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GROWTH OF *ZELKOVA SERRATA* SEEDLINGS IN A CONTAINERISED PRODUCTION SYSTEM TREATED WITH EFFECTIVE MICROORGANISMS AND BIOCHAR

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Improvement in soil physical and chemical qualities in a containerised seedling production system is important to manage the survival of seedlings after transplanting and initial growth for reforestation efforts. The effects of mixed ratio of biochar and effective microorganisms (EM) on the growth of *Zelkova serrata* seedlings were investigated. Five levels of mixed ratio biochar (0–40% v/v with a growth medium) and two levels of fertilisation, either with or without EM, were applied to seedlings for 5 months. Height, root collar diameter and dry weight of each seedling were measured to compare the effects of the treatments. Main effects of the treatments were evident in all parameters. Growth parameters were much higher in seedlings treated with 1 g L^{-1} fertilisation, EM treatment and low biochar ratio than those treated with 0.5 g L^{-1} fertilisation, without EM and with high biochar ratio. Quality index was in the order of as 0% > 10%, 20% > 30%, and 40% mixed ratio biochar. Fertilisation and EM treatment increased quality index by 31 and 13% respectively. No significant interactions were found between treatments. These findings indicate that EM with 1 g L^{-1} fertilisation at low biochar ratio can be applied for better quality of *Z. serrata* seedlings in a containerised production system.

Keywords: Fertilisation, *Quercus acutissima*, biochar ratio, seedling growth, wood chip

INTRODUCTION

Improvement in soil physical and chemical qualities in a containerised seedling production system is important for managing post-transplant survival and initial growth in reforestation. Fertilisation is essential for seedling production in nursery culture, but frequent use of chemical fertilisers can pollute the local environment around the nursery (Park et al. 2012). Alternative measures for enhancing seedling growth, including inoculating with effective microorganisms (EM), have been developed to minimise the negative effects of chemical fertilisers (Ferron & Deguine 2005, Shaxson 2006).

Effective microorganisms are beneficial microorganisms isolated from the soil. Soil inoculation using EM has been employed over a wide range of agro-ecological conditions (Hussain et al. 2002), resulting in enhanced crop productivity, improved soil quality and reduced requirement for chemical fertiliser and pesticides (Hussain et al. 1999). EM increased the yield of wheat in China (Hu & Qi 2013), seed cotton in Pakistan (Khaliq et al. 2006) and onion in New Zealand (Daly & Stewart 1999), nodulation of pea in Pakistan (Javaid 2006), seedling germination and growth of *Albizia saman* in Bangladesh (Khan et al. 2006), and root collar diameter and height growth of black pine in Turkey (Atik 2013). However, there are contradictory effects of EM on crop yields and soil properties. For example, EM application had no effects on corn production in Indonesia (Priyadi et al. 2005) or cotton production in Pakistan (Khaliq et al. 2006).

Biochar is a type of charcoal produced under high temperature and low oxygen conditions using organic waste material, such as crop residues or animal manure. Biochar has been used as soil additive for improving crop productivity, enhancing soil physical and chemical qualities, increasing carbon sequestration in the soil and percolating soil water mainly on agricultural soils (Lehmann & Joseph 2009, Zhang et al. 2012). However, there are potential negative impacts on soil quality, such as N immobilisation and increasing soil pH in alkaline soils (Lehmann et al. 2003, Novak et al. 2009).

Optimal usage of biochars with EM application can enhance seedling growth and reduce chemical utilisation. However, little is known about the consequences of biochar application with EM in a containerised seedling production system (McElligott et al. 2011, Spokas et al. 2012). These effects depend on the properties of the biochar and its interactions with soil and plant communities. As such, it is important to understand the combination effects of biochar and EM application on seedling quality.

This study aimed at investigating the effects of EM in combination with various soil ratios of biochar made from oak (*Quercus acutissima*) chips (byproducts from forestry) on the growth and physiological characteristics of Japanese elm (*Zelkova serrata*) seedlings in a containerised seedling production. This species was selected because of its high economic value as commercial timber and urban decorative enhancement. Our findings will contribute to an understanding of which biochar–EM combinations can be used economically for seedling production in forestry settings.

MATERIALS AND METHODS

Study site and species

Growth experiment was conducted in a greenhouse located in Chungnam National University in Daejeon, South Korea (36° 22' N and 127° 21' E). Temperature and humidity inside the greenhouse were measured from May till September 2015 using a data logger. Mean temperature was 22.5 °C and mean humidity was 78.6%.

