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Optimizing germination and cultivation of edible halophytes using effluents from an IMTA system

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Abstract

BACKGROUND: Halophytes offer nature-based solutions to food insecurity and soil degradation, while their integration into integrated multi-trophic aquaculture (IMTA) systems promotes circular economy practices. This study aimed to optimize the germination and cultivation of edible halophytic species, namely *Limbarda crithmoides*, *Suaeda vera* and *Mesembryanthemum nodiflorum*, using effluents from an IMTA system. Germination was conducted under different substrates (perlite, vermiculite, coco peat, sand and combinations), irrigation (fresh or brackish water) and seed pre-treatments (scarification, gibberellic acid and thermal shock). Cultivation assays assessed plant responses to salinity (25.5–53.4 dS m^{-1}) and density (75–600 plants m^{-2}) using IMTA-derived aquaculture effluents for irrigation.

RESULTS: Salinity significantly reduced seed germination. *Limbarda crithmoides* had the highest germination rate (61.1%) in vermiculite under freshwater irrigation, whereas *S. vera* achieved optimal performance in a substrate of sand, organic peat and perlite. Thermal shock slightly improved *M. nodiflorum* germination. Moderate salinities (35.1–40.7 dS m^{-1}) resulted in higher survival and productivity, particularly for *S. vera*, which showed >86% survival and higher chlorophyll content. At high densities, *L. crithmoides* and *S. vera* maintained >75% survival, while *M. nodiflorum* at 75 plants m^{-2} effectively reduced nitrate and ammonia concentrations in effluents. All species produced biomass with adequate nutritional and microbiological profiles suitable for human consumption, rich in protein, dietary fiber and bioactive compounds. *Suaeda vera* at 300 plants m^{-2} exhibited the highest total content of phenolic compounds.

CONCLUSION: This study outlines a sustainable approach to cultivating edible halophytes in IMTA systems, with applications in saline farming, functional foods and aquaculture wastewater treatment.

Keywords: biosaline agriculture; sustainability; circular economy; phytoremediation

INTRODUCTION

Integrated multi-trophic aquaculture (IMTA) systems, which combine the cultivation of fed aquaculture species with organic and inorganic extractive species, aim to create a balanced system that enhances environmental sustainability, economic stability and social acceptability.[1] IMTA systems have been widely used for the cultivation of different species of trophic levels, including macroalgae (*Gracilaria vermiculophylla*) with turbot (*Scophthalmus rhombus*), sea bass (*Dicentrarchus labrax*) and sole (*Solea senegalensis*).[2]

The integration of salt-tolerant plants and halophytes in IMTA systems has gained increased interest in recent years as a sustainable production of foods with high commercial value.[3] Halophytes are plants capable of tolerating salinity levels exceeding 200 mmol L^{-1} NaCl.[4,5] Their ability to remediate nutrient-rich effluents from marine and coastal aquaculture systems makes them highly valuable for integration into IMTA systems, where

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they contribute to both environmental sustainability and the production of commercially valuable crops.[3,6-8] Additionally, halophytes are gaining popularity as gourmet vegetables, and are rich in nutrients and functional properties that may offer health benefits.[9,10]

To ensure the successful commercial production of edible halophytes, the cultivation procedures must be optimized at all stages of plant growth. However, there is a significant gap in halophyte plant cultivation, such as in knowledge about the germination process of halophytes under field conditions, with most research carried out under laboratory conditions. Intraspecific competition is another important factor to consider for commercial cultivation, as it can influence biomass production, flowering and survival.[11] The effects of plant density on growth and yield have been already investigated in halophyte species such as *Suaeda salsa*, *Inula racemosa* and *Atriplex prostrata*.[12-14] Increasing plant density may lead to size variation among plants, with few large individuals and numerous small plants increasing this difference when there is competition for light, or it may lead to an equal decline in biomass production of plants.[14]

Limbarda crithmoides (L.) Dumort (syn. *Inula crithmoides* L.) (golden samphire), *Suaeda vera* Forssk. ex J.F. Gmel. (shrubby sea-blite) and *Mesembryanthemum nodiflorum* L. (slenderleaf ice-plant) are edible succulent halophytes with commercial interest due to their nutritional and bioactive profiles. *Limbarda crithmoides* has a high protein, is rich in vitamins B_1 and B_6, and exhibits antioxidant properties attributed to the presence of phenolic acids (gallic, syringic, salicylic, caffeic, coumaric and rosmarinic acids) and flavonoids (epicatechin, epigallocatechin gallate, catechin hydrate, quercetin and apigenin).[15-17] Studies on cultivation methods for *L. crithmoides* have primarily focused on agronomic assessments under greenhouse conditions, evaluating various substrates (commercial peat, garden soil, saline soil and sand from a littoral dune)[18] and salinity (0.5–80 dS m^{-1}).[19] *Suaeda vera* thrives in hypersaline environments and has been successfully cultivated both in abandoned salt pans irrigated with estuarine water and in controlled microcosm assays using the same source. In both cases, higher productivities were generally observed at moderate salinities ranging from 23 to 36 dS m^{-1}.[20-22] Additionally, it is rich in α-linolenic, palmitic and linoleic acids, and exhibits notable antioxidant and anti-inflammatory properties.[23,24] *Mesembryanthemum nodiflorum* has a high protein, fiber, sodium and potassium content, along with notable antibacterial, antifungal, cytotoxic and antioxidant properties.[17,25,26] Growing interest in this species as a novel food is driving its commercial cultivation, with recent studies confirming its successful greenhouse production in soilless systems using saline water.[17]

This study aimed to advance sustainable saline cultivation techniques for *L. crithmoides*, *S. vera* and *M. nodiflorum*, conducted with effluents from an IMTA system. Germination conditions were optimized using fresh and saltwater irrigation, different substrates and treatments to enhance germination rates. Following this, optimal salinity levels and plant densities were determined for maximum productivity using effluent from the IMTA system. Key growth metrics (productivity, survival and photosynthesis) and water quality parameters were analyzed, along with a comprehensive evaluation of the plants' nutritional and chemical compositions. This research offers valuable insights for optimizing germination and cultivation conditions, thereby contributing to the large-scale commercial production of these halophytes with high-quality food potential.

MATERIALS AND METHODS

Seed collection/acquisition

Seeds of *S. vera* were purchased from Semillas Cantueso SL (Córdoba, Spain), while those from *L. crithmoides* and *M. nodiflorum* were collected from Olhão and Faro in the South of Portugal (coordinates: 37° 01′ 12.0″ N, 7° 53′ 04.8″ W, and 37°01′ 56.3″ N, 7° 59′ 58.7″ W, respectively). Seeds were separated from the mature inflorescences and stored under dry conditions in darkness at room temperature (RT; approximately 20 °C), and used in the assays throughout April–December 2022.

Germination assays

General germination conditions

Germination was conducted from April to May in a greenhouse located at the University of Algarve (Faro, Portugal). At the time of the experiment, the average air temperature was 25.4 °C, with 8.5 and 58.1 °C as minimum and maximum, respectively, and an average relative air humidity (RH) of 57.1%, with 14.9% and 95.3% as minimum and maximum, respectively. Four replicates of 40 seeds each were sown in Styrofoam trays of 198 cavities (one seed per alveolus, 53 mm deep and 22.05 cm^3 cell capacity) filled with the corresponding substrate and irrigated every 2 days according to the experiment with fresh or saline water from a well (conductivity 20.1 dS m^{-1}). The substrates used were based on the previous work of Castañeda-Loaiza *et al.*;[27] namely perlite, vermiculite, coco peat and sand, alone and in combinations (coco peat and perlite, 1:1 v/v; coco peat and vermiculite, 1:1 v/v; perlite and vermiculite, 1:1 v/v; and sand, organic peat and perlite, 1:1:1 v/v/v). Germination was monitored twice a week for 28 days, and seeds were considered germinated when the radicle emerged 5 mm from the substrate. Results were expressed as germination rate (GR), corresponding to the ratio between the total number of germinated seeds and the total number of seeds multiplied by 100, and the mean germination time (MGT), as the sum of the product of the number of seeds germinated in each interval and the incubation period (in days) at that time point, divided by the total number of germinated seeds.

Seed treatments

Species with GR lower than 50% were submitted to seed treatments to improve germination.[27] Four replicates of 20 seeds were subjected to chemical treatment (soaking in gibberellic acid – GA3, 1 g L^{-1} for 24 h at RT), chemical scarification (soaking in sulfuric acid – H_2SO_4, 50% for 10 min at RT), mechanical scarification (soaking in distilled water and placed for 15 min in an ultrasonic bath (USC-TH, VWR, Carnaxide, Portugal) at RT), water soaking for 24 h at RT, and several thermal shock treatments soaking in distilled water (60 °C for 10 min and 24 h in water: W60; 75 °C for 20 min: W75; 75 °C for 20 min and at −4 °C for 20 min: W75W-4; −4 °C for 20 min and 75 °C for 20 min: W-4 W75; and −4 °C for 20 min: W-4). Seeds submitted to treatments were sown in the substrate with irrigation water salinity having the best GR based on previous results. Germination was monitored twice a week for 28 days, and seeds were considered germinated when the radicle emerged 5 mm from the substrate.

