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Potential of By-Product of *Kappaphycus alvarezii* Derived from Bioethanol Production as Biofertilizer in Growing of *Ocimum basilicum* in an Aquaponic System

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ABSTRACT

Nutrient recycling from biowaste is one of the sustainable approaches to managing waste. The aquaponic system is one of the nutrient recycling methods that can reduce water consumption and reuse the nutrient available in its ecosystem. The nutrient to fertilize the plant in aquaponic depends on the activities of microbes to convert the waste into the nutrient. To enhance the growth of the plants, some aquaponics systems still rely on chemical fertilizers. *Kappaphycus alvarezii* is one of the red seaweeds abundantly found in East Malaysia. After numerous processes such as carrageenan extraction, the biowaste derived from *K. alvarezii* still contains a nutrient that can be recycled. The present study explores the potential of *K. alvarezii* solid waste as fertilizer to grow *Ocimum basilicum* in an aquaponics system. In this study, the macro- and micronutrients in *K. alvarezii* solid waste were determined, and the prevalence of microbes in the aquaponics system was monitored using inductively coupled plasma-optical emission spectrometer (ICP-OES) and 16S metagenomic sequencing method, respectively. Based on the findings, the growth of *O. basilicum* supplemented with *K. alvarezii* biofertilizer was significantly higher than the negative control. For genetic expression study in *O. basilicum*, *cinnamyl alcohol dehydrogenase* (*CAD*), *phenylalanine ammonia-lyase* (*PAL*), and *cytochrome p450 reductase* (*CPR*) genes were upregulated. The *O. basilicum* is free from mycotoxin and heavy metals. Since *K. alvarezii* solid waste is rich with macro- and micronutrients, which are essential for plant growth and can enhance the growth of *O. basilicum*,

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K. alvarezii solid waste produced from bioethanol production could be a potential fertilizer.

Keywords: Aquaponic, basil, biofertilizer, seaweed solid waste, sustainable agriculture

INTRODUCTION

In June 2017, the world population was 7.60 billion, expected to hit 8.60 billion in 2030. According to a new United Nations report, around 83 million people are added to the world's population annually (United Nations Department of Economic and Social Affairs [UN DESA], 2017). As the population grows, world agriculture must raise the demand for crop and livestock products. Currently, most countries still rely on local agriculture for food. However, there is an issue in increasing crop production due to the limitation of local resources under existing technological conditions such as semi-arid areas and soils problem. As such, agricultural research, extension, and infrastructure must be developed to solve the existing problem (Hemathilake & Gunathilake, 2022).

Traditionally, soils are the base for crop production, and large farmable land is required to produce the crop. However, long-term agriculture has resulted in soil damage; thus, the soil is not suitable for agriculture purposes. Besides, environmental pollution has polluted the soil and raised food safety issues. This hydroponic gardening was introduced to replace the conventional farming method. The hydroponic method is growing plants without soil, and the plants obtain the nutrients from the growing medium. The growing medium has become the heated argument about whether the hydroponic is certified as organic or not. The hydroponic system depends on applying nutrients from the concoction of chemicals, salt, and trace elements. Thus, the aquaponics system was introduced to replace hydroponics (Nawaz & Farroq, 2021; Van Gerrewey et al., 2022).

Aquaponics is a combination of recirculating aquaculture and hydroponics in one production system. The aquaponics system does not require soil and chemical pesticides to grow the plants. Therefore, it is a sustainable and intensive food production system and can produce a higher yield than conventional agriculture methods. Furthermore, the aquaponics system is not limited by non-arable lands such as deserts, degraded soil, and sandy islands (Nelson, 2017; Suhl et al., 2016). Therefore, the aquaponics system could be the best future agriculture method for food production. However, although the agriculture system would supply the essential nutrients to grow the plants, the available nutrient might not be sufficient to produce all kinds of plants. Thus, fertilizer is needed to provide a complete nutrient to develop a healthy plant.

