Copper-based-zinc-boron foliar fertilizer improved yield, quality, physiological characteristics, and microelement concentration of celery (Apium graveolens L.)

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ABSTRACT

Celery is one of the most widely cultivated vegetables worldwide. Boron (B), zinc (Zn), and copper (Cu) are essential microelements for plant growth with Cu also important in controlling fungal diseases. But little is known about the combined effects of B, Zn, and Cu fertilizers on celery. A pot experiment was conducted during two growing seasons (2014–2015 and 2015–2016) using a novel foliar fertilizer: a copper-based-zinc-boron foliar fertilizer (Cu-ZnB) based on the Bordeaux mixture (BDM). Celeries were sprayed with water (the control); BDMM; 1.0, 1.5 and 2.0 g L\(^{-1}\) copper foliar fertilizer (CFF1, CFF2, and CFF3), and with 1.0, 1.5 and 2.0 g L\(^{-1}\) the Cu-ZnB (Cu-ZnB1, Cu-ZnB2, and Cu-ZnB3). During both seasons, Cu-ZnB1 increased celery yield and decreased disease index, compared with control and BD M treatments. Application of Cu-ZnB1 also increased vitamin C content while reducing nitrate content of celery compared with BD M and increased leaf Zn and B concentration compared with the control, BD M, and CFF1 treatments. Furthermore, Leaf Cu concentration in Cu-ZnB1 treatment was lower by 76.9% compared with BD M. Spraying Cu-ZnB1 also significantly increased SOD, POD, and CAT activities, while decreasing MDA content of functional leaves. By applying Cu-ZnB during 2015–2016, soil available Cu concentration was reduced by 24.9–42.2%, compared with BD M. Hence, applying 1.0 g L\(^{-1}\) Cu-ZnB to celery is recommended for enhancing plant nutritional quality and yield; simultaneously, it reduces the levels of Cu in the soil and disease index of celery.

1. Introduction

Copper (Cu) plays an important role in the metabolism of plants and also has been frequently used as an active ingredient in agricultural fungicides [1–3]. However, long-term, repeated, and excessive application of copper fungicides has led to excessive accumulations of copper in the vegetation and soil, which may reach toxicity levels for plants and human [4]. The issue is to develop an improved application of a low dose of copper fungicides which could reduce Cu toxicity for plant and environment pollution [5].

Boron (B) is also an essential micronutrient in plants, which maintains the strength and shape of plant cells by enhancing the rigidity and the stability of cell walls [6], and B deficiencies can impair crop quality and reduce yields [7]. By affecting plant physiology and biochemistry, boron also influences the tolerance or resistance mechanisms of plants to pathogens [8]. Boron has been reported for improving yields of wheat, pepper, pomegranate, lemon, etc. [7,9–11]. Zinc (Zn) is another important micronutrient for biological systems and Zn deficiencies are common in both crops and humans [7,12]. Zinc could affect cell division, photosynthesis, the synthesis of tryptophan and proteins, and the activities of many enzymes [13].

Foliar application of microelements is a common practice because it is more efficient than soil application [14]. And foliar applications also could avoid toxicity symptoms while maximizing plant growth [7,14]. Researchers found that foliar application of Zn has nearly doubled the Zn concentration within the grains of wheat, especially in potentially zinc-deficient calcareous soils [12,15]. After spraying ZnSO\(_4\) solution, Zn ions were rapidly absorbed by leaves and transferred to the grain to improve plant growth and yield [16]. However, there has been little research on the effects of integrating copper fungicides with Zn and B foliar fertilizers.

Celery (Apium graveolens L.) ranks with one of the most important green vegetables which widely planted in the greenhouse. It is rich in carotenoids,
flavonoids, carbohydrate, and fibrin, which is beneficial to the cardiovascular system and digestive tract [17]. However, celery is subjected to high pesticide residues compared with other vegetables as disease control [18] and Cu stress has become one of the serious environmental concerns to limit the celery quality and productivity, due to overuse of copper-based fungicides. Thus, a novel foliar fertilizer copper-based-zinc-boron (Cu-ZnB) was developed by our research team to effectively control fungal diseases while providing microelements. It consists of copper hydroxide (Cu(OH)₂), zinc sulfate (ZnSO₄ · 7H₂O), borax (Na₈O₂ · 10H₂O) and additives for improving wetting, dispersal, and avoiding foaming. This novel combination of foliar fertilizer and fungicide, as a single-phase compound, may greatly benefit yield and control disease. The objective of the present study was to test the effectiveness of the copper-based-zinc-boron foliar fertilizer on the yield, quality, disease index, and nutrient uptakes of celery.

2. Material and methods

2.1. Experimental site and materials

The study was conducted during growing seasons of 2014–2015 and 2015–2016 for celery, Apium graveolens L. ‘Ventura’ (produced by Beijing Vegetable Seed Co., China). The celery was grown in pots placed in the intelligent greenhouse of Shandong Agricultural University, and the temperature and humidity were set at 10–25°C and 60–80%, respectively.

A typical soil was collected from a cultivated farmland and is classified as Typic Hapl-Udic Argosols. The soil texture was silt loam with 15.5% clay, 61.2% sand, and 23.3% silt measured using a micropipette method [19]. Basic soil properties were 7.28 pH (soil to water ratio 1:2.5), 11.4 g kg⁻¹ organic matter, 1.08 g kg⁻¹ total N, 30.7 mg kg⁻¹ available P, and 67.5 mg kg⁻¹ available K, and available Cu, Zn, Fe, and Mn were 2.13, 1.05, 12.87, and 5.62 mg kg⁻¹, respectively.

