b. 6 mg L^{-1} (Se) treatment reported the greatest amounts of the contents of EO which recorded the values of 0.6 mL/(100 plant), 192 mL/(feddan) and 480 mL ha^{-1}. The major constituents of EO (dipropyl trisulfide, 1-propenyl propyl disulfide, dipropyl disulfide, methyl propyl disulfide and methyl propyl trisulfide) were changed (increased or decreased) or not changed under Se, chives varieties and their interactions. **Conclusion:** It may be concluded that treated chives varieties with Se doses improved the VGC and EO while PHP and major constituents of EO were changed. Under Se treatments the M2K variety produced higher values of VGC and EO content than M2P while no differences were found in PHP. Different variations were obtained in the major constituents of EO of both varieties under Se applications.

Key words: Chives, plant height, fresh weight, dry weight, chlorophyll a, chlorophyll b, total carotenoids

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Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Chives or *Allium schoenoprasum* (*A. schoenoprasum*) belongs to family *Alliaceae*, it is widely distributed in Europe, Asia and North America regions[1]. It is known for medicinal properties[2-7]. The essential oil (EO) of *A. schoenoprasum* contains sulfur constituents which have antimicrobial and antioxidant activities[8].

Selenium (Se) is an essential element for human, animal and some kinds of microorganisms. Add Se directly to food as food additive not reported by US Food and Drug Administration[9]. So Se can be used in plant nutrition through the soil or as foliar spray that resulted in the accumulation of Se in plant production. Although, Se is not confirmed to be required by higher plants, many investigations indicted that at low doses it plays a necessary role in antioxidative reactions and hormone balance in plant cell such as increasing the activity of glutathione peroxidase (GPX)[10-12]. The GPX roles connected with elevated environmental stress resistance such as salinity and drought stresses[13].

Se treatments caused a significant improvement in vegetative growth characters (VGC) and photosynthetic pigments (PHP) accumulation of cucumber, alfalfa and peanut plants[14-17]. It was found that VGC of cucumber were increased in the 2 mg L^{-1} (Se) level and reduced at the higher level of Se while chlorophyll (Chl) composition and PHP values were not influenced[18].

Se is an important nutrient required for EO production of aromatic plants[19]. Se doses (2-8 mg L^{-1}) caused a highly significant increase in EO extracted from the herb of basil and lemon balm plants[20]. About 0.3 mg (Se) produced the greatest amount of EO and monoterpenes in geranium herb[19]. Khalid[21] investigated the response of salvia EO to Se treatments (2-8 mg L^{-1}) and it was reported that the highest values of major components (α-thujone, β-thujone and camphor) were recorded in the rate of 8 mg L^{-1}, mono and sesquiterpenes were decreased however ketones increased as Se rate increase.

Genotypes of medicinal and aromatic plants play an essential role in medicinal and aromatic plants production systems. They affect growth and productivity by their better yield potentials, resistance against insect pest and diseases under different environmental factors[22]. Enhance yield, active principals and development cultivars of medicinal and aromatic plants is necessary. Producing varieties having resistance against environmental factors can increase yields and productivity of medicinal and aromatic plants to meet world requirement[23].

Growth, yield and active constituents of medicinal and aromatic plants genotypes depend on genetic and environmental factors. Therefore the present study aimed to investigate the possible effects of Se levels on the VGC, EO and PHP of two varieties of chives plant.

MATERIALS AND METHODS

Experimental: Experiments were carried out in arid region at the Experimental Farm of SEKEM Company which located in Belbes city, Egypt, during two successive seasons, 2013-2014 and 2014-2015 {the field experiments were carried out twice during two seasons (18 months) because repeated the experiments lead to reduce the experimental errors and produce comprehensive results}. The chemical analysis, identifications of EO major components and statistical analysis (STA) took more than 1 year. Physical and chemical properties of the soil used in this study were determined according to Margenot *et al.*[24] and Carter and Gregorich[25] which are: Sand (98.8%), silt (0.9%), clay (0.8%), pH (8.2), EC (2.3 dSm^{-1}) and OM (0.1%). Anions and cations (mq L^{-1}): SO_4^{2-} (10.0), Cl^{1-} (10.9), $HCO3^{1-}$ (2.4), Na^{+} (2.3), Mg^{2+} (3.8), Ca^{2+} (0.8) and K^{+} (0.9). Seeds of *A. schoenoprasum*, which were provided by the Pharmasate Company, Germany. Seeds were sown directly in the nursery in the third week of October during both seasons. After 4 weeks from the sowing, the seedlings were transplanted into open field. The experimental design was a randomized complete block (RCBD) with 3 replicates. The experimental area (plot) was 4 m^2 (2 m×2 m) containing 4 rows, the distance between hills was 25 and 50 cm apart. Thinning for three plants/hill was made 45 days after cultivating the plants in the open field. All agriculture practices operations other than experimental treatments were performed according to the recommendations of the Ministry of Agriculture, Egypt. Plots were divided into two main groups. The first group: *A. schoenoprasum* var. Moskurl 2 Petra (M2P) plants were subjected to different levels of Se as foliar spray: Control, 3, 6, 9 and 12 mg L^{-1}. The second group was subjected to the same Se levels but *A. schoenoprasum* var. Moskurl 2 KRA USA (M2K) plants were used. The source of Se was sodium selenate (Na_2SeO_4). The plants were treated by foliar spray twice, the first one was after 2 weeks from transplanting and the second one was after one week from the first one.

