



Research article

Shell structure investigation and utilization of bio-extract fermented from green mussel shell

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Abstract

Green mussel shell left over from seafood processing and consumption is a worthless waste and difficult to eliminate because it takes a long time to decompose. The current study aimed to add value to this garbage item. Green mussel shell was fermented in a non-aerated system using bio-extract as liquid fertilizer for water convolvulus hydroponics culture. The fermentation was divided into two treatments: treatment 1 (based on Land Development Department proportions) and treatment 2 (adding 60% by volume of molasses, based on Land Development Department proportions consisting of molasses, water and Microbial Activator Super LDD 2). The results from scanning electron microscopy showed that the outer structure of the inner and outer shell surfaces was cracked and eroded after fermentation. After 15 wk fermentation, bio-extract treatment 1 had an average calcium oxide content that was greater than for the treatment 2 formula ($p < 0.05$), and the amounts of calcium, chitin and protein in the shells of both treatments had decreased and been transformed into bio-extract. Utilization of bio-extract from the green mussel shells in a bio-extract mixed chemical liquid fertilizer formula resulted in increased height of water convolvulus compared to the control formula (chemical liquid fertilizer). Thus, bio-extract may be suitable as a dietary supplement to hydroponic plant culture to reduce the calcium in liquid chemical fertilizers in the growing crop.

Introduction

Green mussels are an economic aquatic marine shellfish in Thailand due to their low price and their various nutritional components, such as protein, vitamins and minerals (Chakraborty et al., 2016; Department of Fisheries, 2016). The mussels are processed into high value seafood products using different types of processing such as drying, pickling, boiling and freezing (Muraleedharan et al.,

1982; Masniyom et al., 2011; Doncillo and Combras, 2017). They are popular among consumers. Gopalakrishnan and Vijayavel (2009) reported that green mussel (*Perna viridis*) had a better nutritional composition than *Meretrix meretrix* and *Donax cuneatus* regarding the protein, vitamin C and calcium contents. *Perna viridis* has a wet weight of approximately 284.2 µg/mg while the two species have wet weights of approximately 245.3 µg/mg and 275.5 µg/mg, respectively. Green mussel has a vitamin C content of approximately 7.14 µg/mg wet weight compared to the other two species with approximately 5.83 µg/mg wet weight and 6.16 µg/mg wet weight, respectively.

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Furthermore, green mussel has approximately 257 µg/g dry weight of calcium compared to *M. meretrix* with approximately 175 µg/g dry weight and *D. cuneatus* with approximately 236 µg/g dry weight. Fisheries statistics for 2014–2015 indicated that green mussel had the greatest yield (319,229.47 t) amounting to approximately 60% of the total shellfish yield in the past three years (Department of Fisheries, 2018). However, such a large volume of shellfish has caused a problem due to the large amounts of shells and debris after processing and consumption. The fresh mussel from the seafood industry has produced as much as 50,000 t of shell waste per year (Pichayapaiboon, 2015). Martínez-García et al. (2017) reported the shell waste constituted approximately 33% of the total weight which was a worthless byproduct. Most of this shell garbage has been disposed of in landfill and produces a strong odor during decomposition (Shavandi et al., 2015). In addition, the waste mussel shell is hard to degrade and takes a long time to decompose (Pichayapaiboon, 2015).

Green mussel shell consists of calcium carbonate (90–95%) and an organic matrix (5%) (Zhang and Zhang, 2006). The structure of the green mussel shell is composed of three layers (Xu and Zhang, 2014), with the inner periostracum layer consisting of a thin, green or dark brown outer layer containing protein, pigment and chitin, the second middle prismatic layer is the thickest, consisting of calcium carbonate with calcite crystals, while the outer nacre layer has a smooth surface and a shining inner layer composed of calcium carbonate in the form of aragonite crystals attached in several sheets with organic substances arranged together in a perpendicular direction to the shell (Matsushiro and Miyachita, 2004; Xu and Zhang, 2015). The composition of the green mussel shell confers some advantages and various applications have been researched and experimented with to eliminate the shell waste. Hamester et al. (2012) used the calcium carbonate obtained from mussel shell (*Perna perna*) to construct polymer materials for medicine and confirmed that the shell calcium carbonate had comparable properties to commercial calcium carbonate. Shavandi et al. (2015) produced nano-hydroxyapatite, like a component of the bones and teeth in humans, from shell calcium carbonate for medical applications using a microwave irradiation method. Martínez-García et al. (2017) reported using mussel shell waste with different sand and gravel sizes add to concrete mixes. Álvarez et al. (2012) applied ground mussel shell to improve the acidity and lower the effective cation exchange capacity in soil on a pasture area used for grazing and found that mussel shell increased the pH level and exchangeable Ca in the soil.

