Agriculture and Natural Resources 51 (2017) 247–252

Contents lists available at ScienceDirect

Agriculture and Natural Resources

journal homepage: http://www.journals.elsevier.com/agriculture-and-natural-resources/

Original Article

Phytotoxic effects of argan shell biochar on salad and barley germination

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A R T I C L E I N F O

Article history:
Received 20 September 2016
Accepted 12 April 2017
Available online 15 May 2017

Keywords:
Argan shells
Barley germination
Biochar
Phytotoxic tests
Salad germination

A B S T R A C T

Biochar produced from argan shells can be contaminated by toxic substances accumulated during the pyrolysis process. To determine the potential impact of toxic substances and salt stress, this study focused on the effect argan shell biochar had on the germination of salad (0%, 0.5%, 1%, 2%, 4% or 8% biochar dry weight in a sand-biochar mixture) and barley seeds (0%, 1%, 2.5%, 5% or 10% biochar dry weight in a peat-biochar mixture). No negative salt stress effect of argan biochar on the germination of salad was observed nor on the germination rate and fresh weight of seedlings. Additionally, biochar application increased the germination rate and the fresh biomass weight in all of the treatments. No significant difference was observed from the control with the barley germination rate, fresh and dry weights of barley seedlings, water content and water use efficiency of different mixtures (peat-biochar). Thus, for both the salad and barley germination tests, no negative effects of biochar produced from argan shells were identified, providing a preliminary indication that it could be safely used for agriculture.

Introduction

The application of biochar to soils is becoming more and more common and it is now generally accepted as a management tool for the sequestration of carbon and the mitigation of global climate change in soils where the carbon would otherwise have been released into the atmosphere rapidly as carbon emissions (Lorenz and Lal, 2014; Smith, 2016). It has also been used to amend nutrient-poor soil for soil ecological restoration, soil pH, nutrient mobility and water retention (Lehmann et al., 2006; Liu et al., 2012; Wang et al., 2016). It can lower chemical fertilizer usage and prevent eutrophication of groundwater (Xu et al., 2013; Shareef and Zhao, 2017). Some studies have shown that it could also increase the growth of plants and microbial activity (Cui et al., 2013; Zhang et al., 2016), decrease soil bulk density (Brewer et al., 2014; Liu et al., 2016), increase air-filled porosity (Han et al., 2015; Obia et al., 2016) and increase water holding capacity (Abel et al., 2013; Wang et al., 2016). Thus biochar improves the physical, chemical and biological properties of soils, which have an indirect effect on increased yields (Smider and Singh, 2014; Zhang et al., 2016).

Under semi-arid, arid and desert climatic conditions, such as in Morocco, agricultural practices reserve a majority of the water resources for irrigation, with the resultant extent of irrigated agriculture combining with high evaporative rates leading to degradation of soil quality (Badraoui et al., 2000). One possible solution is the addition of biochar, a highly porous pyrolyzed biomass which is well documented to help retain water and nutrients in soils.

In previous research (Bouqbis et al., 2016), argan shells obtained after extraction of argan oil were selected as the biomass source and converted into biochar using a pyrolytic stove fabricated in Morocco based on the design provided by Dr. Claudia Kammann (Institute for Plant Ecology, Giessen University, Germany). The physical and chemical analysis of argan shell biochar revealed a highly alkaline pH (pH = 10.7), a high electrical conductivity (EC = 4.83 mS/cm), high contents of K (1906.25 parts per million; ppm), Na (339.2 ppm), Mg (125.892 ppm) and $NaNO_3$ (100 ppm), and low contents of Ca (4.8 ppm), KH_2PO_4 (0.33 ppm) and of heavy metals compared to sandy soil and peat. Thus, due to its high

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http://dx.doi.org/10.1016/j.anres.2017.04.001

nutrient content and low content of heavy metals, argan biochar can improve plant growth especially in sandy soil.

However, the use of biochar is not without its critics. Research shows that biochar can contain dangerous inorganic (heavy metals) and organic contaminants (Hale et al., 2012; Oleszczuk et al., 2013; Buss and Mašek, 2014; Domene et al., 2015; Kołtowski and Oleszczuk, 2015). Where there are high levels of contaminants, there is a risk of their uptake by plants or migration down the soil profile to groundwater (Kołtowski and Oleszczuk, 2015). This may have negative effects for humans, for the environment and for living organisms. Thus, biochar applied to soils should be free of toxic substances before any future large-scale application.

