

Dose-Dependent Evaluation of *Salicornia* Biomass on Growth Performance and Yield of *Pleurotus sapidus* in Sorghum Stover Substrates

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Abstract

Integrating underutilized sorghum stover and coastal *Salicornia* biomass exemplifies sustainable agriculture within a circular bioeconomy by transforming regional waste into high-value food while addressing Namibia's agricultural and saline-land challenges. This study evaluated the effects of amending sorghum stover with *Salicornia* at different levels (0%, 5%, 10%, and 20%) on substrate physicochemical properties, fungal vegetative growth, and fruiting performance of *Pleurotus sapidus*. A negative correlation was observed between pH and electrical conductivity in both media and substrates. The Petri dish experiment showed that potato dextrose agar amended with *Salicornia* extracts significantly accelerated mycelial growth ($P < 0.05$), with the highest growth recorded at 5% amendment (59.9 ± 1.69 mm/12 days), representing a 47% increase compared to the control. The bag experiment showed no significant differences across the treatments during the primordial stage, whereas a negative correlation was observed in the yield and biological efficiency relative to the inclusion levels of *Salicornia* sp. Significant variation was observed in the second flush ($P < 0.05$), where the 20% treatment produced only 88.2 ± 42.8 g compared to 250-257 g in the 0-10% amended treatments. The total yield was highest at 5% amendment (723 ± 42.6 g), comparable to the control, while the biological efficiency reached $72.3 \pm 4.26\%$ compared to $46.0 \pm 5.30\%$ at 20% amendment ($P < 0.05$). The electrical conductivity threshold for *P. sapidus* yield inhibition was identified as 0.848 S/m, which was consistent with the 20% *Salicornia* treatment group. The findings of this study suggest that the inclusion of *Salicornia* in sorghum stover should be considered primarily to valorize it rather than to enhance the growth or yield of *P. sapidus*. The findings of this study highlight the importance of maintaining substrate electrical conductivity and pH within a suitable tolerance range when integrating halophytic biomass into mushroom cultivation systems. These findings provide practical low-input guidance for integrating halophyte biomass into mushroom cultivation systems, supporting waste valorization and sustainable agriculture in Namibia.

Categories: Food security and sustainability, Field & Horticultural crops sustainable production, Sustainable climate smart agriculture

Keywords: sorghum stover, biological efficiency, food security, salicornia biomass, waste valorization, substrate optimization, salinity stress, mycelial growth, pleurotus species, circular bioeconomy

Introduction

The global challenge of agricultural waste management and food security demands innovative circular bioeconomy approaches that transform waste streams into valuable products [1]. Mushroom cultivation stands out as an efficient bioconversion process that leverages the potent enzymatic machinery of white-rot fungi to degrade lignocellulosic

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residues into nutritious food [2]. Oyster mushrooms (*Pleurotus* spp.) are prominent owing to their robust growth on diverse substrates and significant nutritional and therapeutic properties [3]. However, optimizing substrate formulations to enhance mycelial colonization and fruiting body yield remains a critical research frontier in mycotechnology [4].

Sorghum (*Sorghum bicolor*) stover is a significant, often underutilized agricultural residue with global production exceeding 700 million tons annually; its lignocellulosic composition makes it a promising substrate for mushroom cultivation [5,6]. Concurrently, halophytic plants, such as *Salicornia* species, which thrive in saline environments like Namibia's Walvis Bay lagoon, represent a novel and underexplored resource. In Namibia, native *Salicornia* biomass along the coast currently lacks established commercial or human use and is essentially considered a waste product. These plants accumulate specialized metabolites, minerals, and osmoprotectants that could potentially stimulate fungal metabolism and development [7]. Recent research on the related elm oyster mushroom (*Hypsizygus ulmarius*) demonstrated the viability of *Salicornia* supplementation through enhanced biological yield and improved nutritional profile of fruiting bodies, including increased essential amino acids and beneficial fatty acids such as palmitic, linoleic, and oleic acid [8]. The integration of halophytic biomass with conventional residues, such as sorghum stover, represents an innovative approach to valorizing underutilized resources within sustainable agriculture. Most substrate optimization research has focused on conventional supplements like wheat bran, with limited exploration of halophytic materials [4]. However, in regions where agricultural residues such as sorghum stover are heavily competed for as livestock fodder, there is increasing interest in evaluating alternative biomass resources that can partially substitute conventional substrates without substantially reducing productivity. The unique biochemical profile of *Salicornia*, including its mineral and osmoprotectant content, suggests that it may influence fungal colonization dynamics and substrate physicochemical properties, although its effects on overall mushroom productivity remain poorly understood [7,8].

Therefore, this study investigated the integration of sorghum stover with *Salicornia* biomass for the cultivation of *Pleurotus sapidus*. Specifically, the study evaluated the effects of *Salicornia* amendment on vegetative mycelial growth *in vitro* and assessed the influence of varying amendment levels (0-20% w/w) on substrate physicochemical properties, substrate colonization, fruiting body production, and biological efficiency of *P. sapidus* under bag cultivation conditions. By examining both the potential benefits and limitations of halophytic biomass integration, this work contributes to the development of sustainable substrate diversification strategies for low-input mushroom production and saline biomass valorization in Namibia.

Materials And Methods

Study location and facilities

All experiments were conducted under controlled laboratory conditions at the University of Namibia, Sam Nujoma Campus (Henties Bay, Namibia; 22°5'39"S, 14°15'37"E). Substrate preparation, inoculation, incubation, and harvesting followed standard aseptic protocols.

Biological materials

Fungal Culture

A pure culture of *P. sapidus* [maintained on Potato Dextrose Agar (PDA)] at 4°C was sourced from the Sam Nujoma Campus culture collection. The pure culture was subcultured onto fresh PDA plates using standard mycological techniques to revive its vigor for the purpose of this experiment.

Substrate Materials

Dry *S. bicolor* stover was sourced from a farm in Tsumeb, Namibia, air-dried to ~12% moisture, and chopped into uniform 2-3 cm pieces. Grain spawn was prepared in the laboratory following standard spawn production procedures. Fresh *Salicornia* sp. biomass was harvested from Walvis Bay Lagoon, Namibia; rinsed thoroughly with tap water to remove surface salts and sediment, air dried followed by oven-drying at 60°C for 24 hours to constant weight and chopped into 2-3 cm pieces.

