Journal of Applied Horticulture, 9(1): 62-65, January-June, 2007

Chlorine disinfection: effects on hydroponics lettuce

Zdenka Premuzic[1], Hemilse E. Palmucci[2], Juan Tamborenea[3] and Martin Nakama[4]

[1]Cátedra de Química Analítica, [2]Cátedra de Fitopatología, [3]Investigador alumno. Facultad de Agronomía de la Universidad de Buenos Aires (FAUBA) Av. San Martín 4453. Capital Federal (1416), [4]Instituto de Floricultura –INTA-CASTELAR. De los Reseros y Las Cabañas s/n- CP 1712. Castelar. PROYECTO UBACyT G076. Email: premuzic@agro.uba.ar

Abstract

Disinfection by chlorination was applied to the solution of a soilless closed system of *Lactuca sativa* varieties, Gallega and Mantecosa. The aim was to study the effect of the addition of different doses of chlorine on the production (fresh weight and dry matter), quality (nitrates, vitamin C and nitrogen contents), and phytotoxicity to lettuce (chlorosis) with regard to the chemical properties of the solution (pH, electrical conductivity EC and chlorides). Four treatments were applied: 0.55, 5.5 and 11 mg L^{-1} (ppm) of chlorine and a control without addition of chlorine. The 11 mg L^{-1} treatment produced a decrease in production of Gallega, presenting a 40 % lower fresh weight than the control. Both varieties presented high contents of nitrates. Gallega presented the maximum values (2920- 8158 mg kg^{-1}) and showed values under the permissible limit with the 0.55 and 5.5 mg L^{-1} treatments. Mantecosa showed lower maximum values (3787- 5291 mg kg^{-1}), although with all the values above the limits of permission. The contents of nitrogen for both varieties exceeded the levels of sufficiency in all the treatments. This fact was related to the high nitrogen supply provided by the fertirrigation that contributed to the high nitrate contents. Gallega presented larger contents of vitamin C (19.3-28 mg.$100g^{-1}$) than Mantecosa (15.3-19.98 mg $100g^{-1}$). Chlorination did not affect the chemical properties of the solution (pH and EC remained between the appropriate range for the species). Chloride contents in the nutrient solution were larger at the 11ppm doses; however the values remained under the toxicity levels for the species. For both the varieties, 0.55 mg L^{-1} treatment produced the higest fresh weight and vitamin C contents and the lowest nitrate contents and toxicity symptoms (chlorosis), while 11 mg L^{-1} treatment resulted more chlorosis and necrosis of leaves, diminishing the commercial quality of the plants.

Key words: *Lactuca sativa,* hydroponic, chloride, nitrate, vitamin C, nitrogen, phytotoxicity, pH, electrical conductivity

Introduction

There is a strong environmental legislative pressure worldwide that forces the producer to apply measures that contribute to sustainable and competitive agriculture. The soilless closed culture systems are considered a strategy for alternative production that makes possible a better use of water (20- 30 %) and nutrients (25 and 45%) associated with a less environmental pollution caused by the leaching of fertilizers (www.infoagro.com/abonos/9917asp). Some of the disadvantages of the closed soilless systems are the risk of the fast dissemination of root-infecting pathogens due to the recirculation of the nutrient solution. Different methods (cultural, physical, biological and chemical) are applied to reduce or remove the pathogenic microorganisms or potential pathogens of the nutrient solution (Van Os, 2000; Van Os and Postma, 2000**;** Van Os *et al.*, 2001). Chlorination constitutes a chemical method of low cost and easy application. The oxidation power of the hypochloric acid helps in inhibiting the development of pathogenic organisms, but the addition of high doses can cause damages in the culture. Therefore, it is necessary to establish the effective doses for each plant species and the time of application to control different pathogenic microorganisms without causing damage to the plants or producing undesirable effects on the production and the quality. Tests were performed to select the doses of chlorine, analysing the effects on production, quality and toxicity in plants of *Lactuca sativa,* as a prior step to the effectiveness tests with the presence of *Pythium* sp in lettuce. Two varieties, Gallega and Mantecosa, with different seasonal sensitivity were compared for their response.

