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Research Article

Yield and Essential Oil of Sweet Basil Affected by Chemical and Biological Fertilizers

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Abstract

Sweet basil has been used as a medicinal plant. This study was carried out to describe detailed growth and chemical investigation of sweet basil essential oil affected by chemical and biological fertilizations. The various fresh and dry weights of herb in general changed under the various NPK fertilization+biological fertilization levels. The highest fresh and dry weights of herb (g plant^{-1} and t ha^{-1}) were recoded with NPK (75%)+biological fertilizers treatment during the second harvest with the values of 721.2, 49.8 and 79.4, 4.4, respectively. The highest essential oil contents (0.4%, 0.7 mL plant^{-1}and 57.9 L ha^{-1}) were recorded at NPK (75%)+biological fertilizers treatment during the first harvest compared with other treatments. The highest amount of linalool and estragole were resulted from the NPK (75%) and NPK (50%)+biological fertilization treatment with the values of 51.2, 27.7; 48.2, 24.1; 54.4, 24.1% during first, second and third harvests, respectively. Essential oil constituents belong to two chemical main classes. Oxygenated Monoterpenes (OM) was the major one, the remaining fractions as Monoterpene Hydrocarbons (MH) formed the minor class. The highest amount of OM were obtained from NPK (75%)+biological fertilization treatment with the values of 89.1, 87.7 and 90.3% during first, second and third harvests, respectively.

Key words: Sweet basil, fresh weight, dry weight, essential oil

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INTRODUCTION

Ocimum basilicum L. (*O. basilicum*) or sweet basil belongs to the family Lamiaceae, which includes about 200 species occur in various botanic varieties and forms (Wierdak, 2001). Traditionally, sweet basil has been used as a medicinal plant in the treatment of headaches, coughs, diarrhea, constipation, warts, worms and kidney malfunctions (Simon *et al*., 1999). *O. basilicum* L. is a popular culinary herb and a source of essential oils extracted by steam distillation from the leaves and the flowering tops which are used to flavor foods, in dental and oral products and in fragrances (Akgul, 1989; Guenther, 1952; Heath, 1981). The aromatic character of each type of basil is determined by genotype and depends on the major chemical compounds of essential oils primarily consisting of monoterpenes and phenylpropanoids (Marotti *et al*., 1996; Tateo, 1989). The essential oil has antimicrobial (Lahariya and Rao, 1979), antifungal and insect-repelling (Dube *et al*., 1989), anticonvulsant, hypnotic (Ismail, 2006) and antioxidant (Politeo *et al*., 2007) activities.

One of the methods to increase yield and availability of minerals in the soil, with the decreased use of inorganic fertilizers, is to apply biological fertilization, which also affects the essential oil of aromatic plants. The highest vegetative growth and highest amount of anethole in the essential oil of fennel was found in the case of application of a half rate of NPK and inoculation with *Bacillus megaterium* microorganisms that increase phosphorus availability (Mahfouz and Sharaf-Eldin, 2007). In studying on dill plants, Hellal *et al.* (2011) showed the highest growth characters, oil content and oil yield when biological fertilizers were applied in combination with 500 kg N ha^{-1} in the form of ammonium sulphate. The above relationships resulted from the effect of inoculation of dill seeds with bacteria from the genera Azotobacter, Bacillus and Pseudomonas as well as from the positive effect of nitrogen and the interaction between the investigated factors. Similarly, yield and essential oil in *Artemisia pallens* Wall. were the highest when the medium rate of nitrogen (93.75 kg ha^{-1}) and biological fertilization with Azospirillum were applied (Kumar *et al*., 2009). Growth characters, yield, essential oil and its constituents of medicinal and aromatic plants were significantly affected by adding the biological fertilizers compared with recommended chemical fertilizers (Khalid, 2012). Application of biological fertilizers had a positive effect on the growth characters of sweet basil (Larimi *et al*., 2014). El-Naggar *et al.* (2015) reported that growth, essential oil yield and main chemical components of essential oil of *O. basilicum* were significantly increased due to biological fertilizer treatments relative to non-inoculated plants.

