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Response of Two Species of Black Cumin to Foliar Spray Treatments

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Abstract: Field experiment was carried out to investigate the effect of fertilizer foliar spray treatments on the growth, fixed oil and fatty acids contents of *Nigella sativa* and *Nigella damascena* plants. Foliar spray, black cumin species and their interactions had a significant effects on the growth characters, fixed oil and fatty acids contents of both Nigella *sativa and Nigella damascena.*

Key words: Foliar spray, *Nigella sativa, Nigella damascena*, growth, yield, fixed oil, Fatty acids, Fertilizer.

INTRODUCTION

Black cumin (*Nigella sativa*) belongs to family *Ranunculaceae* and is one of most important medicinal plants. The seeds oil or powder have anti-inflammatory effect (Bhatnagar,1996), reducing effect on blood levels of both glucose and cholesterol (Bamosa *et al,*1997), antibacterial activity (Hussain and Tabji, 1997; Tesaki *et al,*1998), antifertility, galactagogue, anticancer and cardiovascular activities (Siddiqui and Sharma, 1996). El-Komey (1996) reported that black cumin has mammogenic activity and growth promoting ability partly during pregnancy, but mainly during lactation. Both *N. sativa* and *N. damascena* seeds contain a variable amount of oil with linolenic generally recognised as the more abundant fatty acid; relevant amounts of saturated acids such as palmitic, myristic and stearic were found in some cases, as well as the presence of unusual unsaturated c20 acids (Babayan *et al*, 1978).

Recently, it is known that most cultivated areas in Egypt appeared widespread symptoms of Mn, Zn and Fe deficiency (Amberger, 1982). Iron (Fe) is a cofactor for approximately 140 enzymes that catalyze unique biochemical reactions (Brittenham, 1994). Hence, iron fills many essential roles in plant growth and development, including chlorophyll synthesis, thylakoid synthesis and chloroplast development (Miller *et al*, 1995). Iron is required at several steps in the biosynthetic pathways. Zinc (Zn) is an essential element for plant that act as a metal component of various enzymes or as a functional structural or regulatory cofactor and for protein synthesis, photosynthesis, the synthesis of auxin, cell division, the maintance of membrane structure and function, and sexual fertilization (Marschner, 1995). Foliar nutrition is widely used to correct a specific nutrition deficiency or to prove nutrients and it is preferable especially in newly reclaimed soil where this soil is usually poor in their nutrients content. However, the response of plant to foliar micronutrient fertilizer varies according to different factors, such as soil characters, plant species, and environmental condition. Several medicinal and aromatic plants appeared great influence of growth and yield due to application suitable foliar fertilizer rates, e.g. on Majoram (Hafez, 1990), *Hibiscus sabdariffa* (El- Sherbeny and Hussein, 1990) and *Ocimum basilicum.* (El-Kholy *et. Al,* 1994). However, *Nigella sativa* appeared a pronounced growth with foliar application of some macro or micronutrients. Leithy (1998) reported that foliar spray with Fe, Zn and Mn at rate of 25 and 50 ppm stimulated response to growth characterizes and chemical constituents, with different rates according to plant growth stage and doses used. Moreover, Youssef (1998) found that foliar application with 100 ppm Zn to *Nigella sativa* plants resulted in a pronounced growth while increasing the doses to 200 ppm resulted in reverse effect. Khalil *et. Al* (2001) studied the effect of three foliar nutrients where the results showed that the response on *Nigella sativa* to various foliar applications on growth and yield components was very clear.

The regulation of plant growth and biosynthesis of important economic chemical constituents could be achieved through the use of different growth regulating substances. There has been a recent trend to use naturally-occurring compounds (including amino acids) to achieve such regulation. Davies (1982) reported that amino acids as organic nitrogenous compounds are the building blocks in the synthesis of proteins, which are formed by a process in which ribosomes catalyze the polymerization of amino acids. Several hypotheses have been proposed to explain the role of amino acids in plant growth. Available evidence suggests several alternative routes of IAA synthesis in plants, all starting from amino acids (Hashimoto, *et al*, 1994).Amino acids are fundamental ingredients in the process of protein synthesis. Glycine and Glutamic acids play an important role in formation of vegetative tissue and chlorophyll. They also have a chelating effect on micronutrients through making their absorption and transportation easier for the plant. They are precursors or activators of

