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Research Paper

Valorization of *Posidonia oceanica* biomass: Role on germination of cucumber and tomato seeds

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A B S T R A C T

Biostimulants are organic compounds from plant sources such as botanical extracts and bioactive substances that promote plant growth, enhance photosynthesis and increase crop quality. The accumulation of detached *Posidonia oceanica* leaves on coasts of the Mediterranean Sea results in economic problems, due to the rejection of the tourists who frequent the beaches in the summer months. However, it is a plant with high content of secondary metabolites that can be used in sustainable agriculture. In this study we investigated the physicochemical characterization of *Posidonia oceanica* extracts with three different solutions and their application in tomato and cucumber seeds germination. The results showed that the aqueous extract of *Posidonia oceanica* had a high concentration of macro and micronutrients, as well as secondary metabolites with bioactive activity. The aqueous extract had a beneficial effect on both leaf and root growth on tomato seeds, specifically, an increase of 76% for the relative root growth and 73% for the germination index was obtained with respect to the control using the sample with the intermediate dilution (POe0.5). In addition, the extracts did not show toxicity to either germination or growth of the tomato plant. As for cucumber seed germination, the improvement was less significant and did result in a phytotoxic effect on both germination and plant growth. The most diluted extract had better results on seed germination. Therefore, the application of aqueous extracts of *Posidonia oceanica* were suitable to be appropriate for tomato germination and in turn contribute to eliminate the lots of *Posidonia oceanica* remains recovered in summer months in Mediterranean beaches.

1. Introduction

In recent years, the growing demand for sustainable, innovative, safer, and higher quality agricultural production, as well as the increasing awareness of the negative effects that chemical or synthetic pesticides and fertilizers can have on human health and the environment, has forced growers and producers to consider other products such as biostimulants (BST). BST are organic compounds from plant source, e.g. botanical extracts, bioactive substances, protein hydroxylates, chitosan, humic and fulvic acids, which can promote seed germination and plant growth under abiotic stress conditions increase harvest yield and fruit quality, enhance photosynthesis and increase the production of secondary metabolites (Mahdavi and Rahimi, 2013; Mutlu-Durak and Yildiz Kutman, 2021; Sobhy et al., 2014). The main functions of BST in root and foliar application can be summarized as i) nutrient acquisition and mobilization in plant growth promotion (Bhupenchandra et al., 2022), ii) influence in abiotic stress tolerance (i.e. saline, temperature and drought) (Ondrasek et al., 2022), iii) crop quality traits (du Jardin, 2015) and iv) interaction in cellular mechanism and physiological processes (du Jardin, 2015).

This alternative can become important as they can be applied to crops through foliar fertilization, fertigation or direct application to the soil (Van Oosten et al., 2017) and contribute to reducing greenhouse gas emissions by decreasing the use of fertilizers in agriculture (Bhupenchandra et al., 2022). Due to their high potential, the European Commission has fixed a target of replacing 30% of chemical fertilizers with organic-based inputs such as BST or elicitor substances by 2050. Besides, enhanced germination performance due to seed treatments has been reported in several crops such as maize, soybean, spinach, pepper, and wheat in both optimal and stressed environments (Gupta et al., 2022).

Posidonia oceanica (L.) Delile (PO) is an endemic seagrass to the Mediterranean Sea that forms extensive meadows covering approximately 1.5% (Pasqualini et al., 1998) and 300,000 km^2 of the total surface on the planet (Duarte et al., 2013). These meadows are protected through the EU European ‘Habitats’ Council Directive 92/43/EEC

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(European Commission, 1992). PO is a plant with an important ecological role because prevents erosive processes, contributes to maintaining the biodiversity of the Mediterranean Sea and intercede in its oxygen balance and coastal dynamics (Bonanno and Di Martino, 2017; Campagne et al., 2015; Ferrero et al., 2015). The presence of a bench of PO leaves on a beach is an excellent indicator of the high quality of the coastal marine environment as it indicates the presence of healthy grasslands (Shabaka et al., 2021).

Besides the positive aspects mentioned above, it is important to highlight that PO has a high content of secondary metabolites, in particular, 51 natural PO products have been identified (Heglmeier and Zidorn, 2010), including phenolic compounds and derivatives, amino acids, fatty acids, terpenes, sterols, flavonoids, chalcones and cholest-5-enes (Agostini et al., 1998; Vasarri et al., 2021). These molecules play a fundamental role in the growth of seed and plants because they perform active functions in photosynthesis and respiration, as well as its defense and have an ideal chemical structure for scavenging reactive oxygen species (ROS) (Agostini et al., 1998).

