Effect of fucoidan and alginate on germination and growth of mung bean seedling

Efecto de fucoidan y alginato en la germinación y el crecimiento de plántulas de frijol mungo

Dania Andrea Di Filippo-Herrera[1], Dora Luz Arvizu-Higuera[1], Yoloxochitl Elizabeth Rodríguez-Montesinos[1],
Mauricio Muñoz-Ochoa[1], Rosalba Mireya Hernández-Herrera[2], Gustavo Hernández-Carmona[1]*

[1] Centro Interdisciplinario de Ciencias Marinas, Instituto Politécnico Nacional. Av. Instituto Politécnico Nacional s/n. Col. Playa Palo de Santa Rita, La Paz, Baja California Sur, 23096. México

[2] Laboratorio de Biotecnología, Departamento de Botánica y Zoología, Centro Universitario de Ciencias Biológicas y Agropecuarias, Universidad de Guadalajara, Calle Ramón Padilla Sánchez 2100, Col. Nextipac, Zapopan, Jalisco, 45110. México

*Corresponding author:
Gustavo Hernández-Carmona: e-mail: gcarmona@ipn.mx

ABSTRACT

Background. The interest in studying seaweed polysaccharides as plant growth biostimulants is recent and has generally focused on seaweed liquid extracts for compounds content that have positive effect on plant development. Within these compounds are the carbohydrates such as alginate and fucoidan, among others. **Goals.** To evaluate the effect of crude fucoidan and alginate on seed germination and their growth-stimulating activity on seedling of mung bean (*Vigna radiata*). **Methods.** Fucoidan and alginate from *Eisenia arborea* and *Sargassum horridum* at six different doses (0.6, 1.2, 2.5, 5, 10, and 20 mg m⁻¹) were evaluated on seed germination and seedling growth of mung bean. **Results.** Alginate from both algae species in this study did not show a significant effect on mung bean growth. Fucoidan from *S. horridum* produced the significant effect on mung bean growth by increasing root length (16.2%) and seedling total length (11.9%) at a concentration of 0.6 mg mL⁻¹ and an increase in the dry weight of the seedling (31%) at a concentration of 20 mg mL⁻¹.

While fucoidan from *E. arborea* had the higher effect on shoot growth (10.5%) and total length (10.7%), compared to the control when applied at a concentration of 10 mg mL⁻¹. **Conclusions.** Overall, this study showed that fucoidan from both *S. horridum* and *E. arborea* can stimulate seedling growth and increase the germination percentage of mung bean seeds compared to the control.

Keywords: *Eisenia arborea*, polysaccharides, root length, *Sargassum horridum*, shoot length.

RESUMEN

Antecedentes. El interés por estudiar los polisacáridos de algas marinas como bioestimulantes del crecimiento de las plantas es reciente, y generalmente se ha centrado en los extractos líquidos de algas marinas por su contenido de compuestos que tienen un efecto positivo en el desarrollo de plantas. Dentro de estos compuestos se encuentran carbohidratos como el alginato y el fucoidan, entre otros. **Objetivos.** Evaluar el efecto de fucoidan crudo y alginato en la germinación de semillas y su actividad estimulante del crecimiento en plántulas de frijol mungo (*Vigna radiata*). **Métodos.** Fucoidan y alginato de *Eisenia arborea* y *Sargassum horridum* fueron probadas a seis concentraciones diferentes (0.6, 1.2, 2.5, 5, 10 y 20 mg mL⁻¹) sobre la germinación de semillas y el crecimiento de plántulas de frijol mungo. **Resultados.** El alginato de ambas especies de algas de este estudio no mostró un efecto significativo sobre el crecimiento de frijol mungo. El fucoidan de *S. horridum* produjo el mayor efecto en el crecimiento del frijol mungo, incrementando la longitud de la raíz (16.2%) y la longitud total de la plántula (11.9%) a una concentración de 0.6 mg mL⁻¹ y un incremento en el peso seco de la plántula (31%) a una concentración de 20 mg mL⁻¹. Mientras que el fucoidan de *E. arborea* tuvo el mayor efecto en el crecimiento de brotes (10.5%) y la longitud total (10.7%) en comparación con el control, cuando se aplica a una concentración de 10 mg mL⁻¹. **Conclusiones.** En general, este estudio mostró que el fucoidan tanto de *S. horridum* como el de *E. arborea* puede estimular el crecimiento de plántulas y aumentar el porcentaje de germinación de semillas de frijol mungo en comparación con el control.

Palabras clave: *Eisenia arborea*, longitud de plántula, longitud de raíz, polisacáridos, *Sargassum horridum*.

INTRODUCTION

Seaweed liquid extracts have been used in agriculture for centuries as a fertilizer. Currently, studies on seaweed liquid extracts have reported that they have plant growth-promoting activity, therefore, their use in agriculture and horticulture has increased as foliar sprays for various crops such as grasses, cereals, flower species and vegetables; as well as organic manures and fertilizers (Zamani *et al.*, 2013; Battacharyya *et al.*, 2015; Ertani *et al.*, 2018). These extracts contain essential minerals, proteins, polysaccharides, phytohormones, and low molecular weight compounds that stimulate the plant growth and development (Van Oosten *et al.*, 2017; Ali *et al.*, 2019; Mukherjee & Patel, 2020). The set of these compounds can work synergistically, hence understanding their mechanism of action is highly intricate, due the multiple interaction between the substantial numbers of bioactive compound within the same extract (Zamani *et al.*, 2013; EL Boukhari, 2020).

Particularly, the polysaccharides and their oligosaccharides present in seaweed cell walls may directly affect different physiological processes in plants that are related to growth and development (Salachna *et al.*, 2018; Iwasaki & Matsubara, 2000; Mukherjee & Patel, 2020). These oligosaccharides can induce defence responses against potential pests, pathogens (Klarzynski *et al.*, 2003; Falcón & Cabrera, 2007; Mzibra *et al.*, 2018), and abiotic stress (Salachna *et al.*, 2018). In addition, they have been found that they modulate gene expression and multiple hormones signalling pathways (Rolland *et al.*, 2002; Battacharyya *et al.*, 2015; Ertani *et al.*, 2018).

Seaweed liquid extracts involves the three main groups of macroalgae that are red, brown, and green, however, brown seaweeds are the most used commercially to produce liquid or powder extracts, due to their abundance, large distribution and effectiveness. Among the brown seaweeds, the most used are *Ascophyllum nodosum* (Linnaeus) Le Jolis , *Ecklonia maxima* (Osbeck) Papenfuss, *Macrocystis pyrifera* (Linnaeus) C. Agardh, *Durvillaea potatorum* (Labillardière) Areschoug (Khan *et al.*, 2009), *Fucus spp*, *Laminaria* spp., and *Turbinaria* spp. (Hong *et al.*, 2007; Sharma *et al.*, 2012), however, other seaweeds as *Padina gymnospora* (Kützing) Sonder, *Sargassum liebmannii* J. Agardh, and *Sargassum johnstonii* Setchell & N. L. Gardner are also used (Drobek *et al.*, 2019).

Compounds such as alginate, fucoidan, laminaran and their oligosaccharides present in brown algae, have been shown to have growth-promoting activity (González *et al.*, 2013; Rengasamy *et al.*, 2015). Some studies have shown that alginate oligosaccharides improve the growth of rice and peanut plants in hydroponic cultures (Hien *et al.*, 2000), stimulate the growth of lettuce (*Lactuca sativa* Linnaeus) and tomato (*Lycopersicum esculentum* Linnaeus) roots (Iwasaki & Matsubara, 2000; Yabur *et al.*, 2007), increase the biomass in tobacco plants (Laporte *et al.*, 2007); increase shoot and root length of fennel plants (Sarfaraz *et al.*, 2011).

