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Herbal and essential oil yield of Genovese basil (*Ocimum basilicum* L.) grown with mineral and organic fertilizer sources in Egypt

Einfluss mineralischer und organischer Düngung auf Masse- und Ölertrag von Basilikum (*Ocimum basilicum* L.) unter ägyptischen Bedingungen

Abstract

The main objective of this work was to evaluate a measure to decrease the excessive application of chemical fertilizers and to increase the quality of medicinal and aromatic plants grown in Egypt. Basil is one of the most important medicinal plants which grows well under Egyptian conditions and plays a vital role for the export of the country. Two field experiments were conducted on the farm of the National Research Center in Giza during two successive seasons in 2003 and 2004. The treatments of the first experiment were 25, 50, 75 and 100% of the recommended NPK fertilization. In the second experiment different percentages of chemical and organic fertilizers (100% organic, 100% chemical, 50 organic and 50 chemical, 25 organic and 75 chemical and 75 organic and 25 chemical) were applied. The investigation yielded the following main result: increasing NPK rates increased the growth and yield of Genovese basil but the application of only 50% from recommended NPK gave already about 80% of the yield of 100% recommended NPK. Compost as a source of nutrients gave similar yield but higher quality than fertilization with chemical NPK fertilizers alone.

Key words: Genovese basil, *Ocimum basilicum*, essential oils, fertilization

Zusammenfassung

Ziel vorliegender Untersuchungen war es einen Beitrag zur Optimierung des Düngemitteleinsatzes im ägyptischen Basilikumanbau zu leisten und zwar sowohl im Hinblick auf Ertrag und Qualität der Kultur. Basilikum ist für den Export des Landes eine der wichtigsten Medizinalpflanzen, die besonders gut unter ägyptischen Klimabedingungen wächst. Die Versuche wurden in den Jahren 2003 und 2004 auf Feldern des Nationalen Forschungszentrums in Giza durchgeführt. Die Behandlungen des ersten Versuchstyps umfassten eine gesteigerte NPK-Düngung mit jeweils 25, 50, 75 und 100% der offiziell empfohlenen Düngermenge. Im zweiten Versuchstyp wurde das Verhältnis mineralischer zu organischer Anteile an der Gesamtgabe an Nährstoffen sukzessive verändert (100% organisch, 100% mineralisch, 50 organisch und 50 mineralisch, 25 organisch und 75 mineralisch sowie 75 organisch und 25 mineralisch).

Im Ergebnis konnten bereits mit 50% der offiziell empfohlenen Düngermenge bereits 80% des am Standort erzielbaren Höchstertrages erreicht werden. Kompost als Nährstoffquelle erzielte die gleiche Ertragswirkung aber bessere Qualitäten des Basilikums wie mineralische Düngung.

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Stichwörter: Basilikum, *Ocimum basilicum*, essentielle Öle, Düngung

Introduction

Basil (*Ocimum basilicum* L.) is an aromatic herb used extensively to add a distinctive aroma and flavour to food. The leaves can be used fresh or dried for use as a spice. Essential oils extracted from fresh leaves and flowers can be used as aroma additives in food, pharmaceuticals and cosmetics (Simon et al., 1999; Javanmardi et al., 2002; Senatore, 1996). Traditionally, basil has been used as a medicinal plant in the treatment of headaches, coughs, diarrhea, constipation, warts, worms and kidney malfunction (Simon et al., 1999). It also possesses various beneficial effects, e.g., antiseptic, carminative, antimicrobial and antioxidative properties (Baranauskiene et al., 2003). The genus *Ocimum* includes about a dozen species and subspecies native to the tropical and sub-tropical regions of the world. Genovese basil (*Ocimum basilicum* L., var. *Basilicum*). It is widely cultivated for the production of essential oils and is also marketed as an herb, either fresh, dried or frozen (Putievsky and Galambosi, 1999). The essential oil of basil is used as antifungal and insect-repelling and toxic activities (Reuveni et al., 1984; Werner, 1995). Despite the wide use and the importance of basil and its essential oils, little is known about the biosynthesis and developmental regulation of the compounds responsible for the flavour quality of the fresh and dried herbs. The intensive use of manufactured nitrogen fertilizers increased the crops productivity but with low quality which is not acceptable for export (Lain et al., 1996; Wang et al., 1996). The key objectives of this work were: to investigate the performance of Genovese basil growth depending of the sources of nutrients (mineral, organic used for fertilization) and to investigate the effect of fertilization practices on content and quality of essential oils in Genovese basil targeting towards the general recommendation for improving the production of Genovese basil according to high quality standards on small farms.

