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Photosynthetic and growth parameters of *Pfaffia glomerata* in zinc dose cultivation

Parâmetros fotossintéticos e de crescimento de Pfaffia glomerata *em cultivo com doses de zinco*

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ABSTRACT
The objective of the present work was to analyze the influence of zinc doses on biomass production, root biometry and photosynthesis in *Pfaffia glomerata* plants. A genotype of the species was used to obtain the seedlings through *in vitro* propagation. In a greenhouse, the plants were cultivated, using four doses of Zn as treatment: 0.5; 50 75 and 100 μM. The nutrient solutions were applied for 84 days, at the end of which photosynthetic parameters and biomass production were analyzed. From the root system, 25% were frozen for further analysis of root biometry. Data were subjected to statistical analysis using the Scott-Knott test with a 5% significance level. It was observed that the production of fresh biomass was reduced mainly in the aerial part for the treatments of 75 and 100 μM. Root length, surface area and volume were reduced in the 50 and 75 μM treatments. The transpiration and assimilation rate, stomatal conductance, water use efficiency, carboxylation efficiency, were the affected photosynthetic parameters. Visibly, the plants showed variation in leaf color. It is concluded that *P. glomerata* tolerates small excesses of Zn, managing to survive under these conditions, despite showing small changes in its growth and development.
Keywords: metals; mineral nutrition; plant stress.

RESUMO
O objetivo do presente trabalho foi analisar a influência de doses de zinco na produção de biomassa, na biometria radicular e na fotossíntese em plantas de *Pfaffia glomerata*. Foi utilizado um genótipo da espécie para obtenção das mudas por meio de propagação *in vitro*. Em casa de vegetação, realizou-se o cultivo das plantas, utilizando-se, como tratamento, quatro doses de Zn: 0,5, 50 75 e 100 μM. As soluções nutritivas foram aplicadas durante 84 dias, ao final dos quais foi feita a análise de parâmetros fotossintéticos e da produção da biomassa. Do sistema radicular, 25% foram congelados para posterior análise da biometria radicular. Submeteram-se os dados a análise estatística pelo teste de Scott-Knott, com 5% de nível de significância. Observou-se que a produção de biomassa fresca foi reduzida principalmente na parte aérea para os tratamentos de 75 e 100 μM. O comprimento, a área superficial e o volume de raízes foram reduzidos nos tratamentos de 50 e 75 μM. A taxa de transpiração e de assimilação, de condutância estomática, a eficiência no uso da água e a eficiência da carboxilação foram os parâmetros fotossintéticos afetados. Visivelmente, as plantas apresentaram variação na coloração das folhas. Conclui-se que *P. glomerata* tolera pequenos excessos de Zn, conseguindo sobreviver nessas condições, apesar de mostrar pequenas alterações em seu crescimento e desenvolvimento.
Palavras-chave: estresse vegetal; metais; nutrição mineral.

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INTRODUCTION

Zinc (Zn) is a plant nutrient required at low concentrations, but fundamental for many functions (BABAR, 2013). This metal is estimated to be a component of at least 300 enzymes, either as a structural or a catalytic element, participating in metabolic processes such as respiration, chlorophyll biosynthesis, protein and RNA synthesis, cell multiplication and pollen fertility (BROADLEY *et al.*, 2007; PRADO, 2008; GUPTA *et al.*, 2011). Due to increasing industrial, agricultural and urban expansion, soil Zn concentrations are increasing, resulting in toxic levels to plants, and this can affect human health by the consumption of these species (WUANA & OKIEIMEN, 2011).

