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Effect of Copper, Zinc, Cadmium and Chromium in the Growth of Crambe

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Abstract

Crambe is a promising crop for biodiesel production. However, there is no much information available about techniques and research regarding the best performance in different regions to explore its potential. The objective of this study was to evaluate the effect of cadmium, chromium, copper and zinc in the development of crambe. The study was carried out in a greenhouse and it consisted of four independent experiments in completely randomized design, with three replications. Four metals with five levels were applied: 0, 10, 15, 20 and 25 mg·kg^{-1} for copper, cadmium, chromium; and 0, 20, 30, 40 and 50 mg·kg^{-1} for zinc, in order to evaluate the growth of crambe. Data were subjected to analysis of variance and comparison of means by Tukey test at 1 and 5% significance. Despite doses of cadmium, chromium, copper and zinc in growing crambe have influenced in some of the evaluated variables of the plants, in general, their development was similar in relation to doses and the metals applied. The importance of cultivating crambe is related to grain production, based on this, copper and cadmium decreased this production.

Keywords

Heavy Metals, Biodiesel, Oleaginous

1. Introduction

Crambe (*Crambe abyssinica* H.), an oil plant of the cruciferous family, is native of the Mediterranean region

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from Ethiopia to Tanzania, however, it is cultivated in tropical and subtropical regions. Crambe is characterized by being a shrubby plant, short cycle, which blooms once in 35 days and can be harvested between 90 and 95 days with uniform ripening, presenting good production. According to [1], crambe, planted in the "off-season" is an excellent alternative to crop rotation, because it has short cycle, great drought tolerance, hardiness, earliness and mechanized farming, by using the same equipment used for traditional crops grain, maximizing the use of machinery and equipment.

The crambe plant is considered robust, and develops in antagonistic weather conditions, has great tolerance to drought and frost [2]. Contrasting with climate rusticity, crambe is demanding in soil fertility, does not tolerate soil acidity, presenting the best productions in eutrophic soil [3]. According to [4], crambe takes the residual fertilizer left by summer crops, and may increase production with moderate fertilization although there are still no specific recommendations for the crop.

In recent years, this crop has become increasingly important in Brazil because of its suitability for industrial production of biofuels [5], because of its high potential lubricant and oil content. The percentage of the total oil in grains is up to 38%, varying between 30% and 45% depending on climatic and soil conditions [6]. Thus, the use of crambe for the production of cleaner fuels means an important alternative to supplement energy sources more sustainable and less polluting. Moreover, crambe oil cannot be used for human consumption due to the presence of high levels of erucic acid, a monounsaturated long chain fatty acid [7].

Most heavy metals are essential to human beings, animals and higher plants, for example, Co, Cu, Mn, Zn, Ni, among others, except Cd, Hg and Pb. These metals tend to be present in higher concentrations in the upper soil layers, which are a reflection of the addition of the element via atmospheric deposition, application of phosphate fertilizers, humus and also the incorporation of plants used as accumulators of metals [8]. The availability of the heavy metals for plants is governed by several soil factors such as pH, cation exchange capacity, organic matter content, adsorption by clays and phosphorus, calcium and the presence of other metals in the soil system [9].

In general, the plants readily absorb small concentrations of Cd, Cr, Cu and Zn dissolved in soil solution in ionic or chelated form, or in the form of complexes; [10]. The transport of cadmium from roots to leaves is directly proportional to the external concentration of this element. However, the translocation of cadmium from leaves to fruit is low and [11] showed that the cadmium was concentrated more in roots than aerial parts of species such as soybean, carrots, alfalfa, corn and tomato. The absorption and translocation of chromium are very low, although this varies according to the species considered. Because of their affinity for negative charges, chromium is immobilized, mainly in the roots and not on the root surface. The chromium levels in the shoots are therefore very low (0.02 to 1 $mg \cdot kg^{-1}$) and rise only slightly when symptoms of toxicity appear [12].

Copper is absorbed in ionic form and high concentrations of phosphorus, molybdenum and zinc decrease the process that is considered active; translocation occurs within the plants in both the xylem and phloem, which is surrounded by the metal organic nitrogen compounds such as amino acids [13]. Copper is a relatively immobile element in plants. Thus, green leaves can accumulate high concentrations of copper and subsequently not release it to younger leaves and other tissues, such as inflorescences [13]. Some researchers agree with the idea that root uptake of Zn is active, while in the roots about 90% of Zn occur in the exchange sites or absorbed into the walls of the cortical parenchyma cells.