Cultivation

Effect of irrigation salinity on agronomic features and biochemical properties of biomass

Plants were sown in Styrofoam trays and placed in a tank filled with the corresponding salinity water, using the best substrate and irrigation water and with the best seed treatment from previous assays. After 1 month, plants of at least 5 cm height were gradually adapted to the different irrigation salinities obtained by mixing aquaculture effluents of an IMTA system from an outdoor sea bream and sea bass tank (53.4 dS m^{-1}) with well water (25.5 dS m^{-1}). A total of four salinity treatments were used, with the following electrical conductivity values: 25.5, 35.1, 40.7 and 53.4 dS m^{-1}. Irrigation was carried out by refilling the evaporated water with the corresponding salinity water every week until the end of the experiment. Triplicates of a minimum of 20 plants of each species were treated in each experiment, for 12 weeks. Agronomic features were evaluated as survival, expressed as the percentage of plants surviving at the end of the experiment, and productivity, expressed as grams of fresh weight of plant after the experiment per square meter (g m^{-2}, FW). The physicochemical parameters of the water used for irrigation were also determined, namely pH, salinity (g L^{-1}), total dissolved solids (g L^{-1}), total suspended solids (mg L^{-1}), dissolved oxygen (mg L^{-1}), dissolved organic nitrogen (mg L^{-1}), nitrite (mg L^{-1}), nitrate (mg L^{-1}), total ammonium nitrogen (mg L^{-1}), total nitrogen (mg L^{-1}) and phosphate (mg L^{-1}) (Table 1). The shoots of the plants were collected, frozen at −20 °C, freeze-dried and stored in bags in a desiccator until use (see 'Biochemical properties of produced biomass' below).

Effect of plant density on agronomic features and chemical properties of biomass, and the physicochemical parameters of the irrigation water

Plants were cultivated in Styrofoam trays and placed in a tank filled with the corresponding salinity water, using the best conditions, from previous assays, including substrate, seed treatment and irrigation salinity. Plants of at least 5 cm in height were transplanted to pots with a volume of 23.8 L at several plant densities, namely 75, 150, 300 and 600 plants m^{-2}, and triplicates of a minimum of 20 plants of each halophyte were grown for 12 weeks. Irrigation was carried out by refilling the evaporated water with the corresponding salinity water every week until the end of the experiment. Survival was determined as the percentage of plants surviving after the experiment and productivity was expressed as g m^{-2} (FW). The physicochemical properties of irrigation water and post-cultivation water were determined, as detailed under 'Germination', below (Table 1). The aerial parts of the plants were collected, frozen at −20 °C, freeze-dried and stored in bags in a desiccator until use (see 'Biochemical properties of produced biomass', below).

Biochemical properties of produced biomass

Nutritional properties

Fresh biomass was used to determine the moisture content by drying in a ventilated oven at 105 °C for 16 h.[9] Ash was determined by incinerating lyophilized biomass in a muffle furnace at 600 °C for 6 h,[9] while crude fat was determined according to a modified protocol of the Bligh and Dyer method.[28] The total nitrogen content was determined in a CHN Elemental Analyzer Vario EL III (Elementar Analysensysteme GmbH, Langenselbold, Germany), and crude protein was estimated by multiplying the nitrogen content by a factor of 6.25.[29] Total carbohydrates were estimated by difference of ash, crude fat and crude protein.[30] Metabolizable energy (ME) was calculated using the Atwater conversion factors according to FAO, using Eqn (1):

$$\mathrm{ME} = 9\times(\text{crude fat}) + 4\times(\text{protein}) + 4\times(\text{carbohydrate}) \qquad (1)$$

The fiber content in terms of neutral detergent fiber (NDF), acid detergent fiber (ADF) and acid detergent lignin (ADL) was determined according to ISO 16472:2006, ISO 13906:2008 and ISO 13906:2008, respectively. Cellulose was estimated by the difference between ADF and ADL and hemicellulose by the difference between NDF and ADF. Results are expressed as g kg^{-1} (FW) for moisture, as kcal kg^{-1} (DW) for metabolizable energy (ME), and as g kg^{-1} (DW) for the remaining parameters.

Biomass extraction

Freeze-dried biomass was mixed with methanol (1:40 w/w) and extracted overnight, at RT, with constant stirring. Obtained extracts were filtered (Whatman No. 4), the solvent was evaporated and the dried extract resuspended in methanol, to obtain a working solution at a concentration of 10 mg mL^{-1}. This extract was evaluated for pigment content (see 'Pigment content of the produced biomass', below) and for phytochemical profile (see 'Phytochemical profiling of the extract by high-performance liquid chromatographic analysis', below).

Table 1. Physicochemical parameters of the irrigation water from aquaculture effluents used in the experiments

	Conductivity			
Parameter	25.5 dS m^{-1}	35.1 dS m^{-1}	40.7 dS m^{-1}	53.4 dS m^{-1}
Salinity (g L^{-1})	13.0	19.5	20.1	27.7
TDS (g L^{-1})	25.5	35.1	40.9	53.4
TSS (mg L^{-1})	21.2	30.7	22.6	35.7
DO (mg L^{-1})	6.70	6.30	6.10	5.70
DON (mg L^{-1})	1.40	1.40	0.70	1.40
Nitrite (NO_2) (mg L^{-1})	0.04	0.03	0.02	0.01
Nitrate (NO_3) (mg L^{-1})	0.19	0.17	0.11	0.09
TAN (mg L^{-1})	0.20	0.14	0.10	0.04
TN (mg L^{-1})	1.61	1.56	0.80	1.45
Phosphate (PO_4^{3-}) (mg L^{-1})	0.01	0.05	0.01	0.02

Abbreviations: TDS, total dissolved solids; TSS, total suspended solids; DO, dissolved oxygen; DON, dissolved organic nitrogen; TAN, total ammonium nitrogen (TAN = $NH_3 + NH_4^+$); TN, total nitrogen.

Pigment content of the produced biomass

The optical density of the methanol extracts was measured at 470, 653 and 666 nm in a spectrophotometer (U-2000, Hitachi, Tokyo Japan). Chlorophylls *a* and *b* and total carotenoids were determined from Eqns (2)–(4), respectively,[31] and results are expressed as µg g^{-1} DW:

$$\text{Chlorophyll } a = 15.65 \times A_{666} - 7.34 \times A_{653} \quad (2)$$

$$\text{Chlorophyll } b = 27.05 \times A_{653} - 11.21 \times A_{666} \quad (3)$$

$$\text{Total carotenoid} = (1000 \times A_{470} - 2.86 \times C_a - 129.2 \times C_b)/245 \quad (4)$$

where A_{666}, A_{653} and A_{470} are the absorbance values measured at 666, 653 and 470 nm, respectively, and C_a and C_b are the content of chlorophylls *a* and *b*.

Microbiological hygienic parameters

On the same day of the collection, 10 g fresh biomass was cut and immersed in 90 mL Ringer's solution (Himedia, Mumbai, India), previously autoclaved at 121 °C for 15 min. The mixture was homogenized for 1 min in a stomacher (Model 400 Circulator, Seward Ltd, Worthing, UK). Several dilutions were prepared, and aliquots were inoculated using either the pour-plate or spread-plate technique and incubated accordingly, depending on the method. Aerobic mesophilic microorganisms were determined according to ISO 4833:2013 in Plate Count Agar (PCA) culture medium (Scharlau, Barcelona, Spain), inoculating 1 mL of each decimal dilution (up to 10^{-5}) and incubating at 30 °C for 3 days. Aerobic psychrotrophic microorganisms were counted based on ISO 17410:2001 in PCA, by spreading 0.1 mL of the aliquots of each decimal dilution (up to 10^{-5}) and incubating for 10 days at 6.5 °C. Filamentous fungi and yeasts were counted in Dichloran Rose Bengal Chloramphenicol Agar (DRBCA) (Scharlau, Barcelona, Spain) by spreading 0.1 mL of the aliquots of each decimal dilution (up to 10^{-3}) and incubating at 25 °C for 5 days, following ISO 21527-1:2008. *Escherichia coli* was cultured on Tryptone Bile X-Glucuronide (TBX) culture medium (Scharlau), inoculating 1 mL of each decimal dilution (up to 10^{-2}) and incubating at 44 °C for 24 h, according to ISO 16649-2:2001. Coagulase-positive *Staphylococcus* spp. were evaluated using Baird Parker Agar (Scharlau) by spreading 0.1 mL of each aliquot of decimal dilutions (up to 10^{-2}) and incubating at 37 °C for 48 h, following ISO 6888-1:1999. Duplicates were prepared for each sample and each dilution. Results are expressed as $\log_{10}$ colony-forming units (CFU) per gram of fresh weight (log CFU g^{-1}).

