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Nutrients and anti-nutrients in leaf litter of four selected mangrove species from the Sundarbans, Bangladesh and their effect on shrimp (*Penaeus monodon*, Fabricius, 1798) post larvae

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ABSTRACT

The release of nutrients and anti-nutrients from mangrove leaf litter plays an important role in the biogeochemical cycling in aquatic environments and directly or indirectly affects water quality and food availability to shrimp. In this study, we assessed nutrient and anti-nutrient loss during decomposition of leaf litter at a concentration of 1 g/L for four mangrove species (*Avicennia officinalis, Heritiera fomes, Sonneratia apetala, Sonneratia caseolaris*) to monitor water quality and to estimate how leaf litter influences shrimp post larvae (PL) growth and survival. There were significant differences (P < 0.05) between the studied species in terms of mass loss of the leaf litter during the investigation period. There were also significant differences (P < 0.05) between the studied species in terms of loss of nutrients and anti-nutrients in the shrimp PL rearing tank during the four-week experimental period. Decomposing mangrove leaves stimulated availability of natural food for shrimp PLs. There was a strong positive correlation between mass loss and PL production. At the concentrations of leaf litter used, the anti-nutritional factors did not affect the PLs. PL survival with mangrove leaf litter was 75–82%, whereas all the PL died without any leaf litter. PL weight gain ranged from 0.83–3.33 mg/d where *S. apetala* leaf litter resulted in the highest PL growth rate, followed by *A. officinalis, S. caseolaris* and *H. fomes*, in that order (P< 0.05). Overall, mangrove leaf litter had a positive effect on shrimp performance in terms of growth and survival and this effect was highest for *S. apetala* leaf litter.

1. Introduction

Mangroves form a highly productive ecosystem, showing high primary and secondary productivity in intertidal coastal regions of the tropics and subtropics (Nagarajan et al., 2008). Mangrove roots and fallen leaf litter provide substrate for biofilm development and nutrients in the water column and stimulate fish production (Hutchison et al., 2014; Verweij et al., 2008; Nordhaus et al., 2006). Mangroves and aquaculture are not necessarily incompatible though commercial shrimp farming is identified as the main cause of mangrove loss (Hossain et al., 2001). Considering the ecological importance of mangroves as well as the economic value of shrimp culture, mangrove-based shrimp culture (Silvo-aquaculture) is practiced in numerous countries, although not to the extend needed to conserve or restore mangrove biotopes. The first reports on silvo-aquaculture are from Indonesia (Schuster 1952 cited by Primavera, 1993), followed by Vietnam, Malaysia, the Philippines and Thailand (Primavera, 2000). In aquatic waterways the culture of seaweeds, molluscs (Rejeki et al., 2020) and fish in cages is possible adjacent to or between mangroves (Primavera, 1993) while in intertidal mangrove areas different types of silvo-aquaculture can be explored (Bosma et al., 2014; Primavera et al., 2007; Primavera, 2000). The ultimate goal of silvo-aquaculture is to increase the farmer's income while improving environmental and economic resilience. However, from an aquaculture perspective, integration of mangroves with shrimp farming may either be detrimental or beneficial. As mangrove leaf litter is an important influencer of shrimp productivity in silvo-aquaculture, its net effect on shrimp production (either positive or negative) needs to be quantified.

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Leaf litter input rate and composition affect water quality, survival and growth of shrimp. Leaching of nutrients and organic matter from mangrove litter may have positive effects on shrimp performance by supplying nutrients for algal production (Roijackers and Nga, 2002), and by stimulating the food web in shrimp ponds (Gatune et al., 2014; Nga et al., 2006; Hai and Yakupitiyage, 2005). On the other hand, leaf leachates include anti-nutritional substances among which especially tannins, saponins and phytates, and may deteriorate the water quality (Francis et al., 2001). High concentrations of these substances were found to have detrimental effects on shrimp survival and growth by affecting digestibility and hampering mineral utilization (Gemede and Ratta, 2014). Thus, analyses of the nutritional and anti-nutritional profiles of leaves and their decomposition rates in situ are important in determining whether particular mangrove species would be suitable for silvo-aquaculture. While a considerable body of knowledge exists on leaf litter production and decomposition rates in mangrove forests (Srisunont et al., 2017; Gladstone-Gallagher et al., 2014; Kamruzzaman et al., 2012; Imgraben and Dittmann, 2008; Khan et al., 2007; Silva et al., 2007; Bosire et al., 2005), little is known regarding the nutritional and anti-nutritional composition of leaf litter and their potential impacts on aquaculture production.

Different mangrove species might well have different impacts on shrimp production. Selection of the most suitable mangrove species is very important to the successful introduction of shrimp-based silvoaquaculture. For Bangladesh, Rahman et al. (2020) identified 10 mangrove species potentially suitable for silvo-aquaculture. These were Avicennia alba, A. officinalis, A. marina, Bruguiera sexangula, Kandelia candel, Sonneratia apetala, S. caseolaris, Heritiera fomes, Aegialitis rotundifolia, and Lumnitzera racemosa. Among these, A. officinalis, S. apetala, S. caseolaris and H. fomes were selected for further analysis as these mangrove species are common in the mangrove forests and easily grow on the dykes of shrimp farms in the coastal region of the country. The local availability of propagules and seedling, and farmer preference identified by Rahman et al. (2020) also supported the selection process.

