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# Effects of zinc fertilization on growth and leaf nutrient content of *Celosia argentea* L.

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## Abstract

Zinc is an important micronutrient for both plant and human health, and dietary zinc is insufficient in many areas of the world, caused by a diet dominated by grains and vegetables grown on zinc deficient soils. *Celosia argentea* L. is an herbaceous annual adapted to difficult soil conditions, low soil nutrient content, and hot weather and is commonly grown as a leafy vegetable in many parts of Africa. This study was conducted to evaluate zinc fertilization of celosia at levels higher than normally recommended, but below toxic levels, to determine the effect on plant performance and dietary value. Celosia was grown in a controlled environment with four zinc fertilizer treatments (0, 50, 100 and 150 mg Zn·kg$^{-1}$). Zinc fertilization had minor effects on plant growth, with differences only found in plant height and root fresh weight with both decreasing at fertilization levels above 100 mg Zn·kg$^{-1}$. Higher zinc fertilization increased root nitrogen, leaf and root zinc, potassium and magnesium concentrations, though leaf magnesium decreased at the highest zinc fertilizer levels. At 50 mg Zn·kg$^{-1}$, zinc fertilization toxicity symptoms were unexpessed thus it can improve plant performance and provide potentially significant dietary benefits to people with limited access to zinc in their diet.

**Key words:** *Celosia argentea*, cockscomb, zinc deficiency, zinc fertilization, zinc nutrients

Zinc is an important micronutrient, both for plant and human nutrition. In plants, zinc is an integral part of enzymes such as RNA polymerase, CuZn-superoxide dismutase, alcohol dehydrogenase, carbonic anhydrase, and plays important roles in protein synthesis, metabolism of carbohydrates and lipids, and membrane integrity (Cakmak, 2000; Marschner, 1995; Sharma *et al*., 2013). In humans, zinc is part of over 300 different enzymes, including oxido-reductases, transferases, hydrolases, lyases, isomerases and ligases, and is also critical for protein formation and responsible for metabolism of carbohydrates, lipids and proteins (Hambridge, 2000; Tuerk and Fazel, 2009; Awobusuyi *et al*., 2014). Deficiencies result in many varied effects including impaired immune system response, delayed sexual maturation, impaired growth, and many other symptoms (Hambridge, 2000).

In many parts of the developing world, zinc deficiency is a serious nutritional problem across all ages and socioeconomic groups. Bilbis *et al*. (2003) described a population of students in Nigeria where zinc deficiency was found in 38 % of preschool children, and 67 % of children with a Vitamin A deficiency were zinc deficient. In a related study, one-half (50 %) of a group of 212 individuals in a semi-urban Nigerian community were zinc deficient, with the highest serum zinc levels in those over 60 years old and those with the lowest body mass index (Awobusuyi *et al*., 2014). A population is said to be at risk of zinc deficiency when 20 % or more of the population have low serum zinc levels (<7.65 μmol Zn·L$^{-1}$) (De Benoist *et al*., 2007). In rural Nigeria, zinc deficiencies in children can exceed this greatly, with over 87 % of children in one community with low serum zinc levels (Ibeawuchi *et al*., 2017).

Zinc deficiencies are prevalent in populations whose diet is high in cereals, legumes, and nuts and lacking fruits, green leafy vegetables, and animal-based proteins. Cereals, such as rice, are naturally low in zinc, even when grown on zinc-rich soils (Sharma *et al*., 2013). Compounding the problem is that these foods are also high in phytates, decreasing absorption of iron, zinc and manganese (Awobusuyi *et al*., 2014). While urban sites and sites near mines have high zinc levels (Akpa and Agbenin, 2012; Chukwuma, 1994), native soil zinc levels are low (<10 mg Zn·kg$^{-1}$) across virtually all of Nigeria (Kparmwang, 2003). Plants grown in these areas are likely to exhibit zinc deficiency symptoms and contain lower level than sufficient zinc for the diet.

