Current Science International
Volume: 13 | Issue: 04| Oct. – Dec.| 2024

EISSN:2706-7920 ISSN: 2077-4435
DOI: 10.36632/csi/2024.13.4.41
Journal homepage: www.curresweb.com
Pages: 475-486

Comparison between Effects of Two Copper Fertilizers Forms on Onion Plant Quality Grown in Sandy Soil

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Received: 05 Sept. 2024 **Accepted:** 10 Oct. 2024 **Published:** 20 Oct. 2024

ABSTRACT

A field experiment was carried out in the recently reclaimed desert area of Wadi El-Notron station, Beheira Governorate, Egypt. Onion plants (*Allium cepa* L.) were grown during the winter season of 2022 and 2023. Copper is a micronutrient that plants need, so adding it as nano form enhances the plant's absorption efficiency and reduces its loss. Copper nano-particels (CuNPs) were prepared through sub-atomic synthetic methodology technique under tension 1.5 Mpa., and investigated by Transmission Electron Microscopy (TEM) measuring copper oxcide particle sizes. Three treatments as NPK fertilizer (control treatment), copper sulfate fertilizer ($CuSO_4$), copper nano-particles (CuNPs) were applied on onion plants. The aim of the research is to test different sources of copper on onions to select the best form that onions absorb from soil. The data showed that using 10ppm of CuNPS also boosted onion morphological and biochemical constituents. CuNPs were not only safe to use but showed like or superior results to the other Cu forms assess in this experiment.

Keywords: Copper nano-particles, copper sulphate, NPK fertilizers, onion plant, foliar application and physiological and biochemical components.

1. Introduction

1.1. Taxonomy of Alliumcepa (Onion)

Allium cepais of family: Alliaceae, Genus: Allium, Species: cepa. The bulb of *Allium cepaisa* modified stem which is globe-shaped and larger than that of garlic and it is enclosed in white, yellow, or red skin called tunic. It also has a strong smell and pungent taste. They are held together by athin, dry and outer layer. The whole bulb is 3–10 cm in diameter and the plant, each separate leaf, the leaf-base, a leaf-bud, a flower, a flower-bud and the flower are enclosed in a membranous or leathery coat. The roots are fasciculate, soft, and fleshy. The plant has12–15 hollow leaves arranged at four or six to a page. They are very delicate and very difficult to see. The umbraisa cluster of flowers that are attached at the base and the flowers are small, regular and hermaphroditic. It has six tepals, six stamens and a superior ovary. The seeds are small and black and they have a distinct smell and taste. According to the type of onion variety, it is typically grown as a biennial or triennial plant. The cultivation of onions originated in Central Asia, specifically Western Pakistan and Afghanistan from about 5000 years ago. Ancient Egypt had a significant interest in onion cultivation and it is believed that onions spread throughout Iran, as well. Planate is the phylum of the onion plant. This phylum is characterized by plants that are autotrophic and contain chlorophyll a in the vegetative cells, which captures light energy (Brewster, 2008).

1.2. Economic Value and Traditional Uses of *Allium cepa* L. (Onion)

Onion is the second most important vegetable crop after tomato (*Solanum tuberosum*). The nutritional and therapeutic value of onion was recognized for over 4000 years. Nowadays, European Community is the third largest world producer of onions then followed by India and China and accounts for approximately 9% of the world's production. An onion was often intensively farmed on

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cotton land. Onion also contain thio-sulfinates, which are known to provide the health benefits included in traditional medicine. Culinary uses of *Allium cepa* are a main factor in its economic value. Grades of onions are based on dry bulb content and appearance. Premium onions have a single-centered round bulb, minimal off-colored scales, and are usually cured with no thick neck; to be marketed as either "early" or "late" season, requiring mild climate for long day or short day onions. The most notable phyto-chemicals in onion are the flavonoids. The main is quercetin and the sulfur-containing compounds, mainly allyl propyl disulphide and S-alk(en)yl cysteine sulf-oxides. Moreover, Onions are also packed with medicinal effects that are an added benefit to the host of other positive effects they have on the body (Teshika *et al.,* 2019). According to Wysocki *et al*. (2013)"People from 20 to 50 years ago, worldwide, have been eating steadily more onions." This analysis strongly defines the market demand and trend factor for onions. Onion has often been used in traditional societies to cure infections and in the medical industry, it was once utilized to fight flu epidemics. Increased production costs can result from more frequent applications of fungicide or higher rates of application to prevent yield loss. In some cases, it may be more cost efficient to use a variety with genetic resistance to the disease if one is available. As a result, the demand for onions among the types of vegetables grown chemically decreased and increased among the types of vegetables that could be grown without the use of chemicals. Onions are often stored and then exported after the domestic supply for that crop year has decreased. In addition to directly affecting the onion yield, some of pests and diseases prohibit its export from one country to another. For example, USA onion was banned from entering Japan for almost 40 years due to phytosanitary concerns over smut. Measures to control these pests and diseases will increase overall demand for onion crops. Onion by-products have often been a source of disposal problems. Burning onion waste, however, is a cheap and effective means of disposal. Open-air burning has often been incorporated into onion disease control studies as a way to destroy infected crop residues, particularly stubble left in the ground after lifting bulbs (Hamlett, 2017).

