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Effect of Some Plant Growth Biostimulants on Growth and Volatile Oils Productivity of Pelargonium graveolens Plants

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Abstract

The improper application of inorganic fertilizers to improve plant growth and productivity is damaging the environment and human health unlike other natural substances with could be efficient and environmentally friendly. *Pelargonium graveolens* is an aromatic plant that has been used in aromatherapy and traditional medicine. Plants were sprayed with bio-stimulants; brassinosteroids (BRs), seaweed extracts (SW), and chitosan (CH). The BRs were applied at 0, 0.1, 0.2 or 0.3 g/l whereas, SWs or CH at 0, 1 or 2 g/l were treated on the subplot. Plant fresh and dry weights, photosynthetic pigments, and volatile oils (VOs) percentages were significantly increased in the three cuts due to the BRs combined application. However, the total yield was significantly increased for all cuts due to both factors. In both seasons the 3rd cut had the highest herbage yield compared with the other ones. In the 1st season the lowest (427.3 g/plant) and highest (1132.0 g/plant) herb fresh weights in the 3rd cut were for untreated plants and (0.3 g/l BRs + 2 g/l CH) respectively. Also, the study demonstrated that the favorable effect of BRs on VOs% and yield of rose-scented geranium. The highest VO yields (7.35% being for plants treated with (BRs 0.3 g/l + 1g/l of CH or SW), was 184% higher than the lowest value (2.578 ml/plant) for untreated plants. Therefore, the investigation suggested that treated rose-scented pelargonium with BRs 0.3 g/l + SWs 1 g/l or BRs 0.3 g/l + CH 2 g/l for times could improve the VOs production.

Abbreviations: BRs: Brassinosteroids, CH: chitosan, SW: seaweed extracts, and VOs: volatile oils,

1. Introduction

Pelargonium graveolens L'Her (Rose-scented pelargonium, geranium) which belongs to the Geraniaceae family is an aromatic herb with a rose scent. It is native to various parts of Africa and cultivated all over the world, e.g. Russia, Morocco, China, and Egypt. It is cultivated for its valuable volatile oils (VOs) that are used in perfumery industry, and cosmetic products [1]. It is also used in aromatherapy and traditional medicine to relieve dysentery, infections, and cancer. Moreover, the plant has antioxidant, antibacterial, antifungal, and tick-killing properties and the valuable therapeutic features of geranium are mostly related to the presence of VOs, terpenoids, and phenolic complexes [2].

Plant biostimulants are any substance or microorganism that are applied to plants to stimulate their natural processes [3]. Brassinosteroids (BRs) which are a group of polyhydroxyl plant steroid hormones have a role in cell elongation and many other aspects of plant life controlling several important agronomic traits [4 and 5]. Seaweed (SW) is widely used in horticultural crops to promote their growth and development due to their content of complex polysaccharides, fatty acids, vitamins, plant hormones, and mineral nutrients [6]. Chitosan (CH) is a naturally occurring, biodegradable compound with many potential benefits for agriculture. It has been used to improve plant growth resulting in increased yields [7 and 8].

The application of *P. graveolens* with nano-BRs by [9, 10 and 11] significantly increased plant growth and VOs production. Similar observations were achieved on other medicinal and aromatic plants [12 and 13]. Seaweed extracts have been used to improve scented geraniums growth [14 and 15]. A significant increase in photosynthetic pigments, phenols, and proteins in plant tissues of *P. peltatum* following SWs application especially at 2.0% [16]. Chitosan was applied at a concentration up to 5% to many aromatic pants; *Matricaria chamomilla* [17]. and *Mentha arvensis* [18]. They concluded that CH could significantly improve the growth and VOs yield of these plants. Therefore, this study aimed to improve the growth and VOs production of rose-scented geranium plants by using some environment friendly compounds; BRs, SW, and CH.