Harvesting: At vegetative stage, leaves were harvested by cutting the leaves 3 cm above the soil surface. Plant height (PH) (cm/plant), as well as fresh weight (FW) and dry weight (DW) as g/plant were recorded.

Determination of PHP: Chl a, Chl b and total carotenoids (TC) in fresh leaves collected at the first and second seasons of each treatment were determined as mg g^{-1} using the methods described by the Association of Official Agricultural Chemists[26].

EO isolation: Fresh leaves (aerial part) was collected from each treatment during both seasons, air dried and weighed to extract the EO, then 100 g from each replicate of all treatments was subjected to hydro-distillation (HD) for 3 h using a Clevenger-type apparatus[27]. The EO content was calculated as a relative percentage (v/w). In addition, total EO as mL/(100 plant), mL/(feddan) (4200 m^2) and mL ha^{-1} was calculated by using the DW. The EOs extracted from *A. schoenoprasum varieties* were collected in both seasons from each treatment and dried over anhydrous sodium sulfate to identify the chemical constituents.

Gas chromatography-mass spectrometry (GC-MS): The GC-MS analysis was carried out with an Agilent 5975 GC-MSD system. The DB-5 column (60 m × 0.25, 0.25 mm film thickness) was used with helium as carrier gas (0.8 mL min^{-1}). The GC oven temperature was kept at 60°C for 10 min and programmed to 220°C at a rate of 4°C min^{-1} that was kept constant at 220°C for 10 min and followed by elevating the temperature to 240°C at a rate of 1°C min^{-1}. Split ratio was adjusted at 40:1. The injector temperature was set at 250°C. Mass spectra were recorded at 70 eV. Mass range was m/z 35-450.

GC analysis: The GC analysis was carried out using an Agilent 6890N GC system using FID detector temperature of 300°C. To obtain the same elution order with GC-MS, simultaneous auto injection was done on a duplicate of the same column at the same operational conditions. Relative percentage amounts of the separated compounds were calculated from FID chromatograms.

Identification of components: Identification of the EO components was carried out by comparison of their relative retention times with those of authentic samples or by comparison of their retention index (RI) to series of n-alkanes. Computer matching against commercial {Wiley GC/MS Library, Mass Finder 3 Library}[28,29] and inhouse "Başer Library of EO Constituents" built up by genuine compounds and components of known oils. Additionally, MS literature data[30,31] were also used for the identification.

Statistical analysis: In this experiment, 2 factors were considered: Se levels (control, 3.0, 6.0, 9.0 and 12.0 mg L^{-1}) and two *A. schoenoprasum* varieties. For each treatment there were 3 replicates. The experimental design followed a randomized complete block design (RCBD). According to De Smith[32] the averages of data of both seasons were statistically analyzed using two way analysis of variance (ANOVA). Significant values were determined according to p-values ($p<0.05$ = Significant, $p<0.01$ = Moderate significant and $p<0.001$ = Highly significant). The applications of that technique were according to the STAT-ITCF program[33].

RESULTS

Effect of Se, chives varieties and their interactions on VGC: The varieties of chives (M2P and M2K) and Se levels affected plant morphology (Table 1). Thus the VGC {PH (cm/plant), FW (g/plant) and DW (g/plant)} in general were increased in Se levels with or without the various varieties of chives. The greatest values (41, 55.6 and 9.6) of VGC were reported under the treatment of 6 mg L^{-1} (Se) x M2K variety. Regarding the varieties factor, it was found that M2K gave higher values of VGC (36.1, 44.3 and 7.6) than M2P (31, 27.9 and 4.8). The STA indicated that the increases in various VGC were highly significant ($p<0.001$) for Se or chives varieties while Se x various varieties caused insignificant increases in VGC.

