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Effect of Seaweed Liquid Extracts on the Internode Variation of *Lens esculenta* Seedlings

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Abstract: Seaweeds are important sustainable resources by their industrial potential; particularly, because their extracts contain polysaccharides, proteins, polyphenols, nutrient elements and important plant growth hormones or regulators, that make them as an adequate substitutes of biofertilizers and they are actually named biostimulants. Even there are some limitations regarding to their extraction methods; the boiling and soaking extraction method with distilled water is greatly recommended. The aim of this study was to evaluate the effect of two seaweed liquid extracts as growth promoters through the internode variation of *Lens esculenta* seedlings.There was a particular response in shoot elongation of *L. esculenta* seedlings regarding to their internode length measured; where the promotion of shoot growth was evident at 5% of the liquid extracts concentration from both seaweeds species; but the results obtained from *U. fasciata* liquid extracts at 5 and 10% concentrations showed a better growth and appearance of seedlings with a diminish in the internode length and chlorosis at 20% concentration of the liquid extract; showing that the application of low concentrations could be added to plant cultures as a source of growth regulators, can increase the response of plants; particularly, with the application of *Ulva fasciata* extracts.

Keywords: Liquid Seaweed Extracts, Biostimulants, *Lens esculenta*

INTRODUCTION

According to Sridhar and Rengasamy (2011); Wijesinghe and Jeon (2012) and Godlewska, et al. (2016), seaweeds are considered one of the most important sustainable resources because they possess an industrial potential. Particularly, the extracts derived from them contain polysaccharides, proteins, pigments, a variety of polyphenols, nutrient elements and important plant growth hormones (Chojnacka, et al. 2012; Godlewska, et al. 2016). Actually, they are employed as fertilizers in place of conventional synthetic fertilizers; because their management as liquid fertilizers considers them as biostimulants since they contain many growth regulators (Wildgoose, et al. 1978; Crouch and van Staden 1993; Durand, et al. 2003; Stirk, et al. 2003, 2004; Sivasankari, et al. 2006; Hong, et al. 2007; Khan, et al. 2009; Rathore, et al. 2009). It is known by some authors that the wide range of growth responses induced by seaweed extracts implies seeds germination or vegetative growth and flowering (Tay, et al. 1985; Crouch and Van Staden 1993; Moore 2004; Hong, et al. 2007; Khan, et al. 2009). As Godlewska, et al. (2016) noted there are some limitations regarding to the conventional extraction methods to obtain seaweeds extracts and they recommend the boiling and soaking extraction method with distilled water as environment friendly process that do not require organic solvents. The aim of this study was to evaluate the effect of two seaweed liquid extracts as growth promoters through the internode variation of *Lens esculenta* seedlings.

MATERIALS AND METHODS

Preparation of seaweeds liquid extract

For this study, the seaweeds *Sargassum vulgare* and *Ulva fasciata* were hand collected from the intertidal zone at one meter of deep, in “El pulpo beach” located in Barra de Cazones, from Cazones de Herrera municipality in Veracruz, México, (20° 43’ 33.98” N and 97° 11’ 90.72” W), on rainy season (July, 2018). Collected seaweeds were cleaned

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several times with sea water to remove sand, other impurities and epiphytes, then transported to laboratory and again cleaned four times with tap water and finally shade dried. These shade-dried seaweeds were finely chopped and powdered with a Nutribullet®. The aqueous extracts were obtained according to Kumar and Sahoo (2011), Vinoth, et al. (2012) and Godlewska , et al. (2016), as follows: seaweed powders were filter through metallic mesh number 16 (1mm) and 25g of each were deposited in flasks filled with 300mL of distilled water and boiled at 80°C for 45 min in water bath. Then the extracts were allowed to cool at room temperature and filtered through medium pore filter paper. Finally, the filtrates were considered as 100% seaweed extracts according to Bhosle, et al. (1975) and Kumar and Sahoo (2011) and these were stored at 4°C for the bioassays.

***Lens esculenta* with seaweed extracts bioassays**

Commercially seeds of *Lens esculenta* were surface-sterilized with 10% sodium hypochlorite solution for 3 minutes, rinsed with deionized sterile water and placed for 30 minutes in sterile flasks that contained 50mL of the different concentrations of each seaweed liquid extracts (SLE): 5, 10 and 20%, prepared by diluting the concentrate extracts with sterile distilled water.

