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Effects of extracts of two *Ulva* spp. seaweeds on tomato germination and seedling growth

Efeitos dos extratos de duas macroalgas marinhas de *Ulva* spp. na germinação e no crescimento de mudas do tomate

Efectos de extractos de dos macroalgas marinas de *Ulva* spp. na germinación y no crecimiento de plántulas de tomate

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Abstract

Brown seaweed extracts are commercially used as agricultural biostimulants, and the green macroalgae *Ulva* spp. has shown promise for that purpose. We evaluated the ideal dosage of *U. lactuca* ulvan and flour on seed germination invigoration and the effects of *U. flexuosa* and

U. lactuca extracts on tomato seedling growth (*Solanun lycopersicum*). The germination recovery of aged seeds after the application of *U. lactuca* was evaluated by seed germination rates and seedling emergence. Greenhouse cultivated seedlings were irrigated with 0.2 and 0.4 g·L^{-1} of the flour, or an ulvan solution of *Ulva* spp. Seedling growth parameters (height, stem diameter, height/stem diameter ratio, biomass, and number of leaves) were compared with the control (seedlings irrigated with distilled water). All dosages of *U. lactuca* ulvan and flour were found to increase the germination rates of aged seeds as compared to controls. No significant difference in seedling emergence rates were seen. After treatment with *Ulva* spp. extracts no significant differences in seedling growth were detected. We concluded that low doses of the *U. lactuca* extract will increase the germination rates of *S. lycopersicum* seeds and, while different dosages of the extracts of two *Ulva*'s species did not stimulate tomato seedling growth, they were also not lethal.

Keywords: Biostimulant; Eco-friendly agriculture; Seed invigoration; Seedling vigor.

Resumo

Extratos de algas marrons são usados comercialmente como bioestimulantes agrícolas, e as espécies de macroalgas verdes *Ulva* tem se mostrado promissoras para esse propósito. Avaliamos a dosagem ideal da ulvana e da farinha de *U. lactuca* no envigoramento das sementes do tomate *Solanun lycopersicum* e os efeitos dos extratos de *U. flexuosa* e *U. lactuca* no crescimento de mudas A recuperação da germinação de sementes envelhecidas após a aplicação de *U. lactuca* foi avaliada pelas taxas de germinação e emergência de plântulas. Mudas cultivadas em casa de vegetação foram irrigadas com 0,2 e 0,4 g.L^{-1} da solução de farinha ou de ulvana de *Ulva* spp. Os parâmetros de crescimento das mudas (altura, diâmetro do caule, relação altura/diâmetro do caule, biomassa e número de folhas) foram comparados com o controle (mudas irrigadas com água destilada). Todas as dosagens de ulvana e de farinha de *U. lactuca* aumentaram as taxas de germinação das sementes envelhecidas em comparação com os controles. Nenhuma diferença significativa foi observada nas taxas de emergência de plântulas. Após os tratamentos com extratos de *Ulva* spp. não foram detectadas diferenças significativas no crescimento das mudas. Concluímos que baixas doses do extrato de *U. lactuca* aumentam as taxas de germinação de sementes de *S. lycopersicum* e, embora diferentes dosagens dos extratos das duas espécies de *Ulva* não estimulam o crescimento das mudas do tomate, também não são letais.

Palavras-chave: Bioestimulante; Agricultura ecológica; Envigoramento de sementes; Vigor de mudas.