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phytohormones and growth substances. L. Methionine is a precursor of ethylene and growth factors such as espermine and espermidine (Singh, 1999). Gamal El-Din *et al* (1997) found that foliar application of ornithine and phenylalanine 50, 100 mgL$^{-1.}$ On *Cymbopgon citrates* led to significantly increase in vegetative growth, number of leaves and tillers as well as fresh and dry weight of herb. Refaat and Naguib (1998) found that spraying peppermint plant with alpha-alanine at 25 and 50 ppm increased fresh and dry weight of the plant and essential oil content. Harridy (1986) mentioned that the higher alkaloid percentage and yield of *Catharanthus roseus* resulted from foliar application of some amino acids. Similar trend was observed by Awad (1986), studying methionen on *Hyoscyamus muticus.* Talaat and Youssef (2002) found a pronounced increase in vegetative growth of basil plant as a result of lysine and ornithine treatments. However, S-adenosyl – methionine play a role via the methyl group as a donor to produce estragole and t-anethole in cell-free extracts of the bitter fennel plant (Gross *et al,* 2002). Later, Maxwell and Kieber (2004) indicated the link of methionine to the biosynthesis of growth regulating substances, e.g cytokinins, auxins and brassinosteroids in plants. Whereas the link of tryptophan to the biosynthesis of auxins, the phytoalexin camalexin, phenylpropanoids and other related natural products in plants was recently reported (Tao *et al,* 2008). The aim of this study is investigate the effect of foliar spray treatments on the growth fixed oil and fatty acids contents of *Nigella sativa and Nigella damascena* plants.

MATERIALS AND METHODS

Field experiment was carried out at Hakestip Farm belongs to Sekem Co. Seeds of *Nigella sativa* and *Nigella damascena* (obtained from Sekem Co.) were sown on 15th and 18th November -2010 and 2011, respectively , in plots of 2X2 m arranged in complete randomized block design with three replicates for each treatments. Each plot contains 3 rows 60 cm apart , and the distance between plants was (35 cm). Some physical and chemical properties of the soil are shown in Table (1). Complete nutrient fertilizer of commercial named Amino AT manufactured by Misr El Dawlya for Agricultural & Industrial Development Co. The composition of this fertilizer was total Organic Acids + Amino Acids (> 20 %), Iron (2%), Zinc (1.5%), Manganese (0.7%) Magnesium (500 ppm).The plants of both Nigella *sativa* and *Nigella damascena* were sprayed with three doses of aqueous solution of tested nutrient compound (1m, 2ml and 3ml / L.) with water as control at age 30 and 45 days from sowing.

At fruiting stage (during April for both seasons) the growth parameter such as plant height, branches number, capsules number/ plant as well as seed weight (g/plant) were measured.

Table 1: Main Characteristics of soil.

Mechanical Analysis	
Characters	Value
Sand %	80.7
Silt %	6.8
Clay %	12.5
Texture	Sandy Clay Loam
Chemical Analysis	
PH 1:2.5ext.	7.48
Electrical Conductivity 1:2.5ext	0.61
Organic Carbon %	0.89
Organic Matter %	1.55
Total Nitrogen %	0.121
Total Phosphorus %	0.0072
Total Potassium %	0.012
CaC03 %	2.23
Soluble cations meq/L	
Na^+	4.25
K^+	0.10
Ca^{++}	1.21
Mg^{++}	0.49
Soluble anions meq/ L	
$C0^-3$	0.00
HCO^-3	2.68
cl^-	1.64
So^-4	2.55
CECmeq/100g	13.26

Fixed oil Extraction:

The methods described by A.O.A.C. (1965) were used for fixed oil extraction from the seeds of black cumin L. plants. This method involved the extraction of known weights of dried samples of mature seeds repeated three times with n. hexane. The combined extracts were dried over anhydrous sodium sulphate to get rid of water, and then filtrated. The solvent extraction was distilled under reduced pressure. The crude lipids (fixed oil) were weighed and their percentages were finally calculated at the dry weight basis.

Gas-Liquid Chromatographic Analysis of Fatty Acids:

Preparation of Fatty Acids Methyl Ester:

The lipids of ten selected samples were saponified with potassium hydroxide solution (20 % w/v). The mixture was heated on a boiling water-bath for 30 minutes and kept at room temperature over night. The unsaponifiable matter was extracted three times with diethyl ether. The potassium salt of fatty acids was acidified with HCl (6 N) and the liberated fatty acids were extracted three times with HCl diethyl ether. The combined ether extract was washed several times with distilled water, then dried over anhydrous sodium sulphate and filtered. The fatty acids, obtained were converted to methyl esters using diazomethane ethereal solution according to Vogel (1975).

