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The effect of arbuscular mycorrhiza, rice husk compost and biochar on Iranian borage *Echium amoenum* Fisch & C. A. Mey and post-harvesting soil properties

Einfluss vesikulär-arbuskulärer Mykorrhiza, compostierter Reisspreu und Biochar auf Eigenschaften von iranischem Gurkenkraut *Echium amoenum*, Fisch & C. A. Mey und den Bodenzustand nach der Ernte

Abstract

This study was conducted to investigate the effect of rice husk compost (RHC), rice husk biochar (RHB) and mycorrhization (MY) on some properties of Iranian *Echium amoenum* Fisch & C. A. Mey and also on some selected post-harvesting soil properties. A completely randomized design experiment was conducted with six treatments and six replications. Treatments comprised T_1: control, T_2: MY, T_3: RHC, T_4: RHB, T_5: RHC + MY and T_6: RHB + MY. Studied parameters included; shoot and root fresh weights, root and leaf length, shrub height, leaf number, shoot and root NPK content, shoot and root Fe, Zn, Cu and Mn concentration, root colonization percentage, soil NPK status, soil micronutrients concentrations, soil respiration and microbial biomass. Results revealed that application of RHC, RHB and MY individually or in combination with other treatments significantly affected studied parameters. In all cases except for root colonization, combined application (T_5 and T_6) had more satisfied impacts compared with a single application of treatments.

Key words: Rice husk compost, Rice husk biochar, Mycorrhiza, *Echium* growth, Soil properties

Zusammenfassung

In einem Gewächshausversuch wurde der Einfluss von Bio-Düngern, wie vesikulär-arbuskulärer Mykorrhiza, Compost und Biochar aus Azolla-Algen auf Ertrag, Ertragsstruktur sowie die Aufnahme an Haupt- und Spurenelementen von iranischem Gurkenkraut geprüft. Gegenstand der Untersuchung war auch der Nährstoffgehalt der Böden nach der Ernte, sowie deren biologische Aktivität. Alle geprüften Behandlungen zeigten im Vergleich zu den Kontrollen signifikante Effekte auf Ertrag und Nährstoffaufnahme. Höhere Bodenatmung und eine höhere mikrobielle Biomasse indizieren eine Steigerung der Fruchtbarkeit der Böden durch die Behandlungen.

Stichwörter: Iranisches Gurkenkraut, Bio-Dünger, Nährstoffaufnahme, Bodenatmung, mikrobielle Biomasse

Introduction

In recent years, the safe agriculture is one of the main concerns in the world (El-Kouny, 2002). Furthermore, in

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accordance with recent research reports there has been an increasing awareness of the harmful effect of chemical fertilizers on the environment, as well as the potentially dangerous effects of chemical residues in plant tissues on human and animal health (El-Quesni et al., 2010). Low input cropping systems and innovation of modern management of resources are considered in sustainable agriculture, therefore, using organic fertilizers instead of mineral fertilizers is the first step towards sustainability (Salehi et al., 2016). Soil organic matters are generally one of the most important criteria of soil quality and have an influence on the processes occurring in the soil and many soil properties (Gulser and Candemir, 2012; Cercioglu et al., 2014). There are different methods to achieve safe agricultural and using compost is one of them. Composting agricultural residues by supplying the natural microbial flora present on them, their requirements of inorganic nutrients such as nitrogen and phosphorus and applying a proper moistening finally produces a high able production to enhance plant growth (Awad et al., 2003) and improve soil fertility in terms of physical, chemical and biological properties (Evanylo et al., 2008). Using compost increases the organic material contents of processed and unprocessed soil (Adunga, 2016). Rice husk compost as a kind of compost has been used around the world (FAO, 2002). The changes of structural chemistry of rice husk during composting have not yet received attention. It is expected that composted rice husk may be able to improve soil properties by increasing soil organic C, releasing various essential and beneficial elements and suppressing toxic elements at the same time (Markus et al., 2008). Another method that has been implemented nowadays to achieve safe agriculture is biochar application. Biochar, produced through pyrolysis processing, has drawn a lot of international attention as a useful organic material (Anyanwua et al., 2018). In recent years, application of biochar into agricultural soil, which can improve can improve soil fertility, has been increasingly discussed (Glaser et al., 2003; Lehmann et al., 2006). Biochar has been noted to modify soil properties by affecting biological community composition (Egamberdieva et al., 2016), nutrient cycling (Zee et al., 2017), soil structure (Xu et al., 2014) and soil physical characteristics (Downie et al., 2009). Rice husk biochar is a by-product of rice husk gasification (Carter et al., 2013). There are few research reports on the agronomic impacts of rice husk biochar but as a form of biochar it has benefits when it is applied into agricultural soils (Shackley et al., 2012; Basha et al., 2005). The association between plant and fungi is assumed to play an important role in the land colonization by plants due to the ability of the symbiotic organisms in acquiring unavailable nutrients (Simon et al., 1993; Smith and Smith, 2011). A mycorrhizal system helps plants to increase growth and increase the productivity of host plants (Gupta and Janardhanan, 1991; Linderman and Bethlenfalvy, 1992). Nowadays, one third of human demands for medicine is obtained from plants (Agatonovic-Kustrin et al., 2015). Medicinal plant cultivation has been increased during the last two decades across the world (Salehi et al., 2016). Because of their great importance in modern and traditional medicine, they are also used as raw materials for pharmaceutical, cosmetic and fragrance industries (Karthikeyan et al., 2009). Iranian borage (*Echium amoenum* Fisch. & C. A Mey) from Boraginaceae is a valuable medicinal plant native to Iran and Syria (Mehrbani et al., 2005). Petals of *E. amoenum* have been advocated for a variety of effects, such as demulcent, anti-inflammatory and analgesic, especially for common cold, anxiolytic, sedative and other psychiatric symptoms (Morteza-Semnani and Saeedi, 2013). The amount of effective ingredients of medicinal plants depend on yield of these plants and is affected by nutritional status and fertility of soil (Glyn, 2002).

