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Carbon Nanoparticles Reinforced with Titanium Dioxide as Novel Foliar Stimulant for Garlic Plants

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THE CURRENT research work comes within the framework of efforts to overcome the nutritional gap that is increasing over time. The main objective was to develop fertilization management *via* increasing the efficiency of amino acids utilization by loading them onto carbon nanoparticles (CNP). It was hypothesized that this technique would improve absorption, permeability and stability of bioactive compounds within the higher plants. It was also hypothesized that doping these CNP with titanium may play a vital role in stimulating physiological processes and increasing biological activity. In this study, the potential effect of the carbon nanoparticles (CNP) reinforced with titanium dioxide (TiO_2) as a novel foliar stimulant on garlic plants was studied to present an innovative approach, which contributes to increasing the efficiency of amino acids and improving crop quality using smart and advanced fertilization programs. Carbon nanoparticles synthesis was implemented *via* Maillard reaction using four amino acids (β-Alanine, citrulline, proline and taurine), glucose and TiO_2. A field experiment was carried out under complete randomized experimental design aiming at assessing the impact of CNP reinforced with TiO_2 as foliar treatments [$\mathbf{T_1}$ (Tap water as control), $\mathbf{T_2}$ (β-Alanine CNP + TiO_2 at rate of 80 mg L^{-1}), $\mathbf{T_3}$ (Citrulline CNP + TiO_2 at rate of 80 mg L^{-1}), $\mathbf{T_4}$ (Proline CNP + TiO_2 at rate of 80 mg L^{-1}) and $\mathbf{T_5}$ (Taurine CNP + TiO_2 at rate of 80 mg L^{-1})] on garlic growth performance and productivity. Growth criteria such as plant height, photosynthetic pigments such as carotene, leaf nutritional status such as nitrogen content, bulb yield and quality such as vitamin C were measured and determined. The results showed that all CNP treatments reinforced with TiO_2 outperformed the control treatment, as the superior treatment was T_5 (Taurine CNP + TiO_2 at a rate of 80 mg L^{-1}) followed by T_4, T_3 and T_2, respectively. The control treatment came in the last order in terms of the values of growth and productivity criteria. For example, the increasing rate in the bulb yield values due to the studied treatments compared with the control treatment was 35.34, 37.80, 45.17 and 46.12 % with T_2, T_3, T_4 and T_5, respectively. Thus, the obtained results confirmed that the Addition of TiO_2 in the synthesis of CNP adds other features to the final product. Generally, it can be concluded that the CNP reinforced with TiO_2 as a novel foliar stimulant will maintain the sustainability and improve the agricultural sector. However, future research on this point should be intensified under different manufacturing conditions, which may lead us to a better and more effective product in agricultural production.

Keywords: Carbon nanoparticles (CNP), TiO_2, Garlic

1.Introduction

Carbon nanoparticles (CNP) have gained tremendous attention in various fields such as agriculture because of their unique electric, optical, and chemical properties, as well as low toxicity and high biocompatibility, which makes them such a versatile nanomaterial, particularly for the agricultural sector. CNP hold great promise for agriculture due to their water solubility, low toxicity and small size. CNP have a core–shell-like structure CNP and high and biocompatibility, as it is relatively safer for application in agriculture. CNP can augment photosynthesis and utilize solar energy efficiently for increasing crop productivity, making them effective photosynthetic enhancers. Moreover, their antimicrobial and antioxidant properties assist them in relieving biotic and abiotic stresses, improving plant health **(Shojaei *et al.* 2019; Maholiya *et al.* 2023; Pan *et al.* 2024).** CNP migrate around the chloroplasts, where they are absorbed and translocated from the leaf surface. CNP can also enhance the scavenging of reactive oxygen species (ROS) by improving the antioxidant system, chlorophyll content, and biomass accumulation, leading to improved epiphyte resistance to abiotic stress **(Salama *et al.* 2021; Ren *et al.* 2024).**

The use of amino acids and sucrose or glucose to synthesize CNP *via* the Maillard reaction is a model system where the use of safe precursors (amino acids and sugars) from a basic food reaction is beneficial to the safety of these materials, in addition to the vital role amino acids play in higher plant nutrition **(Nguyen *et al.* 2024).** In

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the present, some research has addressed the unique role of titanium in higher plant nutrition **(Ghazi *et al.* 2024).** It has been found that it plays a role in improving the chlorophyll content and scavenging free radicals (ROS), in addition to its role in the process of non-biological nitrogen fixation **(Soliman& El-Sherpiny, 2021; Elsherpiny & Faiyad, 2023).**

To our knowledge, there have been no studies reporting the response of higher plants to CNP reinforced with TiO_2, whereas only a few studies have investigated the response of higher plants to CNP. So, the main objective was to develop fertilization management *via* increasing the efficiency of amino acids utilization by loading them onto carbon nanoparticles (CNP). It was hypothesized that this technique would improve absorption, permeability and stability of bioactive compounds within the higher plants. It was also hypothesized that doping these CNP with titanium may play a vital role in stimulating physiological processes and increasing biological activity. In this study, the potential effect of the carbon nanoparticles (CNP) reinforced with titanium dioxide (TiO_2) as a novel foliar stimulant on garlic plants was studied to present an innovative approach, which contributes to increasing the efficiency of amino acids and improving crop quality using smart and advanced fertilization programs.

2. Materials and Methods

2.1. Materials for synthesis

Table 1 shows the properties of the studied materials for synthesis of carbon nanoparticles reinforced with titanium dioxide. Carbon nanoparticles synthesis was implemented *via* Maillard reaction using 4 amino acids (β-Alanine, citrulline , proline and taurine), glucose and TiO_2 as described by **Nguyen *et al.* (2024).** Each amino acid at a weight of 200 g + glucose at a weight of 200 g + TiO_2 at a weight of 1.0 g was dissolved in 100 ml of distilled water. The mix was heated at 120°C for 12 hours in the autoclave apparatus, as it was obtained about 150 g of CNP reinforced with TiO_2 was obtained as the final product. Then it was naturally cooled to room temperature. Fig 1 illustrates the steps of Maillard reaction.

