Journal homepage
www.ajas.uoanbar.edu.iq
Anbar Journal of Agricultural Sciences
(University of Anbar – College of Agriculture)

RESPONSE OF TWO BLACK CUMIN SPECIES TO FOLIAR ORGANIC FERTILIZATION UNDER SEMI-ARID CONDITIONS

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Article info

Received: 2024-05-03
Accepted: 2024-06-02
Published: 2024-06-30

DOI-Crossref:
10.32649/ajas.2024.147535.1157

Cite as:
Ahmed, R. M., Rashid, A. J. M., Mahmood, B. J., and Ahmad, K. R. (2024). Response of two black cumin species to foliar organic fertilization under semi-arid conditions. Anbar Journal of Agricultural Sciences, 22(1): 197-216.

Abstract

Black cumin is a member of the Ranunculaceae family, it is a popular herb called a miracle remedy to treat various diseases. A factorial experiment was conducted in RCBD with three replicates to evaluate the performance of *Nigella spp.* in response to foliar organic fertilizer (ALGAREN TWIN) in three concentrations (Control, 0.4 and 0.8 mL L^{-1}). Seed yield and volatile oil bioactive composition across two consecutive seasons 2021-2022, and 2022-2023 were evaluated under semi-arid conditions with total precipitations of 310.5 and 715 mm respectively. The results of both seasons revealed a significant variation between the two species in most yield component traits. *N. sativa* showed the highest seed yield (737 kg ha^{-1}), fixed oil (25%), and volatile oil (1.163%). The higher application rate of 0.8 mL L^{-1} generally leads to an increase in the yield of *Nigella spp*. seed and its components with 919 kg ha^{-1} seed yield, 26 % fixed oil, and 1.430% volatile oil. The interaction between *N. sativa* and 0.8 mL L^{-1} fertilization recorded a higher seed yield of 1065 kg ha^{-1}, 28% fixed oil, and 1.430% volatile oil. The 2022-2023 season recorded a higher seed yield of 720 kg ha^{-1} than the first season. The analysis of volatile oils showed a mixture of phytochemical compounds. Terpenoid was the predominant constituent in both black cumin species. Thymoquinone was identified as a major constituent in N. sativa (25%). It could be concluded that the use of high rates of foliar organic fertilization on *N. sativa* gave a superior performance in most yield-related characters.

Keywords: Medicinal plants, N. sativa, N. arvensis, Foliar organic fertilizer ALGAREN TWIN, Phytoconstituents, Thymoquinone.

استجابة نوعين من حبة البركة للسماد العضوي الورقي تحت الظروف شبه الجافة

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الخلاصة

حبة البركة، نبات تابع لعائلة Ranunculaceae، وهو عشب معروف يطلق عليه العلاج المعجزة حيث يستخدم لعلاج مختلف الأمراض. أجريت تجربة عاملية بإستخدم تصميم القطاعات العشوائية الكاملة بثلاث مكررات لتقييم أداء أصناف حبة البركة لأستخدام السماد العضوي الورقي (ALGAREN TWIN) بثلاثة تراكيز (المقارنة، 0.4 و0.8 مل لتر$^{-1}$). تم تقييم حاصل البذور والتركيب الحيوي للزيت الطيار خلال موسمين متتاليين 2021–2022 و2022–2023 في ظل ظروف الشبه قاحلة حيث بلغ إجمالي هطول الأمطار 310.5 و715 ملم للموسمين على التوالي. أظهرت نتائج الموسمين وجود إختلافات معنوية بين النوعين في معظم صفات مكونات الحاصل. أعطى *N. sativa* أعلى إنتاجية للبذور 737 كجم هكتار$^{-1}$، والزيت الثابت 25%، والزيت المتطاير 1.163%. إستخدام معدل 0.8 مل لتر$^{-1}$ من السماد العضوي الورقي بشكل عام أدى إلى زيادة إنتاجية البذور ومكوناتها بواقع 919 كجم هكتار$^{-1}$ من محصول البذور و26% زيت ثابت و1.430% زيت طيار. سجل التفاعل بين *N. sativa* و0.8 مل لتر$^{-1}$ أعلى إنتاجية بذور بلغت 1065 كجم هكتار$^{-1}$، و28% زيت ثابت، و1.430% زيت طيار. أعلى إنتاجية بذور قد سجلت في الموسم الثاني حيث بلغت 720 كجم هكتار$^{-1}$ مقارنة بالموسم الأول. أظهر تحليل الزيوت الطيارة وجود خليط من المركبات الكيميائية النباتية. كان Terpenoid هو المكون السائد في كلا النوعين من حبة البركة. تم تحديد الثيموكينون كمكون رئيسي في *N. sativa* (25٪). ويمكن الاستنتاج أن استخدام معدلات عالية من التسميد العضوي الورقي على نبات *N. sativa* أعطى أداءً متفوقاً في معظم الصفات المرتبطة بالحاصل.

كلمات مفتاحية: السماد العضوي الورقي ALGAREN TWIN، النباتات الطبية، N. sativa, N. arvensis المكونات الكيميائية النباتية، ثايموكيونون.

