

EFFECT OF LOCATION AND ENVIRONMENTAL CONDITIONS ON GROWTH, YIELDS AND CHEMICAL CONSTITUENTS OF SWEET BASIL (*Ocimum basilicum* L.)

Abozeed, A.E.; R.M. El Shafey and Y.A.H Osman.

Medicinal and Aromatic Plants Department, Desert Research Center, El-Matara, Cairo, Egypt.

ABSTRACT

Sweet basil, *Ocimum basilicum* L. is an important medicinal plant, has been used in various uses. The productivity of basil plants of fresh, dry herb and its chemical constituents were determined at two locations in Egypt (Toshka in the south of Egypt and Baloza in the north of Egypt) during the two seasons (2011-2012) to investigate effect of location on the yield and chemical constituents of plants. The obtained results indicated that Baloza location gave the highest significant values of fresh, dry herb and essential oil yield per plant and per feddan , while Toshka location gave the lowest values. The percentages of consequently oils, carbohydrates, nitrogen, protein, flavonoids, alkaloids, phenolics, terpenoids and vitamin C contents reached their maximum values (0.355, 9.03, 0.17, 1.06, 0.73, 0.265, 1.81, 3.56 and 2.065% respectively) in the plant samples collected from Baloza location, while the percentages of total ash and total lipids reached (15.42 and 1.875 % respectively). The mean value of the elements Phosphorus, Potassium, Sodium in the plant samples at the two seasons were higher at Toshka location. By using GC/MS, essential oil composition differed between the two locations. Estragol (54%) was the major compound in the oil of Toshka location.

Meanwhile, linalool (45.62%) and estragol (37.22%) were the main components for the oil of Baloza location. The results of this study revealed that *Ocimum basilicum* growing in Baloza and Toshka locations are a good source of phytochemicals and nutrients ,which can be used in various pharmacological and industrial purposes, but the environmental conditions of Baloza location were more suitable for the growth , yields and chemical constituents than that of Toshka location .

Keywords : sweet basil , essential oil composition , flavonoids , alkaloids , phenolics , terpenoids and vitamin C .

INTRODUCTION

Basil (*Ocimum basilicum* L., family: Lamiaceae) is an herbaceous plant originally from tropical areas such as southern Asia, including India and Africa. In such climates, it can grow as perennial. In much of the world, it is cultivated as an annual. Basil is also an important herb in Asian cuisines. The plant is versatile for so many cuisines partly because its taste varies over different regions. This is due to variation in the types of chemicals found in the plants in various parts of the world (Cumò, 2013).

The economically important parts of basil plants are their leaves and tender shoots and they are used as pharmaceutical agents because of their antimicrobial, antiemetic, antidiabetic, antifertility, antiasthmatic, antistress, insecticidal, diuretic, expectorant, analgesic, hepatoprotective properties (Prakash and Gupta 2005, Bunrathep *et al.* 2007 and Vimala *et al.* 2014).

Some aromatherapists believe that basil oil encourages hair growth (Halpern, 2002). Sweet basil (*Ocimum basilicum*) essential oil has some antimicrobial activity due to primarily to the presence of linalool and methyl chavicol (Naidu, 2000).

Environmental conditions of different agriculture locations are considered as the most important factors that have multiple effects on growth, physiological processes within the plant, which is in the end a large impact on the quantity and quality of the crop. Several authors found that variations of agriculture locations and environmental conditions had a significant effect on growth, production and chemical constituents of cultivated medicinal and aromatic plants in Egypt i.e. Edris *et al.*, (2003) on spearmint (*Mentha spicata* L.), Abd El-Wahab (2009) on spearmint (*Mentha spicata* L.), Abd El-Wahab and Mehasen on Indian fennel (2009), Shalaby *et al.*, (2011) on fennel, Ibrahim *et al.*, (2013) on sweet basil and Al Ahl *et al.*, (2014) on coriander.

Cultivation of the medicinal and aromatic plants extended now to the newly reclaimed area especially in Sinai which is a promising region for cultivation of such plants with its climatic conditions favorable for their growth (Hamed, 2011). Meanwhile Toshka is considered also as promising region for cultivation of medicinal and aromatic plants. (Wahby, 2004).

The two locations Toshka in the south of Egypt and Baloza in the north of Egypt (north Sinai governorate) were chosen for cultivation of basil plants to evaluate the growth, productivity and chemical constituents of basil plants.