2. Materials and methods

2.1. Experimental location and design

A randomized complete block design experiment in split plot arrangement [19] with three replicates was carried out during 2020/2021 and 2021/2022 seasons at the Hort. Dept., Fac. of

Agric., Minia Univ. Terminal stem cuttings about 15 cm long with 4-6 leaves were collected on the 20th of October from mother plants and cultured in 10-cm diameter plastic pots containing a mixture of sand and clay (1:3 v: v). After two months, plants were transferred into 3 x 2 m plots with 60 cm distance between the rows and 40 cm between plants in the row. So, each plot contained 5 rows and 25 plants. The physical and chemical properties of the experimental soil [20] are shown in table 1.

The main plot included BRs treatments at 0.0, 0.1, 0.2, and 0.3 g/l while CH or SWs treatments each at 1 and 2 g/l were applied as a sub-plot. These compounds which obtained from Itan Biotech Limited, India were foliar sprayed by hand spray till run off using triton at 0.05% as a wetting agent. The treatment commenced after 45 days of transplanting and repeated three times with 30 days intervals. The treatments were repeated after 30 days of each cut. Seaweed extracts and CH were sprayed the day after BRs treatment.

All tested plants were fertilized with N as ammonium sulphate (20.6% N) at 300 kg/fad. while, P and K fertilizers, were added at 200 and 50 kg/fad. of calcium superphosphate (15.5% P_2O_5) and potassium sulphate (48% K_2O), respectively. Nitrogen was divided into four equal batches; twice of them were applied after 30 and 45 days of transplanting while one dose was applied after each cut. Potassium fertilizer was added with the first batch of N fertilizer. Whereas P fertilizer was added during preparing the soil to cultivation in both experimental seasons. All other farming practices were carried by the habitats of the farmers.

Table (1): Physical and chemical analysis of the used soil

Soil characters	Value	Soil characters		Value
Soil type	Clay loamy	Avail. P (%)		5.00
Sand (%)	18	Exch. K(mg/100g)		4.70
Silt (%)	29	Exch.Ca (mg/100g)		3.1
Clay	53	Exch. Na (mg/100g).		1.74
(Org. Matt. (%)	2.00		Fe	8.39
			Cu	
CaCo3 (%)	3.60	DTPA Ext.(ppm)	Zn	2.04
PH (1:2.5)	8.27		Mn	2.81
E.C (mmhos/cm)	1.23			8.19
Total N (%)	0.10			

2.2. Data recorded

2.2.1. Herb yield

For each plant the above-ground vegetative growth at 10 cm from the soil surface was cut leaving two branches for regrowth. The harvesting was done 3 times; 8th of May, 8th of Sep., and 8 of Jan during both seasons. For each cut the fresh herb for individual plants was weighted before air-dried to calculate the dry weights. Also, the percentage of volatile oil was assessed using the fresh herb was using hydro-distillation in a Clevenger apparatus for 2 hours using 50 g of fresh material sample according to [21]. Volatile oils yield was calculated based on the fresh weights for each cut then the total yield/plant was estimated.

2.2.2. Photosynthetic pigments

Chlorophyll a and b as well as carotenoids were assessed [22] using 0.5 g of fresh leaves which collected just before the 3rd cut from the recently full expanded leaves. Ethanol 96% was used as an organic solvent and the absorbance was measured at A 665, A649 and A470 for chlorophyll a, b and carotenoids, respectively

2.2.3. Statistical analysis

Obtained data were subjected to analysis of variance (ANOVA) and the difference among the means was compared [23] using MSTAT program (version 4.0) edited by the MSTAT development team, Michigan University and Agricultural University of Norway. The differences were considered significant if P was at least ≤0.05.

3. Results and discussion

3.1. Herb fresh weights

The results showed that all investigated factors significantly affected the fresh herb weights of rose-scented geranium plants in both seasons. Moreover, there was a significant interaction between both investigated factors. Similar observations were estimated for the three cuts but for most treatments the 3rd one had higher weights (Figures 1 and 2).