Five treated *L. esculenta* seeds were placed separately in sterile baby food flasks with Magenta SIGMA caps contained 25mL of mineral medium (0.20 M $NH_4H_2PO_4$, 1.15 M $Ca(NO_3)_2$, 0.4 M $MgSO_4{\cdot}7H_2O$, 1.2 M KNO_3, 1.2×10^{-2} M H_3BO_3, 1.2×10^{-4} M $CuCl_2{\cdot}H_2O$, 2.3×10^{-3} M $ZnCl_2$, 4.4×10^{-4} M $MnCl_2{\cdot}4H_2O$, 6×10^{-6} M $Na_2MoO_4{\cdot}H_2O$, Fe-EDTA (7.1×10^{-3}M $FeSO_4{\cdot}7H_2O$ + 7.2×10^{-3}M EDTA-Na_2), pH = $\pm$ 6.0) with 3g/L of phytagel. All the experiments were performed by quadruplicate for each SLE concentration and maintained in a growth chamber incubated at 28°C with photoperiod of 16 h light /8 h dark with a fluorescent Phillips T8 32 Watts 5000ºK lamp, for 8 days.

After this period, plants were obtained and photographed. The internode length of each plant was measured employing the Motic Images 2000 Ver.1.3 program. All data obtained were analyzed by one-way analysis of variance and the mean differences were compared applying a Tukey-Kramer Method using the statistics program Graph Pad Instat Ver. 2.03.

RESULTS

Internode length of *Lens esculenta* seedlings response to both SLE

Figures 1 and 2 show the *L. esculenta* seedlings appearance after the exposition to the SLE; all of them presented four nodes within variations in their internode lengths and also in the total length of seedlings (data do not show here). Figure 1b shows the internode length variations in the replicates of control seedlings as Figure 3 with the results presented in all the experimental SLE concentrations tested, according to each replicate. Statistical analysis only reported a statistical difference between the internode length of *L. esculenta* seedlings exposed to the liquid extract of *S. vulgare* at 20% ($p < 0.01$) and also between seedlings exposed to the liquid extract of *U. fasciata* at 20% ($p < 0.5$).

DISCUSSION

Comparing the average of the *L. esculenta* internode lengths (Table 1), control plants presented 0.22cm of it; values obtained for seedlings exposed to SLE of both seaweed species showed a particularly response on the internode distance between *S. vulgare* and *U. fasciata* at 5% of their concentration: 0.27 and 0.26 cm, respectively. Between the SLE concentrations tested of *S. vulgare* the *L. esculenta* seedlings response was: 0.27, 0.19 and 0.23, for 5, 10 and 20%, respectively. For *U. fasciata* extracts, the response was: 0.26, 0.24 and 0.18, for 5. 10 and 20%, respectively; where the internode length decreased as the extract concentration increased. Even these values of internode length were higher than the values of control seedlings, it is important to note that they only showed a good appearance at S5%, U5% and U10% SLE concentrations; with green color and less chlorosis compared to the control. There are some reports like Kumar and Sahoo (2011) noted that the application of 20% SLE from *Sargassum wightii* enhanced the shoot length and number of branches of *Triticum aestivum* var. Pusa Gold and beyond this concentration, these variables decreased significantly. Also there are reports of the effect of this LSE concentration from this seaweed on the fresh and dry weigth of *Abelemoschus esculentus* (Jothinayagi and Anbazhagan 2009). Vinoth, et al. (2012) report the effect of extracts from *Gracilaria edulis* and *Sargassum wightii* in shoot elongation and rooting of elongated shoots of *Lycopersicon esculentum* at concentrations of 30% and 50%, respectively; and noted that they have better results than the application of synthetic hormones to the cultures, particularly, through micropropagation technique. Sridhar and Rengasamy (2010) reported the effect of the 1% LSE from *Ulva lactuca* that significantly increased the shoot length, leaf breadth and leaf length in *Arachis hypogea* plants (Sridhar and Rengasamy 2010). Ahmed and Sehrawy (2013), Parthiban, et al. (2013), Pramanick, et al. (2013) and Vijayanand, et al. (2014), noted that in general, the low concentrations of LSE had positive influence and effect on growth and biochemical characteristics of plants, maybe due to the presence not only of mineral nutrients, where it is important to consider that plant growth regulators and vitamins are also present. Some authors reported

the presence of specific phytohormones in seaweeds as follows: auxins in the extracts of *Ascophyllum nodosum* (Sanderson and Jameson 1986) and cytokinins in the extracts of *Ulva* sp., *Durvillaria potatorum* and *Ascophyllum nodosum* (Sekar, et al. 1995; Craft, et al. 2007). In this study, there was a particular response in shoot elongation of *L. esculenta* seedlings regarding to their internode length measured; where the promotion of shoot growth was evident at 5% of the liquid extracts concentration from both seaweeds species, but the results obtained from *U. fasciata* liquid extracts at 5 and 10% concentrations showed a better growth and appearance of seedlings with a diminish in the internode length and chlorosis of them at 20% concentration of the liquid extract.

