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Effects of four year sewage sludge application on some morphological traits and chlorophyll content in basil (*Ocimum basilicum* L.)

Azadeh kashani[1*], Hemmatollah Pirdashti[2] and Keyan Kashani[3]

[1]Faculty of Crop Sciences, Sari Agricultural Sciences and Natural Resources University, Sari, Iran, [2]Genetic and Agricultural Biotechnology Institute of Tabarestan, Sari Agricultural Sciences and Natural Resources University, Sari, Iran, [3]Department of Biology, Islamic Azad University, Sari Branch, Iran

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In order to study the effect of four year enriched and none-enriched sewage sludge application on some morphological traits and chlorophyll contents of basil, an experiment was conducted in randomized complete blocks design with three replications in 2009. Treatments included control (no fertilizers used), chemical fertilizers (urea, potassium sulfate and super phosphate triple 70, 60 and 50 kg ha^{-1}, respectively) and sewage sludge (20 and 40 Mg ha^{-1} alone and plus 50% chemical fertilizer). Results showed that different amounts of fertilizer treatments had a significant effect on the studied traits. The maximum inflorescence number, inflorescence length and dry matter yield obtained by using none-enriched sewage sludge as the rate of 20 Mg ha^{-1}. Maximum plant height, stem diameter, chlorophyll b and chlorophyll a+b obtained by application of 20 Mg ha^{-1} sewage sludge plus 50% chemical fertilizer. Results also showed that increasing the amount of sewage sludge markedly decreased, most of the studied traits where as the minimum leaf area index (LAI) was obtained in both control and 40 Mg ha^{-1} sewage sludge plus 50% chemical fertilizer. Thus, applying 20 Mg ha^{-1} sewage sludge either alone or plus 50% chemical fertilizer, had the best effect on studied traits compared to both control and CF treatments.

Key words: Basil, Chemical fertilizer, Chlorophyll, Dry matter yield, Sewage sludge.

Introduction

Sewage sludge is a rich source of elements that are needed for plants and therefor it is considered as a cheap fertilizer by farmers (Singh and Agraval, 2008). Solid phase of sewage sludge consists of large amounts of elements that are needed by plants like nitrogen, phosphorus and elements and so using them on farms is economically important. According to Magdoff and Weil (2004)

[*] Corresponding author: Azadeh kashani; e-mail: kashani.azadeh@gmail.com; pirdasht@yahoo.com

and Shober *et al*. (2003) the first important element in application of sewage sludge is nitrogen that's been reported between 0 to 56%, but sewage sludge can supply many essential macro and micro elements for plants.

Spnmez and Bozkurt (2006) found that application of sewage sludge can increase the amount of nitrogen, phosphorus, potassium and magnesium in lettuce leaves (*Lactuca sativa*). Warman and Termeer (2005) also reported that sewage sludge contains elements like iron, magnesium, cooper, molybdenum and chlorine which essentially needed by most plants. However, sewage sludge contains a great amount of salt that increases the electrical conductivity of soil. High presence of elements such as sodium and calcium is the reason for increase in sewage sludge's electrical conductivity (Vaseghi *et al.*, 2005). Thus, applying sewage sludge affects the crops yield differently. Haghighi (2011) showed that adding sewage sludge to soil causes a significant increase in dry matter yield in celery, rather than spinage and lettuce. Today medicinal plants cultivation is an important branch of agriculture that is used to extract and production of basic material for many drugs. Usage of medicinal plants in developed countries is highly increasing, as 90% of people in these countries use herbal medicines (Akbarnejad *et al*., 2010).

Basil (*Ocimum basilicum* L.) as a medicinal plant, is of particular importance and it is used for treatment of some heart diseases and migraine and nervous headaches. Since global approaches in production of medicinal plants are going towards sustainable agriculture solutions and management practices like application of organic fertilizers in order to improve quantitative and qualitative yield (Ferreras *et al*., 2006; *Darzi et al*., 2008). Recently in Iran some researches has been done on chemical aspects and pollution of sewage sludge application in farms, but they had less attention to effects of sewage sludge on physiological characteristics of medicinal plants. Therefore, this research has been performed to study the long-term application effect of sewage sludge and chemical fertilizer on chlorophyll and physiological parameters in basil (*Ocimum basilicum* L.)

