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Capacidad de retencion de agua de sustratos conteniendo zeolita y su efecto en el crecimiento, producción de biomasa y contenido de clorofila en plántulas de *Solanum lycopersicum* Mill

Water holding capacity of substrates containing zeolite and its effect on growth, biomass production and chlorophyll content of *Solanum lycopersicum* Mill

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Resumen

Zeolita clinoptilolita es un aluminosilicato hidratado que pertenece a un grupo de minerales de origen volcánico y posee propiedades agrícolas interesantes. El objetivo de este trabajo fue determinar el efecto causado por la zeolita en plántulas de tomate (*Solanum lycopersicum* Mill.) cultivadas en macetas. Por lo tanto, se evaluaron las propiedades físicas de tres sustratos: peat moss (pm), perlita (per) y zeolita (zeo), y sus diferentes mezclas (pm:per:zeo) en los siguientes tratamientos: (T1 o Control) = 100:0:0; T2 = 70:30:0; T3 = 70:20:10; T4 = 70:10:20 y T5 = 70:0:30 (v/v). Comparado con el tratamiento control, los sustratos conteniendo 30% de zeolita incrementaron su capacidad de retención de agua (260%), porosidad total (8.47%), densidad aparente (212%) y la densidad de partículas (230%). En comparación con las plantas desarrolladas en el sustrato control (100% peat moss), las que fueron cultivadas con 30% de zeolita incorporada al sustrato, exhibieron valores estadísticamente superiores en altura (24.2%), área foliar (64.5%), longitud de raíz (63.2%), biomasa seca aérea (62.5%), biomasa seca de raíz (208.9%), diámetro de tallo (28.5%) y número de hojas (92%); sin embargo, el índice de clorofila no mostró efectos significativos. Los resultados claramente indican que la incorporación de zeolita en los sustratos puede promover el crecimiento de las plantas de tomate.

Palabras Clave: clinoptilolita; propiedades fisicoquímicas; fertilizantes; nutrición vegetal; tomate

Abstract

Zeolite-clinoptilolite is a hydrated aluminosilicate, which belongs to a group of minerals of volcanic origin and possesses interesting agricultural properties. The aim of this study was to determine the effect of zeolite-clinoptilolite on *Solanum lycopersicum* Mill. seedling growth, when supplied to substrate in pot culture. An assay was set to test and compare the physical properties of three substrates: peat moss (pm), perlite (per) and zeolite (zeo), and their mixtures (pm:per:zeo) at different proportions: T1 or control = 100:0:0; T2 = 70:30:0; T3 = 70:20:10; T4 = 70:10:20 and T5 = 70:0:30 (v/v). Compared to control plants, substrates containing 30% zeolite increased their water holding capacity (260%), total porosity (8.47%), bulk density (212%) and particle density (230%). Related to control plants grown with 100% peat moss, tomato seedlings cultured in a substrate mixture with 30% zeolite significantly improved shoot length (24.2%), leaf area (64.5%), root length (63.2%), shoot dry weight (62.5%), root dry biomass (208.9%), stem diameter (28.5%) and leaves number (92%), however, the chlorophyll index had no significant effects. The overall outcomes indicated that substrates amendment with zeolite could effectively improve tomato plants growth.

Keywords: clinoptilolite; sustainable agriculture; fertilizer; plant nutrition; tomato

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Introduction

Zeolites are naturally occurring hydrated aluminosilicates comprising about 50 mineral types, including clinoptilolite. Given the natural properties of zeolites, particular attention has been paid in agriculture because they encompass a number of applications (Ramesh *et al.*, 2015, 25). They have a rigid three-dimensional crystal structure with voids and channels of molecular size and a high cation exchange capacity (CEC), arising from the substitution of aluminum (Al) for silicon (Si) in the silicon oxide tetrahedral units that constitute the mineral structure (Figure 1).

