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Environment

Avaliação do efeito tóxico de preservantes de bambu sobre a germinação e crescimento de *Lactuca sativa*

Evaluation of toxic effect of bamboo preservatives on Lactuca sativa germination and growth

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RESUMO

Materiais como madeiras e bambu em suas diversas aplicações estão sujeitos à degradação por organismos deteriorantes como fungos e insetos. Dessa forma, tratamentos químicos são rotineiramente utilizados por profissionais de bambu para controlar essa degradação. No entanto, é usual o descarte dessas soluções no solo após o uso e os riscos ambientais e efeitos sobre à vegetação desse descarte indevido ainda são desconhecidos. Assim, esse estudo visa avaliar a fitotoxicidade de soluções de CCB (composto baseado em cobre, cromo e boro), de Octaborato de sódio tetraidratado e de CB (sulfato de cobre com ácido bórico) sob diferentes concentrações na germinação e desenvolvimento de alface (**Lactuca sativa**). Foram também medidos os valores de pH e condutividade elétrica das soluções preparadas. Os resultados mostram que os três preservantes são fitotóxicos e inibem o desenvolvimento da radícula, mesmo após diluições de 50 vezes a utilizada pelos profissionais. Dessa forma, o descarte desses efluentes no solo ou em corpos d'água pode acarretar impactos ambientais negativos à vegetação, necessitando de tratamento posterior ao uso na preservação dos colmos ou maiores diluições do efluente antes do lançamento.

Palavras-chave: Descarte; Tratamento; Toxicidade

ABSTRACT

Materials such as wood and bamboo in their various applications are subject to degradation by deteriorating organisms like fungi and insects. Chemical treatments are routinely used by bamboo professionals aiming to control this degradation. However, it is usual the dispose of these used solutions on soil and the environmental risks and effects on vegetation of this improper disposal are still unknown. Thus, this study aims to evaluate the phytotoxicity of CCB solutions (compound based on copper, chromium and boron),

tetrahydrate sodium octaborate and CB (copper sulfate with boric acid) under different concentrations in the germination and development of lettuce (**Lactuca sativa**). The pH and electrical conductivity of the prepared solutions were also measured. The results show that the three preservatives are phytotoxic and inhibit root development, even after dilutions of 50 times that used by professionals. Thus, the disposal of these effluents in the soil or water bodies may have negative environmental impacts on vegetation, requiring treatment after use on preservation of the culms or higher dilutions of the effluents before the disposal.
Keywords: Disposal; Treatments; Toxicity

1 INTRODUCTION

Bamboo and wood, natural raw materials of wide applicability, are subject to the destructive attack of xylophagous organisms. Among them, the main ones are fungi and insects such as termites, borers and carpenter ants (SALGADO, 2014). Thus, in order to obtain higher protection against such degradation and, consequently, provide greater durability of bamboo, strategies of treatment have been studied. Among them, chemical treatments are the most efficient and they are the most common method used in Brazil. In general, a preservative solution with boron in its composition is impregnated by immersion or pressure (PEREIRA and BERALDO, 2016).

Among these substances, there are two to highlight: Copper-Chrome-Boron - CCB - commercial solution, with attested efficiency by Espelho (2008) and Tiburtino (2015) and tetrahydrate sodium octaborate ($Na_2B_8O_{13}.4H_2O$), presented by Pereira and Beraldo (2016). In addition, CB, a preservative similar to CCB but without chromium, has also been used by bamboo professionals (SILVA *et al*., 2014).

Despite the efficiency, the treatment creates a barrier in relation to the disposal of the preservative solution. They are often discarded in soil, but there are no studies showing the level of impact or plausible concentration and dilution levels for disposal in the environment yet. In this sense, toxicological characteristics of these solutions must be checked before the discharge into the soil and rivers, as toxic compounds can cause adverse effects on seed germination, plant growth and soil environment. Thus, toxicity tests are an alternative to test how harmful is a specific substance (LUO *et al*., 2018).