Hence, this study was carried out to describe detailed growth and chemical investigation of sweet basil essential oil affected by chemical and biological fertilization.

MATERIALS AND METHODS

Experimental: Experiment was carried out at the Experimental Farm, Faculty of Agriculture, Cairo University, Egypt, during the successive season of 2014 in clay soil. Seeds of sweet basil were kindly provided by the Department of Medicinal and Aromatic Plants, Ministry of Agriculture, Giza, Egypt. Seeds were sown in the third week of February during both seasons. The seedlings were transplanted into the open field 45 days after sowing. The experimental design was a complete randomized block with four replicates. The experimental area (plot) was 6 m^2 (2x3 m); the distance between hills was 25 and 50 cm apart. Thinning for one plant per hill was made 45 days after cultivating the plants in the open field. All agriculture practices operations other than experimental treatments were performed according to the recommendations of the Ministry of Agriculture, Egypt.

Treatments: Plots were divided into five main groups. The first group was subjected to recommended level of NPK. The second, third, fourth and fifth groups were subjected to different levels of NPK (2:1:1) by 75, 50, 25 and 0.0% from recommended dose with biological fertilizers.

Sources of fertilizers: N source was ammonium sulfate (20% N). P source was calcium super phosphate (15% P_2O_5). The K source was potassium sulfate (48% K_2O). Biological fertilizers: The five strains of bacteria; (1) *Azotobacter chroococcum*, (2) *Aospirillum lipoferium,* (3) *Bacillus polymyxa*, (4) *Bacillus medatherium* and (5) *Pseudomonas fluoresens* were used as biological fertilizers which mixed in equal parts after preparation (2.5 L mixture of the strains+22.5 L of tap water).

Harvesting: At full bloom stag, the plants were cut through three times (first, second and third harvest) during the growing season by cutting the plants 5 cm above the soil surface for collect the herb. Fresh and dry weights of herb (g $plant^{-1}$ and t ha^{-1}) were recorded. The first, second and third harvests were collected after 120, 210 and 300 days from transplanting.

Essential oil isolation: The fresh herbs were collected from each treatment, air dried and then 100 g from each replicate of all treatments was subjected to hydro-distillation for 3 h using a Clevenger-type apparatus (Clevenger, 1928). The essential oils were collected and dried over anhydrous sodium sulfate and stored in sealed vials at 4°C until analysis (Joshi *et al*., 2011). The essential oil content was calculated as a relative percentage (v/w). In addition, total essential oil yields (mL plants^{-1} and L ha^{-1}) were calculated by using the dry herbs.

GC/MS: The volatile oil was analyzed on a VG analytical 70- 250S sector field mass spectrometer, 70 eV, using a DB-5 fused silica capillary column 25x30 m, 0.25 µm coating thickness), injector 222°C, detector 240°C, linear temperature 80-270°C at 10°C/min. Diluted samples (1/100, v/v, in n-pentane) of 1 mL were injected, at 250°C, manually and in the splitless mode Flame Ionization Detection (FID) using the HP Chemstation software on a HP 5980 GC with the same type column as used for GC/MS and same temperature program.

Qualitative and quantitative analyses: Identifications were made by library searches combining MS and retention data of authentic compounds by comparison of their GC Retention Indices (RI) with those of the literature (Adams, 1995) or standards available in our laboratories. The retention indexes were determined in relation to a homologous series of n-alkanes (C8–C22) under the same operating conditions. Further identification was made by comparison of their mass spectra on both columns with those stored in NIST 98 and Wiley 5 libraries or with mass spectra from literature. Component relative concentrations were calculated based on GC peak areas without using correction factors.

Statistical analysis: In this experiment, one factor was considered: five fertilization treatments. For each treatment there were 4 replicates. The experimental design followed a complete random block design according to Snedecor and Cochran (1990). The averages of data were statistically analyzed using 1-way analysis of variance (ANOVA-1). Significant values determined according to LSD at 0.05. The applications of that technique were according to the STAT-ITCF program (Foucart, 1982).

