ISSN: 2521-3903

Enhancement of growth and increased productivity of fresh herb and aromatic oil in basil plant by foliar application with stigmasterol

Ahmed A. El-Tantawy¹, Samah N. Azoz^{2*}

¹Ornamental Horticulture Department, Faculty of Agriculture, Cairo University, Giza, Egypt, ²Department of Agricultural Botany, Faculty of Agriculture, Cairo University, Giza, Egypt.

ABSTRACT

The present study was conducted through the two growing seasons of 2017 and 2018 to disclose the impact of foliar application with different concentrations of stigmasterol (0, 25, 50, 75 and 100 ppm) on vegetative growth characters, yield of fresh herb/plant, anatomical structures of vegetative organs (main stem and leaves) and percentage and constituents of aromatic oil of basil plant. The obtained results indicated that stigmasterol application had an enhancing effect on growth and productivity as well as on the percentage and composition of volatile oil of basil plant and the maximum promotion was detected at 100 ppm stigmasterol. Such treatment induced favorable changes in the anatomical structures of vegetative organs.

Keywords: *Ocimum basilicum* L., Growth, Anatomy, Volatile oil, Stigmasterol.

Received: January 14, 2019

Accepted: March 01, 2019

Published: March 05, 2019

*Corresponding Author:

Samah N. Azoz

Email: samah.ali@agr.cu.edu.eg
samahnageeb@gmail.com

INTRODUCTION

Ocimum basilicum L., called sweet basil or basil in English and rehan in Arabic, is one of the most important aromatic and medicinal plant species in the genus *Ocimum* of the family Lamiaceae [16, 21, 26]. It is chosen to be the subject of our present investigation because of its economic importance as an ornamental, spice, culinary and medicinal herb. Basil grows well in Egypt, the cultivated area is about 1091 feddans (one feddan = 4200 m²) produced about 1951 tons fresh herb per year and yielded about 29.363 tons essential oil [6]. Basil herb contains 0.5-1.5% essential oil of varying composition, out of which linalool as the main component [20]. The essential oil, obtained by steam distillation from the fresh herb, is used in perfumery, in making incense and in the food industry [9]. The market for basil oil is dominated by European and Egyptian production [22].

Therefore, increasing productivity of basil plant from fresh herb and essential oil per unit area is highly recommended to meet the demand of human needs and exportation. In this respect, the use of natural and safety growth promoting substances for enhancing plant growth and increasing productivity of aromatic plants from fresh herb and volatile oil is highly

recommended. Stigmasterol is considered as one of the mostly free or conjugated sterols that play essential functions in plant growth and metabolism. [18]. It promoted assimilation of C¹⁴ in leaves and successive translocation of the assimilate into rice panicles [17].

Brassinolides are found in plants with lot of roles in plant growth and development [11]. It has been found that Brassinosteroid enhanced the development of cell walls and thereby speed up the growth cycle [10,25]. In this concern, [1,2,3] reported that sitosterol had a stimulatory effect on growth, yield, photosynthetic pigments and some chemical composition of wheat and maize plants. Also, [5] stated that stigmasterol had a promotive effect on growth, yield and structure of rice plants. Likewise, [23] found that stigmasterol induced promotive effect on vegetative growth, anatomical structure and yield components as well as seed quality of soybean plant. In this connection, [13] stated that increasing stigmasterol concentration up to 200 ppm significantly increased sesame growth, seed yield and number of capsules/plant as well as 1000 seed weight and seed oil percentage. Similar results were also reported by [19] on Egyptian lupine plant. They stated that stigmasterol at concentration of 40 or 80 ppm induced significant promotive effects on lupine plant height and most

Copyright: © The authors. This article is open access and licensed under the terms of the Creative Commons Attribution License (<http://creativecommons.org/licenses/by/4.0/>) which permits unrestricted, use, distribution and reproduction in any medium, or format for any purpose, even commercially provided the work is properly cited. Attribution — You must give appropriate credit, provide a link to the license, and indicate if changes were made.

of yield components. Stigmasterol at 80 ppm had a promotive effect on anatomical structure of stem and leaf of Egyptian lupine.