Table 1. Properties of the studied amino acids and titanium dioxide .

	Properties	**Source**
Titanium dioxide (TiO_2)	Derived from ilmenite mineral ($FeTiO_3$). Its molar mass is 79.866 g/mol Its density is 4.17 g/mL at 25°C Its melting point range from 1830 to 3000°C.	Sigma Company, USA
Glucose sugar		Egyptian commercial market
L-Proline	Pyrrolidine-2-carboxylic acid (cyclic structure)	Purita Chemical Company, Shaanxi, China
L-Citrulline	2-Amino-5-(ureido)pentanoic acid (-NH-C(=O)-NH_2)	
Taurine	Beta-amino sulfonic acid (-SO_3H)	
β-Alanine	3-Aminopropionic acid (-NH_2 on β-carbon)	

Fig. 1. Steps of synthesis of carbon nanoparticles reinforced with titanium dioxide.

2.2. Characterization of Synthesis of Carbon nanoparticles Reinforced with Titanium Dioxide

The characterization of the synthesized carbon nanoparticles (CNP) reinforced with titanium dioxide was done in the Electron Microscope Unit (Faculty of Agriculture, Mansoura University) *via* various analytical techniques (TEM, Zeta potential).

2.3. Trail experimental setup

In a private farm located at Meit-Anter Village, El-Dakahlia Governorate, Egypt, during the winter season of 2024/25, garlic (Seds 40 variety from the Egyptian Ministry of Agriculture and soil Reclamation, MASR) were sown on November 1st at a rate of 450 kg of garlic (including foliage) under a completely randomized design with three replicates. The treatments included **T_1 (**Tap water as control), **T_2** (β-Alanine CNP + TiO_2 at rate of 80 mg L^{-1}**)**, **T_3** (Citrulline CNP + TiO_2 at rate of 80 mg L^{-1}**)**, **T_4** (Proline CNP + TiO_2 at rate of 80 mg L^{-1}) and **T_5** (Taurine CNP + TiO_2 at rate of 80 mg L^{-1}). The soil was thoroughly plowed with adding 20 m^3 of compost and 400 kg of calcium superphosphate. The soil was irrigated then the cloves were sown when the soil was moderately moist, as the planting distance was 10 cm. Fertilization, irrigation, fungal diseases and pest control processes were done according to the guidelines of the MASR. The studied treatments were sprayed 5 times, starting from the 50th day of planting, with a 15-day interval. The harvest process was implemented after 178 days from sowing. Table 2 indicates some information about the field experiment, while Fig 2 shows the flowchart of the fieldwork, including the treatments and measurements.

Table 2. Information about the field location, experiment setup, harvest, measurements and statistical analysis.

Pre-trial procedures	Soil sample was analyzed as routine work, as it was taken at the depth of 0-30 cm (Table 3)
Measurements	Growth criteria, including plant height No. of leaves, leaves fresh and dry weights and leaf area manually measured after 125 days from planting.
	Photosynthetic pigments, including chlorophyll (a & b) and carotene spectrophotometrically determined at 125 days from sowing as described by **Wellburn (1994).**
	Chemical constitutes (NPK) of garlic plant also were determined at 125 days from sowing using Kjeldahl (for N), Spectrophotometr (for P) and Flame photometer (for K), as the plant leaves were digested using mixture of $HClO_4$ + H_2SO_4 **(Peterburgski, 1968; Walinga *et al.* 2013)**.
	Bulb yield parameters (Average bulb weight, bulb diameter, neck diameter, bulbing ratio, number of cloves and total and marketable bulb yield) were manually measured at 178 days after sowing. The bulbing ratio was calculated as the ratio of bulb diameter to pseudostem diameter.
	Bulb quality traits (Carbohydrates, total dissolved solids, dry matter, vitamin C and pungency) at the same age were assessed according to the standard methods mentioned in **AOAC, (2000).**
Statistical analysis	Statistical analysis of the data was conducted using CoStat software (version 6.303, Copyright 1998–2004) at the 0.05 probability level, following the procedure outlined by **Gómez and Gómez (1984).**

Table 3. Analysis of the initial soil using the standard methods described by Dane & Topp, (2020); Sparks *et al.* (2020).

Soil attributes	Values
Sand, silt and clay, respectively	20.5,30.0 and 49.5 %, respectively
Textural class is clay	
Available-N, P and K, respectively	23.6, 7.20 and 201.3 $mgKg^{-1,}$ respectively
pH, EC, WHC and OM, respectively	8.0, 3.4 dSm^{-1},36% and 1.29%

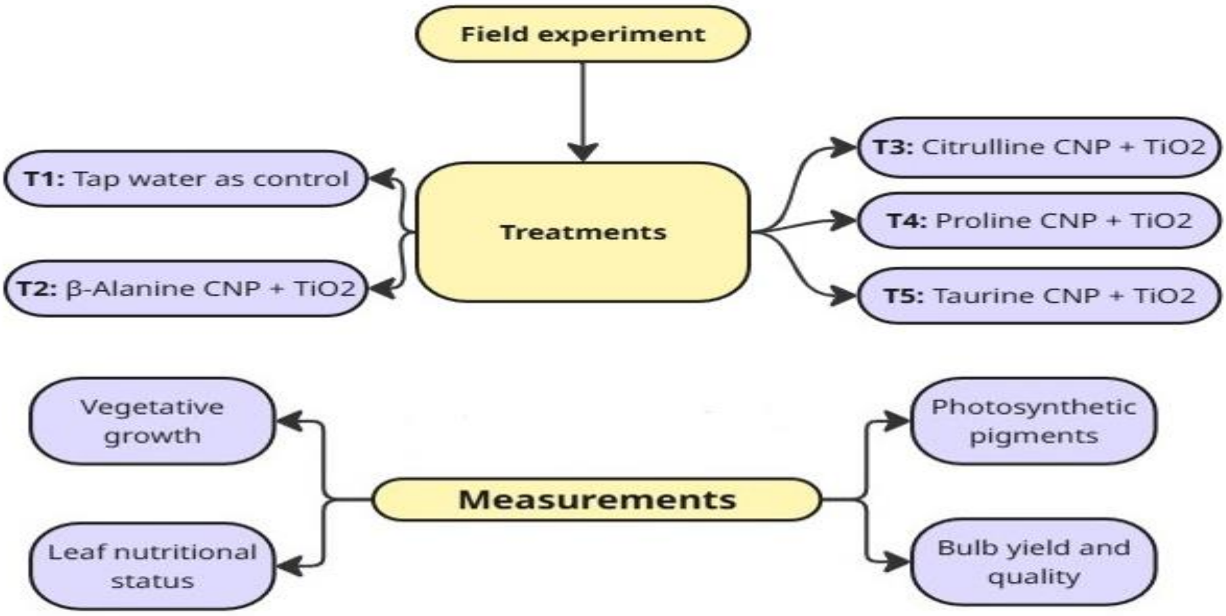


Fig. 2. The flowchart of the fieldwork.