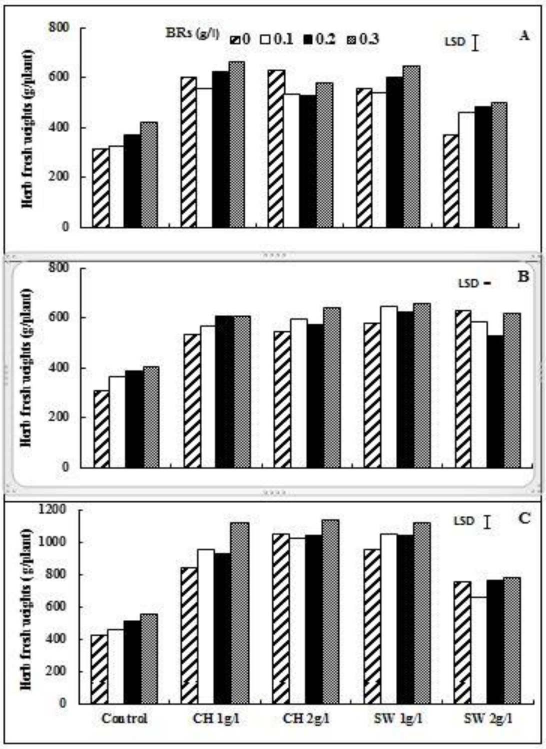

Figure 1: Effect of some plant biostimulants on the herb fresh weights (g/plant) of *Pelargonium garveolens* **(A)** 1st cut, **(B)** 2nd cut and **(C)** 3rd cut of the 1st season

BRs: Brassinolides, **CH:** Chitosan and **SWs:** Seaweed extracts.

Overall rose-scented geranium plants that did not treat with any PBs yielded gave the lowest fresh weight (427.3 g/plant).

However, the highest weights (1132.0 and 1121.0 g/plant being for plants treated with (0.3 g/l BRs + 2 g/l CH and 0.3 g/l BRs + 1 g/l SWs, respectively). These values were approximately double fold these of plants treated only with 0.3 g/l BRs. Interestingly under the same concentration of BRs increasing CH from 1 to 2 g/l significantly increased the herb fresh weights. On the other hand, increasing SWs concentration significantly reduced the herb fresh weight. Similar observation was recorded in the 2nd season with little variations

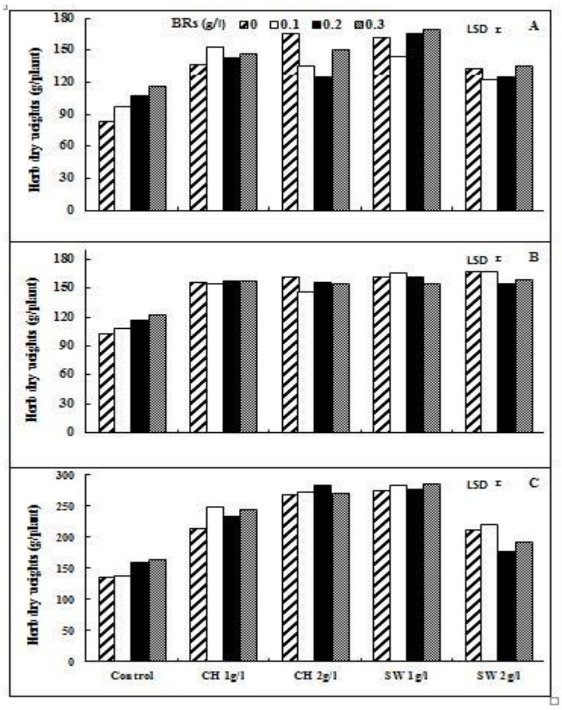

Figure 2: Effect of some plant biostimulants on the herb fresh weights (g/plant) of *Pelargonium graveolens* (**A)** 1st cut, **(B)** 2nd cut and **(C)** 3rd cut in the 2nd season.

BRs: Brassinolides, **CH:** Chitosan and **SWs:** Seaweed extract.

