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Fertilizer Source in Biomass Production and Quality of Essential Oils of Thyme (*Thymus vulgaris* L.)

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Authors' contributions

This work was carried out in collaboration between all authors. Author CRJR designed the study, wrote the protocol, and wrote the first draft of the manuscript. Author JAAC performed the statistical analysis and managed the analyses of the study. Authors MNRM and CRJR managed the literature searches. All authors read and approved the final manuscript.

Original Research Article

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ABSTRACT

Aims: The goal of this study was performed in order to test the effect of the source of crop fertilization and harvest days in the production of biomass and measure the qualitative and quantitative properties of the principal components of the essential oil of thyme.
Study Design: A randomized complete block experimental design with five replications and a factorial arrangement was used.
Place and Duration of Study: The experiment was at the Colegio de Postgraduados, Campus Montecillo (Texcoco, Mexico), during the 2007 spring-summer season.
Methodology: We included three harvests at 60, 90 and 120 days after transplantation. The organic fertilization was with applications of humic acid in the irrigation and by adding a liquid fertilizer combined with biosynthetic amino acids. Steiner solution at 75% concentration was used for the inorganic fertilization as mineral treatment. We evaluated plant height, fresh and dry biomass of the plant, and main stem diameter. Essential oils

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were extracted using steam distillation of water. The concentration (µg/mL) of thymol and carvacrol was determined. Quantitative and qualitative comparisons were carried out by thin layer chromatography (TLC) and gas chromatography-mass spectrometry (GC-MS).
Results: The results show that inorganic fertilization increased plant height (PH) by 36.8%, fresh shoot biomass (FSB) by 72.19%, fresh root biomass (FRB) by 59.27%, stem diameter (SD) by 12.15%, and dry shoot biomass (DSB) and dry root biomass (DRB) by 69.85% and 68.15%, respectively. Days to harvest (DH) influenced positively (p=.05) the evaluated morphological characters but they did not show differences in the total yield of essential oil.
Conclusion: Our data show that fertilizer source modifies fresh and dry biomass production in thyme plants. The total yield of essential oils in thyme was not affected by days to harvest and fertilizer source. However, essential oil quality was higher in the mineral treatment at 90 DH due to the content of thymol and carvacrol in the extract.

Keywords: Carvacrol; concentration; fertilization; quality; thymol; yield.

1. INTRODUCTION

In Mexico, herbs production for the export market has grown steadily in the last decade. The major herbs that are grown for this purpose are basil, mint, oregano, thyme and other from oriental type. However, the trend towards healthy products, safe and exotic has created the need to implement production practices to ensure quality and safety standards for consumers. Thyme (*Thymus vulgaris* L.) belongs to the family Lamiaceae. The useful parts are the leaves and stems [1], from which essential oils are extracted. Thyme leaves can be used fresh or dried as a spice to add aroma and flavor to food [2]. These oils can be used in the pharmaceutical industry for their medicinal properties and are in great demand in food preparation for their culinary applications and use in liqueurs [3].

Some studies have documented that nutritional demand from the plants, such as content and composition of thyme essential oils depend on several factors such as biotype, chemotype, environmental and cultivation conditions [4,5], and harvesting season [6] Among the factors of production that determine the quantitative and qualitative characteristics of aromatic plants is crop nutrition management [7,6]. The values of the nutritional requirements have been reported for the productions of thyme in container nursery stage are: N:2.45-2.61 %; P:0.25 to 0.29%; K:2.17-3.15%; Ca:0.50-1.25%; Mg:0.29-0.40% and S:0.24 to 0.29% [8]. The addition of fertilizers is dependent on the soil type and existing soil fertility. The published results reveal the effects of organic and inorganic fertilizers on yield of thyme [9], and it was found that compost combined with compost tea and rock phosphate increase growth characters, yield and oil percentage in *Thymus vulgaris*. In other hand, [10] reported that nitrogen and phosphorous fertilization had significant effect on the herb yield and essential oil content, but did not change the ratio of thymol. In a Comparative study of organic and mineral fertilization on *Plantago arenaria* cultivation without application of mineral fertilization produces significantly lower yields of leaves, but with highest active substances content [11].