Introduction

The biological activities of most medicinal plant families are always caused by their content of natural bioactive compounds, such as terpenoids, phenols, flavonoids, steroids, tannins, and alkaloids (45). Ranunculaceae is one of the medicinal plant families that is remarkable for having medicinal value and aromatic properties. Black cumin *Nigella spp*. is a member of the Ranunculaceae family, a popular herb called a miracle remedy to treat various diseases such as asthma, inflammation, cough, and headache (19 and 23). It is known as an annual plant and has been used traditionally for a long time as a medicine affecting human health (3). Recent studies have proved that certain herbal supplements have exhibited antiviral activity against similar coronaviruses (2). Black cumin yield and production vary based on neighboring countries due to variations in soil and environmental conditions. It was shown that the highest yield of plant seed was 1250 kg ha^{-1} in Iran (26), while in Turkey and Iraq, the highest yield amounts were 542.3 kg ha^{-1} and 621 kg ha^{-1}, respectively (24 and 40). Nigella seeds consist of 0.5-1.5% volatile oil and 30-35% fixed oil, used as food and in pharmacy (34). The major active constituent of the seed oil is thymoquinone, which is considered to have pharmacological properties (16). Literature also indicated that black cumin extract and seed oil contain many other constituents, including crude fiber, carbohydrates, saponins, fatty acids, minerals, vitamins, and proteins (42). Plant seeds are cultivated mainly in semi-arid regions, like Turkey, India, Egypt, Syria, Pakistan, the Mediterranean, Iran, Saudi Arabia, and southern Europe (43). Because of its flavoring agents, flavor, and aroma, black cumin species are widely used in culinary cuisine and are familiar and widely accepted by consumers. The cultivation of black cumin seeds in Iraq remains limited, therefore, the study of improving and developing this medicinal plant is needed by recognizing and selecting appropriate varieties. The fertilization techniques are also important applications that influence plant growth and overall yield (40). In addition, the study of plant growth management, organic practices, and productivity enhancement are three important keys to progressive advancements for both conventional agriculture and medicinal crop cultivation (37). In plant nutrition, the main important factor is fertilization. Some factors, like application method, amount, and type of fertilizer, directly and indirectly impact plant nutrient availability and physiological and biochemical processes (31). Generally, chemical fertilizers are important and used for the improvement of crop production however if they are used for a long time, they can decrease the benefit of microflora in soil, stop biological systems of soil, pollute water supplies, and change the pH of the soil (15 and 44). Even so, because of the environmental and health benefits of organic production, requesting and asking for these products has recently increased, in particular for the production of pharmacy (15). Recently, organic fertilizers have become increasingly popular as an economical way of meeting the nutrient requirements of crops in sustainable agriculture. Organic fertilizers improve soil fertility and productivity because they contain growth-promoting substances like hormones and enzymes, even though they also contain trace amounts of nutrients. Composting increased the soil's ability to retain water, which improved crop nutrition uptake. Compost also significantly improves the root cover conditions (structure, moisture, etc.), which in turn increases the number of microorganisms in the root cover and promotes plant growth (5 and 39). Additionally, medicinal plant cultivation has been enlarged with organic soil application (10, 15 and 36). Several studies concluded that foliar seaweed stimulates both primary and secondary metabolisms in plants through nutrient uptake and assimilation improvement. It can also promote the accumulation and synthesis of phytochemicals, enhance tolerance to abiotic stressors, and increase crop yields (12 and 35). Despite the favorable environmental conditions in our region for the production of black cumin, the study area of plants has only been practiced. Farmers were not provided with recommendations regarding suitable varieties, nor were they presented with information identifying such varieties for adoption. Based on our region, limited data on black cumin is available concerning the influence of foliar ALGAREN TWIN fertilization on black cumin oil yield, phytochemical constituents, and seed oil contents. Therefore, the main aim of this present study was to determine the effect of foliar ALGAREN TWIN fertilizer on the variation of the yield, fixed oil, volatile oil, and phytochemical constituents of common black cumin species to investigate the sustainability and economic viability of foliar organic fertilization as a cultivation practice in semi-arid regions.

Materials and Methods

Plant materials: Two plant species of black cumin (*N. sativa* and *N. arvensis*) were used in this current investigation; both species were received from (PAKAN BAZR) a company of seed registration in Iran 2020 as shown in Figure 1.

N. sativa L. (Flowers)

N. sativa L. (Capsules and Seeds)

N. arvensis L. (Flowers)

N. arvensis L. (Capsules and Seeds)

Figure 1: *Nigella spp*. flowers, capsules, and seeds.

Field experiment: A factorial experiment 2×3 within 3 replications using CRBD was carried out in Qlyasan Agricultural Research Station under rainfed conditions (Lat. 35^{o} 34' 307"; N, Long. 45^{o} 21' 992"; E, 765 MASL, 2 km northwest of Sulaimani city) during the successive seasons 2021-2022 and 2022-2023 to examine the effect of different levels of ALGAREN TWIN as foliar application compared to control.

Agriculture conditions: In autumn 2021 and 2022, seeds from both species of black cumin were directly planted in 3 m^2 plots. The soil was primed for cultivation by pre-irrigating the field before plowing with a harrow and broad plow in the fieldwork of this study. Weeds were manually controlled whenever necessary. The metrological data for successive years were presented in Figure 2, obtained from (the Ministry of Agriculture and Water Resource).