However, every year the PO leaves are detached from the rhizomes due to the natural cycle of the plant producing a large amount of waste that accumulates, especially on the Mediterranean coast with associated economic problems (Balestri et al., 2006; Mininni et al., 2013; Tarchoun et al., 2019) derived from the uncontrolled decomposition of marine waste accumulated among the PO leaves that, in some occasions, produces a strong odor, thus reducing the possibility of using seaports and beaches for recreational and tourist activities (Cocozza et al., 2011). However, the accumulation of PO remains on the Mediterranean coasts is the most efficient and sustainable way to prevent beach erosion and avoid sand losses. For that reason, PO leaves removal is forbidden from October to March, although allowed during the tourist season. Despite this, during the high season there are many tons of leaves that the councils of the tourist municipalities must handle. The amount of removed PO leaves is especially high in those areas where the PO meadow is in better health and the waters are cleaner. The removal and management of tons of fibrous waste to landfills involves high economic costs (Marilés et al., 2019; Peruzzi et al., 2020).

To provide an effective solution to these problems, different researchers have developed projects for the valorization of PO waste within a circular economy. For instance, PO fibrous balls have been used as an adsorbent material for the removal of inks and ammonium (Wahab et al., 2010) and antibiotics such as oxytetracycline (Ferchichi et al., 2022). It has also served as a precursor material in the production of activated carbon for wastewater and gas treatment, energy applications for supercapacitors and hydrogen storage (Asimakopoulos et al., 2021; Dural et al., 2011; Moltó et al., 2022).

Moreover, due to a large number of phytochemical compounds in its composition, PO has also been evaluated for medical applications as an active principle in drugs due to its antioxidant, antifungal, anti-inflammatory, immunostimulant and vasoprotective properties (Gokce and Haznedaroglu, 2008; Heglmeier and Zidorn, 2010; Vasarri et al., 2020).

PO extracts have also been applied in the field of food preservation decreasing and removing pathogens and microorganisms in food (Kourkoutas and Proestos, 2020). Regarding agricultural applications, PO residues have been used as a constituent in the biofortification of foods to improve their nutritional value (D'Imperio et al., 2021) and as compost in food production crops, such as tomato, lettuce, basil and melon (Mininni et al., 2013, 2012; Peruzzi et al., 2020).

Nowadays, as far as our knowledge, there are not studies using PO plant extract as a BST or elicitor substance, but due to its silicon and secondary metabolites content, this product could be used in plant growth and development. Therefore, the aim of this research was to perform the physicochemical characterization of PO and a PO water extract and evaluate its effect on the germination of tomato and cucumber seeds. These two plants were selected because of their different behavior towards the salinity of the crop. Thus, contributing to solve the problem of the PO remains excess on the beaches during the summer and in the search for natural products for agricultural use.

2. Materials and methods

2.1. Sampling and obtaining of PO extract

The PO residues were collected from the coast of the Mediterranean Sea in Alicante, Spain (latitude: 38°13′18″N; longitude: 0°33′28″W). PO samples were taken to the laboratory and, after removing plastic and other foreign materials, were washed with tap water followed by distilled water to remove sand and salt from the surface. This sample was oven-dried at 55 °C until constant weight. Finally, the sample was ground and sieved using a domestic electric blender until a particle size of < 2 mm. The resulting powder was stored in a dark bottle at room temperature. Water extract, containing BST was made weighting 4 g of PO powder that were introduced into a spherical flask with 100 mL of distilled water of a Soxhlet apparatus kept at 65 °C for 12 h. After that, the extract was cooled to room temperature and filtered through a 0.45 μm filter. The extract was named POe and stored in dark bottles and at 4 °C until its use.

2.2. Physicochemical characterization of PO sample

pH and electrical conductivity (EC) were measured suspending 3 g of powder sample in 50 mL of distilled water, shaked for 30 min and centrifuged for 15 min at 4000 rpm, according to Cocozza et al. (2011), using a simultaneous pH-conductimeter equipment (SevenMulti, Mettler Toledo). Ash content was measured according the ASTM Standard Method Number E1755-01 (ASTM, 2010), total organic carbon (TOC) was analyzed by the Walkley-Black method (Walkley and Black, 1934) and total organic matter (TOM) was calculated by calcination at 540 °C, after carbonate removal. Foliar phosphorus concentration was characterized using the colorimetric method (Kitson and Mellon, 1944) and C and N elemental analysis was performed using an elementary microanalyzer (TruSpec® Micro, LECO Instrumentos SL).

A microwave wet-oxidized digestion with 0.5 g of PO leaves, 2 mL of distilled water and 6 mL of HNO_3 (67%, Merck KGaA) was performed using a specific digestion system (Start D, Milestone SRL) for 30 min at maximum power.

