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NANOFERTILIZER IMPACT ON GROWTH, SEED YIELD, AND ESSENTIAL OIL OF BLACK CUMIN (*NIGELLA SATIVA* L.)

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SUMMARY

The present-day study investigated the effect of different nanofertilizer concentrations on growth and seed yield-related traits and the percentage of essential oil in the seeds of the black cumin (*Nigella sativa* L.), carried out during the crop season of 2019–2020 at the University of Al-Qadisiyah, Al-Diwaniyah, Iraq**.** The experiment was in a randomized complete block design (RCBD) with three replications. The nanofertilizer at a 20 mg/L concentration has a significant impact on the black cumin (*N. sativa* L.) plants and improved the growth, seed yield, and biochemical traits, i.e., plant height (40.38 cm), 1000-seed weight (3.48 g), seed yield per plant (5.55 g), seed essential oil (0.42%), percentage of mineral elements (Nitrogen-5.48%, potassium-1.02%, and zinc-4.00%), and biochemical compounds in the leaves (carbohydrates-4.34%, protein-6.00%, and peroxidase-0.95) compared with the least values for the said traits in the control treatments, i.e., 33.92 cm, 1.65 g, 3.77 g, 0.13%, 3.02%, 0.28%, 2.23%, 2.76%, 4.05%, and 0.23, respectively. However, nanofertilizer levels had nonsignificant effects on the number of branches, leaves, and capsules per plant and the percentage of phosphorus and abscisic acid. Therefore, nanotechnology has established itself as a multidisciplinary and pioneering problem-solving technology in agricultural and allied sciences.

Keywords: Black cumin (*N. sativa* L.), nanofertilizer, growth and seed-yield traits, essential oil, macro-elements, carbohydrates, abscisic acid (ABA), proteins

Key findings: The nanofertilizer (20 mg/L) has significantly impacted the black cumin (*Nigella sativa* L.) plants and improved the growth and yield-related traits, percentage of mineral elements, and biochemical traits in the leaves.

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INTRODUCTION

Nanotechnology is a science that comprising the studies the atomic and molecular scale of materials up to about 10^{-9}(Ghorbani *et al.,* 2011). The properties of the nanomaterials vary from conventional materials in terms of less surface area (Sekhon, 2014). This opens the way for the study of nanomaterials and their applications in biological sciences for the various benefits and positive impact (Roghayyeh *et al.,* 2010). Later on, nanotechnology's different positive uses emerged in biology, medicine, pharmacology, and other diverse fields related to biological sciences (Raab *et al*., 2011; Nemtinov *et al*., 2022).

The global value of nanoparticles was approximately at USD 16.3 billion in 2021, and it is expected that by 2031 it may reach USD 62.8 billion, with a compound at a CAGR (compound annual growth rate) increase of 14.6% from 2022 to 2031. According to global production of nanoparticles, the global value for nanomaterials has an estimated at 11 million tons at a market value of USD 20 billion. However, nanoparticles' global production for 2004 was around 103 tons (Zhang *et al*., 2009).

Nanotechnology can play a significant role in the agriculture sector's economic development. It also improves life through its application in differ fields, such as agriculture and food (Sharon *et al*., 2010). Agriculture occupies the second position using nanotechnology (Shehzad *et al*., 2018). Nanotechnology is vital in increasing and improving crop plants' production and quality (Sherif, 2009). Many believe this technology can provide good health with environmental and economic benefits (Singh *et al*., 2015).

Nanotechnology has proven its value in agricultural sciences as an influential technology in solving problems the farming community faces with crop management (Mousavi and Rezaei, 2011). It also helps obtain high crop productivity while reducing dependence on mineral fertilizer use (Kumar, 2013; Prasad *et al*., 2014). Applying mineral fertilizers in the soil and their foliar application on crop plants at low concentrations may not affect the grain yield because of its disintegration with photolysis and by the microorganisms found in the soil, causing the replicated application of chemicals and mineral fertilizers leading to soil and water pollution (Gutierrez *et al*., 2012).