*Celosia argentea* L. is a tough, rapidly growing annual known as Lagos spinach (Yarger, 2007). Celosia is a member of the Amaranthaceae or Pigweed Family, a family of tropical, subtropical, and temperate herbs and suffrutescent shrubs, many species of which are used as ornamentals (*Celosia, Gomphrena,* and *Iresine*), vegetables (*Beta vulgaris, Spinacea oleracea*, and *Amaranthus*), grains (*Chenopodium* and *Amaranthus*) and medicinal plants (Judd *et al*., 2016). Celosia is found around the world from northern South America, tropical Africa, the West Indies, and tropical Asia, and responds well to good soil fertility (Yarger, 2007). Celosia has high heat tolerance, high drought tolerance, and good pest resistance. The fresh young leaves and tender stems are combined with various green leafy vegetables, onions, eggplant, and peppers in soups, sauces, and stews. The young leaves are nutritionally dense, with 44 kCal, 4.7 g protein, 0.7 g fat, 7.3 g carbohydrate, 1.8 g fiber, 260 mg calcium, 43 mg phosphorus, and 7.8 mg iron per 100 g serving (Leung *et al*., 1968). Leaves are also high in vitamins A and C (NRC, 2006).

It is known that leaf moisture, protein, potassium and zinc of *C. argentea* were higher on fertilized plots than unfertilized (Aboderin, 2000). Soil zinc levels are satisfactory for plant growth at very low levels (28 mg Zn·kg$^{-1}$), and fertilizer recommendations are very low (3.3 to 5.5 kg·ha) for deficient soils. However, fertilization of *C. argentea* with zinc at higher levels than normal may increase total leaf zinc, improving the plant's nutritional value. The purpose of this study was to determine the effect of zinc fertilization on the growth and leaf nutrient content of *C. argentea*.

## Materials and methods

*Celosia argentea spicata* seeds were obtained from a commercial source (Johnny's Selected Seeds, Winslow, ME, USA) and planted in 1801 planting trays filled with a peat-lite planting medium (BM-6, Berger Horticulture, Saint-Modeste, QC, Can.) at the Texas A&M University-Commerce Greenhouse in Commerce, TX, USA. Seedlings were grown for six weeks under natural light and fertilized once weekly with a 20-10-20 general purpose (GP) fertilizer (Peters Professional, Everris, Dublin, OH, USA) at 100 mg N·kg$^{-1}$. At this fertilization rate, the GP fertilizer supplied zinc at 0.25mg Zn·kg$^{-1}$ to prevent deficiency symptoms.

Once fully established, larger seedlings were selected and planted into 3.75 L nursery pots, with one transplant per pot. Once transplanted, the pots continued to receive the GP fertilizer, but were also randomly arranged to receive one of four zinc fertilizer treatments (0, 50, 100 and 150 mg Zn·kg$^{-1}$) using zinc sulfate ($ZnSO_4$) and grown for another six weeks.

Plants were monitored daily for proper moisture and overall plant health. Plant height, width (taken twice, perpendicular to the first), and leaf chlorophyll content were recorded weekly. Leaf chlorophyll content was estimated using a SPAD 502 Plus (Konica-Minolta, Osaka, Japan). After the six weeks, shoots were removed, roots were washed, and fresh weights of both components were recorded. Roots and shoots were dried at 70 °C for 72 hours, and dry weights were recorded. Samples of dried leaves, dried roots, and dried media from each plant were sent to the Texas A&M AgriLife Extension Soil, Water, and Forage Testing Laboratory in College Station, TX, USA for their analysis.

Experimental design was a randomized complete block design with four blocks and each treatment appearing once per block (*n*=16). Data were analyzed using PROC GLM (SAS Institute, Cary, NC, Version 9.3) with Tukey Honest Significant Difference (HSD) used for means separation.

## Results and discussion

**Media properties and nutrition:** All macro and micronutrients were within acceptable ranges for vegetable growth (Table 1), media pH was also within the desired range at 5.77, and media electrical conductivity (EC) was found normal (540 µS·cm$^{-1}$) (Warneke and Krauskopf, 1983). Irrigation water in North-central Texas is often neutral to slightly alkaline and very often has high levels of calcium and magnesium.