Materials and methods

Lettuce was greenhouse grown on wooden benches with galvanized canals, covered with black polyethylene. Pots were placed in each canal and perlite-growing medium was used. A plant and its corresponding drip emitter were placed in each pot. The irrigation took place with Enshi solution (CETTEFFHO, pH: 6.58; EC: 0.016 dS m^{-1} and Cl^{-}: 0. 56 mM L^{-1}), prepared with water previously subjected to reverse osmosis. The canals presented a slope of 5% at their ends to collect the leaching and lead them towards the storage tank of the nutrient solution. Two leafy varieties Mantecosa and Gallega were used in the study. The chlorine stock solution was prepared with 100 ml of commercial sodium hypochloride diluted in 10 L of water. The corresponding doses for each treatment were: 0.55 ppm (0.015mM L^{-1}) with 1 ml stock solution L^{-1}; 5.5 ppm (0.15mM L^{-1}) with 10 ml L^{-1}, and 11ppm (0.30mM L^{-1}) with 20 ml L^{-1}. No sodium hypochloride was added to the control treatment.

According to their seasonal characteristics of growth and production, Gallega variety was studied during July and August (winter), and Mantecosa in October (spring). In both the cases the steps previously described were followed. The plants were placed in the same greenhouse at INTA-CETEFFO-CASTELAR. The phytotoxicity effects for each plant were quantified every four days and measured as number of affected leaves / number of total leaves. Throughout the tests, the presence of disease symptoms by natural infections was assessed. The production as aerial fresh weight (performed with a digital scale ACCULAB, GS200) and production of dry matter (dried for 48 h at 70°C) were evaluated. In addition, the effects in relation to quality were quantified: vitamin C contents (AOAC, 1980); nitrates (Cataldo, 1975), nitrogen (Kjeldahl). Weekly controls of conductivity (conductimetry), pH (potentiometric) and chlorides (volumetric determination with silver nitrate) were performed in the recirculated nutrient solution recovering the consumed volume of nutrient solution and the corresponding doses of chlorine and fertilizer.

The experimental design was completely randomized with four treatments (including control) and 20 replicates for each treatment.

The results were statistically analyzed by ANOVA and means were compared by LSD values ($P \leq 0.05$) using SPSS software (Field, 2000).

Results and discussion

Phytotoxicity: The phytotoxic effects caused by the chlorination were quantified by the percentage of affected leaves based on the total leaves per plant. Mantecosa recorded 15.75 - 42.68 % and Gallega 22.61 - 41.1% of damaged leaves. The phytotoxicity (damage in leaves) was positively correlated to the presence of the largest doses of chlorine for both varieties. Disease symptoms caused by natural infections were not observed during the experiment in any of the varieties. All the observed symptoms of necrosis were caused by phytotoxicity of chlorine, at the beginning, starting at the top of leaves and then moving towards the edges, and finally affecting the whole leaf. Fig. 1 shows the values of percentage of affected leaves corresponding to the last day of evaluation.

Production and quality: Time required to reach the commercial maturity was 45 days for Gallega and 46 days for Mantecosa. There were differences for the fresh weight of both varieties with regard to weight and the response to the chlorination treatments. Gallega presented lower fresh weights (13-33 %) than Mantecosa. The 11 mg L^{-1} dose of chlorine affected the production in Gallega, presenting a negative correlation of the fresh weight and the chlorine addition, while the addition of chlorine did not affect the fresh weight of Mantecosa (Fig. 2).

No significant difference was found for percent dry matter production among treatments. Gallega showed higher values (6.11-7.85%) than Mantecosa (5.52-6.27 %), due to varietal characteristics.

Both varieties presented high contents of nitrates (Fig. 3). Gallega showed the highest contents of nitrates, presenting only the treatments with 0.55 and 5.5 mg L^{-1} of chlorine contents under the 3500 mg kg^{-1} limit values permitted in European legislation (Gazzetta Ufficialle, 1995). The lower content of nitrates of these treatments could be related to a larger fresh weight and an effect of dilution and to larger chloride content compared to control, due to the existence of an antagonism between chloride and nitrates (Behr and Wiebe, 1992). The largest nitrate contents of Gallega could also be related to the environmental conditions, particularly to light or radiation (Blom- Zandstra, 1990), as this variety was harvested 30 days before Mantecosa. Mantecosa over passed the allowed values for nitrates by the European commission (Gazzetta

