

## Effects of biochar on pepperoncini (*Capsicum annuum* L cv. Stavros) germination and seedling growth in two soil types

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### Abstract

Biochar is a solid material obtained from the carbonization of biomass. The effect of biochar on the germination and seedling growth of pepperoncini pepper (*Capsicum annuum* L. cv. Stavros) was studied under controlled growth chamber conditions in two experiments. The substrates in the pre-test experiment (first experiment) were 2%, 4%, 6%, 8%, and 10% biochar (at 24°C or 28°C), while in the second experiment they were acidic (pH 6.1) or alkaline soil (pH 7.2), with or without biochar at 24°C. Three replicates (Petri dishes) for each treatment were placed at random in a growth chamber for 12 days at a 16/8h light/dark photoperiod, 12 klx light intensity and 80% relative humidity. In biochar substrates the seed germination percentage in H<sub>2</sub>O-control was high at 24°C and low at 28°C. At 28°C it was also increased (4°C) and it was higher than the corresponding one in the H<sub>2</sub>O-control. At both temperatures (24°C and 28°C) there was a tendency for increased height growth. The percentage of seed germination in acidic soil substrates was low (42%), while in alkaline soil substrates high (82%). All biochar applications in acidic soil substrates increased the seed germination percentage, which varied between 53% and 67%. Biochar application in alkaline soils substrates did not influence seed germination. Seedling height in acidic soil substrates was 0.15cm, while in alkaline soil substrates it was 1.63cm. Biochar application in acidic soils increased the seedling height, while in alkaline soils it was reduced when compared to the control.

**Keywords:** *Capsicum annuum* L. cv. Stavros; acidic soil, alkaline soil, height.

### Introduction

Biochar is organic, rich carbon, produced through pyrolysis of plant biomass and used as soil amendment. Biochar can be applied to agricultural land as part of agronomic or environmental management (Lehmann and Josephs, 2009; Sparkes and Stoutjesdijk, 2011), and has the potential to become a new technology employed in agricultural systems, since it has the capacity to increase nutrient availability in many soil types. The effects of biochar are specific to soil type and plant species, while studies have focused on changes to soil in agronomic conditions (Xu et al., 2012; Drake et al., 2015). Responses to biochar application depend on the soil physico-chemical characteristics (Agegnehu et al., 2015). Moreover, it can be used as soil amendment to improve soil quality and crop productivity in a variety of soils (Blackwell et al., 2009). It has a positive influence on crop productivity when incorporated into acidic soils with low water holding capacity or low organic matter (Biederman and Harpole, 2013; Jeffery et al., 2011; Liu et al., 2013). Biochar application to acidic soils can increase soil pH to alkaline levels (Ogawa, 1994). Many biochars have the alkaline pH, so, they can provide some benefit in neutralising acidic soils; however, this alkalinity may be detrimental when applied to soils with alkaline pH values, as reductions in plant productivity have been demonstrated when alkaline biochars are added to a Calcarosol (Van Zwieten et al., 2010). Biochar amendment on soil may have agronomic advantages, including improvements in soil quality and plant growth (Jeffery et al., 2011; Chan et al., 2008). Biochar has been reported to increase both (Chan et al., 2008; Yamato et al.,

2006), as well as decrease (Deenik et al., 2010) plant growth, but there was little evidence to support the hypothesis of increased seed germination with the addition of biochar. Researchers observed species-dependent effects of biochar on the germination (Free et al., 2010; Keller et al., 2010; Kwapinski et al., 2010; Van Zwieten et al., 2010; Solaiman et al., 2011; Robertson et al., 2012; Kamara et al., 2014; Soni, et al., 2014). Atkinson et al. (2010) stated the importance of further critical analysis of biochar in temperate soil because of the variability of biochar and the lack of research to date. Most biochars are alkaline in nature (Krull et al., 2012) and offer some degree of acid-neutralising capacity. Meta-analyses conclude that biochar is more likely to be effective in enhancing plant growth in acidic, as opposed to alkaline soil types (Atkinson et al., 2010; Jeffery et al., 2011; Biederman and Harpole 2012; Crane-Droesch et al., 2013). Macdonald et al. (2014) demonstrated that plant growth responses in acidic soils were most evident but demonstrated a strong contrast to one another.

