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Full Length Article

Comparison of Chemical Composition and Growth of Amaranth (*Amaranthus hypochondriacus*) between Greenhouse and Open Field Systems

Jorge L. Chávez-Servín[1*], Héctor F. Cabrera-Baeza[2], Edgar A. Jiménez Ugalde[2], Adán Mercado-Luna[2], Karina de la Torre-Carbot[1], Konisgmar Escobar-García[1], Araceli Aguilera Barreyro[1], Juan Serrano-Arellano[3] and Teresa García-Gasca[1]

[1]*Facultad de Ciencias Naturales, Campus Juriquilla, Universidad Autónoma de Querétaro. Av. de las Ciencias S/N, Juriquilla, Querétaro, Qro. CP 76320, Mexico*

[2]*Departamento de Biosistemas, Facultad de Ingeniería, Centro de Investigación y Desarrollo Tecnológico en Materia Agrícola, Pecuaria, Acuícola y Forestal (CIDAF), Universidad Autónoma de Querétaro, Campus Amazcala. Carretera a Chichimequillas S/N, Amazcala, El Marqués, Querétaro CP 76130, Mexico*

[3]*Departamento de Ingeniería Mecánica, Universidad de Guanajuato. Carretera Salamanca-Valle de Santiago Km 3.5+1.8 Km. Comunidad de Palo Blanco, Salamanca, Gto. C.P. 36885, México*

[*]For correspondence: jorge.chavez@uaq.mx

Abstract

Amaranth can be cultivated in greenhouses or open fields. The aim of this study was to compare the basic chemical composition and growth parameters of *Amaranthus hypochondriacus* grown in a greenhouse in comparison to the open field. Each cropping system was assigned an experimental area of 100 m^2. Cultivation was performed directly in irrigated furrows without fertilizers. The same soil type was used for both cropping systems. Number of leaves, plant height, stem diameter, length of seeds, panicle length, biomass and grain yield, grain area, percentage of popped and sprouted grain, and chemical composition were compared. The variables under greenhouse cultivation yielded superior results were grain yield (26.5%), biomass (61.2%), plant height (87.4%), stem diameter (24.3%), number of leaves (2.4%), panicle length (18.4%), percentage of popped grain (2%) and sprouted grain (7%) than field cultivation. Open field cultivation improved protein, fat and ash (9.0%, 17.6% and 43.1%, respectively), and grain size (22.5%) than greenhouse production. Thus, it may be concluded that greenhouse cultivation of *A. hypochondriacus* crop provides for higher yields, but inferior chemical composition values compared to the open field cultivation system.

Keywords: Amaranth; Greenhouse; Open field; Chemical composition; Growth parameters

Introduction

Amaranth (*Amaranthus hypochondriacus* L.) is a fast-growing annual species with good adaptation and high drought resistance (Liu and Stützel, 2004; Vargas-Ortiz *et al*., 2015). Nearly 20 species of the *Amaranthus* genus grow wild in Mexico (Slabbert and Kruger, 2011). Amaranth can be exploited in many ways: as a grain, vegetable or fodder. It is also a highly efficient crop that thrives under adverse conditions like drought, high temperatures and saline soils (Li *et al*., 2010; Sogbohossou and Achigan-Dako, 2014). The grain is very versatile: it can be used in the preparation of various foods and also has promising potential in industrial applications, including source of bioactive compounds, protein, cosmetics, dyes and even biodegradable plastics (Breene, 1991; Khandaker *et al*., 2010; Ramos-Diaz *et al*., 2013). *A. hypochondriacus* is one of the most robust and best performing for grain and leaves production of the amaranth species (Alfaro *et al*., 1987; Barba de la Rosa *et al*., 2009). Recently, the Revancha and Nutrisol varieties, marketed in Mexico, have become more morphologically uniform and increased grain yield (Steffensen *et al*., 2011).