MATERIALS AND METHODS

This work was carried out during two successive seasons of 2011 and 2012 at two locations (Toshka and Baloza locations) to investigate the effect of location on the yield and chemical constituents of basil (*Ocimum basilicum* L.) plants.

Seeds of *Ocimum basilicum* L. were kindly provided by the Department of Medicinal & Aromatic Plants, Ministry of Agriculture, Giza, Egypt. Seeds were sown in nursery in the med of February at both seasons. After 45 days, the seedlings were transplanted to the field. Drip irrigation system was the prevailing system in both locations, so the seedlings were cultivated under drippers at 30 cm distances and 75 cm between the irrigation pipes.

All agricultural practices were done according to the recommendation of the Egyptian Ministry of Agriculture. At full blooming, the plants were harvested during the growing season by cut the plants and left 5 cm above the soil, and the following data were recorded:

1- Vegetative growth and yield characters: Herb fresh weight/plant (gm) herb fresh weight/feddan (ton), herb dry weight/plant (gm) and herb dry weight/feddan (ton).

2- Chemical analyses:

The following chemical analyses were determined:

Essential oil percentage: Air dried herb (100 g) was subjected to hydro distillation for 3 h using a Clevenger type apparatus (British Pharmacopoeia, 1963).

Essential oil fractionation : The essential oil of each variety was fractionated using GC model HP 6890 Series (Agilent) and Mass Spectrometer HP 5973 with TR-5MS capillary column with 5% phenyl polysil phenylene siloxane (30 mL x 0.25 mm ID x 0.25 film thickness). Oven temperature was programmed at 50 - 180°C with rate of 5°C per minute, then isothermal at 180°C for 10 minute. Both the injector and detector temperatures were maintained at 250°C, and helium was the carrier gas at flow rate of 1 ml per minute. Identification of each compound was carried out by comparing mass spectra of each peak with those of Wile library and with mass spectrum of the standard materials.

pharmacopoeial constants: Water content was estimated according to the method described by Rowell (1994), crude fiber and total ash contents were determined as described Alabi *et al.* (2005).

Quantitative determination of the chemical constituents:

Determination of the total carbohydrates: Total carbohydrates were determined by the method of Chaplin and Kennedy (1994).

Determination of the total lipids, nitrogen and protein contents:

Total lipids, nitrogen and protein contents were determined according to A.O.A.C. (2000).

Determination of total flavonoids: The total flavonoids were determined by the method described by Obadoni and Ochuko (2001) .

Determination of total alkaloids: The total alkaloids were estimated using the method described by Edeoga *et al.* , 2005 .

Determination of total phenolics: The total phenolics were determined according to the methods described by (pulido *et al.* 2000).

Determination of total terpenoids: Quantification of terpenoids was done according to Morigiwe (1986).

mineral analysis : Sodium and potassium were determined in the digested samples of the studied plants using a flame photometer according to Allen (1989). Phosphorus content in the digested samples was determined colorimetrically by the molybdic acid method as described by Humphries (1956). The contents of Ca⁺⁺ were determined using Unicam 929 atomic absorption spectrophotometer.

Determination of vitamin C content : The vitamin C content was determined by iodine titration method of Suntornsuk *et al.* (2002).

3-Statistical Analysis: The experimental layout was a complete randomized block design with three replicates, each replicate contained 10 plants. L.S.D. test at 0.05 was used to compare the average means of treatments, according to Snedecor and Cochran (1982).

4-Meteorological data: The meteorological data of the two locations of 2011 and 2012 years are shown at tables (1&2).

Table (1): Means of the meteorological data of Toshka location during the seasons of 2011 and 2012.

