Journal of Horticultural Science & Ornamental Plants 8 (3): 140-148, 2016
ISSN 2079-2158

DOI: 10.5829/idosi.jhsop.2016.8.3.1181

Effect of Mineral, Biofertilizer (EM) and Zeolite on Growth, Flowering, Yield and Composition of Volatile Oil of *Viola odorata* L. Plants

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Abstract: This experiment was carried out in the open field at the Experimental Farm of Horticulture Department, Faculty of Agriculture, Benha University, Egypt, during two successive seasons 2013-2014 and 2014-2015 to investigate the effect of mineral fertilizers NPK at the rate of 50 kg N/feddan (one feddan=0.42ha) as urea, 25 kg P_2O_5/feddan as calcium superphosphate and 40 kg K_2O/feddan as potassium sulphate (as full dose) and effective microorganisms (EM) at 30 ml/plant (as biofertilizer), as well as Zeolite at the rate of 48.2 kg/feddan (as full dose) and their combination on growth, flowering, yield of volatile oil of violet (*Viola odorata* L.) plants. The results showed that, the vegetative growth, flowering characteristics, as well as yield of concrete of leaves and flowers and its volatile oil composition of violet were favorably affected by different fertilization treatments either full doses of chemical fertilization, biofertilization (EM) or Zeolite separately or in combination with them. The combined treatments of EM at 30 ml/plant + 75 % dose NPK or full dose of NPK gave higher values in both seasons for most characters (number of leaves, fresh and dry weight of leaves, number of flowers, fresh and dry weight of flowers leaves and flowers concrete, minerals (i.e. N, P, K), total carbohydrates and total chlorophylls. Moreover, the combined treatments of EM at 30 ml/plant + 50 % dose NPK induced high significant increments of all the aforementioned parameters in the two seasons. Additionally, the combinations between EM at 30 ml / plant with 75 or 50 % dose Zeolite improved all the studied parameters as compared to the control in the two seasons. Also, the combined treatments of EM at 30 ml/plant + 75 % dose NPK or full dose of NPK have a pronounced effect for improving the yield of concrete in both leaves and flowers which gave the maximum values and its volatile oil recorded 26 compounds in leaves and 23 compounds in flowers which were identified by using GC/MS chromatography analysis. The main component was the Butyl-2 ethylhexyl phthalate (17.22 to 37.11%) in the volatile oil of violet leaves and flowers, respectively.

Key words: Zeolite · Effective microorganisms (EM) · *Viola odorata* L. · Volatile oil · Chemical composition

INTRODUCTION

Viola odorata L. (violet) belongs to family (Violaceae) is an evergreen perennial herb growing in Egypt as ornamental and aromatic plants. It is considered as an important plant due to its volatile natural products. The herb is well known for its pharmaceutical importance in medicinal system. A species of the genus *Viola* native to Europe and Asia, but has also been introduced to North America and Australasia [1]. The analysis of essential oil composition of the leaves of *Viola odorata* L revealed that the major compounds, butyl-2-ethylhexylphthalate and 5,6,7,7a-tetrahydro-4,4,7a-trimethyl-2(4H)-benzofuranone being the two main components, *Viola odorata* also produces macrocyclic peptides [2,3,4]. Recently, unconventional efforts are used to minimize the amounts of chemical fertilizers which applied to medicinal and aromatic plants in order to reduce production cost and environmental pollution without reduction of yield. Therefore, the trend now is using the bio and organic fertilizers. Bio-fertilizers are reasonably safer to the environment than chemical fertilizers and play an important role in decreasing the use of chemical fertilizers. Effective microorganisms which is known as (EM) is a culture containing more than 60 microorganisms including lactic acid bacteria (*Lactobacillus plantarum*, *Lactobacillus casei* and *Streptocous lactis*, Photosynthesis bacteria, yeast and algae.

