



Growth and Certain Biochemical Components of Black Cumin Cultivated under Salinity Stress Factor

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Abstract

Black cumin used as a spice, food additive and medicinal proposes. Cultivation of resistant plants is one way to utilize arid zones in Egypt. So, an attempt was made to follow the effects of salt conditions on growth and some biochemical constituents of black cumin. Black cumin plants were subjected to different levels of NaCl salt, i.e. 0.4, 1.6, 3.1, 4.7 and 6.3 dS m⁻¹. Plant growth characters (PGC) i.e. plant height, PH (cm), number of leaves, NL (plant¹), number of branches, NB (plant¹), number of capsules, NC (plant¹) dry weight of herb (DWH) and yield of seeds, (YS) (plant¹) were decreased as salinity level increased. The biochemical contents such as essential oil (EO), major constituents of EO (p-cymene, α -thujene and γ -terpinene), total soluble sugars (TSS) and proline (PRO) were promoted under salinity treatments while nitrogen (N) and crude protein (CP) were decreased. Greatest PGC for all variables were obtained in the 0.4 dSm⁻¹ treatment with the values of 16.5, 43.9, 4.6, 8.5, 20.8 and 4.4 respectively. The highest values of EO (0.4%), p-cymene (60.3%), α -thujene (7.2%), γ -terpinene (1.5 %), PRO content (23.1 $\mu\text{m g}^{-1}$) and TSS (11.8%) were obtained from the 6.3 dSm⁻¹ treatment. The highest values of N uptake and CP (0.4 $\mu\text{m g}^{-1}$ and 16.5%) were recorded with 0.4 dSm⁻¹ treatment.

Key words: Black cumin (*Nigella sativa* L.), Plant growth characters, Nitrogen, Essential oils, Proline, Total soluble sugars, Crude protein.

1. Introduction

Black cumin (*Nigella sativa* L.) belongs to family *Ranunculaceae*, it used as a spice, food additive (or food preservative) and medicinal proposes [1-2].

Major efforts to breed for traits that confer tolerance of drought, cold, heat, nutrient and salinity stress are already made each year throughout the world. An understanding of the mechanisms that regulate form and function and the significance of those processes to plant physiology, ecology and agriculture must include knowledge of plant stress physiology [3]. Agricultural productivity was affected by salinity stress conditions. Salinity stress has an effect on current plants and positive barriers to the introduction of plants into areas that are not currently being used for agriculture [3].

The effects of salinity stress on black cumin were reported by some previous investigators. PGC, YS, N and CP of black cumin were decreased with salinity treatments but TSS and PRO contents were increased [4-6]. Salinity dose (0.3%) caused a significant reduction in seed germination, PGC, YS, oils, carbohydrates and water content of black cumin [7-9].

On the other hand the influences of salinity on morphological and biochemical accumulation on medicinal plants were investigated. Morphological characters, N and CP content were reduced in response to stress conditions (salinity and drought) while EO, major constituents of EO and carbohydrates were increased in *Salvia officinalis* and *Melissa officinalis* L. [10-11]. Salinity stress resulted in a significant reduction in the PGC and EO content of chamomile, coriander and sage plants [12 - 14].

Most of agricultural soil (90%) in Egypt subjected to arid or semi arid zones and the water availability (water has salt) is a basic problem for medicinal plants production. In such conditions cultivation of resistant crops is one way to utilize these lands and therefore the selection of suitable plants, which could cope with these conditions, is a necessity [15]. Therefore, an attempt was made to follow the effects of salinity stress on growth and some biochemical constituents of black cumin.