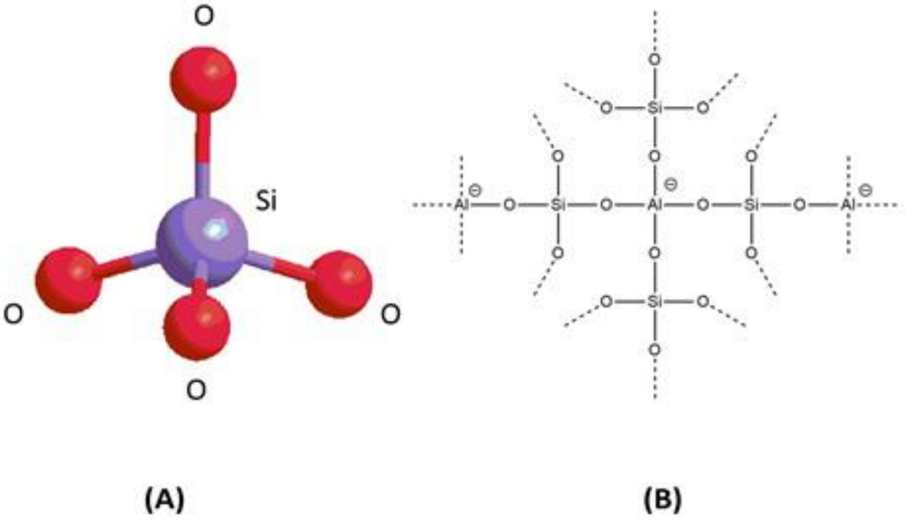

Figure 1. (A) Basic zeolite structure. (B) Tetrahedral representation of a natural zeolite. The main building units zeolites are tetrahedral formed by $[SiO_4]^{4-}$ and $[AlO_4]^{5-}$ linked together forming oxygen bridges.

These unique minerals are being used in agricultural production in order to increase water retention and generate more effective fertilizer availability for plants while minimizing nutrient losses (Ozbahce *et al.*, 2015, 616). Ammonia loss and nutrient leaching from fertilizers can be reduced by the application of materials such as clinoptilolite zeolite, which is high in CEC (Palanivell *et al*., 2016, 709). When zeolites are mixed with fertilizers, they retain nutrients and therefore increase the long-term soil quality by enhancing its absorption readiness. It concerns the most important plant nutrients such as nitrogen, phosphorous, potassium, calcium, magnesium and microelements (Orha *et al.*, 2015, 1).

Furthermore, it has been postulated that zeolite also contributes to sustainable agriculture by preventing the occurrence of environmental problems through increasing N, P, and water-use efficiency in agroecosystems. One way of improving P use efficiency by means of natural zeolite, such as clinoptilolite, would be by increasing soil P availability for plants; zeolite can exchange ammonia to react with phosphate rock substituting calcium in the process and resulting in higher P solubility (Shokouhi *et al*., 2015, 1).

Zeolites are widely used in agriculture either for plant growing on open field, or as substrate for application under greenhouse conditions (Tsintskaladze *et al.*, 2016, 164). Zeolites are a good source for slow release materials of certain mineral elements required as macro or micro nutrients for plants, and its combination with peat moss and perlite is one of the best cultivation media (Eghtedary-Naeini *et al.*, 2016, 523). It is well known that peatmoss as a substrate can be a suitable organic material for growing plants and improve sustainability in soilless culture (Al-Ajlouni *et al.*, 2017, 34).

There are several reports related to the use of zeolites as substrates for hydroponics. Aghdak *et al.* (2016, 967) observed that the incorporation of 25% zeolite in perlite substrate, showed a significant increase in leaf area as well as fresh and dry biomass in pepper plants. Other results also showed that using perlite and zeolite as the growing media produced the highest fruits number and yield. Djedidi *et al*. (2001, 32) observed that tomato plants grown in perlite and zeolite at 2:1 ratio, had the best distribution of fruit size; though total soluble solid and the highest fruit dry matter was found in the treatments with perlite as the substrate.