The salad germination test was selected in the current study to test for salt stress where the biochar is mixed with an inert, fine-sand medium and the germination of a salt-sensitive species (salad, *Lactuca sativa* L.) is evaluated. Thus, negative effects at high application levels on salad germination may indicate high ash contents but no harmful substances. To test for toxic substances, the barley (*Hordeum vulgare* L.) germination test was selected where biochar is mixed into an organic medium (unfertilized peat substrate) and the plant is not sensitive to salt stress. Thus, negative effects at high application levels on barley germination may indicate the potential toxic effects of harmful substances produced during the process of pyrolysis.

Materials and methods

Salad germination test

The biochar was mixed with an inert, fine-sand medium and used in the salad germination test based on ISO–17126 (Busch et al., 2012), where the germination of a salt-sensitive species (*Lactuca sativa* L.) was evaluated. Several different proportions of biochar and fine sand were mixed following geometrical dilution. In accordance with the ISO test, the factor 2 is the maximal allowed, so the series used was: 0% (control), 0.5%, 1%, 2%, 4% and 8%. For one replicate, an amount of fresh weight equal to 100 g of dry weight was taken out of the whole mixture and filled into a Petri dish. Tap water was added to set the water content to 85% of the maximum WHC. Forty seeds of *Lactuca sativa* L. were evenly sowed, leaving a free space of around 1 cm to the border of the Petri dishes. The seeds were pressed softly into the substrate. Afterward, 90 g (dry weight) of coarse sand was distributed on top. The prepared Petri dishes were placed open in a bloated, zippered plastic bag and positioned in the green house. For the first 48 h, black plastic foil covered the dishes.

After 5 d incubation, harvesting commenced and the number of germinated seedlings and the fresh and dry weights of the above ground biomass were determined. The Petri dishes, which still contained the soil mixture were mixed and 25 g of dry weight were taken. Around 100 ml of water were added. After shaking for 1 h at 150 rpm, the substrate was allowed to settle for 30 min and then the pH and electrical conductivity (EC) were measured. All determinations were replicated three times.

Barley germination test

Five biochar-peat mixtures were prepared, using small amounts of biochar (0%, 1%, 2.5%, 5% and 10%) where 1% describes a mixture of 1 g dry biochar with 99 g dry peat. Then, the dry weight and water holding capacity (WHC) of the substrates were determined. To prepare 1000 ml of dry weight for every mixture, the amount of fresh weight of biochar and peat and the amount of water needed to set the mixture to 60% of the maximum water holding capacity (WHCmax) were calculated. Afterward, some textile/filter paper