Resumen

Los extractos de algas pardas se utilizan comercialmente como bioestimulantes agrícolas, y las especies de macroalgas verdes *Ulva* se han mostrado prometedoras para este propósito. Evaluamos la dosis ideal de harina de ulvana y *U. lactuca* en el fortalecimiento de semillas de tomate *Solanun lycopersicum* y los efectos de los extractos de *U. flexuosa* y *U. lactuca* sobre el crecimiento de las plántulas. La recuperación de la germinación de semillas envejecidas después de la aplicación de *U. lactuca* se evaluó mediante las tasas de germinación y emergencia de plántulas. Las plántulas cultivadas en invernadero se irrigaron con 0,2 y 0,4 $g.L^{-1}$ de la solución de harina o ulvana de *Ulva* spp. Los parámetros de crecimiento de las plántulas (altura, diámetro del tallo, relación altura/diámetro del tallo, biomasa y número de hojas) se compararon con el control (plántulas regadas con agua destilada). Todas las dosis de harina de ulvana y *U. lactuca* aumentaron las tasas de germinación de las semillas envejecidas en comparación con los controles. No se observaron diferencias significativas en las tasas de emergencia de las plántulas. Después de tratamientos con extractos de *Ulva* spp. no se detectaron diferencias significativas en el crecimiento de las plántulas. Concluimos que dosis bajas de extracto de *U. lactuca* aumentan las tasas de germinación de semillas de S. *lycopersicum* y, aunque diferentes dosis de extractos de las dos especies de *Ulva* no estimulan el crecimiento de plántulas de tomate, tampoco son letales.

Palabras clave: Bioestimulante; Agricultura ecológica; Vigorización de semillas; Vigor de plántulas.

1. Introduction

From a functional perspective, a plant biostimulant is any product of vegetable, animal, or microorganism origin that enhances plant nutrition efficiency and tolerance to abiotic stress, and thus increases crop quality and yields (du Jardin, 2015; Ricci et al., 2019). Seaweed extracts account for more than a third of the total global biostimulant market for crops (El Boukhari et al., 2020). Those extracts contain macro- and micronutrients, amino acids, vitamins, and hormone-like compounds that can enhance the growth and quality of several crops (Battacharyya et al., 2015; Calvo et al., 2014; Cole et al., 2016; Craigie, 2011; Khan et al., 2009). There is also strong evidence that seaweeds added to the soil can assist gel formation, water retention and soil aeration, and promote cation exchange (du Jardin, 2015; Senthilkumar et al., 2006; Sharma et al., 2018).

Although most macroalgae biostimulant products are obtained from brown algae

(Arioli et al., 2015; Calvo et al., 2014; Craigie, 2011), some green seaweed species belonging to the genus *Ulva* show promising results (Gupta et al., 2011; Hernández-Herrera et al., 2016). Their agricultural benefits vary according to the *Ulva* species, the cultivar, the raw material processing technique used (flour or ulvan), and the dosages employed (Gupta et al., 2011; Khan et al., 2009). Efforts will therefore be needed to examine those variables, choosing algae species of different origins, and comparing the ulvan and flour of *Ulva* spp. – as flour is less costly than ulvan.

Many countries are encouraging local production of *Ulva* spp. through aquaculture (Alaswad et al., 2015; Lehahn et al., 2016; Wade et al., 2020), so that its use as a biostimulant to reinvigorate low-vigor seeds and stimulate horticultural seedlings should be more intensely investigated. Seed germination and seedling development are considered the most vulnerable phases in plant life cycles (Benech-Arnold, R, Sanchez, 2004), and research on that topic can help make those crop production stages more resilient to environmental stress (Chanthini et al., 2019). Being a natural product, the use of *Ulva* extracts as organic alternatives to chemical fertilizers would promote eco-friendly agriculture production.

We tested here the effects of different concentrations of ulvan and flour extracted from two green seaweeds (*Ulva* spp.) on the germination and seedling growth of a tomato (*S. lycopersicum*) cultivar adapted to tropical latitudes. The tomato was chosen as the target species because it is one of the world's most widely cultivated crops, with global production in 2018 reaching 188 million tons, and has an upward production trend conditioned by the expansion of planted areas (faostat, n.d.). The organic tomato market is also an expansion worldwide, avoiding the use of harmful chemicals (IndexBox, n.d.). Increased interest in organic tomato production exemplifies the need to develop less expensive production techniques that lower the costs of organic cultivation (Zoran et al., 2014).