When zeolites are integrated into the soil, they can retain essential nutrients in the root zone, allowing them to be used by plants when required. Consequently, this leads to a more efficient use of fertilizers by reducing their normal application rates, by prolonging their activity, or finally by producing higher yields (Leggo, 2015, 2). During the past 60 years, crop protection and production have relied heavily on synthetic inputs, which damage the environment. In this scenario, synthetic N fertilizer use, combined with other agricultural N inputs (i.e. legumes, crop rotations, and manure applications) to increase productivity over time, has resulted in elevated levels of nitrate in water resources throughout many agricultural regions (Masarik *et al.*, 2014, 241).

Consequently, zeolitic inputs into the soil could relieve environmental pressure such as the increase of N concentration in groundwater recharged by agricultural lands, which has been globally linked to a steady increase of nitrate loading to surface waters; so, the application of zeolites can both prevent nitrogen leaching and improve soils physical properties (Afrous and Goudarzi, 2015, 56). For example, the loading of N and P from primarily Midwestern states in the United States, has been linked to the increase of hypoxic zones in the Gulf of Mexico (Rabalais *et al.,* 2002, 236).

The use of synthetic agrochemicals for plant nutrition or for disease management, pests, weeds, etc., has become a common practice around the world. However, environmental pollution and ecological issues force us to find new strategies, such as the use of bioorganic agrochemicals or the controlled release of fertilizers and pesticides, including a reduction in the amount of active ingredients (De Smedt *et al.*, 2015, 1356). Lately, the interest in restructuring the agricultural supply chain for the development of sustainable agriculture has increased (Priya and Vivek, 2016, 136), and hence the use of natural products such as zeolites to improve physical and chemical properties of the soil (Moraetis *et al.*, 2016, 13274).

Based on the above-mentioned rationale, we hypothesized that by using Mexican zeolite-clinoptilolite (ZC) as substrate, we would increase biomass production and growth of *S. lycopersicum* plants. Therefore, the objective of this study was to analyze the effect of ZC on plant growth and biomass production when incorporated to the substrate at different rates.

Materials and methods

Experimental site and chemical properties of the tested zeolite

The bioassays were conducted during the summer of 2016 at the Center for Applied Chemistry Research (CIQA), located in Saltillo, Coahuila, at 1520 m above sea level, with the coordinates 25° 27′ N and 101° 02′ W, in a portion of the Chihuahuan Desert of Northeast Mexico. Natural ZC was obtained from a mine located in the state of San Luis Potosí, Mexico. The analyses of mineral compounds were performed at the Central Laboratory of the Universidad Autonoma de Chapingo, Estado de Mexico. Zeolite pH was measured with a potentiometer in a distilled water:ZC ratio 2:1; organic matter according to Walkley and Black (1934); N was extracted with potassium chloride 2 N and determined by Kjeldahl. Phosphorous was calculated by the Bray P1 method; potassium (K) was extracted by means of ammonium acetate 1.0 N and pH 7.0 set by spectrometry of flame emission; calcium (Ca) and magnesium (Mg) were extracted with ammonium acetate 1.0 N, pH 7.0 and determined by atomic absorption. This study consisted of two trials conducted inside a commercial greenhouse of intermediate technology.

Experiment 1. Physicochemical properties of the substrate blends

Fifteen porometers were built by using plastic containers of 7.62 x 12.5 cm, diameter and length respectively. On the bottom of the container a plastic cap was fixed in which four holes 5 mm in diameter, were drilled along its perimetral edge. At the other end, a plastic ring was placed without any fixing. The zeolites samples were placed in the porometers and placed vertically inside a water container with the perforated lid downwards, to force the sample saturation during a 24 hr period. Subsequently, the plugs were removed and the volume of water drained throughout a period of 10 minutes was measured. The wet sample was extracted from the porometers to calculate wet and dry weight of the mineral.