The objectives of this study were to: (a) compare the nutritional and anti-nutritional contents of leaf litter from different mangrove species; (b) estimate the leaf litter mass loss over time; (c) assess the impact of the leaf litter on the water quality; and (d) measure and compare survival and growth of shrimp (*Penaeus monodon*, Fabricius, 1978) post larvae (PL), in the presence or absence of mangrove leaf litter.

2. Methodology

2.1. Experimental design

This experiment was split in two parts. In the first part, nutrients and anti-nutrients in leaf litter of four selected mangrove species were analysed in the laboratory of Forestry and Wood Technology (FWT) and the laboratory of Pharmacy, Khulna University. In the second part, the impact of leaf litter on shrimp performances (in terms of survival and growth) and water quality was measured in tank experiments. The latter were carried out at a farm located in Debhata, Satkhira. The tank culture experiments took place under a tent of transparent plastic to prevent the effects of rain water intrusion, while providing ambient lighting. We used five treatments types, executed in triplicate in tanks stocked with PLs; four treatments involved the introduction of the four species of leaf litter while one treatment involved no leaf litter. We did not apply any formulated or supplemental feed as we expected the PLs to feed on the natural food produced based on decomposing leaf litter. A treatment without leaf litter served as control as the natural water source used may have provided an otherwise undocumented and uncontrolled source of nourishment.

In the tank experiment, shrimp were reared in fifteen fibre-enforced polyethylene tanks with a water volume of 1000-L. Natural water from a nearby canal was stocked in a pond and left to settle for one week. The top water layer from this pond was transferred to the tanks through a screen with 25 µm mesh-size net to keep predators and eggs/larvae of predators out. Each tank was aerated using one air stone (diameter 2 cm) connected to an electric air blower (RESUN, LP-100). Mangrove leaf litter collected from Sundarbans, Bangladesh (southern part) was directly added in the culture tanks at a concentration of 1 g/L. This loading rate was standardized following Hai and Yakupitiyage (2005). On the same day, 100 specific pathogens free (SPF) shrimp post larvae (PL15; 0.01 g) obtained from a nearby hatchery (Desh Bangla Hatchery Limited, Khulna, Bangladesh) were stocked in each tank. The survival and growth experiment was conducted over four weeks.

2.2. Collection of leaf litter and sample preparation

Mangrove leaves which became yellowish before falling down naturally, referred to "senescent" leaves, were collected. Leaves were collected by putting 30 litter traps (2mx2m) beneath the selected mangrove species during winter (November 2018-January 2019). At regular intervals, the fallen leaves were recovered from the traps and separated according to the species.

The collected leaves were air dried at room temperature for 48 h. The leaves from each selected mangrove species were weighed (BH 300A, A & D Korea, Ltd.), mixed well and divided into two equal parts; one part was transferred to the shrimp culture tanks on the day of stocking the PLs, the other part was used for analysis of nutrients and anti-nutrients.

To identify the dry matter (DM), five grams of mixed leaves were considered as a sample and three samples (wet weight) of each species were dried in a vacuum drying oven (Vacuum Oven, OV-11, Korea) at 80 °C until a constant weight (Hossain et al., 2011). This low drying temperature was used to minimize possible changes in leaf nutrient and anti-nutrient composition. The average weight was recorded as DM and expressed as g/kg wet weight. The sample for nutrient and anti-nutrient analysis was processed according to Allen (1989). A high speed grinder (Kent 16,003) was used to finely grind the leaf sample. The powdered samples were packed into air-tight plastic bags and stored in the refrigerator (4 °C) until further analysis.

2.3. Quantification of nutrients

2.3.1. Determination of organic matter (OM), ash and ash free calorific value (AFCV)

The organic matter (OM) and ash content was measured according to Allen (1989) using a muffle furnace (Wise Therm Digital Muffle Furnace, FH-05) and the content was expressed as % DM. The gross caloric value (GCV, MJ/kg DM) in leaf litter was measured following the detailed protocol described by Fiori et al. (2015), using an Automatic Isoperible Bomb calorimeter (Parr 6400 Calorimeter). The ash-free calorific value (AFCV) was calculated based on the properties of calorific value and ash content. This was done using the equation described by Islam et al. (2019):

 $\mathsf{AFC} = \mathsf{GCV}/(1 - (\mathsf{Ash}(g)/\mathsf{DM}(g)))$

The value is expressed as MJ/kg DM.

2.3.2. Determination of carbon, nitrogen and phosphorus

The total carbon content of the leaf samples was analysed directly by CHNS Elemental Analyzer Flash 2000 (Thermo scientific, USA). For total nitrogen and total phosphorus per mangrove species, leaf powder was acid-digested according to Allen (1989). Nitrogen (N) and phosphorus (P) concentrations in the sample were measured according to Weatherburm (1967) and Timothy et al. (1984), respectively, using an UV–Visible Recording Spectrophotometer (Shimadzu UV-160A, Japan). The content of C, N and P were expressed as % DM. The C: N ratio was calculated dividing total carbon by total nitrogen content.