1.3. Copper Fertilization from Different Sources

The most common methods to apply fertilizers in acid soils are broadcasting and banding. Broadcasting methods avoid crop injury if the fertilizer is low in salts, while banding exposes roots directly to high salt concentrations in the fertilizer. Controlled release methods, where nutrients are metered through precision meters, may mitigate this. Plants can preferentially de- root under high fertility patches, but de-nitrification can be lower under banding. Using a broadcast application might be more cost-related, but the beneficial effects in manure of end-runoff manure are mainly maintained after banding.

Another method to apply fertilizers is by spraying. Therefore, a capillary interaction between the fertilizer droplets and the soil solution appears to be essential, a result that explains the commonly observed decrease in leaching for low concentrated source drops. Strategies of copper applications are based on the level of copper in tissues, efficiency of applications, and obtaining adequate tree coverage. Foliar standards are higher than soil application ones, and appreciable amounts of copper can be added to the soil without negative effects. For perennial crops, recommendations are based on the quantity of Cu removed with the crop harvests on the basis of the critical soil test Cu value. Further adjustments are made for the known increase of extractable Cu concentrations in successively applied fertilizer. Recommended levels of Cu for citrus with fruits are 2.5-5.0 mg kg^{-1} in leaves as the critical leaf concentration.

Nutritional problems due to Cu deficiency were basically based on the low Cu concentrations from the different sources themselves, low rates or the lack of experiments under uniform and adequate evaluation. Benso *et al.* (2022) did not find positive impacts on fruit characteristic due to the lack of Cu applications in citrus at temperatures below 15 °C in Brazil. Since it is essential for the synthesis of lignin, upon delaying lignin polymerization, the application of Cu on leaves during the summer might lead to changes in maturity of citrus fruit. Different soils, however, have different critical levels for copper, to account for differences in cation exchange capacity and clay mineralogy.

The Cu critical level reported was 0.30 mg kg^{-1} obtained by a correlation of Cu content in the fruit bunch per hectare per annum. Toxic levels of exchangeable Cu are likely to occur if values above 1.5 mg Cu kg^{-1} are attained. The relatively high exchangeable Cu is a result of the large quantities of CuS and $CuFeS_2$, which were present in the upper 80 cm of the profile, thus confirming earlier observations of widespread copper mineralization in the geological formations of the area. Plant-available sources of copper used in fertilizers are copper sulfate, copper oxide, chelated copper compounds, and copper chloride. Copper application timing is also important for optimal plant development. McKenna and Judge (2021) reported that cotton was more responsive to foliar-applied copper in the latter part of the season than in the early season. Citrus has a shallow root system, slow nutrient uptake, and a slow rate of nutrient translocation that cause uniform copper distribution for trees to be difficult to obtain. Thus, annual applications of copper usually. Should be performed as split applications. Yadava *et al.* (2022) found that splitting copper applications for tropical fruit crops was the best way to supply copper fruit trees.

1.4. Copper Nanoparticles in Agriculture

Some studies have pointed out the possible improvement in crop yield through CuNP applications. the details of why such an improvement may occur are difficult to precisely understand as it involves various direct or indirect determinants, including among others, soil-residence time of the nanoparticles, their dissolution rate and thus, the root exposure to the complete mix of dissolved metal and metal oxide cations, the subsequent plant transport and translocation of these cations, specific effects at the cellular level and the eventual influence on various metabolic processes leading to overall plant development. For example, copper is involved in the plant's electron transport process and in defense role by participating in lignin synthesis. Use of easily dissolved formulations such as $Cu(OH)_2$ or CuO NPs or more eco-friendly pro-oxidant coatings on CuNPs may offer quick release of the active component into the root zone and hence lead to immediate plant benefit. However, as discussed later in this section, concerns over overexposure to such active metallic forms have been indicated by numerous authors. It has been shown that chlorophyll production is also increased since there is greater nitrogen and secondarily magnesium uptake following Cu treatment. Another study has found enhanced nitrogen metabolism and increased net CO_2 assimilation upon CuNP application. It may also be speculated that the seeds from such Cu-treated plants, if used as food, might be nutritionally superior due to the accumulation of a mix of these critical minerals during seed development. In addition, a significant increase in root length, a foremost number of flowers per plant, and plants grown in soil impregnated with nanoparticles. A greater dried weight per plant in kernels of maize has also been observed in plants treated with sulfonated copper-polymer nanoparticles. Antioxidant Compounds of Nano Copper in Plant Cells: Copper (or nano copper) uptake by plant roots and the mechanisms involved have been recently reviewed. Copper is essential for plant's growth; however, excess of copper is toxic. In plants, copper functions as a cofactor to many enzymes, but being a transition metal, Cu^{2+} ions may generate cytotoxic reactive oxygen species (ROS) that can overwhelm the fundamental cellular antioxidants (Pătraşcu *et al.*, 2018). Cells thus have developed a terrific sequence of mechanisms to manage the amounts of copper critical for growth and the capability to eliminate ROS.

