Acta Scientiarum

http://www.periodicos.uem.br/ojs/
ISSN on-line: 1807-8621
Doi: 10.4025/actasciagron.v47i1.72076





# Effects of copper nanoparticle treatment on the properties of hybrid corn seeds

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**ABSTRACT.** Corn (*Zea mays* L.), a vital crop economically and nutritionally, benefits from advancements like copper (Cu) nanoparticles, which are engineered to enhance crop growth and grain quality. This study aimed to assess the morphological, physical, chemical, and quality attributes of various corn hybrids (HB 01: 22S18 TOP2®; HB 02: 20A30 VIPTERA®; HB 03: 20A80 TOP2®; HB 04: 22S18 TOP3®, and HB 05: 20A20 TOP2®) treated with copper nanoparticles (900 mg of Cu $L^{-1}$). Hybrid HB 04: 22S18 TOP3® exhibited the highest levels of protein, lipids, fiber, and phosphorus, though calcium levels were comparable to other hybrids ($p > 0.05$). Fatty acid and amino acid profiles remained unchanged. Hybrids not treated with Cu nanoparticles had higher pH levels. The Cu nanoparticles primarily altered the surface, not the interior, of the seeds, with HB 02: 20A30 VIPTERA® showing the highest Cu content. Overall, Cu nanoparticles preserved the physical and chemical properties of the corn grains, confirming their viability for seed treatment.

**Keywords:** adsorption; concentration of copper; fiber; protein; quality.

Received on April 29, 2024.
Accepted on August 23, 2024.

## Introduction

Corn (*Zea mays* L.) is a widely cultivated cereal grain crop, with a global production exceeding 1.1 million tons in 2021/2022 (United States Department of Agriculture [USDA], 2021). In 2023, Brazilian corn production reached 323 million tons, making it one of the most important crops in agribusiness (Companhia Nacional de Abastecimento [CONAB], 2023). Brazil is the third largest corn producer in the world, only behind the United States and China.

Increases in crop production have been achieved primarily through the development and adoption of new technologies (Buso & Silva, 2018; Rigo et al., 2015). Among these technologies, seed treatment, plays a crucial role in managing pests and diseases that affect the initial phase of crop growth. However, before large-scale implementation, studies are necessary to verify their effectiveness and assess any potential harmful effects on the physiological quality of the seed (Silva et al., 2020).

Plant nutrition influences both grain quality and yield. Quality is associated with the nutritional value of food, taking into account physicochemical aspects, organoleptic properties, and the resistance of the grains to damage during storage and transport (Dubal et al., 2020). Regarding nutritional aspects, low copper (Cu) concentration is a limiting factor for grain yield in most soils in Brazil. Therefore, supplying this element via seeds can be a viable and relatively low-cost technique for the initial Cu supply to corn plants, aiming for better crop establishment in the field (Broadley et al., 2012).

Cu is a trace mineral element present in proteins and enzymes essential to plant metabolism. An excess of Cu can lead to increased synthesis of proteins and enzymes involved in defense against oxidative damage, alterations in photosynthetic electron transport, and photoinhibition (Cambrollé et al., 2015).

Cu stress can damage the root structure, reducing the uptake of water and mineral nutrients from the soil and thus slowing plant growth (Bochicchio et al., 2015). The incorporation of Cu nanoparticles in seeds can provide several benefits for plant growth and biological activities (Hoang et al., 2019), as they are stable and inert, not affected by changes in soil pH, and not complexed by other molecules present in the soil, cells, and plant tissues. Nanoparticles can cross cell membranes due to their size of 1 to 100 nm, given that the pore size of plant cell walls is around 4 to 6 nm (Saharan et al., 2016). Metal nanoparticles can exhibit suitable redox potential energy for crop plants through electron transfer reactions, concentrating protons inside the membrane vesicle, and generating an electric field across the photosynthetic membrane (Hoang et al., 2019).

However, there are few studies on the application of Cu nanoparticles to corn seeds, and knowledge is lacking regarding grain quality and nutritional parameters following the application of these nanoparticles. In this sense, the main purpose of this work was to evaluate the morphology, as well as the physical, chemical, and quality properties of various corn hybrids grown from seeds treated with copper nanoparticles (900 mg of Cu $L^{-1}$).

This research demonstrates the effectiveness of Cu nanoparticles in delivering micronutrients to corn grains. This innovative approach offers a precise and efficient method for addressing copper deficiencies in corn-growing regions, ultimately contributing to sustainable agriculture and improved food security.

## Material and methods

### Experimental design

The cupper nanoparticles were provided by Kher Chemical Research and have an average particle size of 25 nm and a purity of 99.5%. The Cu nanoparticles were suspended in deionized water and dispersed by ultrasonic bath (100 W, 40 kHz) for 30 min.

The treatment of corn seeds was carried out the day before sowing at room temperature (25°C), in open glass reactors (Becker type), using 900 mg of Cu $L^{-1}$, as a vehicle for absorption by the seeds.