An assay was set to test and compare the physical properties of three substrates: peat moss (pm), perlite (per) and zeolite (zeo), and their mixtures (pm:per:zeo) at different proportions (T1 or control) = 100:0:0; T2 = 70:30:0; T3 = 70:20:10; T4 = 70:10:20 and T5 = 70:0:30 (v/v), to generate five treatments with three replicates; being a single 1 L container a replicate. Total porosity, water holding capacity, bulk, and particle densities were determined for all substrates mixtures according to Pire and Pereira (2003) by using the following equations (1-4):

$$\text{Total porosity (\%)} = \frac{\text{Dv} + \dfrac{\text{Sfw} - \text{Sdw}}{\text{Wsw}}}{\text{Cv}} \times 100 \qquad (1)$$

$$\text{Water holding capacity (\%)} = \frac{\text{Sfw} - \text{Sdw}}{\text{Cv}} \times 100 \qquad (2)$$

$$\text{Bulk density (Mg m}^{-3}\text{)} = \frac{\text{Sdw}}{\text{Cv}} \times 100 \qquad (3)$$

$$\text{Particle density (Mg m}^{-3}\text{)} = \frac{\text{Bd}}{1 - \dfrac{\text{Tp}}{100}} \qquad (4)$$

Where:

Dv = Drained volume (cm^{-3})

Sfw = Sample fresh weight (g)

Sdw = Sample dry weight (g)

Wsw = Water specific weight (g cm^{-3})

Cv = Container volume (cm^{-3})

Tp = Total porosity (%)

Bd = Bulk density (Mg m^{-3}).

Data collected was processed by analysis of variance (ANOVA) in order to determine statistical differences among a group of variable means.

Experiment 2. Effect of zeolite-clinoptilolite (ZC) on Solanum lycopersicum seedling growth

Plant material and experimental set-up

Floradade tomato seeds (Eden Brothers, USA) were seeded on polystyrene rectangular trays (200 cavities), filled with peat moss as substrate and grown under commercial greenhouse (Netafim™ Greenhouses, USA) conditions. Trays were hand-watered during four weeks before transplanting which was carried out when the seedlings were 9.0±3 cm tall and already showed the first pair of true leaves.

Five treatments were evaluated with twelve replicates each, arranged in a completely randomized design. Before transplanting to 1 L pots (one plant per pot), fertilizers were mixed with the substrate blends (T1–T5) at the rate of 150-80-80 (N-P-K) kg ha^{-1}. Irrigation time was controlled using an automated system, the amount of water applied was regulated according to plant demand; complementary nutrients were supplied every three days by using a Hoagland solution (Fukuyama et al., 1994, 120). During the experimental period, time was allowed for drainage to take place; however, the leached nutrients were not measured.

Data collected were: plant height (from crown to apex), stem diameter (measured 10 cm above the crown), leaf area (LI-COR model LI-300, Lincoln, Nebraska, USA), number of leaves, chlorophyll index (Minolta SPAD 502), primary root length, total root and shoot dry biomass (leaves, stem and roots). Measurements were performed at the end of the experiment (50 days after sowing) and five plants per treatment were harvested.

Leaf area, SPAD units, plant dry biomass and root length

Total leaf area was determined at the end of the experiment by using a leaf area meter (LI-COR 3100, Inc., Lincoln, NE, USA). Four evaluations of 5 different plants per treatment were performed. SPAD units were determined with a Minolta SPAD 502 leaf chlorophyll meter at 50 DAS. For chlorophyll index assessment, data was taken from three homogeneus leaves of five plants per treatment. Fresh biomass was accomplished by weighing the aerial part of three plants per treatment and the number of flowers per plant was counted. To determine total dry biomass the samples were kept in a drying chamber at 75 °C for 96 h. Root length was measured with a 30 cm graduated ruler from the base of the stem.

Statistical analysis

The statistical analysis was performed by one-way ANOVA, and Tukey multiple range test ($p \leq 0.05$) with the statistical software JMP version 5.0.1 (2002).

Results

Experiment 1. Physicochemical properties of substrate blends

The chemical tests of the natural ZC employed in this study (Table 1), point out that this type of aluminosilicate is rich in potassium and calcium, and that it also harbors other macro and micronutrients.

Table 1. Chemical properties of natural zeolite-clinoptilolite (ZC).