3. Results

3.1. Characterization of CNP

TEM of all CNP-TiO_2 (Figures from 3 to 6) show that all of them have a predominantly spherical shape, with a size distribution ranging from 32.19 to 90.95 nm. β-Alanine CNP-TiO_2 particles had the smallest average particle size (32.19–90.90 nm) but the lowest surface potential (-6.2 mV), indicating poor colloidal stability. Citrulline CNP-TiO_2 and proline CNP-TiO_2 had sizes ranging from 53.89–90.88 and 38.05–90.95 nm, with zeta potential values of -9.7 and -10.9 mV, respectively, indicating intermediate stability. While the taurine CNP-TiO_2 exhibited a size range of 43.00 to 90.63 nm, with the highest negative surface potential value (-19.7 mV), reflecting better dispersion stability compared to the other samples.

3.2. Growth performance and yield components

The influence of CNP reinforced with titanium dioxide as foliar application on growth criteria [including plant height (cm), No. of leaves/one plant, leaves fresh and dry weights (g plant^{-1})], photosynthetic pigments [including chlorophyll (a & b) and carotene (mg /g) in leaves F.W], leaf nutritional status [NPK,% in leaves D.W] of garlic after 125 days from sowing was significant (Figs 6 & 7). Additionally, the influence of CNP reinforced with titanium dioxide as foliar application on bulb yield [including average bulb weight (g), bulb diameter (cm), neck diameter(cm), bulbing ratio, number of cloves/one plant and total and marketable bulb yield (Mgfed^{-1})] and bulb quality [including carbohydrates (%), total dissolved solids (%), dry matter (%), vitamin C (mg/100g) and pungency (πmol ml^{-1})] at harvest stage was significant (Table 4 & 5 and Figs7 & 8). The data indicate that all CNP treatments reinforced with TiO_2 outperformed the control treatment (tap water), as the superior treatment was **T_5** (Taurine CNP + TiO_2 at rate of 80 mg L^{-1}) followed by **T_4** (Proline CNP + TiO_2 at rate of 80 mg L^{-1}), **T_3** (Citrulline CNP + TiO_2 at rate of 80 mg L^{-1}) and **T_2** (β-Alanine CNP + TiO_2 at rate of 80 mg L^{-1}), respectively. The control treatment came in the last order in terms of the values of growth and productivity criteria. For example, the increasing rate in the bulb yield values due to the studied treatments comparing with the control treatment was 35.34, 37.80, 45.17 and 46.12% with **T_2, T_3, T_4** and **T_5,** respectively.