Fertilizer is the major significant component in increasing the agricultural product. The public has noticed the disadvantages of chemical fertilizers; therefore, farmers turn towards organic fertilizers. One of the organic fertilizers which have been marketed is seaweed fertilizer. Seaweed extract fertilizer has

been claimed to be beneficial for seed germination, root development, increased frost resistance, increased nutrient uptake, increased resistance to fungal disease, and higher yields (Arioli et al., 2015; Gelli et al., 2020). Seaweed extract was rich in plant growth regulators such as auxins, cytokinins, ethylene, gibberellins, and abscisic acid (Arioli et al., 2015; Benítez García et al., 2020; Ghaderiardakani et al., 2019; Moncada et al., 2022). Besides, seaweed is also rich in minerals and trace elements which is essential for plant development (Petropoulos et al., 2020). However, seaweed extraction creates solid waste as a by-product, considered environmental waste accumulated in the landfill sites. A large amount of waste is generated during extraction as seaweed only accounts for 3–4% of yield, and the remaining are the waste fractions. To reduce the amount of solid waste in the environment, *K. alvarezii* solid waste was recycled and fully reused after bioethanol production as biofertilizers in aquaponics to replace the chemical fertilizers in the market.

Ocimum basilicum is one of the famous aromatic herbs widely used in traditional medicine and culinary herbs. The fresh and dried leaves are widely used as a spice, and the essential oil extracted from the fresh leaves is also used as food aroma additives and in pharmaceuticals and cosmetic products (Shahrajabian et al., 2020). Furthermore, *O. basilicum* is traditionally used as medicine for headaches, coughs, diarrhoea, constipation, warts, worms, and kidney malfunctions (Sonmezdag et al., 2018).

This study investigates the remaining *K. alvarezii* solid waste produced from bioethanol production as a biofertilizer in growing *O. basilicum* in an aquaponics system. Besides, the food safety of *O. basilicum* grown in the aquaponics system was also evaluated. The genetic expression of the genes *CAD*, *PAL*, and *CPR* in *O. basilicum* was also studied.

MATERIALS AND METHODS

Kappaphycus alvarezii Waste Preparation

Kappaphycus alvarezii solid waste from bioethanol production (acid hydrolysis) was collected and rinsed with distilled water. The *K. alvarezii* solid waste was dried until a constant weight was obtained. The dried *K. alvarezii* solid waste was ground into powder form and stored at -20 °C until further usage.

Macro- and Micronutrients in *K. alvarezii* Solid Waste

The macro- and micronutrients in the *K. alvarezii* solid waste were analysed using Association of Official Analytical Chemists (AOAC) Official Method 999.11. The tested macro and micronutrients are nitrogen, phosphorus, potassium, calcium, sulphur, magnesium, boron, chloride, manganese, iron, zinc, copper, molybdenum, and nickel. All the test parameters were expressed in parts per million (ppm).

Aquaponic Design

The aquaponic is consists of a recirculating aquaculture system (RAS) and a hydroponic

system (HS). Water from the fish tank was directed to a settling tank to remove all the solid waste produced from the fish and uneaten food. The settling tank consisted of a pump, biofilter, and filtered water compartment. For HS, the growing bedding consists of a leca medium to support the plants' growth. The filtered water from RAS was directed to HS to supply water and nutrients for plant growth. The used water from HS will be directed back to RAS and form a recycling system. In this study, *Barbonymus schwanenfeldii* fish and *O. basilicum* were grown in the aquaponics system.

Water Parameters in Aquaponic System

Aquaculture tanks' water parameters were determined using LAQUA Twin Compact Meters (Japan) (pH, temperature, ammonia, nitrite, and nitrate), dissolved oxygen (DO) meter, and carbonate hardness meter once a week. All the test parameters were expressed in ppm. In addition, the mean value for water parameters between negative control, positive control, and experimental groups were analysed. The data were statistically different when *P*-value was less than 0.05. This study's statistical analysis was performed using Statistical Package for Social Sciences (SPSS) software (version 20).