In this respect, few reports studied the effect of stigmasterol on growth of medicinal and aromatic plants. It has been indicated that stigmasterol enhanced oil concentration and growth of chamomile plants [4]. Also, [8] investigated the physiological effects of stigmasterol at concentrations of 50 and 100 ppm on growth, flowering, oil yield and some chemical composition of *Tagetes erecta* L. The maximum mean values of growth parameters and flower aspects were detected at 100 ppm stigmasterol. Stigmasterol plays an important role in flower differentiation and development. In this concern, [15] studied the physiological response of *Moringa oleifera* to stigmasterol at concentrations of 50, 100 and 150 ppm. They found that the most effective concentration was that of 150 ppm stigmasterol which recorded the highest growth parameters. Foliar application with 100 or 150 ppm stigmasterol resulted in increasing minerals total sugars and protein contents in leaves of treated plants. In this respect, [14] studied the effect of stigmasterol in form of brassinolide at concentrations of 25 and 50 ppm on vegetative growth, oil yield and oil constituents of marjoram. They observed that morphological parameters were increased significantly due to application of stigmasterol as compared to control. Studying of the effect of stigmasterol on growth of basil plants has not been documented. Thus, the current search was carried out to investigate the effect of foliar spray with stigmasterol on growth, anatomy and productivity from fresh herb and essential oil of basil plant.

MATERIALS AND METHODS

The current research was carried out at the Agricultural Experiments and Researches Station, Faculty of Agriculture, Cairo University, Giza, Egypt during the two growing seasons of 2017 and 2018 in order to investigate the effect of foliar spray with different concentrations (25, 50, 75 and 100ppm) of stigmasterol on growth characters, yield of fresh herb/plant, anatomical structures of vegetative organs and on percentage and composition of essential oil of basil plant (*Ocimum basilicum* L.).

Basil seeds were procured from the Experimental Station of Medicinal Plants, Faculty of Pharmacy, Cairo University, Giza, Egypt. The growth promoter stigmasterol (Stigmasta-5,22-Diene – 3B-OL; (24 S) 24 Ethylcholesta-5, 22 – Diene - 3B - OL.) was obtained from Merck-Co., Germany. It is a powder contain 99.9% active ingredient.

The Procedure of the Experiment

Seeds of basil were sown on 14th March, 2016 in the first season and replicated on 16th March, 2017 in the second one to provide the experimental plant materials. The experiment was made in a randomized complete block design with three replicates. The four levels of stigmasterol beside the control

required that the experimental land of each replicate be divided into five plots, each contain one treatment. The plot was six ridges, four meters long, 60 cm apart. Seeds were sown in hills, spaced 20cm. The plants were thinned to two plants per hill. All field practices were carried out as recommended for basil crop in the vicinity.

The tested concentrations of stigmasterol were applied twice. The first application at seven weeks from sowing date and the second application was done after three weeks from the first application. The control plants were sprayed with tap water, Tween-20 was added as a spreading agent for tested treatments. The volume of spraying solution per plot was almost 1.25 and 2.0 liters in the first and second application; respectively.

Recording of Data

Growth characters and yield of fresh herb per plant

15 plants for each treatment (5 plants from each replicate) were assigned for investigation. Vegetative characters were recorded after 14 weeks from sowing date; i.e.; four weeks after second application of stigmasterol. This age represents full blooming stage. The following characters were studied in both growing seasons.

- Plant height (cm), measured from the cotyledonary node up to the upper most point of the plant.
- Number of primary branches developed per plant.
- Number of leaves per plant.
- Fresh weight of shoot (g) per plant, represents yield of fresh herb in grams per plant.

Anatomical studies

It was intended to carry out a comparative microscopical examination on plant material which showed the most positive response of plant growth to tested treatments with control. Tested materials included the main stem at its median portion and lamina of the corresponding leaf. Specimens were taken throughout the second growing season of 2018 at the age of 12 weeks from sowing date; i.e. two weeks from the second application of stigmasterol. The standard procedure [24] was followed as explained previously [28].

Determination of Percentage and Constituents of Volatile Oil

A chemical analysis was carried out to gain information about the effect of foliar spray with stigmasterol on the percentage and composition of volatile oil of basil herb at full blooming stage of the second growing season 2018 (the age of 14 weeks from sowing date). Hydrodistillation of the volatile oil was conducted using the technique described by [12]. For each studied treatment, plant material was placed in a 2-liter round bottomed flask with distilled deionized water (400 ml for 200g fresh herb) and the volatile oil was extracted by water

distillation using a modified Clevenger trap [7]. For smaller fresh plant sample, the distillation period was one hour and the volatile oil content was determined on an oil volume to tissue weight.