Nair *et al.* (2010) explained that the nanoparticles possess the necessary features for use in agricultural fields, such as high solubility, stability of composition, better effectiveness, safety, and less toxicity, only used in low concentrations to obtain superior productivity from the first application (Valdez *et al*. 2018). By using nAl_2O_3 with three concentrations (400, 2000, and 4000 mg L^{-1}) on *Arabidopsis thaliana* L., results showed a significant impact of nanoparticles in enhancing the seed germination, roots elongation, and seedling growth (Lee *et al*., 2010)**.** Nanotechnology can bring broader developments in the agricultural and food industry because of its ability to increase plants' absorption of micro- and macro-elements well from the soil and crops' protection from pathogens (Joseph and Morrison, 2006; Thul *et al*., 2013).

The black cumin (*N. sativa* L.) plant belongs to the family Hellebeoraceae and is a perennial herbaceous plant (Khattab and Omer, 1999). Flowers mostly form on the trunk and main branches, and the fruit is bisexual. The fruit is a green capsule (a capsule that turns brown or black at maturity and disperses the seeds) (Mousa *et al*., 2001). The seeds are small and polygonal oval. The black cumin is well known for its benefits since the 20th–30th century BC. However, in the present era, the adoption of experimental medicine and development in modern technologies gave the black cumin seed a larger share in its therapeutic uses (Ahmed *et al*., 2004; Al-Jassir, 1992).

Eating *N. sativa* seeds twice a day at a dose of one gram for five days strongly stimulates the immune system of the human by increasing in number of lymphocytes, improving the functional activity of natural killer cells. Micheal (2003) stated that the black cumin seed can remove any obstruction in the genital ducts or the bloodstream in the urethra to treat simple infertility. Vasisht (2004) mentioned that oil extract from its seed is anti-diabetic and antihypertensive. The black cumin seed also helps treat nervous tension, headaches, rheumatic diseases, and joint pain. Nigellone affects chest sensitivity through its effect on mast cells (Chakravarty, 1993). Worthen *et al.* (1998) concluded that black cumin seed oil is an anti-cancer. The black cumin seed oil is stimulating, refreshing, aromatic, aiding digestion, diuretic, milky, irritating, appetizing, tonic anthelmintic, and has other various properties (Micheal, 2003). From the above discussion, the presented study sought to investigate the effect of nanofertilizers on with different concentrations on growth and seed yield-related traits and the percentage of essential oil in black cumin seeds (*N. sativa* L.).

MATERIALS AND METHODS

This pertinent study on black cumin (*N. sativa* L.) with foliar application of different concentrations of nanofertilizer commenced during the crop season of 2019–2020 at the University of Al-Qadisiyah, Al-Diwaniyah, Iraq. The experimental soil of the field was well plowed and leveled, with the soil samples collected at a depth of 0–30 cm to study some of its properties of chemical and physical, i.e., sand (15.3%), silt (55.2%), clay (29.5%), pH (7.5), and E.C. (3.2). The nanofertilizers used in the experiment (consisting of copper, carbon, and zinc nanoparticles) had five different concentrations, including the control treatment (0, 5, 10, 15, and 20 mg/L). For statistical analysis, the treatments' arrangement in a randomized completes block design (RCBD) had a factorial arrangement and three replications. The least significant difference ($LSD_{0.05}$) test helped compare and separate the treatments.

The soil division into experimental subplots proceeded. Sowing the black cumin seeds consisted of keeping 5–6 seeds in each hole, based on the production technology recommendations of this plant (Khattab and Omer, 1990). Practicing thinning two weeks after germination reduced the seedlings to just three per hole. The foliar application of nanofertilizer on black cumin plants followed the specified concentrations early in the morning until complete wetness. Repeating the nanofertilizer foliar spraying ensued after two weeks from the first spray.

Data recorded and statistical analysis

The data recording on the vegetative growth and seed yield-related characteristics of the black cumin plants included plant height (cm), branches per plant), branches on the main stem of plants, leaves per plant, fresh weight of the plant (g), capsules per plant, 1000-seed weight, and the seed yield per plant (g).

Essential oil extraction

In black cumin (*N. sativa* L.), the essential oil extraction from its seeds employed the water distillation method, where 20 g of dry and ground seeds received 250 ml distilled water in a 1 l beaker. Afterward, heating the flask for 4 h ensured obtaining more oil. Adding 35 ml of solvent (diethyl ether) to it continued the mixture's shaking for five minutes, with the vessel left to stand still to separate the two layers. Removal of the upper layer (ether with oil) continued three times until the oil from the bottom layer floated. Then, the collected extract (ether with oil) gained 3–5 g of calcium sulfate to rid the water from the extract. Then, placing the extract in a 37 °C water bath helped remove the ether. Storing acquired essential oil was in well-closed dark bottles at 4 °C (Lachowicz *et al*., 1996).