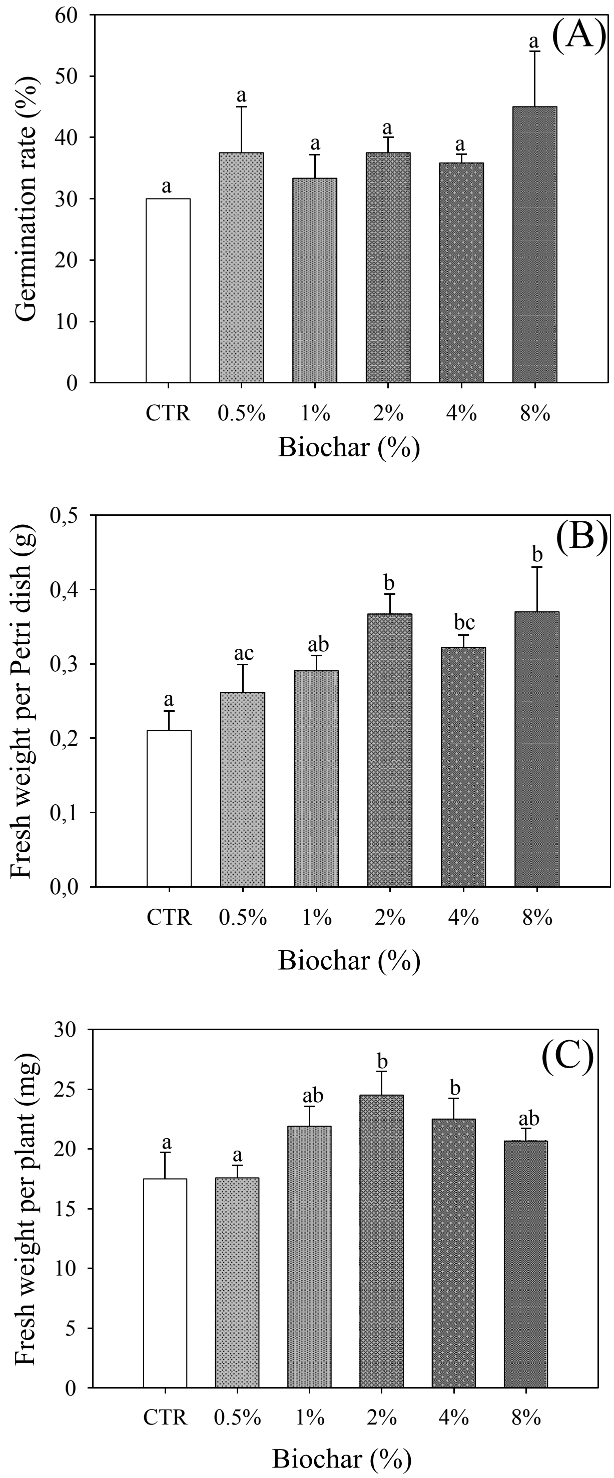

Fig. 1. Salad germination test results: (A) germination rate; (B) fresh weight per Petri dish; (C) fresh weight per plant. Values are mean + SD of the three replicates. Different letters indicate significant differences (one-way analysis of variance) between different mixtures of biochar and the control (CTR).

was placed into the bottom of the plant pots and the mixtures were split into four replicates while leaving sufficient to cover the seeds. After sowing 20 seeds of barely in every pot, the rest of the mixture was distributed over the seeds and the initial weight of the whole construction (pot + mixture with 60% of WHCmax + seeds) was recorded. The plots were then randomly placed in the greenhouse.