CONCLUSIONS

Finally, in this study, the application of low concentrations of seaweed liquid extracts demonstrated that they could be added to plant cultures as a source of growth regulators; increasing the response of plants and considering them as potential biostimulants, particularly, the application of *Ulva fasciata* extracts.

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FIGURES

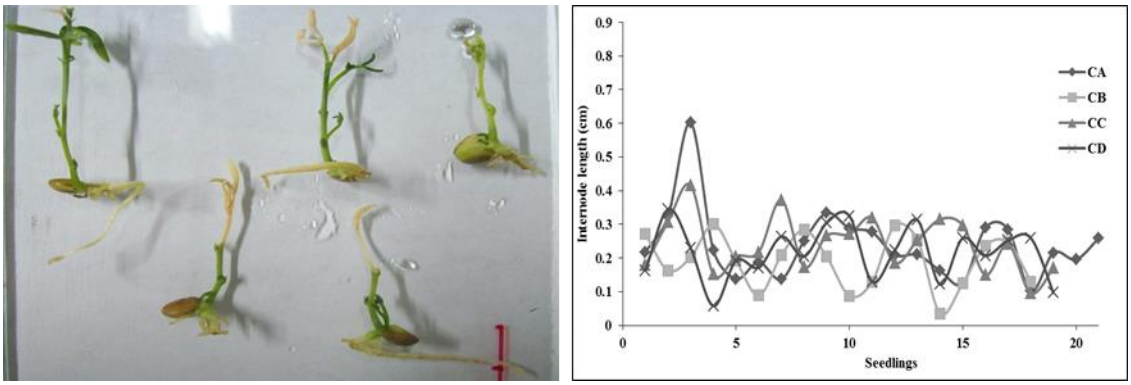

Figure 1: Variations of internode lengths of *Lens esculenta* seedlings with 15 to 23 replicates for control condition.

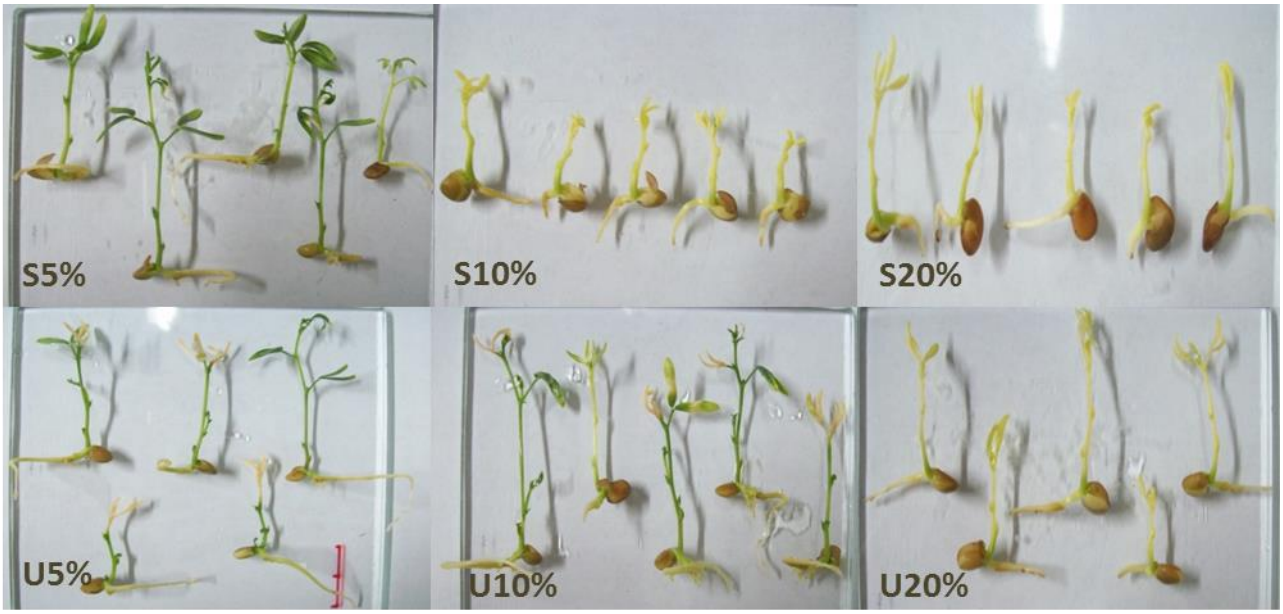

Figure 2: *Lens esculenta* seedlings appearance to seaweed liquid extracts: S= *Sargasum vulgare* and U= *Ulva fasciata*.