Seeds of *Z. serrata* were germinated in March 2015 at the Forest Practice Research Center, National Institute of Forest Science and brought to the greenhouse in early May. *Zelkova serrata* is a deciduous timber tree common in Japan, Korea, Taiwan and China.

Biochar production and biochar analysis

Biochar was made using a wood roasting oven at the College of Agriculture and Life Sciences, Chungnam National University (Cho et al. 2017), which carbonises organic waste materials at temperatures of 200–250 °C (Lee & Kang 2015). For our purposes, *Q. acutissima* wood chips of 2 cm × 2 cm × 0.5 cm were used as production material. These yielded biochar with 3.2% moisture, 16.4% fixed carbon, 79.9% volatility and pH 5.1 (Table 1), with other characteristics in the range of standard biochar (Cho et al. 2017).

Experimental design

A 5 × 2 × 2 factorial experiment was designed with *Z. serrata* seedlings falling into 20 separate treatment groups. Five ratios of soil:biochar were used, either with or without EM, and two levels of fertilisation, with five replicates in each grouping. Seedling assignment to each treatment grouping was randomised.

Planting cells for *Z. serrata* seedlings were 6.8 cm in diameter and 15 cm in depth, for 400 mL volume. Twenty cells each were assigned to trays of 32 cm × 40 cm and placed on a platform 60 cm above the greenhouse floor. An artificial soil mixture of peat, Perlite and Vermiculite at a ratio of 1:1:1 by volume was used as the basis for all control and experimental cultivation (Table 2). Varying levels of biochar were mixed in at 0, 10, 20, 30 and 40%. Seedlings of approximately 6 cm in height were planted in cells and placed

Table 1 Physical and chemical properties of biochar

Moisture content	Ash	Fixed carbon	Volatility	pH	EC (ds m^{-1})
%					
3.2 (0.0)	0.5 (0.0)	16.4 (0.4)	79.9 (0.4)	5.1(0.0)	0.282(54)
N	**P**	**K**	**Na**	**Ca**	**Mg**
%					
0.7 (0.4)	0.94 (0.08)	0.97 (0.12)	0.64 (0.11)	10.3 (1.2)	0.83 (0.09)

Parentheses represent one standard error of the mean (n = 3)

Table 2 Physical and chemical properties of growth media prior to mixing with biochar

Bulk density ($g\ m^{-3}$)	pH	Electric conductivity ($ds\ m^{-1}$)	Organic matter (%)	Total N (%)	P_2O_5 ($mg\ kg^{-1}$)	Exchangeable cations ($cmol_c\ kg^{-1}$)			CEC ($cmol_c\ kg^{-1}$)
						K^+	Ca^{2+}	Mg^{2+}	
0.37	6.1	0.06	4.0	0.08	3.0	0.3	3.3	2.5	24.3

Revised from Cho (2015)

in trays with blank cells separating them, such that each tray contained 10 planted cells and 10 blanks. EM was obtained from the Agricultural Technology Center located at Hongsung-gun, Chungchung-namdo, and applied weekly as 110 mL EM in 1:10 dilution. Half of the seedlings received this treatment and half did not. Fertiliser (20 N:20 P_2O_5:20 K_2O) was also applied weekly at 110 mL per cell. Two levels of fertilisation were used, namely, 0.5 and 1 g L^{-1}. Cells were irrigated with water daily for 20 min. Tray positions were rotated every two weeks in order to reduce the effects of any unknown environmental influences such as unequal water irrigation.

Growth measurements

Height and root collar diameter of seedlings were measured every month over the 5 months of the experiment. Height was measured from the ground to apical meristem, and root collar diameter was measured at 1 cm above the ground. At the conclusion of the experiment, seedlings were harvested and divided into stem, leaf and root parts. Roots were washed with tap water in order to remove any soil particles. All components were then dried at 65 °C for 48 hours to constant weight.

Dickson's quality index

Quality index is a comprehensive index for evaluating seedling quality. Dickson's quality index was used to estimate the quality of seedlings, as follows (Dickson et al. 1960, Johnson & Cline 1991):

$$\text{Quality index} = SD/(HD + SR)$$

where, SD = seedling dry weight (g), HD = height (cm) to root collar diameter ratio (mm) and SR = shoot dry weight (g) to root dry weight (g) ratio (Bayala et al. 2009, Deans et al. 1989, Park et al. 2015).

