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Growth induction of root and tubercle in *Caladium bicolor* with organic fertilizer

Inducción del crecimiento de raíz y tubérculo en *Caladium bicolor* con fertilizante orgánico

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Abstract. The introduction of ornamental crops to the Comarca Lagunera is a great market opportunity for producers of flowers, just as it is the case of *Caladium bicolor* which is exported to Europe, USA, Caribbean islands and Africa. The use of organic products derived from cattle manure for fertilization, improves physical soil conditions and provides significant amount of nutrients to plants and reduce production costs. Therefore, the objective of this study was to quantify the growth of tubercle and root of the ornamental plant *Caladium bicolor* by applying organic fertilizer (OF) extracted from cattle manure. The experimental design was completely randomized and the treatments were three doses of OF: 0 control, 4, y 8 mL/L. The variables were measured with image analysis, and OF was characterized with infrared spectrophotometry. With the data collected was performed ANOVA and treatment means were compared with Tukey test ($P \leq 0.05$). Treatment with 8 mL/L of OF, compared to 4 and 0 mL/L, showed greater tubercle growth (126.61%) with respect to its initial size and root surface (37.485 mm^2) in the period from august to December 2012. Under the experimental conditions described, it includes that the extracted OF bovine manure induces a high adsorption capacity due to its carboxyls, hydroxyls, amines, amides and methyl, which together increase the growth of the tubercle and root in *C. bicolor*.

Keywords: *Caladium bicolor*; Fertilization; Image analysis; Infrared spectrophotometry.

Resumen. La introducción de cultivos ornamentales a la Comarca Lagunera es una gran oportunidad de mercado para los productores de flores, y es el caso del *Caladium bicolor* el cual se exporta a Europa, EE.UU., islas del Caribe y África. El uso de productos orgánicos derivados del estiércol bovino para la fertilización, mejora las condiciones físicas del suelo, aporta una cantidad importante de nutrientes a las plantas y reduce los costos de producción. Por lo anterior, el objetivo del presente estudio fue cuantificar el crecimiento de tubérculo y raíz de la planta ornamental *Caladium bicolor* mediante la aplicación de fertilizante orgánico (FO) extraído de estiércol bovino. El diseño experimental fue completamente al azar y los tratamientos fueron tres dosis de FO: 0 testigo, 4, y 8 mL/L. Las variables fueron medidas con análisis de imagen y el FO se caracterizó con espectrofotometría infrarroja. Con los datos se realizó un ANOVA y las medias de los tratamientos se compararon con la prueba de Tukey ($P \leq 0,05$). El tratamiento con 8 mL/L de FO, comparado con 4 y 0 mL/L, mostró un mayor crecimiento del tubérculo (126,61%) con respecto al tamaño inicial, y la superficie de raíces (37,485 mm^2) en el período de agosto a diciembre de 2012. En las condiciones experimentales descritas se concluye que el FO extraído de estiércol bovino induce una alta capacidad de adsorción debido a sus carboxilos, oxhidrilos, aminas, amidas y metilos, que en conjunto aumentan el crecimiento del tubérculo y de raíz en *C. bicolor*.

Palabras clave: *Caladium bicolor*; Fertilización; Análisis de imagen; Espectrofotometría infrarroja.

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INTRODUCTION

Root absorbs and transports water and essential minerals for plant growth (Taiz & Zeiger, 2010). Under normal conditions, the root growth depends partially on the availability of water, nutrients and growth hormones in soil. Alterations in cell division in meristems, the formation of lateral roots and root hairs formation may have important effects on the architecture of the root system and its ability to grow in soils with limited availability of nutrients and water (Péret et al., 2009).

The reproduction of some plants is from the issue and the multiplication of tubercles, underground stems which are modified to store reserves and are located between an air minimum portion for the stem or leaves, and roots; its function is to store food substances for plant nutrition and reproduction (Péret et al., 2009). The best known tubercles are edible like potatoes (*Solanum tuberosum*), carrots (*Daucus carota*) and radish (*Raphanus sativus*), but there are also common ornamental plants in gardening, as cyclamen or some begonias, gannets and Heart of Christ (C*aladium* spp.).

In organic, ecological or biological agriculture, natural inputs through compost, green manures, associations and crop rotation are used (Márquez et al., 2005). Livestock manure is a basic raw material for compost because it fulfills an important role in nutrient recycling organic function. The characteristics are highly variable depending on the type of animal, its diet and management method (feedlot or pasture), but a good aerobic manure management results in a beneficial product for soil fertility and innocuous (Serrato et al., 2002).

