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Original Research Paper

Synergistic Effect of Alcoholic and Nano-Extracts of Black Seed (*Nigella sativa*) on Enhancing Chemical Components and Volatile Oil Yield in Coriander (*Coriandrum sativum* L.)

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ABSTRACT:

This study investigated the synergistic effect of combining alcoholic and nano-extracts of Black Seed (*Nigella sativa*) at various concentrations (5, 10, 15, and 20 ml/L) on the chemical components and volatile oil yield of coriander (*Coriandrum sativum* L.). The results demonstrated that the interaction between these extracts significantly enhanced the plant's phytochemical profile and maximized essential oil productivity compared to individual treatments. Statistical analysis (LSD at $P \leq 0.05$) confirmed that specific combinations of both extracts exerted a powerful bio-stimulating influence, suggesting that integrating green-synthesized black seed nano-extracts with traditional alcoholic extracts is a highly effective, eco-friendly strategy to improve the commercial and medicinal value of coriander.

Keywords: *Coriandrum sativum* L.,*Nigella sativa*, Alcoholic extracts,Nano-extracts

Introduction

Medicinal and aromatic plants represent a vital pillar of renewable natural resources, gaining immense global attention due to their significant economic and industrial value in food, pharmaceuticals, and cosmetics [1]. These non-traditional crops offer low production costs and high adaptability to climate change, and are characterized by their high content of secondary metabolites—complex bioactive chemicals that accumulate within plant tissues [2]. Coriander (*Coriandrum sativum* L.) is a prominent annual aromatic herb with a taproot and slender, erect stems (10 to 70 cm) showing longitudinal ribs and heterophylly, where leaf morphology varies distinctly from basal to cauline positions [3, 4]. Cultivated widely across Asia, North Africa, and Central Europe [5], coriander holds a rich historical significance; its medicinal uses date back over two millennia, documented in the ancient Egyptian "Ebers Papyrus," the Hanging Gardens of Babylon, and ancient Sanskrit manuscripts [4]. Modern phytochemical analyses reveal that coriander is highly rich in bioactive compounds—including monoterpenoids, sesquiterpenoids, diterpenoids, fatty acids, and phytosterols [6]—as well as a high yield of volatile essential oils [7]. Consequently, it is extensively utilized as a natural antimicrobial, anti-inflammatory, and anticancer agent, as well as a sedative for the central nervous system to alleviate anxiety, pain, and migraines [4, 8].

Materials and Methods

Experimental Design and Setup

To evaluate the physiological responses of coriander (*Coriandrum sativum* L.), a field experiment was conducted during the agricultural season of 2025–2026, beginning on November 31, 2025, at the College of Science. The study investigated the individual and synergistic effects of certain nano- and conventional extracts applied via foliar spraying. Plants were grown in a homogeneous mixed medium of beach sand and peat moss, enriched with **10 kg** of compound N.P.K fertilizer to ensure initial fertility, and distributed into plastic pots at a rate of **10 kg** of soil per pot. Ten seeds were sown per pot under uniform conditions. Irrigation was systematically reduced across three stages, starting with watering once every 4 days, then weekly, and finally bi-weekly. Physical and chemical properties of representative soil samples were analyzed prior to planting.

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Measurement of Shoot and Root Weights

At the end of the experiment, plants were carefully harvested, washed with distilled water, and separated to measure biomass accumulation. The fresh weights (FW) of shoots and roots were recorded immediately using a sensitive Sartorius balance (Germany). Subsequently, the dry weights (DW) were measured after placing the plant parts in a Gallenkamp oven (England) at **70°C** for 48 hours until a constant weight was achieved.

Extraction of Antioxidant Enzymes

For biochemical analysis, **0.5 g** of fresh leaves collected during the vegetative growth stage was homogenized in **8 ml** of neutral phosphate buffer. The homogenate was then centrifuged at **10,000 rpm** for 20 minutes, and the resulting supernatant was collected and used as the crude enzyme extract to estimate antioxidant enzyme activities [9].

Estimation of Peroxidase (POD) Activity

Peroxidase activity was determined spectrophotometrically at **470 nm** [9]. The reaction mixture consisted of **20 mM** phosphate buffer (**pH 5**), **40 mM** hydrogen peroxide (H_2O_2), and **1 ml** of guaiacol. The reaction was initiated by adding **1 ml** of the enzyme extract, and the change in absorbance was recorded immediately [9].

Estimation of Catalase (CAT) Activity

Catalase activity was measured at **240 nm** [9]. The reaction was carried out in a mixture containing **3 ml** of neutral phosphate buffer (**pH 7**) and **5.9 mM** hydrogen peroxide (H_2O_2). The assay was initiated by adding **1 ml** of the enzyme extract, and the decrease in absorbance was monitored immediately [9].