Material and Methods

Two field experiments were carried out on the experimental farm of the National Research Centre in Shalakan Kalubia Governorate, Giza during two successive seasons (March 2003 and March 2004). Genovese basil seeds were sown on 9th March in 2003 and 2004. They germinated after about two weeks. After 45 days from sowing date, the seedlings were transplanted into the field at 20 cm apart. The plots size was 3 x 4 m and plant density was 15 plants per m^2. The plants were cut twice during each growing seasons and then the plants were left for seed setting. The first cut was done during the first week of July and the second one in the middle of October in both seasons.

The experimental soil was a clay loam with the following properties: 5.3% coarse sand, 30.6% fine sand, 38.7% silt and 25.4% clay, E.C. 0.56 dsm-1, 7.25 pH, 3.2% CaCO3, total soluble salts 512 mg kg^{-1}, 0.11% total nitrogen, 41.1, 17.0 and 401 mg kg^{-1} available N, P2O5 and K2O respectively, and 22.2, 3.9, 2.1 and 2.6 mg kg^{-1}, DTPA extractable Fe, Mn, Zn and Cu respectively.

The first experiment was carried out to investigate the effect of NPK fertilization rate on growth, yield, oil content and it's quality of Genovese basil plants. The treatments were: control, 25%, 50%, 75% and 100% of the recommended fertilizer doses: 238 kg ha^{-1} as ammonium nitrate, 128 kg ha^{-1} P as super-phosphate and 114 kg ha^{-1} K as potassium sulfate. The second experiment was carried out to compare the effects of chemical and organic fertilization on the parameters mentioned before. The treatments were 238 kg N per hectare in combination of organic and inorganic fertilizers. Compost was used as organic fertilizer and ammonium nitrate was used as chemical fertilizer. The combinations were 25% N as compost with 75% N as ammonium nitrate, 50% N as compost with 50% N as ammonium nitrate, 75% N as compost with 25% N as ammonium nitrate, 100% N as compost and 100% N as ammonium nitrate. The experimental design was complete randomized with four replicates in both experiments. During the cutting 10 plants were randomly selected for determining plant height, number of branches per plant, plant diameter, plant fresh weight, leaves mass per plant, stem mass per plant and flowers mass per plant (Tab. 1).

Essential oil was isolated using a cleavenger-type apparatus according to Marotti and Piccaglia (1992). 100 g of fresh Genovese basil herb were distilled for three hours in one liter water and then the percentage of essential oil per fresh weight was calculated. The dehydrated oil of each treatment was subjected to gas chromatography. The separation was carried out on a Carbowax 20M column, 25 m length x 0.32 mm, I.D. film thickness 0.3 µm, flow rates nitrogen carrier, hydrogen and air 30, 30 and 300 ml $minute^{-1}$ consecutively. Column temperature from 60°C to 190°C at rate of 8°C per minute, injection temperature 240°C and detector temperature 208°C. The retention times of the chromatogram peaks were compared with those of authentic compounds run under identical conditions.

Tab. 1. Selected physical and chemical data of the compost used in the experiments

Character	Compost
Volume weight kg m^3	521
Water content (%)	35
Organic matter (%)	47
Total carbon (%)	34
C/N ratio	23
Total nitrogen (%)	1.5
Total phosphorus (%)	0.5
Total potassium (%)	2.4

Statistical analysis
The collected data were subjected to statistical analysis using the SAS PROGRAMME (1985), employing the General Linear Model (GLM).