Considering what was described above and also rare investigations about the impact of mycorrhization on studied medicinal plants the objectives of the present study were to determine the effect of rice husk compost, rice husk biochar and mycorrhization on i) growth and nutrient status of the Iranian medicinal plant *E. amoenum* and, ii) on selected post-harvest calcareous soil.

Material and methods

A completely randomized design experiment was conducted with six treatments and six replications in order to investigate the effect of rice husk compost (RHC), rice husk biochar (RHB) and mycorrhization (MY) on growth and nutrient status of the medical plant *E. amoenum* and selected post-harvest calcareous soil properties. The experiment was carried out under greenhouse conditions at the College of Agriculture, Shiraz, Iran between January and August 2017. The greenhouse temperature was 25–27°C in the day and 16–17°C at night. The soil was collected from the top layer of research field of College of Agriculture, Shiraz, Iran and the soil texture was classified as a silt loam. The RHB was produced from the rice husk provided from research field of College of Agriculture, Shiraz University, Iran using a muffle furnace under limited oxygen conditions at 600°C for 3 hours. Previous studies indicated that higher temperature resulted in a higher C content (Liang et al., 2016). In addition, increasing the temperature lead to an increase of the ash and fixed C contents, and to a decrease of the content of volatile materials (Tag et al., 2016). To prepare RHC, rice husk was composted under aerobic conditions in the greenhouse for 2 months. *Claroideoglomus etunicatum*[1] was used to create mycorrhizal system. The treatments of the experiment comprised of T_1: control, T_2: MY, T_3: RHC, T_4: RHB, T_5: RHC + MY and T_6: RHB + MY. Five kilogram pots were used and filled with treated soil. One percent organic matter was applied for both RHC and RHB. Mycorrhizal infection was done by adding 100 g of *C. etunicatum* inoculum (800 spores, root colonization of 75%) into the top layer of soil (3–5 cm) before sowing.

[1]Classification: *Fungi, Mucoromyceta, Glomeromycota, Glomeromycotina, Glomeromycetes, Glomerales, Claroideoglomeraceae, Claroideoglomus*

Two seeds were planted into each pot. Plants were irrigated using distilled water every day and nutrient elements were added to all pots uniformly based on soil testing results. Selected soil and organic matter properties used in the experiment are given in Tables 1 and 2. Plants were grown for 7 months and then harvested along with their roots. Roots were cleaned by dipping them into water 2 to 3 times until the adhering soil particles were removed. To perform plant analysis, the samples of shoots and roots were transferred to the laboratory and prepared after washing and drying in an oven at the temperature of 65°C for 48 hrs. To measure P, K, Fe, Zn, Cu and Mn, the plant samples were ashed using a muffle furnace at 550°C for 2 hrs. Soil samples were air-dried and passed through a 2 mm sieve. Selected soil, plant and organic matter characteristics were determined as follows: soil texture by the method described by Gee and Bauder (1986); soil reaction (pH) in soil saturated paste (Thomas, 1996) and electrical conductivity (EC) in saturated extract (Rhoades, 1996). Calcium carbonate equivalent (CCE) was determined by titration (Nelson, 1982). Total N was determined by the Kjeldahl method (Bremner, 1996). $NaHCO_3$-extactable P was measured by colorimetric method using a spectrophotometer (Olsen, 1954). Amonium acetate extractable K was determined using flame photometer (Page et al., 1982). Organic matter (OM) content was measured by potassium dichromate oxidation method (Nelson and Sommers, 1996). Fe, Zn, Cu and Mn concentrations of soil samples were extracted with diethylene triamine pentaacetic acid (DTPA) method and measured using an atomic absorption spectrophotometer (Shimadzu AA 670 G, Japan) (Lindsay and Norvell, 1978). Soil microbial respiration was determined (mg CO^2 day^{-1}) by bottle closed method (Anderson, 1982). Microbial biomass C (MBC) was estimated following the fumigation extraction (FE) method (Vance et al., 1987). EC and pH of compost and biochar were determined in a 1:10 (water) ratio (Gillman and Sumpter, 1986). Fe, Zn, Cu and Mn concentrations of plant samples, RHC and RHB were determined by dry ashing and dissolving the ashed samples in HCl 2N and measuring using atomic absorption (Chapman and Pratt, 1961). Total concentration of N in plant samples, RHC and RHB were measured by Kjeldahl (Bremner, 1996). Total P in plant samples, RHC and RHB were determined by Vanadate-Molybdate yellow method using spectrophotometer (Chapman and Pratt, 1961). Total K was determined by the flame photometer in the extraction of the dry ash method (Chapman and Pratt, 1961). Ash content of RHB and RHC was measured by standard ASTMD-2866 method on weight basis, by which 1.0 g of oven-dried RHB and RHC was briefly heated at 600°C overnight, cooled and weighed again (Rajkovich et al., 2012). Colonization percent of root was done by coloring method (Kormanik and McGraw, 1982). Furthermore, fresh weigh of shoots and roots, height of shrub, length of roots and leaves and number of leaves were determined in each pot. Analysis of data was performed using SPSS 21 software package. The difference between treatments was determined using Duncan`s Multiple Range Test (DMRT), ($P \leq 0.05$). Prob-

Table 1. Some physicochemical properties of the examined soil (0–30 cm)

Texture	Parameters													
	Sand	Silt	Clay	pH	EC	OM	CCE	N (T)	P (A)	K (A)	Fe (A)	Zn (A)	Cu (A)	Mn (A)
Silt Loam	32.94	50.24	16.82	7.72	0.69	1.2	42.8	0.143	15.4	446.54	4.99	0.73	1.79	9.6
	Unit													
–	%	%	%	–	dS m^{-1}	%	%	%	mg k^{-1}	mg k^{-1}	mg k^{-1}	mg k^{-1}	mg k^{-1}	mg k^{-1}

T: Total value, A: Available value.

