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Productivity of wormwood (*Artemisia abrotanum*) enhanced by trace elements

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Abstract

Background and objective: *Artemisia abrotanum* (*A. abrotanum*) used for against cancer, cough, fever, and tumors; also, it contains essential oil (EO) used in antimicrobial possesses. Trace elements (iron, Fe; magnesium, Mg; and manganese, Mn) play important roles in physiological processes and metabolisms of *A. abrotanum* plants. In this study, growth, yield, EO, and nutrient contents of *A. abrotanum* were evaluated under foliar spray of Fe, Mg, or Mn treatments.

Materials and methods: Plants subjected to different rates of Fe (0, 1, 2, 3, and 4 g/L), Mg (0, 2, 4, 6, and 8 g/L), or Mn (0, 50, 100, 200, and 300 mg/L) as individual experiments. Data were statistically analyzed using ANOVA-1.

Results: Obtained results showed that Fe (3 g/L), Mg (8 g/L), or Mn (300 mg/L) resulted in the greatest values of growth characters (plant height, fresh and dry weights of aerial parts), EO content (% and mL/100 plants), major constituents of EO (2-hydroxy-1, 8-cineole, β -eudesmol, and camphor), nutrient contents, and their uptakes. Different changes were found in various chemical classes of EO (mono and sesquiterpenes).

Conclusion: Significant variations were detected in growth, yield, EO, and nutrient contents due to foliar applications of various trace elements such as Fe, Mg, and Mn.

Keywords: Iron, Magnesium, Manganese, Growth, Essential oil, 2-Hydroxy-1, 8-Cineole, Nutrient

Background

Genus of *Artemisia* belongs to family Asteraceae. It is known as wormwood and comprising 400 species widely distributed in Africa and South America. *Artemisia abrotanum* is a source of essential oil (EO) used as anti-spasmodic, antibacterial, and antifungal (Slokar et al. 1992). It has also been used against cancer, cough, fever, and tumors (Bjork et al. 2002).

Many Egyptian farmers depend on the major elements (nitrogen, N; phosphorus, P; and potassium, K) to feed medicinal and aromatic plants and are neglect the use of trace elements. Trace elements in appropriate dose are essential for plant's growth and development; these play important roles in cell structure and various physiological processes such as photosynthesis, respiration, and different enzymatic activities. Their deficiency or

inappropriate presence or absence disturbs the metabolic activities occurring in plants at different stages of development which finally creates imbalance within and show symptoms like chlorosis, necrosis, stunted growth, mottled leaves, and many more affected morphological parameters (Singh and Dwivedi 2019). As these influence different metabolic pathways so affect final product, intermediate products, primary, and secondary metabolites formation.

On the other hand, scientific work into various methods to improve the productivity of medicinal and aromatic plants must increase as demand for food and natural pharmaceutical raw materials production increases. The application of trace elements is one way of research that has the potential to increase medicinal and aromatic plants productivity (Iskan et al. 2002). Foliar spray, i.e., application of trace elements in liquid forms to the aerial parts of the plant is an effective technique for correcting soil deficiencies and overcoming the soils

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inability to transfer nutrients to the plant under moisture conditions (Rashid and Ryan 2004; Ali et al. 2008).

Iron (Fe), magnesium (Mg), and manganese (Mn) are critical trace elements for healthy plant. Thus, the aim of this study was to evaluate growth, yield, EO, and nutrient content (NPK) in response to foliar application of some individual trace elements (Fe, Mg, and Mn).

Materials and methods

Experimental

Three individual experiments were carried out in a greenhouse at the National Research Centre (NRC), Egypt, during 2018 and 2019. *Artemisia abrotanum* (*A. abrotanum*) seedlings were obtained from the Institute of Medicinal and Aromatic Plants (IMAP), Egypt. Uniform seedlings were transplanted into clay pots (30 cm diameter and 50 cm height). In the first week of February during both seasons, the pots were transferred to a greenhouse adjusted to natural conditions. Each pot was filled with 10 kg of air-dried soil (clay to sand, 1:1). Three weeks after transplanting, the seedlings were thinned to 2 plants per pot. Pots were divided into three main groups. The first group was subjected to different levels of chelated Fe: 0, 1, 2, 3, and 4 g/L. The second group was subjected to chelated Mg: 0, 2, 4, 6, and 8 g/L. The third group was subjected to chelated Mn: 0, 50, 100, 200, and 300 mg/L. Fe, Mg, and Mn were applied twice to run-off to foliage after 15 and 21 days from thinning during both seasons. All agricultural practices were conducted according to the main recommendations by the Ministry of Agriculture, Egypt.

Harvesting

At full bloom (180 days from transplanting), the plants were harvested during the growing seasons by cutting the plants 10 cm above the soil surface. Plant height (cm) and fresh and dry weights of aerial parts (g/plant) were recorded.

EO isolation

Aerial parts were collected from each treatment during both seasons and weighed to extract the EO; then, 200 g from each replicate of all treatments was subjected to hydro-distillation (HD) for 3 h using a Clevenger-type apparatus (Clevenger 1928). The EO content was calculated as a relative percentage (v/w). In addition, total EO as mL/100 plants was calculated by using the dry weight. The EOs extracted from *A. abrotanum* were collected during both seasons from each treatment and dried over anhydrous sodium sulfate to identify the chemical constituents.

GC and GC–MS conditions

GC analyses were performed using a Shimadzu GC-9 gas chromatograph equipped with a DB-5 (dimethylsiloxane, 5% phenyl) fused silica column (J & W Scientific Corporation) (60 m × 0.25 mm i.d., film thickness 0.25 μm). Oven temperature was held at 50 °C for 5 min and then programmed to rise to 240 °C at a rate of 3 °C/min. The flame ionization detector (FID) temperature was 265 °C, and injector temperature was 250 °C. Helium was used as carrier gas with a linear velocity of 32 cm/s. The percentages of compounds were calculated by the area normalization method, without considering response factors.

GC–MS analyses were carried out in a Varian 3400 GC-MS system equipped with a DB-5 fused silica column (60 m × 0.25 mm i.d., film thickness 0.25 μm); oven temperature was 50–240 °C at a rate of 4 °C/min, transfer line temperature 260 °C, carrier gas, helium, with a linear velocity of 31.5 cm/s, split ratio 1:60, ionization energy 70 eV, scan time 1 s, and mass range 40–300 amu.