RESULTS

Effect of the combination between chemical and biological fertilizes on the fresh and dry weights of herb: Fresh and dry weights of herb (g plant^{-1} and t ha^{-1}) were significantly affected by changes in NPK fertilization+biological fertilization treatments. Thus the various fresh and dry weights of herb in general changed under the various NPK fertilization + biological fertilization levels. The highest fresh and dry weights of herb (g plant^{-1} and t ha^{-1}) were recoded with NPK (75%) + biological fertilizers treatment during the second harvest with the values of 721.2, 49.8 and 79.4, 4.4, respectively (Table 1). The changes in fresh and dry weight of herb (g plant^{-1} and t ha^{-1}) were significant for the fertilization treatments and harvests.

Effect of the combination between chemical and biological fertilizes on the essential oil contents: Essential oil contents (%, mL plants^{-1} and L ha^{-1}) increased with various treatments of NPK doses+biological fertilizers compared with recommended NPK and biological fertilizers without NPK treatments. The highest essential oil contents (0.4%) and yield (0.7 mL plant^{-1}and 57.9 L ha^{-1}) were recorded at NPK (75%)+biological fertilizers treatment during the first harvest compared with other treatments (Table 2). The changes in essential oil (% and L ha^{-1}) were insignificant for fertilization treatments, harvests and their interactions. The changes in essential oil (mL plant^{-1}) were significant for insignificant for fertilization treatments as well as the interactions between fertilization treatments and harvests while it was insignificant for harvests.

Effect of the combination between chemical and biological fertilizes on the essential oil constituents: The GC/MS analysis revealed the presence of eleven different compounds identified during the first, second and third harvests (Table 3-5). Linalool and estragole were detected as the major components (accounting more than 70%) of the essential oil which changed under different treatments (Table 3-5). Constituents were identified in essential oil isolated from sweet basil herb belong to two chemical main classes. Oxygenated Monoterpenes (OM) was the major one, the remaining fractions as Monoterpene Hydrocarbons (MH) formed the minor class (Table 3-5). The highest amount of linalool and estragole were resulted from the NPK (75%) and

Table 1: Effect of the combination between chemical and biological fertilizes on fresh and dry weight of herb

		Fresh weight		Dry weight	
Harvest	Treatments	g plant^{-1}	t ha^{-1}	g plant^{-1}	t ha^{-1}
First	NPK (100%)	730.3	40.3	80.6	4.4
	Bio without NPK	390.9	21.5	43.0	2.4
	Bio+NPK (75%)	839.4	46.5	92.7	5.1
	Bio+NPK (50%)	615.2	36.0	69.4	4.1
	Bio+NPK (25%)	475.8	26.0	52.4	3.1
Overall first harvest		610.3	34.1	67.6	3.8
Second	NPK (100%)	666.7	40.3	74.5	4.1
	Bio without NPK	512.1	28.3	56.4	3.1
	Bio+NPK (75%)	721.2	49.8	79.4	4.4
	Bio+NPK (50%)	615.5	41.5	75.1	4.1
	Bio+NPK (25%)	654.5	35.9	71.2	4.1
Overall second harvest		634.0	39.2	71.3	4.0
Third	NPK (100%)	612.1	32.3	67.6	3.1
	Bio without NPK	444.4	23.8	47.6	3.0
	Bio+NPK (75%)	627.3	35.8	70.0	4.3
	Bio+NPK (50%)	593.9	34.5	70.1	4.1
	Bio+NPK (25%)	584.9	33.3	63.3	3.7
Overall third harvest		562.6	31.9	62.8	3.8
Overall treatments	NPK (100%)	698.5	37.6	74.2	3.9
	Bio without NPK	449.1	24.5	49.0	2.8
	Bio+NPK (75%)	729.3	44.0	80.7	4.6
	Bio+NPK (50%)	608.2	37.3	71.5	4.1
	Bio+NPK (25%)	571.7	31.7	62.3	3.6
LSD: 0.05					
Treatment		0.1	0.1	0.2	0.2
Harvest		0.1	0.1	0.2	0.2
Treatment X Harvest		1.0	1.0	1.1	1.2