The mobility of zinc in plants is not large. Normally, the roots contain much more zinc than the shoot, especially if the plants are growing in soils rich in zinc [14] [10]. These metals can express their polluting potential directly on soil organisms, by the availability to plants in phytotoxic levels, besides the possibility of transfer into the food chain through plants themselves or the contamination of surface and subsurface water [15] [16].

Due to the growing interest in the cultivation of crambe in Brazil for biodiesel production, the objective of this work is to evaluate the effect of cadmium, chromium, copper and zinc in the development of crambe.

2. Materials and Methods

The study was carried out from August to November 2012 at the Agricultural Engineering Department of the Federal University of Campina Grande, Campina Grande, Paraíba, Brazil. Temperatures ranged from approximately 32˚C during the day to 27˚C during the night. The pluviometric precipitation was around 700 mm annually.

Each experimental unit consisted of a plastic pot filled with 9.0 kg of a Eutrofic Regosol with the following attributes: sand = 845.6 $g \cdot kg^{-1}$; silt = 47.2 $g \cdot kg^{-1}$; clay = 107.2 $g \cdot kg^{-1}$; pH (H_2O) = 5.0; Ca^{2+} = 0.51 $cmol_c \cdot kg^{-1}$;

Mg^{2+} = 0.20 $cmol_c \cdot kg^{-1}$; Na = 0.05 $cmol_c \cdot kg^{-1}$; K^+ = 0.18 $cmol_c \cdot kg^{-1}$; $H^+ + Al^{3+}$ = 0.56 $cmol_c \cdot kg^{-1}$; organic matter = 0.2 $g \cdot kg^{-1}$; P = 5.4 $mg \cdot kg^{-1}$. The analyses procedures used were those recommended by [17].

The research consisted of four independent experiments, following a completely randomized design to evaluate the performance of four metals (zinc—Zn; Copper—Cu, cadmium—Cd, and chromium—Cr) in five doses, with three replications, totaling 15 experimental units for each metal. The solutions of Zn, Cu, Cd and Cr used as pollutant sources were prepared at concentrations of 0; 10; 15; 20:25 $mg \cdot kg^{-1}$ for Cu, Cd and Cr. For Zn, de concentrations were 0; 20; 30; 40 and 50 $mg \cdot kg^{-1}$. All the solutions were made from Tritisol standard 1000 ppm solution of each metal.

Each experimental unit was fertilized with 10 g of NPK (15:9:20) containing 166.7 mg $N \cdot kg^{-1}$, 100 mg P_2O_5 kg^{-1} and 22.2 mg K_2O kg^{-1}. Then, each pot was irrigated with solutions of Cu, Cd, Cr and Zn, according to the treatment, and distilled water in order to retain the moisture corresponding to 80% of field capacity. In order to provide an opportunity to the soil interact with the metal added, the experimental units remained incubated for 8 days under the same conditions.

Crambe seedlings were prepared in plastic cups with a capacity of 250 ml with substrate for plants using seeds without pericarp. After 8 days after germination (DAG), the seedlings were transplanted to the pots. Ten days after transplanting, the seedlings were harvested and only two plants per pot remained. They were irrigated daily in order to keep the moisture close to field capacity.

Plant height was measured with a graduated ruler, the stem diameter was measured with a digital caliper, and the counting of branches was done at 90 DAG. At the end of the experiment, during the harvest, 100 DAG, the plants were harvested, separated into leaves, stems and roots, washed in water and dried in an oven with forced air circulation at 65˚C until constant weight. The variables evaluated were: plant height, stem diameter, number of branches, dry weight of stem, leaves, shoot and root, root/shoot ratio and grain weight.

The results were analyzed statistically through the analyses of variance (ANOVA) described by [18].

3. Results and Discussion

Table 1 presents the results of analysis of variance of plant height (PH), number of branches (NB), dry weight of leaves (DWL), dry weight of stems (DWSt), dry weight of shoots (DWSh), dry weight of roots (DWR), ratio of root weight by shoot weight (RW/SW) and dry weight of grains (DWG) as a function of doses of cadmium, chromium, copper and zinc. Increasing levels of metals significantly influenced the level of 5% the number of branches and dry weight of grains for cadmium; dry weight of leaves for chromium; plant height, number of branches and dry weight of grains for copper. For zinc, it was significant at 1% in number of branches. The growing importance of crambe is related to the production of grains. Based on this, it can be verified in **Table 1** that only Cd and Cu presented a significant effect for the variable dry weight of grains.

