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Trials on the application of fertilization combined with plant hormone spraying for improving the production of carnation absolute oil

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Abstract

To complete our previous studies on carnation (*Dianthus caryophyllus* L) absolute oil to introduce it as a new sources of essential oils under Egyptian conditions so, this study focused on the effect of fertilization combined with growth regulator on the flowers and absolute oil yield of carnation. Three level of fertilization treatments were used. A recommended fertilization dose was applied as a basic levels (BL = 1.5 g N + 2.25 g K_2O / $m^{2.)}$. Two other treatments, as a low level of fertilization, (LL =BL – ¼ dose of BL) and a high level of fertilization (HL) =BL + ¼ dose of BL) were applied. The application of fertilizer was carried out successively every month. The two growth regulators substances were used in this experiment which, two concentrations of growth retardant (cycocel - CCC) as 2000 and 3000 ppm and one dose of gibberellic acid (GA3) as 100 ppm. were applied at monthly interval starting on 10th November. The treatment for the combined between CCC (2000 ppm) X GA3 (100 ppm) were alternately sprayed also on month intervals. Alternative sprays of growth retardant (CCC) and growth regulators (GA3)was a promising practices in rising the carnation flowers yield especially with using the high level of fertilization. Using basic level of fertilization combined with CCC2 gave the highest percentage of absolute oil compared with the other treatments. Eugenol and benzyl benzoate were found as the main components of carnation absolute essential oil from Egypt.

1. Introduction

Dianthus caryophyllus L, (carnation), belongs to Caryophyllaceae family. Carnation flowers come in second place after roses, so this work amides to evaluate the carnation aromatic flowers in terms of growth and harvest flowers as well as essential oil using soil fertilization and / or growth regulators to work on improving the growth and increment of the yield of flowers and essential oil under Egyptian conditions.

Carnation absolute oil is characterized by a number of therapeutic properties could be taken into consideration. It is an anti-inflammatory and analgesic to muscle aches [1]. Eugenol was found as the major compound of carnation oil [2-4]

Fertilization plays an important role in plant growth and productivity of the active substances. The medicinal and aromatic plants response to the nutrition might differ due to several factors, i.e. kind and quantity of fertilizer, application time and method. as well as the soil texture and conditions of climate. Effect of mineral fertilization on the flowers yield and essential oil production were recorded by many investigators. Mainly the obtained results showed marked effects of the mineral fertilization on growth and volatile oil production [5-10].

There is much information in the literature concerning the influence of growth regulators on growth and productivity of various medicinal and aromatic plants. The majority of these growth substances have exhibited effects by amendment the growth characters. Gibberellins are endogenous plant hormones, which develop the growth of plants. In medicinal and aromatic plants, where active principle is normally present in the leaves or flowers, gibberellins have been used to develop growth and herb yield of the plants [11, 12].

The influence of growth retardant like, chlormequat chloride have been recorded on some aromatic plants and changes in volatile oil formation have been attributed to the result of these materials on enzymes of the biosynthetic pathway [13,14] .

2.Materials and methods:

The experiments included in this study were carried out at the Experimental Farm of Qalyupia governorate, during two successive seasons 2012 /2013 and 20013/ 2014. The layout of the experiments was in complete randomized block design of three replicates. Plant materials were propagated by cutting of carnation (cv. Enfant de Nice) from stock plants of unit of ornamental plants production at the National Research Centre –

Giza- Egypt. All the rooted cuttings were transplanted on 15 th of September. All other practices needed for carnation were carried out. A recommended fertilization dose was applied [15] as a (control) basic level (BL = 1.5 g N + 2.25 g K_2O / $m^{2.)}$. Two other treatments, as a low level of fertilization, (LL =BL – ¼ dose of BL) and a High level of fertilization (HL) =BL + ¼ dose of BL) were applied. The application of fertilizer was carried out successively every month starting on the 15 November 2012. The growth regulators substances used in these experiments were Chlormequat (or cycocel CCC) and Gibberellic acid (GA3).Two concentrations of CCC (2000 and 3000 ppm) and one concentration of GA3 (100 ppm). were applied at monthly interval starting on 10th of November. For the combined CCC X GA3 treatments, the two substances were alternately sprayed also on monthly intervals. Control plants were sprayed with tap water in all cases of spraying

2.1. Soil analysis:

Physical and chemical properties of the soil used in this study were determined according to Jackson [16] (Table 1).

