Evaluation and Influence Nitrogen on Growth, Yield and Chemical Content of *Nigella sativa* L.

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Abstract

The environmental as well as financial impact of (inorganic) nitrogen fertilizer use deserves increased attention. Field data on changes of soil acidity such as pH and nutrient saturation with the application of Ammonium Sulfate and Urea, two major nitrogen ingredients sources, are scarce for Egyptian soils. *Nigella sativa* L. (belongs to family *Ranunculaceae*) is a widely used medicinal plant throughout the world. All nitrogen sources (Ammonium Sulphate and Urea) and quantity at (0.2, 0.4 and 0.6 g pot $^{-1}$) were positively enhanced as compared to the control and significantly improved plant growth characters i.e., plant height (cm), leaf number (plant^{-1}), branch number (plant^{-1}), capsule number (plant^{-1}), herb dry weight (plant^{-1}), seed yield (plant^{-1}) and chemical composition i.e, Fixed oil, Total Carbohydrates, Soluble Sugars, Protein and Nutrient content (NPK) of *Nigella sativa* L. plants. The highest values of plant growth characters were recorded when plants treated with urea at 0.4 g N pot^{-1}. While chemically it was proved the best with the treatment of urea at 0.6 g N pot^{-1}.

Keywords: *Nigella sativa* L., ammonium sulfate, urea, growth characters, chemical composition

Introduction

Nigella sativa L. (belongs to family *Ranunculaceae*) is a widely used medicinal plant throughout the world. It is very popular in various traditional systems of medicine. Seeds and oil have a long history of folklore usage in various systems of medicines and food. The seeds of *Nigella sativa* L. have been widely used in the treatment of different diseases and ailments. The Islamic literature quoted that, it is one of the greatest forms of healing medicine. It has been recommended for using on regular basis in Prophetic Medicine. It has been widely used as antihypertensive, liver tonics, diuretics, digestive, anti-diarrheal, appetite stimulant, analgesics, anti-bacterial and in skin disorders (Ahmad et al., 2013). Plant nutrition one of the most important factors that increase plant production. Nitrogen (N) is the most recognized in plant for its presence in the structure of the protein molecule. Accordingly, N plays an important role in synthesis of the plant constituents through the action of different enzymes (Jones et al., 1991). Nitrogen fertilizers are mostly applied in bands or broadcast. Urea and ammonium sulfate are the two main sources of inorganic N fertilizer (Fageria et al., 2003). Urea has about 46% of N, and ammonium sulfate, while N content is about 21%. In addition, ammonium sulfate also contains about 24% of sulfur (S). Fertilizers with more N content are preferred over low-N fertilizers because of the lower cost of transport and application (Fageria et al., 2010). Nitrogen content is an important criterion; however, other factors should also be taken into consideration when choosing a fertilizer carrier (Fageria et al., 2010). These factors include the content of nutrients other than the principal one, price of the fertilizer, chemical reactions in the soil, and nutrient availability to plants (Fageria et al., 2010).

At present, the environmental as well as financial impact of N fertilizer use deserves increased attention. Field data on changes of soil acidity indices such as pH, calcium (Ca), and magnesium (Mg) saturation, base saturation, aluminum (Al) saturation, and acidity (H + Al) saturation with the application of ammonium sulfate and urea, two major N sources, are scarce for Egyptian soils. The objective of this study was to evaluate influence of ammonium sulfate and urea on growth, yield and chemical content of *Nigella sativa* L. (Fageria et al., 2010).

Materials and Methods

Experimental Site

The present study was carried out at Experimental Farm, Faculty of Agriculture, Ain Shams University, Shubra El-Kheima, Kalubia, Egypt, during two successive seasons of 2006-2007 and 2007-2008.