In the germination process, numerous physiological processes occur in the first days of plant growth, which depend on external (environmental) and internal (dormancy, inhibitors) factors. The presence of toxic solutions can interfere in the plant survival, possibly inhibiting both germination and normal development of the plant (SOBRERO and RONCO, 2004; BRITO, 2010). The inhibition of plant growth can be noticed by the decrease of the process of elongating the radicle and/or the hypoclite, initial organs on plant development (SOBRERO and RONCO, 2004).

In this way, the seeds presented itself as an alternative for toxicological tests to evaluate the quality of an effluent (BRITO, 2010). Bellato *et al*. (2019) used the phytotoxicity test on lettuce (Lactuca sativa) seeds to evaluate the effect of a textile effluent after fungal treatment. This lettuce seed test was also used on to evaluate metals of Polycontaminated industrial effluents (CHARLES *et al*., 2011; PRIAC, 2017; SILVEIRA, 2017), receiving waters near industrial sites (LYU *et al*., 2018), pharmaceuticals (PINO *et al*., 2016).The authors considered the germinative test as a great parameter because of its sensitivity against to the effluent actions.

Peduto *et al*. (2019) also used phytotoxicity tests to evaluate the sensitivity of seeds of different species, including lettuce, against water and potassium dichromate. Like *Sinapis alba, L. sativa* was sensitive to negative control, confirming such seeds as phytotoxicity bioindicators. In addition to showing itself as an efficient test, the authors highlighted other advantages, such as simplicity and low cost to perform the test. Corroborating these findings, Palácio *et al*. (2012) stated that, although lettuce develops in a potentially toxic environment, it has lethal effects, with the inhibition of germination, and sub-lethal effects, inhibiting the development of roots and radicles.

In view of that has been yet exposed, the germinative test of lettuce seeds is an interesting possibility in the evaluation of bamboo preservative phytotoxicity, since the impact caused by their discharges in the soil is still unknown. Being a sensitive, effective and inexpensive method. The damage caused by the most used components in chemical treatment of bamboo could be evaluated, compared and measured. Thus, this study aimed to test the toxicity of the chemical preservatives CCB, tetrahydrate octaborate sodium and CB, used in the treatment of bamboo, on lettuce seeds germination.

2 MATERIAL AND METHODS

The phytotoxicity tests were based on the methodology proposed by Luo *et al*. (2018). They were carried out at the Sanitation Laboratory, Institute of Sciences and Technological and Exact Sciences, Universidade Federal do Triângulo Mineiro. They were made with seeds of L. sativa (cultivar Cinderella), the curly type. The seeds are provided by Feltrin Seeds brand, with 99.6% purity and 89% germination (data informed by the manufacturer). There was no previous treatment to overcome dormancy.

There were 22 test groups with three repetitions: positive control (distilled water) and the three preservatives at 7 groups of different dilutions:

– Tetrahydrate sodium octaborate (SB) at 5%, concentration used to treat bamboo colms, and, from this, disposal dilutions at 1/3, 1/5, 1/10, 1/40, 1/50 and 1/60;

– Cooper Chrome Boron (MOQ OX50 - Montana Química S.A.) (CCB), with the proportions of chromic acid at 30.2% - 34.7%, cupric oxide at 10.0% - 14.2% and boric acid at 29.4% - 33.5%. This preservative was prepared at 3% (concentration of bamboo treatment) and, likewise, were diluted in disposal solutions of 1/3, 1/5, 1/10, 1/20, 1/30 and 1/50;

– Cooper Boron (CB), 2% boric acid with 1% copper sulfate (bamboo treatment concentration), with disposal dilutions of 1/3, 1/5, 1/10, 1/20, 1/30 and 1/50 dilutions.

The concentrations used as reference for the dilutions are values that have been shown to be efficient in the treatment of bamboo and have been used by professionals in the field, with 5% SB, 3% CCB and 2% CB (ESPELHO, 2008; SILVA *et al*., 2014; PEREIRA and BERALDO, 2016).

For the preparation of the solutions, the mass of each preservative was measured in a precision semi-analytical scale of 0.01 g Bel Engineering and diluted in distilled water using 50 mL Becker. After complete dilution, the electrical conductivity and pH parameters were measured using the YSI Professional Plus® multiparameter.

