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Nitrogen-enriched Humic Acid together with Zinc Oxide Nanoparticles to Enhance Growth and Quality of Cabbage

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NANO fertilizers are innovative fertilizers introduced to the agricultural sector to enhance yield production and reduce the known negative impacts of excessive agrochemical use on the ecosystem. Integrating these fertilizers into a conventional farming system, combined with natural growth stimulants, may boost their efficiency and offer a promising approach to addressing poor soil quality in arid and semi-arid regions characterized by low nutrient availability, alkalinity, and reduced productivity. This field study was conducted for two seasons to assess the effectiveness of a combined foliar application of nitrogen-enriched humic acid (NH) and Zinc oxide nanoparticles (nZnO) on the agronomic parameters, quality and yield of cabbage. The experimental treatments include foliar application of two rates of NH (2 and 3 ml l^{-1}), denoted 2NH and 3NH, combined with three fertilization levels of nZnO (25, 50 and 100 mg l^{-1}), referred to as 25 nZnO, 50 nZnO, and 100 nZnO, along with a control treatment aligned in a randomized complete block design. Enhancements of cabbage head weight recorded 46.7 - 53.9% than the control treatment by spraying of NH at 2 or 3 ml l^{-1} with nZnO at 25 mg l^{-1} and reached 80.3-92.5%, after raising nZnO to 50 mg l^{-1}, respectively. Foliar application of 25 nZnO, 50 nZnO, and 100 nZnO increased cabbage yield by 39.3, 59.8 and 25.9% under 2NH relative to the control and 42.9, 62.5 and 28.6% under 3NH, respectively. Comparable results were exhibited from both seasons. Generally, the highest measured trait values were obtained from plants treated with 3NH+50 nZnO and 2NH+50 nZnO, followed by those treated with 3NH+25 nZnO and 2NH+25 nZnO. In contrast, the lowest enhancements were noted from those sprayed with 3NH+100 nZnO and 2NH+100 nZnO. The results showed that spraying nZnO fertilizers under any rate of NH improved the agronomic indices, biochemical constituents and elemental content of cabbage leaves. The study highlighted that to ensure the resilience and sustainable application while maintaining environmental safety, the nZnO application rate must be carefully considered.

Keywords: Natural stimulant, Nano-zinc fertilizer, Cabbage, Plant health, Sustainable agriculture.

1. Introduction

Zinc is an essential micronutrient for living organisms embedded in the structure of several protein compounds. Hence, it plays an intrinsic role in the integrity of cell membranes and several biochemical and physical bioprocesses, besides serving as a cofactor in enzyme activation (Zulfiqar *et al*., 2020; Singh and Singh 2017; Broadley *et al*., 2007). It also has a crucial function in the plants' strategies against different environmental stress conditions like salinity, drought and pathogens (Bastakoti, 2023; Mubarak *et al*., 2021; Dimkpa *et al*., 2019b). Zinc is needed in small amounts; however, its deficiency may cause major nutritional problems for humans and plants (Maxfield *et al*., 2023; Cakmak, 2000). Globally, zinc deficiency grabs the attention related to human health. Gupta *et al*., (2020) stated that it causes growth retardation and impairment in immunity and antiviral immunity, particularly in low and middle-income countries due to malnutrition. They suggested that plant Zn-fortification could be a wise applicable strategy to combat the inadequacy of that vital nutrient in developing countries such as India, China and Egypt. In plants, a deficiency of zinc content leads to a significant reduction in yield production and quality, and it negatively influences photochemical processes, reducing biochemical constituents such as carbohydrates and proteins (Sharma *et al*., 2025; Hajiboland and Amirazad, 2010; Hänsch and Mendel, 2009). The deficit causes disruption of physiological processes and the reactive oxygen species in the plant, which stunts growth (Natasha *et al*., 2022; Cakmak, 2000). Thus, it is imperative to ensure a reliable continuous supply of zinc nutrient to achieve optimal growth and maximize the yield.

Mineral fertilizers are the main source of nutrients for plants. Under Egyptian conditions, mineral fertilization via soil might limit the availability indices values of some nutrients, such as Zn. Where soil fertilization of zinc allows its reaction with soil components under soil conditions like high soil pH and calcium carbonate

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percentage, besides the low organic matter content with hot weather and low annual precipitation that immobilizes Zn-pools and limits their movement, leading to a plant nutrient deficit (Moreno-Lora and Delgado 2020; Alloway 2009). Foliar fertilizer application can be an appropriate approach to overcome the mentioned limitation of soil fertilization and effectively supply plants with lacking nutrients (Al-Shammari *et al*., 2020; Singh *et al*., 2015). Zinc sulfate and chelated zinc are commonly Zn fertilizers used for the foliar technique (Dhaliwal *et al*., 2022). Lately, nutrients in nanoparticle size have demonstrated significant potential as foliar fertilizers (Singh *et al*., 2024; Hussien *et al*., 2015).

Nanofertilizers are innovative materials derived by preparing macro- and micronutrients in tiny particle sizes with dimensions smaller than 100nm (Duhan *et al*., 2017). Nanoscale-sized nutrients, particularly in metal or metal oxide forms, possess the advantages of small size, and engineered surface area, which facilitates their penetration into plant tissues, delivering the needed nutrients for growth (Ijaz *et al*., 2023; El-Ramady *et al*., 2018). Recent studies highlighted the prospective use of nano ZnO, compared to bulk scale, in enhancing plants' growth parameters, and crop yield and quality (Elbanna *et al*. 2025; Nazir *et al*., 2024; Yusefi-Tanha *et al*., 2020; Dimkpa *et al*., 2019a). Furthermore, it is more efficient than conventional Zn fertilizers in reducing environmental stresss-induced physiological damages, thus sustaining plant defense (Singh *et al*., 2025; Fallah *et al*., 2024; Kareem *et al*., 2022; Mubarak *et al*., 2021; Alabdallah and Alzahrani, 2020; Sun *et al*., 2020). Noticeably, the mentioned efficacy of nano-Zn fertilizers was achieved at lower application rates compared to conventional ones, reducing fertilizer consumption (Elbanna *et al*. 2025). Thus, the utilization of nanoparticles can enhance fertilizer use efficiency, impacting economic profit (El-Ramady *et al*., 2018). Nevertheless, precaution must be taken regarding the employed rates of nano-ZnO, as stimulatory or inhibitory effects on the plant may arise depending on the nanoparticle concentrations (Nazir *et al*., 2024; El-Zohri *et al*., 2021; Yusefi-Tanha *et al*., 2020). Therefore, figuring out the appropriate concentration of nano fertilizer that boosts crop growth and yield is crucial to reduce fertilizer consumption, thereby diminishing the risk to the agroecosystem, besides the economic advantage.

