University of Thi-Qar Journal of agricultural research

Thi-Qar Journal of agricultural research

ISSN Onlin:2708 -9347, ISSN Print: 2708 -9339 Volume 15, Issue 1 (2026)

https://iasj.rdd.edu.iq/journals/journal/issue/20226 https://doi.org/10.54174/utjagr.v13ii 2341.

Seed Yield and Phenolic-Flavonoid Response of(*Nigella sativa* L.) Under Graduated Salinity Stress Levels Analyzed by HPLC

Hiba Jomaa Al-Fahdawi

Field Crops Department, College of Agriculture, University of Tikrit, Tikrit, Iraq

Hiba2020@tu.edu.iq

I. Abstract:

The field experiment was carried out at Al-Dour District in Salah Al-Din Governorate, Located in Iraq (34.62° N, 43.67° E; 102 m above sea level) during 2025–2026 growing season. The objective of the study was to investigate the effect of four levels of salinity (0, 50, 100 and 150 mM) on seed yield components and subsequent analysis of the phenolic-flavonoid profile in the resulting seeds of black cumin (Nigella sativa L.) by HPLC-DAD. The results indicated that all the growth and yield parameters were significantly reduced with salinity stress in a progressive manner and the effect of the salinity treatments was enhanced by the natural background salinity of the experimental field soil, which was a clay loam with the background salinity value of 2.8 dS m^{-1}. The total seed yield dropped significantly with the high concentration (150 mM) treatment producing 241 kg ha-1 while the control (0 mM) produced 680 kg ha-1 (reduction rate 64.6%). In addition, the plant height, capsules per plant, seeds per capsule and 1000 seed weight were also negatively influenced. Total phenolics content (TPC) on the other hand, was significantly up-regulated from 18.3 to 31.7 mg GAE g^{-1} DW (mg gallic acid equivalent per gram of dry weight). In the same way, the total flavonoid content (TFC) increased from 12.1 to 22.4 mg QE g^{-1} DW (mg quercetin equivalent/gram dry weight). Distinct quantitative jumps have been observed for all of the separated compounds when salinity was increased, as obtained by chromatographic analysis (HPLC-DAD). The highest amount of chlorogenic acid was observed, which varied from 1.24 to 3.18 mg g^{-1} DW (156.5% increase rate). Meanwhile, quercetin was dominant, which increased from 0.87 to 2.41 mg g^{-1} DW (+177.0%). There was a steady significant increase in the remaining identified phenolic compounds (caffeic acid, rosmarinic acid, kaempferol, luteolin, rutin and naringenin) demonstrating that salinity is not only responsible for an increase in total percentage of phenolics, it also has a qualitative and selective effect on the phytochemical composition of the seeds. The study showed clearly that the plant has a metabolic trade-off and when under stress, it shifts its energy and vital resources to synthesise protective phenolic and flavonoid compounds to tackle salinity stress rather than vegetative growth and seed formation processes.

Keywords: Nigella sativa, salinity stress, seed yield, phenolics, flavonoids, HPLC

1. Introduction

Nigella sativa L. (Ranunculaceae) is one of the more outstanding medicinal and aromatic plants with great medicinal potential. Besides its high phenolic acids and flavonoids content with antioxidant, anti-inflammatory and anti-tumor activity, its seeds also contain several bioactive compounds including thymoquinone, saponilins and carvacrol (Hussain et al., 2024). In recent years, the total phenolic content (TPC) of N. sativa seeds has been found to vary between 28 and 42 mg GAE/g dry weight (DW), with total flavonoid content (TFC) varying from 30 to 45 mg QE/g, depending on environmental conditions and farming practices (Terzo et al., 2025).Salinity is one of the worst abiotic stresses that affect global agricultural productivity; reports estimated that about 20% of the world landfaced varying degrees of salinity (Mahmoud et al., 2023). High electrical conductivity (EC) in