Phytochemical profiling of the extract by high-performance liquid chromatographic analysis

The methanol extracts at a concentration of 10 mg mL^{-1} in a mixture of 90% ultrapure water and 10% methanol were analyzed by high-performance liquid chromatography (HPLC) with diode array detection (1260 Infinity II Series LC system, Agilent Technologies, Waldbronn, Germany), constituted by the following modules: 1260 quaternary pump (G7111B), 1260 Vialsampler (G7129A) and the 1260 diode array detector (G7115A). Data acquisition and instrumental control were performed using OpenLab CDS software (version 2.6, Agilent Technologies). Analyses were performed on a Kinetex C18 column, 15 × 0.46 cm, 5 µm particle size (Phenomenex, Torrance, CA, USA). The mobile phase consists of a mixture of MeOH (solvent A) and 2.5% acetic acid aqueous solution with the following gradient: 0–5 min, 10% A; 5–10 min, 10–30% A; 10–40 min, 30–90% A; 40–45 min, 90% A; 45–55 min, 90–10% A; and 55–60 min, 10% A; using a flow of 0.5 mL min^{-1}. The injection volume was 20 µL with a draw speed of 200 µL min^{-1}. The detector was set at 255, 280, 320 and 350 nm. For identification, the retention parameters of each assay were compared with the standard controls, and the peak purity with the UV–visible spectral reference data. The levels of the different compounds were determined by extrapolating from calibration standard curves. Commercial standards of gallic, protocatechuic, gentisic, 4-hydroxybenzoic, vanillic, syringic, ellagic, salicylic, chlorogenic, caffeic, coumaric and ferulic acids, and catechin, epicatechin, hyperoside, rutin, quercitrin, apigenin and chrysin were prepared in ethanol (1.000 mg L^{-1}) and diluted with ultrapure water in the desired concentration. Results are expressed in milligrams of pigment per gram of dry weight (mg g^{-1} DW).

Statistical analysis

Analysis of variance (ANOVA) was used to test the significance of the parameters and significant ($P < 0.05$) differences were performed using Tukey's HSD. All results were analyzed using the XLSTAT statistical package (v.2015.6.01.23865, Addinsoft, New York, NY, USA). Results were expressed as mean ± standard error of the mean (SEM).

RESULTS AND DISCUSSION

Germination

Results of the influence of salinity, substrate and seed treatment on the germination of *L. crithmoides*, *S. vera* and *M. nodiflorum* are summarized in Table 2. Salinity had a significant negative impact on seed germination. *Limbarda crithmoides* showed the highest germination rate (GR) in vermiculite (61.1%), followed by *S. vera* in sand, organic peat and perlite mixes (40.0%), and *M. nodiflorum* in sand or perlite and vermiculite (18.9%), all under freshwater irrigation. *Mesembryanthemum nodiflorum* exhibited the lowest GR (<20%). When irrigated with saline water, germination occurred only in sand, organic peat and perlite mixtures, with a minimal GR of 2.2% and the longest MGT (28.0 days). A similar trend has been found in other halophytes, such as *Mesembryanthemum crystallinum*, *Medicago marina* and *Ammophila arenaria*, where germination only occurred under freshwater irrigation.[27] *Suaeda vera* germinated when irrigated with saltwater, but, nevertheless, GR remained below 15%. MGT is a measure of the rate and time spread of germination.[32] In this study, a high GR did not correspond to a shorter MGT for *L. crithmoides* and *S. vera*. Instead, the most successful germinations took longer to initiate, indicating that optimal germination required more time to commence. In contrast, germination in saltwater in *L. crithmoides* was superior in sand, organic peat and perlite mixes; however, it took longer to achieve germination (MGT, 23.1 days). Castillo *et al.*[33] have also shown that germination of *L. crithmoides* in high salinities is possible, up to 600 mmol L^{-1} salinity (36 ppt).[33] High temperature and humidity fluctuations in the greenhouse were experienced during the study, which could have affected seed germination. This variability could explain the differences observed compared to studies conducted under controlled conditions.[22] Several treatments were applied to *S. vera* and *M. nodiflorum* to improve their germination rates, as both species exhibited GRs below 50% (Table 2). No significant improvement was observed in the germination of *S. vera*, possibly due to its

Table 2. Germination rate (GR, %) and mean germination time (MGT) of *Limbarda crithmoides*, *Suaeda vera* and *Mesembryanthemum nodiflorum* under controlled greenhouse conditions

		L. crithmoides		*S. vera*		*M. nodiflorum*	
Substrate	Irrigation water	GR	MGT	GR	MGT	GR	MGT
Perlite	Freshwater	22.2 ± 1.5bcde	8.80 ± 0.0b	36.7 ± 0.6abcd	14.6 ± 0.3b	12.2 ± 0.9ab	10.3 ± 0.3c
	Saltwater	1.10 ± 0.3e	28.0 ± 0.3a	3.30 ± 0.0e	23.5 ± 0.3ab	—	—
Vermiculite	Freshwater	61.1 ± 0.9a	12.0 ± 2.5b	34.4 ± 1.5abcd	12.2 ± 0.3b	3.30 ± 0.0b	13.5 ± 0.0bc
	Saltwater	36.7 ± 2.1abcd	22.7 ± 0.7a	4.40 ± 0.3e	15.8 ± 0.0ab	—	—
Coco peat	Freshwater	54.4 ± 2.6a	14.1 ± 0.0b	20.0 ± 1.2abcde	15.8 ± 0.0ab	2.20 ± 0.3b	22.5 ± 0.3ab
	Saltwater	4.40 ± 0.3e	27.3 ± 0.0a	15.6 ± 0.9bcde	26.5 ± 0.7a	—	—
Sand	Freshwater	56.7 ± 1.2a	14.3 ± 0.7b	38.9 ± 0.9ab	19.1 ± 1.2ab	18.9 ± 1.2a	10.7 ± 0.7c
	Saltwater	-	-	1.10 ± 0.3e	24.0 ± 0.0ab	—	—
Coco peat and perlite (1:1 v/v)	Freshwater	56.7 ± 1.0a	13.6 ± 2.3b	37.8 ± 4.1abc	14.5 ± 0.9b	14.4 ± 1.3ab	11.9 ± 0.7c
	Saltwater	18.9 ± 1.8cde	23.4 ± 0.7a	14.4 ± 0.9cde	19.0 ± 0.6ab	—	—
Coco peat and vermiculite (1:1 v/v)	Freshwater	53.3 ± 2.9a	12.9 ± 1.0b	24.4 ± 1.3abcde	20.8 ± 0.7ab	8.90 ± 0.3ab	15.8 ± 0.3bc
	Saltwater	8.90 ± 0.3de	26.0 ± 0.3a	10.0 ± 0.6e	20.2 ± 0.3ab	—	—
Perlite and vermiculite (1:1 v/v)	Freshwater	47.8 ± 2.3ab	11.7 ± 1.7b	23.3 ± 1.2abcde	14.5 ± 0.0b	18.9 ± 0.7a	7.10 ± 0.6c
	Saltwater	21.1 ± 0.9bcde	24.4 ± 0.9a	4.40 ± 0.9e	21.2 ± 0.7ab	—	—
Sand, organic peat and perlite (1:1:1 v/v/v)	Freshwater	37.8 ± 2.6abc	10.6 ± 0.7b	40.0 ± 1.7a	17.6 ± 0.3ab	16.7 ± 1.0a	9.10 ± 0.3c
	Saltwater	40.0 ± 0.6abc	23.1 ± 2.0a	13.3 ± 0.6de	23.6 ± 0.6ab	2.20 ± 0.3b	28.0 ± 0.3a

'—", species without germination. In each column, values followed by different letters are significantly different ($P < 0.05$).

crustaceous, scalariform seed coat, which may act as a physical barrier to germination. This contrasts with previous studies where similar treatments effectively enhanced germination in other halophytes, such as *Polygonum maritimum*, *Medicago marina* and *A. arenaria*.[27,34] The application of thermal shock to *M. nodiflorum*, achieved by soaking the seeds in distilled water at 60 °C for 10 min, followed by 24 h at RT, resulted in an improvement of the germination improvement rate (GIR) of 7.8% (Table 3). Similarly, other authors have used hot water as pre-treatment to break seed dormancy in other halophyte species, such as *Acacia cyclops* A. Cunn. ex G. Dona.[35]

Effect of irrigation salinity on agronomic features and biochemical properties of cultivated plants

Plantlets were cultivated on the previously identified optimal substrate under varying salinity levels and assessed for survival, biomass production and biochemical properties. Table 1 summarizes the physicochemical characteristics of the irrigation water, while Fig. 1 illustrates plants' survival and productivity across different salinity levels. Higher productivity was observed at intermediate salinity levels (950.2 g FW m^{-2} at 35.1 dS m^{-1} for *L. crithmoides*, 5833.3 g FW m^{-2} for *S. vera* and 965.9 g FW m^{-2} at 40.7 dS m^{-1} for *M. nodiflorum*). *Suaeda vera* stood

Table 3. Effect of seed pre-treatments on germination rate (GR, %) and germination improvement rate (GIR, %) of *Suaeda vera* and *Mesembryanthemum nodiflorum*, relative to the untreated control

