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Effect of Biological, Organic and Chemical Fertilizers on Vegetative Growth, Physiological Characteristics and Essential Oils of (*Thymus vulgaris* L.)

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Authors' contributions

This work was carried out in collaboration between both authors. Authors FNB and BP designed the study, performed the statistical analysis, wrote the protocol, and wrote the first draft of the manuscript. Authors FNB and BP managed the analyses of the study. Both authors read and approved the final manuscript.

Original Research Article

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ABSTRACT

Aims: In two successive seasons, the effect of different types of fertilizers on growth, physiological characteristics and oil constituents of thyme plants was studied.

Methodology: The performance of compost and nitroxin were compared with standard, commercial rates of NPK fertilizers with respect to the growth, chemical composition and essential oil production of *Thymus vulgaris* L.

Results: In two successive seasons, plants treated by a mixture of nitroxin, compost showed a significant increase in vegetative growth, total N, P and carbohydrate content and essential oil production.

Conclusion: The findings clearly indicate that Nitroxin, and compost could replace conventional NPK fertilizers in the cultivation of thymus, and consequently minimize environmental pollution by these compounds.

Keywords: *Thymus vulgaris*; bio-fertilizer; compost; essential oil content.

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1. INTRODUCTION

Thyme (*Thymus vulgaris* L.), a member of the Lamiaceae family, is an aromatic and medicinal plant of increasing economic importance for North America, Europe and North Africa [1]. At present time, this plant is cultivated in large scale in Iran. Evidently, thyme continues to command an important place in expanding world market. Thyme volatile phenolic oil has been reported to be among the top 10 essential oils [2,3].

The biosynthesis of secondary metabolites, although controlled genetically, is affected strongly by environmental influences [4]. Fertilizer treatments have a critical effect on quantitative and qualitative characteristics of thyme, which finally result in plant growth and yield increment.

Many researchers have started to give attention to the negative effects of using NPK chemical fertilizers in agriculture, both in agriculture itself and on human beings. The intensive use of chemical fertilizers has side effects in polluting underground water, destroying microorganisms and insects, making plants more susceptible to the attack of diseases and reducing soil fertility. Therefore, the development of satisfactory alternatives for supplying the nutrients needed by crops (so-called "green agriculture") such as bio fertilizers could decrease the problems associated with conventional NPK chemical fertilizers and thereby protect both the environment and human health. *Thymus vulgaris* L. has both medicinal and aromatic properties.

Herbs play an important role in maintaining human health and their extracted oils are used for many medical products [5]. This study was designed to evaluate the effect of using nitroxin, and compost both separately and in a mixture, on the growth characteristics, chemical composition, essential oil content and essential oil yield of thymus plants, and to assess their potential as alternatives to conventional urea fertilizers.

2. MATERIALS AND METHODS

The experiment was conducted under field conditions during 2012 and 2013, at the Experimental field of Urmia, Iran (37°30' 55" N, 45°01' 45" E, and 11.3m of altitude). The climate is classified as semiarid climate, with an average annual temperature of 13.6°C. Field soil is clay loam with pH of 7.5.

Rooted cuttings had been transplanted into experimental pots on 30 and 31 August 2012. Irrigation and other field practices had been done as needed. This experiment has been conducted on the base of factorial experiment in randomized complete block design with three replications.

Table 1 presents information on the chemical characteristics of the soil from the experimental area.

Table 2 shows the chemical properties of the compost.

Table 1. Soil chemical characteristics from in experimental area at 0–20 cm depth (urmia, 2012/2013).

Sand %	Silt %	Clay %	Soil Texture	pH	EC	Organic-C (%)	N %	P %	K %	Ca ppm	Mg ppm
40	31	29	Clay loam	7.09	0.61	0.32	0.03	0.5	0.03	89	37

Table 2. Chemical properties of the compost.