Biochemical compounds

In black cumin plant leaves, the mineral elements) nitrogen, phosphorus, potassium, and zinc) determination engaged the Micro-Kjeldahl method by Singh and Dubey (1995).
Carbohydrates percentage in leaves estimation followed Joslyn's (1970) method. The percentage of the hormone abscisic acid (ABA) in leaves calculation used the approach of Fales *et al*. (1973). Estimating the protein percentage and peroxidase activity (POD) of leaves employed the method of Bates *et al*. (1973).

RESULTS AND DISCUSSION

The different nanofertilizer concentrations significantly affected the black cumin (*N. sativa* L.) plants for the plant height (Table 1). The nanofertilizer with a concentration of 20 mg/L provided taller plants (40.38 cm) than the control treatment, with 33.92 cm. However, the concentrations of nanofertilizer had no significant impact on the number of branches in plant, and number of leaves in plant compared with the control treatment. The results also revealed that nanofertilizer concentrations increased the fresh weight of the plant, which reached the maximum (6.91 g) by foliar application of the nanofertilizer at 20 mg/L. The results further indicated significant effects of nanofertilizer in increasing plant height, perhaps due to the role of nanofertilizer components in enhancing cell division and elongation and stimulating plant growth, thus increasing plant height (Ahmed *et al*., 2004; Thul *et al.,* 2013). The nanofertilizer increases secondary metabolites and facilitates the transfer of photosynthesis products in plants, raising plant weight (Lee *et al*., 2010; Valdez *et al*., 2018).

The results further indicated that nanofertilizer concentration did not considerably affect the number of capsules per plant (Table 2). However, the nanofertilizer significantly increased the 1000-seed weight, with the highest 1000-seed weight (3.48 g) coming from the foliar application of nanofertilizer at 20 mg/L compared with the control treatment, which amounted to 1.65 g. According to seed yield per plant, it was worth noting that nanofertilizer concentrations increased seed yield per plant, which reached the highest (5.55 g) by spraying the black cumin plants with nanofertilizer concentration of 20 mg/L versus the control treatment with the lowest seed yield (3.77 g). In black cumin seeds, the percentage of essential oil enhanced gradually with the increase in the concentrations of the nanofertilizer used. The highest essential oil (0.42%) in the seeds resulted in the foliar application of black cumin plants with nanofertilizer (20 mg/L) compared with the control treatment, giving the minimum seed essential oil (0.13%). The increase in seed yield and its components is due to the efficiency of the photosynthesis process (Zhang *et al*., 2009). The boost in nanofertilizer concentrations enhanced the weight of the seeds due to nutrient enrichment stored inside the seeds (Nair *et al.,* 2010; Kumar, 2013). In addition to raising the activity of enzymes enlarging the metabolic pathways that produce secondary metabolic compounds, it manifested positively in the upsurge in the percentage of oils in the seeds (Mousavi and Rezaei, 2011; Prasad *et al*., 2014; Nemtinov *et al*., 2022).

The nanofertilizer with different concentrations significantly influenced the percentages of mineral elements in the plant leaves (Table 3). The nanofertilizer at the concentration of 20 mg/L showed the highest percentages of nitrogen (5.48%) in leaves compared with the control treatment (3.02%). However, the nanofertilizer concentrations did not significantly affect the phosphorus percentage compared with the control treatment. The results also revealed that the potassium percentage rose with enhanced nanofertilizer concentrations. The maximum percentage of potassium (1.02%) emerged with the foliar application of nanofertilizer (20 mg/L) compared with the control treatment (0.28%). For zinc concentrations in the black cumin leaves, a gradual increase was evident with increased concentrations of nanofertilizer, reaching a peak (4.00%) at the concentration of 20 mg/L versus the control treatment with the lowest zinc value (2.23%). The findings provided significant effects of nanofertilizer on the percentage of mineral elements in the plant leaves due to the role of nanofertilizer components in increasing the efficiency of photosynthesis, boosting the absorption of nutrients from the soil (Valdez *et al*., 2018; Kumar, 2013).