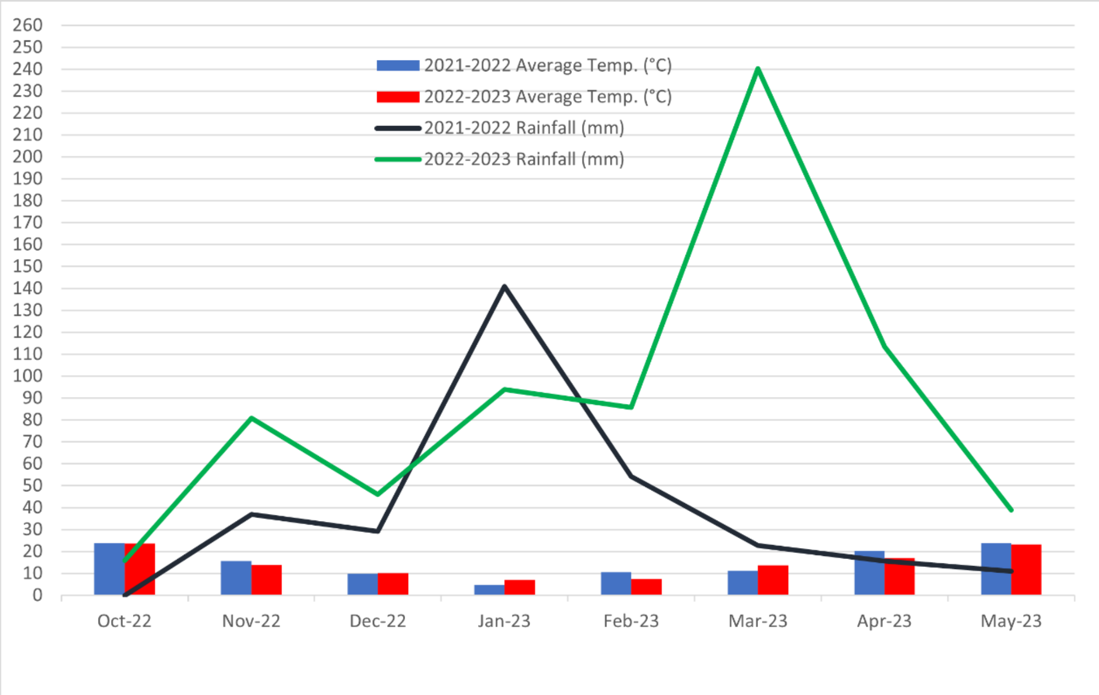


Figure 2: Metrological data of growing seasons 2021-20222 and 022-2023 at Qlyasan.

Fertilizer treatments: Three different levels of liquid organic fertilizers, ALGAREN TWIN, were used which was a mixture of *Ecklonia maxima*, a brown seaweed, and bioactive yeast extract), it consists of organic nitrogen 2.3% w/v, organic carbon 16.1% w/v, and organic matter 34.5% w/v): 0.0 (control), 0.4 and 0.8 mL L^{-1}. When the plants germinated, the foliar was sprayed at three different growth stages seedling, branching, and blooming (the amount of application is 1.5–2 L ha^{-1} for the field crops in general). The soil was also analyzed in Table 1 for the studied area (5).

Experimental parameters: The characters of plant height in cm (PH), number of seeds per capsule (NSC), number of branches per plant (NBP), 1000 seed weight in g (TSW), number of capsules per plant (NCP), and seed yield in kg ha^{-1} (SY) were calculated.

Table 1: Soil analysis of the Qlyasan location.

Soil properties	Qlyasan
% Sand	10.64
% Silt	45.15
% Clay	44.21
Texture	Clay
EC d S m^{-1} at 25°C	0.7
PH	7.85
N %	0.19
Organic matter %	1.25

Volatile oil extraction: To estimate the volatile oil in percentage (VO), 100 g of *Nigella spp*. seed powders were placed into hydro-distillation using a Clavenger-type apparatus (European pharmacopeia) and extracted with 1 L of distillate water for 3 hours. The volatile oil underwent separation from the aqueous phase using anhydrous sodium sulfate and subsequently was subjected to storage at a temperature of 0°C to facilitate subsequent analysis (1 and 28).

Fixed oil content: To calculate the fixed oil in percentage (FO), 20 g of the *Nigella sp.* seed powders from each treatment combination was used for fixed oil extraction using a Soxhlet apparatus and hexane solvent (BDH, UK). The percentage of oil was then determined (20 and 30)

Bioactive components analysis: Agilent Technologies 7890A gas chromatography with a detector of mass selective and an Agilent Technologies 5975C inert XL MSD mass spectrometer was used to analyze the volatile oil of *Nigella spp.* plant samples. The Agilent 190915-433:325°C (30 m × 250 μm × 0.25 μm) GC-MS column was designed to start at 35 °C and increase by 10 °C per minute to a maximum temperature of 280 °C (46). The injector port was set to 290 °C, the temperature of the heater front inlet was 280 °C and helium as the carrier gas was utilized at a 1 mL/min flow rate. The injection mode was split at a ratio of 20:100. At 70 eV, mass spectrometry was also performed in the electron impact mode (EI). The percentages of the phytochemical components were also calculated using the normalization of the GC peak areas, the software automatically normalized the peaks by dividing each peak area by the total area and then multiplying by 100. Using the Wiley Library, the relative proportions of the constituents were calculated (7).

Statistical analysis: A factorial experiment was conducted in two-way ANOVA in triplicate. For the comparison between mean values, the LSD test ($p \leq 0.05$ and $p \leq 0.01$) was performed. SPSS software was utilized to analyze the data, calculating average values and standard deviation (averages ± SD).

Results and Discussion

Table 2 illustrates *Nigella sp.*'s average seed yield and associated traits across seasons. The 2021–2022 season results indicate highly significant species-related variations ($p \leq 0.01$) in PH, NBP, NCP, NSC, TSW, FO%, and VO %. The SY also showed significant differences ($p \leq 0.05$) between species. *N. arvensis* exhibited higher values than *N. sativa* for PH and NCP, with increases of 108.69% and 11.59%, respectively. Conversely, *N. sativa* outperformed *N. arvensis* in terms of NBP, NSC, TSW, SY, FO %, and VO% showing increases of 21.95%, 15.46%, 13.59%, 19.38%, 11.95%, and 16.69%, respectively.

In 2022–2023, a notable species effect was observed at p≤0.01 level for various traits including PH, NBP, NSC, TSW, and FO%. Conversely, the significance level was slightly lower, at p≤0.05, for SY. However, no significant disparity between the two species was noted for the NCP and VO%. Notably, *N. arvensis* exhibited a pronounced superiority over *N. sativa* in PH and NCP by 116.43% and 9.36%. In contrast, *N. sativa* demonstrated superiority over *N. arvensis* in terms of NBP, NSC, TSW, SY, FO, and VO%, with percentages of 23.52%, 15.11%, 24.14%, 33.85%, 16.47%, and 14.17%, respectively.