Production of cattle manure ranks first in La Comarca Lagunera, one of the major milk producing areas of Mexico. In that location, 223,547 cows produce 1,177,370 kg/day of manure, and also lower contributions of manure from goats, horses and rabbits. Therefore, this waste should be exploited to prevent outbreaks of infection such as environmental pollution and water bodies (Fortis et al., 2009; SAGARPA, 2009).

The organic matter (OM) contributes to decompose humic substances (HS) (Schnitzer & Khan, 1978) and is a heterogeneous category of organic compounds (Chefetz et al., 2002) that can be characterized by their yellow to black color, high molecular weight and refractoriness (Aiken, 1985). The definitions of the HS fractions are based on its solubility characteristics in aqueous systems (Hayes & Clapp, 2001), and they represent the total amount of humic acids, fulvic acids and humins. This classification is necessary to isolate and characterize these fractions (Schnitzer et al., 2000).

Humic substances enable better use of foliar fertilizers and radicals that stimulate plant growth, which translates into increased performance and improved crop quality. Therefore, the main of this study was to assess changes in the growth of roots and tubers by applying an organic fertilizer (OF), derived from cattle manure in the Heart of Christ plant.

MATERIALS AND METHODS

Localization of experimental site. The experiment was conducted between August and December 2012 in the greenhouse located in “Las Trojes” neighborhood in Torreon, Coahuila, Mexico, located on old Torreon-San Pedro road Km. 5; the location coordinates are 25° 35’ 31.84” N Latitude and 103° 22’ 39.80” W Longitude at an average height of 1,123 meters above sea level. The climate, according to Köeppen classification modified by García (1981), is a warm, dry desert or steppe with rains in summer and cool winter. The average rainfall is 258 mm and the average annual temperature is 22.1 °C (38.5 °C average maximum to 16.1 °C average minimum). The average annual evaporation is 2,396 mm. Frosts occur from November to March and sometimes in October and April; hailstorms, between May and June.

The greenhouse has an area of 500 m^2, is a rigid metal structure covered with polyethylene plastic on roof and sides. It’s equipped with side curtains, wet wall and extractors for temperature control, which varies from 24 to 35 °C inside, and 20 to 30 °C outside; the relative humidity is around 58% inside and is automatically controlled by foggers. The irrigation system is a drip type spaghetti to provide a uniform humidity to the plant root zone. The average solar irradiance inside is around 276 W/m^2, 47 % minor than outside. The regional photoperiod variations is approximately 13 h/light at summer and 10 h/light at winter.

Sowing and treatment application. Planting Heart of Christ was performed in August 2012. The tubercle was established in Peat moss substrate at a depth of 10 cm in the center of the pot (black plastic with capacity of 11,314.47 cm^3). Before sowing, the size of tubercle was measured manually and it was also measured at the final stage of the vegetative cycle to observe the growth difference of each treatment.

The application of the treatment was performed 8 days after sowing (DAS) and flowering stage (45 DAS). Treatments were applied manually, by dissolving the appropriate dose of fertilizer in one liter of water for each container. The irrigation of plants was carried out daily to keep enough moisture, because water demand of this crop is high.

Infrared Spectrophotometry. The OF used was obtained from cattle manure which was deposited in containers of 3,000 L, using a relationship of 5 g of KOH per kg of compost, and water was added for liquefaction for 7 days. Functional groups were identified by generating spectrograms using a spectrophotometer mid infrared (MIR; Perkin Elmer Model 400 FT-IR Spectrum/FT-NIR Spectrometer) operated at 20 °C and with a wavelength from 4000 to 500 per cm.

Image analysis. To measure root growth, three samples were randomly taken for treatment at 15, 30, 60, 90, 120 DAS during the vegetative cycle; Three samples were taken for treatment to measure the growth of the tubercle only in the initial and final stages of the vegetative cycle. An Olympus digital camera (CCD optical sensor of 4.1 Mpx) was used to scan each of the samples. Before capturing the images, millimeter paper was used as guide of measure for calibration software and the amount and intensity of light was adjusted for sharp and good quality images.

Images treatment and data collection (Fig. 1) were realized with Image Pro Plus® software version 4.5 (Media Cybernetics, Maryland, EE.UU.) on a personal computer (González et al., 2005).

Experimental design. The effect of organic fertilizer on growth, root and tubercle, was studied using a completely randomized design with 25 replications in each of the three treatments, which were: control 0 mL/L OF; T2 4 mL/L OF; T3 8 mL/L OF; doses were applied in L per experimental unit, and only water was applied in the control.

