Application of Biochar to Alleviate Effects of Allelopathic Chemicals on Seed Germination and Seedling Growth

Thobayet Safar Alshahrani and Nurul Iman Suansa *

Allelochemicals are generally harmful to plant species during one or more of the regeneration phases. This study investigated the influence of biochar in mitigating allelopathic effects of *Eucalyptus camaldulensis* in plants (*Acacia gerrardii* and *Acacia ampliceps*). The seed germination was relatively delayed by dried leaf extract (DLE) on the first day. Both DLE and date palm biochar (DPB) relatively decreased radicle hypocotyl development of both the target species (when compared with the control). In the seedling growth trial, most of the parameters were suppressed by either DLE or DPB. The results revealed that the inhibitory effects of DLE was less pronounced in the case of seed germination when compared with seedling growth. Moreover, any DLE treatments that were mixed with DPB allowed the suppressed plants to develop well. This effect was indicated by the positive value of relative allelopathic effect (RAE). The relative growth rates of seedling height (RGR_H) varied for the different treatment combinations. Most of the treatment combinations resulted in a higher RGR_H in test cases than in the control. This study provides meaningful results that support the hypothesis that biochar can be used as an absorptive substance in the immobilization of allelopathic chemicals.

Keywords: Allelopathy, Eucalyptus camaldulensis, Biochar, Seed germination, Seedling growth

Contact information: Plant Production Department, College of Food and Agriculture Sciences, King Saud University, P.O. Box 2460, Riyadh 11451, Saudi Arabia;
** Corresponding author: nsuansa@ksu.edu.sa*

INTRODUCTION

Allelopathy is the harmful effect of one plant on another due to chemicals that are released into the environment; it alters nutrient dynamics, soil organisms, and soil characteristics (Koocheki *et al.* 2013). Allelopathy is a potential mechanism of an invasive species with the intention of invading new habitats by inducing adverse effects on the regeneration of native plant species. Allelopathy involves both inhibitory and stimulatory effects through chemical substances (Willis 2007). This duality of substances is sometimes referred to as hormesis, which means that there is no substance without poisonous qualities, but the dosage determines the poisonous ability of the substance (Duke *et al.* 2006).

The presence of biodiversity in the ecosystem is an important aspect, whether it is natural vegetation or artificial plantation. River red gum (*Eucalyptus camaldulensis*) is planted in plantation forests globally. However, this species exhibits allelopathic effects. Developing a homogenous vegetation results in loss of biodiversity and decreases the productivity of an ecosystem (Vila’ *et al.* 2003; Pretzsch and Schu¨tze 2009). Some researchers have tried to manage the stands by mixing *E. camaldulensis* with *Acacia* sp., which can generate nitrogen fixation (Binkley *et al.* 2003; Forrester *et al.* 2006; Paquette and Messier 2011); as a result, diverse stands create healthier stands. However, the allelopathic effect is still present in the counterpart species. The effect of allelopathy should be reduced to alleviate the allelopathic effects and to decrease its causative factors that could otherwise lead to climate change.

Biochar can absorb allelopathic chemicals and has the following advantages: it is stable in the soil for thousands of years, hence some of the emission of greenhouse gases (GHGs) can be avoided; it can be used to amend soil structure, properties, and improve overall soil health; it preserves the nutrients in soil; it increases the water holding capacity of the soil, which can help plants survive during drought stress; it increases crop productivity; it can suppress soil emissions of methane and nitrous oxide; it absorbs contaminants (pollutants) and reduces nitrate leaching into water bodies; it is a bioenergy source; and it has absorptive properties that can decrease the bioavailability of toxic organic compounds (Lee 2013; Shackley *et al.* 2013; Sujeeun and Thomas 2017; Ussiri and Lal 2017).

Biochar has been used by Sujeeun and Thomas (2017) as an assay to reduce the effects of allelopathy. They observed that biochar leachate positively affects seed germination and initial growth. However, biochar is a complex substance that requires a more comprehensive study of its characteristics, interactions, production, impacts, benefits, and costs. One of the most important factors that influence biochar characteristics is feedstock material (Qin 2012; Lee 2013). Several important issues remain to be explored, such as to what extent the germination and seedling growth stages are affected by biochar leachate and what is the effect of biochar concentration. Furthermore, the effect of allelopathy varies among species and growth stages because it is species specific (An *et al.* 2001; Dadkhah 2013). In this regard, considerable research is required to reveal the effect of allelopathy from different sources on different target species.

This study focused on forest tree species from germination to seedling stages to provide a broad knowledge based on forestry insights. The assumption was that the effect of biochemical compounds (allelochemicals and biochar leachate) may vary during plant growth stages. Additionally, biochar as an adsorptive substance may exhibit an effect on plant growth by alleviating the effect of allelopathy. Therefore, this study was designed to explore the effects of *E. camaldulensis* leaf extracts and leachate of date palm biochar on seed germination and seedling growth of two Acacia species, *Acacia gerrardii* and *Acacia ampliceps*, and to examine the potential of biochar in alleviating the allelopathic effects of *E. camaldulensis* leaf extract on the target species.

EXPERIMENTAL

Materials

In January 2018, fresh leaves of *E. camaldulensis* were collected from Dirab Agricultural Research Station. They were air-dried and soaked in distilled water for 24 h at room temperature. After 24 h, the leaf extract solutions were filtered, and the filtrate was collected (Sujeeun and Thomas 2017). The midribs of date palm leaves were also collected during the same time. They were pyrolyzed at 350 °C for 4 h in a muffle furnace. To avoid any potential negative effects of date palm biochar (DPB) leachates on seed germination and seedling growth, DPB was prewashed with distilled water on an orbital shaker table rotating at 120 rpm for 24 h (Gale *et al.* 2016). The slurries were suction filtered, and the solid residue was collected and dried at 60 °C for 24 h. Thereafter, different dosages of DPB were added to each concentration of dried leaf extracts (DLE) to obtain solutions having final concentrations of 0 g/mL, 0.002 g/mL, and 0.02 g/mL. Each treatment solution containing DPB was mixed on an orbital shaker table rotating at 120 rpm for 24 h. The solutions were then suction filtered, and the filtrate was collected for further procedures (Sujeeun and Thomas 2017). Seeds of *A. gerrardii* and *A. ampliceps* were collected from Dirab Agricultural Research Station in December 2017. To reduce the possibility of seed coat preventing the germination process, seed scarification was done.

Methods

Experimental design

Germination and seedling growth experiments were conducted in a factorial arrangement on the basis of a completely randomized design (CRD) with three replications. Factors included target species (*A. gerrardii* and *A. ampliceps*), concentrations of DLE (0, 0.02, and 0.04 g/mL), and the dosage levels of DPB (0, 0.002, and 0.02 g/mL). The concentrations of DLE and DPB were chosen according to Zhang and Fu (2010) and Sujeeun and Thomas (2017), respectively.

Seed treatment

Bioassays were performed with 5 mL of each treatment solution, on a 9 cm disposable Petri dish containing a filter paper. Ten seeds of each species were placed on each dish. The seeds in each dish were moistened periodically with the different treatment solutions; 5 mL of each treatment solution or 5 mL of distilled water (control) was added to different dishes. The dishes were kept at room temperature for 9 days. Seeds were germinated when the radicle had emerged 1 mm above the seed coat (Dadkhah 2013). The final germination percentage was calculated according to Agrawal (2011). To check early development, lengths (mm) of radicle-hypocotyl were measured for three individual seeds from each dish every 2 days. The vigor index (VI) of early growth development was estimated as suggested by Abdul-Baki and Anderson (1973). The relative allelopathic effect (RAE) index was defined based on the VI, to determine the intensity of the allelopathic effect on seed germination (Hashoum *et al.* 2017). Negative RAE indicated an inhibitory effect in comparison to positive RAE, which indicated the success of biochar in alleviating the allelopathic effect.

Seedling treatments

The pot experiment was conducted in a controlled greenhouse where temperature was approximately 25 °C (D/N). Plastic pots of 9 cm diameter and 10 cm depth were filled with sandy soil. Nine-day old seedlings of *A. gerrardii* and *A. ampliceps* were carefully transplanted in the pots. Each pot was irrigated with a different treatment solution; 25 mL of each treatment solution or 25 mL of distilled water (control) was added to different pots periodically (2 days interval). After 6 weeks, the seedlings were harvested, and parameters including seedling height and diameter, number of leaves, leaf area, root length, average diameter, surface area, volume, and tips were measured. Root images were taken using a flatbed scanner (Canon Unit 101, New York, USA) at 300 dpi, for further analyses with WinRhizo software (Regent Instruments Inc., Quebec, Canada). Relative growth rates of seedling height (RGR_H) were calculated after 35 days and 42 days of seed sowing as reported by Chen *et al.* (2002). The relative allelopathic effect (*RAE*) index (based on seedling height + root length value) was calculated as described earlier.

