Available online at www.notulaebotanicae.ro

Print ISSN 0255-965X; Electronic 1842-4309

Not. Bot. Hort. Agrobot. Cluj 38 (1) 2010, 119-127

Notulae Botanicae Horti Agrobotanici Cluj-Napoca

Potential Activity of Basil Plants as a Source of Antioxidants and Anticancer Agents as Affected by Organic and Bio-organic Fertilization

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Abstract

Sweet basil (*Ocimum basilicum*) is a popular culinary herbal crop grown for fresh or dry leaf, essential oil and seed markets. Recently, basil was shown to rank highest among species and herbal crops for phenolic compounds, essential oils which are associated with decreasing risks of cancer and aging diseases. The current study aimed to evaluate the potential activity of phenolic, flavonoids and essential oil of basil as antioxidant and anticancer activities by application organic and bio-organic fertilization. A pot experiment was conducted. Basil seeds were grown, with three levels of organic fertilizers (compost) in presence or absence of biofertilizer. Growth parameters, pigments content, total phenolics, total flavonoids and antioxidant activity of methanolic plant extract were examined. Application of 50% compost and 50% sand in the presence of biofertilizer resulted in enhancement fresh and dry weights, total phenolics, total flavonoids and pigment content as compared with compost alone. The constituents of essential oils extracted by hydrodistilation of basil leaves were identified by GLC. Eleven components of essential oils were identified. The highest value of antioxidant and anticancer activities were obtained in basil plants grown in 50% and 75% compost treatments in the presence of biofertilizer. These results emphasized the important of bioorganic fertilizers for enhancement the antioxidant activity of phenolics, flavonoids and essential oils of basil plant extract.

Keywords: antioxidant, anticancer, phenolics, flavonoids, organic and bioorganic fertilization, *Ocimum basilicum*

Introduction

Culinary herbes have been reported to posses antioxidant activities (Yanishieva *et al*., 2006) suggesting that they might have potential human health benefits. Basil (family *Lamiaceae*) is a popular herb in the Mediterranean diets an annual herb commonly used in many kinds of food preparations in Mediternean diets. Basil is one of the major essential oils producing species belonging to the genus *Ocimum* (Grayer *et al*., 2002). Its extracts are also used in the manufacturing of cosmotic and pharmaceutical products (Unerie *et al*., 1998).

Basil has shown antioxidant, antimicrobial and antiumer activities due to its phenolic acids and aromatic compounds (Gutierrez *et al*., 2008; Hussain *et al*., 2008).

The main phenolic reported in basil are phenolic acids and flavonol-glycosides (Javanmardi *et al*., 2002; Kividompolo and Hyotylainen, 2007).

The high economic value of basil oil is due to the presence of phenyl propanoids, like eugenol, chavicol and their derivatives or terpenoids like monoterpen alchole linalool, methyl cinnamate, and limonene. The antioxidant activity of phenolic compounds is mainly due to their redox properties, which can be play an important role in absorbing and neutralizing free radicals, quenching singlet and triplet oxygen, or decomposing peroxides (Asami *et al*., 2003). Many of these phytochemicals possess significant antioxidant capacities that may be associated with lower incidence and lower mortality rates of cancer in several human populations.

There is lake information on the effect of different types of fertilizers on antioxidant activity of this plant. Aromatic plants are cultivated from centuries, nevertheless little is known about cultural techniques and recommendation for fertilization which are available for gravers.

Therefore, there has been growing interest in developing simple methodologies to increase polyphenols concentration and essential oil content in basil to further enhance their overall nutritional value (Toor, 2006). Organic farming was recommended as it ensures safety as well as environments (Miele *et al*., 2001a).

Application of organic source of nutrients with no or very little use of inorganic fertilizers is rapidly gaining favour. Organic manuring have beneficial impacts on soil properties and produce safe plants, with good meet sources of better availability of nutrients (Abd-El-Gawwad, 1999). Many investigators obtained best results by using organic compost for several medicinal and aromatic plants.

Application of compost and biofertilizers to improve soil structure, fertility and consensequently development and productivity of basil plants has received little attention.

