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## **EFFECTS OF SODIUM CHLORIDE STRESS ON GROWTH, DEVELOPMENT CHARACTERS AND YIELD OF NIGELLA SATIVA DURING VEGETATIVE, FLOWERING, AND FRUITING STAGES**

### **SUMMARY**

Globally, black cumin (*Nigella sativa* L.) plays a crucial role in human health by being a traditional medicine for treating diseases, and an ingredient for herbal drug formulations and several culinary applications. To maximize its usage, it is important to understand how its growth is affected by stresses. Therefore, an experiment was conducted to determine the effect of saline water irrigation on survival, growth, and yield characteristics of *Nigella sativa*. The plants were treated with four (control=0, 1000, 2000, and 3000 ppm) concentrations of Sodium chloride (NaCl) and its important characteristics (survival, plant height, number of branches/plant, fresh and dry stem weights, fresh and dry leaf weights, total fresh and dry weights, number of umbels/plant, fresh and dry umbels weights, number of capsules/plants, dry capsule weight, dry straw weight, seed weight) were measured during the three growth stages (vegetative, flowering, and fruiting). The results revealed that survival of the plant, its growth, and its productivity significantly decreases with increasing concentration of NaCl at the vegetative and flowering stages. This also caused substantial reduction in the seed yield and yield components at the fruiting stage.

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Our results showed that *Nigella sativa* is sensitive to saline water, and there is a need for irrigating it with minimal salt concentration to reduce the severity of damage in its growth and yield.

**Keywords:** *Nigella sativa*, sodium chloride, saline irrigation, survival, yield

## INTRODUCTION

Traditional herbal medicines, which are naturally occurring plant-based substances used to treat illness, are getting significant attention in therapeutic and nutritional applications (Willcox and Bodker, 2004). More attention for studies on traditional herbal medicine is needed to discover promising medicinal herbs and novel chemical compounds (Tilburt and Kaptchuk, 2008). This is particularly important in Africa because 80% of African populations use some form of traditional herbal medicine (WHO, 2002). There are lots of studies that showed the effectiveness of traditional medicines to treat various diseases such as gastrointestinal diseases, headache and migraine, fertility, diabetes, hyperlipidemia, renal injury, stress and depression, pain, respiratory diseases, neurological disorders, liver disorders, and cancers (Kooti *et al.*, 2016).

One of the most important plants that has been used since ancient times and has a prominent role in folk medicine is black cumin (*Nigella sativa* L.; *Ranunculaceae* family) plant, and it still receives great interest in modern research in the food, cosmetics, and pharmaceutical fields. *Nigella sativa* seed is naturally grown in North Africa, southern Europe, the Middle East, and Southwest Asia; and is widely cultivated for numerous industrial and medicinal purposes (Niu *et al.*, 2020; Shahbazi *et al.*, 2022). *Nigella sativa* seeds stimulate great interest in the food, cosmetics, and pharmaceutical fields. Studies have shown several pharmacological properties of *Nigella sativa* including antibacterial activity, antifungal activity, antimalarial activity, antiviral activity, anti-schistosomal activity, antioxidant activity, anti-inflammatory, anti-hyperlipidemic, anti-cancer activity, anti-diabetic activity, cardiovascular protective activity, gastro-protective, nephro-protective, hepato-protective, neuro-protective, immuno-protective, reproductive system; and healing activities for rheumatoid arthritis, asthma, digestive diseases, anti-hypertensive and wound (Kooti *et al.*, 2016; Oyweri *et al.*, 2019; Khalil *et al.*, 2021; Mukhtar *et al.*, 2021). It also has effects on reproductive, digestive, immune, and central nervous systems; and anticonvulsant and analgesic activities (Kooti *et al.*, 2016). *Nigella sativa* seeds are also employed as preservatives and spices to flavor foods, such as bakery products, cheese, pickles, and a variety of traditional dishes (Salehi *et al.*, 2021; Benazzouz-Smail *et al.*, 2023). *Nigella sativa* seeds are also a substantial source of nutritionally vital constituents, such as fixed oils (32.2–41%), as well as to the fact that seeds are rich in polyunsaturated fatty acids, proteins (13.8–22%), carbohydrates (17%), ashes (4.5–7.5%), dietary fibers (8–16.4%), and vitamins, such as tocopherol and niacin (Kabir *et al.*, 2019; Shahbazi *et al.*, 2022; Benazzouz-Smail *et al.*, 2023). The mentioned biological properties