Studies about fucoidan with application in plants showed that enhance protection against pathogens in tobacco plants (Klarzynski *et al.*, 2003; Lapshina *et al.*, 2006). On the other hand, Bouissil *et al.* (2020) found that fucoidan from *Bifurcaria bifurcata* R. Ross and *Fucus spiralis* Linnaeus stimulate the natural defences of the date palm.

For this study, the brown algae *Eisenia arborea* Areschoug and *Sargassum horridum* Setchell & N. L. Gardner were selected considering the large abundance in the Baja California Peninsula. The extraction of alginate and fucoidan from these algae was carried out, with the aim of evaluating their effect on seed germination and their growth-stimulating activity on seedling of mung bean (*Vigna radiata* (L.) Wilczek).

MATERIALS AND METHODS

Sampling. Seaweeds were harvested (n > 30) from wild populations along the coast of the Baja California Peninsula from March to July 2015. Seaweed collection was conducted by divers at low tide. *Eisenia arborea* was collected from Bahia Magdalena (24° 35' 00″ N–112° 00' 00″ W) and *Sargassum horridum* from Bahia de La Paz (24° 08' 32″ N–110° 18' 39″ W). The fresh seaweeds were washed with tap water to remove epiphytic organisms and sand. They were sun-dried to 10% moisture content and milled to 40-mesh size (0.38 mm^2).

Fucoidan extraction. Twenty-five grams of algae (triplicate) were extracted twice with 350 ml of distilled water at 55 °C and continuous agitation for 2 h. Afterwards, the liquid was recovered by decanting and clarified by centrifugation (1500 × *g* for 15 min; Beckman TJ-6, Palo Alto, CA, USA). Then, 20 mL of 10% CaCl$_2$ was added to precipitate any residual soluble alginate, which was separated by centrifugation. The clarified solution was precipitated with three volumes of ethanol, and crude fucoidan was recovered by centrifugation, dried at 50 °C for 20 h, and stored at –20 °C until analysis**.** The crude fucoidan content was determined as a percentage of the initial algal dry weight (Muñoz-Ochoa *et al.*, 2009).

Alginate extraction. The residual algal tissue obtained from the fucoidan extraction was used for the alginate extraction. The dried algal tissue was hydrated overnight with 180 mL of 0.1% formaldehyde solution. The algae were treated with 300 mL of HCl at pH 4 and subjected to constant stirring for 15 min. After filtration, the algae were treated with 300 ml of Na$_2$CO$_3$ solution, and the pH was adjusted to 10. After which, the algae were heated to 80 °C in a water bath (Precision Scientific, Chicago, IL, USA) with constant stirring for 2 h. The obtained paste was diluted and filtered under vacuum. The alginate was precipitated with ethanol, and the alginate fibres dried at 50 °C for 12 h. Alginate yield was computed as the percentage of the algal dry weight. Viscosity was measured in 1% alginate solution at 22 °C with a viscometer (Brookfield LVT, Middleboro, MA, USA) at 60 rpm using the appropriate spindle. A second measure of viscosity was carried out after adding sodium hexametaphosphate to sequester the residual calcium (Rodríguez-Montesinos *et al.*, 2008). The alginate gel strength was measured by preparing calcium alginate gels using 1% alginate solution and filling a dialysis membrane (9 cm length × 2.9 cm diameter, which was then immersed overnight in a 10% calcium chloride solution. The gels were cut into cylinders (2.9 cm diameter × 3 cm length), and the gel strength measured with a TA.XT Plus texturometer (Stable Micro Systems, Godalming, Surrey, UK) programmed to perform a 2-cm penetration over a 5-s period to measure the gel breaking point (Camacho & Hernández-Carmona, 2012).

Spectroscopic characterization of polysaccharides by Fourier-transform infrared spectroscopy (FT-IR). The Fourier-transform infrared spectroscopy (FT-IR) spectra of the polysaccharides were recorded with a spectrophotometer (Perkin Elmer, TWO, Waltham, MA, USA) equipped with an attenuator of total reflectance (ATR). Each spectrum was obtained from the sum of 14 scans in the spectral range of 4000–500 cm^{-1}. The sulphate–total sugars (SO$_4$/CHOH) ratio was deter-

mined from the intensity of the transmittance bands at 1040 and 1256 cm^{-1} (Lijour *et al.*, 1994).

Bioassays of germination and growth under *in vitro* **conditions.** Certified mung bean (*Vigna radiata*) seeds (High Mowing Organic Seeds, Wolcott, VT, USA) with 95% germination, uniform size, colour, and weight were surface-sterilized using a soap solution for 5 min, followed by immersion in a 4% sodium hypochlorite (NaClO) solution for 10 min. They were then each triple-rinsed in sterilized distilled water for 1 min under aseptic conditions. Mung bean germination was carried out as described by Castellanos-Barriga *et al.* (2017). First, seven groups of 30 mung bean seeds were teste for germination in each of the experimental treatments (fucoidan EA and SH) or (Alginate EA and SH) at different doses of 0.6, 1.2, 2.5, 5, 10 and 20 mg mL^{-1} and control without fucoidan, only distilled water that was used to produce the extracts (fucoidans). The seeds were primed for 15 min in 20 mL in each dose of seaweed polysaccharides (alginate or fucoidans) then they were removed from the polysaccharide doses, wrapped in wet filter paper, and incubated at 25 °C in a 16:8 h light: dark cycle. All experiments were conducted in triplicate. Germination was observed daily over an 8-day period, according to the methods detailed by the Association of Official Seed Analysts (AOSA, 2005). The germination percentage (GP) was determined as the number of germinated seeds/total number of seeds × 100. Seeds were considered germinated when the radicle protruded more than 2 mm.

The effects of the fucoidan and alginate from *E. arborea* and *S. horridum* on the shoot length (from the basal node to the shoot apex), root length (from the basal node to the root tip), and total seedling length (from the root tip to the shoot apex) on 15 days old mung bean seedlings were measured using a Vernier calliper. In addition, mean dry weights were obtained with an electronic balance after oven-drying the seedlings to a constant weight at 50 °C.

Statistical analyses. The data were analysed by a one-way ANOVA and post hoc a least significant differences (LSD) tests after both normality and homogeneity of variance were tested with Shapiro–Wilk and Bartlett tests, respectively. Fucoidan and alginate obtained from brown seaweeds were analysed for their effect on mung bean growth by a one-way ANOVA with the type of algal species and the extract concentration as factors. Statgraphics Centurion XV (The Plains, VA, USA) for Windows (Microsoft Corp., Redmond, WA, USA) was used for all analyses.

RESULTS

The fucoidan yield for *E. arborea* and *S. horridum* was 10.8 ± 0.3% and 11.1 ± 0.2% (w/w), respectively, whereas the alginate yield was 14.9 ± 0.3 and 17.3 ± 0.5% (w/w), respectively.

Spectroscopic characterization of polysaccharides by FT-IR. The FT-IR spectra showed typical absorption bands for fucoidans and alginates. The absorption bands around 3200–3400 and 2937–2945 cm^{-1} are typical of the O—H stretch vibration and C—H stretch vibration of algal polysaccharides, respectively. Absorption bands between 1603–1616 and 1406–1418 cm^{-1} were observed for C=O asymmetric and symmetric carboxyl group oscillations, and the bands around 1125–1130 cm^{-1} were typical of pyranose ring and glycosidic bond C—O stretching vibrations (uronic acids; Table 1).

