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Full Length Article

Effect of Bio- and Nano-fertilization and Growing Media on Essential Oil Production and Chemical Constituents in *Pelargonium graveolens* Plants

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Abstract

The main goal of this work was to study effect of three types of bio-fertilization (Nitrobine, Phosphorine, Potasine) and foliar application of NPK-nano-fertilization on the chemical composition of *Pelargonium graveolens* L., plants grown in two types of soil mixture; sandy soil + compost (SC) and loamy soil + compost (LC) to achieve the desirable essential oil productivity and finding the best fertilization treatment safe for the environment. The results revealed that the highest values of chlorophyll a, b and carotenoids and essential oil percentage were obtained from the treatments of Nitrobine at the rate of 0.05 g for plants grown in the SC and Phosphorine at the rate of 0.05 g for plants grown in the LC, respectively followed by the nano-fertilization treatments 50 mg/L for plant grown in the LC. The main constituents of the oil were found to be. *β*-linalool (7.48%) resulted from Nitrobine at 0.05 g for plants grown in the SS treatment. Citronellol (26.76%) resulted from Nano-fertilization at 50 mg/L for plants grown in the LC. Geraniol (30.44%) resulted from phosphorene at 0.05 gm for plants grown in the LC and I-menthone (11.51%) resulted from nano-fertilization at 50 mg/L for plants grown in the LC. These results referred to the importance of bio- and nano-fertilization and reflect the importance of the interaction between fertilizer concentrations and soil mixture. The comparison between the first and second cuts in the two seasons showed the effect of environmental conditions on essential oil constituents, as these findings can help the plant growers to detect the suitable time for harvesting geranium plants. © 2024 Friends Science Publishers

Keywords: *Pelargonium graveolens*; Bio-fertilizer; Nano-fertilizer; Chlorophylls; Carotenoides; Essential oil

Introduction

Pelargonium gravolens, L. Heirt., which called Rose-Scented Geranium) belongs to the family *Geraniaceae*; it is a perennial herbaceous plant cultivated for medicinal and ornamental purposes (Abd El-Kafee *et al*. 2014). It is native to South Africa and widely cultivated in Egypt as a source of essential oil which is collected by steam distillation of the herb either immediately after harvest or after 24 h to reduce volume and release the oil from the pesticide form of the glycoside. The highest percentage of pharmaceutical components present in the essential oil is citronellol which is concentrated at 40–43%. Geraniol, 10-epi-*γ*-eudesmol, citronyl formate, geranyl formate, and isomenthone components were recorded as the second level (Gupta *et al*. 2016). Previous research reported that the essential oil of *P. graveolens* contains antioxidants and anti-inflammatory constituents (Ghanizadeh *et al*. 2015). Also, *P. gravolens* oil contains rhodinal, which is used for manufacturing of perfumes, soaps, and cosmetics (Mishra *et al*. 2010). It has been reported that there is a close relationship between plant nutrition and increasing herb and essential oil yields in aromatic plants. Nitrogen plays an essential role in the formation of proteins that make up leaf tissue, in addition to promoting plant growth and development. Due to the role of phosphorus in storing energy, it is responsible for nutrient transportation. Likewise, potassium plays an important role in retaining water and carbohydrates in plants (Tanious 2008; Hendawy and Khalid 2011).

Chemical fertilizers play an important role in increasing the yield of agricultural crops (Chaudhary *et al*. 2017) and the impressive results achieved by adding these fertilizers led to their excessive use. However, this has caused many environmental problems such as water pollution, soil fertility deterioration, high levels of heavy metals, and low biodiversity followed by a severe reduction in yield in addition to negative effects on human health (Arora 2018). At the same time, there is still an urgent need to increase agricultural production to feed 70% of the world's population and provide them with appropriate medicines to treat many diseases and epidemics (Mandal and Lalrinchani 2021). Several investigations related to

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long-term experiments with the use of chemical fertilizers and their effects on the production of active substances from medicinal and aromatic plants have been published. Therefore, over the past decade, great efforts have been made to replace chemical fertilizers with bio- and nano-fertilizers that are environmentally friendly and less harmful to human health (Mikhak *et al*. 2017).

Recently, many species of microorganisms are reported to be used as bio-fertilizers since it has been reported that they play a very important role in soil fertility improvement due to its capability to fix atmospheric nitrogen, both in combination with and without plant roots, dissolving insoluble soil phosphate and producing plant growth materials in the soil (Shahwar *et al.* 2023). This is in addition to its effective role in making plants more tolerant to drought and salinity stress in arid and semi-arid regions (Kumar *et al.* 2022). Bio-fertilizers have become a very compelling alternative when compared to chemical fertilizers because they are easy to apply, non-toxic, environmentally friendly, and cost-effective in nature. In the recent past, medicinal and aromatic plant research has mainly focused on the evaluation of bioactive plant molecules. However, there is limited knowledge of the effect of biofertilizers on essential oil quality. Another type of fertilizer that has been used recently is nano-fertilizer, which is a nutrient for plants in the form of nano-granules. Nanoparticles have been used in the field of plant production in the form of agricultural chemicals due to the discovery of their role in improving plant growth and plant tolerance to abiotic stresses since the nanoparticles have the capability of entering and transferring among plant cells because of their extremely small diameters compared to the diameters of plant cells (Kamiab *et al*. 2017; Manzoor *et al*. 2020). Nanomaterials usually have a role in the slow release of fertilizers, as the nanoparticles preserve the fertilizer very strong due to higher surface tension than conventional surfaces (Solanki *et al*. 2015), and thus they solve the problem of nutrients loss and runoff survivors that pollute the environment (Wilson *et al.* 2008). The applications of using nano-fertilizers in agricultural fields are numerous and varied, provided that investigations on the behavior and safety of these fertilizers are completed (El-Ghamry *et al.* 2018). Due to the role of nanomaterials as a catalyst for secondary metabolism, they can be used as new effective abiotic additives in plant biotechnology to stimulate the biosynthesis of secondary metabolic products (Al-Rekaby and Atiyah 2020), including essential oil. Nano-fertilizer applications have been done on the cultivation of medicinal and aromatic plants as in sweet basil (*Ocimum basilicum* L), treatment with F_3O_4 showed that nanoparticles improved plant growth (Elfeky *et al.* 2013; Mandal and Lalrinchani 2021), while treatment with a moderate rate of integrated nano-fertilizer (NPK) showed good results in leaf traits (Alhasan 2020). Also, nano-fertilizers were treated in *Crocus sativus* using nano iron, phosphorous and potassium (Amirnia *et al.* 2014), *Matricaria chamomilla* L. by nano-zeolite treatment (Mikhak *et al*. 2017), and *Mentha piperita* L. by iron. Zinc and potassium nanoparticles (Hassani and Tajali 2015).