	S. vera			*M. nodiflorum*		
Seed treatment	GR	GIR	MGT	GR	GIR	MGT
GA3 1 g L^{-1} for 24 h	20.0 ± 0.6abc	−20.0	18.4 ± 0.1a	18.3 ± 0.9ab	−0.6	18.6 ± 0.9a
SA 50% for 10 min	26.7 ± 0.9a	−13.3	19.0 ± 0.5a	23.0 ± 0.3a	+6.1	18.0 ± 1.1a
Ultrasound for 15 min	—	—	—	8.30 ± 0.3b	10.6	16.3 ± 0.9a
WRT for 24 h	13.3 ± 0.3abc	−26.7	22.5 ± 0.8a	23.3 ± 0.3a	+4.4	16.0 ± 0.4a
W60	—	-	—	26.7 ± 0.9a	+7.8	16.9 ± 0.5a
W75	6.70 ± 0.3c	−33.3	16.7 ± 2.8a	15.0 ± 0.6ab	−3.9	18.0 ± 1.1a
W75W-4	10.0 ± 0.6bc	−30.0	20.5 ± 3.0a	25.0 ± 0.0a	+6.1	18.0 ± 0.4a
W-4 W75	23.3 ± 0.9ab	−16.7	20.3 ± 0.1a	20.0 ± 0.6ab	+1.1	17.2 ± 0.6a
W-4	20.0 ± 0.6abc	−20.0	22.3 ± 0.0a	21.7 ± 0.3ab	+2.8	17.6 ± 0.6a

'—', species without germination. In the same column, values followed by different letters are significantly different at $P < 0.05$.
Abbreviations: GA3, gibberellic acid; SA, sulfuric acid; WRT, water at room temperature (approximately 20 °C); W60, water at 60 °C for 10 min followed by soaking for 24 h; W75, water at 75 °C for 20 min; W75W-4, water at 75 °C for 20 min and at −4 °C for 20 min; W-4 W75, water at −4 °C for 20 min and at 75 °C for 20 min; W-4, water at −4 °C for 20 min.

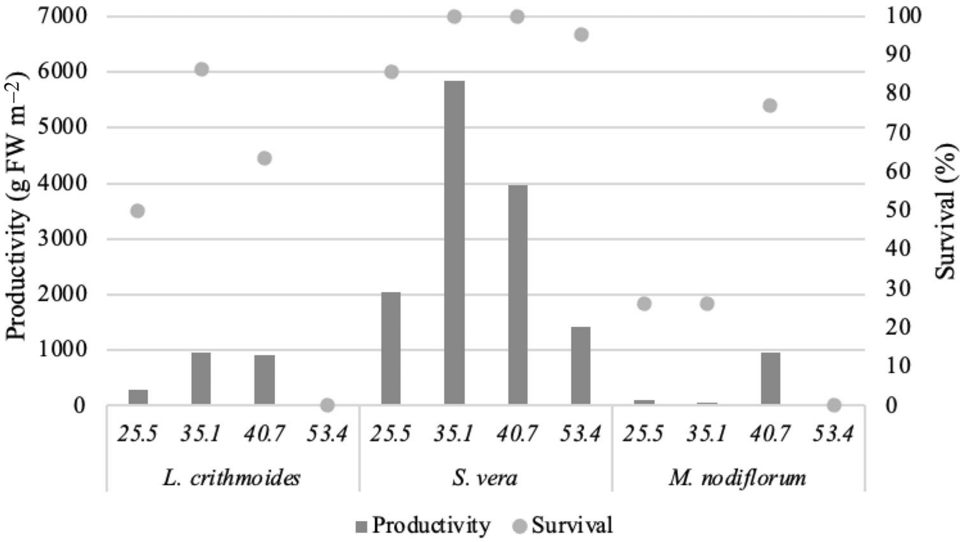

Figure 1. Survival (%) and productivity (g FW m^{-2}) of *Limbarda crithmoides*, *Suaeda vera* and *Mesembryanthemum nodiflorum* irrigated with saltwater dilutions (25.5, 35.1, 40.7 and 53.4 dS m^{-1}) from an integrated multi-trophic aquaculture (IMTA) system.

out in the range of salinities with productivity from 1420.5 to 5833.3 g FW m^{-2}. Other salinity studies in *S. vera* have shown that the highest yields are obtained with salinities of 23–36 dS m^{-1} due to osmotic adjustment by a greater accumulation of ions (Na^+, Cl^- and Ca^{2+}) and organic osmolytes (free amino acids and proline).[20]

High salinity (53.4 dS m^{-1}) resulted in the lowest survival rates for *L. crithmoides* and *M. nodiflorum* (Fig. 1). In a work by Lima *et al.*,[17] *M. nodiflorum* showed the maximum relative productivity in lowest salinities: 35–110 mmol L^{-1} NaCl (100% and 42.9%, respectively).

Halophytes thrive in saline environments through mechanisms such as osmotic stress tolerance, ion exclusion and tissue-level salt accumulation.[36] However, each species has an optimal salinity range, beyond which growth and productivity decline significantly. An increase in survival was observed in *S. vera* at intermediate and high salinity levels (95–100%), which may be linked to its high capacity to translocate salts from the roots to the shoots where they are stored, being able to withstand hypersaline soils of 75.2 dS m^{-1}.[20,37]

Figure 2 shows the pigment content of methanol extracts of biomass of cultivated plants. Chlorophyll *b* showed the highest concentrations, with *S. vera* reaching 153.37 μg g^{-1} DW at 25.5 dS m^{-1}, followed by *M. nodiflorum* at 106.81 μg g^{-1} DW at 35.1 dS m^{-1}, and *L. crithmoides* at 81.60 μg g^{-1} DW at 25.5 dS m^{-1}. Salinity stress resulted in a decrease in the content of chlorophyll *a* and *b* compared to the lowest salinity (25.5 dS m^{-1}) in *L. crithmoides* and *S. vera*. In *M. nodiflorum* the highest chlorophyll *a* and *b* values were detected at 35.1 dS m^{-1}, suggesting different adaptive mechanisms of each species when

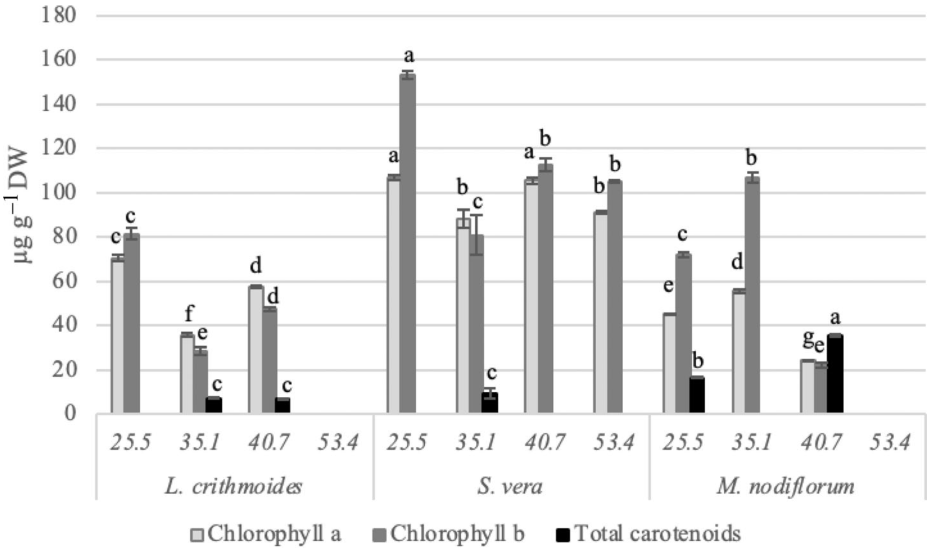

Figure 2. Chlorophylls and total carotenoids (μg g^{-1} DW) of *Limbarda crithmoides*, *Suaeda vera* and *Mesembryanthemum nodiflorum* irrigated with saltwater dilutions (25.5, 35.1, 40.7 and 53.4 dS m^{-1}) from an integrated multi-trophic aquaculture (IMTA) system. Letters highlight significant differences ($P < 0.05$) between all species for the same parameter.

exposed to salinity stress. In a work by Mohammadi and Kardan,[38] the levels of chlorophyll *a* and *b* in *Salicornia europaea* showed the highest values (0.309 and 0.274 mg g^{-1} FW, respectively) in 100 mmol L^{-1} and a decrease was observed when plants were submitted to salinities of 300 mmol L^{-1}.

