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The influence of chlorination on the phytotoxicity and the production of *Zinnia elegans*

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Abstract

Chlorination constitutes a practical and economical chemical control method for the disinfection of recycled nutrient solutions in soil-less growing systems. Although the chlorination can prevent the development of pathogenic organisms, the use of inadequate doses of chlorine could produce damages to the culture and environment. It is necessary to select doses for each plant species that do not cause damages nor produce undesirable effects on the productivity and quality. *Zinnia* sp. in South America has large potential for cultivation as an ornamental potted or vase flower. Tests for disinfection of the recycled nutrient solution were performed with different chlorine quantities (control, 11, 22, 44 and 88 mg L^{-1}) to evaluate the potential phytotoxicity and effects on the flower production (weight and number) of *Zinnia elegans* var. Enana. The production and phytotoxicity were analyzed in relations with the contents of macronutrients (N, P, Ca, and K), sodium and chlorides levels in leaves and related chemical changes (pH, EC and chlorides) in the nutrient solution. The results showed improvement of the development of foliage, roots and the production of flowers with the doses of 11 and 22 ppm, associated to a minor toxicity. The larger doses did not surpass the toxicity levels, although affected the productivity and quality of plants. These results enabled us to select doses under the value of 22 ppm for futures effectiveness test to control pathogens.

Key words: Chlorination, *Zinnia elegans*, phytotoxicity, soil-less culture, disinfection, chemical treatment.

Introduction

From 1980s to 2001, the world-wide area with soil-less cultures production has been increased five times (Rural Industries Research and Development Corporation Program <www.rirdc.gov.au/reports/Ras/01-141.pdf>). The most profitable cultures are tomato, cucumber, pepper, lettuce and cut flowers (roses, gerberas, carnations). Considering Latin American nations, Mexico appears in 17th position with 120 hectares. The future growth of the Closed Soil-less Systems (CSS) in Latin America depends on the development of technology and the adequate assimilation by the production systems, which must be competitive in costs regarding to the technology generated in developed countries.

In many countries, where the soilless culture were developed commercially, the open systems created environmental problems, due to the underground water contamination (Benoit and Cuestermans, 1995; Lopez *et al.*, 1998) caused by leaching of the nutrient solution and resulted into a transition towards the production in closed systems (Tuzel *et al.*, 2000). Some of the advantages of these systems are: diminution of the water consumption (20-30 %), a fertilizer saving (25-45%) and a smaller environmental impact (Magán Cañadas-<www.infoagro.com/abonos/ 917 asp>). However the disadvantages are the demand of a water of superior quality and the risk of a fast dispersion of pathogens through the nutrient solution (pseudofungi such as *Pythium* spp, *Phytophthora* spp, *Plasmopara* spp and *Olpidium* spp), fungi (*Verticillium* spp) or bacteria (*Xanthomonas* spp, *Agrobacterium tumefaciens*, *Erwinia* spp), and virus (Cucumber mosaic virus; Tomato mosaic virus, Lettuce great veins virus and Cucumber mosaic green mottle virus) (George and Biernbaum, 1990; Mac Donald *et al.*, 1994; Stanghellini and Rasmussen, 1994; Van Os *et al.,* 2001). Experiements demonstrated the dispersion of pathogens through the recycled nutrient solution (Magán Cañadas<www.infoagro.com/abonos/docs/9803-3.asp>; George and Biernbaum,1990). The dissemination of diseases is favoured by the homogeneity of variety, the more adequate environmental conditions and the presence of morphologically more adapted waterborne pathogens in the recycled solution (George and Biernbaum, 1990; Mac Donald *et al.*, 1994; Shokes and Mc Carter, 1979; Stanghellini and Rasmussen, 1994).

Chlorination constitutes an alternative chemical control for waterborne pathogens. This method is based on the oxidation capacity of hypochlorous acid. The hypochlorous acid and the ions hypochlorite can prevent the development of pathogenic organisms; their proportion in the water solution depends of the pH and temperature. It is necessary to choose the most effective doses to control the pathogens without causing damages to the cultures productivity and quality. This selection depends on the species, the time of application and the pathogens present in the recycled nutrient solution. The chlorination effect has been studied mainly on pathogens present in cultures of pepper, tomato, cucumber and roses. According to previous studies performed with doses from 0 to 77 ppm of chlorine applied during 12 weeks to some horticultural plants by irrigation, most of the species were not affected by chlorine concentrations under 8 ppm (Frink and Bugbee, 1987). Other studies recommend chlorine doses of 4 to 5 ppm for the control of fungi and bacteria (Escobar *et al.*, 2003; Poncet *et al.*, 2001).