2. Materials and methods

2.1. Plan, site and methodology

Experiments were carried out in a greenhouse at the National Research Centre (NRC), Cairo, Egypt, in 2014 season. Medicinal black cumin seeds were obtained from the Institute of Medicinal and Aromatic Plants (IMAP) located in Kalubia Governorat, Egypt. Uniform seeds were sown into plastic pots (30 cm diameter and 50 cm height) during the first week of November 2014; the pots were transferred to a greenhouse adjusted to 24/18°C, 90/60% RH day/night and light intensity ~3700 $\mu\text{mol.m}^{-2}.\text{s}^{-1}$. Each pot was filled with 10 kg of air-dried soil (sandy soil). Three weeks after sowing, the seedlings were thinned to three plants per pot. 45 days from sowing date plants were divided into five groups were subjected to different levels of saline irrigation water, 0.4 (tap water as control), 1.6, 3.1, 4.7 and 6.3 dS m⁻¹. To prepare irrigation water with different salinity levels, highly soluble NaCl salt were used. This salt was used because it is found naturally in the irrigation water in Egypt [16]. Plants subjected to saline irrigation water every 7 days however all pots were leached by tap water every 28 days (If there was no leaching when irrigating with saline water, it may induce salt build up in pots). All Agricultural practices were done according to recommendations of Ministry of Agriculture, Egypt.

2.2. Harvesting

At the end of the fruiting stage (210 days from sowing), the plants were harvested. PGC measurements [PH (cm), NL (plant⁻¹), NB (plant⁻¹), NC (plant⁻¹), DWH (plant⁻¹) and YS (g plant⁻¹)] were recorded during the end of season.

2.3. EO isolation

Dry seeds were collected from each treatment then 50g from each replicate of all treatments were subjected to hydro-distillation for 3 h using a Clevenger-type apparatus [17]. The EO content was calculated as a relative percentage (v/w). In addition, total EO (ml plant⁻¹) was calculated. The EO were collected from each treatment and dried over anhydrous Sodium Sulphate to identify the chemical constituents of the EO.

2.4. GC-MS analysis

The ADEL SIGLC MS system (Model: GCMS-QP2010 SE; SHIMADZU, Japan), equipped with a BPX5 capillary column (0.22 mm id × 25 m, film thickness 0.25 μm) was used. Analysis was carried out using Helium as the carrier gas, with a flow rate of 1.0 ml/min. The column temperature was programmed from 60 to 240°C at 3°C/min. The sample size was 2 μl , the split ratio 1:20; injector temperature was 250°C; ionization voltage applied was 70 eV, mass range m/z 41-400 amu. Kovat's indices were determined by co-injection of the sample with a solution containing a homologous series of *n*-hydrocarbons in a temperature run identical to that described above.

2.5. Identification of EO components

The separated components of the EO were identified by matching with the National Institute of Standards and Technology (NIST) mass spectral library data, and by comparison with Kovat's indices of authentic components and with published data [18]. Quantitative determination was carried out based on peak area integration.

2.6. PRO determination

PRO content was determined in fresh leaves using the method of Bates *et al* [19].

2.7. TSS determination

TSS contents were determined from plant material (young leaves) collected from each treatment. The method of Dubois *et al* [20] was used.

2.8. N and CP determination

Total N and CP in leaves of each treatment were determined using the methods described by the AOAC [21].

2.9. Statistical analysis

In this experiment, one factor was considered: water salinity [0.4 (tap water as control), 1.6, 3.1 and 4.7 dSm⁻¹]. For each treatment there were 5 replicates, each of which had 8 pots; in each pot 3 individual plants were planted. The experimental design followed a complete randomized block design. According to Snedecor and Cochran [22] the averages of data were statistically analyzed using one-way analysis of variance (ANOVA-1). 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 [23]

3. Results

3.1 Effect of salinity on PGC

PGC i.e. PH, NL, NB, NC, DWH and YS were affected by different salinity levels. Generally the different PGC reduced under the various salinity treatments compared with control. The lowest PGC were obtained at 6.3 dSm⁻¹ with the values of 6.8, 13.5, 2.5, 3.5, 0.8 and 0.7. Greatest PGC for all variables were obtained in the 0.4 dSm⁻¹ treatment with the values of 16.5, 43.9, 4.6, 8.5, 20.8 and 4.4 (Fig. 1-6). ANOVA indicated that the decrease in PGC i.e. PH, NL, NP, NC were highly significant but insignificant for DWH while the reduction in the YS was significant in salinity levels.