The fucoidans from *E. arborea* and *S. horridum* showed some differences in their chemical structure. The *E. arborea* fucoidan showed absorption bands of 840–850 cm^{-1} for C—O—S, and a secondary axial sulphate at C—4 of the fucopyranosyl residue (Fig. 1A) and the *S. horridum* fucoidan did not show. On the contrary, the *S. horridum* fucoidan showed absorption bands at 1725 cm^{-1} for the C—O stretching vibration of O-acetyl groups (Fig. 1A, B) and the *E. arborea* fucoidan did not have. The sulphate—total sugars (SO$_4$/CHOH) ratio was 0.27 and 0.26 for the fucoidans from *E. arborea* and *S. horridum*, respectively.

Table 1. The infrared spectroscopy (IR) spectrum data of crude fucoidan and alginate from *Eisenia arborea* (EA) and *Sargassum horridum* (SH).

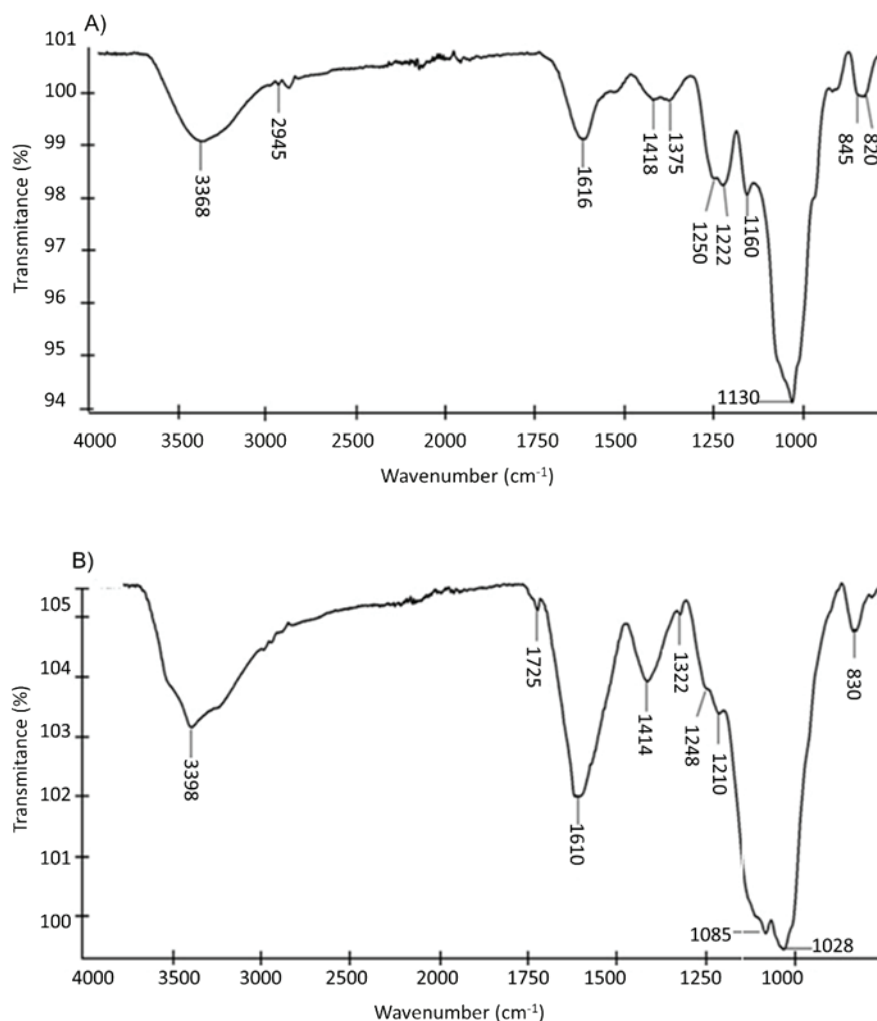| Fucoidan		Alginate		
EA	SH	EA	SH	Characteristic Peaks
Wavenumber (cm^{-1})				
3200–3400	3200–3400	3200–3400	3200–3400	O—H stretch vibration
2945	2941	2937	2937	C—H stretch vibration
N/D	1725	N/D	N/D	C—O stretching vibration of O-acetyl groups
1616	1610	1603	1606	C= O asymmetric and symmetric oscillation of carboxyl group
1418	1414	1404	1412	
1250	1248	*	*	S—O stretching vibration of sulfate
1130	1128	1128	1125	The pyranose ring and glycosidic bond C—O stretching vibrations (uronic acids)
ND	ND	ND	947	C—O stretching of uronic acid residues
850–840	ND	ND	ND	C–O–S secondary axial sulfate at C-4 of the fucopyranosyl residue
ND	ND	813–870	811–880	C1—H deformation vibration of β-mannuronic acid residues
830–20	830	ND	ND	C–O–S secondary equatorial sulfate at C-2 and C-3 position
ND = Not detected, * = Not assigned				

Figure 1. Infrared spectrum (FT-IR ATR) of crude fucoidan from (A) *Eisenia arborea* and (B) *Sargassum horridum.*

The alginate infrared spectrum from *E. arborea* and *S. horridum* showed the typical absorption bands at 811 and 880 cm^{-1} (C1—H deformation vibration of β-mannuronic acid residues) (Fig. 2A, B). However, the *S. horridum* alginate showed C—O stretching of uronic acid residues evidenced by the absorption band at 947 cm^{-1} (Fig. 2B) that the *E. arborea* alginate did not have.

Germination of mung bean seeds under *in vitro* **conditions.** The fucoidan and alginate from *E. arborea* and *S. horridum* promoted the germination of mung bean seeds at all doses up to 96 to 100% (Figure 3A, B). The seeds treated with fucoidan, and alginate germinated at 24 h, whereas the control seeds germinated 48 h later.

Effect of crude fucoidan and alginate on the growth of mung bean seedlings. A significant difference ($p \leq 0.05$) was observed in the growth of mung bean plants treated with crude fucoidans compared to the growth of control plants. *E. arborea* (EA) fucoidan resulted in an increase in shoot length and total length of 10.5 cm and 20.2 cm respectively, and the greater increase in dry weight of seedling (281.3 mg)

with the concentration of 2.5 mg mL^{-1} (Figure 4A, C and Table 2). Greater increases in shoot length (10.6 cm) and dry weight (313.7 mg) were found in plants treated with the *S. horridum* (SH) fucoidan at 20 mg mL^{-1} (Figure 4A, D). In addition, the SH-fucoidan at 0.6mg mL^{-1} showed a significantly higher effect ($p \leq 0.05$) on mung bean root length and total length with an increase of 10.4 cm and 20.6 cm, respectively in comparison to control (9.0 cm and 18.4 cm) (Figure 4B, C and Table 2).

The majority of the doses of alginate did not show beneficial effect in growth compared to those of the control plants. EA-alginate at 1.2 mg mL^{-1} showed significative effect ($p \leq 0.05$) on root length and total length (10.1 cm and 20.6 cm, respectively) respect to control plants (9.2 cm and 18.9 cm, respectively) of mung bean (Figure 5B and C). SH-alginate did not show differences significative in the shoot, root, and total length with respect to control plants (Figure 5A-C and Table 3).

The use of alginates had a negative effect on the dry weights of the mung bean plants (Figure 5D and Table 3).

DISCUSSION

The use of seed priming as a plant growth bio-stimulant method has been shown to be suitable for plant development. Various compounds, such as phytohormones, sugars, and micronutrients, have been used as priming substances for seeds (Hasanuzzaman & Fotopoulos, 2019). However, there are no records of the use of algal polysaccharides as seed priming. This study is the first to report the use of fucoidans and alginates as priming compounds for seeds.