GC-MS technique was used to separate and detect the volatile oil constituents. Analysis was performed at Research Park, Faculty of Agriculture, Cairo University, Giza, Egypt. GC-MS analysis was carried out as explained previously [29].

Qualitative identification of the different constituents was performed by comparison of their relative retention times and mass spectra with those of authentic reference compounds. Also probability merge search software and the NIST MS spectra search program were used.

Statistical Analysis

Data on morphological characters and yield of fresh herb per plant were subjected to conventional methods of analysis of variance according to [27]. The data were statistically analyzed for each season and the homogeneity of experimental error, in both seasons, was tested. Then the combined analysis of the two seasons was done. The least significant difference (L.S.D.) at 0.05 level of probability was calculated for each determined character under different assigned treatments.

Figure 1: Habit of mature plants, at the end of full blooming stage, of Basil as affected by foliar application with stigmasterol. (a) Control plant, (b) Plant treated with 75 ppm stigmasterol, (c) Plant treated with 100 ppm stigmasterol

RESULTS AND DISCUSSION

Growth Parameters

Plant height

Data on plant height of basil plant, at full blooming stage, as affected by foliar application with different concentrations of stigmasterol are presented in Table [1]. These Results reveal that spraying basil plants with the relatively low used concentration of 25 ppm stigmasterol had no statistical effect on plant height. By contrast, treatment with any of the other three assigned concentrations of stigmasterol (50, 75 or 100 ppm) induced significant promotion effect on height of basil plant and the differences among the three used concentrations proved insignificant (Table 1 and Figure 1). The maximum height was observed at 100 ppm stigmasterol being 19.5% more than height of untreated plant.

Number of primary branches/plant

Results regarding the effect of foliar application with different concentrations of stigmasterol on number of primary branches developed per basil plant are given in Table [1]. It is clear from Table (1) that all stigmasterol treatments increased number of primary branches developed per basil plant and the significant increase was started at 50 ppm stigmasterol. Worthy to note that the difference between the two median concentrations of 50 and 75 ppm stigmasterol proved insignificant whereas, the difference between the relatively high used concentration of 100 ppm stigmasterol and any of the two assigned median concentrations of 50 and 75 ppm stigmasterol was significant; consequently the maximum significant increase in number of primary branches/basil plant was detected at 100 ppm stigmasterol, being 45.9% more than number of primary branches developed per untreated plant.

Number of leaves/plant

Results concerning number of leaves developed per basil plant at full blooming stage as influenced by foliar application with various concentrations of stigmasterol are presented in Table [1]. It is realized that the impact of stigmasterol treatments on number of leaves per plant was identical to that previously mentioned about the effect of stigmasterol on number of primary branches developed per basil plant.

Table 1: Average values of the two seasons 2017 and 2018 combined of some growth parameters and yield of fresh herb per basil plant, at full blooming stage, as affected by foliar application with stigmasterol

Treatments	Concentrations (ppm)	Growth characters			Yield of fresh herb (g)/plant
		Height of plant (cm)	No. of primary branches/plant	No. of leaves/plant	
Control	0	69.4 ^b	10.81 ^c	242.9 ^c	418.36 ^c
Stigmasterol	25	71.8 ^b	11.27 ^c	266.3 ^c	434.14 ^c
	50	79.2 ^a	13.14 ^b	309.7 ^b	481.59 ^b
	75	81.5 ^a	13.92 ^b	331.5 ^b	507.72 ^b
	100	82.9 ^a	15.77 ^a	380.6 ^a	556.18 ^a
L.S.D. (0.05)		8.42	1.36	39.3	44.92

Means having the same letter are not statistically different at 0.05 level.

It is noted that all assigned concentrations of stigmasterol increased number of leaves per plant and the significant increase was detected at the relatively low median concentration of 50 ppm stigmasterol. The maximum significant increase in number of leaves per plant was observed when basil plants were treated with the relatively high used concentration of 100 ppm stigmasterol. Such treatment induced 60.8% increment in number of leaves over the untreated plant.

Stigmasterol induced promotion effect on vegetative growth of basil plant. Stigmasterol promoted plant height and increasing significantly number of branches and leaves developed per basil plant. Claims to the promotive effect of stigmasterol on vegetative growth of other different plant species was reported by [5] on soybean, [13] on sesame as well as [19] on Egyptian lupine. The enhancement effect of stigmasterol on vegetative growth was also recorded on other medicinal and aromatic plant species; for instance, [8] on *Tagetes*, [14] on majoram and [15] on moringa. All, being in agreement with the present findings.