Statistical analysis. Data obtained were analyzed by ANOVA and treatment means were compared with Tukey test (P≤0.05) with SAS statistical package Ver. 9.11 (1998).

RESULTS

The organic product after extraction was observed in the spectrogram of Figure 2. The line indicates the signals or functional groups most frequently involved in ion exchange process: the –OH of the extractant occurs in the band of 1361 per cm, N-H amines o amides in 1632 per cm and free functional groups –OH in 3326 per cm; $-CH_3$ group is limited to the band at 2115 per cm.

The results of this analysis may vary according to the type of cattle diet and management where manure is extracted for the OF production.

Tubercle and root growth

Tubercle growth. The application of organic product showed a positive effect on the growth of tubercle. Treatment where 8 ml/L of organic product were applied, presented a growth increase of 126.61% respect to the initial size (Table 1). The initial area of the tubercle was 1,810.94 mm^2, and at the end of the cycle, in the dormancy stage, it was 4,103.83 mm^2. Treatment where 4 mL/L were applied, also showed a significant increase of 56.86 % compared to its initial size; tubercle of control treatment was developed normally show-

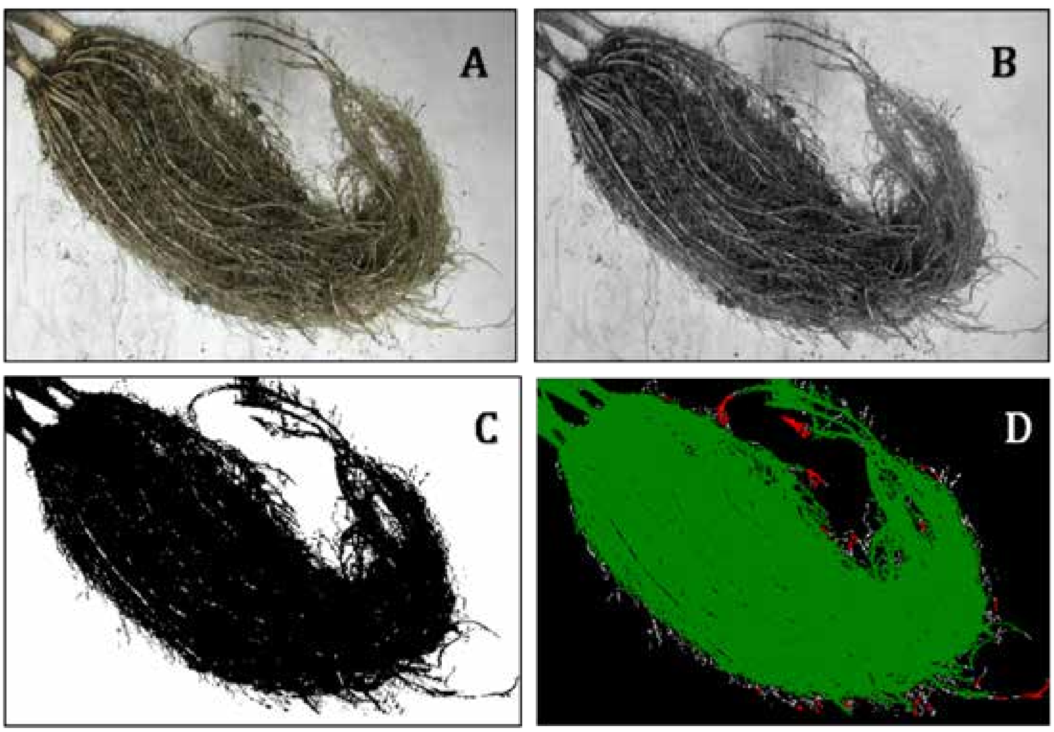

Fig. 1. Root growth analysis by Image Analysis. (A) Real Image; (B) Gray scale Image; (C) Binary Image; (D) Object Classification (Area).
Fig. 1. Análisis de crecimiento de raíz mediante análisis de imagen. (A) Imagen Real; (B) Imagen en escala de grises; (C) Imagen Binaria; (D) Clasificación de objeto (Área).