Phytotoxicity test was according to SOBRERO and RONCO (2004). Transparent glass Petri dishes (100 X 15 mm), previously sterilized, were used for the test. The containers received a layer of filter paper at the base and they were saturated with 3.5 mL of the tested solutions. Then, 10 lettuce seeds were placed under the filter paper and the Petri dishes were wrapped in plastic film. The containers were stored in a refrigerated incubator – Lucadema, with a constant temperature of 20 ± 1 °C for 120 h (5 days), in the dark. During this period, the number of seeds germinated (root protrusion) was daily monitored by observation with naked eye, and the length of the radicle was measured by a 200 mm digital caliper - Malberg.

The following parameters were analyzed: Total germination Index given by Percentage of absolute germination (AG), Relative germination index (RG), Relative root growth (RRG) and Germination Index (GI) (Equation (1) to Equation (4)).

$$AG = \frac{n^{o}\ of\ germinated\ seeds}{total\ n^{o}\ of\ seeds} x100 \quad (1)$$

$$RG = \frac{n^{o}\ of\ germinated\ seeds\ in\ preservative}{total\ n^{o}\ of\ seeds\ in\ positive\ control} x100 \quad (2)$$

$$RRG = \frac{average\ root\ growth\ in\ preservative}{average\ root\ growth\ in\ positive\ control} x100 \quad (3)$$

$$GI = \frac{RG\ X\ RRG}{100} x100 \quad (4)$$

The classification that determines the degree of toxicity present in the sample was made by the values of RRG and GI. For RRG, the methodology based on Lumbaque *et al*. (2017), which evaluated the degradation and ecotoxicity of the Black 5 reagent in front of lettuce (*L. sativa*) seeds. Similarly, GI was based on Kohatsu (2018), which evaluated the phytotoxicity of processes resulting from composting.

2.1 Statistical analysis

In order to verify the statistical differences between the phytotoxicity values results of preservative on their tested concentrations in relation to the lettuce seeds, Kolmogorov-Smirnov tests were performed to assess data normality, nonparametric Kruskal-Wallis tests for comparison between all groups without normality and Dunn's for comparison between groups pairs. The tests were performed by the software Instat v.3.36.

3 RESULTS AND DISCUSSION

Table 1 shows the values of total germination index given by the percentage of Absolute Germination (AG), Relative Germination index (RG), Relative Root Growth (RRG) and Germination Index (GI), in addition to the average length of the radicle corresponding to the dilutions of preservatives and the respective concentrations of the soluble elements present (Copper, Chromium and Boron). It stands out the complete inhibition of germination in all preservatives until the dilution 1/10, showing the toxic effect to the seeds even after making 10 times the concentration used in solutions for the treatment of bamboo.

Germination was observed in concentrations below 0.31 g/L (310 mg/L) of boron. However, Yuri *et al.* (2004) stated that negative results in lettuce were obtained with 15 mg/L. For higher values, symptoms of toxicity manifested in the average total and commercial weights of lettuce heads were significantly minors. In another words, the development of the plant was inhibited. In addition to the negative effect on plant development, boron also inhibited seed germination (CHARLES *et al*., 2011). Similarly, there was germination at chromium concentrations below 0.23 g/L (230 mg/L). Despite germination being observed, Castilhos *et al*. (2001) showed negative effects on the development of plants at a concentration higher than 20 mg/L. The authors showed that the dry matter production of the aerial and root part of soybeans, as well as the fixation and absorption of nutrients decreased with increasing concentration. The same authors also pointed the phytotoxicity of chromium when applied at the aerial part of soybean plants above 5.8 mg/kg. For copper, germinations occurred at concentrations up to 200 mg/L. Plants that grow in environments with high concentrations of copper have reduced biomass, chlorosis and changes in enzymatic activities. Charles *et al*. (2011) evaluated the effect of copper on the germination of *L. sativa* and realized that there was an inhibition in root growth when the copper concentration was higher than 4 mg/L. Values of this element above 20 mg/kg in dry mass interfere with the development and translocation of other plant nutrients (ZAMPIERI, 2010). Thus, the data shows that, although germination is observed, the values are higher than the limits established by literature and, thus, the disposal of preservatives in the soil considerably affects the development of vegetation.