Were used 5 corn hybrids in order to perform the application of Cu nanoparticles. For this, the experimental design used was a randomized complete block design in a factorial setup (5 x 2), with three replications, totaling 30 plots. Five corn hybrids were grown with (900 mg of Cu $L^{-1}$) and without nanoparticle application (0 mg of Cu $L^{-1}$ nanoparticles). The hybrids used were HB 01: 22S18 TOP2®; HB 02: 20A30 VIPTERA®; HB 03: 20A80 TOP2®; HB 04: 22S18 TOP3®; and HB 05: 20A20 TOP2®. The hybrids were previously described by Verdi e tal. (2022). The dose of 900 mg of Cu $L^{-1}$ nanoparticles was chosen because of its physiological relevance for seed quality (Carlesso et al., 2020). The seeds were packed in paper bags and stored in a dry chamber with humidity of 50 ± 5% and temperature of 8 ± 4°C until the following day.

The implementation of the corn crop took place on black oat straw (*Avena strigosa* Scherb), dried on October 18, 2018 with the herbicide Glyphosate (Glyfosato Nortox WG®), at a dose of 2.50 kg $ha^{-1}$, in advance of 21 days before sowing. Sowing was carried out on November 10, 2018, manually with the aid of a seeder known as a “matraca” in order to control the density and distribution of seeds at a depth of 2 to 3 cm. The corn was harvested when the plants reached field maturity (April 20, 2019). The corn cobs were harvested manually, threshed in a manual thresher and the impurities were separated. The grains were analyzed physicochemically and morphologically. For this purpose, all corn grain samples were crushed in a mill (Cuisinart, model DCG-20BKN) and sieved with a 42 mesh sieve (Bertel), corresponding to 355 μm, and stored in polyethylene bags.

### Evaluation of germination

For the germination determination, 8 sets of 50 seeds were placed on germination paper ("germitest"), which had been moistened with distilled water equivalent to 2.5 times the dry paper weight. The paper towels were then rolled and placed in plastic bags to maintain moisture levels. These rolls were positioned vertically in a Mangelsdorf type germinator set to a constant temperature of 25°C and 70% humidity. Germination was monitored over a period of seven days, after which the number of normal seedlings—those with well-developed roots and shoots-was recorded (Brasil, 2009).

### Physic-chemical analyses

The hybrid corn samples were analyzed for pH, moisture, lipids, protein, fiber, and minerals (Ca, P, and Cu) following the methodology of the Institute Adolfo Lutz [IAL] (2008).

Moisture content was determined using an air recirculating oven at 105°C for 4h (IAL, 2008) and pH measurements were carried out with a pH meter (Digimed, model DM-22, São Paulo, Brazil). Lipid content was determined by the Soxhlet method (IAL, 2008). Total nitrogen was obtained through Kjeldahl digestion (IAL, 2008) and multiplied by a factor of 6.25 to obtain the protein quantity. For crude fiber determination, samples were subjected to acid and alkaline digestion, followed by filtration in a Gooch crucible. The resulting organic residue was then burned in an oven at 550ºC. Total mineral content was obtained using a gravimetric method after incineration at 550°C for 6h (IAL, 2008). Calcium (Ca), phosphorus (P), and copper (Cu) concentrations were determined with an atomic absorption spectrometer (AAS Savantaa® Model 6BC 3.11, Braseside, Victoria, Australia). Initially, 3 g of each hybrid grain sample was pre-burned before being placed in an oven at 550°C for 8h. Subsequently, the samples were diluted in nitric acid (Merck® Purity 65%) and analyzed with an ASS equipped with a background corrector (model contraA 700, Analytik Jena, GmbH, Jena, Germany). All analyses were performed in triplicate.

### Fatty acid composition analysis

Oil samples obtained by Soxhlet method were esterified using 0.2 mL of methanolic potassium hydroxide solution (Vetec 0.01 M) and 2.0 mL of n-hexane (Cinética, 95% purity, Jand Industrial Chemical of São Paulo, Brazil). The mixture was stirred for 30 s. Fatty acid methyl esters (FAMEs) were prepared according to the methodology of Adolfo Lutz Institute (IAL, 2008). For gas chromatograph injection, the samples were diluted using 0.1 mL of the supernatant solution and 0.9 mL of n-hexane. The determination of fatty acids was carried out using a Shimadzu gas chromatograph model GC-2010 Plus (Shimadzu Brazil, Barueri, São Paulo State, Brazil) equipped with a split/splitless injector and flame ionization detector (FID). A polar column (Rtx-Wax Restek, 30 m length x 0.25 mm inner diameter x 0.25m thickness) was used. The temperature program was set as follows: 80°C (5 min.), 80 to 200°C at a rate of 20°C $min.^{-1}$ 200°C (5 min.), 200 to 230°C at a rate of 5°C $min.^{-1}$ and 230°C (10 min.). The analysis was performed with a detector temperature of 275ºC and an injector temperature of 250ºC, using a 1:10 split injection. Hydrogen gas (White Martins S.A., 99.999% purity, Brazil) was used as the carrier gas at a flow rate of 1.5 $cm^3$ $min.^{-1}$. The identification of fatty acids in the samples was conducted by comparing them with Sigma standards (Steinheim, Germany).