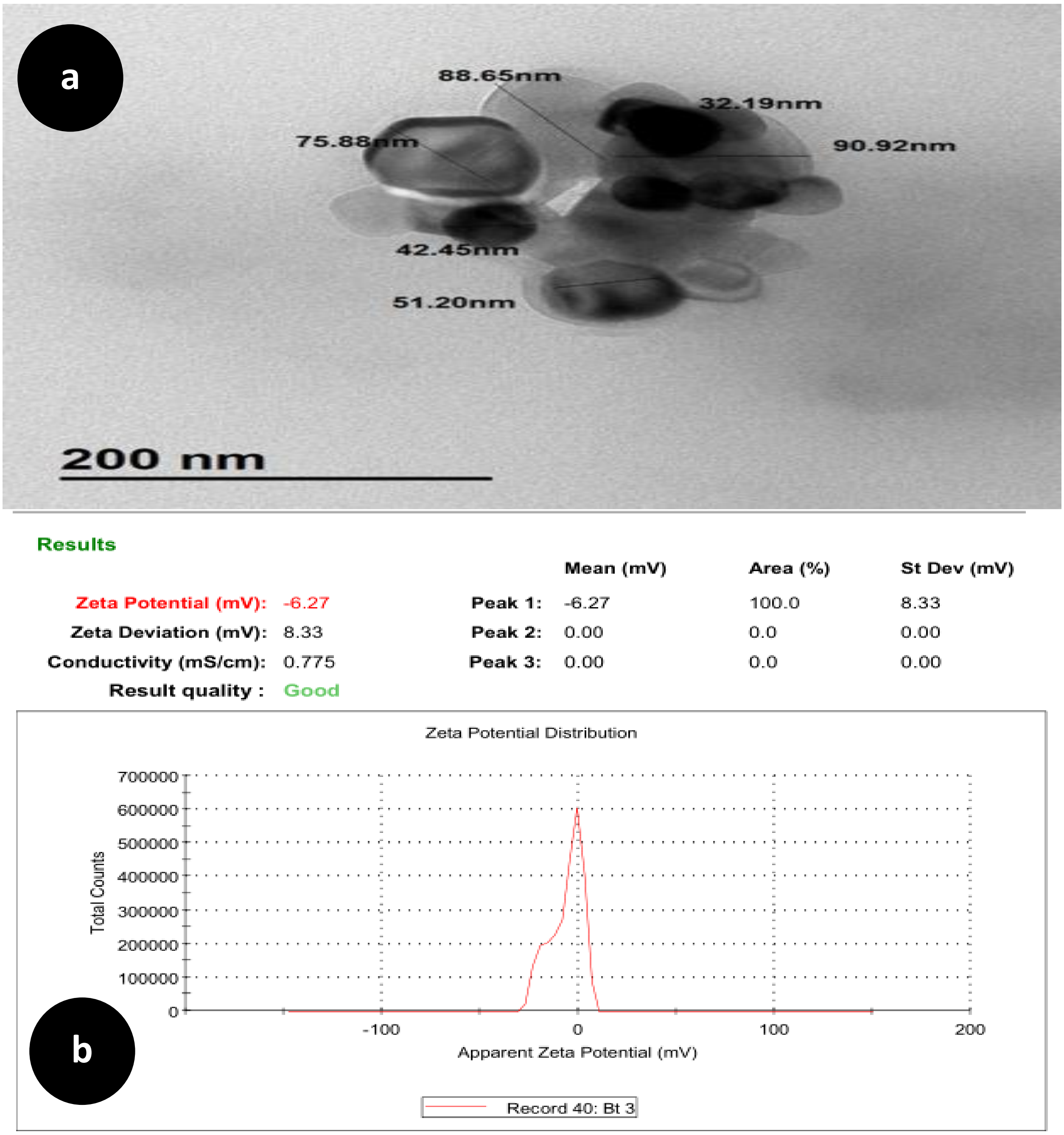


Fig. 3. Characterization of β-Alanine CNP reinforced with titanium dioxide. a, TEM (Transmission electron microscopy). b, ZPA (Zeta Potential).

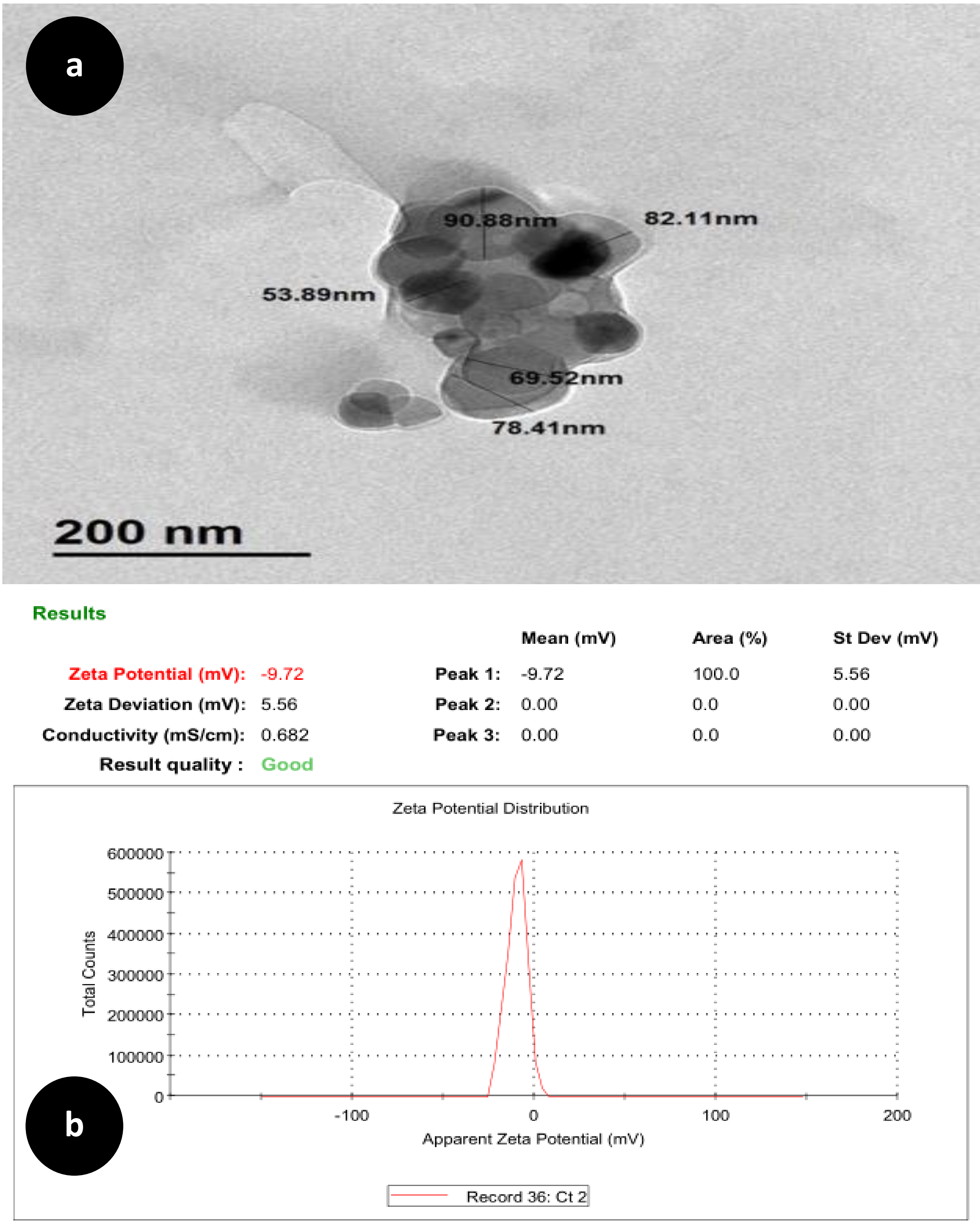
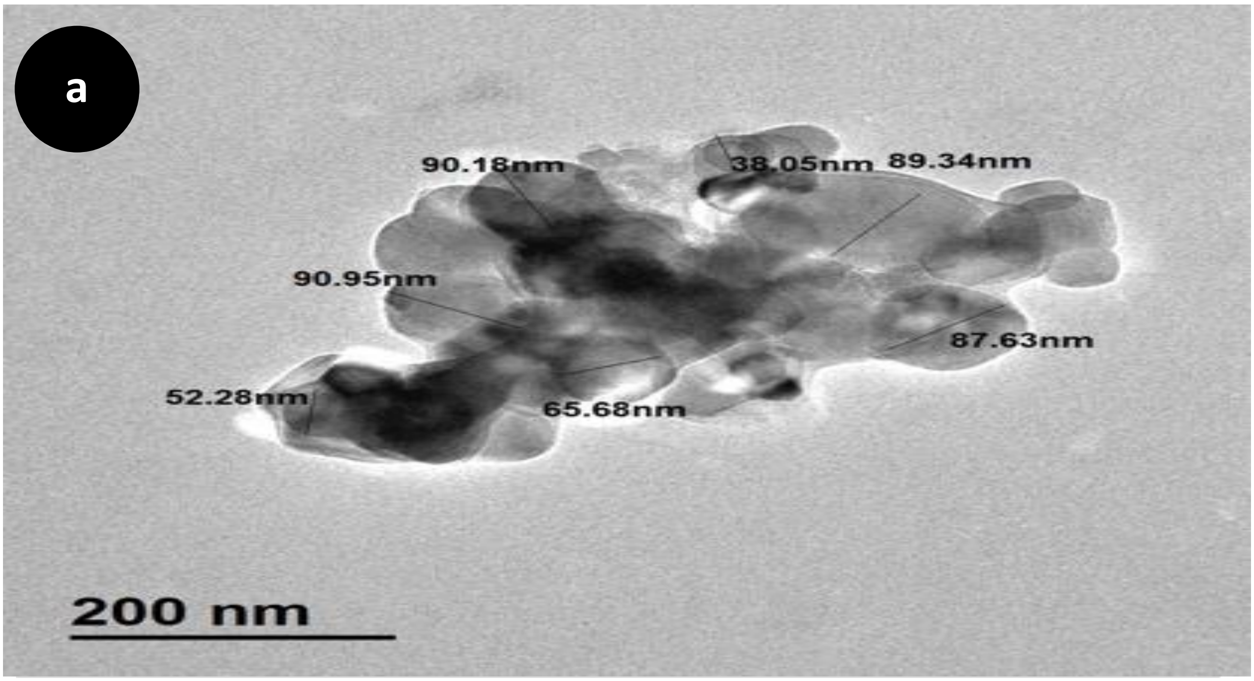