Statistical analysis

The collected data were subjected to analysis of variance (three-way ANOVA, ($p < 0.05$)) using SAS 9.1 for Windows (SAS Institute, Inc., Cary, NC, USA). A least significant difference (LSD) test was used to compare the significant differences among the treatments.

RESULTS AND DISCUSSION

Effects of DLE on Seed Germination and Seedling Growth

Seed germination of the target species (*A. gerrardii* and *A. ampliceps*) was not significantly affected by DLE as analyzed by assessing germination percentage ($p = 0.65$). For all treatments, germination occurred on the first day after sowing. On average, the maximum time taken for germination was less than 2 days. However, DLE relatively delayed seed germination on the first day in testing, when compared to the control (Fig. 1a, 1b).

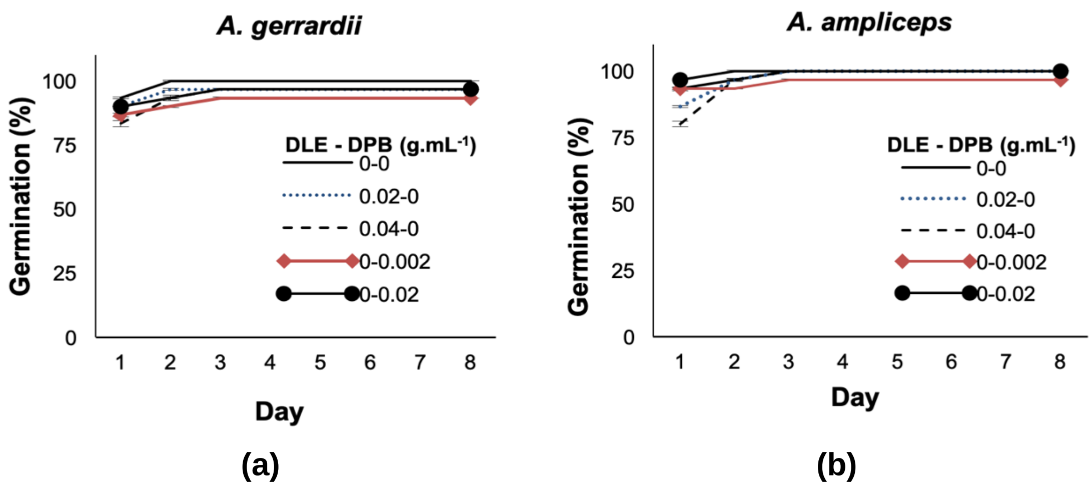


Fig. 1. Germination percentage of (a) *A. gerrardii* and (b) *A. ampliceps* treated with DLE and DPB leachate.

The findings were consistent with another study that reported that the germination of the target species was not significantly affected by aqueous extracts from foliage tissues of *E. camaldulensis* (Dadkhah 2013). However, several other studies have reported that the inhibitory effects of leaf extracts on seed germination are more pronounced than on seedling growth, which is another aspect that could have influenced the result in the application of DLE instead of green leaf extract (Ben-Hammouda *et al.* 1995; Uremis *et al.* 2005). The difference in raw materials might have an influence on the number of allelopathic chemicals and the phytotoxic effects (Silva *et al.* 2014). It is likely that the green leaf extract had a much higher concentration of phenolic compounds. However, this cannot fully explain the results, because allelopathic effects are not only determined by quantitative factors, but also by qualitative factors. In other words, some allelochemicals are present in all species, while some others are specific to a species (Hashoum *et al.* 2017). Hence, further studies are required to compare the application of green leaf extract versus dry leaf extract, and to have a better understanding of other phenological stages.

Other reports provide evidence that support the inhibition of germination by DLE of *E. camaldulensis*. For example, *E. camaldulensis* had an inhibition effect on many plant species such as *Amaranthus caudatus* and *Abelmoschus esculentus* (Igboanugo 1987), *Vigna unguiculata*, *Cicer arietinum*, and *Cajanus cajan* (Ahmed *et al.* 2008), and *Acacia ehrenbergiana* and *Acacia tortilis* (Shetta *et al.* 2017). Several allelochemicals from *E. camaldulensis* including p-menthane-3 and 8-trans-diol (PMD) (Zhang and Fu 2010), α-phellandrene, eucalyptol, p-menth-1-en-8-ol, and α-pinene (Ruwanza *et al.* 2015) have been reported to inhibit plant growth. The effect of allelochemicals might also enhance the seed germination due to the hormesis phenomenon (Duke *et al.* 2006). These varied results are not surprising because effects of these extracts are species specific and more pronounced in affecting the seedling stage (Niakan and Saberi 2009; Cheng and Cheng 2015; Ruwanza *et al.* 2015).

In addition, DLE relatively decreased radicle hypocotyl development of both target species, and the effect was enhanced by increasing the concentration of DLE. The radicle hypocotyl development was decreased by about 9.95% in low concentration and 11.58% in high concentration treatment, when compared to the control. The application of DLE at high concentrations decreased the radicle hypocotyl development to a greater degree than at a low concentration (Fig. 2a, 2b). However, the difference was not that pronounced. Similar results were reported in other studies, which found that the inhibitory effect is increased by raising the concentration of plant extract, *e.g.*, the high concentration of allelochemicals inhibited the water absorption in maize (Oyun 2006) or produced an inhibitory effect on amylase activity in wheat seedlings (Hegab *et al.* 2008). Some other studies reported the opposite effect, such as low concentrations of foliar extract of sugar beet exhibiting a greater inhibitory effect on the growth of purslane than high concentrations of eucalyptus (Dadkhah 2013). This difference may be attributed to allelochemicals' specific inhibitory effect on target species, or the presence of more active phenolic substances in species-specific allelochemicals (An *et al.* 2001; Dadkhah 2013).

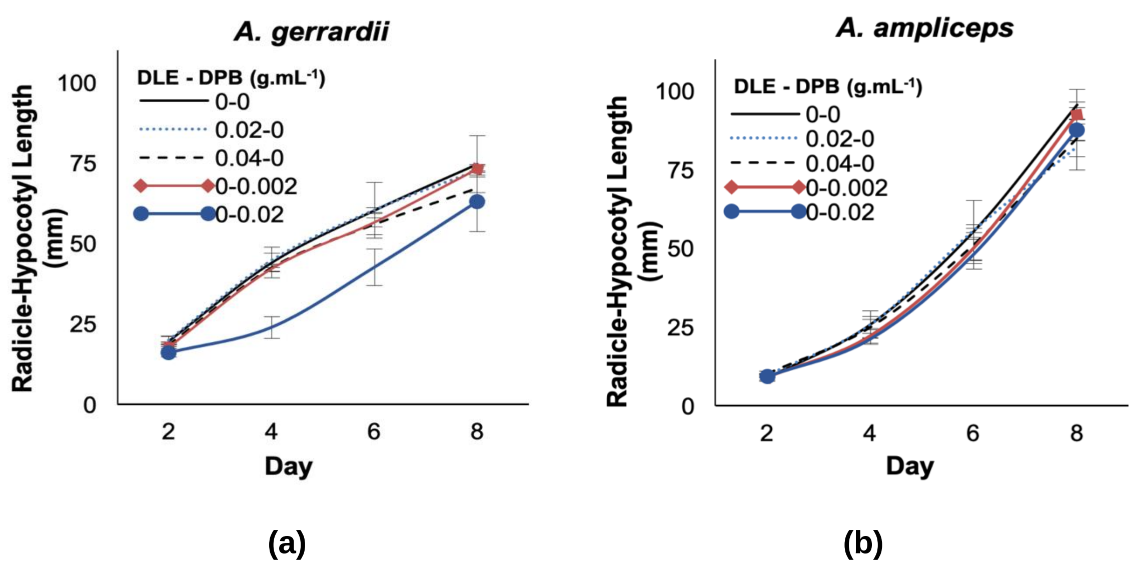


Fig. 2. Radicle hypocotyl length of (a) *A. gerrardii* and (b) *A. ampliceps* on treatment with DLE and DPB leachate

The solution of DLE significantly affected seedling height ($p < 0.00$), seedling diameter ($p < 0.00$), root length ($p < 0.01$), root surface area ($p < 0.00$), root volume ($p < 0.01$), root tips ($p < 0.00$), leaf area ($p < 0.00$), and the number of leaves ($p < 0.00$). However, DLE did not significantly affect root average diameter ($p = 0.2$) (Table 1). Moreover, the RGR_H value in the control treatment for each parameter was generally greater than allelopathic treatments. A higher concentration of leaf extract resulted in a greater decrease in seedling growth (Fig. 3a, 3b).

