Effect of Organic Fertilization on Growth and Development of the Root System of two Medicinal Plants, oregano (*Origanum vulgare* L.) and milk thistle (*Silybum marianum* L. Gaertn.)

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Abstract

Field experiment was conducted in western Greece to determine the effects of organic fertilization on root growth of medicinal plants oregano (*Origanum vulgare* L.) and milk thistle (*Silybum marianum* L. Gaertn.). The experimental design was completely randomized with four replications. Two treatments were applied; compost 2000 kg ha⁻¹ (F1) and compost 4000 kg ha⁻¹ (F2). The results indicated that the soil properties were influenced by organic fertilization. The highest organic matter and total nitrogen content were found under F2 treatment. Also, F2 treatment improves soil porosity. Moreover, there were significant differences in root growth between fertilization treatments. For both medicinal plants the highest root length density, root mass density and root surface density were found under F2 treatment. Furthermore, there were significant differences in arbuscular mycorrhizal (AMF) root colonization between fertilization treatments. The greatest values were observed for F2 treatment. The highest AMF root colonization was found for oregano. A positive correlation was found between AMF root colonization and root surface density. Our results indicated that oregano and milk thistle root growth was improved with increasing compost application rates.

Keywords: medicinal plants, *Origanum vulgare*, organic fertilization, *Silybum marianum*.

INTRODUCTION

Oregano (*Origanum vulgare* L.) and milk thistle (*Silybum marianum* L. Gaertn.) are widely used as medicinal plants and both are native to the Mediterranean basin. In Greece, milk thistle it is considered a weed, in its native range, and is found on roadsides and waste ground, and in cereal crops (Karkanis *et al.*, 2011). The main active constituent of milk thistle seeds is silymarin. Silymarin is used widely in the treatment of liver diseases (Abenavoli *et al.*, 2010). Oregano crop is important for farmers since it brings in significant income. The main

component of oregano essential oils is carvacrol (Olmedo *et al.*, 2014). Oregano plants are known to show antifungal, antioxidant, antibacterial and antimicrobial activities (Vitoratos *et al.*, 2013; Chishti *et al.*, 2013).

Medicinal and aromatic plants respond well to inorganic and organic fertilization. Singh *et al.* (2014) reported that the combined application of 50% of inorganic fertilizers and farmyard manure increase the essential oil yield of basil (*Ocimum basilicum*). In coriander crop, Carrubba (2014) reported that some beneficial effect on yields was

observed after the application of certain organic N fertilizers containing significant amounts of P. There are few references to organic oregano and milk thistle in the literature (Khalil *et al.*, 2007; Kouřimská *et al.*, 2014). Kouřimská *et al.* (2014) observed that there were no significant differences between the organic and conventional farming for oregano (*Origanum vulgare* L. and *Origanum heracleoticum*) and spearmint (*Mentha spicata* L.) antioxidant activity.

The objective of this study was to evaluate the effect of two levels of organic fertilization in the development and characteristics of the root system of medicinal plants *Origanum vulgare* and *Silybum marianum*.

MATERIALS AND METHODS

Field experiment was carried out in western Greece in the region of city Agrinio (38°38'08.43", 21°21'16.62") in 2013. It was established in the 'organic' experimental field of ASKAFEFA cooperative union and certificated by TUV Austria (EN 834/2007). The soil was a clay loam (24.9% clay, 61.2% silt, and 13.9% sand) with pH 7.6 and EC 0.63 mS cm⁻¹. Oregano seedlings were transplanted at the beginning of June 2013 in rows of 75 cm apart. Transplants were set at 35cm between each other. Milk thistle was sown at the beginning of December 2013 by hand and row spacing was 75 cm, with 50 cm between plants in the row.

The experimental design was completely randomized with four replications. Each organic fertilization had two levels (normal and double dose). Compost, consisting of a mixture of farmyard manure and medicinal plants residues, applied at a rate of 2000 kg ha⁻¹ for the normal fertilization dose (F1) and for the double fertilization dose 4000 kg ha⁻¹ (F2). The plot size was 24 m² (3m x 8m). Weeds were controlled by hand, with two hoeings being carried out. The composition of applied compost was N-total 6%, P-olsen 7%, K 9%, Mg 2% and pH 7.2.