3.2. Herb dry weights

Rose-scented geranium herb dry weights of three cuts were varied ($p \leq 0.5$) in both seasons following CH/SWs application with a similar trend as in herb fresh weights. But BRs treatment had a significant effect on this trait only in the 1st and 3rd cuts. Nevertheless, a significant interaction between the two investigated factors was observed in all cases (Figures 3 and 4). Results showed that herb dry weights gradually increased from the 1st cut to last one.

Overall, under the same concentration of BRs any CH/SWs concentration significantly increased herb dry weights. However, unlike CH the lower concentration of SWs was preferable than the higher one. The lowest herb dry weights (about 137 g/plant) were found when plants treated only 0 or 0.1 g/l of BRs without significant difference between them. The best plant dry weights (285.1 g/plant) were estimated with no significant difference among this value and these obtained from plants treated with (0.2 g/l BRs + 2 g/l CH) and (0.1 g/l BRs + 1 g/l SW) (Fig. 3).

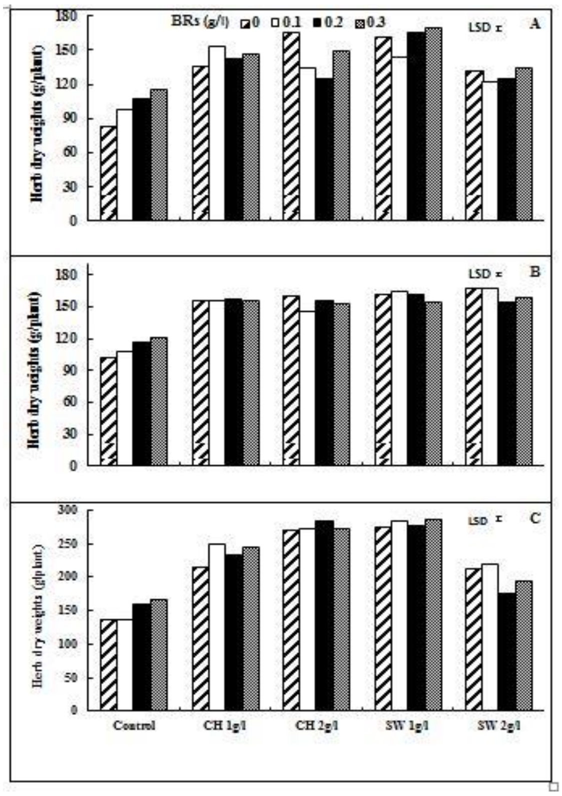

Figure 3: Effect of some plant biostimulants on the herb dry weights (g/plant) of *Pelargonium graveolens* **(A)** 1st cut, **(B)** 2nd cut and **(C)** 3rd cut of the 1st season.

BRs: Brassinolides, **CH:** Chitosan and **SWs:** Seaweed extracts

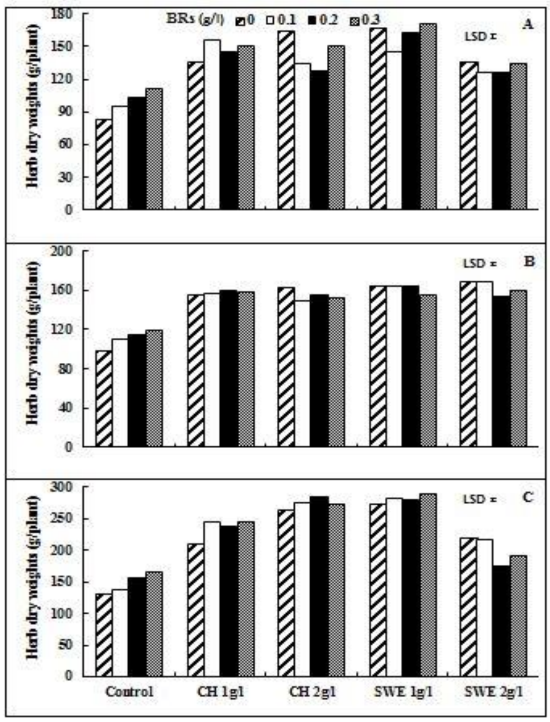

Figure 4: Effect of some plant biostimulants on the herb dry weights (g/plant) of *Pelargonium graveolens* (A) 1st cut, (B) 2nd cut and (C) 3rd cut in the 2nd season.