Table 2. Some properties of the applied organic fertilizers

	Parameters										
	pH	EC	OM	N (T)	P (T)	K (T)	Fe (T)	Zn (T)	Cu (T)	Mn (T)	Ash
RHC	6.77	2.13	11.4	0.653	0.07	0.29	1976	31.6	14.4	132	25
RHB	6.85	2.25	24.3	0.965	0.255	1.45	5636	63.65	20.15	201	44
	Unit										
	–	dS m^{-1}	%	%	%	%	mg k^{-1}	mg k^{-1}	mg k^{-1}	mg k^{-1}	%

RHC: rice husk compost, RHB: rice husk biochar and T: Total value.

ability levels of 1% and 5% ($P \leq 0.01$ or 0.05) were used to test the significance among the treatments. The figures were drawn using Excel 2013 software.

Results and Discussion

Growth attributes of Echium

Application of organic amendments significantly affected shoot and root fresh weights (SFW and RFW) ($P \leq 0.05$) and increased them by the percentage ranged from 13.17%–28.48% and 13.99%–32.03%, respectively. The highest value of SFW was observed in RHB + MY treatments, which was 191.07 g pot^{-1} (Table 3), but there was no significant difference to RHC + MY treatments (Table 3). The similar result was obtained for RFW, whereby the maximum RFW was related to the RHB + MY, which was 263.37 g pot^{-1} (Table 3). In previous studies it is reported that plant growth and yield increased following biochar and compost additions. In most cases, this has been attributed to an optimization of the availability of plant nutrients (Gaskin et al., 2010; Agegnuhe et al., 2016). Fresh weights of shoots and roots were significantly affected using biochar for the medicinal plant *Salvia miltiorrhiza* Bunge (Amei et al., 2016) reasoned by high concentration of nutrients especially nitrogen in the organic fertilizer (Hosseini Vakili and Ghanbari, 2015). Based on our results, mycorrhization had a positive effect on SFW and RFW, which is probably due to the fact that arbsucular mycorrhizal fungi can increase the concentration of micronutrients and other mineral nutrients with low mobility. Similar to our findings, other researchers also reported that inoculation with mycorrhiza affects the development of the rooting system and causes to increase root biomass (Linderman and Bethlenfalvy, 1992; Li et al., 1991; Heikham et al., 2009). Furthermore, improved plant fresh yield may be reasoned by the important impact of mycorrhization on nutrient availability enhancing growth and biomass (Selvakumar and Thamizhiniyan, 2011). With respect to the root and leaf length (RL and LL), our results show that an addition of organic amendments had a positive and significant effect on RL and LL ($P \leq 0.05$) (Fig. 1). Organic fertilizer application caused an increases of RL and LL by 14.28%–28.66% and 17.99%–27.01%, respectively, and the highest values are related to the combination treatments, too. The highest values of RL (15.76 and 16.15 cm were observed in co-application of RHC + MY and RHB + MY, respectively. The highest leaf length (28.38 cm) was measured after the RHB + MY treatment, however, there was no significant difference to the RHC + MY treatment. In addition, Brenann et al. (2014) stated an increase in root and leaf length as a result of biochar application. Biochar application in combination with compost significantly increased leaf length of *Lactuca sativa* L (Dalila et al., 2017). In fact, biochar and compost particles in the rhizosphere improve water retention and nutrient availability, which results in higher growth (Prendergast-Miller et al., 2014). According to the results of the present experiment, it was concluded that mycorrhization along with organic amendments application significantly affected RL and LL reasoned by several causes that the most likely of which is due to ideal conditions in terms of water and nutrient uptake for plant growth (Iverson and Maier, 2008). Furthermore, the results clarified that organic amendment application significantly ($P \leq 0.05$) increased height of shrub and leaf number (HS and LN) by 12.53%–27.07% and 14.37%–32.67%, respectively. The maximum height of plants observed in RHB + MY and RHC + MY treatment was 25.25 and 24.89 cm, respectively and the maximum number of leaves were related to the RHB + MY treatment. However, there was no significant difference to RHC + MY treatments (Fig. 2 and Fig. 3). Our results are in agreement with other research reports (Brenann et al., 2014; Seehausen et al., 2017). Similarly, biochar application increased the number of leaves in medicinal plant

Table 3. Means comparison effect of organic amendments on shoot and root fresh weights (g pot^{-1}).

	Fresh weight	
	Shoot	Root
Control	136.38^{c}	181.89^{d}
MY	160.66^{b}	218.75^{c}
RHC	174.74a^{b}	240.54^{b}
RHB	177.85a^{b}	251.01a^{b}
RHC + MY	189.19^{a}	256.32a^{b}
RHB + MY	191.07^{a}	263.37^{a}

Different letters above the bars indicate statistically significant differences between treatments according to DMRT.
MY: mycorrhizatin, RHC: rice husk compost and RHB: rice husk biochar

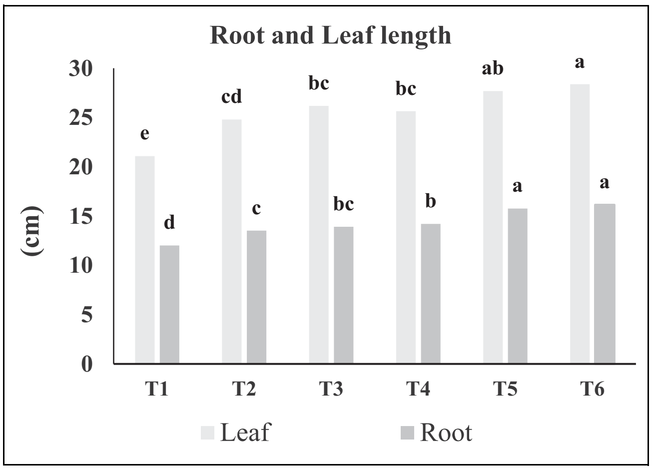

Fig. 1. Means comparison effect of organic amendments on root and leaf length (cm). Different letters above the bars indicate statistically significant differences between treatments according to DMRT ($P \leq 0.05$). T1: Control, T2: MY, T3: RHC, T4: RHB, T5: RHC + MY and T6: RHB + MY.