2.3.3. Determination of crude fibre content

The crude fibre content of the leaf samples was determined according

to Cunniff (1995). Powdered samples (1 g) were taken in a silica crucible and the extractives content was removed first through Soxhlet extraction with petroleum ether. The residue was digested with $1.25\% H_2SO_4$ and 1.25% NaOH solutions. The sample was then dried at 130 °C for 2 h and ignited at 600 °C for 30 min.

Crude fibre content was calculated by following formula:

Crude fibre (%DM) =
$$(W_1-W_2)/W X100$$

where, W = Weight of sample, $W_1 =$ Weight of silica crucible with sample before ignition, $W_2 =$ Weight of silica crucible with sample after ignition;

2.4. Quantification of anti-nutrients

2.4.1. Determination of tannins

Tannin content in the samples was determined by Folin-Denis method described by Saxena et al. (2013) with minor modification of the method of Schanderi (1970). Powdered samples (0.25 g) were extracted with 37.5 ml distilled water and heated in a flask gently and boiled for 30 min. Each sample was centrifuged at 2000 rpm for 20 min and the volume of the supernatant was brought up to 37.5 ml using distilled water in a 100 ml flask. An aliquot of 500 µl of the sample was treated with 1 ml of Folin-Denis reagent followed by 2 ml of sodium carbonate and allowed to stand for color development. The absorbance of the mixture was measured at 700 nm in a spectrophotometer (T80 UV/VIS Spectrometer, PG Instruments). Tannic acid was used as standard. The tannin content was calculated based on spectrophotometer readings of sample concentrations and the standard (theoretical) concentration and expressed as % DM.

2.4.2. Determination of saponins

Saponin content in the samples was determined following the method described by Obadoni and Ochuko (2002). The powdered samples (ca.3 g) were dispersed in 30 ml of 20% aqueous ethanol. The suspension was stirred for 12 h with constant stirring at about 55⁰C on a hotplate. The mixture was filtered (Whatman filter paper 1) and the residue was re-extracted with another 30 ml of 20% aqueous ethanol. The combined extracts (filtrates) were reduced to 15 ml over a water bath at 90⁰C. The concentrated sample extract was transferred into a 250 ml separating funnel and 10 ml of diethyl ether was added and the sample was shaken vigorously. The aqueous layer was recovered while the ether layer was discarded. The purification process was repeated twice. To the combined aqueous sample, 20 ml of n-butanol was added. The combined n-butanol extracts were washed twice with 10 ml of 5% aqueous NaCl. The remaining solution was then heated in a water bath. After evaporation, the concentrated sample was dried in a drying bath to a constant weight and saponin content was calculated according to the formula:

Saponin (%) = $(W_2 - W_1)/W \times 100$

where, W = Weight of sample, $W_1 =$ Weight of evaporating disc, $W_2 =$ Weight of disc + Sample.

2.4.3. Determination of phytates

Phytate content was determined by the method described by Rout et al. (2015) using a minor modification of method of Wheeler and Ferrel (1971). A sample of 3 g was mixed in 25 ml of 10% trichloroacetic acid (TCA) in a 125 ml flask and shaken with mechanical shaker for 2 h. This sample then was centrifuged at 3000 rpm for 20 min. Ten (10) ml of the supernatant was mixed with 4 ml of FeCl₃ solution in a 50 ml centrifuge tube. The resulting solution was then heated in a boiling water bath for 45 min. To make the supernatant clear, one or two drops of 3% sodium sulphate in 10% TCA was added under continued heating. The supernatant was then centrifuged for 10–15 min at 3000 rpm and finally the clear supernatant was discarded. The precipitate so obtained was

washed twice by dispersing it in 25 ml 10% TCA, after which it was heated again in boiling water for 10 min and centrifuged after cooling to room temperature. The precipitate was again dispersed in a few ml of water, followed by addition of 3 ml of 1.5 N NaOH, after which the volume was brought up to 30 ml with distilled water. After heating in boiling water for 30 min, the solution was filtered (Whatman No 2 paper); the precipitate was washed with 70 ml hot water and the filtrate was discarded. The precipitate on the filter paper was then dissolved with 40 ml hot HNO₃ (3.2 N) into a 100 ml volumetric flask. A 5 ml aliquot was taken and placed in a 100 ml volumetric flask and then diluted to 70 ml with distilled water, after which 20 ml of 1.5 M potassium thiocyanate (KSCN) was added. The pinkish-red color obtained was measured immediately (within 1 min) at 480 nm in a spectrophotometer (T80 UV/VIS Spectrometer, PG Instruments) using Ferric nitrate as the standard. The phytate content was calculated based on the spectrophotometer reading of sample concentration and standard (theoretical) concentration and expressed as percentage (%) DM.

2.5. Water quality monitoring

Temperature, salinity, pH, and dissolved oxygen (DO) in each tank were measured daily using, respectively, a Hanna digital thermometer, an Atago (Japan) hand refractometer, a pH (Eutech, Singapore) meter, and a Lutron (Taiwan) DO meter. Total Ammonia Nitrogen (TAN) and Nitrite-N (NO₂-N) were measured weekly by the colorimetric Nessler method, with color card and sliding comparator: HI 3826|TAN, HI 3873| Nitrite test; HANNA instruments.