are related to their richness in several phytochemicals, mainly phenolic compounds, terpenes, essential oils, saponins, and alkaloids (Benazzouz-Smail *et al.*, 2023). As a result of the multiple uses of *Nigella sativa* and its therapeutic benefits, there is a need and interest to study environmental conditions and to identify factors affecting its cultivation and optimal productivity, especially under conditions of salt stress.

To maintain good plant production, there is a need to identify salt-tolerant plants. In studies that aim to achieve this, plants are typically exposed to high levels of salinity to understand the various physiological and biochemical changes, the modifications that occur in the structure, and the function of cell membranes when exposed to salt stress and to enhance salt tolerance of crops (Arzani, 2008; Taarit *et al.*, 2010). Since soil salinity is a global challenge due to the expansion of affected land areas, there are more and more studies that explore the different responses of plants to salinity.

Salinity is one of the main factors limiting plant growth and agricultural production in general (Kheloufi and Mansouri, 2019). Many studies focus on the effects of sodium chloride on medicinal and aromatic plants' anatomical, physiological, molecular, chemical, and protein attributes (Šutković *et al.*, 2011). In general, about three-quarters of the total soluble salt commonly used in irrigation water is sodium chloride. Plants vary greatly in their tolerance to saline water; however, the presence of stunted growth is a sign of salinity stress. As salt level in the soil increases to more toxic level, the leaves start to burn and fall off from the plant, which leads to the death of the plant. In other cases, the leaves may appear yellow, or the crop may show signs of wilting, even though the soil appears adequately moist (Flowers *et al.*, 2015; Geilfus, 2018; Hegazy *et al.*, 2019; Sany *et al.*, 2020).

The negative effects of salinity include causing an imbalance in cellular ions that leads to osmotic stress that makes water absorption difficult (Meloni *et al.*, 2001). It also reduces the absorption of nutrients due to the ability of sodium to compete with the basic cations required for cell function (Tester and Davenport, 2003). Several studies reported the negative effects of salinity on the productivity of various aromatic and medicinal plants, such as coriander (Said-Al Ahl *et al.*, 2014), black cumin (Bourgou *et al.*, 2010), sweet majorana (Baâtour *et al.*, 2013), oregano (Said-Al Ahl and Hussein, 2010), basil (Tarchoune *et al.*, 2013), dill (Said-Al Ahl *et al.*, 2016), and chamomile (Omer *et al.*, 2013).

In Egypt, saline water is used for irrigation in some areas. At the same time, under the arid climatic conditions prevailing in Egypt and associated with the perennial irrigation practices, imperfect drainage system, continuous increase of water table level and the relatively high salinity levels of water sources, particularly in the new reclaimed land, the salinization of Egyptian soils is rapidly becoming an acute problem (Said-Al Ahl *et al.*, 2010). However, in our opinion, this can be overcome by testing the plants' tolerance to salinity and identifying plants with the ability to adapt and have economic productivity. Therefore, the study aimed to investigate the cultivation of *Nigella sativa* in Egypt under soil

salinity conditions to clarify the extent that *Nigella sativa* can tolerate soil salinity conditions. Since salinity is taking an increasing and threatening scale, with NaCl being the most abundant element, the objective of the study is to determine the effect of saline (NaCl) treatment on the growth, development, and production of *Nigella sativa* plants during three growth stages.

## MATERIAL AND METHODS

### Plant material and growing conditions

A pot experiment was conducted in a greenhouse at the Farm Station of the Faculty of Agriculture, Cairo University, Giza, Egypt, during two growing seasons. Seeds of *Nigella sativa* were obtained from Medicinal and Aromatic Research Department, Ministry of Agriculture, Egypt. The seeds were sown on 15<sup>th</sup> of November, during both seasons in pots of 30 cm diameter containing 10 kg of air-dried soil. Chemical and physical analyses of the soil were conducted according to Jackson (1973). Results of the chemical analysis of the soil indicated that pH=8.40; E.C.=0.79 dSm<sup>-1</sup>; total nitrogen=0.13%; available phosphorus=2.18 mg/100 g; and potassium=0.02 mg/100 g.