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Preparation of *Salicornia* Extracts

Aqueous extracts for *in vitro* growth assessment were prepared by boiling 10 g crushed *Salicornia* biomass in 500 mL deionized water (100°C, 10 min), followed by filtration through sterile muslin cloth, following the method described for plant extract preparation in mushroom culture media [9,10]. The stock extract was diluted with deionized water to final concentrations of 0% (control), 5%, 10%, and 20% (v/v) for incorporation into PDA growth media [8].

Experimental design

This study utilized a two-phase completely randomized design (CRD) with four *Salicornia* amendment levels (0%, 5%, 10%, 20%) and five replicates per treatment. Phase 1 assessed vegetative growth; Phase 2 evaluated fruiting body production (Table 7).

Phase and objective	Growth media	Amendment form	Treatment (<i>Salicornia</i> % Concentration)
1. Vegetative growth	Potato Dextrose Agar (PDA)	Aqueous Extract	A: 0% (Control) B: 5% (v/v) C: 10% (v/v) D: 20% (v/v)
2. Fruiting body production	Sorghum Stover Substrate	Dried <i>Salicornia</i>	A: 0% (Control) B: 5% (w/w) C: 10% (w/w) D: 20% (w/w)

TABLE 1: Experimental design of the study.

Vegetative growth assessment on amended PDA

Media Preparation and Physicochemical Analysis

Standard PDA (39 g/L) media were prepared with aqueous *Salicornia* extract replacement at 0-20% (v/v) of the water component, maintaining constant nutrient and agar concentrations across treatments, following established methods for incorporating aqueous plant extracts into mushroom culture media [8,10,11]. The pH and electrical conductivity (EC) of each treatment were measured in triplicate using a calibrated multiparameter meter (HQ40d, Hach), according to standard substrate physicochemical analysis protocols [12]. The media were autoclaved (121°C, 15 min), then poured into sterile Petri dishes, and allowing them to solidify. Mean values are presented in Figure 7.

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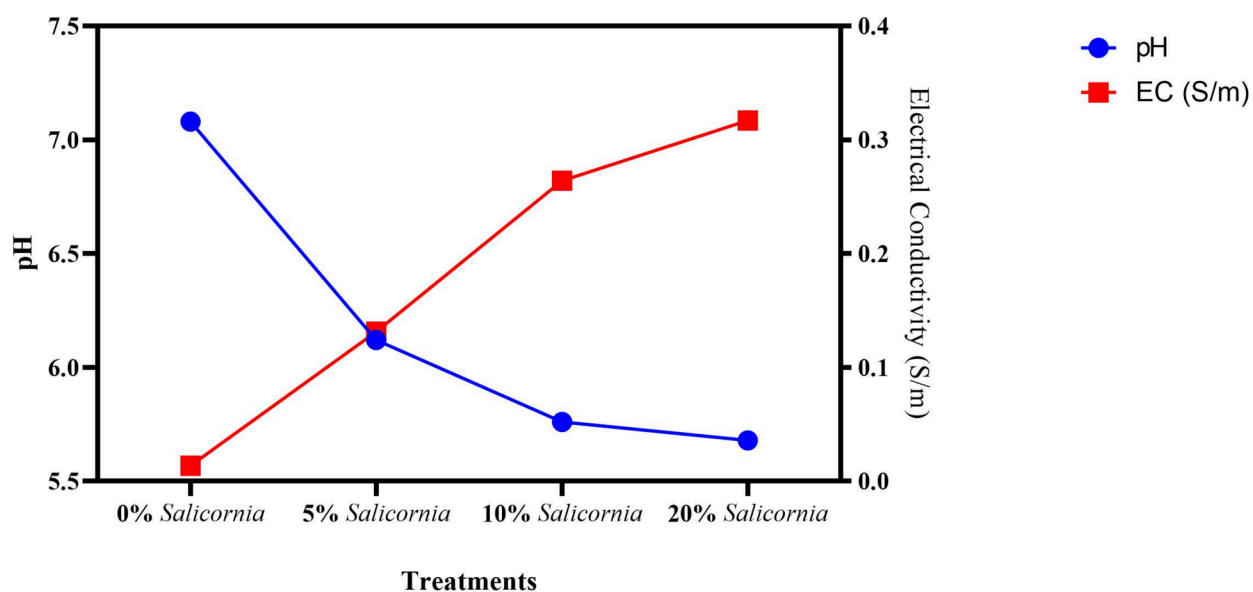


FIGURE 1: pH and electrical conductivity of *Salicornia* extract amended culture media.

EC, Electrical Conductivity

Inoculation and Incubation

Each Petri dish was centrally inoculated with a 5-mm mycelial plug from the margin of an actively growing 7-day-old *P. sapidus* culture. Each treatment was replicated five times, and the Petri dishes were sealed and incubated at $25^{\circ}\text{C} \pm 2^{\circ}\text{C}$ in the dark room [12-14]. The experimental setup is illustrated in Figure 2. Inoculated plates were incubated in the dark at $25^{\circ}\text{C} \pm 2^{\circ}\text{C}$.

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FIGURE 2: Schematic representation of the extraction process from fresh *Salicornia* (drying → boiling → crude extract) and its concentration-dependent evaluation on the vegetative growth of *P. sapidus*.

Growth Measurements and Mycelial Growth Rate

Radial growth was measured every 2 days along two perpendicular axes until the mycelium reached the edge of the plate (full colonization). The mean of the two diameter measurements was recorded for each plate. Mycelial growth rate (MGR) was assessed on PDA media supplemented with *Salicornia* extracts at 0%, 5%, 10%, and 20% concentrations. The radial growth was used to calculate the MGR (mm/day) after 12 days of incubation, the time at which the control treatment (0% extract) reached full colonization, following a previously described method [9]. Representative images of inoculated plates at the start (day 0) and end (day 12) of the growth assessment are shown in Figure 3.

$$\text{MGR in mm/day} = \text{Average final colony diameter (mm)} / \text{Incubation period (days)} \quad (1)$$