Nitro Humic (NH) is a type of nutrient-enriched humic acid produced to enhance its performance and can be considered an organic source of N that can be employed in organic farming (Mirzaei Varoei *et al*., 2023b). Humic acid easily obtained from the dissolution of humic substances in aqueous alkaline media (Mosa *et al*., 2020). It is a macro-organic molecule possesses important functional groups such as carboxyl, hydroxyl, ester and polysaccharides. This organic material can act as a plant biostimulant (Malyushevskaya *et al*., 2023) since it has the behavior of auxin and cytokinin-like activities, which regulate plant growth parameters and quality (Rathor *et al*., 2024). Further, humic acid influence the plant membranes and enzymatic systems and it is able to mimic various growth hormones and enhance antioxidant enzyme activities, inducing plant tolerance against environmental stresses such as salinity and heat (Zhang *et al*., 2022; Salama *et al*., 2020). Abou El Hassan and Husein (2016) indicated that spraying humic acid reduced the occurrence of blossom end rot in tomato fruits, besides inducing plant growth and fruit quality. Additionally, Avinash *et al*., (2017) reported that enriching humic acid with nutrients enhances its value and efficiency, increasing nutrient absorption. In line with this perspective, in this study, humic acid is enriched with nitrogen and tested by incorporation into the foliar fertilization program of a green leafy vegetable (cabbage).

Cabbage is a highly nutritive vegetable valuable for human health as it is rich in fibers, phenolic acids, and organic volatile compounds and contains vitamins A, B1, B2, and C in addition to some essential nutrients such as calcium, magnesium, potassium, and phosphorus. It is preferred for those who improve their diet and manage diabetes because of its low carbs and high fiber content, possessing a low glycemic index. Further, it is a notable source of glucosinolates and anti-oxidative compounds that can boost the body's natural defenses against incurable diseases such as cancer. It also contributes to reducing inflammation and cardiovascular disease (Ağagündüz *et al*., 2022; Xu *et al*., 2020; Novotny *et al*., 2018). This vegetable has great economic importance all over the world. It is ranked third in the aggregate world vegetable production in 2021, recording 71 million tonnes. Egyptian cabbage production has increased annually by 2.7 % reaching 496 in 2022 and has rated 18th among 133 countries monitored in terms of production of cabbage and other brassicas (FAO, 2022; Anonymous, 2024).

Towards achieving sustainable agriculture principles that consider environmental aspects, the study aims to enhance the nutritional value of cabbage plants via the combined foliar application of nitrogen-enriched humic acid (nitro-humic) as a naturally organic stimulant and green-synthesized zinc oxide nanoparticles (nano-Zn). To achieve this goal, the following hypotheses were investigated: i) foliar spraying of niro-humic with nano-Zn improves the growth traits, quality, biochemical parameters and yield production of cabbage. ii) Utilizing the appropriate rate of nano-Zn combined with niro-humic is vital to the foliar fertilization program of cabbage.

2. Materials and Methods

2.1. Experimental materials

2.1.1. Nitro-humic acid

Nitro-humic acid was prepared via two steps following the procedure outlined by Patti *et al*. (1992) and Fong *et al*. (2007): I] Enriching the compost material with nitrogen, II] Alkaline extraction of nitro-humic. In the first step, compost made from medicinal plant residues (French basil and Geranium plants) was stirred with 3 M nitric acid at a 1:10 ratio for one hour under heating; afterward, the material was retrieved and oven-dried at 105°C. Nitrogen enrichment was achieved through nitric acid treatment. Nitric acid acted both as an activating agent (oxidizing the surface of the raw material) and as a source of nitrogen. According to the literature, one hour was sufficient to complete the reaction between the acid and the substrate. In the second step, 100 g of the dried enriched compost was mixed with KOH (0.25 M) and shaken overnight, and later, the precipitate was separated. This process was repeated three times; the supernatants were combined and subjected to acidification to precipitate the nitro-humic acid. The resulting substance was separated by centrifugation, subsequently purified, and denoted NH. The elemental composition of NH material was performed using Vario Elementar CHNS analyzer (Germany) with SD ±0.4%, with the findings recorded in Table 1. The oxygen percentage was estimated by subtracting from 100 the percentages of all other elements (C, H, N, and S). Further, the material was placed in a FTIR spectrometer (NEXUS 670, America) to identify the main functional groups. The spectrum of NH was measured within a range of 400-4000cm^{-1}, and the assigned wavenumbers of the characteristic absorption bands were listed in Table 1.

Table 1. Elemental composition of nitro-humic (NH) acid and its main characteristic FTIR bands with their corresponding functional groups according to the references.

FTIR bands		
Wavenumber (cm^{-1})	**Functional group**	**Reference**
3410	H-bonded OH groups of alcohol, phenol and -COOH.	Stevenson and Goh (1971)
2930	C-H aliphatic and aromatic chains	Patti et al. (1992)
1712	Carbonyl groups impeded in electrophilic molecules	Fong et al. (2007)
1638	C=C, C=O aromatic groups	Mubarak (2017)
1540	Amide indicating asymmetric NO_2 strech	Fong et al. (2007)
1364	Aromatic nitro (symmetric NO_2 stretch)	Smith (2020)

Elemental composition						
Element	**C**	**H**	**N**	**O**	**S**	**C:N**
Percentage (%)	44.7	6.5	4.9	43.3	0.6	9.12

2.1.2. Nano-Zn fertilizer

Three concentrations of foliar nano-fertilizer (25, 50, and 100 mg Zn l^{-1}) were prepared by diluting different aliquots of a 1000 mg l^{-1} stock solution of green-synthesized zinc oxide nanoparticles (nZnO). The morphological features of the used nanomaterial were observed using a scanning electron microscope (Quanta FEG250; SEM) and a transmission electron microscopy (High-resolution JEM 2100, Japan; TEM). In which a thin layer of nZnO suspension was mounted on an aluminium stub, air-dried and coated with gold, then observed by SEM. The scanning electron microscopy coupled with energy dispersive X-ray spectrometry (**EDX**) (Octane Pro, USA). EDX was employed to verify the elemental composition of the used nanomaterial. For TEM analysis, the suspension was sonicated for 10 minutes, then deposited onto a TEM grid and dried before measurement.