2.2. Experimental design and management

The completely randomized design was used for the experiment with four replicates and eight treatments: Control (distilled water), BDM (CuSO₄, CaO and H₂O in a 1:1:200 ratio, respectively), CFF1 (1.0 g L⁻¹ copper-based foliar fertilizer), CFF2 (1.5 g L⁻¹ of copper-based foliar fertilizer), CFF3 (2.0 g L⁻¹ copper-based foliar fertilizer), Cu-ZnB1 (1.0 g L⁻¹ copper-based-zinc-boron foliar fertilizer), Cu-ZnB2 (1.5 g L⁻¹ copper-based-zinc-boron foliar fertilizer), and Cu-ZnB3 (2.0 g L⁻¹ copper-based-zinc-boron foliar fertilizer). The copper-based foliar fertilizer (CFF, 39.1% Cu), copper-based-zinc-boron foliar fertilizer (Cu-ZnB, 39.1% Cu, 8.1% Zn, and 2.1% B) were novel foliar nutritive products produced at the National Engineering Laboratory of Shandong Agricultural University, Shandong, China.

All treatments received same amount of conventional granular fertilizers which were mixed into soil before planting and included urea (2.00 g pot⁻¹), calcium superphosphate for phosphorus (0.87 g pot⁻¹) and potassium chloride for potassium (1.52 g pot⁻¹). The ceramic pots were packed with 3 kg of sand on the bottom, then 12 kg of soil and placed randomly in the greenhouse. Two celery seedlings were transplanted into each pot. At 15, 45, 75 and 105 days after transplant (DAT), the treatments were applied using a small hand-held sprayer with 15, 30, 50, and 50 mL dose of solution, respectively, per pot [3]. Identical irrigation, insect and weed control were conducted using local agronomic practices.

2.3. Sampling and measurement

At 30, 60, 90 and 120 DAT, plant heights, stem diameters, and disease indices were recorded for each pot. The disease index of celery was determined using Ma’s method [20]. Disease index = Σ (relative number of disease leaves × different levels)/(total numbers of leaves × 5) × 100%.

At harvest, celery plants were removed from each pot and the roots were cut off at the surface of the soil, followed by washing with tap water, and rinsing with distilled water. The above-ground fresh weight was recorded for marketable yield.

Soluble solid concentration was determined using an automatic digital (RX-5000a, ATAGO Co., Ltd, Japan) [21]. Vitamin C content was measured with the titration method using ascorbic acid and 2,6-dichlorophenol indophenol sodium. Nitrate content was measured by using the salicylic acid method [22]. Measurements of contact angles of the foliar fertilizers on the celery leaves were also conducted (JC2000C2, Shanghai digital technology equipment Co., Ltd.).

For enzyme analyses, 500 mg frozen celery leaves per replication were crushed and then extracted using an extractant of 10 mL of 50 mM potassium phosphate buffer (pH 7.0), 1% polyvinyl pyrrolidone (PVP) and 1 mmol L⁻¹ EDTA-Na₂. The extracts were centrifuged at 12,000 × g for 20 min at 4°C, and the supernatant was used in enzyme assays [23]. Superoxide dismutase (SOD) activity was determined based on the ability of this supernatant to reduce nitroblue tetrazolium (NBT) using the riboflavin system under illumination. Peroxidase (POD) was measured within a 3.9 mL reaction mixture containing 50 mmol phosphate buffer (pH 7.0), 19 μL H₂O₂, 28 μL guaiacol, and 100 μL of enzyme extract. Absorbance was recorded at least 2 min per 30 s, at 420 nm [24]. Catalase (CAT)
activity was measured by monitoring the consumption of hydrogen peroxide at 240 nm for at least 3 min [25]. For malondialdehyde (MDA) determination, first, 3 mL of 2-thiobarbituric acid in 10% trichloroacetic acid was added to a 1 mL aliquot of the supernatant; then, the mixture was placed in a water bath and heated for 15 min at 98°C, then cooled, followed by centrifuging for 20 min at 4000 × g. Absorbance was measured at 600, 532, and 450 nm [26].

For analyses of dry weight and microelement, plant samples were placed in an oven at 105°C for 30 min and then at 80°C for about 2–4 d until constant weights were obtained. The dry samples were then ground to pass through a 2 mm sieve, digested using a microwave device (Multiwave 3000, Antonpaar, Austria) and analyzed for Cu, Zn, Fe, and Mn using an atomic absorption spectrophotometer (AAS, AA7000, Shimadzu, Japan) [27]. B content was determined by ashing the plant samples at 500°C for 4–5 h, then analyzing using the curcumin method [22].

Soil samples were collected from 0–15 cm depth at a distance of 5 cm from the trunk of a plant at harvest day, then air-dried, ground and sieved to a distance of 5 cm from the trunk of a plant at 500°C for 4–5 h, then analyzing using the curcumin method.

### 2.4. Statistical analyses

Microsoft Excel 2010 and SigmaPlot 12.5 were used to process data and produce figures. All data were subjected to one-way analyses of variance (ANOVA) followed by mean comparisons using the Duncan multiple range test ($P < 0.05$) in Statistical Analysis System version 9.2 (2010, SAS Institute, Cary, NC).

### 3. Results

#### 3.1. Effects of foliar fertilizer on celery yield, disease index, and growth

Compared with the control treatment during two growing seasons, celery yields were significantly enhanced through the application of copper foliar fertilizers and copper-based-zinc-boron foliar fertilizers at all three dilutions (Table 1). The yield of BDM showed no significant difference with that of control, and yields from CFF1, Cu-ZnB1, Cu-ZnB2, and Cu-ZnB3 treatments were even more highly significant compared with BDM. The yield in Cu-ZnB1 treatment was significantly higher than CFF1 treatment. The yield of Cu-ZnB1 treatment was also significantly higher than Cu-ZnB2 and Cu-ZnB3 treatments. There were no significant differences in celery yields among CFF1, CFF2, CFF3, Cu-ZnB2 and Cu-ZnB3. Copper-based-zinc-boron foliar fertilizers at 1.0, 1.5, and 2.0 g L$^{-1}$ resulted in significant improvements in celery dry weight compared with the control (Table 1). Cu-ZnB2 treatment significantly increased dry weight compared with CFF2 treatment.