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Also, EM produces lactic acids [5]. The results of some studies allow one to presume that the use of Effective Microorganisms (EM) technology can be a method for improving the yield and quality of ornamental plants, affecting yields and increasing the content of important chemical constituents in plant raw material [6, 7]. Recently, Zeolite one group of minerals has emerged as having considerable potential in a wide variety of agricultural processes. Zeolite minerals are crystalline hydrated alumino-silicates of alkaline-earth metals, formed by AlO_4 and SiO_4 tetrahydral. More than forty types of Zeolite have been reported by Leggo [8], these minerals have many properties which are interest for agricultural purpose; high cation exchange capacity, high water holding capacity and high adsorption capacity [9]. Roxana [10] confirmed that, the suitability of using natural Zeolite in agriculture had a positive role in plant nutrition and microbial community stability, as evidenced subject experimentation crops. All these unique properties of Zeolite materials promise to contribute significantly too many years of agricultural technology.

The aim of this study is to evaluate the effect of NPK mineral, bio-fertilizer (EM), the natural substance (Zeolite) and their interactions on vegetative growth, flowering characteristics, yield of concrete of leaves and flowers and their fraction of volatile oil of *Viola odorata* L. plants by using GC/MS chromatography analysis.

MATERIALS AND METHODS

Two field experiments were conducted at the Experimental Farm, of the Faculty Agriculture (Moshtoher), Benha University, Egypt during two successive seasons of 2013-2014 and 2014-2015 to investigate the effect of NPK mineral and bio-fertilizer (EM) and Zeolite on growth, flowering, concrete yield and volatile oil composition of violet plant. In the two experiments, uniform plants of *viola odorata* L. were obtained from Horticulture Department, Faculty of Agriculture, Benha University, Egypt. Transplants were sown at 15th September in both seasons. Uniform plants were (each contained 10-12 leaves) and transplanted at the rate of 9 plants per each plot (100 x 100 cm) which considered as replicate. Physical and chemical properties of experimental soil site were analyses according to the method described by Page *et al*. [11] (Table 1). The experimental design was Complete Randomized Block Design (CRBD) according to Snedecor and Cochran [12] including 8 treatments replicated three times and every one represented by 9 plants.

Table 1: Physical and chemical analysis of the experimental soil site

Properties	Value
Physical properties	
Coarse sand %	5.91
Fine sand%	24.73
Silt%	35.22
Clay%	51.14
Textural class	Clayey loam
Chemical properties	
pH	7.90
EC dS/m	0.62
Organic matter %	1.48
$CaCO_3$%	0.52
Total nitrogen %	0.27
Total phosphorus%	0.12
Total potassium%	0.26

Treatments

Chemical Fertilizers: Fertilizers which used were as follow: Zeolite which contains 1.02% N; 0.14% P and 5.35, 3.23, 0.03, 0.07 mg/L Mg, Ca, Zn, Fe, respectively was obtained from Al-Koptan Company for Import & Export, Qalubia Governorate, Egypt and added to soil at a rate of 48.2 kg/feddan (one feddan= 042ha) in three equal doses. Chemical NPK fertilizers were obtained from Research Center of Faculty of Agriculture, Benha University, Egypt. Inorganic nitrogen fertilizer was applied at a rate of 50 kg N/fed as urea. Also, inorganic phosphorus fertilizer was applied at a rate of 25 kg P_2O_5/feddan as calcium superphosphate (15.5% P_2O_5), while, potassium fertilizer was applied at a rate of 40 kg K_2O/feddan as potassium sulphate (48-50 K_2O %) in four equal doses. The first dose was added after one month from planting and then the other doses were applied at monthly intervals.

Effective Microorganisms (EM): EM (each ml contains 0.6 x 10^7 microorganisms) was applied either separately or in a mixture three equal doses to the soil around each plant as 30 ml / plant. The 1st dose was added after 4 weeks from transplanting, while the 2nd dose after 4 weeks from the first dose and also the 3rd dose after 4 weeks from the 2nd dose in both seasons and then plants were irrigated immediately. The treatments were conducted as follows:

- Control (untreated).
- EM at 30 ml/plant
- Full dose of NPK
- Full dose of Zeolite
- EM at 30 ml/plant + 75 % dose NPK
- EM at 30 ml/plant + 50 % dose NPK
- EM at 30 ml/plant + 75 % dose Zeolite
- EM at 30 ml/plant + 50 % dose Zeolite

Data Recorded

Vegetative Growth Parameters: At the 2nd week of May, numbers of leaves/plant, fresh weight of leaves/plant, dry weight of leaves/plant were determined.