Fresh Herb Yield/Plant

Data on yield of fresh herb per basil plant at flowering stage as affected by different levels from stigmasterol are given in Table (1). It is noted that all assigned concentrations of stigmasterol increased yield of fresh herb per basil plant and the significant increase was observed at concentration of 50 ppm stigmasterol. It is clear that the difference between the two used median concentrations of 50 and 75 ppm stigmasterol proved insignificant while, the difference between the relatively high used concentration of 100 ppm stigmasterol and any of the two sprayed median concentrations (50 and 75 ppm stigmasterol) proved significant. The maximum significant increase in yield of fresh herb per plant was found at treatment of 100 ppm stigmasterol, being 32.94% increment over the control.

Previous reports about the effect of foliar application with stigmasterol on yield of fresh herb per basil plant are not available. However, [8] treated *Tagetes* plant with stigmasterol at concentrations of 50 and 100 ppm stating that such treatments increased fresh weight of herb/plant, being in harmony with the present findings. In this respect, [14] studied the effect of stigmasterol at concentrations of 25 and 50 ppm on marjoram plant and they found that such treatments increased significantly fresh weight of herb per plant, being in agreement with the present report.

Anatomical Investigations

Main stem anatomy

Mean values of some histological features in cross sections through the middle part of the main stem of basil plant treated with 100 ppm stigmasterol and those of untreated one are presented in Table (2). Also, microphotographs represented this result are given in Figure (2) which the treatment of 100 ppm stigmasterol resulted in a prominent increase in diameter of

Table 2: Mean values of microscopical measurements (μm) of certain histological aspects in cross sections through the median portion of the main stem of basil plant sprayed with 100 ppm stigmasterol and those of control plant at the age of 12 weeks (Means of three sections from three different specimens)

Histological measurements in micro-meters (μm)	Treatments		
	Control	Stigmasterol (100ppm)	$\pm\%$ to control
Diameter of main stem	3472.30	4522.20	+30.20
Thickness of cortex	171.50	228.60	+33.30
Thickness of fiber strands	68.10	85.70	+25.80
Thickness of phloem tissue	94.70	114.30	+20.70
Thickness of xylem tissue	683.20	1071.80	+56.90
Diameter of vessel	46.90	59.30	+26.44
Diameter of pith	1437.20	1521.40	+5.90

Figure 2: Transverse sections through median portion of the main stem of Basil plant, aged 12 weeks, as affected by foliar spray with stigmasterol (a) From untreated plant (control), (b) From plant treated with 100 ppm stigmasterol. Details: Co, cortex; ep, epidermis; first, fiber strands; ph phloem; pi pith; vs vessel and xy, xylem

the main stem by 30.20% more than that in the control. It is observed that the increment in stem diameter of basil plant by foliar application with 100 ppm stigmasterol was attributed to the prominent increase in thickness of all included tissues including pith diameter, the thickness of cortex, fiber strands, phloem tissue and xylem tissue as well as diameter of the pith were increased by 33.3, 25.8, 20.7, 56.9 and 5.9 % more than those in the stem of untreated plant; respectively. Moreover, vessel diameter was increased by 26.4% more than that of the control.

No report studied the impact of foliar application with stigmasterol on stem anatomy of basil plant. Although, some investigators conformed our present findings using stigmasterol on other different plant species. In this respect, [23] using 100 ppm stigmasterol on soybean plant as well as [19] using 80 ppm stigmasterol on Egyptian lupine plant recorded favorable changes in anatomical structure of the main stem of investigated species due to increase in

Table 3: Mean values of counts and measurements in micro-meters (μm) of certain histological features in transverse sections through the blade of the foliage leaf developed at the median portion of the main stem of basil plants, aged 12 weeks as affected by foliar spray with stigmasterol (Means of three sections from three specimens)

Histological measurements in micro-meters (μm)	Treatments		
	Control	Stigmasterol (100ppm)	$\pm\%$ to control
Midvein thickness	836.20	998.60	+19.4
Lamina thickness	437.90	532.70	+21.7
Palisade tissue thickness	148.60	182.90	+23.1
Spongy tissue thickness	231.20	264.20	+14.3
Dimensions of midvein bundle			
Length	262.4	298.50	+13.8
Width	521.90	701.20	+34.4
No. of xylem rows/midvein bundle	21.80	25.60	+17.4
Vessel diameter	25.20	28.70	+13.9

Figure 3: Transverse sections through lamina of the leaf developed at the median portion of the main stem of Basil plant, aged 12 weeks, as affected by foliar spray with stigmasterol (a) From untreated plant (control). (b) From plant treated with 100 ppm stigmasterol. Details: lep, lower epidermis; mid b, midvein bundle; mid r midvein region; spo, spongy tissue; pal palisade tissue and uep upper epidermis

thickness of most included tissues as a result of treatment with stigmasterol, being in accordance with the present investigation.