Sweet basil (*Ocimum basilicum* L.) has been reported to be cytotoxic to human cancer cells. (Manosroi *et al*., 2006). Also, Lawrence (1993) reported that Holy basil (*Ocimum sanatcutm*) and Sweet basil (*Ocimum basilicum*) possess antitumor activity in mice.

Thus, this study was conducted to evaluate the important role of organic and bioorganic fertilizer in enhancement the production of chemical substances to be used as a bio-agent cancer as well as antioxidants.

Materials and methods

A pot experiment was conducted in the green house of National Research Center, Giza, Egypt. The experiment was conducted in a complete randomized block design with 8 replicates and included 7 fertilization treatment using washed sand, compost and multi-bioorganic fertilizer, as follows:

100% sand (control)
100% compost
100% compost + biofertilizer
75% compost + 25% sand (v/v)
75% compost + 25% sand (v/v) + biofertilizer
50% compost + 50% sand (v/v)
50% compost + 50% sand (v/v) + biofertilizer

Preparation and application of compost

The plant residues were air-dried, sieved to pass through 0.2 mm sieve and moistened to 70% of their water holding capacity and mixed with a chemical accelerator (7 kg rock phosphate, 40 kg ammonium sulphate and 35 kg calcium carbonate and 100 kg fertile soil per ton dry matter). Reacted plant residues were packed plastic bags capacity of 20 kg and were turned off every two weeks. The moisture content during the composting course, 120 days, was kept at a proper level throughout irrigation (Haughe, 1986). The chemical analysis of composted organic wastes was determined (Tab. 1). Composted organic wastes were added and mixed with sand before sowing in composted treatment pots.

Tab. 1. Chemical analysis of composted organic wastes

Character	Values
pH	7.32
EC mmhos/cm	2.85
O.C.%	35.11
Total N%	1.78
Total P%	0.15
C/N ratio	19.72

Preparation and application of bioorganic fertilizer

Highly efficient strains of phosphate dissolving bacteria (*Bacillus megatherium* var. *phosphaticum*, *Azospirillum lipoferum* and *Pseudomonas fleurosences*) were independently grown in nutrient broth (Difco, 1969) for 48 hours at 30°C in a rotary shaking incubator. Liquid broth cultures initially containing 7 x 10^8, 5 x 10^7 and 3 x 10^7 viable cell per ml, respectively were used. In biofertilization treatments, 100 ml of each of tested microorganisms suspension were added to the soil in each inoculated pot just after sowing.

Basil cultivation

Seedlings were sown in plastic pots 30 x 40 cm at the rate of 5 seedlings per pot. The seedlings were thinned to two after 15 days of sowing. Pots of first treatment irrigated with Hoagland solution as inorganic fertilization treatment, while pots of other treatments were irrigated routinely with tap water. The moisture content was kept in the pots at a proper level by eventual irrigation. At harvest time (after 90 days) plants were collected and subjected to analysis.

Plant material

Seeds of *Ocimum basilicum* c.v. 'Grant Vert'. have been kindly provided from the Ornamental Plant Dept. Ministry of Agriculture.

Seeds cultivated in May 2007 for a month, then the uniform healthy basil seedlings (10 cm length). Seedlings cultivated in pots in 13.06.2007.

Selected 10 random plants of each pot were used for recording the vegetative growth parameters in each cut. The plants were harvested in two cuts. The first cut was done on 22.07.2007 and the second cut on 11.09.2007 by cutting the vegetative parts of the plants 10 cm above the soil surface.

Plant height (cm), number of branches per plant, fresh weight of herb (g/plant), dry weight of herb (g/plant), dry weight of leaves (g/plant), chlorophyl a, b and carotenoids were determined according to Saric *et al*. (1967).

Essential oil percent (%) on the basis of fresh weight, and the main constituents of the essential oil of each treatment were recorded for each cut. Fresh weight was recorded immediately after harvesting and the dry weight was determined after an initial drying in the oven at 70°C to a constant weight. The yield of essential oil produced per plant was calculated by multiplying the average of fresh herb weight by the average of oil percentage.