The coefficients of variation (**Table 1**) found in this study ranged from 5.53% to 9.60%, meaning homogeneity and low dispersion between the data. For most variables the coefficients of variation ranged from 10.54 to 19.98% which means average dispersion among the data. The coefficients of greater than 20% and less than or equal to 30% range are considered high, indicating high dispersion of data; and above 30%, which is the case in root dry weights for all elements variable, are considered too high, according to the proposal by [18]. This very high dispersion of data roots may be due to the difficulty of recovering all the roots of the plant, since they had very thin and fragile, causing loss of material during washing.

Increased doses of Cd in the soil caused a decrease in the number of branches of plants and the dry weight of the grain (**Figure 1**). Regarding to the foliage, it was observed that the leaves became large and thick and stunted branches of crop, promoting greater dry weight of leaves. Probably because of this, the number of branches decreased and the grain formation was inhibited or promoted stunted grain, reducing the weight in relation to the increasing levels of Cd.

In plants, the presence of Cd affects absorption, transport and use of macronutrients, such as calcium, phosphorus, potassium [19] [20], nitrate [21] and sulfur [22], as well as trace elements such as iron [23] and chlorine [24]. Due to these effects, the presence of Cd also affects plant growth.

In relation to chrome, only significant effect at 5% probability on the dry weight of the leaves of crambe whose data were best fitted to a quadratic model (**Figure 2**).

In general, the Cr absorbed by plants is accumulated in the roots presenting low mobility in plants. Although no determined the concentration of Cr in plant tissues, it can be inferred that the Cr accumulated in the leaves,

Table 1. Summary of analyzes of variance for the data of plant height (PH), number of branches (NB), dry weight of leaves (DWL), dry weight of stems (DWSt), dry weight of shoots (DWSh), dry weight of roots (DWR), ratio of root weight by shoot weight (RW/SW) and dry weight of grains (DWG) as a function of doses of cadmium, chromium, copper and zinc.

		Mean squares							
Source	DF	PH	NB	DWL	DWSt	DWSh	DWR	RW/SW	DWG
				Cadmium					
LEV	4	368.69^{ns}	200.14^{*}	1.75^{ns}	0.29^{ns}	2.47^{ns}	0.05^{ns}	0.0001^{ns}	2.62^{*}
Lin.		-	358.13^{*}	-	-	-	-	-	8.81^{**}
Qua.		-	342.42^{*}	-	-	-	-	-	0.004^{ns}
ERROR	10	266.98	45.46	0.46	0.99	2.42	0.07	0.0002	0.46
CV (%)		31.18	16.20	19.66	19.98	18.43	30.05	14.96	20.63
GM		52.40	41.63	3.47	4.98	8.45	0.91	0.106	3.30
				Chromium					
LEV	4	18.55^{ns}	84.85^{ns}	0.74^{*}	0.57^{ns}	2.53^{ns}	0.04^{ns}	0.0001^{ns}	0.29^{ns}
Lin.		-	-	0.34^{ns}	-	-	-	-	-
Qua.		-	-	2.57^{**}	-	-	-	-	-
ERROR	10	35.95	26.81	0.22	0.62	1.45	0.05	0.0005	0.28
CV(%)		8.81	11.16	20.38	19.82	19.14	37.68	23.84	11.76
GM		68.03	46.40	2.31	3.97	6.29	0.60	0.094	4.51
				Copper					
LEV	4	55.85^{ns}	98.01^{*}	0.88^{ns}	1.14^{ns}	3.78^{ns}	0.05^{ns}	0.0001^{ns}	0.73^{*}
Lin.		50.17^{ns}	342.96^{**}	-	-	-	-	-	0.59^{ns}
Qua.		1.30^{ns}	9.53^{ns}	-	-	-	-	-	2.31^{**}
ERROR	10	14.25	22.43	0.26	0.49	1.29	0.04	0.0005	0.19
CV (%)		5.53	10.03	23.14	19.01	19.17	34.84	22.52	9.60
GM		68.23	47.20	2.23	3.71	5.94	0.61	0.102	4.54
				Zinc					
LEV	4	23.30^{ns}	223.22^{**}	0.74^{ns}	0.44^{ns}	2.23^{ns}	0.04^{ns}	0.0001^{ns}	0.05^{ns}
Lin.		-	184.06^{**}	-	-	-	-	-	-
Qua.		-	658.50^{**}	-	-	-	-	-	-
Cub.		-	14.48^{ns}	-	-	-	-	-	-
ERROR	10	89.66	16.78	0.37	0.79	2.11	0.06	0.0004	0.30
CV (%)		14.52	10.11	26.97	23.13	23.70	42.59	23.73	12.09
GM		65.20	40.53	2.27	3.86	6.13	0.59	0.09	4.56