The highest levels of total carotenoids (7.04–35.7 μg g^{-1} DW) were generally found at intermediate salinity levels in all species. Similar findings were observed, for example, in *Salicornia* species (2.8 mg g^{-1} FW), which exhibited higher accumulation of carotenoid content when subjected to moderate (100–200 mmol L^{-1} NaCl) salinity stress.[38] Carotenoids are secondary metabolites that play a crucial role in protecting plants from oxidative stress induced by salinity.[39] The results differ from those found in *L. crithmoides*, which decreased the total carotenoid content along with salinities from 3.30 mg g^{-1} DW at 0 mmol L^{-1} NaCl to 1.65 mg g^{-1} DW at 600 mmol L^{-1} NaCl.[40] Based on the results obtained in the agronomic parameters, the following irrigation salinities were established for its growth in the following tests: 40.7 dS m^{-1} for *M. nodiflorum* and 35.1 dS m^{-1} for *L. crithmoides* and *S. vera*.

Effect of plant density on agronomic features and chemical properties of cultivated plants

The highest survival rates (>75%) were observed in *L. crithmoides* in all densities and *S. vera* at 150 and 300 plants m^{-2} (Fig. 3). No relation between survival and productivity was observed. A strong density-dependent effect was observed on plant productivity at higher densities, mainly in *L. crithmoides*, which increased at 600 plants m^{-2} (1597.0 g FW m^{-2}), followed by *S. vera* at 300 plants m^{-2} (863.5 g FW m^{-2}) and *M. nodiflorum* at 600 plants m^{-2} (520.5 g FW m^{-2}). An opposite trend was observed in the halophyte *Suaeda salsa* cultivated in a greenhouse at 200 mmol L^{-1} NaCl, which decreased from 2.9 to 1.7 g biomass when the plant density increased from 1 to 4 plants per pot.[11,12] Similarly, as is discussed later, the post-cultivation water does not exhibit significant differences in nutrient concentrations across plant densities, suggesting limited competition for nutrients supplied by the IMTA system.

The highest total carotenoid content was observed in *M. nodiflorum* biomass at a density of 600 plants m^{-2} (114.1 μg g^{-1} DW), followed by *S. vera* at a density of 300 plants m^{-2} (77.1 μg g^{-1} DW) (Fig. 4). A decrease in carotenoid content is typically expected at higher plant densities due to reduced light availability. Wu *et al.*[41] reported that in *Perilla frutescens* (L.) Britt. increased planting density led to lower light intensity, thereby reducing photosynthetic efficiency. However, in *M. nodiflorum*, a slight increase in carotenoid content was observed, which may be attributed to stress-induced alterations in carotenoid biosynthesis, leading to enhanced accumulation as a protective response.[42] *Limbarda crithmoides* showed the highest values of chlorophyll *a* and *b* when grown at the highest plant density of 600 plants m^{-2} (850.1 and 2017.8 μg g^{-1} DW, respectively). The same tendency was observed in *L. crithmoides* propagated *in vitro* with increasing NaCl concentrations to 200 mmol L^{-1} NaCl.[43] Environmental stress is considered the most critical factor on plant growth parameters, including photosynthetic capacity, which may explain the increase in chlorophyll content when exposed to higher plant densities.[44]

Effect of plant density on the physicochemical parameters of the input irrigation water and output irrigation water

Results regarding the influence of plant density on the physicochemical parameters of the input irrigation water and output irrigation water are shown in Table 4. As expected, electrical conductivity (EC) and total dissolved solids (TDS) increased in the post-cultivation water. This increase can be explained by several factors, such as salt excretion by halophyte roots to maintain osmotic balance, leading to salt accumulation in the surrounding medium.[45,46] Furthermore, water evaporation can concentrate salts in the remaining water and the weekly irrigation procedure, leading to a progressive accumulation of solutes. The conductivities obtained in the post-cultivation water were higher than 60 dS m^{-1}, considered as very saline waters (EC of seawater, 45 dS m^{-2}),[47] reaching the maximum value for *S. vera* in 300 plants m^{-2} (81.6 dS m^{-1}).

Total suspended solids (TSS) increased, reaching maximum values at high plant densities for the three species. In a study by

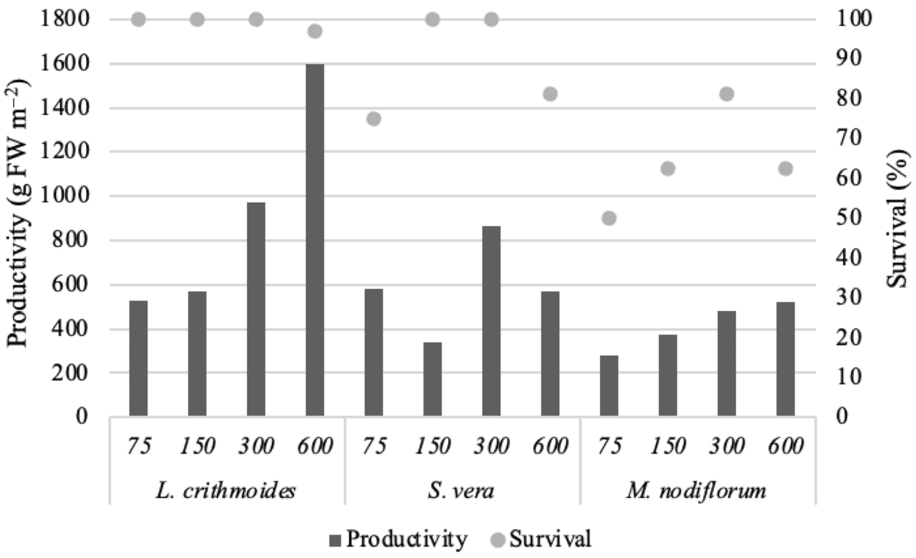

Figure 3. Survival rate (%) and productivity (g FW m^{-2}), of *Limbarda crithmoides*, *Suaeda vera* and *Mesembryanthemum nodiflorum* under varying plant densities (75, 150, 300 and 600 plants m^{-2}), irrigated using effluents from an integrated multi-trophic aquaculture (IMTA) system.

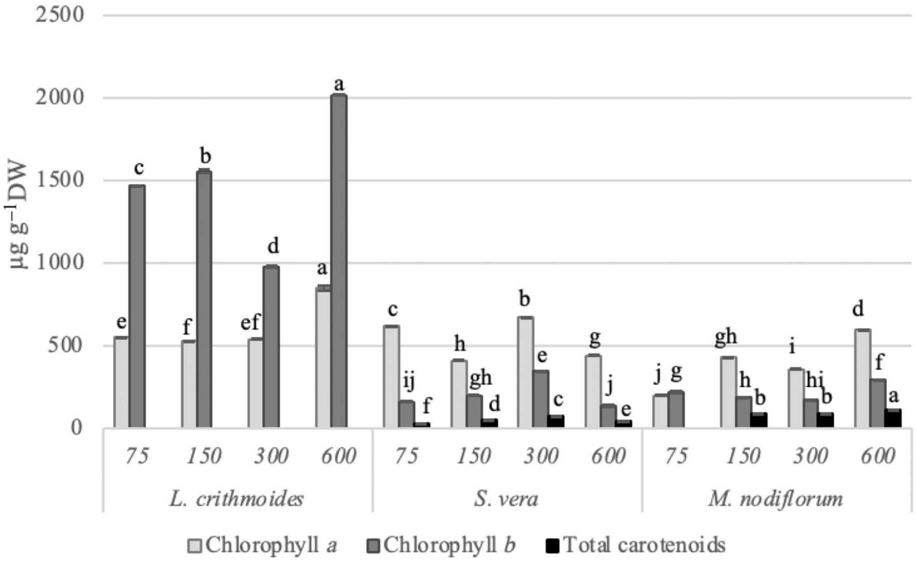

Figure 4. Photosynthetic parameters (μg g^{-1} DW) of *Limbarda crithmoides*, *Suaeda vera* and *Mesembryanthemum nodiflorum* under several plant densities (75, 150, 300 and 600 plants m^{-2}). Letters highlight significant differences ($P < 0.05$) between all species for the same parameter.

Sudarsono *et al.*[48] on the Gajah Mungkur Reservoir, where plant density was monitored in 2013, 2015 and 2017 using satellite images, it was observed that an increase in vegetation density led to higher TSS levels in the water. The relationship between TSS and plant density remains unclear. Coleman *et al.*[49] suggested that it may result from root exudates acidifying the medium, microbial ion release from decomposing roots or increased transpiration concentrating the effluent.