Effect of Se, chives varieties and their interactions on PHP: Chives varieties and applying Se resulted in different changes in the accumulation of PHP {Chl a, Chl b and TC} (Table 2). The highest accumulations of Chl a and TC were recorded with the treatment of 12 mg L^{-1} (Se) with the values of 1.9 and 0.6 for both M2P and M2K varieties, respectively. The greatest amounts of Chl b (0.3 mg g^{-1}) were obtained from the M2P plants treated with 3 and 6 mg L^{-1} (Se) or M2K plants that received 6, 9 and 12 mg L^{-1} (Se). ANOVA reported that the changes in PHP were significant ($p<0.05$) for Se treatments while it was insignificant for varieties or Se x various varieties.

Effect of Se, chives varieties and their interactions on EO contents: The effects of Se, varieties of chives and Se x various varieties on EO content {mL/(100 plant), mL/(feddan) and mL ha^{-1}} are presented in Table 3. Different increases were found in EO contents of Se treatments compared with control except the treatment of 12 mg L^{-1} (Se) which caused no changes in EO extracted from M2P. The highest EO contents

Table 1: Effect of Se, chives varieties and their interactions on VGC

Treatments				
Varieties	Se (mg L^{-1})	PH (cm/plant)	FW (g/plant)	DW (g/plant)
M2P	Control	26.3±1.2	25.0±2.2	4.3±0.4
	3	31.0±2.6	43.8±2.4	7.6±0.4
	6	38.0±3.6	52.8±1.6	9.1±0.3
	9	30.7±3.1	49.2±1.1	8.5±0.2
	12	34.7±2.1	33.1±2.4	5.8±0.3
Overall M2P		32.1±4.7	40.8±1.8	7.1±0.5
M2K	Control	31.0±1.7	27.9±1.0	4.8±0.2
	3	36.3±2.1	46.8±1.4	8.1±0.2
	6	41.0±2.6	55.6±1.4	9.6±0.2
	9	34.0±1.7	54.0±1.8	9.3±0.3
	12	38.3±0.6	37.0±1.9	6.4±0.3
Overall M2K		36.1±3.9	44.3±1.9	7.6±1.9
Overall Se	Control	28.6±3.6	26.5±2.3	4.6±0.4
	3	33.7±3.2	45.3±2.0	7.8±0.4
	6	39.5±2.8	54.2±3.0	9.4±0.5
	9	32.3±2.9	51.6±2.9	8.9±0.5
	12	36.5±2.4	35.0±2.9	6.1±0.3
p-value		0.001	0.001	0.001
Se		19.29***	255.23***	286.72***
Varieties		22.79***	28.48***	30.17***
Se×varieties		NS	NS	NS

***Highly significant, NS: Non-significant, M2P: Moskurl 2 Petra, M2K: Moskurl 2 KRA USA, VGC: Vegetative growth characters, PH: Plant height, FW: Fresh weight, DW: Dry weight, values are given as Mean±SD

Table 2: Effect of Se, chives varieties and their interactions on PHP

Treatments				
Varieties	Se (mg L^{-1})	Chl a (mg g^{-1})	Chl b (mg g^{-1})	TC (mg g^{-1})
M2P	Control	1.1±0.1	0.2±0.0	0.3±0.1
	3	1.4±0.0	0.3±0.0	0.4±0.0
	6	1.5±0.2	0.3±0.0	0.4±0.1
	9	1.8±0.9	0.2±0.1	0.5±0.1
	12	1.9±0.0	0.2±0.0	0.6±0.0
Overall M2P		1.5±0.3	0.2±0.1	0.4±0.1
M2K	Control	1.1±0.1	0.2±0.0	0.3±0.1
	3	1.4±0.0	0.2±0.1	0.4±0.1
	6	1.5±0.2	0.3±0.1	0.4±0.1
	9	1.7±0.1	0.3±0.0	0.5±0.1
	12	1.9±0.0	0.3±0.0	0.6±0.0
Overall M2K		1.5±0.3	0.3±0.1	0.4±0.1
Overall Se	Control	1.1±0.1	0.2±0.0	0.3±0.1
	3	1.4±0.0	0.3±0.1	0.4±0.0
	6	1.5±0.2	0.3±0.0	0.4±0.1
	9	1.8±0.1	0.3±0.1	0.5±0.1
	12	1.9±0.0	0.3±0.1	0.6±0.0
p-value		0.05	0.05	0.05
Se		49.5*	3.0*	28.0*
Varieties		NS	NS	NS
Se×varieties		NS	NS	NS