The major risk of the addition of high quantities of chlorine is the phytotoxic effects that may vary according to the sensitivity of species to chlorine concentration. This phytotoxicity is the result of the oxidation effect of chlorine on the cellular walls and membranes and on the cellular content (plant metabolism) or the sodium ion accumulation in the recycled system. The continuous addition of sodium hypochlorite can lead to sodium ion accumulation and also cause an alteration in the Na/K relation in the nutrient solution. The culture growing media is a complex system and can cause difficulty in the evaluation of chlorine phytotoxicity. The residual chlorine can produce phytotoxicity in cultures grown on inert substrates or with nutrient film techniques (NFT); however in cultures grown on organic substrates the residual chlorine is quickly inactivated.

Zinnia (*Z. elegans* L.) is cultivated during summer when temperature may affect chlorination, susceptible to a great number of pathogens common to other flower species (gerbera, alstroemeria) developed in experimental soilless cultures in Argentina (Chase, 1987; Palacios *et al.*, 1991) and good adapted to irrigation with nutrient solution (fertigation). Preliminary tests with Zinnia determined that 5 and 11 ppm doses elevated the chloride contents in the recycled solutions, although not exhibiting phytotoxicity symptoms, and recommended to test other doses to find out the maximum tolerance to chlorine of this species (Premuzic *et al.*, 2004).

The effects of the addition of different chlorine quantities on phytotoxicity and production (weights and amount of flowers) of *Z. elegans* var. Enana, were studied and related with both content of macronutrients (N, P, Ca, K), sodium and chlorides in plants and the changes in chlorides, pH, EC of the nutrient solution**.**

Materials and methods

A closed soil-less system was developed in a greenhouse of metallic structure and with polyethylene cover with zenithal and lateral ventilation, belonging to the Chair of Floricultura of the FAUBA. The flower pots were kept in the greenhouse and watered with automatically recirculation of drainage. Seeds of *Z. elegans* L. *var.* Enana were germinated and transplanted after 15 days of the emergence to white polyethylene flowerpots of 15 cm Ø and 17 cm height filled with perlite. A plant with its corresponding drip emitter was placed at each flowerpot. A drip irrigation system was installed, applying a volume of 2 L h^{-1} by 6/7 daily irrigations of 4 minutes each one. The pots were placed on wood benches with a 5% slope to allow the flow of leachates to the storage tank at the end of the bench.

Five treatments of sodium hypochlorite including a control (without the addition of sodium hypochlorite) were applied. The chlorine doses of treatments were: (1) control: without hypochlorite; (2) 11 mg L^{-1} or ppm; (3) 22 ppm; (4) 44 ppm and (5) 88 ppm of sodium hypochlorite. The different chlorine doses resulted from a dilution of commercial sodium hypochlorite to 100 ppm (stock solution) and from the corresponding dilutions of the stock solution. Chlorination was done to the spending tank with the nutrient solution. The Hoagland-Arnon solution was used that contributed (ppm): 150 N, 190 K; 60 P; 120 Ca; 40 Mg; S 40; and micronutrients Fe 2; Mn 1.5; B 0.2; Cu 0.07; Zn. 0.07; Mo 0.05; pH was 5.0 and CE 1.5 mS/m^2.

The effects caused by the addition of different chlorine quantities were evaluated on the production (fresh and dry weight), some commercial quality factors (phytotoxicity, number of flowers) and the levels of macronutrients in leaves. All the measured values were confronted with the contents of sodium and chloride in leaves due to the possible toxicity and the chemical changes (pH, electrical conductivity and chloride) in the nutrient solution.

Phytotoxic symptoms were monitored analyzing the relation: number of leaves with necrosis/total number of leaves per plant x 100. The necrosis at the edges of leaves was measured every four days.

A digital balance ACCULAB GS200 was used for the assessment of weight (aerial and root fresh weights, and aerial dry weight on material dried in stove at 70°C for 48 h).

The flowering was quantified as the total number of flowers per plant.

Principal macronutrients levels were determined: N (Kjeldahl), P (colourimetric), Ca (atomic absorption), K and Na (flame photometry). Chlorides were quantified by a volumetric precipitation.