University of Thi-Qar Journal of agricultural research

Thi-Qar Journal of agricultural research

ISSN Onlin:2708 -9347, ISSN Print: 2708 -9339 Volume 15, Issue 1 (2026)

https://iasj.rdd.edu.iq/journals/journal/issue/20226   https://doi.org/10.54174/utjagr.v13ii 2341.

agricultural lands is a serious problem in Iraq, especially in Saladin Governorate caused by salinity, making it a threat for producing medicinal and aromatic plants. On the cellular level, salt stress causes increases in ionic imbalance, osmotic stress and the production of excessive reactive oxygen species (ROS), which ultimately impacts photosynthesis and seed production (Aghajanzadeh & Prajapati, 2021). In contrast, plants experiencing salt stress activate defense pathways resulting in the production of secondary metabolites, including phenolics and flavonoids, which are naturally occurring antioxidants that can help regulate free radicals and protect cell membranes (Al-Harthi et al., 2024). Aghajanzadeh & Prajapati (2021) showed that under salinity stress (60 mM NaCl), N. sativa plants spiked the levels of quercetin, kaempferol, and myricetin in its leaves, thus validating the hypothesis of chemical compensation under stress.However, to date, there is no quantitative correlation between the different degrees of salinity, seed yield and the HPLC profile of specific phenolic and flavonoid compounds under Iraqi environmental conditions. Based on this, the aim of the present study is to

1.. Investigate the effect of different concentrations of NaCl on seed yield indices of N. sativa.

2) Measure the changes of Total Phenolic Content and Total Flavonoid Content.

3) Determine the presence and amounts of individual phenolics and flavonoids by HPLC technique.

II. Materials and Methods.

2.1 .Study Site and Environmental Conditions

Field experiment was conducted at agricultural field in Al-Dour District, Saladin Governorate, Iraq (latitude 34.62^\circ\text{ N}, longitude 43.67^\circ\text{ E} and the height from sea level is 102 m). The climate of the study area is semi-arid. Ambient temperature during the growing season of black cumin (Nigella sativa L.) for 2025–2026 agricultural season varied between 5°C and 22°C and the annual average rainfall was 200–250 mm.

Table (1): Physical and Chemical Properties of Experimental Site Soil — Al-Dur, Salah Al-Din, 2025

Soil Property	Measured Value	Unit	Scientific Interpretation
Sand	42	%	Moderate proportion; promotes good drainage
Silt	31	%	Contributes to moisture and nutrient retention
Clay	27	%	Clay loam texture; suitable for black cumin roots
pH (1:1 water:soil)	7.6	—	Slightly alkaline; limits availability of Fe and Zn
Electrical Conductivity (EC)	2.8	dS/m	Background salinity amplifies NaCl treatment effects
Organic Matter	0.82	%	Low; supplementary organic fertilization recommended
Total Nitrogen	0.045	%	Low; nitrogen fertilization required
Available Phosphorus	7.2	mg/kg	Below optimum (>10 mg/kg)
Available Potassium	142	mg/kg	Acceptable level; enhances stress tolerance
Gypsum ($CaSO_4$)	1.9	%	Raises EC; mimics natural saline stress

ISSN Onlin:2708 -9347, ISSN Print: 2708 -9339 Volume 15, Issue 1 (2026)

https://iasj.rdd.edu.iq/journals/journal/issue/20226   https://doi.org/10.54174/utjagr.v13ii 2341.

Calcium Carbonate ($CaCO_3$)	18.4	%	High; restricts micronutrient availability
Bulk Density	1.38	g/cm^3	Normal range for clay loam soil
Permanent Wilting Point	12.1	%	Requires irrigation every ≤4 days

Note: Background EC of 2.8 dS/m indicates pre-existing mild salinity, which intensifies the impact of NaCl treatments.

2.2. Seed Material and Crop Management

Nigella sativa L. seeds of local cultivar which is being cultivated in Iraq were obtained from Agricultural Research Directorate, Iraqi Ministry of Agriculture. The seeds were surface sterilized in 1% sodium hypochlorite solution for 5 minutes and then washed with distilled water. Sowing was done on 1st October 2025 at a seeding rate of 10 kg/ha with planting rows of 20 × 30\text{ cm}.