Chemical composition (mg kg^{-1})										**Texture %**			**M.O. %**	**pH**
N	P	K	Ca	Mg	Fe	Cu	Zn	Mn	B	Sand	Silt	Clay		
17.2	1.43	17360	2641	385	3.8	0.31	0.64	6.77	0.75	52.2	34	13.8	0.4	8.75

Regarding the substrates physical characterization (Figure 2), treatments containing 20% and 30% ZC, presented a statistical increase ($p \leq 0.05$) of total porosity (Figure 2A), particularly T4 (16.6%) and T5 (8.47%), compared to control treatment (T1). All substrates supplemented with ZC promoted greater water holding capacity (WHC), since T4 and T5 exhibited a superior volume of water retained (35.33% and 33.71%, respectively), followed by T3 (25.85%) (Figure 2B). The lesser WHC was attained by T1 (9.34%) without ZC added. Concerning to bulk density (Figure 2C), this variable was increased significantly ($p < 0.05$) by T4 (0.21 Mg m^{-3}) and T5 (0.25 Mg m^{-3}), compared to T1 (0.08 Mg m^{-3}). The addition of ZC to the substrate also improved particle density (Figure 2D), since T4 and T5 reported 0.37 Mg m^{-3} and 0.43 Mg m^{-3} respectively, compared to T1 (0.13 Mg m^{-3}).

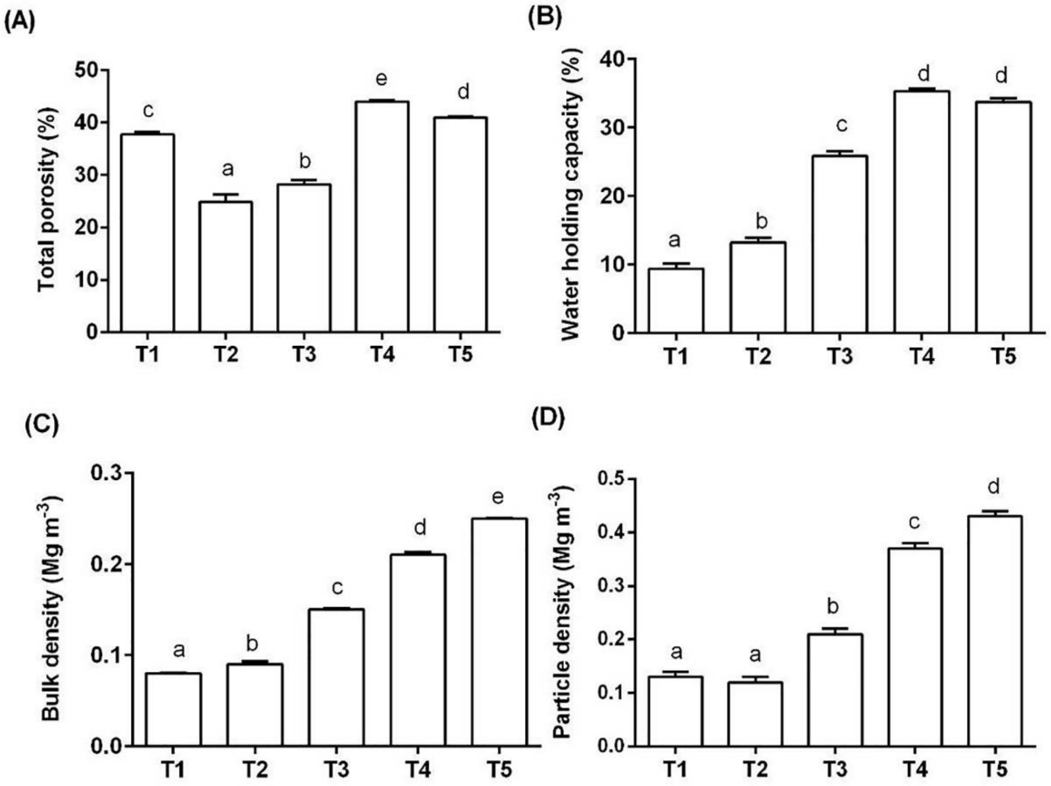

Figure 2. Physical properties of substrate mixtures tested on *S. lycopersicum* seedlings. Error bars represent the standard error of the mean (n=3); columns within the same graph with different letter are statistically different according to Tukey test (α=0.05). An assay was set to test and compare the physical properties of three substrates: peat moss (pm), perlite (per) and zeolite (zeo), and their mixtures (pm:per:zeo) at different proportions. T1 (control) = 100:0:0; T2 = 70:30:0; T3 = 70:20:10; T4 = 70:10:20 and T5 = 70:0:30 (v/v).