At 30 DAT, except for CFF2 treatment, there were no significant differences among other treatments in disease index during two growing seasons (Table 2). However, at 90 and 120 DAT, disease index for the control was significantly higher than for all the other treatments, while the CFF1 and Cu-ZnB1 treatments were each significantly lower than the remaining treatments. But no significant differences occurred in disease index between the copper-based foliar fertilizers and copper-based-zinc-boron foliar fertilizers at the same dilution on any date. At 120 DAT, there were also no statistical differences in disease index among BDM, CFF3, and Cu-ZnB3 treatments.

The foliar fertilizer application rates, celery yield and disease index at 120 DAT were analyzed basing on multiple regression and the dependent and independent variables were related by second-order polynomial equations (Figure 1). The goodness-of-fit for the regression equations were indicated by the adjusted coefficients of determination ($R^2$) and the $R^2$ values indicated high correlations between the foliar fertilizer application rate and celery yield. When the CFF and Cu-ZnB treatments resulted in the highest yields for celery (1017.0 and 1095.1 g pot$^{-1}$), application concentrations for CFF and Cu-ZnB were 1.34 and 1.24 g L$^{-1}$, respectively. And the yield in Cu-ZnB treatment is higher than CFF treatment at same application concentration from 0 to 2 g L$^{-1}$.

The application concentrations for CFF and Cu-ZnB were 1.30 and 1.31 g L$^{-1}$, which results in the lowest disease index at 120 DAT for celery (10.9% and 11.1%).

### Table 1. Celery yield and dry weight. Data shown are means of the two growing seasons.

| Treatment          | Yield (g pot$^{-1}$) | Increment vs. Control (%) | Increment vs. BDM (%) | Dry weight (g pot$^{-1}$) |
|--------------------|----------------------|----------------------------|------------------------|--------------------------|
| Control            | 912.2 ± 42.6d        | 0.00                       | -4.90                  | 77.5 ± 3.1c              |
| BDM                | 959.2 ± 44.1c        | 5.15                       | 0.00                   | 86.0 ± 5.1ab             |
| CFF1               | 1031.7 ± 49.3b       | 13.10                      | 7.56                   | 91.6 ± 9.2ab             |
| CFF2               | 987.3 ± 87.5bc       | 8.24                       | 2.93                   | 87.6 ± 7.0bc             |
| CFF3               | 1002.5 ± 48.9c       | 9.90                       | 4.52                   | 87.1 ± 5.1bc             |
| Cu-ZnB1            | 1188.3 ± 85.5a       | 22.60                      | 16.59                  | 98.0 ± 7.7a              |
| Cu-ZnB2            | 1047.5 ± 57.9b       | 14.84                      | 9.21                   | 96.0 ± 9.2a              |
| Cu-ZnB3            | 1044.2 ± 66.1b       | 14.47                      | 8.86                   | 90.7 ± 8.4b              |
| Significance season| NS                   |                            |                        |                          |

Note: Data are mean ± standard deviations (SD) ($n = 4$). Means with each column followed by the same letter were not significantly different based on a one-way ANOVAs followed with Duncan’s multiple range tests ($P > 0.05$). *, **, and NS are significant at $p ≤ 0.05$, at $p ≤ 0.01$, and not significant, respectively. Control (pure water); BDM (Bordeaux mixture); CFF1, CFF2, and CFF3 (copper-based foliar fertilizer at 1.0, 1.5, and 2.0 g L$^{-1}$, respectively); Cu-ZnB1, Cu-ZnB2, and Cu-ZnB3 (copper-based-zinc-boron foliar fertilizer at 1.0, 1.5, and 2.0 g L$^{-1}$, respectively).
Plant heights and stem diameters of celery plants increased over time during 2015–2016 (Table 3). At 30 DAT, there were no significant differences in plant height or stem diameter among the treatments. However, at 120 DAT for plant height and stem diameter, the control was significantly lower and Cu-ZnB1 treatment was significantly higher than control and BDM treatment. At 120 DAT, plant heights in Cu-ZnB1 treatment had increased 11.2%, 6.0%, and 10.0% compared with BDM, CFF2, Cu-ZnB2, and Cu-ZnB3 (1.0, 1.5, and 2.0 g L\(^{-1}\) copper-based-zinc-boron foliar fertilizer, respectively); DAT (Days after transplant).

### Table 3. Celery plant height and stem diameter in 2015–2016.

| Treatment  | 30 DAT       | 60 DAT       | 90 DAT       | 120 DAT      |
|------------|--------------|--------------|--------------|--------------|
|            | Plant height (cm) | Stem diameter (cm) | Plant height (cm) | Stem diameter (cm) |
| Control    | 14.05 ± 1.13a | 21.06 ± 2.32b | 35.82 ± 1.42c | 55.53 ± 3.70d |
| BDM        | 14.18 ± 1.30a | 24.41 ± 1.97a | 39.57 ± 1.64a | 59.93 ± 2.88c |
| CFF1       | 14.83 ± 1.16a | 25.56 ± 1.33a | 41.96 ± 1.29ab| 63.65 ± 1.72ab|
| CFF2       | 14.63 ± 1.08a | 24.66 ± 1.56a | 39.93 ± 1.88b | 62.85 ± 1.61bc|
| CFF3       | 14.57 ± 1.07a | 25.11 ± 1.23a | 39.73 ± 1.33b | 60.56 ± 1.52bc|
| Cu-ZnB1    | 15.39 ± 1.34a | 25.83 ± 1.70a | 43.10 ± 1.47ab| 66.63 ± 1.03a |
| Cu-ZnB2    | 14.73 ± 0.95a | 25.33 ± 1.41a | 40.80 ± 1.00b | 63.62 ± 1.44ab|
| Cu-ZnB3    | 14.52 ± 1.24a | 25.58 ± 1.16a | 39.80 ± 1.59b | 62.15 ± 1.49bc|