Table1 Mechanical and chemical analysis of the field soil.

Item	Value	Item	Value
Sand	46 %	Total Nitrogen (ppm)	210.0
Silt	29.0 %	Available P (ppm)	90.0
Clay	23.0 %	Available K (ppm)	57.0
		Electronic conductivity (dS m-1)	2

2.2. Methods of chemical analysis:

The nitrogen content and phosphorus percentage of the dried leaves were determined according to methods described by Chapman and Pratt (17). The potassium percentage was determined by using Flame Photometer according to Chapman and Pratt [17].

2.3. Extraction of concrete oils:

Flower samples of carnation were extracted with petroleum ether at room temperature [18]. The samples macerated in3 folds of its weight with pure petroleum ether (40-60 °C) for 12 hours. The maceration was repeated twice applying the same way each time. The combined petroleum ether extract was dried over anhydrous sodium sulphate, then filtered over Whatman No.1 after isolation of any existing matters by decantation. The solution was distilled under vacuum at a temperature not exceeding 35 °C by a Rotavapour apparatus. The completely concentrated extract which called concrete is a solid waxy and dark brown mass containing the odoriferous principles of the natural perfume, plus a considerable amount of plant waxes, albuminous materials and color pigments. The obtained concrete from every sample was weighed and packed separately.

2.4. Extraction of absolute oils:

Carnation absolute oils were extracted from concrete with high – proof ethyl alcohol (ethanol absolute) in three successive washings. The ratio of alcohol by volume to the weight (V/W) of concrete oil was 15:1 in the first washing and 10:1 in the second and third washings. The respective time required for each of the three washings was 20, 15 and 15 hours per each. Then the samples cooled at –20 °C for 20 hours in deep-freezer to facilitate the separation of waxy materials and then were filtered at the same temperature(-20 °C) before the next washing. The filtrate was collected and distilled under vacuum at a temperature not exceeding 35 °C by a Rotavapour apparatus. The obtained absolute oils were then weighed and kept in brown dry bottles

2.5. Gas chromatography analyses (GC)

GC analyses were performed using a Shimadzu GC-9A gas chromatograph equipped with a DB-5 fused silica column (30 m x 0.25 mm i.d., film thickness 0.25 μm). Oven temperature was held at 40°C for 5 min and then programmed until 250°C at a rate of 4°C/min. Injector and detector (FID) temperature was 260°C; helium was the carrier gas with a linear velocity of 32 cm/s.

2.6. Gas chromatography-mass spectrometry (GC-MS)

GC-MS analyses were carried out on a Varian 3400 system equipped with a DB-5 fused silica column (30 m x 0.25 mm i.d.); Oven temperature was 40 to 240°C at a rate of 4°C/min, transfer line temperature 260°C, injector temperature 250°C, carrier gas helium with a linear velocity of 31.5 cm/s, split ratio 1/60, flow rate 1.1 ml/ min, Ionization energy 70 ev; scan time 1 s ; mass range 40-350 amu. The components of the oils were identified by comparison of their mass-spectra with those of a computer library Adams, R. A.[19] or with authentic compounds and confirmed by comparison of their retention indices either with those of authentic compounds. Kovat's indices [20] were determined by co injection of the sample with a solution containing a homologous series of n-hydrocarbons, in a temperature run identical to that described above.

2.7. Qualitative and quantitative analyses

Identifications were made by library searches combining MS and retention data of authentic compounds by comparison of their GC retention indices (RI) with those of the literature [20] or with those of standards available in our laboratories. The retention indices were determined in relation to a homologous series of n-alkanes (C8-C22) under the same operating conditions. Further identification was made by comparison of their mass spectra on both columns with those stored in NIST 98 Libraries or with mass spectra from literature. Component relative concentrations were calculated based on GC peak areas without using correction factors.