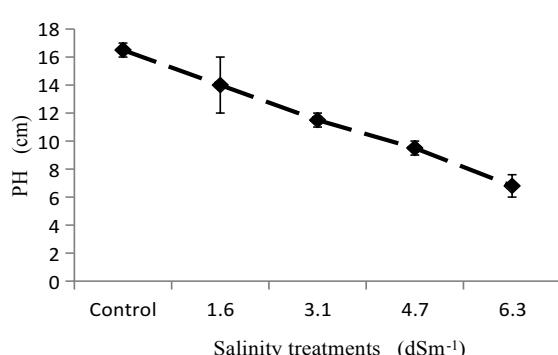


Fig. 1. Effect of salinity on PH.

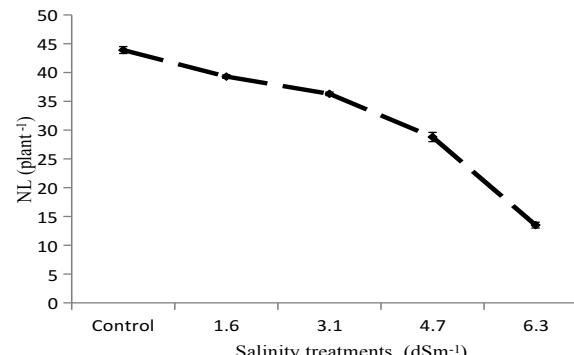


Fig. 2. Effect of salinity on NL.

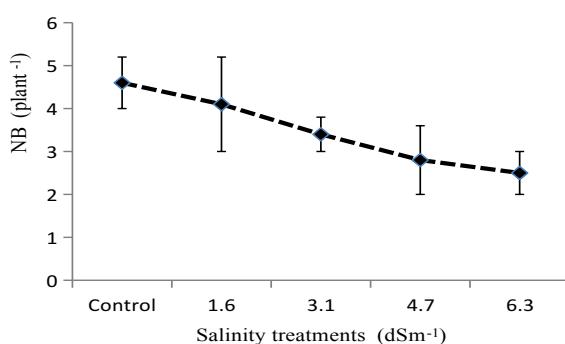


Fig. 3. Effect of salinity on NB.