The effect of biochar on seedling growth varied depending on soil properties (Solaiman, 2012; Wang and Xu, 2013) demonstrated that biochar improves the growth of wheat seedling, while Keller et al. (2010) noted that even with tar-enriched biochar amendment up to 10%, weight in soil, seedling growth of lettuce increased. Kwapinski et al. (2010) showed the growth of maize seedlings was inhibited with Miscanthus biochar made at 400°C, and was stimulated by Miscanthus biochar made at 600°C (Solaiman, 2012). Biochar type and application rate influenced wheat seedling

growth in a similar manner to both the soil-less Petri dish and soil-based bioassay (Wang and Xu, 2013).

Pepperoncini pepper (*Capsicum annuum* L.), commonly known as mildly pungent, pickling pepper, is a processing type grown in Western Greece in limited quantities. Pepperoncini plants grown at the narrowest spacings, produced the smallest plant, leaf, and stem biomass, but resulted in the highest fruit yields and counts per hectare and the lowest fruit yields per plant (Motsenbocker, 1996). Immature fruit of mildly pungent and no pungent pepperoncini are used as a pickled condiment in many cultures. Demand is increasing due to the popularity of salad bars in the retail and food service industries. A pepperoncini crop with biochar incorporation at each stage could be a practical way of achieving soil improvement, however little research has been conducted on pepperoncini (Saamin, 1978).

Major research interests have been directed to study biochar effects on soil quality and crop response, but little information is available about their possible effects on vegetables species. It was hypothesized that these applications of biochar could modify the seed germination and seedling growth of pepper under growth chamber conditions. The purpose of this study was 1) to evaluate the effect of biochar rate on seed germination of pepperoncini and seedling growth in two temperatures, and 2) to determine the effect of biochar on acidic and alkaline soil on seed germination, and seedling growth of pepperoncini.

## Results

### *Seed germination in biochar substrate*

The seed germination observation of pepperoncini pepper (*Capsicum annuum* L cv. Stavros) lasted 12 days at temperatures of 24°C and 28°C in a controlled plant growth chamber, in 2%, 4%, 6%, 8%, and 10% biochar substrates.

The pepperoncini seed germination in the control (H<sub>2</sub>O) was high 82% at 24°C and low 58% at 28°C (Fig.1). In the substrates with 2%, 4%, 6%, 8%, and 10% biochar at 24°C there was a significant increase in the germination of the pepperoncini, when compared to the control. In the substrate with 4% biochar germination was the same (82%) as in the control. At the temperature of 28°C the germination of the pepperoncini in the substrate with biochar was the same as at 24°C. However it was significantly higher than the corresponding one in H<sub>2</sub>O. The germination percentage during the test periods related to the control at 24°C was higher when compared to that at 28°C, and no significant differences were observed between the two temperatures in all biochar substrates, as well as in the seed germination of the pepperoncini (Fig.2).

### *Seedling growth in biochar substrate*

The height of the pepperoncini seedlings in the control (H<sub>2</sub>O) was 1.5cm at 24°C, and 0.9cm at 28°C. With the increasing rate of biochar at 24°C the pepperoncini seedling height was increased (2.4cm to 2.8cm). Moreover at 28°C the seedling height was increased as the biochar rate increased, while it was reduced in the highest (10%) biochar rate in the substrate (2.3cm at 2%, 2.7cm at 4%, and at 6% 2.6cm at 8% and 1.6cm at 10% biochar). At both temperatures there was tendency for increased pepper height growth (Fig. 3). During the growth period at 24°C the pepperoncini seedling height in all biochar rates in the substrates was higher than

the corresponding height in the control (H<sub>2</sub>O) (Fig.4). The same trend was also visible at 28°C for all biochar rates in the substrate during the test periods (Fig.5).