Note: Date are mean ± standard deviations (SD) (n = 4). Means with each column followed by the same letter were not significantly different based on a one-way ANOVA followed by Duncan’s multiple range tests (P > 0.05). Control (pure water); BDM (Bordeaux mixture); CFF1, CFF2, and CFF3 (1.0, 1.5, and 2.0 g L\(^{-1}\) copper-based foliar fertilizer, respectively); Cu-ZnB1, Cu-ZnB2, and Cu-ZnB3 (1.0, 1.5, and 2.0 g L\(^{-1}\) copper-based-zinc-boron foliar fertilizer, respectively); DAT (Days after transplant).

![Figure 1. Copper foliar fertilizer concentration and celery yield and disease index during two growing seasons (n = 32).](image-url)
and CFF3, respectively, while stem diameters increased to 5.9–23.9%, higher than other treatments, respectively.

3.2. Effects of foliar fertilizers on nutritional quality of celery

There were significant differences in soluble solids among fertilizers during 2014–2015 and 2015–2016 (Figure 2(a)). Soluble solid content in 2014–2015, Cu-ZnB1 was significantly higher than the BDM or any dilution of the copper foliar fertilizer, while CFF1 and Cu-ZnB1 treatments were higher than the control treatment. In 2015–2016, soluble solids in Cu-ZnB2 and Cu-ZnB3 treatments were each significantly higher than from control and BDM treatments.

Vitamin C contents of celery provided with Cu-ZnB1 and Cu-ZnB2 treatments were each significantly greater than from all the other treatments except Cu-ZnB3 during both 2014–2015 and 2015–2016 seasons (Figure 2(b)). Cu-ZnB1 increased vitamin C contents by 18.5–21.3% and 9.8–17.1% compared with BDM and CFF1 treatment, respectively, during both seasons. But in either growing season, no significant differences occurred in vitamin C contents between BDM and any dilution of copper foliar fertilizer without zinc and boron.

During both 2014–2015 and 2015–2016, celery nitrate content resulting from the application of the BDM was significantly higher than from Cu-ZnB3 treatment, which was significantly higher than Cu-ZnB1 treatment (Figure 2(c)). Nitrate content in CFF3

Figure 2. Content of soluble compounds from celery leaves in each growing season including solid solids (a), vitamin C (b), and nitrate (c).

Note: Date are mean ± standard deviations (SD) (n = 4). Means within each graph for a soluble compound and season followed by the same letter were not significantly different based on a one-way ANOVAs followed with Duncan’s multiple range tests (P > 0.05). Control (pure water); BDM (Bordeaux mixture); CFF1, CFF2, and CFF3 (1.0, 1.5, and 2.0 g L⁻¹ copper-based foliar fertilizer, respectively); Cu-ZnB1, Cu-ZnB2, and Cu-ZnB3 (1.0, 1.5, and 2.0 g L⁻¹ copper-based-zinc-boron foliar fertilizer, respectively).
treatment was significantly higher than CFF2 and CFF1 treatments, which were higher than the control in 2015–2016. Compared with BDM treatment, CFF1 and Cu-ZnB1 decreased nitrate contents by 37.9–58.8% and 29.4–54.1%, respectively.

### 3.3. Effects of foliar fertilizers on antioxidant enzyme activities and MDA content

During 2015–2016, SOD activity in functional celery leaves treated with Cu-ZnB1 was significantly higher than BDM, CFF1, Cu-ZnB2, and Cu-ZnB3 treatments and all treatments were significantly higher than the control treatment (Table 4). POD activities produced results that were similar to SOD activities: celery leaves treated with Cu-ZnB1 and Cu-ZnB2 were significantly higher than CFF3 and BDM treatments; in turn, all these treatments were significantly higher than the control. For CAT activity, celery leaves treated with the control treatment and with all three dilutions of Cu-ZnB were each significantly higher than CFF3 treatment; in turn, all these treatments were significantly higher than the BDM. There were no significant differences in SOD, POD, and CAT activities among CFF1, CFF2, and CFF3 treatments. BDM, CFF3, and Cu-ZnB3 treatments significantly increased MDA content of celery leaves compared with control, CFF1, Cu-ZnB1, and Cu-ZnB2 treatments. POD activity exhibited trends that were similar among treatments to SOD activity. However, activity of CAT was reduced by applying BDM compared with the control and other treatments.

### 3.4. Contact angles between droplets of foliar fertilizers and celery leaves

The contact angles between droplets of CFF1 and Cu-ZnB1 and celery leaves were 66.5° and 66.8°, which were decreased compared the contact angles of droplets of control and BDM on celery leaves (Figure 3). The results showed that the spraying solutions of CFF1 and Cu-ZnB1 could adhere on the celery leaves more lastly, avoiding dripping from the leaves, compared with those of BDM.