Quantitative analyses of macro and micronutrients, as well as silicon, were carried out by inductively coupled plasma-optical emission spectrometer (model 720-ES, Agilent Technologies) on the digested PO leaves solution as well as on the PO water extract. The experimental conditions were argon as carrier, auxiliary and plasma gas with a flow rate of 0.7, 1.5 and 15 L min^{-1}, respectively. The system for sample introduction was a pneumatic nebulizer and cyclonic spray chamber with a liquid sample flow rate of 1 L min^{-1} and a detection time of 1 s. To quantify the elemental composition, a multielement solution (ICP multielement standard IV, Merck KGaA) was selected as calibration standard.

2.3. Secondary metabolites analysis in PO extract

A standard protocol to identify the presence of groups of secondary metabolites was reference in Table 1.

In addition, the concentration of several secondary metabolites present in the aqueous extract solution was quantified. Anthocyanin content was estimated by the pH differential method (Lee et al., 2005) while the analysis of tannins was performed using the Bate-Smith assay (Bate-smith, 1981). The aluminum chloride procedure was used for total flavonoids content (Pękal and Pyrzynska, 2014) and the total phenolic content in the aqueous extract was calculated using the Folin-Ciocalteu method (Socha et al., 2009). In addition, the Bradford method was used to quantify soluble protein (Bradford, 1976).

Table 1
Detection methods applied to the identification of secondary metabolites in PO extract.

Bioactive substance	Detection method	References
Anthraquinones (total)	Börntrager's reaction	Ajayi et al., (2011)
Anthraquinones (free)	chloroform and 10 % ammonia solution	Ajayi et al., (2011)
Alkaloids	Mayer's reagent (Potassium mercuric iodide solution)	Chakraborty et al., (2010)
Carbohydrates	Barford's reagent method	Chakraborty et al., (2010)
Carotenoids	Chloroform and 85 % sulphuric acid	Ajayi et al., (2011)
Cardiac glycosides	Molisch's reagent and concentrated H_2SO_4	Singh et al., (2012)
Flavonoids	Zn^0 and sodium hydroxide 20 %	Ajayi et al., (2011)
Phenols	Fe(III) chloride	Singh et al., (2012)
Phlobatannins	HCl 1%	Berfad and Alnour, (2014)
Protein	Millon's reagent	Chakraborty et al., (2010)
Reducing sugars	Fehling's reagent	Singh et al., (2012)
Saponin	Foam production after vigorous shaking	Chakraborty et al., (2010)
Tannins	45% ethanol and 10 % ferric chloride solutions	Singh et al., (2012)
Terpenoids	Salkowski test	Singh et al., (2012)

2.4. Seed germination assay

To study the effect of PO extract on seed germination, cucumber (*Cucumis sativus* cv Marketmore 76) and tomato (*Solanum lycopersicum* cv Seny F1) seeds were used. For both species, 12 seeds were placed on a filter paper (Whatman® qualitative Grade 1 No. 1, Merck KGaA) on Petri dishes. Two different assays were performed with 2 mL of PO aqueous extract at three different concentrations, original extract (POe), diluted with distilled water (1:2; POe0.5) and diluted with distilled water (1:10; POe0.1). In addition, a control treatment using distilled water was used for comparison. Seeds were germinated in a growth chamber (MLR-350H, Sanyo Electric Co Ltd) at 25 °C and 44 %HR. During the experimental period, every day, 2 mL of the corresponding extract were added to the Petri dish. Percentages of germination were noted at 24, 48, 72 and 120 h for cucumber or 144 h for tomato. To analyze the effect of PO extract concentration on both tomato and cucumber seeds, 5 Petri dishes with 12 seeds in each were used per treatment. They were considered germinated seeds those whose radicle length was greater than 2 mm (Bagur-González et al., 2011).

The relative percentage of seed germination (RSG), the relative root growth (RRG), as well as the germination index (GI) for each extract, were calculated following the equations described by Hoekstra et al., (2002):

$$\%RSG = \frac{number\ of\ seeds\ germinated\ in\ PO\ extract}{number\ of\ seeds\ germinated\ in\ control} \cdot 100 \tag{1}$$

$$\%RRG = \frac{mean\ root\ length\ in\ PO\ extract}{mean\ root\ length\ in\ control} \cdot 100 \tag{2}$$

$$\%GI = \frac{RSG \cdot RRG}{100} \tag{3}$$

In addition, two parameters that can be useful to stablish the phytotoxicity of POe (Bagur-González et al., 2011) were also considered: normalized residual germination index (NRG) that is calculated as the percentage of germinated seeds in a treatment compared to control after the experimental period (120 h for tomato and 144 h for cucumber) and, residual elongation radical (RER) that is the elongation of the radicle of the germinated seeds per treatment respect to control. Both indexes were calculated following Eqs. (4) and (5) respectively.