The excessive generation of ROS damages cellular proteins, lipids, and nucleic acids, disturbing cellular metabolism. To counteract this, plant cells produce antioxidant compounds. In tomatoes, Francis *et al*. (2024) recently showed that the application of Cu-NP excess the activity of biosynthesis and accumulation of key antioxidant metabolites in response to induced-oxidants. CuO particles were assessed for their fate in soil-plant systems, and found that the activity of CuO nanoparticles was reduced due to agglomeration and formation of bulk-sized aggregates in soil. When compared to the bulk CuO agglomerates, the CuO NPs did not show significant inhibitory influence on the spinach growth and did not significantly enhance Cu-content in the plant parts. Both soil application and foliar treatments of CuO particles resulted in enhanced Cu-content in the spinach. It may be possible that these results were obtained due to the change in particle properties when the nanoparticles interact with the chemical environment and form aggregates. Further, it is difficult to ascertain the influence of CuNPs alone for the reported effects in many such investigations. Soil incorporation of CuNPs has been used in many investigations. In one such study, CuNPs were evaluated to improve wheat growth. Two wheat varieties were used to analyze the genotypic differences in response to nano- and bulk Cu treatments. Apart from the plant growth parameters, the nutrient and Cu contents were also analyzed. Both foliar and soil application of CuNPs boosted the nutrient and Cu contents of the plants. However, with the soil treatment, CuNPs showed a better influence compared to the bulk Cu particles. Similarly, the study reported that the soil application of CuNPs has a more profound influence on growth parameters of wheat, compared to that of coarse Cu. A combination of bulk and nano formulations did not show significant boosting in plant growth parameters. However, in another study, an increase in seed weight and enhancement in soybean growth were reported with a combined soil incorporation of CuNPs and CuO nanoparticles. In liquid suspension the extract is obtained from leaves and stems or roots. A copper nitrate acetyl-acetonate salt was suspended in water and after boiling and, respectively, following a dehydration-rehydration process, nanoparticles obtained were well crystallized with a face-centered cubic crystal configuration.

The research amid to test different copper sources on onions to select the best form that onions absorb from soil

2. Materials and Methods

A field experiment was carried out in the recently reclaimed desert area of Wadi El-Notron station, Beheira Governorate, Egypt (Longitude 28°54E, Latitude28°20N, and Altitude 130 m), over out two seasons 2022/2023 physical and chemical analyses (Table 1) of the reclaimed soil were performed before planting at the Soil, Water, and Environment Research Institute, Agricultural Research Centre (A.R.C.), Egypt, according to Richards, (1954) and Jackson, (1967).

Table1: Some physical and chemical properties of experimental soil before planting

Physical characteristics	Value	Chemical characteristics	Value
distribution of Particle size (%)		**Electrical conductivity (ECds/m)**	1.70
Coarse sand (2000-200µ)	79.20	**pH(1:2.5) soil: water suspension**	7.98
Fine sand(200-20µ)	13.80	**Soluble cations (meq/L)**	
Silt(20-2µ)	4.75	**Ca^{2+}**	5.30
Clay(<2µ)	2.25	**Mg^{2+}**	4.32
Bulk density(g/cm3)	1.56	**K^{+}**	2.39
Total porosity(%)	54.70	**Na+**	5.40
Distributionas of Pore Sizeas % of Total	Porosity	**Soluble Anions(meq/L)**	
Macro(drainable)-pores(>28.8µ)	80.84	**CO_3^{2-}**	Nil
Micropores (<28.8µ)	19.16	**HCO_3^{-}**	1.65
Water holding capacity	21.56	**Cl-**	3.65
Field capacity	8.68	**SO_4^{2-}**	12.11
Wilting percentage	4.15	**Total carbonate (%)**	0.20
Available moisture	4.53	**Organic matter (%)**	0.26

2.1. Soil Preparation and Organic Matter

The soil was ploughed at an approximate depth of 30–40 cm. mechanically for planting. Then, compost (plant residues) was incorporated into the soil 14 days before planting at the rate of 2.5tha^{-1}. After wards, the soil was planked twice until the soil surface had settled. Table (2) shows the chemical characteristics of the compost.