2011						
Month	Wind speed (km/h)	Air temp. (°C)	Humidity (RH%)	Rain fall (mm)	Temp. max (°C)	Temp. min (°C)
April	2.94	26.10	17.46	0.0	34.4	17.8
May	2.60	30.54	15.25	0.0	39.5	21.6
June	2.78	33.01	15.99	0.0	41.8	24.2
July	2.28	33.26	16.50	0.0	46.8	21.7
August	2.61	34.02	17.19	0.0	42.8	25.7
September	2.60	30.35	20.24	0.0	39.2	21.5
October	2.06	29.65	24.22	0.0	38.2	21.2
November	2.22	20.60	21.60	0.0	28.3	12.9
2012						
April	2.66	26.30	17.42	0.0	34.5	18.1
May	2.58	30.50	15.34	0.0	39.5	21.5
June	2.82	33.05	16.07	0.0	41.6	24.5
July	2.24	33.90	16.43	0.0	45.9	22.0
August	2.59	34.60	17.23	0.0	43.5	25.6
September	2.63	30.60	20.21	0.0	39.7	21.5
October	2.10	29.45	24.27	0.0	38.0	20.9
November	2.25	20.75	20.34	0.0	28.4	13.1

Table (2): Means of the meteorological data of Balozza location during the seasons of 2011 and 2012.

2011						
Month	Wind speed (m/s)	Air temp. (°C)	Humidity (RH%)	Rain fall (mm)	Temp. max (°C)	Temp. min (°C)
April	4.38	19.5	69.2	2.07	23.6	16.1
May	4.69	22	71.9	0	25.4	19.1
June	4.39	25	74.1	0	28.3	22.3
July	4.14	27.5	76.2	0	31.3	24.7
August	4.33	27.9	73.3	0	31.5	25
September	3.86	27.1	72.5	0	30.2	24.3
October	4.17	24	66.2	7.11	27.3	21.2
November	3.89	18.9	69.8	17.02	22.1	16.1
2012						
April	4.27	19.5	71.2	0	23.2	16.2
May	4.22	23	73.2	0	26.4	19.9
June	4	25.9	75.1	0	29.2	22.8
July	3.86	28.7	75.7	0	32.2	25.6
August	3.75	28.9	73.7	0	32.7	25.7
September	3.94	27	70.3	0	30.2	24.3
October	3.56	25.4	72.3	1.27	28.7	22.6
November	3.75	22	74.8	1.78	25.2	18.9

RESULTS AND DISCUSSIONS

1- Vegetative growth and yield characters:

- Herb fresh and dry weight:

Data at Table (3) show that Baloza location resulted in the highest weight of fresh herb per plant with average (2157.33 & 2155.00 gm/plant) followed decently by Toshka location with average (1684.00 & 1829.50gm/plant) in both seasons. The differences between the two locations were significant in both seasons.

Table (3): Effect of location on herb fresh weight/plant (gm) during two successive seasons (2011 and 2012).

	First season		Second season	
	Toshka	Baloza	Toshka	Baloza
	1684.00	2157.33	1829.50	2155.00
L.S.D. at 0.05	237.47		187.44	

As for the yield of fresh herb per feddan, Baloza location resulted in the highest values with average (40.27 & 40.23 ton/feddan) where Toshka location resulted in the least values with average (31.44& 34.15 ton/feddan) in both seasons. The differences between the two locations were significant in both seasons (Table 4).

Table (4): Effect of location on herb fresh weight/feddan (ton) during two successive seasons (2011 and 2012).

	First season		Second season	
	Toshka	Baloza	Toshka	Baloza
	31.44	40.27	34.15	40.23
L.S.D. at 0.05	4.43		3.51	

The yield of dry herb per plant or per feddan followed the same trend of fresh weight (Tables, 5 and 6).

Table (5): Effect of location on herb dry weight/plant (gm) during two successive seasons (2011 and 2012).

	First season		Second season	
	Toshka	Baloza	Toshka	Baloza
	302.00	436.00	312.50	438.00
L.S.D. at 0.05	107.06		52.05	

Table (6): Effect of location on herb dry weight/feddan (ton) during two successive seasons (2011 and 2012).

	First season		Second season	
	Toshka	Baloza	Toshka	Baloza
	5.64	8.14	5.83	8.18
L.S.D. at 0.05	2.00		0.98	

Generally, it was noticed from the aforementioned results that Toshka location recorded the lowest vegetative growth and yield parameters

of basil plants which may be attributed to the effect of heat stress on plants due to increased high air temperatures along the cultivation season at Toshka location, which led to a negative effect on plant growth and yield (Wahid et al., 2007).