Antioxidant activity (DPPH assay)

The free Radical scavenging activity using the 1,1-diphenyl-2-picryl-hydrazil (DDPH) reagent was determined according to Brand-Williams *et al*. (1995). The powdered leaves (1g) 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 ug ml^{-1}) was added and stirred. The decolorizing process was recorded after 5 min of reaction at 517 nm and compared with a blank control.

Antioxidant activity = [(control absorbance – sample absorbance /control absorbance x 100 %.)]

Determination of total phenolics

The total phenolics content of ethanol extract of powdered basil leaves was determined according to the method described by Makkar *et al*. (1997). Aliquots of the extract were taken in a test tube and made up to the volume of 1 ml with distilled water. Then 0.5 ml of Folin Ciocalteu reagent (1:1 with water) and 2.5 ml of sodium carbonate solution (20%) were added sequentially in each tube. Soon after overtaxing the reaction mixture, the tubes were placed in the dark for 40 min and the absorbance was recorded at 725 nm against the reagent blank. The amount of total phenolics was calculated as pyrogallol equivalent from a calibration curve.

Determination of total flavonoids

Total flavonoids were estimated using the method of Ordonez *et al*. (2006). To 0.5 ml of Methanolic extract, 0.5 ml of 2% $AlCl_3$ ethanol solution was added. After 1 h at room temperature filtered, then the absorbance was measured at 420 nm. Total flavonoid contents were calculated as quercetin equivalent from a calibration curve.

Essential oil extraction, determination and analysis

The extraction of the essential oil was carried out to extract and quantify the essential oil. A weight of 100 g of fresh herb of each treatment in the two cuts was separately subjected to hydro-distillation for over 3 hours using a modified Clevenger apparatus according to Guenter (1965). The volume of the extracted essential oil was determined and recorded on the basis of the herb fresh weight.

The resulted oil was dehydrated over anhydrous sodium sulfate and stored in glass vials at freezer in the absence of light till used for gas liquid chromatographic (GLC) analysis. GLC analysis of the oil samples was carried out in the first cut using Hewlett Packard gas chromatograph apparatus.

Main compounds of the essential oil were identified by matching their retention times with those of the authentic samples injected under the same conditions. The relative percentage of each compound was calculated from the peak area of the peak corresponding to each compound.

Antitumor effect of basil crude extract and oil

Animals

Female Swiss albino mice, weighing 22-25 g, 8-10 weeks old were used. Animals were kept under normal environmental and nutritional conditions for 2 weeks for adaptation.

Tumor cells

A line Ehrlich ascites carcinoma cells (EACC) resistant to Endoscan (El-Merzabani and Tawfik, 1976) has been used. The tumor line is maintained in the National Cancer Institute (NCI) in female Swiss albino mice by weekly intrapersonal transplantation of 2.5 x 106 cells. For the in vitro studies, the cells were taken from tumor transplanted animals after 7 days of transplantation then the number of cells per ml was calculated by using appropriate microscope.

Preparation of crude extract

One gram of basil fine powder leaves (first cut treatments) was extracted with ethanol (95%). The ethanolic extract was evaporated under reduced pressure at 40°C. The film was dissolve with saline till known volume. The examined concentrations were (1250, 1500, 1750, 2000 ppm) from the ethanolic extract.

Extraction of oils

Extracted oil from the first cut was used (0.04, 0.06, 0.08 and 0.10 mg).

In vitro antitumor activity test

The viability percentage of tumor cells was measured by the modified cytotoxan trypan blue-exclusion technique of Bennett *et al*. (1976).

Procedure

The viability percentage of tumor cells was measured after incubation with the examined extract or saline plus tween 80 as control. Two milliliters of cells (4 x 10^6 cells) were transferred into test tubes, then different volumes of examined extract and oil were added into the appropriate tube, as well as control. The tubes were incubated at 37°C for 2 h under 5% CO_2, then the tubes were centrifuged at 1000 rpm for 5 min and the separated cells were suspended in saline. For each examined tube and control, a new clean, dry small test tube was used and 10 ul of cell suspension, 80 ul saline and 10 ul trypan blue were added and mixed the number of living cells was calculated using a haemocytometer slide.