The stress caused to plants, due to excess Zn in the soil, may reduce the biomass production and growth, because this element is directly linked to the cell division process, causing a delay in root development and reducing pollen fertility (BROADLEY *et al*., 2007; LI *et al.*, 2013). Physiologically, the increase in Zn results in a higher production of reactive oxygen species (ERO's) and changes in the physiological process, as it acts on electron transport and chlorophyll biosynthesis (CHEN *et al.*, 2008; CAMBROLLÉ *et al.*, 2015). There may also be a nutritional imbalance of other nutrients, because the excess of Zn affects the electron transport chain, absorption and translocation of other metals (MAGALHÃES *et al.*, 2004; JIANG & WANG, 2008; WANG *et al.*, 2009). The manner in which the plant will be affected will depend on the species and its stage of development, as many plants have differential defense mechanisms to tolerate soil nutritional changes (LI *et al.*, 2012; MARQUES & NASCIMENTO, 2014).

Medicinal plants suffer directly from metal toxicity (ANNAN *et al*., 2013) and, due to the increasingly frequent consumption of medicinal species, it may result in human intoxication. Medicinal species include *Pfaffia glomerata* (Spreng) Pedersen (Amaranthaceae), native to Brazil (GOSMANN & RATES, 2003). This species is popularly known as Brazilian ginseng because of its use as a substitute for Korean ginseng *Panax ginseng* (VIGO *et al*., 2004; SOUZA & LORENZI, 2005). Its extracts are medicinally characterized by their tonic, aphrodisiac, adaptogenic (anti-stress), antioxidant, tranquilizing and anti-inflammatory properties (FREITAS *et al.*, 2004; MARQUES *et al*., 2004; VIGO *et al*., 2004; NETO *et al*., 2005; SOUZA *et al*., 2005). Such characteristics are due to the chemical composition of the extract of *Pfaffia glomerata*, as it presents glomeric acid (triterpenoid), pfameric acid (nortriterpenoid), β-ecdisone, rubesterone, oleanolic acid and β-glucopyranosyloleanate (SHIOBARA *et al*., 1993).

P. glomerata has been used also for studies on the effects of metals on its development. It has been reported that the species showed moderate tolerance to metals such as mercury (Hg) and copper (Cu) but, due to the wide genetic variability of the plant, this tolerance is not uniform in the species (CALGAROTO, 2009; KAMADA *et al*., 2009; NEIS, 2013). *P. glomerata* has been shown to react negatively at Zn levels of 200 μM, mainly with respect to biomass production and root development.

The objective of this work was to analyze the growth, root development and photosynthetic parameters of *P. glomerata* cultivated with different doses of zinc.

MATERIAL AND METHODS

In this experiment, a *Pfaffia glomerata* genotype called GD was used. This genotype belongs to the collection of medicinal plants of the Federal University of Grande Dourados (UFGD), located in Dourados, State of Mato Grosso do Sul (MS) in Brazil, and is deposited in the Herbarium DDMS (exsicata number 1951).

In vitro clonal propagation was performed, according to the protocol established by Nicoloso *et al.* (2001). One (1) centimeter of nodal segments of the genotype were propagated in MS culture medium (MURASHIGE & SKOOG, 1962) and supplemented with 6 g L^{-1} (grams *per* litre) of agar, 30 g L^{-1} of sucrose and 0.1 g L^{-1} of myo-inositol, for 21 days. The explants were placed for growth in an air-conditioned room, with a temperature of 25°C and photoperiod of 16 hours.

After obtaining the required number of seedlings, they were transplanted to pots lined with plastic bags, to reduce the loss of nutrient solution, and filled with 5 kg^{-1} of previously washed and sieved sand.

All pots were weighed with the dry sand and then their hydric saturation was performed to determine the weight that corresponded to 70% humidity, a condition imposed during the experiment.

During the first week of the experiment, all pots received a modified nutrient solution from Hoagland & Arnon (1950), for acclimation process; after this period, four plants were separated by treatment.

Four different nutrient solutions, containing 0.5 (standard), 50, 75 and 100 μM of zinc, were used.

The plants remained receiving the nutrient solutions in a greenhouse, under controlled conditions, for 84 days. Physiological parameters were analyzed: CO_2 assimilation rate (A), stomatal conductance (GS), internal CO_2 concentration (Ci) and transpiration (E), by means of a Li-Cor 6400XT portable analyzer.