Table 2: Chemical characteristics of utilized compost.

Characteristic	Value
Content of Moisture (%)	25
pH(1:5)	7.5
EC(1:5extract)dsm^{-1}	3.1
Organic C (%)	33.11
Organic matter (%)	70
Total N (%)	1.82
Total K (%)	1.25
C/N ratio	15:1
Total P (%)	1.29
Fe (ppm)	97.5
Mn (ppm)	18.40
Cu (ppm)	5.25
Zn (ppm)	14.33
Total content of bacteria (CFU g^{-1}) **Phosphate dissolving bacteria(CFUg^{-1})**	2.5×10^{7}
Weed seeds	0

2.2. System of irrigation

The system of irrigation water was a drip irrigation net (4 L h^{-1}) according to Karmeli and Keller (1975).

2.3. Material of Plant and Seedlings Transplantation

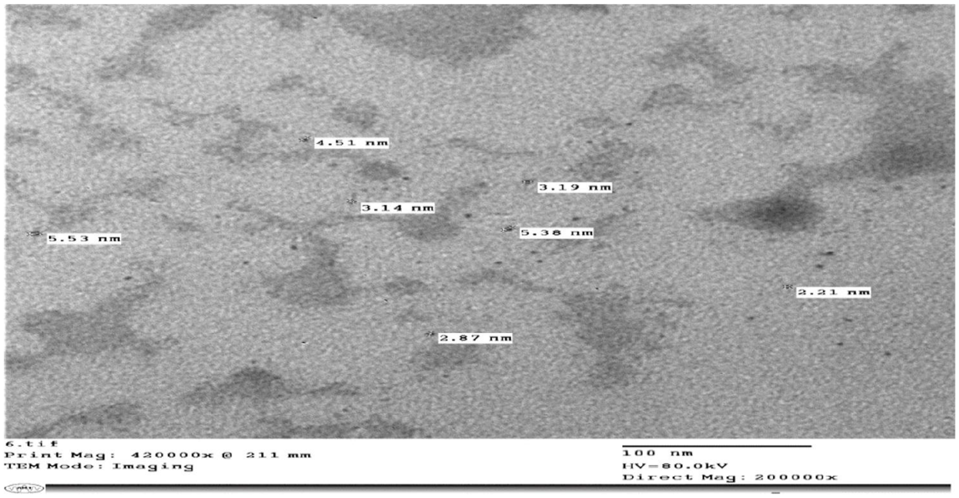
Giza 20 onion seeds were sown in a nursery on October 15 in both seasons. The seed germination medium was a mixture of peat and vermiculite (1:3) (obtained from The Agricultural Research Center, Giza, Egypt). December 1st. from both seasons, 15 cm high seedlings were planted in the open field. The seedlings were treated with fungicides before planting (Topsin M 70% w/w/Vitavax 300% w/w., Wadi El-Nile Co., Giza, Egypt) at a concentration of 2.5 g kg^{-1} to prevent any fungal infection during planting. Seedlings were planted at a distance of 20 cm between plant sand 50 cm between rows. Herbicide (Stomp®, BASF PLC, Cheshire, UK) was sprayed after planting the seedlings, followed by irrigation to check the growth of weeds at the early growth stage. The agriculture practices recommended by the ministry of agriculture were followed to obtained the pest quality onion bulbs.

2.4. Application of Fertilizer and onion bulbs Harvest

The recommended dose of fertilizers was applied as the Ministry of Agriculture. All empirical pieces were adding with ammonium sulfate (20.5%), superphosphate (15.5%), and potassium sulfate (52%) at 690,575, and115 kgha^{-1}, respectively. The bulbs of Onion were harvested in mid-May in both seasons. After of 75% of plants showed neck fall or top die-down. The bulbs were manually harvested. The bulbs were dried (curing) in a naturally ventilated area, then the necks were trimmed, leaving 2–3 cm attached to the bulbs. The bulbs were roughed at this stage based upon their, shape, size and color.