Biochemical (biological) oxygen demand (BOD) was measured weekly (as BOD_5 – i.e. a 5-day incubation). Water samples were collected from the tank at a depth of 10–30 cm from the surface. Two BOD bottles (300 ml) for each replication of treatments were filled carefully with sample water without allowing air bubbles. In one bottle, DO was fixed following the Winkler method to measure initial DO while another bottle was left to incubate for 5 days. Both samples were analysed in the Khulna University water quality laboratory following the method outlined in APHA (1998).

Chemical oxygen demand (COD) was measured bi-weekly. Samples were collected from the middle of the tank at a depth of 10–30 cm from the surface water and transported to the laboratory for analysis. The analysis was done following the open reflux (OR) method outlined in APHA (1998).

2.6. Sampling and analysis of plankton

Phytoplankton and zooplankton samples were collected on day 1 and 28. Samples (15 L per sample) were collected 9.00–11.00 h from three points in each tank and passed through a 45 μ m mesh plankton net and combined. The concentrated samples were preserved in plastic bottles with 1 ml of Lugol's solution. The abundance estimations of plankton (individual.1⁻¹) were done using a one milliliter Sedgewick-Rafter (S-R) counting chamber. One ml sample was put in the S-R cell and left undisturbed for 15 min to allow the plankton to settle. The plankton in 10 randomly selected cells were counted using a compound microscope (Lx 400; magnification-4x-100x, USA) and identified (where possible to genus level) using 5.1 M C-Mount CMOS Camera- Aptina MT9P001 CMOS (Color). Plankton was identified using determination tables by Prescott (1962), Edmondson (1982), Bellinger (1992) and Tomas (1997). Plankton abundance was calculated using the following formula:

 $N = (P \times C \times 100)/V$

where, N = the number of plankton cells or units per liter of original water, P = the number of plankton counted in 10 fields, C = the volume of final concentrate of the sample (ml), V = the volume of the tank water sample in liter.

2.7. Assessment of shrimp larval performances

The growth and survival indices were calculated at the end of the four-week period using the formulas described by Busacker et al. (1990). After harvesting, the shrimp PLs were placed in tissue papers to remove excess water for accurate wet-weight determination. Weight gain was calculated by deduction of initial weight from the final weight. Weight gain per day was calculated from final weight gain divided by experiment duration (days). The formulas for calculation of survival rate (SR) and specific growth rate (SGR) were as follows:

$$\mathsf{SR}(\%) = \frac{\mathsf{Nf}}{\mathsf{Ni}} \times 100$$

SGR (%BW/day) = $(ln (BW_f) - ln(BW_i))/D \times 100$

where SR is the survival rate; N_f is the number of shrimp collected at final sampling time; N_i is the number of PLs stocked; SGR is specific growth rate (% BW day⁻¹); BW_f is the final body weight (g); BWi is the initial body weight (g); and D is the duration of the experiment (days).

2.8. Calculation of leaf mass loss, nutrient and anti-nutrient loss and decomposition rates

The leaf litter remaining in each tank at the end of the 4-week experiment was collected. The samples were prepared and the nutrients and anti-nutrients in the leaf residue also calculated as previously described. Mass loss was calculated on initial dry mass while the decomposition rate was calculated from mass loss divided by the duration of the incubation. The loss of nutrients and anti-nutrients from the leaves over a four-week incubation period was also calculated according to the mass loss during the decomposition process. All the values were expressed as % DM.

2.9. Statistical analysis

All measured values were expressed as mean \pm standard deviation (SD). One-way ANOVA was conducted to compare the dependent variables for the four types of mangrove species. A comparison of growth rates between tanks with the mangrove litter and control tank without mangrove litter was not possible because all shrimp in the control tank died prematurely. For the significant differences, a post-hoc Tukey HSD test was used to determine pair-wise differences (P < 0.05). Correlations among the different variables were assessed using Pearson's correlation coefficient. Linear regression among selected variables were also done. Analyses were conducted using IBM SPSS statistical software (Version 26).

3. Results

3.1. Nutrients and anti-nutrients in leaf litter, decomposition and mass loss

The nutrients and anti-nutrients in leaf litter of four mangrove species (*H. fomes, A. officinalis, S. caseolaris* and *S. apetala*) were identified for both senescent leaves (Table 1) and the leaf litter residue after four weeks in the shrimp PL rearing tanks (Table 2). The loss of nutrients and anti-nutrients through mass loss was also calculated during the incubation of leaf litter over a four week period in the shrimp PL rearing tanks (Table 3).

No significant differences (P > 0.05) between freshly fallen senescent leaves of mangrove species were found for ash free caloric value (MJ/kg DM), tannin or phytate content (% DM) but there were significant differences (P < 0.05) among the species in terms of crude fibre, ash, organic matter (OM), carbon (C), nitrogen (N), phosphorus (P) and saponin content (% DM) (Table 1).

There were also significant (P < 0.01) differences between species in terms of C:N ratios. The highest C:N ratio was found in *H. fomes* (36) followed by *S. apetala* (22), *A. officinalis* (22) and *S. caseolaris* (16) (Table 1). However, for the decomposed leaf litter residues, there were significant differences (P < 0.05) among the species for all types of nutrients and anti-nutrients, except phytate (Table 2). *Heritiera fomes* leaf litter was the highest in crude fibre (33% DM), OM (96%DM), C (48%DM), tannin (1.8%DM) and saponin (1.6%DM) content, whereas *S. apetala* was lowest for all those parameters except for tannin. Among the other species, *S. caseolaris* was the highest in N content (2.8%DM), *A. officinalis* was lowest in P (0.01%DM) and saponin (1.2%DM) content, the latter being similar to saponin contents in *S. caseolaris* and *S. apetala* (Table 1).