Determination of IC50 (determined only for the oil).

IC50, the oil concentration resulting in 50% cytotoxicity was determined from the graph.

Statistical analysis

The data obtained were subjected to standard analysis of variance procedure where as values of LSD were obtained at 0.05% as reported by Snedecore and Cochran (1980).

Results and discussion

Growth parameters

Results in Tab. 2 and Tab. 3 indicated that the growth parameters of basil in terms of plant height, number of branches per plant, as well as fresh and dry weight during the two cuttings. The mean values of all growth parameters of second cut were higher than those of the first cut.

Tab. 2. Effect of organic and bio-organic fertilization on fresh weight, dry weight, plant height and number of branches (first cut)

Treatments	Fresh weight (g)	Dry weight (g)	Plant height (cm)	No. of branches
Control	15.65±0.47	3.33±0.08	42.00±2.64	11.66±1.53
Compost	13.71±0.91	3.29±0.22	49.33±2.08	9.33±1.15
Compost + bio.	13.28±1.46	3.21±0.27	47.67±2.52	14.00±1.73
75% compost	13.41±0.66	2.82±0.14	55.33±3.79	10.33±1.15
75%compost+bio.	13.45±1.76	3.02±0.43	52.33±1.15	11.00±3.00
50% compost	9.05±1.14	2.02±0.27	54.33±2.52	12.00±1.73
50%compost+bio.	14.50±1.52	3.28±0.51	50.00±1.00	11.33±0.58
LSD 0.05	2.12	0.54	4.22	2.98

All values are the mean of three replicates are significantly different at $p<0.05$

Tab. 3. Effect of organic and bio-organic fertilization on fresh weight, dry weight, plant height and number of branches (second cut)

Treatments	Fresh weight (g)	Dry weight (g)	Plant height (cm)	No. of branches
Control	12.40±1.44	3.19±0.31	47.33±2.52	13.67±0.58
Compost	18.93±0.21	3.96±0.30	56.67±2.52	16.66±1.15
Compost + bio.	28.21±0.39	5.96±0.87	50.33±2.31	12.00±0.01
75% compost	34.04±0.85	6.77±0.09	59.33±3.51	12.33±2.08
75%compost+bio.	16.31±1.57	3.06±0.09	59.67±0.58	13.67±0.58
50% compost	24.84±0.81	5.51±0.38	59.67±1.15	13.33±0.58
50%compost+bio.	22.18±1.00	5.07±0.02	57.00±0.001	11.33±0.58
LSD 0.05	1.77	0.69	3.74	1.87

All values are the mean of three replicates are significantly different at $p<0.05$

Plant height

Data tabulated in Tab. 2 and Tab. 3 showed that plant height with concentration of 75% was increased significantly as a result of fertilizer treatments. Increasing organic manure (compost) caused enhancement the values of plant height especially in the second cut.

Tab. 4. Effect of organic and bio-organic fertilization on Chl-a, Chl-b, total Chl a+b and t-carotenoids (first cut)

Treatments	Chl.a (mg/g)	Chl.b (mg/g)	Chl.a+b (mg/g)	Carotene (mg/g)
Control	0.693±0.03	0.272±0.008	0.965±0.042	0.516±0.02
Compost	0.510±0.252	0.204±0.005	0.714±0.006	0.387±0.0076
Compost + bio.	0.616±0.008	0.228±0.009	0.842±0.018	0.450±0.015
75% compost	0.469±0.003	0.18±0.014	0.657±0.002	0.360±0.0015
75% compost +bio.	0.511±0.034	0.207±0.006	0.718±0.039	0.380±0.01
50% compost	0.523±0.021	0.213±0.018	0.786±0.006	0.463±0.026
50% compost +bio.	0.800±0.018	0.280±0.006	1.08±0.023	0.619±0.006
LSD 0.05	0.018	0.006	0.023	0.036

All values are the mean of three replicates are significantly different at $p\leq0.05$

Tab. 5. Effect of organic and bio-organic fertilization on Chl-a, Chl-b, total Chl a+b and t-carotenoids (second cut)