### 3.5. Effects of foliar fertilizers on stem and leaf microelement concentrations

During both 2014–2015 and 2015–2016, Cu concentration in celery stem and leaf resulting from the BDM

| Treatment   | SOD activity (U g⁻¹, FW) | POD activity (U min⁻¹ g⁻¹, FW) | CAT activity (U min⁻¹ g⁻¹, FW) | MDA content (nmol g⁻¹, FW) |
|-------------|------------------------|-------------------------------|-------------------------------|-----------------------------|
| Control     | 237.58 ± 5.16d         | 17.29 ± 0.69d                 | 13.31 ± 0.20ab                | 17.76 ± 1.14c               |
| BDM         | 268.36 ± 7.93c         | 19.57 ± 1.73c                 | 12.36 ± 1.10bc                | 22.32 ± 1.50a               |
| CFF1        | 275.50 ± 10.22bc       | 20.63 ± 0.66bc                | 12.26 ± 1.92bc                | 18.42 ± 1.18c               |
| CFF2        | 269.72 ± 2.99bc        | 20.35 ± 1.47bc                | 11.16 ± 0.43                  | 19.67 ± 1.61bc              |
| CFF3        | 264.62 ± 7.33c         | 20.23 ± 1.29c                 | 13.90 ± 1.21a                 | 21.73 ± 0.60ab              |
| Cu-ZnB1     | 298.03 ± 5.61a         | 22.45 ± 0.70ab                | 13.86 ± 0.61a                 | 17.45 ± 0.78c               |
| Cu-ZnB2     | 279.71 ± 7.50b         | 23.79 ± 1.32a                 | 13.86 ± 0.61a                 | 18.26 ± 1.36c               |
| Cu-ZnB3     | 269.09 ± 6.57bc        | 20.57 ± 1.77bc                | 13.27 ± 0.34ab                | 21.22 ± 2.63ab              |

Note: Date are mean ± standard deviations (SD) (n = 4). Means with each column followed by the same letter were not significantly different based on a one-way ANOVAs followed with Duncan’s multiple range tests (P > 0.05). Control (pure water); BDM (Bordeaux mixture); CFF1, CFF2, and CFF3 (1.0, 1.5, and 2.0 g L⁻¹ copper-based foliar fertilizer, respectively); Cu-ZnB1, Cu-ZnB2, and Cu-ZnB3 (1.0, 1.5, and 2.0 g L⁻¹ copper-based-zinc-boron foliar fertilizer, respectively); FW (fresh weight); SOD (superoxide dismutase); POD (peroxidase); CAT (catalase); MDA (malondialdehyde).

**Figure 3.** Contact angles between droplets of different applied foliar fertilizer treatments and celery leaves: a. control (pure water); b. BDM (Bordeaux mixture); c. CFF1 (1.0 g L⁻¹ copper-based foliar fertilizer); d. Cu-ZnB1 (1.0 g L⁻¹ copper-based-zinc-boron foliar fertilizer).
treatment was significantly higher than from CFF3 and Cu-ZnB3 treatments, which were significantly higher than the CFF2 and Cu-ZnB2 treatments, which in turn were significantly higher than CFF1 and Cu-ZnB1 treatments (Table 5). There were no significant differences between CFF and Cu-ZnB treatments at the same application concentration. The control treatment was always numerically and statistically lower than all other treatments during both seasons for celery leaves and stems.

Zn concentrations in celery stem and leaf resulting from copper-based-zinc-boron fertilizers were consistently and significantly higher than copper foliar fertilizers without zinc and boron, BDM, or the control treatment (Table 5). Similarly, B concentrations within stem and leaf of copper-based-zinc-boron fertilizers were consistently and significantly higher than the copper foliar fertilizers without zinc or boron, BDM, and the control treatment. In addition, there were highly significant correlations between the rate of copper-based-zinc-boron foliar fertilizer and the B and Zn concentration of celery stem and leaf.

There were no significant differences observed in Fe or Mn concentrations within the stem or leaf of celery at harvest stage.

Through the correlation analysis between microelement in celery leaf and disease index, quality, and yield during both seasons, it can be seen that the Zn and B concentrations were mainly positive correlation with celery yield, soluble solid and vitamin C, but disease index was significant negative correlation with yield in either year (Table 6). In addition, Cu concentration was a positive correlation with nitrate content.

### 3.6. Effects of foliar fertilizers on soil microelement concentration

There were no significant differences in soil pH among the treatments during 2014–2015 (Table 7). But in 2015–2016, soil pH in CFF2 treatment was significantly higher than in the Cu-ZnB2, and no significant differences were found in soil pH among the other treatments.

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**Table 5. Microelement concentrations of stems and leaves.** Data shown are means of the two growing seasons.

| Treatment | Cu (mg kg⁻¹) | Zn (mg kg⁻¹) | Fe (mg kg⁻¹) | Mn (mg kg⁻¹) | B (mg kg⁻¹) |
|-----------|--------------|--------------|--------------|---------------|--------------|
| Control   | 16.32 ± 1.91e| 35.93 ± 6.15d| 116.41 ± 9.75a| 57.90 ± 5.42a| 19.46 ± 1.21cd|
| BDM       | 76.29 ± 7.87a| 35.20 ± 5.14d| 115.07 ± 7.74a| 59.99 ± 9.07a| 19.71 ± 1.10cd|
| CFF1      | 23.44 ± 3.54d| 34.95 ± 6.71d| 112.21 ± 7.44a| 59.53 ± 5.79a| 20.18 ± 1.28bc|
| CFF2      | 35.25 ± 6.96c| 37.38 ± 7.95d| 110.98 ± 7.41a| 55.80 ± 4.52a| 19.46 ± 0.86cd|
| CFF3      | 48.05 ± 9.60b| 37.74 ± 8.41d| 114.28 ± 12.41a| 60.46 ± 12.37a| 18.83 ± 1.27cd|
| Cu-ZnB1   | 23.52 ± 4.06d| 45.07 ± 5.14c| 115.84 ± 7.14a| 60.21 ± 4.79a| 21.48 ± 1.10bc|
| Cu-ZnB2   | 33.84 ± 4.60c| 45.07 ± 5.14c| 115.84 ± 7.14a| 60.21 ± 4.79a| 21.48 ± 1.10bc|
| Cu-ZnB3   | 47.31 ± 7.47b| 59.19 ± 9.00a| 113.69 ± 8.59a| 59.05 ± 10.02a| 22.62 ± 0.92ba|
| Significant season | ** | ** | ** | NS | NS |