In *P. graveolens* plants, bio- and nano-fertilizers were used in previous research. It was shown that treating the plant with bacterial chains of tannery sludge led to an increase in the uptake of heavy metals with an increase in the essential oil content and vegetative and root growth (Dharni *et al.* 2014; Gupta *et al*. 2016). Mishra *et al*. (2010) found that treatment of geranium plant with rhizomes leads to an increase in the proportion of essential oil, biomass of leaves and roots, and flavonoid contents (Riahi *et al.* 2020). As for the nano-fertilization, the role of nano-fertilization has been recently studied and it was found that it has the ability to improve the vegetative and root growth of the plant in addition to a significant increase in the chemical content (Osman and Adil 2022). However, studies on the use of nano-fertilizers on the geranium plant are still limited.

The main goal of this research is to determine the response of *P. graveolens* plants to the application of bio- and nano-fertilizers as an alternative to chemical fertilizers in terms of productivity and quality of the essential oil. Also, studying the changes that occur in the active compounds of the essential oil as a result of these treatments is the most important objective of this study. In this context, we performed two conducted experiments, which were fertilizing *P. graveolens* plants with biofertilizers like, Nitrobine, Phosphorine, Potasine, which they release nitrogen, phosphorus, and potassium, respectively while the second experiment was to study the effect of foliar application of nano-fertilizers on the studied plant productivity. To study the essential oil quality, gas chromatography-mass spectrometry (GC-MS) analysis was performed to determine the percentage of volatile oil components in all studied treatments. Due to the relationship between essential oil production and the quality of vegetative growth, the chlorophyll and carotenoid contents in the leaves of *P. graveolens* plants were studied in all studied treatments.

Materials and Methods

Plant materials and growth conditions

This study was conducted in the open field of Ornamental Horticulture Department Nursery, Faculty of Agriculture, Cairo University, Giza, Egypt during the two successive seasons of 2019 and 2020. Rooted cuttings of *P. graveolens* L. Heirt. were provided by Medicinal and Aromatic Plant Research Department, Horticulture Research Institute, Agricultural Research Centre, Cairo, Egypt. Then, they were transplanted into 20 cm diameter pots filled with eight kg of the growing media. After that, the plants were kept in the open field for 10 months per season (starting from March 1st until the end of December) with regular irrigation according to their need until 24 h before starting the treatments.

Growing media

Two mixtures of growing media were tested in this study along with the fertilization treatments which were loam + compost (LC) at the rate of 1: 1 and sand + compost (SC) at the rate of 1: 1. The average total weight for the growing medium was 8.5 and 9 kg, respectively. The physical and chemical components are shown in Table 1–2.

Bio-fertilizers applied

Commercial bio-fertilizers were provided by Agricultural Research Center, Giza, Egypt. Three bio-fertilizers, Nitrobine, Potasine, and Phosphorine, were mixed with the growing media at the rate of 1 or 0.05 g per/pot before transplanting the plants. The effective microorganisms of each bio-fertilizer are shown in Table 3.

Nano-fertilizer preparing, structure of the studied nano-fertilizer treatments

Chitosan solution was prepared by dissolving 0.23 g of chitosan powder (MW 71.3 kDa, degree of deacetylation (89%) Aldrich, Germany) in an aqueous solution of methacrylic acid (0.5%, v/v). Next, polymerization was performed at 75°C until a nanoparticle solution was obtained followed by centrifugation and cooling in a water bath. The NPK nano-fertilizer was obtained by loading the source of each element separately into chitosan nanoparticles (each element was dissolved in 100 mL of chitosan solution). The molecules in the resulting solution were with a size range of 17.5 nm for nitrogen, 16.1 nm for phosphorus, and 15.2 nm for potassium with a crystal structure and purity of 98.5% **(**Fig. 1).

The morphology and size of the nanoparticles were examined under a JEOL 1010 transmission electron microscope at 80 kV (JEOL, Japan). The nanoparticles' sizes were measured using Image-Pro Plus 4.5 software. The prepared NPK-nano-fertilizer, which was in concentration1000 mg/L, was applied in the growing medium every 15 days during one season at the rate of 50 and 100 mL/L of water.

Experimental design

The experiment was conducted in two seasons in 2019 and 2020) starting from March 1st 2019 until the end of December 2019 as a first season, and starting from March 1st 2020 until the end of December 2020 as a second season, with the aim of knowing the effect of two growing media types with different levels of bio-fertilizer, and nano-fertilizers to *P. graveolens* plants. The plants were cut two times per season, firstly at the end of may, and secondly in the end of August remaining 20 cm of the plants. Randomized completely blocks design (RCBD) was adapted to a factorial experiment consisting of two factors and three replicates (2 × 8 × 3). The first factor was two types of growing media, whereas the second factor was 8 levels of fertilization treatments, bio-fertilizer treatments were made at 6 levels of Nitrobine, Phosphorine, and Potasine at the rate of 1 and 0.05 g/pot, respectively, while, the nano-fertilizer treatments were done with two levels at concentrations of 50 and 100 mL/L water (v/v), and three replicates for each treatment distributed randomly in block and became the total units experimental were (2 × 8 × 3 = 48).

Essential oil determination

Essential oil were measured at Medicinal and Aromatic Plants Research Department, Pharmaceutical and Drug Industries Research Division, in National Research Center, Giza, Egypt. For each treatment, the herb was placed in a 2 L round-bottomed flask with distilled deionized water (400 mL for 200 g fresh herb) and the essential oil was extracted by water distillation using a modified Clevenger trap.

