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EFFECTS OF A CITRUS WOOD BIOCHAR ON PHOSPHORUS USE EFFICIENCY OF *SPINACIA OLERACES* IN A CALCAREOUS SOIL

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ABSTRACT
An open-air, pot experiment was conducted to investigate the effects of citrus wood biochar on phosphorus use efficiency (PUE) of spinach in a calcareous, phosphorous (P) deficient soil. Experimental design was completely randomized block with three levels of P fertilizer (0, 50 and 100 kg/ha), three levels of biochar (0, 1 and 3% by weight) and three replications. The results showed that in soils that have not received P fertilizer and have P deficiency, biochar can significantly increase soil available phosphorous levels and spinach dry matter yield, thus reducing the need for P fertilizer application. Biochar application also improved P uptake by spinach plants. Phosphorous fertilizer use efficiency (PUE) at 100 kg/ha was significantly lower than 50 kg/ha P fertilizer rate. Biochar application improved PUE of 100 kg/ha P fertilizer rate and prevented it to be significantly different than PUE of 50 kg/ha P fertilizer treatment.

KEY WORDS: Fertilizer use efficiency, Phosphorous deficiency, Plant nutrition, Spinach.

INTRODUCTION
Phosphorous is one of the most important elements in plant nutrition. Phosphorous deficiency in soil results in significant reduction of plant growth and crop yields. Therefore, phosphorous fertilizers are used widely in agricultural lands around the world to increase soil phosphorous concentration and crop yields. Phosphorous plays and important role in various metabolic processes within plant such as cell division, production and transportation of sugar and starch, structure of cell wall phosphor-lipids, and root growth [Marschner, 1985]. Phosphorous in soils exists in exchangeable, soluble and precipitated forms, as well as, absorbed on the surface of clay minerals. However, only small fraction of it is available for plant uptake. The results of various studies has showed that phosphorous concentration in plant is variable during different growth stages, and its concentration depends on various factors, most importantly concentration of available phosphorous in soil [Bauer et al., 1987; Ziadi et al., 2008]. Calcareous soils are common in arid regions of the world, such as Iran. Phosphorous precipitates rapidly and becomes unavailable to plants in calcareous soils due to high pH and high concentrations of calcium carbonate. Therefore, appropriate phosphorous fertilizer management in calcareous soils of arid regions is important for crop production.

Biochar is a solid, carbon rich material made by chemical-thermal conversion of biomass in an environment with shortage or absence of oxygen [IBI, 2012]. Research has shown that adding biochar to the soil increases the soil fertility and agricultural productivity significantly [Chan et al., 2008; Lehmann and Joseph, 2009; Lehmann *et al.*, 2003; Rondon *et al.*, 2007]. In addition, biochar has been shown to affect soil chemical properties, such as pH, cation exchange capacity and availability of plant nutrients [Amonette and Joseph, 2009; Beesley *et al.*, 2010; Gundale and Luca, 2007; Uchimyia *et al.*, 2010; Warnock *et al.*, 2007]. Biochar application significantly increased pH and soil available phosphorous concentration to lettuce [Nigussie *et al.*, 2012]. Several studies has shown that phosphorous uptake significantly increases by application of biochar [Nigussie *et al.*, 2012; 14, Streubel, 2011] Therefore, it is possible that biochar application in calcareous soils may increase the availability of phosphorous to plants. Considering the importance of phosphorous fertilizer management in calcareous soils in arid regions, the main objective of this study is to investigate the usefulness of biochar application on availability of phosphorous and phosphorous use efficiency in a calcareous soil from Iran.

MATERIALS AND METHODS
An open-air, pot experiment was conducted during early spring of 2014, in city of Forg, Iran. The experimental design was complete randomized block, with two treatments and three replications. The treatments consisted of three levels of phosphorous (P_2O_5) from triple superphosphate fertilizer (0, 50 and 100 kg/ha) and three levels of citrus wood biochar (0, 1 and 3% by weight). The plant under study was spinach (*Spinacia oleraces*). The soil used in this research was