The water use efficiency (WUE) and the instantaneous carboxylation efficiency (A / Ci) were also determined, both obtained by the ratio of the CO2 fixed in photosynthesis and the total water transpired.

The analysis of these parameters was started at 9 am on the third fully expanded leaf of each plant, in the following directions: from the apex to the stem base and from the stem base to the apex (one young leaf and one old leaf, respectively). Then, the number of leaves was counted and the plants were measured. After collecting the plants in the greenhouse, the leaves, stems and roots (previously washed and dried on filter paper) were weighed to obtain the fresh mass. The material was deposited in an oven at a temperature of 65ºC for drying until the dry mass was obtained.

Of the total roots harvested, 25% were frozen for root development analysis. After thawing at room temperature, the roots were digitized with the aid of an Epson 11000 XL scanner and the biometrics analysis was obtained with the support of WinRhizo Pro Software, through which it was possible to determine the total length, surface area, total volume and the average diameter of the roots.

All data were submitted to analysis of variance ($p < 0.05$) and by the means comparison test, of Scott & Knott (1974), at a 5% error probability level, with the aid of the statistical program Sisvar (FERREIRA, 2011).

RESULTS AND DISCUSSION

The nutrient zinc, present in plant cultivation, at higher than ideal concentrations, can cause changes in plant growth and development, reducing biomass production, altering the photosynthetic process and causing leaf chlorosis (BROADLEY *et al.*, 2007).

In this study, the results demonstrate that Zn affected only the stem and the leaf fresh mass. Concerning the stem, the interference occurred only at the highest concentrations (75 and 100 μM), and both did not differ from each other for the values found. The fresh mass of leaves showed a reduction in the Zn 50 and 75 μM treatments, which can be indicated by the reduction in the number of leaves in these treatments (table 1).

Table 1 – Growth parameters of *Pfaffia glomerata* cultivated under zinc doses.

TREATMENTS	MFR (grams plant^{-1})	MSR (grams plant^{-1})	MFC (grams plant^{-1})	MSC (grams plant^{-1})	MFF (grams plant^{-1})
Control	61.00a*	8.12a	65.50a	7.54a	43.50a
Zn 50µM	52.00a	8.12a	60.50a	6.50a	41.00a
Zn 75µM	55.00a	6.15a	53.00b	5.87a	41.00a
Zn 100µM	56.50a	7.89a	57.50b	7.13a	37.50a
AVERAGE	44.00	5.45	38.12	4.37	30.25
CV (%)	19.74	32.69	13.33	20.28	13.53
TREATMENTS	**MSF (grams plant^{-1})**	**MFT (grams plant^{-1})**	**MST (grams plant^{-1})**	**HEIGHT (cm^{-1})**	**LEAVES (number)**
Control	5.46a	170.00a	21.12a	137.30a	135.00b
Zn 50µM	4.65b	153.50a	19.27a	127.02a	130.50b
Zn 75µM	4.22b	145.50a	16.45a	128.25a	124.00b
Zn 100µM	5.41a	155.00a	20.43a	118.30a	164.00a
AVERAGE	4.13	112.37	13.96	92.10	106.72
CV (%)	16.11	14.02	21.19	15.71	13.77

* Averages followed by the same letter in the column do not differ from each other by the Scott & Knott test (1974) at a 5% error probability level. MFR = root fresh mass; MSR = root dry mass; MFC = stem fresh mass; MSC = stem dry mass; MFF = leaves fresh mass; MSF = leaves dry mass; MFT = total fresh mass; MST = total dry mass; CV = coefficient of variation.

In studies conducted by Frizzo (2017), *P. glomerata,* when cultivated with 200 and 400 µM zinc, showed a reduction in leaf and stem dry mass and total dry mass, and also during treatments, the GD access, the same genotype used in this study, showed tolerance to increase in zinc levels, with less reduction in biomass production, leaf area and chlorophyll concentration. The reduction in growth and the alteration of metabolic processes of species exposed to Zn can be explained by the fact that this metal is directly linked to the cell division process, and the plant response will depend on the species and on the level of stress caused (BROADLEY *et al.*, 2007; JAIN *et al.*, 2010).