2.5. Copper Oxide Nanoparticles Synthesis

To prepare CuO nanoparticles from its precursor chemical compound copper sulfate ($CuSO_4$). Were used reagents of analytical grade (Sigma, USA). By top to bottom molecular chemical and physical combined methods, nanoparticles were obtained according to Zhang *et al*. (2014), with some modifications. 1 ML sodium hydroxide (NaOH) solution was added slowly. (NaOH) was added slowly (drop by drop) to aqueous solution of copper sulfate in amolar ratio of 1:3 under vigorous stirring for 10 h, discontinuously (2 days). The obtained precipitate was then mixed with 2 N citric acid monohydrate for 3 h. The obtained precipitate was filtered and washed thoroughly with ionized water in a mixed water/hexane system using a high-speed stirrer, and then washed again with ionized water alone for 2 h. The precipitate was finally dried in an oven at108°C for 24 h, and then exposed to1.5 psi of pressure for 5 days, discontinuously (6 h per day).



2.6. The design of experimental

1- NPK (Control).
2- NPK+ ($CuSO_4$) spray at on leaves with rate of 20ppm L^{-1}
3- NPK+ (CuO-NPs) add with irrigation water, then spray on leaves at a rate of 10ppmL^{-1}.

All copper treatments were applied at 20, 40, 60, and 80 days after planting.

2.7. Plant characteristics

1. The height of Plant (cm), using a measuring tape.
2. The leaves Number per plant.
3. The leaves Fresh weight (g), using a sensitive balance.
4. The bulbs Number of obtained per treatment.

2.8. Chemical analysis

The material of Plant was dried at 70˚C for 24 h according to AOAC (1990), and then finely ground for chemical determination of elements by Andresen *et al.,* (2018) and AOAC (1990). Total nitrogen content of the dried leaves was determined using the modified micro-Kjeldahl method as described by AOAC (1990). Phosphorus was determined colori-metrically according to Jackson (1967). Potassium concentration was determined using the flame photometer apparatus (CORNING M 410, Germany). Fe, Cu, Zn, and Mn Concentrations in plant bulbs were determined using an atomic absorption spectrophotometer with air-acetylene and fuel (PyeUnicam, model SP-1900, USA). Total soluble solids (T.S.S), protein, and fibers were assayed according to AOAC (1990).

By the phosphor-molybdic acid method the total carbohydrates in bulbs were determined according to AOAC (1990). Vitamins were determined in bulbs and estimated per 100 mL fresh weight. Vitamins B1 (thiamin) were determined fluoro metrically, whereas and C (ascorbic acid) were determined colorimetrically according to Nazmul *et al.* (2013).

The bulbs were dried, weighted, and chopped to determine the amino acids. The resulting mixture was and stored at 4°C in sealed vials. The amino acid extraction from all sample of freeze-drie were conducted according to Wayne *et al.*(2012)with some modifications. Twenty ml of distilled water was added to 1.0 g of freeze-dried onion sample. The resulted suspension was preserved under magnetic stirring for 3min at 0 ˚C in an ice bath, then for 15 min, the suspension centrifuge at 10000 rpm. This process was consecutively repeated three times by re-suspending the pellet every time. Under vacuum through a 0.45 μm nylon filter, the final solution was filtered. Each extracted solution was lyophilized again and stored at 4 °C in sealed vials. By HPLC-ELSD methodology was analyzed each extract using Nazmul *et al.*(2013). The prepared samples were at a concentration of 25 mg/mL, filtered through anylon 0.45 µm filter, and analyzed in three coupies. The movabel stage tendency was obtained from eluent A (7 mM HFBA in pure water) and eluent B (net MeCN) as follows: 0–10 min.100%A,10–30 min. from100 upto75% A, 30–38 min from75 up to 70%A, 38–39 min 100% A, 39–70 min 100% A.

Statistical Analysis

The data were analyzed using ANOVA table at the 5% significance level. By Duncan's Test at the 5% significance level, the difference between means of treatments was analyzed.

3. Results

3.1. Growth traits

Data belong to growth traits of onion plant referring to Table (3) disclosed that, copper nano particles (CuNPs) treatment application was considerably augmented growth traits of onion plant understudy as height of plant, leaves number per plant, fresh weight of leaves and number of bulbs in association with advised amount of marketable fertilizers NPK applied as control treatment. The percentages elevated were 9.7% for plant height, 77.8% for number of leaves, and 44.7% for leaves fresh weight in contrast to control treatment NPK. Meanwhile insignificant difference was recorded between Copper sulfate treatment and control plants.

Table 3: Effect of different copper sources treatments on onion plant growth parameters

Treatment	Plant height (cm)	No. of leaves /plant	Fresh weight of leaves/plant (g)	No. of bulbs
NPK	47.6b	5.4c	27.3b	12.8b
$CuSO_4$	45.3b	7.3b	29.5b	13.3b
CuNPS	52.5a	9.6a	39.5a	19.7a

Values with different letters show significant differences at $P \leq 0.05$ (Duncan).