Germplasm Source

Nigella sativa L seeds were obtained from the Department of Medicinal and Aromatic Plants, Ministry of Agriculture, Giza, Egypt. In the first week of November during both the seasons seeds were sown in plastic pots (30 cm diameter and 50 cm height), 10 seed pot^{-1}. The viability of seeds was determined in vitro according to Waheed et al. (2012) and it was observed approximately 92%. In the third week of December during both seasons, the pots were transferred to a greenhouse adjusted to natural conditions. Each pot was filled with 10 kg of air-dried clay loam soil. Physical and chemical properties of the soil used in this study were determined according to Jackson (1973) and Cottenie et al. (1982) presented in Table 1. Eight weeks of post sowing the seedlings were thinned to three plants per pot. Pots were divided into 2 main groups. The first group was subjected to different levels of N (0.2, 0.4 and 0.6 g pot $^{-1}$) as ammonium sulphate [$(NH_4)_2SO_4$] (20.5 % N). The second group was subjected to the same treatments of N but urea [$CO (NH_2)_2$] was added. All cultural practices were practiced according to the recommendations of Egyptian Ministry of Agriculture (EMinAgric).

Table 1 Physical and chemical properties of the soil

Property	Value
Clay (%)	24
Silt (%)	9
Sand	67
Texture	Sand
Soluble cations (mg 100 g^{-1} soil)	
Ca	106
Mg	62
Na	41
K	40
Soluble anions (mg 100 g^{-1} soil)	
HCO_3	2
Cl	5
SO_4	106
Organic matter (%)	1.4
Saturation Pecentage	32
$CaCo_3$ (%)	4.8
pH	7.2
Electronic Cnoductivity (dS m^{-1})	1.8
NO_3 (mg kg^{-1})	20.1
P (mg kg^{-1})	`1.5
Sodium Adsoption Ratio	4.5

Harvesting/Picking

At fruiting stage, the plants were harvested/ picked during the end of both seasons. Vegetative growth characters measurements [Plant height (cm), leaf number ($plant^{-1}$), branch number ($plant^{-1}$), capsule number ($plant^{-1}$), herb dry weight ($plant^{-1}$) and seed yield ($plant^{-1}$)] were recorded.

Total Carbohydrates (TC) and Total Soluble Sugars (TSS) Determination

Total carbohydrates TC and total soluble sugars TSS concentrations in seeds (collected at the both seasons end of each treatment) were determined according to Ciha and Brun (1978) with some modifications. Samples of 100 mg were homogenized with 10 mL of extracting solution [glacial acetic acid: methanol (H_2SO_4 1n): water, 1:4:5, v/v/v.] The homogenate was centrifuged for 10 min at 3.000 rpm and the supernatant was decanted. The residue was re-suspended in 10 ml of extracting solution and centrifuged for another 5 min at 3.000 rpm.

The supernatant was decanted, combined with the original extract and made up to 50 mL with water. For measurement of TC and TSS, a phenol-sulfuric acid assay was used (Dubois et al., 1956). A volume of 0.5 mL of 5% (v/v) phenol solution and 2.5 mL of concentrated sulfuric acid were added to 0.5 mL aliquots. Than the mixture was shaken, heated in a boiling water-bath for 20 min and cooled to room temperature. The absorption was then determined by spectrophotometer at 490 nm.

Fixed oil (FO) Determination

FO extraction: 50g of seeds were crushed to coarse powered and extracted with petroleum ether (40-60^{o}C) in a Soxhlet apparatus (AOAC, 1970).

Nutrients and Protein Determination

Nitrogen N, protein, P and K (in leaves) of both seasons of each treatment were determined using the methods described by the AOAC (1970) as follows: The washed and dried materials were grounded to fine powder with mortar and pestle and used for dried ash. For analysis of K the powdered plant material (0.2g) was taken in pre-cleaner and constantly weighed silica crucible and heated in muffle furnace at 400^{o}C till there was no evolution of smoke. The crucible was cooled in desicator at room temperature.