The characterized chemical changes of the nutrient solution were electrical conductivity (conductimeter), pH (potentiometer) and chloride concentration (volumetric precipitation). The consumed volume of the nutrient solution and the corresponding doses of chlorine and fertilizer were refilled.

The culture was developed between October and February, starting the chlorination of the nutrient solution one week after the transplant of plants from growing media to the pots with perlite.

A totally randomized experimental design with the pots as experimental units was carried out with 15 replicates per treatment. The results were statistically analyzed by comparison of means (ANOVA) and in case of significant differences ($P \leq 0.05$) post doc test (LSD) using the program SPSS (Field, 2000).

Results and discussion

Production: The 44 and 88 ppm treatments affected the flower production and the aerial fresh weight, producing a decrease of 40-70% in the aerial biomass and 50 % in the production of flowers. The treatments with 11 and 22 ppm resulted with the larger values of aerial fresh weight. The root fresh weight decreased with increasing quantities of chlorine, and with a similar trend as in aerial weight, the 44 ppm and 88 ppm treatments presented the minor values. A highly significant positive correlation (P=0.01) among the fresh aerial and root weight and the number of flowers was observed. Percent aerial dry matter increased with the 44 and 88 ppm treatments and 88 ppm showed significant differences with 11 and 22 ppm treatments. The values indicate the presence of larger dry matter % with the larger dose of chlorine (Table 1).

A different behaviour was observed in the macronutrients contents. The contents of P (0.14-0.20 %) and Ca (1.3-1.71%) did not present significant differences among treatments.

The contents of N (2.8- 4.29 %) and K (3.65-5.6 %) presented the larger values with the control and 11 ppm treatments. The high N contents with the 88 ppm treatment were a consequence of a concentration effect due to the minor aerial biomass associated to

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Table 1. Vegetative growth and flowering in *Z. elegans* under different chlorine treatments

Treatments chlorine (ppm)	Aerial fresh weight (g)	Aerial dry matter (%)	Roots fresh weight (g)	Number of flowers
Control	74.1a	22.3ab	9.7bc	1.6b
11 ppm	85.9a	18.4b	15.2ab	2.14a
22 ppm	101.4a	17.5b	17.6a	2.57a
44 ppm	46.8b	25.4ab	8.9bc	1.57b
88 ppm	32.3b	30.2a	4.9c	1.43b

Values are average of 6 units per treatment. Different letters indicate the existence of significant differences (LSD $P \leq 0.05$).

Table 2. P, Ca, N and K content in leaves of *Z. elegans* under different chlorine treatments

Treatments chlorine (ppm)	Ca (%)	P (%)	N (%)	K (%)
Control	1.3a	0.16a	4.3a	5.3a
11 ppm	1.4a	0.15a	3.5a	5.6a
22ppm	1.6a	0.15a	2.9b	4.1b
44 ppm	1.7a	0.14a	2.9b	3.6b
88 ppm	1.3a	0.20a	3.8a	4.1b

Values are average of 6 units per treatment. Different letters indicate the existence of significant differences (LSD $P \leq 0.05$).

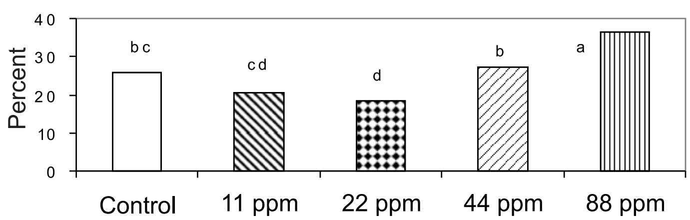

Fig. 1. Phytotoxicity in leaves of *Z. elegans*. Bars represent the average values of 6 units per treatment. Different letters indicate the existence of significant differences (LSD $P \leq 0.05$).

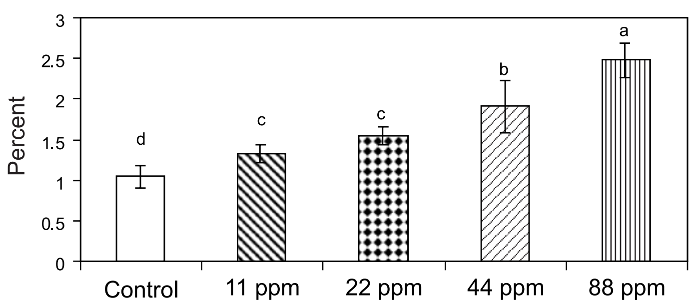

Fig. 2. Contents of chlorides (%) in leaves of *Z. elegans*. Bars represent the average values of 6 units per treatment. Different letters indicate the existence of significant differences (LSD $P \leq 0.05$).

