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Integration of EM Bokashi, Balanced NPK, Nanoparticles of Silicon and Selenium in Sustainable Soil and Physiology of Cabbage

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ABSTRACT

A field experiment was conducted in the Al-Husseiniya region, Babylon Province, Iraq, during the autumn season to assess the environmental and agronomic impact of integrated fertilization strategies on hybrid cabbage (Brassica oleracea L., cv. Top White F1). The study evaluated the effects of soil application of effective microorganism (EM) Bokashi (5 Mg ha^{-1}), balanced NPK (Nitrogen, Phosphorus, and Potassium) (20:20:20 at 300 kg ha^{-1}), and foliar spraying with silicon and selenium nanoparticles (15 mg L^{-1} each), applied in various combinations. The treatments aimed to enhance plant nutrition, promote sustainable soil fertility, and reduce reliance on synthetic inputs. A total of 16 treatments, including a control, were arranged in a randomized complete block design with three replications. Results revealed that the integrated application of EM Bokashi, NPK, Nano-silicon (SiNPs), and Nano-selenium (SeNPs) significantly improved the physiological and nutritional performance of cabbage. The highest concentrations of macro- and micronutrients, as well as essential amino acids, were recorded under this combined treatment. Notably, nitrogen (1.98%), potassium (3.11%), and micronutrients such as iron (185.6 mg kg^{-1}) and selenium (0.102 mg kg^{-1}) were substantially enhanced. Amino acid profiling via HPLC also indicated significant increases in key amino acids like methionine (28.42 mg/100 mg DW) and arginine (42.99 mg/100 mg DW). These findings suggest that integrating organic and nano-based fertilization approaches can improve crop nutritional quality while supporting environmentally sustainable agricultural practices.

Keywords: EM Bokashi; Balanced NPK; Nano-Silicon; Nano-Selenium; Cabbage

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1. Introduction

Calcareous soils in arid and semi-arid regions face structural and nutritional challenges that limit agricultural productivity. The use of bio-organic fertilizers such as EM Bokashi is a promising approach to mitigate soil fertility degradation within the context of sustainable agriculture. EM Bokashi not only provides nutrients but also represents an integrated strategy for enhancing agricultural productivity with efficiency and sustainability [1,2].

Xu et al. [3] reported that EM Bokashi improved photosynthesis and fruit yield, and increased sugar and organic acid concentrations in fertilized fruits. Moreover, fruits from plants fertilized with poultry manure and Bokashi had higher vitamin C levels than those treated with chemical fertilizers alone. EM Bokashi inoculation was found to increase vitamin C content across all fertilization treatments.

Although many fertilization techniques are currently in use, foliar fertilization has emerged as a potential strategy for enhancing plant salt tolerance by directly supplying nutrients through the leaves [4,5]. Some studies have highlighted that integrated mineral fertilization and bio-inoculation significantly influence cabbage yield and quality [6]. Reducing mineral fertilization alongside bio-inoculation improved quality and yield without compromising productivity [7].

Soil fertilization using EM Bokashi and mineral fertilizers (NPK) is considered a complementary and effective approach that contributes to enhancing soil biological fertility and promoting plant growth. Bokashi is a fermented organic mixture containing beneficial microorganisms, and studies have shown that its application improves the physical and chemical properties of the soil and enhances the growth of crops such as cabbage and beet by increasing organic matter content and the availability of essential nutrients [8]. In the same context, foliar spraying with silicon nanoparticles (Si-NPs) plays a prominent role in strengthening plant resistance to environmental stresses such as drought and heavy metal accumulation. It enhances nutrient uptake and activates antioxidant enzymes. Studies have shown that nano-silicon has a positive effect on plant growth and physiological functions under stress conditions [9]. Likewise, nano-selenium (Se-NPs) is a trace element essential for enhancing the plant's physiological defenses by activating antioxidant enzymes such as SOD, CAT, and APX. Research has demonstrated its significant role in improving disease resistance and promoting growth under biotic stress, such as fungal infections [10]. Integrating these factors presents a promising strategy to improve the physiological performance and nutritional quality of cabbage under sustainable agricultural systems. This study aimed to analyze the effects of different fertilization systems, fertilization of bio-organic and balanced mineral (NPK 20:20:20), spraying nanomaterials and their combinations, on the concentrations of macro nutrients (N, P, K) and micronutrients (Fe, Zn, Mn) in cabbage heads, as well as to evaluate the effect of the study treatments on the content of amino acids in cabbage heads under local environmental conditions.

2. Materials and Methods

2.1. Site Description and Experimental Design

The field experiment was conducted in Subdistrict 41, Al-Husseiniya–Al-Taleea District–Babylon Governorate, Iraq, at the geographical coordinates 32.1804358°N latitude and 44.8054460°E longitude during the fall season. The experimental field was prepared by performing perpendicular plowing, harrowing, and laser leveling. A composite soil sample was collected from a depth of 30 cm by combining samples from the four corners and the center of the field to represent its soil characteristics. The soil was classified as silty clay loam, as shown in **Table 1**, which presents the physical, chemical, and biological properties of the soil prior to planting.