Similar results were obtained by Abd El- Wahab (2009) who determined the productivity of *Mentha spicata* of fresh and dry herb in four locations in Upper Egypt (Beni-Suef, Sohag, Qena and Aswan Governorates). The productivity of fresh and dry herb at Beni-Suef (north of Upper Egypt) was the highest followed by Sohag, Qena and Aswan. Abd El-Wahab and Mehasen (2009) investigated the cultivation of Indian fennel in four governorates of Upper Egypt, i.e. El-Minia, Assuit, Sohag and Qena. El-Minia Governorate location resulted in the highest fruits yield followed by Assuit location, where as Sohag and Qena resulted in less fruits yield being the least at Qena.

2- Chemical analyses:

Essential oil percentage and oil yield:

Data at Table (7) show that the highest oil percentage in the dry herb was obtained from Baloza location with average (0.36 & 0.35%) with mean value of 0.355%, while the least essential oil percentage was from Toshka location with average (0.23 & 0.22 %) for the two seasons with mean value of 0.225%.

Table (7): Effect of location on essential oil percentage during two successive seasons (2011 and 2012).

	First season		Second season		Mean value	
	Toshka	Baloza	Toshka	Baloza	Toshka	Baloza
	0.23	0.36	0.22	0.35	0.225	0.355
L.S.D. at 0.05	0.09		0.03			

The differences between the two locations were significant in both seasons. Oil yield/plant at Table (8) show that, Baloza location produced the highest oil yield/plant with average (1.55 & 1.48 ml./plant) with mean value of 1.515 while the least oil yield/plant was from Toshka location (1.31 & 1.37 ml./plant for the two seasons with mean value of 1.33. The oil yield/feddan followed the same trend of oil yield/plant (Table 9). The differences between the two locations were significant in both seasons.

Table (8): Effect of location on essential oil yield/plant (ml.) during two successive seasons (2011 and 2012).

	First season		Second season		Mean value	
	Toshka	Baloza	Toshka	Baloza	Toshka	Baloza
	1.31	1.55	1.37	1.48	1.33	1.515
L.S.D. at 0.05	0.63		0.19			

Table (9): Effect of location on essential oil yield/feddan (L.) during two successive seasons (2011 and 2012).

	First season		Second season		Mean value	
	Toshka	Baloza	Toshka	Baloza	Toshka	Baloza
		24.45	28.93	25.57	27.63	25.01
L.S.D. at 0.05	11.51		7.77			

Similar results were recorded by Ibrahim *et al.*, (2013) on two varieties of basil plants (Egyptian and French varieties) which were cultivated in two locations in Egypt (El-Jammal village, Tokh, Kalubia Governorate and at El-Hammam city, Marsa Matrouh Governorate). They found that oil percent was the most affected character by all the interaction sources of variation; therefore, it is the best trait can measure the environmental variation on the basil plant. Al Ahl *et al.*, (2014) on coriander plant studied the effect of location (Nile Valley and Delta, Giza governorate) and (Sinai Peninsula, North Sinai governorate) on coriander oil content. Generally found that the cultivation of coriander in Giza gave the best results than the cultivation in the North Sinai.

Essential oil fractionation : The essential oil for basil samples grown at both locations was analyzed by GC/MS and shown in table (10). Sixteen components were detected for the basil essential oil . Estragol was the major compound in the oil of Toshka location (54%).

Table (10): GC/MS analysis of essential oil composition for basil at two locations.

Essential oil %			Essential oil %		
	Toshka	Baloza		Toshka	Baloza
Lemonene	2.22	1.26	α - terpeniol	0.80	0.90
Eucalpytol	0.08	0.12	Citronellol	0.65	0.78
1.8 Cineole	1.08	0.98	Graniol	0.03	0.05
Linalool	11.00	45.62	Methyl cinnamate	3.02	2.22
Anethol	3.02	2.20	α - cardinal	2.00	0.00
Estragol	54.00	37.22	α - pinene	0.12	0.40
Eugenol	2.80	1.82	β - pinene	0.07	0.08
Methyl chavicol	7.28	4.64	Myrcene	0.12	0.28

Meanwhile, linalool and estragol (45.62 % and 37.22 % respectively). were the main components for Baloza location. GC/MS analysis of essential oil composition differed between varieties and between locations confirming the results of Grayer *et al.* 1996 and Marotti *et al.* 1996 who reported that basil herb shows significant differences in essential oil content and composition, which depends on genotype, developmental stage or environmental conditions. However, Kruger *et al.* 2002, Trevisan *et al.* 2006 and Gautam *et al.* 2010 found that linalool was the major compound in the basil essential oil. Sakaria , *et al.* , 2007 found that linalool (54%) and methyl chavicol (23%) were the major constituents of basil oil .