Furthermore, concerning the average across both seasons, the influence of species proved highly significant at p≤0.01 for all the examined traits, except NCP, which was significant at p≤0.05. *N. arvensis* demonstrated superiority over *N. sativa* in terms of PH and NCP, surpassing them by 112.42% and 10.44%, respectively. Conversely, *N. sativa* outperformed *N. arvensis* in the NBP, NSC, TSW, SY, FO%, and VO%, with values of 22.83%, 15.27%, 18.72%, 27.06%, 14.18%, and 15.38%, respectively. Overall, *N. sativa* exhibited greater performance than *N. arvensis* across most yield component traits, except for PH and the NCP.

Table 2: The means of seed yield and its components traits of *Nigella spp*.

Species	PH (cm)	NBP	NCP	NSC	TSW (g)	SY (Kg ha^{-1})	FO (%)	VO (%)
2021/2022 Season								
N. sativa	42.222	5.556	3.286	58.128	3.258	650.746	25.769	1.084
N. arvensis	88.111	4.556	3.667	50.344	2.868	545.082	23.018	0.929
LSD (p≤0.05)	2.973	0.683	0.243	2.005	0.128	79.453	1.195	0.081
LSD (p≤0.01)	4.229	0.971	0.345	2.852	0.182	n.s	1.700	0.115
2022/2023 Season								
N. sativa	39.222	7.000	3.556	65.356	3.399	824.502	26.121	1.241
N. arvensis	84.889	5.667	3.889	56.778	2.738	616.002	22.428	1.087
LSD (p≤0.05)	2.578	0.836	n.s	1.786	0.305	150.663	1.185	n.s
LSD (p≤0.01)	3.666	1.189	n.s	2.540	0.435	n.s	1.686	n.s
Average of both Seasons								
N. sativa	40.722	6.278	3.421	61.742	3.328	737.624	25.945	1.163
N. arvensis	86.500	5.111	3.778	53.561	2.803	580.542	22.723	1.008
LSD (p≤0.05)	0.944	0.497	0.292	1.128	0.063	56.742	0.476	0.086
LSD (p≤0.01)	1.288	0.678	n.s	1.538	0.086	77.398	0.650	0.118

The impact of applying foliar organic fertilization on *Nigella sp*. traits' yield components and seed yield is presented in Table 3. The data obtained indicate a highly significant influence of the fertilizer at p≤0.01 across all examined traits for both seasons and their respective averages. In the 2021-2022 season, the application of 0.8 mL L^{-1} significantly outperformed both the control and 0.4 mL L^{-1} treatments in all examined traits, showing increases of 25.94%, and 12.34% for PH, 50%, and 29.18% for the NBP, 45.29%, and 20% for the NCP, 21.04% and 12.20% for NSC, 32.81% and 20.58% for TSW, 136.35% and 62.18% for SY, 15.46% and 7.45% for FO%, and 53.51% and 24.94% for VO%, respectively. The 0.4 mL L^{-1} treatment also exhibited superiority over the control treatments across all traits, showing increases of 12.1%, 29.18%, 20%, 12.2%, 20.58%, 62.18%, 7.45%, and 24.94%, respectively.

In the 2022-2023 season, notable variations were observed attributable to the utilization of foliar organic fertilizer. The highest recorded values for all examined traits were achieved with a fertilizer concentration of 0.8 mL L^{-1}, while the lowest values were noted in the control. The application of 0.8 mL L^{-1} exhibited superior effects compared to both 0.4 mL L^{-1} and the control in terms of PH by 22.26%, and 11.96% for the NBP 84.63% and 19.9 for NCP 58.84% and 17.40%, for NSC 1.14% and 3.33%n, for TSW 29.32% and 8.75%, for SY 132.50% and 32.18% for FO 25.06% and 12.48% and for VO 54.48% and 26.39%, respectively. Furthermore, the use of 0.4 mL L^{-1} fertilizer resulted in higher values for all studied traits compared to the control 9.2 %, 53.87 %, 35.3 %, 8.52 %, 18.92 %, 75.89 %, 11.18 %, and 22.22 %, respectively.

Across both seasons, the application of 0.8 mL L^{-1} was consistently associated with the highest values for all studied traits. Specifically, the application of 0.8 mL L^{-1} resulted in higher values for PH (12.15, 24.12 %), NBP (18.306, 7.99 %), NCP (19.12, 52.04 %), NSC (5.46, 16.24 %), TSW (9.46, 31.1 %), SY (38.12, 134.26 %), FO (9.95, 20.14 %), and VO (24.69, 54.06 %) compared to the application of 0.4 mL L^{-1} and the control. Conversely, the addition of 0.4 mL L^{-1} fertilizer significantly increased all studied traits compared to the control fertilizer (10.67 %, 42%, 27.64 %, 10.22%, 19.76 %, 69.6 %, 9.27 %, and 23.55%, respectively).

Table 3: The means of seed yield and its component traits are affected by foliar organic fertilizer (F.O.F.).