There were also significant differences (P < 0.001) in decomposition rate among the species after four-week incubation in the shrimp rearing tanks (Table 3). The highest decomposition rates (1.8% DM d⁻¹) were found for *S. apetala* and the lowest were for *H. fomes.* Accordingly, the highest percentages of OM (57%), C (57%), N (58%), P (73%) and tannin (64%) losses occurred from *S. apetala* leaves. *Heritiera fomes* leaves showed the lowest loss in percentages. For *S. apetala*, degraded leaves had the lowest OM (77%), C (38%), and P (0.01%) content and highest phytate content (0.33%). *Heritiera fomes* had the highest OM (96%), C (48%), P (0.02%), tannin (1.64%) and saponin (1.29%) content. *Avicennia officinalis* was found to be higher in decomposition rate and mass loss than *S. caseolaris*. As a result, *A. officinalis* was found with higher nutrient and anti-nutrient loss than *S. caseolaris* except for saponin.

Table 1

Nutrients and anti-nutrients contents in senescent leaves of four selected mangrove species.

Nutrients/anti-nutrients (% DM; unless specified within brackets)	Mangrove species				S.E.M.	P-value
	H. fomes	A. officinalis	S. caseolaris	S. apetala		
Energy content (MJ AFDM/Kg)	18.7	19.6	18.7	18.8	0.2	ns
Crude fibre	33.4 ^d	27.7 ^c	22.5^{b}	18.4 ^a	1.7	***
Ash	4.1 ^a	10.8 ^b	11.1 ^b	11.9^{b}	1.0	***
Organic Matter	95.9 ^b	89.0 ^a	88.7 ^a	87.7 ^a	1.0	***
Carbon	48.3 ^c	44.8 ^b	44.7 ^b	44.1 ^a	0.5	***
Nitrogen	1.35^{a}	2.01^{b}	2.79 ^c	1.98^{b}	0.2	***
Phosphorus	0.02^{b}	0.01^{a}	0.03 ^d	0.02 ^c	0.0	***
Tannin	1.84	1.73	1.80	1.79	0.0	ns
Phytate	0.33	0.43	0.36	0.38	0.0	ns
Saponin	1.58^{b}	1.16 ^a	1.23 ^a	1.29 ^a	0.1	***
C:N	35.8 ^d	22.3 ^c	16.1 ^b	22.3 ^a	2.2	**

Small letter used as superscript to indicate significant differences, according to Tukey HSD test (P < 0.05). P value is expressed as a symbol (P < 0.001: ***; P < 0.01: **; P < 0.05: *; ns: not significant, P > 0.05).

Table 2

|--|

Nutrients/anti-nutrients (% DM)	Mangrove species				S.E.M.	P-value
	H. fomes	A. officinalis	S. caseolaris	S. apetala		
Organic Matter	95.5 ^d	81.4 ^b	84.5 ^c	77.3 ^a	2.0	***
Carbon	47.9 ^d	40.8 ^b	42.6 ^c	38.4 ^a	1.1	***
Nitrogen	1.16^{a}	1.83 ^c	2.30 ^d	1.67 ^b	0.1	***
Phosphorus	0.02^{ab}	0.01^{a}	$0.02^{\rm b}$	0.01 ^a	0.0	*
Tannin	1.64 ^b	1.28^{a}	1.18^{a}	1.33 ^{ab}	0.1	*
Phytate	0.24	0.33	0.24	0.33	0.0	ns
Saponin	1.29 ^b	0.73^{a}	0.80^{a}	1.00^{ab}	0.1	*

Small letter used as superscript to indicate significant differences, according to Tukey HSD test (P < 0.05). P value is expressed as a symbol (P < 0.001: ***; P < 0.01: **; P < 0.05: *; ns: not significant, P > 0.05).

Table 3

Mass loss (% DM), nutrient and anti-nutrient loss (% DM) of leaves of four selected mangrove species over a four-week period in shrimp PL tanks.

Loss on Initial weight of leaves (%DM; unless specified within brackets)	Mangrove species				S.E.M.	P-value
	H. fomes	A. officinalis	S. caseolaris	S. apetala		
Mass Loss	23.0 ^a	45.8 ^c	39.4 ^b	50.7 ^c	3.2	***
Decomposition rate (% day $^{-1}$)	0.83^{a}	1.6 ^c	1.4^{b}	$1.8^{\rm c}$	0.1	***
Organic Matter	23.3^{a}	50.3 ^c	42.3 ^b	56.5 ^d	3.8	***
Carbon	23.6 ^a	50.6 ^c	42.3 ^b	57.1 ^d	3.8	***
Nitrogen	33.7 ^a	50.5 ^b	50.1 ^b	58.3 ^c	2.8	***
Phosphorus	36.8 ^a	63.5 ^{bc}	55.8^{b}	73.4 ^c	4.4	**
Tannin	31.4 ^a	60.2^{b}	60.3^{b}	63.5^{b}	4.0	***
Phytate	44.8 ^a	58.2 ^b	60.1^{b}	56.3 ^b	2.0	**
Saponin	37.5 ^a	66.0 ^b	60.5 ^b	62.0^{b}	3.7	**

Small letter used as superscript to indicate significant differences, according to Tukey HSD test (P < 0.05). P value is expressed as a symbol (P < 0.001: ***; P < 0.01: **; P < 0.05: *;)

3.2. Impact of leaf litter decomposition on water quality

No differences (P > 0.05) in water quality parameters were found between the tanks treated with the different mangrove species except for temperature, BOD and phytoplankton concentration (Table 4).