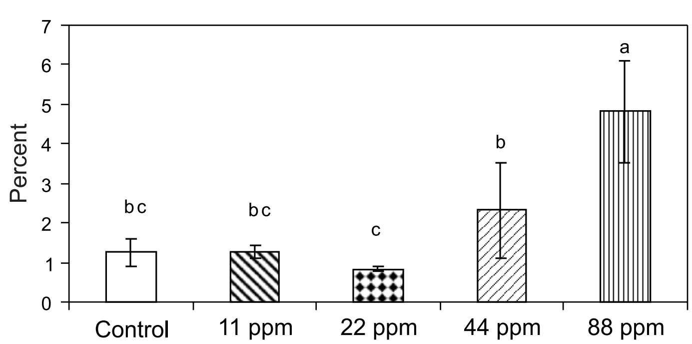

Fig. 3. Sodium (%) in leaves of *Z. elegans*. Bars represent the average values of 6 units per treatment. Different letters indicate the existence of significant differences (LSD $P \leq 0.05$).

the 88 ppm treatment. All values of N, Ca and K were adequate, while the values for P were less than the required for ornamental species (Reed, 1999; Wolf, 1996) (Table 2).

Phytotoxicity: The 88 ppm treatment presented a significantly higher percentage of necrotic leave's tissue as compared to the rest of the treatments while the 22 ppm treatment presented a low percentage of damage, although with no significant differences with the 11 ppm treatment, the last one and the control showed an intermediate damage (Fig. 1).

The damage and necrosis of leaves exhibited a positive correlation with the chloride and sodium content. The 88 ppm treatment induced more than 40 % damage, while with the 11 ppm and 22 ppm treatments the proportion of necrotic leaves was significantly less than the other treatments. The phytotoxicity symptoms caused by chlorine developed first at the tips of the leaves and soon as the toxicity progressed moved throughout the edges. The chloride and sodium contents in leaves increased significantly with the rise of the quantities of applied chlorine. The sodium contents showed differences among treatments and 88 ppm treatment recorded higher values for both sodium and chlorides (Fig. 2 and 3).

The values for chlorides in leaves were between 1.04 and 2.48 %, the 44 ppm and 88 ppm treatments presented contents 100 and 200% larger than the 22 ppm treatment, respectively. There was a negative correlation between chloride contents in the leaves and the fresh weight of plant. The levels of sodium in leaves were between 0.83-4.81%, increasing significantly with the increase in the chlorination dose. The 44 ppm and 88 ppm treatments showed the sodium levels that surpassed the normal levels in vegetative tissue (Chapman and Pratt, 1979).

The chemical properties measured in the recycled solution (Table 3) did not present significant differences among treatments for the electrical conductivity (2.45- 2.7 ds/m). The pH (6.3-6.7) increased significantly with the chlorination, although values were not critical for the culture. The chloride content showed significant differences with two clearly separated treatment groups: in one group control, 11 and 22 ppm treatments were included and another group had 44 and 88 ppm treatment. Although, none of the treatments surpassed the 144 limit of toxicity (Reed, 1999). The addition of chlorine did not affect the values of pH or EC above the ranks recommended for ornamentals plants (Chapman and Pratt, 1979).

Although the doses of 44 and 88 pm did not surpass the toxicity limits they affected the productivity parameters. The 22 ppm and 11 ppm doses favoured the best development of foliage, roots and production of flowers and caused the minor toxicity. The properties measured in the recycled nutrient solution did

Table 3. Chemical properties measured in the recycled solution

Treatments chlorine (ppm)	CE (mS/ m^2)	pH	Chloride (ppm)
Control	2.70a	6.31b	85.9b
11 ppm	2.45a	6.59a	85.2b
22 ppm	2.45a	6.51a	89.1b
44 ppm	2.53a	6.56a	115.0a
88 ppm	2.52a	6.70a	141.0a

Values are average of 6 units per treatment. Different letters indicate the existence of significant differences (LSD $P \leq 0.05$).

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not present critical values for the species. All the contents of macronutrients were adequate for the species. The results suggest not to use chlorine doses higher than 22 ppm for the disinfection of *Z. elegans* culture in closed soilless systems.

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