As the products derived from *Ulva lactuca* L. have had positive effects on germination and seedling growth in some crop species (Castellanos-Barriga et al., 2017; Hassan & Ghareib, 2009; Hernández-Herrera et al., 2014) we tested the dose efficiencies of flour and ulvan derived from *U. flexuosa* and *U. lactuca* on tomato seedling growth. We also tested the dose efficiency of flour and ulvan derived from *U. lactuca* for improving seed germination and seedling emergence in artificially aged (low vigor) tomato seeds. Despite previous research on the use of *Ulva* spp. as a biostimulant in agriculture, no published articles have compared the effects of *Ulva* flour as compared to ulvan. As flour is less expensive, and requires less technology to produce than ulvan, greater focus should be directed towards that product.

2. Methodology

2.1 Collection, identification, and preparation of ulvan and flour from *Ulva spp*.

U. flexuosa and *U. lactuca* were collected at Arpoador Beach, in Rio de Janeiro State, Brazil (22°59'22.71"S - 43°11'29.23"W). According to the taxonomic nomenclature proposed by Guiry & Guiry (2020), *Ulva fasciata* Delile, which we collected on the vertical portion of the rocky shore, is synonymous with *U. lactuca* L., found in the horizontal area of the rocky shore. That nomenclatural position is based on genetic analyses by Hughey et al. (2019), which indicated *U. fasciata* as a heterotypic synonyms of *U. lactuca*.

The algae species were hand-collected, washed with seawater, and dried in an oven (50 ± 2º C). Ulvan extraction was performed according to Castelar et al. (2014). Each sample was obtained from a slurry of 10 g of crushed dried seaweed in 100 mL of distilled water that was autoclaved (120º C) for 40 minutes and centrifuged (3.500 G) for 5 minutes. The polysaccharides were precipitated by adding three volumes of ethanol (98° GL) to the solution, which was then cooled to -20° C for 48 h, and subsequently centrifuged for 5 minutes. The ulvan precipitate was dried at 40° C.

The samples of *Ulva* spp. flour and ulvan were grounded in a ball mill (SOLAB-SL38 Solab Científica) for two minutes and then sieved in screen sifter (0.9 mm). The products were subsequently diluted as stock solutions of 100 g of *Ulva* (ulvan or flour) in 1L of autoclaved distilled water.

2.2 Germination Bioassay

Germination and seedling growth experiments were conducted with tomato seeds (*S. lycopersicum*, cv “Super Marmande Gaúcho”, ISLA Pro Seeds Inc., BRA) purchased from a commercial supplier.

2.3 Effect of *U. lactuca* on the invigoration of tomato seeds

Tomato seed lots with 8% moisture contents and 88% initial germination were partially deteriorated using a rapid ageing method (modified) following Panobianco & Marcos Filho (2001).

Short-term seed storage at high temperatures and high moisture contents accelerate seed deterioration (aging). To that end, the seeds were hydrated for 24 h at 15° C between two sheets of filter paper saturated with distilled water, and subsequently stored in air-tight packages at 35° C for 20 days. The aged seeds were then immersed for ten minutes in distilled water (control treatment), or ulvan, or flour solutions of *U. lactuca* at concentrations of 0.2 $g \cdot L^{-1}$ and 0.4 $g \cdot L^{-1}$.

The imbibed seeds were then sown into Petri dishes (10 cm in diameter) lined with filter paper moistened with 5 mL of the same solutions/dosages described above, and the dishes sealed with Parafilm® to avoid evaporation.

Germination tests were carried out in an incubator (B.O.D. Eletrolab, model 122 FC) under alternating temperature of 20-30° C and a 16-h light/8-h dark regime using fluorescent lamps (120 $\mu mol \cdot photons \cdot m^{-2} \cdot s^{-1}$).