In the seedling growth trial, most of the parameters were strikingly suppressed by DLE (except root average diameter) (Table 1). Generally, the development of root length was more suppressed than that of shoot length. For example, the root length of *A. gerrardii* and *A. ampliceps* was suppressed by 73.61% and 46.41%, respectively, in high concentrations of DLE. On the contrary, shoot length was suppressed by only 51.05% and 34.78%, respectively. This agrees with several studies which have reported that the length and dry weight of roots were more affected than that of the shoot. Examples include the effect of hull extract on *Echinochloa crusgalli* (Ahn and Chung 2000), *Parthenium hysterophorus* on lettuce (Wakjira *et al.* 2006), and the effect of *Mikania micrantha* on both *Oryza sativa* and *Raphanus sativus* (Sahu and Devkota 2013). The robust inhibitory effects of leaf extract on roots might be because the roots are in direct contact with the allelochemical substances (Dadkhah 2013). Moreover, the allelochemicals might inhibit the radicle growth by decreasing mitotic activity (Rice 1984), damaging chromosome structure (Sugiyama *et al.* 2004), and disrupting the phosphorylation pathway and inhibiting ATP synthase activity (Cheng and Cheng 2015). The results of this study have revealed that the inhibitory effect of DLE was less pronounced in the case of seed germination when compared to seedling growth.

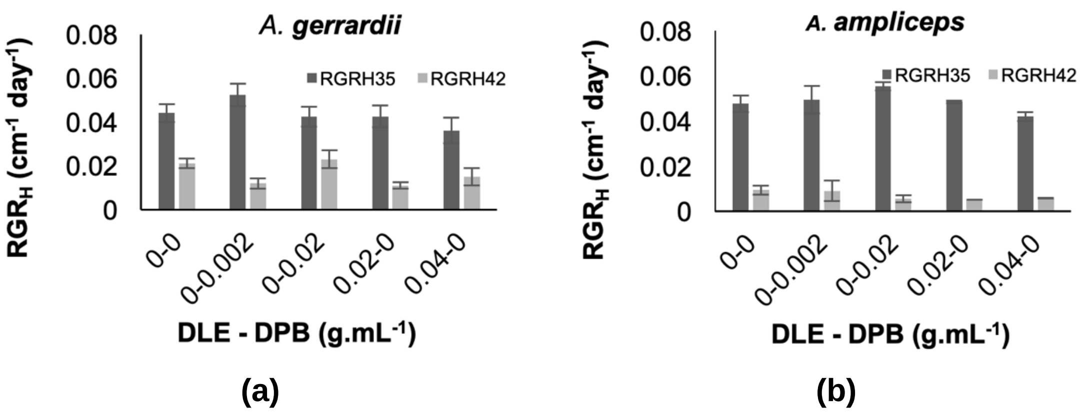


Fig. 3. RGR$_H$ of (a) *A. gerrardii* and (b) *A. ampliceps* treated with DLE and DPB leachate. RGR$_{H35}$: 35 days after sowing, RGR$_{H42}$: 42 days after sowing

Effects of Date Palm Biochar Leachate on Seed Germination and Seedling Growth

The leachate of DPB when used alone, did not significantly affect the germination percentage in *A. gerrardii* and *A. ampliceps* (Fig. 1a, 1b, p = 0.3). It also did not significantly affect radicle hypocotyl development of *A. gerrardii* and *A. ampliceps* (p = 0.31). However, DPB leachate reduced the radicle hypocotyl development, when compared to the control and the effect increased with increasing DPB concentration. The radicle hypocotyl development was decreased by 2.84% in the low concentration treatment and by 12.94% in the high concentration treatment. In addition, DPB leachate resulted in a delay in the radicle hypocotyl development at the beginning of the germination phase. However, it appeared to enhance the development in the following phase (Fig. 2a, 2b). In the pot experiment, the DPB leachate resulted in a significant decrease in seedling diameter ($p <$ 0.00), root tips ($p < 0.05$), leaf area ($p < 0.00$), and the number of leaves ($p < 0.05$) (Table 1). The RGR_H value of DPB leachate treatments was almost like that of the control, yet the effects varied between species and DPB concentration (Fig. 3a, 3b).

Table 1. Means and Standard Errors of Seedling Growth Parameters as Affected by Dry Leaf Extracts and Date Palm Biochar in Two Species of Acacia

Treatment (g.mL^{-1})	Shoot Height (cm)	Stem Diameter (mm)	Root Length (cm)	Root Diameter (mm)	Root Surface Area (cm^{2})	Root Volume (cm^{3})	Root Tips	Leaf Area (cm^{2})	Number of Leaves
DLE									
A. gerrardii	B	A	B	A	B	B	B	B	A
0	9.21^{a} ±0.45	1.91^{a} ±0.06	135.64^{a} ±9.77	0.59^{a} ±0.03	23.96^{a} ±3.72	0.35^{a} ±0.07	359^{a} ±66.46	2.56^{a} ±0.20	7.67^{a} ±0.67
0.02	5.63^{b} ±0.32	1.38^{b} ±0.06	104.46^{b} ±10.36	0.59^{a} ±0.04	19.28^{b} ±3.03	0.29^{b} ±0.07	237^{b} ±44.69	0.87^{b} ±0.38	5.33^{b} ±0.33
0.04	4.51^{c} ±0.3	1.21^{b} ±0.07	35.80^{b} ±3.27	0.63^{a} ±0.08	7.21^{b} ±1.05	0.12^{b} ±0.03	86^{b} ±4.16	0.96^{b} ±0.22	4.00^{c} ±0.58
A. ampliceps	A	B	A	A	A	A	A	A	B
0	9.20^{a} ±0.30	1.39^{a} ±0.08	225.15^{a} ±46.42	0.72^{a} ±0.04	49.29^{a} ±6.64	0.87^{a} ±0.07	1,081^{a} ±147.95	5.31^{a} ±0.85	5.67^{a} ±0.33
0.02	6.47^{b} ±0.38	0.96^{b} ±0.02	103.91^{b} ±27.72	0.51^{a} ±0.09	14.91^{b} ±2.05	0.18^{b} ±0.01	544^{b} ±228.65	2.29^{b} ±0.64	4.67^{b} ±0.67
0.04	6.00^{c} ±0.33	0.96^{b} ±0.08	120.67^{b} ±57.10	0.54^{a} ±0.10	23.81^{b} ±14.91	0.39^{b} ±0.29	451^{b} ±157.75	1.55^{b} ±0.37	3.67^{c} ±0.33
DPB									
A. gerrardii	A	A	B	B	B	B	B	B	A
0	9.21^{a} ±0.45	1.91^{a} ±0.06	135.64^{a} ±9.77	0.59^{a} ±0.03	23.96^{ab} ±3.72	0.35^{a} ±0.07	359^{a} ±66.46	2.56^{a} ±0.20	7.67^{a} ±0.67
0.002	9.74^{a} ±1.10	1.85^{b} ±0.03	147.42^{a} ±21.17	0.64^{a} ±0.03	28.10^{a} ±4.62	0.43^{a} ±0.08	351^{ab} ±67.17	2.07^{b} ±0.43	8.67^{a} ±1.20
0.02	7.86^{a} ±1.69	1.43^{c} ±0.01	126.91^{a} ±25.58	0.54^{a} ±0.01	19.18^{b} ±6.05	0.25^{a} ±0.09	309^{b} ±38.40	1.53^{b} ±0.47	5.67^{b} ±1.20
A. ampliceps	A	B	A	A	A	A	A	A	B
0	9.20^{a} ±0.3	1.39^{a} ±0.08	225.15^{a} ±46.42	0.72^{a} ±0.04	49.29^{ab} ±6.64	0.87^{a} ±0.07	1,081^{a} ±147.95	5.31^{a} ±0.85	5.67^{a} ±0.33
0.002	9.42^{a} ±0.61	1.29^{b} ±0.08	226.10^{a} ±28.88	0.66^{a} ±0.11	47.57^{a} ±12.16	0.85^{a} ±0.35	911^{ab} ±78.03	3.30^{b} ±0.44	4.67^{a} ±0.67
0.02	9.14^{a} ±0.64	1.30^{c} ±0.05	182.40^{a} ±8.39	0.71^{a} ±0.04	39.80^{b} ±5.48	0.72^{a} ±0.14	778^{b} ±25.36	3.83^{b} ±0.58	5.00^{b} ±0.00

Capital letters (A, B) and small letters (a, b, c) in the same column indicate significant differences for each variable between Acacia species and between treatments respectively ($p < 0.05$)

Table 2. Means and Standard Errors of Seeds Germination in *A. gerrardii* and *A. ampliceps*