Microbes Identification in Aquaponics System

DNA Extraction. The water sample from RAS and HS were collected, and the microbes in the water samples were cultured in nutrient broth overnight. The microbes were collected by centrifugation, and the obtained pellet was used for DNA extraction. The microbes' DNA was extracted with G-spin™ Total DNA Extraction Kit (iNtRON Biotechnology, Korea). The steps were conducted according to the manufacturer's protocol. The extracted DNA samples were stored at -80 °C. DNA quality and purity were assessed using fluorescence quantification (Qubit® dsDNA BR Assay Kit, USA) and 0.8% agarose gel electrophoresis (AGE).

Library Construction. The DNA sample was normalised to 30.00 ng per reaction. Next, polymerase chain reaction (PCR) was conducted to obtain the targeted DNA fragments. The PCR product was purified using Agencourt AMPure XP (Beckman Coulter™, USA). All the steps were done according to the manufacturer's protocol. The libraries were validated using the Agilent 2100 bioanalyzer instrument (Agilent DNA 10000 reagents, USA). The samples were then sequenced by using HiSeq 4000 sequencer (HiSeq® 4000 SBS kit, Illumina, USA).

Data Analysis Pipeline. The samples that Illumina MiSeq sequences in 10k tags were then accessed by FastQC v0.11.3 to ensure the quality of the raw sequencing reads. Low-quality reads and bases (lesser than Qv20), ambiguous (Ns), and artefacts were removed. Reads with a longer than 480 bp were also removed based on the estimated hypervariable region size. Finally,

a collective of the high-quality reads was merged as tags for downstream analysis with minimum base overlapping of 15 bp.

The sample was then aligned with tags against the SILVA rRNA reference database, followed by alignment refinement. All the chimeric reads will be identified and removed before performing operational taxonomic units (OUT) analysis. The sample was then assigned sequences to the taxonomy outline using the *k*-nearest neighbour (knn) consensus and the Wang's approach. It also includes reading assignments to OTU. Finally, the obtained information was used to perform diversity analyses using Quantitative Insights Into Microbial Ecology (QIIME).

***Kappaphycus alvarezii* Biofertilizer on Aquaponic System.** Forty-five *B. schwanenfeldii* fish (≈ 20.00 g) and *O. basilicum* (150 germinated seeds) were divided into three groups: Group I — do not supplement with any fertilizer (negative control); Group II — supplemented with 5.00 g/L commercial seaweed fertilizer (positive control); Group III — increased with 5.00 g/L *K. alvarezii* biofertilizer (experimental group). *Ocimum basilicum* was germinated before being transferred to the HS. Commercial seaweed extract fertilizer and *K. alvarezii* biofertilizer were dissolved in Milli-Q ultrapure water with a 5.00 g/L concentration. The fertilizers were applied to the O. *basilicum* leaves once per week. The *O. basilicum* was grown for 60 days. On the 60th day, the *O. basilicum* was harvested, the length and weight of the *O. basilicum* were measured, and the number of leaves and colour of the plant were also recorded. The leaves of *O. basilicum* were stored at -80 °C for molecular genetic analysis.

The mean value for height, dry weight, and the number of leaves between negative control, positive control, and experimental group were analysed. The data were statistically different when *P*-value was less than 0.05. This study's statistical analysis was performed using SPSS software (version 20).

Genetic Expression of *O. basilicum* in Aquaponic System.

***Total RNA Extraction*.** Total RNA was extracted from frozen tissue samples with an easy-Blue™ Total RNA Extraction Kit (iNtRON Biotechnology, Korea). Extracted RNA samples were stored at -80 °C. The RNA quality and purity were determined using NanoVue Plus™ spectrophotometer (United Kingdom).