The totally free ash from carbon moistened with Conc. H_2SO_4 and heated on hot plate till fumes of sulphuric acid (H_2SO_4) get evolved the silica crucible with sulphated ash was again heated at 600^{o}C in muffle furnace till weight of sample was constant (3-4 h) one gram sulphated ash were taken in beaker which dissolved in 100 mL 5% conc. Hydrocholroic acid to obtain solution for determination of K through flame photometry, standard solution of each mineral was prepared and calibration curve drawn for K element using flame photometry.

For determination of protein and Nitrogen using Micro Kjeldahl method, 1 g of plant sample taken in a Pyrex digestion tube and 30 mL of conc. Sulphuric acid (H_2SO_4) carefully added, then 10 g potassium sulphate and 14 g copper sulphate, mixture is placed on sand both on a low flame just to boil the solution, it was further heated till the solution becomes colorless and clear, allowed to cool, diluted with distilled water and transferred 800 mL Kjeldahl flask, washing the digestion flask, 3 or 4 pieces of granulated zinc and 100 mL of 40 % caustic soda were added and the flask was connected with the splash heads of the distillation apparatus. Next 25 mL of 0.1 N Sulphuric acids was taken in the receiving flask and distilled; it was tested for completion of reaction. The flask was removed and titrated against 0.1 N caustic soda solution using Methyl Red indicator for determination of nitrogen, which in turn give the protein content.

For determination of phosphorous 2 g sample of plant material taken in 100 mL conical flask two spoons of Darco-G-60 is added followed by 50 mL of 0.5 M $NaHCO_3$ solution, next flask was corked, and allowed for shaking for 30 min. on shaker. the content was filtered and filtrate was collected in flask from which 5 mL filtrate were taken in 25 mL volumetric flask to this 2 drops of 2, 4-paranitrophenol and 5 N H_2SO_4 drop by drop was added with intermittent shaking till yellow color disappear, content was diluted about 20 mL with distilled water and then 4 mL ascorbic acid was added then the mixture was shacked well and the intensity of blue color at 660 nm on colorimeter was measured. The absorbencies were compared and concentrations of phosphorous using standard value were calculated.

Statistical Analysis

For analyses of the experiments, one factor was considered: nitrogen source (Ammonium sulphate and Urea). Each treatment has 4 replicates, comprised 8 pots; in each pot 3 individual plants. The experimental design followed a completely random block design (RCBD). According to Snedecor and Cochran (1990), the averages of data were statistically analyzed using 1-way analysis of variance and the values of least significant difference (LSD) $P<0.05$.

Results

Nitrogen Affected on Different Growth Traits *Nigella sativa* L

Data in Table 2 shows the response of *Nigella sativa* L plants to the different sources of nitrogen. All nitrogen sources applied in different quantities were significantly improved as compared to control and significantly enhanced plant growth characters [plant height (cm), branch number (plant^{-1}), leaf number (plant^{-1}), capsule number (plant^{-1}), dry weight (g plant^{-1}) and seed yield (g plant^{-1})]. The highest values were recorded when plants treated with CO $(NH_2)_2$ at 0.4g N pot^{-1}. The highest values of plant growth characters were 25.1, 35.3; 11.2, 11.2; 47.3, 47.3; 14.1, 11.9; 18.4, 16.3; 6.4 and 7.6 during both seasons respectively. Analyses of variance indicated that the increases in vegetative growth characters were significant for nitrogen sources and levels treatments (Table 2).

Effect of Nitrogen Sources on Fixed Oil

Data presented in Table 3 shows that intensive the different sources of nitrogen produced an increase in the accumulation of fixed oil (%) extracted from *Nigella sativa* L seeds, highest content of fixed oil obtained with the highest treatment of CO $(NH_2)_2$ (0.6 g N pot^{-1}) with a values of 23.1 and 29.2 % during the both seasons respectively. Analyses of variance indicated that the increase in fixed oil (%) was significant for nitrogen sources and levels of treatments (Table 3).