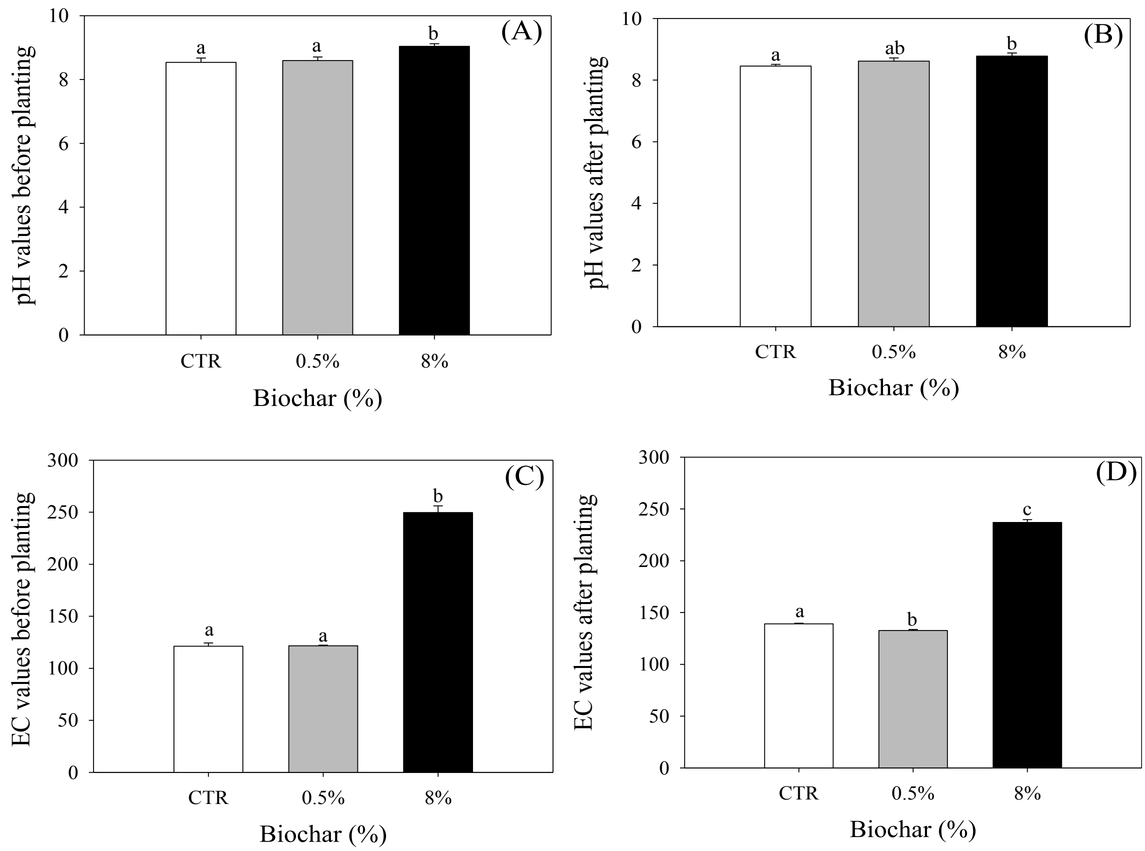

Fig. 2. Effect of biochar application on pH and electrical conductivity (EC) before and after germination of salad: (A) pH values before planting; (B) pH values after planting; (C) EC values before planting; (D) EC values after planting. Values are mean + SD (n = 3). Different letters indicate significant differences (one-way analysis of variance) between different mixtures of biochar and the control (CTR).

The weight of each pot was recorded daily and the difference from the initial weight was made up by adding tap water. The consumption of water (through evapotranspiration) and the produced biomass were used to determine the water use efficiency (WUE). After 9 d, the germination rate, biomass fresh weight, dry weight, water content and WUE (mg dw/g H_2O) were determined.

Statistical analysis

For the barley and salad germination tests, the effects of different biochar additions on all replicated measurements were tested using one-way analysis of variance. The significance of differences among treatment groups was determined using the Tukey test. A result was considered significant at $p < 0.05$. All statistical tests were performed using the SigmaPlot 11 software (Systat Inc.; Chicago, IL, USA).

Results

Salad germination test

In all of the five repeated tests (0.5%, 1%, 2%, 4% and 8% biochar; Fig. 1A), the biochar increased the germination rate of *Lactuca sativa* but not significantly even at a mixture with 0.5% biochar. The highest germination was observed with the highest application rate and represented an increased germination rate of 50% compared to the control. Determining the fresh weight per Petri dish of *L. sativa* seedlings in the biochar test indicated that biochar increased the fresh weight in all of the five repeated tests but the increase was only significant with the three highest application rates (Fig. 1B). Biochar increased the fresh weight by 76% in the mixture with 8% biochar compared to the control. Determining the fresh weight per plant indicated that biochar increased the fresh weight in the four repeated tests while it was nearly similar in the lowest (0.5%) addition. A significant increase was observed with the 2% and 4% biochar applications (Fig. 1C) by 40% and 29%, respectively, compared to the control.

Fig. 2A (before planting) and 2B (after planting) show the pH of each mixture from the highest and lowest rates of application compared to the control. With a higher biochar concentration, the pH before planting was large enough to exceed the pH for the control case while it increased (but not significantly) with the lowest addition. The same results were found after planting. Fig. 2C (before planting) and 2D (after planting) show the EC measured at the highest and lowest rates of application compared to the control. With a higher biochar concentration, the EC was large enough to exceed the EC for the control. With 0.5% biochar, before planting, the EC increased (but not significantly), while it decreased after planting. The water holding capacities were 0.36 g H_2O per g soil (dry weight), 0.39 g H_2O per g soil (dry weight) and 0.43 g H_2O per g soil (dry weight) in the 0%, 0.5% and 8% biochar-sand mixtures, respectively. The biochar application increased the WHC by 7.69% in the 0.5% and by 16.28% in the 8% compared to the control.

