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Uptake of seed-applied copper by maize and the effects on seed vigor

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Abstract

Seed treatment is a low-cost and efficacious method to deliver a diversity of compounds to field crops. This study evaluated the uptake of seed-applied Cu by maize and the effect on seed vigor. The treatments were composed of a control (untreated seeds) and five dosages of Cu: 0.11, 0.22, 0.44, 0.88 and 1.76 mg Cu seed^{-1}, applied as cuprous oxide and copper oxychloride formulations. Seedling emergence and the speed of seedling emergence were determined in three periods: 1, 60 and 120 days after Cu application. Evaluations of root and shoot dry mass, Cu tissue concentration and efficiencies of Cu uptake and incorporation were conducted with two-leaf stage maize plants. Seed-applied Cu reduces the speed of maize seedling emergence, while the final emergence percentage is not affected. Shoot dry mass tends to increase with the application of Cu, while there is no interference on root dry mass within the dosages tested. Cu tissue concentration of both roots and shoots increases as higher dosages of Cu are applied to seeds, with higher accumulation in roots. Cuprous oxide promotes higher uptake of Cu by maize roots compared to copper oxychloride.

Key words: *Zea mays* L., seed treatment, seed coating; seed dressing, micronutrients.

1. INTRODUCTION

Copper is an essential element for plants, mainly for its participation in photosynthesis, respiration, carbon and nitrogen metabolisms, and protection against oxidative stress (Yruela, 2009). More than 50% of Cu atoms in plants are present in chloroplasts, as a component of plastocyanin. Thus, in case of Cu deficiency, photosynthesis is severely affected (Epstein & Bloom, 2004).

The availability of Cu to plants is mainly influenced by soil texture, pH, organic matter content and the amount of other nutrients (Malavolta, 2006). The lack of Cu in soils naturally occurs in many regions worldwide, such as in Brazilian "Cerrado" and other Tropical areas. Moreover, cultivation practices such as superficial liming may contribute to reduce the availability of Cu to field crops, by excessively elevating the soil pH at the surface level; this leads to a deficiency of cationic micronutrients, such as Cu, by early developing seedlings (Fageria & Stone, 2004).

Seed treatment may consist in a feasible option to deliver micronutrients to field crops (Farooq et al., 2012). Considering operational and agronomic aspects, seed treatment contains important characteristics to provide micronutrients to plants, such as relatively low-cost, easy operation, uniform distribution of compounds among plants and availability since the earlier stages of plants growth (Scott, 1998; Taylor et al., 1998).

The initial stages of growth is a crucial phase for the uptake of micronutrients by plants, as higher concentrations of these elements are found in young plant tissues (Cakmak, 2000). A proper supply of micronutrients during this phase may result on a better crop establishment in the field and a faster initial development of plants, which are crucial aspects for obtaining higher grain yields in maize (Dias et al., 2010; Mondo et al., 2013).

Studies involving seed treatment with Cu, in general, presented negative results in terms of seedling emergence and growth. Nazir et al. (2000) and Luchese et al. (2004), studying wheat and maize, respectively, concluded that seed treatment with copper sulphate caused a reduction on seedling emergence. Malhi (2009) and Malhi & Leach (2012) tested Cu-EDTA formulations applied via maize seeds and also reported toxicity to plants. These negative results might be directly associated to the Cu-containing formulation. According to Scott and Blair (1988), water-soluble formulations (such as copper sulphate and Cu-EDTA) are more readily absorbed by plants and, consequently, are more prone to harm the seeds.

This study evaluated the feasibility of using two non-water-soluble formulations containing Cu as maize seed treatment, considering nutritional and seed quality aspects.

2. MATERIAL AND METHODS

A maize seed-lot containing non-treated seeds (hybrid 2B688Hx) was used in the study. The lot was submitted to an initial characterization following the procedures described on the Rules for Seed Analysis (Brasil, 2009), providing the following results: 10.3% of seed moisture content; 330.2 g of a thousand seeds weight; 92% ± 0.40 of germination; 86% ± 2.44 and 80% ± 4.54 of seed vigor, assessed by the accelerated aging and cold test, respectively.