Target Species Concentrations DLE - DPB (g.mL^{-1})	*A. gerrardii* Germination Percentage	*A. gerrardii* Coefficient of Velocity of Germination	*A. gerrardii* Vigor Index	*A. ampliceps* Germination Percentage	*A. ampliceps* Coefficient of Velocity of Germination	*A. ampliceps* Vigor Index
0-0	100.00^{a} ±0.00	93.94^{a} ±3.03	7462.78^{a} ±885.4	100^{a} ±0.0	91.41^{a} ±4.82	9559.00^{a} ±94
0.0-0.002	93.33^{a} ±6.67	92.31^{a} ±7.69	6826.61^{a} ±481.5	96.67^{a} ±3.33	93.94^{a} ±6.06	8973.29^{a} ±1050
0.0-0.02	96.67^{a} ±3.33	91.41^{a} ±4.82	6097.83^{a} ±970	100.00^{a} ±0.0	96.97^{a} ±3.03	8765.78^{a} ±340
0.02-0.0	96.67^{a} ±3.33	93.94^{a} ±3.03	7007.38^{a} ±94.65	100.00^{a} ±0.0	85.86^{a} ±2.53	8219.89^{a} ±739
0.02-0.002	96.67^{a} ±3.33	81.94^{a} ±10.85	7122.74^{a} ±220	100.00^{a} ±0.0	86.25^{a} ±4.66	10305.78^{a} ±283
0.02-0.02	93.33^{a} ±6.67	96.30^{a} ±3.70	7602.29^{a} ±646	100.00^{a} ±0.0	79.06^{a} ±2.14	10030.44^{a} ±856
0.04-0.0	96.67^{a} ±3.33	88.89^{a} ±11.11	6522.42^{a} ±569	100.00^{a} ±0.0	82.83^{a} ±8.08	8503.70^{a} ±587
0.04-0.002	96.67^{a} ±3.33	92.31^{a} ±7.69	7592.18^{a} ±643	100.00^{a} ±0.0	89.28^{a} ±6.71	9881.89^{a} ±541
0.04-0.02	100.00^{a} ±0.00	87.45^{a} ±8.43	8350.11^{a} ±192	96.67^{a} ±3.33	88.97^{a} ±6.68	10156.08^{a} ±971

Different letters (a, b, … *etc*.) if present in the same column indicate significant differences for each variable between concentrations ($p < 0.05$)

Table 3. Interaction Among Treatments Combination and Effect of Main Factors in Seedlings Growth

Parameter		Three-factor Interaction	Two-factor Interaction			Main-Factor		
		V*A*B	V*A	V*B	A*B	V	A	B
Seedling Height	F	2.70	3.72	8.26	15.58	3.07	5.13	51.16
	p-value	0.047	0.035	0.001	<0.0001	0.0885	0.0113	<0.0001
Seedling Diameter	F	7.24	6.00	15.96	39.83	418.44	2.96	54.12
	p-value	0.0002	0.0059	<0.0001	<0.0001	<0.0001	0.0651	<0.0001
Root Length	F	0.77	2.27	0.39	5.55	15.71	3.08	13.14
	p-value	0.5521	0.1187	0.6806	0.0015	0.0004	0.0591	<0.0001
Root Average Diameter	F	1.57	10.67	0.66	3.41	0.46	12.77	3.91
	p-value	0.2050	0.0003	0.5257	0.0189	0.5027	<0.0001	0.0297
Root Surface Area	F	0.52	6.43	0.37	4.29	12.04	2.51	8.93
	p-value	0.7249	0.0043	0.6912	0.0065	0.0014	0.0959	0.0008
Root Volume	F	0.36	9.36	0.26	3.24	11.04	4.42	6.40
	p-value	0.8324	0.0006	0.7743	0.0235	0.0021	0.0196	0.0044
Root Tips	F	3.15	1.30	1.52	7.94	197.39	7.04	6.55
	p-value	0.0263	0.2848	0.2331	0.0001	<0.0001	0.0028	0.0039
Leaf Area	F	2.47	12.15	6.06	43.42	160.07	7.14	26.53
	p-value	0.0629	0.0001	0.0056	<0.0001	<0.0001	0.0026	<0.0001
Number of Leaves	F	5.57	6.36	27.98	17.72	165.75	5.51	38.12
	p-value	0.0015	0.0045	<0.0001	<0.0001	<0.0001	0.0084	<0.0001

V Target species; A Dried leaf extract (DLE); B Date palm biochar (DPB); $p < 0.05$.

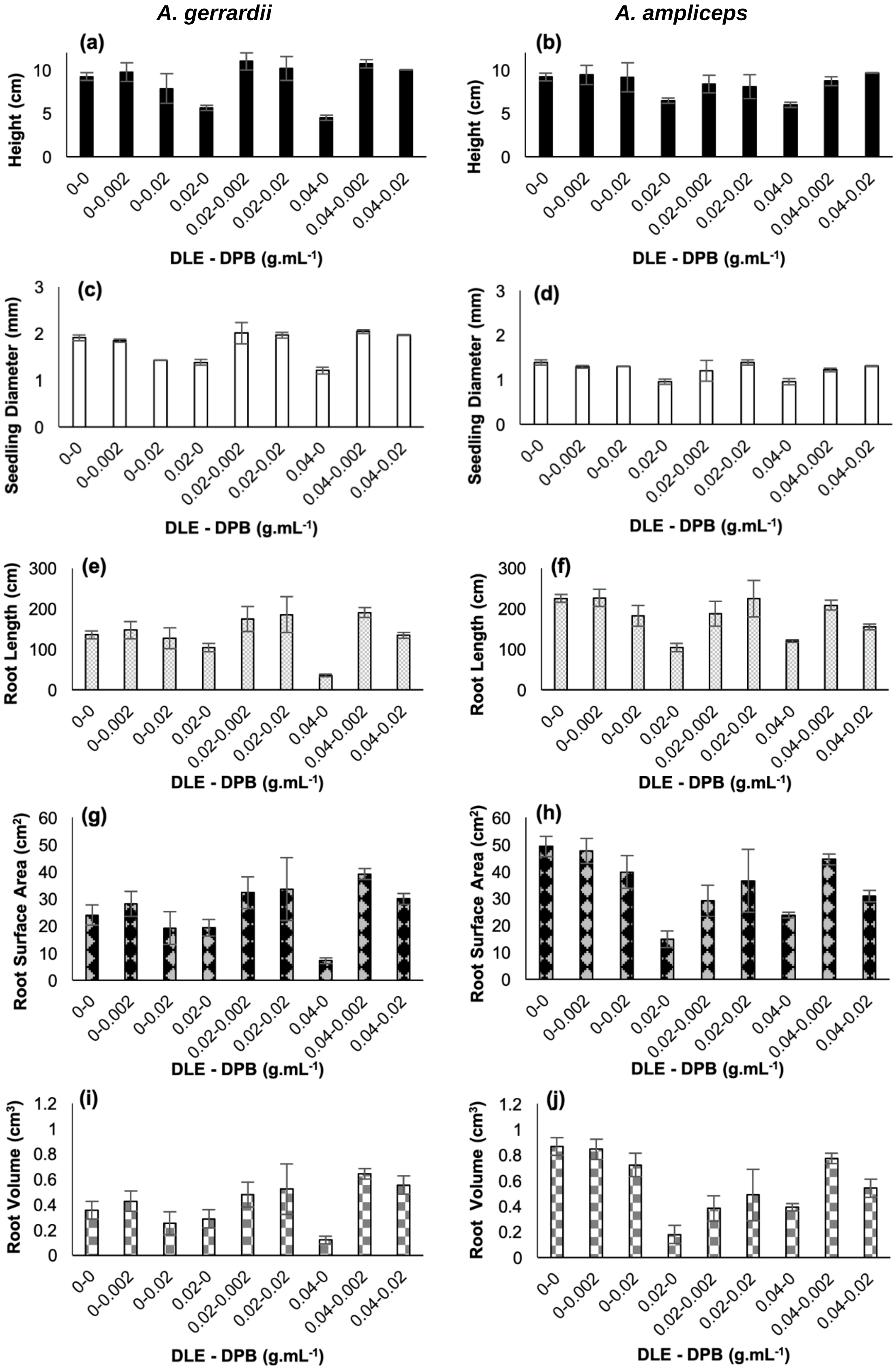
The leachate of DPB also decreased radicle hypocotyl development and seedling growth (except seedling height) (Table 1), and this effect was enhanced by increasing the DPB concentration, especially in *A. gerrardii* (Fig. 6, 7). According to this study, the effect of DPB leachate on radicle hypocotyl development at high concentrations was four times greater than that at low concentrations. Additionally, DPB leachate tended to delay the radicle hypocotyl development at the beginning of the germination phase. Intani *et al.* (2018) discovered that water extracted from biochar shows acute toxic effects on cress (*Lepidium sativum*) seed germination. Several compounds have been reported to be responsible for the potential toxic effects of biochar, such as fatty acids (Shiralipour *et al.* 1997), heavy metals (Li *et al.* 2005), volatile organic compounds (VOCs), polycyclic aromatic hydrocarbons (PAHs) (Rogovska *et al.* 2012), ammonia (Qi *et al.* 2012), and salts (Panuccio *et al.* 2014).