Another option to reduce the amount of shell waste is using the shells to make bio-extract. In Thailand, the Land Development Department, Ministry of Agriculture and Cooperatives has produced and promoted the use of microbial activator substances using the term “Microbial Activator Super LDD2” which can be used as an agricultural input for the production of compost, bio-extracts and for wastewater treatment (Land Development Department, 2016). The Department has reported that Microbial Activator Super LDD2 consists of a group of highly efficient microorganisms that can activate the fermentation

and digestion processes of organic waste such as the residue from vegetable, fish and shells to produce bio-extract. The microorganisms in Super LDD2 include yeast, lactic acid bacteria, proteolytic bacteria, lipolytic bacteria and phosphate-solubilizing bacteria which can use soy bean meal, bran, farmyard manure, rock phosphate and bone meal as sources of substrate for microbes. The ratio of fermentation to produce the bio-extract that Land Development Department (2016) suggests is organic waste:molasses or other sugar:water:Microbial Activator Super LDD2 of 3:1:1:1. Furthermore, Siripoke (2012) reported on using non-aerated fermentation as a fermentation process in a closed system (batch fermentation). This process added the microorganisms in system then provided the substrate for microbes to grow on until the end of the fermentation process, with no added nutrients during fermentation so that the growth of the microorganism was nutrient-limited. Therefore, this fermentation requires the nutrient to contain sufficient carbon for the microbes. Sangon (2017) reported the quantity of microbes used in the industrial fermentation process should be 3–5% and not more than 10% of the nutrient volume on a volume per volume basis. In addition, Rattanasuk and Klinsukon (1981) reported that using non-aerated fermentation as a method for removing organic substances was suitable for tropical areas such as Thailand because the reaction in the fermentation process required a relatively high temperature

The current study investigated the composition of green mussel shell waste in terms of protein and chitin, and studied their utilization from shell waste using a non-aerated fermentation technique, using the bio-extract produced from the green mussel shell waste to culture the water convolvulus (*Ipomoea aquatica*) using a non-circulating hydroponic method. This alternative way to process shell waste is environmental friendly and can add value to a formerly valueless waste product.

Materials and Methods

Preparation of green mussel shell for non-aerated fermentation

Samples of green mussel shell were collected from a community that processed green mussel in Samut Songkhram province, Thailand. The shells were cleaned of tissue scraps and barnacles and then dried before grinding the shell in pieces approximately 2.5 cm in length.

The composition used in fermentation involved: ground green mussel shell, molasses, water and Microbial activator Super LDD 2. The proportions used were based on Land Development Department (2016): green mussel shell:molasses:water:Microbial activator Super LDD 2 were 2:1:1:1. The experiment involving green mussel shell non-aerated fermentation was divided into two treatments with three replications (Table 1): treatment 1, the composition proportions followed the formula of Land Development Department (2016); and treatment 2, added 60% (by volume) of molasses to treatment 1. The proportions for non-aerated fermentation of green mussel shell are shown in Table 1.

Table 1 Proportions used in fermentation of green mussel shell waste

Treatment	Proportion			
	Green mussel shell (g)	Molasses (g)	Water (mL)	Microbial Activator Super LDD 2 (g)
1	2,000	1,000	1,000	2.5
2	2,000	1,600	400	2.5

All ingredients were added and mixed well in each fermentation tank (5 L plastic tank with a screw cap) and sealed with a lid (Rueangsri, 2012). During fermentation, no more nutrients were added (batch fermentation). Each tank was shaken well every 1–2 d to prevent precipitation of the ingredients and to ensure nutrients were in contact with the microbes and the lid was sometimes removed to releasing any gas (Pake, 2013). The tank was kept in a ventilated room for 15 wk. The bio-extract was collected and determined for calcium content using atomic absorption spectroscopy (GBC brand, model SensAA) during weeks 3, 13 and 15 of fermentation. The protein content was analyzed after week 15 of fermentation using Bradford protein assay (Bradford, 1976).