### Determination of amino acids

The amino acid composition of the samples was evaluated according to the method described by Goersch et al. (2019). Essential amino acids including lysine, methionine, and tryptophan, were assessed. Chromatographic separation was conducted using a Hi-Chrom C18 column (250 × 4.6 mm i.d., packed with 5 μm particles) obtained from Hi-Chrom (United Kingdom) with a high-performance liquid chromatography (HPLC) 525 Instrument (Biotech, Germany). A capillary electrophoresis system PNA8C (generously provided by ISB, Brazil) was used, equipped with a laser-induced fluorescence detector for fluorescence detection. The excitation was induced by a 405 nm diode laser, and the sensitive CCD camera facilitated the detection of the fluorescent light. Peak areas were calculated by integrating the chromatograms using Chromophoreasy software. Enzymatic hydrolysis was performed in a hybridization oven (Amersham Pharmacia Biotech, United Kingdom). The results of the amino acids were expressed as mg/g of dry sample.

### Morphological analysis

The morphological and distribution of Cu nanoparticles adsorbed on the corn surfaces, as well as the embedded nanoparticles inside the hybrid grains, were evaluated. Images were obtained using scanning electronic microscopy (SEM) (Zeiss model EVO LS25, Jena, Thuringia, Germany) at an operating voltage of 10 kV. Prior to imaging, the dehydrated samples were coated with a layer of gold using a metallizer (Sputter Coater SCD 050 - Balzers).

### Statistical analysis

The data (n = 3) were subjected to analysis of variance (ANOVA), followed by mean separation with a Tukey test and Student at $p < 0.05$. We used the SISVAR software for analysis of variance.

## Results and discussion

The seeds of the different hybrids treated with Cu nanoparticles exhibited rapid velocity of germination on the 4th day. This result outcome may be attributed to the seed coating, which protects the embryo from external factors while allowing selective permeability. The application of metal nanoparticles appears to have no adverse effects on germination, consistent with findings by Lin and Xing (2008). Previous studies have also indicated that nanoparticles do not influence seed germination (Duran et al., 2017; Wang et al., 2020). Similar to these observations, our results demonstrate that Cu nanoparticles-treated seed exhibited no significant differences in germination potential or rate. The selective permeability of seed coats suggests they can shield the seeds from harmful external factors (Wang & Jablonski, 2016).

The pH results of corn hybrid grains with and without treatment with Cu nanoparticles are shown in Table 1. In general, the pH of hybrid corn with and without Cu nanoparticles ranged between 6.33 and 6.68. This result can be correlated with the findings of Assis et al. (2014), who obtained pH results for 10 types of hybrids, with values ranging from 6.25 to 6.49. According to the authors, the pH is mainly associated with the concentration of protein and minerals in the plant. Thus, it can indirectly contribute to the understanding of how the plant allocates the nitrogen and minerals present in the soil.

The pH of hybrids without the application of Cu nanoparticles was higher than with application, with the exception of HB 03, with a statistical difference ($p \leqslant 0.05$). The pH value depends on the relative concentrations of malic and tartaric acids and the degree of salt formation of acids, which, in turn, are subject to the potassium content (Jackson & Lombard, 1993). No references were found that show the relationship between pH and the micronutrient Cu.

**Table 1.** pH of hybrid corn with and without application of Cu nanoparticles.

| Hybrid | pH | |
|---|---|---|
| | Without Cu nanoparticles | With Cu nanoparticles |
| HB 01: 22S18 TOP2® | 6.68 $^{aA}$ ± 0.06 | 6.41 $^{abB}$ ± 0.05 |
| HB 02: 20A30 VIPTERA3® | 6.60 $^{aA}$ ± 0.05 | 6.45 $^{abB}$ ± 0.06 |
| HB 03: 20A80 TOP2® | 6.42 $^{bA}$ ± 0.08 | 6.50 $^{aA}$ ± 0.08 |
| HB 04: 22S18 TOP3® | 6.46 $^{bA}$ ± 0.07 | 6.33 $^{bB}$ ± 0.07 |
| HB 05: 20A20 TOP2® | 6.55 $^{abA}$ ± 0.07 | 6.47 $^{abA}$ ± 0.06 |

Mean values ± standard error within a row with different lowercase are significantly different by Tukey's test, and within a line with different uppercase are significantly different by Student test, at the 5% level.

The hybrid interaction with Cu nanoparticles in relation to proteins (Table 2) does not show a significant effect ($p > 0.05$). Without the application of Cu nanoparticles, the highest protein content was observed for HB 03: 20A80 TOP2®, whereas with the application, it was for HB 04: 22S18 TOP3®. This is very interesting, as it might result in an increased caloric value for a food. Corn is important for feed formulations because it has a high energy value, and the protein content contributes to feed conversion and reduces production costs (it is cheaper to produce corn than other grains such as soybeans).