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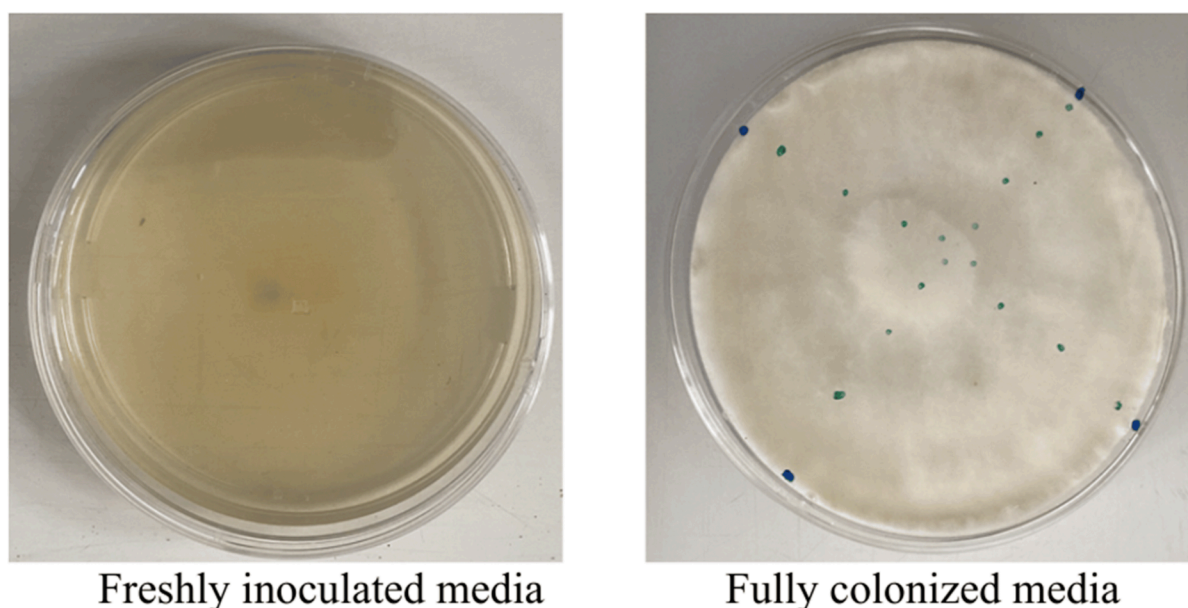


FIGURE 3: Representation of media inoculated with *P. sapidus* at the start (day 0, left) and end (day 12, right) of the vegetative growth.

Fruiting body production on amended sorghum stover

Substrate Preparation and Physicochemical Analysis

For each treatment, sorghum stover was soaked for 24 hours to achieve ~65% moisture content. Dried *Salicornia* sp. biomass was thoroughly mixed into the moist stover at 0%, 5%, 10%, or 20% (w/w, dry weight basis). The pH and EC of each formulation (Figure 4) were measured prior to inoculation using 1:5 (w/v) water suspensions with a calibrated multiparameter meter (HQ40d, Hach) [12]. The wet substrates were packed into 20 × 35 cm polypropylene bags (1 kg/bag) and steam pasteurized at 95°C for 3 hours using an electrical steam pot fitted with a thermometer, followed by overnight cooling prior to inoculation.

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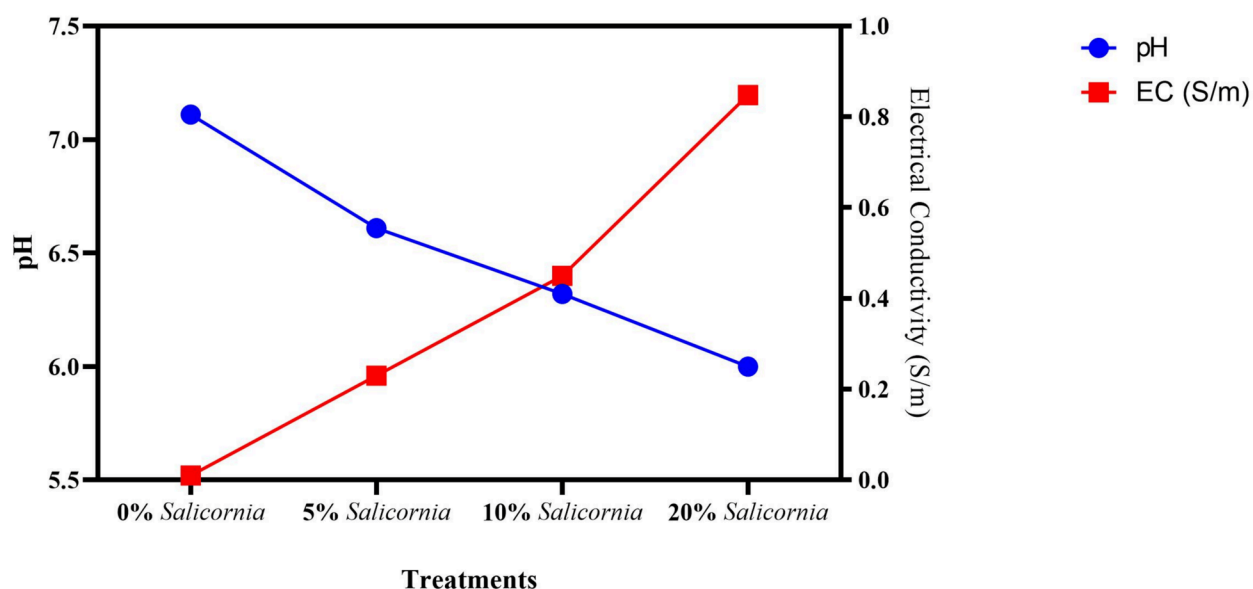


FIGURE 4: pH and electrical conductivity of *Salicornia* amended substrates.

EC, Electrical Conductivity

Inoculation, Incubation, and Fruiting Conditions

Each polypropylene bag was inoculated centrally with 150 g of 14-day-old wheat grain spawn. Bags were incubated in darkness at $26 \pm 2^\circ\text{C}$. After 24 hours, five holes (1 cm diameter) were punched equidistantly around each bag with a sterile scalpel to allow gas exchange and avoid anaerobic conditions in the bags [8]. Pinhead formation in the holes was considered an indication of complete mycelial colonization, and the bags were transferred to a fruiting room maintained at temperatures of $18\text{--}22^\circ\text{C}$ and relative humidity at 85–95% using foggers, without directly irrigating the bags [4]. The fruiting bodies' production process is illustrated in Figure 5.