GC-MS analysis

This analysis was done in Medicinal and Aromatic Plants Research Department, Pharmaceutical and Drug Industries Research division, in National Research Center, Giza, Egypt. The essential oil constituents of each treatment were determined using gas chromatograph (GC) according to Mihajlov-Krstev *et al*. (2011). A TRACE GC Ultra Gas Chromatographs (THERMO Scientific Corp., USA) was coupled with a THERMO mass spectrometer detector (ISQ Single Quadrupole Mass Spectrometer). The GC-MS system was equipped with a TG-WAX MS column (30 m x 0.25 mm internal diameter, 0.25 μm film thickness). Analyses were performed using helium as carrier gas at a flow rate of 1.0 mL/min and a split ratio of 1:10. Diluted samples (1:100 hexane, v/v) of 1 μL of the mixtures were always injected. Mass spectra were obtained by electron ionization (EI) at 70 eV, using a spectral range of m/z 40–450. Most of the compounds were identified using the analytical method: mass spectra (authentic chemicals, Wiley spectral library collection and NSIT library).

Chlorophyll and carotenoid determination

Chlorophyll measurement was performed using of N,N-Dimethylformamide method as described by Moran (1982). Extraction was done by immersing the leaves in N,N-Dimethylformamide. After that, the samples were transferred to the laboratory of Horticultural Research Institute, in Agricultural Research Center, Giza, Egypt. Spectrophotometric measurements were performed by means of CENTRAL LAB model 630 UV-VIS scanning spectrophotometer, calibrated at 703 nm, using the 1.5 nm band width measuring beam. After that, the conversion of the chlorophylls and carotenoids to their acidic derivative was done as described by Lichtenthaler and Buschmann (2001).

Table 1: Physical and chemical analysis of the used soil mixture sand + compost (1:1 ratio)

Method	Elements	Symbol (unit)	Result	Low	Medium	High	Very high
			2.8	0.3–0.7	0.8–1.2	1.3–3	>3
Soluble	Total salts	EC (mmhos/cm)	1792	192–448	512–768	832–1920	>1920
Water 1:2.5	pH	pH	8.4	5.5–6.6	6.5–7.5	7.5–8.2	>8.3
Walkley	Organic Mater	O.M. (%)	3.2	0.1–0.8	0.9–1.5	1.6–5	>5
Olsen	Available P	P (meq/L)	26.2	10–22	23–30	31–36	>36
Soluble	Calcium	Ca (meq/L)	12.4	50–100	101–250	250–450	>450
Soluble	Magnesium	Mg (meq/L)	8.2	0–50	----	51–100	>100
Soluble	Potassium	K (meq/L)	1	41–80	81–120	121–160	>160
Soluble	Sodium	Na (meq/L)	6.4	41–80	81–120	121–160	>160
Soluble	Carbonate	CO_3 (meq/L)	0	0	0	0	0
Soluble	Bicarbonate	HCO_3 (meq/L)	8.8	50–75	76–100	101–250	>250
Soluble	Chloride	Cl (meq/L)	18	30	45	60	>60
Soluble	Sulfur	SO_4 (meq/L)	1.2	4–7	7–11	11–15	>15
Calculation	Sodium adsorption ratio	SAR	1.99	0–5	1–10	10–15	>15
Soluble	Density	Density (g/cm^3)	1.05	----	----	----	----
Soluble	Nitrogen	N (%)	0.16	0	0	0	0
Calculation	Saturation present	SP (%)	21.4	10–15	16–20	21–28	>28

Table 2: Physical and chemical analysis of the used soil mixture loamy + compost (1:1 ratio)

Method	Elements	Symbol (unit)	Result	Low	Medium	High	Very high
			0.85	0.3–0.7	0.8–1.2	1.3–3	>3
Soluble	Total salts	EC (mmhos/cm)	544	192–448	512–768	832–1920	>1920
Water 1:2.5	pH	pH	8.3	5.5–6.6	6.5–7.5	7.5–8.2	>8.3
Walkley	Organic Mater	O.M. (%)	1.2	0.1–0.8	0.9–1.5	1.6–5	>5
Olsen	Available P	P (meq/L)	28.6	10–22	23–22	31–36	>36
Soluble	Calcium	Ca (meq/L)	4	50–100	101–250	250–450	>450
Soluble	Magnesium	Mg (meq/L)	2.4	0–50	----	51–100	>100
Soluble	Potassium	K (meq/L)	0.4	41–80	81–120	121–160	>160
Soluble	Sodium	Na (meq/L)	1.7	41–80	81–120	121–160	>160
Soluble	Carbonate	CO_3 (meq/L)	0	0	0	0	0
Soluble	Bicarbonate	HCO_3 (meq/L)	3	50–75	76–100	101–250	>250
Soluble	Chloride	Cl (meq/L)	4.8	30	45	60	>60
Soluble	Sulfur	SO_4 (meq/L)	0.7	4–7	7–11	11–15	>15
Calculation	Sodium adsorption ratio	SAR	0.95	0–5	1–10	10–15	>15
Soluble	Density	Density (g/cm^3)	1.12	----	----	----	----
Soluble	Nitrogen	N (%)	0.2	0	0	0	0
Calculation	Saturation present	SP (%)	27.8	10–15	16–20	21–28	>28

Table 3: Function and the microorganism's name in the used commercial bio-fertilizers

Name of product	Function	Name of microorganism
Nitrobine	Releasing **nitrogen** nutrient in the soil	*Azotobacter sp.*
Phosphorine	Releasing **phosphorus** nutrient in the soil	*Bacillus megatherium var. phosphaticum*
Potasine	Releasing **potassium** nutrient in the soil	*Bacillus circulans*

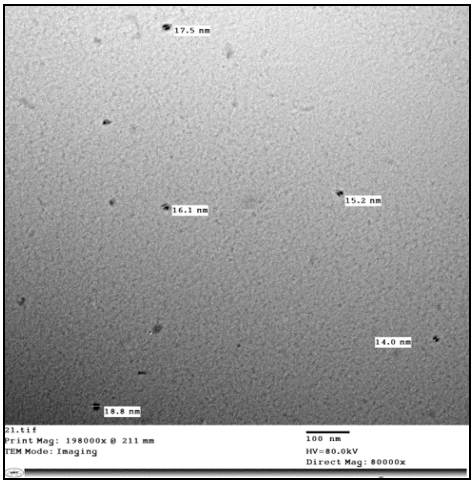

Fig. 1: Details of the NPK nanoparticles preparation

Statistical analyses

The data were subjected to statistical analysis according to Snedecor and Cochran (1969) using MSTAT-C which the variance was analyzed by ANOVA two factors followed by Duncan's multiple range test. The significant differences between mean values were determined at the level of $P \leq 0.05$.