Leaf Blade Anatomy

Results concerning the effect of foliar application with 100 ppm stigmasterol on anatomical structure of leaf blade in basil plant and those in control are shown in Table [3] and figure [3]. These findings show that the treatment of basil plants with 100 ppm stigmasterol resulted in development of thicker leaves compared to leaves of the untreated plants. The increase in leaf thickness was 19.40 % in midvein and 21.70% in lamina more than those of the control. It is noted that the thicker lamina which was resulted from spraying stigmasterol could be attributed mainly to the increase in thickness of both palisade and spongy tissues by 23.10 and 14.30 % more than those of the control; respectively.

Results also indicated that length and width of the main vascular bundle of the midvein was increased in the treated plants by 13.80% and by 34.4% over the control, respectively. Moreover, number of xylem rows/midvein bundle and mean diameter of vessel were increased in treated plants by 17.40 and 13.90 % over the control; respectively. Information about the effect of stigmasterol on the anatomical structure of basil leaves are not available. However, some investigators conformed our present findings using stigmasterol on other different plant species [24] on soybean and [19] on Egyptian lupine.

Essential Oil

The effect of the treatment with 100 ppm stigmasterol on percentage, yield and composition of aromatic oil of basil herb at flowering stage, age of 15 weeks, were evaluated by analyzing the volatile oil components using GC/MS (Table 4; and Figures 4; and 5). It is realized that untreated basil herb recorded 0.59% volatile oil whereas 0.74% volatile oil were observed in plants treated with 100ppm stigmasterol (Table 4). Such percentages yielded 2.46 ml volatile oil/plant in control plants while 100 ppm treated plants yielded 4.116 ml volatile oil/plant. These results proved that stigmasterol had a promotive effect on yield of volatile oil per basil plant. Such treatment increased yield of volatile oil by 66.8% over the control.

Using GC/MS technique in analyzing volatile oil proved the presence of 29 compound in untreated plants against 33 compounds in plants treated with 100ppm stigmasterol. The main constituents are linalool, geranial and neral. The first main component is linalool which comprised 38.46% of the volatile oil of control plants against 49.54% of the volatile oil of plants sprayed with 100ppm stigmasterol. The second main component is geranial which comprised 15.56% of the volatile oil in the control plants versus 6.72% of the volatile oil in treated plants. The third main component is neral which comprised 14.95% of the volatile oil in control plants and 6.27% of the volatile oil in plants treated with 100ppm stigmasterol. It is obvious that such three main components comprised 69.90 and 62.53% of the volatile oil in control and treated plants; respectively. The results of treatment with 100ppm stigmasterol showed extreme effects on the major components of volatile oil in basil herb. Also, such treatment affected on other constituents where 10 different minor components are present in the volatile

Table 4: Volatile oil of basil herb at flowering stage as affected by foliar application with 100 ppm stigmasterol, retention time, components, percentages and yield/plant