Effect of Nitrogen on Total Carbohydrates and Soluble Sugars

Table 3 shows total content of carbohydrates and soluble sugars in *Nigella sativa* L, increased with an increase in nitrogen quantity applications. The highest contents of total carbohydrates and soluble sugars were recorded when plants treated with CO $(NH_2)_2$ at 0.6 g N pot^{-1} with the values of 32.9 and 24.4% (total carbohydrates); 15.3 and 8.4 % (soluble sugars) during both the (1st and 2nd) seasons respectively. Analyses of variance indicated that the increase in total carbohydrates and soluble sugars was significant for nitrogen sources and levels (Table 3).

Effect of Nitrogen on Total Protein

Protein content was positively affected by soil with an application of nitrogen (Table 3). The treatment of CO $(NH_2)_2$ (0.6 g N pot^{-1}) resulted in the highest protein content in *Nigella sativa* L. (22.0 and 26.8%) during both seasons. Analyses of variance indicated that increase in total protein content was significant for nitrogen quantities applications of different sources.

Effect of Nitrogen Sources on Nutrient Contents

It is evident from the Table 3 that NPK content gradually increased in all treatments as compared to the control. With respect to the effect of the nitrogen application rates of different nutrients, data indicated that applying CO $(NH_2)_2$ (0.5 g N pot^{-1}) brought about the highest values of NPK content in *Nigella sativa* L., plants. The highest values were 3.8, 0.5 and 1.6% during the first and second seasons respectively. Analyses of variance indicated that the increases in NK contents were significant while P content was remained not significant.

Table 2 Influence of nitrogen on plant growth characters.

Nitrogen treatment (g pot $^{-1}$)		Growth characters											
		Plant height (cm)		Branch (---------------------		Leaf No. plant $^{-1}$		Capsule ------------------)		Dry weight (-----------		Seed yield g plant $^{-1}$----------)	
		Seasons											
		1st	2nd	1st	2nd	1st	2nd	1st	2nd	1st	2nd	1st	2nd
Control		15.8	19.1	5.8	4.3	17.8	17.5	2.4	3.1	05.7	6.0	3.3	3.6
$(NH_4)_2SO_4$	0.2	16.6	23.2	5.8	5.6	22.0	25.9	9.9	6.7	12.7	7.3	3.9	4.5
	0.4	18.5	26.8	6.2	6.5	24.7	28.7	10.7	8.0	14.5	10.9	4.3	4.7
	0.6	22.7	28.8	6.8	7.3	28.3	33.7	12.9	10.0	16.6	10.9	4.3	4.7
CO $(NH_2)_2$	0.2	19.6	24.3	7.2	6.2	36.9	36.9	10.0	8.0	14.5	14.9	4.6	5.1
	0.4	25.1	35.1	11.2	11.2	47.3	47.3	14.1	11.9	18.4	16.3	6.4	7.6
	0.6	17.7	20.2	5.9	4.4	28.3	28.3	3.0	4.8	7.8	7.2	3.4	3.7
LSD													
0.05		4.3	4.8	0.8	2.8	1.1	0.8	0.4	3.1	5.7	1.5	1.9	0.4
0.01		5.8	6.4	1.1	3.7	1.4	1.1	0.5	4.2	7.5	1.9	2.5	0.5