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Sandy loam, which a pH of 7.6 and salinity (EC_e) of 0.5 dS/m. Phosphorous (P) content of the soil was 5.1 mg/kg, which is considered P deficient for spinach. The biochar used was citrus wood charcoal, which was ground into powder. Some physical and chemical properties of biochar are shown in Table 1. The specific amounts of biochar and phosphorous for each treatment was added to 4 kg of soil, which were packed uniformly in a plastic pot. All pots received urea (250 kg/ha), zinc sulfate (50 kg/ha), copper sulfate (50 kg/ha) and iron sulfate (50 kg/ha), to meet plant nutritional requirements for this soil. 10 spinach seeds were planted in each pot at the depth of 2 cm. Two weeks later the seedlings were thinned to 3 plants per pot. Spinach was harvested 75 days after start of the experiment. Fresh and dry weights of spinach were measured. Soil available phosphorus content was measured according Olsen and Summers [1985]. Phosphorous concentration in spinach plant was measured according to Chapman and Pratt [1982]. Phosphorous uptake by plant was calculated by multiplying dry matter yield to phosphorous concentration in the plant. Phosphorous use efficiency (PUE) was calculated by dividing spinach dry matter yield to the amount of phosphorous fertilizer used per pot. The data were statistically analyzed by SAS statistical software. The comparisons between treatments were performed by Duncan's multiple range test (DMRT) at 5% probability level.

RESULTS AND DISCUSSION

Soil Available Phosphorous

Available P in soil is representative of the amount of P available for plant uptake. The lowest available P was observed in control treatment, which was significantly lower than all other treatments (Fig. 1). The highest plant available P was in 50 kg/ha P fertilizer plus 3% biochar treatment, but it was significantly higher only than control and 1% biochar (no P fertilizer) treatments, and did not have significant differences with the rest of the treatments (Fig. 1).

Table 1: Selected properties of the citrus wood biochar used in the experiment

Biochar properties	Unit	Amount
Ash	%	10.0
Moisture	%	4.25
CEC	cmol/kg	12.84
Phosphorous (P)	mg/kg	21.4
pH (saturated paste)	--	10.41
EC (saturated paste)	dS/m	18.47
Organic carbon	%	88.79

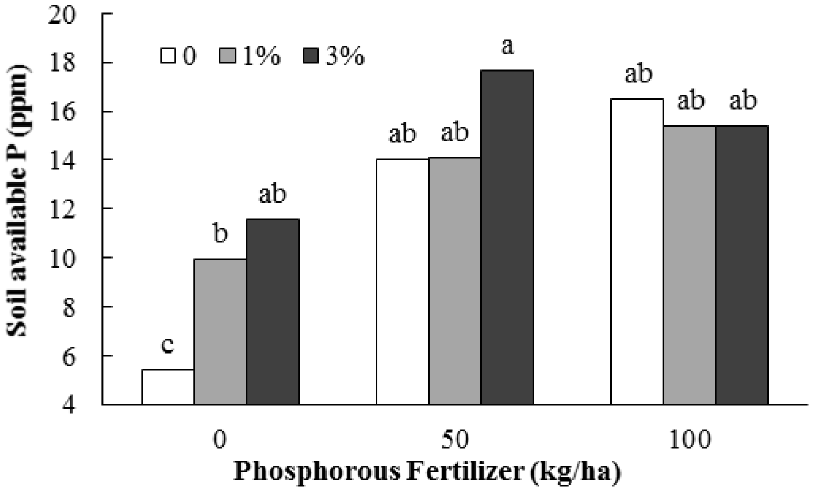

Fig. 1: Interactive effects of P fertilizer and biochar on soil available phosphorous (P). Columns with a letter in common are not significantly different according to DMRT at 5% probability level.

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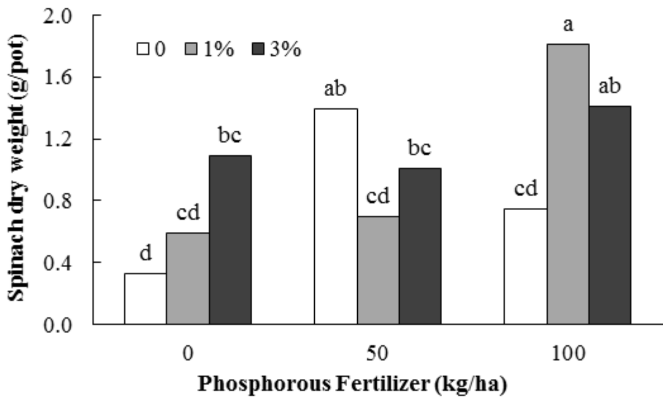

Fig. 2: Interactive effects of P fertilizer and biochar on dry weight of spinach. Columns with a letter in common are not significantly different according to DMRT at 5% probability level.