Flowering Parameters: In both seasons, at the 1st February till 1st May number of flowers was periodically picked and counted at 15-day interval, therefore total number, fresh weight of flowers /plant, dry weight of flowers /plant and concrete % of flowers were separately calculated per an individual plant at the end of each flowering season.

Concrete Extraction with Organic Solvents: Concrete % of leaves and flowers were extracted with organic (n-Hexane) according to Guenther [13]. Redistilled n-Hexane of a boiling range of 65-70°C was used in preparation of the concrete oil. Violet leaves and flowers were placed in large glass container and covered with redistilled n-Hexane. The mixture was allowed to stand at room temperature for 24 hours. After filtration, another quantity of n-Hexane was added to the remaining leaves and flowers which were again soaked for 12 hours. The combined successive n-Hexane extracts separated from the filtrate (a mixture of H_2O and n-Hexane) by means of a separating funnel. The n-Hexane layers were hydrated over anhydrous sodium sulfate, filtered and evaporated under reduced pressure a temperature not exceeding 35°C.The residue (concrete) is a light to dark brown, waxy and quite hard mass.

Volatile Oil Extraction: The violet volatile oil was extracted from the obtained concrete by using purified absolute ethyl alcohol according to Guenther [13]. The residue was shaken three times with ethyl alcohol and filtered, leaving mainly waxy matter. The ratio of alcohol to concrete oil was 8:1v/w. The filtrate, however still contained a small amount of waxes which were separated chilling and filtering at a temperature -15°C. The filtrate was distilled in vacuo at a temperature not exceeding 35°C. The residue (volatile oil) is brownish, very viscous or semiliquid oil.

Plant Chemical Analysis: Total nitrogen, phosphorus, potassium and total carbohydrates were determined in dry violet herbs at the flowering stage according the methods described by Horneck and Miller [14], Hucker and Catroux [15], Horneck and Hanson [16] and Herbert *et al*. [17], respectively. Total chlorophylls as mg/100g fresh weight was determined in the leaves of violet according to the method described by AOAC [18].

Fraction of Violet Volatile Oil: The volatile oil constituents of *Viola odorata* L. leaves and flowers were chosen according to volatile oil percentage (%) in the second season. The GC/MS chromatography analysis was carried out at the Center for Pesticide Residues in Food Laboratory, Dokki, Giza, Egypt. Agilent 6890 gas chromatograph equipped with an Agilent mass spectrometric detector, with a direct capillary interface and fused silica capillary column PAS-5 MS (30m x 0.32mm x 0.25ìm film thickness). Pesticide samples were injected under the following conditions. Helium was used as carrier gas at approximately 1ml/min., pulsed split less mode. The solvent delay was 3 min. and the injection size was 1.0ìm. The mass spectrometric detector was operated in electron impact ionization mode with an ionizing energy of 70 ev scanning from m/z 50 500.The ion source temperature was to 230°C. The electron multiplier voltage (EM voltage) was maintained 1250v above auto tune. The instrument was manually tuned using perfluorotributylamine (PFTBA). The GC temperature program was started at 60°C (2 min.) them elevated to 300°C at rate if 5°C/min. the injector temperature was set at 280°C, respectively. Wiley and Wiley Nist mass spectral data base was used in the identification of the separated peaks.

Statistical Analysis: The obtained data was subjected to statistical analysis according to Snedecor and Cochran [12] and the differences between the mean values of various treatments were compared by Duncan's multiple range tests [19].