The germination performances of the tomato seeds were evaluated based on radicle elongation (> 1 mm) with positive geotropism (total germination, TG), as well as normal seedling formation (normal germination, NG), showing well-developed essential structures (Brasil, 2009). Both evaluations were performed daily until count stabilization.

The Peak value (PV), or the maximum quotient, was derived from the cumulative germination percentages and considered the number of days required to reach that percentage (Czabator, 1962), and was used as the germination speed parameter. Higher values represent more rapid rates of germination. The experimental design was completely randomized using five replicates of 40 seeds.

2.4 Effect of *Ulva* spp. on greenhouse tomato seedling growth

Tomato plants were grown in a greenhouse structure covered with a transparent PVC plastic film under (mean) 1.05 mol·mol − 1 PPFD (~85% total full sunlight, 1.03 red:far-red), and average daily maximum and minimum temperatures of 33° C and 19° C respectively.

Photosynthetic photon-flux density and red:far-red light measurements were made at midday using a radiometer SKR-100 linked to a SpectroSense 2 SKL 904 (Skye Instruments, UK). Air temperatures were recorded using data loggers (ETI model HTD, West Sussex). The chemical characteristics of the sterilized sandy Planosol substrate were as follows: pH, 6.4, organic C, 1.1 %, P, 13.3 $mg \cdot L^{-1}$, K, 88.5 $mg \cdot L^{-1}$. Other nutrients (in mol $c.dm^{-3}$): Ca, 1.0, Mg, 0.7, Na, 0.03, H+Al, 1.98.

The tomato seeds were germinated in polystyrene germination trays (100 cells) for 15 days, with three seeds per cell. After emergence, uniform sized seedlings were transferred to plastic vessels (700 g) as described in item 2.3. The seedlings were then submitted to the following treatments: control (distilled water), *U. lactuca* flour (0.2 and 0.4 g·L^{-1}) or ulvan (0.2 and 0.4 g·L^{-1}), and 0.2 g·L^{-1} of *U. flexuosa* flour.

The seedlings were irrigated with 200 mL of one of the treatment solutions only on the first day of the experiment, after that they were irrigated only with distilled water for 30 days. The parameters used to evaluate seedling growth were: height (cm), stem diameter (in mm, as measured using a digital caliper, Mitutoyo Corp., Tokyo, Japan), the height/stem diameter ratio, number of leaves, and biomass (g dry weight). The experimental design was completely randomized using five seedlings per treatment.

2.5 Statistical analyses

The assumptions of variance homogeneity (Cochran test) of the data were tested prior to the parametric analyses. Differences between each parameter of the different treatments in the seedling and seed experiments were evaluated using unifatorial analysis of variance (one way-ANOVA).

The pos hoc Tukey test was used to identify significant differences between group means from ANOVA. The statistical analyses were performed using Statistical Package Statistica 7.0 software (StatSoft Inc). Data were expressed as mean ± standard deviation, and the confidence interval for significance tests was 95% ($p = 0.05$).

3. Results

3.1 Effect of *U. lactuca* on tomato seed invigoration

The total (26.8 ± 7.6 %) and normal (17.5 ± 13.0%) germination percentages of aged seeds were significantly lower than non-aged seeds (72.8 ± 6.8 % and 21.0 ± 15.7%, respectively).

Liquid extracts of *U. lactuca* significantly increased the germination percentages of tomato seeds previously subjected to accelerated aging (Figure 1), although neither of the ulvan solutions was statistically different from the control treatment ($F = 8.59$, $p < 0.001$, Tukey test). The highest total germination percentage was observed with the *U. lactuca* flour treatment, at 2 g·L^{-1}, only that *U. lactuca* flour concentration (2 g·L^{-1}) significantly increased the germination speed of tomato seeds after accelerated aging ($F = 10.50$, $p < 0.001$, Tukey test).

