



Effects of soilless growing media and extracts of brown seaweed *Ascophyllum nodosum* on the growth of *Robinia pseudoacasia* L.

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Abstract

Ascophyllum nodosum is the most important commercial seaweed. Seaweeds have been applied as soil conditioners in improving plant growth and development in agricultural crops. In current study and for the first time, extracts of brown seaweed *A. nodosum* improved the growth of *Robinia pseudoacasia* L., an ornamental tree. This research investigated the effect of cultivation beds (sand, perlite, compost and cocopeat) with different ratios and extracts of brown seaweed *A. nodosum* on the growth of *R. pseudoacasia* L. in *ex vitro* conditions. Concentrations used from extracts of *A. nodosum* were 0, 1000, 2000, and 3000 mg l⁻¹. The 1000 mg l⁻¹ of extracts of *A. nodosum* developed optimum plant height, node number, longest root, leaf number, dry weight, fresh weight, and plantlets survival when added to culture bed containing sand + perlite + compost with proportion of 1:1:1. About 75% of the propagated plantlets were established successfully in acclimatization medium. Regenerated plantlets were morphologically identical with mother plants.

Keywords: Fabaceae; black locust; plant proliferation; seed germination; culture bed; brown algae

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Introduction

Black locust (*Robinia pseudoacasia* L.) is a fast growing tree belonging to the Fabaceae family. *R. pseudoacasia* L. is an important landscape plant for fall, winter, and spring gardens and parks. *R. pseudoacasia* is the only economic tree from *Robinia* genus and the most widely planted tree worldwide (Zhang et al.,

2007; Kanwar et al., 2009). This species is being cultivated to control erosion and establishment of energy (Zhang et al., 2007). *R. pseudoacasia* L. is able to fix molecular (atmospheric) nitrogen and enrich the soil fertility (Zhang et al., 2007; Kanwar et al., 2009).

Soilless cultures have been successfully applied for several decades to intensify production and reduce cost. The challenge with most modern container mixes is that they rely heavily on organic components such as peat and bark. Significant composting or decomposition in

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the pot causes concerns for deterioration in physical properties. Also, nitrogen that would normally be available for plant growth will be utilized by the microorganisms involved in the composting process (Fitzpatrick, 2001; Wilson et al., 2003). Peat is the most widely used substrate for potted plant production. Also, cocopeat has been considered as a renewable sphagnum peat substitute to use in horticulture. Perlite has frequently been applied in soilless culture. It has a closed cellular structure with the majority of water being retained superficially and released slowly at a relatively low tension, providing excellent drainage of the medium and aeration of rhizosphere. These substrates must be analyzed to verify the physical properties more suitable for the best growth and mineral nutrients of *R. pseudoacasia* L. *R. pseudoacasia* L. can be successfully grown in the soils with proper pH, aeration, and drainage. The soil should be rich in organic matter and retain sufficient moisture for proper growth. Also, maximum yield is obtained by the use of suitable portion of carbon, nitrogen, manganese, magnesium, zinc, copper, iron, phosphorous, and potassium (Alizadeh Zavieh, 2005). Several studies demonstrated that peat can be substituted by various compost types without any negative effects on the plants (Zaller, 2007).

A. nodosum is a brown seaweed known to grow in temperate countries. The most important commercial seaweed, *A. nodosum* has been used as soil conditioners in improving plant growth in agricultural and horticultural species (Hurtado et al., 2009). Commercially, extracts of brown algae such as rockweed are good sources of fertilizer (Hurtado et al., 2009). The extract products of *A. nodosum*, both liquid and powder, are traded globally for agricultural and horticultural farming purposes (Hurtado et al., 2009). *A. nodosum* acts as a plant growth promoter (Rayorath et al., 2008a; Hurtado et al., 2009). Extracts from *A. nodosum* promoted seed germination, seedling vigor, root-tip elongation, and shoot growth in some species (Rayorath et al., 2008a; Hurtado et al., 2009). Jayaraj et al. (2008) showed that extract of *A. nodosum* reduced foliar fungal disease in carrots at 0.2% compared to the control plants. Successful micropropagation of several species has been

reported using different culture media along with extract of *A. nodosum* (Dawes et al., 1993; Hurtado et al., 2009).