RESULTS AND DISCUSSIONS

Effect of NPK Mineral, Bio-fertilizer (EM) and Zeolite on Vegetative Growth of *Viola odorata* L. Plants: Data presented in Table 2 indicated that the vegetative growth characteristics i.e. number of leaves/plant, fresh and dry weight of leaves/plant were affected by all kinds of fertilizer treatments (NPK, bio-fertilizer (EM) and Zeolite) compared to the untreated plants (control) in both seasons. However, the combination of EM + 75 % dose NPK gave the highest values of these parameters in both seasons. Whereas, full dose of NPK ranked the 2nd values in this concern, while the combination of EM + 50 % dose NPK produced the third order in the two seasons. On the other hand, the untreated plants (control) gave the lowest values of these parameters in the first and second seasons. The EM have an advantageous effect on the uptake of nutrients by mung bean, they improve its yielding and the development of the root systems [20].

Moreover, a positive effect was shown on growth, development and yielding of apple trees [21]. Trees treated with the abovementioned microbiological preparation formed more shoots, which were markedly longer and thicker than in case of the control combination. Application of EM had a positive effect on leaf area in apple trees and chlorophyll content in leaves. Application of EM significantly modified nutrient content in leaves, improving nutrient status of plants in terms of nitrogen, phosphorus, potassium, iron, manganese and zinc [22]. In this respect, Abdou *et al.* [23] and Barbara *et al.* [24] on *Ocimum basilicum* L. and Cezary [25] on *Chamomilla recutita* L. stated that the Effective Microorganisms (EM Farming) were not shown to be useful in chamomile growing practices. This may be due to the beneficial effect of Zeolite as those stated by Ippolito *et al*. [26], who found that water retention or soil water contents to be greater in soils to which Zeolite was applied, this effect may play an important role in plant growth activation. Also, Valadabadi *et al*. [27] reported that the addition of Zeolite improved the nutrient status of root zones, especially selective retention of NH_4^+ and K^+ ions which have major role in plant growth improvement. In addition, the use of EM can cause a distinct increase in yield components and plant biometric features and as a consequence, an increase in yield by even 20% [7, 28].

Effect of NPK Mineral, Bio-fertilizer (EM) and Zeolite on Flowering Characteristics of *Viola odorata* L. Plants: Data in presented in Table 3 revealed that all the different treatments increased number of flowers /plant, fresh and dry weight of flowers /plant of *Viola odorata* L. in both seasons. In this concern, the maximum values of these parameters were obtained by using the combination of EM + 75 % dose NPK in the two seasons. On the other hand, the combined treatment of EM + 50 % dose NPK ranked the second value in this concern in the first and second seasons. Regardless the control treatment, the least values of these parameters was recorded by the EM in both seasons.

Effect of NPK Mineral, Bio-fertilizer (EM) and Zeolite on Leaves and Flowers Concrete % of *Viola odorata* L. Plants: Data presented in Table 4 indicated that the concrete percentage of leaves and flowers of *Viola odorata* L. increased generally over control by using all fertilizer treatments. However, the highest concrete percent in leaves and flowers were gained by the combined treatment of EM+ 75 % dose NPK, full dose of NPK and EM+ 50 % dose NPK, respectively in both seasons with none significant differences between them in most cases. On the other hand, the lowest values of these parameters were produced by the control and the treatment of EM, respectively in the first and the second seasons. These results are in agreement with those reported by Barandozi and Pourmaleknejad [29] on *Thymus vulgaris* L., Abd El-Wahab [30] on *Origanum syriacum* var. *sinaicum* and El-Khyat [31] on *Rosmarinus officinalis*. Furthermore, Mohamed *et al.* [32] showed that *Ocimum basilicum* L. cv. Genovese which received biofertilizer showed the highest significant increase of essential oil yield.