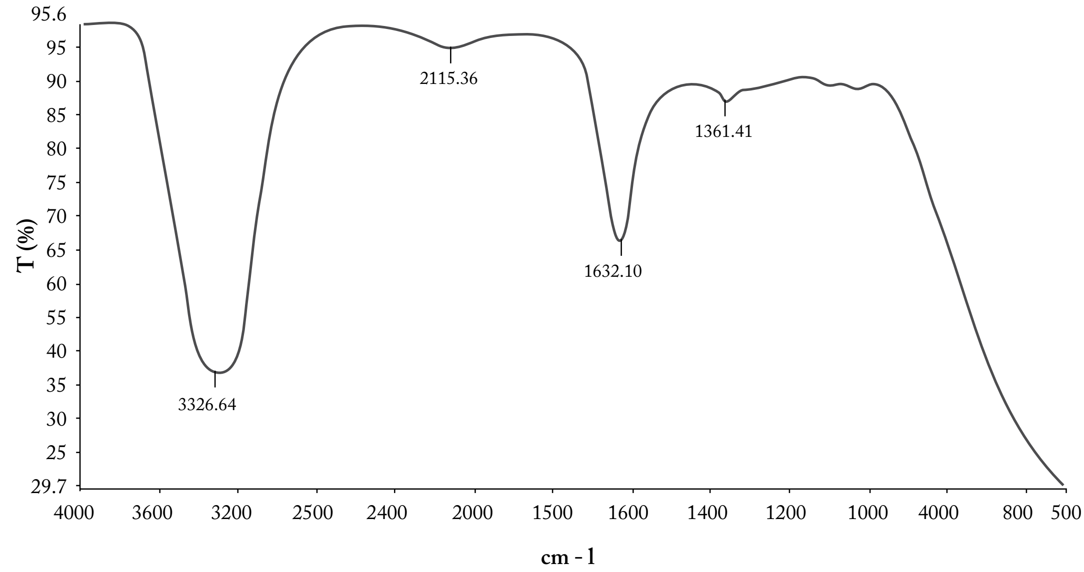

Fig. 2. Mid-infrared spectrum of organic fertilizer. The line indicates the signals of functional groups most frequently involved in the ion exchange process (See text for explanation).
Fig. 2. Espectro en infrarrojo medio del fertilizante orgánico. La línea indica las señales de los grupos funcionales que más frecuentemente están involucrados en el proceso de intercambio iónico (ver texto para una mayor explicación).

Table 1. Tubercle growth with organic treatment.
Tabla 1. Crecimiento de tubérculo con tratamiento orgánico.

Treatment mL/L	Initial area (mm^2)	Final area (mm^2)	Growth (%)
Control	2,012.19	2,039.8	1.37
4	2,219.96	3,482.29	56.86
8	1,810.94	4,103.83	126.61

ing no significant growth, as only its size increased 1.37 % compared to the initial size.

Root growth. Figure 3 shows images of the root development under the same scale, where it's observed that treatment of 8 mL/L produced greater root surface area.

The ANOVA showed that the root growth variable was highly significant ($F \leq 0.0001$). Tukey test showed that treatment with 8 mL/L produced a greater growth surface area (37,485 mm^2) than the control (29,260 mm^2), but it was similar to treatment with 4 mL/L (33,456 mm^2) (Fig. 4).

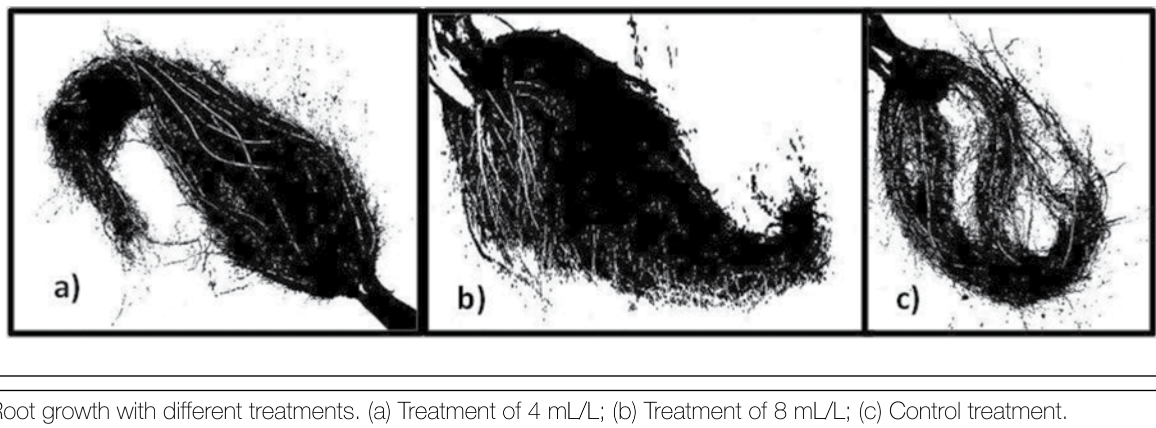

Fig. 3. Root growth with different treatments. (a) Treatment of 4 mL/L; (b) Treatment of 8 mL/L; (c) Control treatment.
Fig. 3. Crecimiento de raíz con diferentes tratamientos. (a)Tratamiento de 4 mL/L (b); Tratamiento de 8 mL/L; (c) Tratamiento testigo.