$$NRG = \frac{germX - germC}{germC} \tag{4}$$

$$RER = \frac{elongX - elongC}{elongC} \tag{5}$$

where germ X is the average percentage of seeds germinated in PO extract and, germ C is the percentage of germinated seeds in the control. Elong X is the average length of the radicle of the germinated seeds in the PO extract and, elong C is the average length of the germinated control seed radicles. When these indexes are negative, germination is inhibited because some toxic effect of the culture media. Both parameters, set toxicity values from −1 to 0 under the following categories: 0 to −0.25 low, −0.25 to −0.5 moderate, −0.5 to −0.75 high and −0.75 to −1.0 very high toxicity.

2.5. Statistical analysis

The data obtained were analyzed using the SPSS® software (23.0 version, IBM). A one-factor analysis of variance (ANOVA) was performed to compare the mean results for different determinations, with three replications, with a significant value of $p < 0.05$ among them according to the Duncan's test.

3. Results

3.1. Physicochemical characterization of PO sample

The physicochemical analysis of PO leaves and aqueous extract is presented in Table 2 and Table 3. PO leaves are highly alkaline and moderately saline. As it was expected, organic carbon is very high, although the organic compounds are poor in N which results in a very high C/N relationship.

Both, PO leaves and aqueous extracts were rich in plant nutrient (Table 2), mainly Ca, Mg and micronutrients. The extraction process resulted in a dilution of these elements compared to the PO powder and, as it was expected, Na was the dominant element in the water extracts. The 40% of Na in PO leaves was solubilized, as well as the 55% of K. Magnesium and Si were also quite soluble with solubilization percentages of 19% and 17% respectively, meanwhile about 6% of Ca passed to the aqueous phase after extraction. The less soluble element was Fe. Only a 0.3% of the Fe present in PO leaves was soluble. As can be seen in Table 2, the concentration of macro and micronutrients present in the aqueous extracts decreases as the dilution is higher.

3.2. Secondary metabolites in PO extract

The preliminary phytochemical study of PO aqueous extract revealed the presence of a total of 9 secondary metabolites. Results are summarized in Table 4. Carbohydrates, cardiac glycosides, flavonoids, phenols, proteins, reducing sugars, saponins, tannins and terpenoids were all

Table 2
Physicochemical characterization of *Posidonia oceanica* (whole plant and aqueous extracts at different concentration). Results are expressed in average ± standard deviation (n = 3).

Nutrient	PO (mg kg^{-1})	POe (mg L^{-1})	POe0.5 (mg L^{-1})	POe0.1 (mg L^{-1})
Ca	28000 ± 700	65 ± 1	33 ± 7	7.2 ± 0.4
Mg	7470 ± 30	49.5 ± 0.7	24.7 ± 0.4	5.1 ± 0.7
Fe	4740 ± 30	0.53 ± 0.03	0.27 ± 0.03	0.06 ± 0.01
Na	6300 ± 10	100 ± 1	49 ± 2	12 ± 2
K	420 ± 9	9.3 ± 0.1	4.7 ± 0.1	1.1 ± 0.2
Mn	19.0 ± 0.3	0.04 ± 0.01	0.021 ± 0.004	0.005 ± 0.003
Zn	16.4 ± 1.4	0.034 ± 0.004	0.016 ± 0.003	0.003 ± 0.001
Cu	15.2 ± 0.2	0.05 ± 0.01	0.02 ± 0.01	0.006 ± 0.002
Si	850 ± 70	6.3 ± 0.1	3.4 ± 0.2	0.73 ± 0.04

Table 3
Physicochemical characterization of aqueous extracts from *Posidonia oceanica* at different concentration. Results are expressed in average ± standard deviation ($n = 3$).

Parameter	POe	POe0.5	POe0.1
pH	8.40 ± 0.05	7.1 ± 0.2	6.8 ± 0.4
EC (dS m^{-1})	4.64 ± 0.07	2.53 ± 0.08	1.87 ± 0.04
Ash content (%w w^{-1})	11.5 ± 0.6	7.4 ± 0.3	4.7 ± 0.3
TOC (%w w^{-1})	37 ± 4	19 ± 3	4.2 ± 0.6
TOMLOI (%w w^{-1})	92 ± 3	47 ± 5	11 ± 4
P$_{available}$ (%w w^{-1})	0.095 ± 0.007	0.04 ± 0.01	0.0017 ± 0.0003
N (%w w^{-1})	0.5 ± 0.1	0.32 ± 0.07	0.16 ± 0.02
C (%w w^{-1})	44.2 ± 0.9	20.4 ± 0.7	6.5 ± 0.4
C/N	88.4 ± 0.4	63.8 ± 0.7	40.6 ± 0.4

Table 4
Secondary metabolites in PO extract.