The foliar application of nanofertilizer substantially impacted the percentage of carbohydrates and proteins (Table 4). The nanofertilizer caused a significant increase in the percentage of carbohydrates in the plant leaves, and the nanofertilizer at 20 mg/L concentration gave the highest percentage

Table 1. Effect of nanofertilizer concentrations on vegetative traits of the *N. sativa* L.

Nanofertilizer Concentrations	Plant height (cm)	Branch plant^{-1}	Leaves plant^{-1}	Plant fresh weight (g)
Control	33.92	5.15	13.18	5.53
5 ml/L	35.27	5.82	13.45	5.82
10 ml/L	35.91	5.88	14.61	6.11
15 ml/L	37.05	6.34	14.87	6.42
20 ml/L	40.38	7.11	14.94	6.91
$LSD_{0.05}$	1.20	N.S.	N.S.	0.52

N.S. = Non-Significant

Table 2. Effect of nanofertilizer concentrations on yield traits and seed essential oil (%) of *N. sativa* L.

Nanofertilizer Concentrations	Capsules plant^{-1}	1000-seed weight (g)	Seed yield plant^{-1} (g)	Essential oil (%)
Control	4.12	1.65	3.77	0.13
5 ml/L	4.35	1.79	4.34	0.22
10 ml/L	4.65	2.60	4.84	0.35
15 ml/L	4.92	3.07	5.08	0.38
20 ml/L	5.21	3.48	5.55	0.42
$LSD_{0.05}$	N.S.	0.18	0.54	0.04

N.S. = Non-Significant

Table 3. Effect of nanofertilizer concentrations on mineral elements (%) in the leaves of *N. sativa* L.

Nanofertilizer Concentrations	N (%)	P (%)	K (%)	Zn (%)
Control	3.02	1.53	0.28	2.23
5 ml/L	3.97	1.62	0.65	2.92
10 ml/L	4.91	2.00	0.68	2.93
15 ml/L	5.00	2.04	0.91	3.12
20 ml/L	5.48	2.06	1.02	4.00
$LSD_{0.05}$	0.62	N.S.	0.03	1.02

N.S. = Non-Significant

Table 4. Effect of nanofertilizer concentrations on biochemical compounds (%) in the leaves of *N. sativa* L.

Nanofertilizer Concentrations	Carbohydrates (%)	Protein (%)	Abscisic acid (%)	Activity of peroxidase (%)
Control	2.76	4.05	1.15	0.23
5 ml/L	2.98	4.82	1.35	0.42
10 ml/L	3.98	5.01	1.49	0.61
15 ml/L	3.94	5.62	1.62	0.82
20 ml/L	4.34	6.00	1.70	0.95
$LSD_{0.05}$	1.00	0.23	N.S.	0.06

N.S. = Non-Significant

(4.34%) compared with the control treatment (2.76%). The nanofertilizer also had the same effect on the protein percentage, and the concentration of 20 mg/L revealed the maximum percentage of protein (6.00%) versus the rest of the treatments and the control (4.05%). The results also revealed the nanofertilizer did not show any significant effects on abscisic acid (ABA) in the black cumin leaves. The activity of the peroxidase enzyme increased with rising concentrations of the nanofertilizer, reaching its utmost level (0.95%) at 20 mg/L of nanofertilizer compared with the control treatment (0.23%). The boost in nanofertilizer concentrations augmented the percentage of carbohydrates and protein in the plant leaves due to enhanced metabolites and the efficiency of the photosynthesis process (Zhang *et al*., 2009; Lee *et al*., 2010). Abscisic acid is one of the hormones that encourage the growth and development of plants and helps boost plant resistance to stress (Prasad *et al*., 2014). The activity of the peroxidase enzyme intensifies with increasing concentrations of the nanofertilizer, perhaps due to the stability of the plasma membrane and stress because raising the concentration of nanofertilizer is crucial in plants resisting stress (Joseph and Morrison, 2006; Singh *et al*., 2015).

CONCLUSIONS

Nanofertilizers are essential in increasing crop plants' production and improving their quality by reducing the farming community's dependence on synthetic chemicals. Using nanofertilizers significantly increased the vegetative growth and seed yield traits and boosted the oil content in the seeds of black cumin (*N. sativa* L.). Nanotechnology has established itself in agriculture as a multidisciplinary and pioneering problem-solving technology.

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