BRs: Brassinolides, CH: Chitosan and SWE: Seaweed extract.

Recent results showed that the economic herb fresh weights were increased in the three cuts by 13, 12 and 26% respectively, by increasing BRs from 0 to 0.3 g/l. However, the increase in herb dry weights (about 5%) due to this application was only significant in the 1st and 3rd cuts. Previous observation on growth of rose-scented geranium [10] and *Mentha piperita* [24].

Current study showed a pronounced effect of CH and SWs in herb fresh and dry weights however, unlike SWs higher concentration of CH had higher effect than the lower one. The growth of *M. arvensis* could be vary depending on the concentrations of CH [25]. The effect of CH in prompting plants responses depends on its degree of deacetylation, molecular weight, and concentration [26]. It improves plant physiological properties via stimulating plant immune system [27]. Moreover, [28 and 29] illustrated that this effect could be attributed to their content of amino acids, vitamins, and growth hormones; including as cytokinin and auxin which encourage a range physiological response, such as biomass. A similar response to SWs on other aromatic plants were previously estimated [14 and 15] on rose-scented geraniums.

3.3. Photosynthetic pigments

In both investigated seasons BRs have a significant effect on chlorophyll a, b and carotenoids content of rose-scented pelargonium plants. Alo, chlorophyll a and b was significantly increased with a similar trend in both seasons due to CH/SWs application.

The ANOVA of chlorophyll a, b and carotenoids showed significant interaction between the BRs and CH/SWs in both seasons. The lowest and highest chlorophyll a content in the 1st season 2.424 and 2.897 mg/g fresh weights were for untreated plants and (0.3 g/l BRs + 1 g/l CH). Almost CH 2 g/l and SWs 2g/l treated plants had a superior effect than other concentrations. Also, the lowest chlorophyll b (1.232 mg/g fresh weights) being for plants treated only with BRs 0.1 g/l nevertheless the highest content (1.768 mg/g fresh weight) was assessed for plants treated with (0.2 g/l BRs + 1 g/l CH). Rose-scented geranium plants had the lowest carotenoids (1.018 mg/g fresh weights) when treated only with 0.3 mg/g fresh weights (Figures 5 and 6).

The BRs application resulted in a significant increase in the CO_2 fixation which associated with elevated chlorophyll contents[10]. Our present study showed a significant increase in chlorophyll a and b and carotenoids of rose-scented geranium plants treated with BRs over untreated plants. So that the improving plant biomass following the BRs application might be due to improvement of carbohydrate profiles [10]. Increasing carotenoids content could improve plant growth and biomass [30]. Carotenoids function as accessory pigments improving light absorbance and photoprotection by their capacity to protect chlorophyll from reactive oxygen species [30].

There are many studies that showed the higher chlorophyll and carotenoids content in different plants as a result for applying BRs application which contributed with increased carbohydrates [10 and 31]. Mukarram *et al*. [32]. estimated an increase in chlorophyll content in lemongrass plants due to CH application. Zhang *et al*. [33] found that CH could suppress genes which encode for chlorophyllase enzyme, a catalyst for chlorophyll degradation, thus enhancing chlorophyll content. However, Salehi and Rezayatmand [34] suggested that CH increased the chlorophyll and carotenoids content by activating the expression of genes in the biosynthesis of photosynthetic pigments. Consequences this will enhance net photosynthetic [35]. Heng *et al*. [36] illustrated that increasing the photosynthesis of the plant by CH caused to increase the dry weight of the plant which was similar to our study.