Table 1. Macronutrient and chemical properties of a commercially available soilless media (Berger BM-6, Berger Hort., Ste. Modeste, QC, Can.)

| Measure | Level |
|---|---|
| pH | 5.77 |
| EC (µS·cm$^{-1}$) | 540 |
| $NO_3$-N ( ppm) | 97.3 |
| P ( ppm) | 15.6 |
| K ( ppm) | 100 |
| Ca ( ppm) | 1398 |
| Mg ( ppm) | 353 |
| S ( ppm) | 133 |
| Na ( ppm) | 25.5 |

Post-harvest electrical conductivity, $NO_3$-N, phosphorus, potassium, calcium, sulfur, sodium, iron, manganese, and copper did not differ among the various fertilizer treatments (Table 2). Media pH increased slightly over the course of the experiment, but stayed within desired ranges. The pH changes differed likely due to the use of $ZnSO_4$, allowing the minor formation of $H_2SO_4$, keeping the pH lower in treated samples. $NO_3$-N and K levels were low, as these nutrients are rapidly leached during irrigation (Marschner, 1995). Calcium levels actually increased during the study, again due to the naturally hard water found in North-central and Northeast Texas. Media magnesium levels differed amongst the various treatments, likely due to the formation of $MgSO_4$ following the application of varied levels of $ZnSO_4$, freeing adsorbed magnesium in the media.

As expected, media zinc levels increased as application rate increased, with media at the highest zinc fertilizer level (150 mg Zn·kg$^{-1}$) at 22.5 mg Zn·kg$^{-1}$ (Table 2). Interestingly, zinc levels did not differ between the treatments receiving 0 and 50 mg Zn·kg$^{-1}$. All plants were fertilized at 100 mg N·kg$^{-1}$ with a 20-10-20 GP water-soluble fertilizer that included ZnEDTA at 0.05 %, resulting in a general fertilizer application that provided zinc at 0.25 mg·kg$^{-1}$. However, the similarities are more likely

Table 2. Fertility of soilless media (Berger BM-6) after a six-week fertilization with zinc sulfate at four different levels

| Measure | 0 ppm Zn | 50 ppm Zn | 100 ppm Zn | 150 ppm Zn |
|---|---|---|---|---|
| pH | 6.43a* | 6.25ab | 6.25ab | 6.18b |
| EC (µS·cm$^{-1}$) | 350a | 381a | 363a | 415a |
| $NO_3$-N ( %) | 0.35a | 0.60a | 1.05a | 0.68a |
| P ( ppm) | 3.35a | 2.88a | 3.25a | 3.33a |
| K ( ppm) | 7.75a | 6.00a | 9.25a | 8.25a |
| Ca ( ppm) | 1156a | 1132a | 1323a | 1481a |
| Mg ( ppm) | 244b | 272ab | 309ab | 351a |
| S ( ppm) | 77.3a | 128a | 135a | 163a |
| Na ( ppm) | 59.3a | 70.3a | 76.5a | 91.8a |
| Fe ( ppm) | 12.9a | 12.9a | 12.4a | 12.2a |
| Zn ( ppm) | 2.13c | 7.46bc | 12.3b | 22.5a |
| Mn ( ppm) | 2.35a | 2.25a | 2.22a | 2.18a |
| Cu ( ppm) | 0.32a | 0.34a | 0.32a | 0.32a |

*Sample means within a row not followed by or not sharing a common letter are statistically different at the α =0.05 significance level using the Tukey Honestly Significant Difference.