Experiment 2. Effect of zeolite-clinoptilolite (ZC) on Solanum lycopersicum seedlings growth

Tomato plants grown on ZC substrates exhibited higher growth (Figure 3), compared to control plants (100% peat moss). Among ZC-containing substrates, peat moss mixed with ZC at a 70:30

ratio (T4), produced higher plants (24.2%, Figure 3A, and Figure 4A); T5 produced greater number of leaves (92.0%, Figure 3B), root length (63.23%, Figure 3C, and Figure 4B); also were increased stem diameter (28.5%, Figure 3D), as well dry weight of aerial part (62.5%), root dry biomass (208.9%) and leaf area (64.5%) than the control plants (Table 2).

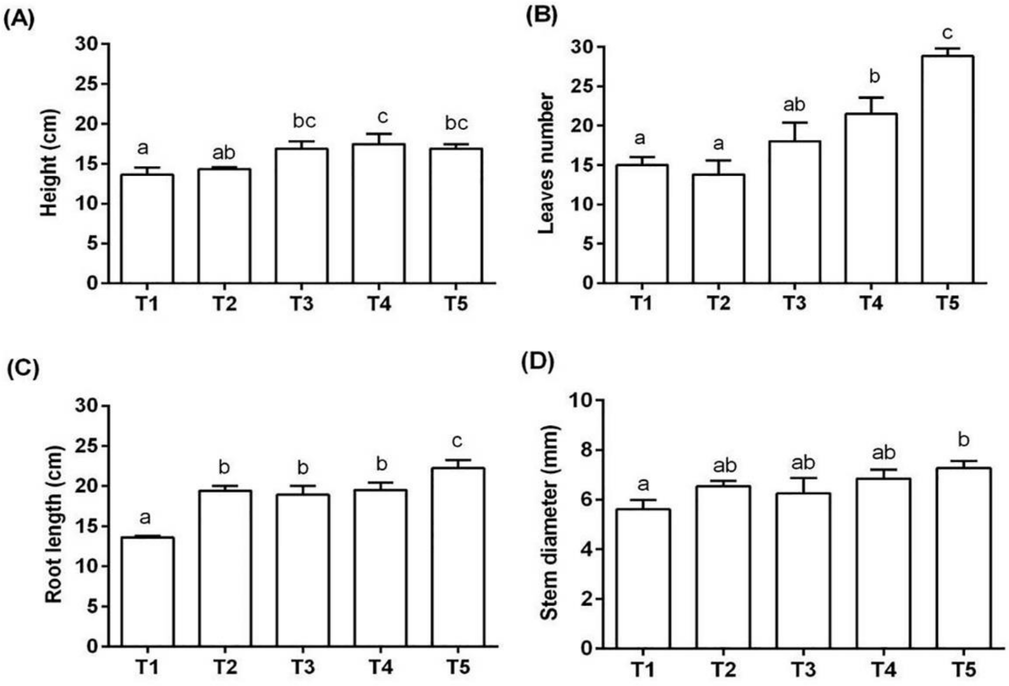

Figure 3. Response of *S. lycopersicum* plants to natural zeolite incorporated to substrates mixtures. T1 = 100:0:0; T2 = 70:30:0; T3 = 70:20:10; T4 = 70:10:20 and T5 = 70:0:30 (peat moss: perlite: zeolite (v:v) respectively). Error bars represent the standard error of the mean (n=12); columns within the same graph with different letter are statistically different according to Tukey test (α=0.05).