Source of standard fatty acids. A set of standard fatty acids consisting of $C_{6:0}$, $C_{7:0}$, $C_{8:0}$, $C_{9:0}$, $C_{10:0}$, $C_{11:0}$, $C_{12:0}$, $C_{13:0}$, $C_{14:0}$, $C_{15:0}$, $C_{16:0}$, $C_{17:0}$, $C_{18:0}$, $C_{18:1}$,$C_{18:2}$, $C_{18:3}$ and $C_{20:0}$, with a stated purity of 99% by Gas-Liquid Chromatography (G.L.C.) was purchased from Nu-Chek-Prep. The purity of each standard compound was checked by G.L.C. and gave one peak.

RESULTS AND DISCUSSION

1-Growth and Some Yield Components:

1-A- Effect of two Species:

Data tabulated in Table (2) show that significant differences were detected between the two species of *Nigella*. Growth and some yield components such as plant height (cm), number of branches / plant, number of capsules / plant and weight of seeds (g/plant) for *Nigella sativa* were recorded 44.70 cm, 4.75, 12.43 and 21.04 g / plant against 48.88 cm, 5.60, 19.68 and 28.81 g / plant for *Nigella damascena* , respectively. Thus, the growth and yield parameters of *Nigella damascena* were higher than those obtained for *Nigella sativa*.

1-B- Effect of Fertilizer:

Most of growth and yield characters were affected by foliar application of fertilizer, whereas, significant differences were detected between fertilizer treatments except for number of branches / plant **(Table 2).** The mean values of plant height were 43.40, 45.95, 47.35 and 50.45 cm with doses 0, 1, 2 and 3 ml / L., respectively. So, the maximum mean value of plant height was produced from plants treated with 3ml / followed by 2 ml / L. The results also, showed that, number of branches recorded 4.75, 4.85, 5.35 and 5.75/ plant as a result of foliar application of fertilizer at 0, 1, 2 and 3 ml/L., respectively. Thus, branches number / plant reached to its maximum mean value (5.75) as a result of foliar application at 3 ml / L. followed by 2ml / L. which recorded 5. 35. Moreover, the data tabulated in Table (2) showed that , most of foliar application treatments had a significant effect on number of capsules / plant that , recorded 12.7, 15.3, 16.5 and 18.7 / plant whereas, the mean values of seed weight (g / plant) were 20.80, 22.93, 27.66 and 28.32 g / plant as a result of foliar application at 0, 1, 2 and 3 ml/L., respectively. The maximum mean values of capsules number / plant and seed weight (g / plant) were obtained as a result of foliar application at 3 ml / L.

The role of amino acids in stimulating growth of several plant species was studied by Phillips (1971), who indicated that several alternative routes of IAA synthesis exist in plants, all starting from amino acids. Russell (1982) reported that, the increase in growth as a result of application of amino acids may be due to their conversion into IAA. Regarding the effect of amino acids on plant growth and yield of this study are in agreement with those obtained by Talaat and Youssef (2002) on *Ocimum basilicum* , Talaat (2005) on *Pelargonium graveolens L.*, Abou- Dahab and Abd El-Aziz (2006) on *Philodendron erubscens*,El-Awadi and Esmat (2010) on *Foeniculum vulgare* Mill and Hendawy and Azza (2010) on *Foeniculum vulgare var. azoricum.* Moreover, the positive effects of Fe, Mn, Mg and Zn on plant may be due to their effects as a metal component of some enzymes or regulatory for the others. Moreover, they have essential roles in plant metabolism (Norman and Albert, 1974 and Abd El-, Wahab,2008). Micronutrients, especially Fe and Zn act as metal components of various enzymes and also are associated with saccharide metabolism, photosynthesis, and protein synthesis and Iron has important functions in plant metabolism, such as activating catalase enzymes associated with superoxide dismutase, as well as in photorespiration, the glycolate pathway and chlorophyll content. Zinc is an essential micronutrient for synthesis of auxin, cell division and the maintenance of membrane structure and function. Zinc deficiency reduces plant growth, pollen viability, flowering, number of fruits and seed production (Sharma *et al*., 1990; Marschner, 1995). Therefore, sufficient amount of these nutrients in the plant is necessary for normal growth, in order to obtain satisfactory yield. Although, foliar application of various macro and micro nutrients has been proved beneficial, foliar feeding is a relatively new and controversial technique of feeding plants by applying liquid fertilizer directly to their leaves (Baloch *et al*., 2008; Yassen *et al*., 2010).Abd El- Wahab (2008) reported that micronutrients such as iron, manganese and zinc have important roles in plant growth and yield of aromatic and medicinal plants. The foliar application of mineral nutrients offers a method of supplying nutrients to higher plants that are more efficiently than methods involving root application when soil conditions are not suitable for nutrients availability (Erdal *et al*., 2004).