Identification of volatile components

The components of EO were identified by comparison of their mass spectra with those of a computer library or with authentic compounds and confirmed by comparison of their retention indices, either with those of authentic compounds or with data published in the literature (Adams 1995). Mass spectra from the literature were also compared (Adams 1995). Further identification was made by comparison of their mass spectra on both columns with those stored in NIST-98 and Wiley-5 Libraries. The retention indices were calculated for all volatile constituents using a homologous series of n-alkanes.

Determination of mineral content

Total nitrogen (N) and phosphorus (P) in dried leaves of each treatment were determined using the methods described by the Anonymous (2016). The samples of leaves were dried, ground, and potassium K extracted by acid digestion (Cottenie et al. 1982). Concentrations were determined by atomic absorption spectrophotometer using a Perkin–Elmer (Gonzalez et al. 1973).

Statistical analysis

In each experiment, one factor was considered: Fe, Mg, and Mn (5 treatments). For each treatment, there were 4 replicates; the experimental design followed a complete random block design. The average data of both seasons were statistically analyzed using one way analysis of variance (ANOVA-1) (De-Smith 2015). Significant values 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 version 10 (Statsoft 2007).

Results

Changes in growth, EO, and nutrient content in response to Fe treatments

The treatments of Fe affected plant growth characters (plant height, fresh and dry weight of aerial parts) in both seasons (Table 1). Thus, the various growth characters in general increased under different Fe doses compared with control. Greatest yields at each season for all variables obtained in the 3 g/L treatment with values of 56.8, 52.3 cm; 183.3, 197.3 g/plant; and 72.8, 63.2 g/plant of the first and second season, respectively. The increases in all growth characters were highly significant ($P < 0.001$) of both seasons.

As shown in Table 1, EO content (%) and EO yield (mL/100 plants) increased at all Fe levels during both seasons. The highest accumulation of EO was recorded at the level of 3 g/L of Fe; this level yielded 0.5 and 0.4% and 36.4 and 25.3 mL/100 plants during the first and second seasons, respectively. The increases in EO (%) were insignificant while it was highly significant ($P < 0.001$) for EO yield of both seasons.

It is evident from Table 1 that all nutrient contents (N, P, and K) and uptake under investigation gradually increased in all Fe treatments as compared with the control treatment in both seasons. Foliar applications of Fe increased the mineral contents and its uptake compared

with untreated plants (control) in both seasons. The level of 3 g/L of Fe resulted in the highest values of NPK content (2.6, 2.5%; 0.3, 0.3%; 3.8, 3.8%) and their uptake (1.9, 1.6 g/plant; 0.2, 0.2 g/plant; 2.8, 2.4 g/plant) during the first and second seasons, respectively. The increases in N and P contents were insignificant while it was highly significant ($P < 0.001$) for K content. On the other hand, the increments in N and K uptakes were highly significant ($P < 0.001$), but it was insignificant for P uptake.

Quality and quantity of the components present with the different Fe doses in the hydro-distilled *A. abrotanum* EO was evaluated. A total of 25 constituents, amounting for 98.1–98.8% of the EO, were detected. The effect of Fe doses on the chemical constituents of EO extracted from *A. abrotanum* is shown in Table 2. The main components were 2-hydroxy-1, 8-cineole, β -eudesmol, and camphor. Moreover, the greatest amounts of the main components obtained from the treatment of 3 g/L of Fe with the values of 42.6, 14.8, and 14.4%, respectively. All detected components belonged to 4 chemical classes; oxygenated monoterpenes (MCHO) and oxygenated sesquiterpenes (SCHO) were the major classes while monoterpene hydrocarbons (MCH) and sesquiterpene hydrocarbons (SCH) formed the minor classes. Untreated plants with Fe produced the highest values of MCH (9.8%) and SCHO (20.8%). The treatment of 3 g/L resulted in the highest amount of MCHO (60.9%) while the greatest value of SCH (9.4%) produced from the treatment of 2 g/L. The changes in all

Table 1 Effect of Fe treatments on growth and chemical content

Fe treatments (g/L)	Growth characters			EO		Nutrient Content (%)			Uptake (g/plant)		
	PH	FW	DW	%	Yield	N	P	K	N	P	K
First season											
0	40.7 ± 0.6	74.1 ± 0.5	27.7 ± 0.3	0.2 ± 0.1	10.1 ± 0.1	2.0 ± 0.1	0.1 ± 0.0	2.9 ± 0.1	0.5 ± 0.1	0.1 ± 0.1	0.9 ± 0.1
1	50.3 ± 0.3	114.7 ± 0.7	38.7 ± 0.3	0.3 ± 0.1	11.6 ± 0.4	2.1 ± 0.1	0.2 ± 0.1	3.1 ± 0.1	0.8 ± 0.1	0.1 ± 0.0	1.2 ± 0.1
2	53.8 ± 0.4	173.1 ± 0.5	55.4 ± 0.7	0.3 ± 0.1	16.6 ± 0.3	2.2 ± 0.2	0.2 ± 0.1	3.5 ± 0.2	1.2 ± 0.1	0.1 ± 0.0	1.9 ± 0.2
3	56.8 ± 0.4	183.3 ± 0.7	72.8 ± 0.4	0.5 ± 0.1	36.4 ± 0.3	2.6 ± 0.2	0.3 ± 0.1	3.8 ± 0.2	1.9 ± 0.3	0.2 ± 0.1	2.8 ± 0.2
4	51.1 ± 0.1	173.9 ± 0.5	65.1 ± 0.2	0.4 ± 0.1	26.1 ± 0.2	2.4 ± 0.1	0.2 ± 0.1	3.2 ± 0.2	1.6 ± 0.1	0.1 ± 0.0	2.1 ± 0.1
F values	763.3***	55510.1***	3621.2***	3.9 ns	4301.7***	2.7 ns	1.8 ns	17.1***	44.3***	3.0 ns	77.3***
Second season											
0	35.1 ± 0.4	66.1 ± 0.8	22.8 ± 0.5	0.2 ± 0.1	4.0 ± 0.3	1.9 ± 0.1	0.1 ± 0.0	2.9 ± 0.2	0.4 ± 0.1	0.1 ± 0.0	0.7 ± 0.2
1	43.1 ± 0.5	114.1 ± 0.9	43.3 ± 0.6	0.3 ± 0.1	13.0 ± 0.4	2.1 ± 0.1	0.2 ± 0.1	3.1 ± 0.2	0.9 ± 0.2	0.1 ± 0.0	1.3 ± 0.1
2	44.7 ± 0.7	182.2 ± 1.1	47.1 ± 0.8	0.3 ± 0.1	14.1 ± 0.3	2.1 ± 0.1	0.2 ± 0.1	3.5 ± 0.3	1.0 ± 0.2	0.1 ± 0.0	1.6 ± 0.1
3	52.3 ± 0.8	197.3 ± 0.8	63.2 ± 0.6	0.4 ± 0.1	25.3 ± 0.3	2.5 ± 0.2	0.3 ± 0.1	3.8 ± 0.3	1.6 ± 0.1	0.2 ± 0.1	2.4 ± 0.1
4	50.7 ± 0.7	185.7 ± 0.9	55.7 ± 0.9	0.3 ± 0.1	16.7 ± 0.3	2.2 ± 0.2	0.2 ± 0.1	3.2 ± 0.3	1.2 ± 0.1	0.1 ± 0.0	1.8 ± 0.1
F values	2422.4***	75424.2***	5237.6***	1.5 ns	400.8***	14.4 ns	1.9 ns	17.1***	36.0***	3.0 ns	53.6**