Table 2: Effect of the combination between chemical and biological fertilizes on essential oil contents

Harvest	Treatments	%	mL plant^{-1}	L ha^{-1}
First	NPK (100%)	0.1	0.4	44.7
	Bio without NPK	0.1	0.2	23.7
	Bio+NPK (75%)	0.3	0.6	55.1
	Bio+NPK (50%)	0.2	0.5	46.6
	Bio+NPK (25%)	0.2	0.5	45.8
Overall first harvest		0.2	0.4	43.2
Second	NPK (100%)	0.1	0.3	47.1
	Bio without NPK	0.1	0.4	36.6
	Bio+NPK (75%)	0.4	0.7	57.9
	Bio+NPK (50%)	0.2	0.5	46.7
	Bio+NPK (25%)	0.2	0.5	45.8
Overall second harvest		0.2	0.5	46.8
Third	NPK (100%)	0.1	0.3	43.2
	Bio without NPK	0.1	0.4	31.6
	Bio+NPK (75%)	0.3	0.5	53.5
	Bio+NPK (50%)	0.2	0.4	42.2
	Bio+NPK (25%)	0.2	0.4	44.1
Overall third harvest		0.2	0.4	42.9
Overall treatments	NPK (100%)	0.1	0.3	45.0
	Bio without NPK	0.1	0.3	30.6
	Bio+NPK (75%)	0.3	0.6	55.5
	Bio+NPK (50%)	0.2	0.5	45.2
	Bio+NPK (25%)	0.2	0.5	45.2
LSD: 0.05				
Treatment		ns	0.1	2.1
Harvest		ns	ns	1.3
Treatment X Harvest		ns	0.2	2.4

ns: Non significant and NPK: Nitrogen, Phosphorus and Potassium

Table 3: Effect of the combination between chemical and biological fertilizes on essential oil constituents during the first harvest

				With biological fertilizers				
Constituents	RI*	Class	NPK (100%)	NPK (0%)	NPK (75%)	NPK (50%)	NPK (25%)	Overall
α-Pinene	939	MH	0.2	0.3	0.3	0.2	0.2	0.2
Sabinene	976	MH	0.2	0.2	0.2	0.2	0.2	0.2
β-Pinene	980	MH	0.3	0.3	0.3	0.3	0.3	0.3
Myrcene	991	MH	5.6	6.1	6.2	5.6	4.4	5.6
Limonene	1031	MH	2.4	2.8	2.9	2.5	2.1	2.5
1,8-Cineole	1033	OM	5.6	6.1	6.4	5.6	7.2	6.2
Linalool	1098	OM	49.2	49.1	51.2	49.8	48.8	49.6
Camphor	1143	OM	0.3	0.3	0.3	0.3	0.3	0.3
Estragole	1195	OM	27.6	27.3	27.1	27.7	25.3	27.0
Geraniol	1255	OM	2.9	2.4	2.3	2.5	2.6	2.5
Eugenol	1356	OM	1.3	1.9	1.8	1.8	1.7	1.7
MH			8.7	9.7	9.9	8.8	7.2	
OM			86.9	87.1	89.1	87.7	85.9	
Total identified			95.6	96.8	99.0	96.5	93.1	

*: Retention indices in elution order from DB-5 column (Adams, 1995), MH: Monoterpene hydrocarbons and OM: Oxygenated monoterpenes

Table 4: Effect of the combination between chemical and biological fertilizes on essential oil constituents during the second harvest