***cDNA Synthesis for qPCR*.** According to the manufacturer's instructions, total RNA (1.00 µg) was used for cDNA synthesis with the AccuScript Hi-Fi cDNA synthesis kit (Agilent, USA). The primers sequence is provided in Table 1. The qPCR was carried out in triplicate using Brilliant III Ultra-Fast SYBR® Green qPCR Master Mix (Agilent, USA). The reactions were performed using an ABI StepOne™ Real-Time PCR Systems (Applied Biosystems, United Kingdom), with universal cycling conditions. The *CAD*, *PAL*, and *CPR* genes were selected

in the study. In addition, *glyceraldehyde-3-phosphate dehydrogenase* (*GAPDH*) was used as a housekeeping gene to normalise gene expression between samples (Table 1).

Table 1
List of primers used in the qPCR

Gene	Forward (5' – 3')	Reverse (5' – 3')
GAPDH	CACCGAGGATGATGTGGTGT	GCAAGATCAGCCTTGGCATC
CAD	CGATGGCAAACCAACCCAAG	AGTAGTGGTGCTGCCTGTTC
PAL	TAAATGGGACAGCAGTGGGG	TCAAGTGGTCCGTGAACTCG
CPR	GCAGCACAAGATGGCACAAA	CCATGCCCTTAGCATCACCA

***Mycotoxin Detection in* O. basilicum.** The *O. basilicum* was dried and ground into powder. The mycotoxins in *O. basilicum* were extracted with 10.00 mL acetonitrile (MeCN) containing 2.00% formic acid. The mixture was then centrifuged, and the supernatant was obtained. The sample was then purified, and the solvent was exchanged with methanol: water [MeOH: H_2O (50:50, v/v)]. For mycotoxins quantification, 10.00 µL of the sample was injected into the High-performance liquid chromatography (HPLC) column, which was heated to 45 °C. A total of 10.00 mM ammonium formate and MeOH were used as mobile phases with a flow rate of 300.00 µL/min. The sample was run for 21 minutes (including 5 minutes of equilibration). The mycotoxin determination method was adapted from the AOAC Official Method 991.31. The tested parameters are aflatoxin (B_1, B_2, G_1, G_2), deoxynivalenol (DON), zearalenone, T-2 toxin, fumonisin (B_1, B_2), and ochratoxin A. All the test parameters were expressed in parts per billion (ppb).

***Heavy Metals Detection in* O. basilicum.** The heavy metals in *O. basilicum* were determined using AOAC Official Method 999.10. The test parameters were arsenic, cadmium, lead, and mercury. All the test parameters were expressed in ppm.

RESULTS AND DISCUSSION

The *K. alvarezii* biofertilizer was rich in phosphorus, potassium, calcium, sulphur, magnesium, and chloride. The macro- and micronutrients in *K. alvarezii* solid waste are shown in Table 2.

In general, green plants can produce their food by photosynthesis. For photosynthesis to take place, it needs nutrients to facilitate the photosynthesis process. Besides, the nutrients are also needed for plant growth and reproduction (Sharkey, 2020). In this study, the *O. basilicum* could obtain the nutrients from *K. alvarezii* biofertilizer and aquaculture system. The nutrients needed by the plants are divided into macro- and micronutrients. Macronutrients (nitrogen, phosphorus,

Table 2

The macro- and micronutrients in K. alvarezii *solid waste*

Test parameters	Reading
Total nitrogen	0.40 ± 0.03 %
Phosphorus	6581.60 ± 1.13 ppm
Potassium	15303.00 ± 0.92 ppm
Calcium	9603.80 ± 0.28 ppm
Sulphur	146064.70 ± 845.63 ppm
Magnesium	1740.70 ± 0.07 ppm
Boron	12.00 ± 0.14 ppm
Chloride	16120.40 ± 0.14 ppm
Manganese	177.10 ± 0.07 ppm
Iron	37.00 ± 0.21 ppm
Zinc	206.10 ± 0.21 ppm
Copper	170.10 ± 0.21 ppm
Molybdenum	ND (< 0.10)
Nickel	3.01 ± 0.06 ppm