Results and Discussion

The vegetative growth of Genovese basil, as indicated by plant height, number of branches and plant diameter (Tab. 2) were not substantially increased by the addition of NPK fertilization rate although the effects were significant compared with control. On an average, the plants fertilized with 25, 50, 75 and 100% from recommended NPK fertilization gave same height, branches and diameter in both cuts and in both growing seasons.

Data given in Tab. 3 show that the fresh weight of Genovese basil was significantly affected by NPK fertilization. No significant differences were observed among plots fertilized with 25, 50, 75 and 100% from recommended doses of NPK, while significant differences were observed between plants fertilized and non fertilized. Plant fresh weight increased by 3.3 g through addition of one kg ha^{-1} NPK in the first cut in 2003, while this increment reached

Tab. 2. Effect of increasing NPK fertilization rate on growth parameters of Genovese basil grown in the Nile valley of Egypt

Treatments	Plant Height (cm)		Branches Numbers (n)		Plant Diameter (cm)	
Year	2003	2004	2003	2004	2003	2004
First Cut						
Control	72 b	81 b	26.3 a	30.1 b	08.1 b	09.8 c
25% NPK	89 a	89 a	29.8 a	33.8 b	10.8 a	11.0 b
50% NPK	87 a	94 a	32.5 a	34.5 b	11.3 a	11.5 b
75% NPK	91 a	97 a	37.5 a	35.5 b	13.0 a	15.1 a
100% NPK	98 a	110 a	40.0 a	37.5 a	13.7 a	15.2 a
Second Cut						
Control	98 b	101 b	35 b	33 c	14.7 b	14.5 b
25% NPK	108 a	115 a	41 b	42 bc	15.8 b	19.3 a
50% NPK	109 a	117 a	43 b	48 b	19.0 ab	20.8 a
75% NPK	111 a	123 a	54 a	51 ab	20.2 ab	21.5 a
100% NPK	119 a	127 a	58 a	57 a	22.3 a	22.3 a

Numbers with different characters are statistically different at the 5% level by the Tukey test

Tab. 3. Effect of increasing NPK fertilization rate on fresh weight of Genovese basil grown in the Nile valley of Egypt

Treatments	whole Plants g $plant^{-1}$		Stems g $plant^{-1}$		Leaves g $plant^{-1}$		Flowers g $plant^{-1}$	
Year	2003	2004	2003	2004	2003	2004	2003	2004
First Cut								
Control	813 b	787 c	123 b	112 d	145 b	141 b	658 b	481 a
25% NPK	1050 a	868 c	141 b	135 c	179 a	165 b	731 a	568 a
50% NPK	1088 a	1082 b	147 a	138 c	190 a	168 b	752 a	776 a
75% NPK	1125 a	1144 b	169 a	161 b	195 a	195 b	761 a	788 a
100% NPK	1186 a	1308 a	173 a	180 a	212 a	252 a	800 a	875 a
Second Cut								
Control	1458 c	1480 c	322 b	351 b	368 b	289 b	651 c	683 c
25% NPK	1462 c	1520 c	346 b	400 ab	374 ab	299 b	742 bc	821 b
50% NPK	1670 bc	1838 c	371 a	436 ab	436 ab	318 b	920 b	1082 b
75% NPK	1787 b	2350 b	391 a	480 ab	480 ab	336 ab	984 b	1533 a
100% NPK	2588 a	2725 a	429 a	520 a	520 a	370 a	1642 a	1835 a

Numbers with different characters are statistically different at the 5% level by the Tukey test

to 10.3 g in the second cut. Also increasing fertilization with NPK by one kg ha^{-1} caused a raise of fresh weight by 5.3 and 13.3 g $plant^{-1}$ in the first and second cuts in 2004 respectively. These results are in agreement with those obtained by Tesi et al. (1997).