Results

Essential oil percentage

Differences among all treatments: Concerning all treatments, we observed that treatments with Phosphorine bio-fertilizer at a concentration of 0.05 g/pot in the mixture of loamy soil + compost (LC) gave the highest percentage of volatile oil, which recorded 2.73 and 1.87% at the first and second cut, respectively during the first season and recorded 2.68 and 1.98% at the first and second cut, respectively during the second season (Table 4). The followed by treatment was NPK nano-fertilizer at a concentration of 50 mg/L in LC soil, which recorded 2.00 and 1.57% at the first and second cut, respectively during the first season and recorded 2.01 and 1.78% at the first and second cut, respectively during the second season. Nitrobine at the rate of 1 g/pot in LC soil recorded the lowest value after control (0.60 and 0.45% during the first season, and 0.57 and 0.58% during the second season) as shown in Table 4.

For comparative study between sandy and loamy soil mixture, the produced essential oil percentage was recorded from *P. graveolens* herbs after cutting for two times. Plants grown in the mixture of sandy soil + compost (SC) gave higher percentage of volatile oil at the first and second cut during first season (1.47 % and 1.08 %) compared with the percentage of produced oil from the plants grown in LC soil (1.31 and 0.98 %) (Table 4). At the second season, the mean essential oil percentage was 1.37 and 1.16% for the sandy soil and 1.34 and 1.09% in the first and second cut, respectively.

Differences between the efficiency of the mixture of SC and LC soils was dependent on the fertilization treatments. Significant (P value ≤ 0.05) differences were found in all fertilization treatments between sandy and loamy soils with the treatments of Nitrobine at 1 and 0.05 g/pot, Phosphorine at 0.05 g/pot, and NPK nano-fertilizer at 100 mg/L at the first cut, whereas at the second cut, significant differences were found between sandy and loamy soils except the treatments of control, and NPK nano-fertilization at 50 mg/L. During second season, similar results were found at the first cut except for the treatment with Potasine at 1 and 0.05 g/pot, NPK nano-fertilizer at the rate of 100 mg/L. At second cut, significant differences were noted between sandy and loamy soils with NPK nano-fertilizer at the rate of 100 mg/L.

Comparison between first and second cuts: Second cut performed at the end of July showed higher volatile oil percentage compared with the first cut performed at the end of May, which indicated the role of temperature in changing in the essential oil quantity. In the mixture of SC soil, significant differences (P value ≤ 0.05) between first and second cut in both two seasons were observed. No significant differences were observed in control, Nitrobine at 1 g/pot, Potasine at 1g/pot, and Potasine 0.05 g/pot in the first and second season. In the mixture of LC soil, significant differences (P value ≤ 0.05) between first and second cut in both seasons were also observed except control, Nitrobine 1 g/pot, Phosphorine 1 g/ pot, and Potasine 1 g/pot (Table 4).

Essential oil constituents

The total ion chromatogram recorded by GC–MS of the *P. graveolens* essential oil and the total chemical composition, which contained 32 identified compounds (Table 5). Control plants indicated the lowest percentage of the essential oil (Fig. 2). The treatment with nitrobeine @ 0.05 g/pot in SC soil gave the best result (Fig. 3). The main constituents of the essential oil were citronellol (12.44%), geraniol (26.96%), b-Linalool (7.48%), I-menthone (7.58%), citronellyl formate (6.94%), geraniol formate (6.45%), (-)-b-Brurbonene (0.92%) and geranyl tiglate (2.61%). The second-best result was recorded with the treatment of Phosphorine 0.05 g/pot in mixture of LC soil (Fig. 4). The main constituents of the essential oil were citronellol (12.56%), geraniol (30.44%), β-Linalool (3.87%), I-menthone (4.79%), citronellyl formate (0.59%), geraniol formate (9.90%), (-)-b-Brurbonene (1.21%) and geranyl tiglate (3.64%). The treatment of Potasine 0.05 g/pot in LC soil recorded as the third best result (Fig. 5). The main constituents of the essential oil were citronellol (11.52%), geraniol (27.23%), β-Linalool (1.98%), I-menthone (7.60%), citronellyl formate (8.21%), geraniol formate (7.85%), (-)-b-Brurbonene (1.39%) and geranyl tiglate (4.41%). The treatment with NPK nano-ferilizer 50 mg/L/pot in the mixture of LC soil ranked as 4th best treatment in respect of percentages of the chemical constituents (Fig. 6). The main constituents of the essential oil were citronellol (26.76%), geraniol (18.93%), β-linalool (1.7), I-menthone (3.1%), citronellyl formate (22.25%), geraniol formate (4.75%), (-)-b-brurbonene (0.38%) and geranyl tiglate (2.0%).

