

# Investigation of Vermicompost Fertilizer Effect on Chemical Composition of Essential Oil of *Ziziphora tenuior* in Weather Conditions of Kahnoojin Iran

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**Abstract** Recent tendencies in utilizing organic products have grown owing to the negative effects of using the chemical fertilizers. Meanwhile, animal manures have a devastating impact on the soil and the excessive uses of them can also reduce the agricultural soil quality. So a normal fertilizer produced with distinctive and certain types of properties that this manure is produced using the earthworms (from *Eisenia Foetida* species). Meanwhile *Ziziphora tenuior* plants are very fragrant and have great food and pharmaceutical applications. In this study, we tried to extract it from the plant after a period of fertilization by vermicompost and compare it with essential oils of plant compounds in vermicompost without fertilization to evaluate changes of vermicompost upon the secondary compounds. For this, we used of the essential oil by hydro distillation by Clevenger method and essential oil with a yield of 1/1% (g/g) which was obtained by GC-MS were analyzed to determine the composition of the essential oils. The present of compounds in essential oils *Ziziphora tenuior* shows 21 effective combinations that have the significant phenolic compounds. Also, we could cite the pulegone compounds with 69.9% before fertilization, and 81.3% after fertilization, limonene with 4.8% before fertilization and 6.7% after fertilization and cyclohexane with 4.1% before the fertilization and 1.3% after fertilization. Given the abundant phenolic compound and oxygenated terpenes in the essential oil of this plant (*Ziziphora tenuior*), can be used as a natural source for organic and natural use of this material normally that usage of vermicompost fertilizers significantly improve the quality of essential oils.

**Keywords** *Ziziphora tenuior*, Vermicompost, Essential Oils

## 1. Introduction

Nowadays, researchers suggest that excessive use of chemical fertilizers and other agricultural field causes many diseases and problems of the society. Can be noted that a rise in the general level of cancer statistics. That is why a growing consumer interest in organic products has emerged. Meanwhile, animal manures have the high percentage of organic compounds containing nitrogen and mineral salts which can be cited. So a normal and healthy fertilizer with distinctive properties of the manure is produced by certain types of earthworm's species as *Eisenia Foetida*. [1]

Thus, earthworms eat the organic matters consisting of plant waste and livestock waste and convert into vermicompost fertilizer during a process. Vermicompost is a

well changed-form material and its structure, porosity, ventilation, drainage and water holding capacity is at its highest level. [2]

Vermicompost fertilizers minerally are much richer than conventional manures so that the amount of nitrogen in manure is averagely five times more than the animal manure, the average amount of phosphorus in manure is seven times more than the average amount of animal manure, where its potassium is eleven times more than the average amount of animal manures. Even moderate amounts of calcium and magnesium within them are two times more than the average amount of animal manures. [3] However, consumption of chemical fertilizers and even animal manures also are higher in rank in comparison to the vermicompost.

*Ziziphora tenuior* is from *Labiatae* family and is part of the subfamily of *Ziziphora*. This plant is very aromatic plants which are used for food and medicine and its aroma is near to Thyme and Oregano. It is a perennial herb and its bushes are thick and its height is 20-50 cm and has purple flowers. [4] *Labiatae* family plants as flavoring or spice foods as well as

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Published online at <http://journal.sapub.org/ijaf>

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medicines for digestion and to combat with viral diseases have been used a century ago at most countries. The family has the 46 genera and 410 species and subspecies. This plant in the mountainous areas of Iran as a spice widely used in foods and cosmetics and its aroma is very close to Oregano and thyme and a bit too similar to Pennyroyal. New shepherds and mountaineers gather *Ziziphora tenuior* and drink it as a tea. [5]

In this study we tried to evaluate the effect of vermicompost fertilizer on plant's secondary compounds to show that by improving the quality and quantity of oil is better to use the vermicompost fertilizer for fertilization.

