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Combined effect of biostimulants and potassium humate on the growth and yield characteristics of black seed plant

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ABSTRACT

To test the influence of biostimulants and potassium humate on growth and yield traits of black cumin cultivated on soil obtained from an agricultural station in Al-Shafi'iyah, Diwaniyah Governorate, Iraq, at a depth of 0-30 cm, the experiment was carried out in a randomized complete block design (RCBD) as a two-factor factorial experiment with three replications. The pots were irrigated just one time before sowing. Planting was done with local black cumin seeds received from an agricultural office in Sulaymaniyah Governorate on October 16, 2022. Seeds were 10 in number, and 5 were thinned after germination. The first factor of the experiment was biostimulants applied at three levels denoted by (A0, A1, and A2), and the second was potassium humate supplied at four levels, denoted by (HK0, HK1, HK2, and HK3). Results of the experiment are as follows: - Among the studied growth and yield traits (plant height, number of flowers per plant, number of seeds per capsule, 1000-seed weight, and percentage of oil in the grains), the binary combination of biostimulants and potassium humate gave the best results. The binary combination A2HK2 gave the highest values of these traits with 58.88 cm plant^{-1}, 29.55 flowers plant^{-1}, 97.05 seeds Wallet^{-1}, 75.17 g, and 1.653% in comparison to the control treatment, which gave the lowest values as 32.96 cm plant^{-1}, 13.92 flowers plant^{-1}, 70.85 seeds Wallet^{-1}, 41.08 g, and 0.803%.

1- Introduction

Nigella sativa L., commonly known as black seed or black cumin, is an annual herbaceous plant belonging to the Ranunculaceae family, renowned for its historical, medicinal, and economic significance [1]. Its seeds and fixed oil contain a plethora of bioactive compounds, most notably thymoquinone, which confer a wide range of pharmacological properties, including antimicrobial, anti-inflammatory, antioxidant, and anti-diabetic effects [2]. Consequently, there is a growing global demand for both its seeds and oil, driving the need to enhance cultivation practices to maximize yield and bioactive content [3].

In modern agriculture, particularly within the framework of sustainable intensification, there is a paradigm shift towards minimizing reliance on synthetic chemical inputs. Instead, the focus is increasingly on utilizing natural, eco-friendly substances that can improve plant performance, stress tolerance, and resource use efficiency [4]. Among these, biostimulants have emerged as a promising category [5]. These are diverse mixtures of substances, microorganisms, or compounds that, when applied to plants or the rhizosphere, stimulate natural processes to enhance nutrient uptake, efficiency, abiotic stress tolerance, and crop quality, irrespective of their own nutrient content [6]. Concurrently, humic substances, such as potassium humate—a salt derived from humic acids—are recognized for their profound impact on soil health and plant physiology. They improve soil structure, cation exchange capacity, and microbial activity, while directly influencing plant hormonal activity, membrane permeability, and nutrient assimilation [7].

While the individual effects of biostimulants and potassium humate on various crops have been documented, their combined, or synergistic, application on *Nigella sativa* remains an underexplored area of research, particularly under specific edaphic and climatic conditions [8]. The interaction between these two classes of bio-enhancers could potentially unlock additive or synergistic effects on the plant's morphological development, reproductive success, and ultimately, seed yield and quality parameters such as oil content [9].

Therefore, this study was designed to investigate the combined effect of biostimulants and potassium humate on the growth and yield characteristics of black seed plants [10]. The experiment, conducted under the soil conditions of Al-Shafi'iyah, Diwaniyah Governorate, Iraq, employed a factorial design to systematically evaluate this interaction. Key parameters assessed included plant height, number of flowers per plant, number of seeds per capsule, 1000-seed weight, and the percentage of oil in the grains. The primary hypothesis was that the co-application of these organic amendments would significantly outperform their individual applications or the control, leading to marked improvements in the agronomic and qualitative traits of *Nigella sativa*, thereby offering a viable strategy for sustainable production enhancement.

2- Materials and Methods

2.1. Experimental Site and Setup

The pot experiment was conducted during the 2022–2023 agricultural season within a controlled shade house environment. The study was designed to investigate the individual and interactive effects of biostimulants and potassium humate on the growth, yield, and oil content of black cumin (*Nigella sativa* L.) (Fig1).

Figure 1. Stages of plant development.