Note. Values = Mean ± standard deviation; ND = Not detected

potassium, calcium, magnesium, and sulphur) are the nutrients that are needed by the plants in relatively large amounts, whereas micronutrients (boron, chloride, iron, manganese, copper, molybdenum, nickel, and zinc) are the nutrients needed in trace amount (De Bang et al., 2020). *Kappaphycus alvarezii* biofertilizer was rich in phosphorus, potassium, calcium, sulphur, and magnesium, which are the macronutrients needed for the *O. basilicum* to grow. Furthermore, a significant amount of boron, chloride, iron, manganese, copper, nickel, and zinc was also found in *K. alvarezii* biofertilizer.

In *K. alvarezii* biofertilizer, the nitrogen content was 0.40 ± 0.03%, and molybdenum was not found. Although the nitrogen content in the supplemented *K. alvarezii* biofertilizer is relatively low and molybdenum was not found, the *O. basilicum* in the negative control, positive control, and experimental group were still growing healthily. The *O. basilicum* do not have any nitrogen deficiencies symptoms such as yellowing of older leaves, thin stems, and poor vigour (McCauley et al., 2011). Nitrogen is needed for building structures, photosynthesis, cell growth, metabolic process, and chlorophyll production. Both nitrogen and molybdenum needed by the *O. basilicum* were obtained from the aquaculture system. The nitrogen in the aquaculture system exists in the nitrate form, and the *O. basilicum* can

utilise a moderate amount of ammonia and even the free amino acid in the aquaculture system. For molybdenum, it is correlated to nitrogen function, where the plants use molybdenum to catalyse redox reactions with different forms of nitrogen. The absence of molybdenum in the plants could lead to nitrogen deficiency symptoms even with nitrogen (De Bang et al., 2020; McCauley et al., 2011). Thus, the low content of nitrogen and molybdenum in the *K. alvarezii* biofertilizer could be supplemented from the aquaculture system and can still grow healthy plants.

Water Parameters in Aquaculture Tanks

The water parameters (pH temperature, ammonia, nitrite, nitrate, dissolved oxygen [DO], and carbonate hardness) in the negative control, positive control, and experimental aquaculture tanks showed no significant difference. The water quality for the aquaculture tanks is shown in Table 3.

The aquaculture system's water quality also plays an important role in growing *O. basilicum*. In general, the pH in the aquaculture system would interfere with the plant's access to nutrients. The pH beyond the tolerance range could cause the plant to be unable to take up the nutrients in the water even though the desirable nutrients exist. The desirable pH in an aquaculture system is between 5.50 to 7.50 (Oladimeji et al., 2018). The pH in the negative control (7.32 ± 0.30), positive control (7.55 ± 0.14), and experimental group (7.58 ± 0.21) were in the desirable range, which allows the *O. basilicum* to grow. Furthermore, a pH lower than 6.00 will reduce the nitrifying bacteria activities to convert the ammonia to nitrate. When the ammonia level increases, it will lead to an unbalanced system which is stressful and toxic to the fish (Sahrawat, 2008).

Ocimum basilicum is a warm climate plant which grows in temperatures of 17 °C to 30 °C. The temperature for the three

Table 3
Water quality of the aquaculture tanks

Water parameters	Negative control	Positive control	Experimental
pH	7.32 ± 0.30	7.55 ± 0.14	7.58 ± 0.21
Temperature (°C)	23.80 ± 1.24	23.90 ± 1.04	23.90 ± 1.08
DO (ppm)	6.59 ± 0.34	6.52 ± 0.18	6.73 ± 0.06
Ammonia (ppm)	0.06 ± 1.78	0.06 ± 0.18	0.06 ± 0.18
Nitrite (ppm)	0.06 ± 1.78	0.06 ± 0.18	0.06 ± 0.18
Nitrate (ppm)	143.13 ± 10.33	151.38 ± 3.96	148.50 ± 4.38
Carbonate hardness (ppm)	71.88 ± 5.94	75.63 ± 3.20	71.88 ± 2.59