Statistical analysis

Analysis of variance (ANOVA) with Duncan's multiple comparison tests was used to test the effects of biochar, EM treatment and fertilisation on seedling height, root collar diameter, dry weight and quality index. Differences were considered to be significant at $p \leq 0.05$.

RESULTS

Growth response

Significant differences were observed between treatments in several measures (Table 3). Mean height in seedlings treated with 1 g L^{-1} fertilisation level was higher by 35% than those treated with 0.5 g L^{-1} level ($p < 0.01$) (Table 3, Figure 1). Mean height was not significantly different between biochar treatment levels of 0–20%, but seedlings in these treatment levels had significantly greater height than those in the 30 and 40% levels. Treatment with EM increased mean height by 8% ($p = 0.03$).

Root collar diameters were also significantly influenced by fertilisation, EM treatment and biochar treatment (Table 3, Figure 2). Average root collar diameter at 1 g L^{-1} fertilisation was significantly higher than that at 0.5 g L^{-1} treatment by 21%. Average root collar diameter was highest at 0% biochar, then at 10 and 20%, and lowest at 30%. EM treatment increased average root collar diameter growth by 6%.

Leaf, stem and root biomass were significantly influenced by fertilisation, EM and biochar treatments with trends similar to those of height and root collar diameter growth (Table 3, Figure 3). Average total seedling dry weight at 1 g L^{-1} fertilisation was higher than at 0.5 g L^{-1} fertilisation treatment by 50%. Average dry weight was highest at 0% biochar, followed by at 10 and 20% and lowest at 30 and 40%. Even though there were no significant interactions

Table 3 ANOVA table for growth parameters of *Zelkova serrata* seedlings treated with combinations of fertilisation, effective microorganisms (EM) and different levels of biochar treatments

Source of variation	Degrees of freedom	Probability (Pr > F)						
		Height	Root collar diameter	Dry weight				Quality index
				Leaf	Stem	Root	Total	
Fertilisation	1	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01
EM	1	0.03	0.02	0.53	< 0.01	< 0.01	< 0.01	0.03
Biochar	4	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01
Fertilisation × EM	1	0.13	0.29	0.37	0.04	0.60	0.39	0.75
Fertilisation × biochar	4	0.52	0.09	0.69	0.49	0.33	0.36	0.16
EM × biochar	4	0.49	0.68	0.89	0.56	0.97	0.77	0.87
Fertilisation × EM × biochar	4	0.90	0.89	0.66	0.63	0.86	0.77	0.75