This study showed that the crude fucoidans obtained from *S. horridum* and *E. arborea* act as bio-stimulants for seed germination and seedling growth.
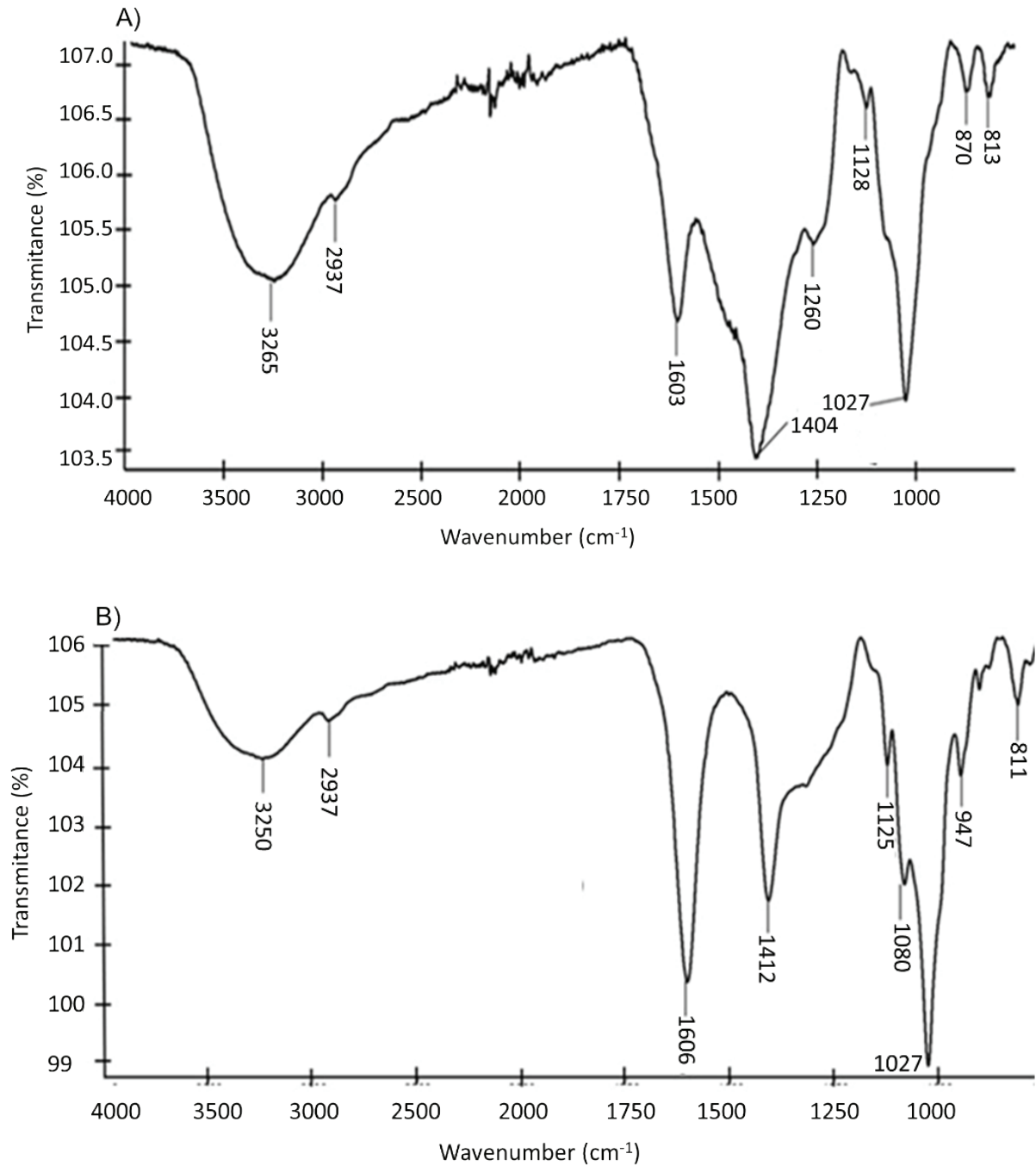

Figure 2. Infrared spectrum (FT-IR ATR) of alginate from (A) *Eisenia arborea* and (B) *Sargassum horridum.*

Table 2. Mung bean growth using different doses of crude fucoidan from *Eisenia arborea* (EA) and *Sargassum horridum* (SH).

Doses (mg mL⁻¹)	Shoot length (cm)	Root length (cm)	Total length (cm)	Dry weight (mg)
Control	9.4 ± 1.4a	9.0 ± 2.2a	18.4 ± 2.9a	239.4 ± 32.5c
EA 0.6	10.3 ± 1.7a	9.5 ± 2.1a	19.8 ± 2.9a	237.4 ± 17.6c
EA 1.2	10.1 ± 1.8a	8.9 ± 2.5a	19.0 ± 3.5a	235.9 ± 9.2b
EA 2.5	10.5 ± 1.5b	9.8 ± 3.2a	20.2 ± 3.8b	281.3 ± 25.9e
EA 5.0	9.1 ± 1.6a	8.6 ± 2.1a	17.7 ± 2.9a	224.8 ± 27.0a
EA 10	10.4 ± 2.2b	10.0 ± 2.5a	20.4 ± 3.7b	241.8 ± 16.1c
EA 20	10.2 ± 1.9a	10.1 ± 2.9a	20.2 ± 3.3b	266.6 ± 32.7d
Control	9.4 ± 1.4a	9.0 ± 2.2a	18.4 ± 2.9a	239.4 ± 32.5c
SH 0.6	10.2 ± 1.7a	10.4 ± 2.8b	20.6 ± 3.8b	232.5 ± 12.3b
SH 1.2	9.9 ± 1.5a	8.9 ± 3.6a	19.8 ± 4.4a	257.8 ± 43.9d
SH 2.5	10.1 ± 1.3a	10.0 ± 2.9a	20.0 ± 3.6a	295.3 ± 8.6e
SH 5.0	9.5 ± 1.7a	9.2 ± 2.3a	18.7 ± 3.2a	230.0 ± 5.9b
SH 10	9.6 ± 1.6a	10.2 ± 2.3a	19.8 ± 3.1a	219.9 ± 15.7a
SH 20	10.6 ± 1.8b	9.0 ± 2.0a	19.6 ± 3.0a	313.7 ± 25.1f

Different letters (a–f) indicate significant differences between the concentrations from each treatment of crude fucoidan EA or SH according to the Least Significant Difference (LSD) mean comparison test (p ≤ 0.05). Values represent the mean of n = 30 plants; ± standard errors.

Table 3. Mung bean growth using different doses of alginate from *Eisenia arborea* (EA) and *Sargassum horridum* (SH).

Doses (mg mL⁻¹)	Shoot length (cm)	Root length (cm)	Total length (cm)	Dry weight (mg)
Control	9.7 ± 1.9a	9.2 ± 2.3a	18.9 ± 3.0a	256.4 ± 29.2e
EA 0.6	10.0 ± 1.7a	9.7 ± 2.0a	19.5 ± 3.0b	209.1 ± 15.0a
EA 1.2	10.5 ± 1.6a	10.1 ± 2.1b	20.6 ± 2.5b	218.7 ± 13.9b
EA 2.5	10.3 ± 1.6a	9.8 ± 2.2a	20.1 ± 3.2b	234.5 ± 9.20c
EA 5.0	9.9 ± 1.7a	9.3 ± 2.1a	19.2 ± 2.7a	250.4 ± 21.6d
EA 10	9.5 ± 1.3a	9.0 ± 1.6a	18.5 ± 2.5a	216.6 ± 26.6b
EA 20	9.6 ± 1.1a	9.6 ± 1.1a	19.1 ± 2.4a	242.6 ± 36.5c
Control	9.7 ± 1.9a	9.2 ± 2.3a	18.9 ± 3.0a	256.4 ± 29.2d
SH 0.6	10.2 ± 1.3a	9.2 ± 2.2a	19.4 ± 2.7a	226.2 ± 17.6b
SH 1.2	9.7 ± 1.5a	9.3 ± 2.4a	19.0 ± 3.1a	220.5 ± 9.20a
SH 2.5	9.5 ± 1.8a	9.1 ± 2.8a	18.6 ± 3.9a	229.0 ± 25.9b
SH 5.0	9.2 ± 2.1a	9.3 ± 2.9a	18.6 ± 4.1a	225.0 ± 27.0b
SH 10	9.9 ± 1.5a	9.3 ± 2.5a	19.2 ± 3.1a	225.8 ± 16.1b
SH 20	9.7 ± 1.5a	9.3 ± 2.7a	19.0 ± 3.3a	237.4 ± 32.7c

Different letters (a-d) indicate significant differences between the concentrations from each treatment of alginate EA or SH according to the Least Significant Difference (LSD) mean comparison test (p ≤ 0.05). Values represent the mean of n = 30 plants; ± standard errors.