Null and negative effects of biochar on plant growth were found in most instances. One potential reason is that the volatile or mobile organic compounds produced during pyrolysis were exposed to the target species (Buss *et al.* 2015; Koltowski and Oleszczuk 2015; Gale *et al.* 2016). These volatile or mobile compounds are trapped as gases within the pore of biochar or re-condensed as liquids on the surface of biochar (Spokas *et al.* 2010, 2011; Hale *et al.* 2012). The types and amounts of these compounds depend on the feedstock and the pyrolysis conditions (Qin 2012; Lee 2013). Hence, post-treatment procedures might be important to diminish the potential toxicity of volatile or mobile organic compounds (Gale *et al.* 2016). In addition, pre-treatment techniques for feedstock before processing the biochar might be necessary, but enough research has not been done on this yet. Thus, a supplementary study is needed for better understanding. The negative effects can include binding and deactivation of agrochemicals in soil, release of toxicants that may be present in biochar, oversupply of nutrients, increase in EC and pH, and the effects on germination and soil biological processes (Kookana *et al.* 2011; Rogovska *et al.* 2012; Buss *et al.* 2015). In addition, biochar produced below 500 °C may be initially hydrophobic (Masiello *et al.* 2015), and this is likely to impact the ability of water to enter the soil column. Further studies are needed to clarify this effect.

Effects of Treatment Combinations on Seed Germination and Seedling Growth

The germination percentage and germination speed of both target species were not affected by treatment combinations of DLE and DPB (Table 2). Effects of all treatment combinations were not significantly different from the germination percentage ($p > 0.05$) and for germination speed ($p > 0.05$). However, the combination of treatments showed a significant interaction between DLE and DPB on the vigor index (VI) for *A. gerrardii* and *A. ampliceps* ($p < 0.05$). In the seedling growth trial, the treatment combinations exhibited significant two-factor and three-factor interactions (Table 3, Fig. 4a). Three factor interactions occurred in seedling height and diameter, root tips, and the number of leaves. An interesting result occurred in a two-factor interaction between DLE (A) and DPB (B), which exhibited pronounced differences in all parameters. The presence of DPB on DLE reduced the inhibitory effect and the impact varied by increasing the DPB concentrations.

Furthermore, the RGR_H value significantly differed among the treatment combinations ($p < 0.01$). The highest value was shown by any leaf extract concentration that contained a high dose of biochar, while the lowest value was shown by the treatment combination having the highest dose of raw leaf extract of *E. camaldulensis*. Most of the treatment combinations resulted in a higher RGR_H value than the control, while the opposite effect was shown by the raw leaf extracts of *E. camaldulensis* (Fig. 5).



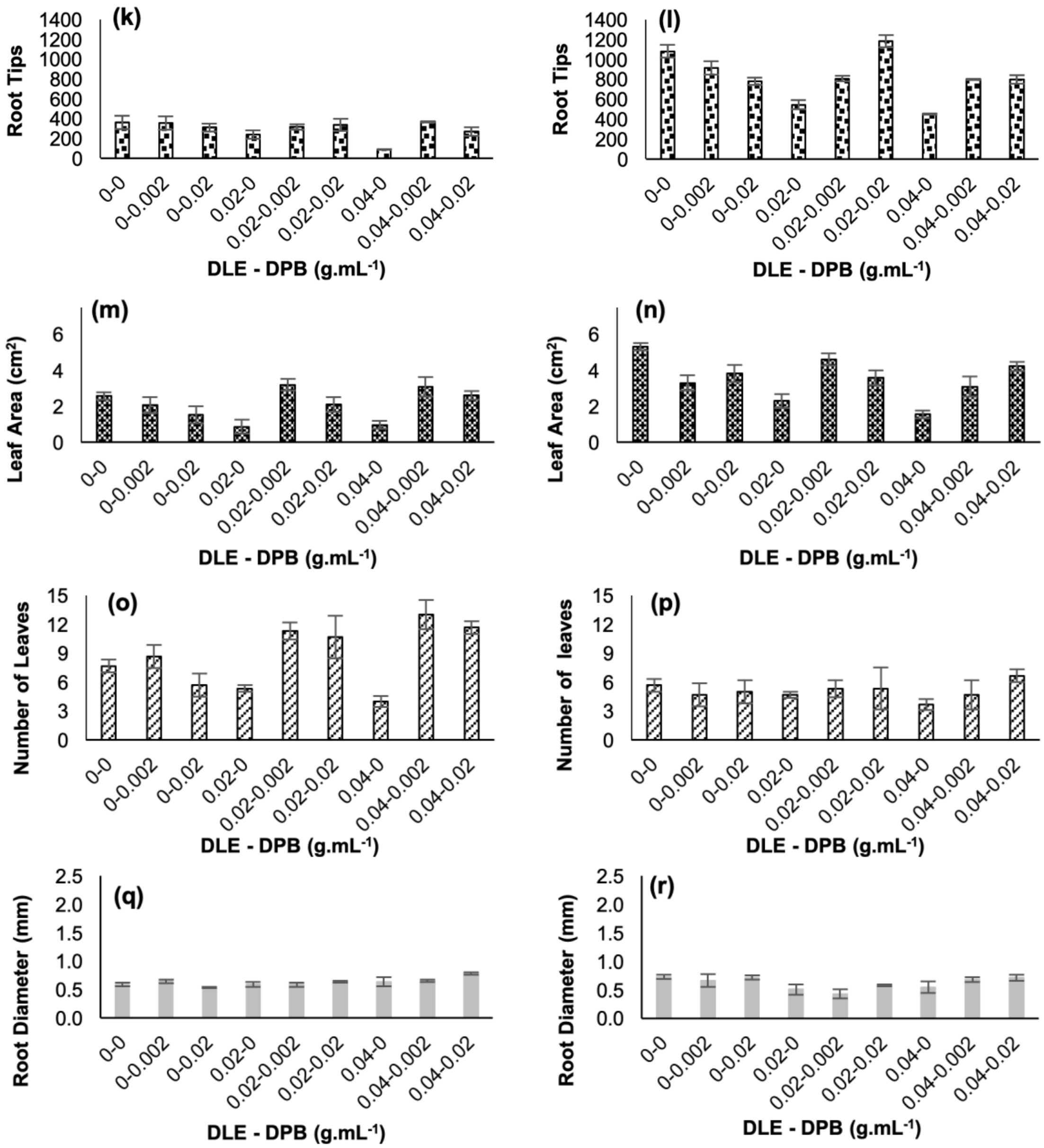


Fig. 4. (a)(b) Seedling height; (c)(d) seedling diameter; (e)(f) root length; (g)(h) root surface area; (i)(j) root volume; (k)(l) root tips; (m)(n) leaf area; (o)(p) number of leaves (q)(r) root diameter of *A. gerrardii* and *A. ampliceps* treated with DLE and DPB leachate

The RGR_H was strikingly different for the different treatments. The mean seedling height in the DLE treatment was smaller than in other treatments (Fig. 5). Any DLE treatments that were mixed with DPB showed the opposite result. The reason for this might be that seedlings in favorable conditions (without DLE or DLE+DPB combinations) have a higher potential speed of growth than those in unfavorable conditions (Lambers and Poorter 1992). The inhibitory effect of DLE on target species might be correlated with the accumulation of allelochemical compounds. The allelochemicals triggered unfavorable conditions that affected the physiological processes, thereby reducing the speed of seed growth (Dadkhah 2013). Furthermore, DLE was reported to decrease chlorophyll content (Mohamadi and Rajaie 2009; Siyar *et al.* 2019) by degrading the chlorophyll pigments and decrease the number of leaves (Dadkhah 2013). In line with the results of the present study, leaf area and the number of leaves were drastically reduced by the DLE addition.