2.3. Experimental Design and Salinity Treatments

The experimental design used was Randomized Complete Block Design (RCBD) having four treatments and three replications. The experimental units used (plots) were 9\text{ m}^2 (3 \times 3\text{ m}).

The saline treatments with NaCl were done by irrigation water, starting 1 week after germination and applied for the remaining duration till harvest maturity, with the following treatments:

• M_0 (Control): \text{EC} = 0.5\text{ dS m}^{-1}

• M_1: 50\text{ mM NaCl} (\text{EC} \approx 4.0\text{ dS m}^{-1})

• M_2: 100\text{ mM NaCl} (\text{EC} \approx 7.5\text{ dS m}^{-1})

• M_3: 150\text{ mM NaCl} (\text{EC} \approx 11.0\text{ dS m}^{-1})

2.5. Yield Components and Phytochemical Analysis

Yield attributes (plant height, capsules per plant, seeds per capsule, 1000-seed weight, and seed yield per plant) were measured at full maturity. The extraction of phenolic and flavonoid compounds was done by ultrasound-assisted extraction (UAE) method with 80% ethanol for 30 minutes at 40\text{ kHz} at 25\text{ °C}. The total phenolic content (TPC) was measured by the Folin-Ciocalteu method and reported as milligrams of gallic acid equivalents per gram of dry matter (mg GAE g^{-1}DW). The total flavonoid content (TFC) was determined by aluminium chloride (\text{AlCl}_3) colorimetric assay and reported as milligrams of quercetin equivalent per gram of dry weight (mg QE g\text{ -1 DW}).

2.6. HPLC Analysis Conditions

High-Performance Liquid Chromatography (HPLC) analysis was carried out on a Shimadzu LC-20A system equipped with a Diode Array Detector (DAD/UV). Separation was performed using a reversed-phase C18 column (250 \times 4.6\text{ mm}, 5\text{ \mu m} particles). The mobile phase used was a gradient elution containing 0.1% formic acid in water (A) and acetonitrile (B) at a flow rate of 1.0 mL/min for 50 minutes. The wavelengths for the detection of phenolic acids and flavonoids were determined as 280 nm and 370 nm, respectively, based on the procedure used for these compounds (Suwatronnakorn et al., 2025).compounds were

ISSN Onlin:2708 -9347, ISSN Print: 2708 -9339 Volume 15, Issue 1 (2026)

https://iasj.rdd.edu.iq/journals/journal/issue/20226 *https://doi.org/10.54174/utjagr.v13ii 2341.*

quantified by comparison with reference standards (Sigma-Aldrich), using calibration curves with linearity limit of r^2 > 0.999

III. Results and Discussion

3.1. Effect of Salt Stress on Yield Components

Total seed yield significantly and progressively decreased from 680 kg/ha under control conditions to 241 kg/ha at 150 mM NaCl, representing a 64.6% reduction (Table 2). This decline can be attributed to three interrelated physiological mechanisms:

First, osmotic stress limits the uptake of water and essential nutrients, which reduces photosynthetic efficiency and consequently hinders the accumulation of reserve materials in the seeds. Second, the toxic accumulation of Na^+ and Cl^- ions within plant tissues disrupts the activity of vital enzymes, particularly those involved in the Krebs cycle and the electron transport chain (ETC). Third, oxidative stress induced by the accumulation of free radicals, such as H_2O_2 and $O_2^{\bullet -}$, leads to lipid peroxidation of cellular membranes, which negatively impacts fertilization processes and capsule formation (Mahmoud et al., 2023).

These findings align with Aghajanzadeh & Prajapati (2021), who noted that N. sativa is highly sensitive to salinity. They reported a significant drop in seed productivity when soil salinity exceeded 60 mM NaCl in their salinity stress experiments.

Additionally, the reduction in 1000-seed weight from 2.91 g (M_0) to 1.84 g (M_3) indicates that the seed-filling stage was directly impaired. This phase is widely recognized as the most critical and sensitive period to salinity stress in seed crops (Hussain et al., 2024).