2.2. Soil Preparation and Characterization

Soil was collected from the top layer (0–30 cm) of an agricultural research field located in Al-Shafi'iyah, Diwaniyah Governorate, Iraq. The soil was air-dried, homogenized, and sieved through a 4 mm mesh. A representative subsample was analyzed for key physicochemical properties (Table 1). The prepared soil was packed into plastic pots lined with polyethylene bags at a rate of 8 kg per pot.

Table 1. Initial physicochemical properties of the experimental soil.

Attribute	Value	Unit
pH (1:1 soil:water)	7.59	-
EC (1:1 soil:water)	2.17	ds m^{-1}
Cation Exchange Capacity (CEC)	23.25	Cmolc Kg^{-1} soil
$CaCO_3$	233.75	g Kg^{-1} soil
Available N	32.06	mg Kg^{-1} soil
Available P	145.12	mg kg^{-1} soil
Available K	28.04	mg kg^{-1} soil

2.3. Plant Material and Sowing

Seeds of a local black cumin variety, obtained from the Agricultural Directorate of Sulaymaniyah Governorate, were surface-sterilized with a 1% sodium hypochlorite (NaOCl) solution for 5 minutes, followed by rinsing with distilled water. Ten seeds were sown per pot on 16 October 2022. One week after germination, seedlings were thinned to maintain five uniform plants per pot.

2.4. Experimental Design and Treatments

The experiment was arranged in a two-factorial Randomized Complete Block Design (RCBD) with three replications. The factors were:

1. **Biostimulant (Factor A):** Applied at three levels:
   - **A_0:** Control (no biostimulant).
   - **A_1:** 3 L ha^{-1}.
   - **A_2:** 6 L ha^{-1}.

   The commercial biostimulant (specify product name if available) was diluted in distilled water and applied as a soil drench at sowing. The application volume was adjusted per pot based on soil surface area equivalents.
2. **Potassium Humate (Factor B):** Applied at four levels:
   - **HK_0:** Control (0 kg ha^{-1}).
   - **HK_1:** 2 kg ha^{-1} (~0.006 g pot^{-1}).
   - **HK_2:** 4 kg ha^{-1} (~0.012 g pot^{-1}).
   - **HK_3:** 6 kg ha^{-1} (~0.018 g pot^{-1}).

   Potassium humate was thoroughly mixed into the soil prior to sowing.

This resulted in 12 treatment combinations (3 × 4), with a total of 36 experimental units (pots).

2.5. Agronomic Management

A uniform basal fertilization was applied to all pots: triple superphosphate at 100 kg P_2O_5 ha^{-1}, urea (46% N) at 240 kg N ha^{-1} (split into two equal applications at 21 and 51 days after sowing), and potassium sulfate at 100 kg K_2O ha^{-1} applied prior to the flowering stage. Pots were irrigated once prior to sowing. Post-sowing, three consecutive irrigations were applied at three-day intervals to ensure uniform germination. Subsequent irrigation was performed based on plant demand (see Figure 1 for phenological stages). Manual harvesting was conducted on 5 May 2023 upon observing full physiological maturity.

2.6. Data Collection

The following parameters were recorded at harvest:

- **Plant Height (cm $plant^{-1}$):** Height from soil surface to the plant apex was measured for all five plants per pot using a measuring tape, and the mean was calculated.
- **Number of Flowers per Plant:** The total number of flowers was counted per pot, and the mean per plant was determined.
- **Number of Seeds per Capsule:** A random sample of capsules from each pot was used to count seeds, and the mean was calculated.
- **1000-Seed Weight (g):** One thousand seeds from each pot were randomly selected, and their weight was recorded after standardizing moisture content to 15.5% [10].
- **Seed Oil Content (%):** Oil was extracted from a known weight of ground seeds using a Soxhlet apparatus with petroleum ether as the solvent, following the standard method of the Association of Official Analytical Chemists [11]. Oil content was expressed as a percentage of seed dry weight.

2.7. Statistical Analysis

All collected data were analyzed using a two-factor analysis of variance (ANOVA) for a randomized complete block design (RCBD) in SPSS software (Version 26, IBM Corp., Armonk, NY, USA). Treatment means were separated using Fisher's Least Significant Difference (LSD) test at the 5% probability level (α = 0.05). Interaction effects between the main factors (biostimulant and potassium humate) were examined, and when found to be statistically significant ($P \leq 0.05$), they are presented and discussed in detail within the results section.