According to Mexico's National Commission of Arid Zones (CONAZA), the states considered arid (ratio of precipitation/ evapotranspiration: 0.00–0.20) are Baja California Norte, Baja California Sur, Sonora, Chihuahua, Coahuila, Nuevo Leon, Tamaulipas, Durango, San Luis Potosi, Zacatecas, Guanajuato, Queretaro, Hidalgo, Jalisco, Aguascalientes, Michoacan, Oaxaca, Guerrero and Puebla-covering most of the national territory. Therefore, cultivation of this plant represents a good potential source of food (Barba de la Rosa *et al*., 2009). Part of amaranth's importance lies in its high protein content, which is 13–

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18%, compared with cereals like corn (10.3%), rice (8.5%) and wheat (14%). It also contains starch (58–66%), dietary fiber (9–16%) and lipids (3.1–11.5%) (Akanbi and Togun, 2002; Caselato-Sousa and Amaya-Farfan, 2012). On the other hand, in Mexico, encouraging primary sector production is one of the greatest challenges of our time (Salomon, 2014). This makes it crucial to find ways to increase productivity and monitor important aspects such as the chemical composition of the harvested product. Using greenhouses for high-potential crops like amaranth is a strategy that has attracted considerable interest in recent years. For example, in the state of San Luis Potosi in 2011 a pilot program developed by the government to produce amaranth in 20 hectares of greenhouses was launched. Greenhouses can intensify agricultural production if appropriate measures are taken to accelerate the development of crops and increase the amount of biomass per unit of cultivated area, compared with open-field conditions (Fuller and Zahnd, 2012). As greenhouses allow for crops to develop with little risk to production, protect plants unlike open-field crops, which are exposed to greater environmental changes and depend on natural factors. Greenhouses permit a more efficient use of water and inputs, and a better control of pests, weeds and diseases (Davies, 2005). There are various factors that can affect the chemical composition of amaranth, including weather, direct sunlight, growth conditions and the degree of plant maturity. Environmental stress is also known to stimulate plant's production of antioxidant compounds. Thus, an environment favorable for growth can alter a plant's composition and performance (Wilkinson, 2009). Therefore, aim of this study is to compare chemical composition, growth parameters, grain composition and performance of the plant Amaranth cultivated in two systems: greenhouse vs. open field.

Materials and Methods

Experimental Details and Treatments

Location, irrigation and cropping systems: The study was conducted at the Amazcala Campus of the Querétaro State University School of Engineering, located in the municipality of El Marques, state of Queretaro, Mexico 76130. Amazcala is situated at 20° 42' 20 " north and 100° 15' 37" west, 1,921m above sea level. The region has a semi-dry weather. A single 100 m^2 Gothic style greenhouse was used, equipped with an electric ventilator (50 in and 0.5 hp). The greenhouse is oriented north to south. The cladding material was a single layer of long-term polyethylene plastic. An area of 100 m^2 was used for the open-field cultivar. For both cropping systems, Eurodrip irrigation driplines (diameter: 8000) were used, with high turbulent flow emitters and a coefficient variation of <2%. The distance between drippers was 8 in, with a flow rate of 0.4 GPH (USA, Eurodrip, Guanajuato, Mexico). An automatic watering system was used, with 5 irrigations every 8 minutes from the day when the plant emerged until day 95, and 2 irrigations every 8 minutes between 95 and 115 days after sowing. The production cycle (spring–summer) was 5 months (March–July) for both cropping systems. The soil type used was the same for both cropping systems. Amaranth seeds of species *A. hypochondriacus*, var. Revancha, certified by INIFAP (the National Forestry and Livestock Research Institute) were used. For each cropping system-open field and greenhouse-direct seeding was performed. In both cropping systems, soil was loosened using a backhoe, while furrows and land leveling were sown manually. Furrows were spaced about 50 cm apart, and were 10.5 cm deep. Crop density was established at 15 plants per m^2 (150, 000 plants/ha). Analysis of the soil used revealed the following characteristics: pH: 7.9; total carbonates: 8.45%; organic matter: 3%; inorganic N: 24.6 mg kg^{-1}; P: 35.7 mg kg^{-1}; K: 1505 mg kg^{-1}; Ca: 5258 mg kg^{-1}; Mg: 154 mg kg^{-1}; Na: 211 mg kg^{-1}; Fe: 4.42 mg kg^{-1}; Zn: 1.54 mg kg^{-1}; Mn: 17.4 mg kg^{-1}; and Cu: 0.93 mg kg^{-1}. Temperature (°C) and relative humidity (%) in the greenhouse and open field were monitored using a sensor from Spectrum Technologies, INC. (Micro Stations WatchDog 1000 Series).

