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# Effects of chicken manure and vermicompost teas on herb yield, secondary metabolites and antioxidant activity of lemon basil (*Ocimum × citriodorum* Vis.)

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*Key words:* essential oil, flavonoids, organic agriculture, sustainable agriculture, total phenolics.

**Abstract:** Effects of chicken manure tea (CMT) and vermicompost tea (VCT) as soil drench on vegetative growth, herb yield, essential oil content, total phenolics, total flavonoids and antioxidant activity of lemon basil (*Ocimum × citriodorum* Vis.) was evaluated in a two-year field experiment. The greatest plant height, number of leaves and flowers, shoot fresh and dry weight and leaf chlorophyll content were obtained using CMT at either 1:5 or 1:10 dilutions with no significant differences. The highest number of lateral branches and flavonoid content were obtained when CMT at 1:5 dilution was applied. Essential oil content was at its highest level (0.618%) when CMT or VCT were used at 1:10 dilution, while the greatest total phenolic content and total antioxidant activity were obtained at 1:5 dilution of VCT. The results emphasize the possibility of using organic-based compost teas for enhancing herbal yield and important secondary metabolites in aromatic medicinal plants.

## 1. Introduction

The genus *Ocimum* (*Lamiaceae* family), collectively called basil, comprises between 50 and 150 species of herbs and shrubs (Darrah, 1980). Basil is native to Asia (India, Pakistan, Iran, Thailand, and other countries) and can be observed growing wild in tropical and sub-tropical regions (Makri and Kintzios, 2008). The essential oil profiles of this group of plants are extremely variable, such that several aroma compounds can be found in chemotypes of basil such as citral, eugenol, linalool, methylchavicol, and methylcinnamate that are traded in the international essential oil market (Simon *et al.*, 1999). The diversity within basil species has been accentuated by centuries of cultivation and cross compatibility, which has led to great variation in morphology and chemical profile (Javanmardi *et al.*, 2002). Basil species have antioxidant, antimicrobial and antitumor activities that are due to the presence of phenolic acids and aromatic compounds (Hussain *et al.*, 2008).

Lemon basil (*Ocimum × citriodorum* Vis.), a hybrid of sweet basil (*Ocimum basilicum*) and American basil (*Ocimum americanum*), is a herb grown primarily in northeastern Africa and southern Asia (Fisher and Phillips, 2006). It is naturalized in Asia and cultivated for its lemon-scented leaves due to the essential oils citral and neral as pre-

dominant compounds (Grayer *et al.*, 1996). Lemon basil is characterized by its small stature, early flowering, and small, narrow leaves.

Application of organic sources of nutrients, with no or very little use of inorganic fertilizers, is rapidly gaining favor (Anwar *et al.*, 2005). Compost tea is a highly concentrated microbial solution produced by extracting beneficial microbes from compost. Compost tea is produced by mixing compost with water and incubating it for a defined period, either actively aerating (aerated compost tea, ACT) or not (non-aerated compost tea, NCT) and with or without additives that are intended to increase microbial population densities during production (Scheuerell and Mahaffee, 2002; Ingham, 2005). It is a source of foliar and soil nutrients, contains chelated micronutrients for easy plant absorption and the nutrients are in biologically available forms for both plant and microbial uptake (Hendawy, 2008). Many researchers have pointed out the efficacy of organic manures, compost and compost teas in increasing vegetative growth, biomass and essential oil yield of sweet marjoram (Gharib *et al.*, 2008), cumin (Safwat and Badran, 2002), fennel (Azzaz *et al.*, 2009) and sweet basil (Khalid *et al.*, 2006). Improvements in yield and quality following application of these organic-based substances has been attributed to an enhancement of the beneficial microbial communities in soil, an improvement of mineral absorption conditions for plants, and a stimulation of defense compounds, growth regulators or phytohormones in

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plants (Pant *et al.*, 2009). Various liquid manures or their extracts are known to serve primarily as a source of soluble plant nutrients, growth stimulants and disease suppressors (Khalid *et al.*, 2006).

Several studies have reported the effects of compost tea on suppression of certain plant diseases such as damping-off caused by *Phytophthora ultimum* (Scheuerell and Mahaffee, 2004), gray mold (*Botrytis cinerea*) (Scheuerell and Mahaffee, 2006), *Alternaria solani* and *Phytophthora infestans* (Koné *et al.*, 2010). However, relatively little work has been done to investigate the effect of vermicompost or manure teas on yield, nutritional and quality factors, secondary metabolites and antioxidant activity of vegetable herbs.