Effect of NPK Mineral, Bio-fertilizer (EM) and Zeolite on N%, P%, K%, Total carbohydrates and Total Chlorophylls of *Viola odorata* L. Plants: Data presented in Tables 5 and 6 indicated that N%, P%, K%, total carbohydrates and total chlorophylls in leaves of *Viola odorata* L. plants was more affected by using all the fertilizer treatments compared to control in the first and second seasons. However, using full dose of NPK was the most effected fertilizer for increasing N%, P%, K% and total chlorophylls, followed by the combination of EM + 75 % dose NPK which ranked the second in this concern in both seasons. While, the combination of EM + 75 % dose NPK gave the highest value of total carbohydrates percentage compared to the other ones in the 1st and 2nd seasons. Besides, the combination of EM + 50 % dose NPK gave the third value in this respect in both seasons. On the contrary, the untreated plants produced the lowest values in this regard in the two seasons. In this concern, Gendy *et al.* [33] found that the application of biofertilizers increased N, P and K in leaves of Roselle plants. Also, Barandozi and Pourmaleknejad [29] showed that *Thymus vulgaris* L. treated by nitrogen fixing bacteria showed a significant increase in total N, P and carbohydrate content. Mohamed *et al.* [32] showed that bio fertilizers application increased N, P, K, chlorophyll a, b and carotenoids and total carbohydrates contents of *Ocimum basilicum* L. cv. Genovese leaves compared to control plants.

Volatile Oil Composition of *Viola odorata* L. Leaves and Flowers by Using GC- MS Analysis: Data in presented in Tables 7 & 8 and Figs 1 & 2 indicated that, The GC-MS analysis composition of the volatile oil of violet leaves and flowers produced 60 and 40 compounds the volatile oil of violet leaves and flowers, respectively.

Table 2: Effect of NPK mineral, bio-fertilizer (E.M.) and zeolite on No. of leaves/plant, fresh and dry weight of leaves/plant of *Viola odorata* L. during 2014/2015 and 2015/2016 seasons.

	Parameters					
	No. of leaves/plant		Fresh weight of leaves/plant		Dry weight of leaves/plant	
Treatments	1st season	2nd season	1st season	2nd season	1st season	2nd season
Control	35.00g	39.67h	73.80h	78.83h	15.30h	15.37g
EM at 30 ml	53.33f	59.33g	98.27g	105.0g	26.50g	28.40f
full dose of NPK	71.67b	76.00b	174.3b	179.7b	39.93b	41.33b
Full dose of Zeolite	60.67e	62.63f	158.1f	162.3f	30.53f	31.30e
EM at 30 ml + 75 % dose NPK	77.33a	80.53a	181.0a	186.5a	42.50a	44.43a
EM at 30 ml + 50 % dose NPK	70.67bc	73.36c	172.2c	175.5c	39.17c	40.60c
EM at 30 ml + 75 % dose Zeolite	69.33c	65.43e	170.6d	168.4e	37.50d	36.63d
EM at 30 ml + 50 % dose Zeolite	66.00d	69.35d	167.6e	171.0d	36.27e	36.43d

Table 3: Effect of NPK mineral, bio-fertilizer (E.M.) and zeolite on No. of flowers /plant, fresh and dry weight of flowers /plant of *Viola odorata* L. during 2014/2015 and 2015/2016 seasons.

	Parameters					
	No. of flowers/plant		Fresh weight of flowers/plant		Dry weight of flowers/plant	
Treatments	1st season	2nd season	1st season	2nd season	1st season	2nd season
Control	31.73f	32.00h	10.87g	11.57h	2.83e	3.43e
EM at 30 ml	45.33e	48.67g	13.87f	15.17g	3.90d	4.57d
full dose of NPK	66.33b	69.00c	21.63c	22.47c	6.40b	6.47b
Full dose of Zeolite	50.67d	55.67f	14.43f	16.70f	4.00d	4.57d
EM at 30 ml + 75 % dose NPK	70.67a	74.67a	25.47a	27.37a	7.27a	4.53a
EM at 30 ml + 50 % dose NPK	70.67a	70.67b	23.33b	23.87b	7.00a	7.53a
EM at 30 ml + 75 % dose Zeolite	60.33c	65.33e	18.67d	21.23d	5.50c	6.43b
EM at 30 ml + 50 % dose Zeolite	59.33c	62.00e	17.37e	19.63e	5.47c	5.87c

Table 4: Effect of NPK mineral, bio-fertilizer (EM) and zeolite on Leaves and flowers concrete % of *Viola odorata L.* during 2014/2015 and 2015/2016 seasons.

