GREEK OREGANO ESSENTIAL OILS PRODUCTION, PHYTOTOXICITY AND ANTIFUNGAL ACTIVITY

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ABSTRACT
In this research the properties, the phytotoxicity and the antifungal activity of four Greek oregano essential oils were discussed. In a field trail the oregano essential oil production was remarkable higher at the first year at the second harvest (spring harvest) compared to the first harvest (autumn). Compared to the origin less total phenols composition were observed from the oregano biotypes originated from the mountain Olympus compared to the oregano derived from Central Greece and Ios island. At the applied doses of 97, 256 and 610 µl oregano essential oils per plant, less plant development and yield were observed whereas the applied dose of 16 µl caused no damage and promote tomato plant yield. In vitro all oregano biotypes proved a strong antifungal effect against important tomato plant pathogens. The essential oil from the mountain Olympus, "Leptokaria" biotype, was proved the most effective.

Keywords: *Origanum vulgare*, essential oil, soil borne pathogens

Introduction
Organic fertilizers (10) and particular plants extracts based to natural products as *Oreganum vulgare* L., are some of the material considered appropriate to play an important role in the establishment of sustainable agriculture; their impacts on soil properties affect soil productivity, and should be deeply evaluated. Oregano (*Origanum vulgare*) is a native species in Europe especially in Mediterranean region. Oregano is high in antioxidant, activity due to a high content of phenolic acids and flavonoids (5, 8). The leaves, the flowers and the stems have a strong anticeptic and antimicrobial activity mainly due to the basic constituents including carvacrol, thymol, γ-terpinene and *p*-cymene (8). Concerning our research, Oregano essential oil has been reported to be effective against soil-borne pathogens (6) and plant parasitic nematodes (7). Hence, in a field experiment with corn and cotton cultivations the application of oregano essential oil showed promising results to control those pathogens (4). The main objective of this study was to determine the most effective period of oregano essential oil production of four oregano biotypes derived from different regions in Greece, to investigate their phytotoxic effects and to determine their antifungal activity against major tomato soil borne pathogens.

Materials and Methods

Oregano biotypes
Oregano plants of three biotypes (*Origanum vulgare*) and one biotype of *O. onites* L. were collected from different regions in Greece (**Table 1**) and cultivated at the Technological Education Institute of Larissa (TEI/L) in field trials.

Extraction of the essential oil (Yield)
Oregano plants from the field trials, were collected in two cultivate periods: spring and autumn at the years 2004 and 2005 (**Fig. 1**). In all harvests the top plant parts (stems, leaves and flowers) were collected and were air-dried separately in shadow. Further, the essential oil of each biotype was collected by submitted the dried oregano top parts for 2h into water distillation using a type of Electrothermal (UK) apparatus. Finally the yield v/w of the obtained essential oil of each biotype per harvest period (year, autumn or spring harvest time) was estimated as presented in (**Fig. 1**).

TABLE 1

Oregano origin, taxonomy and percentage of total phenols composit

a/a	Oregano region	Oregano biotype classification	% composition of GAE
1	Ios (Agean island)	*Origanum onites* L.	68.8
2	Itea (Central Greece)	*Origanum vulgare* L.	68
3	Leptokaria (middle - mountain Olympus)	*Origanum vulgare* L.	51
4	Olymbos (foot - mountain Olympus)	*Origanum vulgare* L.	40.85

Quantitative composition of oregano biotypes essential oils

The total phenols of each oregano biotype essential oil was performed as a percentage of gallic acid (G.A.E.) content according to Singleton and Rossi (12), with Folin-Ciocalteu reagent (Merek) on a UV-9200 spectrophotometer in 750 nm.

Fungi isolates

Four soil borne pathogens were used in this study, *Pythium* spp., *Verticillium dahliae*, *Fusarium oxysporum* f. sp. *lycopersici* and *Sclerotinia sclerotiorum*. All pathogens were isolated from infected tomato plants.

Essential oils' antifungal activity

The antifungal activity of all four oregano essential oils was tested *in vitro* against mycelia plugs of the above mentioned soil born plant pathogens. The experiment was contacted in Petri dishes amended with Potato dextrose agar (PDA) as follows. A drop of 4 μl essential oil of each oregano biotype was placed in the centre of the petri dish. At the same time a 5-mm mycelial plug which has been received from the edge of 7 days old mycelium (of each fungus) was placed at the centre of the Petri dishes. Mycelia plugs without any essential oil were kept as controls. Each treatment was replicated ten times. Treated and untreated plates were incubated at 25^0C for 144h. After the incubation period, the mycelia radius growth was measured. Each fungus mycelia radius growth was presented in Excel graphs.