Table 3. Average growth and morphological traits of *C. argentea spicata* at harvest with zinc sulfate at four fertilization levels six weeks after beginning of treatments

| Measure | 0 ppm | 50 ppm | 100 ppm | 150 ppm |
|---|---|---|---|---|
| Plant Height (cm) | 55.5b* | 64.1a | 62.9a | 57.8b |
| Number of Leaves | 54.0a | 54.0a | 58.0a | 58.0a |
| SPAD | 43.1a | 45.5a | 45.8a | 46.5a |
| Shoot Fresh Weight (g) | 6.63a | 6.14a | 6.83a | 6.58a |
| Shoot Dry Weight (g) | 1.13a | 0.93a | 0.98a | 0.93a |
| Root Fresh Weight (g) | 14.2c | 15.7bc | 18.9a | 16.9ab |
| Root Dry Weight (g) | 2.48a | 2.63a | 2.68a | 2.53a |

*Different letters within a row indicate sample means are statistically different at the α =0.05 significance level using the Tukey Honestly Significant Difference.

related to $ZnSO_4$ leaching rather than GP fertilizer contamination.

**Celosia growth and morphological traits:** Overall, the addition of various levels of zinc fertilizer did not affect most aspects of plant growth. No differences were found among treatments in plant width, leaf number, shoot fresh weight, shoot dry weight or root dry weight (Table 3). SPAD readings were also similar across all treatments.

Across all treatments, plant height ranged from 55 to 65 cm, normal for celosia (Akinfasoye *et al*., 2008). Fertilization at 50 and 100 mg Zn·kg$^{-1}$ led to plants that were taller ($P \le 0.05$), 64.1 cm and 62.9 cm, respectively, than either 0 or 150 mg Zn·kg$^{-1}$ (55.5 and 57.8 cm, respectively) (Table 3). The decrease in height at 150 ppm may be symptomatic of mild zinc toxicity.

Fresh root weight was highest ($P \le 0.05$) in plants fertilized at 100 mg Zn·kg$^{-1}$ (18.9 g), followed by 150 (16.9 g) and 50 mg Zn·kg$^{-1}$ (15.7 g) while the lowest fresh root weights were found in the control (14.2 g). However, this trend disappeared after drying, and no statistical differences were found in root dry weights.

**Leaf nutrient analysis:** Addition of $ZnSO_4$ did not affect leaf nitrogen, phosphorus, calcium, sodium, iron, copper, manganese, sulfur or boron levels (Table 4).

Leaf potassium increased as zinc levels increased, with highest levels (33, 559 and 38, 491 mg K·kg$^{-1}$), when fertilized at 100 and 150 mg Zn·kg$^{-1}$, respectively ($P \le 0.05$). Lowest level was found in the control (26, 276 mg K·kg$^{-1}$). Ciećko *et al*. (2003) found a strong, positive correlation between potassium and zinc in radish, lupine, and maize leaves. Because zinc plays important roles in dehydrogenase, proteinase, and peptidase enzymes, optimal zinc levels improve performance in phloem and xylem transport systems, increasing potassium uptake (Malvi, 2011).

As expected, leaf zinc levels were higher in the treated plants than the control ($P \le 0.05$), with leaf zinc levels of 261, 300, and 303 mg Zn·kg$^{-1}$ in the 50, 100 and 150 mg Zn·kg$^{-1}$, respectively, as compared to the control at 165 mg Zn·kg$^{-1}$ (Table 4). However, leaf zinc levels maximized at the lowest rate, as no differences were found among the three treatments.

Leaf magnesium levels were higher ($P \le 0.05$) in the control and samples with the two lowest zinc fertilizer levels of 50 and 100 mg Zn·kg$^{-1}$ (17, 979, 19, 597 and 19, 175 mg Mg·kg$^{-1}$, respectively) (Table 4). Magnesium was lowest in the 150 mg Zn·kg$^{-1}$sample, likely due to displacement of magnesium by zinc, a similar sized ion (Cakmak, 2000).

**Root nutrient analysis:** The addition of $ZnSO_4$ did not affect phosphorus, calcium, iron, copper, manganese, sulfur or boron levels in the roots (Table 5).