Our study found a total protein content of around 6.5%, which is significantly higher than the 4.8% reported by Shafiq et al. (2024) in their evaluation of copper oxide nanoparticles (CuO-NPs) on growth and biochemical characteristics in corn hybrids YH-5427 and FH-1046. This notable difference may be attributed to varying experimental conditions such as soil composition, nutrient availability, and environmental factors. Additionally, differences in maize hybrid genetics and specific nanoparticle application methods likely contributed to the higher protein content observed in our study.

**Table 2.** Value of protein in hybrid corn with and without application of Cu nanoparticles.

| Hybrids | Protein (%) | |
|---|---|---|
| | Without Cu nanoparticles | With Cu nanoparticles |
| HB 01: 22S18 TOP2® | 10.11 $^{aA}$ ± 0.09 | 9.97 $^{bA}$ ± 0.06 |
| HB 02: 20A30 VIPTERA3® | 9.80 $^{bA}$ ± 0.07 | 9.92 $^{bA}$ ± 0.07 |
| HB 03: 20A80 TOP2® | 10.03 $^{aA}$ ± 0.08 | 9.95 $^{bA}$ ± 0.09 |
| HB 04: 22S18 TOP3® | 9.96 $^{abA}$ ± 0.08 | 10.13 $^{aA}$ ± 0.09 |
| HB 05: 20A20 TOP2® | 10.07 $^{aA}$ ± 0.08 | 9.91 $^{bA}$ ± 0.08 |

Mean values ± standard error within a row with different lowercase are significantly different by Tukey's test, and within a line with different uppercase are significantly different by Student test, at the 5% level.

Changing the average composition of corn grain without losing productivity, stability, and disease tolerance is a challenge. The protein content of corn is between 8.0 and 11.5%, with an average of 10.0%. The protein content of corn depends on the variety, growing conditions, and environmental factors of cultivation (Mutlu et al., 2018). Assis et al. (2014) found protein results for different hybrids between 8.06 and 9.10. These differences observed in protein content may be related to differences in the efficiency of nitrogen absorption available in the soil for the plant, in which the different hybrids and varieties require different amounts of nitrogen, according to their yield potential. The cultivation conditions in the present study were identical for both hybrids, with and without the application of Cu nanoparticles. Thus, the different protein contents found may be due to different hybrids. The application of Cu nanoparticles, in general, did not contribute to the increase in protein.

There was no significant effect ($p > 0.05$) of the interaction of hybrids and Cu nanoparticles on lipids (Table 3). Without and with the application of Cu nanoparticles, the highest lipid content was found in HB 03: 20A80 TOP2®. The difference in lipid content between the hybrids may be due to the genetic differences between them. Thus, it is verified that the treatment of seeds with Cu nanoparticles did not influence the lipid content of the hybrids. These results correspond with the findings of Vázquez-Carrillo et al. (2015), who determined the lipid content in maize hybrids (5.8%).

**Table 3.** Value of lipids of hybrid corn with and without the application of Cu nanoparticles.

| Hybrid | Lipids (%) | |
|---|---|---|
| | Without Cu nanoparticles | With Cu nanoparticles |
| HB 01: 22S18 TOP2® | 4.49 $^{bA}$ ± 0.09 | 4.58 $^{bA}$ ± 0.07 |
| HB 02: 20A30 VIPTERA3® | 4.46 $^{bA}$ ± 0.07 | 4.49 $^{bA}$ ± 0.13 |
| HB 03: 20A80 TOP2® | 5.02 $^{aA}$ ± 0.08 | 5.06 $^{aA}$ ± 0.10 |
| HB 04: 22S18 TOP3® | 4.45 $^{bA}$ ± 0.09 | 4.62 $^{bA}$ ± 0.14 |
| HB 05: 20A20 TOP2® | 4.48 $^{bA}$ ± 0.12 | 4.67 $^{bA}$ ± 0.09 |

Mean values ± standard error within a row with different lowercase are significantly different by Tukey's test, and within a line with different uppercase are significantly different by Student test, at the 5% level.

The analysis of variance did not present a significant effect ($p > 0.05$) of the Cu nanoparticles application factor in relation to the crude fiber. On the other hand, the analysis showed a significant effect ($p \leqslant 0.05$) of the hybrid factor in relation to crude fiber (Table 4). Fiber content in corn is an important parameter affecting both nutritional and industrial uses of the crop. Fiber content in corn depending on several factors, such as genetics, environmental conditions, and agricultural practices (Singh et al., 2000).

**Table 4.** Value of crude fiber of hybrid corn with and without the application of Cu nanoparticles.

| Hybrid | Crude fiber (%) | |
|---|---|---|
| | Without Cu nanoparticles | With Cu nanoparticles |
| HB 01: 22S18 TOP2® | 2.77 $^{aA}$ ±0.08 | 2.76 $^{aA}$ ±0.06 |
| HB 02: 20A30 VIPTERA3® | 2.81 $^{aA}$ ±0.06 | 2.82 $^{aA}$ ±0.05 |
| HB 03: 20A80 TOP2® | 2.78 $^{aA}$ ±0.08 | 2.81 $^{aA}$ ±0.05 |
| HB 04: 22S18 TOP3® | 2.73 $^{aA}$ ±0.07 | 2.71 $^{aA}$ ±0.05 |
| HB 05: 20A20 TOP2® | 2.82 $^{aA}$ ±0.08 | 2.83 $^{aA}$ ±0.04 |

Mean values ± standard error within a row with different lowercase are significantly different by Tukey's test, and within a line with different uppercase are significantly different by Student test, at the 5% level.