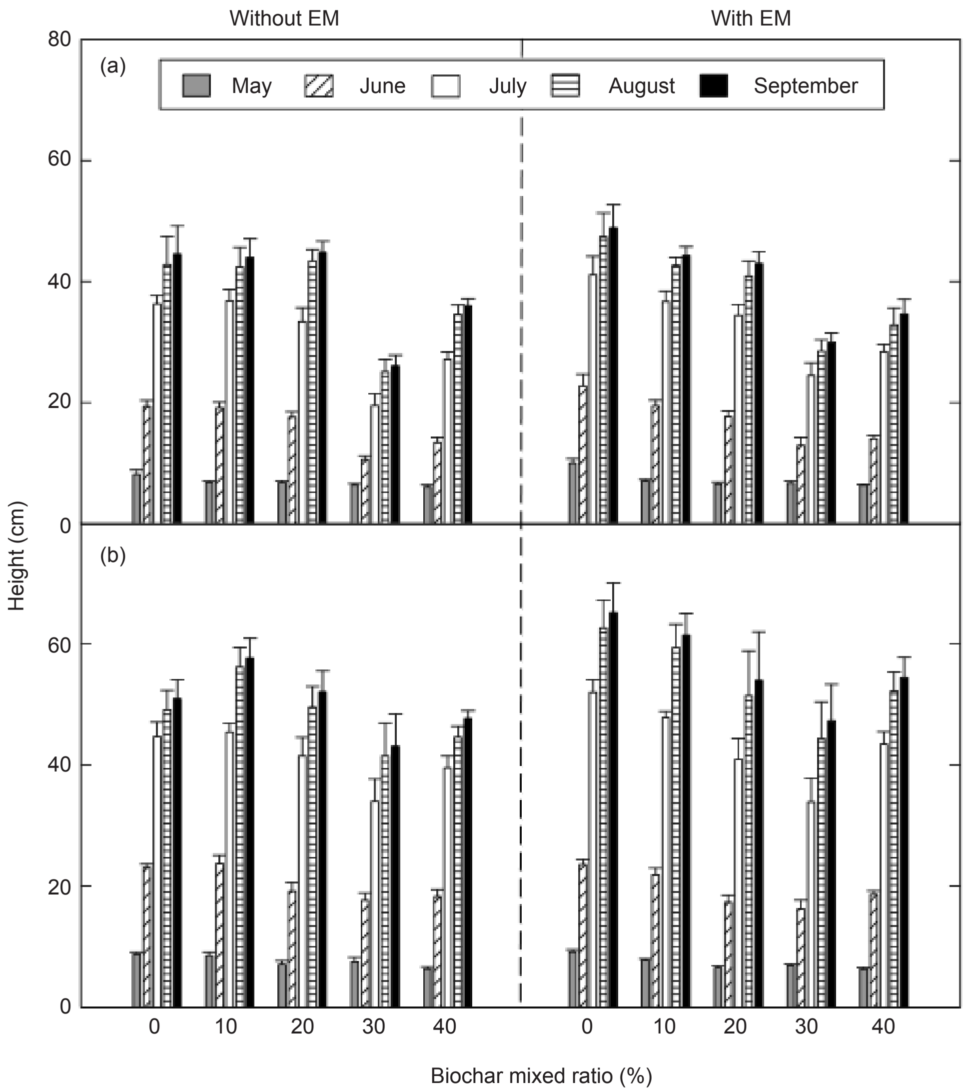

Figure 1 Height of *Zelkova serrata* at (a) 0.5 g L^{-1} fertilisation and (b) 1 g L^{-1} fertilisation, with and without effective microorganisms (EM) and five levels of biochar mixed ratio in a containerised production system; vertical bars represent one standard error of the mean (n = 5)