The temperature of tank water ranged from 27.9–28.0 °C where the temperature in the tank waters incubated with *S. apetala* was slightly but significantly different (P < 0.001) from the other three species of mangrove leaf litter. The highest BOD was measured in the tanks with *S. apetala* leaves (2.41 mg/L) and the lowest was measured in tanks with *H. fomes* litter (1.98 mg/L). The BOD in the tanks with *H. fomes* litter was lower (P < 0.001) than for the other mangrove species. There were also significant differences (P < 0.001) in phytoplankton concentrations between tanks treated with different mangrove species. The highest concentration of phytoplankton was found with *S. apetala* (9.2 cells/ml) and the lowest number with *H. fomes* (2.50 cells/ml). In general, tank waters with *H. fomes*-incubated water had the highest concentration observed. The highest pH was measured in the tanks with *S. apetala* (9.2 cells/ml) and the lowest number with *M. fomes* (2.50 cells/ml).

leaves (7.94) and the lowest was measured in tanks with *H. fomes* litter (7.87). DO levels also showed no significant differences (P > 0.05) between tanks incubated with the different leaf litter species. The COD was the highest in tanks with *A. officinalis* (49 mg/L) and the lowest with *H. fomes* (40 mg/L) but no significant difference could be demonstrated (P > 0.05). The TAN concentrations were higher in tanks with *H. fomes* litter (0.13 ppm) but lower for those with *S. apetala* (0.1 ppm). For neither TAN nor NO₂-N concentrations were there significant differences (P > 0.05) in concentration among the mangrove species. There were also no significant differences in zooplankton concentrations between the different mangrove treatments (P > 0.05).

3.3. Impact of decomposing leaf litter on PL survival, weight gain and specific growth rate (SGR)

The survival rate in the tanks with leaf litter ranged between 75% and 82% (Fig. 1a) and did not differ significantly between mangrove leaf treatments (P > 0.05). In contrast, the shrimp PL in the control treatment without any leaf litter started to die on day 3 and on day 8 all the

Table 4

Average water quality parameter values observed in shrimp PL rearing tanks during a four-week incubation period, with leaf litter from four different mangrove species.

Water quality parameter	Mangrove specie	es	S.E.M.	P-value		
	H. fomes	A. officinalis	S. caseolaris	S. apetala		
Temperature (°C)	28.01 ^b	27.99 ^b	27.98 ^b	27.93 ^a	0.01	***
pH	7.87	7.89	7.91	7.94	0.01	ns
DO (mg/L)	5.36	5.43	5.38	5.38	0.10	ns
BOD (mg/L)	1.98^{a}	2.34 ^b	2.13^{b}	2.41^{b}	0.05	***
COD (mg/L)	40.0	48.9	45.6	46.7	1.71	ns
TAN (ppm)	0.13	0.1	0.1	0.1	0.01	ns
NO ₂ -N (ppm)	0.07	0.27	0.17	0.23	0.03	ns
Phytoplankton (Cell/ml)	2.50^{a}	5.83 ^b	4.17 ^{ab}	9.17 ^c	0.80	***
Zooplankton (Cell/ml)	2.50	5.0	3.33	5.0	0.57	ns

Small letter on the superscript indicate significant differences, according to Tukey HSD test (P < 0.05). P value is expressed as a symbol (P < 0.001: ***; P < 0.01: **; P < 0.05: *; ns: not significant, P > 0.05).



Fig. 1. (a-d): Impact of leaf litter of four selected mangrove species on shrimp performances: (a) Survival rate (%) of Shrimp PL with four types of leaf litter, (b) Survival rate (%) decreased with time (day 01 to day 08) in the control (without any leaf litter), (c) Weight gain (mg d⁻¹) of shrimp PL with four types of leaf litter; (d) SGR (%BW d⁻¹) of shrimp PL with four types of leaf litter; Values are means (\pm SD) of three replicate tanks per treatment. Different letters above data points indicate significant differences.

shrimp PL had died (Fig. 1b).

The growth rates of shrimp PL did differ significantly (P < 0.05) depending on the species of mangrove species used in the tanks. The average daily growth rate was highest for the larvae incubated with *S. apetala* leaf litter and the lowest for those incubated with *H. fomes* litter (Fig. 1c). The SGR was highest for larvae reared with *S. apetala* (10.6) leaf litter and lowest in larvae reared with *H. fomes* (6.2) (Fig. 1d) (P < 0.05). This concurred with the highest final shrimp size reached in *S. apetala* tanks and lowest in *H. fomes* tanks.