Treatments	Chl.a (mg/g)	Chl.b (mg/g)	Chl.a+b (mg/g)	Carotene (mg/g)
Control	0.680±0.55	0.058±0.007	0.686±0.108	0.184±0.014
Compost	0.510±0.004	0.077±0.004	0.630±0.077	0.203±0.005
Compost+bio.	0.907±0.015	0.08±0.006	0.991±0.014	0.255±0.023
75% compost	0.858±0.040	0.080±0.003	0.939±0.043	0.264±0.015
75% compost+bio.	0.861±0.021	0.122±0.045	0.984±0.082	0.261±0.025
50% compost	0.768±0.040	0.071±0.011	0.887±0.049	0.268±0.024
50% compost+bio.	0.942±0.021	0.056±0.003	0.943±0.097	0.282±0.01
LSD 0.05	0.056	0.032	0.129	0.032

All values are the mean of three replicates are significantly different at $p\leq0.05$

Number of branches

The number of branches tended to increase with 100% organic manure (compost) and biofertilizer (14.00 ± 1.73 branches per plant) compared to control. The mean values of number of branches at the second cut were higher than those of the first cut, which could be attributed to the fact that cutting stimulated branching and increased new shoots which in corporate into more accumulation of dry matter.

Dry weight

Tab. 2 and Tab. 3 show that leaves dry weight of basil plant was significantly affected when the plant treated with chemical, organic and bioorganic fertilization. From obtained results it could be noticed that 75% compost alone significantly increased the dry weight of basil plant at the second cut (6.77 g/plant), followed by compost 100% and bioorganic fertilization (5.96 g/plant).

The highest numbers for leaves, plant height and dry weight of basil were obtained by application of organic fertilizer (Khalil, 2002; Arisha *et al*., 2003). This variation might be due to the availability of nutrients especially N_2 and could be due to improvement of soil water holding capacity as mentioned earlier by (El-Sherbeny *et al*., 2005). Furthermore, organic manure activates many species of living organisms which release phytohormones and may stimulate the plant growth and absorption of nutrient (Naguib and Aziz, 2004).

Leaf pigments content

Basil had much higher concentration of chlorophyll content (on a fresh weight basis). Average chlorophyll pigments values were significantly higher with organic manure (compost) adding with biofertilizer (Tab. 4 and Tab. 5).

Thus, we believe that the higher basil leaf carotenoids values found with adding 50% compost with biofertilizer. A promotion effect of organic and biofertilizer on chlorophyll and carotenoids contents might be attributed to the fact that N_2 is a constituent of chlorophyll molecule.

Moreover, nitrogen is the main constituent of all amino acids in proteins and lipids that acting as a structural compounds of the chloroplast (Badr and Fekry, 1998; Arisha and Bradisi, 1999; Al-Tarwneh, 2005).

Total phenolics and flavonoids

Application of organic farming with different compost levels with biofertilizer showed the highest values for total phenolics and total flavonoids as compared with inorganic treatment (Tab. 6 and Tab. 7). Asami *et al*. (2003) reported that organic fertilization alone did not stimulate the biosynthesis of phytochemicals as phenolics in strawberries, lettuce.

Whereas Sousa *et al*. (2005) found that the agriculture production of organic farming techniques showed the highest level for phenolics content than in conventionally techniques. They may be attributed to the higher pathogenic pressures in organic farming which in turn may have a biotic stress, and caused an increase in the level of phenolics grown organically.

Biofertilizers play a major role for increasing the levels of phenolics and flavonoids of basil plants grown organically. Adding biofertilizers to 50% or 75% compost results in great enhancement effect on total phenolics and total flavonoids contents compared to inorganic fertilizer or organic fertilizer alone. Adding biofertilizers to compost resulted in an increase in phenolics content in mung bean because of multi-biofertilizer are usually play as a growth promoting rhizobacteria (Javanmardi *et al*., 2002). Thus microorganisms can fix N_2 and supply it to the plant they synthesis siderophores which play a key role for solublization of minerals such as phosphorus and iron which become readily and available for plants.