The objectives of the present study was to determine the efficiency of different growing media (sand, perlite, compost, and cocopeat) and extracts of brown seaweed *A. nodosum* on improvement of *ex vitro* proliferation of *R. pseudoacasia* L.

Materials and Methods

Seed explants were collected from 15-year-old *Robinia pseudoacasia* L. plants grown in a greenhouse at the suburb of Amol, Mazandaran province, Iran. To investigate the effect of cultivation beds and extracts of brown seaweed *Ascophyllum nodosum*, 30-day-old plantlets were transferred to cultivation beds (sand, 1/3 sand + 1/3 perlite + 1/3 compost, 1/3 sand + 1/3 cocopeat + 1/3 compost, and 1/4 sand + 1/4 perlite + 1/4 compost + 1/4 cocopeat) and different concentrations of extracts of *A. nodosum* (0, 1000, 2000 and 3000 mg l⁻¹).

Measured characters consisting plant height, node number, longest root, leaf number, dry weight, fresh weight, and plantlets survival were calculated after 30 days. For determination of plant fresh weight, the plants were weighed by a digital balance. After recording fresh weight, plants were dried in oven at 103 °C for 24 h, and their dry weight was obtained by a digital balance.

Statistical analysis

A complete randomized block (CRD) design was used for the experiments with three replicates. After 30 days from the start of the experiments, the data were recorded and analyzed. Analysis of variance (ANOVA) was done using SPSS and MSTAT-C statistical software and means were compared using LSD test at 0.05 level of probability.

Results

Our data revealed that there are differences in the effect of different cultivation beds, different concentrations of extracts of *A. nodosum*, and interaction between these two

Table 1

Mean comparison for the effect of different cultivation beds and different concentrations of extracts of *A. nodosum* on some traits of *Robinia pseudoacasia* L.*