Significant correlations (P < 0.05) were identified between different pairs of variables. We found a negative correlation between crude fibre content and decomposition rate of leaf litter, and positive correlations between decomposition rate of leaf litter and BOD, mass loss of leaf litter and phytoplankton concentration, and PL weight gain and phytoplankton concentration (Fig. 2).

4. Discussion

4.1. Decomposition, mass loss and biochemical changes (nutrient and anti-nutrient composition) in leaf litter

In this study we found differences in the biochemical composition of freshly fallen leaf litter of different mangrove species (Table 1). Mangrove leaves vary in their organic and inorganic constituents according to species, age, season and physical or morphological characteristics of the leaves (Hossain et al., 2011; Basak et al., 1998, 1996; Tam

et al., 1998). The leaves of the mangroves species studied differed in their tendencies to lose mass and release biochemical components during decomposition (Table 3). Rajendran and Kathiresan (2000) previously studied biochemical changes in decomposing leaves of two mangrove species, Rhizophora apiculata and Avicennia marina, and found that different rates of leaf decomposition between species led to different rates of mass loss of the decomposing leaves. This was in part due to the rapid leaching of water-soluble organic and inorganic substances during the initial stages of the decomposition process (Hossain et al., 2009; Ashton et al., 1999) and to microbial breakdown (Hossain et al., 2014). We observed lower mass loss for H. fomes among the four studied species. Hossain et al. (2014) observed the similar tendency of mass loss for H. fomes in comparison to three other mangrove species Excoecaria agallocha, Ceriops decandraand and Xylocarpus mekongensis from the Sundarbans. The variation in crude fibre contents (%) might be a determinant of variation in decomposition rate. In our study, the crude fibre content was highest in H. fomes (33%) and lowest for S. apetala (18%), and negatively correlated (Fig. 2a) to the decomposition rate of the different species of mangrove litter, as also previously reported by Du et al. (2020) and Ibrahima et al. (2008).

4.2. Impact of dry matter, carbon, nitrogen and phosphorous loss from mangrove leaf litter on water quality and shrimp performance

Mangrove leaf litter is an important source of organic matter in tropical and subtropical aquatic environments, supporting the



Fig. 2. (a-d): Linear regression of (a) Crude fibre content in leaf litter and decomposition rate, (b) Decomposition rate and biological oxygen demand, (c) Mass loss of leaf litter and phytoplankton concentration, (d) Phytoplankton concentration and PL weight gain.

microbial-based food web and providing natural food to PLs (Gatune et al., 2014; Nga et al., 2006). Considering the efficiency (30-36%) of microbial conversion of the portion of mangrove leaves lost to decomposition, it appears that a significant percentages of mangrove detritus is relatively rapidly assimilated into microbial biomass and thus potentially available to the aquatic food web (Benner et al., 1986). Mangrove litter releases nutrients and supports periphytic biofilm growth, a good food source for PLs (Gatune et al., 2012). In our study, the mangrove species with higher decomposition rates contributed more nutrients through mass loss in the shrimp culture tank (Table 3). Faster weight loss by the leaves meant that more organic and inorganic compounds became available for microbiota development (Wetzel, 1995), resulting in better PL growth, and illustrated by the positive correlation between leaf litter mass loss and PL weight gain (Fig. 2c). The results clearly showed that the mangrove litter supplied to the tanks served as a needed food source for the PL. Decomposing mangrove leaf litter stimulates natural food production (Rejeki et al., 2019; Nga et al., 2006). Natural food can contribute up to 50-70% of nutritional requirements of shrimp held in culture ponds (Martinez-Cordova and Enriquez-Ocana, 2007; Enríquez, 2003; Tacon, 2002). Thus the natural food produced from decomposed leaf litter helped the PLs to survive and gain weight. It cannot be excluded that the difference in survival rate between treatment with leaf litter and the controls without leaf litter could have partially been due to the leaf litter serving as shelter and reducing cannibalism (Hai and Yakupitiyage, 2005)

We found no significant differences (P > 0.05) in water quality parameters between the types of leaf litter, except forf biological oxygen demand (BOD mg L¹) and algal biomass (cells ml⁻¹). A higher BOD indicated more decomposition and conversion of litter into

phytoplankton as shown by significant correlations between leaf litter mass loss and phytoplankton concentration, and between PL weight gain and phytoplankton concentration (Fig. 2 (b-d)). In our study large quantities of leaf litter were available in comparison to the PL biomass, supporting PL production, while the water quality remained good. Clearly, in cases with much higher litter stocking densities or lower levels of aeration, the PLs could just as well have experienced detrimental conditions, leading to higher mortality by sudden depletion of DO (Rejeki et al., 2019; Nga et al., 2006; Hai and Yakupitiyage, 2005). Hence, while our results show a positive effect of mangrove litter, the outcome of leaf litter addition is situation-specific, so the results cannot be generalized. In our experiment, aeration kept the water volume in rearing tanks aerobic. The PLs prefer a well oxygenated environment, which is found in estuaries. The shallow water in estuaries ensure increase of DO concentration in water through constant wave action (Bozkurt and Kabdasli, 2013). An estuary on a mangrove coast provides a lot of food, substrate and protection to young penaeids (Vance et al., 1990; Zimmerman and Minello, 1984). In our results, higher BOD and algal biomass, were found in tanks treated with S. apetala litter followed by A. officinalis, S. caseolaris and H. fomes. This differences might be due to the quality of organic matter in the water as influenced by decomposition of leaf litter, as indicated by the BOD: COD ratio (Rojas-Tirado et al., 2017). There was a strong positive correlation (P < 0.01; r =0.820) between OM and BOD. As the water quality was not affected by mangrove species, no significant difference in survival rate of the shrimp was observed.

