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Supplementing Commercial Media with Shrimp Fecal Waste Enhances Productivity in *Salicornia* Grown in a Hydroponic System

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HIGHLIGHTS

- The final biomass of *Salicornia europaea* was influenced by the composition of the growth media.
- Yields varied with concentration ratios, emphasizing nutrient source importance.
- Including shrimp fecal waste in commercial media resulted in a substantial enhancement of *Salicornia* performance.
- Shrimp waste holds potential as a bioavailable nutrient for hydroponic halophyte productivity.

Abstract: The aim of this work was to evaluate the use of shrimp waste as a bioavailable source of nutrients for the growth of *Salicornia europaea*, before and after a cavitation process and either in combination with or without commercial fertilizer. Fecal wastes were collected from the drum filter in a recirculating system for shrimp *Penaeus vannamei* culture. The two-factorial experimental design was pretreatment of fecal waste with cavitation (and control without cavitation) and nutrient source (commercial nutrient media (NM) or fecal waste (FW)) at different concentration ratios (100%/0%; 65%/35%; 35%/65% and 0%/100% of nutrient media or fecal waste respectively). The growth experiment lasted 62 days. Media composition had a significant effect on final biomass, and yields ranged from 0.29 to 0.62 kg m^{-2} at 0%/100% and 65%/35%, respectively. The commercial media evaluated does not appear to be well suited for cultivation of *Salicornia*, and performance can be significantly increased if the commercial media is partially replaced with fecal waste (65%/35% ratio). However, whether pretreatment of fecal sludge is necessary to make nutrients available to *Salicornia* cannot be answered because it had no significant effect on the final biomass.

Keywords: halophyte; *Penaeus vannamei*; cavitation; mineralization; plant nutrition.

INTRODUCTION

Over the past fifty years, the decrease in freshwater and groundwater has led to a continuing degradation of agricultural land by salinization [1,2]. Therefore, there is some interest in conducting further studies with plants typically referred to as halophytes because they are more salt resistant than conventional crops [3]. Halophytes are plants that grow in environments affected by periodic flooding by salt water, such as mangroves and salt marshes, and not only can they tolerate high salinity, but also require salt for better growth [4,5].

The halophyte genus *Salicornia*, popularly known as sea asparagus, samphire, or glasswort, is of great interest in the world market of haute cuisine for its slightly salty taste and high nutritional value [6]. These plants have a nutritional profile suitable for human consumption, containing protein and n-3 polyunsaturated fatty acids, and are also a valuable source of natural antioxidants and phenolic compounds for the food and pharmaceutical industries [6–9]. Regarding cultivation, different degrees of success have been observed in different parts of the world. *Salicornia* species are being farmed at a commercial scale for biodiesel, animal feed, and salt and oil extraction [10–12]. Therefore, integrating food production systems can reduce input and waste and increase yield and sustainability [13].

With the progressive flooding of land masses and the growing scarcity of fresh water due to global warming [14], the potential of marine aquaculture to produce high-value food in salinized areas has emerged. This can be achieved by combining traditional crops with halophyte plants, which can thrive in salt-rich environments and yield a secondary species with considerable market demand. Some authors have achieved positive results producing vegetables irrigated with low salinity effluents from shrimp farms [15–18]. The successful development of *Salicornia* in marine aquaponics with shrimp *Penaeus vannamei* in biofloc system [19–21], and the treatment of marine aquaculture effluent using halophyte plants have already been validated [22–24].

The development of recirculating aquaculture systems (RAS) in Europe allows the recovery and utilization of fecal wastes for fertilizer, since the solid fraction can commonly carry up to 32% and 84% of the total nitrogen and total phosphorus in the wastewater, respectively [25]. However, fecal composition also differs among species, feed quality and management. Despite all the differences between the culture methods, most of the nitrogen waste (60-90%) is in dissolved form (mainly ammonia), whereas the phosphorus is excreted within the fecal waste (25-85%) [26]. In addition, approximately 70% of the feed nutrients given to aquatic animals are not retained in their bodies and can be potentially available plant nutrients, and there are substantial proportions of essential nutrients bound in solids relative to the respective amounts that appear dissolved [27,28].