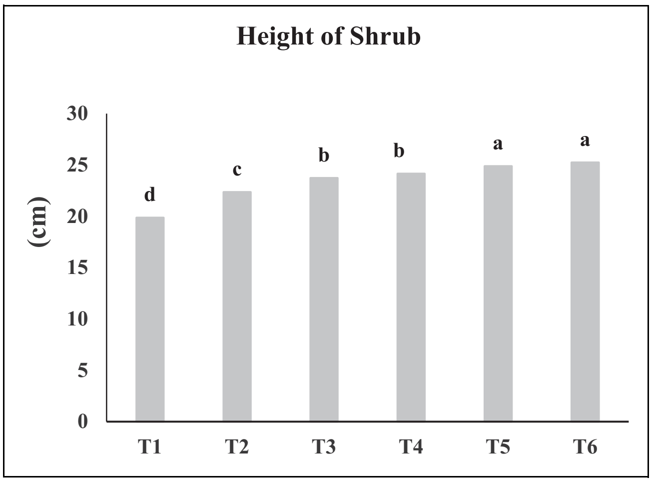

Fig. 2. Means comparison effect of organic amendments on height of shrub (cm). Different letters above the bars indicate statistically significant differences between treatments according to DMRT ($P \leq 0.05$). T1: Control, T2: MY, T3: RHC, T4: RHB, T5: RHC + MY and T6: RHB + MY.

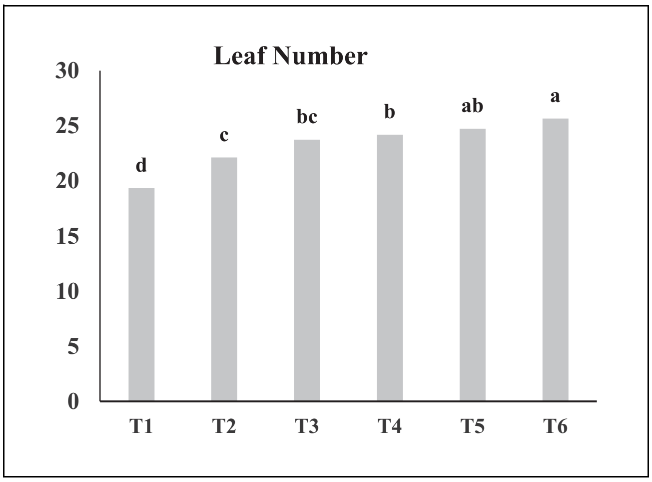

Fig. 3. Means comparison effect of organic amendments on leaf number. Different letters above the bars indicate statistically significant differences between treatments according to DMRT ($P \leq 0.05$). T1: Control, T2: MY, T3: RHC, T4: RHB, T5: RHC + MY and T6: RHB + MY.

S. miltiorrhiza (Amei et al., 2016). The conducted study with *L. sativa* and *Brassica chinensis* showed that biochar application increased both plant height and leaf number (Carter et al., 2013). In the present study mycorrhization had a significant effect on HS and LN (Fig. 2 and Fig. 3). In addition, it was observed that mycorrhization with arbuscular mycorrhiza increased plant height in both *Zea mays* and *Lycopersicon esculentum* (Taylor et al., 2008). Mycorrhizal infection in association with an important medicinal herb (*Artemisia annua*) significantly affected plant height, leaf number and other growth attributes (Raei and Weria, 2013). Furthermore, obtained results by Aguin et al. (2004), indicated that mycorrhizal inoculation increased leaf number of Grapevine compared to the control.

Root colonization percentage

The percentage of root colonization was significantly ($P \leq 0.05$) influenced in mycorrhizal treatments compared to the non-inoculated treatments. Non-inoculated treatments, control, RHC and RHB had only 9.21%, 15.13% and 15.12% colonization, respectively, from indigenous mycorrhizal fungus. The percentage of root colonization levels in mycorrhizal treatments ranged from 29.24% to 38.32%. The highest percentage was observed in single applications of MY (Fig. 4). In conformity to our findings Thapa et al. (2015), documented a significant variation in percentage of root colonization following mycorrhization, which can be due to some components of the rhizospheric soil that might have favored arbuscular mycorrhizal fungi growth. Similarly, the results of a carried out study indicated more percentage of root colonization in a medicinal plant *Valutina A. Juss* compared to non-mycorrhizal plants (Samina et al., 2015). The same was reported by Ohsowski et al. (2018). Based on the results of the present study, an effect of organic amendments on soil P status, that was 13.50, 15.48,

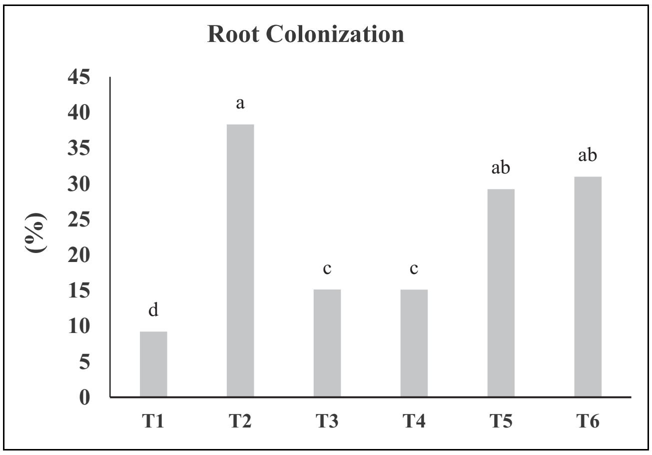

Fig. 4. Means comparison effect of organic amendments on of root colonization (%). Different letters above the bars indicate statistically significant differences between treatments according to DMRT ($P \leq 0.05$). T1: Control, T2: MY, T3: RHC, T4: RHB, T5: RHC + MY and T6: RHB + MY.