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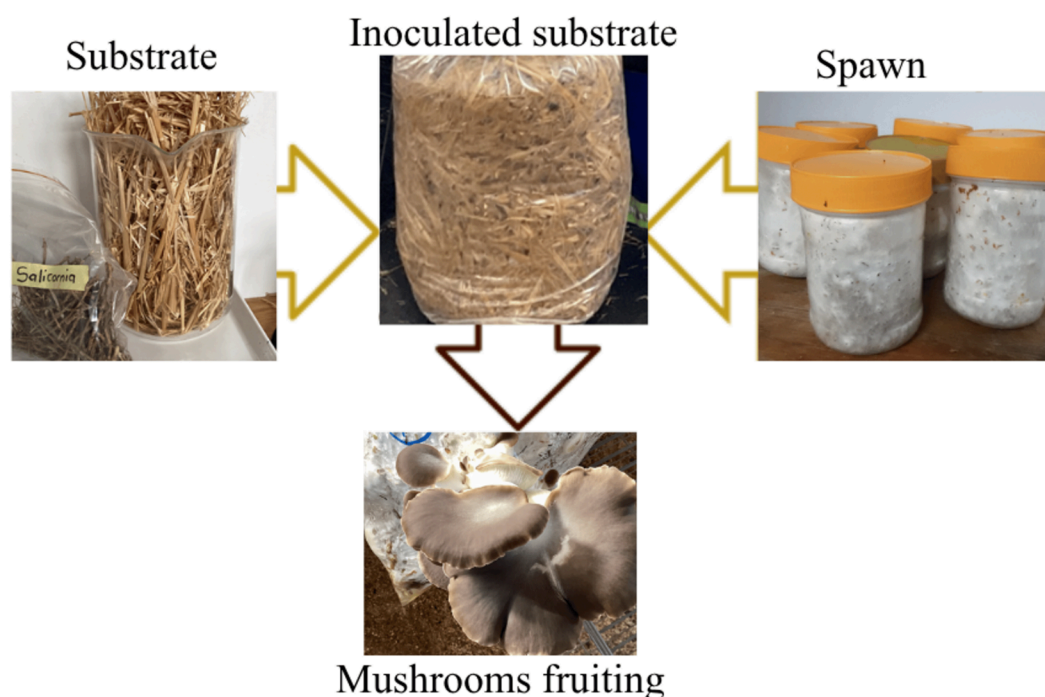


FIGURE 5: Fruiting body production process.

Harvest Parameters, Biological Efficiency, and Dry Mass Determination

Mushrooms were harvested at optimal maturity from the two production flushes. The total fresh biological yield per treatment was calculated as the sum of the harvests from all five replicate bags. Biological efficiency (BE) was calculated using the total fresh mushroom weight following the standard method for oyster mushroom cultivation [15]:

$$(\text{BE} (\%)) = ((\text{Total fresh mushroom weight (g)} / (\text{Dry substrate weight (g)}) \times 100 \text{ (2)})$$

Freshly harvested fruiting bodies from each replicate bag and flush were weighed and oven-dried at 60°C for 48 hours until constant weight was achieved. The dry mass of the fruiting bodies from the respective treatments was then determined using a digital analytical balance (0.01 g precision).

Statistical analysis

All statistical analyses were conducted using Jamovi v2.4.14 (The Jamovi project, 2023). Data were reported as mean $\pm$ standard error (SE). Normality and homogeneity of variance were verified prior to analysis using Shapiro-Wilk and Levene's tests, respectively. Vegetative growth (mycelial colony diameters over time) was analyzed by repeated-measures analysis of variance (ANOVA). Fruiting body production parameters (biological yield, biological efficiency) underwent one-way ANOVA. Significant effects were followed by Tukey's Honestly Significant Difference (HSD) post-hoc tests for pairwise comparisons. Data visualization was performed using GraphPad Prism v8.0 (GraphPad Software, San Diego, CA, USA).

Ethical considerations

Salicornia sp. biomass was harvested from Walvis Bay Lagoon using sustainable practices compliant with Namibian environmental regulations. All experiments with plant materials adhered to the University of Namibia's ethical standards and relevant international guidelines. Material procurement, handling, and disposal minimized environmental impact

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and followed biosafety protocols.

Results And Discussion

Vegetative growth performance

Amendment of PDA with *Salicornia* aqueous extracts significantly enhanced the vegetative growth of *P. sapidus* (Figure 6). Repeated-measures ANOVA revealed significant main effects of time ($F_{4,64} = 285.4$, $P < 0.001$) and treatment ($F_{3,16} = 36.6$, $P < 0.001$), as well as a significant time $\times$ treatment interaction ($F_{12,64} = 18.9$, $P < 0.001$). Mycelial colonization was fastest with the 5% *Salicornia* extract, reaching 80.4 ± 1.60 mm in diameter by day 8, compared to 51.0 ± 1.67 mm in the control. All treatments achieved full Petri dish colonization (90 mm) by day 12.

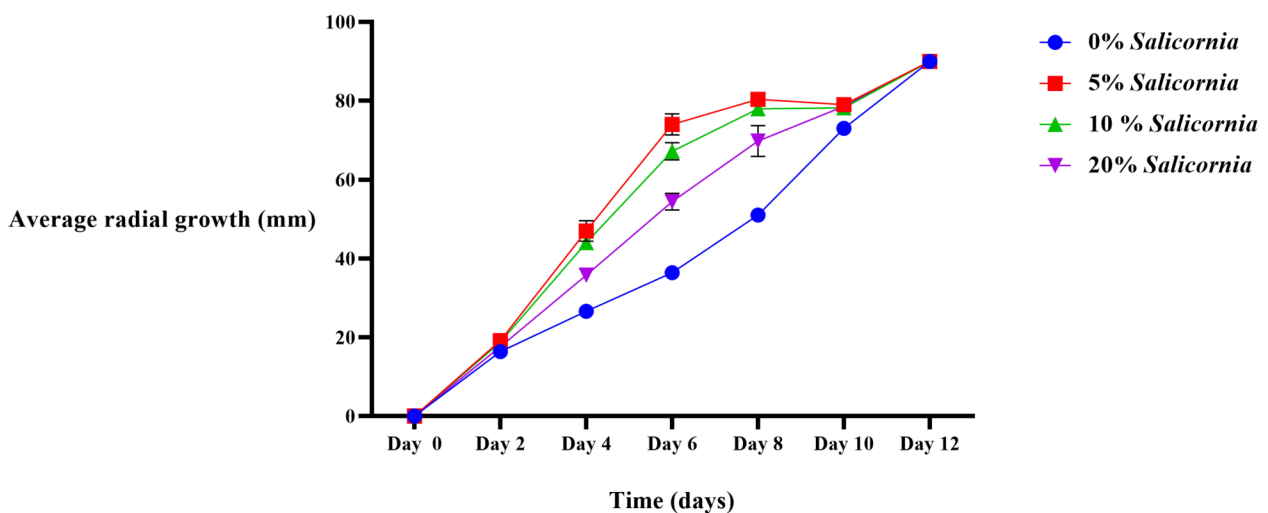


FIGURE 6: Radial mycelial growth of *P. sapidus* on *Salicornia* extract-amended PDA over 12 days. Data show mean colony diameter (mm) $\pm$ SE ($n = 5$ replicates per treatment).