Fig. 4. Characterization of citrulline CNP. CNP reinforced with titanium dioxide. a, TEM (Transmission electron microscopy). b, ZPA (Zeta Potential).



Results

			Mean (mV)	Area (%)	St Dev (mV)
Zeta Potential (mV):	-10.9	Peak 1:	-10.9	100.0	3.53
Zeta Deviation (mV):	3.53	Peak 2:	0.00	0.0	0.00
Conductivity (mS/cm):	0.541	Peak 3:	0.00	0.0	0.00
Result quality :	Good				

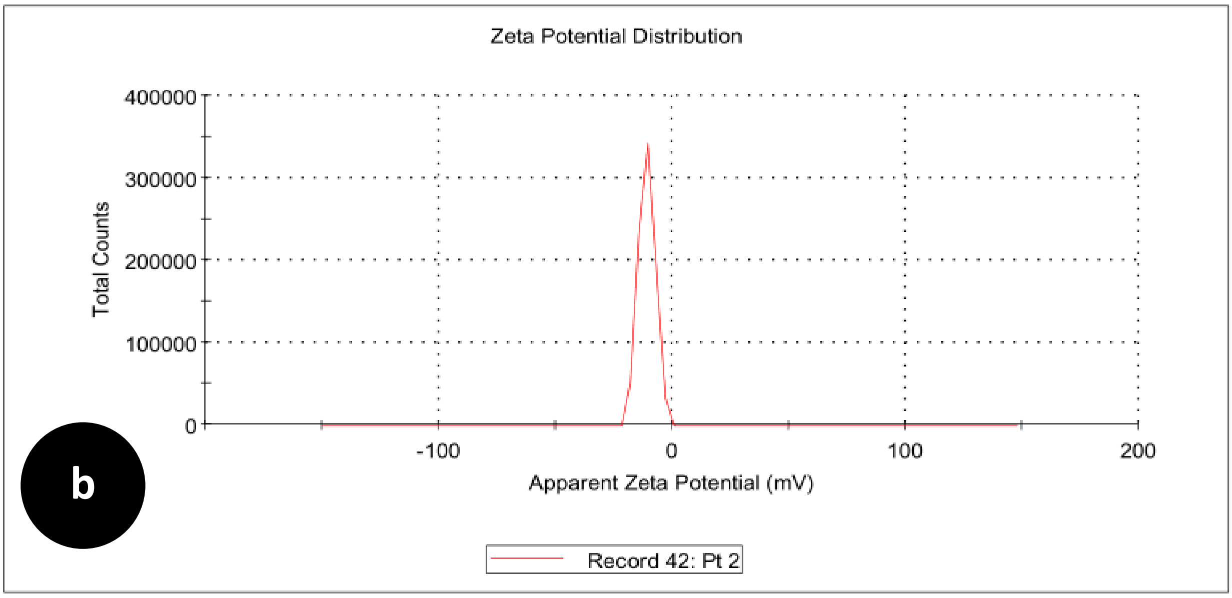


Fig. 5. Characterization of proline CNP. CNP reinforced with titanium dioxide. a, TEM (Transmission electron microscopy). b, ZPA (Zeta Potential).