The seeds were coated with two liquid formulations containing Cu, cuprous oxide (density: 1.49 g cm^3; 501.3 g Cu dm^{-3}) and copper oxychloride (1.72 g cm^3; 589.0 g Cu dm^{-3}). The formulations were applied with a laboratory scale conventional pan coater, equipped with a Leroy-Somer rotating motor (model LS71 0.75 Kw). This equipment allowed an uniform coverage of maize pericarp.

The dosages of Cu corresponded to: 0.11, 0.22, 0.44, 0.88 and 1.76 mg Cu $seed^{-1}$, for both formulations. After coated, seeds were placed in paper bags and stored under controlled conditions (20 °C, 45% R.H.) along the experiment.

Seed vigor was assessed through the seedling emergence (SE) and speed of seedling emergence tests (SSE), installed at three periods after Cu application: 1, 60 and 120 days. Both tests were conducted in the same experimental unit, composed of four replicates of 50 seeds per treatment, sowed in polyethylene trays (0.47 × 0.30 × 0.11 m) filled with 8 dm^3 of sand moistened at 60% of water holding capacity. The trays were maintained in a greenhouse and irrigated as needed. The speed of seedling emergence (index) was calculated according to Maguire (1962), based on a daily counting of emerged seedlings.

Root and shoot dry mass and Cu tissue concentration were evaluated with four replicates of 10 plants, cultivated in sand, in the same conditions described previously; in this test, only the dosages 0, 0.11, 0.44 and 1.76 mg Cu $seed^{-1}$ were considered. At the two-leaf stage, plants were carefully removed from sand and rinsed in deionized water. Afterwards, roots and shoots were separated and oven-dried at 60°C until constant mass, followed by weighing in analytical scale (0.0001 g). The root and shoot samples were submitted to a tissue analysis of Cu concentration, according to the procedures described by Malavolta et al. (1997).

Values of root and shoot dry mass and Cu tissue content allowed calculating the uptake and incorporation efficiencies of this element by maize, following the method described by Baligar et al. (2001). The uptake efficiency (UE) was calculated as the ratio of total Cu content in the plant, in mg, and root dry weight, in g; the incorporation efficiency (IE) was based on the Cu content in shoots, in mg, divided by the total Cu content in the plant, in mg.

Data was analyzed using the JMP® statistical software (SAS Institute, version 10). Results from seed vigor tests were submitted to ANOVA and, in case of significance, means were compared by Tukey test ($p<0.05$); data of tissues dry mass and Cu uptake was submitted to regression analysis.

3. RESULTS AND DISCUSSION

Results of seedling emergence (SE) are presented on table 1, specified by each treatment and evaluation period. In the first and third periods (1 and 120 days), no difference was verified between dosages and Cu formulations, while in the second period (60 days), the treatment containing 0.44 mg Cu $seed^{-1}$ presented lower SE compared to non-treated seeds and did not differ from the other dosages.

The speed of seedling emergence (SSE) was affected by the treatments containing Cu, with variable results among dosages, formulations and periods of testing (Table 2). The

Table 1. Maize seedling emergence evaluated with different dosages of Cu, applied to seeds as cuprous oxide (CuOxi) and copper oxychloride (CuOxy). The evaluations were conducted in three periods: 1, 60 and 120 days after Cu application

Cu dosages (mg Cu $seed^{-1}$)		0	0.11	0.22	0.44	0.88	1.76
Period (days)	Treatment	%					
1	CuOxi	99Aa1	98Aa	99Aa	98Aa	99Aa	99Aa
	CuOxy	99Aa	99Aa	99Aa	98Aa	99Aa	98Aa
60	CuOxi	99Aa	99Aab	99Aab	95Ab	97Aab	96Aab
	CuOxy	99Aa	99Aab	97Aab	95Ab	97Aab	97Aab
120	CuOxi	100Aa	98Aa	99Aa	99Aa	95Aa	97Aa
	CuOxy	100Aa	99Aa	97Aa	100Aa	99Aa	98Aa

[1]Means followed by the same uppercase letter in the column and lowercase letter in the row do not differ by Tukey test ($p<0.05$).