It is widely approved that the use of polysaccharide-enriched extracts (PPEs) showed contradictory effects on seed germination mainly with a dose dependent response (Hernández-Herrera et al. 2016; Castellanos-Barriga et al. 2017). In the present study, the presence of bioactive compounds in PEEs that can stimulate or inhibit seed germination may explain this finding.

In another study, seed treated with alginate and ulvan-derived oligosaccharides resulted from *Ulva lactuca* Linnaeus and *Padina gymnospora* PEE-treatments was proposed as a potential mechanism of increased germination percentage under *in vitro* conditions (Hernández-Herrera et al. 2016). Such oligosaccharide can increase activities of several germination enzymes and accelerate seed metabolic activity (Hu et al. 2004). Water uptake by seeds treated with PEEs was more efficient and can also explain the increased germination percentage over the untreated seeds mainly in the first 3 days.

The use of *S. horridum* fucoidan resulted in greater benefits for root and overall growth at a concentration of 0.6 mg mL⁻¹, while higher dry weight was obtained with a concentration of 20 mg mL⁻¹. This result suggests that the use of crude fucoidan as a biostimulant is a viable option to improve plant growth. However, the alginates in this study did not show bio-stimulant activity on mung bean growth.

The variation in bio-stimulant activity reflected in growth depended on the polysaccharide concentration. These effects have also been demonstrated in tomato plants with the use of polysaccharide-enriched extracts (PPEs) from *Ulva lactuca* and *Padina gymnospora* in neutral and alkaline conditions (Hernández-Herrera *et al.*, 2016).

Recently Zou *et al.* (2021) reported the use of fucoidans from the brown alga *Macrocystis pyrifera* as growth promoter for wheat plants. Therefore, this is the first study that has explored the use of crude fucoidans with this purpose from *Eisenia arborea* and *Sargassum horridum*. The results of this work have shown that fucoidans have the potential growth stimulation activity.

The mung bean seeds treated with polysaccharides were able to maintain their humidity for a longer period compared to control plants. In addition, when the mung bean root emerges, it maintains the growth and development availability, because of the polysaccharide effect. In the case of the mung bean plants treated with SH-fucoidan, shoot length showed the greatest growth with the maximum fucoidan concentration (20 mg mL⁻¹). However, mung bean root length and total plant length showed maximum values with the lower fucoidan concentration of 0.6 mg mL⁻¹. Whereas the greatest growth stimulation activity of EA-fucoidan was observed in both shoot length and total plant length, at the concentration of 2.5 and 10 mg mL⁻¹, respectively. However, these values were lower compared to those of *S. horridum*. Although both extracts belong to the same family of fucoidans, variations in chemical structure or molecular weight can result in different activities (Lim *et al.*, 2014; Zhao *et al.*, 2018;; Fitton *et al.*, 2019; Koh *et al.*, 2019).

The plant growth stimulation activity observed with fucoidans in this study may be because fucoidans are sulphated polysaccharides and alginates are not. The ability of fucoidans to act as plant growth promoters can be explained because the sulphate groups (Rachidi *et al.*, 2020). The sulphate groups present in PPEs have shown positive correlations with shoot and root length, dry weight, and chlorophyll content in tomato plants (Mzibra *et al.*, 2018).
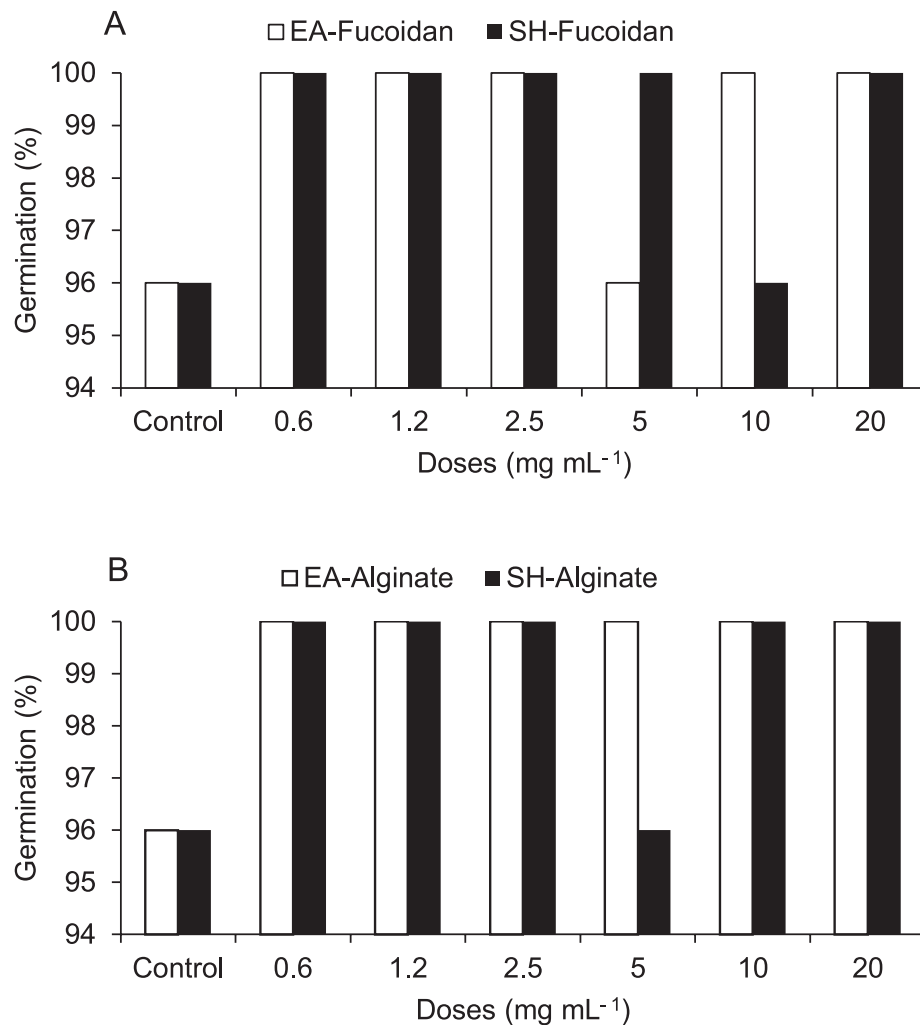
Figure 3. Effect of (A) crude fucoidan and (B) alginates from *Eisenia arborea* (EA) and *Sargassum horridum* (SH) at different doses on the germination percentage.