In the present work the effects of chicken manure and vermicompost teas as soil drench on growth characteristics, chlorophyll content, essential oil yield, total phenolics, total flavonoids and antioxidant activity of lemon basil were evaluated. The significance of this study lies in the possibility of applying compost teas as soil amendments to improve yield components and secondary metabolites in organic agriculture.

## 2. Materials and Methods

### Site description, plant material and experimental design

The experiment was carried out in two subsequent years, 2010 and 2011, in a field 1810 m above sea level in a silty-loam soil. Three soil samples from 0-30 cm depth were collected and sent to a certified local soil laboratory for analysis. The chemical properties of the soil are shown in Table 1. The local maximum-minimum mean temperatures and relative humidity during the growing period were 28.7-12.6°C and 47.3%, respectively.

Seeds of lemon basil (*Ocimum × citriodorum* Vis.) were sown in double rows (20 cm apart) with 50 cm spacing. Seedlings were thinned three weeks after sowing to 15 cm between plants within the rows. Plants were harvested at ground level when they were at full bloom stage (90 days after sowing) for further analyses. Irrigation (using a drip-tube system), hand weeding, and other management practices were performed when required throughout the growing period.

The experiment was carried out in a complete randomized block design with three replicates per treatment, each of which consisted of 20 plants. Five fertilization treatments were applied as soil drench of de-ionized water (control), chicken manure tea (CMT) at 1:5 and 1:10 water

dilution (v/v) and vermicompost tea (VCT) at 1:5 and 1:10 water dilution (v/v).

### Tea preparation and application method

Vermicompost and chicken manure teas were prepared as described by Javanmardi (2010). Briefly, vermicompost and chicken manure were separately mixed with tap water at ratios of 1:5 and 1:10 (v/v) in loosely covered 14 l plastic containers. Water was allowed to stand for 24 h for passive chlorine removal before mixing. The mixtures were aerated using an aquarium pump for 72 h brewing time in a shaded area. Solutions were filtered through cheesecloth before application. Chemical properties for teas are presented in Table 2. Treatments were started four weeks after sowing and applied five times as soil drench with 600 ml of solution per plant at weekly intervals. Fresh solutions were prepared for each application interval.

### Vegetative growth parameters

At full bloom stage ten central plants from each replicate (to avoid marginal effect) were cut at ground level and plant height (cm), number of branches per plant, number of flowers, number of leaves, and fresh and dry weight of herb (g per plant) were recorded.

### Chemical analysis

**Chlorophyll content.** Chlorophyll content was determined as described by Saini *et al.* (2001). Randomly selected samples of fully expanded leaves (0.5 g) were used. Samples were homogenized with 5 ml of acetone (80% v/v) using a pestle and mortar and filtered through filter paper (Whatman No. 2). The process was conducted in the dark to avoid photo bleaching. Absorbance was measured with a UV-visible spectrophotometer (Camspec M108, Spectronic Instruments, Leeds, UK) at 652 nm and total chlorophyll content calculated using:

$$\text{Total chlorophyll (mg}\cdot\text{g}^{-1}\text{ FW)} = [D_{652} \times V] \times V/W$$

where: V is the total volume of acetone extract (ml) and W, the fresh sample weight (g).

**Essential oil content.** Quantitative determination of the essential oil obtained from lemon basil subjected to the different treatments was achieved by placing the air-dried herbage in a 2 l flask with distilled water (1:15 w/v) and using a Clevenger apparatus, as described by Charles and

Table 1 - Chemical properties of soil

Organic matter (%)	Total N (%)	Available phosphorus as P (mg·kg <sup>-1</sup> ) Bray method	Available potassium as K (mg·kg <sup>-1</sup> )	Fe (mg·kg <sup>-1</sup> )	Cu (mg·kg <sup>-1</sup> )	Mn (mg·kg <sup>-1</sup> )	Zn (mg·kg <sup>-1</sup> )	pH	EC (ds·m <sup>-1</sup> )
1.21	0.05	13.50	540	4.88	1.19	0.39	0.23	7.73	1.50