Pharmacopeial constants:

Data presented in Table (11) indicated that, the moisture and the crude fiber content reached their maximum value of 50.76 and 13.50% respectively at Baloza location, while the total ash content reached its maximum value of 15.42% at Toshka location. The decrease of moisture content in Toshka may be due to increasing in temperature and wind velocity.

Table (11): Pharmacopeial constants and chemical constituents and vitamin C of *Ocimum basilicum* at two locations and two seasons .

Item %	First season		Second season		Mean value	
	Toshka	Baloza	Toshka	Baloza	Toshka	Baloza
Moisture content	42.10	50.62	43.26	50.90	42.68	50.76
Crude fiber	10.26	13.20	9.98	13.80	10.12	13.50
Total ash	16.02	12.40	14.82	12.00	15.42	12.20
Total carbohydrates	6.03	9.07	6.07	8.99	6.05	9.03
Total lipids	1.87	1.52	1.88	1.65	1.875	1.585
Total nitrogen	0.16	0.18	0.15	0.16	0.155	0.17
Total protein	1.00	1.12	0.93	1.00	0.965	1.06
Total flavonoids	0.33	0.71	0.32	0.75	0.325	0.73
Total alkaloids	0.17	0.26	0.17	0.27	0.17	0.265
Total phenolics	0.67	1.79	0.62	1.83	0.645	1.81
Total terpenoids	2.16	3.50	2.08	3.62	2.12	3.56
Vitamin C content	2.050	2.120	1.900	2.010	1.975	2.065

The ash content, which is an indication of the mineral in the leaves, was higher than that of *O. gratissimum* leaves (6.88%) as reported by Edeoga et al. (2006).

This was an indication of the richness of *O. basilicum* in minerals of nutritional importance. The crude fiber in the diet has been reported to lower the blood cholesterol and sugar levels, reduce the risk of hypertension and other cardiovascular related diseases. The high crude fiber content implies the samples of *O. basilicum* can be a good dietary supplement especially for the hypertensive and obese patients.

Quantitative determination of the chemical constituents:

It is clear from Table (11) that the percentages of total carbohydrates, nitrogen, protein, flavonoids, alkaloids, phenolics, terpenoids and vitamin C contents reached their maximum values of 9.03, 0.17, 1.06, 0.73, 0.265, 1.81, 3.56 and 2.065% respectively in the plant samples collected from Baloza location, while the percentages of total ash and total lipids reached their mean maximum values of 15.42 and 1.875% respectively in the plant samples from Toshka location . The results obtained in Table (11) were confirmed with the findings of Abokassem et al. (2002) which reported that the high salt concentration can result in osmotic adjustment by regulating the accumulation of solutes especially carbohydrates and proteins.

Carbohydrate is a pivotal nutrient that is required in adequate amounts in the diet to produce energy for the smooth functioning of the body. Its content in *O. basilicum* as observed in this study makes the plant a rich

source of vegetable carbohydrate. Dietary fats are flavoring agents that are used to increase the palatability of food (Antia et al., 2006).

However, *O. basilicum* may not be a good source of protein because the level (1.12 ± 0.93 g/kg) observed in this study is very low when compared with other leafy vegetables like *O. gratissimum* leaves (1.21%) and Cabbage (1.40%). This observation is in agreement with the findings of Tull (1996) who reported that leafy vegetables are usually poor sources of proteins.

Flavonoids are polyphenolic compounds that are biologically active against liver toxins, microorganisms, inflammation, tumor and free radicals (Okwu, 2004). They have also been reported to inhibit the growth of cataracts in diabetic patients (Okwu and Omodamiro, 2005). Alkaloids are chemicals which help plants to repel some predators.

Phenolic compounds are potent antioxidants and free radical scavengers with inhibitory activities against some pathogenic microorganisms (Khoobchandani et al., 2010). The concentration of phenolics in *O. basilicum* as observed in this study was higher than that of *Ocimum gratissimum* (0.049%) and *Hyptis suaveolens* (0.05%) as reported by Edeoga et al. (2006).