Chlorophyll and carotenoids content

Data showed clearly that the plants grown in SC soil and the mixture of LC soil produced the lowest record in chlorophyll a, chlorophyll b, and carotenoids in non-fertilized plants (control), while the plants grown in sandy soil gave the highest record when it was inoculated with Nitrobine at the rate of 0.05 g/pot in both first and second seasons, whereas the best result was given by the mixture of LC soil (Table 6). Treatment with Phosphorine at the rate of 0.05 g/pot also recorded the highest value in all fertilization treatments in

Table 4: Effect of bio- and nano-fertilization and their interaction with the soil types on essential oil percentage (%) of *P. graveolens* plants

Treatment	First cut		Second cut	
	SC	LC	SC	LC
			First season	
Control	0.20 ± 0.03^{dA}	0.23 ± 0.06^{eA}	0.14 ± 0.02^{gA}	0.18 ± 0.04^{fA}
Nitrobine 1 g/pot	1.73 ± 0.29^{bA}	0.60 ± 0.10^{dB}	1.20 ± 0.05^{cA}	0.45 ± 0.04^{eB}
Nitrobine 0.05 g/pot	2.40 ± 0.06^{aA}	0.70 ± 0.10^{dC}	1.69 ± 0.04^{aB}	0.47 ± 0.04^{eD}
Phosphorine1 g/pot	1.50 ± 0.06^{cA}	1.53 ± 0.07^{bA}	1.10 ± 0.03^{dC}	1.18 ± 0.03^{cB}
Phosphorine 0.05 g/pot	1.70 ± 0.06^{bB}	2.73 ± 0.50^{aA}	1.13 ± 0.03^{cC}	1.87 ± 0.05^{aB}
Potasine 1 g/pot	0.87 ± 0.24^{cA}	1.13 ± 0.31^{cA}	0.62 ± 0.05^{fB}	0.91 ± 0.04^{dA}
Potasine 0.05 g/pot	1.10 ± 0.21^{cA}	1.30 ± 0.02^{cB}	1.01 ± 0.02^{eC}	1.07 ± 0.03^{cB}
NPK Nano 100 mg/L	1.85 ± 0.01^{bA}	1.60 ± 0.20^{bA}	1.39 ± 0.01^{bB}	1.11 ± 0.06^{cC}
NPK Nano 50 mg/L	1.89 ± 0.06^{bA}	2.00 ± 1.00^{aA}	1.43 ± 0.03^{bA}	1.57 ± 0.06^{bA}
Mean	1.47	1.31	1.08	0.98
			Second season	
Control	0.22 ± 0.04^{fA}	0.25 ± 0.06^{fA}	0.18 ± 0.01^{gA}	0.19 ± 0.04^{hA}
Nitrobine 1 g/pot	1.71 ± 0.51^{cA}	0.57 ± 0.02^{eC}	1.36 ± 0.04^{cB}	0.58 ± 0.01^{fC}
Nitrobine 0.05 g/pot	2.38 ± 0.11^{aA}	0.69 ± 0.01^{eC}	1.80 ± 0.05^{aB}	0.41 ± 0.02^{gC}
Phosphorine1 g/pot	1.46 ± 0.10^{dA}	1.51 ± 0.54^{bA}	1.18 ± 0.03^{dC}	1.36 ± 0.01^{cB}
Phosphorine 0.05 g/pot	1.66 ± 0.11^{cC}	2.68 ± 0.45^{aA}	1.27 ± 0.03^{dD}	1.98 ± 0.03^{aB}
Potasine 1 g/pot	0.82 ± 0.32^{eB}	1.28 ± 0.24^{dA}	0.70 ± 0.04^{fB}	1.05 ± 0.04^{eA}
Potasine 0.05 g/pot	0.88 ± 0.35^{fC}	1.66 ± 0.23^{bA}	0.78 ± 0.02^{eC}	1.15 ± 0.03^{dB}
NPK Nano 100 mg/L	1.87 ± 0.2^{bA}	1.41 ± 0.01^{cB}	1.48 ± 0.01^{bB}	1.27 ± 0.04^{cB}
NPK Nano 50 mg/L	1.98 ± 0.08^{bA}	2.01 ± 0.01^{aA}	1.55 ± 0.03^{bB}	1.78 ± 0.02^{bB}
Mean	1.37	1.34	1.16	1.09

SC: sand + compost; LC: loam + compost. Mean values followed by the same uppercase letter in the row and lowercase in the column within the same factor are not significantly different according to the Duncan's multiple test ($P \leq 0.05$)

Table 5: Effect of bio- and nano-fertilization and their interaction with the soil types on essential oil constituents (%) of *P. graveolens* plants

Retention time (min)	Identification of essential oil	Soil type				
		Control (no addition)	Nano.50 mg/L Loamy soil + compost	Phos. 0.5 g Loamy soil + compost	Potas. 0.5 g Loamy soil + compost	Nitro. 0.5 g Sandy soil + compost
4.10	α-Pinene	0.05	--	--	--	0.69
5.47	β-Myrcene	--	--	0.48	--	0.37
9.16	β-Linalool	1.13	1.78	3.87	1.98	7.48
9.38	Rose oxide	0.73	5.84	--	3.57	1.27
11.25	Citronellal	0.17	--	--	0.48	--
11.67	l-Menthone	3.94	11.51	4.79	7.60	7.58
14.24	Citronellol	35.64	26.76	12.56	11.52	12.44
14.86	cis-Citral	--	--	--	0.79	1.28
15.25	Geraniol	14.1	18.93	30.44	27.23	26.96
15.94	Citronellyl formate	8.04	22.25	0.59	8.21	6.94
16.17	trans-Citral	--	--	4.98	3.71	3.72
17.07	Geraniol formate	1.41	4.75	9.90	7.85	6.45
19.41	Geraniol, trimethylsilyl ether	--	--	1.38	--	0.55
19.67	à-ylangene	0.20	--	--	--	--
19.74	Propanoic acid, 2-phenylethyl ester	--	--	0.51	--	0.35
19.94	β-Cubebene	1.29	--	0.49	0.43	0.35
19.99	(-)-β-Bourbonene	0.61	0.38	1.21	1.39	0.92
20.45	Nerol acetate	--	--	3.34	0.73	2.66
21.38	β-Phenylethyl butyrate	--	--	0.80	--	0.67
22.91	6-Octen-1-ol, 3,7-dimethyl-, propanoate	--	--	--	0.46	--
24.21	2,6-Octadien-1-ol, 3,7-dimethyl-, propanoate, (E)-	--	--	1.16	1.72	1.03
24.40	Germacrene D	0.15	--	1.92	0.62	1.67
25.61	Geranyl isobutyrate	--	--	2.41	0.67	0.68
26.15	cis-Calamene	0.46	--	--	1.38	0.50
27.56	Geranyl butyrate	0.26	--	--	--	0.41
28.85	Phenylethyl tiglate	--	--	5.94	7.68	6.47
30.37	γ-Eudesmol	15.87	--	4.13	3.88	5.20
31.48	Guaiol	1.14	--	0.66	0.55	0.40
32.62	Geranyl tiglate	2.21	0.23	3.64	4.41	2.61
49.28	Tetradecanamide	--	--	1.81	--	--
54.33	9-Octadecenamide, (Z)-	--	--	1.29	--	--
54.95	Octadecanamide	--	--	0.52	--	--