The resulting SEM image revealed that the nanoparticles had a hexagonal and near-spherical to oval shape and tended to aggregate (Fig. 1a), which is a characteristic commonly associated with biosynthesized nZnOs (Chanthapong et al., 2025). TEM analysis provided further information on the shape and size of the particles, revealing well-dispersed particles with an average size of less than 100nm (Fig. 1b). Comparable morphological features were reported in the literature (Muhammad et al., 2019). The EDX pattern depicted diagnostic peaks (Zn, O) attributed to nZnOs and a minor peak for C referred to the biological origin, as well as an Al peak related to the SEM aluminium stub. The absence of any other peak confirmed the good purity of the nanomaterial (Fig. 1c).

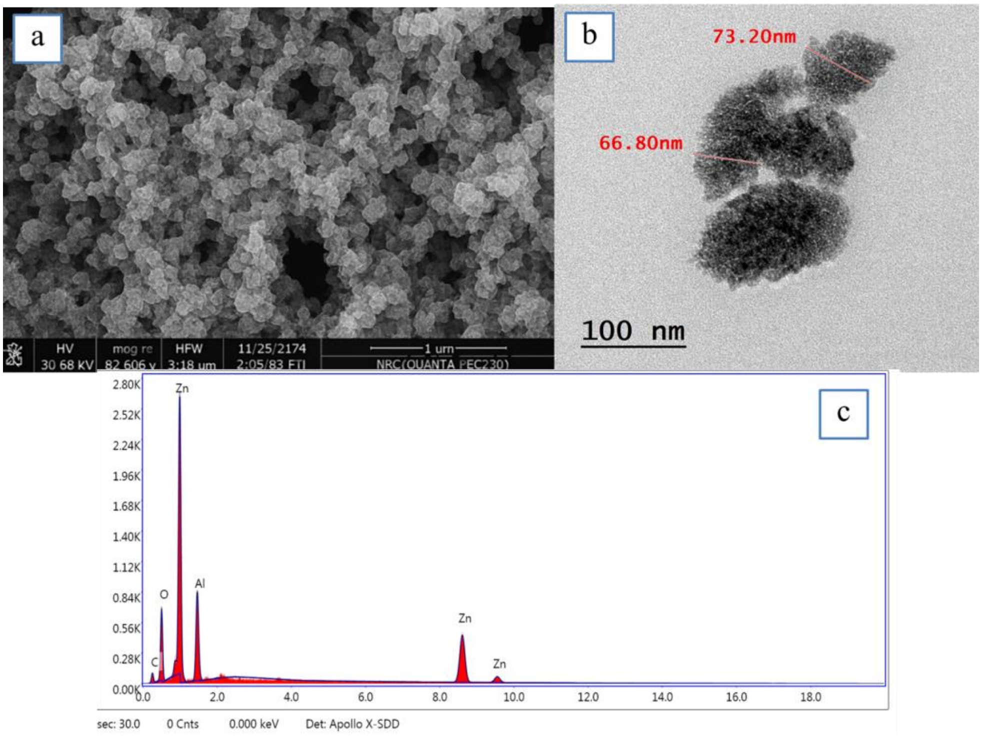

Fig. 1. SEM (a) and TEM (b) images and EDX pattern (c) of the used green-synthesized zinc oxide nanoparticles (nZnOs).

2.2. Experimental site and soil analysis

A field experiment was carried out during two successive seasons, 2021-22 and 2022-23, to assess the influence of combined foliar application of NH as a natural biostimulant and nZnO fertilizer on the growth and quality of cabbage. The experimental site took place at the Research and Production Station of the National Research Centre, located in El-Nubaria (at 30° 30’ N latitude and 30°20’ E longitude), Beheira Governorate, Delta Egypt. The field characterized by its sandy soil texture and low calcium carbonate content. The main chemical characteristics of the experimental soil namely pH and EC were measured according to Jackson (1973), calcium carbonate determined by Collin’s calcimeter (Allison and Moodie, 1965). Available macro- and micronutrient in soil were determined by the methods stated by Cottenie (1980). The obtained data are recorded in Table 2 and Table 3. The irrigation water used in this study was also analyzed for some chemical properties and it is classified as moderately to high saline water (EC 2.73 dS m^{-1}; Table 2).

Table 2. Characteristics of the irrigation water and the experimental soil at the beginning of the experiment.

Parameters	Irrigation water	Experimental Soils 2021-22	Experimental Soils 2022-23
CaCO3 (%)	-	3.3	3.5
pH	7.3	8.22*	8.31*
EC (dS m^{-1})	2.73	2.3**	2.1**
	Soluble ions (meq l^{-1})		
Ca^{++}	7.3	9.2	9.1
Mg^{++}	3.6	3.4	3.7
Na^{+}	15.8	6.9	5.7
K^{+}	0.6	3.5	2.5
CO_3^{--}	-	-	-
HCO_3^{-}	4.9	1.7	1.8
Cl^{-}	15.1	9.6	9.2
SO_4^{--}	7.3	11.7	10.0

*measured in 1:2.5 soil:water suspension. ** measured in 1: 5 soil:water extraction

Table 3. Available macro- and micronutrients in the experimental soil.