Secondary metabolites	POe
Anthraquinones	–
Free Anthraquinones	–
Alkaloids	–
Carbohydrates	+
Carotenoids	–
Cardiac glycosides	+
Flavonoids	+
Phenols	+
Phlobatannins	–
Proteins	+
Reducing sugars	+
Saponins	+
Tannins	+
Terpenoids	+

+ (presence of bioactive compound), - (absence of bioactive compound).

positive. On the other hand, anthraquinones (total and free), alkaloids, carotenoids and phlobatannins were not detected in the aqueous extract owed to their low water solubility (Cepeda et al., 1989; Yalkowsky et al., 2010).

3.3. Quantitative analysis of secondary metabolites in PO extracts

Five secondary metabolites were quantified in the dried powder PO sample and in the PO extract (Table 5). The values for the secondary metabolites were similar to those reported by other authors in plants (Agostini et al., 1998; Cannac et al., 2006; Rotini et al., 2013). The extract was rich in soluble protein, phenolics and flavonoids and very poor in tannins.

3.4. Effect of aqueous extract of *Posidonia oceanica* in germination experiments

Figs. 1 and 2 illustrate that the PO aqueous extract had a beneficial effect on both leaf and root growth in cucumber (Fig. 1) and tomato (Fig. 2) seeds. In the cucumber germination test, for shoot growth, the treatments with PO extracts showed an improvement compared to the control. In particular, the sample treated with POe0.5 exhibited the largest SL after 122 h of test, followed by the experiments carried out with POe and POe0.1. As for the RL, it was higher and significantly different in the control group compared with POe, but very similar to the growth obtained with the diluted extracts. For the tomato germination experiment, the same trend as for cucumber was observed, where the POe0.5 extract showed the best and significant results for both SL (Fig. 2a) and RL (Fig. 2b), followed by the POe0.1 and POe samples. In this case, the worst behavior was associated to the control treatment with distilled water. Moreover, it's worth noting the increase in shoot length with the diluted extract for both crops, although it had a greater impact on shoot development in cucumber.

Table 5
Concentration of secondary metabolites in aqueous extract of *Posidonia oceanica*. Results are expressed in average ± standard deviation ($n = 3$); CE, cyanidin-3-glucoside equivalent; GAE, gallic acid equivalent; QE, quercetin equivalent; BSA, bovine serum albumin.

Secondary metabolites	POe	Units
Anthocyanins	2.6 ± 0.3	mg CE L^{-1}
Tannins	0.41 ± 0.02	mg L^{-1}
Phenolic compounds	33.0 ± 0.6	mg GAE L^{-1}
Flavonoids	28 ± 2	mg QE L^{-1}
Soluble protein	65 ± 4	mg BSA L^{-1}

The values for %RSG, %RRG, and %GI, provided information on the relative germination of the seeds, meanwhile NRG and RER were used to evaluate the phytotoxicity of PO extracts (Table 6). PO extracts did not affect cucumber seeds germination, although a slight negative trend was observed for the POe extract. However, although the number of germinated tomato seeds did not increase respect to control, PO extracts had a significant effect on %RRG and %GI, specially POe0.5 extract, derived from the differences in root length between the aqueous extracts and control.

For the cucumber test, NRG values revealed a low toxicity gradient (0 to −0.25) but an RER of −0.22 (moderate toxicity) for the concentrated PO extract (POE). These results, combined with the Na and K values reported in Table 2, suggest that a possible saline stress induced by the PO extracts was affecting the growth of these seeds. However, for tomato seeds, both NRG exhibited values nearly 0 indicating that the extract solutions did not show toxicity with respect to seed germination. Regarding RER, as can be seen in the Table 6, all samples exhibited positive values with statistically significant differences for the four treatments, indicating a stimulation of root growth (Bagur-González et al., 2011), mainly with the POe0.5 extract, which was found to be the most optimal concentration for this germination study.

4. Discussion

The physicochemical characteristics of PO were similar to those obtained by different authors (Cocozza et al., 2011; Grassi et al., 2015; Khiari et al., 2010). The extract of PO obtained obeys the maximum levels imposed by the European Parliament and Council (European Comission, 2019) for fertilizer products made from raw materials from animal or plant origin.