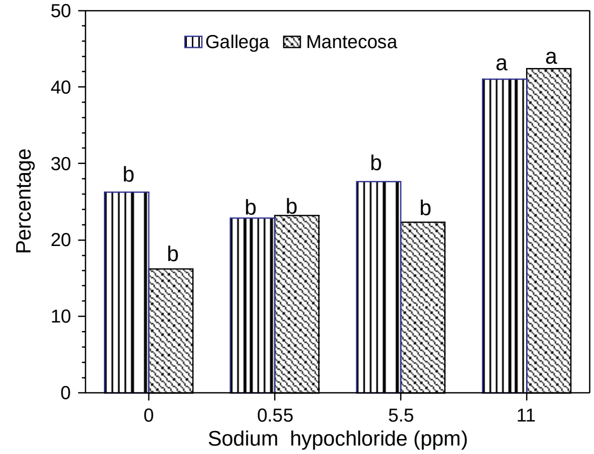

Fig. 1. Effect of the addition of sodium hypochloride in hydroponics on *Lactuca sativa* leaves damage (%) in varieties Gallega and Mantecosa. Different letters represent significant differences ($P \leq 0.05$)

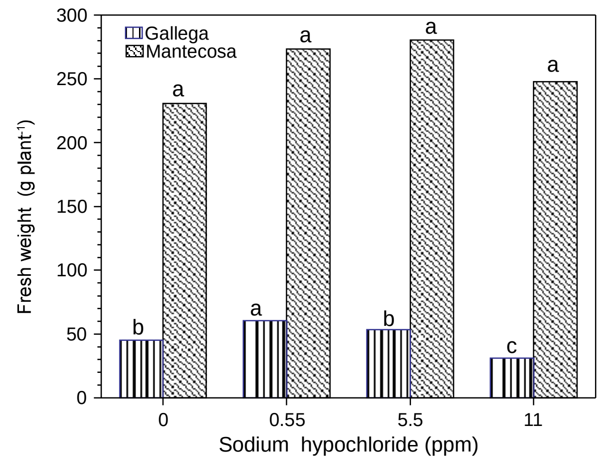

Fig. 2. Effect of the addition of sodium hypochloride in hydroponics on *Lactuca sativa* fresh weight of plants in varieties Gallega and Mantecosa. Different letters represent significant differences ($P \leq 0.05$)

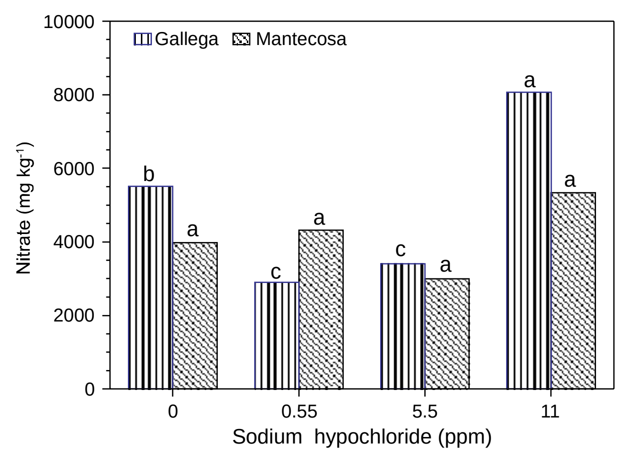

Fig. 3. Effect of the addition of sodium hypochloride in hydroponics on *Lactuca sativa* nitrate content in varieties Gallega and Mantecosa. Different letters represent significant differences ($P \leq 0.05$)

Ufficialle, 1995), for all treatments. The high levels of nitrates of both varieties could be related to a high doses in the nitrogen fertilization (Rincón Sanchez *et al.*, 2002), as well as to the NO_3/NH_4 relation in the nutrient solution (Van Der Boon *et al.*, 1990), resulted by the replacements of nutrients in the recirculating solution. The content of nitrates presented a negative correlation with the fresh weight of plants in both varieties.