Growth Parameters

To determine the growth parameters, 250 plants from each cropping system were randomly sampled 28, 42, 78 and 98 days after sowing. The total number of true leaves was counted in each of the randomly-selected plants. Plant height was measured in centimeters (cm) using a 5 m Mark Truper measuring tape, FH-3m (Truper, Taiwan), with an accuracy of 0.01 mm, placed vertically on the substrate surface. The measurement was taken at the apical meristem. The stem diameter was taken with a hardened stainless 190 mm vernier caliper (Metromex, Mexico). The stem diameter was measured (mm) on the main stem of the plant, 1 cm above the substrate. The grain lengths were measured using a scanning microscope EVO 50 series (Zeiss, Germany). Seeds selected at random from each culture system were placed on an EVO slide for identification and evaluation. The slide was then placed in the scanning microscope, where grain-to-grain equatorial ends were measured (Fig. 1). The analysis conditions were EHT = 15.00 Kv, WD = 8.0 mm. Signal A = CZBSD and Mag = 130 X. Sixty randomly-selected seeds from each group-greenhouse and open field-were analyzed for both systems.

Chemical Composition

The chemical composition of grains was analyzed using AOAC methods (AOAC, 2000). Protein was estimated using the Kjeldahl method (Büchi digester distillation K-436 and K-370 Büchi unit, Switzerland) by determining the nitrogen and using the corresponding conversion factor (N × 6.25) (960.52). Fat was estimated using the Soxhlet method (extraction unit 810 Büchi, Switzerland), (920.39). Ash was determined by organic matter calcination (Lindberg furnace chamber 51894, Mexico) (923.03). Moisture was determined by drying in a forced-air drying oven (air re-circulating oven, Carbolite, United Kingdom) (934.01). Crude fiber was determined after digestion with dilution acids and alkalis (fiber analyzer, Ankom 200, USA) (920.86). Carbohydrates were calculated by difference. The calculations were performed in triplicate from previously milled composite samples.

Determining Popped and Sprouted Grain

The germination test was performed by placing a known number of randomly-chosen grains (200 from each cultivation system) in several petri dishes, each with moistened cotton. After 10 days, the percentage of germinated and un-germinated grains was evaluated (Moncaleano-Escandon *et al*., 2013). The popped grain test was conducted by placing a given number of randomly-obtained grains (300) on the surface of a preheated pan. Subsequently, the number of popped and unpopped grains was evaluated.

Determination of Biomass and Grain Yield

Biomass was determined by weighing vegetable material extracted from 1 m^2 of randomly-selected area at 5 different sites within each cultivation area. This test excluded the weight of the root. Grain yield was determined as follows: plants were harvested when physiologically mature (around 150 days after seeding). The panicle was cut and kept in a drying room until it lost sufficient moisture to be threshed (after 15 to 25 days, during which time the panicles were moved daily to avoid overheating). During this drying process, the moisture content decreased from 52% to 14%. To prevent excess moisture fermentation, harvesting was performed on three different days sequentially. The grains were collected using a vacuum cleaner and sieved using a mesh, then stored in 3 kg paper bags. The grain was stored in a cool, dry and ventilated area. A primary sample of approximately 500 g was taken from each bag to comprise a composite sample and homogenized by mixing. For laboratory measurements, the grain was ground in a mill (Thomas Wiley Model 4, USA) and then sieved through a 0.5 mm opening. The samples were stored in a deep freezer (-70°C) until analysis.