PDA, Potato Dextrose Agar

The overall MGR results over the 12-days' incubation period showed that *Salicornia* amendment affected media colonization differently across concentrations, as shown in Figure 6. The 5% *Salicornia* treatment resulted in the highest MGR, which was significantly higher at day 8 compared to the control and 20% treatment ($P < 0.001$) but not significantly different from that of the 10% treatment ($P = 0.551$).

This significant enhancement in mycelial colonization at the 5% amendment level suggests that bioactive compounds in *Salicornia*, such as minerals and osmoprotectants, preferentially stimulate hyphal extension and metabolic activity [7,16]. The optimal growth at moderate (5-10%) concentrations aligns with established principles of substrate amendment for *Pleurotus* spp., where moderate levels of nutrient-rich additives maximize performance without inducing imbalances [17,18]. The decline in MGR at the 20% level suggests the onset of inhibitory factors, foreshadowing the stress effects observed in later fruiting stages.

Fruiting body production and yield efficiency

Primordial Initiation and Pinhead Formation

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The duration required for primordial initiation differed among substrate formulations (Figure 7). Low-to-moderate *Salicornia* amendment (5% and 10%) did not significantly alter the time required for pinhead formation relative to the control treatment ($P > 0.05$). In contrast, the 20% *Salicornia* treatment significantly delayed primordial initiation compared with the 5% ($P = 0.028$) and 10% treatments ($P = 0.037$), although it did not differ significantly from the control treatment ($P = 0.140$).

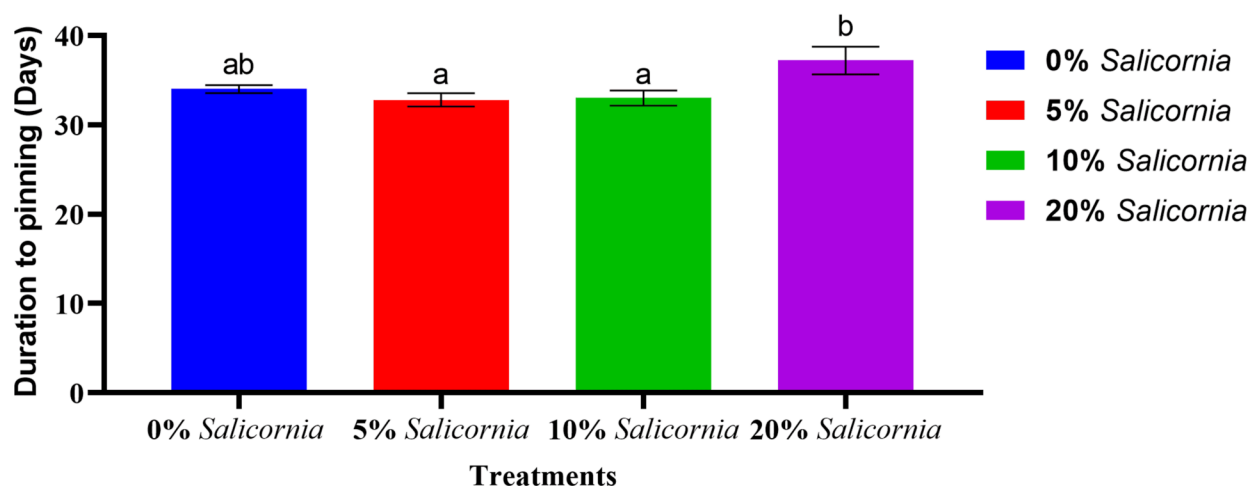


FIGURE 7: Duration from substrate inoculation to primordial formation of *P. sapidus* evaluated on sorghum stover amended with *Salicornia* substrates. Bars represent mean values $\pm$ SE ($n = 5$ replicates per treatment). Different lowercase letters indicate significant differences among treatments according to Tukey's HSD test ($P < 0.05$)

HSD, Honestly Significant Difference

The slight delayed pinning observed at 20% amendment level may suggest that elevated substrate salinity and associated osmotic stress may impair the transition from vegetative mycelial growth to reproductive development. Similar responses have been reported in other *Pleurotus* species exposed to saline conditions, where excessive salt concentrations suppress metabolic activity and prolong the colonization period prior to fruiting initiation [17,19]. Delayed primordial formation has been attributed to high concentrations of soluble salts that may divert fungal metabolism toward osmotic regulation rather than reproductive development [20]. Conversely, moderate *Salicornia* amendment has been shown to be tolerated without negatively affecting fruiting initiation under bag-cultivation conditions [8]. This may suggest that sorghum stover and low-dose *Salicornia* biomass provide sufficient structural carbohydrates and trace minerals to support mycelial development without inducing inhibitory salinity stress [4,5]. These findings support the potential use of *Salicornia* biomass as an amendment for mushroom substrate in circular agricultural systems when applied at moderate inclusion levels.

Fresh yield performance

First-flush yields showed no significant treatment effects ($P = 0.148$; Table 2). The absence of statistical significance in the first flush, despite the variation in substrate chemistry, suggests that the mushroom mycelium was able to utilize internal moisture and pre-colonized nutrient reserves to initiate pinhead formation, temporarily buffering short-term environmental stresses during early fruiting [12,20,21]. In contrast, second-flush yields differed significantly, as reflected in Table 2.

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Flush	ANOVA F(3,16)	P-value	η^2	ω^2	Key findings
Flush 1	2.05	0.148	0.277	0.136	No significant treatment effect
Flush 2	5.79	0.007	0.521	0.418	Strong treatment effect

TABLE 2: Summary of fruiting yield.