Antioxidant activity

Antioxidant activities of basil extract are shown in Tab. 6 and Tab. 7. The total antioxidant activity (TAA) ranged from 57.85% to 63.15% grown organically in presence or absence of biofertilizer.

There were no significant statistical differences between treatments. Our results compared favourably with previous studies on *Lamiaceous* plants (Zhang and Wang, 2001) and showed equivalent or higher antioxidant activity. It can be concluded that phenolics and the presence of other antioxidant secondary metabolites such as volatile oils, carotenoids, vitamines contributed to the antioxidant activity. The antioxidant activity of phenolics is mainly due to their redox properties, which allow them to act as reducing agent, hydrogen donors and single oxygen quenchers. They may also have a metal chelating activity. (Rice-Evans *et al*., 1995).

Essential oil yield

Data in Tab. 6 and Tab. 7 indicated that, the significantly differed and ranged from 0.50% to 0.81% and from 0.42% to 0.69% in the first and second cut respectively.

Tab. 6. Effect of organic and bio-organic fertilization phenolic, flavonoids, volatile oil content and antioxidant activity of basil (first cut)

Treatments	Total phenols mg/g gallic acid	Total flavonoids mg/g quercetine	Antioxidant activity (%)	Volatile oil (%)
Control	39.51±0.80	7.03±0.34	57.85±0.72	0.50±0.02
Compost	51.44±1.32	8.28±0.16	62.16±0.61	0.71±0.01
Compost + bio.	58.20±0.28	11.48±0.48	63.79±0.55	0.75±0.01
75% compost	56.97±0.80	8.46±0.35	62.59±0.55	0.73±0.01
75% compost+bio.	57.64±0.30	9.15±0.69	63.15±0.13	0.77±0.03
50% compost	60.41±0.57	9.42±0.57	61.46±0.44	0.77±0.02
50% compost+bio.	65.30±1.04	12.86±1.11	62.43±0.42	0.81±0.02
LSD 0.05	1.42	1.05	1.00	0.03

All values are the mean of three replicates are significantly different at $p \leq 0.05$

The essential oil increased in the first cut than in the second cut. The highest values were recorded with (compost and Bio.; 75% compost and Bio.; 50% compost; 50% compost and Bio.) 0.75%, 0.77%, 0.77% and 0.81% respectively. This data are in contrast with Omer *et al*. (1994) who found that the percentage of essential oil increased in the second cut than the first cut**.**

Essential oil composition

Chemical compositions of the essential oil for sweet basil under different types of fertilizers was reprehensive in Tab. 8, in the order of the retention times of the constituent. Eleven constituents were identified in *O. basilicum* by GLC. Considerable variation in chemical composition was recorded as shown in Tab. 8.

The essential oil from sweet basil contained α- pinene, β-pinene as the most monoterpenic hydrocarbons. Among the monoterpenic hydrocarbons in basil oil, α-pinine, β pinene were the most important, also the amounts of

Tab. 7. Effect of organic and bio-organic fertilization on phenolic, flavonoids, volatile oil content and antioxidant activity of basil (second cut)

Treatments	Total phenols mg/g gallic acid	Total flavonoids mg/g quercetine	Antioxidant activity%	Volatile oil %
Control	46.73±1.29	8.53±0.24	52.60±0.35	0.42±0.015
Compost	51.36±0.20	9.82±0.48	61.22±1.00	0.55±0.021
Compost + bio.	54.82±0.28	11.25±0.54	62.64±1.00	0.51±0.021
75% compost	47.38±0.52	10.43±0.17	61.00±0.11	0.55±0.006
75% compost+bio.	58.57±0.38	11.30±0.514	64.38±0.77	0.63±0.03
50% compost	48.66±0.46	10.96±0.37	61.53±0.49	0.60±0.012
50% compost+bio.	52.84±0.68	11.91±0.04	62.32±0.26	0.69±0.021
LSD 0.05	1.12	0.662	1.14	0.034

All values are the mean of three replicates are significantly different at $p<0.05$

Tab. 8. Effect of organic and bioorganic fertilization on profiling of essential oils of basil

