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Evaluation the Effects of Salicylic Acid and Methyl Jasmonate on the Scent of Purple Coneflower (*Echinacea purpurea* L. Moench) Flowers

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Abstract: Flowers of many plants emit scents, which are almost always a complex of small volatile organic compounds such as essential oils that they are the ones who give the fragrance of flowers and also have medicinal curative properties. So, the most common topic in the field of plant sciences, has been focused on improving flower's quality and quantity by application of plant growth regulators (PGRs) to modify growth and flowering patterns. For this purpose, a research was conducted at the research field of agricultural faculty of Zanjan university on purple coneflower during farming years of 2017-2018 and 2018-2019 by application of salicylic-acid (0 (control), 50, 100 and 150 mM) and methyl-jasmonate (0 (control), 50, 100 and 200 µM). In the first year of experiment, treatments were sprayed on plants (four plants per plot) in two stages (20 days apart). In the second year, they were also sprayed on remaining plants as the first year. The results indicated that the highest percentage of essential oils in the flower heads of purple coneflower was related to treatment of 100 mM salicylic acid and 50 µmol methyl jasmonate in the first year and treatments of 100 mM salicylic acid and 50 and 100 µmol methyl jasmonate in the second year. The highest percentage of total compounds of essential oils in the first year was related to treatment of 50 mM salicylic acid and 50 µmol methyl jasmonate and in the second year was related to treatment of 100 mM salicylic acid and 100 µmol methyl jasmonate. The identified compounds in the essential oils of purple coneflower samples included hydrocarbon monoterpenes, oxygenated monoterpenes, hydrocarbon sesquiterpene, oxygenated sesquiterpenes and other compounds. The highest percentage of total compounds of essential oils in the first year was related to treatment of 50 mM salicylic acid and 50 µmol methyl jasmonate and in the second year was related to treatment of 100 mM salicylic acid and 100 µmol methyl jasmonate. Also, most of compounds increased in the second year compared to the first year.

Keywords: Essential Oils, Fragrance, Purple Coneflower, Terpenes, Scent

1. Introduction

One of the plants application in urban landscape designing, is utilization of aromatic plants (flower and foliage aroma) for more citizens uses and pay more attention to the importance of landscape that they can use all the elements in the parks, such as beautiful scenery, sounds of water and birds and wind movement between trees foliage, and especially the pleasant aroma of flowers and aromatic plants, because the importance of these plants planting, in addition to the beautiful appearance of their flowers, also includes their fragrance and create calmness for humans with joy and happiness. Therefore, over the last decade, numerous studies have been conducted on flower fragrance production, their pattern differences between different flowers, biosynthesis, release and therapeutic effects (aromatherapy) of aromatic volatiles released from flowers [23, 4].

It is believed that in ornamental flowers, flower aroma is

one of the important characteristics for flowers evaluation and is an important part of volatile compounds. It has been reported that flower aroma plays a key role in flower development in flowering plants [10, 16]. For this purpose in recent years, an increasing number of studies have focused on floral fragrances [14, 17].

Essential oils are low molecular weight compounds that are responsible for creating the specific aromas in plants. These compounds include terpenoid and non-terpenoid hydrocarbons and their oxygenated derivatives that the ratio of one component or a group of specific components to other components is one of the essential oils properties that affects their aroma and these compounds are found in more than 60 plant families such as Lauraceae, Myrtaceae, Umbelliferae, Labiatae and Compositae [20].

Coneflower genus is an important herbaceous perennial-ornamental plant belonging to the Asteraceae family, originated in north America and widely used for planting in landscapes, creating perennial orchards and sometimes as cut flowers [35]. Among coneflower species, *Echinacea purpurea* L. Moench (Purple coneflower) is one of the main commercial species that has been introduced and cultivated in some parts of Iran.

Terpenes form a large group of essential oils that are made from acetyl coenzyme A via mevalonic acid pathway by binding to five carbon units with an isopronoid structure (C_5H_8) and based on the number of isoprene units that are divided into monoterpene (C_{10}), sesquiterpene (C_{15}), diterpene (C_{20}), triterpene (C_{30}), tetraterpene (C_{40}) and polyterpenes and are secondary volatile metabolites that make plants fragrant [32].