Investigation of composition of green mussel shell before and after non-aerated fermentation

The amounts of calcium, chitin and protein in the green mussel shell were analyzed before and after 15 wk of fermentation. Quantification of calcium content in the green mussel shell followed Association of Analytical Chemists (2000). A sample (1 g) of the shell was added to 69% HNO₃ and HClO₄ (ratio 1:2) and heated on a hot plate set up in a fume hood for digestion of the shell. Diluted shell digested solution was made up to 100 mL with deionization water. The wavelength for calcium analysis was 422.7 nm and the radiation of the calcium absorbed was compared with a calibration curve and the calcium content in the shell was shown as a calcium oxide (CaO) value (Kostecka, 2000; Palma et al., 2017).

Chitin and chitosan were determined according to Abdulkarim et al. (2013) and Rinaudo (2006). A sample of crushed green mussel shell (100 g in 1 cm pieces) was mixed with 1M NaOH (ratio 1:10 weight per volume, w/v) and left on a magnetic stirrer at 100°C for 4 hr. The sample was filtered, washed with distilled water and adjusted to pH 7 before being dried in an oven at 50°C for 24 hr. Then, the green mussel shell residue was weighed. The shell residue was added to 0.68 M HCl (ratio 1:10 w/v), soaked for 6 hr and then filtered with the reaction with the 0.68 M HCl repeated 5–6 times or until no gas bubbles were produced. After washing the sample with distilled water and adjusting to pH 7 (based on a pH paper test), the pigments were removed by soaking in 99.5% acetone and then in 100% methanol. The soaking was repeated until no color was evident in the solvent. Then, the green mussel shell residue was dried in a hot-air oven at 50°C for 24 hr, weighed and the amount of chitin was determined according to Equation 1:

$$\% \text{ Chitin} = \frac{\text{Weight of chitin}}{\text{Weight of green mussel shell}} \times 100 \quad (1)$$

To make chitosan, the chitin was added with 50% NaOH (ratio 1:10 w/v) and mixed well using a magnetic stirrer at 100°C for 1 hr. The mixture was allowed to cool at room temperature, washed with distilled water and adjusted to pH 7 before drying in a hot-air oven at 60°C for 24 hr. The sample was weighed the amount of chitosan was calculated based on Equation 2:

$$\% \text{ Chitosan} = \frac{\text{Weight of Chitosan}}{\text{Weight of chitin}} \times 100 \quad (2)$$

The total protein content in the green mussel shell was determined using the Kjeldahl method (Association of Analytical Chemists, 2000).

Investigation of characteristics of green mussel shell before and after non-aerated fermentation

The structures of the inner and outer shell surfaces of green mussel shell were compared to the characteristics before and after fermentation using scanning electron microscopy (SEM). The pieces of green mussel shell (up to 1 mm in size) were washed with distilled water five times, dried with hot air and kept in a desiccator. Then, each sample was stuck on a stub using carbon tape and kept in a vacuum desiccator before being coated with platinum in a sputter coater and observed using SEM (Goldstein et al., 2003)

Utilization of bio-extract from green mussel shell waste to culture water convolvulus (Ipomoea aquatica)

Water convolvulus seeds were initially placed on a wet filter cloth and protected from exposure for 24 hr. After the seeds had produced their roots, the sprouts were transferred to the planting materials (cubes of sponge, 1 cm³ in size, 2 sprouts/sponge) and placed in containers filled with water to the depth of the sponge (one planting with sprouts/container). When the roots of the sprouts had grown through the sponge, the water in the containers was changed to liquid fertilizer. The liquid fertilizer in each treatment was based on the mixture of bio-extract from green mussel shell waste and chemical liquid fertilizers for hydroponic culture in the ratio suitable for 500 mL per container (Table 2) and placed in a shady area to protect from direct sunlight (Phibunwatthanawong and Riddech, 2019). The heights of the water convolvulus were measured daily for 30 d using a piece of string from the base of the planting material to the highest point of the plant and the string length was then measured using a ruler (Heady, 1957). The proportions of bio-extract from green mussel shell waste and the different chemical liquid fertilizers for hydroponic water convolvulus culture are shown in Table 2.