Moreover, vitamin C is a water soluble antioxidant whose high dietary intake is associated with reduced risk of colon cancer and inflammatory diseases (Pattison et al., 2004). The sufficient amount of vitamin C in *Ocimum basilicum* is an indication of the ability of the leaves to prevent the formation of carcinogens and scavenge free radicals which are formed during metabolic processes in humans.

mineral analysis : Table (12) showed that, Calcium is the most abundant macro element in the plant samples . It is a vital element in building and maintaining strong bones and teeth. It is essential for blood clotting, maintenance of blood pressure and as a cofactor in enzymatic processes (Akubugwo et al., 2007). The macro elements (potassium and sodium) are required for the normal functioning of the nervous system and play a vital role in regulating blood pressure . These elements are essential components of immune system and are vital for the synthesis of hemoglobin (Akubugwo et al., 2007). Iron is involved in oxygen binding to hemoglobin thereby playing a vital role in blood formation . the results of this study showed that all the elements were higher in plant samples collected from Toshka than the plant samples collected from Baloza location .

Table (12): Mineral analysis contents of *Ocimum basilicum* at two seasons of the two locations :

Element g/kg	First season		Second season		Mean seasons value	
	Toshka	Baloza	Toshka	Baloza	Toshka	Baloza
Calcium	5.320	4.770	5.400	5.610	5.360	5.190
Phosphorus	0.710	0.640	0.700	0.680	0.705	0.660
Potassium	3.040	2.700	3.040	2.740	3.040	2.720
Sodium	2.980	2.080	2.900	2.080	2.940	2.080
Iron	0.080	0.080	0.070	0.050	0.075	0.065

CONCLUSIONS

The amount of oils, flavonoids, alkaloids, phenolics, , terpenoids and vitamin C were found to be higher in the plants collected from Baloza when compared to the plants collected from Toshka location and hence can be utilized to meet various pharmaceutical and industrial purposes.

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تأثير الموقع والظروف البيئية على النمو والانتاجية والمكونات الكيميائية لنبات الريحان

عاطف السيد أبو زيد ، رمضان محيي الدين و ياسر عادل حنفي
قسم النباتات الطبية والعطرية

نبات الريحان نبات طبي هام متعدد الإستخدامات الطبية. تم زراعة النبات في منطقتين مختلفتين اولاهما وهي منطقة توشكى (جنوب مصر) والثانية وهي منطقة بالوظة (شمال مصر) خلال موسمين متتاليين (٢٠١١-٢٠١٢)، حيث تم تقدير انتاجية النبات الطازج والجاف كما تم تقدير المكونات الكيميائية للنبات. واتضح من الدراسة ان منطقة بالوظة اعطت اعلي انتاجية للنبات الطازج والجاف وكذلك اعلي انتاجية من الزيت . وقد تم فصل ستة عشر مركبا من الزيت الطيار لنبات الريحان في كلا المنطقتين باستخدام جهاز التحليل الكروماتوجرافي المزود بمطياف الكتلة وأوضحت النتائج ان مركب الاستراجول هو الاعلى نسبة في منطقة توشكى (٥٤%) بينما كان مركبي اللينالول (٤٥,٦٢%) والاستراجول (٣٧,٢٢%) هما المكونان الرئيسيان للزيت في النبات في منطقة بالوظة . ودلت النتائج ايضا علي ان النسبة المئوية لكل من الكربوهيدرات- النيتروجين - البروتين - الفلافونيدات - القلويدات - الفينولات- التربينات وكذلك فيتامين سي كانت اعلى في منطقة بالوظة (٩,٠٣، ٠,١٧، ١,٠٦، ٠,٧٣، ٠,٢٦٥، ٠,٨١، ٣,٥٦، ٢,٠٦٥ على التوالي) بينما اعطت منطقة توشكى اعلي قيم بالنسبة للرماد والدهون وكذلك نسبة الاملاح الموجودة بالنبات . وقد اثبتت هذه الدراسة ان نبات الريحان النامي في كلا المنطقتين يعتبر مصدر جيد للمواد الفعالة والعناصر لذا يمكن استخدامه في العديد من الاغراض الطبية والصناعية . ولكن الظروف البيئية في منطقة بالوظة كانت أكثر ملائمة لنمو وانتاجية والمكونات الكيميائية لنبات الريحان عنها في منطقة توشكى .