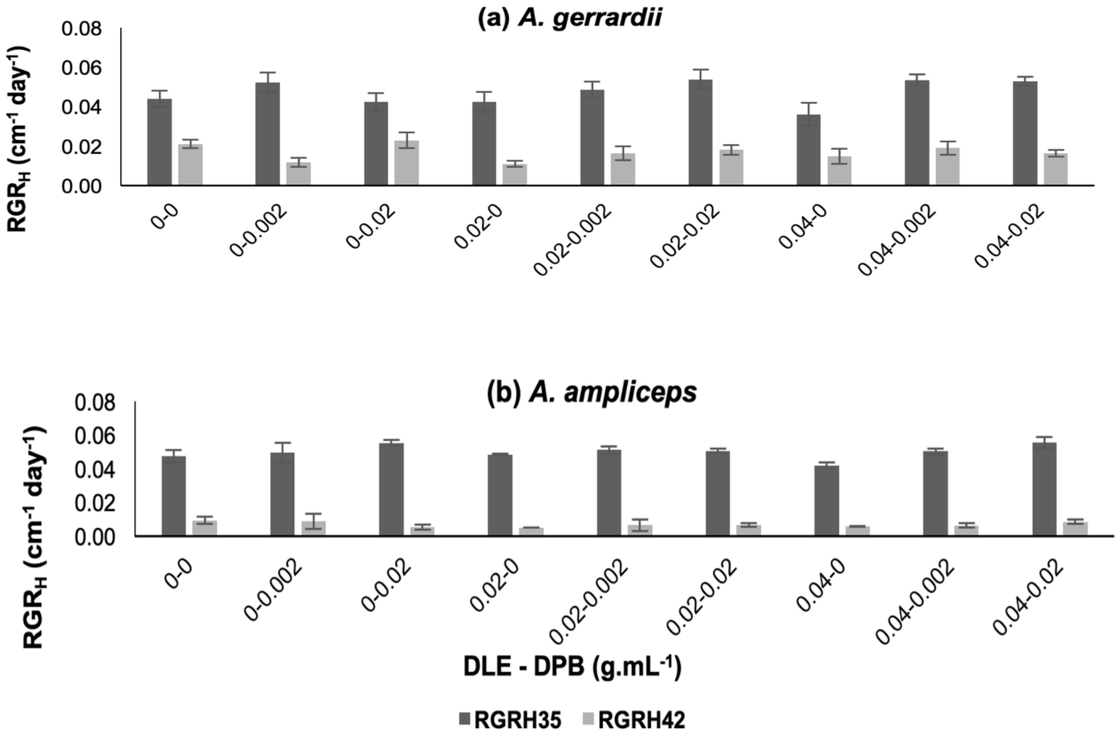


Fig. 5. Effect of DLE and DPB leachate on RGRH of (a) *A. gerrardii* and (b) *A. ampliceps*

Biochar Alleviates the Allelopathic Effect on Seedling Growth

Decrease in radicle hypocotyl development and seedling growth because of allelopathic effect of DLE was alleviated by DPB treatments. DPB reduced the inhibitory effects of DLE, and this was enhanced by increasing DPB concentrations (Fig. 6, 7). In *A. gerrardii*, DPB improved radicle hypocotyl development by 1.65% in low concentrations and by 8.49% in high concentrations for 0.02 g DLE/mL. For the combination having a concentration of 0.04 g DLE/mL, the radicle hypocotyl development was improved by 16.4% in low concentrations and by 28.02% in high concentrations of DPB. In *A. ampliceps*, radicle hypocotyl development was improved by 25.38% in low concentrations and by 22.03% in high concentrations of DPB for 0.02 g DLE/mL. Similarly, for 0.04 g DLE/mL, the improvement was by 16.21% in low concentrations and by 19.43% in high concentrations of DPB.

The inhibitory effect of DLE on seedling height and root length was also ameliorated by biochar treatments. In *A. gerrardii*, the inhibitory effect was improved by 69.16% in low concentrations and by 77.76% in high concentrations of DPB for 0.02 g DLE/mL. Likewise, it was 399.13% in low concentrations and 258.92% in high concentrations of DPB for 0.04 g DLE/mL. In *A. ampliceps*, the inhibitory effect was improved by 77.02% in low concentrations and by 110.64% in high concentrations of DPB for 0.02 g DLE/mL. For 0.04 g DLE/mL, it was 71.5% in low concentrations and 29.93% in high concentrations of DPB.

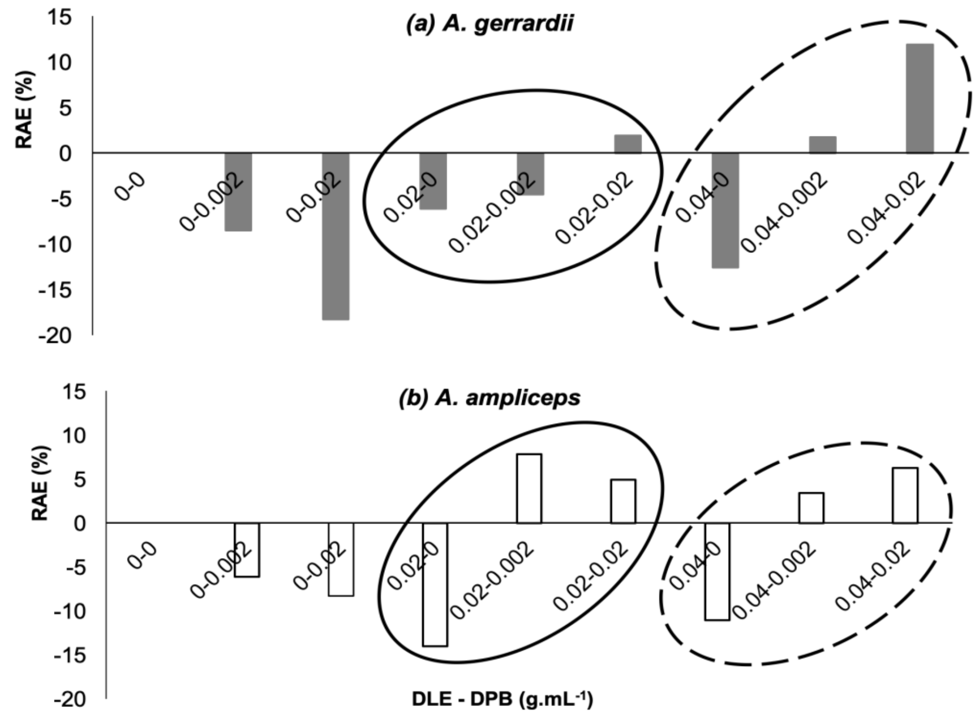


Fig. 6. RAE on VI of (a) *A. gerrardii* and (b) *A. ampliceps* based on the effect of treatment combination on the response of target species. Negative values of RAE indicated an inhibitory effect, while positive values assumed an influence of biochar to alleviate the allelopathic effect.

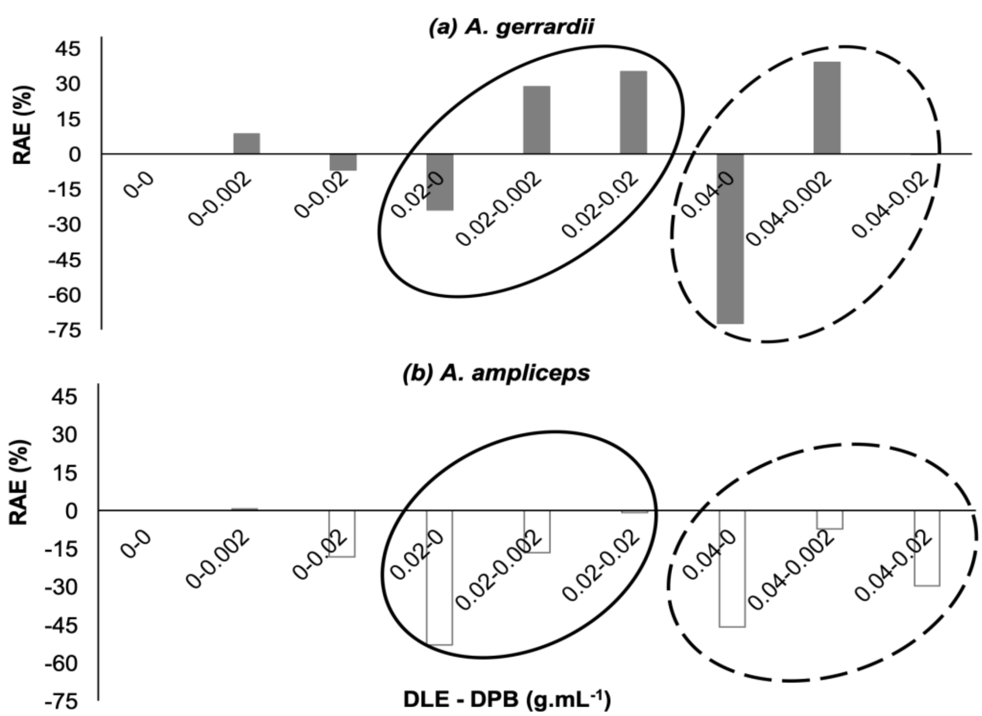


Fig. 7. Relative allelopathic effect on seedling height + root length of (a) *A. gerrardii* and (b) *A. ampliceps* based on the effect of treatment combination on the response of target species. Negative values of RAE indicated an inhibitory effect, while positive values assumed an influence of biochar to alleviate the allelopathic effect.

There are less data available on the interactions of biochar with allelopathic chemicals. Previous studies suggest that biochar can absorb allelochemicals. For instance, biochar exhibits positive effects on the growth of tree seedlings in boreal forests (Wardle *et al.* 1998), absorption of allelochemicals by corn crop residues enhanced the radicle and shoot length of corn seedlings (Rogovska *et al.* 2012), and acts as a tool for combatting invasive species in tropical ecosystems (Sujeeun and Thomas 2017). Similarly, this study showed the obvious positive effect of biochar in alleviating the inhibitory effect of *E. camaldulensis* leaf extract in early development (radicle hypocotyl) and seedling growth.

