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Agrochemical Studies on *Dianthus caryophyllus* L. Grown in Egypt

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Abstract: Through our intensive study to detect new sources of absolute essential oils under conditions of Egypt we decided to evaluate the carnation (*Dianthus caryophyllus* L) absolute essential oils content *cv*. Enfant de Nice as a new source of absolute oil in Egypt. Also,to study the effect of nitrogen and potassium fertilizers at different rates on the yield of carnation absolute essential oil. Data showed that, nitrogen and potassium fertilizers had significant effects on growth characters and absolute oil yield of carnation during growing seasons. The constituents of carnation absolute essential oils isolated from flowers were identified by GC and GC-MS. Seventeen compounds were identified in the carnation absolute oil. They represent approximately 80.1 %. Eugenol 15.29 % and Benzyl benzoate (14.12 %) were found as the major constituents of carnation oil. The carnation absolute essential oil was characterized by its high content of calamene, benzyl salicylate, limonene and elemol.
Keywords : *Dianthus caryophyllus L*, Solvent extraction Essential oil, Concrete, GC-MS, Constituents, Eugenol, Benzyl benzoate. Nitrogen, Potassium, fertilization.

Introduction

Egypt has the potential for the growth of many types of flowering plants for the purpose of domestic and external marketing. Carnation absolute oil extracted from flowers of *Dianthus caryophyllus* L plant could be considered as a source of hard currency. This point itself needed a comprehensive and accurate study of carnation plant from both agricultural and chemical points of view, especially when using new varieties in order to extract volatile oils. Also Carnation (*Dianthus caryophyllus* L, Fam. Caryophyllaceae) flowers occupy the second place after the roses and this also needs to a further study under Egyptian conditions

Absolute oil is a concentrated volatile oil extracted from aromatic plants. Usually, it is used in perfumery rather than for aromatherapy, because it is isolated using chemical solvents, such as hexane (used to create a concrete) and then ethyl alcohol (to remove the plant waxes and other compositions)[1,2]. These two-step methods results in a brilliantly aromatic liquid that displays a fragrance profile often quite similar to the original plant except more concentrated and strong.

There are many therapeutic benefits of carnation absolute oil can be taken into consideration, these benefits contain an anti-inflammatory effect, helping with hair loss and be active as a muscle relaxant Robert.[2]

There are few studies on carnation absolute oil in terms of its constituents. Eugenol, benzyl benzoate and benzyl salicylate were found as the main constituents of carnation absolute oil.[1]

It is known that production of the aromatic plants and their essential oil is significantly affected by fertilizer application .The response of the aromatic plants to nutrition might vary due to many factors , i.e. type and quantity of fertilizer method and application time. Also the soil texture aeration and climatic conditions. Studies on the effect of mineral nutrition on the growth and essential oil content were reported by many investigators. Most of the obtained results revealed marked effects of the nutrition on growth and essential oil production.[3-8]

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 design of three replicates. Plant materials were propagated by cutting of carnation (cv. Enfant de Nice) from stock plants of unit production of ornamental plants at the National Research Centre – Giza- Egypt. All the rooted plants were transplanted on 15th of September. All other practices needed for carnation were carried out. A recommended fertilization dose was applied[9] as medium level (ML = 1.5 g N + 2.25 g K_2O / $m^{2.)}$. Two other treatment, as low level of fertilization, (LL =ML – ¼ dose of ML) and High level of fertilization (HL) =ML + ¼ dose of ML) were applied. The application of fertilizer was carried out successively every month starting on the 15 November 2012 and stopped at the end of the experiments.

Methods of soil analysis:

Physical and chemical properties of soil used in this study were determined according to Jackson[10] in Table 1.

Table (1) 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

Methods of Chemical Analysis

The nitrogen content and phosphorus percentage of the dried leaves were determined according to methods described by Chapman and Pratt[11]. The potassium percentage was determined by using Flame Photometer according to Chapman and Pratt[11]

Extraction Methods

Extraction of concrete oils:

Plant samples of Carnation were extracted with petroleum ether at room temperature[12]. The samples macerated 3 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 ratio between plant material and petroleum ether was 1: 3 w/v. The combined petroleum ether extract was dried over anhydrous sodium sulphate, and 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 containing the odoriferous principles of the natural perfume, plus a considerable amount of plant waxes, albuminous materials and color pigments therefore; it was a solid waxy and dark brown mass. The obtained concrete from every sample was weight and packed separately.

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

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.

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.[13] or with authentic compounds and confirmed by comparison of their retention indices either with those of authentic compounds. Kovat's indices (14) 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.

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[14] 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.