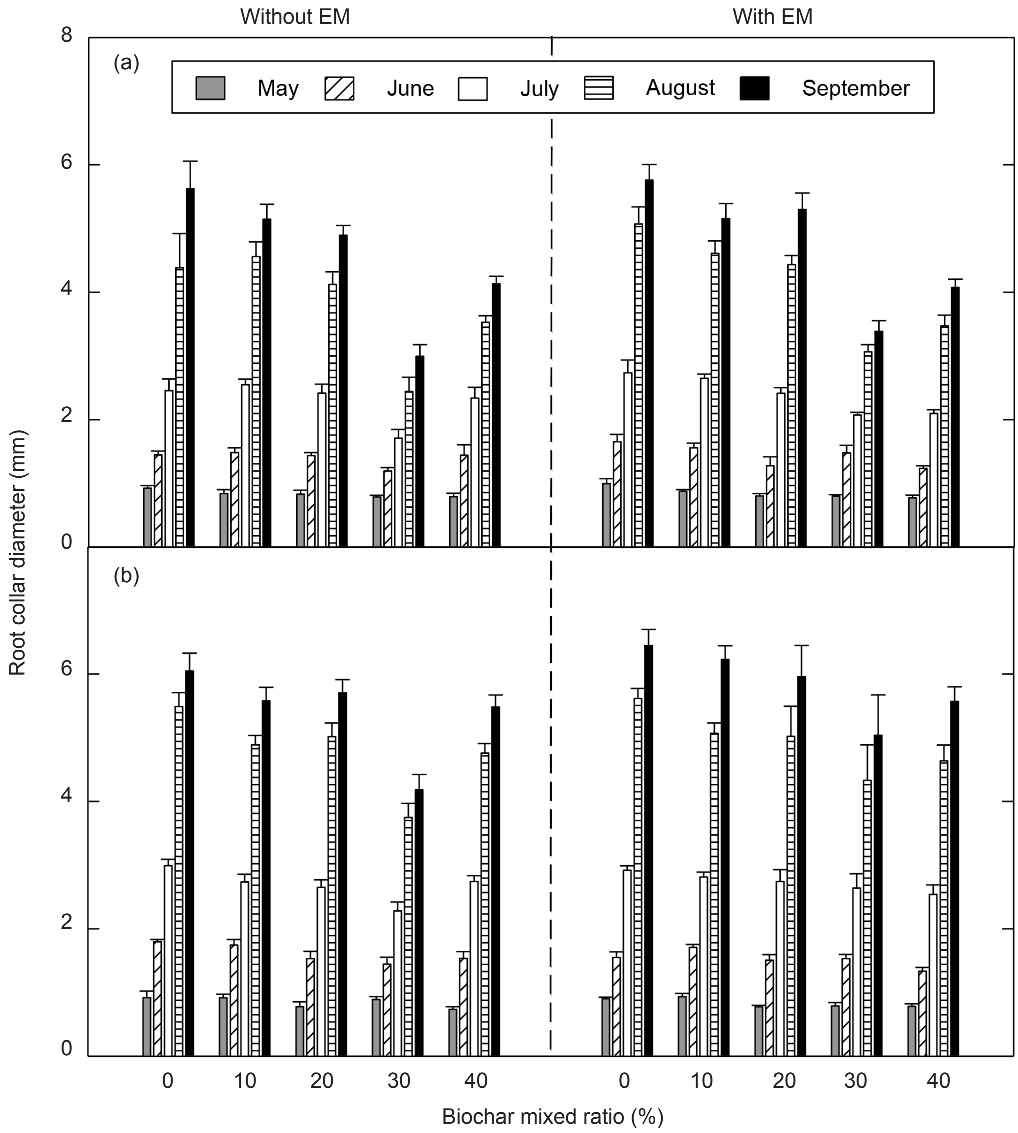

Figure 2 Root collar diameter of *Zelkova serrata* at (a) 0.5 g L^{-1} fertilisation and (b) 1 g L^{-1} fertilisation, with and without effective microorganisms (EM) and five levels of biochar mixed ratio in a containerised production system; vertical bars represent one standard error of the mean (n = 5)

between treatments, average total biomass dry weights at 30 and 40% biochar in non-EM and EM treatments were much smaller than those from other biochar levels. For example, total dry weight at 1 g L^{-1} fertilisation was less than a third of those measured at lower biochar levels, and total dry weight at 0.5 g L^{-1} fertilisation was roughly half of the mean at lower biochar levels.

Quality index

Quality indices were highest at 0% biochar ratio followed by 10, 20, 30 and 40% ratios (Table 3, Figure 4). A higher level (1 g L^{-1}) of fertilisation and EM treatment increased quality index by 31 and 13% respectively.

DISCUSSION

The greatest seedling growth was observed under EM treatment with 1 g L^{-1} fertilisation, with zero or low levels of biochar in the soil. Further, EM effects on growth parameters were not as pronounced as those from fertilisation application (Table 3, Figure 3). When fertiliser is applied to soil media, they form solutions in the soil and yield ions available for direct uptake by the plant (Schachtman et al. 1998). EM indirectly improves nutrient use efficiency and microbial activity, both of which help to decompose organic matter to allow greater solubility. As a result, plants can use nutrients released from organic matter (Higa & Parr 1994).