**; * and ^{ns}: Significant at 1 and 5% of probability, and no-significant, respectively. LEV = levels; CV = coefficient of variation; GM = general mean.

since increased levels of this element influencing their development [25]. Crambe plants grown in soil supplied, on average, with 13 mg Cr kg^{-1}, resulted in a 20% reduction in dry weight of leaves compared to control. Although some studies show that Cr produce some stimulatory effects on plant growth [26] [27] its essentiality has not been proven. As [28] the reduction in leaf growth of tomato plants, due to the presence of chromium, just started to occur at concentrations greater than 100 mg Cr^{+3} L^{-1}.

The number of branches and the weight of the grains of crambe were influenced at 5% probability with increasing levels of copper (**Table 1**). The data of these variables were best fitted to models linear and quadratic, respectively (**Figure 3**).

The presence of branches is responsible for having the function of sustaining the fruits besides being a source of minerals and assimilates drain. Therefore, reducing the number of branches can increase grain yield by reducing the sap to drain them, as can be seen in **Figure 3**. For the dry weight of grains there was a small increase

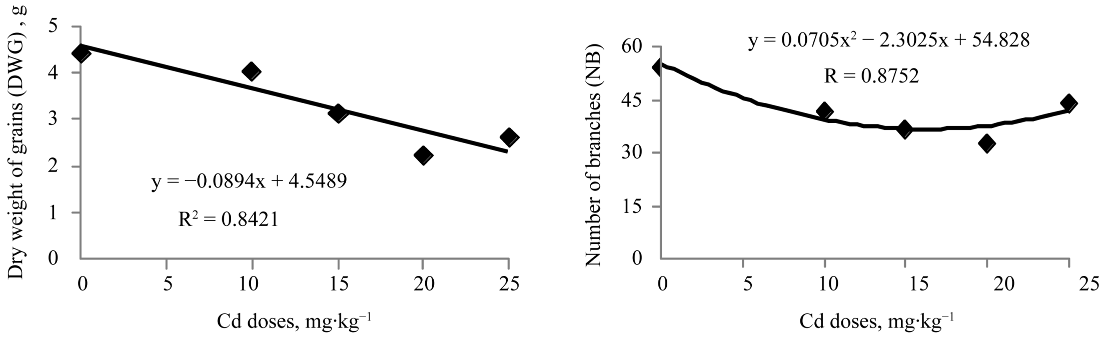

Figure 1. Dry weight of grains and number of branches of crambe according to the doses of cadmium.

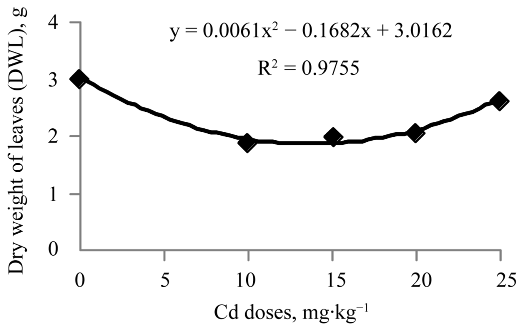

Figure 2. Dry weight of leaves of crambe according to the doses of chromium.