Table 2 - Chemical properties of vermicompost tea (VCT) and chicken manure tea (CMT)

	vermicompost tea (1:5)	vermicompost tea (1:10)	chicken manure tea (1:5)	chicken manure tea (1:10)
Organic matter (%)	-	-	-	
Total N (%)	6.30	3.14	2.21	1.10
Phosphorus as P (%)	1.01	0.50	1.31	0.65
Potassium as K (%)	6.18	3.08	5.61	2.81
Fe (mg·l <sup>-1</sup> )	9.20	4.51	9.00	4.48
Cu (mg·l <sup>-1</sup> )	0.07	0.03	5.00	2.48
Zn (mg·l <sup>-1</sup> )	25.0	12.0	3.20	1.60
Mn (mg·l <sup>-1</sup> )	0.18	0.89	0.08	0.04
pH	8.22	8.22	8.29	8.29
EC (ds·m <sup>-1</sup> )	4.41	2.20	2.82	1.41

Simon (1990). The average essential oil content of aerial parts is reported as percent of plant dry matter.

*Sample preparation for total phenolic and flavonoid content determination.* Samples were prepared using the method described previously by Javanmardi *et al.* (2003). Briefly, 250 mg of dried plant material from each replicate were ground and dissolved in 10 ml of 80% acetone. Sample extracts were rotated for 1 h in the dark and centrifuged at 5400 g for 10 min. One ml of supernatant was dried under vacuum at 45°C and kept at -18°C for further use. Each sample was dissolved in 1 ml acetone prior to analysis for total phenolic and flavonoid determination.

*Total phenolic compound analysis.* The amount of total phenolics in extracts was determined with the Folin-Ciocalteu reagent using the method of Spanos and Wrolstad (1990), as described by Javanmardi *et al.* (2003). To 50 ml of each sample, 2.5 ml of 1/10 dilution of Folin-Ciocalteu reagent and 2 ml of Na<sub>2</sub>CO<sub>3</sub> (7.5%, w/v) were added and incubated at 45°C for 15 min. The absorbance of all samples was measured at 765 nm using a UV-visible spectrophotometer (Camspec M108, Spectronic Instruments, Leeds, UK). Gallic acid was used as standard and results are expressed as mg of gallic acid equivalent per g of dry weight (mg GAE/g dw).

*Total flavonoid analysis.* The method described by Adom and Liu (2002) was adopted for total flavonoid content analysis. To 0.5 ml of extract, 2.5 ml distilled water were added followed by 0.15 ml of 5% NaNO<sub>2</sub> solution. The mixture was left to stand for 6 min at room temperature before adding 0.3 ml 10% AlCl<sub>3</sub>·6H<sub>2</sub>O solution. The mixture was left for an additional 5 min, then 1 ml of 1 M NaOH added and made up to 5 ml with distilled water. The solution was vortexed and the UV absorbance at 510 nm was recorded against catechin as reference. The result is expressed as µg/g DW.

*Total antioxidant activity assay.* The antioxidant activity of samples was determined by free radical scavenging

activity assay using 1,1-diphenyl-2-picryl-hydrazil (DDPH) reagent according to Brand-Williams *et al.* (1995). The ground leaves (1 g) were extracted with 50% methanol, 50% water. To 0.75 ml of the extract sample, 1.5 ml of freshly prepared methanolic DPPH solution (20 µg·ml<sup>-1</sup>) were added and stirred. The decolorizing process was recorded after 5 min of reaction at 517 nm and compared with a blank control. The total antioxidant activity is expressed in % calculated as (control absorbance - sample absorbance / control absorbance) × 100.

#### Statistical analysis

The experiment was carried out for two years in a randomized complete block design (RCBD) with three replicates, each of which consisted of 10 plants. Data were analyzed using one-way analysis of variance (one-way ANOVA) and means of two years for each trait were compared with Least Significant Difference (LSD) at p≤0.05 by SPSS12 (SPSS Inc., Chicago, IL) computer software for Windows. The data presented in tables and figures are mean values ± standard errors of two years of data for three replicates.

Pearson correlation analysis using SPSS12 (SPSS Inc., Chicago, IL) was performed to assess the relationship between total phenolics, total flavonoids and essential oils with total antioxidant activity.

### 3. Results and Discussion

The combined analysis of data from the experiment did not show any significant differences for all traits (data not shown).

#### Growth parameters

Analyses of variance showed that the growth parameters of lemon basil including plant height, number of lateral branches, leaves and flowers, shoot fresh and dry

weight at full bloom stage were significantly affected by VCT and CMT (Table 3).