A decrease in the nitrate and total ammonia nitrogen (TAN) content was expected since these compounds are assimilated by plants.[50] An effective absorption of TAN was observed in all three species, especially *M. nodiflorum* at 75 plants m^{-2}. However, the effective reduction in nitrate and nitrite content were only reached by *M. nodiflorum* at 75 plants m^{-2} and *L. crithmoides* at 300 and 600 plants m^{-2}. A similar effect was observed in IMTA systems integrating the halophytes *Avicennia officinalis* and *Bruguiera gymnorhiza* with crustaceans, fish and bivalves. Nitrate concentrations were significantly decreased from approximately 0.70 mg L^{-1} in the absence of plants to 0.40 and 0.50 mg L^{-1} in the presence of *A. officinalis* and *B. gymnorhiza*, respectively. Similarly, nitrite levels dropped from around 0.05 mg L^{-1} to 0.025 and 0.03 mg L^{-1}, in *A. officinalis* and *B. gymnorhiza*, respectively.[8] In plants, nitrate is absorbed by roots and reduced to nitrite in the cytoplasm by nitrate reductase, then further reduced to ammonium by plastidic nitrite reductase.[50] The varying nitrate uptake observed across plant densities may reflect differences in root system development, where higher densities increase competition for resources like nitrate and nitrite, potentially limiting uptake efficiency.[51]

Phosphorus is an essential macronutrient for the plant, related to plant growth and development.[52] Despite phosphate having a low plant absorption, its concentration decreased in post-cultivation water for the three species, being found below the quantification limit, suggesting that the halophytes acts as a natural biofilter for phosphate content in the effluent. Other halophyte species have efficiently removed phosphate content from marine aquaculture wastewater, such as *Sarcocornia neei* (0.44 g m^{-2} per day) or *Salicornia europaea* (20.70 mmol m^{-2} per day).[53,54]

Effect of plant densities on the nutritional properties of produced biomass

Figure 5 shows the nutritional composition of *L. crithmoides*, *S. vera* and *M. nodiflorum* cultivated under several plant densities. High moisture content was observed in *M. nodiflorum* (Fig. 5(C)), with significant variation across planting densities. Similar values (86.8–89.8 g 100 g^{-1} FW) were previously reported for this species under different salinities in soilless systems.[17] The high ash content is a typical characteristic of halophytes due to their high capacity to absorb minerals.[55] An increase in ash content was observed in *M. nodiflorum* with increasing plant densities, reaching the maximum value (4.57 g kg^{-1} DW) at 600 plants m^{-2}. While specific studies on *M. nodiflorum* are limited, the ash content of halophytes varies due to several factors, including species type, cultivation system, environmental salinity levels, seasonal changes and geographic location.[9,56,57]

Plant density had a significant impact on the protein content of *S. vera* (Fig. 5(B)) and *M. nodiflorum* (Fig. 5(C)), with both species showing higher protein levels at a density of 600 plants m^{-2} (0.98 g 100 g^{-1} DW and 0.85 g kg^{-1} DW, respectively). Other halophytes had shown similar protein content, such as *Suaeda salsa* at 20 g L^{-1} NaCl (9.45%) or *Salicornia ramosissima* (7.2%).[58,59] A decrease in total carbohydrates with increasing plant density was observed in *M. nodiflorum*, from 0.52 to 0.42 g kg^{-1} DW. Gil *et al.*[60] noted that halophytes tend to accumulate more soluble carbohydrates under salt stress, a trend reflected in this study, where *M. nodiflorum* showed higher carbohydrate levels at increased planting densities and associated competitive stress. Obtained values are within the range of the total carbohydrate determined for other plants, including *Crithmum maritimum* L. leaves (66.5% DW).[61-63] The metabolized energy (ME) showed a decrease along plant density, especially in *S. vera* and *M. nodiflorum*, with the best results (29.7 and 23.6 kcal kg^{-1} DW) for 75 plants m^{-2}. Since ME is closely linked to carbohydrate content, even slight reductions, such as those observed in *M. nodiflorum*, may lead to lower ME values across planting densities. Dietary fiber plays a key role in reducing the risk of conditions such as heart disease, stroke, obesity and gastrointestinal disorders.[64] Fiber composition, including NDF, ADF, ADL, cellulose

Table 4. Physicochemical properties of irrigation water and post-cultivation water used in the cultivation of *Limbarda crithmoides*, *Suaeda vera* and *Mesembryanthemum nodiflorum* under different plant densities

	L. crithmoides					*S. vera*					*M. nodiflorum*				
		Post-cultivation water					Post-cultivation water					Post-cultivation water			
		Plant density (plants m^{-2})					Plant density (plants m^{-2})					Plant density (plants m^{-2})			
Parameter	Irrigation water	75	150	300	600	Irrigation water	75	150	300	600	Irrigation water	75	150	300	600
Conductivity (dS m^{-1})	29.3	71.4	66.7	71.8	76.4	29.3	76.4	73.5	81.6	79.4	41.5	82.1	69.4	74.2	75.1
Salinity (g L^{-1})	14.0	37.3	39.8	43.7	46.6	14.0	47.4	44.6	50.8	48.8	14.3	51.3	42.3	45.5	46.3
TDS (g L^{-1})	29.3	71.5	66.8	72.2	76.3	29.3	76.6	73.7	81.7	79.4	41.5	82.2	69.4	74.4	75.1
TSS (mg L^{-1})	6.2	88.2	86.6	96.7	63.5	6.2	44.4	55.2	53.3	76.0	7.0	56.8	103	105	119
DON (mg L^{-1})	0.16	15.6	29.0	13.6	11.7	0.16	4.24	15.0	14.4	25.2	9.44	42.8	8.85	13.2	5.41
TAN (mg L^{-1})	0.40	0.04	0.01	0.03	0.04	0.40	0.02	0.03	0.07	0.03	0.22	<LOQ	0.03	0.04	0.03
Nitrite (NO_2) (mg L^{-1})	0.08	0.39	0.75	0.05	0.04	0.08	1.05	2.37	0.95	0.74	0.11	0.05	0.71	0.67	0.71
Nitrate (NO_3) (mg L^{-1})	45.1	49.3	37.8	<LOQ	<LOQ	45.1	35.9	56.5	47.4	41.9	20.5	0.03	64.4	68.0	67.9
TN (mg L^{-1})	7.90	26.9	37.8	13.6	11.7	7.90	12.6	28.5	25.4	35.0	14.2	42.8	23.6	28.8	21.0
Phosphate (PO_4^{3-}) (mg L^{-1})	0.04	<LOQ	<LOQ	<LOQ	<LOQ	0.04	<LOQ	<LOQ	<LOQ	<LOQ	0.05	<LOQ	<LOQ	<LOQ	0.03

LOQ (TAN): 0.006 mg L^{-1}, LOQ (NO_2): 0.007 mg L^{-1}, LOQ (NO_3): 0.016 mg L^{-1}, LOQ (PO_4^{3-}): 0.026 mg L^{-1}.
Abbreviations: TDS, total dissolved solids; TSS, total suspended solids; DO, dissolved oxygen; DON, dissolved organic nitrogen; TAN, total ammonium nitrogen (TAN = $NH_3 + NH_4^+$); TN, total nitrogen; LOQ, Limit of quantification.