Values are given as mean ± SD

ns insignificant

*** $P < 0.001$

Table 2 Effect of Fe treatments on EO constituents

No.	Components	RT	RI ^L	RI ^C	Fe treatments (g/L)					F values
					0	1	2	3	4	
1	α -Pinene	2.2	939	939	1.3 \pm 0.1	0.7 \pm 0.2	0.6 \pm 0.1	1.9 \pm 0.1	0.5 \pm 0.1	65.6***
2	Camphene	2.5	953	955	1.8 \pm 0.1	0.6 \pm 0.1	1.5 \pm 0.1	0.9 \pm 0.1	1.7 \pm 0.2	37.5***
3	Sabinene	2.8	976	978	0.8 \pm 0.1	1.5 \pm 0.1	0.4 \pm 0.1	0.7 \pm 0.2	1.1 \pm 0.1	32.8***
4	Myrcene	3.2	991	991	2.1 \pm 0.1	1.9 \pm 0.1	0.9 \pm 0.1	1.4 \pm 0.1	0.6 \pm 0.1	122.1***
5	ρ -Cymene	3.8	1026	1026	0.7 \pm 0.2	1.8 \pm 0.2	1.2 \pm 0.2	1.8 \pm 0.2	0.7 \pm 0.2	22.7***
6	Limonene	4.3	1031	1032	1.6 \pm 0.1	1.3 \pm 0.2	0.6 \pm 0.1	1.9 \pm 0.1	1.4 \pm 0.2	31.8***
7	γ -Terpinene	5.3	1062	1062	1.5 \pm 0.1	1.4 \pm 0.1	2.4 \pm 0.1	0.8 \pm 0.1	0.8 \pm 0.1	81.0***
8	Camphor	5.7	1143	1143	12.1 \pm 0.3	12.9 \pm 0.3	13.7 \pm 0.4	14.2 \pm 0.4	12.8 \pm 0.4	72.1***
9	Borneol	6.1	1165	1166	0.5 \pm 0.1	0.7 \pm 0.1	1.8 \pm 0.2	0.9 \pm 0.1	0.5 \pm 0.1	54.8***
10	Terpinen-4-ol	6.8	1177	1177	0.8 \pm 0.1	1.9 \pm 0.1	0.7 \pm 0.2	2.1 \pm 0.1	2.3 \pm 0.2	103.9***
11	Myrtanal	7.2	1188	1189	1.1 \pm 0.1	0.4 \pm 0.1	0.5 \pm 0.1	1.3 \pm 0.3	1.7 \pm 0.2	28.1***
12	α -Terpineol	7.9	1189	1189	1.9 \pm 0.1	1.8 \pm 0.2	0.7 \pm 0.2	1.6 \pm 0.1	0.7 \pm 0.1	37.8***
13	2-Hydroxy-1,8-cineole	8.8	1219	1219	38.7 \pm 0.3	39.8 \pm 0.4	41.2 \pm 0.4	42.6 \pm 0.4	39.1 \pm 0.5	114.1***
14	Terpenyl acetate	9.1	1352	1352	2.2 \pm 0.2	2.5 \pm 0.1	2.1 \pm 0.1	1.1 \pm 0.1	1.2 \pm 0.1	54.1***
15	Eugenol	9.5	1356	1356	1.6 \pm 0.1	2.3 \pm 0.2	0.8 \pm 0.1	1.6 \pm 0.1	2.6 \pm 0.2	92.2***
16	β -Elemene	9.8	1375	1376	1.7 \pm 0.2	2.4 \pm 0.1	2.1 \pm 0.1	0.5 \pm 0.1	0.7 \pm 0.1	97.1***
17	α -Copaene	11.1	1376	1377	2.4 \pm 0.1	2.5 \pm 0.1	2.4 \pm 0.1	1.2 \pm 0.2	2.9 \pm 0.2	79.1***
18	β -Caryophyllene	11.3	1418	1418	2.8 \pm 0.2	0.8 \pm 0.1	2.1 \pm 0.1	1.3 \pm 0.3	2.1 \pm 0.1	47.9***
19	Germacrene D	11.6	1480	1480	1.8 \pm 0.2	3.5 \pm 0.5	2.8 \pm 0.2	0.7 \pm 0.1	1.8 \pm 0.1	45.3***
20	Davanone	12.1	1586	1688	1.4 \pm 0.2	1.4 \pm 0.2	0.7 \pm 0.1	1.7 \pm 0.2	1.8 \pm 0.2	16.3***
21	T-Muurolol	12.6	1632	1634	1.6 \pm 0.1	0.8 \pm 0.1	1.8 \pm 0.2	1.4 \pm 0.1	1.7 \pm 0.1	29.4***
22	Methyl cis-Jasmonate	13.1	1647	1648	1.9 \pm 0.1	0.5 \pm 0.1	0.9 \pm 0.1	1.6 \pm 0.1	2.9 \pm 0.3	6.4***
23	β -Eudesmol	13.6	1649	1649	12.4 \pm 0.4	13.1 \pm 0.3	13.9 \pm 0.4	14.8 \pm 0.5	12.7 \pm 0.9	54.6***
24	α -Cadinol	14.1	1653	1653	1.8 \pm 0.2	0.9 \pm 0.1	1.2 \pm 0.2	0.4 \pm 0.1	1.5 \pm 0.1	40.0***
25	α -Bisabolol	14.6	1683	1684	1.7 \pm 0.2	1.4 \pm 0.1	1.5 \pm 0.1	0.4 \pm 0.1	2.3 \pm 0.2	44.3***
MCH, 1–7					9.8 \pm 0.2	9.2 \pm 0.2	7.6 \pm 0.1	9.4 \pm 0.2	6.8 \pm 0.3	66.5***
MCHO, 8–15					58.9 \pm 0.5	62.3 \pm 0.3	61.5 \pm 0.5	65.4 \pm 0.4	60.9 \pm 0.5	162.4***
SCH, 16–19					8.7 \pm 0.2	9.2 \pm 0.2	9.4 \pm 0.1	3.7 \pm 0.1	7.5 \pm 0.2	231.0***
SCHO, 20–25					20.8 \pm 0.2	18.1 \pm 0.2	20.0 \pm 0.9	20.3 \pm 0.3	22.9 \pm 0.4	38.7***
Total detected					98.2	98.8	98.5	98.8	98.1	