The soil texture consisted of 45.00% sand, 28.25% silt, 26.75% clay, and 0.85% organic matter. The experimental layout was a randomized blocks design (RBD) with 3 blocks within each season, which makes the design for the statistical analysis RBD with 6 blocks (combinations of the 3 blocks and the 2 seasons). Each block contained 10 pots of 5 plants each. The NaCl saline irrigation treatments used in the study were control (0.40 dSm<sup>-1</sup>, tap water=256 ppm), 1000 ppm (1.563 dSm<sup>-1</sup>), 2000 ppm (3.125 dSm<sup>-1</sup>), and 3000 ppm (4.688 dSm<sup>-1</sup>). The use of salt solutions started one month after sowing. Data on survival, growth characteristics, and yield components were measured during three stages of development as follows; (1) survival (%), plant height (cm), number of branches/plant, fresh and dry stem weights (g/plant), fresh and dry leaf weights (g/plant), and total fresh and dry weights (g/plant) at the vegetative stage (75 days after sowing); (2) survival (%), plant height (cm), number of branches/plant, number of umbels/plant, fresh and dry stem weights (g/plant), fresh and dry leaf weights (g/plant), fresh and dry umbels weights (g/plant), and total fresh and dry weights (g)/plant at the flowering stage (120 days after sowing); and (3) survival (%), number of capsules/plant, dry capsule weight (g/plant), dry straw weight (g/plant), seed weight (g/plant), and total dry weight (g/plant) at the fruiting stage (165 days after sowing).

### Statistical analyses

Analysis of Variance (ANOVA) of a RBD was completed to determine the presence of significant differences among saline irrigation treatments at each of the three growth stages of *Nigella sativa*. Since the experiment was designed as RBD with 3 blocks within each of the two growing seasons, the 6 combinations of the 3 original blocks and the 2 seasons were used as blocks. The saline treatments during the vegetative stage were Control, 1000 ppm, 2000 ppm, and

3000 ppm; during the flowering stage were Control, 1000 ppm, and 2000 ppm; and during the fruiting stage were Control and 1000 ppm. The number of saline treatments decreased as the growth stage of the plants change because fewer and fewer plants survived at the highest level of saline treatment.

The statistical analysis was completed using the Mixed Procedure of SAS (SAS, 2014). Since the effect of Saline treatment was highly significant (P-value < 0.01) on all response variables except dry straw weight at fruiting stage, further multiple means comparison was completed using Tukey's multiple range test at 5% level of significance and letter groupings were generated. For each response variable, the validity of model assumptions (normal distribution and constant variance assumptions on the error terms) was verified by examining the residuals as described in Montgomery (2020).

## RESULTS AND DISCUSSION

The ANOVA results showed that the effect of irrigation with saline water significantly affected survival and all other growth characteristics at the vegetative stage. The multiple means comparison results shown in Tables 1 and 2 reveal that irrigation with saline water has a negative effect on all growth characteristics at the vegetative stage. The mean survival, fresh stem weight, dry leaf weight, and both fresh and dry total plant weight obtained from the four treatments were all significantly different from each other. The plants that received the control treatment gave the best values compared to the rest of the treatments. Increasing saline in the irrigation water led to a decrease in the values of all growth characteristics.

For plant height and number of branches, the differences among the treatments were significant, except between 1000 and 2000 ppm saline irrigation treatments. Generally, plants treated with tap water (Control treatment) gave the best results compared to the rest of the treatments. Also, plants irrigated with 3000 ppm NaCl gave the lowest mean values of plant height and number of branches.

As shown in Table 1, there was a significant difference between the mean fresh weight of leaves from the control treatment and the rest of the treatments. While the differences among the other treatments were not significant, a decreasing trend was observed as the concentration of saline water increases. However, for dry stem weight, there was no significant difference between 2000 and 3000 ppm, but the difference between the control and 1000 ppm, and between 1000 and 2000/3000 ppm were significant (Table 2). In general, all the mean values of survival and all growth characteristics decreased with increasing NaCl concentration, and the best values were obtained from the control treatment at the vegetative growth stage.