In experimental studies, supplemented aquaponic nutrient solution (i.e., after adding missing nutrients) has been shown to promote plant growth compared to hydroponics [29]. Therefore, sludge mineralization is also a promising way to improve aquaponics system performance, as the recovered nutrients are used to supplement the aquaponics solution [30]. Hence, the combined use of cavitation and biological processes for wastewater treatment is economically and ecologically very promising [31].

Cavitation is the process of formation, growth, and collapse of vapor cavities within microseconds. Intense shock waves are generated, causing the nanobubbles to implode and decompose the organic material by releasing high pressure and temperature [32]. In this way, when a cell of the fecal waste is lysed due to cavitation, its intracellular material, which contains soluble organic matter and nutrients, is released to the liquid phase and becomes available as a growth substrate [33]. Fecal waste could, therefore, be an effective mineral substitute if used in crop production.

Shrimp farming has recently attained great attraction because of its high price and market demand. Reproduction and rearing technologies are available, and more and more shrimp farms are producing throughout Europe [34]. Since typical European shrimp farming is based on RAS, an increasing amount of shrimp feces will be produced in the near future. Thus, this work aimed to evaluate the use of shrimp waste as a bioavailable source of nutrients for the growth of *Salicornia*, before and after the cavitation process, and either in combination or not with commercial fertilizer.

MATERIAL AND METHODS

Origin of plants, fecal waste, and commercial nutrient media

Fecal waste was originated from the culture of shrimp *Penaeus vannamei* fed a commercial shrimp feed (Le Gouessant Aquaculture, France). Fecal waste was collected from drum filter residue in two-day intervals and several times prior to the experiment and stored at -4 °C before use. The nutrient media used in this study was the "Blaumarke" manufactured by YARA GmbH & Co. KG (Dülmen, Germany). The nutrient composition of both fecal waste (after cavitation) and nutrient media is presented in Table 1.

Table 1. Composition of the nutrient sources tested: fecal waste and commercial nutrient media (Blaumarke, Yara GmbH).

Nutrient composition (mg L^{-1})	Nutrient source	
	Shrimp fecal waste	**Commercial nutrient media**
NO_3-N	40.0	119.0
NH_4-N	250.0	71.0
Phosphorus (P), total	52.5	60.0
Potassium (K)	216.5	166.03
Sodium (Na)	3271.0	-
Iron (Fe)	0.049	0.7
Copper (Cu)	0.0135	0.1
Molybdenum (Mo)	< 0.0005	0.04
Zinc (Zn)	0.4535	0.25
Manganese (Mn)	0.083	0.4
Magnesium (Mg)	455.0	18.0
Boron (B)	0.4535	0.25
Calcium (Ca)	187.0	-
Sulfur (S)	164.0	24.0

Values for NO3-N, NH4-N and Phosphorus (P) obtained by a single measurement according to DIN ISO 6878 2004, DIN EN 6878 / D11. All other values were analyzed in duplicate samples, according to DIN by Labor Iben GmbH.

The seeds of *Salicornia europaea* were obtained from the company Rühlemann's (Horstedt, Germany). They were sowed in December 2019 and transferred to the experimental units in May 2020. In total, 24 growth plastic trays measuring 30 x 20 x 7 cm (OBI Eurobox-System Tauro Box Vollwand, Wermelskirchen, Germany) were setup on a table in a greenhouse by random order. Trays were covered with shading nets (70% light removal) about 20 cm above the trays. Seeds were planted on growth taps made from coconut fiber (n= 5 per tap).

Experimental design

The growth trial was performed for 62 days, between May and July 2020. All nutrient media were filled into 1-liter bottles and stored at -4°C prior to use. Individual bottles were removed from the freezer and thawed one day prior to deployment into the experimental units. The experiment was completely randomized in a two-factor (2x4) experimental design, with three replicates totaling 24 experimental units. The factors were a pretreatment of fecal waste with cavitation (and control without cavitation) and the nutrient source (commercial nutrient media (NM) or fecal waste (FW)) at different concentration ratios (100%/0%; 65%/35%; 35%/65% and 0%/100% of nutrient media or fecal waste respectively).

