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Research Article

Effect of Foliar Nutrition on *Nigella sativa* L. Plants Cultivated under Ammonium Sulfate Treatments

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Abstract

The effects of Ammonium Sulfate (AS), Foliar Nutrition (FN) and their interaction on the growth and chemical constituents of *Nigella sativa* L. (*N. sativa*) were investigated. The highest plant growth characters [Plant Height (PH) (cm), Leaf Number (LN) (plant^{-1}), Branch Number (BN) (plant^{-1}), Capsule Number (CN) (plant^{-1}), Herb Dry Weight (HDW) (plant^{-1}) and Seed Yield (SY) (g plant^{-1})] were obtained in the 3 g pot^{-1} of AS+FN treatment with values of 27.7, 30; 46.9, 38.7; 9.3, 8.9; 13.2, 11.3; 45.8, 43.6; 4.6, 5.1 during the first and second seasons, respectively. The highest accumulations of Fixed Oil (FO), Total Carbohydrates (TC), Soluble Sugars (SS), Crude Protein (CP), Nitrogen (N), Phosphorous (P) and potassium (K) contents were recorded at the highest AS level (3 g pot^{-1})+FN with the values of 27.7 and 27.5%; 36.3 and 27.3%; 16.3 and 8.7%; 27.8 and 25.1%; 2.7, 3.5%; 0.1%; 0.6, 0.8% during the first and second seasons, respectively.

Key words: Ammonium Sulfate (AS), Foliar Nutrition (FN), *Nigella sativa* L. (*N. sativa*), growth and chemical constituents

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Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Mineral nutrients applied basally or through foliar application enhance the plant productivity and could be used as tool to ameliorate the quality of herbal medicines. Ammonium Sulfate (AS) is the main source of inorganic nitrogen (N) fertilizer (Fageria, 2003). It has about 21% of N and also contains about 24% sulfur (S). The N is the single most important limiting nutrient for crop growth and hence ample N availability acts as a key contributing factor towards wholesome improvement in agriculture production (Singh, 2009). It is considered to be of prime importance as it plays several important roles in metabolic and regulatory processes in plants. The AS treatments increased herb and essential oil yields and changed the level of secondary metabolites in dragonhead plants. Highest oil yields were obtained with AS at 200 kg N ha⁻¹ (Aziz *et al.*, 2010). The most effective dose of AS was 200 kg ha⁻¹, resulting in a positive increase in vegetative growth characters and content of essential oil, fixed oil, total carbohydrates, soluble sugars, protein and nutrients (NPK) of anise, coriander and sweet fennel plants (Khalid, 2013).

The soils of Egypt are generally sandy with low Cation Exchange Capacity (CEC) values. This means that the soil does not have the ability to hold on to many of the nutrients allowing them to be easily leached out of the rooting zone during irrigation. Foliar feeding is a technique of feeding plants by applying liquid fertilizer directly to their leaves (George, 2003). Foliar Nutrition (FN) is an effective method for correcting soil deficiencies and overcoming the soils inability to transfer nutrients to the plant under moisture conditions. On the other hand soil application of nutrient on calcareous soils is less efficient as these nutrients remain inaccessible to plant roots due to the higher soil pH (Rashid and Ryan, 2004; Ali *et al.*, 2008). However, an alternative approach under such circumstances is FN of nutrients (Rab and Haq, 2012) primarily for two reasons. First, it eliminates the effects of soil pH on the availability of these nutrients (Ali, 2012). Second, it is more effective and less costly (Ali *et al.*, 2007). Kandeel (1991) reported that using FN at 2000 mg L⁻¹ had a significant effect on plant height, fresh weight, dry weight, fruit yield and essential oil content of parsley (*Petroselinum crispum* Mill). The FN had a significant effect on anise, coriander and sweet fennel plants which positively affect on growth and chemical constituents (essential oils, fixed oil, total carbohydrates, soluble sugars, protein and nutrient content) of these three plants grow under arid regions in Egypt (Khalid, 2012).