The experiment was laid out in a randomized complete block design (RCBD) with a one-direction layout and three replications. Each replication included 16 treatments, which were randomly distributed within each block. A total of 48 experimental units were established.

Soil fertilization treatments included:

Application of EM Bokashi (EM B) bio-organic fertilizer at a rate of 5000 kg ha^{-1},

Application of balanced mineral fertilizer NPK (20:20:20) at a rate of 300 kg ha^{-1}.

Foliar spraying treatments included:

Nano-silicon (SiNPs) at a concentration of 15 mg L^{-1},

Nano-selenium (SeNPs) at a concentration of 15 mg L^{-1}.

The foliar sprays were applied as individual treatments, binary combinations, ternary combinations, and one quaternary combination, in addition to a control treatment. The nanomaterials were sprayed four times, using a spraying volume of 400 L ha^{-1} per application. The fertilizer treatments, their respective quantities, foliar spray concentrations, and spraying schedule are detailed in **Table 2**. The experiment was arranged using a Randomized Complete Block Design (RCBD) with three replications. Each replication included 16 treatments randomly assigned to experimental units. The treatments included: EM Bokashi (5000 kg ha^{-1}), balanced mineral fertilizer NPK (20:20:20) at 300 kg ha^{-1}, foliar sprays of nano-silicon (SiNPs) and nano-selenium (SeNPs) at 15 mg L^{-1} individually and in combinations (binary, ternary, and quaternary), in addition to a control treatment. This resulted in a total of 48 experimental units. **Table 2** details the fertilizer treatments, quantities, concentrations, and number of foliar sprays.

Table 1. Physical and Chemical Properties of the Field Soil before Planting.

Parameter	Value	Unit
Soil Ph	7.71	-
Electrical conductivity (EC)	2.65	dS m^{-1}
Cation exchange capacity (CEC)	27.80	cmol(+) kg^{-1} soil
Organic matter content	9.7	g kg^{-1} soil
Carbonate minerals	217	g kg^{-1} soil
Bulk density	1.38	Mg m^{-3}
Available nitrogen	18.26	mg N kg^{-1} soil
Available phosphorus	10.30	mg P kg^{-1} soil
Available potassium	257.39	mg K kg^{-1} soil
Sand	134	g kg^{-1} soil
Silt	560	g kg^{-1} soil
Clay	306	g kg^{-1} soil
Soil texture	Silty clay loam	-

Table 2. Fertilization Treatments Applied in the Experiment, Including the Amount of Added Fertilizers (kg ha^{-1}), Concentrations of Sprayed Nanomaterials (mg L^{-1}), and the Number of Foliar Spray Applications.

No.	Fertilizer Treatment Description	Treatment Code	Added Fertilizer Amounts (kg ha^{-1})	Nanomaterial Spray Concentration (mg L^{-1})			
				Spray 1	Spray 2	Spray 3	Spray 4
1	Control (water spray only)	C.O.	0				
2	Soil application of EM Bokashi	EM B.	5000	0	0	0	0
3	Soil application of balanced NPK	NPK	300	0	0	0	0
4	Foliar application of Si-NPs	SiNPs	0.024	15	15	15	15
5	Foliar application of Se-NPs	SeNPs	0.024	15	15	15	15
6	Foliar application of Si-NPs + Se-NPs	SiNPs+SeNPs	0.024 + 0.024	15 + 15	15 + 15	15 + 15	15 + 15
7	NPK + foliar spray of Si-NPs	NPK+SiNPs	300 + 0.024	15	15	15	15
8	NPK + foliar spray of Se-NPs	NPK+SeNPs	300 + 0.024	15	15	15	15
9	NPK + foliar spray of Si-NPs + Se-NPs	NPK+SiNPs+SeNPs	300 + 0.024 + 0.024	15 + 15	15 + 15	15 + 15	15 + 15
10	EM B. + foliar spray of Si-NPs	EM B.+SiNPs	5000 + 0.024	15	15	15	15
11	EM B. + foliar spray of Se-NPs	EM B.+SeNPs	5000 + 0.024	15	15	15	15
12	EM B. + foliar spray of Si-NPs + Se-NPs	EM B.+SiNPs+SeNPs	5000 + 0.024 + 0.024	15 + 15	15 + 15	15 + 15	15 + 15
13	EM B. + balanced NPK fertilizer	EM B.+NPK	5000 + 300	0	0	0	0
14	EM B. + NPK + foliar Si-NPs	EM B.+NPK+SiNPs	5000 + 300 + 0.024	15	15	15	15
15	EM B. + NPK + foliar Se-NPs	EM B.+NPK+SeNPs	5000 + 300 + 0.024	15	15	15	15
16	EM B. + NPK + foliar Si-NPs + Se-NPs	EM B.+NPK+SiNPs+SeNPs	5000 + 300 + 0.024 + 0.024	15 + 15	15 + 15	15 + 15	15 + 15

2.2. Field Layout and Crop Establishment

The total field area was 8642 m², divided into three equal replications. Each replication included 16 experimental units, with each unit measuring 3 m × 4 m (12 m²). A buffer of 1 m separated the replications, and 0.5 m separated the individual treatments. Cabbage seedlings (hybrid cultivar: Top White F1) were transplanted on 15/9/2024 at a spacing of 0.75 m between rows and 0.5 m between plants, with an estimated planting density of 13,364 plants ha^{-1}. Furrows were prepared for irrigation, and drainage channels were constructed to remove excess water. Standard field management practices were followed, including irrigation as needed. Missing seedlings were replaced on 22/9/2024 using same-aged transplants. Details of the field treatments and fertilizer applications are presented in **Table 3**.