The aqueous extract of PO contains nutrients and metabolites with proven effects on germination and plant development. Even Na, can promote seed germination at concentrations lower than 100 mM. although at higher concentrations may cause damages (Wang et al., 2022). However, Ca can mitigate the toxic effects of high Na concentrations by inhibiting Na uptake (Salahshoor and Kazemi, 2016; Shaikh et al., 2007; Zehra et al., 2012). Mulaudzi et al., (2020) considered that this alleviation of the saline stress by Ca was caused through modulation of osmoprotectants and regulation of some genes in stress response. Seeds treated with Ca, improved bioactive substances in brown rice and antioxidant capacity (Choe et al., 2021). Silicon has been shown to play a BST role in plant growth and development, being able to mitigate both biotic and abiotic stresses (Savvas and Ntatsi, 2015). Specifically, Si can favor the germination of tomato seeds in drought conditions, which is a similar situation that of saline excess. The presence of Si in the growth medium of tomato seeds under water stress reduced oxidative stress (Shi et al., 2014). Seed priming with Si alleviates abiotic stress in plants, improving various parameters such as tomato growth, photosynthetic pigment and soluble protein contents, photosynthetic rate, and root morphological traits (Gupta et al., 2022; Li et al., 2015). The occurrence of Ca and Si implies that although the Na is the major element in the extract, the presence of abundant Ca and Si can prevent the possible toxic effects of Na and salinity, specially in tomato seeds and may help to

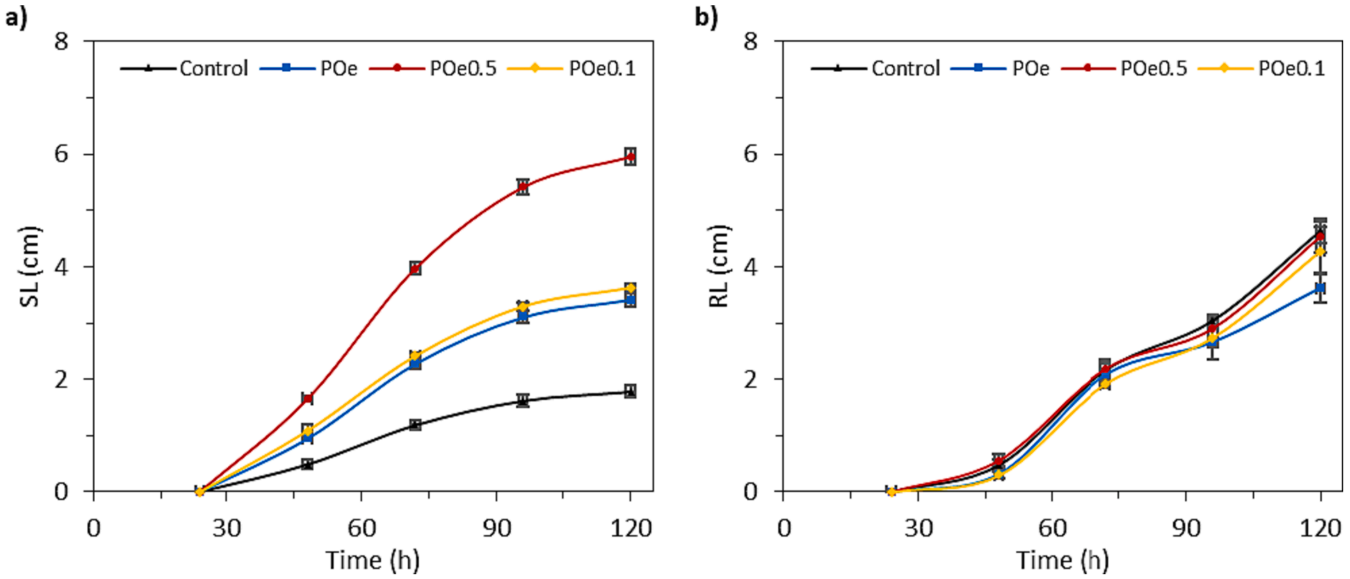

Fig. 1. Effect of concentration of aqueous extract from *Posidonia oceanica* in the germination of cucumber for shoot (a) and root (b) length. Results are expressed in average ± standard deviation (n = 12). All experiments were done in quintuplicate.