2.8. Statistical analysis

Data subjected to statistical analysis according to Snedecor and Cochran [21]

3. Results and discussion:

3.1. Flower yield (g/plant)

Application of fertilization with the high level gave the best results which recorded 364.80 g / plant against 256.5 g/ plant for the low level. Also using CCC with low level of fertilization increased the flowers yield of carnation. The flower yields in this respect recorded 300.5 and 312.5 g/ plant for the CCC1 an CCC2, respectively. On the other hand decreasing effect was observed due to application CCC with the high level of fertilization, this means that, carnation plants may benefit from CCC application in case low fertilization and the plants do not need to be sprayed with cycocel with the application of a high level of fertilization

GA3 was very effective in increasing the yield of flower when applied on plants receiving the basic level of fertilization. The increases in this respect were slightly less than the use of CCC on similar plants. Using GA3 with a high level of fertilization had limited impact on the flowers yield of carnation. At the same time, alternating sprays of CCC combined with GA3 may be promising practice in increasing the flower yield of carnation especially when fertilization is under the low level

Table 2 Effect of soil fertilization combined with plant hormones spraying on carnation flower yield g/plant.(Mean value of the two seasons)

Nutrition	Growth regulators ppm/L					
Treatments	(Control)	CCC1 (2000)	CCC2 (3000)	GA3 (100)	GA3 +CCC1	Mean
Basic (B)	256.0	342.0	228.2	298.0	247.0	247.24
Low (L)	256.5	300.5	312.5	250.0	263.0	276.5
High (H)	364.8	323.0	300.5	356.5	345.0	337.96
Mean	292.4	321.8	280.4	301.5	285.0	296.2
L.S.D for Fertilizer at 0.05		12.30				
L.S.D for Growth regulators at 0.05		15.88				
L.S.D for Interaction at 0.05		22.83				

3.2. Determination of minerals carnation leaves. (mean of the two years)

3.2.1. Nitrogen:

The results showed the convergence of nitrogen percentage in the carnation leaves due to application fertilization only or combined with growth regulators. The percentage of nitrogen element ranged between 1.9 to 2.6 % under all treatments. Using fertilizer treatments only gave the high concentration of nitrogen compared with other treatments except the (CCC2X BL) treatment which recorded 2.6 %.

3.2.2. Phosphorus:

Data in Table 3 recorded that, the percentage of phosphorus element ranged from 0.27 to 0.45 % in the leaves of carnation under the conditions of all treatment. Using fertilizer treatment only gave the high percentage of phosphorus element which recorded 0.45, 0.44 and 0.40 % for the low, basic and high level of fertilization, respectively, while the rest of the results were close in the percentage of phosphorus in the other treatments

3.2.3. Potassium:

Potassium ratio varied in the leaves of the plant, due to application of growth regulators or adding soil fertilization only or both together. The high percentage of potassium in this respect recorded 3.1 % for (H), (BL X CCC2) and (BLXGA3) treatments, respectively, while the lowest amount of potassium were obtained with all treatments of GA3 + CCC1. There is a positive relationship between plant nutrients (N,P and K) content and use of soil fertilization or application of plant hormone, the beneficial effect is the improvement of NPK elements content. Many investigators reported that, there was a close relationship between the leaf NPK content and the quality and yield of the plant [22-24]

Table 3 Effect of soil fertilization combined with plant hormones spraying on (minerals content) of carnation leaves

Nitrogen						
Nutrition	Growth regulators ppm/L					
Treatments	(Control)	CCC1 2000	CCC2 3000	GA3 100	GA3 +CCC1	Mean
Basic (B)	2.6	2.2	2.6	1.9	2.5	2.4
Low (L)	2.6	2.2	2.3	2.3	1.9	2.3
High (H)	2.7	2.3	2.3	2.3	1.9	2.3
Mean	2.6	2.2	2.4	2.2	2.1	2.3
Phosphorus						
Basic (M)	0.44	0.34	0.35	0.38	0.40	0.36
Low (L)	0.45	0.40	0.34	0.35	0.35	0.38
High (H)	0.40	0.27	0.34	0.40	0.38	0.36
Mean	0.43	0.33	0.34	0.38	0.38	0.37
Potassium						
Basic (B)	2.8	2.8	3.1	3.1	2.1	2.78
Low (L)	2.7	2.8	2.4	2.5	2.3	2.54
High (H)	3.1	2.3	2.4	2.1	2.2	2.42
Mean	2.9	2.6	2.6	2.6	2.2	2.54

3.3. Concrete oil %:

Concretes are produced mainly from flowers. They are containing the odoriferous principles of the normal perfume, plus a significant amount of plant albuminous, waxes materials and color pigments so; they were a hard waxy and dark brown mass . Data in Table 4 reported that, basic level of fertilization gave the highest concrete oil percentage (0.40 %), compared with low (0.29%) and high fertilization level (0.34 %) ,respectively. Obvious effect due to interaction of both growth regulators was observed. The high amounts of concert oil percentage in this respect were obtained from BCCC2 (0.52 %), LCCC2 (0.45 %) and GA1+CCC1 with basic fertilization (0.43) treatments, respectively. Similar results were proved by Ahmed and Eid, (1975) on . umbelliferae plants and Khalil (1979) on *Achilles millefollum* L.) [25-26].