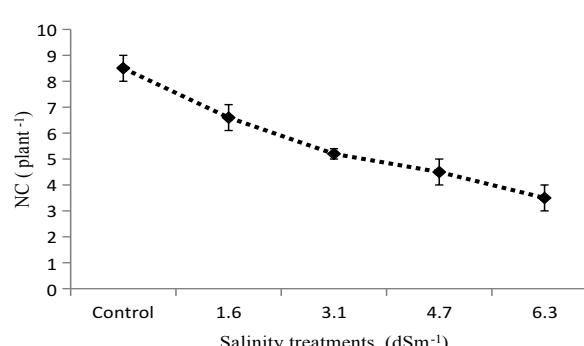
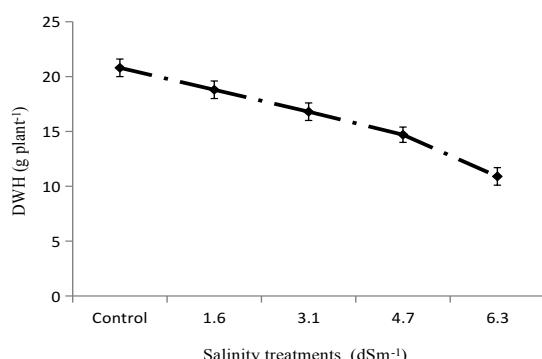
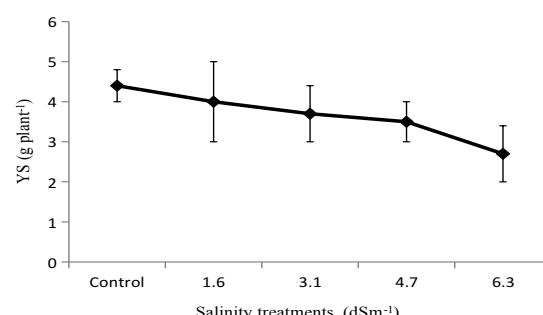
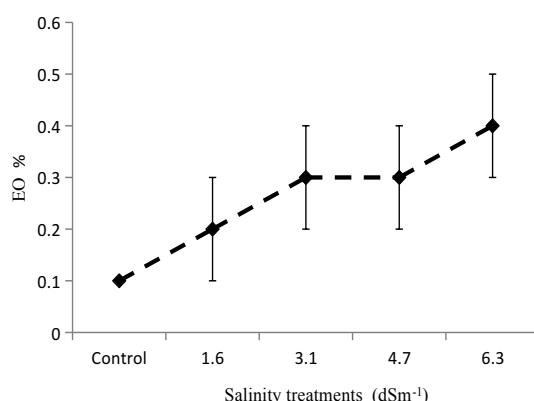
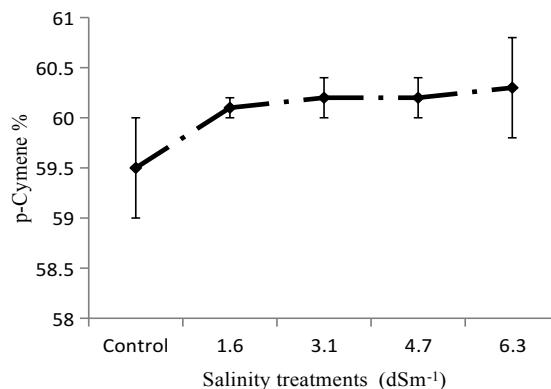
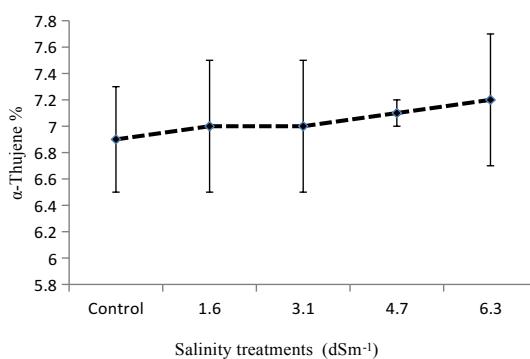
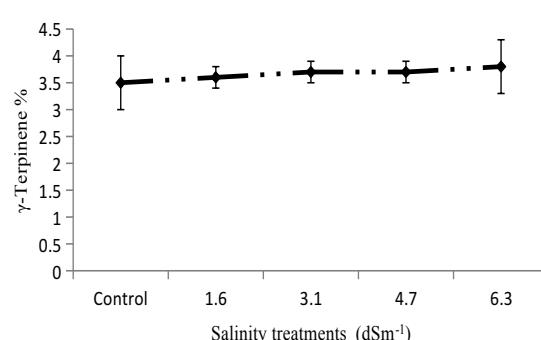


Fig. 4. Effect of salinity on NC.

**Fig. 5.** Effect of salinity on DWH.**Fig. 6.** Effect of salinity on YS.

3.2. Effect of salinity on EO composition

As shown in Fig. 7, EO percentage increased at all salinity levels. Salinity treatment of 6.3 dSm⁻¹ resulted in greatest EO content (0.4%) while the untreated plants resulted in the lowest value (0.1%). ANOVA indicated that EO (%) was insignificant for salinity treatments. Fig. 8-10 shows the major components (p-cymene, α -thujene and γ -terpinene) of EO extracted from black cumin seeds as detected by GC-MS. The highest amounts of major constituents (60.3, 7.2 and 1.5 %) were obtained from the 6.3 dSm⁻¹ treatment (Fig. 8-10). ANOVA indicated that the main constituents of *N. sativa* were significant except the γ -terpinene was insignificant for salinity treatments.