Note. Values = Mean ± standard deviation

groups was ≈24 °C. For *O. basilicum*, the optimum temperature was suggested at 25 °C. The *O. basilicum* grows at 25 °C resulting in higher volatile oil content and taller plant (Chang et al., 2005; Saha et al., 2016). Furthermore, the DO content in the negative, positive, and experimental groups fell between 6.52 ± 0.18 ppm to 6.73 ± 0.06 ppm. Plants require high DO content in the water (> 3.00 ppm); Low DO content might result in rotting of the root, which will cause the growth of fungus.

Ammonia, nitrite, and nitrate are nitrogen sources for plants. Three nitrogen sources are available; however, nitrate is the most accessible nitrogen form (B. Z. Wang et al., 1989; Raven et al., 1992) because ammonia and nitrite are toxic to the fish, thus, it should always maintain below 1.00 ppm (McCauley et al., 2011). The ammonia and nitrite content in the three groups maintained an average of 0.60 ppm in the study. The nitrate content in the three groups was found in the range of 143.13 ± 10.33 ppm to 148.50 ± 4.38ppm. The nitrate in the water served as the nitrogen source for the *O. basilicum*; thus, even though the biofertilizer did not contain a high amount of nitrogen, the *O. basilicum* was still able to obtain the nitrogen from the water.

The last important water parameter in the aquaculture system is carbonate hardness. The optimal level for the aquaponic is 60.00 ppm to 140.00ppm, and the carbonate hardness in the three aquaculture systems was between 71.88 ± 5.94 ppm to 75.63 ± 3.20 ppm. Carbonate hardness is correlated with the pH in the aquaculture system, where it acts as a buffer (or resistance) to lowering pH. When nitrification occurs, nitric acid will be produced, dissociating in water into two components (hydrogen ion and nitrate). When the hydrogen ion increases in the water, it will reduce the pH of the water. The presence of carbonate and bicarbonate would stabilise against the acidification caused by the nitrification process (Russell, 2009; Wurts & Durborow, 1992). Therefore, the optimal conditions of the water parameters in the systems can create a healthy ecosystem for the fish, vegetables, and bacteria.

Metagenomics Analysis

The total throughput for the sample is 49,146 raw reads or ~14.50 Mbp of data, as depicted in Table 4. Over 86.71% of the sequencing data were retained after the quality filtering and merging process (Table 5), indicating that the sequencing reads are of moderate quality.

Table 4

Total throughput for the water samples collected from recirculating aquaculture system (RAS) and hydroponic system (HS)

Sample	Read size distribution	Total reads	Total bases	GC%
Recirculating Aquaculture system	291-297	24,340	7,180,300	54.00
Hydroponic system	294-297	24,806	7,330,017	53.50
Total	-	49,146	14,510,317	-

Note. GC = Guanine-cytosine content

Table 5

The sequencing statistics for the samples from recirculating aquaculture system (RAS) and hydroponic system (HS)

Sample		Before pre-processing		After pre-processing	
		Number of reads	%	Number of reads	%
Recirculating aquaculture system (RAS)	Forward	12,170			
	Reverse	12,170			
	Total	24,340	100.00	10,533	86.71
		Read size (bp)	%	Read size (bp)	%
	Forward	3,565,810			
	Reverse	3,614,490			
	Total	7,180,300	100.00	4,464,931	
		Number of reads	%	Number of reads	%
Hydroponic system (HS)	Forward	12,403			
	Reverse	12,403			
	Total	24,806	100.00	11,901	89.42
		Read size (bp)	%	Read size (bp)	%
	Forward	3,646,483			
	Reverse	3,683,691			
	Total	7,330,173	100.00	4,562,491	

A total of 241 OTUs were identified and assigned with taxonomy information up to the genus level. The top 4 OTU in RAS and HS and their respective assigned taxonomy and abundance are shown in Table 6.