According to the CONAMA Resolution (2011), which provides the conditions and standards for the discharge of effluents into receiving water bodies, it is established that the maximum values for the elements tested are: 5 mg/L, 1.0 mg/L and 1.0 mg/L for boron, copper and chromium, respectively. Thus, it is clear that in all groups the concentrations exceeded the maximum levels established by CONAMA, even in the groups where the solutions were most diluted. The group with the lowest concentrations was the CCB at the highest dilution (1/50). For boron, the value obtained was 40 mg/L, a value eight times higher than the maximum concentration recommended by the standard. Copper, in its turn, exceeded the maximum value by 70 times (70 mg/L) and chromium by more than 90 times (91.8 mg/L). The disposal of the CCB at bamboo colm treatment concentration in the water body would be possible if it were diluted up to 4500 times. In this way, none of the preservatives tested in any dilution can be discarded in the water.

The AG results (Table 1), show that the highest values were observed in the most diluted preservatives. However, even at the highest dilutions tested (1/50 and 1/60), germination rates did not exceed 75%, reinforcing the toxic effect on the same seeds (Figure 1).

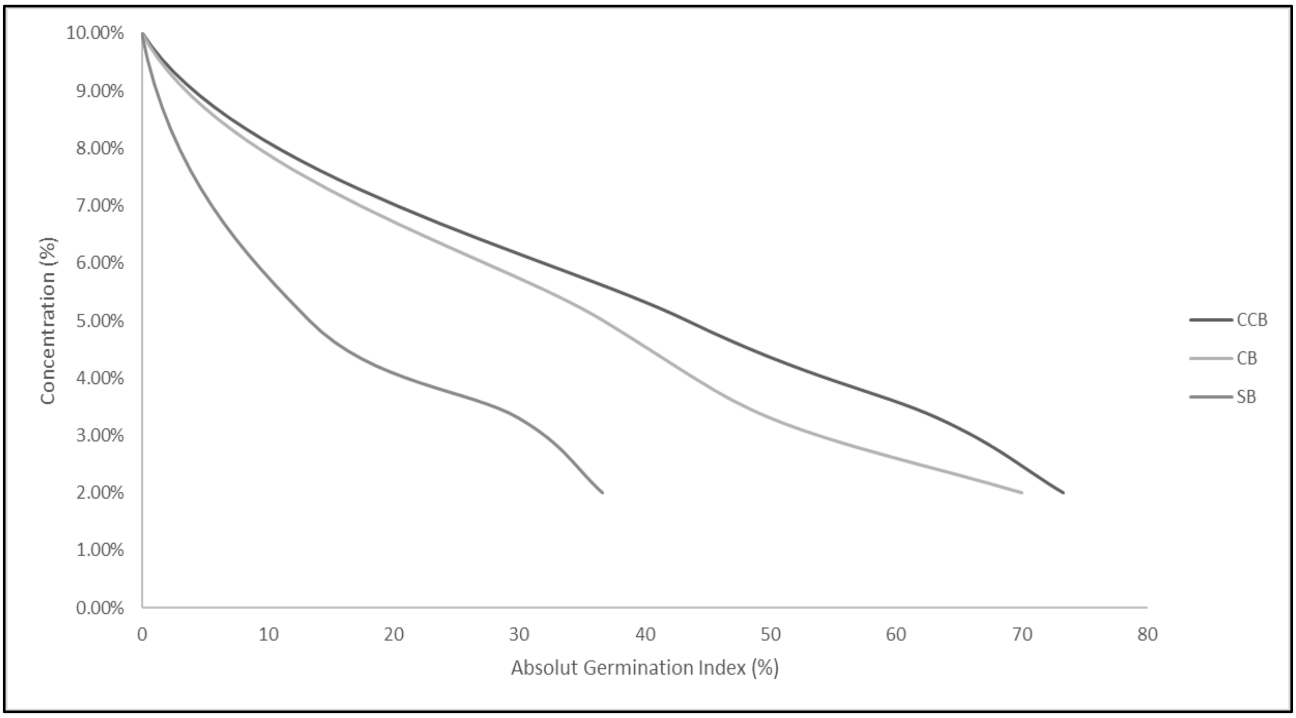
Table 1 – Medium values and standard errors of germination indexes and radicle length after phytotoxicity tests of preservatives SB, CBB and CB under different dilutions