Table 2 Proportion of bio-extract from green mussel shell waste and chemical liquid fertilizers for hydroponic water convolvulus culture

Treatment	Proportions of liquid fertilizer			
	chemical liquid fertilizer A (clfA)* (mL)	chemical liquid fertilizer B (clfB)* (mL)	bio-extract (mL)	Water (mL)
Treatment 1 (bio-extract from treatment 1)				
Formula 1	1.25	2.50	1.25	500.00
Formula 2	0.00	2.50	2.50	500.00
Formula 3	0.00	0.00	2.50	500.00
Treatment 2 (bio-extract from treatment 2)				
Formula 4	1.25	2.50	1.25	500.00
Formula 5	0.00	2.50	2.50	500.00
Formula 6	0.00	0.00	2.50	500.00
Control formula	2.50	2.50	0.00	500.00

* bio-extract in each treatment was mixed with chemical liquid fertilizers; A (clfA) provided calcium as the main nutrient in the fertilizer. Ratios of bio-extract mixed with chemical liquid fertilizer A (clfA) were: bio-extract and liquid chemical fertilizer A at 1:1 (formulas 1 and 4), bio-extract instead of liquid chemical fertilizer A (formulas 2 and 5) and bio-extract only (formulas 3 and 6).

All experiments had three replications.

The chemical liquid fertilizers (clf) consisted of commercial inorganic nutrients for a hydroponic system (Churilova and Midmore, 2019): A (clf A), 20 g/L CaO and 0.5 g/L Fe; B (clf B), 10 MgO g/L, 5 g/L S, 0.5 g/L Fe, 2 g/L Mn, 0.5 g/L Cu, 0.5 g/L Zn and 0.5 g/L B. The application ratio was 5 mL of part A or 5 mL of part B per liter of water.

Statistical analyses

Data were analyzed using one-way analysis of variance with significance tested at $p < 0.05$ for the components in green mussel shell before and after fermentation, the calcium content in bio-extract each week of fermentation and increased height of water convolvulus. Means were compared using post-hoc test. All statistical analyses were performed using the SPSS software (version 11, SPSS Inc.; Chicago, IL, USA).

Results and Discussion

Composition of green mussel shell before and after non-aerated fermentation

Before fermentation, the mean amounts (\pm SD) of calcium, chitin and protein were $53.97 \pm 6.56\%$ CaO%, $26.28 \pm 2.12\%$ and $3.58 \pm 0.14\%$, respectively, while the contents after fermentation (treatments 1 and 2) had decreased at week 15. The amounts of all shell components in each treatment are shown in Table 3.

Chitosan is derived from the chitin deacetylation process under standard conditions (Martinou et al., 1995). The dry weight of chitosan decreased correspondingly to the chitin content. The mean dry weight (\pm SD) of chitosan before fermentation was 20.12 ± 1.45 g and after fermentation was 5.14 ± 0.49 g (treatment 1) and 11.70 ± 2.08 g (treatment 2). Before fermentation, the mean percentage chitosan (\pm SD%) was $76.61 \pm 1.20\%$, while after fermentation it was $63.93 \pm 15.89\%$ (treatment 1) and $85.97 \pm 2.00\%$ (treatment 2), respectively.

The total protein contents in green mussel shell before and after fermentation were not significantly different. Comparison of the shell components before and after fermentation indicated that the quantities of both calcium oxide (CaO) and chitin in the shell for treatments 1 and 2 significantly decreased.

Calcium plays an important role in plant growth by supporting cell synthesis and cell structure, promoting the shape of the cell wall (Hepler, 2005). If the plant lacks calcium, it will easily rot and turn black. Chitin acts as a protective structure and strengthens the structure of living things as it prevents insect, rot and microbial spoilage (Rinaudo, 2006) and can also accelerate the growth of plants and act as a hormone to stimulate the roots (Rinaudo, 2006). Currently, there is no report on the impacts of using different concentrations of calcium, chitin and chitosan in hydroponic culture of water convolvulus. The current research could indicate that using extracts from fermented green mussel shells in growing water convolvulus will improve the strength, promote fast rooting and growth and disease prevention.