Table 3 Influence of nitrogen on chemical constituents

Nitrogen treatment (g pot $^{-1}$)		Chemicals constituents (%)													
		Fixed oil		T. Carbohydrates		S. Sugars		N		Protein		P		K	
		Seasons													
		1st	2nd	1st	2nd	1st	2nd	1st	2nd	1st	2nd	1st	2nd	1st	2nd
Control		17.1	16.9	17.5	15.1	6.7	3.8	2.7	3.5	16.8	21.6	0.1	0.1	0.6	0.8
$(NH_4)_2SO_4$	0.2	17.2	18.3	22.9	18.8	7.4	4.9	2.9	3.6	17.8	21.8	0.2	0.2	0.8	1.1
	0.4	17.3	19.6	25.4	22.7	8.9	5.2	3.0	3.6	18.7	22.5	0.3	0.3	1.2	1.4
	0.6	18.2	23.3	31.3	23.8	12.1	6.7	3.1	3.7	19.3	23.1	0.4	0.4	1.3	1.5
$CO(NH_2)_2$	0.2	18.7	21.2	22.9	16.8	8.7	5.2	2.9	3.5	17.8	21.9	0.3	0.3	1.1	0.9
	0.4	20.1	23.6	24.6	20.8	12.9	6.3	3.4	3.7	21.3	22.8	0.4	0.4	1.3	1.0
	0.6	23.1	29.2	32.9	24.4	15.3	8.4	3.8	3.8	22.0	26.8	0.5	0.5	1.6	1.6
LSD															
0.05		0.7	1.3	3.4	1.5	0.8	0.5	0.4	0.1	1.4	0.8	NS	NS	0.6	0.6
0.01		0.9	1.8	4.6	1.9	1.1	0.7	0.5	0.2	1.8	1.2	NS	NS	0.9	0.8

Discussion

It has been reported earlier that by use of Ammonium Sulfate and Urea, the positive effect of different nitrogen nutrients may be due to decrease in soil pH; (Hetrick and Schwab, 1992). Stumpe and Vlek (1991) revealed that a pH decrease in tropical acid soils (Oxisols, Ultisols, and Alfisols) due to use of three N fertilizers was in the order of Ammonium Sulfate > Urea > Ammonium Nitrate (Fageria, 1989; Bolan and Hedley, 2003).The positive effects of N fertilization quantity may be due to the important physiological role of N on molecule structure as porphyrin. The porphyrin structure is found in such metabolically important compounds as the chlorophyll pigments and the cytochromes, which are essential in photosynthesis and respiration. Coenzymes are essential to the function of many enzymes.

Accordingly, Nitrogen plays an important role in synthesis of the plant constituents through the action of different enzymes activities and protein synthesis (Jones et al*.,* 1991) that reflected in the increase in growth parameters of plants such asanise, coriander and sweet fennel plants. Also, these results are in accordance with those obtained by Khalid (1996; 2001; Khalid and Shedeed 2015) on some *Apiaceae* and *Nigella sativa* L. plants; Ashraf et al., 2006 on cumin; Akbarinia et al., 2007 on coriander*;* Hellal et al., 2011 on dill (*Anethum graveolens* L.), all of whom reported that N fertilizer treatments were superior to the control and significantly improved the vegetative growth characters of family *Apiaceae.*

The results of fixed oil were similar to those of Khalid (1996) on some *Apiacea* plants; Khalid (2001) on *Nigella sativa* L.; Ashraf et al. (2006) on *Nigella sativa* L.; and Akbarinia et al. (2007) on coriander (*Coriandrum sativum* L.); Mohammed et al., 2016 on sweet basil. The increase in total carbohydrate and soluble sugars was significant. These results may be due to the increase in chlorophyll content, and consequently, photosynthesis efficiency, induced by N. So it showed that total carbohydrates and soluble sugars contents increased with of application of N (Jones et al*.,* 1991).

The results of protein content may be due to the influence of N on the ribosome structure and the biosynthesis of some hormones (gibberellines, auxins, cytokinins) involved in protein synthesis (Jones et al*.,* 1991; El-Wahab and Mohamed, 2007).The increase in essential minerals according to the N treatments may be due to increase in dry matter of plant materials (Khalid, 2012; El-Wahab and Mohamed, 2007).

Conclusions

It may be concluded that all nitrogen nutrients (Ammonium Sulphate and Urea) and their quantity were positively higher as compared to the control and significantly enhanced plant growth characters and chemical composition of *Nigella sativa* L. plants. Significant plant growth characters were recorded when plants treated with urea at 0.4g N pot^{-1}. While chemically it proved the best with urea at 0.5 g N pot^{-1} treatment. However some deep study will also be required to investigate comprehensively in other climatic zones to compare the results achieved so far.

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