The highest moisture contents, with and without the application of Cu nanoparticles, were observed in the hybrids HB 02: 20A30 VIPTERA3® and HB 04: 22S18 TOP3®. The moisture results did not show a significant effect ($p > 0.05$) of the interaction of hybrids and Cu nanoparticles (Table 5). All samples met the moisture specifications as recommended by the current legislation, Normative Instruction (NI) No. 29, of June 8, 2011, which is 13%, in relation to the storage moisture of corn grains (Universidade Estadual de Campinas [UNICAMP], 2011).

**Table 5.** Value of moisture of hybrid corn with and without the application of Cu nanoparticles.

| Hybrid | Moisture (%) | |
|---|---|---|
| | Without Cu nanoparticles | With Cu nanoparticles |
| HB 01: 22S18 TOP2® | 10.15 $^{aA}$ ±0.63 | 10.89 $^{aA}$ ±0.29 |
| HB 02: 20A30 VIPTERA3® | 11.13 $^{aA}$ ±0.56 | 11.30 $^{aA}$ ±0.39 |
| HB 03: 20A80 TOP2® | 10.52 $^{aA}$ ±0.36 | 10.55 $^{aA}$ ±0.44 |
| HB 04: 22S18 TOP3® | 11.19 $^{aA}$ ±0.49 | 11.28 $^{aA}$ ±0.37 |
| HB 05: 20A20 TOP2® | 11.04 $^{aA}$ ±0.38 | 11.07 $^{aA}$ ±0.24 |

Mean values ± standard error within a row with different lowercase are significantly different by Tukey's test, and within a line with different uppercase are significantly different by Student test, at the 5% level.

The application of Cu nanoparticles in the seeds had no significant effect ($p \leqslant 0.05$) in relation to the phosphorus and calcium minerals. However, it showed a significant effect ($p \leqslant 0.05$) of the hybrid factor in relation to phosphorus, but not a significant effect ($p > 0.05$) for calcium (Table 6). The results show that the process of treating corn seeds with Cu nanoparticles is efficient. It was found that the Cu residues in the grains were far less than the safety limits recommended by Food and Agriculture Organization/World Health Organization [FAO/WHO] (2001), that is, 73.3 mg $kg^{-1}$.

**Table 6.** Value of minerals (phosphorus and calcium) in hybrid corn with and without the application of Cu nanoparticles.

| Hybrid | Phosphorus (%) | |
|---|---|---|
| | Without Cu nanoparticles | With Cu nanoparticles |
| HB 01: 22S18 TOP2® | 0.24 $^{aA}$ ±0.02 | 0.22 $^{bA}$ ± 0.02 |
| HB 02: 20A30 VIPTERA3® | 0.18 $^{bA}$ ±0.01 | 0.17 $^{cA}$ ± 0.01 |
| HB 03: 20A80 TOP2® | 0.23 $^{aA}$ ±0.01 | 0.24 $^{abA}$ ± 0.02 |
| HB 04: 22S18 TOP3® | 0.25 $^{aA}$ ±0.02 | 0.27 $^{aA}$ ± 0.03 |
| HB 05: 20A20 TOP2® | 0.23 $^{aA}$ ±0.01 | 0.22 $^{abA}$ ± 0.02 |
| Hybrid | Calcium (%) | |
| HB 01: 22S18 TOP2® | 0.08 $^{aA}$ ± 0.02 | 0.09 $^{aA}$ ± 0.01 |
| HB 02: 20A30 VIPTERA3® | 0.09 $^{aA}$ ± 0.01 | 0.09 $^{aA}$ ± 0.02 |
| HB 03: 20A80 TOP2® | 0.09 $^{aA}$ ± 0.02 | 0.09 $^{aA}$ ± 0.02 |
| HB 04: 22S18 TOP3® | 0.09 $^{aA}$ ± 0.03 | 0.08 $^{aA}$ ± 0.01 |
| HB 05: 20A20 TOP2® | 0.08 $^{aA}$ ± 0.02 | 0.09 $^{aA}$ ± 0.02 |

Mean values ± standard error within a row with different lowercase are significantly different by Tukey's test, and within a line with different uppercase are significantly different by Student test, at the 5% level.

The hybrids HB 02: 20A30 VIPTERA3® and HB 05: 20A20 TOP2® with Cu nanoparticles applied had a higher concentration of Cu mineral than those without (Table 7). Duarte et al. (2019) measured macro- and micronutrient contents in corn grains from different regions across both the first (summer) and second (fall) crops. They observed Cu values ranging from 0.13 to 0.28 mg 100 $g^{-1}$, which corroborates with the findings of the present study.