Groups	Dilution	Concentration [1] of solution	pH	EC (uS/cm)	Concentration of elements (g/L)			AG[3] (%)	RG (%)	RRG (%)	GI (%)	Length of radicle (mm)
					Boron	Cooper	Chrome					
Control		-	7.0					-	-	-	-	3.303 ± 0.649
SB[4]	1/1	5%	5.38	6843	12.57	-	-	0.00 ± 0.00	0.00 ± 0.00	0.00	0.00	0.000 ± 0.000
	1/3	1.67%	5.7	3222	4.19	-	-	0.00 ± 0.00	0.00 ± 0.00	0.00	0.00	0.000 ± 0.000
	1/5	1%	5.63	2303	2.514	-	-	0.00 ± 0.00	0.00 ± 0.00	0.00	0.00	0.000 ± 0.000
	1/10	0.5%	5,58	1220	1.26	-	-	0.00 ± 0.00	0.00 ± 0.00	0.00	0.00	0.000 ± 0.000
	1/40	0.125%	7.74	344.2	0.31	-	-	13.33 ± 11.55	16.67 ± 14.43	21.02	3.50	0.077 ± 0.056
	1/50	0.1%	7.64	343.3	0.25	-	-	30.00 ± 0.00	37.50 ± 0.00	27.42	10.28	0.100 ± 0.074
	1/60	0.083%	7.46	259.2	0.21	-	-	36.67 ± 5.77	45.83 ± 7.22	53.54	24.54	0.683 ± 0.296
CB	1/1	2%/1%[2]	1.28	3495	3.55	4.00	-	0.00 ± 0.00	0.00 ± 0.00	0.00	0.00	0.000 ± 0.000
	1/3	0.67%/0.33%	1.82	1777	1.18	1.33	-	0.00 ± 0.00	0.00 ± 0.00	0.00	0.00	0.000 ± 0.000
	1/5	0.4%/0.2%	2.42	1175	0.71	0.80	-	0.00 ± 0.00	0.00 ± 0.00	0.00	0.00	0.000 ± 0.000
	1/10	0.2%/0.1%	3.33	653	0.36	0.40	-	0.00 ± 0.00	0.00 ± 0.00	0.00	0.00	0.000 ± 0.000
	1/20	0.1%/0.05%	4.69	446.5	0.18	0.20	-	36.67 ± 20.82	45.83 ± 26.02	9.32	4.27	0.013 ± 0.013
	1/30	0.067%/0.033%	4.9	312.6	0.12	0.13	-	50.00 ± 10.00	62.50 ± 12.50	15.93	9.96	0.290 ± 0.081
	1/50	0.04%/0.02%	5.4	238.7	0.07	0.08	-	70.00 ± 10.00	87.50 ± 12.50	16.55	14.49	0.310 ± 0.073
CCB[5]	1/1	3%	0.22	5818	1.78	3.41	4.59	0.00 ± 0.00	0.00 ± 0.00	0.00	0.00	0.000 ± 0.000
	1/3	1%	0.95	2510	0.59	1.14	1.53	0.00 ± 0.00	0.00 ± 0.00	0.00	0.00	0.000 ± 0.000
	1/5	0.6%	1.55	1750	0.36	0.68	0.92	0.00 ± 0.00	0.00 ± 0.00	0.00	0.00	0.000 ± 0.000
	1/10	0.3%	2.6	995	0.18	0.34	0.46	0.00 ± 0.00	0.00 ± 0.00	0.00	0.00	0.000 ± 0.000
	1/20	0.15%	3.7	543	0.09	0.17	0.23	43.33 ± 5.77	54.17 ± 7.22	7.31	3.96	0.303 ± 0.088
	1/30	0.1%	4.5	198	0.06	0.11	0.15	63.33 ± 11.55	79.17 ± 14.43	13.25	10.49	0.407 ± 0.094
	1/50	0.06%	4.83	233	0.04	0.07	0.09	73.33 ± 5.77	91.67 ± 7.22	13.45	12.33	0.543 ± 0.123

*[1]concentrations refer to the preservative solution in the respective dilution.
[2]2% boric acid and 1% copper sulfate.
[3]AG = Absolute Germination Index; RG = Relative Germination Index; RRG = Relative Root Growth; GI = Relative Germination Index.
[4]CCB = chrome, cooper, boron.
[5]SB = Sodium octaborate tetrahydrate.