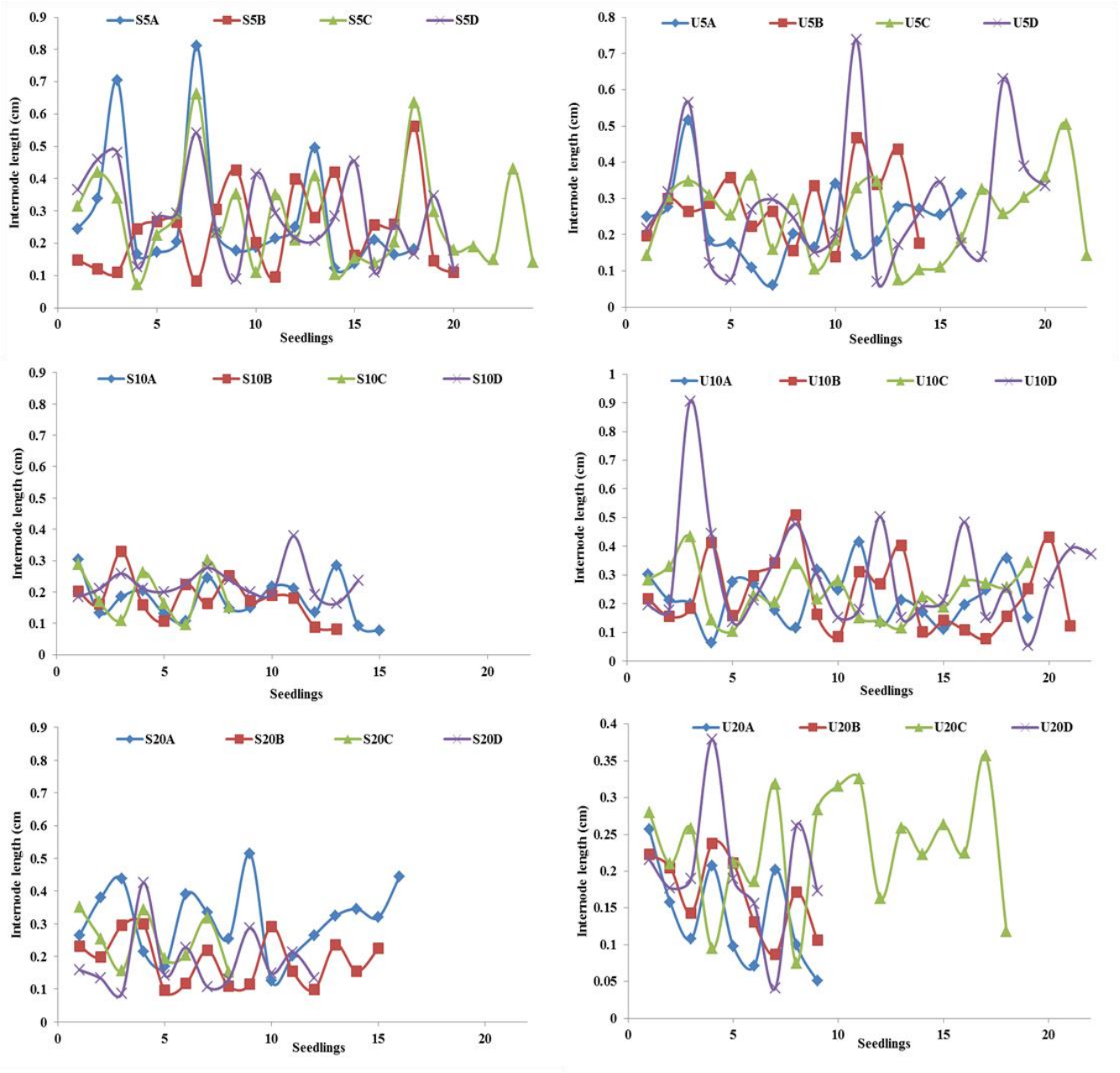

Figure 3: Variations of internode lengths of *Lens esculenta* seedlings with 15 to 23 replicates for each experimental condition.

Table 1. Internode length average of *Lens esculenta* seedlings exposed to SLE from *Sargasum vulgare* and *Ulva fasciata*

	Biosssay treatments						
	Control	**S5%**	**S10%**	**S20%**	**U5%**	**U10%**	**U20%**
Internode length(cm)	**0.22±0.02**	**0.27±0.01***	**0.19±0.02**	**0.23±0.05**	**0.26±0.02**	**0.24±0.03**	**0.18±0.03***

***** Asterisks showed the significant differences between treatments ($p < 0.05$).