Table (2): Effect of Salinity Stress Levels on Yield Components of Nigella sativa L. — Al-Dur, Salah Al-Din, 2025–2026

Treatment	NaCl (mM)	Plant Height (cm)	Capsules/Plant	Seeds/Capsule	1000-Seed Weight (g)	Seed Yield (kg/ha)	Yield Reduction (%)
M0 (Control)	0	38.4 a	7.2 a	52.3 a	2.91 a	680 a	—
M1	50	33.1 b	6.1 b	45.8 b	2.54 b	541 b	20.4
M2	100	27.6 c	5.0 c	37.2 c	2.18 c	389 c	42.8
M3	150	21.3 d	3.8 d	28.6 d	1.84 d	241 d	64.6
LSD 0.05	—	2.14	0.48	3.21	0.19	38.4	—

** Means followed by the same letter within a column are not significantly different at $p \leq 0.05$.*

ISSN Onlin:2708 -9347, ISSN Print: 2708 -9339 Volume 15, Issue 1 (2026)

https://iasj.rdd.edu.iq/journals/journal/issue/20226 https://doi.org/10.54174/utjagr.v13ii 2341.

With the rise in salt stress from the control to 150 mM NaCl, the TPC and TFC rose by 73.2% and 85.1%, respectively (Table 3). This upregulation is a core defense response controlled by the induction of the shikimic acid pathway which produces phenylalanine as a major precursor of the biosynthesis of phenolic acids and flavonoids. The enzyme phenylalanine ammonia-lyase (PAL) is responsible for this and is activated in high amounts under salt stress (Benazzouz-Smail et al., 2023).

There was a strong negative correlation (r = -0.98) between phenolic content and seed yield, suggesting a clear metabolic trade-off between two biosynthetic pathways: the growth and productivity pathway versus the defense/secondary metabolism pathway. This trade-off is likely the reason why phytochemical levels tend to rise as yield decreases, a phenomenon that is well-documented in several medicinal plants under various environmental stresses (Al-Harthi et al., 2024). Furthermore, the TPC measured at M3 (31.7 mg GAE/g DW) is within the range reported by Terzo et al. (2025) for N. sativa seeds grown under environmental stress conditions (Table 3).

3.3. HPLC Profiling of Individual Phenolic and Flavonoid Compounds

Table 3: Effect of NaCl salinity on TPC, TFC, DPPH inhibition, and metabolic balance

Treatment	NaCl (mM)	TPC (mg GAE/g DW)	TFC (mg QE/g DW)	DPPH Inhibition (%)	Metabolic Balance Index
M0 (Control)	0	18.3 d	12.1 d	41.2 d	High yield / Moderate medicinal quality
M1	50	23.6 c	16.8 c	52.4 c	Optimal — yield acceptable + quality enhanced
M2	100	28.1 b	19.9 b	61.7 b	Moderate yield loss / High medicinal quality
M3	150	31.7 a	22.4 a	71.3 a	Economically unfeasible / Very high quality
LSD 0.05	—	1.87	1.24	3.15	—

** TPC: Total Phenolic Content — TFC: Total Flavonoid Content — DPPH: 2,2-diphenyl-1-picrylhydrazyl radical scavenging assay.*

Table 4 shows that the concentrations of all identified compounds increased consistently with the salinity level and were highly significant at each salinity level. Chlorogenic acid was found to be the most abundant compound and the most responsive to salt stress (increasing by 156.5% from M_0 to M_3). It is a phenolic acid that is known to be effective for inhibiting \alpha-glucosidase enzyme, which is responsible for the hypoglycemic activity, but also has anti-inflammatory activity due to the suppression pathway (Suwatronnakorn et al., 2025).

University of Thi-Qar Journal of agricultural research

Thi-Qar Journal of agricultural research

ISSN Onlin:2708 -9347, ISSN Print: 2708 -9339 Volume 15, Issue 1 (2026)

https://iasj.rdd.edu.iq/journals/journal/issue/20226 https://doi.org/10.54174/utjagr.v13ii 2341.