16.07 and 16.17 mg kg^{-1} for treatments of control, MY, RHC + MY and RHB + MY, respectively, it was concluded that lower root colonization in combined treatments (RHC + MY and RHB + MY) may cause an enhancement in presence of P in easily forms in the root zone of plants induced by organic amendment applications (Kowalska et al., 2015). Furthermore, Rayan and Graham suggested that high availability of P limits root colonization in mycorrhizal plants (Rayan and Graham, 2002). The same results were reported by Jimenze-Morenoa et al. (2018) who demonstrated a negative correlation between P level and root colonization in seedlings of *Poncirus trifoliate*.

Concentration of N, P, and K in roots and shoots

The concentration of N, P, and K in plant shoots and roots was significantly affected by single or combined application of organic amendments ($P \leq 0.05$). The highest values were observed in co-application of RHB + MY. Maximum N, P and K concentrations in plant shoots were 2.32%, 0.22% and 2.39%, respectively. The similar result was observed for roots showing the highest concentrations of N, P and K (3.11%, 0.30% and 2.45%, respectively) (Fig. 5, 6 and 7). These findings are in agreement with other studies whose authors reported that bio-fertilizers are able to enhance plant macronutrient availability (MARKUS et al., 2008). According to the results of a further study it was revealed that organic amendments significantly influenced shoot N and P concentrations (AGEGNUHE et al., 2016). Moreover, ZHANG et al. (2016) documented that separate or combined application of biochar and compost had a significant influence on plant N, P and K concentrations in comparison with inorganic amendments. This observation was due to the impact of biochar on increasing fertilizer use efficiency (SCHULZ et al., 2013) and also temporary altering pH for low available nutrients (AMEI et al., 2016). HETIKOTTER and MARSCHNER (2015) revealed that the chemistry of biochar can lead to the retention of K by cation exchange capacity associated with acidic functional groups formed during oxidation process on biochar surfaces, hence, K can become more available for plants. The results of a conducted study showed more availability of N, P and K for plants treated with organic amendments in comparison with plants treated only with mineral fertilizers. This was due to the fact that supplied compost and biochar added nutrients to the soil and also improved their availability to plants by reducing the sorption and leaching potential of nutrients (AGEGNUHE et al., 2015). Additionally, SUBRAMANIUM et al. (2018) stated that improved soil N content following organic matter application may be because of more reduction of organic matter compared to the NH_3 loss which usually increases N concentration. They also reported that the concentration of P and K through the plant showed an increase at the period of compost application, which was due to mineralization. In general, improvement of plant nutrition by using of organic amendments could be reasoned by the hypothesis describing that their application helps slowly releasing nutrients to plants and improveing soil ability to provide plant required nutrients (INAL et al., 2015). Mycorrhization has an influence on plant nutrition and our findings are in agreement with others who described that nutrient concentration was enhanced by mycorrhization due to the ability of mycorrhiza to translocate nutrients via extending hyphae and increasing root absorbing surface (ZHANG et al., 2016; KOTHARI et al., 1991). Mycorrhizal fungi absorb non-mobile nutrients from the soil and translocate them to host plants. Beside this, they facilitate inter plant transfer of nutrients and beneficially modify plant water relations (MONDAL and DUTTA, 2017). Furthermore, KUMAR et al. (2015), concluded that higher P content in mycorrhizal plants was attributed to higher influx of P into the plant system via an increase in P efficiency by mycorrhizal plants. They assumed that this may be because

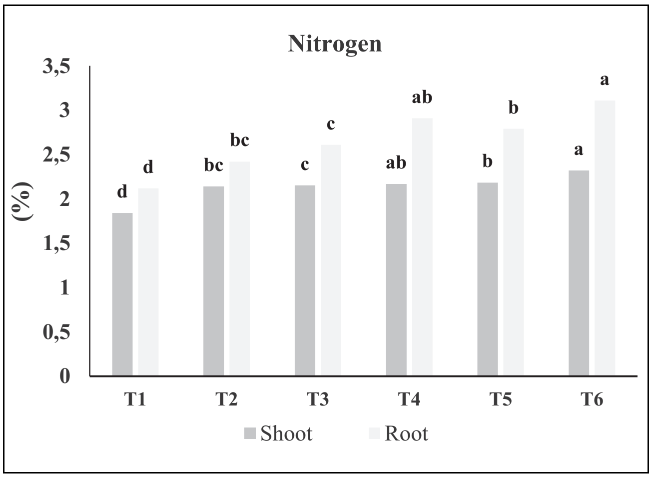

Fig. 5. Means comparison effect of organic amendments on shoot and root N content (%). Different letters above the bars indicate statistically significant differences between treatments according to DMRT ($P \leq 0.05$). T1: Control, T2: MY, T3: RHC, T4: RHB, T5: RHC + MY and T6: RHB + MY.