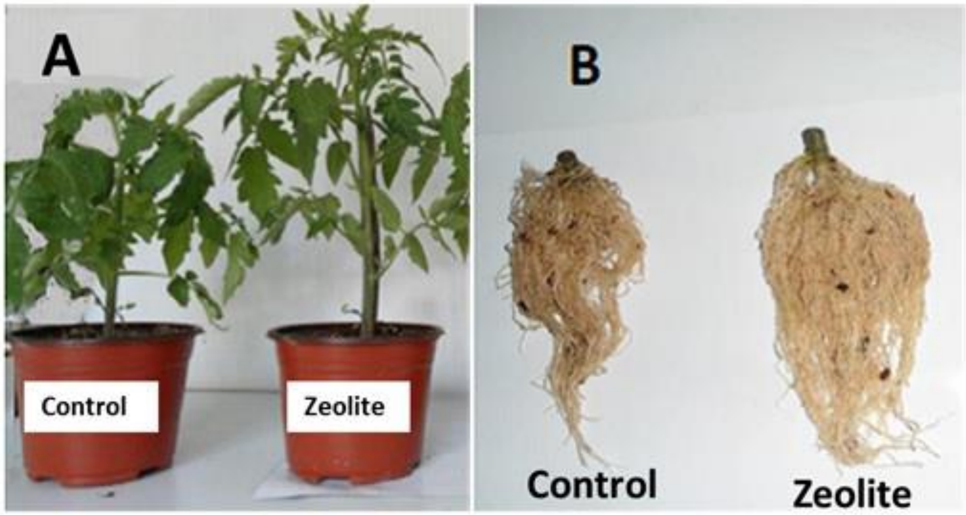

Figure 4. (A) Aerial biomass and root length (B) of tomato seedlings grown on substrates containing zeolite clinoptilolite.

Nevertheless, pm: per: zeo at 70:20:10 (T3) and 70:10:20 (T4) ratios were the substrates that produced plants with superior root growth (Table 2). On the contrary, relative chlorophyll content of leaves (SPAD index), was the only parameter which showed no significant differences (p>0.05) among plants grown on any of the substrates tested.

Table 2. Traits of tomato plants grown on substrates containing different proportions of peat moss, perlite and zeolite-clinoptilolite.

Treatments							
Traits	**T1 100:0:0**	**T2 70:30:0**	**T3 70:20:10**	**T4 70:10:20**	**T5 70:0:30**	**CV (%)**	**p>F**
Leaf area/plant (cm^2)	478.80±42b	706.41±86a	701.14±88a	695.82±34a	787.89±46a	22.2	0.020^*
Aerial part dry weight (g)	2.49±0.34a	3.51±0.33ab	3.66±0.37ab	3.52±0.20ab	3.94±0.27b	21.4	0.025^*
Root dry biomass (g)	0.67±0.07a	1.87±0.44ab	2.47±0.43b	2.34±0.62b	2.07±0.20ab	50.0	0.025^*
Chlorophyll content‡	39.12±2.0a	40.26±1.0a	40.70±2.0a	38.78±1.0a	41.25±1.0a	11.4	0.866_{ns}

Means (n=12); ± SE in the same row with different letters, are statistically different (Tukey's, p≤0.05); * = Significantly different; ‡Spad value; ns= No significance between treatments; CV= Variation coefficient.

Discussion

Substrates containing ZC significantly increased the water holding capacity compared to substrates without ZC added, this means water in the treated substrate can be more readily adsorbed by crop plants. Thus, ZC has special properties that can be potentially applied to increase water use efficiency in agriculture. Several experiments carried out in pots at the seedling stage agree with our results. The results of Yılmaz *et al*. (2014, 2771) point out that mixtures of peat moss; peat moss and zeolite (20 % and 100% v / v), significantly promoted better germination, plant height, stem diameter, fresh weight and nutrient content in dry biomass of cucumber seedlings. The outcome of Manolov *et al*. (2006, 487) stated that zeolite mixed with perlite (1:1 v/v), stimulated greater dry biomass, root length and leaf area of tomato and cucumber seedlings after 25 days of evaluation. Likewise Gul *et al*. (2005, 464) detected a growth increase of N and K content in lettuce plants, and a reduction of K leaching on substrates containing zeolite at 50 and 70%. Bernardi *et al*. (2010, 435) documented the highest increase in growth and dry biomass production of lettuce, tomato and rice plants, with the incorporation of 20, 40, 80 and 160 grams per 3 kilo of perlite substrate added.