Table 3. Fertilizers and Nano-Materials Used in the Experiment.

Fertilizer Name	Type	Composition and Purity
EM Bokashi	Organic Biofertilizer	Nocardia (45.45%), Streptomyces (36.36%), Terrabacter (9.1%), Micromonospora (4.54%), Mycobacterium (4.54%), fungi species; O.M (82.5%), pH (4.90), NaCl (0.25%), Na (0.10%), Moisture (7.74%), EC (7.30 dS m^{-1}), CFU (1.45×10^6)
NPK	Chelated Mineral Fertilizer	20% N, 20% P, 20% K + micronutrients
SeNPs	Dry Nanomaterial	99.9% Selenium (**Figure 1**)
SiNPs	Dry Nanomaterial	99.9% Silicon (**Figure 2**)

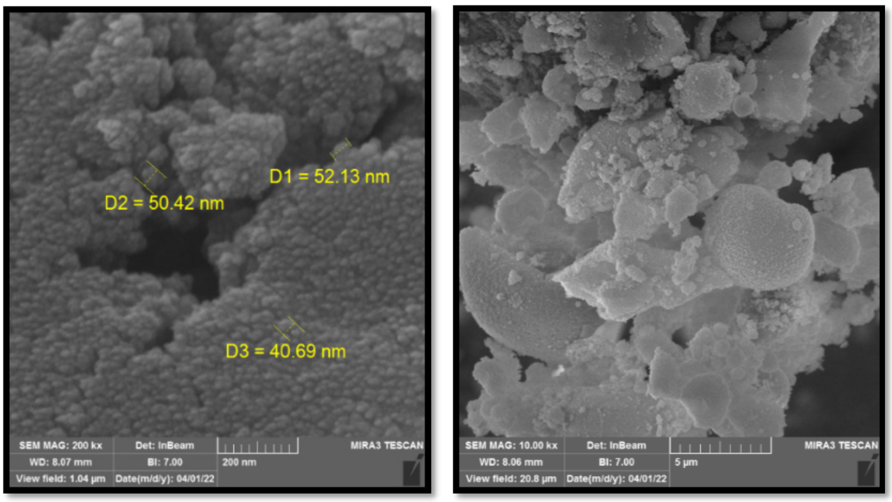

Figure 1. SeNPs under FE-SEM Microscope.

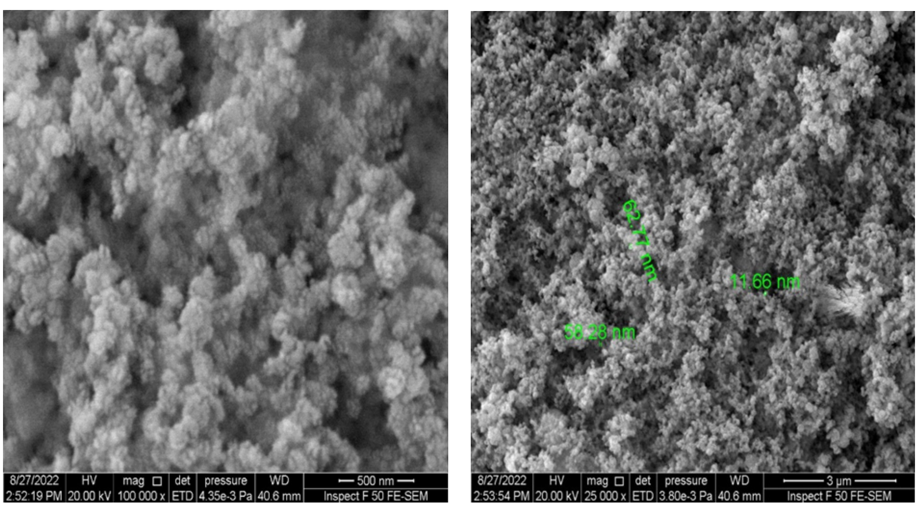

Figure 2. SiNPs under FE-SEM Microscope.

Organic Fertilizer (EM Bokashi): Applied at a rate of 5000 kg ha^{-1}, 15 days after transplanting, in a single dose. The fertilizer was incorporated into the soil in 5 cm deep trenches, placed 5 cm from the planting line.

Mineral Fertilizer (NPK): Applied at a total rate of 300 kg ha^{-1}, split into four equal doses during the growth period.

Nano-Fertilizer Foliar Application: Silicon and selenium nanoparticles were sprayed at 15 mg L^{-1}, starting one month after transplanting. A total of four foliar sprays were applied at weekly intervals using a foliar sprayer at 400 L ha^{-1}. All sprays were carried out in the late evening to minimize evaporation and enhance absorption.