Experimental Soils	N	P	K	Fe (mg kg^{-1})	Mn	Zn	Cu
2021-22	10	2.1	67	4.1	0.95	0.5	0.6
2022-23	12.2	2	60	3.9	1.1	0.6	0.56

2.3. Experimental design and treatments

In the current experiment, two rates of NH (2 and 3 ml l^{-1}), denoted 2NH and 3NH, were applied in combination with three fertilization levels of nZnO (25, 50 and 100 mg l^{-1}), referred to as 25 nZnO, 50 nZnO, and 100 nZnO, alongside a control treatment (DW was used as foliar solution), resulting in a total of seven foliar treatments. The experiment was set up in a randomized complete block design with three replicates. The experimental flowchart is presented in Fig 2.

Cabbage seedlings (*Brassica oleracea* L.) were obtained from the vegetable Dept., Egyptian Ministry of Agriculture. They were planted in the field on the 31st and 29th of December for the first and second seasons, respectively. The plot area was 16.8 m^2 and had three rows with 8 m length and 0.7 m width. The irrigation system was drip and the soil was irrigated continuously for three days before transplanting. The basal fertilizers, which included farmyard manure at a rate of 8 tons fed^{-1}, as well as mineral fertilizers: calcium superphosphate at a rate of 100 kg P_2O_5 fed^{-1} and ammonium sulfate at a rate of 20 kg N fed^{-1}, were incorporated before sowing. Potassium sulfate at a rate of 25 kg K_2O kg fed^{-1} and the remaining ammonium sulfate fertilizer (80 kg N fed^{-1}) were divided into two equal portions: the first applied two weeks after transplanting and the second two weeks later beside the plants. The seedlings were spaced 0.5 meters apart along one side of the irrigation line, wherein each was placed next to one irrigation point. The spraying processes of treatments were executed in the early morning, starting 28 days after planting and repeated every two weeks. Before foliar application, nZnO suspensions were sonicated for 20 minutes to have good homogeneity and improve dispersion stability. Small and more evenly distributed nZnO particles will have a greater ability to diffuse into the leaf tissue. All other agricultural practices were performed similarly for all plants as the recommended and conventional cultural services of weeding, control, and irrigation, in order to promote growth and productivity.

2.4. Measurement of vegetative growth, yield parameters and plant analyses

The vegetative growth parameters included stem weight (g), stem diameter and length (cm), number of wrapped leaves per head, as well as head height and circumference (cm). To assess the yield parameters, the outer leaves of the cabbage head were removed, and the fresh weight of the plant's head was recorded, which was then utilized to estimate the yield per ton fed^{-1}. The measurements were carried out for each season.

To evaluate the nutritional status of cabbage plants, leaves samples were collected, oven-dried, and wet-digested according to Cottenie (1980). The digestion solutions were used to estimate macro- and micronutrients. Phosphorous was analyzed spectrophotometrically using nitrovanadomolybdate reagent and potassium content was measured using a flame photometer. Nitrogen content in leaves was distilled and determined using Kjeldahl method. Micronutrients, Fe, Mn, Zn, and Cu, were assessed using atomic absorption spectroscopy in an air-acetylene flame.

Plant leaves were also used for the biochemical analyses, wherein total soluble sugars and total carbohydrates were determined by the phenol-sulphuric method, adopting the procedure described by DuBois *et al.* (1956). Total proteins was measured following the protocol of A.O.A.C (1995) and vitamin "C" was determined by UV-spectrophotometry according to the modified method by Riemschneider et al. (cf. Rahman *et al.* 2007).

2.5. Statistical analyses

The collected data were statistically analyzed using analysis of variance (ANOVA) test at P≤0.05. Duncan's test was adopted to compare differences between treatment means, using MSTAT software (ver.4, 1987).