Murillo-Amadot *et al.* (2006) tested twenty-five genotypes of *Vigna unguiculata* for salt-tolerance at the vegetative growth stage at salinity levels of 0, 85, and 170 mM NaCl. Also, a study by Murillo-Amadot *et al.* (2002) showed

that seedling survival of *Vigna unguiculata* decreases linearly as salinity increases. A study by Kheloufi *et al.* (2019) on *Acacia karroo* reported that the germination % under 400 mM NaCl decreased by up to 66%, while the seed germination % of *Acacia saligna* at 150 mM decreased by only 18%. Cuba-Díaz *et al.* (2017) found that, NaCl decreased germination, survival seedlings % and shoot formation in *Colobanthus quitensis*. Also, NaCl inhibited germination, growth, and seed production of *Cakile maritima* (Debez *et al.*, 2004).

Table 1. Mean survival (%), plant height (cm), number of branches/plants, fresh stem weight (g/plant), and fresh leaf weight (g/plant) during the vegetative stage obtained from the 4 saline treatments.

Saline treatment (ppm)	Survival (%)	Plant height (cm)	Number of branches	Fresh stem weight (g/plant)	Fresh leaf weight (g/plant)
Control	100 a*	20.3 a	1.97 a	0.308 a	0.393 a
1000	90 b	16.2 b	1.22 b	0.191 b	0.037 b
2000	81 c	14.1 b	0.98 b	0.101 c	0.024 b
3000	48 d	11.0 c	0.69 c	0.056 d	0.015 b

\*within each column, means sharing the same letter are not significantly different at the 5% level of significance

Table 2. Mean total fresh weight (g/plant), dry stem weight (g/plant), dry leaf weight (g/plant), and total dry weight (g/plant) during the vegetative stage obtained from the 4 saline treatments.

Saline treatment (ppm)	Total fresh weight (g/plant)	Dry stem weight (g/plant)	Dry leaf weight (g/plant)	Total dry weight (g/plant)
Control	0.702 a*	0.032 a	0.039 a	0.071 a
1000	0.228 b	0.021 b	0.024 b	0.045 b
2000	0.125 c	0.012 c	0.018 c	0.029 c
3000	0.069 d	0.009 c	0.011 d	0.019 d

\*within each column, means sharing the same letter are not significantly different at the 5% level of significance

Salinity is a major abiotic stressor that restricts crop growth and development. Li *et al.* (2023) concluded that NaCl adversely affects the survival rate, growth, and development of cucumber. The study showed that cucumber plant height and growth are significantly inhibited by 150 mM NaCl, resulting in leaf wilting or even death. Li *et al.* (2023) also reported that the leaves of cucumber turn yellow under the 100 mM NaCl treatment, and most of the plants under the 150 mM NaCl treatment die. The symptom of salinity injury in plants is growth retardation due to the inhibition of cell elongation (Yasar *et al.*, 2008). Generally, the higher the salt damage index, the lower the salt tolerance. The salt damage index is divided into five levels, including normal leaf growth, drying of a small amount of the leaf edge, drying of less than 50% of the leaves, drying of more than 50% of the leaves, and entire plant death (Li *et al.*, 2023). Li *et al.* (2023) also showed that salt stress significantly reduces the total chlorophyll

contents. The reduced production or increased breakdown of chlorophyll molecules under salinity stress limits photosynthetic activity. Moreover, the toxicity of  $\text{Na}^+$  or salt-induced oxidative damage triggers the disintegration of the chloroplast ultrastructure. Decreased photosynthetic pigment, stomatal conductance, impaired enzyme activity, and reduced photosynthetic activity limit the carbon fixation capacity of plants under salt stress conditions (Gul *et al.*, 2023). The photosynthetic pigments and chlorophyll contents decreased significantly due to a salt-induced increase in the activity of the chlorophyll-degrading enzyme, chlorophyllase. Lovelock and Ball (2006) explained that the reduction of carbon fixation and the biomass allocation between leaf, stem, and root could alter the balance of photosynthesis and respiration. Other possibilities related to osmotic adjustment are inability to accumulate and/or distribute sufficient nutrients or synthesize sufficient organic solutes, and the futile cycling of ions (Britto and Kronzucker 2006).