Materials and methods

This experiment has been conducted in research farms of Sari Agricultural Science and Natural Resources University in 2009, Iran, located in north of Iran (34˚33'N; 52˚6'E and altitude 16 m). The characteristics of soil and non-enriched sewage sludge used in this study are presented in Table 1. The soil of the experiment site was silty clay (10.33% sand, 46.33% clay and 43.33% silt) with 2.43% organic matter. Experiment was arranged in randomized complete block design with six treatments and three replications. Treatments included control (no fertilizers used), chemical fertilizer (urea, potassium sulphate, super phosphate triple, 70, 60 and 50 kg ha^{-1} respectively), SS_{20}: sewage sludge 20 Mg ha^{-1}, SS_{20}+½ CF: sewage sludge 20 Mg ha^{-1}+50% chemical fertilizer (NPK:35-30-25), SS_{40}: sewage sludge 40 Mg ha^{-1}, SS_{40}+½ CF: sewage sludge 40 Mg ha^{-1}+50% chemical fertilizer (NPK:35-30-25). All treatments has been added to experimental plots during 2006-2009 (four continuous years). The sewage sludge and fertilizer P and K were applied as pretreatment before planting in the plots (3×1/5 m^2) and then incorporated in soil. Nitrogen was applied in two equal splits, half at the time of planting, and the remaining half 50 days after planting. Standard irrigation, weeding, and other management practices were followed when required throughout the growth period. Basil plants were harvested from the central part of each plot on 65 days after planting at full bloom. At the end of growth period (beginning of anthesis) three middle rows of each plot has been taken after eliminating the edge effects. Some agronomical parameters like plants height, stems diameter, inflorescence number and length, leaf area index, chlorophyll a+b, chlorophyll a/b ratio, chlorophyll content (SPAD value) and dry matter yield have been determined. Leaf surface of each plant determined using leaf area meter (USA LICOR Model LI-3000). Total leaf chlorophyll contentwas recorded using chlorophyll meter (SPAD – 502, Minolta, Japan). For measuring the amount of chlorophyll a and b (Equation 1-3) with methanol and measuring the absorbed light spectrum from the solution by spectrophotometer (6405.UV/Vis – Jenway – England) in 652 and 665.2 wavelengths (Porra *et al*., 1989).

$$[\text{Chl a}] = 16.29\ E^{665.2} - 8.45\ E^{652}$$

$$[\text{Chl b}] = 30.66\ E^{652} - 13.58\ E^{665.2}$$

$$[\text{Chls a+b}] = 22.12\ E^{652} + 2.71\ E^{665.2}$$

Statistical analysis of data and orthogonal comparisons were done using SAS (SAS Institute, 1997) software and means were compared using least significant difference (LSD) test ($P \leq 0.05$).

Table 1. Chemical properties of soil and vermicompost in this study

Properties	Electrical conductivity ($dS.m^{-1}$)	pH	Organic matter	N	P	K	Sand	Clay	Silt	Soil Texture
			(%)		mg kg^{-1}		(%)			
(Soil)	1.52	7.81	2.43	0.26	10	270	10.33	46.33	43.33	Silty-Clay
Sewage Sludge	10.75	7.44	4.50	0.58	43	480	-	-	-	-

Results

Morphological trait

Variance analysis of data showed that the type of fertilizer significantly affects plants height, stems diameter, inflorescence number and lenght, leaf area index and dry matter yield (Table 2).

Results represented that in SS_{20}+50%CF treatment, plants height significantly increased (11% and 6%, respectively) compared to both control and chemical fertilizer treatments (Fig 1: a). In addition, in chemical fertilizer treatment and SS_{20}+50% CF treatment highest stem diameter (9.42 and 9.66mm, respectively) was obtained (Fig 1: b). On the other hand, increasing the amount of sewage sludge increase (from SS_{20} to SS_{40}+50% CF), significantly decreased plants height and stems diameter by 11% and 34% respectively (Fig 1: a and b). According to results of this research, maximum inflorescence number and length (43 and 39 cm, respectively) and dry matter yield (5947 kg ha^{-1}) were recorded when SS_{20} treatment was applied. Application of this treatment (SS_{20}) increased the inflorescence number and length by 2-3 times and 41% compared to control, respectively (Fig 1: c and d). Furthermore, over application of sewage sludge (both SS_{40} and SS_{40}+50% CF treatments) markedly reduced inflorescence number and dry matter yield up to 50% and 54%, respectively. Meanwhile, the lowest leaf area index (LAI) was observed in control and SS_{40}+50% CF (Fig 1: e and f).