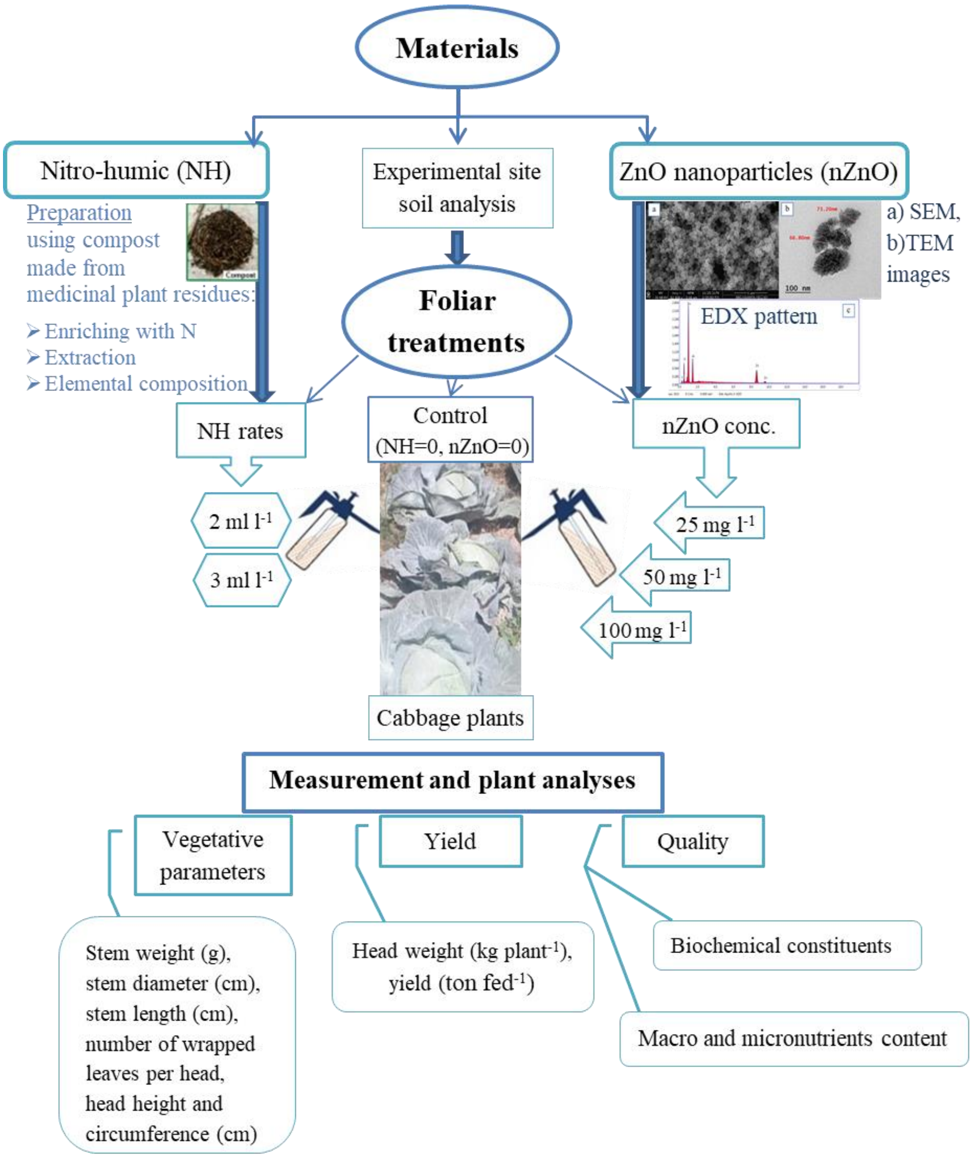

Fig. 2. Experimental flowchart.

3. Results

The elemental analysis of the prepared NH revealed a nitrogen content of 4.9% (Table 1), indicating nitrogen enrichment, as it surpassed that found in conventionally extracted humic acid from compost (1.8-2.7%) (Mahgoub and Abdelhameed, 2023). Further confirmation was provided by the diagnostic bands of the aromatic nitro functional groups assigned at 1540 – 1364 cm^{-1}, corresponding to the asymmetric and symmetric NO_2 stretching (Smith, 2020; Fong et al. 2007).

3.1. Vegetative growth, yield parameters

Influence the prepared NH together with the green-synthesized nZnO on the vegetative growth of cabbage is presented in Table 4 and Table 5. Under any concentration of NH, spraying of nZnO significantly increased the stem length, diameter, and weight in comparison to the control treatment. A similar trend was observed for head growth parameters such as head circumference and height, as well as the number of wrapped leaves per plant. The obtained enhancements were continued by elevating the sprayed rate of NH and concentration of nZnO as well, when it compared to control.

Table 4. Effect of nitro-humic acid and ZnO nanoparticles (N-ZnO) on vegetative growth of cabbage plants.

Treatment	Nitro-humic (NH) rate (ml l^{-1})	nZnO conc. (mg l^{-1})	Stem weight (g)	Stem diameter (cm)	Stem length (cm)	Number of wrapped leaves
			1st season			
Control	0.0	0.0	71.1d	2.99g	4.33g	76.5g
2NH+25 nZnO	2	25	90.8b	3.88d	6.11d	93.2d
2NH+50 nZnO	2	50	99.5a	4.66b	7.2b	100.1b
2NH+100 nZnO	2	100	80.2c	3.56f	5.22f	88.6f
3NH+25 nZnO	3	25	92.2b	3.92c	6.45c	95.1c
3NH+50 nZnO	3	50	103.3a	4.8a	7.56a	104.3a
3NH+100 nZnO	3	100	81.8c	3.62e	5.3e	89.1e
			2nd season			
Control	0.0	0.0	71.2g	2.85d	4.28f	75.2d
2NH+25 nZnO	2	25	89.1d	3.75c	6.15d	95.2bc
2NH+50 nZnO	2	50	96.3b	4.58a	7.23b	102.1ab
2NH+100 nZnO	2	100	81.1e	3.55cd	5.20e	85.6cd
3NH+25 nZnO	3	25	91.3c	3.89bc	6.47c	94.2bc
3NH+50 nZnO	3	50	101.2a	4.77a	7.52a	105.1a
3NH+100 nZnO	3	100	80.5f	3.55cd	5.21e	87.8cd

Different letters indicate a significant difference among foliar treatments under each season ($P \leq 0.05$).

Table 5. Effect of nitro-humic acid and ZnO nanoparticles (nZnO) on head growth parameters.

Treatment	Nitro-humic rate (NH) (ml l^{-1})	nZnO conc. (mg l^{-1})	Head circumference (cm)	Head height (cm)	Head circumference (cm)	Head height (cm)
			1st season		2nd season	
Control	0.0	0.0	36.3c	13.6d	35.9c	13.5d
2NH+25 nZnO	2	25	40.2b	16.3b	40.4b	16.3b
2NH+50nZnO	2	50	46.8a	19.1a	47.0a	19.2a
2NH+100 nZnO	2	100	39.1b	14.9c	38.4b	14.5c
3NH+25 nZnO	3	25	41.3b	16.8b	41.2b	16.6b
3NH+50 nZnO	3	50	47.3a	19.8a	48.1a	19.7a
3NH+100 nZnO	3	100	40.1b	15.0c	39.2b	14.9c

Different letters indicate a significant difference among foliar treatments under each season ($P \leq 0.05$).

All treatments enhanced the yield and yield attributes of cabbage by various ratios. Compared to the control, the combined application of 2NH or 3NH with nZnO at 25 mg l^{-1} increased the head weight by 46.7 - 53.9%, respectively, as a mean value of the two seasons. These values were augmented by raising the sprayed concentration of nZnO to 50 mg l^{-1} under the same NH levels, reaching 80.3-92.5%, respectively (Table 6). Foliar application of nZnO at 25, 50 and 100 mg l^{-1} under 2NH improved the yield of cabbage by 39.3, 59.8 and 25.9%, respectively, in the 1st season; 41.4, 62.2 and 26.1% in the 2nd season relative to the control (Table 6). Elevating the rate of humic to 3NH combined with the same nZnO concentrations raised the obtained cabbage yield by 42.9-43.2%, 62.5-64.9% and 28.6-27.0 %, respectively, in the first and second seasons.

Table 6. Influence of foliar nitro-humic (NH) and ZnO nanoparticles (nZnO) on the yield of cabbage plants.