In the flowering stage, *Nigella sativa* plants were affected significantly by saline treatments. The severity of the negative effect resulted in the death of all plants irrigated with 3000 ppm NaCl. Survival, plant height, number of branches, number of umbles, and fresh and dry weights of umbles, leaves, stem as well as total weights were affected by saline irrigation. Moreover, there were significant differences among the three treatments in all growth characteristics except number of branches, where the difference between saline irrigation at 1000 ppm and 2000 ppm was not significant (Tables 3 and 4).

Table 3. Mean survival (%), plant height (cm), number of branches/plant, number of umbles/plant, fresh umbles weight (g/plant), and dry umbles weight (g/plant) during the flowering stage obtained from the 3 saline treatments.

Saline treatment (ppm)	Survival (%)	Plant height (cm)	Number of branches/plant	Number of umbles/plant	Fresh umbles weight (g/plant)	Dry umbles weight (g/plant)
Control	100 a*	34.5 a	5.08 a	3.78 a	0.855 a	0.177 a
1000	79 b	26.7 b	4.68 a	2.60 b	0.415 b	0.092 b
2000	27 c	18.8 c	2.78 b	1.02 c	0.147 c	0.040 c

\*within each column, means sharing the same letter are not significantly different at the 5% level of significance

Table 4. Mean fresh stem weight (g/plant), fresh leaf weight (g/plant), total fresh weight (g/plant), dry stem weight (g/plant), dry leaf weight (g/plant), and total dry weight (g/plant) during the flowering stage obtained from the 3 Saline treatments.

Saline treatment (ppm)	Fresh stem weight (g/plant)	Fresh leaf weight (g/plant)	Total fresh weight (g/plant)	Dry stem weight (g/plant)	Dry leaf weight (g/plant)	Total dry weight (g/plant)
Control	0.425 a*	0.718 a	1.66 a	0.051 a	0.112 a	0.313 a
1000	0.318 b	0.477 b	1.05 b	0.046 b	0.095 b	0.226 b
2000	0.177 c	0.238 c	0.52 c	0.035 c	0.080 c	0.155 c

\*within each column, means sharing the same letter are not significantly different at the 5% level of significance

In general, plants treated with the control treatment gave the highest values of all growth characteristics, followed by those treated with 1000 ppm NaCl, and

plants treated with 2000 ppm NaCl gave the lowest values for all studied characteristics.

The negative effect of saline irrigation continued in the fruiting stage of *Nigella sativa* plants, which resulted in the death of the plants when treated with 2000 ppm NaCl. Here, at the fruiting stage only the plants that received the control and 1000 ppm NaCl survived to the end of the experiment and gave seed yield. While the differences among the treatments were significant in all studied traits (survival percentage, number of capsules, dry weights of capsules, as well as seed weight), the mean straw weights from the control and the 1000 ppm NaCl treatments were not significantly different (Table 5).

In general, the control plants gave higher mean values of all studied characteristics at fruiting stage. The results also showed that with increasing age, and increasing number of saline irrigation times, the harmful effect of salt increases because of the increased accumulation of salts in the vicinity of the roots and consequently leading to the death of plants.

Table 5. Mean survival (%), dry straw weight (g/plant), dry capsule weight (g/plant), total dry weight (g/plant), number of capsules/plant, and seed weight (g/plant) during the fruiting stage obtained from the 2 Saline treatments.