Among these compounds, terpene hydrocarbons, due to early oxidation, only partially contribute to essential oils flavor, but their oxygenated derivatives, i.e oxygenated terpene compounds, are highly aromatic due to their high resistance to oxidative oxidation. Therefore, oxygenated derivatives of monoterpenes and sesquiterpenes are more important as aromatic chemicals than terpene hydrocarbons, so that the specific odor of many essential oils represents a combination of oxygenated compounds aroma [6].

One of the ways to increase secondary compounds in plants, is plant hormones application [3] that they play an important role in their production cycle [2]. So, the most common topics in the field of plant sciences, is exploitation the commercial potential of ornamental plants that has been focused on improving flower's quality and quantity by application of plant growth regulators (PGRs) to modify growth and flowering patterns in some horticultural products regarding to ornamental importance that can have a profound effect on their morpho-physiological and phytochemical reactions [22].

Salicylic acid and methyl jasmonate are phytohormonal elicitors used in most studies, because they biologically elicit important active compounds [31] and they are important phytohormones that actively involved in plant defense mechanisms through octadecanoid and phenylpropanoid pathways, respectively [30]. Salicylic acid is a naturally occurring hormone with chemical formula $C_7H_6O_3$ that is present in plants and acts as a potential plant regulator and plays important roles in regulating a number of morphophysiological and biochemical processes. Methyl jasmonate is a natural plant growth regulator that can affect many morphological, physiological and biochemical processes in plants [33, 21] and with chemical formula $C_{13}H_{20}O_3$ is a colorless liquid that is effective in gene expression regulation, metabolite pathways regulation, defensive responses induction and reproduction as a plant hormone [28] and it can affect a wide range of morphophysiological and biochemical processes [11]. Therefore, the aim of this study was to investigate the effects of different concentrations of salicylic acid and methyl jasmonate elicitors on odor production in flowers of purple coneflower (*Echinacea purpurea*) plant.

2. Materials and Methods

2.1. Field Experiment

This study was conducted in the research farm of faculty of agriculture, university of Zanjan, Iran, during 2017~2018 and 2018~2019. Meteorological information of experiment years is listed in Table 1.

Table 1. *Meteorological information of Zanjan Station in cultivation years.*

Cultivation Year (2017~2018)												
Month	**Sep.**	**Oct.**	**Nov.**	**Dec.**	**Jan.**	**Feb.**	**Mar.**	**Apr.**	**May**	**Jun.**	**Jul.**	**Aug.**
Average Temperature (°C)	15.7	12.7	3.8	5.5	2.3	7.5	14.4	10.6	21.2	25.5	26.3	23.9
Average Rainfall (mm)	0	11.7	20	17.5	70.3	26.8	39.8	22.1	0	1.3	4.8	0
Relative Humidity (%)	45	54	58	66	66	61	58	51	37	42	38	37
Cultivation Year (2018~2019)												
Month	Sep.	Oct.	Nov.	Dec.	Jan.	Feb.	Mar.	Apr.	May	Jun.	Jul.	Aug.
Average Temperature (°C)	16.1	8	5.3	1.1	2	3.8	11.8	13.3	19.3	26.3	26.3	21.8
Average Rainfall (mm)	8.8	26.5	67.2	26.3	45.5	28.2	14.2	62	23.2	0	0.7	1.8
Relative Humidit (%)	55	68	72	69	69	62	47	61	54	36	41	42

Table 2. *Physical and chemical properties of soil, research field of Zanjan University.*

Soil Texure				**Total N (%)**	**K (ppm)**	**P (ppm)**	**$CaCO_3$ (%)**	**Organic Matter (%)**	**EC (mmohs cm^{-1})**	**pH**
Clay (%)	**Silt (%)**	**Sand (%)**	**Silty-Clay**							
37	38	25		0.07	286	9.60	7.20	0.94	1.49	7.4