Black seed *N. sativa* plant belongs to the family Ranunculaceae and is most extensively investigated for

therapeutic purposes (Aggarwal *et al.*, 2008). Pharmacological studies on *N. sativa* found analgesic, bronchodilator, hypolipidemic, anti-tumor, diuretic, immunopotentiator, hypotensive, calcium antagonist, antidiabetic, histamine release inhibitor, antioxidant, hepatoprotective, anthelmintic, antifungal, antibacterial, anticancer and anti-inflammatory activities (Al-Logmani and Zari, 2011).

In this study, the possible effects of AS, FN and their interaction on the growth and chemical constituents of *N. sativa*, an important medicinal plant were investigated.

MATERIALS AND METHODS

Experiments were carried out in a greenhouse at the National Research Centre (NRC), Cairo, Egypt, during, 2013/2014 and 2014/2015 *Nigella sativa* L. seeds were obtained from the Ministry of Agriculture, Egypt. Uniform seeds were sown into plastic pots (30 cm diameter and 50 cm height) during the first week of November 2013 and 2014 the pots were transferred to a greenhouse adjusted to 24-18°C, 90-60% R.H., day-night and light intensity ~3700 µmol m⁻² sec⁻¹. Each pot was filled with 10 kg of air-dried soil. Physical and chemical properties of the soil used in this study were determined according to Jackson (1973) and Cottenie *et al.* (1982) and are presented in Table 1. Three weeks after sowing, the seedlings were thinned to three plants per pot. After 45 days from sowing date plants were divided into two main groups. The first group was subjected to different levels of AS (0, 1, 2 and 3 g pot⁻¹) (21% N). The second group was subjected to the same levels of AS but FN was added (1 g L⁻¹). Foliar nutrition was solution commercially known as agronal, which consists of the following minerals: N (120 mg L⁻¹), P₂O₅ (40 mg L⁻¹), K₂O (40 mg L⁻¹), Mg (2 mg L⁻¹), S (2 mg L⁻¹), Fe (1200 mg L⁻¹), Zn (1200 mg L⁻¹), Mn (1000 mg L⁻¹), Cu (500 mg L⁻¹), Ni (1 mg L⁻¹) and Co (1 mg L⁻¹).

Harvesting: At the end of fruiting stage (215 day from sowing), the plants were harvested. Vegetative growth characters measurements [Plant Height (PH) (cm), Leaf Number (LN) (plant⁻¹), Branch Number (BN) (plant⁻¹), Capsule Number (CN) (plant⁻¹), Herb Dry Weight (HDW) (plant⁻¹) and Seed Yield (SY) (g plant⁻¹)] were recorded.

Table 1: Physical and chemical properties of soil

Items	Value	Items	Value (meq L ⁻¹)	Items	Value (meq L ⁻¹)
Sand	77	Ca	5.1	Cl	17.6
Silt	13	Mg	7.8	SO ₄	2.1
Clay	7.7	Na	11.9	Fe	8.8
Gravel	2.3	K	0.9	Cu	1.2
pH	8.9	CO ₃	2.1	Zn	0.7
EC (dS m ⁻¹)	25	HCO ₃	1.8	Mn	1.8

Fixed Oil (FO) isolation: For the FO extraction, 50 g of seeds were crushed to coarse powered and extracted with petroleum ether (40-60°C) in a Soxhlet apparatus (AOAC., 1970).

Total Carbohydrates (TC) and Soluble Sugars (SS) determinations: The TC and SS contents were determined from plant material (young leaves) collected from each treatment. The method of DuBois *et al.* (1956) was used.

Nutrients (NPK) and Crude Protein (CP) determination: The N, CP, P and K (in the leaves) of both seasons of each treatment were determined using the methods described by AOAC (1970).

Statistical analysis: In this experiment, two factors were considered: AS (0, 1, 2 and 3 g pot⁻¹) and FN (1 g L⁻¹). For each treatment there were 4 replicates, each of which had 8 pots; in each pot 3 individual plants were planted. The experimental design followed a Complete Random Block Design (CRBD). According to Snedecor and Cochran (1990). The averages of data from each season were statistically analyzed using 2-way analysis of variance (ANOVA-2). Significant values determined according to p-values (p<0.05; significant, p<0.01; moderate significant and p<0.001; highly significant). The applications of that technique were according to the STAT-ITCF program (Foucart, 1982).