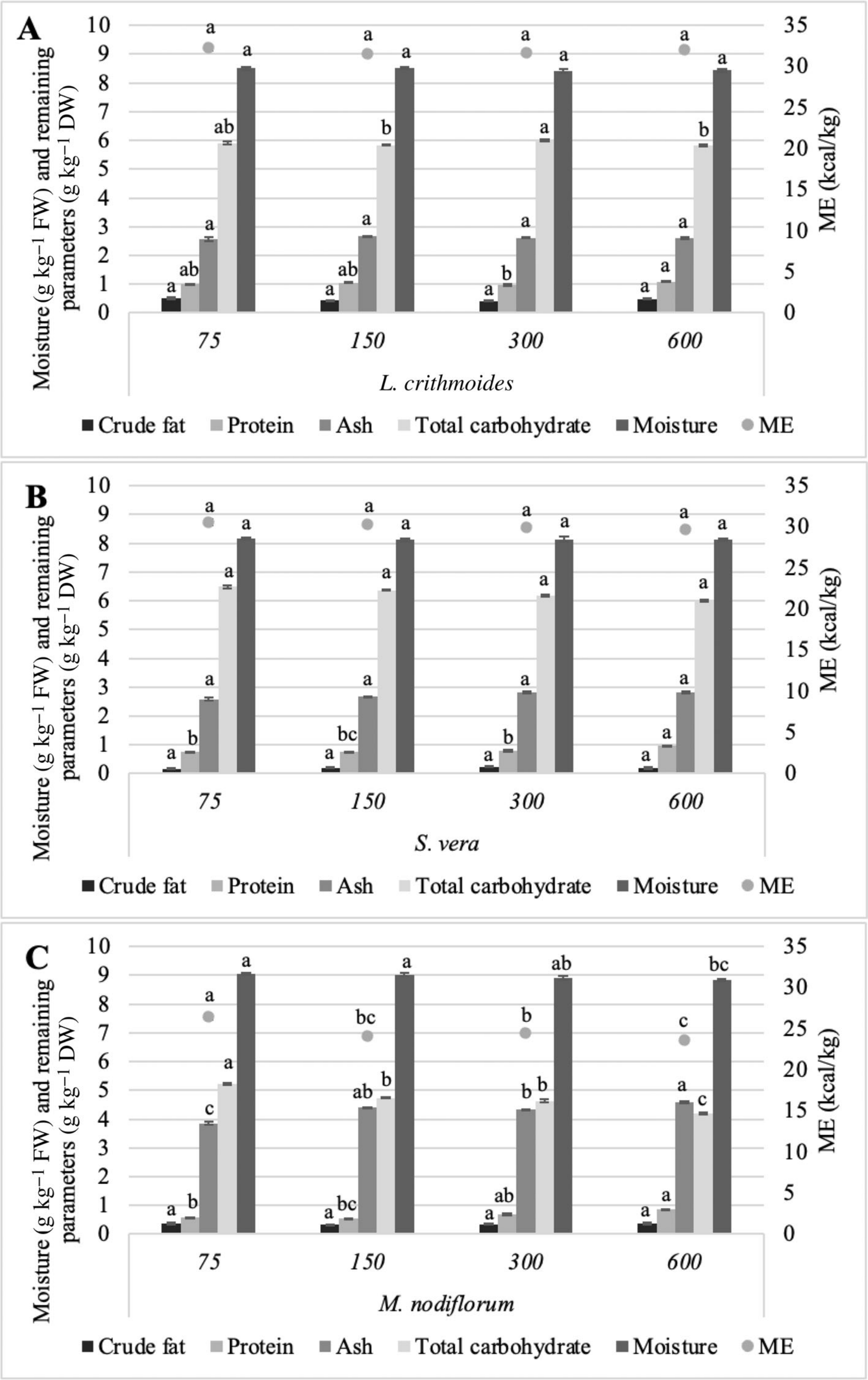

Figure 5. Nutritional composition of (A) *Limbarda crithmoides*, (B) *Suaeda vera* and (C) *Mesembryanthemum nodiflorum* under several plant densities (75, 150, 300 and 600 plants m^{-2}) irrigated using effluents from an integrated multi-trophic aquaculture (IMTA) system. Letters highlight significant differences ($P < 0.05$) between all species for the same parameter.

and hemicellulose, was analyzed in the studied halophytes under different planting densities (Fig. 6). *Suaeda vera* exhibits the highest NDF at 150 plants m^{-2} (4.01 g kg^{-1} DW), with results comparable with other halophytes, such as *Atriplex undulata* (32.9% DW).[65] The lowest fiber content in all species was in ADL, ranging from 0.16 (*M. nodiflorum* at 600 plants m^{-2}) to 0.68 g kg^{-1} DW (*S. vera* at 150 plants m^{-2}). Cellulose, along with lignin, are structural constituents of vascular plants that confer toughness to plant tissues.[66] The lowest plant densities (75 plants m^{-2}) resulted in the highest levels of cellulose in *L. crithmoides* and *M. nodiflorum* (1.42 and 0.80 g kg^{-1} DW, respectively) and in *S. vera* at 150 plants m^{-2} (1.85 g kg^{-1} DW). Values obtained are lower than found in other cultivated halophytes such as *Juncus rigidus* (approximately 35% DW) or *Thespesia populnea*

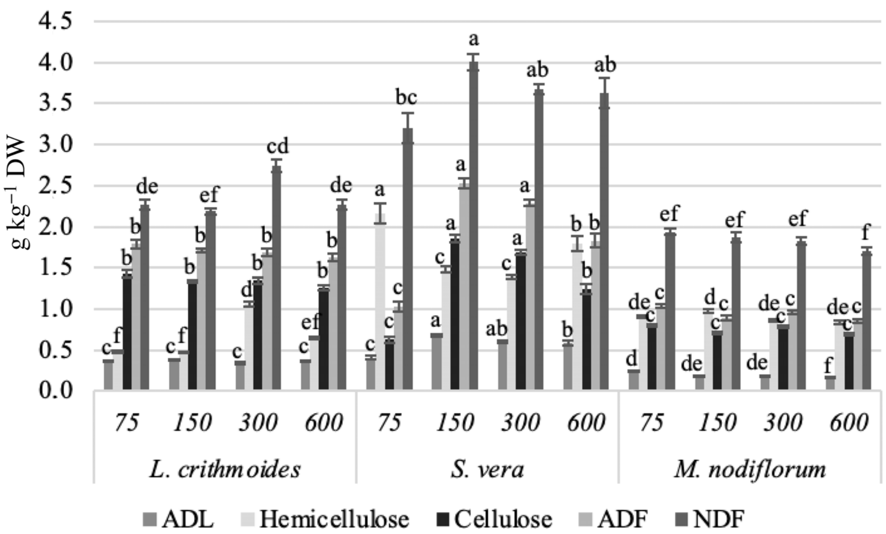

Figure 6. Fiber content of *Limbarda crithmoides*, *Suaeda vera* and *Mesembryanthemum nodiflorum* under several plant densities (75, 150, 300 and 600 plants m^{-2}) irrigated with effluents from an integrated multi-trophic aquaculture (IMTA) system. NDF, neutral detergent fiber; ADF, acid detergent fiber; ADL, acid detergent lignin.

(approximately 37% DW).[67] On the other hand, ADF is an insoluble dietary fiber composed of lignocellulose and silica fractions in plant fiber.[68] The highest ADF values were reached in *S. vera* at 150 plants m^{-2} (2.53 g kg^{-1} DW) and *L. crithmoides* in all plant densities (1.641.90 g 100 g^{-1} DW). No clear correlation was observed between plant density and fiber content in the studied species. A similar lack of consistent trends has been reported under varying saline irrigation conditions. For instance, *Tetragonia decumbens* showed increased fiber content with rising salinity to 200 mmol L^{-1} NaCl,[69] whereas *Suaeda salsa* (irrigated with water containing 0–40 g L^{-1} of NaCl) and *Trachyandra ciliata* (irrigated with saline solutions ranging from 0 to 200 mmol L^{-1} of NaCl) exhibited fiber content that appeared independent of salinity levels.[58,70] Fiber content is influenced by phenological stage, primarily due to processes such as lignification. As plants progress in development and stem elongation, cellulose and other structural carbohydrates accumulate in the cell walls, leading to increased fiber content at maturity.[71,72]

Effect of plant density on the microbiological hygienic parameters of produced biomass

Table 5 summarizes the impact of plant density on the microbiological quality of the harvested halophytes. Neither *E. coli* nor coagulase-positive staphylococci were detected under any of the tested cultivation conditions. Yeasts were found in low abundance (1.85–2.65 log CFU g^{-1}) in *L. crithmoides* at densities ranging from 75 to 300 plants m^{-2}. *Mesembryanthemum nodiflorum* exhibited the lowest filamentous fungi counts (2.71–3.61 log CFU g^{-1}), while the highest content was observed in *S. vera* at 150 plants m^{-2} (5.46 log CFU g^{-1}), followed by *L. crithmoides* at 150 and 300 plants m^{-2} (5.38 log CFU g^{-1}). These values exceed those reported for *S. ramosissima* cultivated under varying salinities (2.62–3.11 log CFU g^{-1}).[73] According to the Commission Regulation (EC) No. 2073/2005 on microbiological criteria for foodstuffs, no legal limits are currently set for filamentous fungi content in foods. Mold contamination may arise from several sources, including the growing substrate, contaminated cuttings, airborne or waterborne propagules, as well as residual inoculum from previous crops.[74]

Aerobic mesophilic counts decreased across all species at the highest plant density (600 plants m^{-2}), ranging from 1.86 to 2.92 log CFU g^{-1} – values notably lower than those reported for *S. ramosissima* and *Crithmum maritimum* (3.43–4.74 and 5.24–5.40 log CFU g^{-1}, respectively) under different salinity levels.[73,75] A similar trend was observed for psychrotrophic aerobes, with *M. nodiflorum* showing the most pronounced decrease, from 4.81 to 4.04 log CFU g^{-1}. In comparison, *C. maritimum* has been reported to exhibit psychrotroph counts as high as 5.81 log CFU g^{-1}.[75] Phyllosphere bacterial abundance is influenced by nutrient-rich exudates from leaves, including sugars, amino acids and organic acids. At higher planting densities, increased resource competition may reduce exudation, thereby limiting microbial proliferation.[76]

Phytochemical profile of the produced biomass

The phytochemical profile of methanol extracts from the studied species cultivated under several plant densities was performed by HPLC analysis, and results are summarized in Table 6. The main compounds detected belong to three phenolic groups, namely hydroxybenzoic acids (gallic, protocatechuic, gentisic, 4-hydroxibenzoic, vanillic, syringic, ellagic and salicylic acids), hydroxycinnamic acids (chlorogenic, caffeic, coumaric and ferulic acids) and flavonoids (catechin, epicatechin, hyperoside, rutin, quercitrin, apigenin and chrysin). *Suaeda vera* grown at 300 plants m^{-2} exhibited the highest content of the total compounds (1.48 mg g^{-1} DW), mostly due to chlorogenic, 4-hydroxybenzoic and gentisic acids. *Limbarda crithmoides* showed the highest values of 4-hydroxybenzoic acid (0.62 mg g^{-1} DW at 75 plants m^{-2}) – a polyphenolic compound related to several biological activities such as antioxidant, antifungal, antidiabetic, anticancer, cardioprotective and antimicrobial activities.[77,78] Chlorogenic acid was detected in higher quantity (up to 0.70 mg g^{-1} DW) in *S. vera* extracts. This hydroxycinnamic acid is involved in regulating glucose, reducing the risk of type 2 diabetes and cardiovascular diseases, among others, and has antioxidant, anti-inflammatory, antibacterial and antiviral activity.[79] The flavonoid detected in higher quantity was epicatechin in *S. vera* cultivated at 600 plants m^{-2} and *L. crithmoides* at