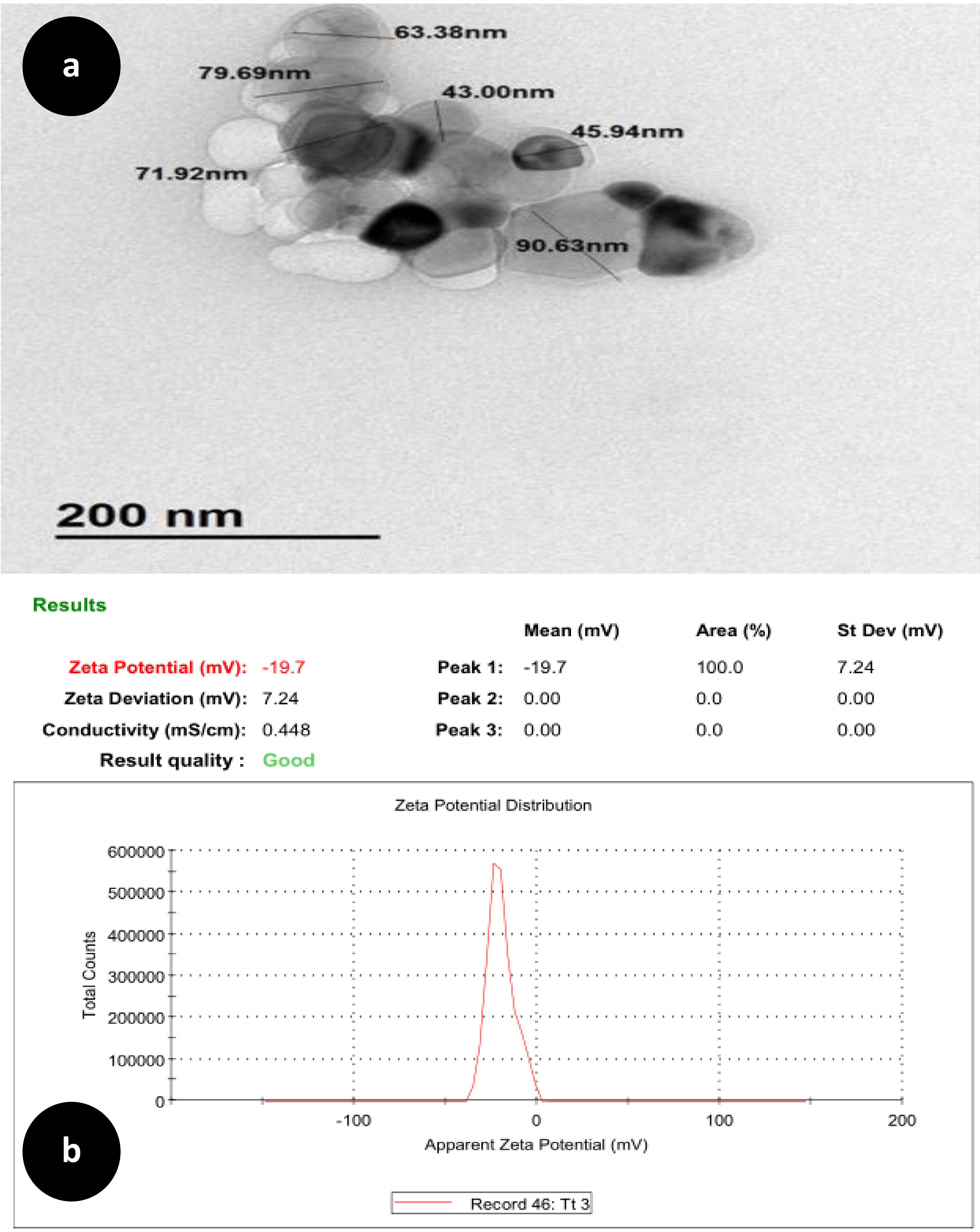


Fig. 6. Characterization of Taurine CNP. CNP reinforced with titanium dioxide. a, TEM (Transmission electron microscopy). b, ZPA (Zeta Potential).

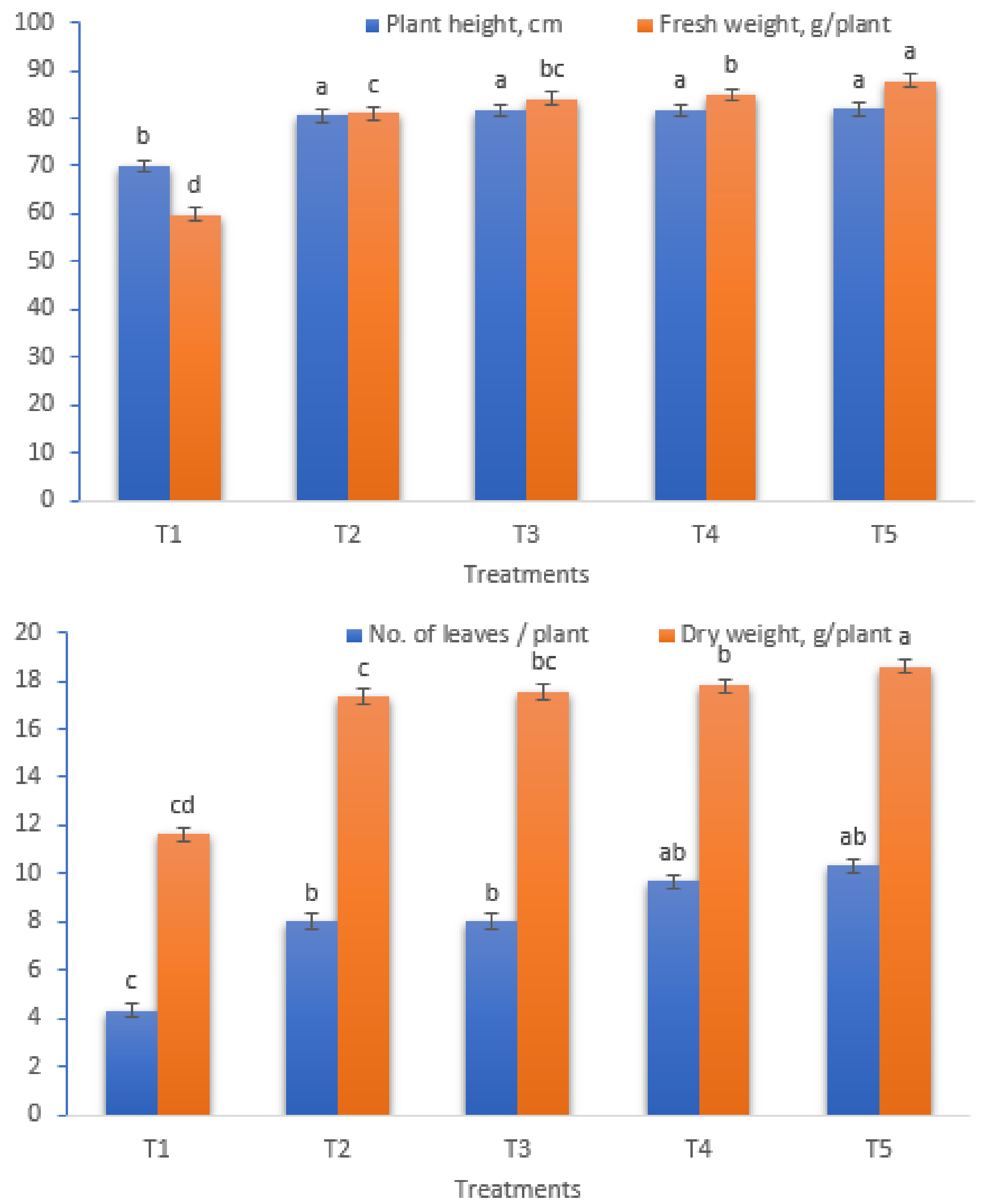


Fig. 7. Impact of CNP reinforced with titanium dioxide on growth parameters of garlic after 125 days from sowing.