**Fig. 7.** Effect of salinity on EO (%).**Fig. 8.** Effect of salinity on .p-cymene**Fig. 9.** Effect of salinity on α -thujene**Fig. 10.** Effect of salinity on γ -terpinene

3.3 Effect of salinity on PRO content

PRO, which increases proportionately faster than other amino acids in plants under stress, has been suggested as an evaluating parameter for irrigation scheduling and for selecting drought stress – resistance varieties [19]. The accumulation of PRO in black cumin leaves was promoted by applying various levels of salinity (Fig. 11). The highest PRO content ($23.1 \mu\text{m g}^{-1}$) resulted from 6.3 dSm^{-1} treatment compared with control ($7.8 \mu\text{m g}^{-1}$). ANOVA indicated that the increase in PRO contents were highly significant for salinity treatments.

3.4. Effect of salinity on TSS content

Salinity levels caused a significant increase in TSS content compared with control (Fig. 12). However, the highest TSS (11.8%) content obtained from 6.3 dSm^{-1} treatments however the lowest value (5.4%) resulted from untreated plants. The increase in TSS was highly significant for salinity treatments.

3.5. Effect of salinity on N uptake and CP content

The accumulations of N (or CP) in black cumin leaves were promoted without applying various levels of salinity (Fig. 13 and 14). The highest values of N uptake and CP ($0.4 \mu\text{m g}^{-1}$ and 16.5%) were recorded with control ($0.4 \mu\text{m g}^{-1}$). ANOVA indicated that the decreases in N uptake and CP were significant for salinity treatments.