Content of bacteria	Organic matter %	pH	EC	Organic -C (%)	N %	P %	K %	Fe Ppm	Mn ppm	Cu ppm	Zn Ppm
2.5 x 10 ⁷	65	7.3	2.9	30.21	1.45	1.26	1.20	1010	102	167	230

1. Chemical fertilizer N, P, K (control): The manufacturer's recommended rate of nitrogen for sandy soils was applied at a rate of 100kg N / ha using ammonium nitrate (33.5% N). Phosphorus was applied at a rate of 50kg P / ha using calcium super phosphate (15.5% P₂O₅). Potassium was applied at a rate of 50kg / ha using potassium sulphate (48% K₂O).
2. Nitroxin: Nitrogen-fixing bacteria, azotobacter chroococcum and phosphate solubilising bacteria, *Bacillus megaterium*, were used. Cutting of *Thymus vulgaris* L. were dipped in a solution of these microorganisms for 5 min using a liquid culture from each strain at a rate of 5ml/litre before planting (1ml contain 10⁹ cells of bacteria) as recommended by [6].
3. Compost: a rate of 25t/ha of compost was applied as recommended by the manufacturer.
4. A mixture of the same nitroxin and the same compost was applied at the same rates as above.

In the two seasons, plant height measured the harvested portion, number of branches per plant; number of inflorescences and plant fresh and dry weight were measured. Determination of N, P and K was done on the dry herb. Total nitrogen was determined using the micro-Kjeldahl method as described by [7].

Phosphorus was estimated colorimetric using the chlorostannous reduced molybdophosphoric blue colour method as described by [8]. Potassium was determined using a flame photometer as described by [9]. The concentration of total carbohydrate was determined in the dried herb and root system using the phenolsulphuric acid method [10].

Plants interred blooming, full-blooming and fruit set stages on 2, 11 and 23 May 2012/2013, respectively. The first cut had been done at the above-mentioned dates. Plants of the first stage (beginning of blooming) had reflowered again on 20 June 2012/2013. On 13 October 2012/2013, irrespective of flowering stage, plants were harvested. Plants were cut at a height of 10cm above soil levels and dried in a shaded area. Fresh and dry weight of aerial parts, oil content, thymol and carvacrol were determined. Oils were extracted by hydrodistillation for 3h, of the aerial parts using Clevenger-type apparatus [11]. The oils were dried over anhydrous sodium sulphate and kept at -4°C until it was analyzed. GLC analysis of the volatile oil of each treatment was performed separately with a Hewlett-Packard model 5890; a fused silica capillary column (Carbowax 20M measuring 20m x 0.32mm internal diameter, thickness of 0.17µm) was used. The temperature program adopted was maintained at 75°C for 5 min⁻¹ with an increase of 4°C min⁻¹ until 220°C (10min). The carrier gas was Helium and the working flow rate was 1.0ml/min, with a detector of 9144 HP. The identification of the compounds of the essential oil was achieved by matching their retention times with those of authentic samples injected under the same conditions. All data were statistically analyzed using LSD 0.05.

3. RESULTS AND DISCUSSION

In general, plants treated with the mixture of nitroxin and compost showed significant increases in plant height, number of branches, fresh and dry weights, and number of flowers, compared to those treated with NPK fertilizer, especially in the second season Table 3.

In this respect, it is possible that the favourable effect of compost and nitroxin on growth characteristics may be due to their ability to enhance the physical, chemical and biological properties of the soil. A similar suggestion was made on rocket plants [12]. Furthermore, this stimulative effect may be related to the good equilibrium of nutrients and water in the root medium [13] or to the beneficial effects of bacteria on vital enzymes and hormonal, stimulating effects on plant growth [14].

As regard to the effect of treatments on plant chemical composition, a small but significant increase was found in N, K and total carbohydrate content in plants treated with the mixture of compost and nitroxin, while no significant differences were observed in the P content between treatments Table 4. Similar results were obtained by [15] on wheat and [16] on lupinus termis. On the other hand, the control plants had higher levels of all chemicals compared to plants treated by nitroxin, and compost alone. Furthermore, both [17], studying *glossostemon bruguieri*, and [18], studying marjoram, mentioned that both compost and biofertilizer led to an increase in carbohydrate percentage and some macronutrients.

These increases might be related to the positive effect of compost and nitroxin in increasing the root surface area per unit of soil volume, water-use efficiency and photosynthetic activity, which directly affects the physiological processes and utilization of carbohydrates. These suggestions are confirmed by the data in Tables 2, which illustrate the higher levels of nutrients and organic matter in compost.