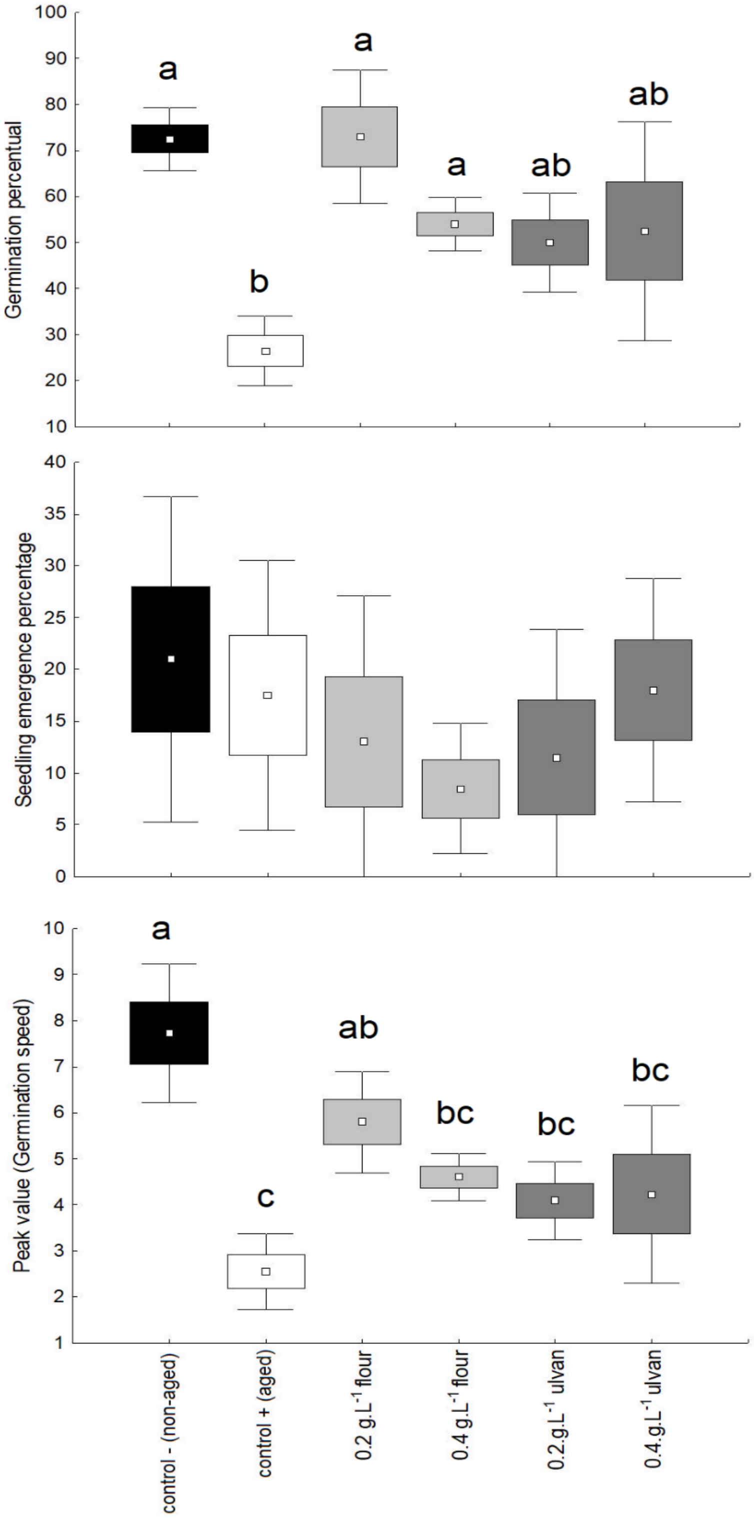
There were no differences between the *U. lactuca* treatments in terms of tomato seedling emergence percentages (F = 0.72, p = 0.62). Visual analysis of the roots of the germinated seeds after application of the seaweed liquid extracts revealed no apparent damage during the nine days of the experiment.