1- C- Effect of the Interaction:

The combination treatments between species and foliar fertilizer application had a significant effect on number of capsules / plant as well as weight of seeds (g / plant). These interaction treatments had no pronounced effect on plant height and number of branches / plant. It can be noticed that the maximum mean values of plant height, branches number, capsules number and weight of seeds (g/plant)) were 53.2 cm, 6.5/plant, 24.7 / plant and 32.87 g / plant were obtained as a result of the combination between *N. damascena* and foliar fertilizer at 3ml / L. The lowest values of plant height, branches number, capsules number and weight of seeds (g/plant) for the combination treatments between *N. sativa* and foliar fertilizer were 42.2 cm, 4.6 / plant, 10.9 / plant and 18.36 g/ plant were obtained as a result of growing plants of *N. Sativa* without foliar fertilizer. The variation in plant height , branches number, capsules number and weight of seeds (g/plant) between plants produced the maximum and the lowest one for *N. damascena* are reached to 19.28%, 32.65%, 70.34% and 41.50 %, respectively.

Table 2: Growth and Some Yield Components for Two Species of *Nigella* as Affected by Foliar Application of Amino A T. Fertilizer (Mean values of two successive seasons).

Species	Fertilizer	Plant Height (cm)	No. of Branches / Plant	No. Capsules / plant	Weight of Seeds (g/plant)
N. Sativa L.	Control	42.2	4.6	10.9	18.36
	1ml/L	43.6	4.7	12.0	18.77
	2ml/L	45.3	4.7	12.10	23.27
	3ml/L	47.7	5.0	14.7	23.77
Mean Value of *N. Sativa*		44.7	4.75	12.43	21.04
N. Damascena	Control	44.6	4.9	14.5	23.23
	1ml/L	48.3	5.0	18.6	27.08
	2ml/L	49.4	6.0	20.9	32.04
	3ml/L	53.2	6.5	24.7	32.87
Mean Value of *N.damascena*		48.88	5.6	19.68	28.81
Mean values of Fertilizer:	Control	43.40	4.75	12.7	20.80
	1ml/L	45.95	4.85	15.3	22.93
	2ml/L	47.35	5.35	16.5	27.66
	3ml/L	50.45	5.75	18.7	28.32
LSD at 0.05 for :	Species (S)	0.81	0.78	0.81	2.30
	Fertilizer(F)	1.13	N.S.	1.15	1.32
	S X F	N.S.	N.S.	2.01	2.31

2- Fixed oil Content (%) and Yield (g/Plant):

2- A- Effect of two Species:

Data tabulated in Table (3) clear that there are significant differences between *N. sativa* and *N. damascana* for both fixed oil percentage and yield (g / plant). It can be noticed that the maximum mean values of fixed oil percentage (26.58%) and yield (7.76 g / plant) were obtained from *N. damascena* plants.In this respect, under Turkish conditions, Pertrand and Mehmet (2011) found that, total oil content of Nigella sativa seeds was ranged from 30.4 – 36.4 % while, total oil content for N. damascena was found to be low (28.0%).The differences between the values of the seed oil content can be probably due to growing, climatic, and environmental conditions as well as analytical conditions and localities.

2- B- Effect of Foliar Fertilizer:

Foliar fertilizer had a significant effect on fixed oil percentage and yield (g / plant). It is clear that the highest mean values of fixed oil percentage (28.70%) was obtained from plants sprayed with 3 ml / L followed by 2 ml / L which recorded 26.90 % . Moreover, the same trend was observed for fixed oil yield, where foliar fertilizer at 3 ml / L. gave the maximum mean value of fixed oil yield (8.30 g / plant) and followed by 2ml / L., which recorded 7.53 g / plant. The influence of amino acids on fixed oil content may be due to the alternation in levels and / or relevant enzymes. Moreover, micronutrients, especially Fe and Zn, act either as metal components of various enzymes or as functional, structural, or regulatory cofactors. Thus, they are associated with saccharide metabolism, photosynthesis, and protein synthesis and Iron has important functions in plant metabolism, such as activating catalase enzymes associated with superoxide dismutase, as well as in photorespiration, the glycolate pathway and chlorophyll content. Zinc is an essential micronutrient for synthesis of auxin, cell division and the maintenance of membrane structure and function.

Effect of the Interaction:
2- C- Effect of the Combination:

Data illustrated in Table (3) show effect of the interaction treatments on fixed oil percentage and yield. It can be noticed that the interaction between every specie and different levels of foliar fertilizer (1, 2, 3 ml / L.) significantly increased both fixed oil percentage and yield comparing with untreated plants.