Saline treatment (ppm)	Survival (%)	Dry straw weight (g/plant)	Dry capsule weight (g/plant)	Total dry weight (g/plant)	Number of capsules/plant	Seed weight (g/plant)
Control	100 a*	0.378 a	2.78 a	3.15 a	5.77 a	0.315 a
1000	65 b	0.233 a	2.38 b	2.61 b	3.80 b	0.207 b

\*within each column, means sharing the same letter are not significantly different at the 5% level of significance

Rebey *et al.* (2017) observed significant decreases in plant growth with increasing severity of salt treatment. Compared to the control treatment, while the application of 50 mmol NaCl caused a slight drop (6.11%) in plant height, the application of 75 mmol and 125 mmol of NaCl reduced the plant height by about 32.15% and 54%, respectively. Also, they noted that salinity led to 18.5%, 35.61%, and 29.12% decline in dry matter with the application of 50, 75, and 125 mmol of NaCl, respectively. The results indicate that salinity limits the biomass production of cumin seeds.

Results of the current study showed that, at the flowering stage, continuous irrigation with NaCl has a fatal effect, especially at concentrations of 3000 ppm or above, which led to the death of all plants. This shows that severe sensitivity of *Nigella sativa* to NaCl happens at 3000 ppm. Therefore, it is not recommended to irrigate *Nigella sativa* with 3000 ppm or above NaCl.

Salty irrigation water affects plant growth in two ways: 1) Salinity effect; plant roots take up moisture through membranes in root cells by osmosis; and water passes through a semi-permeable membrane and moves from a solution of low levels of dissolved salts to one with higher salts until the plant cells become full. If the irrigation water is moderately saline, then the growth of the plant is



slowed, which leads to a reduced yield. If it is highly saline, the process of osmosis can reverse, and the plant loses moisture and suffers stress. 2) Toxicity effect; excessive concentrations of sodium and chloride ions in irrigation water can cause toxicities in plants. Sodium and chloride toxicity symptoms are leaf burn. There is another effect of sodium, which happens when sodium is high compared to calcium and magnesium leading to waterlogging because of the degradation of well-structured soils and poor plant growth (Hegazy *et al.*, 2019; Sany *et al.*, 2020).

The depressive effect of salt on seed yield of several aromatic and medicinal plants including *Foeniculum vulgare* (Rahimi *et al.*, 2012) and *Trachyspermum ammi* (Ashraf and Orooj, 2006) has been reported. One cause of this yield reduction under saline constraint is an inadequate photosynthesis owing to stomatal closure that limits carbon dioxide uptake (Zhu, 2001). A decrease in seed yield could be due to a reduction in flower production and/or a decrease in their fertility.

Many studies conducted on medicinal and aromatic plants concluded that the growth, productivity, and seed yield of the plants are affected negatively by saline water irrigation. Among the previous results that confirmed this are by Said-Al Ahl and Omer (2011) on different medicinal plants; Sany *et al.* (2020) on *Plectranthus amboinicus*; Hegazy *et al.* (2019) on *Satureja montana*; Said-Al Ahl *et al.* (2016) on dill; and Said-Al Ahl *et al.* (2014) on coriander. Rashed *et al.* (2017) reported that salinity treatments significantly decrease growth and yield parameters of *Nigella sativa*. Ashraf and Akhtar (2004) also reported that increasing the concentration of NaCl leads to a significant decrease in fresh and dry masses of both shoots and roots of sweet fennel. A study by Hassanzadehdelouei *et al.* (2013) showed that salinity stress has a significant inhibition of Cumin's growth stages (e.g., vegetables, flowering, and seed filling stage) where it has a significant impact on fresh weight, height and seed and yield.

## CONCLUSIONS

The present work extends our knowledge about the effect of NaCl on the production of *Nigella sativa* seed, which is a source of natural products, mainly in the food industry due to its several useful compounds. From this point of view, our results revealed that NaCl decreases the seed yield of *Nigella sativa*. Since the negative impact of NaCl on the growth and development of *Nigella sativa* increases with increasing concentration of NaCl, it is recommended to irrigate *Nigella sativa* with very low concentration of NaCl. However, NaCl treatment may represent an effective method of improving the nutritional quality of *Nigella sativa* seeds through induced biochemical changes and may function as a potential source of oil and antioxidant compounds, which could support the utilization of this plant in a large field of applications including in the agroalimentary sector. Therefore, future studies should focus on working towards increasing the productivity of *Nigella sativa*, its adaptation to environmental

stress, and the utilization of its biologically active compounds for therapeutic and nutritional applications as well as the development of new drugs.

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