Values are given as mean \pm SD

ns non significant

* $P < 0.05$

*** $P < 0.001$

components and various chemical classes were highly significant ($P < 0.001$) except the changes in methyl cis-jasmonate were moderate significant ($P < 0.01$) for Fe treatments.

Changes in growth, EO, and nutrient content in response to Mg treatments

Data presented in Table 3 showed the response of growth characters (plant height, fresh and dry weight of aerial parts) in *A. abrotanum* plant to the different rates of Mg. The data indicated that all Mg

treatments overcame the control treatment and improved the vegetative growth characters during both seasons. Greatest values of growth characters at each season for all variables obtained in the 8 g/L rate with values of 58.7, 51.3 cm; 219.4, 48.6 g/plant; and 48.6, 58.7 g/plant during the first and second seasons, respectively. The increments in all growth measurements were highly significant ($P < 0.001$) for Mg treatments of both seasons.

Responses of EO extracted from *A. abrotanum* plant to the different rates of Mg are presented in Table 3.

Table 3 Effect of Mg treatments on growth and chemical content

Mg treatments (g/L)	Growth characters			EO		Nutrient					
	PH	FW	DW	%	Yield	Content (%)			Uptake (g/plant)		
						N	P	K	N	P	K
First season											
0	42.8 ± 0.4	81.7 ± 0.3	28.7 ± 0.3	0.1 ± 0.0	2.9 ± 0.1	2.1 ± 0.1	0.1 ± 0.0	2.9 ± 0.1	0.6 ± 0.1	0.1 ± 0.1	0.8 ± 0.2
2	55.3 ± 0.3	162.3 ± 0.3	39.7 ± 0.4	0.2 ± 0.1	7.9 ± 0.2	2.3 ± 0.1	0.2 ± 0.1	3.1 ± 0.1	0.9 ± 0.1	0.1 ± 0.0	1.2 ± 0.3
4	55.7 ± 0.7	193.3 ± 0.9	43.8 ± 0.2	0.2 ± 0.1	8.8 ± 0.2	2.4 ± 0.1	0.2 ± 0.1	3.2 ± 0.1	1.0 ± 0.2	0.1 ± 0.0	1.4 ± 0.2
6	57.1 ± 2.1	199.2 ± 3.2	44.3 ± 0.9	0.2 ± 0.1	8.9 ± 0.1	2.6 ± 0.2	0.2 ± 0.1	3.5 ± 0.3	1.2 ± 0.4	0.1 ± 0.0	1.6 ± 0.3
8	58.7 ± 2.1	219.4 ± 4.8	48.6 ± 7.2	0.3 ± 0.1	14.6 ± 0.5	2.7 ± 0.1	0.4 ± 0.1	3.7 ± 0.3	1.3 ± 0.1	0.2 ± 0.1	1.8 ± 0.4
F values	506.2***	93232.2***	2058.3***	1.8 ns	1126.8***	10.6***	4.5*	19.1***	10.2***	3.0 ns	15.8***
Second season											
0	43.7 ± 2.8	88.1 ± 6.7	29.2 ± 2.1	0.1 ± 0.1	2.9 ± 0.1	1.8 ± 0.6	0.1 ± 0.0	2.6 ± 0.4	0.5 ± 0.1	0.1 ± 0.0	0.8 ± 0.2
2	45.1 ± 4.4	129.1 ± 5.1	32.5 ± 0.6	0.2 ± 0.1	6.5 ± 0.5	2.1 ± 0.2	0.2 ± 0.1	2.7 ± 0.5	0.7 ± 0.3	0.1 ± 0.0	0.9 ± 0.2
4	48.7 ± 7.1	200.3 ± 9.3	44.8 ± 4.1	0.2 ± 0.1	9.1 ± 0.7	2.3 ± 0.3	0.3 ± 0.1	2.9 ± 0.6	1.0 ± 0.1	0.1 ± 0.0	1.3 ± 0.3
6	49.2 ± 6.1	212.7 ± 5.3	47.8 ± 5.9	0.2 ± 0.1	9.6 ± 0.6	2.5 ± 0.5	0.3 ± 0.1	3.1 ± 0.1	1.2 ± 0.1	0.1 ± 0.0	1.5 ± 0.3
8	51.3 ± 0.1	281.7 ± 9.8	58.7 ± 7.6	0.3 ± 0.1	17.6 ± 1.6	2.6 ± 0.2	0.5 ± 0.2	3.6 ± 0.4	1.5 ± 0.3	0.2 ± 0.1	2.1 ± 0.2
F values	456.1***	239083.2***	4668.4***	1.8 ns	748.0***	19.3***	8.2*	29.4***	16.8***	3.0 ns	58.2***

Values are given as mean ± SD

ns insignificant

***P < 0.001

The data revealed that various Mg rates improved the EO content (% or yield). The highest values of EO contents (0.3, 0.3%; 14.6, 17.6 mL/100 plants) were recorded with the rate of 8 g/L. The increases in EO contents were insignificant.