				With biological fertilizers				
Constituents	RI*	Class	NPK (100%)	NPK (0%)	NPK (75%)	NPK (50%)	NPK (25%)	Overall
α-Pinene	939	MH	0.2	0.3	0.2	0.3	0.2	0.2
Sabinene	976	MH	0.2	0.2	0.2	0.2	0.2	0.2
.β-Pinene	980	MH	0.3	0.3	0.3	0.4	0.4	0.3
Myrcene	991	MH	1.1	1.3	2.2	1.6	1.8	1.6
Limonene	1031	MH	1.2	1.0	1.2	1.1	1.1	1.1
1,8-Cineole	1033	OM	9.5	9.2	9.1	8.5	10.7	9.4
Linalool	1098	OM	42.3	46.8	48.2	43.4	42.4	44.6
Camphor	1143	OM	0.4	0.3	0.4	0.4	0.3	0.4
Estragole	1195	OM	21.5	23.6	20.1	24.5	21.6	22.3
Geraniol	1255	OM	4.5	4.2	6.1	5.3	3.3	4.7
Eugenol	1356	OM	3.2	3.3	3.8	1.1	2.8	2.8
MH			3.0	3.1	4.1	3.6	3.7	
OM			81.4	87.4	87.7	83.2	81.1	
Total identified			84.4	90.5	91.8	86.8	84.8	

*: Retention indices in elution order from DB-5 column (Adams, 1995), MH: Monoterpene hydrocarbons and OM: Oxygenated monoterpenes

Table 5: Effect of the combination between chemical and biological fertilizes on essential oil constituents during the third harvest

				With biological fertilizers				
Constituents	RI*	Class	NPK (100%)	NPK (0%)	NPK (75%)	NPK (50%)	NPK (25%)	Overall
α-Pinene	939	MH	0.3	0.2	0.3	0.2	0.3	0.3
Sabinene	976	MH	0.1	0.2	0.2	0.1	0.1	0.1
.β-Pinene	980	MH	0.3	0.3	0.3	0.3	0.3	0.3
Myrcene	991	MH	2.9	5.6	3.8	3.7	3.5	3.9
Limonene	1031	MH	0.2	0.3	0.2	0.3	0.5	0.3
1,8-Cineole	1033	OM	2.9	5.6	3.4	3.8	6.3	4.4
Linalool	1098	OM	51.6	52.6	54.4	42.3	42.2	48.6
Camphor	1143	OM	0.3	0.3	0.3	0.3	0.3	0.3
Estragole	1195	OM	22.8	20.3	23.9	24.1	22.9	22.8
Geraniol	1255	OM	3.9	3.4	3.8	5.6	3.6	4.1
Eugenol	1356	OM	2.2	3.2	4.5	4.9	1.1	3.2
MH			3.8	6.6	4.8	4.6	4.7	
OM			83.7	85.4	90.3	81	76.4	
Total identified			87.5	92.0	95.1	85.6	81.1	

*: Retention indices in elution order from DB-5 column (Adams, 1995), MH: Monoterpene hydrocarbons and OM: Oxygenated monoterpenes

NPK (50%)+biological fertilization treatment with the values of 51.2, 27.7; 48.2, 24.1; 54.4, 24.1% during first, second and third harvests, respectively compared with other treatments. The highest amount of OM were obtained from NPK (75%)+biological fertilization treatment with the values of 89.1, 87.7 and 90.3% during first, second and third harvests, respectively.

DISCUSSION

This increase in the fresh and dry weights of herbs, essential oil and essential oil major constituents can be because of release nutrition material by effective microorganisms in the soil and followed by increased plant growth and essential oil composition (Ashour, 1998).