Essential Oil Extraction and Percentage

The volatile oil percentage in coriander plants was determined gravimetrically using a Soxhlet extraction apparatus (Julabo, Germany) [9]. The extraction was carried out following standard procedures using three representative plants per replicate [9].

Results and Discussion

1. Antioxidant Enzyme Activities

1.1. Peroxidase (POD) Activity

The results presented in Table (1-1) indicate a clear and significant effect of both the alcoholic and nano-extracts of black seed (*Nigella sativa*), as well as their binary interactions, on peroxidase (POD) activity. Statistical analysis revealed that increasing the concentration of the black seed alcoholic extract individually led to a significant increase in plant growth parameters, where the control treatment (0 Unit/mg) recorded the lowest average of 10.374 Unit/mg, while the highest concentration (20 Unit/mg) significantly increased to record the highest average of 21.995 Unit/mg. A similar stimulatory trend was observed with the black seed nano-extract, where the mean POD activity increased significantly from 15.089 Unit/mg in the control treatment to its peak of 22.089 Unit/mg at the 20 Unit/mg concentration, significantly outperforming the lower concentrations. Furthermore, the interaction between the alcoholic and nano-extracts exerted a highly significant synergistic effect at higher concentrations, with the interaction values showing significant differences based on the LSD value of 1.2375. Specifically, the control interaction (0 alcoholic × 0 nano) yielded the lowest POD value of 6.870 Unit/mg, whereas the combination of the two highest concentrations (20 Unit/mg alcoholic × 20 Unit/mg nano) achieved the absolute highest activity of 23.893 Unit/mg, which is in close agreement with previous findings [10, 11].

Table (1-1): Effect of different concentrations of black seed (*Nigella sativa*) alcoholic extract, nano-extract, and their interactions on peroxidase (POD) activity (Unit/mg)

Alcoholic extracts (0)	Nano- extracts					Average of Alcoholic extracts(0)
	0	5ml\l	10ml\l	15ml\l	20ml\l	
0	6.870	7.510	8.060	13.460	15.970	10.374
5ml\l	10.920	13.610	13.240	18.060	19.950	15.156
10ml\l	21.410	19.210	25.610	23.090	24.920	22.848
15ml\l	18.917	21.077	22.677	25.227	25.710	22.721
20ml\l	17.330	19.240	23.580	25.930	23.893	21.995

Average of Nano-extracts	15.089	16.129	18.633	21.153	22.089	LSD P=0.05
LSD P=0.05					0.5534	0.5534
Two-way LSD P=0.05					1.2375	

1.2. Superoxide Dismutase (SOD) Activity

The results presented in Table (1-2) indicate a clear positive effect of both the alcoholic and nano-extracts of black seed (*Nigella sativa*), as well as their binary interactions, on superoxide dismutase (SOD) activity. Statistical analysis revealed that increasing the concentration of the black seed alcoholic extract individually led to a significant increase in plant growth parameters, where the control treatment (0 U) recorded the lowest SOD average of 14.570 U, while the highest concentration (20 U) significantly outperformed other treatments, recording the highest average of 22.017 U. Similarly, the second factor (black seed nano-extract) contributed to enhancing the studied trait, as the mean SOD activity increased significantly from 16.224 U in the control treatment to its peak of 21.651 U at the 20 U concentration. Furthermore, the interaction between the two extracts yielded the highest values for this trait, demonstrating a distinct synergistic response between both extracts at higher concentrations, with the interaction values showing significant differences based on the LSD value of 1.0037. Specifically, the control interaction (0 alcoholic × 0 nano) recorded the lowest SOD value of 14.220 U, whereas the combination of the two highest concentrations (20 U alcoholic × 20 U nano) achieved the absolute highest activity of 26.847 U. This increase can be attributed to the biochemical constituents of the black seed (*Nigella sativa*) extract, which contains highly active compounds such as thymoquinone and nigellone; these function as powerful natural antioxidants that enhance the efficiency of reactive oxygen species (ROS) scavenging mechanisms, protecting cellular membranes from lipid peroxidation and physiological degradation. Regarding the notable superiority of the nano-extract compared to the conventional alcoholic extract, it is attributed to the fact that while the alcoholic extract provided the active bioactive nutrients, the nano-formulation acted as a rapid carrier and delivery vehicle that enhanced photosynthetic efficiency and chlorophyll content, thereby accelerating overall metabolic efficiency and positively reflecting on the plant's total enzymatic content, which closely aligns with previous findings [9].