	Parameters			
	Leaves concrete %		Flowers concrete %	
Treatments	1st season	2nd season	1st season	2nd season
Control	0.067c	0.071e	0.077d	0.087e
EM at 30 ml	0.096b	0.090d	0.103c	0.097d
full dose of NPK	0.119a	0.118ab	0.121a	0.124a
Full dose of Zeolite	0.111ab	0.113bc	0.113b	0.116c
EM at 30 ml + 75 % dose NPK	0.119a	0.120a	0.122a	0.122ab
EM at 30 ml + 50 % dose NPK	0.116a	0.117abc	0.120a	0.117bc
EM at 30 ml + 75 % dose Zeolite	0.112ab	0.115abc	0.114b	0.115c
EM at 30 ml + 50 % dose Zeolite	0.110ab	0.112c	0.113b	0.112c

Table 5: Effect of NPK mineral, bio-fertilizer (E.M.) and zeolite on N%, P% and K%, of *Viola odorata* L. during 2014/2015 and 2015/2016 seasons.

	Parameters					
	N%		P%		K%	
Treatments	1st season	2nd season	1st season	2nd season	1st season	2nd season
Control	2.13g	2.05f	0.221c	0.229d	1.74f	1.83h
EM at 30 ml	2.35f	2.42e	0.252bc	0.260cd	2.19e	2.27g
full dose of NPK	3.14a	2.96a	0.381a	0.371a	2.63a	2.76a
Full dose of Zeolite	2.65a	2.65d	0.269bc	0.281bcd	2.35d	2.33f
EM at 30 ml + 75 % dose NPK	2.90b	2.87b	0.379a	0.367a	2.58b	2.69b
EM at 30 ml + 50 % dose NPK	2.82c	2.85b	0.376a	0.362a	2.54b	2.60c
EM at 30 ml + 75 % dose Zeolite	2.71d	2.69d	0.302b	0.317ab	2.43c	2.50d
EM at 30 ml + 50 % dose Zeolite	2.70d	2.78c	0.297b	0.305bc	2.41c	2.42e

Table 6: Effect of NPK mineral, bio-fertilizer (EM) and zeolite on total carbohydrates and total chlorophylls (mg/100g FW) of *Viola odorata* L. during 2014/2015 and 2015/2016 seasons.

	Parameters			
	Total carbohydrates (%)		Total chlorophylls (mg/100g FW)	
Treatments	1st season	2nd season	1st season	2nd season
Control	8.08h	9.83h	148.9g	159.9h
EM at 30 ml	10.08g	11.53g	160.9f	170.9g
full dose of NPK	14.88c	16.30b	221.2a	238.3a
Full dose of Zeolite	11.45f	12.91f	161.1f	177.5f
EM at 30 ml + 75 % dose NPK	16.32a	16.89a	218.3b	231.2b
EM at 30 ml + 50 % dose NPK	15.18b	15.47c	215.3c	226.8c
EM at 30 ml + 75 % dose Zeolite	13.83d	14.28d	176.7d	187.0d
EM at 30 ml + 50 % dose Zeolite	12.92e	13.40e	168.1e	180.8e