Treatments	Characters						
	Longest root length (cm)	Plant height (cm)	Node No.	Leaf No.	Dry weight (g)	Fresh weight (g)	Viability (%)
Control (S ₁)	7.98 ^c	5.78 ^h	12.88 ^c	10.88 ^c	1.50 ^c	2.54 ^c	46.06 ^c
1/3 sand + 1/3 perlite + 1/3 compost (S ₂)	10.31 ^a	10.54 ^a	18.73 ^a	16.50 ^a	2.55 ^a	4.53 ^a	69.69 ^a
1/3 sand + 1/3 cocopeat + 1/3 compost (S ₃)	10.22 ^b	9.30 ^b	16.23 ^b	14.78 ^b	1.87 ^b	3.48 ^b	54.17 ^b
1/4 sand + 1/4 perlite + 1/4 compost + 1/4 cocopeat (S ₄)	11.22 ^a	11.28 ^a	19.35 ^a	16.54 ^a	1.97 ^a	4.17 ^a	67.50 ^a
Control (A ₁)	9.22 ^c	8.73 ^c	15.33 ^c	13.32 ^b	1.46 ^c	3.17 ^c	51.66 ^c
1000 mg l ⁻¹ <i>A. nodosum</i> (A ₂)	12.40 ^a	12.38 ^a	21.25 ^a	18.30 ^a	2.31 ^a	4.45 ^a	75.00 ^a
2000 mg l ⁻¹ <i>A. nodosum</i> (A ₃)	10.23 ^b	9.98 ^b	17.32 ^b	14.23 ^b	1.73 ^b	3.62 ^b	60.00 ^b
3000 mg l ⁻¹ <i>A. nodosum</i> (A ₄)	9.44 ^c	8.70 ^c	15.00 ^c	13.20 ^b	1.48 ^c	3.11 ^c	50.83 ^c
S ₁ × A ₁	8.96 ^{fg}	7.65 ^g	13.40 ^g	11.25	1.30 ^{gh}	2.52 ^{gh}	43.33
S ₁ × A ₂	10.26 ^{def}	9.61 ^{de}	16.85 ^{de}	14.11	1.74 ^{def}	3.28 ^e	60.00
S ₁ × A ₃	8.33 ^{gh}	7.14 ^{gh}	12.44 ^g	11.28	1.16 ^h	2.51 ^{hg}	40.00
S ₁ × A ₄	7.01 ^h	7.57 ^c	10.14 ^h	9.15	0.88 ⁱ	2.10 ^h	33.33
S ₂ × A ₁	8.64 ^g	9.17 ^{ef}	16.13 ^{ef}	15.13	1.49 ^{fg}	3.39 ^{de}	56.66
S ₂ × A ₂	14.56 ^a	15.24 ^a	25.95 ^a	22.88	2.94 ^a	5.52 ^a	93.33
S ₂ × A ₃	10.86 ^{cde}	11.24 ^c	19.34 ^c	15.83	1.98 ^{cd}	4.03 ^c	73.33
S ₂ × A ₄	11.18 ^{bcd}	10.50 ^{cd}	18.32 ^{cd}	15.31	1.80 ^{cde}	3.60 ^{cde}	63.33
S ₃ × A ₁	10.34 ^{def}	8.31 ^{ef}	14.35 ^{fg}	12.25	1.52 ^{fg}	3.60 ^{ef}	46.66
S ₃ × A ₂	12.18 ^{bc}	11.22 ^c	19.63 ^c	16.74	2.02 ^c	4.07 ^c	66.66
S ₃ × A ₃	9.39 ^{efg}	10.34 ^{cde}	18.20 ^{cde}	14.31	1.82 ^{cd}	3.86 ^{cd}	60.00
S ₃ × A ₄	8.55 ^{gh}	7.72 ^g	13.53 ^g	11.81	1.35 ^{gh}	2.71 ^{fg}	43.33
S ₄ × A ₁	8.94 ^{fg}	9.80 ^{de}	17.47 ^{cde}	14.66	1.54 ^{efg}	3.64 ^{cde}	60.00
S ₄ × A ₂	12.61 ^b	13.45 ^b	22.60 ^b	19.46	2.53 ^b	4.93 ^b	80.00
S ₄ × A ₃	12.33 ^{bc}	11.20 ^c	19.33 ^c	15.49	1.96 ^{cd}	4.10 ^c	66.66
S ₄ × A ₄	11.01 ^{cd}	10.70 ^{cd}	18.02 ^{cde}	16.55	1.88 ^{cd}	4.03 ^c	63.33

*In each column, means with the similar letters are not significantly different at 5% level of probability using LSD test.

factors on measured characters (Tables 1 and 2). Produced plantlets were transferred to experimental cultivation beds enriched with extracts of *A. nodosum*. Treatment of 1000 mg l⁻¹ *A. nodosum* and 1/3 sand + 1/3 perlite + 1/3 compost increased plant height by 15.25 cm. This treatment increased plant height to more than twice that of the control (5.78 cm) (Table 1). Comparison between each one of these two treatments separately showed precedence of 1000 mg l⁻¹ *A. nodosum* and 1/3 sand + 1/3 perlite + 1/3 compost over the other treatments (Table 1). There were significant differences in plant height between plants treated with extracts of *A.*

nodosum and cultivation beds and control plants (Table 2).

ANOVA analysis showed that the node number was significantly affected by cultivation beds, inoculation with extract of *A. nodosum*, and interaction of cultivation beds and inoculation with extract of *A. nodosum* ($p < 0.01$) (Table 2). Visual observations revealed that node number in beds containing 1000 mg l⁻¹ *A. nodosum* and 1/3 sand + 1/3 perlite + 1/3 compost (25.95) was higher than that of other treatments (Table 1). Node number obtained in this condition was twice more than that of the control.

ANOVA analysis showed that the leaf number was significantly affected by cultivation

that of the control (3.17 g) (Table 1). Treatments 1/3 sand + 1/3 perlite + 1/3 compost with 2.05 g

Table 2

Analysis of variance for the effect of different cultivation beds and concentrations of extracts of *A. nodosum* on the longest root length, plant height, node number, leaf number, dry and fresh weight, and viability percent of *Robinia pseudoacacia* L.