Table 3 Green mussel shell components before and after non-aerated fermentation

Component	Before fermentation (mean \pm SD%)	After fermentation (mean \pm SD%)	
		treatment 1	treatment 2
CaO	53.97 ± 6.56^a	48.37 ± 1.96^b	46.23 ± 2.55^b
Chitin	26.28 ± 2.12^a	8.19 ± 1.27^b	13.65 ± 2.71^b
Protein	3.58 ± 0.14^a	3.71 ± 0.12^a	3.58 ± 0.06^a

mean values superscripted with different lowercase superscript letters within each row indicate significant differences ($p < 0.05$) between groups

The amount of calcium oxide, chitin and protein that had accumulated in the shell decreased after fermentation perhaps due to the action of enzymes from Microbial Activator Super LDD 2 that decomposed the shell and cracked the shell as was evident in the SEM images (Fig. 1).

Characteristics of outer structure of green mussel shell before and after non-aerated fermentation

Samples both the outer and inner shell components of green mussel shell were observed using SEM. The outer shell side before the fermentation had a smooth surface (Fig. 1A) while inside surface accumulated many crystals of calcium carbonate (Fig. 1C), as was also reported by Xu and Zhang (2015). After fermentation, the outside of the green mussel shell had increased cracking (Fig. 1B), while the calcium carbonate crystals on the inside of the shell side looked eroded and broken (Fig. 1D).

The SEM images of the mussel shell showed shell that both the outside and inside of the shell was broken after fermentation. Xu and Zhang (2014) reported the outer shell layer (periostracum) of green mussel shell could be divided into three layers which the outermost and innermost layers consisting of protein, while the middle layer was minerals such as calcium carbonate (CaCO_3) and magnesium phosphate $\text{Mg}_3(\text{PO}_4)_2$; thus, it was possibly the proteolytic bacteria and phosphate-solubilizing bacteria in Microbial Activator Super LDD 2 that had decomposed the mussel shell resulting in more cracks.

The non-aerated fermentation occurred under acid conditions since the metabolic activities of the microorganisms produced organic acids with for example, the proteolytic bacteria producing amino acid and the lactic bacteria producing lactic acid (Land Development Department, 2016). Fermentation under acid conditions may have

caused the shell erosion. Wan et al. (2019) tested the resistance to acidic conditions of mussel shell (*Mytilus edulis*) using 0.1 mol/L and 0.2 mol/L HCl (pH 0.99) in pipette tips that was then dropped on the periostracum, prismatic and nacreous surfaces for 1 hr prior to visualizing using SEM. The results showed that the periostracum had been destroyed and subsequently the prismatic layer had corroded pitting. The current study suggested that the degradation of the mussel shell may have been due to both biological and physical causes in the fermentation process.

Chemical components in bio-extracts from green mussel shell fermentation

The bio-extracts from green mussel shell non-aerated fermentation were dark brown in color. Testing the pH value of the bio-extract using pH paper indicated a value of 3.5, which corresponded to Land Development Department (2016) which reported completion of the fermentation process when the pH of the bio-extract was 3–4 and had an alcoholic smell. The calcium content in the bio-extract is shown in Table 4.

Table 4 Mean percentage (\pm SD, n = 3) of calcium oxide content in terms of CaO in bio-extract from green mussel shell

Weeks of fermentation	Calcium content (CaO%)	
	Bio-extract of treatment 1	Bio-extract of treatment 2
3	1.35 \pm 0.50 ^{Bb}	1.14 \pm 0.43 ^{Ba}
13	5.76 \pm 0.24 ^{Aa}	1.11 \pm 0.31 ^{Ba}
15	5.08 \pm 1.05 ^{Ab}	1.42 \pm 0.09 ^{Bb}

mean values superscripted with different uppercase superscripts within each column indicate significant differences ($p < 0.05$) between time points; different lowercase superscripts within each row indicate significant differences ($p < 0.05$) between treatments