Barley germination test

In the three repeated test runs (1%, 2.5% or 5% biochar-peat mixture), the biochar increased the germination rate of barley but not significantly (Fig. 3A) while at the high level of biochar (10%), a small but not significant decrease in the germination rate was

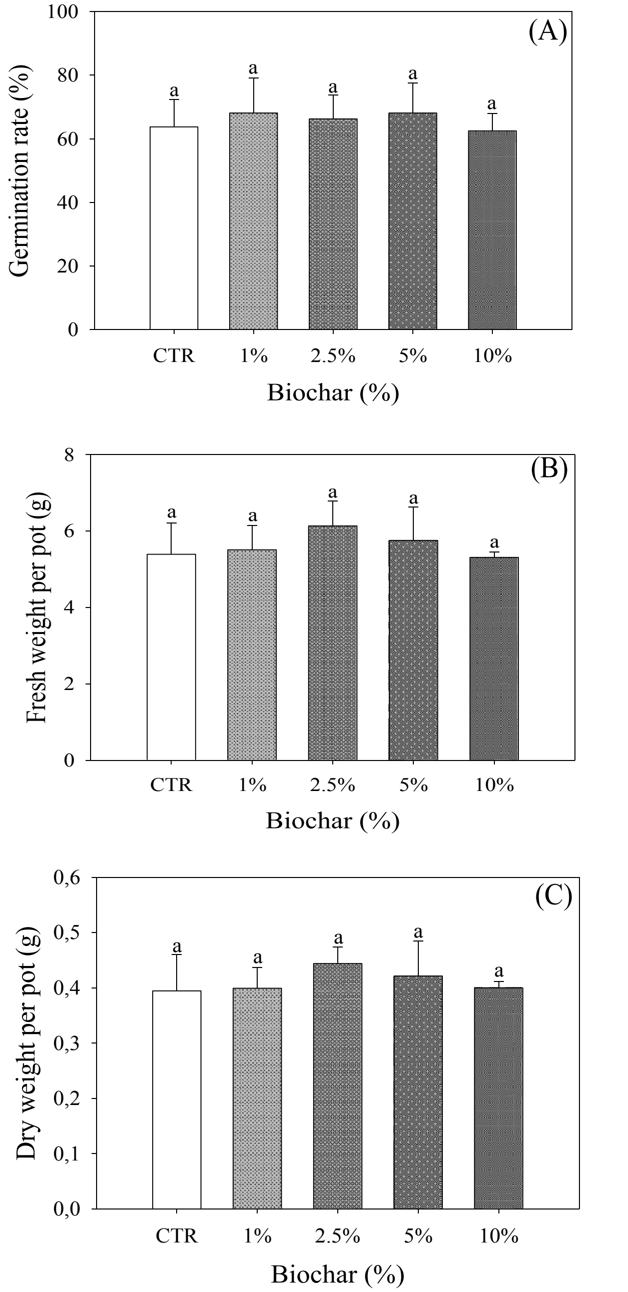

Fig. 3. Effect of argan shell biochar on germination of barley: (A) germination rate; (B) fresh weight per pot; (C) dry weight per pot. Values are mean + SD of the four replicates. Different letters indicate significant differences (one-way analysis of variance) between different mixtures of biochar and the control (CTR).

observed. Fig. 3B shows the fresh weight of barley seedlings in the four biochar tests compared to the control. The results indicated that the biochar increased the fresh weight of barley in three repeated tests (1%, 2.5% or 5% biochar-peat mixture); but the largest positive effect was observed in the 2.5% biochar in which biochar increased the fresh weight by 14% compared to the control. At the higher level, biochar decreased the fresh weight of barley seedlings, but none of the biochar additions resulted in a significant difference to the control. Fig. 3C shows the dry weight of barley seedlings in the four biochar tests compared to the control. In all repeated test runs, the biochar had a positive effect on the seedling dry weight. It increased the dry weight even with the lowest application rate; but none of the biochar additions resulted in a significant increase. The largest positive effect was observed in the 2.5% biochar where the biochar increased the dry weight by 13% compared to the control.

Argan shell biochar had a positive effect on the water content (Fig. 4A) at three biochar-peat mixtures (1%, 2.5%, 5%) while it decreased the water content at the higher level (10%). The largest positive effect was observed with the 2.5% biochar where the water content increased by 14% compared to the control but none of the biochar additions resulted in a significant difference compared to the control. In all of the four repeated tests: 1%, 2.5%, 5% and 10% biochar of the biochar-peat mixture (Fig. 4B), there was an increase (but not significantly) in the water use efficiency even with a mixture with 1% biochar. The largest difference was observed with the 2.5% application rate where the biochar increased the water use efficiency by 11% compared to the control. The water holding capacities were 5.89% g H_2O per g soil (mixture) dry weight, 6% g H_2O per g soil (mixture) dry weight and 6.39% g H_2O per g soil (mixture) dry weight in the 10%, 5% and 0% biochar-peat mixtures, respectively.