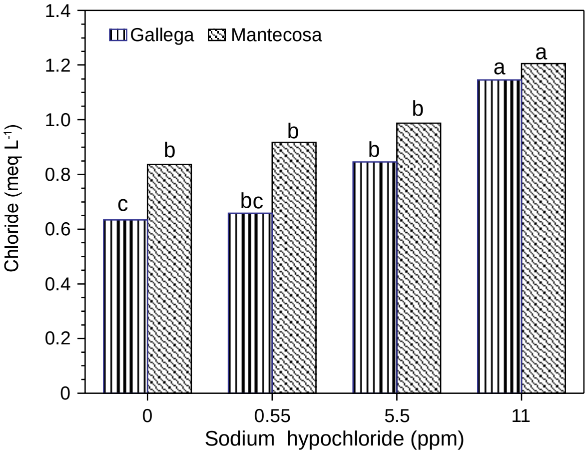

Fig. 4. Effect of the addition of sodium hypochlorite in hydroponics on *Lactuca sativa* chloride content of recirculated solutions. Different letters represent significant differences ($P \leq 0.05$).

No differences for the contents of vitamin C among treatments were observed in Mantecosa (15.3-19.98 mg 100g^{-1}). Gallega (19.3-28 mg 100g^{-1}) presented differences among treatments (Table 1) and showed a negative correlation between the nitrate (in leaves) and the chloride concentrations (in the nutrition solution). The larger levels of vitamin C of Gallega could be related to a concentration effect due to the smaller size of the plants and to the larger dry weight of this variety. The vitamin C contents of both varieties presented negative correlation with the fresh weight (Drews *et al.*, 1997) and a positive correlation with the dry matter. These correlations were related to the presence of light and consequently with a higher photosynthesis rate and a larger presence of carbohydrates; and therefore a larger production of dry matter. The synthesis of vitamin C is improved in the presence of light (Blom- Zandstra *et al.*, 1985). A higher photosynthesis is associated with a higher production of biomass and a larger fresh weight; which, in turn, is associated with a larger number of leaves (in leaf type lettuce) and a higher shading effect, so the synthesis of the vitamin C is affected.

Table 1. Effect of the addition of sodium hypochloride to *Lactuca sativa* var. Gallega and Mantecosa on vitamin C (mg 100g^{-1}) content

Treatments of chlorine (mg L^{-1})	Variety	
	Gallega	Mantecosa
0 (Control)	28 ± 5ab	18 ± 6a
0.55	24 ± 5b	20 ± 7a
5.50	31 ±6a	15 ± 5a
11.00	19 ± 2c	15 ± 4a

The values represent the average of 20 replications and different letters indicate significant differences among treatment means (LSD, $P \leq 0.05$)

The nitrogen contents in both varieties (3.83-4.6%) were similar. In Mantecosa, in spite of showing no differences among treatments, the lowest content of nitrogen was with 11 mg L^{-1} of chlorine. The nitrogen contents were over the values of sufficiency for all the cases (Hochmuth, 1994). This fact could be related to the high nitrogen contribution supplied with the fertilization.

For both the varieties, an increase in pH of the recirculated solution appeared before the addition of increasing doses of chlorine, although only significant in the Mantecosa variety with the 11 ppm treatment compared to the control and 0.55 ppm treatment. The addition of chlorine did not produce modifications in pH outside the range recommended for the species (Table 2) in any variety.

Table 2. Effect of the addition of sodium hypochloride on the pH of recirculating nutrient solution

Treatments (mg L^{-1})	Variety	
	Gallega	Mantecosa
0 (Control)	6.06 ± 0.49a	5.36 ± 0.70b
0.55	6.24 ± 0.28a	5.72 ± 0.81b
5.50	6.32 ± 0.20a	5.91 ± 0.74ab
11.00	6.37 ± 0.21a	6.14 ± 0.52a

The values represent the means and standard deviation of 20 replications. Different letters indicate significant differences ($P \leq 0.05$)

The recirculated solution of variety Mantecosa (Table 3) presented a larger electrical conductivity with the values negatively correlated to the values of pH.

Table 3. Effect of sodium hypochloride addition on electrical conductivity of the recirculated nutrient solution

Treatments (mg L^{-1})	Variety	
	Gallega	Mantecosa
0 (Control)	2.13 ± 0.06a	2.62 ± 0.23ab
0.55	1.94 ± 0.13a	2.62 ± 0.44ab
5.50	1.95 ± 0.21a	2.67 ± 0.45a
11.00	2.07 ± 0.09a	2.41 ± 0.48b

The values represent the means and standard deviation of 20 replications. Different letters indicate significant differences ($P \leq 0.05$)

The contents of chloride in the recirculated nutrient solution for both varieties (Fig. 4) were larger with the dose of 11 ppm, although none of the values surpassed the toxicity levels of the species (Reed, 1999)

For both lettuce varieties, the addition of 0.55 mg L^{-1} chlorine produced the largest fresh weight and vitamin C contents and the lowest nitrate contents and toxicity symptoms (chlorosis), suggesting this dose as the most suitable (among the tested ones) for this species. Doses between 0.55 and 5.5 mg L^{-1} may be selected for a future evaluation of the effectiveness for the control of *Pythium* sp in hydroponics culture of lettuce.

