The effect of Organic fertilizers and Biostimulants on Oregano (*Origanum vulgare*) Yield and Essential Oil

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Abstract. The purpose of this study was to investigate the effect of biological fertilizers and a natural origin stimulant in oregano cultivation. For the purposes of the study a field experiment was established in Larissa (central Greece). There was used a factorial experimental design with three factors: a) T_1: Control, b) T_2: Application of 1500 kg ha^{-1} Italpollina + 2 sprays with Auxym and c) T_3: Application of 1500 kg ha^{-1} Solfero + 2 sprays with Auxym under four replications. The fresh and dry total weight of T_2 and T_3 were reduced to rates of approximately 4% and 6% respectively, compared to those of T_1. Contrary to the above observation, the dry drogue weight of T_1 plants was reduced by approximately 13.5% compared to T_2 and by 7% compared to T_3. Finally, essential oil yield was higher in T_2, at rates of 21% and 15% compared to T_1 and T_3 respectively.

Keywords: Oregano; biological fertilizers; bio-stimulants; yield; essential oil.

1 Introduction

Organic farming is one of the alternative forms of agricultural production and refers to a management system that provides the consumer with food while respecting the balance of ecosystems. The growing interest in organic farming has been the subject of many global initiatives since the 1920s.

Aromatic and Medicinal Plants (AAF) have a special place in the life of all peoples. The trend in recent years for the "return to nature" and the application of more environmentally friendly cultivation methods has led, in Greece and worldwide, to an increase in demand for natural products and especially for AAF.

AFF due to low input requirements can be grown as organic without special infestations by enemies and diseases producing satisfactory yields of biomass and essential oil.

One of the most cultivated AFF in the world and especially in Greece and the Mediterranean basin is oregano. Oregano belongs to *Lamiaceae* family and it is a

Proceedings of the 9th International Conference on Information and Communication Technologies in Agriculture, Food & Environment (HAICTA 2020), Thessaloniki, Greece, September 24-27, 2020.

genus of herbaceous perennials and sub-shrubs. *Origanum* genus is native to dry, rocky calcareous soils and its leaves and flowers have a strong aroma.

Origanum genus includes many species and the most cultivated species (native in Greece; Kokkini et al., 1994) is *Origanum vulgare ssp. hirtum* (Skoula and Harborne, 2002) which is also known as Greek oregano. *Origanum vulgare ssp. hirtum* has been reported to have a high essential oil concentration of a high quality (1.1–8.2%, v/w; Goliaris and Skroumpis, 1992; Baser et al., 1993; Franz and Novak, 1997).

There are few studies where the effect of fertilization on growth and development of oregano species has been investigated (Ozgóven et al., 2006; Omer, 1999) and it is reported the positive effect in harvested yield. Furthermore, in case of nitrogen fertilization, it is reported that dry biomass yield and essential oil yield is affected without affecting the essential oil content (Dordas, 2009).

Furthermore, there are many studies on the essential oil of Greek oregano and its medicinal properties, where it is reported that the main compound is carvacrol (Kokkini and Vokou, 1989; Adam et al., 1998; Zheng and Wang, 2001; Esen et al., 2007; Mastelic et al., 2008) while they are also high in content: thymol and γ-terpinene (D'Antuono et al., 2000).

Finally, there is no literature on the cultivation practices of this plant, especially under the organic production regime, while there is a growing interest in finding alternative crops and food (Kintzios, 2002). The aim of this study was to investigate the effect of biological fertilizers Solfero (12%Fe + 5% MgO + Zn + Mn + 34% SO_3), Italpollina (4-4-4 + 70.7% Organic) and natural origin stimulator Auxym in oregano cultivation biomass yield, essential oil content and its quality characteristics.

2 Materials and Methods

A field experiment was established at the Experimental Farm of the General Department of the University of Thessaly (central Greece) on 2018 (39°62′69″ N, 22°38′14″ E) and the results refer to the measurements occurred in 2019 where oregano cultivation was in its second growing year.

A factorial experimental design with three factors a) T_1: Control, b) T_2: Application of 1500 kg ha^{-1} Italpollina (4-4-4+70% organic substance) + 2 sprays with Auxym and c) T_3: Application of 1500 kg ha^{-1} Solfero (12%Fe+5%MgO+Zn+Mn+34%SO3) + 2 sprays with Auxym under four replications.

Harvest took place on 22/06/2019 at a height of 30 cm above ground where the plants were in flowering stage. Plant height ranged from 60 to 70 cm. Further measurements of the fresh and dry weight of shoots and leaves, the essential oil content (%),the weight of oregano quality product (grated and sieved dry oregano herbal substance), as well as a chemical analysis of the produced essential oil were carried out.

The soil of the experimental site is characterized as calcareous (pH = 7.53), of low fertility (organic matter content 1.21%) and low salinity (0.33 dSm^{-1}) at a depth of 30 cm.

Bio-fertilizer or bio-stimulant composition is given in details:

- Italpollina is an organic plant growth aid, 100% natural with composition: N 4%, P_2O_5 4%, K_2O 4%, organic matter 70.7%, MgO 0.5%, Fe & B 1% and 12% fulvic acids.
- Solferro is a mixture that consists: Fe water soluble 12%, Mn water soluble 0.55%, Zn water soluble 0.49%, MgO 5.1% and SO_3 water soluble 37%.
- Auxym is a liquid plant growth aid with composition: B water soluble 0.4%, Fe hydrogen EDTA 6%, Mn EDTA water soluble 0.5%, Zn EDTA water soluble 0.4%, Cu EDTA water soluble 0.2%.