T_1 (Tap water as control), **T_2** (β-Alanine CNP + TiO_2 at rate of 80 mg L^{-1}**)**, **T_3** (Citrulline CNP + TiO_2 at rate of 80 mg L^{-1}**)**, **T_4** (Proline CNP + TiO_2 at rate of 80 mg L^{-1}) and **T_5** (Taurine CNP + TiO_2 at rate of 80 mg L^{-1})

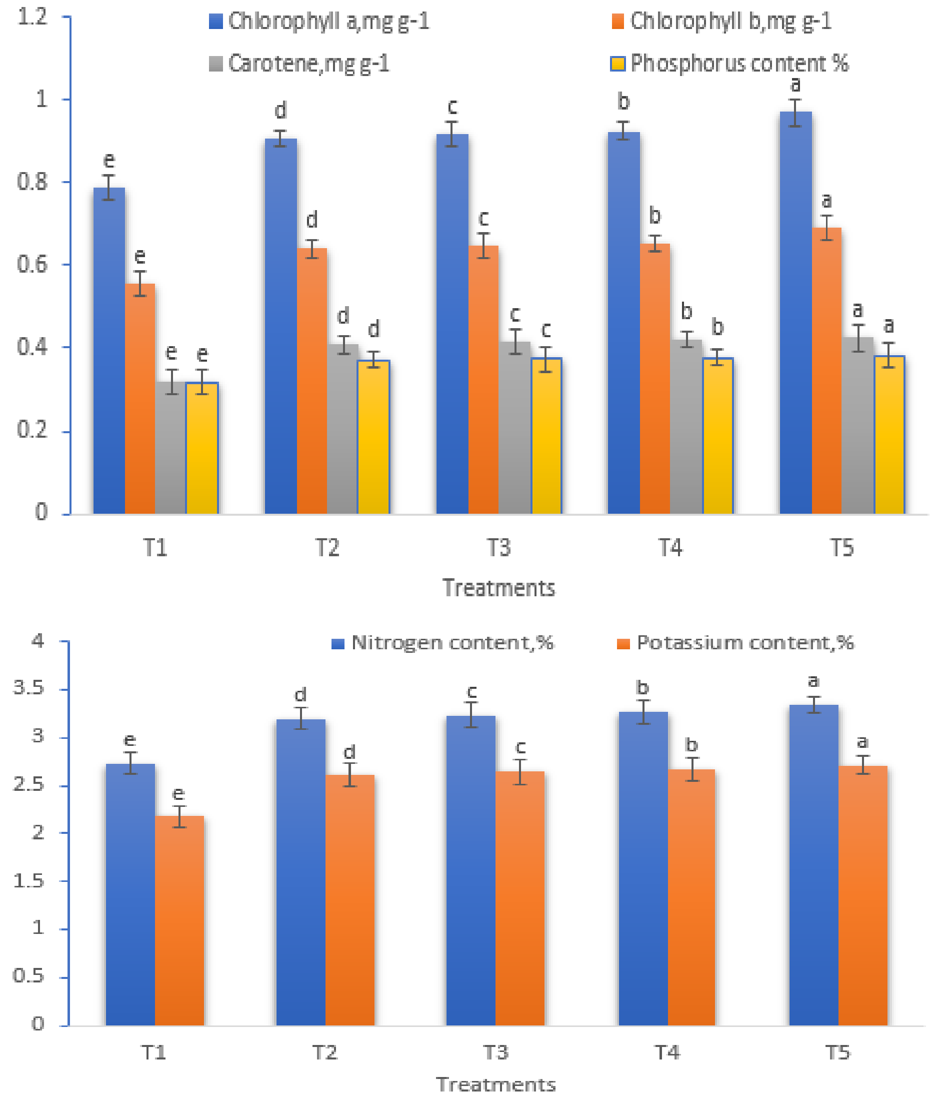


Fig. 8. Impact of CNP reinforced with titanium dioxide on photosynthetic pigments and chemical contents (NPK) of garlic leaves after 125 days from sowing .

T_1 (Tap water as control), **T_2** (β-Alanine CNP + TiO_2 at rate of 80 mg L^{-1})**, T_3** (Citrulline CNP + TiO_2 at rate of 80 mg L^{-1})**,** **T_4** (Proline CNP + TiO_2 at rate of 80 mg L^{-1}) and **T_5** (Taurine CNP + TiO_2 at rate of 80 mg L^{-1})

Table 4. Impact of CNP reinforced with titanium dioxide on garlic bulb yield of garlic at harvest stage (176 days from sowing).

Treatments	Weigh of average bulb (g)	Bulb diameter	Neck diameter	Bulbing ratio	No. of cloves/ bulb	Total bulb yield	Marketable yield
		(cm)				(Mg fed^{-1})	
T_1	33.04c	4.00d	0.50c	0.12c	26.67d	5.29e	4.38e
T_2	44.78b	5.20c	1.30b	0.25b	32.00c	7.16d	6.14d
T_3	45.57b	5.40b	1.33b	0.25b	33.67c	7.29c	6.35c
T_4	48.02a	5.47b	1.53a	0.28a	35.00b	7.68b	6.48b
T_5	48.33a	5.70a	1.53a	0.27a	37.00a	7.73a	6.63a
F test	**	***	**	**	***	***	***

Means within a row followed by a different letter (s) are statistically different at a 0.05 level
T_1 (Tap water as control), T_2 (β-Alanine CNP + TiO_2 at rate of 80 mg L^{-1}), T_3 (Citrulline CNP + TiO_2 at rate of 80 mg L^{-1}), T_4 (Proline CNP + TiO_2 at rate of 80 mg L^{-1}) and T_5 (Taurine CNP + TiO_2 at rate of 80 mg L^{-1})

Table 5. Impact of CNP reinforced with titanium dioxide on garlic bulb quality of garlic at harvest stage (176 days from sowing).