ANOVA, Analysis of Variance

The 20% *Salicornia* treatment produced significantly lower second-flush yields than the control, 5%, and 10% treatments ($P = 0.002$, $P = 0.001$, and $P = 0.027$, respectively; Table 2). The emergence of this significant statistical effect on the yield from 20% in the second flush could be attributable to the high EC (0.848 S/m) and acidic pH (6.00) measured in the 20% treatment substrate. After the first flush, the mycelium required rehydration and fresh nutrient uptake to produce a second pinset; however, the potentially high-soluble salt concentration may have created an inconducive osmotic gradient that prevented water uptake, while the suboptimal pH likely inhibited the enzymatic activity required for substrate degradation and recovery [18,22]. Consequently, total fresh yields across both flushes showed strong treatment effects ($P < 0.001$; Figure 8). The 20% treatment produced significantly lower total yields than the control and 5% treatments ($P = 0.002$ and $P = 0.001$, respectively; Table 2), whereas the 5% *Salicornia* treatment remained statistically comparable to the control ($P = 0.994$; Table 2).

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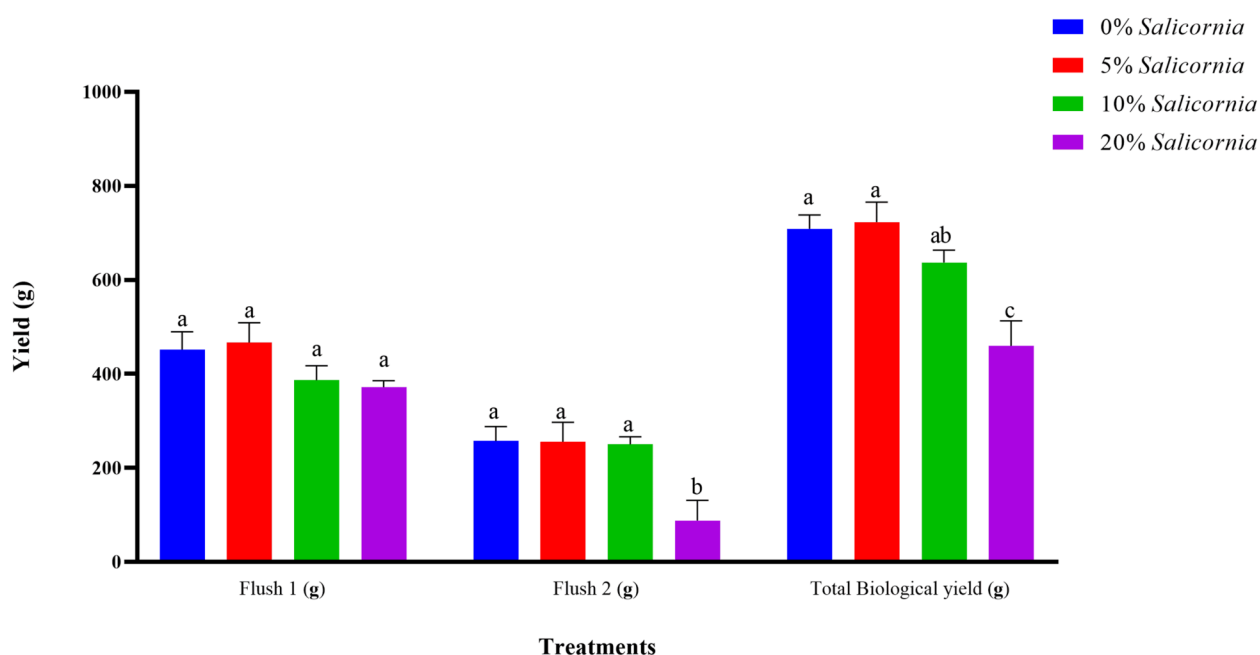


FIGURE 8: Fresh yield of *P. sapidus* grown on sorghum stover amended with *Salicornia* biomass. Columns show mean fresh weight $\pm$ SE ($n = 5$ replicates per treatment). Different lowercase letters indicate significant differences between treatments (Tukey's HSD, $P < 0.05$).

SE, Standard Error; HSD, Honestly Significant Difference

The substantial 66% reduction in yield during the second flush at the 20% amendment level represents an important observation that correlates directly with substantial substrate acidification (pH 6.00) and elevated salinity (EC 0.848 S/m). Although *Pleurotus* species are robust, they exhibit limited salt tolerance, and the observed pH shift approaches the lower limit of their optimal growth range (pH 6.0-7.5) [4]. This pattern aligns with the established salinity thresholds for fungi; for instance, a study on terrestrial basidiomycetes reported conductivity values causing 50% growth inhibition (EC_{50}) within a range of 1.49 to 2.20 S/m for NaCl. While the EC values are higher than the 0.848 S/m recorded here, the significant yield suppression in the 20% treatment indicates that *P. sapidus* is particularly sensitive during the reproductive (fruiting) phase, even at sub-lethal vegetative concentrations, and that the elevated EC from high *Salicornia* amendment likely induced osmotic and ionic stress [23-25]. The disproportionate impact on the second flush points to a cumulative stress mechanism, likely salt accumulation stress, where soluble salts and acidic compounds concentrate over time and are taken up by the fungus, progressively inhibiting water and nutrient uptake [13,25]. The ability of the 5% treatment (EC 0.230 S/m) to match the control yield suggests a hormetic effect, where low-level salinity provided trace minerals that slightly stimulated growth without crossing the inhibitory threshold [26]. This is consistent with cultivation studies on other *Pleurotus* species, where higher concentrations of saline or other amendments often show diminishing returns or inhibitory effects, while moderate levels (typically 5-10%) optimize yield and biological efficiency [13,27,28]. The data from this study suggest that the critical EC threshold for sustained production in this system lies between 0.450 S/m and 0.848 S/m.

Biological efficiency and dry mass production

BE was not significantly improved by *Salicornia* amendment (Figure 9). Although the 5% *Salicornia* treatment produced a nominal mean BE of 72.3%, this value was statistically equivalent to the unamended control ($P = 0.994$). The BE results of this study is contrary to what have been previously reported in elm oyster mushrooms [8], suggesting that substrate chemical/physical properties tolerance in oyster mushrooms is species specific. Moreover, the observed results suggest

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that low-level halophytic inclusion may function more effectively as a partial substrate substitute than as a yield-enhancing supplement. Any nutritional contribution from the *Salicornia* biomass, including minerals or organic nutrients, appeared insufficient under the present conditions to significantly improve substrate conversion efficiency [24,29].