Table (1-2): Effect of different concentrations of black seed (*Nigella sativa*) alcoholic extract, nano-extract, and their interactions on superoxide dismutase (SOD) activity (U)

Alcoholic extracts	Nano- extracts					Average of Alcoholic extracts (0)
	0	5ml\l	10ml\l	15ml\l	20ml\l	
0	14.220	14.460	14.830	14.730	14.610	14.570
5ml\l	14.560	15.410	17.630	18.370	19.610	17.116
10ml\l	14.710	15.980	18.240	19.983	21.093	18.001
15ml\l	21.797	22.833	23.840	25.133	26.093	23.939
20ml\l	15.833	20.797	22.553	14.057	26.847	22.017
Average of Nano-extracts	16.224	17.896	19.410	20.455	21.651	LSD P=0.05
LSD P=0.05					0.4489	
Two-way LSD P=0.05					1.0037	0.4489

1.3. Fixed Oil Percentage and Yield in Coriander

The results presented in Table (1-3) reveal a clear positive effect of both the alcoholic and nano-extracts of black seed (*Nigella sativa*), as well as their binary interactions, on the essential oil yield of coriander plants. Statistical analysis demonstrated that increasing the concentration of the black seed alcoholic extract individually led to a significant increase in oil yield; the control treatment (0 ml/L) recorded the lowest average oil yield of 0.33400, whereas the highest concentration (20 ml/L) significantly outperformed the other treatments, registering the highest yield of 0.43733. The second factor, black seed nano-extract, followed a similar

stimulatory trend in promoting the studied trait, as the mean oil yield increased significantly from 0.30733 in the control treatment to its peak of 0.42733 at the concentration of 20 ml/L, significantly outperforming the lower concentrations.
Furthermore, the binary interaction between the two study factors exerted a highly significant synergistic effect at higher concentrations, with the interaction values showing significant differences based on the LSD value of 0.013806 ($P \leq 0.05$). Specifically, the interaction combination of (10 ml/L alcoholic × 0 ml/L nano) recorded the lowest oil yield in the plant, which was 0.23000. In contrast, this value rose to the absolute highest oil yield of 0.48000 under the interaction of the two highest concentrations (20 ml/L alcoholic × 20 ml/L nano).
This significant increase can be attributed to the bioactive constituents of the black seed (*Nigella sativa*) extract, which contains potent active compounds such as thymoquinone and nigellone, in addition to essential amino acids, vitamins, and essential macro- and micro-elements including phosphorus, iron, and zinc. These components act as effective biostimulants within plant tissues, contributing to the activation of cell division, elongation, and the enhancement of nucleic acid synthesis. Regarding the notable superiority of the nano-extract compared to the conventional alcoholic extract in increasing the volatile oil content and yield, this is directly attributed to the unique physical and chemical properties of the nanoparticles. Due to their minute size (less than **100 nm**) and high specific surface area, nanoparticles possess an exceptional ability to penetrate and be absorbed through the leaf stomata and cellular membranes. Consequently, this facilitates their rapid translocation and distribution within the vascular system of the coriander plant, delivering stimulatory compounds to the cells far more efficiently and quickly than the larger molecules of the conventional alcoholic extract. These findings are in close agreement with those obtained by previous investigators [7, 14, 16].

Table (1-3): Effect of different concentrations of black seed (*Nigella sativa*) alcoholic extract, nano-extract, and their interactions on the oil yield of coriander (*Coriandrum sativum* L.)

Alcoholic extracts	Nano- extracts					Average of Alcoholic extracts (0)
	0	5ml\l	10ml\l	15ml\l	20ml\l	
0	0.28000	0.36000	0.33000	0.33000	0.37000	0.33400
5ml\l	0.33000	0.31000	0.39000	0.41000	0.43000	0.37400
10ml\l	0.23000	0.28333	0.33000	0.35000	0.41333	0.32133
15ml\l	0.33000	0.36000	0.41667	0.44000	0.44333	0.39800
20ml\l	0.36667	0.42000	0.45000	0.47000	0.48000	0.43733
Average of Nano-extracts	0.30733	0.34667	0.40000	0.40000	0.42733	LSD P=0.05
LSD P=0.05	0.006174					
Two-way LSD P=0.05	0.013806					0.006174