Prior to the experimental period, trays were randomly assigned to treatments and filled with nutrient media or fecal waste. The trays were kept in a greenhouse, and the water temperature in the hydroponic system ranged between 26.6 °C and 28.1°C. On the first day of the experiment, each *Salicornia* seedling was weighed, randomly assigned, and transferred to an experimental tray (9 plants per tray). The mean initial weight of the plants (including tabs) was 38.25 ± 4.38 g (SD). Trays were filled with thawed nutrient at a rate of 0.4 L per tray (so that the bottom of each tray was covered with nutrient by about 1 cm). The liquid that evaporated during the week was manually replaced with fresh water and the volumes were recorded. Nutrient sources were replaced with fresh media once per week. For weekly biometrics, plants were removed from trays prior to application of the new media and excess water was drained from the tabs for one minute. Then each plant (including the tabs) was weighed and returned to the original tray, and the fresh media was applied.

Plant production indexes

At the end of the experimental time, all plants were weighed individually. The average final weight (g), final biomass (kg), specific growth rate (SGR), and production (kg m^{-2}) were then calculated. SGR was calculated according to the formula:

$$SGR = 100*(\ln(FW_{end}) - \ln(FW_0))/d,$$

where FW_{end} is the final mean fresh weight of plant per pot, FW_0 is the initial mean fresh weight of plant per pot, and *d* is the number of experimental days.

Statistics

There were 207 observations, distributed similarly among the different subgroups. However, for the 100%/0% NM/FW treatment, it is worth noting that all the observations collected (58) were accumulated in the control factor, as there was no shrimp fecal waste to treat in this ratio combination. The descriptive analysis showed bimodality of the data, indicating that the variances in the collected observations in each group were not homogeneous. The difference between the pretreatment and control groups was caused by this pronounced bimodality. Therefore, a two-sample Kolmogorov-Smirnov (KS) test [35] was performed in R [36]. Multiple testing was adjusted using the Holm-Bonferroni correction [37]. All tests were performed at a significance level of 0.05.

RESULTS

Media composition significantly affected the final biomass. Except for 100/0 versus 0/100 (P= 0.18), all direct treatment comparisons were significantly different (P < 0.03) (Figure 1). Pretreatment had no significant effect on final *Salicornia* biomass (P= 0.18) (Figure 2). The final mean weights (in g ± SD) obtained were 2.31 ± 0.13, 4.77 ± 1.80, 2.52 ± 0.15 and 2.31 ± 0.11 for 100%/0%, 65%/35%, 35%/65% and 0%/100% respectively. Accordingly, the final biomasses of the respective treatments were 55.46 g, 200.24 g, 68.03 g, and 39.28 g in the treatments 100%/0%, 65%/35%, 35%/65% and0%/100% respectively.

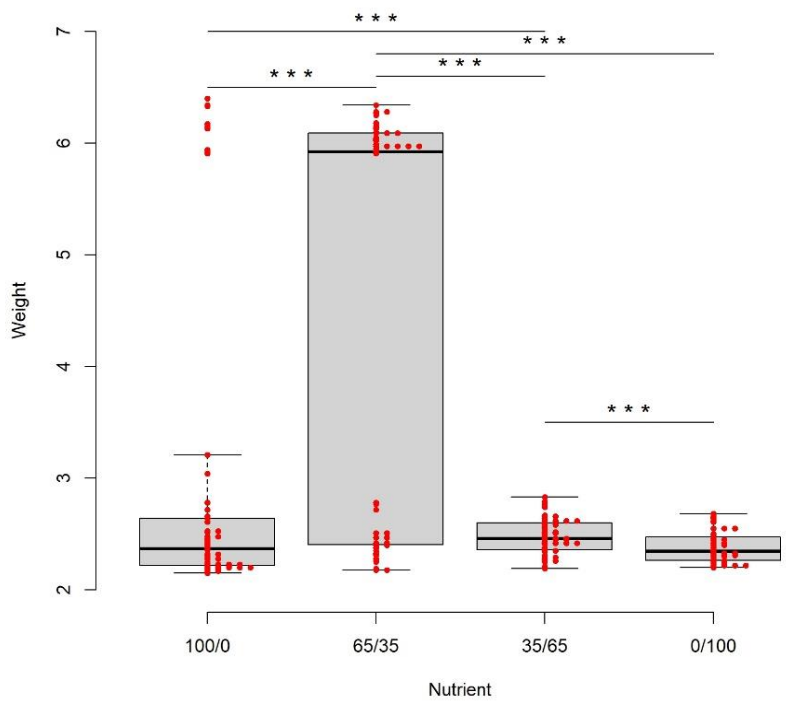

Figure 1. Results of the Kolmogorov-Smirnov test for the weight of the plants (g), after adjustment for multiple testing using the Holm correction for the nutrient ratio (%) group. The three asterisks represent the groups for which the p-values are less than 0.05.