The dilutions tested for the same preservative did not show statistical differences between themselves ($p > 0.05$), showing that a possible dilution without impact on seed germination was not achieved.

Figure 1 – Absolute Germination Index of lettuce seeds compared to preservatives in concentrations below 10% of those used in the treatment of bamboo



The RG analyzes the percentage of seeds germinated in the solutions compared to the control (Table 1), where it showed statistical differences between all groups ($p < 0.05$). Higher index values are observed in the most diluted samples: around 90% for CCB and CB and 45% for SB, showing that it is more toxic to seed germination. This fact can be explained by the higher concentration of boron when compared to the other groups: for the same 1/50 dilution, SB has 250 mg/L of boron, a concentration higher than three times when compared to CB and more than five times in relation to the CCB. It is also noteworthy the greater toxicity in the germination of CCB when compared to CB, a fact that may be related to the presence of chromium in the CCB.

Rodrigues *et al*. (2016) analyzed the toxicity of the herbicide glyphosate (Roundup), an agricultural pesticide widely used in agriculture, whose action causes enzyme inhibition (EPSPS) on plant. In view of the lettuce seeds, this herbicide in its highest concentration (382.70 mg./L) had around 60% of RG. This demonstrates the negative impact of these substances on the germination process of the seeds.

The RRG evaluates the growth of the radicle compared to the control group and the highest values are observed in the most diluted preservatives. However, they were less than 55%, presenting the effect "Inhibition on root growth" (RRG <80) and indicating changes in plant metabolism (LUMBAQUE *et al*., 2017). Similarly, Roundup´s RRGs values vary from around to 40% at higher concentration to 80% at the lowest concentration (3.60 mg/L) (RODRIGUES *et al*., 2016). The highest RRG value for preservatives was 53.54% for SB at the highest dilution (1/60). A similar result was found by Silva and Mattiolo (2011) in the tests with potassium dichromate (50 mg/L). It is a compound used in antifungal treatment of wood, with acute toxic characteristics.

The concomitant analysis of RG and RRG (Table 1) shows that the radicle development of plants is more sensitive than germination, with roots generally more vulnerable to allelochemical substances than other seedling structures, such as seeds. The allelopathic effects do not occur on germination in the physiological sense, but, in the development of the plant, primarily at its root (GONÇALVES *et al*., 2016). The roots are responsible for the absorption of bioactive compounds present in the environment (MELO *et al*., 2016).

The highest values of GI are also observed in the most diluted preservatives: 12.33% to CCB at 1/50,14.49% to CB at 1/50 and a greater value in SB at 1/60 with 24.54%. According to Kohatsu's classification (2018), they are very phytotoxic (GI <30) and, thus, interfere in the physiological processes of lettuce germination.

Corroborating that was yet presented, the radicle length of the germinated seed in front of the solutions also pointed out difficulty in the root development. The highest mean radicle length occurred in the most diluted SB solution (0.683 mm ± 0.296 mm), a value 4.83 times lower than that observed in the control group (3.303 mm ± 0.649 mm), highlighting the toxic action of these compounds for the plant. Potassium dichromate at 50 mg/L gave a similar result (0.3 mm ± 0.2 mm) (SILVA and MATTIOLO, 2011). In the statistical analysis of the radicle length, some groups did not pass the normality test and it showed a statistical difference between all groups ($p < 0.05$). Besides that, there was difference between all groups in relation to control group ($p < 0.05$).