Therefore, the aim of this research was to determine the accumulation of aerial biomass and the amount and quality of essential oils that can be obtained from thyme (*Thymus vulgaris* L.) under organic and mineral fertilization based on days to harvest.

2. MATERIALS AND METHOD

The experiment was conducted in a greenhouse at the Colegio de Postgraduados, Campus Montecillo located in the State of Mexico at 19°29'N and 98°53'W and at an altitude of 2,250m. Thyme seedlings were obtained in 200-cavity polystyrene containers using commercial seed. The transplantation was performed at 30 days after emergence. Seedlings of 5cm in height were placed in flexible black plastic bags (20X20cm) containing a mixture of soil, compost and perlite (50:30:20) for the organic treatments. The physiochemical properties of this mixture were: O.M.:20%; pH:6.88; Nt:0.59%; P:26.64mgkg^{-1}; K:11.12 cmol$_{(+)}$kg^{-1}; Ca:5.08cmol$_{(+)}$kg^{-1} and Mg:4.44cmol$_{(+)}$kg^{-1}. In the treatment with mineral fertilizer, scoria with particle size from 0.3 to 0.7cm was used.

A completely random design with five replicates and a factorial arrangement was used. The factors were days to harvest (60, 90 and 120) and fertilizer source. The fertilizer was: Organic I (irrigation with 1% humic acid) every 8 days; Organic II (irrigation with 1% organic liquid fertilizer every 8d and foliar sprays containing biosynthetic acids every 15 days; and mineral (Steiner nutrient solution (SS) at 75% [12].

At each sampling date, plant height (PH), stem diameter (SD), fresh shoot biomass (FSB) and fresh root biomass (FRB) were evaluated. The plant material was dried in an oven at 70°C for 72h to obtain dry shoot biomass (DSB) and dry root biomass (DRB). Essential oil components were also extracted and quantified.

Essential oils were extracted by simple distillation by steam (50 grams of the aerial parts of fresh material). 150ml of the distillate collected was made three extractions with dichloromethane. With a separatory funnel the organic phase was obtained. To remove water from this phase using anhydrous sulfate sodium. The solvent was recovered and the yield of essential oil was determined by weight difference and then kept at 4°C until analysis. The characterization of the components was by thin layer chromatography following the method described by [13] to develop color. Quantitative analysis was performed with the GC-MS technique using the Hewlett Packard 5973 system (HP5MS capillary column, 5% phenyl methyl siloxane, 30mX250µmX0.253µm) under the conditions indicated by [14]. The quantification of the components was performed by means of the retention times that were confirmed with external standards of thymol and carvacrol.

SAS [15] was used for analysis of variance and means were compared using Tukey's test (p=.05).

3. RESULTS AND DISCUSSION

From transplant until 60 DH, the plants developed their main stem and some lateral branches. From 60 to 90 DH, the main stem reached its maximum height (30.26cm/plant) and stem diameter (6.61mm), while all other variables also increased their size and therefore increased the weight of fresh and dry biomass. From this stage until 120 DH, the plants developed a greater number of lateral branches and roots, which made the FSB and DSB increase significantly Table 1. In this study, root length was favored with mineral nutrition compared to organic treatments, possibly for the substrate and the frequency of irrigation as indicated by [1], because the scoria is a highly porous material with respect to the organic substrates used, which may exhibit a reduced availability of oxygen in the roots due to soil compaction and high moisture retention capability.

Table 1. Mean values of the variables evaluated in thyme (*Thymus vulgaris* L.) plants

	PH (cm)	FSB g/plant	FRB g/plant	SD (mm)	DSB g/plant	DRB g/plant
DH						
60	19.24c	24.83c	17.38c	4.91b	4.77c	3.08c
90	30.22a	58.51b	39.96b	6.61a	12.26b	9.63b
120	26.94b	83.75a	77.18a	5.74a	22.79a	23.00a
FS						
Organic 1	21.96b	25.88b	24.82b	5.30b	6.54b	4.70b
Organic 2	20.68b	33.84b	35.56b	5.41ab	8.44b	9.20b
Inorganic	33.77a	107.38a	74.14a	6.09a	24.84a	21.82a
HSD	1.66	8.64	11.68	0.72	3.26	4.65

Means with different letters in each column for each factor are statistically different (p=.05).