2.3. Nutrient Content Determination in Cabbage Heads

The nitrogen concentration (%) was measured using the Kjeldahl method as described by Akol et al. [11] and Hassan et al [12]. Approximately 5 g of dried sample was digested with concentrated H_2SO_4 and a digestion mixture of potassium sulfate and copper sulfate. The digested sample was distilled using the Kjeldahl apparatus, and the released ammonia was trapped in 20% boric acid and titrated with 0.1 N HCl. A blank sample was also prepared. Nitrogen percentage was calculated as:

Nitrogen percentage (%) = (Volume of HCl consumed × Normality × 0.014) / Sample weight × 100

Phosphorus Concentration (%): Determined using the colorimetric method as described by Chapman and Pratt (1962). A 0.5 g sample was digested using 5 mL H_2SO_4 and 2 mL $HClO_4$. Molybdate and ascorbic acid reagents were used, and absorbance was measured at 700 nm using a spectrophotometer.

Potassium (%), Micronutrients (Mn, Zn, Fe, Cu, Se in mg kg^{-1}), and Silicon (µg kg^{-1}): These elements were determined using wet digestion (APHA, 2017). Three grams of plant powder were digested using $HClO_4$ and HNO_3 on a hot plate. After the digest turned light-colored, 5 mL of diluted HCl (1:1) was added to dissolve any residue, followed by filtration and volume adjustment to 50 mL. Samples were analyzed using an Atomic Absorption Spectrophotometer (SHEMADZU AA 7000).

Determination of Amino Acids in Cabbage Heads: The analysis was conducted at the Scientific Research Authority Laboratories / Environmental and Water Research Center using a high-performance liquid chromatography (HPLC) system (German-made). The method was adopted, in which the mobile phase consisted of methanol, acetonitrile 5% formic acid in the ratio (20:60:20), with a flow rate of 1 mL/min. An HPLC column (C18–NH2, 250 mm × 4.6 mm) was used for amino acid separation, and a fluorescence detector was employed for detection at wavelengths of Ex = 445 nm and Em = 465 nm.

1. Extraction Process: Amino acids were extracted according to the method described by Dahl-Lassen et al. [13]. A 3 g sample was weighed and placed in a 25 mL volumetric flask. Then, 25 mL of 1 M hydrochloric acid was added, and the mixture was heated at 55°C for 3 hours. The sample was subsequently evaporated to dryness using a rotary evaporator. Afterward, 5 mL of sodium citrate buffer (pH 2.2) was added. The solution was filtered using a 0.45 µm plastic filter and injected into the HPLC system.
2. Derivatization Process: One milliliter of the extracted sample was mixed with 200 µL of 5% ortho-phthaldehyde (OPA), and the mixture was vortexed for 2 min. Subsequently, 100 µL of the resulting solution was injected into the HPLC system for analysis.
3. Statistical Analysis: The experimental data were statistically analyzed using a randomized complete block design (RCBD) to study the effect of the investigated treatments on various traits. Significant differences between means were compared using Duncan's Multiple Range Test (1955) at a probability level of $p \leq 0.05$. The SAS software (2012) was used for statistical analysis based on the following mathematical model:

Yij = µ + Ti + Pj + eijY_{ij} = \mu + T_i + P_j + e_{ij}Yij= µ + Ti+ Pj+ eij

3. Results

3.1. Effect of EM Bokashi, NPK, and Nano-Silicon and Nano-Selenium Sprays on Macro- and Micronutrient Concentrations and Silicon Content in Cabbage Heads

3.1.1. Nitrogen Concentration (%) in Cabbage Heads

As shown in **Table 4**, the highest significant ($p \leq 0.05$) nitrogen concentration in cabbage heads was recorded in the combined treatment EM B+NPK+SiNPs+SeNPs (1.98%). This was followed by the triple combinations EM B+NPK+SeNPs and EM B+NPK+SiNPs, which recorded 1.90% and 1.93%, respectively, both statistically similar to EM B+NPK (1.89%). All other treatments outperformed the control (1.41%).

3.1.2. Phosphorus Concentration (%)

The highest significant phosphorus content was observed in the EM B+NPK+SiNPs+SeNPs treatment (0.125%), followed by the triple combinations EM B+NPK+SeNPs and EM B+NPK+SiNPs, both with equal values (0.122%). These treatments significantly surpassed the control (0.101%).

3.1.3. Potassium Concentration (%)

Similar to phosphorus, the EM B+NPK+SiNPs+SeNPs treatment achieved the highest potassium concentration (3.113%), significantly higher than the control (2.587%). The triple treatments EM B+NPK+SeNPs and EM B+NPK+SiNPs followed with 3.047% and 3.073%, respectively. All other treatments outperformed the control except for the sole or dual nano-fertilizer treatments (SiNPs, SeNPs, and SiNPs+SeNPs), which were statistically similar to the control.

3.1.4. Zinc Content (mg Zn kg^{-1} Dry Weight)

All treatments significantly improved zinc content compared to the control (11.11 mg Zn kg^{-1}). The highest value was observed in the EM B+NPK+SiNPs+SeNPs treatment (16.51 mg Zn kg^{-1}), followed by the various triple, double, and single treatments of EM Bokashi, NPK, SiNPs, and SeNPs.