The parameters that were determined were: root length density (RLD), root mass density (RMD), root surface density (RSD), arbuscular mycorrhiza (AMF), soil porosity (SP), soil organic matter and soil N-total. Root samples were collected from the 0–35 cm layer by using a cylindrical auger (25 cm length, 10 cm diameter) at the midpoint between successive plants within

a row. Root measurements were made at the beginning of July 2014, at flowering stage and at maturity stage for oregano and milk thistle, respectively. Firstly, roots were separated from the soil by soaking the samples overnight in 30 ml of a 0.5% solution of sodium hexametaphosphate. Afterwards, the samples were stirred for 5 min and washed over a 5 mm mesh-sieve. The roots thus held on the sieves were decanted into a 0.1% trypan blue FAA staining solution (mixture of 10% formalin, 50% ethanol and 5% acetic acid solutions). For the determination of root length density (RLD) and root surface (RS), the stained root samples were placed on a high resolution scanner (Hewlett Packard 4c, Palo Alto, CA, USA) and images captured using Delta-T software was used, (Delta-T Scan version 2.04; Delta-T Devices Ltd, Burwell, Cambridge, UK). The root dry mass density (RMD) was determined after drying for 48 h at 70 °C.

The second root samples were cleaned and stained with trypan blue in lactophenol, according to the method of Phillips and Hayman (1970). The percentage of root length colonized by AM fungi was determined microscopically with the gridline-intersection method at a magnification of × 30-40 (Giovannetti and Mosse, 1980). Total porosity of the soil was determined by 1-Db/Dp, where Dp is the particle density (2.5 g cm⁻³) and D_b is the soil bulk density. Soil bulk density was determined for each plot by taking undisturbed soil cores with 100 cm³-cylinders from a depth of 0-10 cm. The Wakley and Black method (1934) was used to determine the soil organic matter content. The soil N-total determined by Kjeldahl Method (Bremner, 1960).

For analysis of variance (ANOVA), comparisons of means and 3D graphs, the software Statsoft (2007), was used. All comparisons were made at the 5% level of significance.

RESULTS AND DISCUSSION

Organic fertilization influences the soil properties. The highest organic matter and soil total nitrogen were obtained with double rate compost (F2) treatment (Tab. 1). According to Efthimiadou *et al.* (2010) organic matter content was proportionate to the amount of manure applied. Furthermore, the highest soil porosity was found under double rate compost (F2) treatment. The addition of organic materials (i.e.

Tab. 1. Effect of organic fertilization (F1: compost 2000 kg ha⁻¹ and F2: compost 4000 kg ha⁻¹) on soil quality and root properties of oregano and milk thistle plants. *F values and p-levels are also shown.*

Oregano crop	Soil Porosity %	Soil N total	Organic Matter	RMD g/cm ³	RLD cm/cm ³	RSD mm ² /cm ³	AMF %
F1	41.25	0.185	1.800	0.135	0.341	1.071	46.25
F2	43.75	0.217	2.058	0.171	0.442	1.388	54.50
F value	6.52	11.80	11.54	15.56	2.70	52.86	14.14
p level	0.043	0.014	0.015	0.008	0.151	0.0003	0.009
Milk thistle crop	Soil Porosity %	Soil N total	Organic Matter	RMD g/cm ³	RLD cm/cm ³	RSD mm ² /cm ³	AMF %
F1	40.50	0.140	1.449	0.232	0.873	0.637	32.75
F2	44.00	0.197	1.818	0.293	1.064	0.901	37.00
F value	5.44	9.03	27.93	3.22	16.02	17.90	11.56
p level	0.058	0.024	0.002	0.123	0.007	0.005	0.014

compost, manure) to agricultural soils improves soil properties and health (Brown and Cotton, 2011; Celik *et al.*, 2004). Mylavarapu and Zinati (2009) also reported that the addition of compost resulted in improvement of both soil physical and chemical properties. Celik *et al.* (2004) observed that compost and manure treatments increased available water content of soils by 86 and 56%, respectively. Moreover, composts from aromatic plant wastes can be used to enhance the soil microbial activity (Kayikcioglu, 2013).

Compost application improves soil quality and also the crop growth and yield. For both medicinal plants, there were significant differences in root growth between compost treatments (Tab. 1). Oregano responds well to organic fertilization. Corrêa *et al.* (2010) found that cattle and chicken manure significantly influenced oregano plant growth. Our results indicated that the highest oregano root mass density and root length density was observed for double rate compost treatment (0.171 g/cm³ and 0.441 cm/cm³, respectively). Mbatha *et al.* (2014) also reported that as organic fertiliser rate increased, parsley root fresh mass increased.