Fig. 1 shows that the highest essential oil yields were obtained from plants treated by the mixture of nitroxin and compost (58, 75kg ha⁻¹), followed by plants treated by NPK chemical fertilizers (52, 63kg ha⁻¹), in both seasons, respectively. In this respect, it can be suggested that the stimulative effect of the mixture of compost and nitroxin on increasing essential oil yield might be attributed to their enhancing effect on vegetative growth characteristics and plant chemical composition. In addition, this favourable effect could be related to increasing the number of glands. However, in this respect, the physiological activity of the glands had a greater effect on essential oil levels than simply their number [19]. Similar results have been reported by [20] on *Mentha arvensis*, [21] on thyme and on marjoram [18].

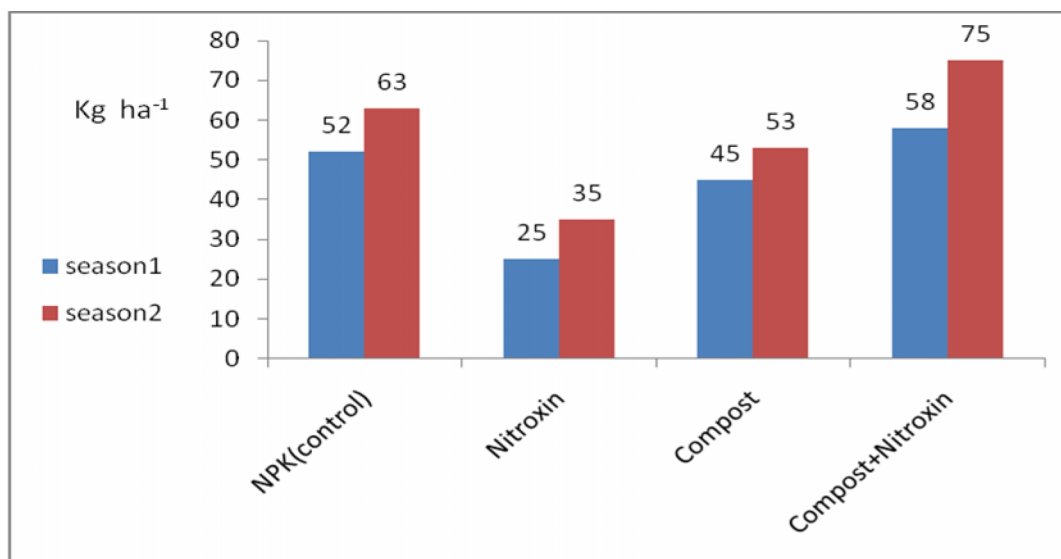
Nitroxin affected plant growth, plant height, thymol and carvacrol content, and yields of fresh and dry herbage, oil, thymol and carvacrol per unit area.

Table 3. Effect of different fertilizers on growth characters of thymus plants.

Growth characters	Plant height (mm)		Number of branches		Fresh weight (g)		Dry weight (g)		Number of inflorescences	
	1 st	2 nd	1 st	2 nd	1 st	2 nd	1 st	2 nd	1 st	2 nd
NPK (Control)	720	811	38	49	510	535	105	116	20	19
Nitroxin	510	605	20	25	320	359	68	70	8	15
Compost	650	630	32	29	422	474	85	92	11	15
Nitroxin× Compost	790	850	40	57	510	605	112	130	22	26
L. S. D. 0.05	10.28	9.45	3.69	5.12	22.68	25.25	8.13	9.22	2.38	3.10

Table 4. Effect of different fertilizers on N, P, K and total carbohydrates of of thymus plants.

Growth characters	N %		P%		K%		Total carbohydrates mg.g ⁻¹ DW	
	1 st	2 nd	1 st	2 nd	1 st	2 nd	1 st	2 nd
NPK (Control)	1.5	2.1	0.52	0.56	3.5	4.1	45	51
Nitroxin	1.1	1.5	0.47	0.51	2.7	3.2	46	42
Compost	1.5	1.8	0.50	0.52	3.1	3.6	48	52
Nitroxin× Compost	2.2	2.4	0.52	0.56	3.9	4.3	51	55
L. S. D. 0.05	0.04	0.07	NS	NS	0.30	1.20	3.15	4.32

**Fig. 1. Effect of NPK, Nitroxin and compost on essential oil production of thymus plants. *Intervals represent LSD 0.05**

4. CONCLUSION

The results point to the beneficial effects of a compost and nitroxin mixture as alternative nutrition systems on the growth characteristics, total N, P and carbohydrate content, and essential oil yields of thymus. With application of nitroxin and compost, we found the highest dry herbage. Alternative nutrition systems could have environmental advantages when compared to conventional NPK fertilizers

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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