3.2. Photosynthesis, Transpiration rate and Water use efficiency

Onion (*Allium cepa* L.) plant leaf photosynthesis rate under different treatments as publicized in Table (4) unquestionably revealed that, CuNPs treatment significantly augmented biological process of net photosynthesis rate by 20.5% compared to plant leaves under control. As shown in vegetative traits; Copper sulfate treatment recorded insignificant difference with control plants regarding photosynthesis rate. For the time being, CuNPs treatment application increased stomatal conductance by 7% over marketable fertilizers NPK (control treatment). Moreover, CuNPs treatment significantly boosted intercellular CO_2 concentration by 18% compared to control treatment plants. Additionally, water use efficiency was the highest 97% with CuNPs treatment compared to control (NPK). Meanwhile, marketable fertilizers dose NPK (control) donated the highest value of transpiration rate 63.9% in comparison to CuNPs treatment.

Table 4: Effect of different copper sources treatments on photosynthetic characters of onion Plant

Treatment	Photosynthetic rate ($\mu mol\ m^{-2}s^{-1}$)	Stomatal conductance ($mmol\ m^{-2}s^{-1}$)	Transpiration rate ($mmol\ m^{-2}s^{-1}$)	Water-use efficiency ($\mu mol\ mmol^{-1}$)	Intercellular CO2 concentration (ppm)
NPK	17.52b	222.5c	4.72a	3.71c	141.2c
$CuSO_4$	18.63b	225.8b	3.97b	4.69b	159.5b
CuNPs	21.11a	238.6a	2.88c	7.32a	166.6a

Values with different letters show significant differences at $P \leq 0.05$ (Duncan).

3.3. Elements components

3.3.1. Macro elements (N, P, K)

Macro-elements Nitrogen (N), Phosphrus (P) and Potassium (K) component as showed from chemical analysis and exposed in Table (5) divulged that, submission of CuNPs treatment significant raised macro-elements (N, P and K) within plant tissue by 18.5% for N, 35.7 % for P and 12% for K overmarketable chemical fertilizers (NPK) as control treatment. Meanwhile $CuSO_4$treatment recorded significant increase 14% over control treatment concerning phosphorus and insignificant difference regarding potassium.

Table 5: Effect of different copper sources treatments on macro and micro elements of onion Plant

Treatment	N%	P%	K%	Zn (ppm)	Cu (ppm)	Fe (ppm)	Mn (ppm)
NPK	1.46c	0.14c	1.33b	21.85c	3.69c	133.27b	18.16c
$CuSO_4$	1.61b	0.16b	1.36b	24.11b	5.77b	135.41b	20.49b
CuNPs	1.73a	0.19a	1.49a	29.42a	7.69a	139.59a	24.68a

Values with different letters show significant differences at $P \leq 0.05$ (Duncan).

3.3.2. Microelements (Zn, Fe, Cu, Mn)

On the subject of microelements analysis as exposed in Table (3) found that implementation of CuNPs treatment considerably increased concentrations of micro-elements within plant tissue by 34.7% for zinc, 108% for copper, 4.7% for iron and 35.9% for manganese balanced to commercial chemical fertilizers (NPK) as control treatment. While results from iron recorded insignificant differences between both treatments.

3.3.3. Chemical constituents

Data in Table (6) showd that the reflect of appliance of CuNPs treatment appears significantly elevated biochemical compounds represented in total chlorophyll by 83%, 11% for total phenolic, 29% for total carbohydrate, 19% for TSS, 67% for vitamin C and 26% for thiamin in comparison with commercial chemical fertilizers (NPK) as control treatment. At the same time there were significant differences in the favour of $CuSO_4$ treatment compared to commercial chemical fertilizers (NPK) control treatment regarding most previous biochemical compounds.

Table 6: Effect of different copper sources applications on chemical components of onion Plant

Treatment	Chlorophyll content (mg/g FW)	Total phenolic (mg GAE g^{-1} DW)	Total carbohydrates (mg/g)	TSS (%)	Vitamin C (mg)	Thiamin (mg)
NPK	3.61c	198.51c	61.4c	5.8b	5.2c	0.038c
$CuSO_4$	4.84b	211.36b	70.5b	6.3a	6.7b	0.042b
CuNPs	6.62a	220.79a	79.2a	6.9a	8.7a	0.048a

Values with different letters show significant differences at $P \leq 0.05$ (Duncan).