Treatments	Control	Compost	Compost+bio.	75% compost	75% compost+bio.	50% compost	50% Compost+bio.
α-Pinene	0.620	0.416	0.471		0.200	0.608	0.941
β-Pinene	1.209	0.904	0.561		0.468	1.30	1.326
Methyl chavicol	7.691	4.173	5.294	2.988	4.257	6.285	1.787
1,8 Cineole	7.490	0.802	1.287			2.687	6.960
Llinalool	81.618	92.923	91.104	94.507	92.403	86.831	87.543
Ocimene	0.105	0.081	0.164	0.050		0.452	0.579
Borneol	0.387	0.506	0.133	0.324	1.206	0.578	0.503
Geraneol	0.425	0.108	0.479	0.826	0.138	0.717	0.363
B-Caryphyllone	0.456	0.221	0.232	0.580	0.537	0.285	
n-Cinnamate		0.149	0.180	0.580	0.339	0.117	
Eugenol		0.210	0.095	0.146	0.453	0.144	
Total	100%	100%	100%	100%	100%	100%	100%

oxygenated compounds such as 1, 8 cineole linalool were found to be high. It was previously reported that the oil of basil contained linalool 81-94%.

The dominant constituent in basil oil with organic and inorganic fertilizers was linalool, ranging from 81.618% to 94.507% of total oil. This agrees with data produced by Marotti *et al*. (1996). Methyl chavicol and 1,8 cineole also prevalent in almost all treatments except in the case of 75% compost and 75% compost adding biofertilizer.

Ocimene, n-cinnamate and Eugenal were also detected, although at lower concentrations. A previous study showed that the variability in aromatic compounds composition depending on growth stage (Miele *et al*., 2001a; Miele *et al*., 2001b; Jawanmardi *et al*., 2002). Factors which may potentially affect essential oil composition may differ, in the same genotype, depending on the light rigeme (Skrubis and Markakis, 1976) or on whether plants have been grown is green house or in open field (Morales *et al*., 1993). Type of fertilizer this finding is important and should be investigated further, in view of possible risks for human health, as it has been shown to be carcinogenic (Philips, 1994). Eugenol and Methyleugenol is predominant in plant up to 10 cm height while eugenol is prevalent in taller plants (Johnson *et al*., 1999). Marotti *et al*. (1996) reported the presence of linalool, methyl chavicol and eugenol as main components of basil. In another study, the major components reported were linalool and methyl chavicol (Lochowicz *et al*., 1996).

The results published on the chemical composition of basil oil reveal that linalool 94.5% represent the main compound in basil oil followed by methyl chavicol and 1,8 cineole 7.2%. The observed differences may be due to different environmental and genetic factors, differing types of fertilization and the nutritianol status of the plants. Actually the high quantities of linalool make it a most interesting species from the economic perpoint of view.

In vitro antitumor activity

The viability of tumor cells after incubation with different concentrations of *Ocimum* ethanolic extract (OEE) treatments and their oil (first cut) are reported in Fig. 1 and Fig. 2. Data showed that incubation with different concentrations of (OCC) and oil affected the viability of Ehrlich Ascites Carcinoma Cells compared with untreated cells. The dead cells were increased with increasing concentrations of (OEE) and oil. With ethanolic extract, the treatments 50% compost and bio. and 75% compost and bio. recorded the highest percentage of dead cells (59.83% and 59.37% respectively).

The data in Fig. 2 indicated that the reducing effect on the viability were higher with *Ocimum* oil and the same two treatments recorded the highest inhibition in the viability the dead cells percentage were (81.05% with IC50 0.0616 ppm and 77.89% with IC50 0.0642 ppm) for 50% compost plus bio. and 75% compost plus bio. respectively. These findings corporate the findings by Manosroi *et al*. (2006) who reported that sweet basil leaf oil gave the IC50 value of 0.0362 in P388 cell line. Parduman *et al*. (2008) found that the essential oil from a lemon grass variety of *Cymbopogon flexuosus* recorded tumor growth inhibition