The low growth stimulation activity of alginates may be the result of their chemical structure or that activity occurs only when their oligosaccharides are available. It has been shown that hydrolysed alginate (mono and oligosaccharides) increases bio-stimulant activity, which is reflected in plant growth (Iwasaki & Matsubara, 2000; Idrees *et al*., 2011; González *et al*., 2013). The sodium alginate oligosaccharides from *Lessonia trabeculata* Villouta & Santelices and *L. vadosa* Searles (= L. flavicans Bory), have been found to result in length and weight increases in tobacco plants (Laporte *et al*., 2007), and the same has been found with alginates from *L. vadose* (= *L. flavicans*), in wheat plants (Chandía *et al*., 2004).

In a previous study, it was observed that the application of *S. horridum* liquid extract on mung bean seeds had a negative effect (Di Filippo-Herrera *et al*., 2019). However, in this study it was found that applying only fucoidan has a positive effect on the growth of both seeds and seedlings. Moreover, both the liquid extract from *E. arborea* (Di

Filippo-Herrera *et al*., 2019) and the fucoidan obtained in this study showed higher activities at a higher concentration, especially regarding promoting root length (21 and 10.1%) and total length (27 and 20.2%), respectively.

The best result of the polysaccharide pre-treatment on mung bean seeds was observed in dry weight. Similar effects have been previously reported with the lengths of tomato plants treated with PPEs from the brown seaweed *Padina gymnospora* (Hernández-Herrera *et al*., 2016) and with sodium alginate-treated lettuce (*Lactuca sativa*) (Iwasaki and Matsubara, 2000). Mzibra *et al*. (2018) showed that the PPEs from the brown seaweeds *Fucus spiralis* and *Bifurcaria bifurcata* resulted in significant differences in tomato plant length (growth of 338 and 337 mm, respectively) compared to the growth of control plants. Similar results were obtained regarding dry root weight with the PPE from *F. spiralis* (0.26 g) and in dry shoot weight with the PPE from *B. bifurcate* (0.81 g), both of which were significantly different from those of the control.
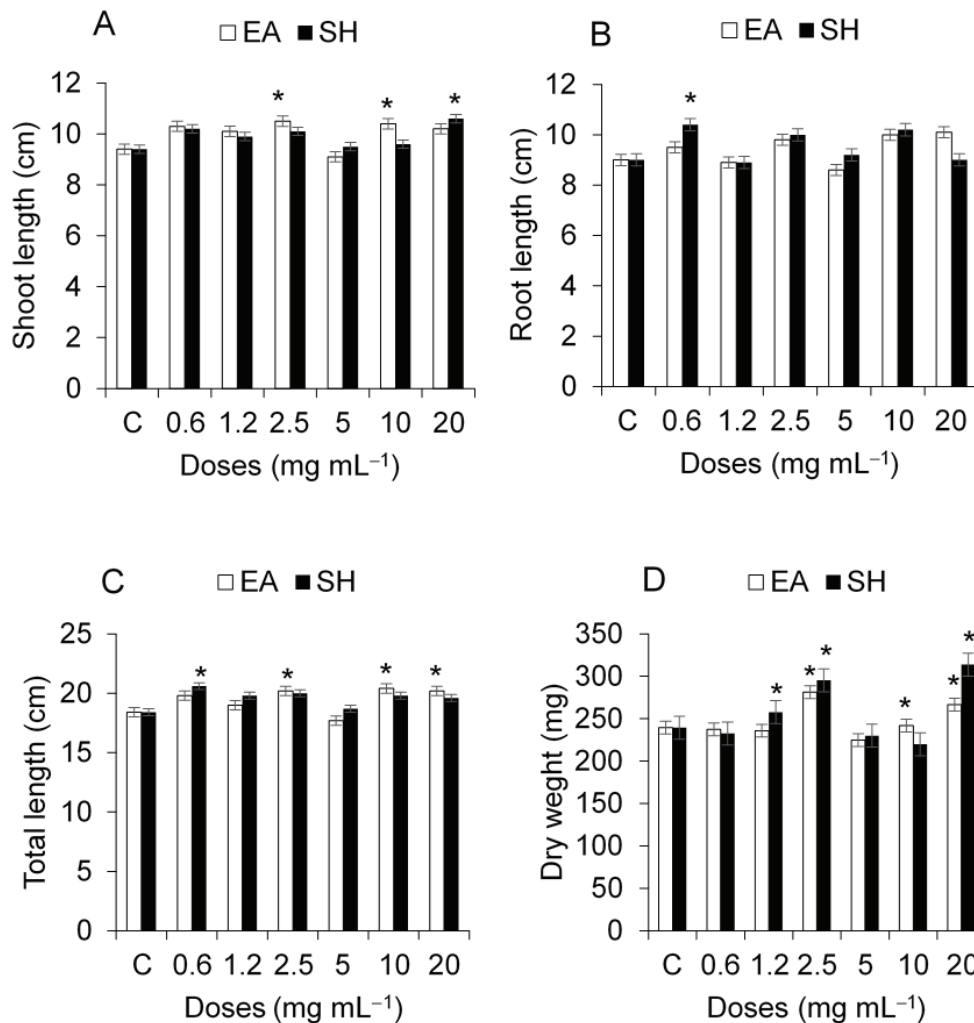
Figure 4. Effect of crude fucoidan from *Eisenia arborea* (EA) and *Sargassum horridum* (SH) at different doses on (A) shoot, (B) root, (C) total length and (D) Dry weight of mung bean. Columns denoted by asterisk are significantly different to control. Values represent the mean of n = 30 plants; bars represent standard errors.

ACKNOWLEDGEMENTS

REFERENCES

Ali, O., A. Ramsubhag & J. Jayaraman. 2019. Biostimulatory activities of *Ascophyllum nodosum* extract in tomato and sweet pepper crops in a tropical environmental. *PLoS ONE* 14(5): e0216710. DOI: 10.1371/journal.pone.0216710

AOSA (Association of Official Seed Analysts). 2005. *In*: A. Capashew (ed.). *Rules for testing seeds*. 4-10, 4-11. Las Cruces, NM. (Seventh Edition 2008).

Battacharyya, D., M. Z. Babgohari, P. Rathor & B. Prithiviraj. 2015. Seaweed extracts as biostimulants in horticulture. *Scientia Horticulturae* 196: 39-48. DOI: 10.1016/j.scienta.2015.09.012

Bouissil, S., Z. E. Alaoui-Talibi, G. Pierre, H. Rchid, P. Michaud, C. Delattre & C. El Modafar. 2020. Fucoidans of Moroccan brown seaweed as elicitors of natural defenses in date palm roots. *Marine Drugs* 18(12): 596. DOI: 10.3390/md18120596

Camacho, O. & G. Hernández-Carmona. 2012. Phenology and alginates of two *Sargassum* species from the Caribbean coast of Colombia. *Ciencias Marinas* 38(2): 381-393. DOI: 10.7773/cm.v38i2.1963