3.4. Chemical soil conditions with use of compost in Onion plant agriculture

The information in Table (7) demonstrated that adding compost improves the chemical properties of soil, enriches agricultural soil with nutrients, and creates an ideal environment for plants. Recycle as demonstrated in Table 1, adding to soil during the preparation stage typically results in lower soil pH values when compared to the chemical characteristics of the experimental soil prior to planting. This could be a reference to the acidic functional groups that are generated when compost oxidizes, which can lower the pH of the soil (Liu and Zhang, 2012).

Data from the same study revealed that the planting soil's electric conductivity (EC) changed from 1.70 (before planting) to 3.11 dS $m.^{-1}$ (after planting) due to the compost impact (compost EC 3.10 dS m^{-1} as shown in Table 2), which is consistent with the findings of Wagida and Seddik (2018).

Table 7: Effect of compost on some soil chemical properties after planting

Soil status	pH (1:2.5)	EC dSm^{-1}	Macronutrient availability (mg kg^{-1})		
			N	P	K
Before planting	7.98	1.70	14.56	7.13	47.11
After planting	7.10	3.11	197.40	30.50	63.00

4. Discussion

4.1. Growth traits

Cu with iron, zinc, boron, chlorine, manganese, and molybdenum are regarded as vital micronutrients, playing crucial roles in plants physiological process and metabolic pathways. At the cellular stage, Cu absence hinder selection transport within the thylakoid is affected by plastocyanin proteins (Pilon *et al.,* 2006). Also, plants deficient in Cu- show a decreased cellular capability to scavenger reactive oxygen species (ROS) due to Cu/ZnSO4 enzymes be altered (Yamasaki *et al.,* 2008). Such damages influence plant development and crop quality. One of the causes leading to the appearance of copper shortage symptoms is a species cultivated in sandy soils of little organic matter content. This turns into worsen by other ecological factors, including aspects like the pH of the soil, calcium concentration presence, and the type of crop grown (Fageria, 2016). While the total amount of copper in most soils is adequate, ranging 20–250 ppm, the texture of sandy soils are more likely to be copper-lacking than clays, with Cu contents varying between 1–20 ppm soil (Schoffer *et al.,*2022). This suggests a sub-optimal value for normal plant growth and development as per the aforementioned studies ranges.

Sensitivity to copper absence is at variances widely between plant species. Kong *et al.* (2019) reported that a number of field crops such as wheat are highly sensitive to copper (Cu) deficiency, while others like rapeseed exhibit lower sensitive. Among vegetable crops, onions and carrots appears to be the most sensitive species to copper shortage. Commonly, copper shortage symptoms in plants reflect the crucial roles that copper performs. Copper is a transition element plays a vital role as an enzyme co-factor that catalyzing redox reactions. Furthermore, copper is involved in several key metabolic process including photosynthesis and respiration, protein synthesis, cell lignification, auxin regulation, and protection against oxidative stress (Broadley *et al.,* 2012). In general plants, copper deficiency initially manifests in the young leaves due to Cu is an immobile element.

As the deficiency progresses, the tips become necrotic and turn dark-brown. In severe cases, new leaves emerge needle-shaped as they fail to unroll.

In onion, the leaf tips turn white and become twisted or bend at right angles as copper shortage worsens, the leaves may also turn yellow and experience dieback. As for the onion bulbs, they become light colored with thin and brittle skins. This could be due to of variations in expression of cell wall-related genes, as previously demonstrated in alfalfa (Printz *et al.,* 2016). It is important to note that the root system is the first to experience soil a biotic stress, including nutrient deficiency and its traits are affected to a higher degree compared to other plant organs. Consequently, onion bulbs are often significantly impacted by these stresses. Copper deficiency in onion leads to premature plant maturation, characterized by smaller dried tops and bulbs that lack firmness and solidity displaying greenish-bronze hues (El-Ghamrya, *et al.,* 2024).

It is widely recognized that plant biomass increases with an enhanced photosynthetic rate up to a certain threshold. Copper deficiency influences both PSI and PSII activities in the chloroplast, reduce carbohydrate synthesis by almost 50% by affecting both chlorophyll content and leaf area. These reduction was revealed to be correlated with a decrease in plasto-cyanin level copper-deficient leaves. Also, copper deficient plants exhibit declines in photosynthetic rates which are associated with disruptions in thylakoids function reduced stomatal conductance, and decrease Rubisco activity and RuBP regeneration (Morales and Kaiser, 2020). The decrease in bulb weight was observed under the control conditions of our experiment (Table 2) in comparison to plants sprayed with copper nano-particles(CuNPs). This may indicate that copper-deficient conditions limit the maximal reduction in photosynthesis, which in turn decreased carbohydrate, fibers, and TSS % in affected bulbs (Table 5). As stated above, plants vary in their sensitivity to Cu deficiency shortage and, so, which influences their response to copper supplementation and revealed differential yield increases of crops (Zeb *et al.,* 2024).