3- Results

3.1 The effect of humic acid, agricultural sulfur, and their interaction on plant height (cm plant^{-1}).

Results in Table (2) proved the proven significance of biostimulants added to the potting soil at different levels and potassium humate added at different levels. The height of the black seed plant increased its increase significantly when biostimulants were added at level A2. It recorded a height of 49.36 cm plant^{-1}. An increase compared to the check without biostimulants added recorded the lowest height of 32.96 cm plant^{-1}. The addition of potassium humate added an increase in the average height of black seed plants. From 32.96 cm plant^{-1} for the check treatment to 43.75 cm plant^{-1} for plants treated with HK3. The interaction between biostimulants and potassium humate significantly influenced plant height in a two-way manner. All the treatments resulted in significantly taller black seeded plants than the control, which was the most stunted with an overall mean of 32.96 cm plant^{-1}. Treatment combination A2HK2 gave the best mean height with 58.88 cm plant^{-1}.

Table 2. How biostimulants and potassium humate affect the height of black seed plants (cm plant^{-}).

bio-growth stimulants (A)	(HK) potassium humate				
	HK0	HK1	HK2	HK3	Average
A0	32.96	36.7	40.95	43.75	38.59
A1	36.96	46.61	49.00	52.15	46.18
A2	49.36	54.26	58.88	57.66	55.04
Lsd .05		1.72			0.86
Average	39.76	45.86	49.61	51.19	
Lsd .05			0.99		

3.2 Number of flowers (flower plant^{-1})

Results in Table (3) clearly show the significant increase in the number of black seed flowers per addition of biostimulants at levels to potting soil and add potassium humate at different levels. The number of black seed flowers increased significantly in the treatment where biostimulants were added at level 2. This is an allusion to 18.58 flower plant^{-1}; this being the highest compared to the treatment without biostimulants, which registered the least rate of 13.92 flower plant^{-1}. A2 level biostimulants gave a very significant increase in the number of flowers that the control treatment with no biostimulants. The application of potassium humate showed a significant effect on the mean number of black seed flowers. They increased from 13.92 flower plant^{-1} in the control (HK0) treatment to 23.21 flower plant^{-1} in the HK3-treated plants.

There was a significant two-way interaction between biostimulants and potassium humate for flowers traits. All the treatments registered a significant number of black cumin flowers over and above their comparison with the check treatment (A0HK0). The latter recorded the lowest mean regarding this trait with 13.92 flower plant^{-1}. The combination A2HK2 performed better over all other combinations in recording the highest mean number of flowers with 29.55 flower plant^{-1}.

Table 3. Effect of biostimulants and potassium humate on the number of black seed flowers (flower plant^{-1})

bio-growth stimulants (A)	(HK) potassium humate				
	HK0	HK1	HK2	HK3	Average
A0	13.92	16.59	18.97	23.21	18.17
A1	18.99	24.82	25.86	26.88	24.14
A2	22.84	27.19	29.55	27.71	26.82
Lsd .05		0.98			0.49
Average	18.58	22.87	24.791	25.93	
Lsd .05			0.57		

3.3 Number of seeds per capsule (seed Wallet^{-1}).

Results of Table (4) were very significant (P<0.05) in the addition of bio-growth stimulants to potting soil at different levels and potassium humate at different levels for all parameters observed. The seeds per Wallet of black cumin plants increased significantly when bio-growth stimulant was applied at level A2. The highest rate of 80.65 seed Wallet^{-1} was recorded compared to the control treatment without the application of bio-growth stimulant (A0), which recorded the least rate of 70.85 seed Wallet^{-1}. The application of potassium humate resulted in a very highly significant increase ($P \leq 0.05$) in the mean seeds per pod of black seed plants to 81.14 seed Wallet^{-1} for HK3 over 70.85 seed Wallet^{-1} for HK0 control. The highly significant two-way interaction between the biostimulants and humic potassium salt resulted in highly significant differences in the number of seeds per tray. All treatments resulted in a highly significant number of seeds per tray when compared to the control treatment A0HK0, which had the least mean for the trait; this stood at 70.85 seed Wallet^{-1}. The A2HUM2 combination gave the highest mean number of seeds per tray at 97.05 seed Wallet^{-1}; this being seeds per tray.