No of peaks	Retention time (min)	Components (%)	Treatments	
			Control	Stigmasterol (100 ppm)
1	8.42	Alpha-Pinene	0.73	0.56
2	9.79	Sabinene	-	0.63
3	10.43	Beta- Myrcene	0.78	0.62
4	10.94	Alpha-Terpinen	0.66	0.32
5	11.12	Limonene	0.64	0.51
6	11.50	Eucalyptol (1,8-Cineol)	0.99	3.95
7	11.66	Beta- Ocimene	-	0.30
8	11.86	Gamma-Terpinene	1.18	0.70
9	12.54	Alpha-Terpinolene	0.23	0.16
10	12.76	Cis-Sabinene Hydrate	0.82	0.39
11	13.17	Linalool	38.46	49.54
12	14.89	Trans-Chrysanthemal	1.40	0.65
13	15.04	2-(2-Methylenecyclohexyl) ethanal	0.59	-
14	15.27	4-Terpineol	7.05	3.10
15	15.99	Methyl chavicol	0.64	0.15
16	16.06	Alpha-Terpineol	0.21	-
17	16.11	Allylmethrone	-	0.56
18	16.26	Acetic Acid	0.99	0.34
19	16.64	Nerol	2.01	0.53
20	16.81	Z-Citral (Neral)	14.95	6.27
21	17.41	Geraniol	-	0.65
22	17.55	E-Citral (geranial)	16.56	6.72
23	20.05	Phenol (Iso-eugenol)	1.06	6.86
24	20.41	Neryl Alcohol	0.58	-
25	20.42	Neryl Acetate	0.59	0.29
26	20.65	Alpha-Copaene (Alpha-cubebene)	-	0.43
27	21.04	Beta-Elementene	0.32	1.30
28	21.59	Trans-Caryophyllene	1.65	1.52
29	21.83	Bicyclo[3.1.1]Heptene	1.52	1.69
30	22.60	Alpha- Humulene	0.68	0.95
31	23.02	Germacrene-D	1.92	3.05
32	23.60	Beta-Bisabolene	-	1.12
33	23.68	Naphthalene	0.39	1.13
34	24.12	Cis-Alpha-Bisabolene	1.81	1.76
35	26.01	Alpha-copaene	-	0.39
36	26.67	Alpha-Amorphene (Delta Cadinen)	0.51	2.85
% of volatile oil in Basil herb			0.59	0.74
Yield of volatile oil (ml)/ plant			2.468	4.116

Figure 4: GC/MS of volatile oil of basil herb at full blooming stage of untreated plants (control)

Figure 5: GC/MS of volatile oil of basil herb at full blooming stage of plants treated with 100 ppm stigmasterol

oil in which 3 minor components were recorded in control plants comprising 1.38% of its volatile oil (Table 4 and Figure 4). Also, seven other different minor components in treated plants and comprised 3.43% of its volatile oil (Table 4 and Figure 5).

Therefore, it could be stated that treating basil plants with 100ppm stigmasterol reduced 3 minor components in the constituents of volatile oil which were found in control and induced 7 minor components in the composition of volatile oil which were not found in control. Information about the impact of foliar application with stigmasterol on the percentage and composition of volatile oil of basil plants has not been reported.

According to our results, it can be concluded that treating basil plant with 100 ppm increased the yield of fresh herb and volatile oil improving economically the basil plant production in Egypt.

ACKNOWLEDGMENT

We would like to express our gratitude towards prof. Mohamed Abd EL-Aziz Nassar (Department of Agricultural Botany, Faculty of Agriculture, Cairo University) for his assistance with the technique, methodology and comments that greatly improved the manuscript.