RESULTS

Effect of AS, FN and their interaction on growth characters:

Plant growth characters such as PH, LN, BN, CN, HDW and SY of *N. sativa* were affected by changes in AS, FN and their interaction treatments (Table 2). Thus the various growth characters in general increased under the various AS levels+FN compared AS treatments. The highest plant growth characters were obtained in the 3 g pot⁻¹ of AS+FN treatment with values of 27.7, 30; 46.9, 38.7; 9.3, 8.9; 13.2, 11.3; 45.8, 43.6; 4.6, 5.1 cm during the first and second seasons, respectively. The lowest plant growth characters were obtained in the control treatment with the values of 15.8, 19.1 cm; 17.8, 25.9 plant⁻¹; 5.8, 4.3 plant⁻¹; 2.4, 3.1 plant⁻¹; 22, 22.1 g plant⁻¹; 3.3, 3.6 g plant⁻¹ during the first and second seasons, respectively. The increases in plant height and leaf number were highly significant for AS, FN and their interaction. The increases in branch number were highly significant for AS and FN while it were significant for the interaction between AS and FN. The increases in capsule number were highly significant for AS and FN (during the first season) but insignificant for FN (during the second season) and the interaction between AS and FN. The increases in herb dry weight were highly significant for AS and FN but insignificant for the interaction between AS and FN. The increases in seed yield were highly significant for AS but insignificant for FN and the interaction between AS and FN.

Table 2: Effect of AS, FN and their interaction on growth characters

		Growth characters											
		Plant height (cm) (Plant ⁻¹)		Leaf number (Plant ⁻¹)		Branch number (Plant ⁻¹)		Capsule number (Plant ⁻¹)		Herb dry weight (g Plant ⁻¹)		Seed yield (g Plant ⁻¹)	
Treatments		1st	2nd	1st	2nd	1st	2nd	1st	2nd	1st	2nd	1st	2nd
FN (1 g L ⁻¹) AS (g Pot ⁻¹)		(Season)											
Without FN	0.0	15.8	19.1	17.8	25.9	5.8	4.3	2.4	3.1	22.0	22.1	3.3	3.6
	1.0	16.6	23.2	22.0	28.7	5.9	5.4	9.9	6.7	38.7	33.5	4.0	4.5
	2.0	18.5	26.8	24.7	33.7	6.2	6.5	10.7	8.0	41.5	38.0	4.3	4.7
	3.0	22.7	28.8	28.3	25.9	6.8	7.1	12.9	10.0	44.1	39.1	4.4	4.8
Overall without FN		18.4	24.5	23.2	28.6	6.2	5.9	9.0	7.0	36.6	33.2	4.0	4.4
With FN	0.0	19.4	25.3	24.9	24.4	6.5	7.2	3.6	6.2	25.3	27.3	3.5	3.9
	1.0	22.1	26.9	25.2	32.1	6.8	7.6	11.6	7.9	41.0	38.5	4.1	4.7
	2.0	26.2	27.8	30.2	33.9	7.3	8.2	11.8	8.9	44.8	41.1	4.4	4.9
	3.0	27.7	30.0	46.9	38.7	9.3	8.9	13.2	11.3	45.8	43.6	4.6	5.1
Overall with FN		23.9	27.5	31.8	32.3	7.5	8.0	10.1	9.1	39.2	37.6	4.2	4.7
Overall AS	0.0	17.6	22.2	21.4	25.2	6.2	5.8	3.0	4.7	23.7	24.7	3.4	3.8
	1.0	19.4	25.1	23.6	30.4	6.4	6.5	10.8	7.3	39.9	36.0	4.1	4.6
	2.0	22.4	27.3	27.5	33.8	6.8	7.4	11.3	8.5	43.2	39.6	4.4	4.8
	3.0	25.2	29.4	37.6	32.3	8.1	8.0	13.1	11.6	45.0	41.4	4.5	5.5
F-ratio													
AS		220.9***	67.1***	358.4***	168.0***	17.5***	41.9***	269.0***	11.0***	680.5***	538.0***	5.9***	127***
FN		584.3***	64.9***	513.0***	163.6***	40.5***	198.5***	15.0***	1.3	47.9***	190.9***	0.5	2.8
ASXFN		14.3***	11.1***	81.5***	120.1***	4.0*	3.7*	1.1	2.1	1.3	2.2	0.0	0.0