3.2 Effect of *Ulva* spp. on tomato seedling growth under greenhouse conditions

No significant differences [stem diameter (F = 1.40, p =0.26), height/stem diameter ratio (F = 1.26, p = 0.31), number of leaves (F = 1.47, p = 0.24), and dry mass (F = 1.22, p = 0.33)] or low (height (F=3.09, p = 0,03) were detected in the growth of tomato seedlings irrigated on the first day of cultivation with different concentrations of *U. lactuca* flour or ulvan.

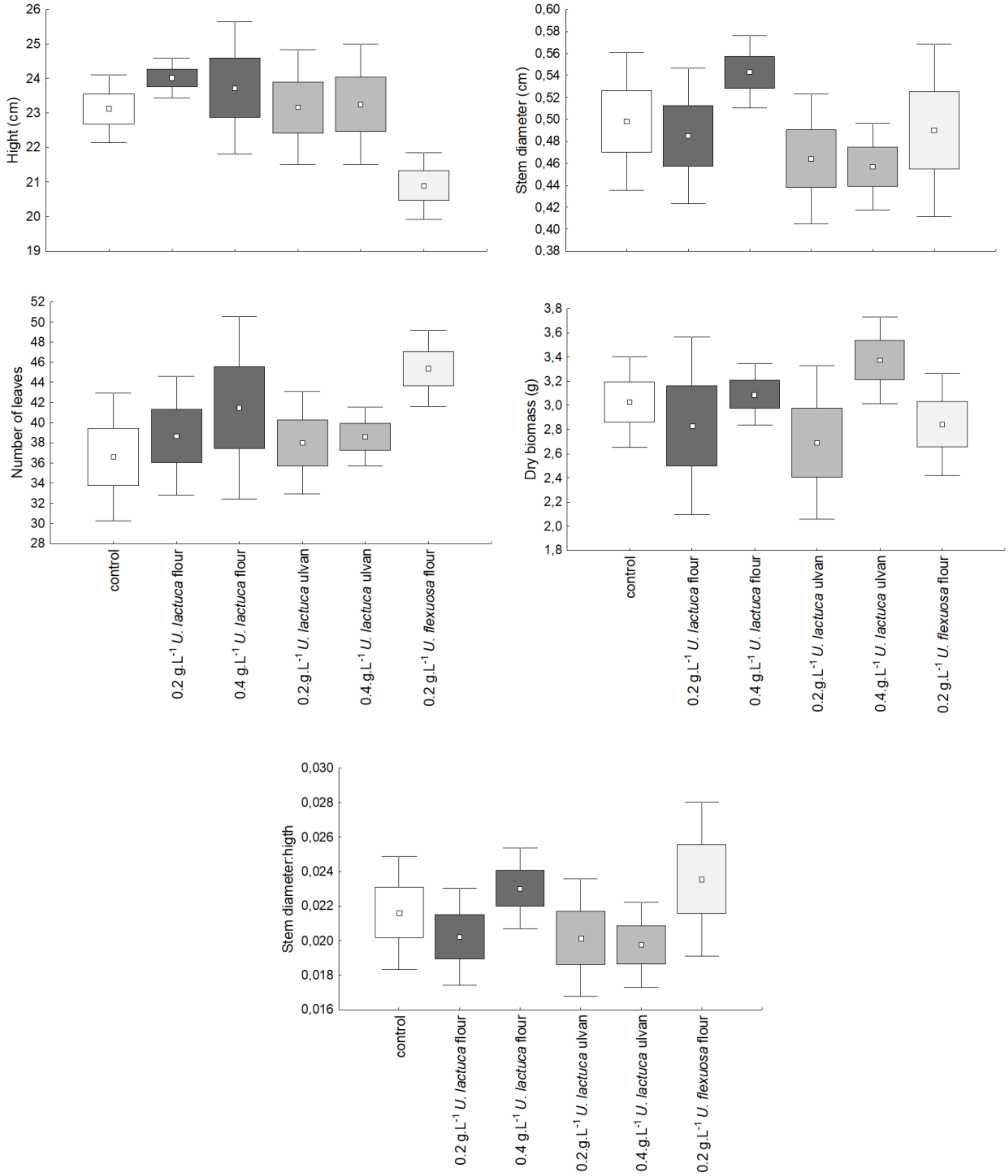
The heights of the *S. lycopersicum* seedlings were lower than the control when irrigated with *U. flexuosa* extracts (Figure 2). None of the seedlings were lost.