Statistical Analysis

Descriptive statistics were used to present the results in terms of means and standard deviations. Student's *t-test* was used for comparing the two cropping systems, using a significance level of p <0.01 and a confidence interval of 95%. The contrasting variables were greenhouse vs. open field. The software used for the statistical tests was SPSS v18.0 for Windows.

Results

Growth Parameters

For comparing growth parameters in the two cultivation systems, sampling was carried out 28, 42, 78 and 98 days after sowing (Table 1). Based on the number of leaves, growth was always superior in the greenhouse, with a significant difference (p <0.01) for the first three samplings. Similar results were observed regarding height and diameter with higher values (p<0.01) for greenhouse-grown plants (Table 1). The average height of greenhouse plants, after 98 days, was almost twice the height of plants in the open field. Plants grown in greenhouses showed a faster and more robust development with taller and thicker stems.

Grain Length and Area

The grains obtained from open-field crops were larger with greater leaf area (30%) than grains obtained from greenhouse crop (Table 2). This feature is important for the grain industry: larger grains are more in demand for the production of Mexican traditional sweets such as *alegría*s, a combination of popped amaranth grains, honey and molasses shaped into a bar known as *palanqueta*. Greenhouse grains were significantly larger (30%) than those obtained in the open field. Panicle length was also greater, 18% more in greenhouse plants, with an average of 55 cm.

Estimated Chemical Composition

Regarding the chemical composition analysis in the two cropping systems, we observed significant differences (p<0.01) in the content of lipids, protein and ash, with higher values in open-field cultivation (Table 3). This means the chemical composition of open field crops is superior, despite their inferior growth values.

Crop Yield

Greenhouse cultivation yielded 26% more biomass than the open-field system (Table 4). The amount of grain harvested in greenhouses was also 61% greater (2.19 ton/ha) than obtained in the open field (1.36 ton/ha). This is consistent with the growth parameter variables observed throughout our study (Table 1).

Percentage of Popped Grain and Germination

Popped grain percentages were similar for greenhouse and open field amaranth. The germination rate was 8% higher in grains from greenhouse plants (Table 4), although both results exceeded 90%, which is considered an optimal rate.

Table 1: Comparison of growth parameters (*A. hypochondriacus*) over time in the two cropping systems: greenhouse vs. open field

Variable	Time[a] (days)	Greenhouse	Open field
Number of leaves	28	8.0 ± 1.8*	6.2 ± 1.4
	42	19.6 ± 5.4*	16.0 ± 3.8
	78	45.6 ± 9.9*	26.1 ± 5.6
	98	44.8 ± 10.7	43.7 ± 8.9
Plant height (cm)	28	28.0 ± 7.3*	9.4 ± 2.6
	42	55.5 ± 17.4*	25.1 ± 5.9
	78	187.2 ± 28.6*	52.4 ± 11.3
	98	192.4 ± 29.2*	102.6 ± 25.9
Stem diameter (mm)	28	5.6 ± 2.1*	2.2 ± 0.8
	42	8.9 ± 2.5*	7.3 ± 2.0
	78	21.4 ± 3.9*	5.8 ± 1.6
	98	24.2 ± 4.8*	17.2 ± 5.2

The results are shown as the average of 250 measurements ± one standard deviation
[a] Time after sowing
*Means significant difference ($p < 0.01$) in the same variable when comparing greenhouse vs. open field

Table 2: Length and area of the grains and panicle length (*A. hypochondriacus*) in the two cropping systems: greenhouse vs. open field

Variable	Greenhouse	Open field
Length A[a] (mm)	1.28 ± 0.1*	1.44 ± 0.1
Length B[b] (mm)	1.09 ± 0.1*	1.25 ± 0.1
Grain area (mm^2)	4.44 ± 0.7*	5.73 ± 0.7
Panicle length (cm)	55.02 ± 3.8	46.46 ± 3.9

The results are shown as the average of 60 measurements ± one standard deviation
*Means a significant difference ($p < 0.01$) in the same variable when comparing greenhouse vs. open field
[a]Longitudinal axis
[b]Transverse axis