Discussion

Salad germination test

The results revealed a positive effect of argan shell biochar on the germination rate of salad in all biochar treatments. The same result was observed for the fresh weight of seedlings which is a more sensitive parameter to the biotoxic substances in the biochar (Fig. 1B and C). Even with 8% biochar, a positive effect was observed. Argan shell biochar has a highly alkaline pH, high EC, high contents of K, Na and Mg and low contents of Ca and heavy metals compared to the soil (Bouqbis et al., 2016). Previous research has revealed that the contents of water soluble K, Ca, Na and Mg increased significantly with biochar application because of the higher content of the cations contained in the biochar relative to soil (Biederman and Harpole, 2013; Jung et al., 2016; Wang et al., 2016). Moreover, biochar increases the retention of mineral nutrients which decreases lixiviation and is likely to increase nutrient availability in the long term (Lehmann and Rondon, 2006; Lehmann et al., 2006; Sigua et al., 2016).

Furthermore, changes in the nutrient and carbon availability after biochar addition to soils may have a positive impact on microbial abundance, rhizobial nodulation and soil fauna because biochar contains macro- and micro-pores (Lehmann et al., 2011; Zhang et al., 2016). Such changes in soil fauna may well have effects on nutrient cycles, soil structure and indirectly affect plant growth (Lehmann et al., 2011).

Based on these studies, the current results (increasing germination rate, fresh weight of biomass, pH and EC) can be explained by the presence of more water-soluble nutrients in the mixture which mainly originated from the argan shell biochar. Thus, mixing argan biochar and soil would facilitate cation retention in the soil.

The application of biochar at an increasing rate significantly increased the pH before and after planting. Similar to pH, biochar addition at high level increased significantly the EC of the mixture before and after planting. The high pH of argan shell biochar indicated that a substantial portion of the nutrient salts sequestered in argan waste was concentrated during the pyrolysis process. The same results were reported by Singh et al. (2010), Biederman and Harpole (2013) and Wang et al. (2016). Given its high pH value, the argan biochar could contain high levels of both macro- and micro-nutrient elements, which were concentrated in the biochar during the pyrolysis process. Collectively, the results indicated that argan shell biochar would be beneficial to soil at these levels and will be useful to increase the pH of acidic soils. The biochar application increased the WHC by 16.28% at 8% compared to the control.