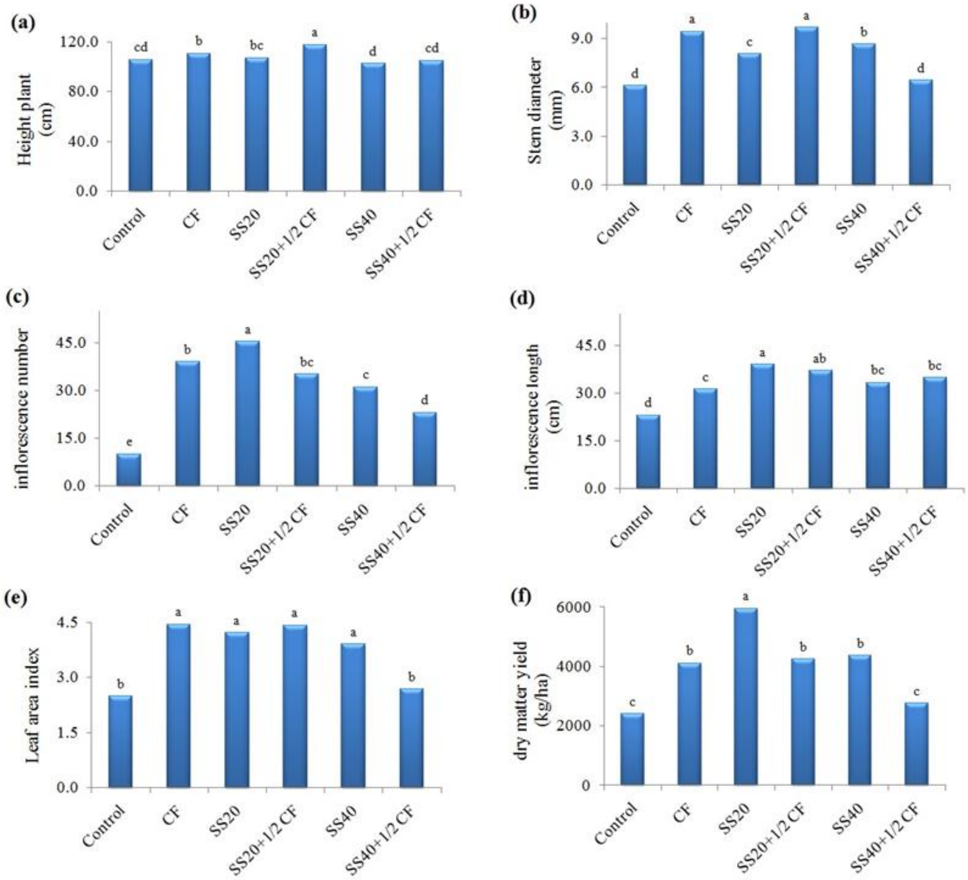

Fig. 1. Effects of different amounts of fertilizers on morphological traits in basil
CF: chemical fertilizer; SS20 : sewage sludge 20 Mg ha-1; SS20+½CF : sewage sludge 20 Mg ha-1+ 50% Chemical fertilizer; SS40: sewage sludge 40 Mg ha-1; SS40+½CF : sewage sludge 40 Mg ha-1 + 50% Chemical fertilizer.

Physiological traits

Results showed that sewage sludge, significantly affects the amount of chlorophyll a, chlorophyll b, chlorophyll a+b, chlorophyll a/b ratio and leaf chlorophyll content (SPAD) (Table 2).

The highest amount of chlorophyll a (4.88 mg ml^{-1}) observed in control (Table 2). Results in table 4 also showed that chlorophyll a has the highest positive and significant correlation with chlorophyll a+b (r = 0.97**) and a negative and significant correlation with LAI (r = - 0.62**). There was a weak negative linear regression (Fig 2) between chlorophyll a and leaf area index during flowering stage. On the other hand the highest amount of chlorophyll b (2.14 mg ml^{-1}) obtained by application of SS_{20}+50%CF treatment (Table 2).

Results also showed that highest amount of chlorophyll a+b recorded in control and SS_{20}+50% CF treatment (6.78 & 6.86 mg ml^{-1}, respectively) whereas increasing amount of sewage sludge up to SS_{40} and SS_{40}+50% CF dramatically decreased chlorophyll a+b content by 17% & 8%, respectively. Highest chlorophyll a/b ratio obtained by applying SS_{40}+ 50%CF treatment (Table 2). Results suggested that leaf chlorophyll content increased in chemical fertilizer and sewage sludge treatments, as the highest leaf chlorophyll content (48.07) was observed in SS_{20} treatment (Table 2).