Table 3: Chemical composition of amaranth grains (*A. hypochondriacus*) from two cropping systems: greenhouse vs. open field

Variable	Greenhouse	Open field
Moisture (%)	9.35	5.22
Ether extract[a]	5.0 ± 0.1*	5.9 ± 0.1
Crude protein[b]	14.8 ± 0.0*	16.2 ± 0.2
Crude fiber[a]	2.1 ± 0.0	2.0 ± 0.6
Crude ash[a]	2.1 ± 0.0*	3.0 ± 0.1
Nitrogen free extract	66.6 ± 0.1	67.1 ± 0.7

The results are shown as the average of 3 measurements ± one standard deviation
*Means a significant difference ($p < 0.01$) in the same variable when comparing greenhouse vs. open field
[a](% dry matter)
[b](% dry matter N x 6.25)

Temperature and Humidity Records

The high and low temperatures in the open field vary widely, which is typical for this arid region in Queretaro (Table 5). As expected, during all the months of our analysis, average heat and humidity were higher in the greenhouse, leading to a hot thermal environment. Average temperature in the greenhouse from March to July was 21.8 to 25.2°C, with highs of 46.5–52.0°C. Thermal environmental conditions in the greenhouse favored an increase in biomass.

Table 4: Amaranth yield (*A. hypochondriacus*), percentage of popped grain and percentage of germination in the two cropping systems: greenhouse and open field

Variable	Greenhouse	Open field	Difference
Biomass (ton/ha)	67	53	14
Grain yield (ton/ha)	2.2	1.4	0.8
Popped grain (%)*	84	82	2
Germinated grain (%)*	98	91	7

*Results from 300 grains randomly obtained in each case

Table 5: Temperature and humidity recorded in the two cropping systems: greenhouse and open field

Variable	Greenhouse	Open field
Temperature (°C)		
March	21.8 ± 12.2	17.9 ± 5.5
April	23.6 ± 14.2	18.6 ± 6.1
May	25.5 ± 10.3	23.1 ± 2.4
June	24.3 ± 13.5	18.7 ± 5.3
July	25.2 ± 08.1	23.4 ± 6.3
Humidity (%)		
March	52.5 ± 23.4	50.6 ± 24.7
April	47.8 ± 22.5	44.9 ± 25.3
May	52.6 ± 18.3	40.4 ± 15.3
June	52.3 ± 17.4	50.7 ± 21.5
July	51.8 ± 15.5	46.1 ± 24.6

Data from March to July. The results are shown as the average of 3034 measurements (each hour) ± one standard deviation

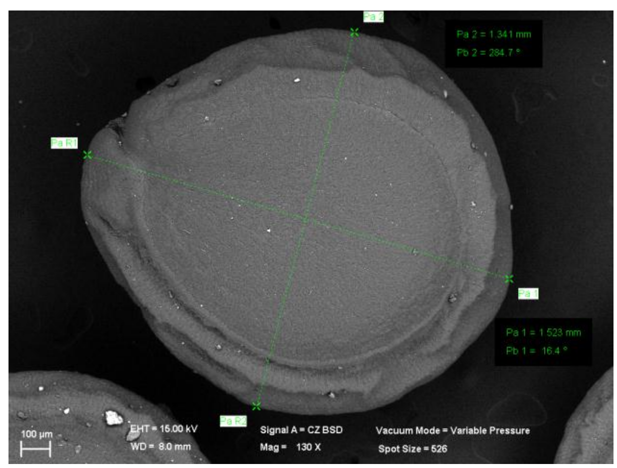

Fig. 1: Image of the determination of longitudinal measures of grain amaranth using a scanning electron microscope (SEM)