Castellanos-Barriga, L. G., F. Santacruz-Ruvalcaba, G. Hernández-Carmona, E. Ramírez-Briones & R. M. Hernández-Herrera. 2017. Effect of seaweed
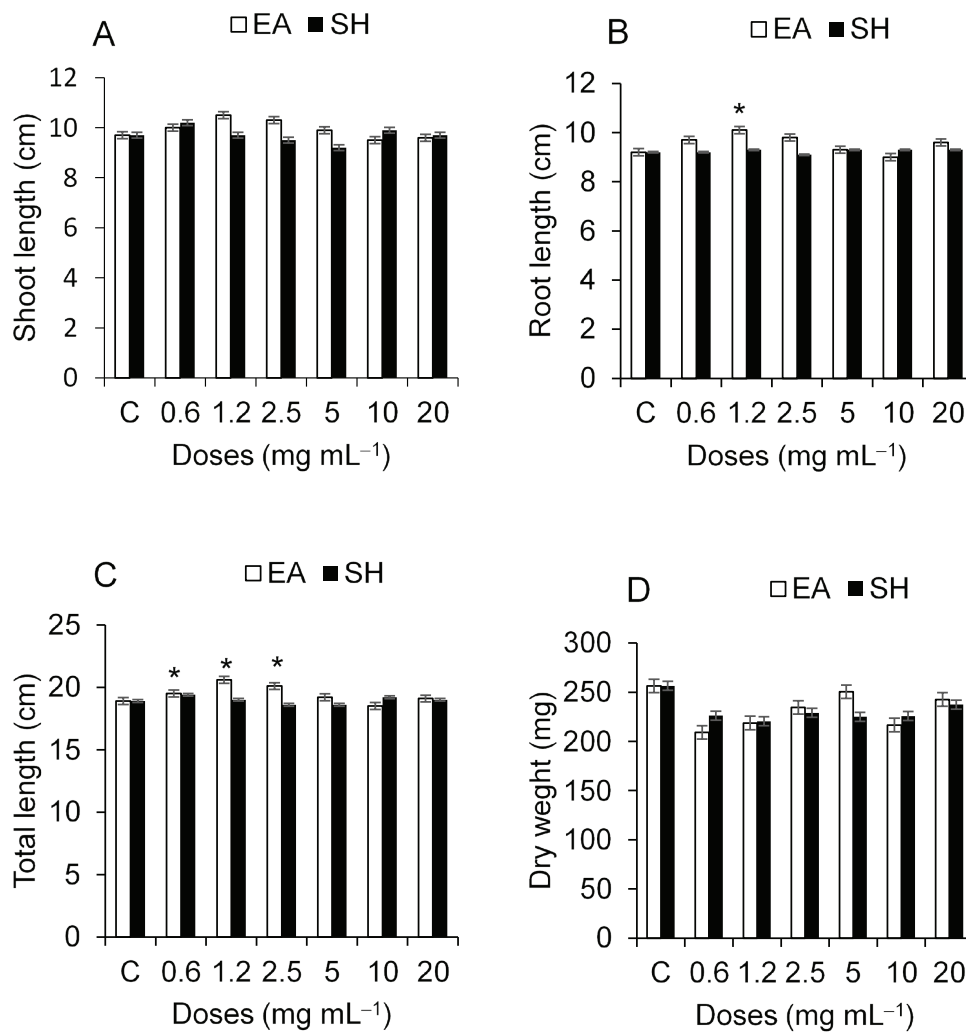
Figure 5. Effect of alginate from *Eisenia arborea* (EA) and *Sargassum horridum* (SH) at different doses on (A) shoot, (B) root, (C) total length and (D) Dry weight of mung bean. Columns denoted by asterisk are significantly different to control. Values represent the mean of n = 30 plants; bars represent standard errors.

extracts from *Ulva lactuca* on seedling growth of mung bean (*Vigna radiata*). *Journal of Applied Phycology* 29: 2479-2488. DOI: 10.1007/s10811-017-1082-x

Chandía, N. P., B. Matsuhiro, E. Mejías & A. Moenne. 2004. Alginic acids in *Lessonia vadosa*: Partial hydrolysis and elicitor properties of the polymannuronic acid fraction. *Journal of Applied Phycology* 16(2): 127-133. DOI: 10.1023/B:JAPH.0000044778.44193.a8

Di Filippo-Herrera, D., M. Muñoz-Ochoa, R. M. Hernández-Herrera & G. Hernández-Carmona. 2019. Biostimulant activity of individual and blended seaweed extracts on the germination and growth of the mung bean. *Journal of Applied Phycology* 31(3): 2025-2037. DOI: 10.1007/s10811-018-1680-2

Drobek, M., M. Frąc & J. Cybulska. 2019. Plant biostimulants: Importance of the quality and yield of horticultural crops and the improvement of plant tolerance to abiotic stress–A review. *Agronomy* 9(6): 335. DOI: 10.3390/agronomy9060335

EL Boukhari, M. E. M., M. Barakate, Y. Bouhia & K. Lyamlouli. 2020. Trends in seaweed extract based biostimulants: manufacturing process and beneficial effect on soil-plant systems. *Plants* 9(3): 359. DOI: 10.3390/plants9030359

Ertani, A., O. Francioso, A. Tinti, M. Shiavon, D. Pizzeghello & S. Nardi. 2018. Evaluation of seaweed extracts from *Laminaria* and *Ascophyllum nodosum* spp. as biostimulants in *Zea mays* L. using a combination of chemical, biochemical and morphological approaches. *Frontiers in Plant Science* 9: 428. DOI: 10.3389/fpls.2018.00428

Falcón, A. B. & J. C. Cabrera. 2007. Actividad enraizadora de una mezcla de oligogaracturónidos en pecíolos de violeta africana (*Saintpaulia ionantha*). *Cultivos Tropicales* 28(2): 87-90.

Fitton, H. J., D. S. Stringer, A. Y. Park & S. N. Karpiniec. 2019. Review. Therapies from fucoidan: new developments. *Marine Drugs* 17(10): 571. DOI: 10.3390/md17100571

González, A., J. Castro, J. Vera & A. Moenne. 2013. Seaweed oligosaccharides stimulate plant growth by enhancing carbon and nitrogen assimilation, basal metabolism, and cell division. *Journal of Plant Growth Regulation* 32: 443-448. DOI: 10.1007/s00344-012-9309-1

Hasanuzzaman, M. & V. Fotopoulos. 2019. *Priming and pretreatment of seeds and seedlings: Implication in plant stress tolerance and enhancing productivity in crop plants.* Springer, Singapore. 604 p.

Hernández-Herrera, R. M., F. Santacruz-Ruvalcaba, J. Zañudo-Hernández & G. Hernández-Carmona. 2016. Activity of seaweed extracts and polysaccharide-enriched extracts form *Ulva lactuca* and *Padina gymnospora* as growth promoters of tomato and mung bean plants. *Journal of Applied Phycology* 28(4): 2549-2560. DOI: 10.1007/s10811-015-0781-4

Hien, N. Q., N. Nagasawa, L. X. Tham, F. Yoshii, V. H. Dang, H. Mitomo, K. Makuuchi & T. Kume. 2000. Growth promotion of plants with depolymerized alginates by irradiation. *Radiation Physics and Chemistry* 59(1): 97–101. DOI: 10.1016/S0969-806X(99)00522-8

Hong, D. D., H. M. Hien & P. N. Son. 2007. Seaweeds from Vietnam used for functional food, medicine and biofertilizer. *Journal of Applied Phycology* 19(6): 817-826. DOI: 10.1007/s10811-007-9213-4

Hu, X., X. Jiang, H Hwang, S. Liu & H. Guan. 2004. Promotive effect of alginate-derived oligosaccharides on maize. Promoting effects of alginate-derived oligosaccharides on maize seeds germination. *Journal of Applied Phycology* 16:73-76.