Moreover, in milk thistle crop, the highest root mass density and root length density were again found in F2 treatment (0.293 g/cm³ and 1.064

cm/cm³, respectively). Moreover, the root length density was lower in oregano crop than that in milk thistle crop. In addition, the root surface density was higher in oregano crop than that in milk thistle crop. According to Zhang *et al.* (2014) the compost application increases rhizosphere soil carbon mineralization and stimulates root growth in cucumber crop. The root surface density had positive and significant correlation (Tab. 2) with soil nitrogen total ($r=0.797$, $p<0.05$ and $r=0.971$, $p<0.001$, for oregano and milk thistle crop, respectively). Sotiropoulou and Karamanos (2010) found that oregano root surface density in the top 25 cm of soil was positively affected by nitrogen. Moreover, root surface density had positive and significant correlation with organic matter content in soil ($r=0.796$, $p<0.05$ and $r=0.789$, $p<0.05$, for oregano and milk thistle crop, respectively).

Furthermore, in oregano crop, the multiple regression analysis indicates that there is statistically significant relationship between root surface density, soil N total and soil organic matter as described by the equation given in Fig. 1. Similarly, in milk thistle crop (Fig. 2), multiple regression analysis between root surface density soil N total and soil organic matter was also significant. The R-squared statistic indicates that

Tab. 2. Correlations coefficients¹ between soil properties and root parameters of oregano (*Origanum vulgare* L.) and milk thistle (*Silybum marianum* L. Gaertn).

Oregano Crop	Soil Porosity	Soil N total	Organic matter	RMD	RLD	RSD	AMF
Soil Porosity	-	-	-	-	-	-	-
Soil N total	0.413ns	-	-	-	-	-	-
Organic matter	0.836**	0.458ns	-	-	-	-	-
RMD	0.670ns	0.890**	0.665ns	-	-	-	-
RLD	0.589ns	0.431ns	0.413ns	0.632ns	-	-	-
RSD	0.6481ns	0.797*	0.796*	0.858**	0.534ns	-	-
AMF	0.813**	0.665ns	0.896**	0.747*	0.471ns	0.763*	-
Milk thistle crop	Soil Porosity	Soil N total	Organic matter	RMD	RLD	RSD	AMF
Soil Porosity	-	-	-	-	-	-	-
Soil N total	0.756*	-	-	-	-	-	-
Organic matter	0.670ns	0.707*	-	-	-	-	-
RMD	0.561ns	0.526ns	0.537ns	-	-	-	-
RLD	0.788*	0.749*	0.647ns	0.357ns	-	-	-
RSD	0.680ns	0.971***	0.789*	0.559ns	0.755*	-	-
AMF	0.870**	0.733*	0.604ns	0.560ns	0.948***	0.725*	-

¹r was calculated using the linear equation. Significant at *P=0.05, ** P=0.01 and ***P=0.001, respectively. ns: not significant.

Fig. 1. Multiple regression analysis between root surface density (RSD) of oregano crop, soil organic matter and soil N total.

Fig. 2. Multiple regression analysis between root surface density (RSD) of milk thistle crop, soil organic matter and soil N total.

the two models explain the variability in root surface density of oregano and milk thistle crop by 81% and 95%, respectively.

Concerning the AMF root colonization (Tab. 2) of oregano and milk thistle plants, there were significant differences between the compost treatments. The highest AMF root colonization was found under the double rate compost treatment (54.50% and 37% for oregano and milk thistle plants). Khaosaad *et al.* (2006) also observed that oregano plants were well colonized. Cavagnaro (2014) observed that while the compost addition at low rates of application had little effect on root colonization by AM, at higher rates a small decrease in colonization of roots by AMF was observed. The AMF root colonization was lower in milk thistle crop than that in oregano crop. The AMF root colonization had positive and significant correlation (Tab. 2) with root surface density ($r=0.763$, $p<0.05$ and $r=0.725$, $p<0.05$, for oregano and milk thistle crop, respectively). Moreover, AMF root colonization had positive and significant correlation with soil porosity ($r=0.813$, $p<0.01$ and $r=0.870$, $p<0.01$, for oregano and milk thistle crop, respectively).

CONCLUSION

Our results indicated that the soil properties were influenced by organic fertilization. The highest organic matter and total nitrogen content were found with double compost treatment. Moreover, there were significant differences in root growth between fertilization treatments. For both medicinal plants the highest root length density, root mass density and root surface density were found under F2 treatment. Furthermore, the highest values of AMF root colonization were observed for F2 treatment. The AMF root colonization was lower in milk thistle crop than that in oregano crop. Our results indicated that the compost at high rate improve oregano and milk thistle root growth.

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