#### *Plant height and number of leaves*

The greatest plant height and leaf number were observed at 1:5 CMT, which was not significantly different from 1:10 dilution. Other treatments did not show significant differences for plant height and leaf number (Table 3). The same result has been reported in *Plantago* plants under 300 ml·l<sup>-1</sup> compost tea application (Hendawy, 2008). The increasing effects of organic manure and bio-fertilizers on plant height on fennel (Azzaz *et al.*, 2009) and peppermint (Swafey *et al.*, 2007) have been previously reported.

#### *Number of lateral branches*

The highest lateral branch number was observed at 1:5 CMT dilution, however it was not significantly different from 1:10 CMT. The differences among other treatments (1:5 and 1:10 VMT and control) with regard to plant lateral branches were not significant (Table 3). A promoting effect of organic fertilizers on the number of branches was observed also by Azzaz *et al.* (2009) in fennel plants, especially when organic fertilizers were used in combination with bio-fertilizers.

#### *Number of flowers*

The highest flower numbers were observed in lemon basil plants treated with CMT (1:5 and 1:10 dilutions) and VCT (1:5 dilution) with no significant differences. Control plants and VCT-treated plants at 1:10 dilution showed lower flower numbers (Table 3). An increased number of flowers in *Plantago* plants under 300 ml·L<sup>-1</sup> compost tea application has previously been reported (Hendawy, 2008).

#### *Shoot fresh and dry weight*

The highest shoot fresh and dry weights were observed at 1:5 CMT, which was not significantly different from 1:10 dilution. Other treatments did not show significant differences for these parameters (Table 3). The promoting effect of chicken manure tea on herb yield may be attributed to the micronutrient content and to the action of living micro-organisms and microbial metabolites which stimulate plant growth (Diver, 2002; Carpenter, 2005). Higher

fresh and dry weights have also been attributed to the availability of macronutrients, especially nitrogen, and/or to the improvement of soil water-holding capacity (El-Sherbeny *et al.*, 2005). Furthermore, it has been stated that organic manure activates many species of living organisms which release phytohormones and may stimulate plant growth and absorption of nutrients (Naguib and Aziz, 2003). The same increased vegetative growth characters (including shoot fresh and dry weights) of basil plants under organic farming has been previously reported (Khalid *et al.*, 2006).

#### *Chemical parameters*

*Leaf chlorophyll content.* The highest leaf chlorophyll content was observed at 1:5 CMT which was not different from 1:10 CMT dilution. Other treatments did not show significant differences for this parameter (Table 3). The promoting effect of highly N-containing chicken manure tea on chlorophyll contents might be attributed to the fact that N is a constituent of chlorophyll molecule. Moreover, nitrogen is the main constituent of all amino acids and lipids that act as structural compounds of the chloroplast (Al-Tarwneh, 2005). In sweet basil, chlorophyll content was significantly higher when organic manure compost was applied than in non-fertilized control plants (Taie *et al.*, 2010).

*Essential oil content.* The essential oil content of lemon basil was affected by dilution levels of organic compost teas. The 1:10 dilution of VCT and CMT was higher in essential oil content compared to 1:5 dilutions (Fig. 1). The highest essential oil content was found in the 1:10 VCT treatment and it was about 3.12 times higher than in control plants. Previously, the highest essential oil content in *Ocimum basilicum* was obtained following soil application of vermicompost at 10 t·ha<sup>-1</sup> level compared to application of 10 t·ha<sup>-1</sup> farmyard manure and control treatments (Anwar *et al.*, 2005). In marjoram plants, aqueous extract of compost increased essential oil percentage and yield (Gharib *et al.*, 2008). In basil as a source of essential oils and aroma compounds (Simon *et al.*, 1990), the increase in essential oil yield has been attributed to increase in vegetative growth or changes in leaf oil gland population (Gharib *et al.*, 2008). Our data are in agreement with a previous work that found a higher essential oil percentage in ba-

Table 3 - Effect of different dilutions of chicken manure tea (CMT) and vermicompost tea (VCT) on plant height, number of leaves, lateral branches and flowers, shoot fresh and dry weight and leaf chlorophyll content of lemon basil plants