References

AOAC. 1980. *Official Methods of Analysis*, 13th ed. Association of Official Analytical. Chemists. Washington D.C.

Behr, U. and H.J. Wiebe, 1992. Relation between photosynthesis and nitrate content of lettuce cultivars. *Scientia Horticulturae*, 49: 175-179.

Blom-Zandstra, M. and J. Lampe, 1985. The role of nitrate in the osmoregulation of lettuce (*Lactuca sativa*) grown at different light intensities. *Journal Experimental Botany*, 39: 1043-1052.,

Blom-Zandstra, M. 1990. Manuring- Nitrate accumulation in vegetables: Four alternatives which can lead to lower contents. *Groenten-en-Fruit*, 45(36): 18-19.

Cataldo, D., M. Haroon, L. Schrader and V. Young, 1975. Rapid colorimetric determination of nitrate in plant tissue by nitration of salicylic acid. *Communication in Soil Science Plant Annals,* 6: 71-80.

Drews, M., I. Schonhof and A. Krumbein, 1997. Contents of minerals, vitamins and sugars in Iceberg lettuce (*Lactuca sativa* var capitata L.) grown in greenhouse dependent on cultivar and development stage. *Gartenbauwissenschaft*, 62(2): 65-72.

Field, A. 2000. SPSS C8057 Research Methods Contrast and post Hoc Tests.

Gazzetta Uffic.dell´Aliment.Umana, 1995. 26ª ser. Com.Lussem. ISBN92-826-3465-5.

Hochmuth, G., I. Secker and R. Jones, 1994. N requirements of Crisphead lettuce grown with drip irrigation on polyethylene mulched beds. *25th National Agricultural Plastics Congress*. Proceedings of Conference Held in Lexington, KY, USA: pp: 96-100.

Reed, D.W.M. 1999. Agua, sustratos y nutrición en los cultivos de flores bajo invernadero. Ed. HortiTecnia Ltda., Bogotá, Colombia. p.31-42.

Rincón Sanchez, L., A. Peréz Crespo, C. Pellicer Botía, J. Sáez Sironi and A. Abadía Sánchez, 2002. Influencia de la fertilización nitrogenada en la absorción de nitrógeno y acumulación de nitratos en la lechuga iceberg. *Invest. Agri. Prod. Prot. Veg.,* 17(2): 303-318.

Van Der Boon, J., J.W.Y. Steenhuizen and E.G. Steingrover, 1990. Growth and nitrate concentration of lettuce as affected by total nitrogen and chloride concentration, NH_4 / NO_3 ratio and temperature of the recirculating nutrient solution. *Journal Horticultural Science,* 65: 309-321.

Van Os, E.A. 2000. New Developments in recirculation systems and disinfection methods for greenhouse crops. *The 15th Workshop on Agricultural Structures and ACESYS (Automation, Culture, Environment & System)*. IV Conference. December 4-5. Tsukuba, Ibaraki, Japan. p. 81-91.

Van Os, E.A and J. Postma, 2000. Prevention of root diseases in closed soilless growing systems by microbial optimisation and slow sand filtration. Proc. IS Chemical and Non- Chemical. Soil and substrate disinfestation. Eds. M.L. Gullino, J. Katan, A. Matta. *Acta Horticulturae,* 532: 97-102.

Van Os, E.A., M. Bruins, W. Wohanka and R. Seidel, 2001. Slow Filtration: a technique to minimise the risks of spreading root-infecting pathogens in closed hydroponic systems. *Acta Horticulturae*, 559: 495-502.

www.infoagro.com/abonos/9917.asp. Magán Cañadas, J.J. Sistemas de cultivo en sustrato: a solución perdida y con recirculación del lixiviado. Estación Experimental Las Palmerillas- Caja Rural de Almería. Cultivos sin suelo II. Curso Superior de Especialización. pp. 173-205.