One limitation of our experiments is that the duration (4 weeks) was short, allowing only limited time to develop any potential negative effects of organic matter decomposition. Sustainable accumulation and decomposition of organic matter might lead to a decline of water quality, cause stress, reduce growth and increase the susceptibility to disease and mortality of fish and shrimp (Jackson et al., 2003). Therefore, additional studies are needed to look at the longer-term effects of prolonged accumulation of organic load so as to develop insight into how to benefit from mangrove leaf decomposition without experiencing its potentially negative effects at higher leaf densities and for longer periods of exposure. Considering the positive effect of *Sonneratia apetala* leaf litter, it is recommended to perform a leaf litter dose-response follow-up experiment for this mangrove species.

4.3. Impact of tannin, saponin and phytate from mangrove leaf litter on water quality and shrimp performance

Along with nutrients, tannin, phytate and saponin were released in the shrimp PL rearing tank through decomposition of the leaves. Fitzgerald (1999) reported that higher concentrations of tannin might be toxic to shrimp in silvo-aquaculture systems. Hai and Yakupitiyage (2005) identified higher amounts of tannin (ranged 8.2-28.7 mg/L) in the water column leached from leaves of R. apiculata, A. officinalis, Excoecaria agallocha and Acacia auriculiformis in shrimp experimental tanks and their effects on shrimp growth and survival depending on the loading rate of leaves and leaf concentrations of tannin. However, some researchers also stated that anti-nutrients sometimes act as non-nutritive compounds with positive effects. For instance, Sudheer et al. (2011) found the mangrove species Ceriops tagal to be effective against white spot syndrome virus (WSSV) disease of shrimp. Thus, the resistance properties of tannins to microbial degradation and their anti-bacterial, antiviral, antifungal activity (Krzyzowska et al., 2017) might be interesting topics for further research. As we found considerable concentrations of tannin in leaf litter of all four species in comparison to other nutrients (N, P) and anti-nutrients (phytate, saponin) it might also be interesting to have a challenge test to study how tannin help protect against shrimp diseases. Though anti-nutrients have been found to impact the water quality and shrimp performance in other studies (Rejeki et al., 2019), we found no significant impact based on the (lower) litter densities used, the time frame of the growth experiment and the level of aeration used in our study.

Other work also suggests that phytate affect PL performance by affecting the mineral utilization and reducing enzymatic activities in post larvae (Gemede and Ratta, 2014). On the other hand, phytate sometimes play a positive role by supplying available P through breakdown of phytate-P (Kumar et al., 2012). Though termed antinutrients, saponins also sometimes play a positive role (Freeland et al., 1985). Saponins increase digestibility of carbohydrate-rich food because of their detergent-like activity by reducing viscosity and preventing obstruction of movement of digesta in fish intestines (Hajra et al., 2013) and possible also for shrimp PLs. Our results show a positive correlation (P > 0.05; r = 0.016) between phytates and weight gain. This suggests but does not prove a causal relationship. The correlation (P >0.05; r = -0.086 (survival), r = -0.319 (weight gain)) between saponin and shrimp performances (survival and weight gain) was negative but insignificant. As a result, there was no mentionable negative impact of phytate and saponin contents on the shrimp performances.

Considering the overall impact of nutrients and anti-nutrients of mangrove leaf litter on shrimp PL performance in this study, it appears that the leaf litter of selected mangrove species can be of use to enhance shrimp survival and growth performances.

5. Conclusions and recommendations

There is an urgent need to develop more sustainable and ecologically and socio-economically resilient approaches to food production. This is particularly the case for vulnerable tropical muddy coastlines where mangrove vegetation has been cleared in the past for large-scale shrimp pond culture. A case in point are the Sundarbans-associated muddy mangrove coasts of Bangladesh, the country that has worldwide been shown to be most vulnerable to climate change risks (World Bank, 2018). In this study we demonstrate positive effects of different species of mangrove leaves on water quality, shrimp growth and survival rate under controlled conditions at a concentration of 1 g (fresh leaves) /L. *Sonneratia apetala* was found to perform better in terms of nutrients return to the aquatic environment through mass loss during decomposition and gave the most positive effect on shrimp growth rate. *Heritiera fomes* showed positive effects on survival but (compared to control tanks without mangroves) but growth was the lowest of all four species tested. *Avicennia officinalis* and *S. caseolaris* showed similar and intermediate growth performance of shrimp PL. Finally, to introduce silvoaquaculture using the studied species we recommend further research on:

- 1. How to optimize growth performance by combining supplementary feeding with leaf litter addition;
- 2. The performances of PL using mixed mangrove species leaf litter;
- 3. The effect of mangrove litter in ponds as compared to tanks.

Author statement

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Declaration of Competing Interest

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

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