Treatment	Plant height (cm)	No. of leaves	No. of lateral branches	No. of flowers	Shoot fresh weight (g)	Shoot dry weight (g)	Chlorophyll (mg·g <sup>-1</sup> fw)
Control	27.30±0.89	130.03±12.15	6.80±0.84	6.57±0.90	8.10±1.01	1.45±0.18	0.71±0.016
CMT (1:5)	30.47±1.27	225.93±22.80	10.23±0.92	11.80±1.62	17.63±2.44	2.64±0.36	0.82±0.012
CMT (1:10)	29.23±1.09	197.30±22.50	8.07±0.85	10.27±1.48	14.27±2.41	2.51±0.48	0.80±0.013
VMT (1:5)	27.57±0.64	136.70±13.58	6.77±0.93	10.47±1.80	8.37±0.92	1.60±0.18	0.74±0.016
VMT (1:10)	27.30±1.01	131.13±12.88	5.20±0.72	6.84±0.91	7.87±1.07	1.53±0.17	0.74±0.015
LSD value ( <i>p</i> =0.05)	2.65	47.11	2.34	3.85	4.58	0.79	0.039

Data (per plant) are mean values of 3 replicates ± standard errors.

oil due to application of organic fertilizers as compared to control plants (Taie *et al.*, 2010).

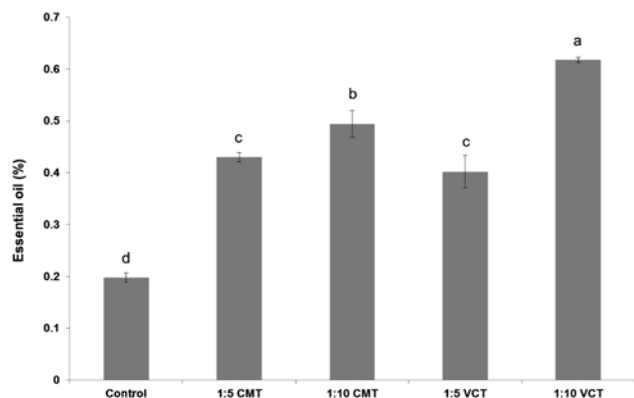


Fig. 1 - Effect of different dilutions of chicken manure tea (CMT) and vermicompost tea (VCT) as soil drench on essential oil percent of lemon basil. Vertical bars show standard errors of the means (n=3).

**Total phenolic content.** Application of VCT at 1:5 dilution gave the highest total phenolic content. The differences between CMT and 1:10 dilution of VCT were not significant (Fig. 2). Previously, Sousa *et al.* (2005) and Taie *et al.* (2010) reported that total phenolic contents achieved by organic culture were higher than those from conventional practice in troncuda cabbage (*Brassica oleracea* L. var. costata DC) and sweet basil. Asami *et al.* (2003) and Wang and Lin (2002) also observed consistently higher levels of total phenolics in organically-grown crops compared with those produced by conventional agricultural practices. The amount of total phenolic content of lemon basil in this experiment is in the range of previously reported total phenolic content of different sweet basil accessions (Javanmardi *et al.*, 2003). Plant phenolics constitute one of the major groups of compounds acting as primary antioxidants or free radical terminators (Lukmanul-Hakim *et al.*, 2008).

**Total flavonoids.** The highest total flavonoid content was observed in 1:5 CMT-treated lemon basil plants and

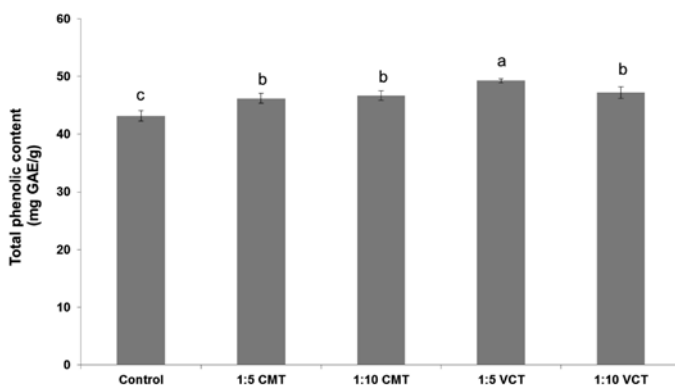


Fig. 2 - Effect of different dilutions of chicken manure tea (CMT) and vermicompost tea (VCT) as soil drench on total phenolic content of lemon basil. Vertical bars show standard errors of the means (n=3).

it was over 1.4 times higher than that produced in control plants. The differences between the two dilutions of VCT were not significant (Fig. 3). In previous studies, the amounts of surface flavonoids in *Ocimum x citriodorum* specimens were reported in a range of 0.2 to 5.7 mg g<sup>-1</sup> (Grayer *et al.*, 2004). Our finding is in agreement with a previous work that reported a significant increase in flavonoid content of *Ocimum basilicum* due to compost or compost tea application in comparison with control plants (Khalid *et al.*, 2006). It has been stated that the antioxidant activity in basil is largely due to the presence of phenolic components, including flavonoids and phenylpropanoids (Juliani and Simon, 2002).