Root nitrogen increased as zinc levels increased, with nitrogen highest levels (0.99 and 1.19 mg N·kg$^{-1}$) when fertilized at 100 and 150 mg Zn·kg$^{-1}$, respectively ($P \le 0.05$). The leaf N concentration of the control was approximately the same as 50 mg Zn·kg$^{-1}$ (0.87 mg N·kg$^{-1}$). Synergistic relationships between root nitrogen and zinc have been previously described in rice (Hakoomat *et al*., 2014) and wheat (Verma and Bhagat, 1990). Since zinc is required for growth of the root and nitrogen stored in the roots and aids the transport of water and other nutrient into the plant, increasing available zinc will increase root nitrogen.

Root potassium also increased as zinc increased. Root potassium reached 18, 233 mg K·kg$^{-1}$ at 150 mg Zn·kg$^{-1}$, higher ($P \le 0.05$) than the control and 50 mg Zn·kg$^{-1}$fertilized plants. This is likely due to the strong enzymatic impact of zinc increasing potassium uptake, similar to results found in soybean (Weisany *et al*., 2014).

As zinc increased, root magnesium increased. Roots had 9, 785, 12, 175, and 13, 015 mg Mg·kg$^{-1}$ in 50, 100 and 150 mg Zn·kg$^{-1}$ applications, respectively, all greater ($P \le 0.05$) than the control. These results are consistent with those of previous studies (Samreen *et al*., 2013)

Samples fertilized at 150 mg Zn·kg$^{-1}$ had higher root sodium levels ($P \le 0.05$) than all other treatments. Zinc plays an important

Table 4. Leaf nutrients in celosia with zinc sulfate at four fertilization levels

| Levels | Leaf N (%) | Leaf P (ppm) | Leaf K (ppm) | Leaf Ca (ppm) | Leaf Mg (ppm) | Leaf Na (ppm) | Leaf Zn (ppm) | Leaf Fe (ppm) | Leaf Cu (ppm) | Leaf Mn (ppm) | Leaf S (ppm) | Leaf B (ppm) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 0 ppm Zn | 3.07a* | 2753.7a | 26276a | 15827a | 17979ab | 130a | 165b | 63a | 12.5a | 122a | 4074a | 66.7a |
| 50 ppm Zn | 3.24a | 2944.7a | 28440ab | 15828a | 19597a | 121a | 261a | 60a | 13.0a | 119a | 4036a | 70.5a |
| 100 ppm Zn | 3.47a | 3117.4a | 33559bc | 17442a | 19175a | 91a | 300a | 59a | 12.7a | 113a | 3941a | 67.2a |
| 150 ppm Zn | 3.81a | 3341.9a | 38491c | 16464a | 16678b | 71a | 303a | 56a | 13.9a | 107a | 4204a | 65.6a |

*Different letters within a column indicate sample means are statistically different at the α =0.05 significance level using the Tukey Honestly Significant Difference.

Table 5. Root nutrients in celosia with zinc sulfate at four fertilization levels

| Levels | Root N (ppm) | Root P (ppm) | Root K (ppm) | Root Ca (ppm) | Root Mg (ppm) | Root Na (ppm) | Root Zn (ppm) | Root Fe (ppm) | Root Cu (ppm) | Root Mn (ppm) | Root S (ppm) | Root B (ppm) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 0 ppm Zn | 0.87b* | 893a | 13587b | 4112a | 9519b | 1619b | 65.9c | 29.4a | 5.20a | 20.7a | 1939a | 18.8a |
| 50 ppm Zn | 0.87b | 894a | 13473b | 3285a | 9785b | 1659b | 101.9c | 45.4a | 4.85a | 20.4a | 1581a | 18.1a |
| 100 ppm Zn | 0.99ab | 957a | 15878ab | 3865a | 12175a | 1754b | 153.7b | 34.7a | 6.03a | 20.2a | 1734a | 19.2a |
| 150 ppm Zn | 1.19a | 997a | 18233a | 4069a | 13015a | 2296a | 195.3a | 40.1a | 5.24a | 21.5a | 1939a | 20.5a |

*Different letters within a column indicate sample means are statistically different at the α =0.05 significant level using the Tukey Honestly Significant Difference.

role in cell membrane integrity and typically reduces sodium uptake and translocation (Aktas *et al*., 2006; Alpaslan *et al*., 1999). However, the application of high rates of $ZnSO_4$ may have allowed for the formation of the relatively stable $Na_2SO_4$. This may have increased the availability of sodium in the media, increasing sodium levels in the root. However, as described in Aktas *et al*. (2006) and Alpaslan *et al*. (1999), excess sodium, in the presence of zinc, did not have a significant negative effect on plant growth.