Table 4. Effect of the Application of EM Bokashi Bio-organic Fertilizer, Balanced NPK Mineral Fertilizer, and Foliar Spray with (Si-NPs) and (Se-NPs) on the Concentrations of Macronutrients (N, P, K, %) and the Contents of Iron, Zinc, Copper, Manganese, and Selenium (mg kg^{-1} Dry Matter) and Silicon (µg kg^{-1} Dry Matter) in Cabbage Heads.

No.	Treatments	N (%)	P (%)	K (%)	Fe (mg kg^{-1})	Mn (mg kg^{-1})	Zn (mg kg^{-1})	Cu (mg kg^{-1})	Se (mg kg^{-1})	Si (µg kg^{-1})
1	Control (C.O.)	1.417 I	0.101 J	2.587 E	173.4 I	20.64 d	11.11 N	18.09 N	0.086 Fg	0.008 G
2	EM Bokashi	1.587 G	0.112 Efgh	2.823 D	178.9 Efg	22.10 bcd	14.04 G	19.99 I	0.091 Defg	0.110 Efg
3	NPK	1.607 Fg	0.111 Fghi	2.837 D	176.7 Gh	21.46 cd	13.31 J	18.99 K	0.089 Efg	0.098 Efg
4	Si-NPs	1.427 Hi	0.106 Ij	2.603 E	174.7 Hi	20.80 d	11.35 M	18.37 M	0.085 G	0.143 Efg
5	Se-NPs	1.463 Hi	0.107 I	2.623 E	174.6 Hi	20.97 d	11.72 L	18.42 M	0.091 Defg	0.090 Fg
6	Si-NPs + Se-NPs	1.470 H	0.109 Hi	2.637 E	174.8 Hi	20.84 d	12.32 K	18.66 L	0.097 Abcd	0.152 Efg
7	NPK+Si-NPs	1.650 Ef	0.113 Defgh	2.850 D	176.4 Gh	21.67 cd	13.59 I	19.22 J	0.093 Cdef	0.212 Def
8	NPK+Se-NPs	1.760 C	0.110 Ghi	2.897 Cd	177.0 Fgh	21.95 bcd	13.69 Hi	19.14 J	0.095 Abcde	0.478 Ab
9	NPK+Si-NPs+Se-NPs	1.723 Cd	0.117 Cde	2.910 Cd	177.4 Fgh	22.81 abc	13.87 Gh	20.17 H	0.098 Abc	0.262 Cde
10	EM B.+Si-NPs	1.630 Fg	0.114 Cdefg	2.847 D	179.7 Def	22.10 bcd	14.30 F	20.81 G	0.095 Bcde	0.327 Bcd
11	EM B.+Se-NPs	1.683 De	0.116 Cdef	2.893 Cd	180.4 Cde	22.48 bc	14.36 F	22.56 F	0.101 Ab	0.092 Fg
12	EM B.+Si-NPs+Se-NPs	1.727 Cd	0.118 Bcd	2.907 Cd	181.1 Bcde	22.82 abc	14.74 E	22.90 E	0.095 Bcde	0.374 Abc
13	EM B.+NPK	1.897 B	0.119 Bc	2.987 Bc	182.2 Bcd	22.04 bcd	15.00 D	23.17 D	0.096 Abcde	0.143 Efg
14	EM B.+NPK+Si-NPs	1.930 B	0.122 Ab	3.073 Ab	182.6 Bc	22.90 abc	15.34 C	23.92 C	0.097 Abcd	0.389 Abc
15	EM B.+NPK+Se-NPs	1.900 B	0.122 Ab	3.047 Ab	183.5 Ab	23.17 ab	15.88 B	24.13 B	0.101 Ab	0.153 Efg
16	EM B.+NPK+Si-NPs+Se-NPs	1.980 A	0.125 A	3.113 A	185.6 A	24.06 a	16.51 A	24.96 A	0.102 A	0.526 A

3.1.5. Copper Content (mg Cu kg^{-1} Dry Weight)

The EM B+NPK+SiNPs+SeNPs treatment again showed the highest copper content (24.96 mg Cu kg^{-1}). Close values were observed in the EM B+NPK+SeNPs and EM B+NPK+SiNPs treatments (15.88 and 15.34 mg Cu kg^{-1}, respectively). All other treatments outperformed the control (18.09 mg Cu kg^{-1}).

3.1.6. Manganese Content (mg Mn kg^{-1} Dry Weight)

Manganese results followed a similar trend to iron and copper. All treatments significantly outperformed the control (20.64 mg Mn kg^{-1}), except the individual foliar applications of SiNPs and SeNPs. The highest Mn content was recorded in EM B+NPK+SiNPs+SeNPs (24.06 mg Mn kg^{-1}), followed by EM B+NPK+SeNPs (23.17 mg Mn kg^{-1}).