### *Response of pepperoncini seed germination to biochar application in acidic and alkaline soils*

The seed germination observation of pepperoncini lasted 12 days at a temperature of 24°C in a controlled plant growth chamber in acidic and alkaline soil substrates with 2%, 4%, 6%, 8%, and 10% biochar application (Fig.6). The seed germination of pepperoncini in the acidic soil substrates (control) was low (42%), while in the alkaline soil substrates (control) high (82%). The 2%, 4%, 6%, 8%, and 10% biochar application in the acidic soil substrates increased significantly the seed germination percentage of pepperoncini, and varied between 53% and 67%. In acidic soil substrates with high 8% and 10% biochar applications, low reduction of seed germination was observed (53% and 58% respectively). The biochar application in alkaline soils substrates did not influence the seed germination, with the exception of the 6% biochar application in alkaline soils substrates, where the seed germination amounted to 88% (Fig.6).

### *Response of pepperoncini seedling growth to biochar application in acidic and alkaline soils*

The height of the pepperoncini seedlings in acidic soil substrates (control) was low (0.15cm), while in the alkaline soil substrates (control) high (1.63cm). The biochar application in acidic soil substrates increased significantly the seedling height, while in the alkaline soil substrates it was significantly reduced when compared to the control (acidic soil substrates and alkaline soil substrates respectively) (Fig.7). At 24°C the biochar application in acidic soil substrates had the tendency to increase the height of the pepperoncini seedlings. The height of the pepperoncini seedlings in all biochar applications during the test periods in acidic soil substrates was higher when compared to the control (acidic soil substrates). In the 4%, 6%, and 10% biochar applications in acidic soil substrates the height of the pepperoncini seedlings varied between 0.35 and 0.41cm, and it was smaller (0.25cm) in the 2%, and 8% biochar application in acidic soil substrates. During the test periods the height of the pepperoncini seedlings in all biochar applications in alkaline soil substrates was smaller when compared to the control (alkaline soil substrates). In the 2% 4%, 6%, and 8% biochar applications in alkaline soil substrates the height of the pepperoncini seedlings was increasing (from 0.56, 0.63, 0.84 up to 1.10cm) when the biochar application rate was increasing. In the 10% biochar application in alkaline soil substrates the height of the pepperoncini seedlings was smaller, i.e. 0.91cm (Fig.7).

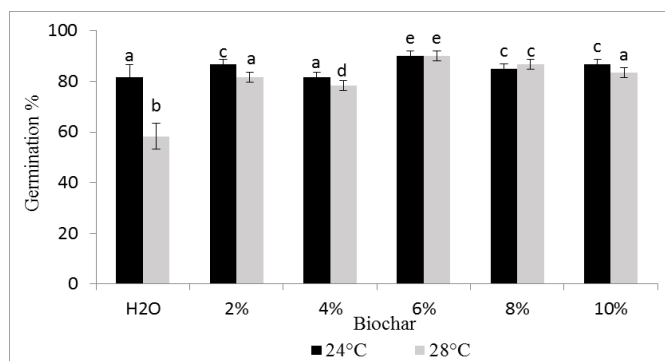
## Discussion

Temperature is one of the most significant environmental conditions affecting germination (Mayer and Poljakoff-Mayber, 1989; Marcos-Filho, 2012). The pre-test with two temperatures showed that the highest seed germination percentage was achieved at 24°C and 28°C, because the pepper species seeds germinate well in a constant temperature range between 15°C and 30°C (Kaymak, 2014); however the highest germination rate of pepperoncini was

**Table 1.** Analytical parameters of the biochar used in the experiments. Analytical methods followed the guidelines in the European Biochar Certificate (EBC, 2013).