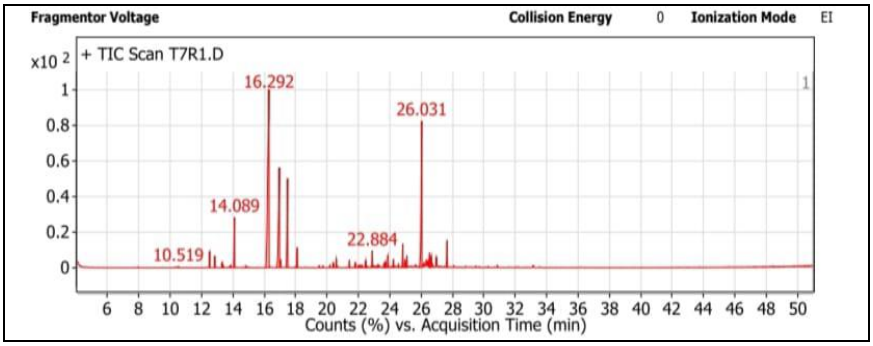

Fig. 2: GC-MS determination for the essential oil of *P. graveolens* harvested from the control plants

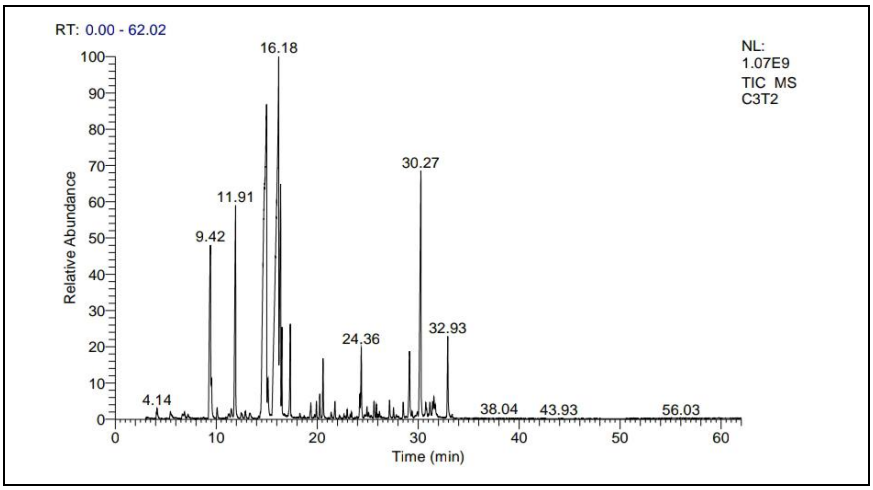

Fig. 3: GC-MS determination for the essential oil of *P. graveolens* harvested from the plants treated with Nitrobeine 0.05g /pot in sandy soil

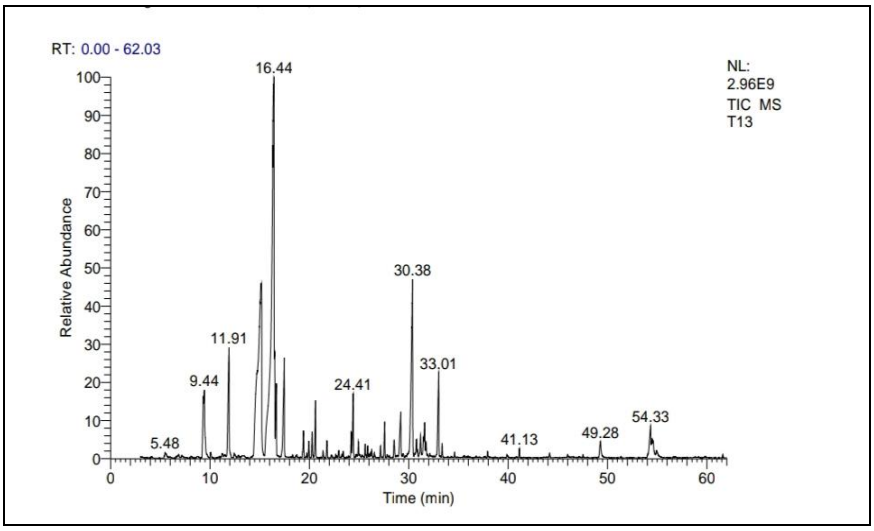

Fig. 4: GC-MS determination for the essential oil of *P. graveolens* harvested from the plants treated with Phosphorine 0.05g/pot in loamy soil

both first and second seasons. Nano-fertilizer treatments recorded as a second level after Phosphorine bio-fertilizer treatments which Nano-NPK fertilizer treatment at the concentration of 50 mg/L gave higher record compared with the fertilization ate the concentration of 100 mg/L (Table 6).