Treatment	Nitro-humic rate (NH) (ml l^{-1})	nZnO conc. (mg l^{-1})	Head weight (kg)	Yield (ton fed^{-1})	Head weight (kg)	Yield (ton fed^{-1})
			1st season		2nd season	
Control	0.0	0.0	2.95d	11.2d	2.89d	11.1d
2NH+25n-ZnO	2	25	4.33c	15.6b	4.24b	15.7b
2NH+50N-ZnO	2	50	5.23ab	17.9a	5.30a	18.0a
2NH+100n-ZnO	2	100	3.11d	14.1c	3.01d	14.0c
3NH+25 nZnO	3	25	4.55bc	16.0b	4.44b	15.9b
3NH+50 nZnO	3	50	5.66a	18.2a	5.58a	18.3a
3NH+100 nZnO	3	100	3.32d	14.4c	3.21c	14.1c

Different letters indicate a significant difference among foliar treatments under each season ($P \leq 0.05$).

3.2. Effect of treatments on cabbage quality (biochemical constituents and elemental status)

The quality of cabbage is correlated to its nutritional status and chemical constituents, which are primarily affected by the fertilization and associated agricultural practices. The study revealed that the combined application of NH with nZnO increased protein content in the leaves by 22 - 73% than the control and the highest value was signified for the plant treated by 3NH+50nZnO (Table 7). A comparable trend was exposed from the data of the second season. The carbohydrate content as well as the total soluble sugars content in leaves increased with most treatments, being as follows: 3NH+50 nZnO > 2NH+50 nZnO > 3NH+25 nZnO > 2NH+25 nZnO > 3NH+100 nZnO > 2NH+100 nZnO > Control. A similar tendency was pronounced regarding vitamin C content in the fresh leaves. Among the treatments, 2NH+50 nZnO and 3NH+50 nZnO achieved the highest values for the tested biochemical constituents (Table 7).

The concentrations of macro and micronutrients in cabbage leaves were increased by spraying NH and nZnO combination treatments as compared with the control (Table 8). The highest content of N and K were recorded from the plant treated with 3NH+100 nZnO treatment while the highest value of P showed by 3NH+50 nZnO. On the other side, the maximum micronutrient (Fe, Mn, and Cu) content was detected in plants treated with 3NH+50 nZnO, followed by those treated with 2NH+50 nZnO. Regards Zn concentration the highest value achieved by 3NH+100 nZnO treatment. The lowest content of nutrients was noticed in the plants under the control treatment. Both seasons exhibited analogous patterns.

Table 7. Effect of nitro-humic acid and ZnO nanoparticles (nZnO) on yield quality of cabbage plants.

Treatment	Nitro-humic (NH) rate (ml l^{-1})	nZnO conc. (mg l^{-1})	Total protein	Total carbohydrate	Total soluble sugars	Vitamin C
				(%)		mg/100 g FW
		1st season				
Control	0.0	0.0	4.00e	12.9d	7.36e	30.7c
2NH+25 nZnO	2	25	5.80c	14.5b	8.45c	36.6b
2NH+50 nZnO	2	50	6.73b	16.2a	9.25b	38.2ab
2NH+100 nZnO	2	100	4.88d	13.3c	7.66d	31.1c
3NH+25 nZnO	3	25	5.90c	15.1b	8.65c	37.1b
3NH+50 nZnO	3	50	6.91a	16.5a	9.70a	38.7a
3NH+100 nZnO	3	100	4.92d	13.5c	7.88d	32.0c
		2nd season				
Control	0.0	0.0	3.98g	12.7d	7.22d	30.6c
2NH+25 nZnO	2	25	5.78d	14.3c	8.42b	36.1b
2NH+50 nZnO	2	50	6.72b	16.1a	9.24a	37.9ab
2NH+100 nZnO	2	100	4.82f	13.3d	7.58cd	30.7c
3NH+25 nZnO	3	25	5.87c	15.2b	8.61b	37.0b
3NH+50 nZnO	3	50	6.90a	16.6a	9.68a	38.5a
3NH+100 nZnO	3	100	4.90e	13.4d	7.79c	31.9c

Different letters indicate a significant difference among foliar treatments under each season ($P \leq 0.05$).

Table 8. Effect of nitro-humic acid and ZnO nanoparticles (nZnO) on on the nutritional status of cabbage leaves.

Treatment	Nitro-humic (NH rate) (ml l^{-1})	nZnO conc. (mg l^{-1})	N	P	K	Fe	Mn	Zn	Cu
				(%)			mg kg^{-1}		
			1st season						
Control	0.0	0.0	1.13c	0.29c	2.44e	177d	36.1f	23.2e	35.6e
2NH+25 nZnO	2	25	1.14c	0.35b	2.51d	182bc	38.5cd	26.1d	41.3c
2NH+50 nZnO	2	50	1.95b	0.37ab	2.54cd	191ab	40.1b	28.2b	45.4a
2NH+100 nZnO	2	100	2.02a	0.30c	2.55cd	181c	37.8e	30.6a	39.3d
3NH+25 nZnO	3	25	1.13c	0.36ab	2.60bc	183bc	39.0c	26.6d	42.5b
3NH+50 nZnO	3	50	2.00a	0.38a	2.69ab	192a	41.1a	28.3b	45.6a
3NH+100 nZnO	3	100	2.06a	0.31c	2.72a	182bc	38.0de	30.8a	40.1d
			2nd season						
Control	0.0	0.0	1.17c	0.29d	2.45e	175e	36.0f	23.1f	34.9f
2NH+25 nZnO	2	25	1.16c	0.33bc	2.55d	178d	37.7de	25.9e	41.0c
2NH+50 nZnO	2	50	1.95b	0.36ab	2.58cd	188b	40.0ab	28.1c	44.8a
2NH+100 nZnO	2	100	2.03a	0.29d	2.59cd	180cd	36.8ef	30.6b	39.0e
3NH+25 nZnO	3	25	1.14c	0.35b	2.64bc	182c	38.8bc	26.4d	42.1b
3NH+50 nZnO	3	50	2.02a	0.38a	2.71ab	191a	40.2a	28.1c	45.0a
3NH+100 nZnO	3	100	2.07a	0.30cd	2.74a	180cd	38.1cd	31.4a	40.2d

Different letters indicate a significant difference among foliar treatments under each season ($P \leq 0.05$).