It had been demonstrated that zeolite mixed with other substrates can hold macro and micro nutrients, such as K and N from the chemical fertilization, therefore reducing its loss by percolation, favoring its availability, and functioning as a slow release fertilizer in grain crops such as *Triticum aestivum* and *Avena sativa* (Orha *et al.*, 2015, 4). This fact is of great significance for tomato and other vegetable crops, since K has a very important function in pH stability, enzymatic activation, photosynthesis, protein synthesis, cellular elongation and stomatal aperture (Najafi-Ghiri, 2014, 33; Orha *et al.*, 2015, 4).

Results reported by Xiubin and Zhanbin (2001, 45) show that soil treated with zeolite, compared to normal soil, increase infiltration by 7-30% on gentle slope land and more than 50% on steep slope land. In addition, soil amended with zeolite increased soil moisture by 0.4-1.8% in extreme drought condition and 5-15% in general situation. Consequently zeolites improves water use efficiency, as well the effectiveness of fertilizers such as nitrogen, phosphorous and organic manure incorporated to the soil (Ramesh *et al.*, 2011, 228).

In the present study the growth enhancement showed by *S. lycopersicum* plants cultured in the presence of ZC incorporated into the substrate, agrees with several reports related to different crop plants grown with this aluminosilicate as substrate. For example, Kavoosi (2007, 69) reported that zeolite applied to substrates at the rate of 8, 16 and 24 t ha^{-1} increased the yield of rice plants by 19%, and all levels of zeolite with N added, statistically increased grain yield; zeolite also increased significantly soil-available K and its uptake by rice straw.

Incorporation of zeolite to substrates enhances N uptake by plants (Lija *et al.*, 2014, 284), and since it is a major plant nutrient involved in protein and chlorophyll chemical structures, as well in many more important molecules, its availability impacts crop yield (Malekian *et al.*, 2011, 970). Zeolite also acts as a mineral fertilizer, because it is a natural source of N, K, Ca, Mg, Fe and other minerals. In the present study the high K content might have been assimilated by *S. lycopersicum* plants and then positively impacted photosynthesis by improving the water dynamics of plants, namely stomata conductance and transpiration (Abdi *et al.*, 2006, 388).

An improved yield effect due to zeolite addition also was found in *Brassica napus* plants by Shahsavari *et al*. (2014, 1813), since the greatest proportions of all studied traits were attained by applying Z_2Zn_2 (15 t ha^{-1} zeolite and 0.1% Zn sulfate). The rates of grain yield, biological yield, and harvest index improved by 43.8 %, 73.9 %, and 30.0%, respectively, by the pooled application of Z and Zn.

In a similar way, Yilmaz *et al.* (2014, 2771) described that soil-applied zeolite had a positive effect on nutrients contents of *Cucumis sativus* plants. Likewise, Malekian *et al.* (2011, 973) described that grain yield, grain N content, dry matter, and N uptake of *Zea mays* were significantly greater in soil amended with 60 g kg^{-1} of zeolite-clinoptilolite. Najafinezhad *et al*. (2014, 234) reported that for corn and sorghum crops, treatments with zeolite had the highest forage yields and the lowest cadmium concentrations in the tissue. Using 4.5 t ha^{-1} residues, significantly increased relative water content and decreased leaf malondialdehyde and proline; consequently, they recommend planting corn using 5 t ha^{-1} zeolite and 4.5 t ha^{-1} residues in a double-cropping system.

In consideration of our results, we conclude that the zeolite-clinoptilolite, which was obtained from a Mexican natural land deposit, promoted plant growth and biomass accumulation of *S. lycopersicum* seedlings, by means of substrate amendment, since the ionic mobility of major cations is greatly increased in the zeolitic substrates used for greenhouses or shade houses. However, our results have to be validated under open field conditions or in protected agriculture.

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