Bernardy *et al.* (2016) observed, in hydroponic cultivation, after seven days of treatment, that this access showed an increase in biomass production in response to moderate doses of Zn (50, 36 and 40 µM), however, at 14 days, such responses decreased. Other authors have reported the increase in biomass production in relation to the moderate increase of zinc in other species, since it is a micronutrient and its presence can stimulate the growth of stress tolerant species, stressed by this metal (DISANTE *et al.*, 2010). In *Phyllostachys pubescens*, root dry mass in treatments from 10 to 100 µM is not affected, but, when there is an increase in doses (200 and 400 µM), the dry mass of the species was affected (LIU *et al.*, 2014).

Natale *et al.* (2002) demonstrated that high doses of Zn reduce the production of dry biomass of roots and shoots of passion fruit (*Passiflora edulis* Sims) seedlings. According to Malavolta *et al.* (1997), the reduction in biomass production may be explained by buffer accumulation in the xylem. These buffers contain Zn and make it difficult to lift raw sap.

In *Beta vulgaris* L., with increasing levels of Zn, there was a reduction in root dry mass, number of leaves and leaf area, in addition to the occurrence of visible symptoms such as chlorosis (SAGARDOY *et al.*, 2009), on the contrary of this study, where the highest concentration of Zn increased the number of leaves. However, visibly, it was possible to observe the variation in leaf color resulting from the increase in zinc concentrations (figure 1).

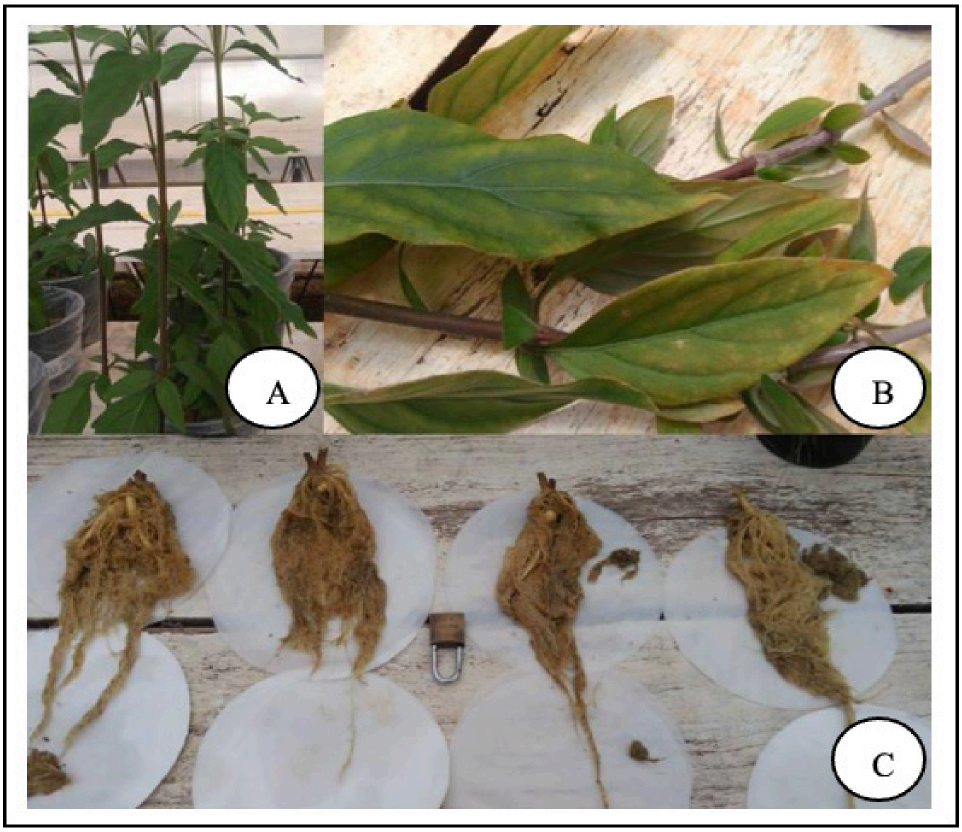

Figure 1 – Visual changes of *Pfaffia glomerata* grown with 100 μM zinc levels. (A) Leaves without changes in pigmentation; (B) Leaves colored red – orange; (C) Variations in root growth and development (from left to right: standard treatment, 50, 75 and 100 μM Zn).