Figure 1. Germination percentage (left) and speed of germination (peak value, right) of non-aged (control -) and aged (control +) seeds of *S. lycopersicum* treated with different concentrations of *U. lactuca* flour and ulvan (g.L^{-1}). Squares represent the means, boxes the standard errors, and vertical lines the standard deviations. Letters shows significant similarity.



Source: Authors.

Figure 2. Parameters used to evaluate the growth of *S. lycopersicum* seedlings in response to exposure to *U. lactuca* and *U. flexuosa* flour and ulvan (g.L^{-1}). Squares represent the means, boxes the standard errors, and vertical lines the standard deviations.



Source: Authors.

4. Discussion

Previous studies have reported the use of seaweed extracts as a potentially stimulatory seed reinvigoration treatment, promoting faster and more constant germination and plant growth. Slight stimulatory effects of *Ulva* spp. extracts have been observed in germination and seedling growth performance in high quality seeds of certain crops: okra (Castellanos-Barriga et al., 2017), and cherry tomatoes (Chanthini et al., 2019).

To evaluate the effects of *U. lactuca* extracts on the recovery of the germination capacity of deteriorated tomato seeds, we first subjected those seeds to accelerated aging conditions, as natural seed deterioration processes can be very slow under standard storage conditions (Argerich et al., 1989; Nigam et al., 2019). That induced aging process caused the physiological deterioration of tomato seeds, with drastic reductions in their germination performances as compared to control (non-aged) seeds. We were then able to do detect positive effects of all *U. lactuca* extracts on the recovery of seed germination percentages and speed (peak value), although both parameters recovered to levels similar to the control (non-aged) seeds only when exposed to the lowest concentrations of *U. lactuca* flour (2 $g \cdot L^{-1}$).

Both of the evaluated parameters are important attributes that can predict crop performances, as high germination percentages are directly related to successful seedling establishment (Matthews et al., 2012). Speed of germination is an important indicator of seed quality, as it predicts the ability to achieve maximum and uniform seedling emergence in the field (Finch-Savage & Bassel, 2016).

Seaweed extracts can either stimulate or inhibit seed germination processes, depending on the concentrations of their soluble compounds (Hernández-Herrera et al., 2014, 2016; Mzibra et al., 2018). The main active compounds identified in seaweed extracts include macro- and micronutrients, plant growth regulators (PGRs), and other endogenous compounds that influence specific physiological processes related to plant development (Calvo et al., 2014; Craigie, 2011; du Jardin, 2015; Khan et al., 2009). Some studies, for example, have indicated externally applied PGRs as central to stimulating germination processes, mainly through their regulation of PGR gene expression in the plant tissues themselves and, to a lesser extent, through the actual PGRs concentrations in the seaweed extracts (du Jardin, 2015; Yakhin et al., 2017). Recovery of seed quality has also been attributed to the incorporation of ulvan oligosaccharides during the water absorption process, boosting amylase activity, and accelerating the metabolic activities of the seeds (Hernández-Herrera et al., 2016).

Our study showed that the highest concentrations (0.4%) of *U. lactuca* extracts tested inhibited both tomato seed germination and vigor. At high concentrations, the extracts can delay germination onset or impact root formation (Hernández-Herrera et al., 2014, 2016; Mzibra et al., 2018). Similar responses have been reported in crop studies (Castellanos-Barriga et al., 2017), including of tomatoes (Hernández-Herrera et al., 2014), and are probably due to moderate to high sensitivity of some tomato cultivars to the high levels of salinity (Cuartero et al., 2006; Singh et al., 2012) detected in the highest concentrations of *U. lactuca* extracts (Hernández-Herrera et al., 2014).