*p<0.05 according to F-values of the 2-way analysis of variance (ANOVA-2), ***p<0.001 according to F-values of the 2-way analysis of variance (ANOVA-2)

Table 3: Effect of AS, FN and their interaction on biochemical constituents

		Biochemical constituents (%)													
Treatments		Fixed oil		T. carbohydrates		Soluble sugars		Crude protein		N		P		K	
		1st	2nd	1st	2nd	1st	2nd	1st	2nd	1st	2nd	1st	2nd	1st	2nd
FN (1 g L ⁻¹)	AS (g Pot ⁻¹)	(Season)													
Without FN	0.0	17.1	17.0	17.5	15.1	6.7	3.9	16.9	21.6	2.7	3.5	0.1	0.1	0.6	0.8
	1.0	17.2	18.3	22.9	18.8	7.4	9.9	17.8	21.9	2.9	3.5	0.2	0.2	0.8	1.1
	2.0	17.3	19.6	25.4	22.7	9.0	5.3	18.8	22.5	3.0	3.6	0.2	0.2	1.2	1.4
	3.0	18.2	23.3	31.3	23.8	12.1	6.7	19.4	23.1	3.1	3.7	0.3	0.3	1.3	1.5
Overall without FN		17.5	20.0	24.3	20.1	8.8	4.5	18.2	22.3	3.0	3.6	0.2	0.2	0.2	1.2
With FN	0.0	18.7	18.1	20.4	17.6	8.1	5.2	20.0	23.8	3.2	3.8	0.2	0.2	0.8	1.3
	1.0	18.9	20.6	25.0	21.8	9.4	5.6	19.7	23.9	3.3	3.8	0.3	0.3	1.0	1.5
	2.0	19.0	24.1	29.2	26.7	13.6	6.9	21.4	24.3	3.4	3.8	0.3	0.3	1.5	1.7
	3.0	27.7	27.5	36.3	27.3	16.3	8.7	22.8	25.1	3.7	3.9	0.4	0.4	1.7	2.2
Overall with FN		21.1	22.6	27.7	23.4	11.9	6.6	21.0	24.3	3.4	3.8	0.3	0.3	0.3	1.7
Overall AS	0.0	17.9	17.6	19.0	16.4	7.4	4.6	18.5	22.7	3.0	3.7	0.2	0.2	0.7	1.1
	1.0	18.1	19.5	24.0	20.3	8.4	7.8	18.8	22.9	3.1	3.7	0.3	0.3	0.9	1.3
	2.0	18.2	21.9	27.3	24.7	11.3	6.1	20.1	23.4	3.2	3.7	0.3	0.3	1.4	1.6
	3.0	23.0	25.4	33.8	25.6	14.2	7.7	21.1	24.1	3.4	3.8	0.4	0.4	1.5	1.9
F-ratio															
AS		48.7***	108.5***	332.7***	259.1***	192.8***	89.3***	16.0***	6.2***	2.7*	0.2	0.2	0.2	12.5***	6.6***
FN		105.7***	87.7***	102.0***	150.9***	193.8***	1.7	80.0***	64.4***	16.9***	2.9	0.2	0.2	6.7***	12.7***
AS×FN		30.8***	6.3***	3.3*	1.5	14.7***	81.3***	1.1	0.1	0.2	0.0	0.3	0.3	0.2	0.4

*p<0.05 according to F-values of the 2-way analysis of variance (ANOVA-2), ***p<0.001 according to F-values of the 2-way analysis of variance (ANOVA-2)

Effect of AS, FN and their interaction FO: The FO content increased at all various AS levels+FN compared AS treatments (Table 3). The highest accumulations of FO content were recorded at the highest AS level (3 g pot⁻¹)+FN with the values of 27.7 and 27.5% during first and second seasons. The lowest FO contents were obtained in control treatment with the values of 17.1 and 17% during first and second seasons. The increases in FO contents were highly significant for AS, FN and their interaction.