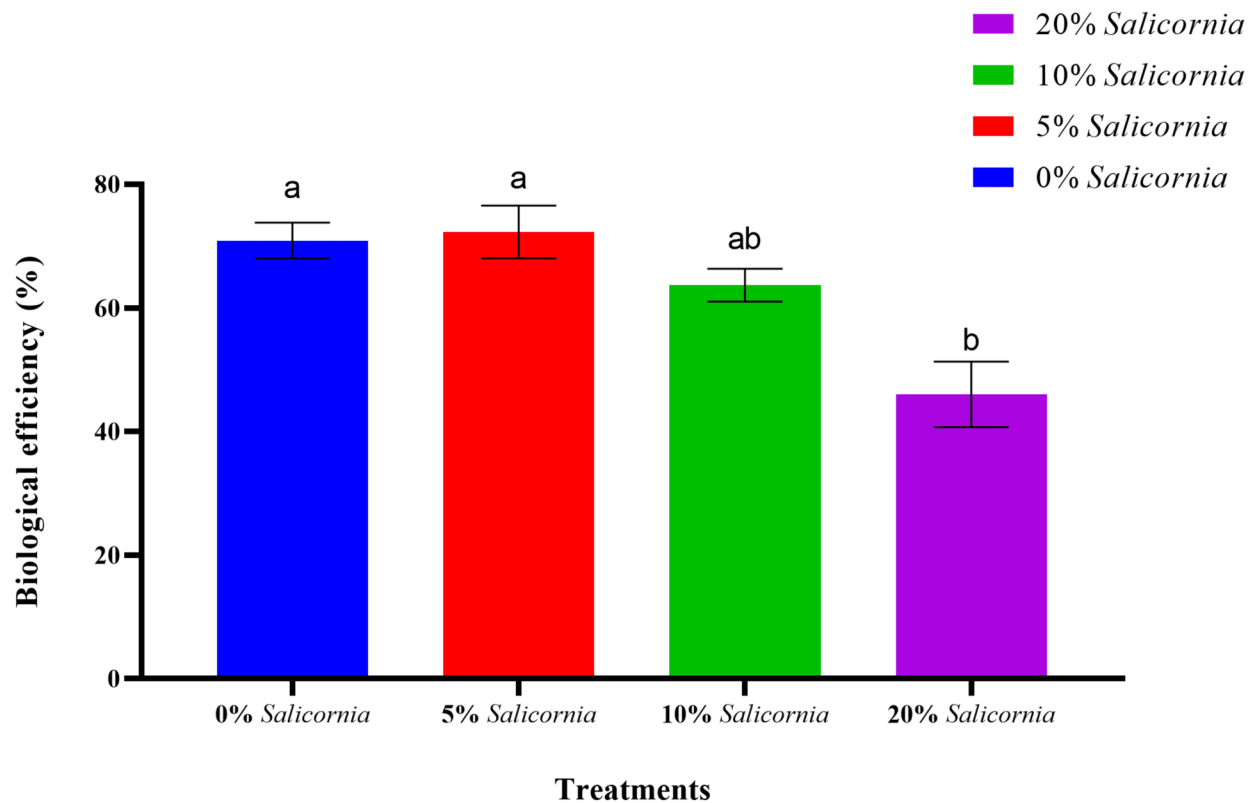


FIGURE 9: Biological efficiency (BE) of *P. sapidus* on sorghum stover amended with *Salicornia* biomass. Columns show mean BE (%) $\pm$ SE ($n = 5$ replicates per treatment). Different lowercase letters denote significant differences (Tukey's HSD, $P < 0.05$).

SE, Standard Error; HSD, Honestly Significant Difference

Similar to the fresh yield trends, first-flush dry mass did not differ significantly among treatments ($P > 0.05$; Table 3). This suggests that early reproductive development was not substantially affected by *Salicornia* amendment during the initial flush, possibly because the fungus relied on pre-colonized substrate reserves and internal carbohydrate storage during early basidiocarp formation [20,30].

In contrast, second-flush dry mass differed significantly among treatments ($P < 0.05$), primarily due to the marked reduction observed in the 20% *Salicornia* treatment (Table 3). This decline again corresponded with elevated substrate salinity (0.848 S/m) and lower pH (6.00), indicating that increasingly unfavorable physicochemical conditions may have limited post-flush substrate utilization and sustained fruiting performance. Such conditions could potentially reduce water availability and interfere with extracellular enzymatic activity involved in lignocellulose degradation and nutrient mobilization required for subsequent flush development [20,21].

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Treatment (% of <i>Salicornia</i>)	First flush dry mass (g) ± SE	Second flush dry mass (g) ± SE	Total dry mass (g) ± SE
0%	55.8 ± 3.76 ^a	29.4 ± 4.78 ^a	85.2 ± 3.15 ^a
5%	52.2 ± 4.19 ^a	32.0 ± 4.77 ^a	84.2 ± 3.18 ^a
10%	48.2 ± 4.62 ^a	33.2 ± 4.68 ^a	81.4 ± 4.18 ^a
20%	59.0 ± 2.74 ^a	11.2 ± 5.93 ^b	70.2 ± 6.51 ^a

TABLE 3: Dry mass of *P. sapidus* from first and second flushes on sorghum stover amended with *Salicornia* substrate. Values represent mean dry weight (g) ± SE (*n* = 5 replicates per treatment). Different lowercase letters within columns indicate significant differences (Tukey's HSD, *P* < 0.05).
SE, Standard Error; HSD, Honestly Significant Difference

Although total cumulative dry mass across both flushes showed no statistically significant differences among treatments (*P* = 0.108), the control, and 5% *Salicornia* treatments maintained the highest overall yields. The absence of significant differences despite the poor second-flush performance at 20% likely reflects the relatively high first-flush production observed in this treatment. These findings suggest that while higher *Salicornia* inclusion levels may still support initial fruiting, they appear less suitable for sustained production across multiple flushes.

The reduction in second-flush dry mass at 20% *Salicornia* reflects cumulative salt stress. After the first flush, mycelial rehydration was likely impaired by a strong osmotic barrier (EC 0.848 S/m; estimated osmotic potential ≈ −0.8 MPa), while the acidic pH (6.0) may have inhibited key extracellular lignocellulolytic enzymes, including laccase and manganese peroxidase, required for efficient breakdown of residual substrate and initiation of second pinhead formation [19,20]. Total dry mass across both flushes showed a non-significant trend (*P* = 0.108), with the control and 5% *Salicornia* treatments producing the highest yields. The lack of statistical significance in total dry mass, despite the pronounced collapse in second-flush production at 20%, is explained by compensatory first-flush output (59.0 ± 2.74 g), which was numerically the highest among treatments. This pattern suggests that extreme substrate chemistry (EC 0.848 S/m; pH 6.0) still permitted initial fruiting but was not conducive to sustained production across flushes.