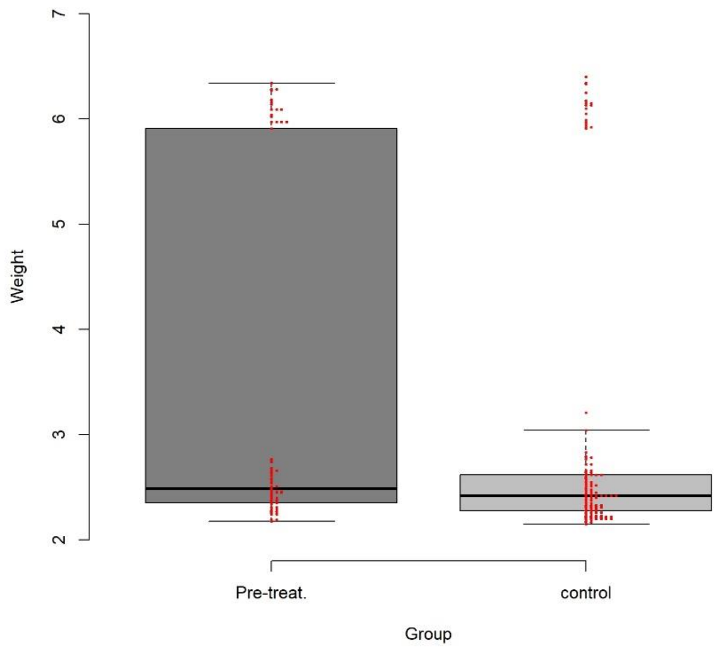

Figure 2. Box plots along with dot plots comparing the control and the pretreatment (cavitation) group's final weight (g). Pretreatment had no significant effect on final *Salicornia* biomass (P = 0.18).

DISCUSSION

The current yields of 2.92 to 6.17 tons ha^{-1} are similar to yields previously reported for *Salicornia brachiata* in the growing season in tidal marshes (2.51 to 6.07 tons ha^{-1} [38]. However, much higher values are also given in the literature. For example, yields of 10 to 20 tons ha^{-1} have been reported for *S. europaea* grown in aquaculture effluent [39], and yields as high as 23.7 tons ha^{-1} have been reported for *Salicornia* sp. [40]. These differences in yields can be manifold, including species, cropping system, season, nutrient supply, flooding interval, salinity, or pH [2]. When growing seasons are included, the results become clearer. Current yields in 62 days ranged from 0.29 to 0.62 kg m^{-2} (in 0%/100% and 65%/35%, respectively). For *S. ambigua* grown in an aquaponic system with the marine shrimp *L. vannamei*, the average production yield was 8 kg m^{-2} of fresh biomass after 73 experimental days [19]. The genus *Salicornia* grown in hydroponic culture with fertilizer-enriched seawater yielded a production of 6 kg m^{-2} [3]. In an experimental culture of *S. ambigua* irrigated with wastewater from shrimp farms in Brazil, up to 2 kg m^{-2} of fresh biomass was obtained after 150 days of culture [41]. In our study, only nine plants were grown in each container, and the yield per unit area could be increased by increasing the planting density. Another factor that may have contributed to the low overall performance compared to the literature is the high variation in weights of individual plants per pot (data not shown). Some individuals show severely inhibited growth or even no growth, which reduces the overall yield. This could be due to poor seed quality. In fact, the seedlings showed that only approximately 10% of the seeds germinated. In order to ensure optimal results, it is advised that future experiments be conducted following a germination pre-test, utilizing viable seeds and taking measures to avoid any growth-related issues throughout the cycle.