4. Discussion

Towards a safe environment and sustainability, this study used NH substance extracted from medicinal plant residues compost as an eco-friendly growth stimulator combined with a green-synthesized nZnO fertilizer to enhance the growth and quality of cabbage. Compared to the control and regardless of the applied concentration of nZnO, spraying of NH improved the measured growth parameters, including stem weight, diameter, and length, as well as head growth attributes: head circumference, height, and the number of wrapped leaves. Additionally, the best values resulted from treatments with the highest NH rate. These findings were in accordance with those of Jan *et al*. (2020) who indicated that the humic acid rate significantly affected stem diameter, plant height, number of branches, fruit diameter, number of fruits per plant, fruit weight and total yield of Chilli. A similar influence was stated by Turan *et al*. (2022) on spinach plants and Abou El Hassan and Husein (2016) on tomato.

Among all treatments, notable enhancements in all studied growth parameters and yield attributes resulted from increasing nZnO concentration up to 50mg l^{-1} compared to control; this was true under any rate of applied NH. Conversely, the lowest increases in these parameter values were noted in plants treated with 2NH+100 nZnO and 3NH+100 nZnO. The results of this study are in accordance with those reported recently on the positive influences of foliar application of nano-Zn fertilizer on the agronomic traits of soya bean, wheat (AbdElAziz *et al*. 2021), safflower (Mubarak *et al*. 2021) and maize (Azam *et al*. 2022). It is known that the enhancement of any morphological and physiological parameters of the plant growth reflects a positive impact on the yield. The aforementioned illustrated the positive response of the cabbage head and stem growth parameter values to the foliar application of NH and nZnO compared to the control, ultimately improving the marketable yield, thereby highlighting the importance of the sprayed materials. As self-observation at the laboratory scale, we observed that humic acid extracted from pre-acid-treated substrate exhibited good storage stability and high resistance to microbial activity, consistent with the findings of Avinash et al. (2017).

The significant enhancements of all evaluated indices in cabbage listed in this work were consistent with the findings of Abed and Sallume (2020) who recorded enhancements in shoot height, leaf number per plant, leaf area, root length, bulb dimensions, protein content and overall yield in onion crops following the application of humic acid at 500 mg l^{-1} with nZnO at 20 mg l^{-1}. The remarkable improvements in growth attributes and yield of cabbages under this study can be ascribed to the role of both sprayed materials (NH and nZnO) in increasing nutrient absorption and stimulating various physiological activities within the plants.

Nitro-humic acid possesses a structure similar to conventional humic acid with additional nitrogen concentration and is characterized by a large organic molecular structure containing active function groups and several elements. The effectiveness of this substance in promoting plant growth is associated with its origin, composition, biological activity, application rate and method of use (Ampong *et al*. 2022; Nardi *et al*. 2021). A debate exists concerning the link between the structural features of humic substances and their stimulating effects in plants. Some studies have shown that the activity of growth processes in plants is closely related to chemical structure rather than to the distribution of molecular weights in humic aggregates, while others support the conclusion that the molecular size of humic substances, their hydrophilicity, and specific functional groups are strongly linked to their activity (Wang et al., 2023; Aguiar et al., 2009). van Tol de Castro *et al*. (2021) stated that the aromatic and aliphatic functional groups in humic acids were responsible for increasing the nutrient uptake and soluble sugar content in rice crops, which enhanced the obtained yield. Humic acid acts as a biostimulant for several bioprocesses in plants such as stomatal conductance, respiration rate, photosynthesis, hormonal activities, and nitrogen metabolism, impacting plant growth, reproduction, and development, hence improving yield quality and quantity (Ichwan *et al*. 2022; García *et al*. 2016). De Hita *et al*. (2020) observed a significant impact on the root architecture and enhancements in both shoot and root growth of cucumber plants sprayed with humic acid. They attributed these effects to alterations in the hormonal balance and the activation of signaling pathways involving several cytokinins, jasmonoyl-isoleucine, jasmonic acid, and salicylic acid in plant tissues, which might explain the enhancements observed in cabbage plants under this study. Moreover, the nitrogen inclusion in NH; as in the case of this study, might enhance the capability of humic acid to promote plant growth. Khan et al. (2019) highlighted the auxiliary impact of humic acid on the physiological processes and growth of maize when incorporated with nitrogen. Mirzaei Varoei et al. (2023a) reported improvements in all the physiological parameters, including plant height, and fresh and dry weight, of savory plants treated with nitro-humic in comparison with those treated with traditional humic acid. Hemati et al. (2022) indicated that nutrients enriched humic acid enhanced growth indices, nutrients uptake and vitality of canola. The study highlighted the preparation method of NH and its potential application as a nutrient-enriched humic acid. The organic nature of nitro-humic suggests its suitability as a source of organic nitrogen for transitional farming systems, where it may partially replace mineral nitrogen fertilizers and may also serve as a potential slow-release nitrogen source (Mirzaei Varoei et al. 2023b).