Table 5. Microbiological evaluation (log CFU g^{-1} FW) of *Limbarda crithmoides*, *Suaeda vera* and *Mesembryanthemum nodiflorum* cultivated at different plant densities

Species	Plant density (plants m^{-2})	Microorganisms		Fungi		Bacteria	
		Mesophilics	Psychrotrophics	Molds	Yeast	*Staph. aureus*	*E. coli*
L. crithmoides	75	3.79 ± 0.17ab	4.24 ± 0.08bcde	5.21 ± 0.13a	2.65 ± 0.35a	< 2	< 1
	150	3.18 ± 0.11bcd	4.62 ± 0.08ab	5.38 ± 0.05a	2.85 ± 0.24a	< 2	< 1
	300	3.05 ± 0.13bcd	4.48 ± 0.09abcd	5.38 ± 0.05a	2.65 ± 0.17a	< 2	< 1
	600	2.92 ± 0.18cde	3.97 ± 0.09ef	5.05 ± 0.14a	<2	< 2	< 1
S. vera	75	3.33 ± 0.18bc	3.76 ± 0.09fg	3.74 ± 0.15bc	< 2	< 2	< 1
	150	4.41 ± 0.5a	4.77 ± 0.05a	5.46 ± 0.21a	< 2	< 2	< 1
	300	2.43 ± 0.04def	4.17 ± 0.12cdef	4.15 ± 0.15b	< 2	< 2	< 1
	600	2.18 ± 0.14ef	4.54 ± 0.11abc	3.55 ± 0.33bcd	< 2	< 2	< 1
M. nodiflorum	75	2.45 ± 0.14def	4.81 ± 0.06a	3.61 ± 0.24bcd	< 2	< 2	< 1
	150	1.54 ± 0.10f	3.49 ± 0.10g	3.15 ± 0.12bcd	< 2	< 2	< 1
	300	1.51 ± 0.27f	3.48 ± 0.09g	3.03 ± 0.33cd	< 2	< 2	< 1
	600	1.86 ± 0.02ef	4.04 ± 0.14def	2.71 ± 0.25d	< 2	< 2	< 1

In the same column, values followed by different letters are significantly different at $P < 0.05$.

75 plants m^{-2} (0.16 and 0.14 mg g^{-1} DW, respectively). Epicatechin (flavan-3-ols) exhibits, for example, antioxidant, antimicrobial, anti-inflammatory, antitumor and cardioprotective properties.[80] The reduction of photosynthetic pigments produced by the decrease in light availability at high salinities, together with salt stress, leads to excessive production of reactive oxygen species (ROS).[81] To mitigate ROS overproduction, plant cells develop powerful enzymic and non-enzymic antioxidant mechanisms that lead to phenolic accumulation as part of the antioxidant defense system.[82] In this work, *L. crithmoides* grown at 300 plants m^{-2}, which showed the lowest content of photosynthetic parameters, exhibited the highest content of total compounds (1.33 mg g^{-1} DW). A limited number of studies have explored the effects of plant density on the phytochemical profile of halophytic plants,

Table 6. HPLC-based quantification (mg g^{-1} DW) of metabolites in methanolic extracts of *Limbarda crithmoides*, *Suaeda vera* and *Mesembryanthemum nodiflorum* cultivated at different plant densities

Compound		RT (min)	*L. crithmoides*				*S. vera*				*M. nodiflorum*			
			Plant density (plants m^{-2})				Plant density (plants m^{-2})				Plant density (plants m^{-2})			
			75	150	300	600	75	150	300	600	75	150	300	600
Hydroxybenzoic acids	Gallic acid	4.3	0.13	0.14	0.14	0.14	0.14	0.13	0.13	0.13	0.15	0.14	0.14	0.14
	Protocatechuic acid	7.1	0.05	0.07	0.04	0.04	<LOQ	0.02	0.02	0.02	—	—	—	—
	Gentisic acid	11.4	0.06	0.05	0.07	0.07	0.14	0.09	0.16	0.08	0.03	0.03	0.02	0.02
	4-Hydroxybenzoic acid	11.7	0.62	0.49	0.51	0.51	0.27	0.34	0.21	0.26	0.20	0.18	0.23	0.20
	Vanillic acid	13.9	0.05	0.04	0.04	0.04	0.04	0.04	0.05	0.05	0.02	0.02	0.02	0.01
	Syringic acid	14.9	0.03	0.02	0.02	0.02	<LOQ	<LOQ	<LOQ	<LOQ	—	—	—	—
	Ellagic acid	20.8	0.05	0.05	0.04	0.05	—	—	—	—	—	—	—	—
	Salicylic acid	21.8	0.04	0.04	0.04	0.04	0.02	0.02	0.13	0.02	—	—	—	—
Hydroxycinnamic acids	Chlorogenic acid	12.7	0.03	0.02	0.02	0.02	0.62	0.43	0.64	0.70	<LOQ	<LOQ	<LOQ	<LOQ
	Caffeic acid	13.8	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	—	—	—	—
	Coumaric acid	17.2	0.04	0.02	0.02	0.02	0.02	0.01	0.03	0.03	—	—	—	—
	Ferulic acid	18.2	0.02	0.02	0.02	0.02	0.01	0.01	0.01	0.01	—	—	—	—
Flavan-3-ols	Catechin	11.3	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
	Epicatechin	14.1	0.14	0.13	0.12	0.12	0.13	0.12	0.15	0.16	0.05	0.05	0.05	0.05
Flavonols	Hyperoside	20.3	0.01	0.01	0.01	0.01	—	—	—	—	0.01	0.01	0.01	0.01
	Rutin	20.4	0.03	0.03	0.02	<LOQ	—	—	—	—	—	—	—	—
	Quercitrin	22.6	0.03	0.03	0.03	0.03	—	—	—	—	—	—	—	—
Flavones	Apigenin	29.6	—	—	—	—	<LOQ	0.01	0.02	0.01	—	—	—	—
	Chrysin	34.4	—	—	—	—	0.01	0.02	0.01	0.01	—	—	—	—
Total compounds			1.33	1.16	1.14	1.13	1.40	1.24	1.56	1.48	0.46	0.43	0.47	0.43

RT, retention time; LOQ, Limit of quantification; '—' not detected; LOQ = 0.01 mg g^{-1} DW.

and do not include the species addressed in this study. For example, in the halophyte *Cressa cretica* L., higher plant densities under irrigation with 850 mmol L^{-1} NaCl were associated with increased proline content (from ~5 to 9 mol m^{-3} plant) and decreased acid-soluble oxalate levels (from 7.8 to 2.7 meq L^{-1}) in above-ground tissues.[83] In contrast, most studies investigating the influence of plant density on phytochemical traits have been conducted on glycophytes. For instance, Lombardo *et al*.[84] reported that in the non-halophyte *Cynara cardunculus* L. var. *scolymus* (L.) Fiori increasing plant density from 1.0 to 1.8 plants m^{-2} led to a rise in total polyphenol content from 444 to 671 mg CAE 100 g^{-1} FM. Similarly, Kałużewicz *et al*.[85] found that in *Brassica oleracea* L. var. *botrytis* higher planting densities resulted in elevated levels of quercetin (from 33.9 to 83.1 mg 100 g^{-1} DW) and kaempferol (from 25.8 to 66.7 mg 100 g^{-1} DW).

CONCLUSIONS

This study provides the first comprehensive evaluation of the germination and cultivation performance of the edible halophytic species *L. crithmoides*, *S. vera* and *M. nodiflorum* under varying salinities and planting densities using effluents from IMTA system. Fluctuations in temperature and humidity of the greenhouse could limit germination of halophytes. *Limbarda crithmoides* and *S. vera* demonstrated strong germination (higher than 40%) under non-saline conditions. High survival and productivity were maintained at elevated planting densities, supporting their suitability for intensive cultivation. *Suaeda vera* also exhibited optimal growth at moderate salinities (35.1–40.7 dS m^{-1}), reinforcing its robustness across cultivation conditions. *Mesembryanthemum nodiflorum* cultivated at 75 plants m^{-2} significantly reduced nitrate and total ammonia nitrogen levels in aquaculture effluents, highlighting its potential role in bioremediation. Across all species and densities, biomass met the nutritional and microbiological standards for human consumption. *Suaeda vera* at 300 plants m^{-2} showed the highest accumulation of phenolics (1.56 mg g^{-1}), including chlorogenic and 4-hydroxybenzoic acids, as well as epicatechin. Plant density did not significantly modify phenolic profiles. Our results underscore the viability of cultivating these halophytes as sustainable food sources and functional components of IMTA systems. Future studies should further explore their bioactive properties and contributions to ecosystem services to support their commercial-scale adoption.

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DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available on request from the corresponding author. The data are not publicly available due to privacy or ethical restrictions.

CONFLICT OF INTEREST

The authors declare no conflicts of interest.

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