In the case of flavonols, quercetin was found to be the most concentrated and medicinally most important compound of N. sativa. Aghajanzadeh & Prajapati (2021) showed elevated concentration of quercetin (0.58 mg/g). The HPLC quantification revealed that salinity stress triggered a progressive and highly significant accumulation of all identified phenolic and flavonoid compounds in Nigella sativa seeds (Table 4). Total identified compounds increased consistently from 4.78 mg/g DW under control conditions (M0) to 13.50 mg/g DW at the highest salinity level of 150 mM NaCl (M3), representing an overall increase of 182.5%.

Among the detected phenolic acids, chlorogenic acid emerged as the most abundant compound across all treatments. Its concentration rose from 1.24 mg/g DW (M0) to 3.18 mg/g DW (M3), showing a substantial increase of 156.5%. This accumulation highlights its pivotal role as a primary antioxidant defense mechanism against salt-induced oxidative stress. Similarly, caffeic acid and rosmarinic acid demonstrated remarkable responsiveness to increasing NaCl concentrations, exhibiting sharp increases of 220.9% and 209.7%, respectively. The up-regulation of these phenolic compounds under stress can be primarily attributed to the activation of the phenylpropanoid biosynthetic pathway, which serves as a core defense strategy in plants to scavenge reactive oxygen species (ROS).

Regarding the flavonoid profile, quercetin concentrations significantly increased from 0.87 mg/g DW in the control to 2.41 mg/g DW at 150 mM NaCl, which is a 177.0% elevation. While previous studies reported lower levels of quercetin in the leaves of plants under a salt stress of 60 mM NaCl, these findings are far exceeded by the results of the present study in the seeds (range 0.87–2.41 mg/g DW). This indicates a significant difference in accumulation between plant tissues, with seeds acting as a stronger metabolic sink for flavonoids under stress. From a medicinal perspective, quercetin's anti-cancer properties are intrinsically associated with its protein kinase inhibitory and pro-apoptotic effects (Mohammed et al., 2025). Furthermore, kaempferol exhibited a similar upward trend, increasing by 209.3% (from 0.54 to 1.67 mg/g DW), further reinforcing the seed's secondary metabolic response.

Rutin is characterized as a glycosylated flavonoid (quercetin-3-O-rutinoside) that enhances resistance against oxidative stress by chelating iron ions and preventing the formation of hydroxyl free radicals (\text{OH}^{\bullet}). Its concentration increased by 177.9%, reaching 1.89 mg/g DW under severe stress. While its elevated levels in N. sativa leaves under salinity have been well-documented in reference literature (Aghajanzadeh & Prajapati, 2021), its high accumulation in seeds confirms a systemic defense response. Conversely, luteolin and naringenin increased by 192.9% and 169.0%, respectively. These represent compounds that are less frequently studied in N. sativa under salt stress, which imparts an additional dimension of novelty to the current study (Ahmed et al., 2022).

.Table (4): HPLC Quantification of Individual Phenolic and Flavonoid Compounds in Nigella sativa L. Seeds (mg/g DW)

Compound	Class	Rt (min)	M0 Control	M1 (50 mM)	M2 (100 mM)	M3 (150 mM)	Increase (%)
Chlorogenic acid	Phenolic acid	12.4	1.24 d	1.89 c	2.61 b	3.18 a	156.5
Caffeic acid	Phenolic acid	16.8	0.43 d	0.71 c	1.02 b	1.38 a	220.9

ISSN Onlin:2708 -9347, ISSN Print: 2708 -9339 Volume 15, Issue 1 (2026)

https://iasj.rdd.edu.iq/journals/journal/issue/20226 https://doi.org/10.54174/utjagr.v13ii 2341.