A single application of the liquid extracts (flour and ulvan) derived from *U. lactuca* and *U. flexuosa* did not act as biostimulants for tomato seedling growth, while more frequent applications of the liquid extracts from *U. lactuca* did promote seedling growth, even at high concentrations (1%) (Hernández-Herrera et al., 2014, 2016). In general, positive responses to seaweed liquid extracts will depend on the frequency of their application, on the duration of their use, soil fertility, and the cultivar species involved (Craigie, 2011). It must be remembered that our studies showed no deleterious effect of *U. flexuosa* or *U. lactuca* extracts on tomato seedling growth, which suggests that their frequencies of application could be increased with that cultivar.

Seaweed extract concentrations influenced their biostimulatory effects on seedling growth, as well as plant mortality. Positive results were observed at low dosages, while higher concentrations inhibited plant growth, as was previously reported for *U. lactuca* with both lettuce and tomato plants (Castellanos-Barriga et al., 2017; Hassan & Ghareib, 2009; Hernández-Herrera et al., 2014) and for *Sargassum myriocystum* extracts with *Vigna mundo* (Kalaivanan et al., 2012).

Many studies have focused on environmentally friendly products to improve crop productivity (Cole et al., 2016) and increase the food stocks that will be necessary to feed an estimated nine billion people by the mid-21st century (Arioli et al., 2015), and a better understanding of the modes of action of biostimulants could improve agricultural outputs (Craigie, 2011; du Jardin, 2015; Khan et al., 2009) We therefore recommend further investigations into producing more robust tomato seedling using 0.4 $g \cdot L^{-1}$ of *U. lactuca* flour and 0.2 $g \cdot L^{-1}$ of *U. flexuosa* flour. It will be crucial to establish the correct irrigation regime with *Ulva* spp. flour or ulvan to increase plant growth, and to survey the effects of those seaweeds on plant roots, and search for other potential uses. Paulert et al. (2009) and Hernández-Herrera et al. (2014) noted that *Ulva* extracts protected the plant against pathogens. Reis et al. (2018) suggested that the addition of *Ulva fasciata* (a current synonym of *U. lactuca*) extracts (ulvan and flour) could suppress the growth of *Stemphylium solani* Weber (a fungal pathogen commonly infecting tomatoes) by delaying its melanization, and consequently reducing host penetration.

As commercial macroalgae biostimulants can reduce environmental stresses, with consequent plant remediation (du Jardin, 2015; Van Oosten et al., 2017), we recommend further studies with *U. lactuca* using lower concentrations for reinvigorating deteriorating crop seeds, and to explore its influence on tomato DNA repair mechanisms. According to Matthews et al. (2012), germination recovery in deteriorated seeds is related to DNA repair mechanisms for effective root emergence, and the growth of healthy and resistant seedlings.

Even though precise analytical techniques have identified plant biostimulants in *Ulva* spp. extracts, such as phytohormones, their roles in plant metabolic pathways remain poorly understood (Gupta et al., 2011), so that multidisciplinary efforts will be needed to better understand those mechanisms and establish more productive cultivar management techniques (Gupta et al., 2011; Van Oosten et al., 2017). Those efforts must also consider the environmental and economic sustainability of plant production (Calvo et al., 2014).

5. Conclusion

The treatment with 0.2 $g.L^{-1}$ *U. lactuca* flour increases the germination rate of deteriorated of *S. lycopersicum* seeds and is more effective than ulvan. The irrigation of *S. lycopersicum* seedlings during the first day of experiment with 0.2 and 0.4 $g.L^{-1}$ of *U. flexuosa* and *U. lactuca* flour or ulvan do not stimulate tomato seedling growth and is not lethal to them.

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