Growth rate is of great importance when evaluating the commercial potential of a species of interest. Species of the genus *Sarcocornia*, for example, are characterized by slow growth and low productivity when irrigated with seawater. It was not the scope of this study to maximize yields. Such studies require a complete nutrient screening of all media. Rather, the goal was to find simple solutions to reduce reliance on commercial media and to use aquaculture waste streams that are becoming increasingly available. It is therefore quite interesting to note that a) commercial media does not seem to be well suited for culturing *Salicornia* and b) performance can be significantly increased if commercial media is partially replaced by fecal waste (65%/35% ratio). The average final biomass in this treatment was almost 3 times higher than in all other treatments. The poor performance of *Salicornia* grown in fully commercial media cannot be fully explained by a lack of salinity. Salinity present in the form of sodium ions in fecal waste is known to be a key factor affecting the growth of *Salicornia*. Optimal growth of *Salicornia europaea* is between 200 and 400 mM NaCl, while growth is limited at 0, 800, and 1000 mM NaCl [42]. Adequate nutrient composition may have counteracted this positive effect on salinity in these treatments. Thus, we conclude that the observed differences in growth performance are indeed because of the positive effects of fecal sludge.

Fecal sludge from aquaculture comprises nutrients and other promoting substances such as bacteria, which are known to enhance the transfer of nutrients to plant roots. The temperature of the water in the hydroponic system, and organic substances from the fecal waste, may have favored bacterial growth in the nutrient film [43] and therefore may have hence contributed to nutrient liberalization.

The lack of a pretreatment effect known to increase the bioavailability of organic sources to plants may be related to bacterial mineralization [44]. Cavitation can release organically bound nutrients in fish feces [31], but in this case did not result in improved growth of *Salicornia*. However, the effect of higher nutrient levels may have been counteracted by inadequate experimental culture conditions. At the same time, the lack of a positive effect of cavitation could be related to the fact that this method does not release enough ions or releases only selective ions. The lack of an effect can be explained by several effects. Not all nutrients are released during the application of the cavitation method and are increasingly available to the plants. For example, although the concentration of orthophosphate and iron doubles, the concentration of other elements, such as K, Mg or Mn, increases only slightly (unpublished data). Potassium, in particular, is needed as a macronutrient that limits growth when it is present in small amounts of the growth media.

Whether fecal sludge needs pre-treatment to make nutrients available to *Salicornia* cannot be answered. Organically bound nutrients in fecal sludge could have resulted in slow release, as bacterial mineralization was sufficient to support nutrient supply. However, numerically there was a tendency for *Salicornia* to grow better in pre-treated fecal sludge than *Salicornia* growing in commercial media. This could be related to the weekly supply of new media, which does not allow substantial release of elements by bacteria.

Fecal waste has the potential to reduce the environmental impact of *Salicornia* production. Conventional phosphate fertilizer used in mineral media contributes 27MJ/kg P_2O_5 to the non-renewable cumulative energy input from fossil and nuclear resources (KEA), 1.2kg CO_2 eq/kg P_2O_5 to 100a global warming potential (GWP), 19g SO_2 eq/kg P_2O_5 to terrestrial acidification potential (TAP), 24g P eq/kg P_2O_5 to freshwater eutrophication potential (FEP), 0.4g N eq/kg P_2O_5 to marine eutrophication potential (MEP), and 4.5kg 1,4-DCBEq/kg P_2O_5 to human toxicity potential (HTP) [45]. In the present study, 19.2 liters of the nutrient media were used for the highest yield during the experimental period of 60 days. The 65%/35% media contained 0.21 mg P_2O_5 less than the full conventional media (100%/0%). This corresponds to a reduction of 18.7 mg P kg^{-1} fresh weight of *Salicornia* biomass produced, which is equivalent to 0.02 kg P per ton of fresh biomass produced.

CONCLUSION

Present results showed that *Salicornia* growth performance can be significantly increased if the commercial media is partially replaced with fecal waste (65%/35% ratio). Fecal waste can reduce the environmental impact of *Salicornia* production by 18.7 mg P kg^{-1} fresh weight equivalent to 0.02 kg P per ton of fresh *Salicornia* biomass produced.

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