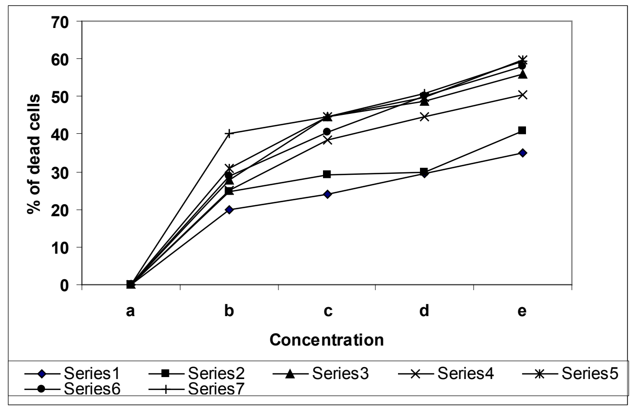

Fig. 1. Effect of organic and bioorganic fertilization on the viability of Ehrlich ascites carcinoma cells in basil ethanolic extract; LSD 0.05 =0.395; 1: Control, 2: Compost, 3: Compost + bio, 4: 75% Compost, 5: 75% Compost + bio., 6: 50% Compost, 7: 50% Compost + bio., a: 0, b: 1250 ppm, c: 1500 ppm, d: 1750 ppm, e: 2000 ppm

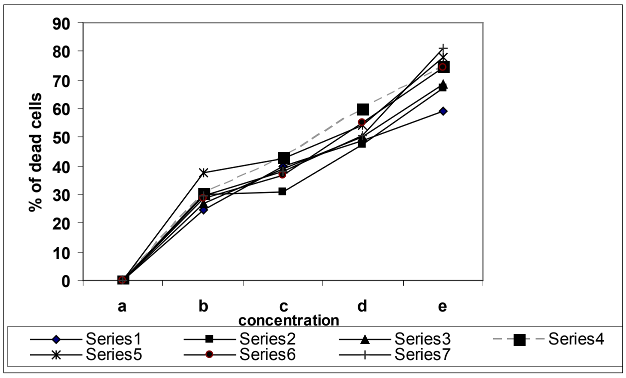

Fig. 2. Effect of organic and bioorganic fertilization on the viability of Ehrlich ascites carcinoma cells in basil oil; LSD 0.05=0.2985; 1: Control, 2: Compost, 3: Compost + bio., 4: 75% Compost, 5: 75% Compost + bio., 6: 50% Compost, 7: 50% Compost + bio., a: 0, b: 0.04 mg, c: 0.06 mg, d: 0.08 mg, e: 0.1 mg

at 200 mg/Kg (ip) of the oil observed with both ascetic and solid tumor forms of Ehrlich ascites carcinoma was 97.34% and 57.83% respectively.

The previous cytotoxic effect of basil ethanolic extract and oil may be due to that *Ocimum* spp. contains essential oil based primarily on monoterpene derevatives such as camphor, limonene, hymol, citral, geraniol and linalool (Lawrence, 1993; Simon *et al.*,1990; Charles and Simon, 1992; Martins *et al.*,1999). Sweet basil oil possess antifungal, insect-repelling and toxic activities (Werner, 1995).

The phenolic compounds and flavonoids such as cinnamic acid, caffeic acid, sinapic acid, ferulic acid and rosemarinic acid in *O. basilicum* have also been reported (Grayer *et al.*,1996).

They are potent antioxidants, free radical scavengers and metal chelators. Rosemarinic acid is a phenolic compound widely distributed in *Labiatae* herbs such as rosemary, sweet basil, and perilla. It can inhibit the cell proliferation induced by platelet-derived growth factor or tumor necrosis factor (Grayer *et al.*,1996; Loughrin and Kasperbauer, 2001; Gerhardt and Schroter, 1983; Baritaux *et al.*,1991; Teda *et al.*,1996; Makino *et al.*, 2000).

Conclusions

The findings of this study indicated that organic and bioorganic fertilization can have a significant increase on the antioxidant activity, anticancer activity, phenolics, flavonoids and essential oil profile of *Ocimum basilicum* plant extract. These findings suggest that basil oil might be good innovative therapeutic strategies against cancer. The results from this study will be advantageous for further development of new chemotherapeutic agents.

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