The first application of Auxym carried out on 21/05/2019 where oregano was on the beginning of the flower stage (5% of the plants had flowers) and the second application with Auxym took place on 03/06/2019 where the 40% of the plants were at flowering stage.

Oregano harvest took place on 22/06/2019 at 30 cm above ground and in case to avoid any border effect plants were cut from the 1 m^2 of the inner plot of each replication. Oregano plants during the harvest period were 75% flowered. Plants height ranged from 60 to 70 cm. There after the harvested biomass was air dried in the laboratory for a week and sanding in a small sander. The essential oil content was measured using a Clevenger-type distillation apparatus where 12.5 g of dry oregano were subjected to 105 minutes of hydro distillation (250 ml of water).

The average obtained essential oils of its treatments were further analyzed using a Gas chromatograph interfaced with a mass spectrometer using GC-MS on a fused silica DB-5 column. Each compound's relative content was calculated as percentage of the total chromatographic region and the results are expressed as the mean percentage (%) of three replicates (Sarrou et al., 2017; Tsivelika et al., 2018).

The statistical package GenStat (7th Edition) was used for the analysis of variance (ANOVA) and the $LSD_{.05}$ was used as the test criterion for assessing differences between means (Steel and Torrie, 1982).

3 Results and Discussion

Although there were not observed statistically significant differences between the treatments for the total fresh and dry weight (table 1), it was found that treatments 2 and 3 produced reduced yield by approximately 4% and 6%, respectively, compared to control.

In contrast to the above observation, the dry drogue weight of the control (Table 1) was reduced by approximately 13.5% compared to treatment 2 and by 7% compared to treatment 3.

The essential oil content was similar between control and treatment 3, while the plants in treatment 2 had an increased content of approximately 10% compared to both Witness and Transplant 3 plants (Table 1).

Multiplying the essential oil content with the dry drogue yield is calculated the production of essential oil per hectare. It was found that the essential oil yield per hectare was higher for treatment 2, at a rate of 21% and 15% compared to the control and treatment 3, respectively.

The values of all measured parameters except total fresh and dry weight of treatment 2 have a numerically superiority compared to control and treatment 3. This is probably due to the fact that the plants in treatment 2 received an extra nitrogen application of about 60 kg ha^{-1} compared to the treatments of control and treatment 3.

It is reported in literature (Ozguven et al., 2006) that the application of 40 kg N ha^{-1} increased significantly the total fresh and dry weight. Furthermore, it is reported that the N-application in aromatic-medicinal plants increases the essential oil content due to the increase in biomass per unit area, the leaf area and the photosynthesis degree (Ram et al., 1995; Sangwan et al., 2001).

Table 1. The effect of the different treatments on the total fresh - dry weight of oregano, on dry drogue and in the produced essential oil.

	Total Fresh Weight	**Total Dry Weight**	**Dry Drogue Weight**	**Essential oil content**	**Essential oil yield**
	t ha^{-1}			**%**	**kg ha^{-1}**
Treatment 1	10.01	4.95	2.85	0.96	27.2
Treatment 2	9.63	4.65	3.29	1.05	34.4
Treatment 3	963	4.66	3.06	0.95	29.1
$LSD_{0.05}$	ns	ns	ns	ns	ns
CV (%)	11.2	12	8.9	12.4	12.6

Finally, in the produced essential oil were identified 25 ingredients where the main ones (Table 2) are: carvacrol (81.78%), thymol (0.15%), p-Cymene (4.42%), a-Thujene (1.51%) and c-Terpinen (4.81%).

Table 2. The main essential oil compounds found to the tested treatments.

	Carvacrol	**Thymol**	**p-Cymene**	**a-Thujene**	**c-Terpinen**
	%				
Treatment 1	83.247	0.168	4.290	1.300	4.418
Treatment 2	81.413	0.068	4.345	1.587	5.128
Treatment 3	80.676	0.223	4.615	1.636	4.873

It was observed that in Treatment 1 the percentage in Carvacrol (83.247%) is higher than the other treatments, while the content of Thymol is higher in Treatment 3 (0.223%). In the rest compounds treatment 1 (control) has lower content.

4 Conclusions

There was not found any significant statistical effect of the tested treatments on the measured parameters (fresh-dry weight and essential oil yield).

Although the found values of the fresh and dry weight in control were slightly higher than those of treatments 2 and 3, the dry drogue yield in control was the lowest of all. This may be due to the higher percentage of shoots in the control plants

The slightly increase observed in the dry drogue weight and in the essential oil content of the plants in treatment 2 (Italpollina treatment), may be attributed to the additional nitrogen application.

In order to draw more complete conclusions, it is essential to extend the time of experimentation by at least one year, where oregano plants will have entered the fully productive years of its biological cycle. Finally, it is suggested that in order to strengthen the conclusions in the coming research, there must be analyzed few more physiological parameters such as: the leaf area index, as well as the photosynthetic efficiency.

Acknowledgments. This work was supported by the General Department, University of Thessaly, Greece.

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