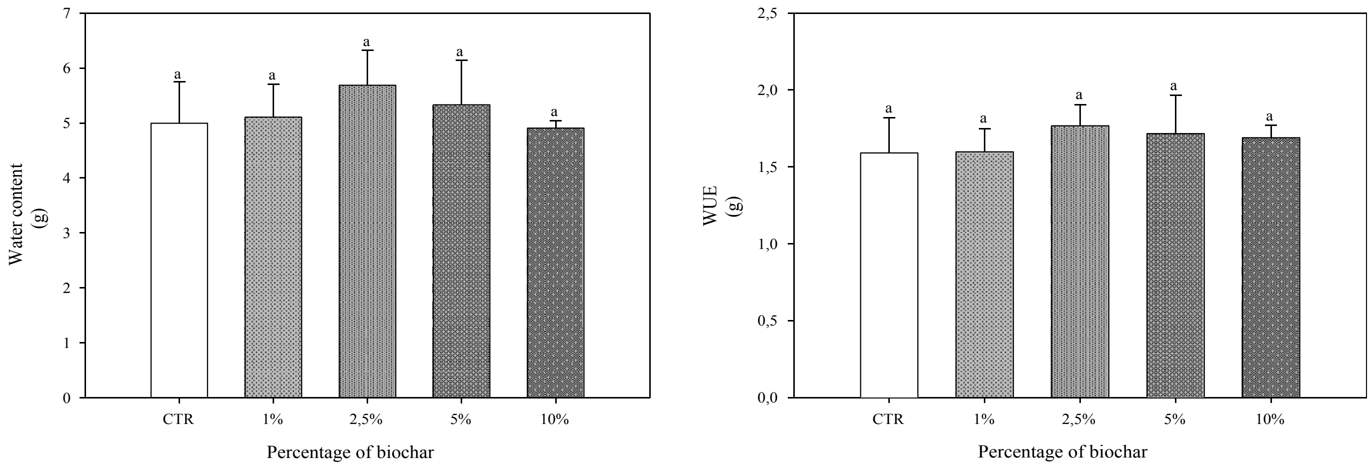

Fig. 4. Effect of argan shells biochar on water content and water use efficiency (WUE) 9 d after germination of barley: (A) water content; (B) water use efficiency. Values are mean + SD (n = 4). Different letters indicate significant differences (one-way analysis of variance) between different mixtures of biochar and the control (CTR).

An increase in the WHC with biochar application has been reported by Abel et al. (2013), Cao et al. (2014) and Wang et al. (2016).

Barley germination test

The germination results of different mixtures (peat-biochar) were compared with the germination results of the control (unfertilized peat, 0% biochar) where the fresh/dry weight was the most sensitive parameter to indicate any negative effect of biochar on seedlings. In all biochar treatments, no negative effects of argan shell biochar were observed on the germination rate of barley (Fig. 3A). The same result was observed for the more sensitive parameters of the fresh and dry weight of seedlings to biotoxic substances in biochar (Fig. 3B and C). No negative effect of peanut hull biochar on the germination of barley was reported by Busch et al. (2012). Furthermore, argan shell biochar had a positive effect on the germination rate, seedling fresh/dry weight, water content and water use efficiency in 1%, 2.5% and 5% biochar-peat mixtures (Figs. 3 and 4) with increases in all the parameters, even at low levels of biochar treatment. At the highest level (10% biochar-peat mixture), biochar increased the dry weight of the biomass and the water use efficiency.

However, at 10%, biochar decreased (but not significantly) the germination rate and fresh weight of barley seedlings compared to the control. The reduction of fresh weight could be attributed to delayed germination that could have resulted in reduced time for growth (9 d) and so led to reduced fresh weight. Delayed germination was also reported for barley seeds from different biochars (Bargmann et al., 2013). Thus, detailed research utilizing long-term experiments will be a logical next step.

Comparing the chemical properties of argan shells to the peat, there was a high content of K, Na and Mg and a low content of heavy metals; the observed increases, even though low, mainly in the fresh and dry weights of biomass could have been the results of the high level of water soluble nutrients and the absence or low content of harmful substances in the mixture with biochar application. Thus argan shell biochar could provide potentially better plant growth.

Argan shell biochar has also a positive effect on the water content and water use efficiency (Fig. 4A and B) with increases in these two parameters even at the lowest application rate, although none of the biochar additions resulted in a significant increase. As with the present study, improvement in the water retention capacity after biochar addition has previously been observed (Cao et al., 2014; Obia et al., 2016) and reported to be due to its porous nature (Downie et al., 2009; Yu et al., 2016). Furthermore, Laird et al. (2009); Sun and Lu (2014) and Obia et al. (2016) have reported that the use of biochar as a soil amendment was anticipated to increase both nutrient and water retention and thereby crop productivity where there is less drainage which was in line with the current observations.

Biochar use as a soil conditioner is currently an important topic of research because of the benefits to crop yield and to the soil, by increasing the water holding capacity, pH, soil organic matter levels and favoring microbial activity. However, biochars can contain toxic compounds produced during the process of pyrolysis or accumulated in the feedstock used in the production of biochars. Thus, for future large-scale application of biochar, it is important to ensure that it will neither show toxic effects nor otherwise pose a short- or long-term threat to soil and the environment.

To determine the potential impact of toxic substances and salt stress, this study focused on the effect of argan shell biochar has on seed germination. Two different germination tests were selected: 1) a barley germination test and 2) a salad germination test. The results obtained from the two phytotoxicity tests revealed no negative effects of argan shell biochar on germination, which provides a preliminary indication that it could be safely used for agriculture. However, future research in long-term experiments will be a logical next step and should consider changes in nutrient availability and plant growth.

Conflict of interest

None.

Acknowledgements

This work was funded by the Hassan II Agronomic and Veterinary Institute, Agadir, Morocco. The authors would like to acknowledge Mr Mohamed Doulhousne for his contribution to the experimental work.

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