The maximum mean value of oil percentage (30.60 %) and oil yield (10.06 g / plant) was observed because of the combination between *Nigella damascena* and foliar fertilizer at 3 ml / L.

Table 3: Fixed Oil Percentage and Yield for Two Species of *Nigella* as Affected by Foliar Application of Amino A T. Fertilizer. (Mean values of two successive seasons).

Species	Fertilizer	Fixed Oil (%)	Fixed Oil (g/plant)
N. Sativa L.	Control	21.04	3.86
	1ml/L	24.82	4.66
	2ml/L	25.00	5.82
	3ml/L	26.80	6.37
Mean Value of *N. Sativa*		24.42	5.18
N. Damascena	Control	21.30	4.95
	1ml/L	25.60	6.80
	2ml/L	28.80	9.23
	3ml/L	30.60	10.06
Mean Value of *N.damascena*		26.58	7.76
Mean values of Fertilizer:	Control	21.17	4.41
	1ml/L	25.21	5.73
	2ml/L	26.90	7.53
	3ml/L	28.70	8.30
LSD at 0.05 for :	Species (S)	0.80	0.63
	Fertilizer(F)	1.13	0.98
	S X F	2.05	1.03

3- Fatty Acids Content:

Fats are classified into saturated and unsaturated fats. Saturated fats tend to increase blood cholesterol levels, while unsaturated ones show the reverse direction; they are mostly from plant sources. The most common saturated fatty acids found in plant lipids that contain 16 or 18 carbon atoms. Low content of saturated fatty acids is desirable for edible uses (Anderson and Beardall, 1999).

Eleven fatty acids [accounting for 97.82 % to 98.96% of total fatty acids (Table 4).] were identified in fixed oil extracted from the seeds produced from the plants treated with foliar application and control treatments for *Nigella sativa and Nigella damascena*. The main fatty acid in both of *Nigella sativa and Nigella damascena* fixed oils were linoleic (60.03 – 60.82%), oleic (19.94 – 20.37%), palmatic (12.24 – 13.48%) and eicosadienoic (3.30 – 4.42%). The highest amount of saturated fatty acids resulted from the treatments of 0 mg L^{-1} (13.83%) and 3 mg L^{-1} (1.59%) for *Nigella sativa and Nigella damascena* fixed oils, respectively. On the other hand the highest amount of unsaturated fatty acids resulted from the treatments of 1 mg L^{-1} which recorded (86.95 and 85.27%) for *Nigella sativa and Nigella damascena* fixed oils. The obtained fatty acids of fixed oil extracted from *Nigella sativa* and *Nigella damascena* seeds were also found by Pertrand and Mehmet,(2011). In this context, Burbott and Loomis (1969) reported that variations in fatty acids of *Nigella sativa and Nigella damascena* could be due to its effect of our treatments on enzymes activity and metabolism improvements.

Table 4: Fatty acid Two Species of *Nigella* as Affected by Foliar Application of Amino A T. Fertilizer.

Fatty acids (%)	Treatments							
	Nigella sativa				*Nigella damascena*			
	control	T1	T2	T3	control	T1	T2	T3
Lauric	0.02	0.04	0.05	0.04	0.02	0.05	0.03	0.06
Myristic	0.16	0.36	0.25	0.27	0.28	0.24	0.12	0.27
Palmatic	12.24	13.28	10.54	12.82	12.76	12.66	12.48	12.97
Oleic	20.37	19.98	21.29	20.35	19.94	20.68	20.89	20.32
Linoleic	60.60	60.03	61.18	60.77	60.83	59.99	59.68	59.97
Linolenic	0.46	0.44	0.71	0.43	0.45	0.69	0.49	0.44
Arachidic	0.18	0.19	0.15	0.18	0.17	0.18	0.19	0.20
Eicsenoic	0.36	0.36	0.34	0.39	0.31	0.37	0.38	0.37
Eicosadienoic	3.47	3.38	3.30	3.42	3.30	3.43	3.43	3.71
Behenic	0.10	0.11	0.12	0.15	0.11	0.08	0.20	0.09
Eruic	0.14	0.18	0.13	0.15	0.13	0.11	0.12	0.13
Saturated fatty acids	13.83	10.92	13.45	13.17	13.17	13.21	13.02	13.59
Unsaturated fatty acids	84.37	86.95	85.51	85.51	85.12	85.27	85.04	84.94
Total identified	98.20	97.87	98.96	98.68	98.29	98.48	98.06	98.53

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