The supply of mineral nutrition overtook the organic in all the variables evaluated, such as plant height which increased by 36.5%; this was because the essential elements in the nutrient solution were readily available [16], and favored the growth of plants in comparison to plants to which organic nutrients were applied. However, these treatments showed homogeneous growth, perhaps because the plants used inorganic fractions of organic substrates that were available to the roots as indicated by [17], and because the composition of organic fertilizers contain, in addition to nutrients, other substances that stimulate and regulate plant growth. This is because, compost is a source of organic nutrients, contain chelated micronutrients for easy plant absorption and the nutrients is in biologically available form for plant uptake [11]. In this way, some studies have documented that the fresh herb positively responded to increased levels of either organic or chemical fertilizers [18], and improve growth of plant foliage and roots [19].

Using the TLC technique, seven components of thyme essential oil were identified, highlighted by: geraniol with a retention factor (Rf) value of 0.21 and blue color; linalool with an Rf of 0.29 and blue color; thymol with an Rf of 0.51 and a red-violet color; and carvacrol with a Rf of 0.53 and red color. The total yield of essential oils obtained from thyme did not vary in the different sections and by fertilizer source Table 2. While the thymol yield showed highly significant differences (p=.05) due to the fertilizer source and days to harvest, it was the interaction of these factors that was significant. The carvacrol yield showed significant differences in the factors evaluated. Days to harvest influenced the content of thymol and carvacrol, being at 90 DH when the highest content of these active compounds was recorded. This may indicate that under greenhouse conditions these compounds are available in larger quantities without reaching the flowering stage, which is the period of greatest production of volatile constituents [20,21].

The highest content of thymol (22.967µg/mL) and carvacrol (1.12µg/mL) was obtained from plants under mineral nutrition Table 2. These components define the quality of thyme essential oil for their pharmacological properties [22] and for giving the characteristic aroma of Thymus species [21]. In the similar way, fertilization source affected the composition of the essential oils by increasing the percentage of thymol and carvacrol. These results support previous observations [18] regarding to the total oil yield per plant that remained comparable with the lower levels of both organic and chemical fertilization. The effect of organic fertilization treatments (application of compost and humic acids) on growth parameters and essential oil yield was reported in several species such as: *Thymus vulgaris*

[9], *Origanum vulgaris* [19], *Borago officinalis* [23], *Mentha aquatica* and *Mentha pulegium* [24].

Table 2. Effect of fertilization and days to harvest on essential oil yield and thymol and carvacrol content in *Thymus vulgaris* L.

	Essential oil yield (g)	Thymol yield (µg/mL)	Carvacrol yield (µg/mL)
DH			
60	0.0143a	12.714b	0.677b
90	0.0134a	21.627a	1.161a
120	0.0167a	9.973b	0.674b
FS			
Organic 1	0.0157a	8.927b	0.648b
Organic 2	0.0137a	12.421b	0.739b
Inorganic	0.0152a	22.967a	1.126a
HSD	0.0039	4.360	0.364

Means with different letters between columns for each factor are statistically different (p=.05).

Under conditions of mineral fertilization can obtain greater economic return in terms of weight of fresh biomass, representing an increased amount of essential oil, thymol and carvacrol per unit area, representing an advantage over organic treatments. But, production thyme with different organic fertilizers in the form of compost is recommended to add value to fresh produce and the essential oil.

4. CONCLUSION

The fertilizer source modifies fresh and dry biomass production in thyme plants, with mineral fertilization favoring their development. The total yield of essential oils in thyme was not affected by days to harvest and fertilizer source. Essential oil quality was higher in the mineral treatment at 90 DH due to the content of thymol and carvacrol in the extract.

CONSENT

Not applicable.

ETHICAL APPROVAL

Not applicable.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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