In the first year of experiment (2017), at first after preparation of the soil according to soil test results (Table 2), purple coneflower transplants at four leaf and 15 cm high stage, were purchased and planted according to planting plan on May 20. Experimental design was conducted as factorial based on complete randomized block design (CRBD) with three replications. In the first year of experiment after completely establishment of plants, different concentrations of salicylic acid and methyl jasmonate were sprayed on plants at 24 hours interval and in two stages (20 days apart). Treatments included salicylic acid at four levels of 0 (control), 50, 100 and 150 mM and methyl jasmonate at four levels of 0 (control), 50, 100 and 200 µM. In the second year, treatments were sprayed on remaining plants (four plants per plot) as in the first year. Four plants were selected in the first year and four remaining plants in the second year from each plot (totally 8 plants per plot).

2.2. Laboratory Experiment

Essential Oils Content (Total Essential Oils and Chemical Components of Essential Oils)

The essential oils of *Echinacea purpurea* flowers were extracted by Clevenger apparatus by water distillation. This method is used for dry plant organs [27]. To do this, at first dried flowers were ground with a milling machine and 50 grams of each sample was weighed and was poured into the one liter round bottom balloon and 500 ml of distilled water was added. Then the device heater was turned on and plant materials were boiled for extraction of essential oil for 2.5 hours. The volume of extracted essential oils was recorded in milliliters and recorded as a percentage. The essential oils were then carefully collected and stored in the refrigerator with aluminum foil covering until further investigation. The essential oils yield was also calculated using the following equation:

$$\text{Essential oil yield} = \frac{\text{Essential Oil\%} \times \text{Dried Flower Weight}}{100}$$

GC $_{mass}$ device (Agilent 7890B model, made in USA, MS detector model: 5977A) was used to analyze the essential oils and identify the active ingredients.

Data analysis and mean comparison based on Duncan level test were performed using SAS software version 9.1 and MSTAT-C at five percent level.

3. Result and Discussion

3.1. Essential Oils Percentage of Flowers

The results of salicylic acid and methyl jasmonate application on essential oils percentages in *Echinacea purpurea* flowers in two years of experiment are shown in Table 3. The highest percentage of essential oils in the first year (0.186) was related to treatments of 100 mM salicylic acid and 50 µM methyl jasmonate.

The highest percentage of essential oils in the second year was obtained from 100 mM salicylic acid and 100 µM methyl jasmonate (0.243) and 100 mM salicylic acid and 50 µM methyl jasmonate (0.242) treatments. Also, the highest average percent of essential oils (0.193) was in the second year that shows the significant effects of salicylic acid and methyl jasmonate on essential oils production.

Table 3. *Essential oils Percentage of Echinacea purpurea flowers in two years of experiment.*

Treatments		Essential oils percentage	
Salicylic Acid (mM)	**Methyl jasmonate (µM)**	**Year 1**	**Year 2**
0	0	0.036	0.080
	50	0.095	0.193
	100	0.176	0.179
	200	0.131	0.168
50	0	0.141	0.169
	50	0.157	0.195
	100	0.167	0.175
	200	0.170	0.237
100	0	0.169	0.206
	50	0.186	0.242
	100	0.175	0.243
	200	0.123	0.235
150	0	0.124	0.200
	50	0.159	0.168
	100	0.100	0.194
	200	0.149	0.207
Mean		0.141	0.193

One of the ways to increase secondary compounds in plants is use of plant hormones [3], that they are applied in all aspects of plant life cycle, so that these substances can have a profound effect on their physiological and phytochemical reactions [5].

Research has shown that salicylic acid and methyl jasmonate, as signaling molecules, like other stimuli, induce the expression of genes involved in the production of secondary metabolites in plants. In fact, salicylic acid and methyl jasmonate are key messengers in activating plant defense responses and in addition to reducing the raw materials production, also lead to biosynthesis and accumulation of various plant secondary compounds [8, 7].

When plant cells are treated with these elicitors, the transmission of a message from the surface to the plasma membrane begins and it caused to produce of reactive oxygen species that stimulate the plant's defense response and increase the activity of key and specific enzymes that catalyze chemical reactions and biosynthesis of target secondary metabolites, in fact, accumulation of secondary metabolites in plants occurs mainly in response to stresses or stimulus molecules [37] to produce low molecular weight defense compounds such as essential oils (terpenoids) in plants [19, 36].