Source of variations	df	MS						
		Longest Root Length	Plant height	Node No.	Leaf No.	Dry weight	Fresh weight	Viability (%)
Cultivation beds (S)	3	17.540**	42.832**	124.110**	83.197**	1.320**	6.770**	1912.183**
<i>A. nodosum</i> (A)	3	27.333**	34.867**	97.178**	70.045**	1.681**	4.190**	1527.678**
S × A	9	4.610**	2.333**	6.282**	4.550 ^{ns}	0.175**	0.370*	79.866 ^{ns}
Error	32	0.982	0.550	1.728	2.378	0.025	0.127	47.716
Total	47	-	-	-	-	-	-	-
CV (%)	-	9.197	7.550	7.616	11.465	10.460	10.536	11.258

** : Significant at $\alpha = 1\%$, * : Significant at $\alpha = 5\%$, ^{ns}=Not significant

beds and inoculation with extract of *A. nodosum* ($p < 0.01$) (Table 2). Visual observations demonstrated that leaf number in beds containing 1000 mg l⁻¹ *A. nodosum* and 1/3 sand + 1/3 perlite + 1/3 compost (21.26) was maximum (Table 1). Leaf number produced in beds containing 1/3 sand + 1/3 perlite + 1/3 compost (17.30), and 2000 mg l⁻¹ *A. nodosum* (17.33) was apparently higher than the leaf number produced in the control beds (10.88).

The production of longest root length differed significantly when the beds supplemented with 1000 mg l⁻¹ *A. nodosum* and 1/3 sand + 1/3 perlite + 1/3 compost (14.57 cm) were employed for rooting. Explants cultured on beds supplemented with 1000 mg l⁻¹ *A. nodosum* and 1/3 sand + 1/3 perlite + 1/3 compost, separately produced roots with 12.40 and 11.31 cm long (Table 1), and thus were better than the control (7.98 cm). ANOVA analysis showed that the root length was significantly affected by cultivation beds, inoculation with extract of *A. nodosum*, and interaction of cultivation beds and inoculation with extract of *A. nodosum* ($p < 0.01$) (Table 2).

Medium containing 3000 mg l⁻¹ *A. nodosum* and 1/3 sand + 1/3 cocopeat + 1/3 compost and also 1000 mg l⁻¹ *A. nodosum* and 1/3 sand + 1/3 perlite + 1/3 compost displayed a strong effect on the fresh (4.93 g) and dry weight (2.94 g) (Table 1). Treatments 1/3 sand + 1/3 cocopeat + 1/3 compost with 4.18 g fresh weight and inoculation by 1000 mg l⁻¹ *A. nodosum* with 4.45 g fresh weight induced more weights than

dry weight and inoculation by 1000 mg l⁻¹ *A. nodosum* with 2.31 g dry weight induced more weights than that of the control (1.46 g) (Table 1). The data presented in Table 2 indicated that the effect of cultivation beds and inoculation with extract of *A. nodosum* were significant on fresh weight at 1% probability level. But, the interaction of cultivation beds and inoculation with extract of *A. nodosum* was significant at 5% probability level. Table 2 also showed that the highest dry weight was significantly affected by cultivation beds, inoculation with extract of *A. nodosum*, and interaction of cultivation beds and inoculation with extract of *A. nodosum* ($p < 0.01$).

The interaction effect of cultivation beds and inoculation with extract of *A. nodosum* was not significant on plant viability percent, but the effect of these two factors, separately were significant ($p < 0.01$) (Table 2). Comparing these treatments revealed that the plant viability percent of the medium containing 1/3 sand + 1/3 perlite + 1/3 compost with 71.67% viability and the medium with 1000 mg l⁻¹ *A. nodosum* with 75.00% viability were superior to the control medium (Table 1).

Discussion

Our results showed that the effect of different cultivation media on all measured characteristics was significant. Many studies revealed that the different cultivation media had significant effect on the most vegetative growth characteristics (El-Naggar and El-Nasharty, 2009; Mahgoub et al., 2006; Abouzari et al., 2012).