Table 2. Speed of maize seedling emergence evaluated with different dosages Cu, applied to seeds as cuprous oxide (CuOxi) and copper oxychloride (CuOxy). The evaluations were conducted in three periods: 1, 60 and 120 days after copper application

Cu dosages (mg $seed^{-1}$)		0	0.11	0.22	0.44	0.88	1.76
Period (days)	Treatment	Index					
1	CuOxi	21.9Aa1	19.7Aa	19.5Aa	19.3Aa	20.0Aa	19.6Aa
	CuOxy	21.9Aa	20.3Aab	20.5Aab	19.1Ab	20.3Aab	20.1Aab
60	CuOxi	21.3Aa	20.4Aab	20.3Aab	19.0Ab	18.8Ab	18.7Ab
	CuOxy	21.3Aa	20.3Aab	19.2Ab	18.7Ab	19.7Aab	19.9Aab
120	CuOxi	19.7Aa	19.5Aab	19.6Aab	19.4Aab	18.6Aab	18.6Ab
	CuOxy	19.7Aa	19.3Aa	19.2Aa	19.5Aa	19.2Aa	18.7Aa

[1]Means followed by the same uppercase letter in the column and lowercase letter in the row do not differ by Tukey test ($p<0.05$).

control treatment (non-treated seeds) presented higher absolute values for SSE among all treatments and periods, and significant differences occurred randomly with the dosages of 0.22, 0.44, 0.88 and 1.76 mg Cu seed^{-1}.

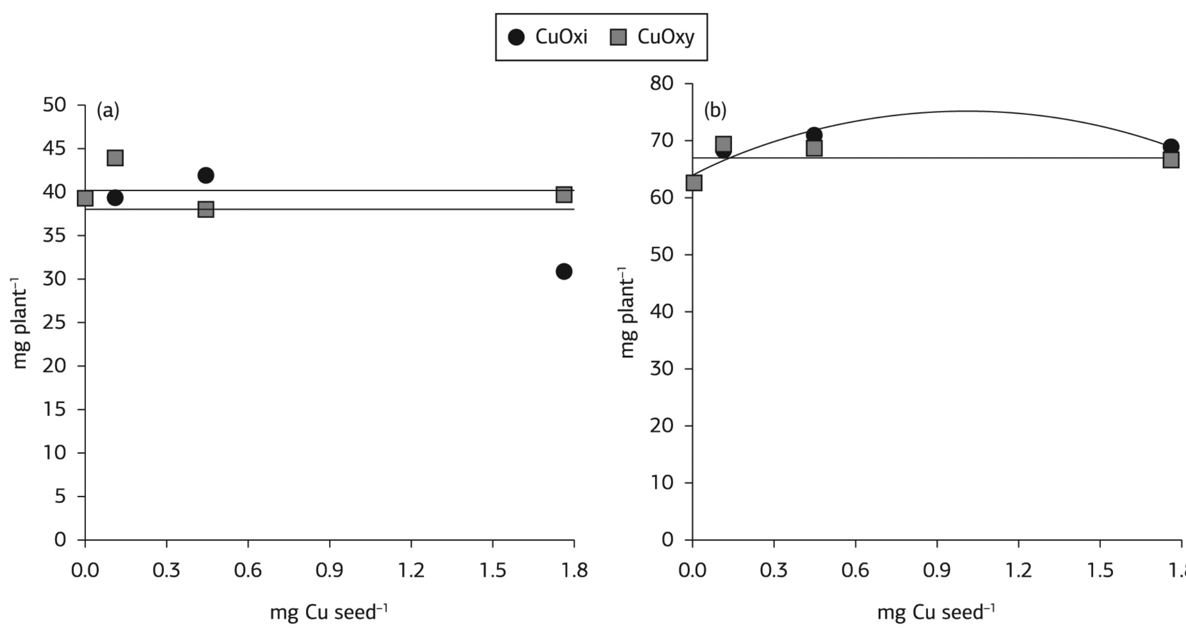
Data of root and shoot dry mass is presented on figure 1. Root dry mass values did not significantly differ among the dosages of Cu, for both formulations, although a lower mean value was obtained at the dosage of 1.76 mg Cu seed^{-1} applied as cuprous oxide. For shoots, only cuprous oxide significantly increased the dry mass, however, the difference were almost non-significant at the 5% significance level ($p=0.047$).