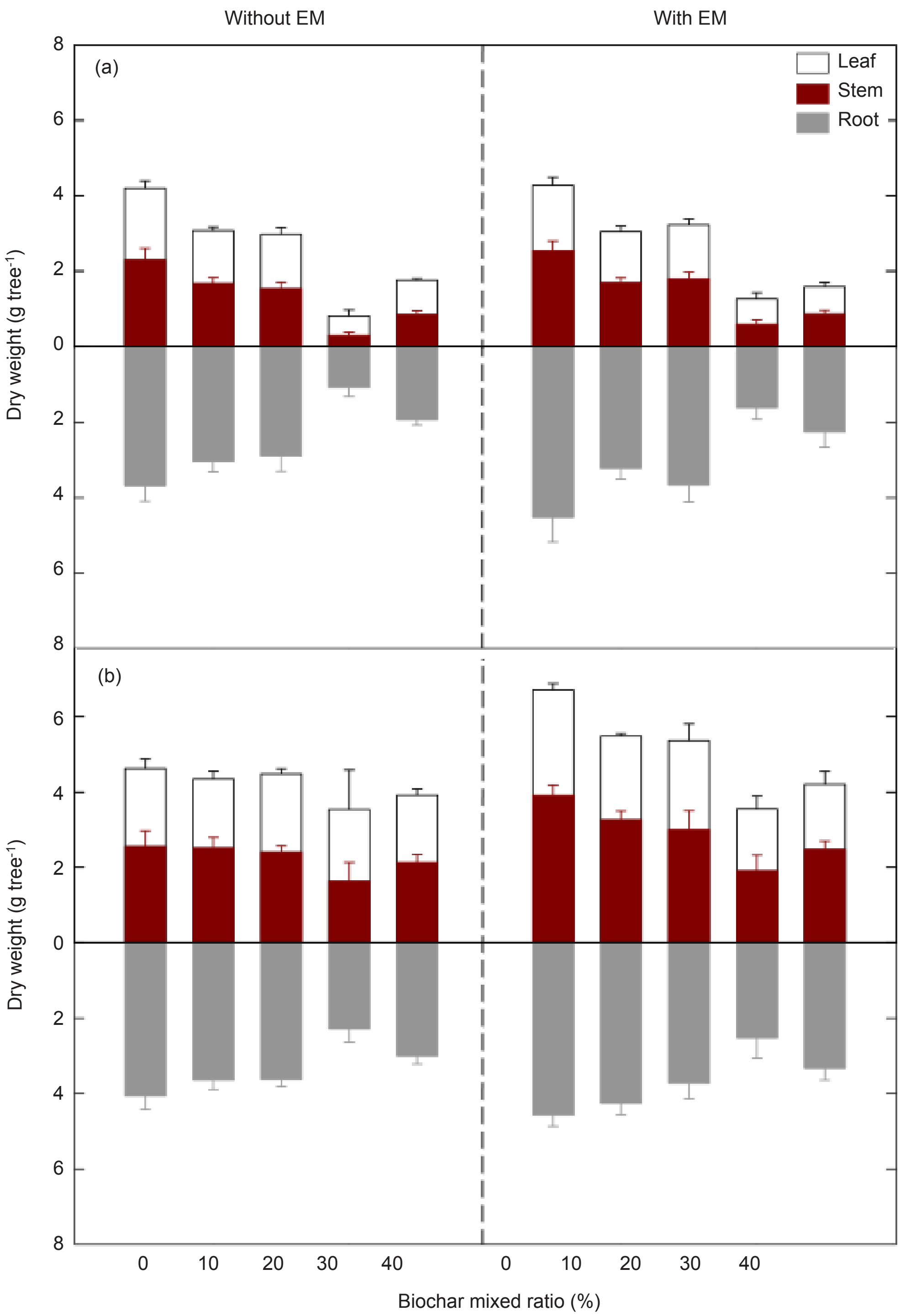

Figure 3 Dry weight of *Zelkova serrata* at (a) 0.5 g L^{-1} fertilisation and (b) 1 g L^{-1} fertilisation, with and without effective microorganisms (EM) and five levels of biochar mixed ratio in a containerised production system; vertical bars represent one standard error of the mean (n = 5)

EM influences plant growth by enhancing microbial activity of the indigenous microflora. Application of EM to onion, pea, and sweetcorn cultures increased yields by 29, 31 and 23% respectively (Daly & Stewart 1999). EM application also increased the germination of peanut plants by 2.5% and crop yield by 8.4% in China (Zhao 1992).

Amending soils with biochars from various feedstocks results in differing effects on soil properties and subsequent effects on plant growth (Amonette & Joseph 2009). Biochars made from animal manure and corn stove increased corn biomass respectively by 43 and 30% (Rajkovich et al. 2012). However, some investigations showed