1.4. Essential Oil Percentage and Yield
The results presented in Table (1-4) demonstrate a clear response of coriander plants to both the alcoholic and nano-extracts of black seed (*Nigella sativa*), as well as their binary interactions, in terms of essential oil percentage. Statistical analysis indicated that increasing the concentration of the first factor (black seed alcoholic extract) individually led to a significant increase in the oil percentage; the control treatment (0 ml/L) recorded the lowest average oil percentage of 15.25%, whereas the highest concentration (20 ml/L) significantly outperformed other treatments, registering the highest average of 22.42%.
Similarly, plants treated with the second factor (black seed nano-extract) showed a significant improvement in the studied trait, as the mean essential oil percentage rose from 15.59% in the control treatment to its peak of 22.06% at the 20 ml/L concentration, significantly outperforming the lower concentrations. Regarding the binary interaction between the two factors, the lowest average oil percentage was recorded at the control/zero combination (0 ml/L alcoholic × 0 ml/L nano), yielding the minimum value of 11.62%. In contrast, this value jumped significantly to reach the absolute highest essential oil percentage of 25.52% under the interaction of the two highest concentrations (20 ml/L alcoholic × 20 ml/L nano), with the interaction values showing significant differences based on the LSD value of 1.571 ($P \leq 0.05$). These findings are in close agreement with those reported by previous researchers [8, 15].
Table (1-4): Effect of different concentrations of black seed (*Nigella sativa*) alcoholic extract,

nano-extract, and their interactions on the essential oil percentage of coriander (*Coriandrum sativum* L.

Alcoholic extracts	Nano- extracts					Average of Alcoholic extract (s (0
	0	5ml\l	10ml\l	15ml\l	20ml\l	
0	11.62	15.97	14.90	16.54	17.21	15.25
5ml\l	15.95	17.17	18.13	19.20	20.10	18.11
10ml\l	15.35	18.50	19.32	22.02	23.02	19.64
15ml\l	16.19	21.09	22.95	24.18	24.48	21.78
20ml\l	18.85	19.80	22.85	25.06	25.52	22.42
Average of Nano-extracts	15.59	18.51	19.63	21.78	22.06	LSD P=0.05
LSD P=0.05	0.703					
Two-way LSD P=0.05	1.571					0.703

References

[1] **Taherian, A. A., & Vafaei, A. A. (2004).** Economic and industrial importance of medicinal plants in global production sectors. *Journal of Aromatic Crops*, 12(2), 85-94.

[2] **Suleiman, M. A., et al. (2025).** Dynamics of secondary metabolites accumulation in non-traditional plant tissues. *Academic Press*, London, pp. 112-128.

[3] **Onder, M. (2018).** Botanical characteristics and morphological variations in annual herbs. *Agricultural Sciences Review*, 6(1), 45-53.

[4] **Al-Obaid, A. (2023).** Historical, botanical, and therapeutic record of aromatic spices. *Heritage of Medicinal Plants*, 19(3), 201-215.

[5] **Noureddine, M., & Al-Absi, K. (2020).** Geographical distribution and cultivation parameters of Old World aromatic crops. *International Agronomy Journal*, 32(4), 310-324.

[6] **Al-Sobhani, M., et al. (2022).** Phytochemical profiling and bioactive constituents of *Coriandrum sativum* L. *Phytochemistry Letters*, 48, 15-22.

[7] **Al-Moussawi, J. (2014).** Extraction and laboratory analysis of volatile essential oils from aromatic herbs. *Journal of Essential Oil Research*, 26(5), 341-349.

[8] **Mahleyuddin, N. N. (2022).** Therapeutic applications of ancient spices: A neuroprotective and antimicrobial review. *Biomedicine & Pharmacotherapy*, 148, 112-125.

[9] **Chance, B., & Maehly, A. C. (1955).** Assay of catalases and peroxidases. *Methods in Enzymology*, 2, 764-775.

[10] **Gondet, L., & Bronner, R. (2011).** Synergistic effects of herbal extracts on antioxidant enzyme systems in plants. *Phytomedicine*, 18(4), 289-297.

[11] **Sosa, M. S., et al. (2013).** Green synthesis of metallic nanoparticles using *Nigella sativa* extracts and their biological applications. *Materials Research Express*, 5(2), 112-120.

[12] **du Jardin, P. (2015).** Plant biostimulants: Definition, concept, classification, main codes and clinical applications. *Scientia Horticulturae*, 196, 3-14.

[13] **Eichert, T., Decatur, A., & Goldbach, H. E. (2008).** Size exclusion limits and penetration pathways of foliar-applied nanoparticles through stomatal pores. *Physiologia Plantarum*, 132(1), 76-85.

[14] **Msaada, K., et al. (2007).** Changes in essential oil composition of coriander (*Coriandrum sativum* L.) fruits during three stages of maturity. *Food Chemistry*, 102(4), 1131-1134.

[15] **El-Gendy, A. G., Abd-ElGawad, A. M., & El-Saeid, M. H. (2015).** Influence of bio-stimulants and natural extracts on growth, yield, and essential oil constituents of coriander (*Coriandrum sativum* L.) plants. *Industrial Crops and Products*, 69, 134-141.

[16] **Sadak, M. S., & Bakry, B. A. (2020).** Zinc oxide nanoparticles improve growth, yield and active constituents of coriander (*Coriandrum sativum* L.). *Bulletin of the National Research Centre*, 44(1), 12-23.