Data in Tab. 4 show that the effect of chemical and organic fertilization on Genovese basil growth parameters was significance in both cuts and both growing seasons. In many cases fertilization with 50% organic fertilizer and 50% chemical fertilizer gave higher yield than fertilization with compost or ammonium nitrate alone.

Data given in Tab. 5 show that the effect of chemical and organic fertilization ratio on biomass of Genovese basil at the first and second cuts during two successive seasons 2003 and 2004 was insignificant in first cut except stem fresh weight which fertilization with 50% compost and 50% ammonium nitrate gave higher yield g $plant^{-1}$ than fertilization with compost or ammonium nitrate alone. Fertilization Genovese basil with compost alone or ammonium nitrate alone gave the heaviest leaves fresh weight g $plant^{-1}$ in the second cut in both seasons. In general flowers fresh weight was not affect by

Tab. 4. Effect of chemical and organic fertilization ratios on growth parameters of Genovese basil grown in the Nile valley of Egypt

Chemical/Organic (%)	Plant Height (cm)		Branches Numbers (n)		Plant Diameter (cm)	
Year	2003	2004	2003	2004	2003	2004
First Cut						
25/75	111 a	118 a	39.0 a	40.0 a	14.0 a	14.0 a
50/50	102 b	102 b	41.0 a	42.3 a	11.3 a	11.8 a
75/25	99 b	104 b	42.0 a	45.0 a	14.5 a	17.3 a
100 chemical	97 b	104 b	40.0 a	46.5 a	13.8 a	15.3 a
100 organic	116 a	124 a	43.0 a	45.5 a	14.0 a	15.8 a
Second Cut						
25/75	109 ab	105 b	58.7 a	61.0 a	20.0 b	21.5 b
50/50	106 b	100 b	54.5 a	57.8 a	283 a	36.8 a
75/25	107 ab	112 b	47.8 b	49.8 b	20.3 b	22.8 b
100 chemical	119 ab	127 a	58.0 a	57.3 a	21.3 b	22.3 b
100 organic	121 a	134 a	48.8 b	54.3 ab	21.5 b	20.8 b

Numbers with different characters are statistically different at the 5% level by the Tukey test

Tab. 5. Effect of chemical and organic fertilization ratios on fresh weight of Genovese basil grown in the Nile valley of Egypt

Chemical/ Organic (%)	Plant g $plant^{-1}$		Stem g $plant^{-1}$		Leaves g $plant^{-1}$		Flowers g $plant^{-1}$	
Year	2003	2004	2003	2004	2003	2004	2003	2004
First Cut								
25/75	1250 a	1275 a	220 a	239 a	204 a	211 a	791 a	825 a
50/50	1313 a	1400 a	182 a	250 a	251 a	213 a	880 a	936 a
75/25	1262 a	1235 a	195 b	203 b	232 a	217 a	864 a	814 a
100 chemical	1186 a	1308 a	174 b	180 b	212 a	252 a	800 a	875 a
100 organic	1275 a	1350 a	186 b	197 b	220 a	231 a	889 a	921 a
Second Cut								
25/75	1937 b	2137 ab	331 bc	332 b	295 b	293 c	1311 a	1314 a
50/50	2250 a	2425 a	362 bc	357 b	325 b	312 b	1562 a	1455 a
75/25	1788 b	1912 b	275 c	268 b	319 b	299 bc	1294 a	1346 a
100 chemical	2475 a	2150 ab	428 b	520 a	516 a	370 b	1530 a	1260 a
100 organic	2525 a	2225 ab	569 a	518 a	588 a	501 a	1368 a	1205 a

Numbers with different characters are statistically different at the 5% level by the Tukey test

different nitrogen fertilization treatments in both cuts and in both seasons.