Table 6

Top 4 abundant microbe identified in recirculating aquaculture system (RAS) and hydroponic system (HS)

Sample	Taxonomy	Relative abundance (%)
Recirculating aquaculture system (RAS)	Aeromonas	39.47
	Clostridium_sensu_stricto_3	24.85
	Unclassified Pseudomonadaceae	13.63
	Unclassified Enterobacteriaceae	9.92
Hydroponic system (HS)	Bacillus	34.74
	Unclassified Pseudomonadaceae	29.03
	Unclassified Enterobacteriaceae	12.16
	Aeromonas	6.76

A comparison of the OTU richness at the genus level between the samples was plotted. Between the RAS and HS samples, it was observed that 34 OTUs were shared between them, while 83 and 90 OTUs were specific to the former and latter, respectively (Figure 1). The number of species in the group RAS was 117, and HS was 124. The number of species shared between groups RAS and HS were 34 (16.43%).

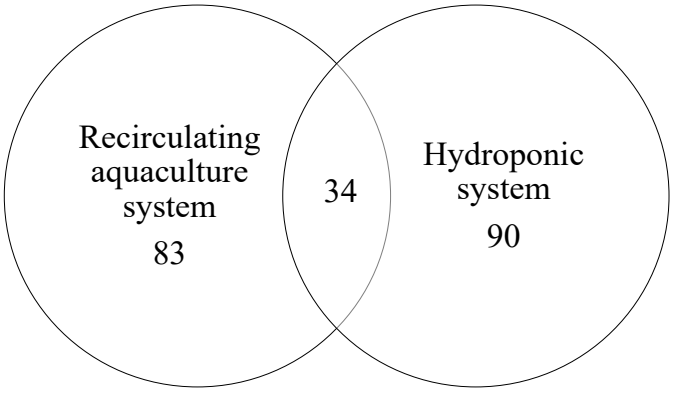

Figure 1. Venn diagram of OTUs at genus level between recirculating aquaculture system (RAS) and hydroponic system (HS)

A total of 241 OTUs bacteria were identified in the aquaponics system. The most abundant bacteria in the aquaponics system are *Aeromonas*, *Clostridium sensu stricto*, Pseudomonadaceae, Enterobacteriaceae, and *Bacillus*. These bacteria are heterotrophic and nitrifying bacteria that assist in converting fish waste into nitrate, an important nutrient for the plants (Chen et al., 2015; D. Xu et al., 2017a; He et al., 2016; Kathiravan & Krishnani, 2014; Y. Xu et al., 2017b) A total of 60.00–70.00% of the waste produced from the fish and released into the water is ammonia, the organic mix containing proteins, carbohydrates, fats, vitamins, and minerals. Nitrifying and heterotrophic bacteria metabolise the waste and convert it into macro- and micronutrients for the plants.

In the RAS and HS, the different prevalence of the bacteria observed is due to the changes in the environmental conditions which affect the attachment processes of the bacteria. The abundance of the bacteria depends on environmental conditions, such as changes in nutrient concentrations. Besides, the presence of the bacteria also depends on the characteristics and the species of the bacteria. Since the bacterial samples were collected from two environments, thus, the environmental conditions and nutrient contents were also different. Therefore, the prevalence of bacteria in these two systems was also different.

Ocimum basilicum Growth in Aquaponics System

The *O. basilicum* was grown in the aquaponics system and designated into negative control, positive control, and experimental group. After 60 days of growth in the aquaponics system, the height, dry weight, and the number of leaves in the positive control and experimental group were significantly higher than in the negative control group, with a *p*-value less than 0.05 ($P < 0.05$). The mean height, weight, and the number of leaves for *O. basilicum* are shown in Table 7. Furthermore, the *O. basilicum* appeared in healthy, bright green colour in all the groups.