Parameter of biochar					
Water, 4h, 103°C (%)	15.6	Cr (g/t)	10.6	K (g/kg)	7.01
Ash (%)	9.9	Ni (g/t)	7.71	Mg (g/kg)	2.5
Volatile matter (%)	77.2	F mg/kg)	<80 <sup>pe</sup>	Ca (g/kg)	22.9
H (%)	1.05	Cu (g/t)	10	P (g/kg)	0.57
C (%)	76.8	Zn (g/t)	34.5		
N (%)	1.4	Mn mg/kg)	626		
TOC (%)	71.3	Pb (g/t)	1.1		
ρB (g/cm <sup>3</sup> )	0.14	Cd (g/t)	0.1		
pH (H <sub>2</sub> O)	9.98	Hg (g/t)	<0.02		
pH-Wert (CaCl <sub>2</sub> )	10.8	Ar (g/t)	0.27		

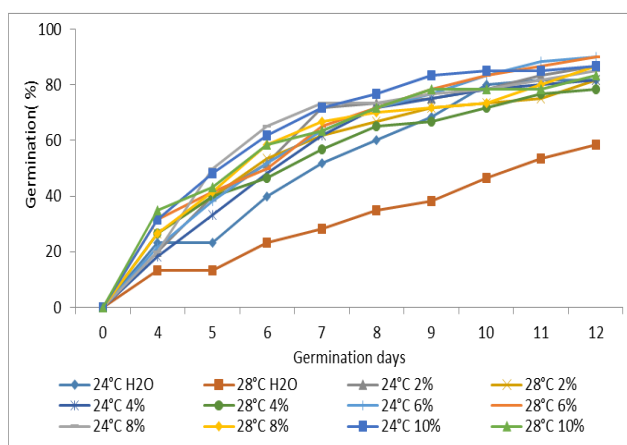
TOC = soil organic carbon content, ρB = bulk density



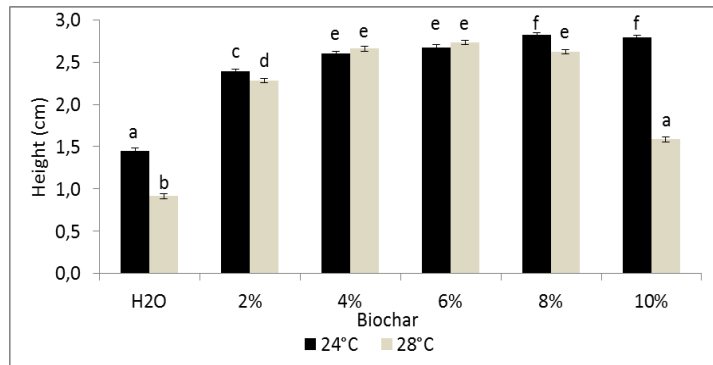
**Fig 1.** Effect of biochar rate on seed germination (%) of pepperoncini at different temperatures. Different letters indicate statistically significant differences among treatments (Duncan test,  $P \leq 0.05$ ).