**Table 7.** Value of Cu mineral in hybrid corn with and without the application of Cu nanoparticles.

| Hybrid | Cu (mg 100 $g^{-1}$) | |
|---|---|---|
| | Without Cu nanoparticles | With Cu nanoparticles |
| HB 01: 22S18 TOP2® | 0.1113 $^{aB}$ ± 0.04 | 0.1195 $^{bA}$ ± 0.01 |
| HB 02: 20A30 VIPTERA3® | 0.0947 $^{aB}$ ± 0.02 | 0.1285 $^{abA}$ ± 0.01 |
| HB 03: 20A80 TOP2® | 0.1048 $^{aB}$ ± 0.01 | 0.1240 $^{bA}$ ± 0.01 |
| HB 04: 22S18 TOP3® | 0.1095 $^{aB}$ ± 0.01 | 0.1295 $^{abA}$ ± 0.01 |
| HB 05: 20A20 TOP2® | 0.0920 $^{aB}$ ± 0.02 | 0.1343 $^{aA}$ ± 0.01 |

Mean values ± standard error within a row with different lowercase are significantly different by Tukey's test, and within a line with different uppercase are significantly different by Student test, at the 5% level.

The results for fatty acids quantification from the hybrids showed linoleic acid was the most abundant (50.02 – 51.92%), followed by oleic, palmitic, and stearic acids (Table 8). The linolenic acid content increased significantly ($p < 0.05$) in the sample with Cu nanoparticles. The palmitic, stearic, and oleic acids showed similarity between the hybrids without a significant difference ($p > 0.05$). Studies reported similarity in the fatty acid composition of hybrid corns. In a study conducted in isogenic and genetically modified hybrid corns, the dominant fatty acid was linoleic acid, accounting for 45 – 50% of the composition (Jiménez et al., 2009). Likewise, three corn varieties have linolenic acid (49.7 - 62.7%), oleic acid (23.5 – 34.9%), and palmitic acid (9.5 - 11.5%) as the main fatty acids (Saoussem et al., 2009). The results obtained from the analysis of fatty acids in hybrid corn treated with Cu nanoparticles can provide insights into any potential changes or modifications in the fatty acid composition. These findings can contribute to a better understanding of the effects of Cu nanoparticles on the nutritional value and overall quality of corn kernels.

It is important to note that the investigation of nanoparticles in agricultural applications, including their potential effects on crops, is an active area of research. Strict adherence to relevant safety guidelines and regulations should be followed to ensure the responsible use and evaluation of nanoparticles in agriculture.

Table 9 shows the amino acid compositions in hybrids with and without the application of Cu nanoparticles. It can be seen that there is no significant ($p > 0.05$) difference in the amino acid compositions. The most abundant amino acids were lysine and tryptophan, whereas the content of methionine was the lowest. Essential amino acids play a crucial role in the nutritional composition of corn. These amino acids possess special physiological functions within the human body and are referred to as medicinal amino acids.

**Table 8.** Fatty acids content (%) in hybrid corn with and without the application of Cu nanoparticles.

| Hybrid | Palmitic acid | |
|---|---|---|
| | Without Cu nanoparticles | With Cu nanoparticles |
| HB 01: 22S18 TOP2® | 11.32 $^{aA}$ ± 0.15 | 11.47 $^{aA}$ ± 0.13 |
| HB 02: 20A30 VIPTERA3® | 11.47 $^{aA}$ ± 0.12 | 12.65 $^{aA}$ ± 0.11 |
| HB 03: 20A80 TOP2® | 11.48 $^{aA}$ ± 0.17 | 11.77 $^{aA}$ ± 0.14 |
| HB 04: 22S18 TOP3® | 11.35 $^{aA}$ ± 0.16 | 11.75 $^{aA}$ ± 0.17 |
| HB 05: 20A20 TOP2® | 11.30 $^{aA}$ ± 0.18 | 11.80 $^{aA}$ ± 0.21 |
| | Stearic acid | |
| HB 01: 22S18 TOP2® | 2.02 $^{aA}$ ± 0.07 | 2.05 $^{aA}$ ± 0.09 |
| HB 02: 20A30 VIPTERA3® | 2.17 $^{aA}$ ± 0.06 | 2.20 $^{aA}$ ± 0.05 |
| HB 03: 20A80 TOP2® | 2.07 $^{aA}$ ± 0.07 | 2.08 $^{aA}$ ± 0.08 |
| HB 04: 22S18 TOP3® | 2.05 $^{aA}$ ± 0.06 | 2.07 $^{aA}$ ± 0.07 |
| HB 05: 20A20 TOP2® | 2.09 $^{aA}$ ± 0.08 | 2.11 $^{aA}$ ± 0.07 |
| | Oleic acid | |
| HB 01: 22S18 TOP2® | 29.32 $^{aA}$ ± 0.13 | 30.47 $^{aA}$ ± 0.18 |
| HB 02: 20A30 VIPTERA3® | 30.20 $^{bA}$ ± 0.16 | 30.95 $^{aA}$ ± 0.17 |
| HB 03: 20A80 TOP2® | 30.48 $^{aA}$ ± 0.17 | 30.88 $^{aA}$ ± 0.14 |
| HB 04: 22S18 TOP3® | 30.35 $^{aA}$ ± 0.18 | 30.75 $^{aA}$ ± 0.18 |
| HB 05: 20A20 TOP2® | 30.11 $^{aA}$ ± 0.18 | 30.80 $^{aA}$ ± 0.19 |
| | Linolenic acid | |
| HB 01: 22S18 TOP2® | 50.02 $^{bB}$ ± 0.13 | 50.99 $^{bA}$ ± 0.16 |
| HB 02: 20A30 VIPTERA3® | 51.20 $^{aB}$ ± 0.12 | 51.92 $^{aA}$ ± 0.12 |
| HB 03: 20A80 TOP2® | 50.48 $^{bB}$ ± 0.14 | 50.93 $^{bA}$ ± 0.10 |
| HB 04: 22S18 TOP3® | 50.35 $^{bB}$ ± 0.15 | 50.96 $^{bA}$ ± 0.15 |
| HB 05: 20A20 TOP2® | 50.20 $^{bB}$ ± 0.10 | 50.93 $^{bA}$ ± 0.16 |