Note: Date are mean ± standard deviations (SD) (n = 4). Means with each column and season followed by the same letter were not significantly different based on a one-way ANOVAs followed with Duncan’s multiple range tests (P < 0.05). *, **, and NS are significant at p ≤ 0.05, at p ≤ 0.01, and not significant, respectively, Control (pure water); BDM (Bordeaux mixture); CFF1, CFF2, and CFF3 (1.0, 1.5, and 2.0 g L⁻¹ copper-based foliar fertilizer, respectively); Cu-ZnB1, Cu-ZnB2, and Cu-ZnB3 (1.0, 1.5, and 2.0 g L⁻¹ copper-based-zinc-boron foliar fertilizer, respectively).

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**Table 6. Correlation analyses of leaf microelement concentrations, disease index, quality, and yield for celery at harvest (n = 80).** Data shown are means of the two growing seasons.

| L. Cu | L. Zn | L. Mn | L. B | Sol. solid | Nitrates | Vit. C | Dis. index |
|-------|-------|-------|------|------------|----------|-------|-----------|
| L. Fe | 0.018 | -     | -    | -          | -        | -     | -         |
| L. Zn | −0.003| 0.130 | -    | -          | -        | -     | -         |
| L. Mn | 0.121 | 0.120 | 0.223| -          | -        | -     | -         |
| L. B  | −0.118| 0.125 | 0.824**| 0.116 | -        | -     | -         |
| Sol. solid | 0.022 | 0.306* | 0.469** | 0.321** | 0.358** | -     | -         |
| Nitrates | 0.723** | −0.236 | 0.036 | −0.066 | −0.012 | −0.246 | -         |
| Vit. C | −0.194| 0.141**| 0.034 | 0.508** | 0.301* | −0.112 | -         |
| Dis. index | −0.144| 0.015 | −0.125| 0.071 | −0.187 | −0.228 | −0.273* | −0.436** |
| Yield | −0.129| 0.129 | 0.329**| 0.015 | 0.378** | 0.311* | −0.115 | 0.479** | −0.508** |

Note: * and ** are significant at p ≤ 0.05 and at p ≤ 0.01, respectively. Abbreviations: L. (Leaf total), Sol. (Soluble), Vit. (Vitamin), Dis. (Disease).
During 2015–2016 season, soil available Cu concentration resulting from BDM was significantly higher than from all the other treatments including CFF3 and Cu-ZnB3, which were significantly higher than the control and Cu-ZnB1 treatments (Table 7). Available soil Cu contents in BDM treatment were 51.8% and 95.8% higher than from the control during 2014–2015 and 2015–2016, respectively. The soil Cu concentration in 2015–2016 season forms all treatments except CFF1 and Cu-ZnB1 was significantly increased than the control and Cu concentration was significantly different between the two seasons. During both seasons, there was no significant difference comparing CFF and Cu-ZnB in same spray dilutions.

During both 2014–2015 and 2015–2016, available Zn concentration resulting from Cu-ZnB3 was significantly higher than from all other treatments including Cu-ZnB2, which was significantly higher than CFF1 and CFF2 treatments (Table 7). In 2015–2016, available B content in Cu-ZnB3 treatment was significantly higher than all the other treatments except the Cu-ZnB1 and Cu-ZnB2 treatments. There were no significant differences among treatments in available soil Fe or Mn concentration.

### 4. Discussion

#### 4.1. Effects of copper-based-zinc-boron foliar fertilizer on celery disease indices and yields

Foliar applications of Zn and B fertilizers have been found to improve quality and yield of pomegranate fruits [7]. Similarly, B fertilizers have increased fruit yields in peaches, pears, apples, and persimmons [29–32]. Increased yields also occurred after spraying Zn fertilizer in apple, pistachio, and orange groves [33,34]. In the present study, a comparison among eight treatments during both growing seasons showed that Cu-ZnB1 increased celery yield, compared with the control, BDM, CFF2 and CFF3 treatments. Boron plays an important role in plant growth enhancing the rigidity and stability of cell walls and thus maintaining the strength and shape of plant cells [8]. Zn is also considered essential in plants because of its importance in the activity of many enzymes, and it helps with cell division, photosynthesis, and the synthesis of tryptophan and proteins [13]. Increased celery yields in the present study appear to have resulted from adding Zn and B to the copper fertilizers.