The Cu tissue concentration in roots presented a linear response for both formulations, increasing as Cu dosages increased, while shoot concentration presented a quadratic response, with maximum values obtained at the dosages of 1.16 and 1.10 mg Cu seed^{-1} for cuprous oxide and copper oxychloride, respectively (Figure 2). The Cu tissue concentration ranged from 61.65 to 677.33 mg Cu kg^{-1} in roots and 5.87 to 25.93 mg Cu kg^{-1} in shoots.

The Cu uptake efficiency (UE) presented a linear response for both formulations, with higher UE values as higher dosages of Cu were applied to seeds (Figure 3); cuprous oxide presented higher UE values at all dosages compared to copper oxychloride. For the incorporation efficiency (IE), also in figure 3, there was a quadratic response, with decreases of IE values with increasing amounts of seed-applied Cu. Both formulations presented similar IE values, and could not be differentiated by this parameter.

To a certain extent, results of seed vigor (Table 2) indicate a negative effect of Cu only for SSE; SE values ranged from 95 to 100% and the treatments containing the highest dosages Cu (0.88 and 1.76 mg Cu seed^{-1}) did not differ from the control. By evaluating the mean values of SSE, it's possible to verify that non-treated seeds provided higher results than any treatment containing Cu-treated seeds, although significant difference started only at the dosage of 0.22 mg Cu seed^{-1}.

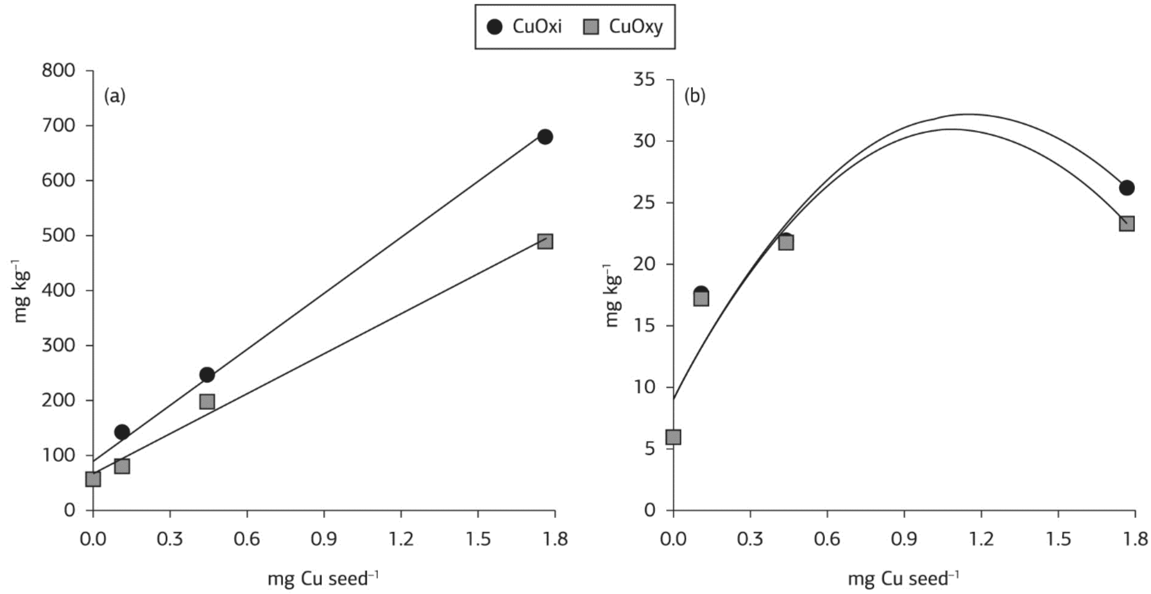
According to Mondo et al. (2013), variation in maize SE affects subsequent plant growth, with late-emerging plants being in disadvantage compared to earlier emerging plants. Thus, a fast and uniform initial development of plants is highly desired for maize crop, especially under stressful conditions. Foti et al. (2008) found that small amounts of Cu applied via seed priming on maize increased the number of germinated seeds compared to the control. On the contrary, Luchese et al. (2004) reported a toxic effect