Discussion

There are some studies on the production of different varieties of amaranth, all of which are made in the open field. At the moment no studies are available in the scientific literature on the cultivation of amaranth in the greenhouse. García-Pereyra *et al.* (2009) tested four *A. hypochondriacus* genotypes and one *A. cruentus* genotype at four population densities: 31,250; 41,666; 62,500 and 125,000 plants/ha, during spring–summer 2000 and autumn–winter 2001 and 2002 in Nuevo León, Mexico. They evaluated grain yield, plant height, stem diameter and panicle length. Besides the crude protein, ashes, acid detergent fiber and neutral detergent fiber were evaluated for stems and leaves. They reported than the best grain yield was obtained with 125,000 plants/ha in all genotypes. Also, the best yields for the different *A. hypochondriacus* genotypes were obtained during the spring–summer cycle (1.3–2.2 ton/ha) compared to autumn-winter cycles (0.07–1.3 ton/ha). In our study, we used a population density of 150, 000 plants/ha. The height and stem diameter values in the five varieties of amaranth exceeded our results from open-field cultivation, with average values of 117–172 cm in height, and for stem diameter of 15, 17, 19, 25 and 30 mm for the 5 varieties tested. Regarding the yield reported in our open field study (1.4 ton/ha), the results are within the range reported in this study.

Peiretti and Gesumaria (1998) studied the influence of line spacing on amaranth growth and yield (*Amaranthus spp*). Among the cultivars used in their study was *A. hypochondriacus*. They evaluated four distances between lines that correspond to densities of 1,100,000 (0.30 m); 740,000 (0.45 m); 550,000 (0.60 m) and 470,000 (0.70 m) plants/ha. Plant height, stem diameter, panicle length and seed production per plant, reporting a decrease in the values of these parameters as line spacing decreases and plant density increases. In our study, the panicle length values for open field (46.46 cm) and stem diameter (17.2 cm) were higher than reported in this study at different densities. This may be due to the fact that a lower density of plants (150,000 plants/ha) was used in our study.

Greenhouse grain yields in our study surpassed yields reported from open field systems in other amaranth studies, for example maximum yield of 1.67 ton/ha and a yield of 1.44 ton/ha for the *A. hypochondriacus* (var. Revancha) (Ramírez *et al*., 2010), and 1.5 ton/ha (Peiretti and Gesumaria, 1998). However, Gimplinger *et al.* (2008) study under semiarid conditions (9.8°C, 546 mm) how crop density affects amaranth morphology, biological grain production and combine yield in two adapted genotypes of *A. cruentus* and *A. hypochondriacus* at different plant densities (80,000; 170,000; 350,000; 700,000 and 140 plants per hectare) reported that plant height decreased with rising density and grain production and seed number decreased in parallel way. It was also concluded that amaranth stands produce highest grain yields at low plant populations. In order of increasing plant density average grain yields of 2.73, 2.74, 2.64, 2.37 and 2.39 ton/ha was reported.

In another study, a morphological characterization in ten different Amaranth varieties was carried out (Ramírez *et al*., 2010) and average height of the *A. hypochondriacus* (var. Revancha) 137.7 cm was observed. According to our results, the average height of greenhouse plants was greater than reported in various open-field studies. An average height of 185 cm is reported for amaranth crops in open fields, where different doses of N, P and K were used (Ramírez *et al*., 2010). One study (Peiretti and Gesumaria, 1998) reported a maximum height of 157 cm, and other study (García Pereyra *et al*., 2009) reported heights from 124–185 cm at a density of 125,000 amaranth plants per hectare. Regarding the stem diameter of greenhouse plants, we obtained higher values (+2.42 cm) then another study, where a maximum value of 1.065 cm was found (Peiretti and Gesumaria, 1998).