Treatments	*TC, %	*TDS, %	*VC, mg 100g^{-1}	*DM, %	Pungency, purvate content πmol ml^{-1}
T_1	19.16c	21.17c	11.84c	17.91d	8.06c
T_2	20.50b	22.73b	12.63b	20.12c	9.31b
T_3	20.69b	22.82b	12.64b	20.30b	9.61b
T_4	21.05a	23.04a	12.82a	20.37b	9.75ab
T_5	21.16a	23.21a	12.88a	20.69a	9.81a
F test	***	***	***	***	***

Means within a row followed by a different letter (s) are statistically different at a 0.05 level
T_1 (Tap water as control), **T_2** (β-Alanine CNP + TiO_2 at rate of 80 mg L^{-1})**, T_3** (Citrulline CNP + TiO_2 at rate of 80 mg L^{-1})**,**
T_4 (Proline CNP + TiO_2 at rate of 80 mg L^{-1}) and **T_5** (Taurine CNP + TiO_2 at rate of 80 mg L^{-1})
***TDS= Total dissolved solids**
***TC= Total carbohydrates**
***VC= Vitamin C**
***DM= Dry matter**

4. Discussion

The obtained results can be attributed to the unique role of Carbon nanoparticles (CNP), as it is characterized by water solubility, low toxicity and small size. All of these unique traits may have made all treatments of CNP enhanced the garlic plant performance and productivity. Carbon nanoparticles (CNP) may have protected the molecules and reduced their degradation or loss, thus increasing their effectiveness. Furthermore, their CNP form may have given them a high ability to penetrate cell walls and penetrate the inside of garlic plant cells, thus ensuring that amino acids quickly reach their sites of action within the garlic plant. In other words, CNP can act as smart carriers **(Shojaei *et al.* 2019; Salama *et al.* 2021).** The obtained findings support the hypothesis that CNP may have enhanced the scavenging of reactive oxygen species (ROS) *via* improving the antioxidant system, chlorophyll content and biomass accumulation, leading to improved garlic resistance to abiotic stress **(Shojaei *et al.* 2019; Salama *et al.* 2021; Ren *et al.* 2024).** The difference among the CNP treatments to the type of amino acid used in the Maillard reaction, as each amino acid performs in specific role.

Taurine and proline amino acids came in the first and second order in terms of the improvement of garlic performance and this may be attributed to that, these amino acids may have raised the garlic plant's resistance to any biotic and abiotic stress. Additionally, they may have improved the osmotic balance in the garlic cells or helped maintain water content within the garlic leaves. The usage of these amino acids in the formation of the CNP may optimize their vital roles. Taurine outperformed proline, and this may be due to its role in scavenging ROS from garlic plant cells. Perhaps the soil EC value (3.4 dSm^{-1}), although it does not indicate that the soil is saline, it may have caused salt stress on the growing garlic plant, and thus caused the role of these two amino acids (taurine and proline) to become prominent.

Citrulline came in the third order in terms of its effect, as it possesses a role in removing excess ammonia within the garlic plant cell, while β-alanine, which came in the fourth order, has a role as an important mediator in garlic energy pathways. Also, the usage of the citrulline and β-alanine in the formation of the CNP may optimize their vital roles **(Li *et al.* 2024; Karunarathne *et al.* 2025).**

On the other hand, the addition of TiO_2 in the synthesis of CNP may have added to them other unique properties, as Ti, as currently known, plays a role in improving the chlorophyll content and scavenging free radicals (ROS), in addition to its role in the process of non-biological nitrogen fixation **(Soliman& El-Sherpiny, 2021).** The addition of titanium may have enhanced the bioactivity of all aminoacids CNP when spraying on garlic plants. The presence of Ti may have increased the activity of the enzyme RuBisCO, improved nutrient uptake, enhanced root and shoot growth and increased metabolic efficiency, regulated the opening and closing of stomata and improved water and carbon dioxide use efficiency. Moreover, TiO_2 possess photocatalytic properties, making it to reduce oxidative stress in the garlic plants by stimulating ROS scavenging. Carbon nanoparticles reinforced with titanium dioxide may have enhanced cellular permeability and distribution within plant tissues, maximizing the positive influences on plant performance **(Elsherpiny & Faiyad, 2023; Ghazi *et al.* 2024).**

5. Conclusion

Building on the obtained results, the current investigation confirms the effectiveness of spraying CNP reinforced with titanium dioxide (TiO_2) as a novel foliar stimulant for garlic plants. The results confirmed that all CNP treatments reinforced with TiO_2 outperformed the control treatment, as the superior treatment was taurine CNP + TiO_2 at a rate of 80 mg L^{-1}. Also, the β-Alanine, citrulline and proline CNP+ TiO_2 possess the unique effect. Addition of TiO_2 in the synthesis of CNP add other features of the final product , as it plays a role in improving the chlorophyll content and scavenging free radicals (ROS), in addition to its role in the process of non-biological nitrogen fixation. The control treatment came in the last order in terms of the values of growth and productivity criteria. For example, the increasing rate in the bulb yield values due to the studied treatments compared with the control treatment was 35.34, 37.80, 45.17 and 46.12 % with T_2, T_3, T_4 and T_5, respectively. Thus, the obtained results confirmed that the Addition of TiO_2 in the synthesis of CNP adds other features to the final product. Generally, it can be concluded that the CNP reinforced with TiO_2 as a novel foliar stimulant will maintain the sustainability and improve the agricultural sector. However, future research on this point should be intensified under different manufacturing conditions, which may lead us to a better and more effective product in agricultural production.

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