The effects of Mg doses on N, P, and K content and its uptake by *A. abrotanum* plant are shown in Table 3. The data show that the application of different doses of Mg gave a slight increase in N, P, and K content and its uptakes as compared to the control in both seasons. Eight grams per liter rate resulted in the highest amounts of mineral contents (2.7, 2.6%; 0.4, 0.3%; 3.7, 3.6%) and their uptakes (1.3, 1.5 g/plant; 0.2, 0.2 g/plant; 1.8, 2.1 g/plant) of both seasons. The increases in N and K contents were highly significant (P < 0.001) for Mg rates while the increases in P content were significant (P < 0.05). Regarding to the increments in N and K uptakes were highly significant (P < 0.001), but the increases in P uptakes were insignificant.

Results in Table 4 reveal an effect of Mg on the constituents of EO extracted from *A. abrotanum* herb. A qualitative and quantitative comparison of the components present with the Mg doses in the hydro-distilled *A. abrotanum* EO was performed. The identified components and their percentages are given in Table 4. The variations in all components are important between the Mg doses. The major components (2-hydroxy-1, 8-cineole, β-eudesmol, and camphor) increased under various Mg treatments. The highest values of major constituents (42.1, 15.7, and 14.2) resulted from the plants treated with

8 g/L of Mg. Control treatment resulted in the greatest amounts of MCH (14.1%) and SCH (8.5%) classes. The highest values of SCHO (24.0%) and MCHO (64.8%) obtained from the treatments of 2 and 6 g/L of Mg, respectively. The changes in all identified components and chemical classes were highly significant (P < 0.001) except in camphene components were significant (P < 0.05).

Changes in growth, EO, and nutrient content in response to Mn treatments

The responses of growth measurements (plant height, fresh and dry weight of aerial parts) to Mn levels are presented in Table 5. Highly significant increases (P < 0.001) were found in all growth measurements due to Mn application compared with control in both seasons. 300 milligrams per liter level resulted in the highest values of plant height (52.6 and 49.8 cm), fresh weight (173.1 and 174.7 g/plant), and dry weight (65.2 and 55.9 g/plant) during the first and second seasons, respectively.

As shown in Table 5, EO content increased at all Mn levels during both seasons. The highest accumulations of EO (0.3, 0.3%; 19.6, 16.8 mL/100 plants) were recorded at the highest Mn level (300 mg/L) compared with control treatment (0.0) during the first and second seasons. The increase in EO (%) was insignificant, but it was highly significant (P < 0.001) in EO yield.

The application of Mn levels caused an increase in measured nutrient content such as N, P, and K (Table 5), in both seasons. Mn (300 mg/L) resulted in the highest nutrient accumulation (1.4, 1.5%; 0.3, 0.4%; 3.1, 2.9%) and

Table 4 Effect of Mg treatments on EO constituents

No.	Components	RT	RI ^L	RI ^C	Mg treatments (g/L)					F values
					0	2	4	6	8	
1	α-Pinene	2.2	939	939	1.9 ± 0.1	0.7 ± 0.1	0.6 ± 0.1	1.3 ± 0.1	0.8 ± 0.2	54.9***
2	Camphene	2.5	953	955	1.2 ± 0.2	1.1 ± 0.1	1.1 ± 0.5	1.5 ± 0.3	1.3 ± 0.3	5.3*
3	Sabinene	2.8	976	978	1.6 ± 0.1	1.3 ± 0.1	2.2 ± 0.2	1.5 ± 0.1	0.8 ± 0.2	35.1***
4	Myrcene	3.2	991	991	2.7 ± 0.2	0.7 ± 0.1	2.7 ± 0.2	1.1 ± 0.1	1.1 ± 0.1	126.5***
5	p-Cymene	3.8	1026	1026	1.5 ± 0.1	1.2 ± 0.2	1.3 ± 0.1	0.7 ± 0.1	1.2 ± 0.3	11.9***
6	Limonene	4.3	1031	1032	2.3 ± 0.3	2.2 ± 0.2	0.9 ± 0.1	1.8 ± 0.2	1.1 ± 0.2	31.8***
7	γ-Terpinene	5.3	1062	1062	2.9 ± 0.1	1.4 ± 0.1	1.7 ± 0.3	1.5 ± 0.3	2.4 ± 0.3	78.2***
8	Camphor	5.7	1143	1143	11.8 ± 0.4	12.1 ± 0.5	12.8 ± 0.8	13.6 ± 0.5	14.2 ± 0.9	108.2***
9	Borneol	6.1	1165	1166	2.2 ± 0.2	1.5 ± 0.2	2.4 ± 0.3	1.8 ± 0.2	0.7 ± 0.2	61.0***
10	Terpinen-4-ol	6.8	1177	1177	0.9 ± 0.1	0.9 ± 0.1	1.3 ± 0.1	1.9 ± 0.1	0.8 ± 0.2	39.0***
11	Myrtanal	7.2	1188	1189	0.4 ± 0.1	1.9 ± 0.1	0.9 ± 0.1	2.5 ± 0.2	2.3 ± 0.3	249.0***
12	α-Terpineol	7.9	1189	1189	1.3 ± 0.1	0.8 ± 0.1	1.2 ± 0.2	1.8 ± 0.3	0.8 ± 0.2	23.5***
13	2-Hydroxy-1,8-cineole	8.8	1219	1219	37.9 ± 0.5	38.4 ± 0.4	38.9 ± 0.8	41.3 ± 0.9	42.1 ± 0.9	186.0***
14	Terpenyl acetate	9.1	1352	1352	0.7 ± 0.2	2.6 ± 0.1	0.6 ± 0.1	0.8 ± 0.2	2.1 ± 0.1	135.4***
15	Eugenol	9.5	1356	1356	2.1 ± 0.1	1.6 ± 0.1	0.5 ± 0.1	1.1 ± 0.1	2.4 ± 0.4	109.3***
16	β-Elementene	9.8	1375	1376	2.4 ± 0.2	1.1 ± 0.1	3.2 ± 0.2	0.8 ± 0.2	0.9 ± 0.2	121.8***
17	α-Copaene	11.1	1376	1377	1.5 ± 0.2	2.8 ± 0.1	0.8 ± 0.1	1.8 ± 0.3	1.4 ± 0.4	73.4***
18	β-Caryophyllene	11.3	1418	1418	1.9 ± 0.1	1.6 ± 0.1	0.8 ± 0.1	0.7 ± 0.2	1.2 ± 0.2	49.3***
19	Germacrene D	11.6	1480	1480	2.7 ± 0.1	1.5 ± 0.2	2.1 ± 0.2	1.9 ± 0.2	1.6 ± 0.3	68.4***
20	Davanone	12.1	1586	1688	2.1 ± 0.1	2.2 ± 0.2	1.6 ± 0.1	0.9 ± 0.1	0.8 ± 0.2	80.1***
21	T-Muurolol	12.6	1632	1634	0.4 ± 0.1	1.3 ± 0.1	1.6 ± 0.1	0.8 ± 0.2	0.7 ± 0.3	31.8***
22	Methyl cis-Jasmonate	13.1	1647	1648	2.6 ± 0.1	2.1 ± 0.2	2.2 ± 0.2	0.6 ± 0.1	0.9 ± 0.3	143.8***
23	β-Eudesmol	13.6	1649	1649	12.9 ± 0.3	13.8 ± 0.6	14.8 ± 0.4	15.1 ± 0.7	15.7 ± 0.6	35.9***
24	α-Cadinol	14.1	1653	1653	0.7 ± 0.1	2.9 ± 0.3	1.6 ± 0.1	0.9 ± 0.2	1.1 ± 0.1	185.7***
25	α-Bisabolol	14.6	1683	1684	0.5 ± 0.1	1.7 ± 0.2	1.6 ± 0.1	1.4 ± 0.3	1.3 ± 0.3	42.2***
MCH, 1–7					14.1 ± 0.3	8.6 ± 0.3	10.5 ± 0.5	9.4 ± 0.4	8.7 ± 0.8	149.5***
MCHO, 8–15					57.3 ± 0.5	59.8 ± 1.2	58.6 ± 0.7	64.8 ± 1.2	65.4 ± 2.1	69.8***
SCH, 16–19					8.5 ± 0.2	7.0 ± 0.5	6.9 ± 0.7	5.2 ± 0.2	5.1 ± 0.5	23.1***
SCHO, 20–25					19.2 ± 0.3	24.0 ± 0.4	23.4 ± 0.5	19.7 ± 0.7	20.5 ± 0.5	46.6***
Total detected					99.1	99.4	99.4	99.1	99.7	