This study provided meaningful results that confirm the potential of biochar as an absorptive substance to immobilize the allelopathic chemicals. All the combination treatments that contained DLE and DPB exhibited pronounced interactions (Table 3, Figs. 4, 6, and 7). It is possible that biochar either alleviated the inhibitory effect of allelopathy (Rogovska *et al.* 2014) or absorbed and immobilized the allelochemicals (Sujeeun and Thomas 2017), thus improving seedling growth (Bonanomi *et al.* 2015). However, the effect was not linearly or reversely related to the changes in concentration for either biochar or leaf extract. However, the better understanding of specific substances, the source of feedstock, pyrolysis process, and large-scale trials are needed to increase comprehensive knowledge of these issues. Thus, field experiments are essential to examine the ability of biochar in alleviating the allelopathic effects, as there will be multifarious interactions between biochar and various soil components. Another important point to consider is that the effects of biochar may result in inconsistencies in plant development, which can be negative, null or positive. Hence, there should be proper investigation of the pre-treatment of feedstock and post-treatment of biochar product. Appropriate treatments may remarkably reduce the amount of the mobile organic compounds which are present in biochar. Pre-weathering application, production and storage methods, water leaching, and convection heating can be used to decrease or eradicate concentrations of toxic organic compounds.

CONCLUSIONS

1. The obvious positive effect of biochar in alleviating the inhibitory effect of *E. camaldulensis* leaf extract in early development (radicle hypocotyl) and seedling growth was showed in the present study.
2. DLE relatively delayed seed germination on the first day, but there were no significant variations in germination percentage and germination speed on addition of either DLE or DPB. Both DLE and DPB relatively decreased radicle hypocotyl development of both the target species (when compared to the control), and the effect was enhanced by increasing their concentrations. In the seedling growth trial, most parameters were significantly suppressed by either DLE (except root average diameter) or DPB (except seedling height). Hence, the results revealed that the inhibitory effects of DLE was less pronounced in the case of seed germination when compared to seedling growth.
3. The better understanding of specific substances, the source of feedstock, pyrolysis process, and large-scale trials are needed for improving this comprehensive knowledge. Thus, field experiments are essential to examine the ability of biochar in alleviating the allelopathic effects, as there will be multifarious interactions between biochar and various soil components.

ACKNOWLEDGMENTS

The authors are thankful to the Deanship of Scientific Research, King Saud University and the Agricultural Research Center, College of Food and Agricultural Science, for funding this research.

REFERENCES CITED

Abdul-Baki, A. A., and Anderson, J. D. (1973). "Vigour determination in soybean seed by multiple criteria," *Crop. Sci.* 13, 630-633. DOI: 10.2135/cropsci1973.0011183X001300060013x

Ahmed, R., Hoque, R., and Hossain, M. K. (2008). "Allelopathic effects of leaf litters of *Eucalyptus camaldulensis* on some forest and agricultural crops," *J. For. Res.* 19(1), 19-24. DOI: 10.1007/s11676-008-0003-x

Agrawal, R. (2011). "*Seed Technology*, (2nd Ed.), Oxford IBH Publ., New Delhi, India.

Ahn, J. K., and Chung, I. M. (2000). "Allelopathic potential of rice hulls on germination and seedlings growth of barnyard grass," *Agron. J.* 92, 1162-1167. DOI: 10.2134/agronj2000.9261162x

An, M., Pratley, J. E., and Higa, T. (2001). "Phytotoxicity of *Vulpia residues*, III. Biological activity of identified allelochemicals from *Vulpia myuros*," *J. Chem. Ecol.* 27, 383-394. DOI: 10.1023/A:100564070

Ben-Hammouda, M., Kremer, R. J., Minor, H. C., and Sarwar, M. (1995). "A chemical basis for differential allelopathic potential of Sorghum hybrids on wheat," *J. Chem. Ecol.* 21, 775-785. DOI: 10.1007/BF02033460

Binkley, D., Senock, R., and Cromack, K. J. (2003). "Phosphorus limitation on nitrogen fixation by Falcataria seedlings," *Forest Ecol. Manag.* 186, 171-176. DOI: 10.1016/S0378-1127(03)00240-8

Bonanomi, G., Ippolito, F., and Cala, F. (2015). "A 'black' future for plant pathology? Biochar as a new soil amendment for controlling plant diseases," *J. Plant. Pathol.* 97(2), 223-234. DOI: 10.4454/jpp.v97i2.3381

Buss, W., Masek, O., Graham, M., and Wust, D. (2015). "Inherent organic compounds in biochar–their content, composition and potential toxic effects," *J. Environ. Manag.* 156, 150-157. DOI: 10.1016/j.jenvman.2015.03.035

Chen, S., Li, J., Fritz, E., Wang, S., and Hutterman, A. (2002). "Sodium and chloride distribution in roots and transport in three poplar genotypes under increasing NaCl stress," *Forest Ecol. Manag.* 168, 217-230. DOI: 10.1016/S0378-1127(01)00743-5

Cheng, F., and Cheng, Z. (2015). "Research progress on the use of plant allelopathy in agriculture and the physiological and ecological mechanisms of allelopathy," *Front. Plant Sci.* 6, 1020. DOI: 10.3389/fpls.2015.01020

Dadkhah, A. (2013). "Allelopathic effect of sugar beet (*Beta vulgaris*) and eucalyptus (*Eucalyptus camaldulensis*) on seed germination and growth of *Portulaca oleracea*," *Russ. Agr. Sci.* 39(2), 117-123. DOI: 10.3103/S106836741

Duke, O., Cedergreen, N., Vellini, E. D., and Belz, R. G. (2006). "Hormesis: Is it an important factor in herbicides use and allelopathy?," *Out. on Pest Manag.* 19, 29-33. DOI: 10.1564/16feb10

Forrester, D. I., Bauhus, J., Cowie, A. L., and Vanclay, J. K. (2006). "Mixed-species plantations of *Eucalyptus* with nitrogen-fixing trees: A review," *Forest Ecol. Manag.*

233, 211-230. DOI: 10.1016/j.foreco.2006.05.012

Gale, N. V., Sackett, T., and Thomas, S. C. (2016). "Thermal treatment and leaching biochar alleviates plant growth inhibition from mobile organic compounds," *Peer J.* 4, e2385. DOI: 10.7717/peerj.2385

Hale, S.E., Lehmann, J., Rutherford, D., Zimmerman, A.R., Bachmann, R. T., Shitumbanuma, V., Toole, A., Sundqvist, K. L., Arp, H. P. H., and Cornelissen, G. (2012). "Quantifying the total and bioavailable polycyclic aromatic hydrocarbons and dioxins in biochars," *Environ. Sci. Technol.* 46(5), 2830-2838. DOI: 10.1021/es203984k

Hashoum, H., Santonja, M., Gauquelin, T., Saatkamp, A., Gavinet, J., Greff, S., Lecareux, C., Fernandez, C., and Bousquet-Me´lou, A. (2017). "Biotic interactions in a Mediterranean oak forest: role of allelopathy along phenological development of woody species," *Eur. J. For. Res.* 136, 699-710. DOI: 10.1007/s10342-017-1066-z

Hegab, M. M., Khodary, S. E. A., Hammouda, O., and Ghareib, H. R. (2008). "Autotoxicity of chard, its allelopathic potentiality on germination and some metabolic activities associated with growth of wheat seedlings," *Afr. J. Biotechnol.* 7, 884-892. DOI: 10.5897/ajb07.919

Igboanugo, A. B. I. (1987). "Effect of *Eucalyptus* on growth and yield of *Amaranthus Caudatus* and *Abelmoschus esculenthus*," *Agric. Ecosyst. Environ.* 18, 243-250. DOI: 10.1016/0167-8809(87)90088-0

Intani, K., Latif, S., Islam, M. S., and Müller, J. (2018). "Phytotoxicity of corncob biochar before and after heat treatment and washing," *Sustainability* 11(1), 1-18. DOI: 10.3390/su11010030

Koltowski, M., and Oleszczuk, P. (2015). "Toxicity of biochars after polycyclic aromatic hydrocarbons removal by thermal treatment," *Ecol. Eng.* 75, 79-85. DOI: 10.1016/j.ecoleng.2014.11.004

Koocheki, A., Lalegani, B., and Hosseini, S. A. (2013). "Ecological consequences of allelopathy," in: *Allelopathy: Current Trends and Future Applications*, Z. A. Cheema, M. Farooq, and A. Wahid (eds.), Springer-Verlag, Berlin, pp. 23-38.

Kookana, R. S., Sarmah, A. K., Van Zwieten, L., Krull, E., and Singh, B. (2011). "Biochar application to soil: Agronomic and environmental benefits and unintended consequences," *Adv. Agron.* 112, 103-143. DOI: 10.1016/B978-0-12-385538-1.00003-2

Lambers, H., and Poorter, H. (1992). "Inherent variation in growth rate between higher plants; a search for physiological causes and ecological consequences," *Adv. Ecol. Res.* 23, 188-242. DOI: 10.1016/S0065-2504(08)60148-8

Lee, J. W. (2013). "Introduction: An overview of advanced biofuels and bioproducts," in: *Advanced Biofuels and Bioproducts*, J.W. Lee (ed.), Springer Science Business Media, New York, pp. 3-12.