The maintenance of comparable biological efficiency and total dry mass at the 5% amendment level indicates that low-level *Salicornia* inclusion can partially substitute conventional substrate material without significantly compromising productivity. This may have practical relevance in regions where agricultural residues such as sorghum stover are limited or heavily competed for. Furthermore, stable dry mass production is particularly important for rural mushroom production systems in Namibia, where drying is commonly used to extend shelf life in the absence of reliable cold-chain infrastructure [20,31-33].

Integrated performance summary

A comprehensive overview of treatment performance across all measured parameters is presented in Table 4. The 5% *Salicornia* amendment consistently showed the most balanced performance among treatments, with enhanced *in vitro* mycelial growth (47% faster than the control) and overall fruiting body production that remained comparable to the control. This response at 5% amendment (0.230 S/m, pH 6.61) suggests that low-level inclusion of *Salicornia* biomass may allow for the incorporation of soluble mineral fractions without adversely affecting productivity, unlike the inhibitory effects observed at higher inclusion levels [29].

Parameter	Optimal inclusion level of <i>Salicornia</i> (%)	Key finding
Vegetative growth rate	5%	59.9 mm/10 days (47% higher than control)
First flush yield	5%	467.0 g (No significant difference from control)
Second flush yield	0%	Yield drastically reduced by 66% at higher (20%) inclusion levels
Total fresh yield	5%	723.0 g (Highest among all treatments)
Total dry mass	0%	No significant difference with <i>Salicornia</i> -amended substrate
Biological efficiency	5%	72.3% (Highest among all treatments)
Overall optimal	5%	Provides the best balance across all growth and yield metrics

TABLE 4: Integrated performance of *P. sapidus* on sorghum stover amended with *Salicornia* biomass at 0%, 5%, 10%, and 20% (w/w). Values represent key metrics (MGR, fresh/dry yields, BE).

MGR, Mycelial Growth Rate; BE, Biological Efficiency

Crucially, substrate chemistry suggests that the observed acceleration in mycelial growth at low amendment levels can be achieved without exceeding the *P. sapidus* reproductive salinity threshold (~0.450 S/m), provided the *Salicornia* inclusion remains at or below 5% (EC ≤ 0.230 S/m). The pronounced reduction in second-flush yield observed at the 20% amendment level (0.848 S/m, pH 6.00) indicates that elevated conductivity is associated with reduced reproductive performance, particularly during later flushes. In contrast, the comparatively stable performance at intermediate levels (e.g., 10%) suggests that inhibitory effects may become more apparent only beyond a certain cumulative salinity range, likely linked to increased osmotic and ionic stress between flush cycles [20].

These findings are broadly consistent with the literature reporting that alternative substrate formulations can modify production dynamics and potentially improve resource use efficiency, although not necessarily by increasing total yield [13,33,34]. From a sustainability perspective, the integration of sorghum stover and *Salicornia* biomass provides a locally relevant approach for valorizing underutilized agricultural and halophytic resources in Namibia, contributing to circular bioeconomy objectives [13,35].

The measured physicochemical range (pH 6.00-7.11; EC 0.090-0.848 S/m) suggests indicative bounds for substrate formulation in this system. While low-level supplementation (≤5%; EC ≤ 0.230 S/m) appears compatible with normal production performance, higher inclusion levels were associated with reduced second-flush productivity, highlighting

the importance of monitoring salinity dynamics across successive flushes. Overall, this co-cultivation approach supports the conversion of regional biomass residues into edible fungal protein, although maintaining substrate salinity within a suitable range appears critical for sustaining multi-flush productivity in arid production systems.

Conclusions

This study demonstrated that the amendment of sorghum stover with *Salicornia* sp. had varying effects on the vegetative phase, with neutral to negative effects on the fruiting phase. In the media experiment, a positive effect was observed in the initial stage of mycelial colonization in the Petri dishes, which eventually leveled out towards the final stage of the incubation period, resulting in no significant differences in the MGR. In the bag experiment, a neutral to negative correlation was observed with the progression of the experiment. A neutral effect was observed during the primordial formation stage, as no significant difference was observed between the treatments with and without *Salicornia* sp. A decrease was noted in the biological yields, biological efficiencies, and total dry mass with an increase in *Salicornia* sp. levels in the treatment, suggesting a prohibitory effect on the effectiveness of *P. sapidus* in substrate utilization and in-fruiting performance. Overall, the findings suggest that the inclusion of *Salicornia* sp. in sorghum-based stover should primarily be used to valorize *Salicornia* sp. and not necessarily to enhance growth or yield parameters. When *Salicornia* is incorporated into a sorghum-based substrate for the cultivation of *P. sapidus*, its level should be maintained within 5% (w/w). These findings highlight the importance of maintaining substrate electrical conductivity and pH within a suitable tolerance range when integrating halophytic biomass into mushroom cultivation systems. Future research should evaluate pretreatment approaches to reduce the salt content of *Salicornia* biomass and investigate whether amendments influence the nutritional composition of *P. sapidus* fruit bodies.

Additional Information

Author Contributions

All authors have reviewed the final version to be published and agreed to be accountable for all aspects of the work.

Concept and design: Martha K. Hausiku-Ikechukwu, Ndamononghenda David

Acquisition, analysis, or interpretation of data: Martha K. Hausiku-Ikechukwu, Ndamononghenda David

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Supervision: Martha K. Hausiku-Ikechukwu

Disclosures

Plant subjects: All authors have confirmed that this study did not involve any herbarium specimen. **Human subjects:** All authors have confirmed that this study did not involve human participants or tissue. **Animal subjects:** All authors have confirmed that this study did not involve animal subjects or tissue. **Conflicts of interest:** In compliance with the ICMJE uniform disclosure form, all authors declare the following: **Payment/services info:** This work was supported by third-party institutional research funding from the University of Namibia (UNAM), project reference CA52/2901. **Financial relationships:** All authors have declared that they have no financial relationships at present or within the previous three years with any organizations that might have an interest in the submitted work. **Other relationships:** All authors have declared that there are no other relationships or activities that could appear to have influenced the submitted work.

Data Availability Statements

The datasets (and/or code) supporting this study are available from the corresponding author upon reasonable request.

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