Salicylic acid, as a transmitter of stress messages, activates the plant's defense system. Given that stressors stimulate essential oils production, it can be acknowledged that use of salicylic acid increases production of essential oils by creating false stress. In a study, Gharib [12] indicated that the effect of salicylic acid on basil and marjoram at a

concentration of 10^{-4} mM increases quantity and quality of essential oils. Also, Rowshan, and Bahmanzadegan [26] reported that external application of salicylic acid at concentrations of 200 and 400 mg l^{-1} may alter yarrow secondary metabolites and their pathways by affecting plastids and chlorophyll content that represents stress conditions.

3.2. Components of Chemical Compounds of Essential Oils in Echinacea Flowers

According to the results, essential oils of six top treatments were selected along with control and their chemical components were analyzed by GC_{mass} method (Figure 1). The essential oils of samples contained 82-98 compounds that 50 important compounds were identified.

In general, identified compounds in *Echinaceae* flowers included hydrocarbon monoterpenes, oxygenated monoterpenes, hydrocarbon sesquiterpenes, oxygenated sesquiterpenes and other compounds.

According to results of Table 4, in the first year, the highest percentage of hydrocarbon monoterpenes (2.17%) was related to treatment of 50 mM of salicylic acid and 100 µM of methyl jasmonate, the highest percentage of oxygenated monoterpenes (1.9%) was related to treatment of 50 mM of salicylic acid and 100 µM of methyl jasmonat, the highest percentage of hydrocarbon sesquiterpenes (18.24%) was related to treatment of 50 mM of salicylic acid and 100 µM of methyl jasmonate and the highest percentage of oxygenated sesquiterpenes (33.74%) was related to treatment of 50 mM of salicylic acid and 50 µM of methyl jasmonate.

In the second year, the highest percentage of hydrocarbon monoterpenes (2.89%) was related to treatment of 100 mM of salicylic acid and 100 µM of methyl jasmonate, the highest percentage of oxygenated monoterpenes (3.23%) was related to treatment of 50 mM of salicylic acid and 50 µM of methyl jasmonate, the highest percentage of hydrocarbon sesquiterpenes (13.58%) was related to treatment of 100 mM of salicylic acid and 0 µM of methyl jasmonate and the highest percentage of oxygenated sesquiterpenes (58.31%) was related to treatment of 100 mM of salicylic acid and 100 µM of methyl jasmonate. Also, the highest percentage of total compounds (50.11%) in the first year was related to treatment of 50 mM of salicylic acid and 50 µM of methyl jasmonate and in the second year (80.53%) was related to the treatment of 100 mM of salicylic acid and 100 µM methyl jasmonate.

Table 4. *Chemical Components of Echinacea purpurea Flower's Essential Oils.*

Compound Name	year	Essential oils percentage of *Echinacea* flowers in treatments						
		Control	$SA_{(50)}\times mJA_{(50)}$	$SA_{(50)}\times mJA_{(100)}$	$SA_{(100)}\times mJA_{(0)}$	$SA_{(100)}\times mJA_{(50)}$	$SA_{(100)}\times mJA_{(100)}$	$SA_{(100)}\times mJA_{(200)}$
Hydrocarbon	1	0.99	0.42	0.32	0.42	0.95	0.95	0.94
Monoterpenes	2	1.17	0.5	2.17	0.14	0.42	2.89	1.9
Oxygenated	1	1.74	1.64	1.9	0.92	0.97	0.82	0.38
Monoterpenes	2	2.36	3.23	2.2	2.28	1.69	2.33	1.9
Hydrocarbon	1	12.59	9.98	18.24	10.19	10.47	13.45	10.29
Sesquiterpenes	2	11.83	11.87	10.82	13.58	11.67	10.18	12.38
Oxygenated	1	22.92	33.74	22.91	23.2	21.67	31.96	18.88
Sesquiterpenes	2	51.77	54.08	54.35	52.14	56.34	58.31	54.76
Other	1	1.42	4.33	0.85	2.3	1.47	0.47	1.4
Compounds	2	7.45	6.73	6.73	5.69	6.05	6.82	6.39
Total Detected	1	39.66	50.11	46.07	37.07	35.53	47.53	31.89
Compounds	2	74.58	74.95	74.42	73.83	76.17	80.53	75.43

SA: Salicylic Acid (m M); mJA: Methyl Jasmonate (µM).