The responses of *P. glomerata* to other metals have already been determined; thus, as for mercury (Hg) and aluminum (Al), these reduced the main growth parameters, unlike treatments with cadmium (Cd), where the species was not negatively affected (CALGAROTO, 2009; GOMES *et al.*, 2013; MALDANER *et al.*, 2015).

Regarding root biometry, the 50 and 75 μM treatments showed reduction in length, surface area and volume of roots of *P. glomerata*, not differing from each other (table 2). Root diameter was not changed between treatments. In studies by Frizzo (2017), GD access reduced surface area and volume only at 400 μM Zn level, while root diameter was affected, whereas in the present study lower concentrations have already changed the roots parameters and the highest level (100 μM) did not affect such characters. Bernardy *et al.* (2016) reported the same inhibitory effect on *P. glomerata* root growth, surface area and root volume in response to Zn treatments. The last authors demonstrated that the root system of the species is sensitive to Zn excess and this may be related to the fact that the roots are in direct contact with the stressor, and the reduction in root growth is one of the fastest responses to the excess of metals (DEGENHARDT & GIMMLER, 2000). In the *Arabidopsis thaliana* model plant, changes induced by cadmium, copper and zinc in the roots, were related to changes in the auxin/ catechin relationship, due to the functions exerted by these metals (SOFO *et al.*, 2013).

Table 2 – Root biometry of *Pfaffia glomerata* cultivated under zinc doses.

Treatments	Length (cm^{-1})	Surface area (cm^{-2})	Volume (cm^{-3})	Diameter (mm)
Control	8146.08a*	1055.27a	10.93a	0.82a
Zn 50µM	5625.80b	707.36b	6.88b	1.10a
Zn 75µM	6397.05b	703.79b	6.17b	1.31a
Zn 100µM	7640.50a	929.28a	9.03a	1.06a
Average	5181.6	629.92	6.13	0.84
CV (%)	22.90	24.50	26.28	26.81

* Averages followed by the same letter in the column do not differ from each other by the Scott & Knott test at a 5% error probability level. CV = coefficient of variation.

In *Phyllostachys pubescens,* low levels of Zn also decreased root growth, whose response by 100 µM was higher because the roots were much shorter in this treatment, no differences in responses in treatments between 100 and 400 µM being noticed and low levels (10 µM) increased root parameters considerably (LIU *et al.*, 2014). Similar results were found in seven rice varieties (*Oryza sativa* L.) as, in these varieties, the length, average diameter, surface area and volume increased at low Zn concentrations, but decreased as these concentrations increased (SHARIFIANPOUR *et al.*, 2014).

Assimilation rate (A), stomatal conductance (GS), CO_2 concentration (Ci), transpiration rate (E), water use efficiency (WUE) and carboxylation efficiency (A\Ci) correspond to the physiological parameters and were measured on an upper and lower leaf of each plant (table 3). In general, the parameters were in better situation when measured in the upper leaf in the control treatment, being these leaves the youngest. This result is justified by the intense photosynthetic activity of these organs.

The assimilation rate was higher in upper leaves, with greater influence of 50 µM Zn treatments on both (upper and lower leaves). Regarding stomatal conductance, the lower leaves reduced values independently of the applied treatments and, among the upper leaves, all levels of Zn reduced GS, with 100 µM being the most reduced. The results found for CO_2 concentration differed only when analyzing the origin of the leaves, since the upper leaves were more efficient than the lower leaves, but the results did not differ between treatments. Treatment of Zn 50 µM in lower leaves was responsible for the largest reduction in *P. glomerata* transpiration rate and this level was also the one that most reduced this value in upper leaves. Water use efficiency was the only factor in which the lower leaves presented better results than the upper leaves and, in this case, the 50 and 75 µM Zn treatments in lower leaves were the most efficient. The carboxylation efficiency was reduced in the treatments of 50 and 100 µM in the lower leaves and, among the upper leaves, they were also the treatments that most affected this parameter.