Data given in Tab. 6 show that the effect of NPK fertilization rate on yield of Genovese basil per hectare during two successive seasons 2003 and 2004 was significant. Addition of one percent from the recommended dose of NPK fertilization increased the yield of Genovese basil by 133 and 118 kg respectively. No significant differences were observed between plots fertilized with 75 and 100% of the commended NPK fertilization in the second season. Although fertilization with the recommended dose of NPK gave the highest Genovese basil yield in the first season, already 75% of the recommended dose was sufficient to obtain a best yield in the second season. With increasing prices for NPK fertilizers it could be useful for the Egyptian farmers to produce Genovese basil by with only 75% of the recommended NPK dose to save 25% from the cost of chemical fertilizer with loss of only less than 10% of the income. Similar results were obtained by KHATAB and GOMAA (2003).

Fertilization with different combinations of chemical and organic fertilizer did not effect the yield of Genovese. CARIA and MARTINETTI (1996) also studied the effect of organic and mineral fertilization on basil, and found that among different N sources the organic form gave the best results when nitrogen rates exceeded 120 kg ha^{-1} N.

The concentration of average essential oil in Genovese basil was 0.5% and was not effected by the rate of NPK fertilization. OMER et al. (1998) found that the concentration of oil in all basil varieties grown in Egypt were higher in the first cut than in the second cut. These differences could probably be attributed to differences in environmental factors i. e. temperature, moisture and light levels (ALI et al., 1986; ELBALAL et al., 1983; OMER et al., 1994).

Increasing rates of NPK fertilization significantly increased the biomass (Tab. 5) and consequently also the oil yield of Genovese basil in both seasons (Tab. 7). No significant differences were obtained between plots fertilized with 75% from recommended dose of NPK and the plots fertilized with recommended dose of NPK in the second season 2004 (Tab. 6). But different NPK fertilization rates had no effects on the concentration of essential oils in Genovese basil.

Data in Tab. 8 show that, fifteen hydrocarbon compounds were detected in the essential oil of Genovese basil. The main constituent found was linalool at a relative percent of (55 to 60%) followed by 1,8 cineol with (12 to 13.5%), methyl chavicol (3.3 to 6.17%), fugenol (2.76 to 3.37%), farnesol (2.56 to 3.41), methyl eugenol (1.21 to 1.86%), iso eugenol (1.32 to 1.67%), myrcene (0.59 to 1.89%) and other constituents were less than 1%. These results agree with the findings of KARAWYA et al. (1974) and OMER et al. (1998) who found that linalool is the most prominent component in Genovese basil grown in Egypt and also the results of CONAN (1977) who commutated the essential oil of basil cultivated in Egypt contained 48% linalool, 3.04% methyl chavicol and 5.9% eugenol. The linalool content was about 5% higher in plots fertilized with 75% of the recommended dose of NPK compared with those fertilized with 100% NPK. But 50% of the recommended NPK gave the lowest linalool content. Ocimum, contains essential oils based primarily on monoterpene derivatives such as linalool (LAWRENCE, 1993). Interestingly, other members of the genus, including sweet basil (*O. basilicum L.*), contain an essential oil based primarily on high proportions of phenolic derivatives, such as eugenol, methyl chavicol (estragole) and methyl cinnamate, often combined with various proportions of linalool, a monoterpenol (FLEISHER and FLEISHER, 1992). In general increasing methyl chavicol means low quality of the essential oil. In this study we found that fertilization with 50 or 75% of the recommended NPK fertilization gave the lowest estragole (methyl chavecol) content and by this increased the quality of essential oil in Genovese basil.

Data in Tab. 9 show the effect of combinations of chemical and organic fertilization on the compositions of essential oil in Genovese basil. The main essential oil constituents were only slightly effected by fertilization with different ratios of organic and chemical nitrogen fertilizers. Plots fertilized with 50% compost and 50%

Tab. 6. Effect of NPK fertilization rate on yield of Genovese basil grown in the Nile valley of Egypt

Fertilization rate	Herb fresh weight ton ha^{-1}	
Year	2003	2004
Control	19.8 e	19.3 e
25% NPK	24.3 d	23.4 c
50% NPK	27.7 c	26.8 b
75% NPK	29.7 b	28.7 a
100% NPK	33.8 a	31.4 a