Table 4 Effect of soil fertilization combined with plant hormones spraying on Concrete oil % of carnation flowers

Nutrition	Growth regulators ppm/L					
Treatments	(Control)	CCC1 2000	CCC2 3000	GA3 100	GA3 +CCC1	Mean
Basic (B)	0.40	0.36	0.52	0.28	0.43	0.40
Low (L)	0.33	0.33	0.45	0.36	0.39	0.37
High	0.29	0.40	0.33	0.37	0.39	0.36
Mean	0.34	0.36	0.46	0.34	0.37	0.38

3.4. 1. Absolute oil %:

Absolute oil of carnation is extracted by ethanolic extraction of the concrete, it is produced by solvent extraction of the *Dianthus caryophyllus* flowers; the absolute essential oil is then added with perfumery ingredients to create high grade perfume oil. The result in Table 5 has the same trend as a result of concrete oil percentage (Table 4). The carnation absolute oil varied between o.16 percent to 0.29 % under all treatments.

The maximum absolute oil percentage was obtained with BCCC2 treatment which amounted to 0.29 %. Also significant amounts of percentage of absolute oil were observed due to application of LCCC2 (0.25 %) followed by GA3+CCC1 with BL fertilizer (0.24) treatments, respectively.

3.4.2. Yield of absolute oil g/plant:

Comparing the results of the absolute oil percentage with the results for fresh yield of flowers per plant, it could be noticed that, the basic level of fertilization combined with CCC2 gave the highest fresh yield of flowers per plant; this effect was reflected on the production of the carnation absolute oil per plant. The amount of absolute oil per plant in this respect recorded (0.92 g/plant) Also, the plants treated with the high level of fertilization companied with GA3+CCC1 with high level of fertilization gave significant amount of carnation absolute oil yield (0.71 g/plant). At the same time Reasonable amounts of carnation oil were recorded due to application of fertilization combined with growth regulators. Some investigators found that, application of fertilization and or growth regulators was enhanced essential oil yield per plant [5, 25, 27].

Table 5 Effect of soil fertilization combined with plant hormones spraying on carnation absolute oil production

Absolute oil %						
Nutrition	Growth regulators					
Treatments	control	CCC1 2000	CCC2 3000	GA3 100	GA3 +CCC1	Mean
Basic (B)	0.22	0.2	0.29	0.15	0.24	0.22
Low (L)	0.18	0.18	0.25	0.2	0.22	0.21
High (H)	0.16	0.22	0.18	0.2	0.22	0.20
Mean	0.19	0.2	0.25	0.19	0.21	0.21
Absolute oil ml / plant						
Basic (B)	0.56	0.6	0.92	0.38	0.63	0.61
Low (L)	0.46	0.62	0.57	0.6	0.54	0.52
High (H)	0.58	0.71	0.54	0.71	0.76	0.68
Mean	0.56	0.64	0.7	0.57	0.6	0.62
L.S.D for Fertilizer at 0.05=0.037		L.S.D for Growth regulators at 0.05=0.48		L.S.D for Interaction at 0.05 = 0.69		

3.5. Constituents of carnation absolute essential oils

As show in Fig (1) Eugenol (15.3 %) and benzyl benzoate (14.1 %) were found as the main constituents of carnation absolute oil under Egyptian condition.