REFERENCES

1. Abd El-Wahed, M.S.A.; Amin, A.A. and Ali, Z.A. Effect of different concentrations of stigmasterol on growth, yield and its components of maize plants. J. Agric. Sci., Mansoura Univ., 2000; 25(1): 201-215.
2. Abd El-Wahed, M.S.A., Sitosterol stimulation of root growth, yield and some biochemical constituents of maize. 17th International conference on plant growth substance, Brno, Czech Republic, July, 2001; 1-6, p.175.
3. Abd El-Wahed, M.S.A.; Ali, Z.A.; Abd EL-Hady, M.S. and Rashad, S.M. Physiological and anatomical changes on wheat cultivars as affected by sitosterol. J. Agric. Sci., Mansoura Univ., 2001; 26(3):4823-4839.
4. Abd El-Wahed, M.S.A. and Gamal El Din, K. M. Stimulation effect of spermidine and stigmasterol on growth, flowering, biochemical constituents and essential oil of chamomile plant (*Chamomilla recutita* L., Rausch). Bulg. J. Plant Physiol, 2004; 30 (3-4): 48 – 60.
5. Ali, Z.A.; Abd El-Wahed, M.S.A. and Amin, A.A. 2002. Effect of stigmasterol on growth, productivity and anatomical structure of rice plants. Egypt. J. Appl. Sci., 2002;17(12): 508-530.
6. Arafa, G.K. Optimum drying conditions for thin-layer drying of sweet Basil. Misr J. Ag. Eng., 2007;24(3):540-556.
7. ASTA., Official Analytical Method of American Spice Trade Association ASTA, Englewood cliffs, 1968; N.J.pp:8-11.
8. Balbaa, L. K.; Abd El Aziz, N. G. and Youssef, A.A. Physiological Effects of Stigmasterol and Nicotinamide on Growth, Flowering, Oil Yield and Some Chemical Compositions of *Tagetes erecta* L. Plant. Journal of Applied Sciences Research, 2008;3(12): 1936-1942.
9. Bunney, S. The illustrated encyclopedia of herbs (their medicinal and culinary uses). Chancellor Press, London., 1992; p:200.
10. Clouse, S.D. and Sasse, J.M. Brassinosteroids: Essential Regulator of plant growth and Development. Annu.Rev. plant Physiol.plant. Mol. Biol., 1998; 49:427.
11. Culter, H.G.; Yokota, T. and Adam, G. Brassinosteroids: Chemistry, Bioactivity and Applications. American Chemical Society, Washington, D.C.1991.
12. Denys, J.C. and Simon, J.E. Comparison of extraction method for the rapid determination essential oil content and composition of Basil. Soc Hort,Sci., 1990; 115(3):458-462.
13. El-Greedly, Nadia H.M. and Mekki, B.B. Growth, yield and endogenous hormones of two sesame (*sesamum indicum* L.) cultivars as influenced by stigmasterol. Journal of Applied Science Research, 2005;1(1): 63-66.
14. El-Khateeb, M. A.; El-Attar, A.B. and Nour, R. M. Application of Plant Biostimulants to Improve the Biological Responses and essential oil production of marjoram (*Majorana hortensis*, Moench) plants. Middle East Journal of Agriculture Research. 2017; 6(4): 928-941.
15. El-Moursi, A.; Talaat I. M.; Bekheta, M.; Abdel- Ghany and Gamal EL-Din, K. Physiological response of *Moringa oleifera* to stigmasterol and chelated zinc. Nusantara Bioscience, 2012; 4(3):118-123.
16. Evans, W.C. Trease and Evans Pharmacognosy. 4th. Edit. WB Saunders Company LTD., London, 2001; p: 48.
17. Fujii, S., Hirai, K. and Saka, H., Growth regulating action of brassinolide in rice plants. Acs. Symposium-series. American Chemical Society, 1991;474: 306-311.
18. Gens, J.M.C., Steroids Hormones and growth and development. Phytochem., 1978; 17:1-44.
19. Helal, S.H.M. and Gomaa, E. F. Response of Egyptian lupine plant to treatment with stigmasterol. J. Agric. Sci., Mansoura Univ., 2007; 32(10): 8315-8331.
20. Hiltunen, R. Chemical composition of *Ocimum* species. H orwood Academic Publishers, 1999; pp: 67-75.
21. Hugh, T.W Herbs and spices of Thailand. National Library Board., Singapore., 2005; p:79.
22. Nahak, G.; Mishra, R. C. and Sahu, R. k. Taxonomic distribution, medicinal properties and drug development potentiality of *Ocimum* (Tulsi)., Drug Invention Today, 2011; 3(6):95-113.
23. Nassar, Dalia, M.A. Effect of stigmasterol on morphological, anatomical and yield characters of soybean plant (*Glycine max* (L.) Merrill). J. Agric. Sci., Mansoura Univ., 2004; 29(5): 2459-2474.
24. Nassar, M.A. and El-Sahhar K.F. Botanical preparations and Microscopy (Microtechnique). Academic bookshop, Dokki, Giza, Egypt. 1998; 219 pp. (In Arabic).
25. Sasse, J.M., "Brassinosteroids-are they endogenous plant hormones?". PGRSA Quarterly, 1991;19:1-18.
26. Singh, M.P. and Panda, H. Medicinal herbs with formulations. Daya Publishing House. Delhi., 2005; pp:607-610.
27. Snedecor, G. W. and Cochran, W. G. Statistical Methods (7th Edit., 2nd printing). The Iowa State University Press, Ames, Iowa, U.S.A., 1982; pp:507.
28. Salama AM, Yousef RS. Response of basil plant (*Ocimum sanctum* L.) to foliar spray with amino acids or seaweed extract. Journal of Horticultural Science and Ornamental Plants. 2015;7(3):94-106.
29. Hajhashemi V, Sajjadi SE, Heshmati M. Anti-inflammatory and analgesic properties of *Heracleum persicum* essential oil and hydroalcoholic extract in animal models. Journal of Ethnopharmacology. 2009;124(3):475-80.