F. O. F. (mL L^{-1})	PH (cm)	NBP	NCP	NSC	TSW (g)	SY (Kg ha^{-1})	FO (%)	VO (%)
2021/2022 Season								
Control	57.833	4.000	2.855	48.825	2.600	359.810	22.663	0.798
0.4	64.833	5.167	3.426	54.783	3.135	583.528	24.352	0.997
0.8	72.833	6.000	4.148	59.100	3.453	850.403	26.166	1.225
LSD ($p\leq0.05$)	3.641	0.836	0.297	2.456	0.157	97.310	1.464	0.099
LSD ($p\leq0.01$)	5.179	1.189	0.423	3.493	0.223	138.412	2.082	0.141
2022/2023 Season								
Control	56.167	4.333	2.833	57.133	2.643	425.026	21.658	0.927
0.4	61.333	6.667	3.833	62.000	3.143	747.566	24.080	1.133
0.8	68.667	8.000	4.500	64.067	3.418	988.165	27.085	1.432
LSD ($p\leq0.05$)	3.157	1.024	0.730	2.187	0.374	184.524	1.451	0.212
LSD ($p\leq0.01$)	4.490	1.456	1.039	3.111	0.532	262.465	2.065	0.302
Average of both Seasons								
Control	57.000	4.167	2.844	52.979	2.621	392.418	22.161	0.862
0.4	63.083	5.917	3.630	58.392	3.139	665.547	24.216	1.065
0.8	70.750	7.000	4.324	61.583	3.436	919.284	26.625	1.328
LSD ($p\leq0.05$)	1.157	0.609	0.357	1.381	0.077	69.494	0.584	0.106
LSD ($p\leq0.01$)	1.578	0.830	0.487	1.884	0.105	94.793	0.796	0.144

Table 4 delineates the interactive impact between species and foliar organic fertilizer on black cumin's yield components and seed yield across both seasons and their respective averages. In the initial season, the interaction effect achieved significance at $p\leq0.05$ solely for PH, with insignificance observed for other traits. Notably, the interaction involving *N. arvensis* and the application rate of 0.8 mL L^{-1}

yielded the highest recorded PH at 96.667 cm, contrasting with the minimum of 37.667 cm resulting from the interaction of *N. sativa* and the control. Similarly, the interaction between *N. sativa* and the 0.8 mL L^{-1} application demonstrated superior performance, yielding the highest values for TSW, FO%, SY, and VO%, recording at 3.710 g, 972.773 kg h^{-1}, 26.805%, and 1.350%, respectively. Conversely, the lowest values were observed for *N. arvensis* and the control across these traits, recording 2.487 g, 343.425 kg h^{-1}, 20.500%, and 0.729%, respectively.

In the subsequent season 2022-2023, the combined effect of species and fertilizer did not exhibit any significant impact on the studied traits, except for TSW, which maintained significance at $p \leq 0.05$. The interaction between *N. sativa* and 0.8 mL L^{-1} fertilizer yielded the maximum values for TSW 3.920g, SY 1158.780 kg h^{-1}, FO 29.927%, and VO 1.510%. Conversely, the minimum values were observed with *N. arvensis* and the control level, with 1000 seed weight at 2.602g, SY at 416.269 kg h^{-1}, FO at 20.130%, and VO at 0.857%.

Across both seasons, the interaction effect between species and foliar organic fertilizer significantly impacted PH, TSW, and SY ($p \leq 0.01$). The highest PH 93.500 cm was recorded with the interaction between *N. arvensis* and 0.8 mL L^{-1}, while the lowest 35.833 cm occurred with the interaction between *N. sativa* and the control level. Conversely, the interaction between *N. sativa* and 0.8mL L^{-1} resulted in maximum values for TSW 3.815 g, SY 1065.776 kg h^{-1}, FO 28.366%, and VO 1.430%. Conversely, the lowest values for TSW 2.545 g, SY 379.847 kg h^{-1}, FO 20.315%, and VO 0.793% were obtained with the interaction between *N. arvensis* and the control level.

Table 4: The means of seed yield and its component traits are affected by the interaction between *Nigella spp*. and foliar organic fertilizer.

Species	Foliar Organic Fertilizer (mL L^{-1})	PH (cm)	NBP	NCP	NSC	TSW (g)	SY (Kg ha^{-1})	FO (%)	VO (%)
2021/2022 Season									
N. sativa	**Control**	37.667	4.333	2.637	52.583	2.713	376.195	24.827	0.867
	0.4	40.000	5.667	3.110	57.933	3.350	603.269	25.677	1.037
	0.8	49.000	6.667	4.111	63.867	3.710	972.773	26.805	1.350
N. arvensis	**Control**	78.000	3.667	3.073	45.067	2.487	343.425	20.500	0.729
	0.4	89.667	4.667	3.742	51.633	2.920	563.787	23.027	0.957
	0.8	96.667	5.333	4.184	54.333	3.197	728.033	25.527	1.100
LSD ($p\leq0.05$)		5.149	n.s	n.s	n.s	n.s	n.s	n.s	n.s
LSD ($p\leq0.01$)		n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s
2022/2023 Season									
N. sativa	**Control**	34.000	5.000	2.667	60.933	2.683	433.782	23.187	0.997
	0.4	36.667	7.333	3.667	67.000	3.592	880.945	25.250	1.217
	0.8	47.000	8.667	4.333	68.133	3.920	1158.780	29.927	1.510
N. arvensis	**Control**	78.333	3.667	3.000	53.333	2.602	416.269	20.130	0.857
	0.4	86.000	6.000	4.000	57.000	2.694	614.187	22.910	1.050
	0.8	90.333	7.333	4.667	60.000	2.917	817.550	24.243	1.353
LSD ($p\leq0.05$)		n.s	n.s	n.s	n.s	0.529	n.s	n.s	n.s
LSD ($p\leq0.01$)		n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s
Average of both seasons									
N. sativa	**Control**	35.833	4.667	2.652	56.758	2.698	404.989	24.007	0.932
	0.4	38.333	6.500	3.388	62.467	3.471	742.107	25.463	1.127
	0.8	48.000	7.667	4.222	66.000	3.815	1065.776	28.366	1.430
N. arvensis	**Control**	78.167	3.667	3.037	49.200	2.545	379.847	20.315	0.793
	0.4	87.833	5.333	3.871	54.317	2.807	588.987	22.968	1.003
	0.8	93.500	6.333	4.426	57.167	3.057	772.792	24.885	1.227
LSD ($p\leq0.05$)		1.636	n.s	n.s	n.s	0.109	98.280	n.s	n.s
LSD ($p\leq0.01$)		2.231	n.s	n.s	n.s	0.149	134.058	n.s	n.s