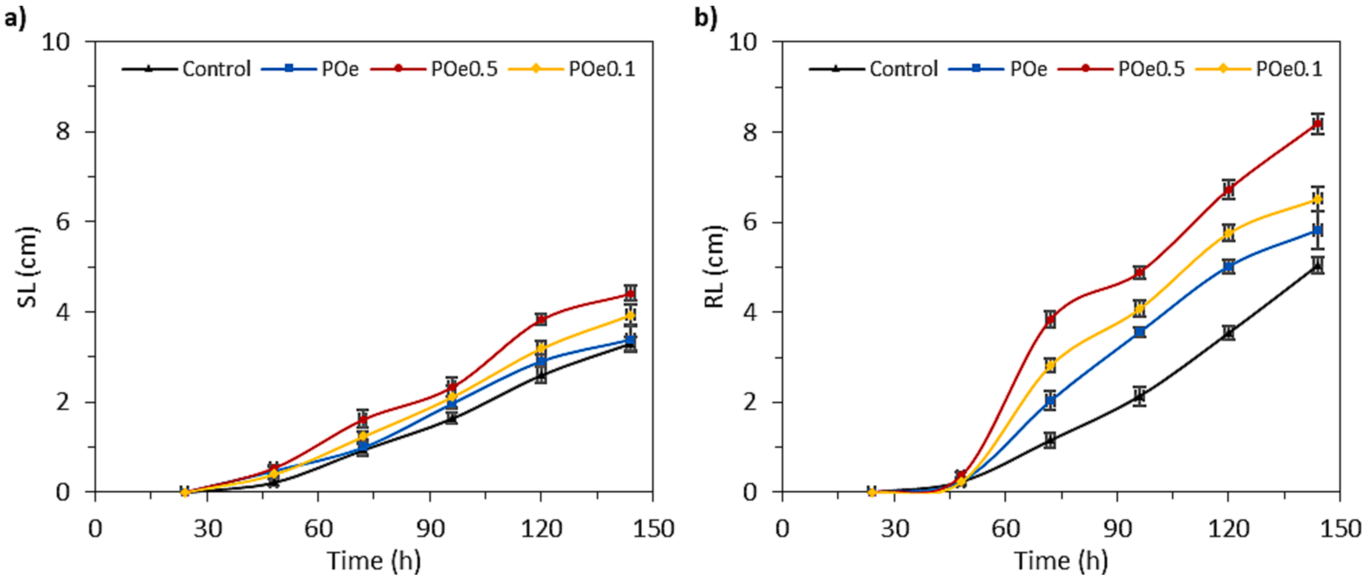

Fig. 2. Effect of concentration of aqueous extract from *Posidonia oceanica* in the germination of tomato for shoot (a) and root (b) length. Results are expressed in average ± standard deviation (n = 12). All experiments were done in quintuplicate.

Table 6

Effect of *Posidonia oceanica* extracts on germination and phytotoxicity of cucumber and tomato seeds experiment. Results are expressed in average ± standard deviation (n = 12). All experiments were done in quintuplicate. Mean values followed by the same letter in the same line do not differ significantly ($p < 0.05$) according to Duncan's test.

Variable	Cucumber				Tomato			
	Control	POe	POe0.5	POe0.1	Control	POe	POe0.5	POe0.1
%RSG	100±4a	93±7a	90±12a	92±9a	100±4a	95±7a	98±8a	98±7a
%RRG	100±5a	78±6b	97±6a	92±9a	100±7d	125±9c	176±5a	140±6b
%GI	100±6a	72±7b	88±11a	84±10a	100±4c	118±8b	173±13a	138±15b
NRG	0a	-0.07±0.02b	-0.10±0.03b	-0.08±0.01b	0a	-0.05±0.01c	-0.02±0.01b	0.02±0.01b
RER	0a	-0.22±0.06d	-0.06±0.02c	-0.02±0.01b	0a	0.26±0.03c	0.77±0.05a	0.41±0.04b

reduce toxicity in cucumber seeds. The secondary metabolites detected in the aqueous PO extract also play important roles on seed germination. Carbohydrates enhanced germination and seedling development by fulfilling energy requirements during seed germination (Johnson et al., 2011). This effect can be especially useful in small seeds with low storage capacity as tomato seeds meanwhile in larger seeds this effect could be less important. On the other hand, although phenolic compounds play a key role for plant stress self-protection (Heglmeier and Zidorn, 2010; Pratyusha, 2022; Zidorn, 2016), they may also inhibit germination (Tadic et al., 2014; Williams and Hoagland, 1982). This inhibition could be owed to the natural increase in phenol and flavonoid substances in germinated seeds (Chen et al., 2022). The excess of these active substances in the germination media could limit their synthesis so limiting germination, although this inhibitory effect has also been found in natural media to self-control of seed dormancy (Bhattacharyya et al., 1999). Polyphenol inhibited the glycolysis enzyme activity and the oxidative pentose phosphate pathway, impairing pine seeds germination. The phenol production during germination differs between plant species (Cevallos-Casals and Cisneros-Zevallos, 2010), as well as the effect of phenols on their germination ability which explains the different behavior of cucumber and tomato seeds. Indeed, not all polyphenols negatively affect germination. Some of them act promoting germination and plant development. Polyphenols extracted from spruce bark favored tomato seed germination although they shortened root length (Tanase et al., 2019). Those beneficial effects had been observed at concentrations of 0.25–1.0 g/L. Much lower than for PO extract. No negative effects were observed in our experiment, except for the moderate toxicity in cucumber plants despite their phenolic and flavonoid composition. Another issue to take into account is the osmotic pressure of the extract, due to the high solute concentration. This could slow down the entry of water into the seeds, which, in any case, would be easier in tomato seeds than in cucumber seeds, due to the differences in size. The diluted POe0.5 extract would be better to seed wetting and water absorption which is essential to germination This behavior was previously observed in tomato seeds treated with liquid seaweed extracts of *Ulva lactuca* and *Padina gymnospora* which showed improved germination at lower concentrations compared to the more concentrated extracts (Hernández-Herrera et al., 2014). In other experiment with foliar and seed application of aqueous extracts of the green algae *Acutodesmus dimorphus* was observed that there is a threshold concentration above which higher extract concentrations produces a decrease in the overall growth and development of tomato plants (García-González and Sommerfeld, 2016). In addition, similar effect has been observed in cucumber seeds with the application of garlic bulb extracts (Dong et al., 2012).