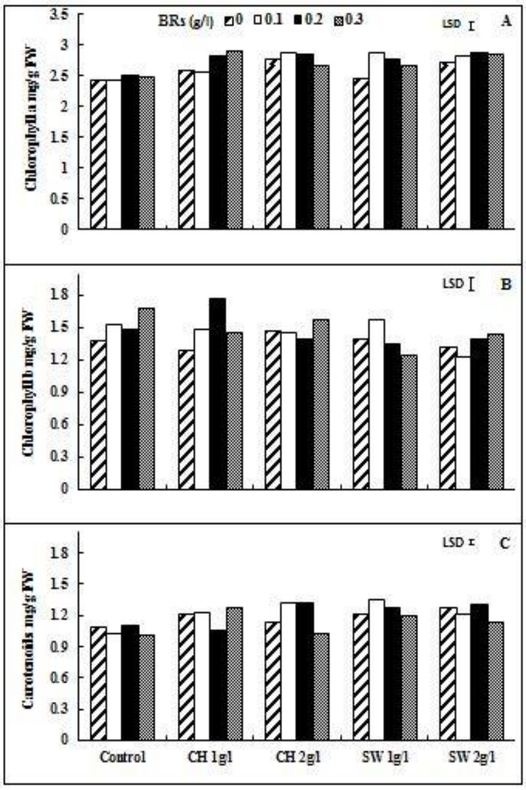

Figure 5: Effect some plant biostimulants on the **(A)** chlorophyll a , **(B)** chlorophyll b and **(C)** carotenoids (mg/g FW) of *Pelargonium graveolens* of the 1st season.

BRs: Brassinolides, **CH**: Chitosan and **SWs:** Seaweed extracts

3.4. Volatile oils

3.4.1. Volatile oils percentages

Unlike CH/SWs results showed that VO% of all cuts were significantly varied following the application of BRs with similar data in both seasons. Moreover, there was no significant interaction between the two factors. Also, overall results showed that VO% was increased in both seasons in the 2nd cut (0.260-0.337 %) than the 1st one (0.23 0.310%) but it decreased again in the 3rd one (0.217-0.290%) which had generally the slightly higher values for all treatments than the 1st one. In the 2nd cut of 1st season VO% was increased ($p \leq 0.5$) from 0.276 to 0.324% by increasing BRs concentration from zero to 0.3 g/l. Overall, plants which treated only with 2 g/l SWs had the lowest volatile oils (0.260%) however the highest percentage

(0.337%) was for plants treated with 0.3 g/ BRs +1g/l SW1) (Figures 7 and 8).

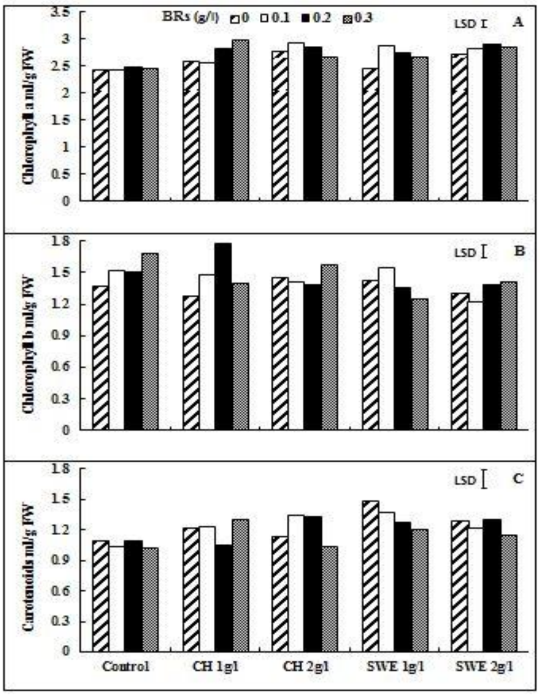

Figure 6: Effect some plant biostimulants on the **(A)** chlorophyll a , **(B)** chlorophyll b and **(C)** carotenoids (mg/g FW) of *Pelargonium graveolens* of the 2nd season.