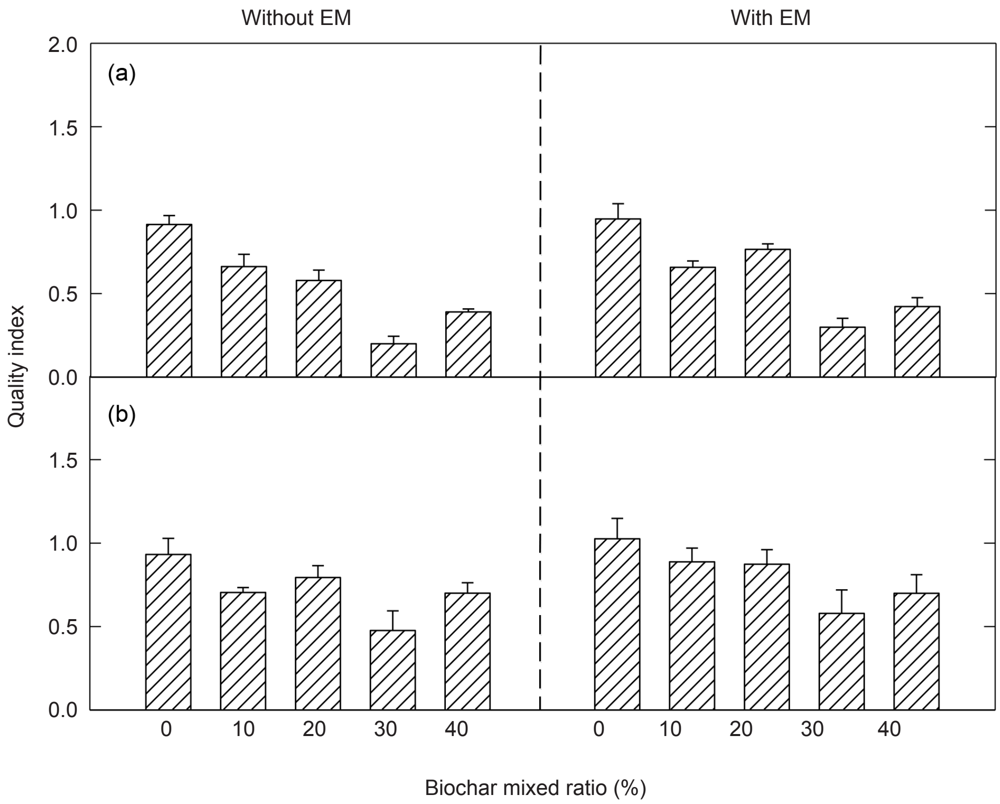

Figure 4 Quality index of *Zelkova serrata* at (a) 0.5 g L^{-1} fertilisation and (b) 1 g L^{-1} fertilisation, with and without effective microorganisms (EM) and five levels of biochar mixed ratio in a containerised production system; vertical bars represent one standard error of the mean (n = 5)

no effects at all or even negative effects from biochar application (Cornelissen et al. 2013, Kloss et al. 2014). Biochar effects in forestry also varied by target species and biochar source material. In a degraded restoration field in Laos, biochar treatment increased diameter and height growth of all species tested especially the slow-growing trees (*Dipterocarpus alatus, D. cochinchinensis* and *Pterocarpus macrocarpus*) (Sovu et al. 2012). Biochar made from bamboo applied at a rate of 0.5 kg m^{-2} increased tea tree biomass by 20% and height by 40% (Hoshi 2001).

Biochar effects are significantly improved by applying mineral fertilisation simultaneously. Schulz and Glaser (2012) showed that biochar application along with mineral fertiliser increased plant growth by 65–90% for *Avena sativa* in Germany. Biochar made from wood chips increased crop yield by 39% when fertilised at a concentration of 91 kg N ha^{-1} and by 44% at 215 kg N ha^{-1}. Biochar made from corn cobs increased crop yield by 54% when fertilised at 91 kg N ha^{-1} and 72% at 215 kg N ha^{-1} (Zheng et al. 2010). These findings are in agreement with our study, wherein biochar application with standard fertiliser treatment improved plant growth significantly.

Some studies have shown that biochar application over 20% was more effective for the growth of plants such as *Avena sativa* in Central Amazonia (Schulz et al. 2013). Zhang et al. (2012) found that soil treated with 10% biochar improved the yield of rice. However, in our study, biochar treatment at less than 20% yielded higher growth for *Z. serrata* seedlings. Our data suggested that EM with a low ratio of biochar and 1 g L^{-1} fertilisation increased the growth of plants in a containerised seedling production system. Since the quality index was positively correlated with seedling outplanting performance (Dickson et al. 1960, Johnson & Cline 1991), seedlings treated with low biochar mixed ratio, fertilisation and EM treatment in this study were expected to have high survival rate and vigorous growth in harsh environmental conditions.

CONCLUSIONS

The investigation presented in this study indicated some distinct benefits of combined application of EM and biochar in a certain amount. Treating soils with EM in combination with biochar improved soil quality and seedling growth in a containerised production system. This can be economically beneficial and eco-friendly as EM reduces the usage of chemical fertiliser and biochars are locally available as byproducts from forestry. This study showed that treating soil with EM and low levels of biochar will be useful in improving seedling quality in nursery production and directly save costs.

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