The number of leaves per plant is a necessary variable to compare the potential of the plant. More leaves mean more leaf area, which is physiologically important because it provides a larger surface for active photosynthesis. This favors increased production of carbohydrates, which when combined with the water and minerals assimilated by the plant, intervenes directly in the synthesis of protein and other organic compounds, producing plants with increased biomass (Liu *et al*., 2010). Our results are comparable with those of Peiretti and Gesumaria (1998), who studied *A. hypochondriacus* and reported an average of 35 leaves in an open-field cultivation system. The larger the grain, the more attractive the product is to consumers (Zapotoczny *et al*., 2006). It has been reported that *A. hypochondriacus* grains typically measure about 1.1–1.4 mm long by 1.0–1.3 mm wide (Corke *et al*., 2016), which coincides with the values reported in the present study, where it was observed that the grain area was greater ($p<0.01$) in the open field crop (Table 2). A study reported the panicle length of *A. hypochondriacus* cultivated in open field was 41 cm (Peiretti and Gesumaria, 1998), whereas in our study we found values of 55.02 and 46.46 cm in greenhouse and open field, respectively (Table 2). The germination rate is an estimate of the maximum number of optimal grains that can germinate and is a quality parameter of grains susceptible to subsequent cultivation (Hampton, 1981; Riis *et al*., 1995). Environmental interactions also play a role in actual grain germination—for example, different temperature, humidity, light, and space conditions, soil type, contaminants, pesticides, etc. (Aufhammer *et al*., 1998)— so the actual percentage of germinated grain may vary considerably (Table 4).

Greenhouses are known to represent favorable conditions for the plant. But in the open field, environmental conditions are much more variable, inducing plants to react accordingly to survive. In this regard, the greater amount of protein in open field plants can be attributed to the importance of the macromolecules that play a role in every aspect of plant growth and development. For example, proteins are involved in processes such as storage proteins, catalytic chemical reactions (enzymes), facilitating membrane transport, intracellular structure and energy generation, including electron transport reactions (Aubry *et* *al*., 2011). The protein and lipid contents found in our study were within the range reported in the literature (protein: 12–18%, lipids: 1.9–9.7%) (Caselato-Sousa and Amaya-Farfan, 2012).

A significant difference ($p < 0.01$) was observed in every growth parameter analyzed, with greenhouse plants growing more values than open field plants (Table 1). A study on amaranth reported temperatures ranging between 14°C and 16°C from January to April, with yields of about 1.5 ton/ha (Peiretti and Gesumaria, 1998). Another study reported with low temperatures of 5°C in March and high temperatures of 42°C in May green forage yields about 22.75 to 72.50 ton/ha were obtained in different amaranth genotypes (García Pereyra *et al*., 2004). These values are comparable with those obtained in the open field portion of our experiment. Amaranth belongs to a group of fast-growing plants and its photosynthesis is very efficient, which enables the available carbon dioxide in the air to concentrate in chloroplasts of specialized cells. Their photosynthetic pathway is C_4, which differs from the majority of plants with C_3 photosynthesis (Calvin pathway) (Long *et al.*, 1994). It has been reported that *Amaranthus* photosynthetic activity peaks at 40°C with an optimum temperature of 35°C (Lin and Ehleringer, 1983). Therefore, one can infer from this study that environmental conditions in the greenhouse (Serrano-Arellano *et al.*, 2015) favored higher growth parameters in amaranth plants. It should be mentioned that in our study, no fertilization was used in any of the cropping systems. The application of fertilizers in crop cycles could increase both biomass and grain yield. The amaranth requires various nutrients found in the soil, or that must be provided by the producer. By increasing plant height, growth and maturity, as well as plant density, the amounts of nutrients that the soil must provide proportionally increase to meet the nutritional requirements of plants. If the soil does not provide them, their potential development will be affected (Ramírez-Vázquez *et al*., 2011). In that way the lower nutritional quality observed in the amaranth grown in greenhouse, can be due to the lack of fertilization.

Conclusion

In the conditions tested, in terms of growth, biomass and grain yield, greenhouse amaranth plants performed better than open field plants. But quality was lower than open-field counterparts, meaning their chemical composition was negatively affected. Furthermore, the grain area of greenhouse plants was lower, but this did not negatively affect the percentage of popped grains or the germination rate. Further studies are required in which fertilizers are used in both cultivation systems, in order to determine the true potential of plants grown in greenhouses from a production point of view. An evaluation of the production of secondary metabolites of plants is also necessary.

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