Table 4. Effect of bio-growth stimulants and potassium humate on the number of seeds per capsule of black cumin plant (seed Wallet^{-1})

bio-growth stimulants (A)	(HK) potassium humate				
	HK0	HK1	HK2	HK3	Average
A0	70.85	74.03	77.58	81.14	75.90
A1	77.23	80.25	83.15	85.63	81.57
A2	80.65	92.94	97.05	94.92	91.39
Lsd .05		1.59			0.80
Average	76.24	82.41	85.93	87.23	
Lsd .05			0.92		

3.4 Weight of 1000 seeds (g)

Table 5 results proved the very highly significant ($p < 0.05$) effect of adding bio-stimulants to the potting soil at different levels and potassium humate at different levels on the weight of 1000 black seeds. The weight of 1000 black seeds increased significantly to 60.27 g in bio-stimulant treatment at level A2 compared to the 41.08 g weight recorded in the control (no bio-stimulant) treatment A0. The addition of potassium humate increased the average 1000-seed weight of black seed from 41.08 g in the control treatment of HK0 to 56.26 g in the plant treatment of HK3. This marked increase is due to the effect of potassium humate on the absorption of major nutrients in the soil; such nutrients include general nitrogen followed by phosphorus, iron, manganese, and copper in general order of preference, which results in better and more yield. The two-way interaction between the biostimulants and potassium humate gave significant differences for the 1000-seed weight trait. Nearly all treatments showed a significant difference in 1000-seed weight over the control treatment A0HK0. It had the lowest significant mean for this trait at 41.08 g. The combination A2HK2 gave the highest significant mean for 1000-seed weight at 75.17 g.

Table 5. Effect of biostimulants and potassium humate on the 1000-seed weight of black seed (g).

bio-growth stimulants (A)	(HK) potassium humate				
	HK0	HK1	HK2	HK3	Average
A0	41.08	44.67	51.55	56.26	48.39

A1	54.57	62.39	67.37	68.82	63.29
A2	60.27	64.85	75.17	70.64	67.73
Lsd .05		1.35			0.67
Average	51.97	57.30	64.70	65.24	
Lsd .05			0.75		

3.5 Oil content in grains (%).

Results in Table (6) show significant significance in the levels of adding biostimulants to potting soil and supplying potassium humate. Black seed oil content significantly increased when biostimulants were applied at level A2, registering the highest value of 1.437%, over treatment without biostimulants (A0) with the least value of 0.803%. The treatment of potassium humate resulted in a highly significant increase ($P < 0.001$) in average oil percentage in black seed from 0.803% for the control to 1.337% for HK3. There was a highly significant two-way interaction between biostimulants and potassium humate ($P < 0.01$) in oil percentage in grains. Most of the treatments registered a highly significant difference in oil percentage in grains compared to control A0HK0. The latter exhibited the lowest significant mean for the trait at 0.803%. The A2HK2 combination resulted in the highest significant mean for grain oil percentage at 1.653%.

Table 6. Effect of biostimulants and potassium humate on the percentage of oil in black seed seeds (%)

bio-growth stimulants	(HK) potassium humate				
(A)	HK0	HK1	HK2	HK3	Average
A0	0.803	0.970	1.180	1.337	1.073
A1	1.273	1.373	1.430	1.560	1.409
A2	1.437	1.580	1.653	1.597	1.567
Lsd .05		0.025			0.013
Average	1.171	1.308	64.70	1.498	
Lsd .05			0.015		

4. Discussion

The combined application of biostimulants (BS) and potassium humate (KH) elicited significant and often synergistic improvements in the growth, yield, and seed quality of black cumin (*Nigella sativa* L.). The most pronounced effects were consistently observed with the dual treatment of A_2 (6 L ha^{-1} BS) and HK_2 (4 kg ha^{-1} KH), underscoring the potential of integrated bio-stimulation strategies.

4.1. Plant Height

The significant increase in plant height with BS and KH application, culminating in the maximum height (58.88 cm) under A_2HK_2, can be attributed to their combined role in enhancing fundamental physiological processes. Biostimulants, rich in amino acids and plant growth-promoting compounds, act as precursors or enhancers of endogenous hormones involved in cell division and elongation [12]. Concurrently, potassium humate, a source of humic substances, functions as a bioregulator, improving membrane permeability and nutrient uptake efficiency [13]. This synergy likely enhanced the availability and assimilation of essential nutrients, thereby accelerating meristematic activity and internodal elongation. The results align with findings that humic substances mimic auxin-like activity, promoting cell expansion and overall vegetative growth [14, 2].