The CuNPs form proved to be the most effective treatment for boosting onion yields. This could indicate a major impact to CuNPs on the photosynthetic machinery as in contrast to copper sulfate. Recent research showed a 31% more in total onion yield when plants were treated with 50 ppm of copper sulphate (Fouda, 2016). However the high doses from Copper are not sustainable for use in future plantations as they may lead to toxicity in other crops that are more sensitive to copper. As well, our experiment granted evidence which applying copper dose (i.e.,10 ppm CuNP treatment) was really efficient in a achieving large- scale vegetative growth improvement compared with higher doses from Cu sulfate and control plants.

4.2. Macro and micro elements concentrations

Even though the morphological response of Cu-deficient onion plants are well-documented in the literature, little is known about its response to refer the accumulation of mineral and biochemical compounds in its tissues. Our experiment clearly demonstrates that onion plants were significantly impacted by copper deficiency. Copper risk known to have numerous interactions with macro-andmicro-elements which may consequently influence crop yields. Regarding macronutrients, the submission of nitrogen and/or phosphorus has been accounted to aggravate Cu-deficiency in wheat (Sagwal *et al.*, 2022). While CuNPs demonstrated a notable effectiveness in escalating the accumulation of macro-elements in onion bulb tissues was evaluated in comparison to Cu sulfate and control plants (Table 4), the reasons at the back of this remain to be explained. Relating to micro-nutrients, evidence suggests that copper uptake is also hindered by application or presence of Zn (Fageria, 2016). Plant requisites for Copper (Cu) is lower than the rest of the micronutrients. Commonly, sufficient the concentration of copper in plant dry tissues is generally between 2 and 20 ppm (Mengel, 2001). In onion, usual copper levels in the whole plant ranges from 8 and 45 ppm in dry matter (Mottaleb *et al.,* 2021). Onion leaves and stems appear to accumulate more copper than the bulbs when Cu supplementation is high. Our data are correspond with the previously mentioned tissue copper levels.

4.3. Chemical constituents

A new result from our study reveals the impact of copper deficiency on the levels of bioactive components that encourage a lot of beneficials for humans (Teshika, 2019). It is well known that onions are a rich source of vitamins B6 (pyridoxine), C (ascorbic acid), and B9 (folate). These vitamins essential and have a crucial role in plant metabolism and various molecular events associated with acquired resistance. These function include, but are not limited to, serving as a co-enzyme in chemical reactions and promoting the synthesis of DNA and proteins. In fact, the vitamins found in onions appear to have a valuable effect in treating or preventing hyper-homo cyteinemia and providing protection against oxidative damage in humans. Ascorbic acid (vitamin C) is the most extensively studied vitamin in relation to copper supplementation. The effect of Cu supplementation on plant tissue levels of ascorbic acid appears to be species-dependent. For example, in vegetables, the levels of ascorbic acid in cabbage, carrots, cauliflower, eggplants, lettuce, and spinach increase with Cu supplementation. On the other hand, Cu supplementation had no effect on ascorbic acid levels in mandarin and tomato plants, while it led to decreased in ascorbic acid levels in spleen amaranth plants (Mozafar, 2018). Before starting the present study, it was expected that onion plants would respond to Cu supplementation and increase its vitamin C contents in the same way as other Cu- responsive plants, as spinach and carrots. Never the less, our data on onion showed that, although the level of vitamin C increased in all Cu forms used compared to control treatment, therefore, further studies using higher concentrations of Cu are needed to confirm its effect on ascorbic acid contents in onion plants. A similar conclusions can be drawn for the levels of vitamin B1 (Table 5).

5. Conclusions

Recovery from copper deficiency symptoms can be achieved through the use of various Cu sources and forms. $CuSO_4$ is commonly used as Cu source to achieve this goal. Nevertheless, the results presented in this experiment provide evidence supporting the hypothesis that a low concentration of CuNPs is not only safe for application to onion plants, but also promotes higher yield, antioxidant activity and phytochemical accumulation compared to $CuSO_4$ and control treatment. Using 10ppm of CuNPs also enhanced the morphological and biochemical constituents of onion plants. CuNPs were not only safe to use but also showed results that were either similar to or superior to the other Cu forms evaluated in this experiment.

6. Acknowledgement

Exceptional thanks for plant physiology lab (Nanotechnology project) Faculty of agriculture-Cairo university which support present research work.

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