Table 2. Effects of Different amounts of fertilizer treatments on basi leaf chlorophyll content

Treatment	**Chlorophyll**			**Chlorophyll a/b ratio**	**SPAD Value**
	a	b	a+b		
	(mg/ml)				
Control	4.88 ^{a}	1.90 ^{b}	6.78 ^{a}	2.57 ^{c}	35.90 ^{d}
CF	3.36 ^{f}	1.36 ^{e}	4.71 ^{d}	2.48 ^{d}	46.85 ^{ab}
SS20	4.02 ^{e}	1.61 ^{c}	5.63 ^{c}	2.50 ^{d}	48.07 ^{a}
SS20+1/2 CF	4.72 ^{c}	2.14 ^{a}	6.86 ^{a}	2.21 ^{e}	41.25 ^{c}
SS40	4.17 ^{d}	1.51 ^{d}	5.68 ^{c}	2.76 ^{b}	43.20 ^{bc}
SS40+1/2 CF	4.76 ^{b}	1.56 ^{c}	6.32 ^{b}	3.04 ^{a}	45.80 ^{ab}
F test					
Mean Squares (MS)	1.03**	0.25**	2.01**	0.24**	60.17**
Coefficient of Variation (CV)	0.4	1.7	0.8	1.1	5.2

In each columns means with similar letters were not significantly different based on LSD test.
**: significant at 1% levels

Table 3. Simple correlation between leaf area index and chlorophyll (n=18)

	Chlorophyll a+b	**Chlorophyll a**	**Chlorophyll b**	**Leaf area index**
Chlorophyll a+b	1			
Chlorophyll a	0.97**	1		
Chlorophyll b	0.87**	0.73**	1	
Leaf area index	-0.47*	-0.62**	0.09^{ns}	1

ns, * and ** non- significant and significant at 5 and 1% levels, respectively

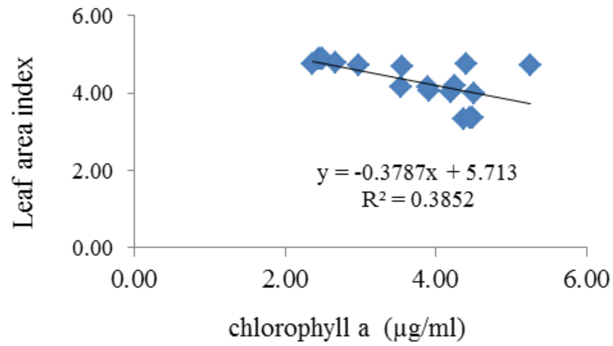

Fig. 2. Regression relationship between chlorophyll a and leaf area index (LAI)