## 2. Materials and Methods

Samples of plant for pot cultivation have been delivered from the greenhouse of Islamic Azad University of Jiroft and transferred to the Islamic Azad University of Kahnooj. This species was determined its scientific name as the garden thyme by team science professors of Kahnooj Branch Islamic Azad University. [6]

Fourteen different samples of plants were grown in pots and seven pots were considered as the control sample and other seven samples were fertilized four times and with the frequency of seven days. Ten grams of milled vermicompost fertilizer after mixing and stirring in 40 ml of water at a temperature of 35°C was added to the pot. After fertilization, the plant species were collected and transferred to the University Chemical Laboratory of Kahnooj and then separately were flattened for fast drying under indirect sunlight. Essential oils obtained by hydro distillation using Clevenger apparatus were performed. For this purpose, plant samples can be milled to proper levels.

All essential oils samples were doing at temperatures of 80-90°C and 210 min, 300 ml of twice distilled water and 50 grams within 2-liter flask. The extracted essential oils after each dewatering operation by NaSO<sub>4</sub> were poured into special containers and to identify the compounds in the essential oils, were used of GC-MS instruments of Shahid Beheshti University made in the USA model (HP Agilent Technology). Column (HP-5MS) with a length of 60m, in which helium gas was used at 1ml/min speed. In the temperature programming, initially the temperature of beginning of column was kept constant at 50°C for five minutes and then temperature increases to 270°C with speed of 4 °C /min. In the temperature program, because the temperature variations speed was high, the peaks were pressed close together.

In addition, before the injection of sample of *Ziziphora tenuior* essential oils, a standard of 6-27 carbon were injected and then the oil samples in the separate programs were injected for 4 times. The program used to identify compounds in the oils is Wiley Version 7.

## 3. Results and Discussion

*Ziziphora tenuior* samples were collected after completion of fertilization and after initial preparation isolated from the aboveground plant parts and plant samples preparation by water distillation using Clevenger apparatus after determining the optimal conditions for making essential oil was obtained. Essential oil with an efficiency of 1/1 percent (g/g) was obtained and by GC-MS to determine the composition of the essential oils was analyzed.

Results from the plant oils, indicates 21 effective combinations of *Ziziphora tenuior* essential oils that have a considerable amount of the phenolic compounds that this indicates that there is anti-microbial nature of the essential oils of *Ziziphora tenuior*. Major compounds in the oils of this plant include:

- 1 - Pulegone with 69.9% before fertilization, and 81.3% after fertilization
- 2 - Limonene with 4.8% before fertilization and 67% after fertilization
- 3 - Cyclohexane with 4.1% before fertilization, and 1.3% after fertilization
- 4 -  $\alpha$ -pinene with 3.4 percent before fertilization and 1.2% after fertilization
- 5 - Iso-pulegone with 1.2% before fertilization and 2.8 percent after fertilization

**Table 1.** Chemical composition of essential oils of *Ziziphora tenuior* (Before and after fertilization)

No.	Compounds	RI	% before fertilization	% after fertilization
1	Tricyclene	925	0.7	0.1
2	$\alpha$ -thujene	930	0.5	0.5
3	$\alpha$ -pinene	937	3.4	1.2
4	Sabinene	968	1.5	0.8
5	$\beta$ -pinene	977	1.8	0.3
6	Myrcene	993	2.2	0.9
7	Limonene	1021	4.8	6.7
8	Isopulegone	1066	1.2	2.8
9	Terpinolene	1081	3.1	1.1
10	Pulegone	1213	69.9	81.3
11	2-cyclohexen	1248	4.1	1.3
12	$\alpha$ -copaene	1334	0.2	0.4
13	$\beta$ -bourbonene	1388	0.3	0.1
14	Benzenepropanoic acid	1392	1.2	0.7
15	trans-caryophyllene	1398	0.1	0.2
16	$\alpha$ -humulene	1417	0.1	0.7
17	$\beta$ -cubebene	1431	1.4	0.1
18	Germacrene D	1492	1.8	0.4
19	$\gamma$ -cadinene	1516	0.9	0.2
20	Caryophyllene oxide	1578	0.5	0.1
21	Hexadecanoic acid	1682	0.3	0.1