Phytotoxicity

The phytotoxicity of each oregano essential oil was determined by exposing tomato plants, at six true leave stages, to six oregano essential oil doses 0, 16, 64, 97, 256 and 610 μl. Oregano doses were pipetted separately one dose to each tomato plant. Treatments were 10 folds. Tomato plants were kept in a glasshouse and after 45 days tomato top fresh stem weights (stem and leave fresh weight) and fruits weight were used to estimate the oregano biotype phytotoxicity effect.

Data analysis

All data were analysed in an analysis of variance (ANOVA), using the statistical package *GenStat7*.

Results and Discussion

Oregano biotypes

Table 1 presents the origin of the four oregano biotypes used in this study. The percentage of total phenols (expressed as a percentage of gallic acid) is also shown in **Table 1**. The total phenols was varied from 40.85 and 51% in "Olymbos" and "Leptokaria" oregano region biotypes up to 68 and 68.8% in "Ios" and "Itea" oregano region biotypes (**Table 1**). The highest percentage of total phenols was determined in "Ios" followed by "Itea" oregano region biotypes.

Extraction of the essential oil (Yield)

Concerning the essential oil yield/oregano biotype the results in **Fig. 1** show that: a) at the first harvest (autumn) the yield of the essential oil is moderate and was not affected by the year. That is not observed for the second harvest (spring) where a significantly more essential oil was received especially at the first cultivation year. b) the oregano essential oil yield is not affected by the year except the "Itea" biotype which gave more essential oil from the first cultivation year (**Fig. 1**).

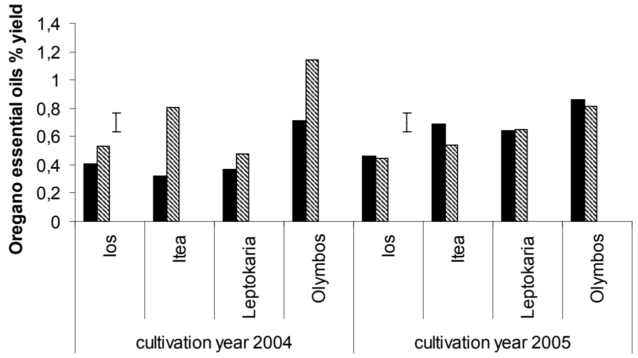

Fig. 1. Oregano essential oils yield per origin, summarized into two different group year (2004 and 2005) and harvest period (autumn(■) and spring(▧) harvest).

Phytotoxicity

From the results in **Fig. 2**, we can conclude that: a) when oregano essential oil was applied at a concentration of 610 μl/plant, a significantly high damage occurs to the tomato plant, especially for "Ios" and "Itea" oregano biotypes. Oregano essential oil application dose of more than 64 μl e.g 16 μl is less phytotoxic to tomato plants (**Fig. 3**). "Leptokaria" and "Olymbos" oregano biotype essential oils at the applied dose of 16 μl showed plant promotion effects producing a higher yield (**Fig. 4**).

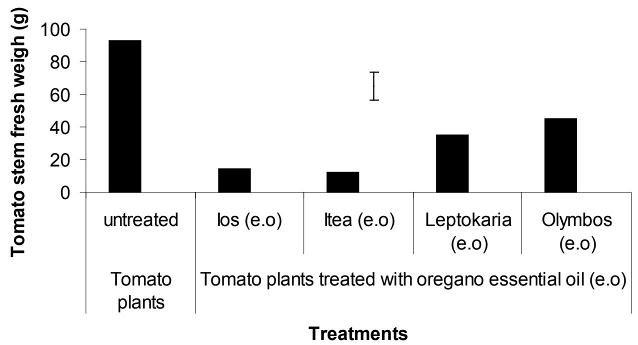

Fig. 2. The effect of oregano essential oil applied dose at a concentration of 610 μl/plant /biotype onto tomato stem fresh weigh compared to the untreated (Control).

Essential oils antifungal activity

The results in **Fig. 5** show that 4 μl of oregano essential oil for all four tested oregano biotypes, significant inhibits the growth of all tested fungi mycelia. The "Leptokaria", biotype proved as the most effective, as the mycelia of all tested fungi were completely inhibited. Compare to "Leptokaria" essential oil all other three oregano biotypes showed moderate antifungal inhibition effects (**Fig. 5**).