Numbers with different characters are statistically different at the 5% level by the Tukey test
First season y = 20377 + 133 kg NPK ha^{-1} and second season y = 20030 + 118 kg NPK ha^{-1}

Tab. 7. Effect of NPK fertilization rate on essential oil yield of Genovese basil grown in the Nile valley of Egypt

Fertilization rate	Essential oil yield l ha^{-1}	
	Year 2003	Year 2004
Control	95 d	93 d
25 NPK	134 c	131 c
50 NPK	150 b	145 b
75 NPK	156 b	158 ab
100 NPK	183 a	170 a

Numbers with different characters are statistically different at the 5% level by the Tukey test

Tab. 8. Effect of NPK fertilization rates on essential oil composition (%) of Genovese basil grown in the Nile valley of Egypt (means of growing seasons 2003 and 2004)

Compound %	25% NPK	50% NPK	75% NPK	100% NPK	LSD 5%
α-pinene	0.80	0.84	0.75	0.12	0.25
β-pinene	1.93	0.15	0.82	0.74	0.51
Myrcene	0.59	1.89	1.85	1.76	0.47
1,8 Cineol	13.5	13.8	12.6	12.7	0.52
α-terpinene	0.11	0.19	0.16	0.15	0.02
β-terpinene	0.68	0.73	0.67	0.65	0.02
Linalool	57.9	55.8	60.6	57.8	1.29
Linalyl acetate	0.84	1.08	0.90	0.78	0.09
Methyl chavicol	6.17	3.30	4.43	5.84	1.07
α-terpineol	0.12	0.16	0.15	0.19	0.02
β-terpineol	0.42	0.52	0.38	0.52	0.06
Methyl eugenol	1.58	1.86	1.21	1.51	0.18
Eugenol	3.25	2.99	3.37	2.76	0.22
Iso eugenol	1.32	1.67	1.59	1.44	0.13
Farnesol	2.77	3.41	2.56	3.06	0.29

Tab. 9. Effect of chemical and organic fertilization ratio on the composition (%) of essential oils in Genovese basil grown in the Nile valley of Egypt (means of growing season 2003 and 2004)

Compound	25 chem. 75 org.	50 chem. 50 org.	75 chem. 25 organic	100 organic	100 chemical	LSD 5%
α-pinene	0.80	0.84	0.75	0.13	0.12	0.32
β-pinene	1.93	0.15	0.83	0.82	0.74	0.41
Myrcene	0.59	1.89	1.85	1.85	1.76	0.40
1,8 Cineol	13.5	13.8	12.6	12.6	12.7	0.51
α-terpinene	0.11	0.18	0.16	0.15	0.15	0.02
β-terpinene	0.68	0.73	0.67	0.53	0.65	0.05
Linalool	58	59	61	58	58	1.07
Linalyl acetate	0.84	1.08	0.89	0.85	0.78	0.08
Methyl chavicol	6.17	3.30	4.43	5.71	5.84	0.98
α-terpineol	0.12	0.16	0.15	0.19	0.19	0.02
β-terpineol	0.42	0.52	0.38	0.33	0.52	0.07
Methyl eugenol	1.58	1.86	1.21	2.03	1.51	0.24
Eugenol	3.25	2.99	3.37	0.20	2.76	0.93
Iso eugenol	1.32	1.67	1.59	1.57	1.44	0.11
Farnesol	2.77	3.41	2.56	3.08	3.06	0.25

ammonium nitrate gave with 3.3% the lowest estragole content compared to 5.7% and 5.9% from those fertilized with compost or ammonium nitrate respectively. Fertilization with compost or ammonium nitrate gave nearly the same amount of linalool percentage, while fertilization with 75% ammonium nitrate and 25% compost gave with 60.6% the highest linalool content. The farnesol content was higher in plots fertilized with 50:50 (organic:inorganic) than plots fertilized with organic or inorganic alone. In general compost as organic fertilizer gave similar qualities essential oil in Genovese basil like those fertilized with inorganic fertilizer. These results were in agreement with those reported by KARAWYA et al. (1974) on sweet basil.

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