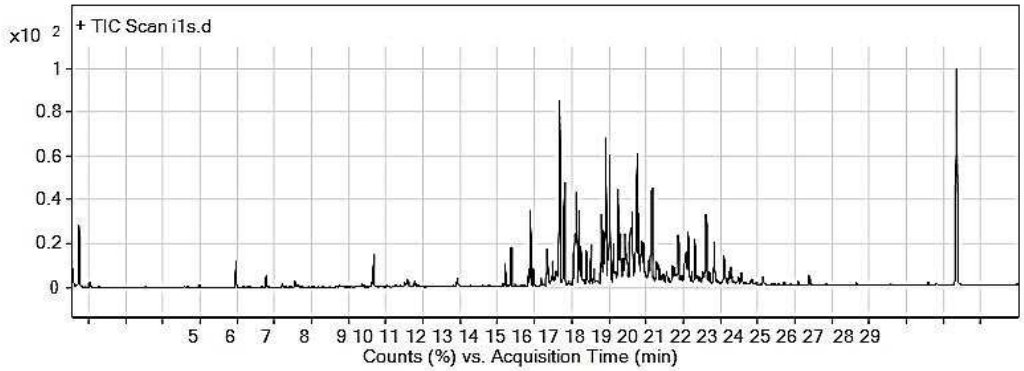

Figure 1. *GC_{mass} Chromatogram of Chemical components of Echinacea flower's essential oils.*

In a study, Mirjalili *et al*. [18] did not identify oxygenated monoterpenes in essential oils of *Echinacea* species, but they identified 6.4 percent of monoterpene hydrocarbons, 70.9 percent of Sesquiterpene hydrocarbons and 15.4 percent of oxygenated sesquiterpenes that is in accordance with the results of present study. Recently, some studies have shown that salicylic acid signaling pathways are involved in the biosynthesis of terpenoids, including monoterpenes and

sesquiterpenes [1], diterpenes [34] and triterpenes [29]. The results have shown that the percentage of response and essential oils yield in leaves and flowers to salicylic acid is dependent on concentration, to the extent that an increase in salicylic acid concentration increases its effects on essential oils production. This effect may be due to the activity of phenylalanine ammonia lyase enzyme involved in the production of secondary metabolites [9].

According to research records, it seems that methyl jasmonate affects the genetically regulation of enzymes activity in the metabolic pathway involved in secondary metabolites synthesis [25, 15] and probably improving of essential oils yield by using of methyl jasmonate is due to the prolongation of growth period, increasing the nutrient uptake and changes in the number of leaf glands and synthesis of terpenes [13]. Therefore, relationship between salicylic acid and methyl jasmonate in the regulating of plant responses to the abiotic stresses, may describes the role of these plant growth regulators in the synthesis of secondary metabolites and changes in essential oils composition [24].

4. Conclusions

Based on the study, the highest percentage of essential oils in *Echinacea purpurea flower's* was related to the treatment of 100 mM salicylic acid and 50 µM of methyl jasmonate in the first year and the treatments of 100 mM salicylic acid and 100 µM of methyl jasmonate in the second year. Also, the highest average percent of essential oils was obtained in the second year, which showed a significant effect of salicylic acid and methyl jasmonate on the essential oils production.

The identified compounds in essential oils included hydrocarbon monoterpenes, oxygenated monoterpenes, hydrocarbon sesquiterpene, oxygenated sesquiterpenes and other compounds. The highest percentage of total compounds (50.11%) in the first year was related to the treatment of 50 mM of salicylic acid and 50 µM of methyl jasmonate and in the second year (80.53%) was related to the treatment of 100 mM of salicylic acid and 100 µM of methyl jasmonate. Also, most of the secondary compounds increased in the second year compared to the first year.

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