BRs: Brassinolides, **CH**: Chitosan and **SWs:** Seaweed extracts

3.4.2. Volatile oil yield

All investigated applied PBs significantly improved VOs yield because of increasing the herb fresh weight as well as VOs% (Figure 9). Moreover, there were no significant interactions between the two investigated factors with the same trend in the two seasons. In the 1st season the VOs yield was significantly and gradually augmented from 4.488 ml/plant for untreated ones to 6.186 ml/plants for the highest BRs concentration. Similarly, CH/SWs treatments significantly increased VOs yield compared to control plants which had the lowest value (3.407 ml/plant). However, the variation between the lower and higher concentration of these compounds was significant in SW-treated plants than the CH-one. The lower SWs concentration was more effective than the higher one as the VOs yield for them was (4.480 and 6.343 ml/plant) respectively (Figure 9). Overall, the lowest VOs yield (2.578 ml/plant) being for untreated plants however the highest ones (7.351 and 7.347 ml/plant) was estimated for plants treated with 0.3 g/l BRS + 1g/l CH1 or WS respectively.

The favorable effect of BRs on VOs% and yield of rose-scented geranium plants was similar to these of [10] on the same plants suggesting that BRs increase VOs yield through its effect on growth and secondary metabolism. They might activate the plant genetic potentiality to synthesis more VOs. Higher levels of chlorophyll of BRs-treated plants might activate photosynthesis and their possible impact in the secondary metabolism [10]. The increase in the VOs content in BRs-treated lavender and mint plants was obtained [31 and 37]. Moreover, the capability of BRs to activate rooting of rose-scented geranium cuttings was recorded [11]. Improving rooting might correlated with higher nutrient uptake which could increase the herbage and plant secondary metabolites.

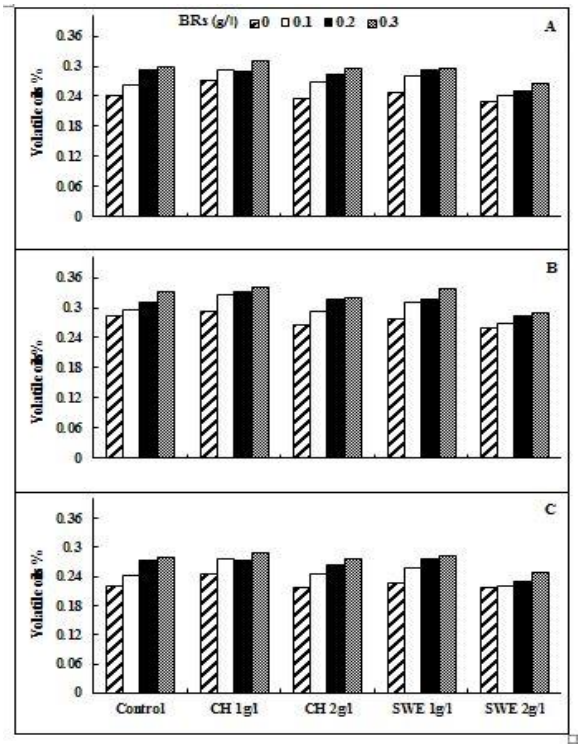

Figure 7: Effect some plant biostimulants on the volatile oils % of *Pelargonium graveolens* **(A)** 1st cut, **(B)** 2nd cut and **(C)** 3rd cut of the 1st season.

BRs: Brassinolides, **CH:** Chitosan and **SW:** Seaweed extracts.