**Table 2.** Some characteristics of used soils

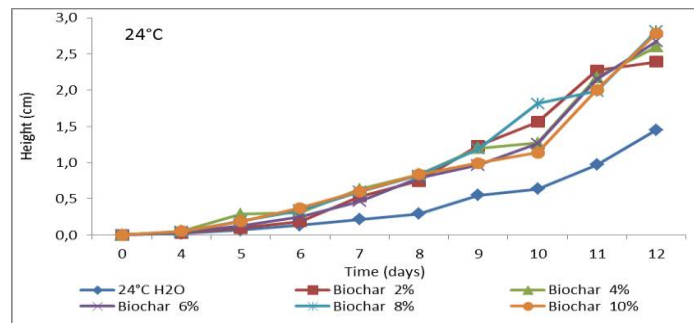
Site	Lappa Achaiais	Amaliada Ilias
soil type	Acidic soil	Alkaline soil
Texture	SCL	CL
EC ( $\mu\text{S cm}^{-1}$ )	180	264
CEC ( $\text{mmolc kg}^{-1}$ )	15.6	17.2
pH	6.1	7.4
pH-value (CaCl <sub>2</sub> )	6.0	7.2
C <sub>inorg</sub> <sup>-</sup> content (%)	-	0.42
C <sub>org</sub> (%)	0.5	0.7
N <sub>tot</sub> (%)	0.4	0.5
ρB ( $\text{g cm}^{-3}$ )	1.16	1.20
WHC (g/100g)	38.2	41.6



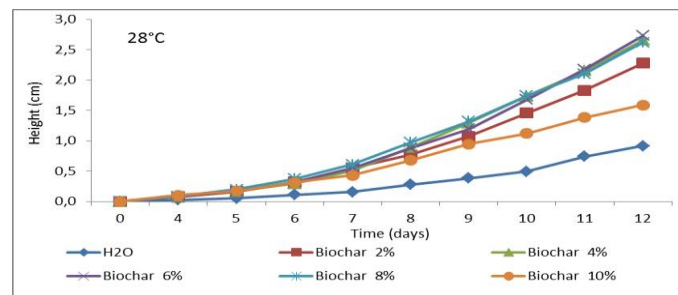
**Fig 2.** Effect of biochar rate on seed germination (%) of pepperoncini at different temperatures during the germination period. Different letters indicate statistically significant differences among treatments (Duncan test,  $P \leq 0.05$ ).



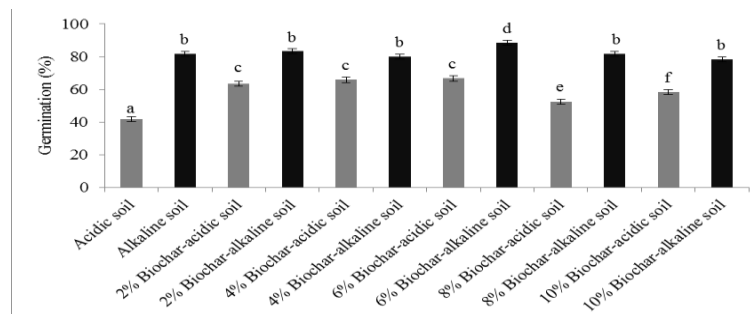
**Fig 3.** Effect of biochar rate on the height ( $\pm$ s.e.) of pepperoncini at different temperatures. Different letters indicate statistically significant differences among treatments (Duncan test,  $P \leq 0.05$ ).



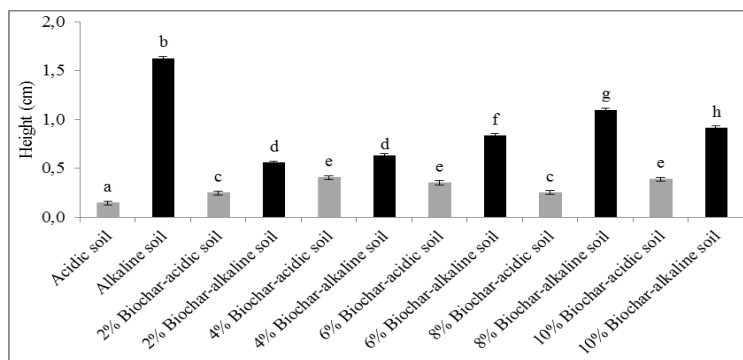
**Fig 4.** Effect of biochar rate on the height of pepperoncini at 24°C during the seedling growth period. Different letters indicate statistically significant differences among treatments (Duncan test,  $P \leq 0.05$ ).