3.1.7. Silicon Content (µg Si kg^{-1} Dry Weight)

The EM B+NPK+SiNPs+SeNPs treatment significantly increased silicon content to 0.526 µg Si kg^{-1} compared to the control (0.008 µg Si kg^{-1}). This treatment outperformed all individual and combined applications. For example, Si content in EM Bokashi, NPK, SiNPs, and SeNPs was 0.110, 0.098, 0.143, and 0.090 µg Si kg^{-1}, respectively, while the dual treatment NPK+SiNPs showed a notable increase (0.212 µg Si kg^{-1}).

3.1.8. Selenium Content (mg Se kg^{-1} Dry Weight)

Selenium content was highest in the EM B+NPK+SiNPs+SeNPs treatment (0.102 mg Se kg^{-1}), significantly exceeding the control (0.086 mg Se kg^{-1}). Elevated values were also recorded in EMB+SeNPs, EMB+SiNPs+SeNPs, and EMB+NPK+SeNPs (0.101, 0.095, and 0.101 mg Se kg^{-1}, respectively). Some treatments showed no significant difference from the control.

3.1.9. Iron Content (mg Fe kg^{-1} Dry Weight)

Table 4 also shows that all treatments significantly improved iron content over the control. The EM B+NPK+SiNPs+SeNPs treatment recorded the highest value (185.6 mg Fe kg^{-1}), followed by triple combinations EM B+SiNPs+SeNPs, EM B+NPK+SiNPs, and EM B+NPK+SeNPs with values of 181.1, 182.6, and 183.5 mg Fe kg^{-1}, respectively. All exceeded the control (173.4 mg Fe kg^{-1}).

3.2. Effect of EM Bokashi, NPK, and Foliar Si-NPs and Se-NPs, Alone or Combined, on Amino Acid Content in Cabbage Heads

The results in **Table 5** revealed that all soil-applied treatments of EM Bokashi, balanced NPK, and foliar spray of Si-NPs and Se-NPs whether individually or in dual, triple, or quadruple combinations-significantly ($p \leq 0.05$) increased the amino acid content in cabbage heads compared to the control, with the exception of the Si-NPs treatment for Methionine, Arginine, and Glutamic acid, which recorded (19.87, 32.53, and 15.51 mg 100 mg^{-1} dry matter, respectively) and did not differ significantly from the control. Also, neither the individual nor combined application of Se-NPs with Si-NPs significantly affected Arginine compared to the control. The quadruple combination (EMB+NPK+SiNPs+SeNPs) recorded the highest and most significant increase ($p \leq 0.05$) in all amino acids tested, reaching (20.54, 14.65, 12.65, 28.42, 42.99, and 21.48 mg 100 mg^{-1} dry matter), respectively, compared to the control (12.03, 9.22, 7.11, 19.76, 32.04, and 15.08). It was followed by the triple combination (EMB+NPK+SeNPs), then (EMB+NPK+SiNPs), and the dual combination (EMB+NPK), respectively.

Table 5. Effect of Various Fertilizer Applications (Organic, Mineral, Nano, and Their Combinations) on the Amino Acid Content in Cabbage Heads after Harvest (mg 100 mg^{-1} Dry Matter).

No.	Treatments	Tryptophan	Alanine	Glycine	Methionine	Arginine	Glutamic Acid
1	Control (C.O.)	12.03 J	9.22 o	7.11 O	19.76 I	32.04 I	15.08 J
2	EM Bokashi	16.80 d	11.92 h	9.28 J	23.03 E	36.13 G	17.79 Ef
3	NPK	15.37 g	11.36 k	9.00 K	21.56 G	35.24 H	17.21 H

Table 5. *Cont.*

No.	Treatments	Tryptophan	Alanine	Glycine	Methionine	Arginine	Glutamic Acid
4	Si-NPs	12.33 ij	9.44 n	7.72 N	19.87 I	32.13 I	15.12 J
5	Se-NPs	12.62 hi	9.58 m	7.91 M	19.92 Hi	32.46 I	15.61 I
6	Si-NPs+Se-NPs	12.87 h	9.84 L	8.40 L	20.21 H	32.53 I	15.51 I
7	NPK+Si-NPs	15.75 fg	11.53 J	9.47 I	21.38 G	35.78 G	17.37 Gh
8	NPK+Se-NPs	15.87 F	11.65 I	9.63 Gh	21.95 F	35.90 G	17.57 Fg
9	NPK+Si-NPs+Se-NPs	16.30 e	11.94 h	10.04 F	21.96 F	36.26 G	18.41 D
10	EM B.+Si-NPs	16.94 d	12.24 g	9.58 Hi	23.21 E	36.86 F	17.43 Gh
11	EM B.+Se-NPs	17.08 d	12.39 F	9.69 G	23.53 D	37.18 Ef	17.96 E
12	EM B.+Si-NPs+Se-NPs	17.22 d	12.58 e	10.18 E	23.80 D	37.51 De	17.77 Ef
13	EM B.+NPK	17.90 c	12.80 d	10.57 D	24.24 C	38.00 D	18.93 C
14	EM B.+NPK+Si-NPs	17.80 c	12.93 c	11.26 C	26.30 B	39.41 C	19.44 B
15	EM B.+NPK+Se-NPs	18.66 b	13.48 b	11.76 B	26.58 B	40.72 B	19.72 B
16	EM B.+NPK+Si-NPs+Se-NPs	20.54 a	14.65 a	12.65 A	28.42 A	42.99 A	21.48 A

Notes: Significance level: * ($p < 0.05$); Different letters within the same column indicate significant differences between treatments.