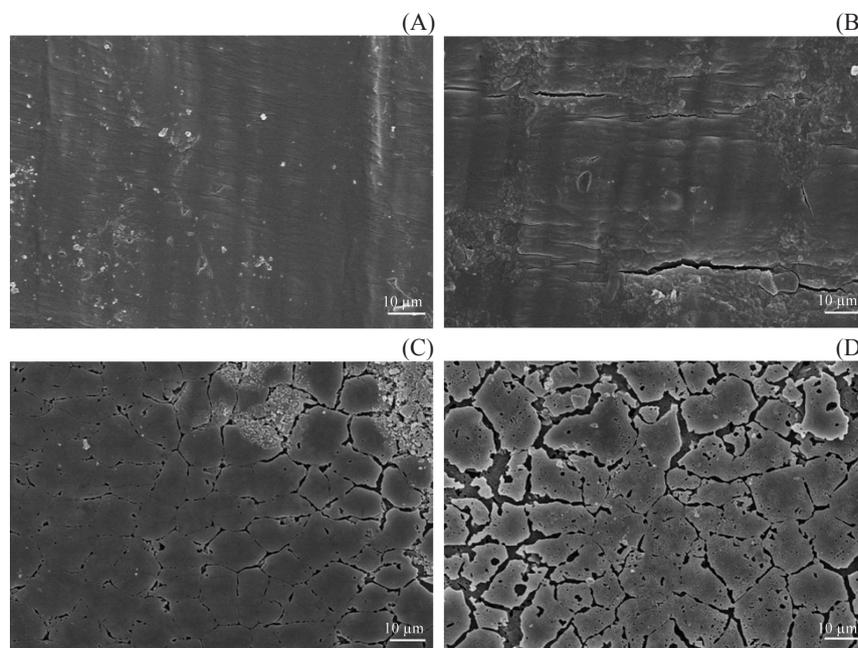


Fig. 1 Characteristics of outer surface of green mussel shell: (A) before fermentation; (B) after fermentation process, and of inner shell surface; (C) before fermentation; (D) after fermentation, where scale bar = 10 µm

It was noticed that the calcium oxide content in bio-extract treatment 1 in week 13 was significantly higher than in week 3, while the content in week 15 was not significantly different from week 13 (Table 4). In contrast in treatment 2, the calcium oxide content in week 3 and week 13 were not significantly different, while in week 15, the calcium oxide content in the bio-extract had significantly increased. The calcium oxide content in the bio-extract from green mussel shell fermentation in weeks 3, 13 and 15 indicated that the bio-extract obtained from treatment 1 had a significantly greater mean calcium oxide content from treatment 2. This implied that increasing the amount of molasses, which acted as a carbon source for the microbial fermentation process, did not affect the decomposition of calcium in green mussel shells. However, the total protein content in bio-extract treatment 2 was higher (3.24 ± 0.49 mg/mL) than in treatment 1 (2.89 ± 0.11 mg/mL).

Utilization of bio-extract fermented from green mussel shell to hydroponic water convolvulus culture

The impact was studied of bio-extract from green mussel shell non-aerated fermentation as liquid fertilizer mixed with commercial chemical liquid fertilizers to grow water convolvulus. The height of the water convolvulus increased during the 30 d of growth, as shown in Fig. 2.

At day 2 of culture, the mean (\pm SD) height increases of the water convolvulus growing in chemical liquid fertilizer (clf) were 4.42 ± 0.40 cm, 4.02 ± 1.35 cm, 4.83 ± 1.24 cm, 1.53 ± 0.06 cm, 3.73 ± 0.89 cm, 3.40 ± 2.86 cm and 2.20 ± 1.14 cm in the control treatment (clf A, clf B), formula 1 treatment (clf A, clf B, bio-extract treatment 1), formula 2 treatment (clf B, bio-extract treatment 1), formula 3 treatment (bio-extract treatment 1), formula 4 treatment (clf A, clf B, bio-extract treatment 2), formula 5 treatment (clf B, bio-extract treatment 2) and formula 6 treatment (bio-extract treatment 2), respectively. The mean height increases of water convolvulus growing in formulas 4, 5 and the control were significantly greater than for the other formulas, while formulas 3 and 6 had mean height increases that were significantly less than the other formulas.