Idrees, M., M. Naeem, M. Alam, T. Aftab, N. Hashmi, M. M. A. Khan, Moinuddin & L. Varshney. 2011. Utilizing the γ-irradiated sodium alginate as a plant growth promoter for enhancing the growth, physiological activities, and alkaloids production in *Catharanthus roseus* L. *Agricultural Sciences in China* 10(8): 1213-1221. DOI: 10.1016/S1671-2927(11)60112-0

Iwasaki, K. & Y. Matsubara. 2000. Purification of alginate oligosaccharides with root growth-promoting activity toward lettuce. 2000. *Bioscience, Biotechnology, and Biochemestry* 64(5): 1067-1070. DOI: 10.1271/bbb.64.1067

Khan, W., U. P. Rayirath, S. Subramanian, M. N. Jithesh, P. Rayorath, D. M. Hodges, A. T. Critchley, J. S. Craigie, J. Norrie & B. Prithiviraj. 2009. Seaweed extracts as biostimulants of plant growth and development. *Journal of Plant Growth Regulation* 28(4): 386-399. DOI: 10.1007/s00344-009-9103-x

Klarzynski, O., V. Descamps, B. Plesse, J. C. Yvin, M. Kopp, B. Kloareg & B. Fritig. 2003. Sulfated fucan oligosaccharides elicit defense responses in tobacco and local and systemic resistance against tobacco mosaic virus. *Molecular Plant-Microbe Interactions* 16(2): 115-122. DOI: 10.1094/MPMI.2003.16.2.115

Koh, H. S. A., J. Lu & W. Zhou. 2019. Structure characterization and antioxidant activity of fucoidan isolated from *Undaria pinnatifida* grown in New Zealand. *Carbohydrate Polymers* 212: 178-185. DOI: 10.1016/j.carbpol.2019.02.040

Laporte, D., J. Vera, N. P. Chandía, E. A. Zúñiga, B. Matsuhiro & A. Moenne. 2007. Structurally unrelated algal oligosaccharides differentially stimulate growth and defense against tobacco mosaic virus in tobacco plants. *Journal of Applied Phycology* 19(1): 79-88. DOI: 10.1007/s10811-006-9114-y

Lapshina, L. A., A. V. Reunov, V. P. Nagorskaya, T. N. Zvyagintseva & N. M. Shevchenko. 2006. Inhibitory effect of fucoidan from brown alga *Fucus evanescens* on the spread of infection induced by tobacco mosaic virus in tobacco leaves of two cultivars. *Russian Journal of Plant Physiology* 53(2): 246-251. DOI: 10.1134/S1021443706020154

Lim, S. J., W. M. W. Aida, M. Y. Maskat, S. Mamot, J. Ropien & D. M. Mohd. 2014. Isolation and antioxidant capacity of fucoidan from selected Malaysian seaweeds. *Food Hydrocolloids* 42(Part 2): 280-288. DOI: 10.1016/j.foodhyd.2014.03.007

Lijour, Y., E. Gentric, E. Deslandes & J. Guezennec. 1994. Estimation of the sulfate content of hydrothermal vent bacterial polysaccharides by Fourier transform infrared spectroscopy. *Analytical Biochemistry* 220(2): 244-248. DOI: 10.1006/abio.1994.1334

Mukherjee, A. & J. S. Patel. 2020. Seaweed extract: biostimulator of plant defense and plant productivity. *International Journal of Environmental Science and Technology* 17(1): 553-558. DOI: 10.1007/s13762-019-02442-z

Muñoz-Ochoa. M., J. I. Murillo-Álvarez, Y. E. Rodríguez-Montesinos, G. Hernández-Carmona, D. L. Arvizu-Higuera, J. Peralta-Cruz & J. Lizardi-Mendoza. 2009. Anticoagulant screening of marine algae from México, and partial characterization of the active sulphated polysaccharide from *Eisenia arborea*. *CICIMAR Oceánides* 24(1): 41-51. DOI: 10.37543/oceanides.v24i1.52

Mzibra, A., A. Aasfar, H. El Arroussi, M. Khouloud, D. Dhiba, I. Meftah Kadmiri & A. Bamouh. 2018. Polysaccharides extracted from Moroccan seaweed: a promising source of tomato plant growth promoters. *Journal of Applied Phycology* 30(5): 2953-2962. DOI: 10.1007/s10811-018-1421-6

Rachidi, F., R. Benhima, L. Sbabou & H. El Arroussi. 2020. Microalgae polysaccharides bio-stimulating effect on tomato plants: Growth and metabolic distribution. *Biotechnology Reports* 25: e00426. DOI: 10.1016/j.btre.2020.e00426

Rengasamy, K. R. R., M. G. Kulkarni, W.A. Stirk & J. Van Staden. 2015. Eckol - a new plant growth stimulant from the brown seaweed *Ecklonia maxima*. *Journal of Applied Phycology* 27(1): 581-587. DOI: 10.1007/s10811-014-0337-z

Rodríguez-Montesinos, Y. E., D. L. Arvizu-Higuera & G. Hernández-Carmona. 2008. Seasonal variation on size and chemical constituents of *Sargassum sinicola* Setchell et Gardner from Bahía de La Paz, Baja California Sur, Mexico. *Phycological Research* 56(1): 33-38. DOI: 10.1111/j.1440-1835.2008.00482.x

Rolland, F., B. Moore & J. Sheen. 2002. Sugar sensing and signalling in plants. *Plant Cell* 14(Supp. 1): 185-205. DOI: 10.1105/tpc.010455

Salachna, P., M. Grzeszczuk, E. Meller & M. Soból. 2018. Oligo-alginate with low molecular mass improves growth and physiological activity of *Eucomis autumnalis* under salinity stress. *Molecules* 23(4): 812. DOI: 10.3390/molecules23040812

Sarfaraz, A., M. Naeem, S. Nasir, M. Idrees, T. Aftab, N. Hashmi, M. M. A. Khan, Moinuddin & L. Varshney. 2011. An evaluation of the effects of irradiated sodium alginate on the growth, physiological activities and essential oil production of fennel (*Foeniculum vulgare* Mill.). *Journal of Medicinal Plants Research* 5(1): 15-21. DOI: 10.5897/JMPR.9000071

Sharma, S. H. S, G. Lyons, C. McRoberts, D. McCall, E. Carmichael, F. Andrews, R. Swan, R. McCormack & R. Mellon. 2012. Bio-stimulant activity of brown seaweeds species from Strangford Lough: compositional analyses of polysaccharides and bioassay of extracts using mung bean (*Vigno mungo* L.) and pak choi (*Brassica rapa chinensis* L.). *Journal of Applied Phycology* 24(5): 1081-1091. DOI: 10.1007/s10811-011-9737-5

Van Oosten, M. J., O. Pepe, S. De Pascale, S. Silletti & A. Maggio. 2017. The role of biostimulants and bioeffector as alleviators of abiotic stress in crop plants. *Chemical and Biological Technologies in Agriculture* 4:5. DOI: 10.1186/s40538-017-0089-5

Yabur, R., Y. Bashan & G. Hernández-Carmona. 2007. Alginate from the macroalgae *Sargassum sinicola* as a novel source for microbial immobilization material in wastewater treatment and plant growth promotion. *Journal of Applied Phycology* 19(1): 43-53. DOI: 10.1007/s10811-006-9109-8

Zamani, S., S. Khorasaninejad & B. Kashefi. 2013. The importance role of seaweeds of some characters of plant. *International Journal of Agriculture and Crop* Sciences 5(16): 1789-1793.

Zhao, Y., Y. Zheng, J. Wang, S. Ma, Y. Yu, W. L. White, S. Yang, F. Yang & J. Lu. 2018. Fucoidan extracted from *Undaria pinnatifida*: Source for nutraceuticals/functional foods. *Marine Drugs* 16(9): 321. DOI: 10.3390/md16090321

Zou, P., X. Yang, Y. Yuan, C. Jing, J. Cao, Y. Wang, Y. L. Zhang, C. Zhang & Y. Li. 2021. Purification and characterization of a fucoidan from the brown algae *Macrocystis pyrifera* and the activity of enhancing salt-stress tolerance of wheat seedlings. *International Journal of Biological Macromolecules* 180: 547-558. DOI: 10.1016/j.ijbiomac.2021.03.039