Mean values ± standard error within a row with different lowercase are significantly different by Tukey's test, and within a line with different uppercase are significantly different by Student test, at the 5% level.

**Table 9.** Amino acids composition (mg $g^{-1}$) in hybrid corn with and without the application of Cu nanoparticles.

| Hybrid | Lysine | |
|---|---|---|
| | Without Cu nanoparticles | With Cu nanoparticles |
| HB 01: 22S18 TOP2® | 1.08 $^{aA}$ ± 0.18 | 1.18 $^{aA}$ ± 0.13 |
| HB 02: 20A30 VIPTERA3® | 1.47 $^{aA}$ ± 0.12 | 1.55 $^{aA}$ ± 0.10 |
| HB 03: 20A80 TOP2® | 1.28 $^{aA}$ ± 0.11 | 1.37 $^{aA}$ ± 0.14 |
| HB 04: 22S18 TOP3® | 1.25 $^{aA}$ ± 0.09 | 1.35 $^{aA}$ ± 0.11 |
| HB 05: 20A20 TOP2® | 1.22 $^{aA}$ ± 0.13 | 1.30 $^{aA}$ ± 0.15 |
| | Methionine | |
| HB 01: 22S18 TOP2® | 0.62 $^{aA}$ ± 0.07 | 0.65 $^{aA}$ ± 0.09 |
| HB 02: 20A30 VIPTERA3® | 0.67 $^{aA}$ ± 0.06 | 0.75 $^{aA}$ ± 0.05 |
| HB 03: 20A80 TOP2® | 0.67 $^{aA}$ ± 0.07 | 0.68 $^{aA}$ ± 0.08 |
| HB 04: 22S18 TOP3® | 0.66 $^{aA}$ ± 0.09 | 0.70 $^{aA}$ ± 0.06 |
| HB 05: 20A20 TOP2® | 0.65 $^{aA}$ ± 0.08 | 0.69 $^{aA}$ ± 0.04 |
| | Tryptophan | |
| HB 01: 22S18 TOP2® | 1.24 $^{aA}$ ± 0.07 | 1.26 $^{aA}$ ± 0.08 |
| HB 02: 20A30 VIPTERA3® | 1.27 $^{bA}$ ± 0.06 | 1.34 $^{aA}$ ± 0.07 |
| HB 03: 20A80 TOP2® | 1.28 $^{aA}$ ± 0.07 | 1.30 $^{aA}$ ± 0.05 |
| HB 04: 22S18 TOP3® | 1.25 $^{aA}$ ± 0.05 | 1.29 $^{aA}$ ± 0.07 |
| HB 05: 20A20 TOP2® | 1.28 $^{aA}$ ± 0.05 | 1.30 $^{aA}$ ± 0.09 |

Mean values ± standard error within a row with different lowercase are significantly different by Tukey's test, and within a line with different uppercase are significantly different by Student test, at the 5% level.

Amino acids exhibit a diverse range of physiological activities and can be utilized for source identification purposes, making amino acid evaluation highly relevant for tracing the origin and ensuring the quality assessment of agricultural products (Li et al., 2022). Lysine synthesis in plants occurs via the lysine branch of the asparagine family pathway. Tryptophan has particular significance due to its involvement in several metabolic pathways and its impact on plant growth and development. So, the treatments with Cu nanoparticles did not interfere with the amount of this amino acid in the corn.

In morphological analysis, the presence of nanostructures on the surface of corn seeds was verified (Figure 1). Knowing the nutrient absorption of a culture allows one to directly infer the process of nutrient installment during the life cycle (Zhao et al., 2015). The quantities of these in the dry matter of each part of the plant and the absorption of nutrients that occurred in the different parts of the plant are combined. The nutrients are absorbed by the root or leaf, and the choice of the supply of nutrients is related to the real need for each plant.