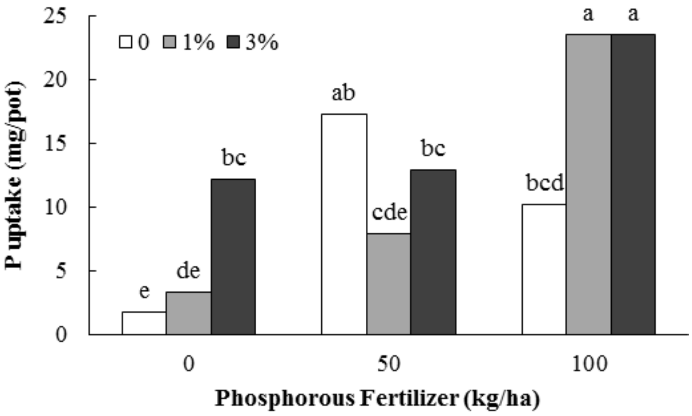

Fig. 3: Interactive effects of P fertilizer and biochar on phosphorous (P) uptake by spinach. Columns with a letter in common are not significantly different according to DMRT at 5% probability level.

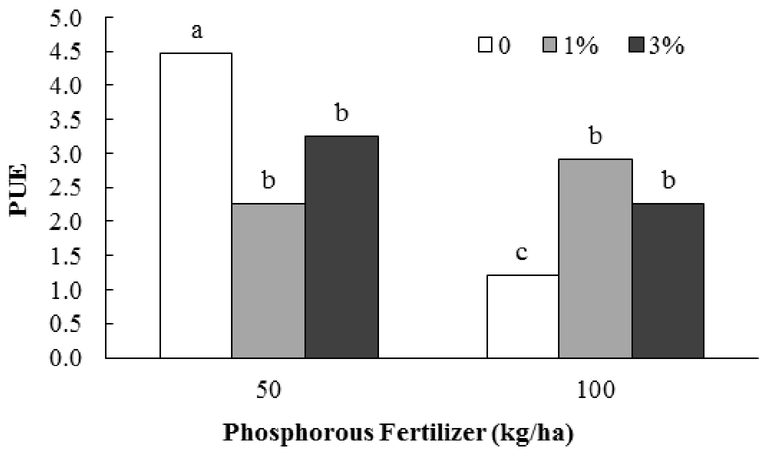

Fig. 4: Interactive effects of P fertilizer and biochar on phosphorous use efficiency, PUE, (g dry weight/g P fertilizer). Columns with a letter in common are not significantly different according to DMRT at 5% probability level.

When no biochar was used, available P increased by P fertilizer application, but there was no significant differences between 50 and 100 kg/ha P fertilizer treatments. When biochar was used, there was no significant differences in available P of control plus biochar and P fertilizer plus biochar treatments (Fig. 1). In 50 and 100 kg/ha P fertilizer treatments, biochar application at any rate did not have significant effect on P availability, but in control treatment, 1 and 3% biochar application significantly increased available P as compared to the control by 83.9 and 114.3%, respectively, but there was no significant difference between them (Fig. 1). Streubel [2011], also observed that biochar application significantly increased soil avail P for plant. Nigussie et al. [2012] observed the same for lettuce plant, and concluded that biochar application is beneficial for increasing uptake of plant nutrients and soil fertility. These findings are consistent with the observations of this experiment. By considering the results, it seems that in soils that do not have P deficiency or have received adequate P fertilizer, biochar application does not have significant effect on soil available P. However, in soils with P deficiency or no fertilizer application, biochar significantly improves P availability to spinach.