4. Discussion

The results presented in **Table 4** indicate that the application of bio-organic fertilization (EM Bokashi) had a positive impact on nutrient uptake. The addition of EM Bokashi led to a noticeable increase in nitrogen, phosphorus, and potassium absorption, consistent with previous research highlighting its role in enhancing soil microbial activity, thereby increasing nutrient availability to plants [14]. Moreover, EM Bokashi application increased the concentration of nitrogen, phosphorus, and potassium in cabbage leaves. This improvement is attributed to the presence of beneficial microorganisms in EM Bokashi, as well as its high organic matter content, including lactic acid-producing bacteria and yeast capable of decomposing sugars and carbohydrates in organic materials. Such microbial activity contributes to plant growth enhancement by improving plant height, leaf area, and nutrient uptake, resulting in higher concentrations of these elements in plant tissues [15].

This improvement in nutrient availability also positively influenced plant metabolic processes. The absorbed nutrients were translocated to the root zone, stimulating the release of specific compounds into the rhizosphere, which in turn activated beneficial microorganisms that produce organic acids and bioactive compounds. These processes enhance the availability of essential elements such as nitrogen, phosphorus, and potassium to plants [16]. Treatments that included NPK also showed superiority in all studied nutrients, confirming the significance of nutrient balance in promoting plant growth and improving nutrient uptake, as supported by Fouda [17].

Additionally, treatments involving silicon and selenium had a positive effect on nutrient uptake, aligning with studies that report the role of these elements in enhancing stress resistance and nutrient use efficiency [18]. A synergistic effect was observed when combining nano-elements with EM Bokashi, resulting in increased nutrient concentrations compared to individual treatments. Biofertilizer treatments such as EM Bokashi also positively influenced the uptake of elements like silicon, selenium, and iron, although this effect was more pronounced when nano-elements were applied, indicating that bio-organic fertilization in conjunction with nano-elements leads to higher nutrient absorption efficiency.

These findings agree with the study of Ola and Al-Juthery [19] on hybrid broccoli (HAPA F1), where EM Bokashi application significantly improved soil fertility by increasing post-harvest nutrient levels and activating soil microbial communities, which positively affected crop yield. The combined use of EM Bokashi and nano inputs achieved the highest agricultural efficiency, attributed to enhanced nutrient absorption and improved plant productivity. These results clearly demonstrate the vital role of EM Bokashi as an effective bio-input for promoting the sustainability of agricultural systems by positively impacting soil properties, vegetative growth, and crop yield.

The results also confirmed the importance of balanced mineral fertilization (NPK) in improving nutrient uptake by plant roots, as evident from the highest values recorded in the quadripartite and tripartite combination treatments involving NPK, compared to the control. Hamid [20] reported that integrated fertilization using both organic and inorganic inputs, such as NPK, improved nutrient concentrations in maize and wheat plants in central and southern China. Similarly, Hamid [21] found that this combined fertilization strategy was effective in enhancing crop sustainability, although it may lead to the accumulation of certain nutrients in the soil, emphasizing the need for balanced management practices to maintain long-term soil fertility.

The use of nano-elements such as selenium and silicon also contributed to increased nutrient uptake, potentially due to their larger surface area, which enhances absorption and improves plant stress resistance [22,23]. The differences observed in **Table 4** reveal significant variations among treatments, with increased concentrations of manganese, copper, and zinc resulting from the application of EM Bokashi in combination with mineral and nano fertilizers.

Manganese contributes to stomatal development and enhances growth indicators by stimulating carbon assimilation, cell division, and overall plant growth, in addition to maintaining nutrient balance [24]. Treatments that included nano-elements and bio-organic fertilizers demonstrated improved uptake of micronutrients compared to chemical fertilization or no fertilization. This improvement is attributed to enhanced bioavailability of nutrients through the stimulatory effects of nano-elements, which may include increased enzymatic activity and enhanced root absorption capacity [25].

Balanced NPK fertilization also improved overall plant nutrition, thereby increasing manganese uptake efficiency. Meanwhile, EM Bokashi enhanced microbial activity and organic matter decomposition, increasing manganese availability in the soil. Silicon and selenium may play a role in improving manganese uptake by enhancing plant resistance to environmental stress and stimulating micronutrient absorption. Studies have shown that silicon can enhance manganese uptake by reducing oxidative stress, improving root architecture, and enhancing plant resistance to heavy metal toxicity [26,27].

Zinc is essential for many physiological processes, and its deficiency leads to growth disorders and oxidative stress. Copper is necessary for the formation of enzymes responsible for redox reactions in plants [28]. Improved zinc and copper concentrations in EM Bokashi treatments may be attributed to improved soil conditions and enhanced microbial activity that promotes the decomposition of organic matter and release of plant-available zinc and copper [29]. Silicon and selenium contributed to enhanced zinc and copper uptake by improving plant resistance and metabolic efficiency in treatments involving NPK, silicon, and selenium.