(a)	CuOxi	$y = 38.0 \pm 1.80$	
	CuOxy	$y = 40.2 \pm 1.18$	
(b)	CuOxi	$y = 64.232 + 22.001x - 10,933x^2$	$R^2 = 0.49$*
	CuOxy	$y = 67,0 \pm 0,96$	

*Difference between means are significant by F-Test ($p<0.05$). Numbers following the signal "±" represents the Standard Mean Error (SME).

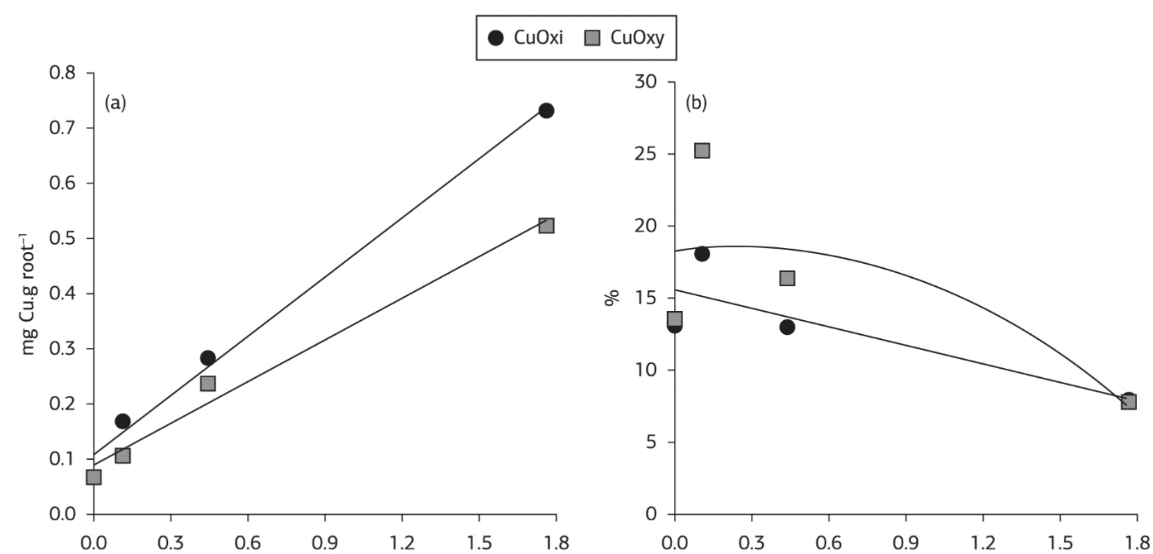
Figure 1. Root (a) and shoot (b) dry mass of two-leaf maize plants (hybrid 2B688Hx). Treatments correspond to different dosages of seed-applied Cu, provided via cuprous oxide (CuOxi) and copper oxychloride (CuOxy).