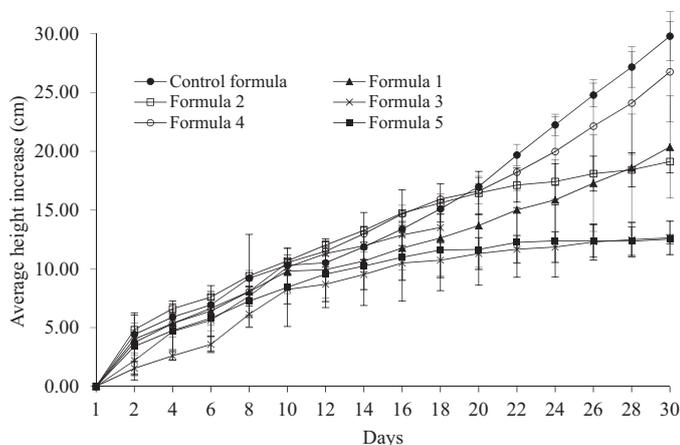


Fig. 2 Average height increase of water convolvulus (*Ipomoea aquatica*) cultured using bio-extract from green mussel shell fermentation mixed with commercial chemical liquid fertilizers in various ratios, where error bars indicate \pm SD

For days 3–10 of culture, the mean height increase of the water convolvulus growing in formula 6 (bio-extract treatment 2) was significantly less than for the other formulas, while the mean height increases of the water convolvulus in all treatments were not significantly different during days 12–18. At day 20, the increase in height with formula 4 was not significantly different from the control formula while the water convolvulus growing in formula 6 (bio-extract treatment 2) had withered and died. At day 22, the increases in height of the water convolvulus growing in formulas 1, 2 and 3 were significantly less than those in formula 4 and the control formula. On the other hand, the height increases of the water convolvulus growing in formula 4 and the control formula were significantly higher than for the other formulas. At day 26, the control formula had the significantly greatest height, while the mean height from growing in formula 4 was significantly greater than for formulas 1, 2, 3, 5 and 6. After 30 d of culture, the mean (\pm SD) heights increases of the water convolvulus were 29.80 ± 2.07 cm, 20.37 ± 4.36 cm, 19.13 ± 0.95 cm, 12.63 ± 1.43 cm, 26.77 ± 4.28 cm and 12.53 ± 0.39 cm for the control, formula 1, formula 2, formula 3, formula 4 and formula 5 treatments, respectively. These results suggested that the water convolvulus growing in bio-extract treatment 2 only (formula 3) had the least growth, while the water convolvulus cultured in clf A plus bio-extract treatment 2 had the greatest growth along with the control formula.

The growth of water convolvulus in the first period (days 1–20) showed that using the proportions of clf A plus bio-extract treatment 1 and clf A plus bio-extract treatment 2 (formulas 1 and 4), containing calcium which was a major nutrient needed by the plants, was the most suitable. The pH levels of the hydroponic media in formulas 1 and 4 measured using the pH meter were 6.38 ± 0.02 , which were approximately the same as for the control formula (6.56 ± 0.02) which corresponded with Sardare and Admane (2013) who reported the optimum pH in hydroponics was in the range 5.5–6.5. It was possible to utilize both formulas as nutrient supplement in growing crops and to provide an alternative to reduce the amount of chemical liquid fertilizers and to decrease the cost of hydroponics crops without using soil. Furthermore, Department of Environment and Primary Industries (2010) reported that the time for harvesting the water convolvulus was in the range 25–30 d or when the plants had reached about 30 cm in height, which in the current study was when the water convolvulus were taller than 25 cm.

The results showed that water convolvulus could grow in a mixed solution of bio-extract and chemical liquid fertilizer. Growing water convolvulus using bio-extract only (formulas 3 and 5) resulted in poorer development, perhaps due to the bio-extract not containing sufficient nutrients resulting in a low growth rate and thus requiring chemical liquid fertilizer parts A and B (Sardare and Admane, 2013).

The results suggested that growing water convolvulus hydroponically using a mixture bio-extract from green mussel shell containing calcium together with chemical liquid fertilizer part B, containing other nutrients needed for plant growth, could reduce the amount of calcium in the chemical liquid fertilizer used for hydroponic plants. This would also provide an alternative to utilize green mussel shell waste to add value.

Conflict of Interest

The authors declare that there are no conflicts of interest.

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