*Significant, NS: Non-significant, M2P: Moskurl 2 Petra, M2K: Moskurl 2 KRA USA, Chl: Chlorophyll, TC: Total carotenoids, values are given as Mean±SD

were obtained from M2K plants that treated with 6 mg L^{-1} (Se) with the values of 0.6 mL/(100 plant), 192 mL/(feddan) and 480 mL ha^{-1}. The M2P plants produced lower accumulations of EO {0.4 mL/(100 Plant), 128 mL/(Feddan) and 320 mL ha^{-1}} than M2K {0.5 mL/(100 plant), 160 mL/(Feddan) and 400 mL ha^{-1}}. The increments in EO contents were highly significant ($p<0.001$) for Se, chives varieties and Se x various varieties.

Table 3: Effect of Se, chives varieties and their interactions on EO contents

Treatments				
Varieties	Se (mg L^{-1})	mL/(100 plant)	mL/(Feddan)	mL ha^{-1}
M2P	Control	0.3±0.0	96.0±4.0	240.0±10.0
	3	0.4±0.0	128.0±2.0	320.0±10.0
	6	0.5±0.0	160.0±5.0	400.0±5.0
	9	0.5±0.0	160.0±5.0	400.0±5.0
	12	0.3±0.0	96.0±5.0	240.0±5.0
Overall M2P		0.4±0.0	128.0±6.7	320.0±8.4
M2K	Control	0.3±0.0	96.0±4.0	240.0±7.0
	3	0.5±0.0	160.0±4.0	400.0±7.0
	6	0.6±0.0	192.0±4.0	480.0±7.0
	9	0.5±0.0	160.0±4.0	400.0±7.0
	12	0.4±0.0	128.0±4.0	320.0±7.0
Overall M2K		0.5±0.0	160.0±6.7	400.0±5.1
Overall Se	Control	0.3±0.0	96.0±3.3	240.0±8.4
	3	0.5±0.0	160.0±3.3	400.0±8.4
	6	0.6±0.0	192.0±3.3	480.0±8.4
	9	0.5±0.0	160.0±3.3	400.0±8.4
	12	0.4±0.0	128.0±3.3	320.0±8.4
p-value		0.001	0.001	0.001
Se		45.73***	412.8***	858.2***
Varieties		71.43***	172.8***	359.2***
Se x varieties		11.84***	28.8***	59.9***

***Highly significant, NS: Non-significant, M2P: Moskurl 2 Petra, M2K: Moskurl 2 KRA USA, EO: Essential oil, Values are given as Mean±SD

Table 4: Effect of Se, chives varieties and their interactions on EO major constituents

Treatments		Major constituents (%)					
Varieties	Se (mg L^{-1})	A	B	C	D	E	TI
M2P	Control	14.9	10.0	18.8	7.1	12.2	63.0
	3	4.3	1.0	85.4	1.0	1.8	93.5
	6	18.1	9.6	23.1	4.0	13.9	68.7
	9	17.4	7.3	19.9	6.1	11.7	62.4
	12	14.9	1.4	58.5	1.3	2.9	79.0
Overall M2P		13.9	5.9	41.1	3.9	8.5	73.3
M2K	Control	22.9	6.5	19.5	4.7	7.0	60.6
	3	24.5	7.3	23.2	3.0	15.5	73.5
	6	34.4	9.0	13.0	5.2	12.0	73.6
	9	24.1	8.4	19.8	4.6	9.2	66.1
	12	21.0	5.1	7.8	5.1	7.3	46.3
Overall M2K		25.4	7.3	16.7	4.5	10.2	64.1
Overall Se	Control	18.9	8.3	19.2	5.9	9.6	61.9
	3	14.4	4.2	54.3	2.0	8.7	83.6
	6	26.3	9.3	18.1	4.6	13.0	71.3
	9	20.8	7.9	19.9	5.4	10.5	64.5
	12	18.0	3.3	33.2	3.2	5.1	62.8

A: Dipropyl trisulfide, B: 1-propenyl propyl disulfide, C: Dipropyl disulfide, D: Methyl propyl disulfide, E: Methyl propyl trisulfide, M2P: Moskurl 2 Petra, M2K: Moskurl 2 KRA USA, TI: Total identified, EO: Essential oil