Successful greenhouse and nursery production of container-grown plants is largely dependent on the chemical and physical properties of the growing media (Fitzpatrick, 2001; Wilson et al., 2003). A number of critical chemical and physical properties need to be evaluated before making a final media decision (Fitzpatrick, 2001; Wilson et al., 2003). During the last ten years, peat consumption in horticulture has remarkably increased and the level of imports has multiplied by ten (Abouzari et al., 2012). Researchers have shown immense potential of medicinal and ornamental plants used in various traditional systems (Kaufman, 1999; Dhanukar et al., 2000). Selection of bed type and its effect on vegetative and reproductive growth in tuberose are the main challenge for procedures. Light soils containing high sand warm faster than heavy soils and cause acceleration of the germination of bulbs in sand and fine gravel cultivation beds. The main reason for the significant increase of the number and length of leaves in perlite is that this cultivation bed absorbs water about 3 to 4 times more than its volume and includes enough pores for ejection of additional water, thus there is proper aeration (Hartmann et al., 1990). El-Naggar and El-Nasharty (2009) indicated the superiority of using composted leaves medium for increasing of total fresh and dry weight/plant in *Hippeastrum vittatum*. These results are similar with those obtained by Ali (1998) on *Lawsonia inermis*. Studies of El-Naggar and El-Nasharty (2009) on the effect of growing media (clay, composted leaves, and sand + composted leaves) on growth of *H. vittatum* revealed that different growing media had significant effect on the most vegetative growth characteristics. Applying the complete fertilizer of nitrogen, phosphorus, and potassium grown in composted leaves medium or its mixture with sand had the maximum effect on the growth characteristics like leaf length and width. Contrary to our results, El-Naggar and El-Nasharty (2009) showed that there is no significant difference in the number of leaves/plant as a result of using different growing media in plantation, while, fertilizer treatments significantly increased number of leaves/plant. Studies of Mahgoub et al. (2006) on response of Iris bulbs grown in sandy soil to nitrogen and potassium fertilization showed that the plant

height, number of leaves as well as fresh and dry weight/leaves increased when bulbs were fertilized with suitable levels of nitrogen and potassium in sandy soil medium. These results may be due to the growth and production of plants affected by soil type and may be sandy soil is poor in nutrient content (Mahgoub et al., 2006). These findings are similar to studies of Ramesh Kumar et al. (2002) on *P. tuberosa* Linn cv. Single. In conclusion, the type of cultivation beds due to their different fertilizers and other factors are effective on growth characteristics.

Extracts of *A. nodosum* improved growth and developmental conditions of *R. pseudoacasia* L. Several reports have shown the positive effect of extracts of *A. nodosum* on quantity and quality features, pest and disease resistance, and environmental stress tolerance of some species (Rayorath et al., 2008a; 2008b; Jayaraj et al., 2008; Hurtado et al., 2009). Chemical analysis of extracts of *A. nodosum* showed that they act as plant growth regulators such as auxins, cytokinins, and gibberellins (Jameson, 1993; Craft et al., 2007; Hiltz et al., 2007). They are a source of major and minor nutrients, carbohydrates, and amino acids (Hurtado et al., 2009). Several studies have shown the positive effect of products of *A. nodosum* on tissue culture conditions (Dawes et al., 1994; Hurtado et al., 2009). Extracts of *A. nodosum* stimulated root and shoots of growth of *Arabidopsis thaliana* (Rayorath et al., 2008a), barley (Rayorath et al., 2008b), and *Kappaphycus* varieties (Hurtado et al., 2009). Our study revealed that the use of extracts of *A. nodosum* can improve the acclimatization process and growth of *R. pseudoacasia* L. Current work is similar to Hurtado et al. (2009). These researchers demonstrated the efficiency of extracts of *A. nodosum* as a culture medium for the regeneration of young plants of *Kappaphycus* varieties. The positive effect of some other microorganisms such as bacteria on improvement of shoot and root growth and development have been shown for *R. pseudoacasia* L. (Boine et al., 2008; Ewald, 2008).

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