In all images (Figure 1), spherical particles were observed on the surfaces and edges of the constituent cells in the pericarp. Larger clusters are formed on cell edges, and small clusters are formed on cell surfaces. The results show that Cu nanoparticles are adsorbed by the fibrous cells of the pericarp and form nanostructured agglomerates on the surface, preferentially anchored at the cell interfaces. Because they have nanometric dimensions, they form clusters that can serve as nanonutrient reserves for the seed during germination and the following stages.

It is notorious for the formation of agglomerates in the cavities of the cell interfaces and is composed of Cu nanostructures with dimensions smaller than those of the cavities. These characteristics favor the migration of Cu nanostructures inside the corn grain (pericarp) and transform the agglomerates into viable Cu reserves for the seed, with the availability of Cu throughout its development stages. Thus, it is possible to predict that during seed germination and the plant's growth and production phases, Cu nanoparticles with dimensions on the order of 1 to 4 nm will migrate from the agglomerates to the interior of the seed. In addition, Cu nanoparticles, serving as nanonutrient reserves, can contribute to the antimicrobial protection of the seed, especially with antifungal protection.

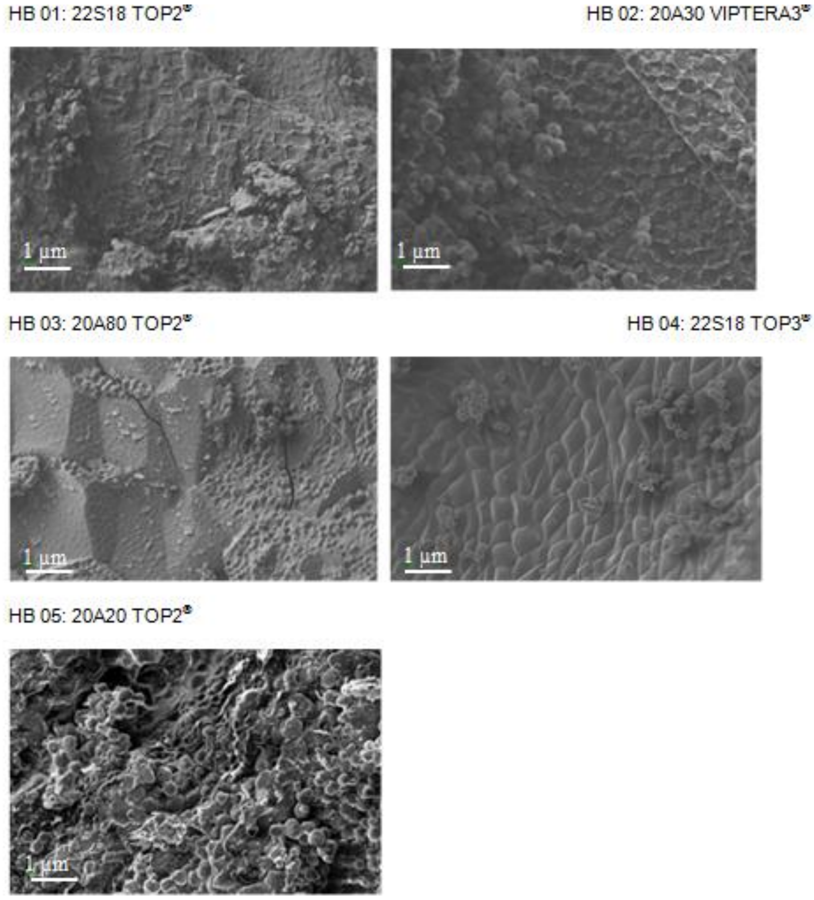


**Figure 1.** Scanning electron microscopy (SEM) images of hybrid corn with application of Cu nanoparticles.

Correlating mineral results with SEM images for seeds treated in reaction medium with 900 mg $L^{-1}$ Cu nanoparticles show that the inner cells of seeds maintain their structural integrity (Figure 1). This feature is a strong indication of the presence of Cu nanoparticles.

## Conclusion

The HB 02: 20A30 VIPTERA3® and HB 04: 22S18 TOP3® show better performances in physicochemical quality compared to other hybrids, an expressive result due to the morpho-physiology of the type of grain. Cu mineral content was higher in HB 02: 20A30 VIPTERA3®. The treatment with Cu nanoparticles does not affect the mineral contents of potassium, calcium, fatty acids, and amino acids. These findings suggest that corn seed treated with Cu nanoparticles is an effective tool for the delivery of Cu micronutrients to corn grains. Further research and optimization of this delivery system can provide valuable insights into the potential benefits of using Cu nanoparticles in agriculture, specifically for corn production. By enhancing nutrient availability and utilization, this approach supports sustainable farming practices and contributes to food security and nutrition.

## Acknowledgements

The authors would like to thank the National Council for Scientific and Technological Development (CNPq), Coordination for the Improvement of Higher Education Personnel - Brazil (CAPES) - Finance Code 001, Research Support Foundation of the State of Rio Grande do Sul (FAPERGS), and URI for the financial and structural support.

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