Spinach Yield

The effects of biochar and P fertilization treatments on spinach dry matter yield were variable. The lowest and highest spinach dry matter weight (DM) were in control and 100 kg/ha P fertilizer + 1% biochar, respectively (Fig. 2). When no biochar was used, both 50 and 100 kg/ha P fertilizer applications significantly increased DM but there was no significant differences between the two P fertilizer treatments. At 1% biochar level, DM significantly increased only in 100 kg/ha P fertilizer treatment, while at 3% biochar level, there was no significant differences in DM of any P fertilizer treatments (Fig. 2). In control treatment (no P application), 1 and 3% biochar application increased DM by 78.8 and 230.3%, respectively, but the increase was significant only at 3% biochar treatment, and there was no significant differences between 1 and 3% biochar levels (Fig. 2). At 50 kg/ha P treatment, 1 and 3% biochar application reduced DM as compared to no biochar application, which was significant at 1% biochar level. However, at 100 kg/ha P fertilizer application, both biochar levels significantly increased DM as compared to 100 kg/ha P with no biochar application (Fig. 2). Considering the results it seems that under the conditions of this experiment, 50 kg/ha P fertilizer is recommendable for spinach. The results showed that spinach DM of no P fertilizer + 3% biochar application was not significantly different than 50 and 100 kg/ha P fertilizer application (both without biochar) (Fig. 2). In addition, spinach DM in no P fertilizer + 1% biochar application was not significantly different than 100 kg/ha P fertilizer treatment (without biochar). Therefore, it seems that in soils with P deficiency, as was the case with our soil, 1 and 3% biochar application can significantly increase spinach DM and reduced P fertilizer application.

Phosphorous Uptake

The trends in the effects of P and biochar applications on phosphorous uptake by spinach were similar to their effects on spinach DM yield. P uptake increased significantly by P fertilizer application at all levels of biochar (Fig. 3). The highest P uptakes were in 100 kg/ha P fertilizer + 1 or 3% biochar application, which were significantly higher than all other treatments, but were not significantly different than each other (Fig. 3). In control treatment (no P fertilizer), 1 and 3% biochar application increased P uptake significantly by 90 and 596%, respectively. At 50 kg/ha P fertilizer, biochar application reduced P uptake, which was significant at 1% biochar level (Fig. 3). However, at 100 kg/ha P fertilizer level, both 1 and 3% biochar levels increased P uptake significantly as compared to all other treatments (Fig. 3). P uptake in 3% biochar application treatment (no P fertilizer) was not significantly different than 50 and 100 kg/ha P fertilizer treatments (without biochar) (Fig. 3). Therefore, under the conditions of this experiment, 3% biochar application can improve P uptake by spinach significantly and reduce the need for P fertilizer application.

Phosphorous Use Efficiency

The results showed that when no biochar was used, phosphorous use efficiency (PUE) of spinach decreased significantly by increasing P fertilizer rate from 50 to 100 kg/ha (Fig. 4). At 1 and 3% biochar application rates, there was no significant differences in PUE of 50 and 100 kg/ha P fertilizer rates (Fig. 4). Therefore, under the conditions of this experiment, utilization of biochar caused PUE improvement in 100 kg/ha P fertilizer application, and prevented significant reduction of PUE as compared to the 50 kg/ha P fertilizer treatment. The highest PUE was observed at 50 kg/ha P fertilizer application + no biochar treatment, which was significantly higher than all other treatments (Fig. 4).

CONCLUSION

Based on the results, it seems that biochar application in soils that have not received P fertilizer and have P deficiency can significantly increase soil available phosphorous levels. In addition, in the above mentioned soil conditions, 1 and 3% biochar application rates can increase spinach dry weight significantly, and thus, reduce the need for P fertilizer application. Biochar application also improved P uptake by spinach plants. Phosphorous fertilizer use efficiency (PUE) at 100 kg/ha was significantly lower than 50 kg/ha P fertilizer rate. Biochar application improved PUE of 100 kg/ha P fertilizer rate and prevented it to be significantly different than PUE of 50 kg/ha P fertilizer treatment. Under the conditions of this experiment, 50 kg/ha P seems to be the sufficiency level for spinach and is recommended. If the soil is P deficient, it is recommended to apply at least 1% citrus wood charcoal instead of P fertilizer. This would be a more environmental friendly management practice, and due to special characteristics of biochars, the benefits may last for many decades.

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