A reduction in the disease index, or estimate of infection level, also may help to the increased celery yield because the reduction of damage from diseases often leads to greater availability of water and nutrients, which help with plant growth and yield. Hence, the present study showed that foliar application of copper fertilizers can effectively reduce the severity of diseases in celery. Both the CFF1 and Cu-ZnB1 treatment during each growing season at 120 DAT reduced the disease index by 45.4–78.7%, compared with BDM and control treatments. Copper fungicides have been found to lessen the spore germination rate of pathogenic bacteria and the growth of fungal hyphae [35]. When copper fungicides were sprayed on the leaves, Cu ions were released and destroyed cell protoplasm, thus killing the pathogenic bacteria. Cu has a role in many cellular processes and is an essential ligand for the activities of certain enzymes, such as cytochrome oxidase, superoxide dismutase, dopamine b-hydroxylase, which are important in cell differentiation and growth [36]. Because they are

### Table 7. Soil pH and available microelement concentrations in 2014–2015 and 2015–2016.

| Treatment  | pH     | Cu (mg kg⁻¹) | Zn (mg kg⁻¹) | Fe (mg kg⁻¹) | Mn (mg kg⁻¹) | B (mg kg⁻¹) |
|------------|--------|--------------|--------------|--------------|--------------|-------------|
| **2014–2015** |
| Control    | 7.92 ± 0.02a | 2.18 ± 0.19d | 1.07 ± 0.08bc | 12.49 ± 0.74a | 5.48 ± 0.58a | 0.61 ± 0.04a |
| BDM        | 7.93 ± 0.12a | 3.31 ± 0.61a | 1.13 ± 0.11bc | 13.34 ± 0.82a | 5.39 ± 0.74a | 0.59 ± 0.04a |
| CFF1       | 7.85 ± 0.08a | 2.29 ± 0.45d | 1.00 ± 0.08bc | 12.46 ± 1.30a | 5.71 ± 0.66a | 0.58 ± 0.05a |
| CFF2       | 7.95 ± 0.10a | 2.70 ± 0.22bc | 1.01 ± 0.07c  | 12.89 ± 1.00a | 5.86 ± 0.60a | 0.61 ± 0.05a |
| CFF3       | 7.97 ± 0.06a | 2.97 ± 0.17ab | 1.10 ± 0.09bc | 12.52 ± 1.40a | 5.90 ± 0.78a | 0.62 ± 0.05a |
| Cu-ZnB1    | 7.92 ± 0.07a | 2.54 ± 0.11bc | 1.16 ± 0.06bc | 12.37 ± 0.57a | 5.89 ± 0.63a | 0.62 ± 0.05a |
| Cu-ZnB2    | 7.93 ± 0.08a | 2.77 ± 0.13abc | 1.22 ± 0.07b  | 13.56 ± 0.62a | 6.09 ± 0.67a | 0.62 ± 0.04a |
| Cu-ZnB3    | 7.98 ± 0.08a | 2.88 ± 0.16abc | 1.50 ± 0.20a  | 13.37 ± 1.16a | 6.11 ± 1.13a | 0.63 ± 0.04a |
| **2015–2016** |
| Control    | 8.02 ± 0.02a | 2.18 ± 0.19d | 1.07 ± 0.08bc | 12.49 ± 0.74a | 5.48 ± 0.58a | 0.61 ± 0.04a |
| BDM        | 8.10 ± 0.15ab | 4.17 ± 0.38c  | 0.95 ± 0.05c  | 12.12 ± 1.05a | 5.39 ± 0.61a | 0.59 ± 0.03b |
| CFF1       | 7.96 ± 0.08ab | 2.53 ± 0.52bcd | 0.99 ± 0.09c  | 11.26 ± 0.62a | 5.69 ± 0.48a | 0.58 ± 0.04b |
| CFF2       | 8.09 ± 0.10a | 2.98 ± 0.17bc | 0.93 ± 0.10c  | 12.18 ± 1.09a | 5.34 ± 0.33a | 0.61 ± 0.03b |
| CFF3       | 8.12 ± 0.03ab | 3.21 ± 0.26b  | 0.91 ± 0.07c  | 11.62 ± 0.92a | 5.22 ± 0.39a | 0.59 ± 0.04b |
| Cu-ZnB1    | 8.03 ± 0.13ab | 2.41 ± 0.39cd | 1.33 ± 0.07b  | 12.06 ± 1.78a | 5.51 ± 0.46a | 0.64 ± 0.02ab |
| Cu-ZnB2    | 7.92 ± 0.13ab | 2.90 ± 0.52bc | 1.42 ± 0.10b  | 12.25 ± 1.42a | 5.81 ± 0.52a | 0.64 ± 0.03ab |
| Cu-ZnB3    | 8.11 ± 0.10ab | 3.13 ± 0.65b  | 1.67 ± 0.08a  | 12.12 ± 1.07a | 5.83 ± 0.47a | 0.67 ± 0.04a |

Note: Date mean ± standard deviations (SD) (n = 4). Means with the same column and season followed by the same letter are not significantly different based on one-way ANOVAs followed with Duncan’s multiple range tests (P > 0.05). **, **, and NS are significant at P ≤ 0.05, P ≤ 0.01, and not significant, respectively. Control (pure water); BDM (Bordeaux mixture); CFF1, CFF2, and CFF3 (1.0, 1.5, and 2.0 g L⁻¹ copper-based foliar fertilizer, respectively); Cu-ZnB1, Cu-ZnB2, and Cu-ZnB3 (1.0, 1.5, and 2.0 g L⁻¹ copper-based-zinc-boron foliar fertilizer, respectively).
micronutrients affecting plant physiology and biochemistry, B and Zn may also influence the tolerance or resistance mechanisms within plants to pathogens [8]. Besides the Zn and B, the Cu(OH)₂ of Cu-ZnB formula has smaller particle size and larger specific surface area under the grinding of grinding machine. So CFF1 and Cu-ZnB1 had better effects of antimicrobial than BDM. In the present study, a reduction in disease index apparently helped to increase celery yield; however, there was no significant difference in disease index between copper foliar fertilizers and copper-based-zinc-boron foliar fertilizer at the same dilution.