Values are given as mean ± SD

ns non significant

* $P < 0.05$

*** $P < 0.001$

uptakes (0.9, 0.8%; 0.2, 0.2%; 0.2, 1.6%) while the lowest mineral content was observed in the control treatment. The increases in N and K contents were highly significant ($P < 0.001$) for Mn rates while the increases in P content were insignificant. On the other hand, the increments in N and P uptakes were insignificant, but the increases in K uptakes were highly significant ($P < 0.001$).

The application of different levels of Mn affected the EO constituents and various chemical classes of *A. abrotanum* (Table 6). The major constituents (2-hydroxy-1,

8-cineole, β-eudesmol, and camphor) gradually increased with Mn treatments. The greatest amounts of major constituents (42.7, 14.7, and 13.9%) resulted from the plants treated with 300 mg/L of Mn. The highest values of hydrocarbons classes (MCH, 11.9% and SCH, 8.7%) were detected under control treatment while the greatest amounts of oxygenated classes (MCHO, 64.6% and SCHO, 22.5%) resulted from the treatments of 50 and 300 mg/L. The changes in all components and chemical classes were highly significant ($P < 0.001$) except the changes in α-bisabolol were significant ($P < 0.05$).

Table 5 Effect of Mn treatments on growth and chemical content

Mn treatments (mg/L)	Growth characters			EO		Nutrient					
	PH	FW	DW	%	Yield	Content (%)			Uptake (g/plant)		
						N	P	K	N	P	K
First season											
0	38.9 ± 3.8	68.4 ± 6.7	25.7 ± 2.1	0.1 ± 0.0	2.5 ± 0.5	0.8 ± 0.2	0.1 ± 0.0	1.4 ± 0.2	0.2 ± 0.1	0.1 ± 0.0	0.4 ± 0.1
50	42.3 ± 6.4	116.7 ± 8.9	39.3 ± 4.4	0.2 ± 0.1	7.9 ± 0.8	0.9 ± 0.2	0.2 ± 0.1	1.9 ± 0.3	0.4 ± 0.1	0.1 ± 0.9	0.7 ± 0.2
100	43.7 ± 9.1	134.1 ± 8.3	42.8 ± 9.2	0.2 ± 0.1	8.6 ± 0.6	1.1 ± 0.1	0.2 ± 0.1	2.2 ± 0.4	0.5 ± 0.1	0.1 ± 0.0	0.9 ± 0.3
200	50.9 ± 8.3	158.3 ± 11.1	48.9 ± 8.4	0.2 ± 0.1	9.8 ± 0.7	1.2 ± 0.1	0.2 ± 0.1	2.8 ± 0.3	0.6 ± 0.2	0.1 ± 0.0	1.4 ± 0.3
300	52.6 ± 5.1	173.1 ± 9.8	65.2 ± 7.1	0.3 ± 0.1	19.6 ± 5.1	1.4 ± 0.1	0.3 ± 0.1	3.1 ± 0.2	0.9 ± 0.2	0.2 ± 0.1	2.0 ± 0.1
F values	1421.3***	19626.0***	11543.7***	1.8 ns	2225.6***	7.7**	1.9 ns	50.0***	1.1 ns	3.0 ns	54.1***
Second season											
0.0	35.1 ± 7.4	67.1 ± 7.1	20.6 ± 3.4	0.1 ± 0.0	2.2 ± 0.2	0.7 ± 0.1	0.1 ± 0.0	1.7 ± 0.2	0.1 ± 0.0	0.1 ± 0.0	0.4 ± 0.1
50	37.2 ± 6.5	108.7 ± 8.4	25.1 ± 4.1	0.2 ± 0.1	5.0 ± 0.3	0.8 ± 0.1	0.2 ± 0.1	1.9 ± 0.1	0.2 ± 0.1	0.1 ± 0.0	0.5 ± 0.2
100	38.9 ± 6.2	114.3 ± 9.6	29.6 ± 4.4	0.2 ± 0.1	5.9 ± 0.2	1.2 ± 0.2	0.2 ± 0.1	2.2 ± 0.2	0.4 ± 0.1	0.1 ± 0.0	0.7 ± 0.2
200	44.7 ± 4.4	168.8 ± 7.9	41.3 ± 7.8	0.2 ± 0.1	8.3 ± 0.3	1.4 ± 0.2	0.3 ± 0.1	2.5 ± 0.3	0.6 ± 0.1	0.1 ± 0.0	1.0 ± 0.2
300	49.8 ± 9.1	174.7 ± 8.4	55.9 ± 7.9	0.3 ± 0.1	16.8 ± 0.9	1.5 ± 0.3	0.4 ± 0.1	2.9 ± 0.3	0.8 ± 0.2	0.2 ± 0.1	1.6 ± 0.3
F values	3142.9***	72206.9***	6860.8***	1.9 ns	393.9***	6.6**	4.8 ns	21.4***	17.6 ns	3.0 ns	69.9***