Sprent and Sprent (1990) reported that nitrogen fixing bacteria (Azotobacter, Azospirillum and Pseudomonas) by symbiotic to the plants root causes increased uptake moisture level and this extensive network of roots by uptake water and salts and transfer to the host plant is causes increased leaf area that as a result increases amount of chlorophyll and improved photosynthesis. It happens as a result of production more assimilate and increased cell division and size cells (Selossey *et al*., 2004). Effect of biofertilizer (Azotobacter, Azospirrillum and Pseudomonas) on leaf are might be attribute to its efficiency in supplying the growing plants with biologically fixed nitrogen, dissolved immobilized phosphorus and produced phytohormones, which could simulate nutrients absorption as well as photosynthesis process which increased plant growth (Hewedy, 1999). Qurbanly *et al*. (2006) reported a positive correlation between the nitrogen and the chlorophyll content of leaves, mainly due to the presence of nitrogen in the structure of chlorophyll molecules. In addition, Chandrasekar *et al*. (2005) observed an increase in chlorophyll content of white millet and wheat leaves, respectively, after inoculation by Azosperilium bacteria, probably due to nitrogen fixation which increases nitrogen content of vegetative tissues. Higher correlation between leaf area and nitrogen compound with chlorophyll a. than chlorophyll b. is because of conversion chlorophyll a. pigments to chlorophyll b. Found that addition of biofertilizer combined with chemical fertilizers improved vegetative growth. The superiority of using the required N in biologic form and 75% in mineral form on improving vegetative growth may be due to the favorable effect of the chemical nitrogen application on the activity of microorganisms responsible for biofertilizer decay in the soil which increased available N in soil, N-uptake and consequently encouraged vegetative growth of the plant. The superiority in plant growth due to inoculating with biofertilizer (N-free living bacteria+phosphorus dissolving bacteria) is in agreement with the results obtained by Alian (2005) using Azospirillum, Azotobacter and Bacillus. They mentioned that N-fixing bacteria increased the available N in the soil. Moreover, the role of N-free living bacteria in production of phytohormones and/or improving the availability and acquisition of nutrients or by both, may explain the encouraged growth of plants inoculated with these non-symbiotic N-fixing bacteria (Barakat and Gabr, 1998). In addition, many organic acids produced by rhizosphere micro-organisms are effective in solubilizing soil phosphates (Ashour, 1998). Furthermore, Azotobacter and Azospirillum could produce IAA and Cytokinins which increase the surface area per unit root length and were responsible for root hair branching with an eventual increase in acquisition of nutrients from the soil (Jain and Patriquin, 1985).

Our results agree with those obtained by previous literature. According to Hellal *et al*. (2011) applying bio-fertilizer treatment alone (*Azotobacter chroococcum*, *Azospirillum lipoferum*, *Bacillus polymyxa, Bacillus megatherium* and *Pseudomonas fluorescens*) or in combination with chemical N fertilizer increased the growth, yield, essential oil and chemical constituents of dill plant compared to the untreated control; the highest values of vegetative growth and oil yield were recorded by the treatment of bio-fertilizer plus two third of recommended dose of nitrogen fertilizer; the lowest values in this respect were obtained by control plants during two seasons; the GC analysis of volatile oil indicated that the main components were carvone, limonene and apiol; these components were affected by bio-fertilization and chemical N treatments; partial substitution of mineral nitrogen fertilizer by bio-fertilizer was recommended to increase the yield as well as the quality of dill plant. Bio-fertilizer treatments increased the growth characters and essential oil composition of coriander compared with the chemical fertilizers treatments (Hassan *et al*., 2012). Bio-fertilizer treatments increased the seed yield and essential oil of fennel plants compared with vermicompost treatments (Darzi, 2012). Application of phosphate bio-fertilizer and phosphorus were significant on the vegetative growth characters of *Tagetes erecta* L. plants (Hashemabad *et al*., 2012). The maximum fresh weight (3.96 g plant^{-1}) and essential oil yield (0.82%) were observed in the *Pseudomonas* + Azotobacter + *Azosprillum* treatment. All factors were higher in the Pseudomonas+Azotobacter+Azosprillum and Azotobacter+Azosprillum treatments (Khalid, 2012). Application of bio fertilizers had a positive effect on the growth characters of sweet basil (Larimi *et al*., 2014).

El-Naggar *et al.* (2015) reported that growth, essential oil yield and main chemical components of essential oil of *Ocimum basillicum* L. were significantly increased due to bio-fertilizer treatments relative to non-inoculated plants.

The overall the results of this study was suggested that application of chemical and biological fertilizers mixing together, have a positive effect on development of fresh weight, dry weight and essential oil composition of sweet basil.

CONCLUSION

According to necessity production of sweet basil plant need attention to medicinal plant cultivation in low chemical fertilizes application appear biological fertilizers are suitable for production medicinal plants.

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