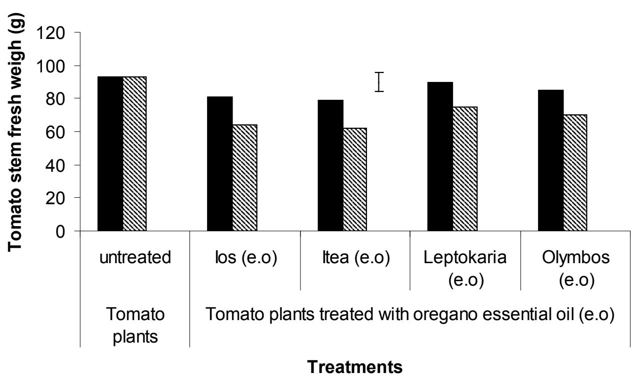

Fig. 3. The effect of oregano essential oil applied dose at a concentration of 16(■)and 64 (▧) μl/plant /biotype onto tomato stem fresh weigh compared to the untreated (Control).

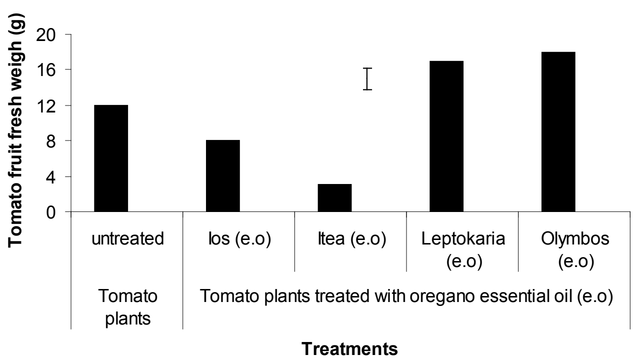

Fig. 4. The effect of 16 μl oregano essential oil application dose onto tomato fruit fresh weigh compared to the untreated tomato plants (Control).

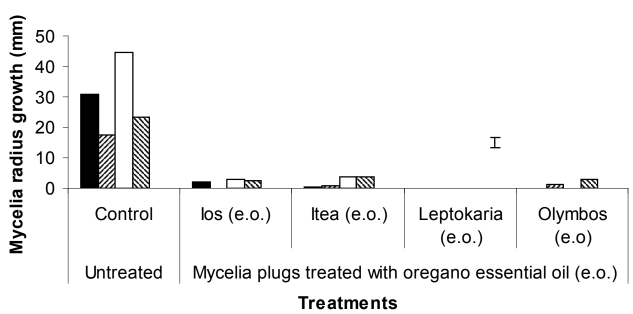

Fig. 5. The effect the essential oils of "Ios", "Itea", "Leptokaria" and "Olymbos" biotype onto *Pythium* spp.(■), *Verticillium dahliae* (▨), *Sclerotinia sclerotiorum* (□) and *Fusarium oxysporum* f. sp. *lycopersici* (▧) mycelia growth.

Conclusions

The results in this study proved the oregano essential oil antifungal activity at a low application dose of 4 μl against important tomato plant pathogens. The essential oil derived from oregano is known to produced antifungal activities against plant pathogens (3), post harvest pathogens (11), food pathogens (2) and human pathogenic fungi (1), mainly due to the properties of carvacrol and thymol (9). In our study tomato plant growth and yield promotion effects were clearly resulted by the application dose of 16 μl of oregano essential oil/plant especially for the "Leptokaria" biotype, a low phenol essential oil oregano biotype. Same results presented by Vokou *et al.* (13), showed that essential oils of *Origanum* species with low phenolic contents, proved unable to exert any substantial potato sprout suppression probably due to low phenolic contents, but the same essential oils remarkably inhibited *Erwinia carotovora* due to carvacrol. Some results presented by us in this research for the "Leptokaria" oregano biotype, a low phenol essential oil oregano species from the mountain Olympus, demonstrated a strong antifungal activity against important tomato plant pathogens. Overall in this research oregano seems to play an appreciable role, acting as a biopesticide against important soil borne pathogens. Further research is needed in order to characterise those phenols as we assume that the percent of the carvacrol and the thymol of the total phenolic contents probably has a potential role to be used in an effective management strategy for control of soil born greenhouse pathogens.

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