**Fig 5.** Effect of biochar rate on the height of pepperoncini at 28°C during the seedling growth period. Different letters indicate statistically significant differences among treatments (Duncan test,  $P \leq 0.05$ ).



**Fig 6.** Final germination of pepperoncini seeds at biochar rate ( $\pm$ s.e.) in acidic and alkaline soil substrates at 24°C. Different letters indicate statistically significant differences among treatments (Duncan test,  $P \leq 0.05$ ).



**Fig 7.** Effect of biochar rate on height ( $\pm$ s.e.) of pepperoncini in acidic and alkaline soil substrates at 24°C. Different letters indicate statistically significant differences among treatments (Duncan test,  $P \leq 0.05$ ).

found in the control ( $H_2O$ ) at 24°C. At both temperatures the seed germination of pepperoncini in biochar substrates was significantly higher than the corresponding one in  $H_2O$ . This pre-test revealed that used substrates of biochar had positive impact on the pepperoncini seed germination at both temperatures. Other reports on related studies found positive and negative effects of biochar on the germination. Solaiman et al. (2011) observed species-dependent effects of biochar on the germination of wheat, mung bean, and subtterranean clover. Soni et al. (2014) showed that biochar increased germination of palmer amaranth but had no effect on sicklepod and southern crabgrass. The application of maize stover biochar or rice straw biochar did not have an adverse effect on the germination (Kamara, et al 2014). Free et al. (2010) reported that maize seed germination was not affected by biochars made from a range of organic sources.

The results of this study showed that the effect of biochar in the substrate had a positive effect on the height of pepperoncini at both temperatures, but the effect of 10% biochar at 28°C decreased the seedling growth. Wang and Xu (2013) mentioned that biochar improves the growth of wheat seedling. It appears that seed germination of pepperoncini features in the biochar application in acidic soil substrates is the result of not only one factor, but of several different factors. Biochars are predominantly alkaline and have consistently been shown to have a liming effect when added to acidic soils (Krull et al., 2012). Application of biochar to acidic soils can increase soil pH to alkaline levels (Ogawa, 1994). Indeed the 2%, 4%, 6%, 8%, and 10% biochar applications in acidic soil substrates increased significantly the seed germination percentage of the pepperoncini, which varied between 53% and 67%. This study shows that the germination responses as a result of biochar application in acidic soil substrates were higher, mainly in the 2% to 6% biochar applications.

The results of this study showed that during the test periods the height of the pepperoncini seedlings in all biochar applications in acidic soil substrates was higher when compared to the control. The relation between the biochar application in acidic soil substrates and the seedling growth features are obviously complex and hard to assess.

Most of the studies on biochar research have been done in acidic soils and therefore critical lack of research on alkaline soils exists. Qayyum et al. (2014) investigated the effects of an application of various biochars (1%) to alkaline soils on the germination of maize. They reported that the germination percentages ranged from 69% to 85% in different treatments, but none of the biochars caused detrimental effect. Our results show that the germination percentage of pepperoncini seeds in alkaline soils substrates with 2%, 4%, 8%, and 10%

biochar generally increased slightly above the control, although the increase was not significant. Qayyum et al. (2014) referred significant effect of biochar (1%) application to alkaline soil on seedling shoot and root lengths. This paper shows that the height of pepperoncini in all biochar applications in alkaline soil substrates was smaller when compared to the control. Furthermore, the pepperoncini height was increasing when the biochar application rate was increasing from 2% to 8% of biochar application in alkaline soil substrates.

More studies are needed to give further insight into the causes of the differential germination and seedling growth responses of pepperoncini to biochar. Finally, the results suggest that multidisciplinary attempts are fundamental to unravel the complexity of the interactions between biochar seed germination and seedling growth behaviour in the field.

## Materials and methods

### Plant materials

This study was conducted in the Laboratory of the Department of Agricultural Technology of the Technological Educational Institute of Western Greece. Seeds of pepperoncini pepper (*Capsicum annuum* L cv. Stavros) were used nine months after they had been harvested. The seeds are being used in commercial horticulture and were obtained from a company (Ilis Quality Foods SA Paraskevopoulos) in Amaliada, Greece.