Discussion

In this work, we found that bio- and nano- fertilization increased the productivity of the essential oil compared with the control (Table 4–5). Likewise, a variation among all our

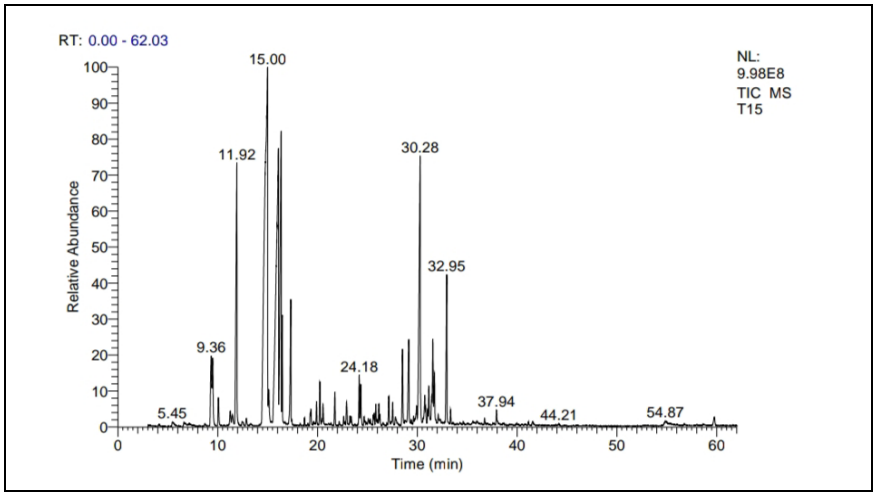

Fig. 5: GC-MS determination for the essential oil of *P. graveolens* harvested from the plants treated with Potasine 0.05g/pot in loamy soil

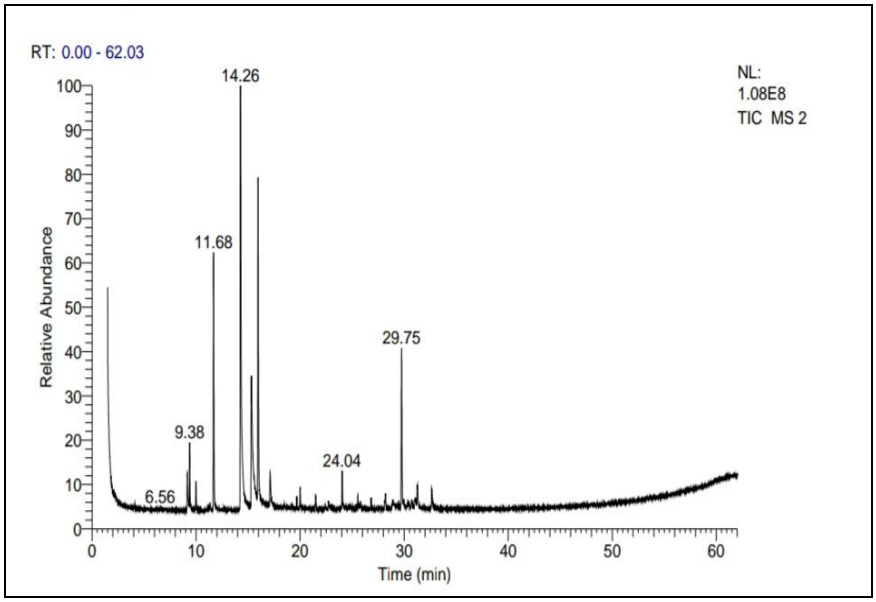

Fig. 6: GC-MS determination for the essential oil of *P. graveolens* harvested from the plants treated with NPK nano-fertilizer 50 mg/L/pot in loamy soil

treatments was found which the bio-fertilizer Phosphorine, the source of phosphorus, in the mixture of loamy soil + compost showed the highest record followed by Nitrobine, the source of nitrogen, in the mixture of loamy soil + compost, the mixture of sandy soil + compost (Table 4). The role of phosphorus and nitrogen in essential oil production has been studied in many research studies; e.g., it has been reported that the accumulation of essential oil constituents is influenced by the nitrogen and phosphorus fertilizer treatments in *Satureja montana* L. (Said-Al Ahl and Hussien 2016). Phosphorus is required for metabolic processes, which can enhance the amino acid production (Kisko *et al*. 2018). Hence, phosphorus fertilization has the ability to increase plant growth and essential oil yield in medicinal and aromatic plants (El-Ghandour *et al*. 2009; Erbaş *et al*. 2017). In *P. graveolens*, high oil yield was obtained with an increment of phosphorus concentrations (Sedibe and Allemann 2012; Abd El-Kafee *et al*. 2014). In this work, the phosphorus bio-fertilization (Phosphorine) increased the essential oil percentage suggesting the crucial role of phosphorus nutrient in essential oil production and the possibility of using this type of fertilizer instead of the chemical form (Table 4). Regarding nano-fertilization, it has been indicated that nanoparticles have a key role in increase of biochemical activities and reactivity (El-Ansary and Faddah 2010). It has been demonstrated that NPK nano-

Table 6: Effect of bio- and nano-fertilization and their interaction with the soil types on chlorophyll a and b, and carotenoid contents of *P. graveolens* plants

Treatment	First cut		Second cut	
	SC	LC	SC	LC
			Chlorophyll a (mg/g fresh weight)	
Control	0.24	0.24	0.26	0.25
Nitrobine 1 g/pot	0.38	0.29	0.40	0.30
Nitrobine 0.05 g/pot	0.69	0.32	0.71	0.33
Phosphorine1 g/pot	0.34	0.35	0.37	0.37
Phosphorine 0.05 g/pot	0.37	0.88	0.4	0.89
Potasine 1 g/pot	0.28	0.35	0.3	0.37
Potasine 0.05 g/pot	0.31	0.36	0.33	0.37
NPK Nano 100 mg/L	0.47	0.41	0.50	0.43
NPK Nano 50 mg/L	0.51	0.55	0.53	0.57
Mean	0.40	0.432	0.42	0.43
			Chlorophyll b (mg/g fresh weight)	
Control	0.05	0.07	0.06	0.07
Nitrobine 1 g/pot	0.34	0.14	0.35	0.15
Nitrobine 0.05 g/pot	0.58	0.21	0.59	0.22
Phosphorine1 g/pot	0.31	0.25	0.32	0.25
Phosphorine 0.05 g/pot	0.34	0.72	0.34	0.72
Potasine 1 g/pot	0.14	0.24	0.15	0.25
Potasine 0.05 g/pot	0.22	0.27	0.23	0.28
NPK Nano 100 mg/L	0.39	0.35	0.40	0.34
NPK Nano 50 mg/L	0.42	0.38	0.44	0.38
Mean	0.31	0.29	0.32	0.30
			Carotenoids (mg/g fresh weight)	
Control	0.09	0.06	0.11	0.07
Nitrobine 1 g/pot	0.24	0.10	0.26	0.09
Nitrobine 0.05 g/pot	0.43	0.13	0.45	0.15
Phosphorine1 g/pot	0.22	0.15	0.24	0.17
Phosphorine 0.05 g/pot	0.25	0.43	0.26	0.46
Potasine 1 g/pot	0.13	0.14	0.15	0.16
Potasine 0.05 g/pot	0.19	0.18	0.22	0.19
NPK Nano 100 mg/L	0.27	0.21	0.29	0.23
NPK Nano 50 mg/L	0.28	0.21	0.31	0.24
Mean	0.23	0.18	0.25	0.20