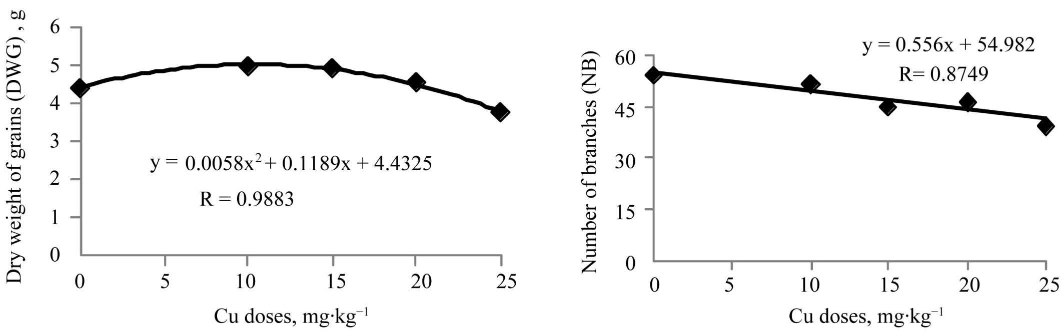

Figure 3. Dry weight of grains and number of branches of crambe according to the doses of copper.

in the first dose of Cu compared to the control (4.45 g per plant) decreasing with increasing doses to 3.76 g per plant.

Even with the aggravation of soil contamination, results are superior to those obtained by [29] evaluated the effect of base saturation in the development and yield of crambe grown in medium textured soil where found 2.22 g per plant. On the other hand [30], evaluating organic fertilization in crambe achieved a mean grain weight of 5.64 g per plant. Increasing levels of Zn influenced the 1% probability the number of branches of crambe (**Table 1**), whose data of this variable were best fitted the quadratic model (**Figure 4**). There was a tendency to decrease compared to control up to level 20 mg Zn kg^{-1}, returning to growth in higher levels studied, up to 50 $mg{\cdot}kg^{-1}$, reaching 44 branches. However, this number is still lower than the control (54) within the range cited by [31], in which the plant crambe has 30 or more branches.

In general, small changes in the data for plant height (from the control to highest level) were similar for all metals (**Table 2**). Only with the increase in cadmium levels, there was a trend to reduce plant height. However, data of crambe height, reported in **Table 2**, were lower than some results reported in the literature.

According to [31] the height of crambe can reach up to 100 cm; however, [32] mentioned that the height va-

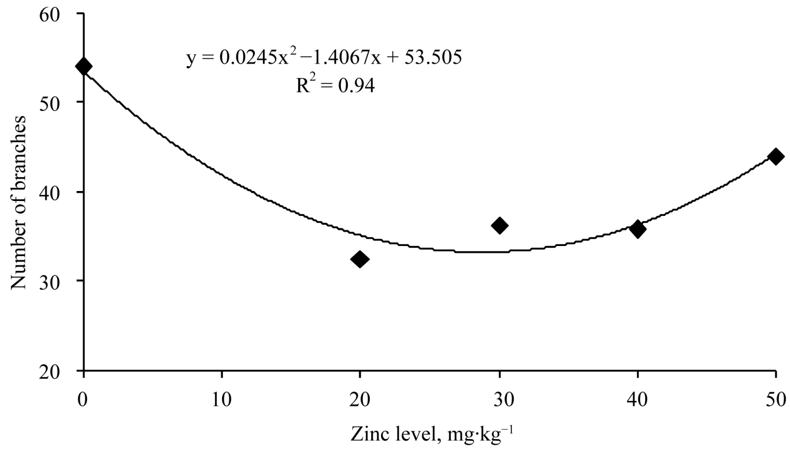

Figure 4. Number of branches according to zinc level in the planting of crambe.

Table 2. Average data of plant height (PH), stem diameter, number of branches (NB), dry weight of leaves (DWL), dry weight of stems (DWSt), dry weight of shoots (DWSh), dry weight of roots (DWR), ratio of root weight by shoot weight (RW/SW) and dry weight of grains (DWG) as a function of doses of cadmium, chromium, copper and zinc.