When germinative capacity (AG and RG) is compared with seedling development (RRG and radicle length): it is noted that in the SB treatment the seeds suffered a negative effect on the germination process and, at the same time, the seedlings developed better when compared to the other treatment groups. This stimulus was probably due to hormesis, a phenomenon that occurs when a toxic substance in large quantities is, in a way, useful to organisms that are in an optimal situation. In sodium octoborate tetrahydrate, there are micronutrients present necessary for the development of the plant, such as sodium and boron. However, this does not indicate that the effluent is not toxic (BARBOSA, 2017). For the CB and CCB groups, the opposite occurred: there was a higher germination index with less seedling growth, which may be due to the presence of copper (absent in SB).

The observed effects are due to the concentrations of elements above the limits. At appropriate levels, boron and copper play a positive role in plants. Boron in the soil occurs as boric acid and borate and helps vegetables transporting sugars from the leaves to the other organs, formation of cell walls, cell division, synthesis of lignin and cellulose, root absorption, among others. The lack of this element appears in the new leaves, which are deformed, small and with rounded edges (GUPTA, 1979; MALAVOLTA *et al*., 1997). In the other hand, copper helps the plant in its metabolism, acting in the control of DNA and RNA synthesis, and participating in various physiological processes, such as photosynthesis, respiration and metabolism of carbohydrates, lipids and nitrogen (MELO, 2016).

Chrome is not considered essential for plant nutrition, being classified as a toxic element. However, there are studies that indicate that, in minimal amounts, this element can assist in nitrogen fixation and nutrient absorption in plants (SOUSA, 2018).

The measured results of pH and electrical conductivity (CE) of the preservative solutions shows that the values of the two variables decreased as the dilution increased, confirming Beltrão *et al*. (1997) when they state that increases in conductivity lead to a decrease in the production of dry lettuce material.

Costa *et al*. (2001) related the productivity of L. sativa in mediums with different values of conductivity and they could perceive a better development of the plant occurred with CE around 2.46 mS/cm, value close to the CE of SB groups at 1/5 dilution (2.30 mS/cm), CB to 1/3 (2.51 mS/cm) and CCB to 1/3 (1.78 mS/cm). However, in these three dilution groups all the germination indexes were zero. The solution prepared by Costa *et al*. (2001) had its composition balanced with twelve elements, like Nitrogen and Potassium, which are essential to the development of the plant, unlike bamboo treatment solutions, in which there are high concentrations of few elements (Bo, Cr and Cu), bringing toxicity primarily by the action of the high availability of the element than by salt stress.

Under extreme conditions of acidity or alkalinity, germination and plant development are negatively affected. Inhibition and decrease effects on root growth were not manifested at pH less than 3.5 or greater than 6.4 in Koszo *et. al*. (2007). CONAMA Resolution nº 430 establishes that the pH of the effluent must be between 5 and 9. However, the results found in this work did not allow the relationship of pH values with germination indexes, since for SB, in which the values were less acidic, there was inhibition similar to that of the other groups in which had high acidity. Besides that, Koszo *et. al*. (2007) also states that the amount of the element had greater interference in the environment when compared to the Ph values because the response of plants to metals is dose dependent. At the same pH value, different solutions did not change the germination capacity of the seeds as they did with others. For electrical conductivity, germinations only occurred for groups with values less than 543 uS/cm. For values higher than this, germinations were inhibited.

4 CONCLUSIONS

The phytotoxicity tests allowed to evaluate the effects on germination and growth of *L. sativa* seeds when exposed to three preservatives routinely used for bamboo treatment: CCB (chrome, cooper, boron), Sodium octaborate tetrahydrate (SB) and CB (cooper, boron).

It was observed that with the three tested products, both the germination of the lettuce seeds and the growth of the plant's roots were totally inhibited in dilutions up to 10% of concentrations used in the treatment. In addition, even after being diluted 50 times in relation to the concentration used by bamboo professionals, damage was observed in germination and root growth. Thus, the disposal of these effluents may cause negative effects on the vegetation both in the soil and in the water body, which makes a previous treatment of the effluent or greater dilutions necessary to avoid environmental imbalances.

The phytotoxicity tests performed are unprecedented with bamboo preservatives. In addition to showing its high toxicity, it allows future work on the effect on other living beings and it encourages studies of preservative treatments before their disposal in the environment.

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