The impacts of seasonal variations on the observed traits have been illustrated in Table 5. Highly significant effects were observed at a level of $p\leq0.01$ for traits such as PH, the NBP, and the NSC. Conversely, variables such as SY and VO% exhibited significant responses at a $p\leq0.05$ concerning location, while the effect was found to be insignificant for traits including the NCP, TSW, and FO%. In 2021-2022, traits such as PH, and FO% were notably predominant, accounting for 5.01%, and 0.49% respectively. In the subsequent season of 2022-2023, traits such as NBP, NCP, NSC, TSW, SY, and VO% accounted for 25.26%, 7.08% 12.59%,0.163%, 20.46, and 15.59% respectively.

Table 5: The means of seed yield and its components characters are affected by different seasons.

Seasons	PH (cm)	NBP	NCP	NSC	TSW (g)	SY (Kg ha^{-1})	FO (%)	VO (%)
2021/2022	65.167	5.056	3.476	54.236	3.063	597.914	24.394	1.007
2022/2023	62.056	6.333	3.722	61.067	3.068	720.252	24.274	1.164
LSD $_{(p\leq0.05)}$	0.488	0.308	n.s	0.705	n.s	119.191	n.s	0.096
LSD $_{(p\leq0.01)}$	0.809	0.512	n.s	1.170	n.s	n.s	n.s	n.s

Analysis of bioactive components: The analysis of *N. sativa* volatile oil by gas chromatography-mass spectra in the 2021-2022 season indicated fifteen compounds. Terpenoids were the main components, which consist of 66.667% of compounds, and monoterpenes were made of 26.667% of total terpenoids, including α-Thujene, α-Pinene, β-Pinene, and p-Cymene, as shown in Table 6. At the same time, the GC mass analysis did not detect any sesquiterpene. The highest portion of terpenoids was oxygenated monoterpenes (40%) with the structure $C_{10}H_nO$ (n=$_{12}$, $_{14}$, $_{16}$, and $_{18}$) as shown in peaks no. 6, 7, 8, 9, 10, and 12 represented as Fenchone, Terpinen-4-ol, Nerol, Estragole, Carvone, and Trans-Anethole, respectively. Thymoquinone is the major constituent of *Nigella*, and it was the most abundant chemical constituent (18.367%) as shown in peak no. 11. The compositions of the volatile oils extracted from *N. sativa* seeds in the 2022- 2023 season. The results show that the volatile oil composition is relatively similar to the 2021-2022 season. Out of the twelve compounds identified, 50% were total terpenoids, with 33.333% being monoterpenes, including α-Thujene (5.02%), α-Pinene (2.023%), β-Pinene (3.008), and p-Cymene (18.57%). Oxygenated monoterpene, D-fenchone, and dihydrocarvone accounted for 16.667% of the total area. Additionally, other aromatic compounds accounted for 50% of the total area.

Table 6: Bioactive components of *N. sativa* volatile oil in both seasons.

Peaks No.	Compound Names	Molecular Formula	R. time	Area (%)
2021/2022 Season				
1	α-Thujene	$C_{10}H_{16}$	2.611	2.103
2	a-Pinene	$C_{10}H_{16}$	5.00	4.154
3	b-Pinene	$C_{10}H_{16}$	5.93	3.154
4	n-Decane	$C_{10}H_{22}$	7.531	0.423
5	p-Cymene	$C_{10}H_{14}$	10.058	11.04
6	D-Fenchone	$C_{10}H_{16}O$	10.584	10.018
7	Terpinen-4-ol	$C_{10}H_{18}O$	11.095	3.017
8	Nerol	$C_{10}H_{18}O$	11.405	5.01
9	Estragole	$C_{10}H_{12}O$	13.86	4.06
10	Carvone	$C_{10}H_{14}O$	15.942	6.066
11	Thymoquinone	$C_{10}H_{12}O_2$	16.107	18.367
12	Trans-Anethole	$C_{10}H_{12}O$	17.243	5.215
13	9-Octadecenoic acid, methyl ester, (E)-	$C_{19}H_{36}O_2$	19.755	5.961
14	9-Octadecenoic acid, (E)-	$C_{18}H_{34}O_2$	21.908	6.034
15	Uvidine	$C_{15}H_{24}O_3$	22.598	5.377
2022/2023 Season				
1	α-Thujene	$C_{10}H_{16}$	2.64	5.02
2	α-Pinene	$C_{10}H_{16}$	2.847	2.023
3	β-Pinene	$C_{10}H_{16}$	3.322	3.008
4	n-Decane	$C_{10}H_{22}$	6.001	7.078
5	*p*-Cymene	$C_{10}H_{14}$	7.274	18.57
6	D-Fenchone	$C_{10}H_{16}O$	7.541	11.145
7	Dihydrocarvone	$C_{10}H_{16}O$	10.538	4.005
8	Thymoquinone	$C_{10}H_{12}O_2$	14.051	25.34
9	1-Naphthalenol, 4-methoxy-	$C_{11}H_{10}O_2$	17.025	0.413
10	Formic acid, 3,7,11-trimethyl-1,6,10-dodecatrien-3-yl ester	$C_{16}H_{26}O_2$	21.461	2.627
11	Myristicin	$C_{11}H_{12}O_3$	21.645	6.604
12	Apiole	$C_{12}H_{14}O_4$	24.272	11.993