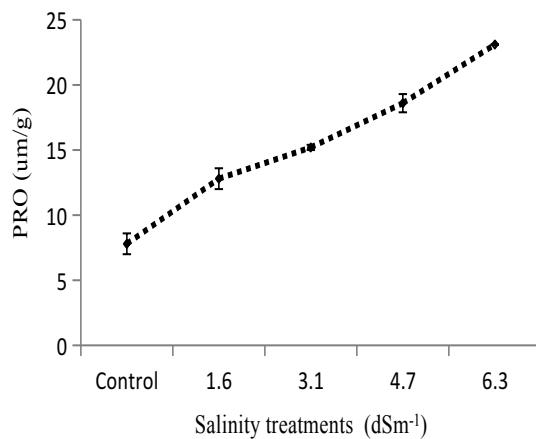


Fig.11. Effect of salinity on PRO

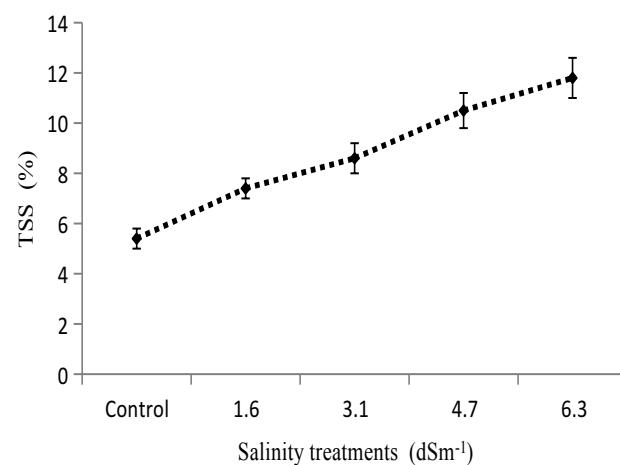


Fig.12. Effect of salinity on TSS

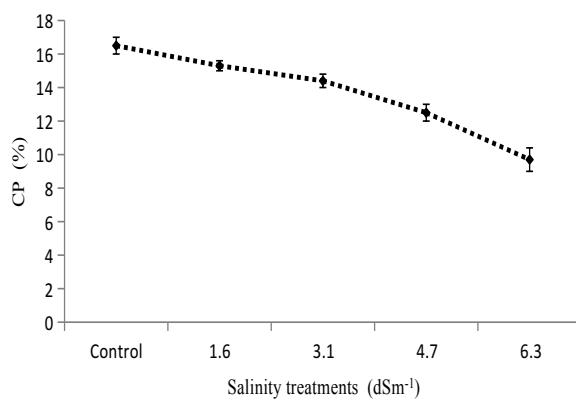


Fig.13. Effect of salinity on CP

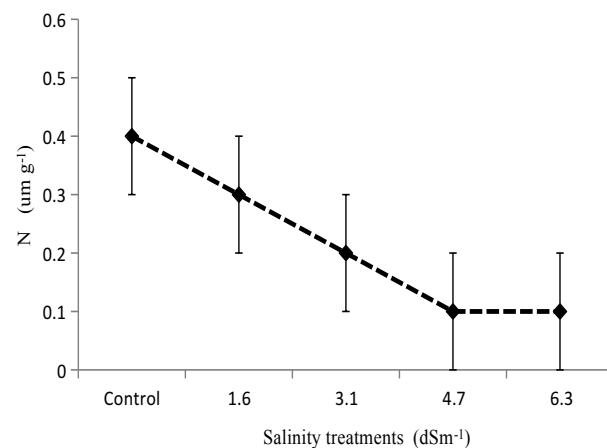


Fig.14. Effect of salinity on N uptake

4. Discussion

Under salinity conditions, osmotic adjustment is usually achieved by the uptake of Na^+ and Cl^- from the soil solution. Balibrea *et al.* [24] suggested that a great deal of harmless and compatible solutes were synthesized and accumulated in plant leaves, thus maintaining the osmotic balance. Osmotic adjustment by inorganic ions accumulation is less energy and carbon-demanding than adjustment by organic solutes [25]. Inorganic solutes formed the largest component contributing to osmotic adjustment in grapevines. The production of sufficient organic osmotica is metabolically expensive and potentially limits plant growth by consuming significant quantities of carbon that could otherwise be used for growth [26]. An alternative to producing organic osmotica is for the plants to accumulate a sufficiently high content of ions from the soil. The energetic cost of osmotic adjustment using inorganic ions is much lower than that of using organic molecules synthesized in the cells [25, 27]. Thus by using this alternative mechanism of inorganic ion accumulation to adjust their osmotic potential, grapevines seem to save energy, which enables them to grow in less favorable conditions.

The high accumulation in *N. sativa* essential oil composition under salinity stress levels may be due to its effect of salinity on enzyme activity and metabolism of essential oils production [28]. The high accumulation in proline and soluble sugars with salinity stress levels may be due to proline is regarded as a source of energy, carbon, and nitrogen for recovering tissues under salt stress conditions [29]; salinity levels enhanced the plant to preserve sugars for sustained metabolism, prolonged energy supply, and for better recovery after stress relief [30-34]. The decrease in N uptake and protein contents during salinity stress conditions may be due to low availability of N to plants [16].

On the other hand some new studies indicated that salinity treatment reduced plant growth characters and increased the menthone and pulegone contents of mint herb [35]. The positive or negative effects of salt stress on basil depend on the degree of tolerance of the different genotypes [36]. The accumulative effect of increasing salinity reduced stem height and elongation [37].

Conclusion

It has been concluded from present research study that PGC, N and CP were reduced as salinity stress increased. Salinity stress enhanced the EO, main components of EO, TSS, and PRO composition. *N. sativa* plants grow well under moderate levels of saline irrigation water. We can recommend that *N. sativa* can grow under low levels of salinity.

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