### Biochar and soils characterization

Biochar is charred organic matter used as a valuable soil amendment. The biochars used in the study were purchased from a company located in Augsburg city, Germany (Carbon Terra GmbH), and obtained from woody chips (80% coniferous, 20% deciduous wood) made at 700°C for 36h. The biochar had a pH of 9.98, a carbon content of 77.2% and  $H/C_{org}$  molar ratio of 0.014. Its specific surface (BET) area was  $320 \text{ m}^2 \text{ g}^{-1}$ . Further characteristics of the biochar used in this study are summarized in Table 1.

Two soil samples, acidic and alkaline, were collected from two sites, Lappa Achaiais and Amaliada Ilias, respectively, in Western Greece, while the soil samples were collected from the surface layer (0-30cm). The soil samples were air-dried and ground to pass through a 2mm sieve for laboratory experiments. Table 1 shows some characteristics of used soils.

## Seed germination and seedling growth

In order to study seed germination and seedling growth in a growth chamber, two experiments were conducted: The first experiment was a pre-test with two temperatures (24°C and 28°C) in order to gather a first impression of a possible temperature impact on the seed germination and seedling growth of pepper under the effect of biochar. Used substrates were 2%, 4%, 6%, 8%, and 10% (w/v) biochar (CarbonTerra, Augsburg, Germany) and (0%) distilled H<sub>2</sub>O-control. Table 1 shows some properties of biochar. After testing, the second experiment was carried out in the same way, but with a 24°C temperature in acidic and alkaline soil substrates with biochar amendment of 2%, 4%, 6%, 8%, and 10%, acidic (control) and alkaline soil (control).

In the first experiment each of the Petri dishes (Ø 9cm) was filled with 0% 2%, 4%, 6%, 8%, and 10% (w/v) biochar and 15ml H<sub>2</sub>O, while 20 pepperoncini seeds were placed.

In the second experiment every Petri dish contained i) acidic soil (control), ii) alkaline soil (control), iii) acidic soil with 2%, 4%, 6%, 8%, and 10% biochar, and iv) alkaline soil with 2%, 4%, 6%, 8%, and 10% biochar, moistened with 15ml H<sub>2</sub>O, and 20 pepperoncini seeds were sown. Table 2 shows some properties of the acidic and alkaline soils.

Three Petri dishes for each treatment were placed in a completely randomized design in a controlled plant growth chamber at a 24/8h light/dark photoperiod, 12 klx light intensity and 80% relative humidity. Distilled water was added to each Petri dish during the experiment, according to their water requirements. The duration of the test was 12 days.

The germination percentage is an estimate of the viability of seeds. The equation to calculate the germination percentage is: GP = seeds germinated / total seeds x 100.

## Statistical analysis

The seed germination and growth of the pepperoncini seedling data were statistically analyzed using the Analysis of Variance (ANOVA) (Steel & Torrie, 1984). A three-way analysis of variance (ANOVA) was used where temperature, biochar rate, test period were the factors for the first experiment and soil types, biochar rate, and test period for the second experiment. Differences between means were considered to be significant at  $P \leq 0.05$  by Duncan's multiple range test. All statistical analyses were done using the software SPSS package, Version 21.0.

## Conclusion

Our results showed that in biochar substrates at both temperatures (24°C and 28°C) there was a tendency for increased height growth of pepperoncini peppers. All biochar applications in acidic soil substrates increased the seed germination percentage and did not influence seed germination in alkaline soils substrates. Moreover, biochar application in acidic soils increased the seedling height, while in alkaline soils it reduced it when compared to the controls. Thus, biochar applications in acidic soil is beneficial for seed germination and growth of pepperoncini pepper.

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