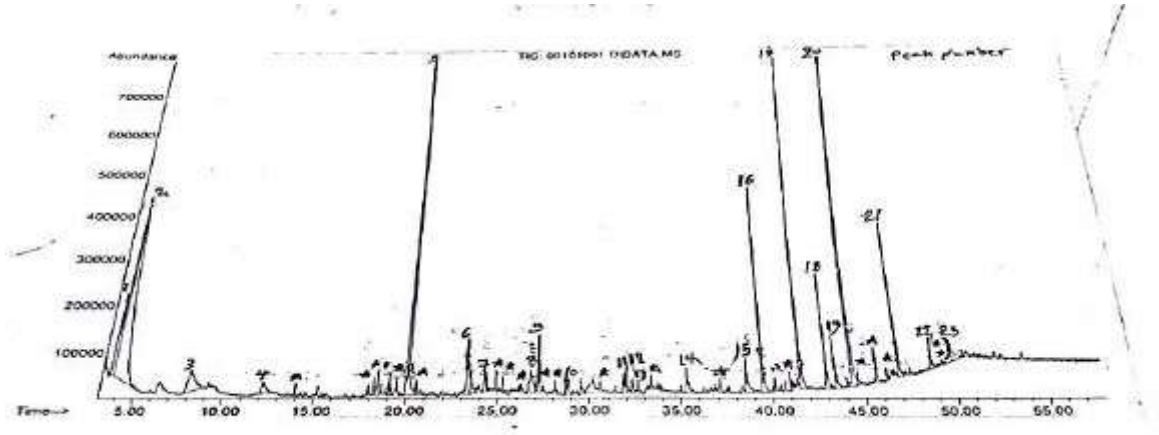
Table 7: The GC- MS analysis of *Viola odorata* L. leaves volatile oil component.

Peak No.	Component name	Area %
1	Ethyl benzene	7.94
2	1-Octen-3-ol	1.04
3	3-Hexenyl Acetate	9.30
4	Benzyl alcohol	0.68
5	Methyl salicylate	1.01
6	Butyl-2 ethylhexyl phthalate	17.22
7	2-Propionic acid, trimethylsilyl ester	1.19
8	Phytol	1.16
9	1,2-Benzenedicarboxylic acid, dibutyl ester	1.26
10	Hexadecanoic acid	3.24
11	2,4-Dimethyl-10-methoxyfuro2,3:3,4 cycloheptaisochromene-one	0.66
12	2-Hexadecen-1-ol, 3,7,11,15-tetramethyl	3.24
13	9,12,15-octadecatrienoic acid	3.09
14	Methyl 8,11,14-heptadecatrienoate	0.99
15	Icos-1-ene	1.03
16	Phthalic acid, di (2-propylpentyl)ester	1.85
17	Heptacosane	1.26
18	2,4-Dimethoxy-3-(prop-2-enyl)benzaldehyde	1.88
19	Squalene	1.27
20	Nonacosane	2.47
21	Tetracosane	2.18
22	á-Tocopherol	1.28
23	Urs-12-ene	6.22
24	Lupeol	1.70
25	ethyl-1,4a,7-trimethyl-perhydro-phenanthrene	10.36
26	4-epi-Friedelin	5.08
*	Unidentified	11.40
Total		100.00

Table 8: The GC- MS analysis *of Viola odorata* L. flowers volatile oil component.