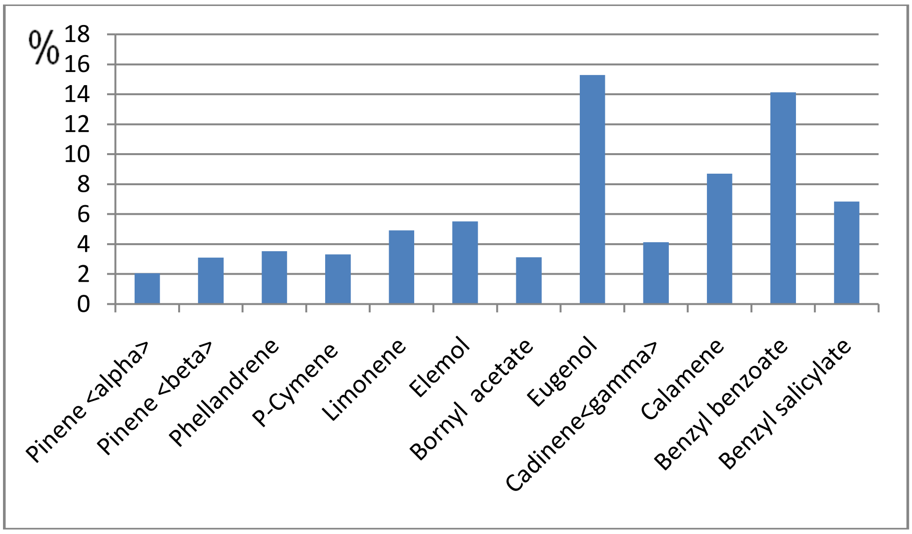

Figure 1 The major constituents of Carnation oil

Also significant amount of elemol (5.51%) and bornyl acetate (3.12%) were found as major composition. carnation absolute oil under Egyptian conditions contains four chemical fractions i.e. monoterpene hydrocarbons (19.6 %), oxygenated monoterpene (26.7 %), sesquiterpene hydrocarbons (12.83 %) and various compounds fraction (21.0 %). It was characterized by a high oxygenated monoterpene percentage. Eugenol was fount as the main constituent of this group. It is used in perfumes, volatile oils and flavoring. Eugenol is also used as a local antiseptic [27]. Limonene compound is one of the most excellent known aroma compositions of carnation absolute essential oil. It is one of terpenes and distributed widely, occurring in much essential oil particularly in citrus oils. Similar results were reported by Ibrahim [3] and El-Ghorab *et al* [4].

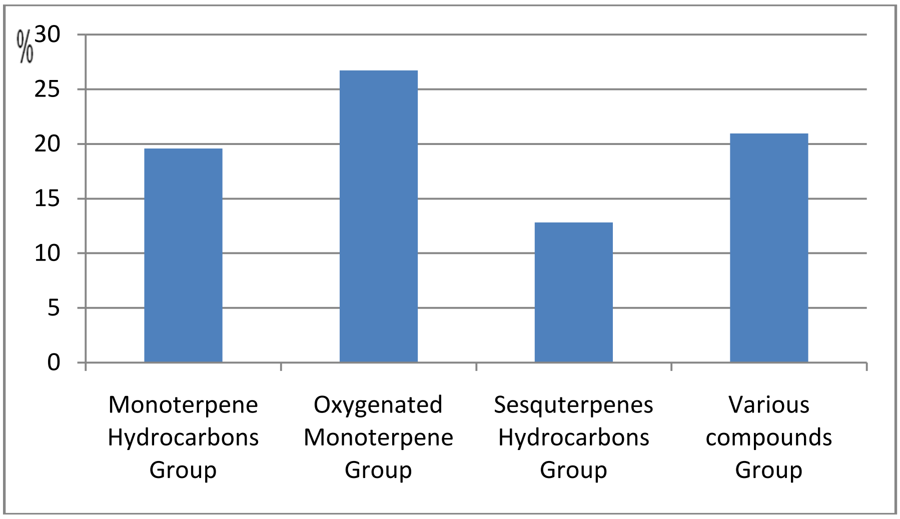

Figure 2 Classification of the oil constituents

Conclusions

Carnation (*Dianthus caryophyllus* L) flowers come in second place after roses, so this work aimed to evaluate the carnation aromatic flowers in terms of growth and harvest flowers as well as essential oil using soil fertilization and / or growth regulators to work on improving the growth and increasing the yield of flowers and essential oil under Egyptian conditions.

It may be concluded that alternating sprays of CCC with GA3 was a promising practice in rising carnation flower yield especially when fertilization is under the accurate level.

There is a positive relationship between plant nutrients (N, P and K) content and the use of soil nutrition or application of plant hormone, which is the improvement of NPK content.

Basic level of fertilization gave the highest concrete and absolute oil percentage, compared with other fertilizer treatments. Obvious effect due to interaction of both growth regulators was observed.

Eugenol and benzyl benzoate were found as the main constituents of carnation absolute oil under Egyptian condition. Also significant amounts of elemol and bornyl acetate were observed as major constituents of carnation absolute oil from Egypt.

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