Li, W., Khan, M., Yamaguchi, S., and Kamiya, Y. (2005). "Effects of heavy metals on seed germination and early seedling growth of *Arabidopis thaliana*," *Plant Growth Regul.* 46(1), 45-50. DOI: 10.1007/s10725-005-6324-2

Masiello, C., Dugan, B., Brewer, C., Spokas, K., Novak, J., Liu, Z., and Sorrenti, G. (2015). "Biochar effects on soil hydrology," in: *Biochar for Environmental Management Science, Technology and Implementation*, 2nd Edition, J. Lehmann, S. Joseph (eds.), Routledge, London, UK, pp. 541-560.

Mohamadi, N., and Rajaie, P. (2009). "Effects of aqueous eucalyptus (*E. camaldulensis* Labill) extracts on seed germination, seedling growth and physiological responses of

Phaseolus vulgaris and *Sorghum bicolor*," *Res. J. Biol. Sci.* 4, 1292-1296. DOI: 10.3923/rjbsci.2009.1292.1296

Niakan, M., and Saberi, K. (2009). "Effects of *Eucalyptus* allelopathy on growth characters and antioxidant enzymes activity in *Phalaris* weeds," *Asian J. Plant Sci.* 8, 440-446. DOI: 10.3923/ajps.2009.440.446

Oyun, M. B. (2006). "Allelopathic potentialities of *Gliricidia sepium* and *Acacia auriculiformis* on the germination and seedling vigor of maize (*Zea mays* L.)," *Am. J. Agric. Biol. Sci.* 1, 44-47. DOI: 10.3844/ajabssp.2006.44.47

Panuccio, M. R., Jacobsen, S. E., Akhtar, S. S., and Muscolo, A. (2014). "Effect of saline water on seed germination and early seedling growth of the halophyte quinoa," *AoB Plants*, 6, plu047. DOI: 10.1093/aobpla/plu047

Paquette, A., and Messier, C. (2011). "The effect of biodiversity on tree productivity: From temperate to boreal forests," *Glob. Ecol. Biogeogr.* 20, 170-180. DOI: 10.1111/j.1466-8238.2010.00592.x

Pretzsch, H., and Schütze, G. (2009). "Transgressive over yielding in mixed compared with pure stands of Norway spruce and European beech in Central Europe: evidence on stand level and explanation on individual tree level," *Eur. J. For. Res.* 128, 183-204. DOI: 10.1007/s10342-008-0215-9

Qi, X., Wu, W., Shah, F., Peng, S., Huang, J., Cui, K., Liu, H., and Nie, L. (2012). "Ammonia volatilization from urea-application influenced germination and early seedling growth of dry direct-seeded rice," *Sci. World J.* 857472, 1-7. DOI: 10.1100/2012/857472

Qin, H.Z. (2012). *Study on Properties of Biochar Made from Household Waste,* Ph.D. Dissertation, Nanjing Agricultural University, Nanjing, JS.

Rice, E. L. (1984). *Allelopathy* (2nd Ed.), Academic Press, New York, NY.

Rogovska, N., Laird, D., Cruse, R. M., Trabue, S., and Heaton, E. (2012). "Germination tests for assessing biochar quality," *J. Environ. Qual.* 41, 1014-1022. DOI: 10.2134/jeq2011.0103.

Rogovska, N., Laird, D.A., Rathke, J., and Karlen, D. L. (2014). "Biochar impact on Midwestern Mollisols and maize nutrient availability," *Geoderma* 231, 340-347. DOI: 10.1016/j.geoderma.2014.04.009

Ruwanza, S., Gaertner, M., Esler, K. J., and Richardson, D. M. (2015). "Allelopathic effects of invasive *Eucalyptus camaldulensis* on germination and early growth of four native species in the Western Cape, South Africa," *South. For.* 77(2), 91-105. DOI: 10.2989/20702620.2014.965985

Sahu, A., and Devkota, A. (2013). "Allelopathic effects of aqueous extract of leaves of *Mikania micrantha* H.B.K. on seed germination and seedling growth of *Oryza Sativa* L. and *Raphanus Sativus* L," *Scien. World* 11(11), 90-93. DOI: 10.3126/sw.v11i11.8559

Shackley, S., Sohi, S., Ibarrola, R., Hammond, J., Masek, O., Brownsort, P., Cross, A., Prendergast-Miller, M., and Haszeldine, S. (2013). "Biochar, Tool for climate change mitigation and soil management," in: *Geoengineering Responses to Climate Change,* T. Lenton and N. Vaughan (eds.), Springer Science Business Media, New York, NY, pp. 73-140.

Shetta, N., Alshahrani, T. S., Aref, I. M., and Nasser, R. A. (2017). "Allelopathic potential of *Calotropis procera* and *Eucalyptus* species on germination and growth of some timber trees," *Allel. J.* 40(1), 81-94. DOI: 10.4197/Sci.19-1.9

Shiralipour, A., McConnell, D. B., and Smith, W. H. (1997). "Phytotoxic effects of a short-chain fatty acid on seed germination and root length of *Cucumis sativus* cv.'Poinset'," *Compost Sci. Util.* 5(2), 47-52. DOI: 10.1080/1065657X.1997.10701873

Silva, E. R., Overbec, G. E., and Soares, G. L. (2014). "Phytotoxicity of volatiles from fresh and dry leaves of two Asteraceae shrubs: Evaluation of seasonal effects," *S. Afr. J. Bot.* 93, 14-18. DOI: 10.1016/j.sajb.2014.03.006

Siyar, S., Majeed, A., Muhammad, Z., Ali, H., and Inayat, N. (2019). "Allelopathic effect of aqueous extracts of three weed species on the growth and leaf chlorophyll content of bread wheat," *Acta Ecol. Sin.* 39(1), 63-68. DOI: 10.1016/j.chnaes.2018.05.007

Spokas, K. A., Baker, J. M., and Reicosky, D. C. (2010). "Ethylene: Potential key for biochar amendment impacts," *Plant Soil* 333(1), 443-452. DOI: 10.1007/s11104-010-0359-5

Spokas, K. A., Novak, J. M., Stewart, C. E., Cantrell, K. B., Uchimiy, M., DuSaire, M. G., and Ro, K. S. (2011). "Qualitative analysis of volatile organic compounds on biochar," *Chemo.* 85(5), 869-882. DOI: 10.1016/j.chemosphere.2011.06.108

Sugiyama, S., Yoshino, T., Kanahara, H., Shichiri, M., Fukushi, D., and Ohtani, T. (2004). "Effects of acetic acid treatment on plant chromosome structures analyzed by atomic force microscopy," *Anal. Biochem.* 324, 39-44. DOI: 10.1016/j.ab.2003.09.026

Sujeeun, L., and Thomas, S. C. (2017). "Potential of biochar to mitigate allelopathic effects in tropical island invasive plants: Evidence from seed germination trials," *Trop. Conserv. Sci.* 10, 1-14. DOI: 10.1177/1940082917697264

Uremis, I., Arslan, M., and Uludag, A. (2005). "Allelopathic effects of some Brassica species on germination and growth of cutleaf ground-cherry (*Physlis angulata* L.)," *J. Biol. Sci.* 5, 661-665. DOI: 10.3923/jbs.2005.661.665

Ussiri, D., and Lal, R. (2017). *Carbon Sequestration for Climate Change Mitigation and Adaptation*, Springer International Publishing, Cham, Switzerland.

Vila', M., Vayreda, J., Gracia, C., and Ibanez, J. J. (2003). "Does tree diversity increase wood production in pine forests?," *Oecol.* 135, 299-303. DOI: 10.1007/s00442-003-1182-y

Wakjira, M., Berecha, G., and Bulti, B. (2006). "Allelopathic effects of *Parthenium hysterophorus* extracts on seed germination and seedling growth of lettuce," *Trop. Sci.* 45(4), 159-162. DOI: 10.1002/ts.21

Wardle, D. A., Zackrisson, O., and Nilsson, M. C. (1998). "The charcoal effect in Boreal forests: Mechanisms and ecological consequences," *Oecol.* 115, 419-426. DOI: 10.1007/s004420050536

Willis, R.J. (2007). *The History of Allelopathy*, Springer, Amsterdam.

Zhang, C., and Fu. S. (2010). "Allelopathic effects of leaf litter and live roots exudates of *Eucalyptus* species on crops," *Allelopathy J.* 26(1), 91-100.

Article submitted: September 14, 2019; Peer review completed: November 9, 2019; Revised version received and accepted: November 23, 2019; Published: November 26, 2019.
DOI: 10.15376/biores.15.1.382-400