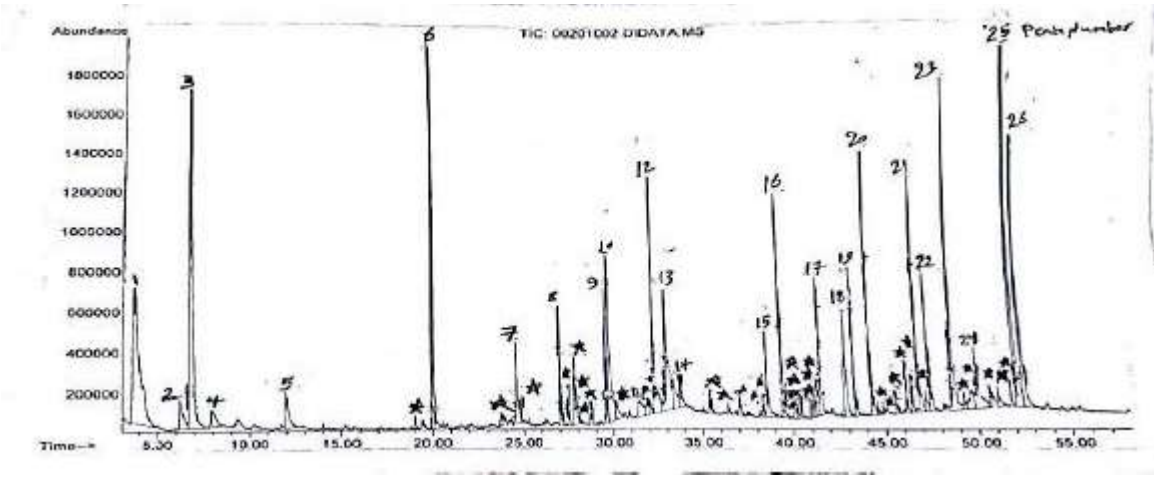
Peak No.	Component name	Area %
1	Ethyl benzene	4.63
2	*p*-Xylene	12.06
3	Benzene acetaldhyde	1.84
4	Methyl salicylate	0.65
5	Butyl-2 ethylhexyl phthalate	37.11
6	6,8-Nonadien-2-one,6-methyl-5-(1-methylethylidene	1.40
7	2-Hydroxyphenyl-1-hexanone	0.71
8	Bisabolene	0.94
9	2-Pentadecanone,6,10,14-trimethyl	0.75
10	Hexadecanoicacid,methyl ester	0.38
11	Octadecanoic acid	0.86
12	Octadecadienoic acid	0.69
13	Phytol	0.31
14	Heptadecenal	0.60
15	Octadec-1-ene	0.82
16	1,2-Benzenedicarboxylic acid,bis2-ethyl ester	2.60
17	Heptacosane	4.50
18	Icos-1-ene	1.43
19	Squalene	0.87
20	Nonacosane	17.09
21	Tetracosane	1.94
22	1,30-Triacontanediol	0.63
23	2,4-Dimethylbenzaquinoline	0.69
*	Unidentified	6.50
Total		100.00

The volatile oil constituents of violet leaves included 26 compounds were identified. The main constituents of the volatile oil of violet leaves was Butyl-2 ethylhexyl phthalate (17.22), followed by ethyl-1,4a,7-trimethyl-perhydro-phenanthrene (10.36), 3-Hexenyl Acetate (9.30), Ethyl benzene (7.94), Urs-12-ene (6.22), Hexadecanoic acid (3.24) and 2-Hexadecen-1-ol, 3,7,11,15-tetramethyl (3.24). While, the volatile oil constituents of violet flowers included 23 compounds were identified. The concentration of the main constituents of the volatile oil of violet flowers (Butyl-2 ethylhexyl phthalate) is higher than that in absolute oil of violet leaves as (37.11). The major constituents of the absolute oil of violet flowers were Nonacosane (17.09), p-Xylene (12.06), Ethyl benzene (4.63), Heptacosane (4.50) and 1, 2-Benzenedicarboxylic acid, bis2-ethyl ester (2.60). Also, the unknown component with values (11.40 and 6.50) in the volatile oil of violet leaves and flowers, respectively. Similar findings were obtained by Craik *et al.* [2], Akhbari *et al.* [3] and Mittal *et al.* [4], who stated that, the analysis of essential oil composition of the leaves of *Viola odorata* L. revealed the presence of 25 identified compounds, representing 92.77% of the oil with butyl-2-



The peak number indicated to the peaks number in Table 7.

Fig. 1: The GC- MS analysis of *Viola odorata* L. leaves the volatile oil constituents



The peak number indicated to the peaks number in Table 8.

Fig. 2: The GC- MS analysis of *Viola odorata* L. flowers the volatile oil constituents.

thylhexylphthalate (30.10%) and 5,6,7,7a-tetrahydro-4,4,7a-trimethyl-2(4H)-benzofuranone (12.03%) being the two main components. *Viola odorata* also produces macrocyclic peptides.

CONCLUSIONS

Conclusively, it is preferable from the previous results that treating violet plants with EM at 30 ml/plant + 75% dose NPK or full dose of NPK for enhancing vegetative growth characteristics, flowering characteristics, yield of concrete of leaves and flowers and composition of the volatile oil of violet.

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