(a)	CuOxi	y = 87.059 + 337.71x	R^2 = 0.97*
	CuOxy	y = 68.343 + 242.02x	R^2 = 0.87*
(b)	CuOxi	y = 8.879 + 39.792x + 17.125x^2	R^2 = 0.85*
	CuOxy	y = 8.748 + 39.721x + 17.967x^2	R^2 = 0.84*

*Difference between means are significant by F-Test (p<0.05).

Figure 2. Cu tissue concentration of roots (a) and shoots (b) of two-leaf maize plants (hybrid 2B688Hx). Treatments correspond to different dosages of seed-applied Cu, provided via cuprous oxide (CuOxi) and copper oxychloride (CuOxy).



(a)	CuOxi	y = 0.107 + 0.360x	R^2 = 0.96*
	CuOxy	y = 0.090 + 0.250x	R^2 = 0.87*
(b)	CuOxi	y = 15.616 –3.730x –0.827x^2	R^2 = 0.54*
	CuOxy	y = 19.907 –3.030x –4.952x^2	R^2 = 0.52*

*Difference between means are significant by F-Test (p<0.05).

Figure 3. Cu uptake (a) and incorporation (b) efficiencies determined at the two-leaf stage of maize (hybrid 2B688Hx). Treatments correspond to different dosages of seed-applied Cu, provided via cuprous oxide (CuOxi) and copper oxychloride (CuOxy).

of Cu applied via maize seeds, obtaining lower seedling emergence percentages.

Despite the similar values of shoot dry mass obtained among all treatments, visual symptoms of Cu toxicity could be observed with the highest dosages (0.88 and 1.76 mg Cu seed^{-1}), such as leaf necrosis and plant stunting, which are common symptoms of Cu toxicity in maize (Yruela, 2005).

Under Cu toxicity, root growth usually is inhibited before shoot growth, as the first are a preferential site of Cu accumulation (Broadley et al., 2012). The mechanisms to resist excess Cu by plants include: binding the element to cell walls, restrict the influx through plasma membrane, stimulation of efflux from the cytoplasm, compartmentalization of Cu in the vacuole and chelation at the cell wall-plasma membrane interface (Yruela, 2009). In this experiment, roots accumulated considerably higher proportions of Cu than shoots, however, non-significant effect between roots dry mass values were verified.

The linear positive response of Cu UE to the increasing dosages of Cu can be mainly explained by the root accumulation, as Cu shoot concentration represented a considerably lower amount of Cu in the entire plant. In the case of IE, which basically considers the amount of Cu transported to shoots, there was a reduction as higher amounts of Cu were applied. This is an expected response, as plants ability to remobilize Cu from roots to shoots is limited (Broadley et al., 2012); also, the plants ability to utilize any nutrient tends to decreases as their availability increases (Baligar et al., 2001).

Copper seed treatment may consist in a good option for maize cultivated in soils with low Cu availability (Karamanos & Goh, 2004; Malhi et al., 2005; Karamanos et al., 2005; Malhi, 2009). However, soil or foliar applications may also be needed in order to totally supply the crop demand for this micronutrient. Moreover, the application of Cu may present advantages in terms of plant's disease management, mainly by controlling certain types of fungal pathogens (Peruch & Bruna, 2008)

This research demonstrates that both cuprous oxide and copper oxychloride can be used as maize seed treatment. The formulations did not negatively affect the final seedling emergence and the tissues dry mass, while provided a significant uptake of Cu by plants. However, a delayed seedling emergence is expected.

4. CONCLUSION

Seed-applied Cu reduces the speed of maize seedling emergence, while the final emergence percentage is not affected. Shoot dry mass tends to increase with the application of Cu, while there is no interference on root dry mass within the dosages tested. Cu tissue concentration of both roots and shoots increases as higher dosages of Cu are applied to seeds, with higher accumulation in roots. Cuprous oxide promotes higher uptake of Cu by maize roots compared to copper oxychloride.

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