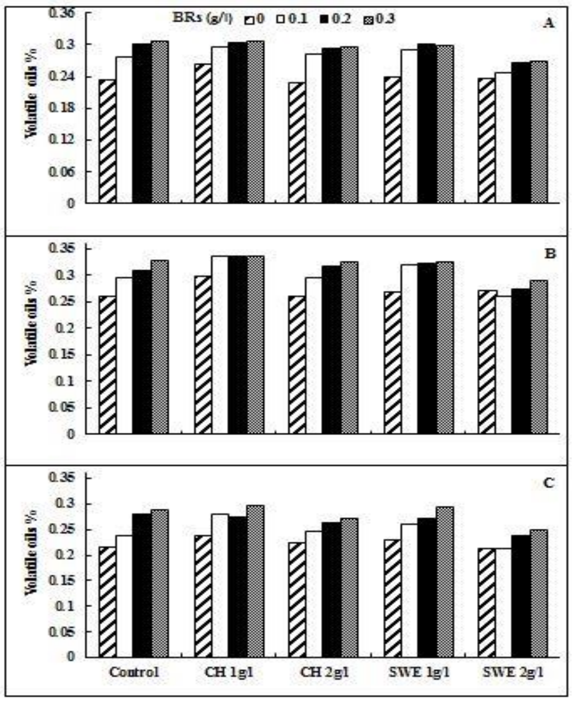

Figure 8: Effect some plant biostimulants on the volatile oil % of *Pelargonium graveolens* **(A)** 1st cut, **(B)** 2nd cut and **(C)** 3rd cut of the 2nd season.

BRs: Brassinolides, **CH:** Chitosan and **SW:** Seaweed extracts.

The role of SWs in different secondary plant production including Vos has been emphasized by [38 and 39]. In the current study, the increase in VOs content could be attributed to some factors such as the presence nutrient elements, amino acids, vitamins, and plant hormones as well as secondary metabolite elicitors on SWs [40]. The presence of N, P and micronutrients was correlated with increased VOs production in several plants [40 and 41]. The efficiency of CH in the raise of plant growth and secondary metabolites biosynthesis in numerous aromatic species has been reviewed [42] for example [32] on lemongrass, [43] on sweet basil, and [44] on salvia plants. They suggested that an elicitor such as CH can efficiently induce different phytochemicals including VOs. Many mechanisms regarding the role of CH as an elicitor such as oligochitosan which modulates various genes, in addition to their activity as antioxidants agent has been cited [42].

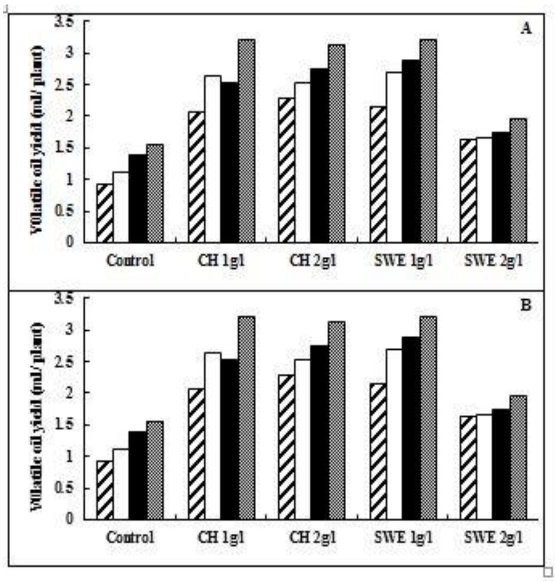

Figure 9: Effect some plant biostimulants on the total volatile oils **(ml/plant**) of *Pelargonium graveolens* (A) of the 1st season and (B) in the 2nd season.

BRs: Brassinolides, **CH:** Chitosan and **SW:** Seaweed extracts

The effectiveness of BRs CH and SWs at low concentration and cost and environmental safety. On the basis of showing such considerable growth and secondary metabolites, there is increasing attention on the application of these compounds in horticulture and agriculture productions [45]. The present study confirms these suggesting however, [41] suggested further studies to provide more understanding the role of brassinosteroids in plant secondary metabolism. Therefore, its recommended to treat rose scent-geranium plants with BRs at 0.3 g/l in addition to 1g/l SWs or 1 g/l CH.

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