Doses	PH	NB	DWL	DWSt	DWSh	DWR	RW/SW	DWG
$mg \cdot kg^{-1}$	cm		g/plant	g/plant	g/plant	g/plant		g/plant
				Cadmium				
0	66.17	54.00	3.03	4.47	7.50	0.80	0.10	4.45
10	60.00	41.50	2.88	5.12	8.01	0.93	0.11	4.05
15	50.00	36.50	3.17	5.26	8.42	0.84	0.10	3.14
20	37.50	32.50	4.78	5.16	9.93	1.14	0.11	1.63
25	48.33	43.67	3.53	4.91	8.43	0.87	0.10	2.64
				Chromium				
0	66.17	54.00	3.03	4.47	7.50	0.80	0.10	4.45
10	66.50	42.33	1.86	3.36	5.22	0.48	0.09	4.46
15	67.67	40.67	2.00	3.91	5.90	0.58	0.10	4.81
20	72.33	46.17	2.06	3.82	5.88	0.58	0.10	4.05
25	67.50	48.83	2.64	4.33	6.97	0.60	0.09	4.81
				Copper				
0	66.17	54.00	3.03	4.47	7.50	0.80	0.10	4.45
10	62.17	51.50	1.52	3.13	4.66	0.43	0.09	4.99
15	73.00	44.83	2.30	4.05	6.35	0.64	0.10	4.94
20	71.50	46.17	2.27	3.89	6.16	0.68	0.11	4.57
25	68.33	39.50	2.05	3.02	5.07	0.54	0.11	3.76
				Zinc				
0	66.17	54.00	3.03	4.47	7.50	0.80	0.10	4.45
20	61.83	32.50	1.76	3.75	5.51	0.49	0.09	4.48
30	68.00	36.33	2.48	4.01	6.49	0.55	0.08	4.78
40	62.67	35.83	1.98	3.50	5.47	0.59	0.10	4.62
50	67.33	44.00	2.12	3.62	5.74	0.54	0.09	4.48

ries between 70 and 90 cm. Almeida *et al*. [33] and [34] studied the cultivation of crambe in the field, under different fertilization and found the greatest heights of 123 cm and 109.54 cm, respectively. Santos *et al*. [35] analyzed different levels of water for irrigation of crambe and observed the largest height of 107.63 cm.

Carvalho *et al*. [36] found inferior results to the present study *i.e*., they observed 45.25 cm of plant height grown in the Oxisol with 75% base saturation and [37] observed 58 cm height plant grown for different spacing in Hapludox Haplortox.

According to [32], the height varies depending on the time of planting and plant density. In the present study, the decrease in plant height may have been due to plant fertilization, and/or the effect of metals and/or because the planting season (August to November) which is related to climate, *i.e*. plants were developed at a higher ambient temperature than in other traditional growing regions of crambe (April or May).

Increasing levels of metals significantly influenced the number of branches, with the exception of chromium (**Table 1**). The average of number of branches ranged from 40.53 for Zn to 47.20 for Cu, values within cited by [31], in which the plants presented 30 or more branches.

The dry weight of the leaves ranged from 3.03 g to 2.05 g, and 3.03 g to 2.12 g, corresponding to increasing levels of Cu and Zn, respectively, being not affected by application of these elements in growing crambe, corroborating [38] that analyzed increasing doses of zinc in bean plants.

The dry weight of the stems as well as the production of dry matter of shoots and roots of crambe was not affected by the application of all doses of Cd, Cr, Cu and Zn to the soil, whose values were statistically similar to those obtained in the absence these metals (**Table 2**). Tito *et al*. [38] analyzing increasing doses of zinc in beans, also found no significant effect on the weight of the roots. However, it had a significant effect on the dry mass of stems and grains, corroborating [39] that researched about corn, and disagreeing with [40] for the cultivation of sorghum. Regarding Cr, [41] observed that the reduction in dry matter of shoots and roots of soybeans occurred at 40 mg Cr^{+3} L^{-1}. *i.e*. up to this dose, chromium does not affect these variables of the plant. Observing the values of the dry weight of stems, dry matter production of shoots and roots even without statistical analysis, apparently, the values corresponding to increasing levels of Cd were higher than with the application of Cr, Cu and Zn, disagreement with [38] who observed lower biomass production of beans in the presence of Cd compared to Cu and Zn.

The application of metals to soil did not affect root/shoot crambe plants ratio (**Table 1**), corroborating [29]. The behavior observed in this ratio with increased levels of all elements used (**Table 2**) may be associated with similar roots and aerial parts of the plants.

The dry weight of the grains of cultivated plants with Cr and Zn were not affected by increasing doses of these elements, corroborating [30]. These authors, applying increasing doses of poultry litter in growing crambe, no significant effect of this material on the weight of the grains was observed, that ranged from 4.77 g (zero g of poultry litter) to 6.40 g (140 g poultry litter).

4. Conclusions

Although the dosages of cadmium, chromium, copper and zinc in growing crambe have influenced in some of the variables of the plant in general, their development was similar in both in relation to doses as the metal used. Increasing levels of metals influenced significantly number of branches, with the exception of the metal chromium, which showed no significant difference.

The production of grain was harmed only with copper and cadmium in the conditions adopted for this study.

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