Statistical analysis

Data subjected to statistical analysis according to Snedecor and Cochran.[15]

Results and Discussion:

Data in Table (1) recorded that application nitrogen and potassium fertilization at high level gave highly significant increase in the fresh flowers yield of carnation. The mean value of fresh flowers yield in this respect recorded 366.4 gms/plant for the high fertilization level against 307.7 and 256.1 gms / plant for the medium and low fertilizer level respectively. Similar results were observed on Carnation[16] and on Iris.[17]

Concerning the effect of nitrogen and potassium fertilization on accumulation of the NP K in leaves of carnation It was observed from the data that, in most cases, increasing the percentage of N and K elements in soil media with fertilization did not affect the Uptake by plant as indicated from the average percentage of the element in carnation leaves. At the same time, a slight increasing was observed in the phosphorus content due to the application of potassium and nitrogen fertilization at low- and middle levels. The percentage of P in this respect recorded 0.46, 0.43 and 0.38% for the low, medium and high level respectively. On the other hand application of nitrogen and potassium fertilization on accumulation of potassium (K) in carnation leaves did not affect the K uptake by plant. Adding nitrogen fertilization and potassium to the soil at the medium fertilization level led to an increase in both the percentage of concrete oil as well as the carnation absolute oil content, which is reflected in the yield of carnations absolute essential oil per plant. The yield of absolute oil in this respect amounted to 0.68 gms / plant for medium level against 0.56 and 0.44 gms / plant for the high and low level fertilization respectively. These results are in agreement with those obtained by Kazimirova[18]. He found correlation between soil nutrient content and carnation composition. He reported that nutrient uptake by carnation was greatest during full bloom. Table (2) Effect of fertilization on the flowers yield, NPK content and oil production

Table (2) Effect of fertilization on the flowers yield, NPK content and oil production

Character	Fertilizer Level			LSD	
	Low	Medium	High	0.05	0.01
Flowers Yield (gms/plant)	256.1	307.7	366.4	28.6	47.4
Nitrogen content (N)	2.6	2.6	2.7	NS	NS
Phosphorus content (P)	0.46	0.43	0.38	NS	NS
Potassium content (K)	2.5	2.6	2.9	NS	NS
Concrete oil %	0.33	0.47	0.32	0.10	0.14
Absolute Oil %	0.17	0.22	0.15	0.05	0.06
Oil Yield (gms/plant)	0.44	0.68	0.56	0.15	0.17

Table (3) Constituents of Carnation absolute oil extracted by organic solvent

Compound	%	(DB5) KI
Monoterpene Hydrocarbons Group		
Tricyclene	0.17	919
Pinene <alpha>	2.05	933
camphene	0.98	953
Pinene <beta>	3.11	981
Phellandrene	3.52	1005
P-Cymene	3.32	1026
Limonene	4.91	1031
Terpinene <gamma>	1.53	1059
Total	19.59	
Oxygenated Monoterpene Group		
Elemol	5.51	1199
Citronellol	1.11	1228
Bornyl acetate	3.12	1285
Eugenol	15.29	1356
Methyl Eugenol	1.68	1401
Total	26.71	
Sesquterpenes Hydrocarbons Group		
Cadinene<gamma>	4.12	1513
Calamene	8.71	1563
Total	12.83	
Various compounds Group		
Benzyl benzoate	14.12	1762
Benzyl salicylate	6.85	1863
Total	20.97	

Constituents of Carnation Absolute Essential Oils

The composition of carnation absolute essential oils from Egypt was found in Table (3), where the techniques used for identification of components were numbered according to the sequence of elution on capillary column DB5. It is obvious from the data that, Seventeen compounds representing 80.1% of the Carnation absolute oil were identified under the Egyptian conditions. The main components were Eugenol 15.29 % and Benzyl benzoate (14.12 %). The two compounds were found as constituents in distilled carnation oil from Egypt[19]. Egyptian carnation absolute oil contains four chemical groups i.e. monoterpene hydrocarbons group (19.59 %), oxygenated monoterpene group (26.71 %), sesquiterpene hydrocarbons group (12.83 %) and various compounds group (20.97 %). The carnation absolute essential oil composition was characterized by a high percentage of Oxygenated monoterpene group. The main constituents in this respect were Eugenol (15.29%), Elemol (5.51%) and Bornyl acetate (3.12%) followed by Methyl Eugenol (1.68%), and Citronellol (1.11%). Limonene, Phellandrene, P-Cymene, β-Pinene and α- Pinene are the major monoterpene hydrocarbon compounds, since their intensities were 4.91, 3.52, 3.32, 3.11 and 2.05 % respectively. Limonene is one of the best known aroma constituents of carnation absolute oil. It is one of the most widely distributed terpenes, occurring in much volatile oil especially in citrus oils. On the other hand two other important constituents were found in the group of sesquiterpene hydrocarbon. The first compound was Calamene (8.71%) and - Cadinene (4.12 %)

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