Discussion

The significant effects of fertilizers type in different amounts on morphological traits (plants height, stems diameter, inflorescence number and length, leaf area index and dry matter yield) and leaf chlorophyll amounts (chlorophyll a, chlorophyll b, chlorophyll a+b, chlorophyll a/b ratio and leaf chlorophyll content (SPAD) showed that, sewage sludge, which is a kind of organic waste, increases macro and micro elements like nitrogen, phosphorus, potassium, iron, manganese, cooper and zinc (Baran *et al*., 2001). Meanwhile, using it plus chemical fertilizers, improves physical characteristics of soil, and increases the production by using water and nutrients efficiently (Bhattacharyya, 2008). Thus by applying sewage sludge, nitrogen absorption increases in roots which raises the growth and production of more leaves and inflorescences (Sharifi *et al*., 2010) and therefore, causes an increase in dry matter yield. Delgado *et al.* (2003) reported that adding 1000 kg sewage sludge plus 350 kg urea per hectare, increases the corns yield by 20% compared to control. Shabanian Borujeni *et al*. (2005) also noticed that 50 Mg ha^{-1} and 100 mg ha^{-1} of sewage sludge treatments causes a significant increase in aboveground dry matter weight and plant height in snapdragon (*Antirrhinum majus*) compared to control. This researchers also found that number of florets in snapdragon inflorescences also increased from 13 in control treatment to around 19 in 50 Mg ha^{-1} sewage sludge treatment. They suggested that presence of organic matter and nutrients like nitrogen, phosphorus, potassium, iron and other nutrients in sewage sludge caused the increase in snapdragon growth. Similarly, Perez-Murcia *et al*. (2006) found that the maximum fresh and dry weight of Broccoli (*Brassica oleracea*) obtained in a 30% sewage sludge combination. They suggested that adding an appropriate amount of sewage sludge to soil can supply the plants with nutrients in a better situation. In Spinach (*Spinacia oleracea*) Vaseghi *et al*. (2005) also observed an increase in the dry and fresh matter yield along with increase of sewage sludge application. They believed that the main reason is the increase of nitrogen absorption by roots and production of more leaves. Higher light absorption and photosynthesis following the increase in number of leaves lead to production of more hydrocarbons in leaves and caused the increase in number of blossoms and dry matter yield. Consistently, Gardio *et al*. (2005) noticed that application of sewage sludge caused the faba bean plant to be more green and have higher leaf surface and dry matter yield compared to control. Findings by Sabye *et al*. (1990) sage (*Artemisia* spp) and Atriplex (*Atriplex* spp) showed that leaf area index were significantly higher in the sections in which sewage sludge was applied; compared to control sections with no sewage sludge. Shabanian Borujeni *et al*. (2005) found that in clove gilly flower (*Dianthus spp*) chlorophyll density increased from 478 mg kg^{-1} in control treatment to 596 mg kg^{-1} and 726 mg kg^{-1} in 50 Mg ha-1 and 100 Mg ha^{-1} sewage sludge treatments, respectively. According to the low leaf area index, they believed that this increase in chlorophyll is because of increased chlorophyll density in leaf surface unit and/or increase in minerals needed in chlorophyll production (high magnesium density in sewage sludge). Because magnesium is one of the main elements in chlorophyll structure.

Organic matter in sewage sludge improves soil characteristics including hydraulic conductivity, soil particles stability, aeration and moisture and thus improves growth and increases crops yield (Debosz *et al*., 2002) but on the other hand, inordinate application, increases the probability of toxic densities of elements and causes aggregation of heavy elements in soil (Amini *et al*., 2008). Shirani *et al*. (2010) found that over application of sewage sludge causes excessive salinity of soil and decreases crops yield. Kassray and Saedi (2010) also noticed that application of 5 and 10 Mg ha^{-1} sewage sludge treatments increased the fresh and dry weight of aboveground in tomato compared to control. Application of higher levels of sewage sludge a severe increase in yield and plant toxicities; as this decrease in 30 Mg ha^{-1} level was estimated around 20% for fresh weight and 44% for dry weight compared to control. Parida and Das (2005) also found that one reasons of chlorophyll reduction in high salinity conditions is increased amount of chlorophyllase that degrades the chlorophyll. High salinity prevents the composition of chlorophyll and carotenoids in many plant species leaves and also induces senescence, chloroplast infraction and reduction of leaves chlorophyll in plants and this reduction causes less efficiency in photosynthesis.

Consistently, in the present study results showed that in higher amounts of sewage sludge application either enriched or none-enriched with chemical fertilizers (SS_{40} or SS_{40}+50%CF treatments) most of the studied traits (plants height, stems diameter, inflorescence number and length, leaf area index, dry matter yield, chlorophyll b, and chlorophyll a+b) were significantly reduced. This reduction is the consequence of excessive soil salinity due to effects of residuals of some elements particularly chloride, because in this research fertilizer treatments added to soil in four continuous years (2006-2009) and also effects of remaining elements during four years increased the probability of toxic densities of elements in soil or caused higher salinity and interruption in nutrients balance and finally reduced the plants growth. Hence application of SS_{20} and SS_{20}+50% CF treatments could cause better improvement in the growth and increase of the yield in basil compared to SS_{40} and SS_{40}+50% CF treatments.

Conclusionffgfg

Generally it is observed that 20 Mg ha^{-1} of sewage sludge enriched or none-enriched with chemical fertilizer treatments have the best effect on studied traits compared to control and chemical fertilizer treatment. As application of this treatment caused an increase in some traits (plants height, inflorescences number and length, leaf area index, dry matter yield and chlorophyll a+b) in basil leaves compared to control and chemical fertilizer treatment. Thus application of this fertilizer (sewage sludge) instead of a part of chemical fertilizer in basil cultivation, will reduce fertilizer consumption and optimize the usage of sewage sludge in addition to promotion of morphological traits and chlorophyll content.

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