The role of nZnO in boosting the agronomic traits and head quality indices of cabbage in the current study could be attributed firstly to its small size that enabled easy penetration into plant tissues, ensuring the existence at the plant' needed concentration as an indispensable nutritional element, even though it was sprayed at a lower concentration than the conventional zinc. This interpretation is supported by what was observed by Howladar (2022), as he compared the efficacy of various zinc fertilizer sources on growth characteristics, leaf relative content of water, membranes stability index, photosynthesis performance, chlorophyll, nutrient content, and the activities of non-enzymatic and enzymatic antioxidants in eggplant, and concluded that spraying of nano-sized ZnO at 25 mg l^{-1} showed same efficiency as that attained by normal sized ZnO at 200 mg l^{-1}. Secondly, the beneficial impact of nZnO is also due to the vital role that Zn plays in multiple facets of plant growth and bio-physiological processes. Zinc acts as a regulatory cofactor and engages in several enzymatic activities that are responsible for catalyzing several metabolic processes (Brown et al. 1993). It contributes to photosynthesis and sugar transformation, impacting carbohydrate metabolism (Garza-Alonso et al. 2023). Further, Zn has a direct effect on protein synthesis since it is embedded in its structural components and an indirect effect as an activator for enzymes involved in protein modification. Generally, nanomaterials penetrate plants through the pores of the cell wall. This occurs when their size matches the pore dimensions; however, when the materials are larger than the pore size, they interact with cell wall components (proteins and polysaccharides), initiating the formation of new pores (Yashveer et al., 2022). Foliar nanofertilizers enter plant tissues via the cuticle and stomata. The cuticle contains both hydrophilic pathways (for ionic molecules) and lipophilic pathways (for nonpolar and non-ionic particles). The stomata act as entry gates for suspended materials. In their study on the foliar absorbability and translocation of metal oxide nanoparticles (iron, copper, and zinc) in maize, Li et al., (2024) reported that nZnO has relatively greater hydrophilicity and solubility than the other investigated metal oxide nanoparticles, which facilitates its penetration and distribution within the extracellular space of mesophyll cells in treated leaves. They also noted that a hydrophobic surface and a negative charge assist the foliar absorption of nanoparticles smaller than 200 nm. The boosting mechanism of nZnO in plants can be described in two phases. The first phase involves the interaction of the nanomaterial with the cell membrane, which is modulated by the nanomaterial characteristics such as surface charge, morphological features, and hydrophobicity. This interaction can disrupt membrane integrity and alter signaling pathways involving redox balance, membrane potential, protein synthesis, and gene expression. A biostimulatory effect results from the dissemination of these signals between cells. A comparable response can also occur once the nanomaterials enter the cell via active processes such as diffusion or endocytosis, where they interact with chloroplasts, mitochondria, or the nucleus. he second phase entails transformation processes. Following the internalization of nanomaterials into cells, transformation and ionization occur, leading to the release of elemental components, including Zn^{2+} ions. These processes are governed by the physicochemical characteristics of the nanomaterials, as well as by plant-specific factors such as species, developmental stage, and growth rate, in addition to prevailing environmental conditions. The resulting free ions in the cytoplasm of plant cells can engage in various ongoing metabolic processes (Garza-Alonso et al., 2023).

In the present study, nZnO-containing treatments showed superiority in the measured growth indices as well as biochemical constituents: carbohydrate, soluble sugars, vitamin C, and protein content, along with macro and micronutrient concentrations; in cabbage leaves, when compared to the control treatment. These findings align with those stated by Garza-Alonso *et al*. (2023), illustrating that foliar nZnO promoted antioxidant compounds including chlorophylls, flavonoids, and vitamin C, increasing absorption of Ca, Mg, S, Fe, Mn, Zn, and Si and improving fresh and dry weight in lettuce. A similar influence was observed in sweet basil, tomato and Chinese mustard (Singh *et al*. 2025; Faizan *et al*. 2018; El-Kereti *et al*. 2013). It is worth mentioning that spraying 2NH+100 nZnO and 3NH+100 nZnO yielded the lowest values for the measured parameters in cabbage among all foliar treatments, yet these values were still higher than those obtained from the control. This was related to using nZnO at 100 mg l^{-1} which might cause a kind of stress to the plant resulting from accumulation of Zn. El-Zohri et al. (2021) illustrated that the high rate of Zn application increased reactive oxygen variables as a defense mechanism concomitant with exposing plants to stress. Furthermore, the excess of Zn might lead to an antagonistic relationship with other nutrients, as shown in the case of phosphorus; this phenomenon affects nutrient uptake, translocation and absorption (Xian et al., 2024). Therefore, attention to selecting the appropriate concentration of applied nano-fertilizers and managing nutrient interaction is crucial.

Moreover, the proposed NH and nZnO materials were applied foliarly. It is worth mentioning that their absorption efficiency can be influenced by several factors associated with this technique (Hu et al., 2023). Some of these factors relate to the sprayed materials themselves, such as molecular size, solubility, application rate, functional groups, and material characteristics, which may affect the foliar uptake in the case of NH. Factors such as homogeneity, particle size distribution, surface charge, composition, and particle dispersion can also contribute to the foliage uptake of nZnO. Other factors that can modulate foliar absorption are associated with plant characteristics, including plant species and features of the leaf surface (such as cuticles, stomata, wax layers, lenticels, and trichomes), as well as environmental factors (light, temperature, and humidity). Future studies addressing the mentioned factors will be essential to enhance the foliar absorbability of the proposed materials and thereby maximize their use efficiency.

In addition, cabbage has been rated as moderately to sensitive to salinity (Pavlović et al. 2019; Shannon and Grieve 1998). The irrigation water used in this study falls into the moderately to high saline category (Table 2), which requires careful management practices (Zaman et al. 2018; Abou El-Defan et al. 2016; Rhoades et al. 1992). As observed in this study with the abovementioned conditions, the treated cabbage plants tended to increase the measured indices over those untreated. Hence, foliar application of NH combined with nZnO fertilizer could be an effective approach to support plants' resilience to environmental stress, along with improving nutritional and quality aspects of the yield, thereby ensuring sustainable quality of crop production.

5. Conclusions

The study investigates the influence of co-foliar application of green-synthesized ZnO nanoparticles (nZnO) and nitro-humic acid (NH) on the growth, quality and yield of cabbage plants. All nZnO rates with both NH levels positively influenced the measured growth indices and biochemical constituents (carbohydrate, soluble sugars, vitamin C, and protein content), as well as macro and micronutrients in cabbage leaves, compared to the control treatment. The best results for yield and yield attribute values were recorded for the plants treated with 3NH+50 nZnO and 2NH+50 nZnO. The results remarked that employing the highest level of nZnO may cause an antagonistic relationship between nutrients, affecting their absorption.

The study provides a viable method can be applied within an integrated nutrient management strategy, optimizing nutrient utilization and delivery to plants, along with reducing environmental impact which are priorities for sustainable agriculture.

List of abbreviations:
NH: Nitrogen-enriched humic acid (nitro-humic)
nZnO: Green-synthesized zinc oxide nanoparticles
SEM: Scanning electron microscope
EDX: energy dispersive X-ray spectrometry
TEM: Transmission electron microscopy
DW: Distilled water

Declarations
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