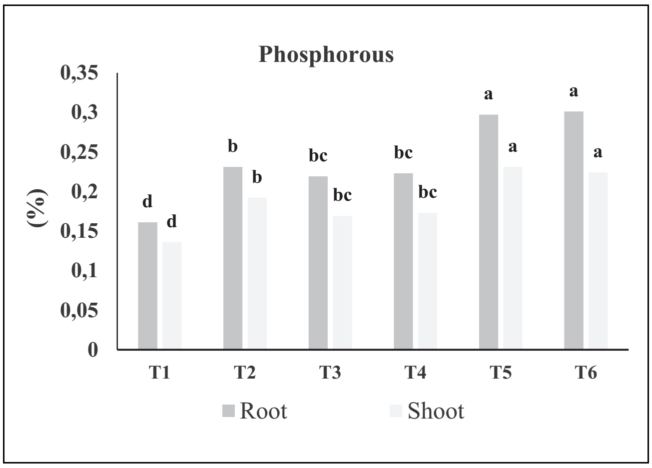

Fig. 6. Means comparison effect of organic amendments on shoot and root P content (%). Different letters above the bars indicate statistically significant differences between treatments according to DMRT ($P \leq 0.05$). T1: Control, T2: MY, T3: RHC, T4: RHB, T5: RHC + MY

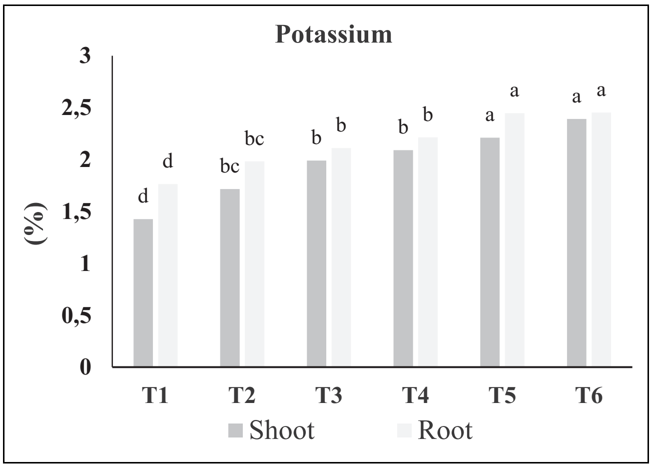

Fig. 7. Means comparison effect of organic amendments on shoot and root K content (%). Different letters above the bars indicate statistically significant differences between treatments according to DMRT ($P \leq 0.05$). T1: Control, T2: MY, T3

of morphological changes in the plant and a provision of an additional or more efficient absorbing surface in the fungal hyphae with subsequent transfer to the host and ability of the mycorrhizal root or hyphae to utilize the source of P not available to non mycorrhizal roots. Besides what mentioned above, the positive role of mycorrhiza in mobilization of macronutrients from the organic substrate via secretion of extracellular enzymes that mobilize mineral nutrients in the soil is also considered as an important effect of mycorrhization on plant macro-nutrients concentration (Hodge and Fitter, 2010; Kumar et al., 2017).

Concentration of micronutrients in roots and shoots

The results shown in Table 4, demonstrate that single or combined treatments had a positive and significant effect on shoot and root concentration of micro-nutrients and the highest values were observed in the RHB + MY treatment ($P \leq 0.01$). The maximum concentration of Fe, Zn, Cu and Mn in shoots were 334.6, 33.02, 11.12 and 39.68 mg kg^{-1}, respectively. The same was observed for roots where the highest concentrations of Fe, Zn, Cu and Mn were 365.1, 48.53, 13.19 and 41.73 mg kg^{-1}, respectively. Improvements in plant performance are consistent with other studies, which indicated that RHC may be able to improve essential element status in plant (Basha et al., 2005). Several research findings have shown that improving plant nutrition using of organic amendments for plant production is a promising approach and their application helps slowly releasing nutrients to plants and improving soil ability to provide plant required nutrients (Inal et al., 2015). Based on the results of a further study, processed poultry manure and its biochar application significantly increased the concentrations of Zn, Cu and Mn in maize plants, which may be attributed to improved availability of nutrients and soil conditions (Inal et al., 2015, Zhang et al., 2016; Adunga, 2016). More plant available nutrients, particularly micro-elements, may be obtained since organic acids are provided as a decomposition product of organic matters (Conversa et al., 2015). With respect to the influence of mycorrhization, our findings are in agreement with those observed by other researchers who reported improved plant nutrition following mycorrhization (Zhang et al., 2016). According to a further experiment result, it was concluded that mycorrhization has a considerable ability to translocate nutrients including Zn, S, Ca, Cu, Mn, Fe and N via extending hyphae and increasing root absorbing surface. (Raei and Weria, 2013). One assessment indicated that increase in Zn and Cu concentration related to mycorrhization is due to higher mycorrhizal colonization on previously treated soils (David et al., 1998). One reason for high nutrient concentration after mycorrhization might be due to the ability of mycorrhiza to colonize roots completely (Neumann et al., 2009), which enhances nutrient content by the extraradical mycelium (Kumar et al., 2017). The same conclusion was reported by Briccoli et al. (2015), who studied the effect of mycorrhization and documented an increase in the percentage of Mn translocation to the leaves from 14% to 22%. This led to an increase of the photosynthetic activity in the mycorrhizal plants since Mn is a constitutive element of photosystem II (Enami et al., 2008). The carried out study by Ortas (2010) showed increased Cu and Zn value in inoculated plants. Mycorrhization create an elaborate web of hyphae that im-

Table 4. Effect of organic amendments on micronutrients concentration in shoot and root (mg kg^{-1})

Treatments	Shoot			
	Fe	Zn	Cu	Mn
Control	316.38 d	26.19 e	9.167 d	34.523 d
MY	323.47 c	28.69 c	9.52 c	36.331 c
RHC	325.41 bc	27.81 d	9.793 b	37.481 b
RHB	328.11 b	28.33 cd	9.836 b	38.69 ab
RHC + MY	332.97 ab	31.26 b	11.033 a	39.265 a
RHB + MY	334.59 a	33.02 a	11.175 a	39.683 a
		Root		
Control	321.08 d	40.458 d	11.163 d	37.56 c
MY	354.75 c	44.775 b	11.522 c	38.35 bc
RHC	357.56 b	43.36 c	12.746 b	39.92 b
RHB	358.94 b	43.517 c	12.798 b	40.74 ab
RHC + MY	360.13 ab	46.841 ab	13.007 ab	41.688 a
RHB + MY	365.1 a	48.525 ab	13.193 a	41.733 a

Different letters above the bars indicate statistically significant differences between treatments according to DMRT
MY: mycorrhizatin, RHC: rice husk compost and RHB: rice husk biochar

proves absorption of nutrients including Fe, Cu, Mn and Zn (Briccoli et al., 2015).