Effect of Se, chives varieties and their interactions on the major constituents of EO: Five constituents were detected by GC/MS analysis in EO isolated from two varieties of chives leaves (Table 4), accounting 46.3-93.5% of total constituents. The major constituents were dipropyl trisulfide, 1-propenyl propyl disulfide, dipropyl disulfide, methyl propyl disulfide and methyl propyl trisulfide. Varieties of chives, Se doses and varieties of chives x Se doses resulted in different changes in EO major constituents. The highest amounts of 1-propenyl propyl disulfide and methyl propyl disulfide were detected with the control treatment in EO extracted from M2P plants with the values of 10 and 7.1%. Treatments of 3 and 6 mg L^{-1} (Se) caused the greatest accumulation of dipropyl disulfide, methyl propyl trisulfide and dipropyl trisulfide in EO isolated from M2K plants which recorded the values of 23.2, 15.5 and 34.3%, respectively. EO of M2K plants resulted in the highest values of dipropyl trisulfide (25.4%), 1-propenyl propyl disulfide (7.3%), methyl propyl disulfide (4.5%) and methyl propyl trisulfide (10.2%) while the greatest value of dipropyl disulfide (41.1%) were recorded with M2P plants.

DISCUSSION

Different variations were found in VGC, PHP and EO composition when the varieties of *A. schoenoprasum* plants (M2P and M2K) subjected to various levels of Se. Improving the VGC under Se treatments may be due to Se can increase the plant PHP especially Chl content, respiration rates in leaves, glutathione peroxidase (GSH-Px) activity in mitochondria and dry matter content which suggested by some investigators[34-40]. Regarding the changes in PHP some previous investigators supported these results, they indicted that PHP such as Chl and TC affected by Se levels and low levels of Se can increase the PHP[14,41-43]. On the other hand Se can affect the PHP by increase the uptake of some nutrients such as magnesium (Mg) in leaves which led to increasing in PHP[18]. Opposite trends were found in PHP under Se treatments i.e., PHP including Chl, TC and xanthophylls were reduced in the leaves of coffee, *Phaseolus vulgaris* L. and *Sinapis alba* L. under Se doses[44-47]. The increases in EO content in response to Se treatments may be due to the Se can increase the secondary products in aromatic plants especially EO[19]. Se has a significant effect CO_2 assimilation level, PHP content and ultimately the accumulation of EO (quantity and quality) isolated from geranium[19]. Obtained results are in accordance with those obtained by Lee *et al.*[20], they revealed that Se caused an increase in basil and lemon balm EO from two to three folds compared with control. The changes in sulfur components (EO constituents) under Se conditions may be due to its effect of Se on enzymes activity and metabolism improvement[48]. Sulfur components are known for medicinal and biological activities such as anticancer and antiproliferative activities as well as antimicrobial (having effects against different bacteria, fungi and yeasts)[49,50]. The changes in VGC, PHP and EO (contents and major components) of different *A. schoenoprasum* varieties under Se conditions may be due to the genetic variations between the various varieties[51]. It may be concluded that the type of nutrient (Se) and plant variety in the production system should be considered in the growth, yield and EO composition produced from aromatic plants.

CONCLUSION

It can be reported that the VGC and EO of Chives varieties were increased under Se treatments while the PHP and major constituents of EO (dipropyl trisulfide, 1-propenyl propyl disulfide, dipropyl disulfide, methyl propyl disulfide and methyl propyl trisulfide) were changed. The highest values of VGC were reported under the treatment of 6 mg L^{-1} (Se) x M2K Varity. The treatments of 3, 6 and 12 mg L^{-1} (Se) produced the highest values of PHP in both varieties. The greatest values of EO contents were recorded with M2K plants x 6 mg L^{-1} (Se). Control, 3 and 6 mg L^{-1} (Se) resulted in the highest amounts of EO major constituents for both varieties.

SIGNIFICANCE STATEMENTS

This study discovered that a cultivation of *A. schoenoprasum* plant is required. Treatments of Se improved the VGC, EO contents and PHP of both *A. schoenoprasum* varieties. The application of Se resulted in different changes in the major constituents of EO extracted from *A. schoenoprasum*. This study help the farmers, ministry of agriculture and pharmaceutical companies to improve yield and chemical contents of *A. schoenoprasum* (important medicinal and aromatic crop) as a natural source of drugs and pharmaceutical industries.

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