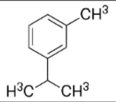
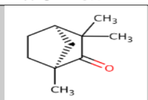
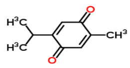
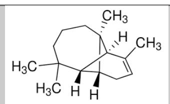
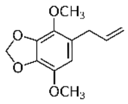
Data obtained from GC-MS analysis of *N. arvensis* volatile oilseeds in 2021-2022 are presented in Table 7. The total terpenoid composition was found to be 68.75%. The monoterpenes, including α-Pinene, Sabinene, β-Pinene, Myrcene, α-Phellandrene, *p-cymene*, D-Limonene, Limonene, D-Fenchone, 2,4,6-octatriene, 2,6-dimethyl-, (E, Z), made up 56.25% of the total area. Meanwhile, the oxygenated monoterpene D-Fenchone constituted 6.25% of the total area. Additionally, the α-Longipinene components of the volatile oil made up 6.25% of the total area, representing oxygenated sesquiterpene. Additionally, the aromatics and other components of the volatile oil made up 31.25% of the total area. The results of the GC-MS analysis conducted on volatile oil extracted from *N. arvensis* seeds in the 2022-2023 season. The analysis identified fourteen compounds, with terpenoids being the most abundant (85.714%) of the total area. Among all terpenoids, 64.286% were monoterpenes, with Anethole being the most prevalent (50.234%), followed by Estragole (6.259%). Only limonene was detected as a monoterpene (1.384%). Sesquiterpene made 19.241% of the total terpenoids, while, isolongifolene was the most abundant (12.476%). Furthermore, the diterpene (Eicosyne) was detected (8.896%). The remaining 13.986% of the total essential compounds comprised phenol and other aromatic compounds.

Table 7: Bioactive components of *N. arvensis* volatile oil in both seasons.

Peaks No.	Compound Names	Molecular Formula	R. time	Area (%)
2021/2022 Season				
1	3-Methyl Nonane	$C_{10}H_{22}$	2.625	1.158
2	α-Pinene	$C_{10}H_{16}$	3.59	1.104
3	Sabinene	$C_{10}H_{16}$	4.697	1.035
4	β-Pinene	$C_{10}H_{16}$	5.299	2.161
5	Myrcene	$C_{10}H_{16}$	5.995	1.476
6	α-Phellandrene	$C_{10}H_{16}$	7.261	3.057
7	*p*-Cymene	$C_{10}H_{14}$	7.524	18.097
8	D-Limonene	$C_{10}H_{16}$	10.084	5.534
9	Limonene	$C_{10}H_{16}$	10.542	5.038
10	D-Fenchone	$C_{10}H_{16}O$	11.139	10.304
11	2,4,6-Octatriene, 2,6-dimethyl-, (E, Z)-	$C_{10}H_{16}$	11.451	3.144
12	Thymoquinone	$C_{10}H_{12}O_2$	12.264	12.189
13	Benzeneacetic acid, α-hydroxy-4-methoxy-	$C_{10}H_{12}O_4$	14.042	1.5
14	α-Longipinene	$C_{15}H_{24}$	16.96	10.990
15	9,12-Octadecadienoic acid (Z, Z)-, methyl ester	$C_{19}H_{34}O_2$	22.636	4.106
16	*n*-Hexadecane	$C_{16}H_{34}$	25.359	11.507
2022/2023 Season				
1	3-Methyl Nonane	$C_{10}H_{22}$	3.317	5.017
2	a-Pinene	$C_{10}H_{16}$	3.595	4.063
3	Sabinene	$C_{10}H_{16}$	4.704	4.021
4	b-Pinene	$C_{10}H_{16}$	6.001	1.283
5	Myrcene	$C_{10}H_{16}$	6.805	4.011
6	a-Phellandrene	$C_{10}H_{16}$	7.269	5.059
7	*p*-Cymene	$C_{10}H_{14}$	7.53	10.142
8	Ethyl-2,3-dimethyl benzene-1	$C_{10}H_{14}$	8.748	7.07
9	Limonene	$C_{10}H_{16}$	9.242	4.051
10	Terpinen-4-ol	$C_{10}H_{18}O$	10.533	3.029
11	2,4,6-Octatriene, 2,6-dimethyl-, (E, Z)-	$C_{10}H_{16}$	11.449	7.052
12	Thymoquinone	$C_{10}H_{12}O_2$	14.021	20.17
13	Benzeneacetic acid, α-hydroxy-4-methoxy-	$C_{10}H_{12}O_4$	16.9	3.871
14	α-Longipinene	$C_{15}H_{24}$	21.863	10.292

The major constituent variation of black cumin species' volatile oil in successive seasons is summarized in Table 8. P-Cymene was present in both black cumin species' volatile oils. The highest amount was exhibited by *N. sativa* with 18.57±0.392 in the second season, and the lowest was obtained by *N. arvensis* with the amount of 10.142±0.160. A higher amount of D-fenchone was detected in *N. sativa* with 11.145±0.070 in the second season, while this compound was not detected in *N. arvensis* in the second season. Thymoquinone was found as a major bioactive compound in black cumin volatile oil, *N. sativa* exhibited the maximum amount with 25.34±0.400 in the second season, while the lowest was observed in *N. arvensis* with 12.189±0.056 in the first season. α-Longipinene was only detected in *N. arvensis* in both seasons. The GC-MS analysis also detected Apiol only in *N. sativa*, and *n*-Hexadecane in *N. arvensis*.

Table 8: The major bioactive components of *Nigella spp.* volatile oil in the 2021/2022 and 2022/ 2023 seasons.