Table 3 – Photosynthetic parameters of *Pfaffia glomerata* cultivated under zinc doses.

TREATMENTS	A µmol CO_2 $mol^{-1}s^{-1}$	GS mol H_2O m^{-1} s^{-1}	Ci µmol CO_2 mol^{-1}
Control (ll)	15.64b*	0.49c	310.65b
Zn 50µM (ll)	10.04d	0.29c	316.96b
Zn 75µM (ll)	13.82c	0.36c	305.03b
Zn 100µM (ll)	12.43c	0.37c	312.33b
Control (ul)	19.98a	1.32a	332.99a
Zn 50µM (ul)	15.30b	0.77b	335.14a
Zn 75µM (ul)	18.34a	0.98b	330.37a
Zn 100µM (ul)	16.33b	0.90c	330.34a
AVERAGE	15.23	0.69	322.17
CV (%)	13.33	18.60	1.94

to be continued

Continuation of table 3

TREATMENTS	E mmol H_2O $m^{-1}s^{-1}$	WUE mol CO_2 mol H_2O^{-1}	A/Ci µmol m^{-1} s^{-1} Pa^{-1}
Control (ll)	6.10c	2.57b	0.05b
Zn 50µM (ll)	3.44e	2.96a	0.03c
Zn 75µM (ll)	4.70d	2.94a	0.04b
Zn 100µM (ll)	5.06d	2.46b	0.03c
Control (ul)	8.86a	2.24b	0.06a
Zn 50µM (ul)	5.97c	2.60b	0.04b
Zn 75µM (ul)	7.36b	2.49b	0.05a
Zn 100µM (ul)	7.51b	2.16b	0.04b
AVERAGE	6.13	2.55	0.04
CV (%)	10.53	10.79	13.99

* Averages followed by the same letter in the column do not differ from each other by the Scott & Knott test at a 5% error probability level. ll = lower leaf; ul = upper leaf; A = assimilation rate; GS = stomatal conductance; Ci = CO_2 concentration; E = transpiration rate; WUE = water use efficiency; A\Ci = carboxylation efficiency; CV = coefficient of variation.

Increased availability of Zn may lead to reduced photosynthetic efficiency by several factors, such as inhibition of chlorophyll biosynthesis, reduction of carbon assimilation, interference with the electron transport chain and absorption of other nutrients (MAGALHÃES *et al.*, 2004; CHEN *et al.*, 2008; DHIR *et al.*, 2008; WANG *et al.*, 2009). The change in leaf coloration presented by plants in this experiment (figure 1) is a factor that demonstrates modifications in the concentration of photosynthetic pigments. Studies with species such as *Canavalia ensiformis* (L.) DC, *Zea mays* (L.) and *Avena strigosa* (Schreb) have shown leaf chlorosis and decreased chlorophyll and carotenoids (SANTANA *et al.*, 2015; TIECHER *et al.*, 2016a, 2016b). This reduces the energy uptake and, consequently, the growth of species.

In *Limoniastrum monopetalum* (L.), Zn levels higher than 90 µM reduced photosynthetic activity (CAMBROLLÉ *et al.*, 2013) but, in this study, a reduction at 50 µM can be seen. In a study with *P. glomerata* by Frizzo (2017), photosynthetic alterations of the species were also reported, but at levels higher than those used in this study.

CONCLUSION

From the results analyzed, it can be concluded that biomass production and growth of *Pfaffia glomerata* are little affected by the tested zinc levels, indicating that the species has zinc tolerance under these conditions. Root biometry and photosynthetic parameters were the most affected by excess zinc.

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