Dahham et al. (2025) [29] reported that applying silicon to roots or leaves of cucumber plants under varying zinc conditions did not significantly affect zinc levels during sufficiency or deficiency phases but helped plants recover quickly after stress, particularly when applied foliarly, indicating its role in enhancing stress tolerance. Stated Dahham et al. (2025) that nano-selenium (SeNPs) possesses greater biological activity compared to bulk forms and is more effective in biofortification, enhancing stress tolerance and metabolic processes. Additionally, the combined application of selenium and silica reduces arsenic accumulation in rice plants compared to arsenic treatment alone, and enhances antioxidant enzyme activity, helping mitigate oxidative stress. These improvements were associated with increased plant tolerance to arsenic-induced stress. The results confirm that balanced application of selenium and silica can be an effective strategy to alleviate arsenic toxicity in plants [23]. According to Jin et al. [30], SeNPs can enhance plant resistance to oxidative stress, stimulate growth, improve soil nutritional status, increase antioxidant levels in plants, and contribute to transpiration. Moreover, these particles rely on readily available, biodegradable reducing agents, making them environmentally friendly.

According to the results in **Table 5**, bio-organic fertilization (EM Bokashi), balanced fertilization (NPK), and foliar application of nano-silicon and nano-selenium had the most pronounced effects in increasing the concentrations of the studied amino acids. Tryptophan and other amino acids were significantly influenced by the integrated treatments, where the combination of NPK, EM Bokashi, and nano-silicon and selenium enhanced metabolic activity and amino acid biosynthesis. Treatments involving silicon and selenium showed improved nutrient uptake and stimulated physiological processes, boosting amino acid production [31–33].

Bokashi application improves soil quality by stimulating microbial activity and is considered a highly biologically efficient organic fertilizer. The microbial fermentation in Bokashi enhances organic matter decomposition, improves soil structure, reduces nutrient loss, and increases nutrient use efficiency, ultimately enhancing amino acid production. It also promotes plant resistance to environmental stresses such as drought and nutrient deficiency. Using Bokashi as a sustainable organic fertilizer can be an effective alternative to chemical fertilizers, contributing to soil and plant health in an eco-friendly and sustainable manner [19]. It also improves seedling germination, biomass production, and nutrient uptake, particularly of nitrogen, phosphorus, and potassium, which positively influences amino acid synthesis and plant productivity [34].

Hamid et al. [35] showed in a study on pepper plants that silicon application (60 mg L^{-1}) significantly increased amino acid accumulation during early growth stages, enhancing physiological development. In contrast, higher concentrations (125 and 250 mg L^{-1}) reduced amino acid levels, indicating a dose-dependent hormonal response. This suggests that optimal silicon levels can enhance amino acid biosynthesis and plant growth. Furthermore, AL-Hamandi et al. [36] demonstrated in their study on Medicago intertexta that treatment with selenium nanoparticles (25 mg L^{-1}) enhanced the effects of beta-aminobutyric acid (BABA, 30 mM) on plant growth, physiology, nitrogen metabolism, and bioactive compound synthesis. BABA and SeNPs together increased photosynthesis, providing the precursors for primary and secondary metabolite synthesis, leading to increases in essential amino acids, phenolics, glutathione (GSH), and ascorbic acid (ASC).

Hussein et al. [37] found that soaking wheat seeds in arginine solutions at various concentrations improved morphological growth traits such as root length, leaf number, and both fresh and dry biomass. It also enhanced pigment content (chlorophyll a, b, and carotenoids) and physiological and biochemical aspects by increasing soluble sugars, free amino acids, proline, phenolics, flavonoids, and activating metabolic defense mechanisms that support drought tolerance.

Furthermore, **Table 5** shows that bio-organic and nano-fertilization increased nutrient uptake efficiency, enhancing methionine content in plants. Methionine is an essential amino acid involved in protein synthesis and plays a vital role in regulating plant growth and development. These findings are consistent with those reported by Jinwen et al. [38], who observed that applying organic and chemical fertilizers (NPK) significantly improved various growth, productivity, and biochemical quality parameters in coriander, including root and shoot biomass, SPAD chlorophyll index, ascorbic acid content, mineral elements, and flavonoids.

5. Conclusions

The findings of this study demonstrate that the integrative application of bio-organic fertilizer (EM Bokashi), balanced mineral fertilizer (NPK), and foliar sprays of silicon and selenium nanoparticles significantly enhances the physiological performance and nutritional quality of cabbage. The combined (quadruple) treatment outperformed all other treatments in improving macro- and micronutrient concentrations as well as essential amino acid content ($p \leq 0.05$). This synergy between organic, mineral, and nano-based fertilization improves nutrient use efficiency, elevates the nutritional value of the crop, and reduces dependence on chemical inputs, offering a promising strategy for sustainable agriculture. The study contributes a novel approach by integrating three fertilization technologies into a single, coherent system that supports environmentally sound practices with clear agronomic and economic benefits.

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The data that supports the findings of this study will be available on request from the corresponding author.

Conflicts of Interest

The authors declare no conflict of interest.

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