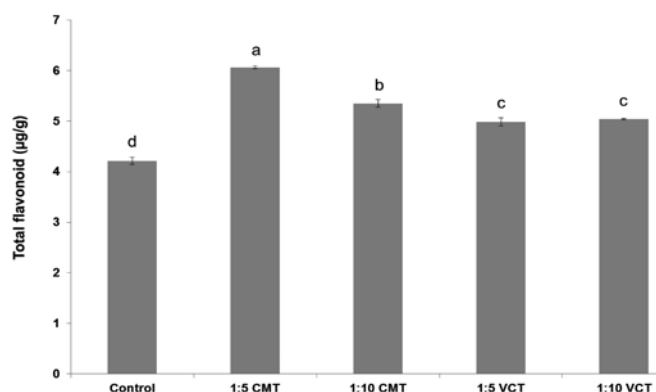


Fig. 3 - Effect of different dilutions of chicken manure tea (CMT) and vermicompost tea (VCT) as soil drench on total flavonoids of lemon basil. Vertical bars show standard errors of the means (n=3).

**Total antioxidant activity.** The antioxidant activity of lemon basil extracts is shown in figure 4. The total antioxidant activity (TAA) ranged from 48.28% (control) to 58.42% (VCT at 1:5 dilution). There were no statistically significant differences between CMT dilutions and 1:10 dilution of VCT.

Pearson correlation analysis between secondary metabolites (total phenolics, total flavonoids and essential oils)

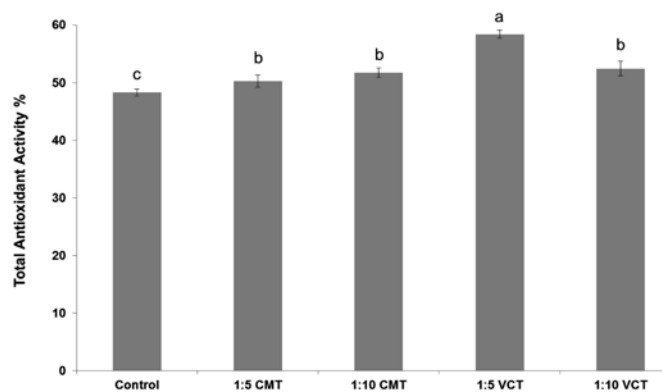


Fig. 4 - Effect of different dilutions of chicken manure tea (CMT) and vermicompost tea (VCT) as soil drench on total antioxidant activity of lemon basil. Vertical bars show standard errors of the means (n=3).

with total antioxidant activity showed a significant positive correlation coefficient of  $R^2=0.91$  between the amount of total phenolics and total antioxidant activity (Table 4). This means that about 91% of total antioxidant activity in lemon basil plants was due to phenolic compounds. Other secondary metabolites did not show significant contributions to the antioxidant activity. The same correlation was previously reported in sweet basil (*Ocimum basilicum* L.) accessions (Javanmardi *et al.*, 2003). The antioxidant activity of phenolics is mainly due to their redox properties, which allow them to act as reducing agent, hydrogen donors, single oxygen quenchers and having possible metal chelating activity (Rice-Evans *et al.*, 1995).

Table 4 - Pearson's correlation coefficients between total phenolics, total antioxidant activity, total flavonoids and essential oil percent of treated lemon basil plants

	Total phenolics	Total flavonoids	Essential oil content
Total antioxidant activity	0.919	0.071 NS	0.320 NS

Data (per plant) are mean values of 3 replicates  $\pm$  standard errors.

#### 4. Conclusions

The findings of this study indicate that fertilization with chicken manure and vermicompost organic teas improves herbal and essential oil yields as well as antioxidative agents such as total phenolics in lemon basil plants. The results point to the beneficial effects of compost tea as possible nutrition sources on growth characteristics and essential oil yields of basil. Organic nutrition systems have environmental advantages such as a beneficial impact on soil properties and the production of safe plants.

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