Values are given as mean ± SD

ns insignificant

***P < 0.001

Discussion

In this investigation, obtained results revealed that treated *A. abrotanum* plants by individual trace elements (Fe, Mg, and Mn) resulted in positive effects on the growth, yield, EO composition, nutrients, and their uptake. These effects may be due to trace elements improve plant growth characters and metabolism leading to improve the quantity of the secondary metabolites, i.e., EO (Singh and Dwivedi 2019). Obtained results confirmed by previous literature. Fe plays critical roles in some metabolic processes such as chlorophyll and DNA synthesis and respiration; it serves as a constituent of various vital enzymes such as cytochromes of the electron transport chain, and it is thus required for different of biological activities (Rout and Sahoo 2015). Black cumin oil and its constituents were significantly affected in response to foliar application of Fe (Mohammadi et al. 2016). Different changes were found in fresh and dry mass, EO, and major constituents of EO of lemon balm due to Fe application (Yadegari 2017a, 2017b). The effect of Fe on growth and chemical composition of anise (*Pimpinella anisum* L.) was investigated (Pirzad and Barin 2018); obtained results recorded that foliar application of Fe enhanced growth, seed yield, EO, anethole, and NPK compared with control. Fresh and dry weights of *W. Murcott* mandarin were significantly increased with Fe application under high PH (Incesu et al. 2015). Significant increases were detected in fresh weight, EO composition, menthol, menthofuran, and nutrient uptake of mint and peppermint in response to Fe

supply (Pande et al. 2007; Lafmejani et al. 2018). Mg is required for chlorophyll molecule, mitochondrion, structural development of the chloroplast, and several of enzymes involved in energy transfer, particularly those utilizing ATP (Hall et al. 1972). Mg has influences on the ribosome structure and biosynthesis of some hormones such as gibberellines, auxins, and cytokinins which involved in protein synthesis. Marjoram plants treated with Mg resulted in higher biomass production, EO values, and nitrogen (N) contents than those untreated control (Cheol et al. 2001). Significant increments were detected in the quality and quantity of chamomile EO production in response to Mg application (Eva et al. 2004). The EO constituents of *Trachyspermum ammi* L. and nutrient contents (NPK) showed enhanced response to Mg application (El-Wahab and Mohamed 2007). Adding Mg caused significant increases in dry matter and nutrient uptake of Guinea grass (Fajemilehin et al. 2008). Khalid et al. (2009) showed that *Thymus vulgaris* L. plants treated with Mg produced significant increases in growth measurements, EO content, major components of EO, and nutrient contents (NPK). Mn is involved in photosynthesis, as an enzyme antioxidant cofactor and as a catalyst in the oxygen-evolving complex of photosystem (Millaleo et al. 2010; Schmidt et al. 2016). Kanwal et al. (2017) indicated that application of Mn could improve the fresh weight of herb and EO composition of *Ocimum sanctum* plant. Lemon balm (*Melissa officinalis* L.) plants were subjected to different treatments of Mn (0, 150, and 300 mg/L) (Yadegari