Soil parameters

Status of macro-nutrients in soil. Data in Table 5 show that, all three organic amendments in a single or combined form significantly affected the concentration of soil macro-nutrients ($P \leq 0.05$). The highest values of total concentrations of N, P and K were observed in the RHB + MY treatment, and were 0.15%, 16.17 mg kg^{-1} and 500 mg kg^{-1}, respectively. Improvements in soil macro-element concentration are consistent with other studies, which indicated that combined application of compost and biochar significantly influenced concentrations of N, P, and K in soil (Schulz et al., 2013). Previous experiments have cleared that amending soil with biochar decreased leaching and improved soil nitrogen availability (Zhu et al., 2015). Similar results were obtained by Demir and Gulser (2015), who described that values of K and P concentration in soil increased by 9.49% and 25.56%, respectively, after application of 3% RHC. It was concluded that the amount of available N, P and K concentrations in soil increased as a result of compost application (Adunga, 2016). Furthermore, there was an increase in K concentration of planted soil with *Cocoa* following RHC application (Markus et al., 2008). Subramanium et al. (2018) documented that RHC application in soil had a positive impact on available P and K in comparison with control. They assumed that this was due to the fact that organic acids are formed during the decomposition of RHC, which helps to release P and K from a mineral bound insoluble form and also reduces the fixation processes. As a soil conditioner, biochar application can positively influence P and K concentrations in soil (Deluca et al., 2015). There are several mechanisms describing how biochar increases P value in soil. Biochar acts as a P source providing available P for soils, alerts P solubility via the alteration of soil pH, adsorbs specific chelates and improves the process of P mineralization and phosphatase enzyme activities (Madiba et al., 2016; Deluca et al., 2015). The chemistry of biochar demonstrates that it can lead to the retention of K by cation exchange capacity associated with acidic functional groups formed during oxidation process on biochar surfaces, thus retains K (Hetikotter and Marschner, 2015). Our results indicated that application of RHC or RHB in combination with mycorrhizal fungi was more effective in comparison with non-mycorrhizal treatments. Mycorrhiza alliance with other soil microbes improves soil fertility by mobilization of nutrients from the organic substrates (Kumar et al., 2017) and could perhaps perform a major function in mobilization of macro-nutrients from the organic substrate via secretion of extracellular enzymes, which mobilize, mineral nutrients in soil (Hodge and Fitter, 2010).

Concentration of micro-nutrients in soil. Application of organic amendments significantly ($P \leq 0.05$) enhanced soil micro-nutrients status (Table 5). Similar to what was observed for macro-nutrients, in combined treatments (RHC + MY and RHB + MY) the highest values for micro-nutrients occurred. The maximum concentrations belonged to RHC + MY and RHB + MY and a higher concentraton of Fe, Zn, Cu and Mn (50.35, 1.90, 1.90 and 10.05 mg kg^{-1}, respectively) was observed in RHB + MY. Our findings

Table 5. Effect of organic amendments on micro and macro-nutrients concentration of planted soil

	Micro-nutrients			
	Fe (mg kg^{-1})	Zn (mg kg^{-1})	Cu (mg kg^{-1})	Mn (mg kg^{-1})
Control	3.688 ^{c}	0.923 ^{d}	1.113 ^{d}	7.258 ^{d}
MY	4.113 ^{b}	1.655 ^{b}	1.343 ^{c}	8.813 ^{c}
RHC	4.527 ^{ab}	1.492 ^{c}	1.679 ^{b}	9.128 ^{bc}
RHB	4.696 ^{ab}	1.513 ^{c}	1.728 ^{b}	9.236 ^{bc}
RHC* MY	4.897 ^{a}	1.885 ^{a}	1.871 ^{a}	9.972 ^{a}
RHB* MY	5.035 ^{a}	1.9 ^{a}	1.895 ^{a}	10.054 ^{a}
		Macro-nutrients		
		N (%)	P (mg kg^{-1})	K (mg kg^{-1})
Control		0.099 ^{e}	13.495 ^{d}	432.97 ^{c}
MY		0.128 ^{d}	15.483 ^{b}	484.35 ^{ab}
RHC		0.131 ^{cd}	14.676 ^{c}	473.06 ^{b}
RHB		0.138 ^{b}	14.752 ^{c}	476.84 ^{b}
RHC* MY		0.146 ^{ab}	16.066 ^{a}	499.11 ^{a}
RHB* MY		0.148 ^{a}	16.174 ^{a}	500.06 ^{a}