We did not find any description of the role of proteins or anthocyanins on seed germination. However, these substances are able to complex metal (Landi et al., 2015) favoring iron nutrition. Iron is essential in the synthesis of chlorophylls (Römheld et al., 1982), so the presence of chelating agents could be important in the first days of plant development. In the same way, Mg applied to seeds may not play a direct role on seed germination. Magnesium deficiency in plants gives low quality seeds and then, low germination rates (Ceylan et al., 2016), i.e., its main role is in seed production and not on seed activation, but as a nutrient and its central role in chlorophyll molecule, its occurrence in the culture media is important on seedling growth.

In recent years, BST derived from plant extracts rich in secondary metabolites have gained popularity as a means to improve seed germination and activate defense mechanisms against pests and diseases, thanks to their antifungal, antimicrobial, and antioxidant properties (Al-aghabary et al., 2005; García-Sánchez et al., 2012; Khan et al., 2010). This approach has helped to promote sustainable agriculture.

The results presented in this work showed that PO aqueous extracts had an impact on seed germination and vigor. Interestingly, the product with the intermediate concentration of the three proposed of secondary metabolites and inorganic elements showed the best values. This could be because, as mentioned above, a high concentration of secondary metabolites can be harmful for seed germination, but a low concentration of secondary metabolites due to dilution of the initial POe induced a minor impact on the different growth parameters.

The toxic effect observed with the concentrated extracts may be due to the higher amount of Na present in the form of NaCl. In particular, under saline stress, Na^+ can compete with other cations, such as Ca^{2+}, for binding sites in the root cell wall, which can hinder root development (Hoffmann et al., 2020). To counteract this effect, it has been suggested that in this regard, the PO extracts used this study had 6.3 and 3.3 mg/LSi (Table 2). Despite this, it appears that the higher Na concentration was more beneficial for the development of pepper and tomato seeds. This finding supports the conclusion reached by Bulgari et al. (2019), which suggested that the effectiveness of BST is not due to a single molecule but rather to the synergistic action of many bioactive molecules and compound.

Furthermore, the finding that diluted BST, which contained lower concentrations of Na, K, and phenolic compounds, performed better than the original BST in both types of seeds reinforces the hypothesis of the development of saline stress and phytotoxicity in the seeds. In this regard, different authors have reported that tomato seeds and plants exhibit a higher resistance to this type of stress compared to cucumber due to the weaker capacity of cucumber membranes to restrict excess uptake in salinity (Alpaslan and Gunes, 2001). However, these results disagreed with Abdel-Farid et al. (2020), who reported that tomato seeds are more sensitive to salinity stress than cucumber seeds.

5. Conclusions

The aqueous extract of *Posidonia oceanica* remains was effective in improve tomato seeds germination and had little effect on cucumber seeds. Specially promising results were obtained with the diluted extract Poe0.5. It demonstrated to be the extract with the optimum concentration as it showed the best germination parameters with the lowest toxicity. In spite of the different BST substances with contradictory effects on seed germination, this pool of compounds has resulted in an appropriate mean to improve tomato and cucumber plants development and in turn contribute to eliminate the lots of *Posidonia oceanica* remains recovered in summer months.

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CRediT authorship contribution statement

Borja Ferrández-Gómez: Conceptualization, Investigation, Writing – original draft. **Juana D. Jordá:** Supervision, Writing – review & editing. **Mar Cerdán:** Conceptualization, Methodology, Writing – review & editing. **Antonio Sánchez:** Conceptualization, Methodology, Supervision, Funding acquisition, Writing – review & editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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