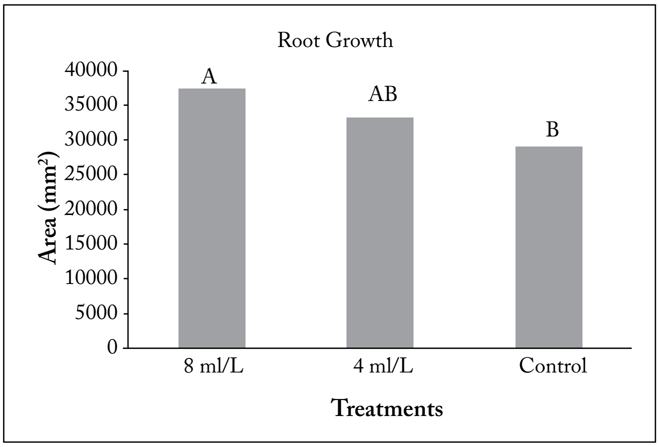

Fig. 4. Comparison of root growth means. Means with different letter are statistically different (P≤0.05).

Fig. 4. Comparación de medias de crecimiento de raíz. Medias con letras diferentes son estadísticamente distintas (P≤0,05).

DISCUSSION

The results obtained in this study are consistent with those reported by Chassapis et al. (2010), about the use of a typical infrared spectrum, which shows that humic substances, absorption, adsorption or associations of different phenolic groups of importance are distributed in the regions of 1050, 1650 y 3400 per cm. It is also noted that the physicochemical properties of humic substances vary according to their origin (Fukushima & Tatsumi, 2006). They might be amorphous multifunctional biopolymers, composed of hundreds of organic compounds including carbohydrates and fused aromatic rings, which may be substituted by phenolic, carboxyl, hydroxyl and methyl groups.

Concerning the application of the OF, the results in this experiment coincide with those published by Peuravuori et al. (2006) and Evangelou et al. (1999), who claim that the higher the concentration of OF applied, the higher is the growth obtained in these plant organs. This is because the OF shows a high adsorption capacity (due to carboxyls, hydroxyls, amines, amides and methyls) which is provided through this fertilizer. These groups, because of chelation, become more complex cations, which are transported together with nutrients through the xylem to the points of vegetative growth as roots and tubercles. Carboxyls which are provided by the OF, are directly involved in various physiological processes that significantly benefited the development of these two plant organs. They positively influence the yield and quality of crops due to the increase in stomatal conductance, which in turn increases oxygen exchange and photosynthetic performance, resulting in an increased production of carbohydrates that are stored in the tubercle (Román & Gutiérrez, 1998). Nutrient absorption by roots is also strongly increased by providing sources of H^+ ions, which are root exudates. These H^+ ions are exchanged with cations of the soil solution that bind to carboxylic radicals, which in turn alters the structure of membranes, causing further opening of the same, thus facilitating the access to nutrients. Moreover, Lopez et al. (2002) indicate that humic substances have positive influences on the development and growth of different plant organs. The application of humic substances can positively impact on the physiology of the plant, a greater degree in the root system (Dobbss et al., 2007) and, in the case of the current study, also in the tubercle. The study by Chen et al. (2004), confirms the findings in this paper, noting that the addition of humic substances to the substrate stimulates root growth because they cause cell growth by stimulating morphological changes in plants, similar to those promoting by auxins (Dominguez et al., 2010).

CONCLUSIONS

The use of infrared spectrophotometry and image analysis allowed to identify and compare the functional groups of organic substances and growth of roots and tubercles of *Caladium bicolor*. Organic fertilizer exhibits high absorptivity due to carboxyls, hydroxyls, amines, amides and methyl. Hydroxyls free radicals are detected at high-interval wavenumbers, differing from oxydryls from the diluent because it produces low-interval wavenumbers. Tubercle size directly influenced the development of the plant root through treatments using organic fertilizers, whose nutrients change positively and significantly the vegetative crop development, reflected by healthy and larger plant leaves.

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