Components	Chemical Structures	*N. sativa* volatile Area (%) Mean/SD		*N. arvensis* volatile Area (%)	
		2021-2022	2022-2023	2021-2022	2022-2023
***p*-Cymene**		11.04±0.078	18.57±0.392	18.097±0.039	10.142±0.160
D-Fenchone		10.018±0.235	11.145±0.070	10.304±0.159	/
Thymoquinone		18.367±0.095	25.34±0.400	12.189±0.056	20.17±0.047
α-Longipinene		/	/	10.990±0.100	10.292±0.171
Apiole		/	11.993±0.102		/
***n*-Hexadecane**		/	/	11.507±0.162	/

Discussions: Data indicated that *N. sativa* surpassed *N. arvensis* in most of the yield components, except for the no. of capsule plant^{-1} and plant height characters in both years and their average. Whereas *N. sativa* exhibited a higher no. of branches plant^{-1}, number of seeds plant^{-1}, no. of capsules plant^{-1},1000 seeds weight, which resulted in higher total seed weight, fixed oil %, and volatile oil% in successive seasons. The black cumin plant height varied significantly between studies, ranging from 27.9 cm to 95.1 cm (17 and 43). Plant morphology is a characteristic associated with plant genotype and is readily influenced by agricultural practices and ecological differences in growth environments. Therefore, it is reasonable to expect variations in plant morphology across various ecological and soil conditions with various applications of fertilizer (33). The same results were obtained by the studies conducted by (29 and 30), who reported that *N. sativa* exceeded *N. arvensis* in most of the yield component characters, seed yield, fixed oil, and volatile oil yield.

Due to their capacity to enhance vegetable crop performance, mineral uptake, and resistance to mineral stress, recently ALGAREN TWIN is known as a plant biostimulant (11 and 13) Throughout two years of investigation, both black cumin species yield component characters responded significantly to foliar organic fertilizer at the level of 0.8 mL L^{-1}, which resulted in a higher seed yield, fixed oil %, and volatile oil%. (38) reported that the yield component characteristics of black cumin significantly responded to the application of fertilizer. (6) also, reported that black cumin crops treated with nitrogen fertilizer resulted in a greater yield component. The application of both organic and chemical fertilization resulted in a higher no. of capsules plant^{-1}, no. of seeds plant^{-1}, no. of branches plant^{-1}, and 1000 seed weight (29 and 30). Studying different varieties of black cumin with the application of foliar fertilization gave significant increases in growth and yield attributes (9). In an investigation on black cumin, the result indicated a significant increase in growth and seed production and fixed and volatile oil when the plant was treated with foliar spray of seaweed extract (21).

The results of both seasons indicated that the 2022- 2023 season surpassed the 2021-2022 season in most of the yield components, seed yield, fixed oil %, and volatile oil. This may be due to the amount and distribution of rainfall during the growing period in the second season (Figure 1). Rainfall in 2022–2023 enhances seed production because plants' ability to absorb nutrients is increased by the soil's sufficient moisture content. This is in agreement with those detected by (24 and 25), who reported that seed yield component characters varied depending on the climatic condition of the year.

The GC-MS analysis indicated the black cumin species' volatile oil extract in successive seasons. Terpenoid was the main component, and D-fenchone, thymoquinone, and P-cymene were the major bioactive constituents (8 and 21). As a result, the most volatile oil bioactive composition was detected in *N. sativa* compared to *N. arvensis*. The variation in qualitative and quantitative characteristics of black cumin volatile oil could be related to the variation in macro- and microclimatic conditions. Similarly, (27 and 32) confirmed that the variation in the black cumin volatile oil composition was affected by plant genotype, growth conditions, environmental and climatic conditions, and the methods of extraction. Regarding the volatile oil bioactive component from the genus *Nigella*, particularly *N. arvensis*, the composition closely resembles that of *N. sativa* more than any other species within the genus (22). An investigation by (18) also reported that among the 31 compounds detected in *N. sativa* volatile oil, terpenoids were the main components. A higher amount of the major components, p-cymene, and thymoquinone were obtained. Furthermore, (4 and 41) revealed that thymoquinone, p-cymene, longifolene, α-thujene, α, β-pinene, and carvacrol were found as major compounds of *N. sativa*. Another study by (22) reported that the volatile oil analysis of *N. arvensis* detected 69 compounds, of which monoterpene was the main component. The most abundant components were α, β-pinene, carvacrol, and *n*-undecane. (14) reported the main constituents identified in the *Nigella* essential oils were p-cymene (45.26%) and thymoquinone (35.35%). The GC-MS analysis of black cumin essential oil detected p-cymene, γ-Terpinene, β-Thujene, trans-4-methoxy thujene, and Longifolene as the main constituents (21).

Conclusions

The results concluded that this study highlights the importance of species selection and fertilization strategies in optimizing black cumin seed yield and quality. *N. sativa* demonstrated superior performance in most yield-related characters, making it a preferred choice for cultivation. Foliar organic fertilization proved to be an effective agronomic practice for enhancing yield components in black cumin cultivation, with a higher application rate leading to significant improvement in various characteristics. The interaction between black cumin species and organic foliar fertilization underscored the importance of tailored management practices for maximizing yield potential. The composition analysis of volatile oils provided valuable insight into the chemical profile of black cumin seeds. Terpenoids and other aromatic compounds were found as major constituents. These findings contributed to a better understanding of the factors influencing black cumin cultivation and offer practical recommendations for enhancing productivity and quality in commercial production systems.

Supplementary Materials:

No Supplementary Materials.

Author Contributions:

Author R. M. Ahmad; methodology, writing—original draft preparation, Author A. J. M. Rashid, Author B. J. Mahmood and Author K. R. Ahmad writing—review and editing. All authors have read and agreed to the published version of the manuscript.

Funding:

This research received no external funding.

Institutional Review Board Statement:

Non.

Informed Consent Statement:

No Informed Consent Statement.

Data Availability Statement:

No Data Availability Statement.

Conflicts of Interest:

The authors declare no conflict of interest.

Acknowledgments:

The authors are thankful for the help of the Department of Biotechnology and Crop Science, College of Agricultural Sciences, University of Sulaimani for their valuable help and technical assistance in conducting this research.

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