Different letters above the bars indicate statistically significant differences between treatments according to DMRT
MY: mycorrhizatin, RHC: rice husk compost and RHB: rice husk biochar

were confirmed by other researches who illustrated that organic fertilization influences micro-nutrients contents such as Cu, Fe, Mn and Zn (RUTKOWSKA et al., 2009). DEMIR and GULSER (2015) showed an increase in the availability of soil micro-nutrients induced by decomposition of organic acids after an application of rice husk compost. An increased concentration of micro-nutrients induced by compost and biochar application was reasoned by a high concentration of these elements in RHC and RHB. The same was found by ADUNGA (2016), who revealed that increased available micro-nutrient concentrations in soil as a result of compost application was due to a high content of these elements in compost. AGEGNUHE et al. (2015) described that a combined application of compost and biochar significantly affected soil micro-nutrients. Micro-element mobility strongly depends upon soil pH. Fertilization with organic N resources, such as biochar and compost results in soil acidification through nitrification of this ion. Hence, by increasing soil acidification an increase in the availability of Cu, Fe, Mn and Zn content is observed in soil (RUTKOWSKA et al., 2014). Regarding the lower pH of RHC and RHB compared to the bulk soil in the present research, it can be concluded that application of RHC and RHB in soil might have altered soil pH temporary and affected the availability of micro-nutrients. The results of further conducted experiments indicated the enhanced soil conditions in terms of Fe, Zn, Cu and Mn following biochar application suggested the importance of biochar's pH altering capability of increasing the availability of micro-nutrients for plants (LENTZ and IPPOLITO, 2011). Biochar may promote or inhibit microbial activity that influences micro-nutrients availability via changes in microbial populations and activities (KHODADAD et al., 2011). Biochar is a promising resource for soil fertility management, which enhances the soil fertility status by a slow release of micro-nutrient (YANG et al., 2016). In conformity to our results, there are numerous reports on the enhancement of micro-nutrients content by mycorrhiza infection (MARSCHNER and DELL, 1994). Mycorrhization positively affects the availability of micro-nutrients, such as Fe, Cu, and Zn. It provides a significant C sink in soil, which can be considered as a critical impact on cycling of microelements within the soil. Mycorrhizal fungi are also accountable for dynamically mobilizing nutrients from the mineral particles and the rock surfaces by means of weathering. This may occur either by mycorrhizal fungi alone or in alliance with other microbes like bacteria or any other fungi (NIKPEY and NIKPEY, 2016).

Soil respiration and microbial biomass. A positive relationship exists between soil respiration rate and organic amendments application ($P \leq 0.05$). The same was true for microbial biomass. The highest values of soil respiration and microbial biomass was observed in combined treatments of RHB + MY and were 14.53 mg CO^2-C kg soil^{-1} h^{-1} and 26.92 mg C kg soil^{-1}, respectively (Fig. 8). The results are in agreement with other studies describing that an increase of organic materials, such as compost and bichar in soil, a maximum respiration and microbial biomass in soil occurs via improving soil physical, chemical and biological properties and increasing different enzyme and growth hormone secretion by microbes (NIKPEY and NIKPEY, 2016; ZHANG et al., 2014). The conducted study by URBANKOVA et al. (2014), indicated that biochar application significantly affected soil respiration in both rhizosphere and non-rhizosphere soil. Similarly, the results of a further study suggested that cattle compost changed microbial biomass in soil bacteria by increasing the carbon pool of soil and improving the living conditions for indigenous microbial populations (ZHEN et al., 2014). Furthermore, SLAPAKOVA et al. (2018), observed significantly higher respiration rates following biochar treatment. As expected, mycorrhization significantly increased soil respiration and microbial biomass which might be reasoned by the role of mycorrhiza in food sources for soil microbes, which increases soil microbial community and also soil respiration (CHENGA and KENDRA, 2006). On the other hand, STAMOU et al. (2017), illustrated that total microbial biomass was affected by mycorrhization because of promoting soil microbial activity causing development of microbial community in soil.

Conclusion

The findings of the present research revealed that all examined treatments significantly affected nutrient element (N, P, K, Fe, Zn, Cu and Mn) concentration, growth/yield indexes of the plants, soil nutrient status (N, P, K, Fe, Zn, Cu and Mn), soil respiration and microbial biomass in soil. An important result of the present study is the considerable effect of enriched RHC and RHB with mycorrhiza on the studied parameters. Combined application of organic amendments was superior to individual application of treatments. A positive role of my-

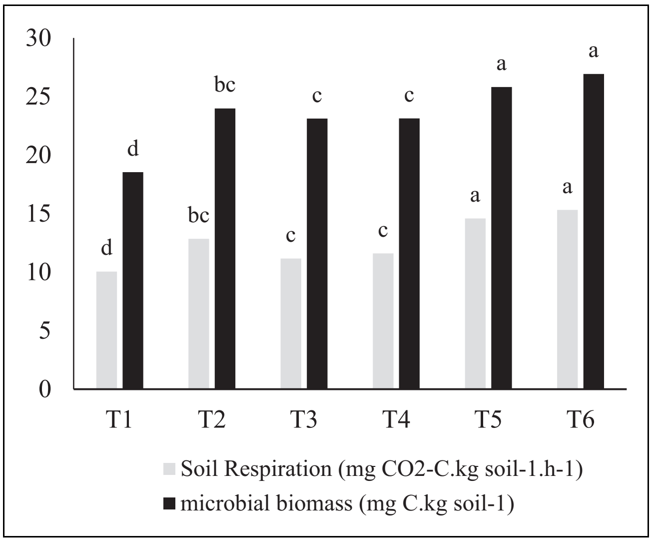

Fig. 8. Means comparison effect of organic amendments on of soil respiration and microbial biomass. T1: Control, T2: MY, T3: RHC, T4: RHB, T5: RHC + MY and T6: RHB + MY.: RHC, T4: RHB, T5: RHC + MY and T6: RHB + MY.

corrhization on nutrient concentration in plants was observed via creating an elaborate web of hyphae, which enhanced nutrient uptake by the plant. Furthermore, mycorrhization affected soil nutrients status due to the role of mycorrhiza in mobilization of nutrients from organic substrate via secretion of extracellular enzymes, which mobilized mineral nutrients in soil. Moreover, mycorrhization significantly increased soil respiration and microbial biomass, which might be reasoned by the role of mycorrhiza in food sources for soil microbes, which increases the soil microbial community and also soil respiration. Our results suggest that RHC, RHB, and MY application in soil led to enhanced soil properties and improved plant growth/yield indices. Further research is needed to determine long-term impacts of these soil conditioners on the soil and plant traits.

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