Table 6 Effect of Mn treatments on EO constituents

No.	Components	RT	RI ^L	RI ^C	Mn treatments (mg/L)					F values
					0	50	100	200	300	
1	α -Pinene	2.2	939	939	1.6 \pm 0.1	0.6 \pm 0.2	0.5 \pm 0.1	0.9 \pm 0.1	0.8 \pm 0.1	35.0***
2	Camphene	2.5	953	955	1.5 \pm 0.2	0.7 \pm 0.1	1.7 \pm 0.3	1.7 \pm 0.2	1.7 \pm 0.2	14.1***
3	Sabinene	2.8	976	978	1.2 \pm 0.2	1.7 \pm 0.3	1.6 \pm 0.3	1.1 \pm 0.1	2.1 \pm 0.3	22.2***
4	Myrcene	3.2	991	991	2.4 \pm 0.4	1.3 \pm 0.2	1.1 \pm 0.2	1.4 \pm 0.3	0.6 \pm 0.1	20.9***
5	p -Cymene	3.8	1026	1026	1.1 \pm 0.1	2.7 \pm 0.4	1.4 \pm 0.1	1.7 \pm 0.1	1.6 \pm 0.2	68.4***
6	Limonene	4.3	1031	1032	1.9 \pm 0.1	1.1 \pm 0.1	2.1 \pm 0.1	0.9 \pm 0.1	1.4 \pm 0.1	49.1***
7	γ -Terpinene	5.3	1062	1062	2.2 \pm 0.2	2.1 \pm 0.1	0.9 \pm 0.2	1.8 \pm 0.2	2.4 \pm 0.3	37.2***
8	Camphor	5.7	1143	1143	11.9 \pm 0.8	12.5 \pm 0.6	13.1 \pm 0.9	13.6 \pm 0.8	13.9 \pm 0.9	22.5***
9	Borneol	6.1	1165	1166	1.4 \pm 0.2	2.8 \pm 0.3	1.1 \pm 0.4	1.1 \pm 0.1	1.1 \pm 0.3	102.2***
10	Terpinen-4-ol	6.8	1177	1177	0.9 \pm 0.3	1.1 \pm 0.1	2.7 \pm 0.4	0.9 \pm 0.1	0.9 \pm 0.2	39.8***
11	Myrtanal	7.2	1188	1189	0.8 \pm 0.4	2.3 \pm 0.3	1.2 \pm 0.2	0.8 \pm 0.1	0.7 \pm 0.2	34.9***
12	α -Terpineol	7.9	1189	1189	1.6 \pm 0.2	1.9 \pm 0.2	0.7 \pm 0.1	1.5 \pm 0.3	0.9 \pm 0.3	75.6***
13	2-Hydroxy-1,8-cineole	8.8	1219	1219	38.3 \pm 0.5	38.9 \pm 0.9	39.6 \pm 0.8	41.8 \pm 0.8	42.7 \pm 0.7	138.9***
14	Terpenyl acetate	9.1	1352	1352	1.5 \pm 0.3	2.3 \pm 0.2	2.2 \pm 0.2	0.9 \pm 0.2	1.1 \pm 0.4	37.5***
15	Eugenol	9.5	1356	1356	1.9 \pm 0.2	2.8 \pm 0.5	0.7 \pm 0.1	1.3 \pm 0.2	2.1 \pm 0.3	59.8***
16	β -Elemene	9.8	1375	1376	2.1 \pm 0.1	1.9 \pm 0.3	2.3 \pm 0.3	1.8 \pm 0.3	0.5 \pm 0.1	67.1***
17	α -Copaene	11.1	1376	1377	1.9 \pm 0.1	2.2 \pm 0.2	2.7 \pm 0.4	0.6 \pm 0.1	1.2 \pm 0.2	73.6***
18	β -Caryophyllene	11.3	1418	1418	2.4 \pm 0.4	1.7 \pm 0.1	1.7 \pm 0.1	1.7 \pm 0.2	0.5 \pm 0.1	35.3***
19	Germacrene D	11.6	1480	1480	2.3 \pm 0.2	1.5 \pm 0.3	1.7 \pm 0.1	2.4 \pm 0.3	0.7 \pm 0.1	54.5***
20	Davanone	12.1	1586	1688	1.9 \pm 0.1	1.1 \pm 0.2	1.7 \pm 0.3	2.3 \pm 0.2	2.1 \pm 0.3	24.5***
21	T-Murolol	12.6	1632	1634	1.1 \pm 0.4	0.5 \pm 0.1	1.4 \pm 0.2	0.8 \pm 0.1	2.3 \pm 0.2	23.1***
22	Methyl cis-Jasmonate	13.1	1647	1648	2.3 \pm 0.3	0.8 \pm 0.1	1.4 \pm 0.3	1.7 \pm 0.4	1.9 \pm 0.3	25.1***
23	β -Eudesmol	13.6	1649	1649	12.7 \pm 0.9	12.9 \pm 0.9	13.2 \pm 0.7	13.9 \pm 0.9	14.7 \pm 0.8	35.8***
24	α -Cadinol	14.1	1653	1653	1.3 \pm 0.1	0.8 \pm 0.2	1.4 \pm 0.1	1.8 \pm 0.3	0.3 \pm 0.1	14.9***
25	α -Bisabolol	14.6	1683	1684	1.1 \pm 0.3	0.7 \pm 0.2	1.4 \pm 0.1	1.1 \pm 0.2	1.2 \pm 0.4	4.2*
MCH, 1–7					11.9 \pm 0.9	10.2 \pm 0.3	9.3 \pm 0.9	9.5 \pm 0.5	10.6 \pm 0.7	29.3***
MCHO, 8–15					58.3 \pm 2.1	64.6 \pm 4.1	61.3 \pm 5.1	61.9 \pm 0.8	63.4 \pm 1.1	168.1***
SCH, 16–19					8.7 \pm 0.8	7.3 \pm 0.4	8.4 \pm 0.4	6.5 \pm 0.6	2.9 \pm 0.4	102.9***
SCHO, 20–25					20.4 \pm 0.4	16.8 \pm 0.7	20.5 \pm 0.6	21.6 \pm 0.5	22.5 \pm 0.5	82.0***
Total detected					99.3	98.9	99.5	99.5	99.4	

Values are given as mean \pm SD

ns non significant

* $P < 0.05$

*** $P < 0.001$

2017a, 2017b); the treatment of 150 mg/L resulted in the greatest values of growth characters, EO composition, biosynthesis of terpenes, citronellal, and chavicol compared with control and other treatments. Senbayram et al. (2015) indicated that Mn treatments resulted in high availability of various elements in soil solution which causing an increase in the nutrient contents and plants uptake. On the other hand, the positive changes under various trace elements may be due to other environmental factors such as irrigation (Khalid and Ahmed 2017), fertilization (Yassen and Khalid 2009; Khalid

2013), soil properties (Ahmed et al. 2017), location (Ibrahim et al. 2014), and meteorological conditions (Khalid and El-Gohary 2014); all of them can induce changes in growth, yield, and chemical composition of medicinal and aromatic plants.

This study discovered that production of *A. abrotanum* with trace elements is required because the application of various trace elements produced significant variation in the growth, yield, and chemical composition *A. abrotanum*, and this study helps the farmers and pharmaceutical companies to increase the yield and

active ingredients (EO) of *A. abrotanum* as a natural source of drug industry.

Conclusion

It may be concluded that treated the *A. abrotanum* plants with trace elements (Fe, Mg, and Mn) produce significant variation in growth, yield, EO, major constituents of EO, and element contents. Plants treated with Fe (3 g/L), Mg (8 g/L), or Mn (300 mg/L) resulted in the highest values of growth, yield, and chemical composition of *A. abrotanum*. The effect of Fe, Mg, and Mn on the productivity of *A. abrotanum* has not been investigated before under Egyptian conditions, so this study will help the farmer and producers to increase the production and active substances from *A. abrotanum* as a source of natural products.

Abbreviations

DW: Dry weight (g/plant); EO: Essential oil; Fe: Iron; FW: Fresh weight (g/plant); GC: Gas chromatography; GC/MS: Gas Chromatography Mass Spectrometry; K: Potassium; MCH: Monoterpene hydrocarbons; MCHO: Oxygenated monoterpenes; Mg: Magnesium; Mn: Manganese; N: Nitrogen; P: Phosphorous; PH: Plant height; RI^C: Retention index calculated; RI^L: Retention index from literature; RT: Retention time; SCH: Sesquiterpene hydrocarbons; SCHO: Oxygenated sesquiterpenes

Acknowledgements

The authors would like to thank the National Research Centre (NRC) for its facilitates during this scientific work.

Authors' contributions

All authors have contributed significantly to the conception and design of the study, the interpretation of data, and the drafting and revision of the manuscript. All authors read and approved the final manuscript.

Funding

No specific fund was supplied for this work.

Availability of data and materials

The datasets supporting the results are included within the article.

Ethics approval and consent to participate

The manuscript does not contain studies involving human participants, human data, or human tissue.

Consent for publication

The authors declare that the work has consent for publication.

Competing interests

The authors declare that they have no competing interests.

Received: 30 November 2019 Accepted: 13 July 2020

Published online: 20 July 2020

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