4.2. Reproductive Parameters: Flowers and Seeds per Capsule

The enhanced floral production (29.55 flowers $plant^{-1}$ under A_2HK_2) and seed set (97.05 seeds $capsule^{-1}$) directly reflect improved plant nutritional and physiological status. Biostimulants are known to improve nitrogen metabolism and chlorophyll synthesis, leading to greater photosynthetic capacity and carbohydrate reserves available for reproductive development [15]. Furthermore, the application of KH improves soil structure and cation exchange capacity, facilitating the mobilization and root absorption of critical nutrients like magnesium, which is central to chlorophyll formation and flower initiation [16]. The synergistic interaction likely amplified these effects; BS may enhance metabolic pathways for flower and seed development, while KH ensures a sustained supply of nutrients from the rhizosphere. This is consistent with research showing that such bio-effectors can increase the number of reproductive sites and improve seed filling by optimizing source-sink relationships [17, 18, 19].

4.3. 1000-Seed Weight and Oil Content

The substantial increase in 1000-seed weight (75.17 g under A_2HK_2) and seed oil concentration (1.653%) represents a critical qualitative improvement. Seed weight is a direct function of assimilate partitioning and nutrient accumulation during the grain-filling stage. The BS+KH combination appears to have prolonged photosynthetic activity and enhanced the translocation of photoassimilates and minerals (e.g., N, P, K, S) to the developing seeds [18, 21]. Specifically, the role of biostimulants in improving nutrient translocation and the function of humates in chelating micronutrients may have collectively boosted the synthesis of storage compounds [22].

The elevated oil content is of particular economic and pharmacological importance. The synthesis of fatty acids and fixed oils in seeds is a nutrient-intensive process heavily reliant on nitrogen and carbon skeletons. The treatments, especially A_2HK_2, likely created optimal conditions for this biosynthesis. Biostimulants provide amino acid precursors for protein and lipid synthesis [23], while potassium humate enhances nitrogen availability and uptake—nitrogen being a fundamental component of enzymes and co-factors involved in oil biosynthesis [24]. The synergistic improvement in soil physicochemical properties and root growth induced by these amendments would further support a more efficient and prolonged period of oil accumulation in the seeds [1, 25, 26].

4.4. Conclusion of the Discussion

In conclusion, the positive and often interactive effects of biostimulants and potassium humate on black cumin can be mechanistically linked to their complementary modes of action: (1) direct biochemical stimulation of plant metabolism and hormone-like activity, and (2) indirect improvement of soil health and nutrient bioavailability. The optimal dosage combination identified (A_2HK_2) suggests a balanced application that maximizes these benefits without inducing potential inhibitory effects that might occur at higher doses. This study strongly advocates for the integrated use of these organic amendments as a sustainable strategy to enhance both the productivity and the quality of *Nigella sativa*, potentially increasing its economic yield (seed weight) and the concentration of its valuable bioactive oil.

5. Conclusion

This study demonstrates that the integrated application of bio stimulants and potassium humate is a highly effective and sustainable strategy for enhancing the agronomic performance and seed quality of Nigella sativa L. The factorial experiment revealed significant main effects and, more importantly, synergistic interactions between the two factors across all measured parameters. The optimal treatment combination of bio stimulants at 6 L ha^{-1} (A_2) and potassium humate at 4 kg ha^{-1} (HK_2) consistently produced the most favorable outcomes. This treatment yielded the tallest plants (58.88 cm), the highest number of flowers (29.55 $plant^{-1}$) and seeds per capsule (97.05), the greatest 1000-seed weight (75.17 g), and the maximum seed oil content (1.653%). These values represent substantial increases over the control, underscoring the potent combined effect of these amendments. The observed improvements are attributed to the complementary physiological and edaphic mechanisms of the two inputs. Bio stimulants likely enhanced intrinsic metabolic processes, including photosynthesis, hormone regulation, and nutrient translocation. Concurrently, potassium humate improved soil nutrient availability, root development, and overall plant vigor. Their synergy amplified these benefits, leading to superior vegetative growth, robust reproductive development, and enhanced accumulation of biomass and storage compounds (oil) in the seeds. Therefore, the co-application of bio stimulants and potassium humate is recommended for the cultivation of black cumin. This practice aligns with the principles of sustainable agriculture by utilizing natural substances to reduce dependency on synthetic inputs while significantly boosting yield and the concentration of valuable phytochemicals. Future research should investigate the long-term soil health benefits, economic feasibility, and the efficacy of this approach under different environmental conditions and field settings.

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7- Transparency of data

- Data not available: The data set that

supports the results of this study is not publicly available.

8- Conflicts of interest

This work does not address or conflict with any particular interests.

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