Root zinc levels were highest ($P \leq 0.05$) in the 150 mg Zn $kg^{-1}$ fertilized plants, followed by the 100 mg $Zn{\cdot}kg^{-1}$ fertilized plants (Table 5). No difference was found between the 50 mg $Zn{\cdot}kg^{-1}$ fertilized plants and the control. The similarity between the control and the lowest treatment level was likely due to zinc present in the general purpose fertilizer used.

Zinc application did not have profound effects on celosia growth. Plant height was reduced at fertilizer levels above 100 mg $Zn{\cdot}kg^{-1}$. Root fresh weights increased up to 100 mg $Zn{\cdot}kg^{-1}$, but declined above. These results are consistent with zinc toxicity, which may occur in leaves with as low as 100 mg $Zn{\cdot}kg^{-1}$ dry weight to 300 mg $Zn{\cdot}kg^{-1}$ or more (Marschner, 1995). Though we did not see chlorosis, other toxicity symptoms were similar to those found by previous authors (Ren *et al*., 1993; Vijayarengan and Mahalakshmi, 2013), with stunted shoot growth as leaf zinc reached and exceeded 300 $mg{\cdot}kg^{-1}$ and decreased root growth as root tissues approached 200 mg $Zn{\cdot}kg^{-1}$. Therefore, fertilization at levels above 100 mg $Zn{\cdot}kg^{-1}$ is contraindicated.

Zinc fertilization did not affect the concentration of most leaf nutrients, but changes in important nutrients did occur. Leaf zinc levels increased by over 30 % with the addition of $ZnSO_4$, at even the lowest levels. Zinc fertilization increased leaf potassium concentration and magnesium, though leaf magnesium levels decreased at 150 mg $Zn{\cdot}kg^{-1}$, likely an induced reduction as zinc displaces the more mobile magnesium (Boardman and McGuire, 1990). Cakmak and Marschner (1988) linked potassium efflux into the growing medium to zinc deficiency, and higher tissue potassium is likely related to improved cell membrane performance associated with high zinc levels (Aktas *et al*., 2006). Though differences between treatments were not significant, as zinc fertilization increased, leaf sodium decreased from 130 to 71 mg $Na{\cdot}kg^{-1}$, and increased the potassium to sodium ratio, an important mechanism of salt stress resistance (Wakeel, 2013), likely due to membrane stability associated with zinc (Cakmak, 2000).

Considering the leaf zinc at the lowest $ZnSO_4$ levels and the potential benefits to human nutrition, raising soil zinc levels to 50 mg $Zn{\cdot}kg^{-1}$ would provide the most benefit. Assuming a plow layer of 15 cm, this would be equivalent to 90 kg $ZnSO_4{\cdot}ha^{-1}$ or 0.9 kg $ZnSO_4{\cdot}100\ m^{-2}$, though a soil test should be conducted to determine the appropriate zinc fertilization levels. Given a zinc dietary reference intake (DRI) of 11 mg for men and 8 mg for women (Del Valle *et al*., 2011); celosia fertilized at 50 mg $Zn{\cdot}kg^{-1}$ would meet the DRI through consumption 42 g of celosia leaves by men and 31 g by women, less than half of a typical 100 g serving of celosia leaves, though further study is needed to determine the bioavailability of zinc from celosia. Therefore, while minimum zinc soil fertility needs are very low for healthy plant growth, a slight increase in zinc fertilization avoids toxicity symptoms, but can improve plant performance and provide potentially significant dietary benefits to people with limited access to zinc in their diet.

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