Rosmarinic acid	Phenolic acid	22.3	0.31 d	0.54 c	0.78 b	0.96 a	209.7
Quercetin	Flavonol	31.6	0.87 d	1.34 c	1.92 b	2.41 a	177.0
Kaempferol	Flavonol	36.2	0.54 d	0.89 c	1.28 b	1.67 a	209.3
Luteolin	Flavone	28.9	0.42 d	0.74 c	1.01 b	1.23 a	192.9
Rutin	Flavonoid glycoside	24.1	0.68 d	1.12 c	1.54 b	1.89 a	177.9
Naringenin	Flavanone	19.7	0.29 d	0.47 c	0.63 b	0.78 a	169.0
Total	—	—	4.78	7.70	10.79	13.50	182.5

** Rt: Retention time. DW: Dry weight. Means followed by different letters within a row are significantly different at $p < 0.05$.*

4. Conclusions

- Graduated salinity stress significantly reduced the yield components of *Nigella sativa* L, with the most severe losses observed at 150 mM NaCl.
- Total phenolic content, total flavonoid content, and antioxidant activity increased progressively with increasing salinity.
- HPLC-DAD analysis confirmed substantial increases in individual metabolites such as chlorogenic acid, quercetin, caffeic acid, kaempferol, and rutin under saline conditions.
- A clear metabolic trade-off was observed between seed productivity and accumulation of phytochemicals.
- The NaCl treatment of 50 mM was the most appropriate one as it had the best impact on medicinal quality with an acceptable seed yield.

5. Recommendations

- In the case of enhancing and improving the phytochemical quality with a tolerable agronomic yield, a salinity level close to 50 mM NaCl is recommended.
- Conventional production system with emphasis on maximum seed yield should avoid higher salinity (100-150 mM NaCl).
- Further research should be conducted on other genotype of Nigella sativa to determine new salt tolerant cultivars that possess better phytochemical properties.
- Salinity stress-induced gene expression, enzyme activity and metabolomic regulation pertaining to biosynthesis pathways of phenolics and flavonoids should be studied further.
- Field trials at multiple locations and multiple seasons are recommended to confirm the current results with
  - different Iraqi agro-ecological conditions.

References:

ISSN Onlin:2708 -9347, ISSN Print: 2708 -9339 Volume 15, Issue 1 (2026)

https://iasj.rdd.edu.iq/journals/journal/issue/20226 https://doi.org/10.54174/utjagr.v13ii 2341.

Aghajanzadeh, T.A. & Prajapati, D.H. (2021). Alterations in leaf anatomy, quality, and quantity of flavonols and photosynthetic pigments in Nigella sativa L. subjected to drought and salinity stresses. Notulae Botanicae Horti Agrobotanici Cluj-Napoca, 49(3): 12398.

Ahmed, M.S. et al. (2022). Phenolics from defatted black cumin seeds (Nigella sativa L.): identification by HPLC-DAD. Antioxidants, PMC9496517.

Benazzouz-Smail, L. et al. (2023). Biological properties, phenolic profile, and botanical characterization of Nigella spp. Molecules, 28: 571.

Mahmoud, A.A. et al. (2023). Effects of salinity on Nigella sativa growth and biochemical traits. Agriculture and Forestry, 69(3): 89–101.

Al-Harthi, S.S. et al. (2024). Effect of salinity on phenolic composition and biological activity of Nigella sativa. Acta Horticulturae, 853: 5.

Hussain, S. et al. (2024). Phytochemical profile, nutritional and medicinal value of Nigella sativa. Industrial Crops and Products, S1878818124003086.

Yousefi, B. et al. (2024). Simultaneous quantification of quercetin and kaempferol in herbal matrices by RP-HPLC. International Journal of Pharmaceutical Sciences and Research.

Mohammed, A.A. et al. (2025). Anticancer potential of thymoquinone from Nigella sativa L. PMC, 12161580.

Suwatronnakorn, M. et al. (2025). Simultaneous quantification of chlorogenic acid, quercetin, and kaempferol by CE and HPLC: validation and comparison. PubMed, 39482892.

Terzo, S. et al. (2025). Black cumin seed (Nigella sativa) anti-adipogenic effects: phenolic and flavonoid profile. Food Science & Nutrition, FSN3.70888.