Given the proven antimicrobial effect of these compounds and since these five combinations constitute 83.4% of total essential oils *Ziziphora tenuior* before fertilization, and

93.3% after fertilization, it can be expected that *Ziziphora tenuior* have antimicrobial properties. Meanwhile, the comparison of the compounds showed a significant increase in compounds such as pulegone, Iso- pulegone and Limonene. Meanwhile, a comparison of the amounts of these compounds specially pulegone, iso-pulegone and limonene showed a significant increase in the antimicrobial compounds inessential oils of *Ziziphora tenuior* and presence of those in this oils and plant alone can justify the use of a viable local population of this herb based for treatment of various diseases.

In Table 1, the effective compounds in essential oils of *Ziziphora tenuior* according to the Coates' coefficient and Wiley libraries, GC-MS identified and respectively Coates' coefficients are included in the table.

## 4. Conclusions

*Ziziphora tenuior* is one of herbs which several studies in various fields have been done on it, especially in Iran. One such study was conducted by P. Babakhanlou et al. that the phytochemical studies on the one species of *Ziziphora tenuior* around Tehran that used organs were aerial parts of the plant and using the water and steam distillation method of Kasier & Lang was done essential oils making that the extracted essential oils with yield about 80% relative to the dry weight of plant and key combinations were as: pulegone (82.6 %), limonene (6.8%), cineol (1.9 %) which was extracted. [7]

Another study on *Ziziphora tenuior* took part by Khanikiet al. showed that the essential oil of this plant shows considerable antimicrobial effect. [8] Khaniki and colleagues in this study collected a kind of *Ziziphora tenuior* from four habitats in the western provinces of the country (Hamadan, Kurdistan) which were in full bloom. Results showed that the habitat conditions caused to significant differences in yields of essential oils at one percent level. The compounds identified in the oils showed that the main compounds of the essential oils (pulegone, 1 -8 cineole) and other compounds in the sample vary which shows the effect of habitat condition on the quality of essential oil. [8]

H. Azimi similarly with research on *Ziziphora tenuior* of West Azerbaijan Province of Iran showed that *Ziziphora tenuior* essential oils can show significant antimicrobial properties. In this research, for quantitative and qualitative methods to investigate the essential oil of this plant, samples of appropriate plant selected in 1450, 1800, 2000 and 2200 m elevation then the oil was analyzed by GC-MS apparatus. According to the results, it was found that the highest percentages of essential oils were in 2200m height and the lowest amount in 1450 meters height. Essential oils ingredients at the height of 2200 meters, including 98.5% of monoterpene compounds and 53.2% of monoterpene is composed of Cineole, pulegone beta Pentene, Parmanta. [9]

Compounds identified in essential oils of the aerial parts of *Ziziphora tenuior* is close to the research conducted by

Babakhanlou et al. However, there are significant differences in the amount of compounds that can be caused by climatic differences, environmental factors and harvest time. Meanwhile, due to the significant increase of these compounds after fertilization by vermicompost, if you wish to use the properties of these compounds by vermicompost fertilizer can improve the quality of essential oils and thus the therapeutic and antimicrobial effect. Given to the texts in which talked about the existence of abundant phenolic and oxygenated terpenes compounds inessential oils of *Ziziphora tenuior*, we can use this plant as a source for natural source for extraction or use of these materials naturally and organic form, in which case the use of vermicompost fertilizers is enough to significantly improve the quality of essential oils.

With regards to improving the quality of essential oil of *Ziziphora tenuior* after a period of one month fertilizing with manure vermicompost, which represents a step forward to produce secondary compounds or in other words increase the conversion rate of primary compounds, secondary compounds in plant photosynthesis is *Ziziphora tenuior* ,It can be stated that if we used of this fertilizer for production and plant operation of *Ziziphora tenuior*, it causes we have products with higher quality and more importantly organic and away from the hazards of chemical fertilizers.

## ACKNOWLEDGEMENTS

The authors appreciate the Research office of Islamic Azad University, Kahnooj Branch because this research was financial support by the Research office of Islamic Azad University, Kahnooj Branch.

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