SC: sand + compost; LC: loam + compost

fertilizer has positive effects on essential oil yield in *Ocimum basilicum* (Elfeky *et al*. 2013; Alhasan 2020), and *Nigella sativa* (Azizi and Safaei 2016). Our results showed that NPK nano-fertilizer treatments increased the essential oil percentage in *P. graveolens* L. plants compared with control (Table 4). In comparison between bio- and nano-fertilization, we found that fertilization with Phosphorine and Nitrobine gave higher records than in nano-fertilizer treatments. This finding agrees with the results of Hegab *et al*. (2018) who tested the differences between bio- and nano-fertilization treatments in *Salvia officcinalis* L. plants. On the other hand, the treatments of NPK nano-fertilization gave a higher percentage of essential oil than Phosphorine in the mixture of sandy soil + compost, Nitrobine in the mixture of loamy soil + compost, and Potasine (Table 4).

Soil is the main source for providing plants with essential nutrients and water, in addition to the vital functions of many forms of terrestrial life, as it contains microorganisms that play a major role in the various biogeochemical cycles of nutrients (Singh 2015). Thus, soil components play essential role in soil ecosystem sustainability, and plant productivity including the essential oil components (Tuğrul 2019). So, the effects of bio- and nano- fertilization on the percentage of essential oil in *P. graveolens* should be dependent on the soil type. In this work, we found that loamy soil + compost recorded higher essential oil percentage than in the misxture of sandy soil + compost with the biofertilizers Phosphorine and Potasine whereas mixture of loamy soil was better with Nitrobine (Table 4).

It has been reported that high concentration of nitrogen fertilization reduces essential oil yield in *Juniperus horizontalis* (Robert and Francis 1986). However, high N concentration increased the essential oil content in *Thymus vulgaris* L. (Baranauskiene *et al*. 2003) and in *Cuminum cyminum* (Azizi and Kahrizi 2008). Also, high concentration of phosphorus decreased the volatile oil quantity of *Matricaria chamomilla* L. (Emongor *et al*. 1990) but increased in *Tanacetum parthenium* L. (Saharkhiz and Omidbaigi 2008) and in *Salvia officinalis* L. (Nell *et al*. 2009). In this work, lower concentration of bio-fertilization (0.05 g/pot compared with 1 g/pot) and nano-fertilization (50 mg/L compared with 100 mg/L) gave better result compared with higher (Table 4). It has been reported that nanoparticles and bio-fertilizers may be toxic to soil organisms and cultivated

plants both of them could be transported and up-taken by plants causing phytotoxicity (Vachan and Tripathi 2017). Our results found that a high concentration of bio- and nano-fertilizers gave a lower percentage of essential oil in *P. graveolens* (Table 4), indicating the toxicity of high rates of bio- and nano-fertilization.

The first two main oil compounds (citronellol and geraniol) produced from same progenitor (geranyl pyrophosphate) but it is assumed that different enzymes produced these two alcohols. This could be the reason for variation in the content of citronellol and geraniol with respect to the employed treatments. Our results clearly indicated the superiority of the low concentration of bio-fertilizers that gave the highest percentages of the main components of essential oil (Citronellol, Geraniol, β-Linalool, I-menthone, Citronellyl formate, Geraniol formate, and geranyl tiglate) which is in an agreement with the results of several scientists (Abdou *et al*. 2015; Negi *et al*. 2022). Also, the results of this research showed that the low concentration of Nano-fertilizer treatment gave high percentages of the essential oil constituents compared with the higher concentration. This result agreed with some previous investigations in other plant species (Ostadi *et al*. 2020; Bahmanzadegan *et al*. 2022).

Also, bio- and nano-fertilization treatments increased chlorophyll and carotenoid contents in *P. graveolens* plants (Table 6). Similarly, many investigations proved that chlorophyll a, b, and carotenoid increased with bio-fertilizers in many plant species, for example, but not limited, *Moringa oleifera* (Mazher *et al*. 2014), and *Cupressus sempervirens* (Youssef and Gharib 2013) since the bio-fertilizer treatments induce leaf photosynthesis and sucrose biosynthesis that is transported to the root and released into their rhizosphere (Trevisan *et al*. 2010). Regarding high records of the treatment with Phosphorine bio-fertilizers, phosphorus has a key role in the improvement of photosynthesis (Bisht and Chandel 1991); therefore, it can act as a way to influence the growth of the key enzyme of photosynthesis – carboxydismutase (Rao and Terry 1995) and thereby the photosynthetic activity of the plant.

Conclusion

In comparison between bio- and nano-fertilization, we found that bio-fertilizer treatment is the most recommended compared with NPK nano-fertilizers. Although, this is not a final indicator to make this type of comparison as more investigations are needed to find the differences in both fertilizations in respect of phosphorus sources. Concerning the comparison between the first and second cut, we can consider that this study is to show the effect of the environmental conditions on the essential oil constituents. It is extremely important for the farmers and plant breeder to detect the best time for harvesting *P. graveolens* plants according to the target of this cultivation.

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Author Contributions

Sarhan AZ conceived and designed the experiments; Emam KA; Magdy A performed the experiments and El-Tantawy AA analyzed the data.

Conflicts of Interest

The authors declare that no conflicts of interest to disclose.

Data Availability

All data will be available upon request.

Ethics Approval

The authors declare that all treatments for the studied plant follow the required research ethics.

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