4.2. Effects of copper-based-zinc-boron foliar fertilizers on the levels of micronutrients and quality within celery plants

Although Cu is an essential micronutrient for most living organisms, excess Cu or its deficiency can produce adverse effects on crops. It is therefore important to determine the content and bioavailability of Cu to permit an estimation of adverse environmental effects [1]. In the present study, celery Cu concentration was increased following application of copper foliar fertilizers, unlike with the control treatment. Simultaneously, application of CFF and Cu-ZnB helped to reduce the accumulation of metallic Cu in celery, while had the similar effects of control disease, when compared with BDM. A major disadvantage of using BDM is the resulting wide fluctuation in Cu ions of spray solution, which can range from high concentrations immediately after application leading to a risk of phytotoxicity, to low concentrations later causing an inadequate supply for plants [37]. The high Cu concentration of celery in BDM treatment significantly decreased the CAT activity and increased MDA content of leaves, which was bad for celery growth (Table 4).

Application of B has been shown to increase leaf B concentration in pepper, sweet orange, lemon, and pomegranate trees [7,9,11,38]. Similarly, Zn applications have increased leaf concentrations of Zn in wheat, and in pomegranate and ocean nut trees [7,13,39,40]. We found that spraying copper-zinc-boron fertilizers significantly increased Zn and B concentrations of celery leaves when compared with control, BDM, and the copper-based foliar fertilizers. Similar results were found by Cakmak [41] and Yadav [32]. However, among the various dilutions of applied Cu-ZnB, the final leaf concentration of B did not differ between Cu-ZnB2 and Cu-ZnB3 treatments. This may have been the result of variation in the absorptive capacity of celery leaf for different concentrations of different nutrient elements. The finding of the present study suggested that application of 15 – 50 mL Cu-ZnB1 per pot could be absorbed readily into celery leaf with no harmful effects. Compared with the other treatment options, this led to higher Zn and B concentrations within celery stems and leaves without excessive Cu accumulation.

Vegetable quality mainly refers to the chemical composition of the portions consumed, including soluble solids, acids, vitamin C, nitrate, and other aromatic compounds [42]. This present study used the contents of soluble solids, vitamin C, and nitrate as celery nutritional quality indices. Spraying B or Zn nano-fertilizer on pomegranate has been shown to improve fruit quality by increasing the levels of soluble solid and titratable acid in addition to the maturity index and juice pH [7]. Denre et al. [11] performed similar research on pepper plants, which could improve pepper quality, and the result was consistent with our study. In the present study, applying Cu-ZnB1 increased the soluble solid and vitamin C content compared with the control, BDM, or copper-based foliar fertilizers. Zn plays an important role in the activities of enzymes involved in biochemical reactions associated with nucleic acids and starches [13], and B is important in carbohydrate metabolism and transport of sugars. For Cu-ZnB2 and Cu-ZnB3, compared with the lowest concentration (Cu-ZnB1), the contents of vitamin C were not increased, whereas nitrate contents increased. High nitrate content can be dangerous to human health; hence, some governments have imposed limits on the levels of nitrate permitted in some vegetable products [43]. Application of BDM resulted in higher Cu concentration in celery compared with Cu-ZnB1 and the excessive Cu ions could increase nitrate content of plants by reducing nitrate reductase activities, which could promote the conversion of NO₃⁻ → NO₂⁻ [44]. Compared with BDM, we found that applying Cu-ZnB reduced the levels of nitrate within celery plants without sacrificing crop yield or quality, thus avoiding possible adverse effects on livestock or human health.

4.3. Effects of copper-based-zinc-boron foliar fertilizers on soil microelements

A large accumulation of copper was observed in soils of apple and grapevine plantations resulting from long-term application of BDM and this has led to detrimental effects on soil fertility and local ecologies [35]. Long-term foliar application of copper fungicides can lead to excessive Cu accumulation within the soil, through direct application, drift, or dripping from leaves [45,46]. The extra Cu can change the soil enzyme properties and processes mediated by microbes [47] and hence is detrimental to the quality and productivity of soil ecosystems. We found that after applying the CFF1 and Cu-ZnB1, soil available Cu concentrations were reduced by 39.3% and 42.2%, respectively, compared with BDM in 2015–2016. Because agents for wetting,
dispersing, and antifoaming were added to improve adhesion capacity of CFF and Cu-ZnB, the contact angles between celery leaves and droplets of these fertilizers were smaller than from the BDM (Figure 3). Compared with BDM, this permitted spray solutions from CFF and Cu-ZnB, to adhere and be more readily absorbed by celery leaf instead of dripping wastefully onto the soil. Available soil Zn concentration following application of Cu-ZnB at three dilutions was 1.33–1.67 mg kg\(^{-1}\), significantly higher than BDM. Copper-based-zinc-boron fertilizer which sprayed on celery plants continuously increased the soil availability Zn and B concentration.

5. Conclusions

Given the results of tests spanning two growing seasons in the present study, celery microelement concentration, quality, and yield were markedly affected by the application of copper-based-zinc-boron foliar fertilizer. In many tests, Cu-ZnB1 produced better results than the Cu-ZnB2 and Cu-ZnB3. Yield following application of Cu-ZnB1 was higher than from the control, BDM, CFF1, CFF2 and CFF3. In addition, Cu-ZnB1 increased the levels of vitamin C and antioxidant enzyme activities within the leaves, while reducing the nitrate content and disease index. Furthermore, compared with BDM treatment, foliar application of copper-based-zinc-boron fertilizers increased Zn and B concentrations of celery, while lowering the level of Cu. Within the soil in 2015–2016 season, these novel fertilizers also increased the concentrations of available Zn and B, while reducing Cu accumulation compared with BDM.

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Author Contribution

Min Zhang and Zhiguang Liu conceived and designed the research. Jinzhao Ma carried out the experiment and wrote the first draft. All authors contributed to read and approved the manuscript.

Disclosure statement

No potential conflict of interest was reported by the authors.

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