Effect of AS, FN and their interaction on TC and SS: The TC and SS content increased with AS, FN and the AS×FN interaction in both seasons (Table 3). However, the highest TC and SS content resulted from 3 g pot⁻¹ of AS+FN, 108.6, 80.8%; 143.3, 123.1% higher than the control for the first and second seasons, respectively. The increases in TC were highly significant for AS and FN while it were significant for AS×FN during the first season and insignificant during the second season. The increases in SS were highly significant for AS, FN and AS×FN except during the second season for FN (were insignificant).

Effect of AS, FN and their interaction on CP: The CP contents of *N. sativa* were affected by changes in AS, FN and their interaction treatments (Table 3). Thus the CP in general

increased under the various As levels+FN compared AS treatments. The highest CP contents were obtained in the 3 g pot⁻¹ of AS+FN treatment with values of 27.8 and 25.1% in both seasons. The lowest CP contents were obtained in the control treatment with the values of 16.9 and 21.2% during the first and second seasons, respectively. The increases in CP contents were highly significant for AS and FN, while it was insignificant for AS×FN interaction.

Effect of AS, FN and their interaction on NPK: The accumulation of NPK in *N. sativa* leaves during the first and second seasons was promoted by applying various levels of AS, FN and AS+FN (Table 3). The NPK increase by adding AS with FN compared with AS alone. Control treatment resulted in the lowest nutrient accumulation on NPK while the highest mineral content was observed in the control treatment with 3 g pot⁻¹+FN. The lowest values of NPK contents were 2.7, 3.5%; 0.1%; 0.6, 0.8%, while the highest values were 3.7, 3.9%; 0.4%; 1.7, 2.2% during the first and second seasons. The increases in N content were significant for AS, highly significant for FN but it were significant for AS+FN during the first season while it were insignificant during the second season for AS, FN, AS+FN. The increases in P content were insignificant. The increases in K content were highly significant for AS and FN and insignificant for AS+FN.

DISCUSSION

The positive effect of AS on *N. sativa* may be due to it the main source of inorganic N fertilizer. 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 as anise, coriander and sweet fennel plants. Also, these results are in accordance with those obtained by Khalid (1996, 2001) on some Apiaceae and *N. sativa* plants; Ashraf *et al.* (2006) on cumin, Akbarinia *et al.* (2007) on coriander and Hellal *et al.* (2011) on dill (*Anethum graveolens* L.) all of whom reported that N fertilizer treatments were superior to the control treatment 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) and Ashraf *et al.* (2006) on *N. sativa* and Akbarinia *et al.* (2007) on coriander (*Coriandrum sativum* L.). 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 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 and cytokinins) involved in protein synthesis (Jones *et al.*, 1991; El-Wahab and Mohamed, 2007). The increase in the essential minerals according to the N treatments may be due to the increase in the dry matter of plant materials (El-Wahab and Mohamed, 2007).

On the other hand, increasing N rates by ammonium sulfate fertilizers have a tendency to decrease soil pH, this means that the fertilizers increase soil acidity which causing an increase in the availability of other nutrients for *N. sativa* plants (Fageria *et al.*, 2010).

The results of FN treatments were agreement with some previous investigators. Kandeel (1991) reported that using FN application at 2000 mg L⁻¹ had a significant effect on plant height, fresh weight, dry weight, fruit yield and oil content of parsley (*Petroselinum crispum* Mill). The FN had a significant effect on anise, coriander and sweet fennel plants which positively affect growth and chemical constituents of these three plants grown under arid regions in Egypt (Khalid, 2012).

CONCLUSION

It may be concluded that the various growth characters and chemical constituents of *N. sativa* were increased under

the various AS levels+FN compared AS treatments. The highest values of growth characters and chemical constituents obtained from the treatment of 3 g Pot⁻¹ of AS+FN.

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