Table 7

Mean height, dry weight, and number of leaves of O. basilicum *in the negative control, positive control, and experimental group*

Group	Height (cm)	Dry weight (g)	Number of leaves
Negative control	10.50 ± 1.15^{a}	0.71 ± 0.07^{a}	12.00 ± 2.11^{a}
Positive control	18.90 ± 0.88^{b}	3.59 ± 0.17^{b}	30.00 ± 1.69^{b}
Experimental	20.00 ± 1.41^{b}	3.73 ± 0.21^{b}	31.00 ± 1.69^{b}

Note. Values = mean ± standard deviation of n = 40 plants in each group. Different superscript letters in each row indicate a statistical significantly different at $P < 0.05$

The *O. basilicum* was grown in a stable aquaponics system. The height, dry weight, and the number of leaves of *O. basilicum* in positive and experimental groups were significantly higher than the *O. basilicum* grown in the negative group. Although the fish waste would be converted to nutrients by the bacteria, the nutrients produced in the water system might not be sufficient for the plants to grow. Besides, the conversion rate from waste to nutrients is slower than the uptake rate by the plants; thus, supplemented fertilizer would assist in plant growth (Bindraban et al., 2015; Han et al., 2016; Hussain & Abbasi, 2018; Liu et al., 2014). Therefore, the positive control and experimental groups were supplemented with a fertilizer, where the fertilizers are rich in macro- and micronutrients. Thus, *O. basilicum* grown in the positive control and experimental group was bigger than in the negative control group.

mRNA Expression in *O. basilicum* by qPCR

Three genes (*CAD*, *PAL*, and *CPR*) were selected to study the growth of *O. basilicum*. Compared to the negative control, *CAD*, *PAL*, and *CPR* genes were upregulated in the positive and experimental groups. *PAL* and *CPR* were not significantly different between the positive and experimental groups. However, *CAD* gene expression was significantly higher in the positive control group than in the experimental group. The genetic expression of *O. basilicum* is shown in Figure 2.

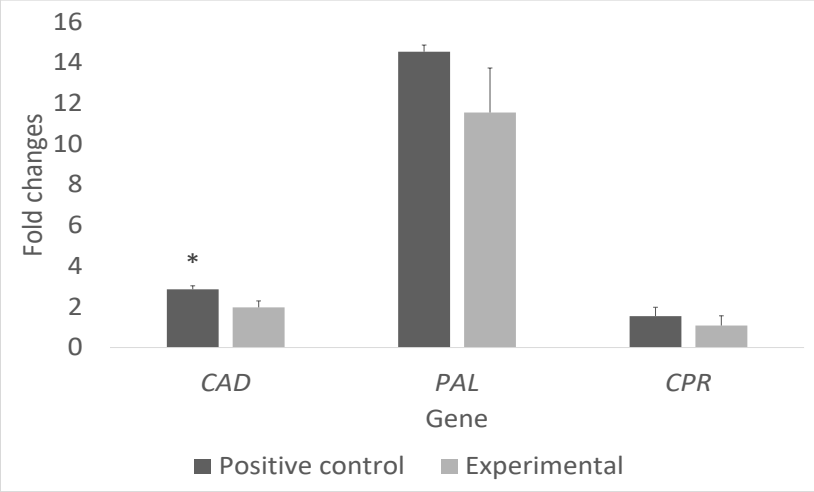

Figure 2. Fold changes in mRNA expression for the positive and experimental group on the 60th day. * in the bar chart indicate a statistical significantly different between positive control and experimental groups at $P < 0.05$. The x-axis indicates the genes of the *cinnamyl alcohol dehydrogenase* (*CAD*), *phenylalanine ammonia-lyase* (*PAL*), and *NADPH-cytochrome p450 reductase* (*CPR*)

In this study, the *O. basilicum* has been supplemented with commercial seaweed fertilizer and *K. alvarezii* biofertilizer, respectively. Thus, the expression of secondary metabolites in the *CAD*, *PAL*, and *CPR* will interfere. Both *CAD* and *PAL* genes are responsible for essential oil and phenylpropanoid production. The essential oil gives the plants good aroma and flavour (G. J. Wang et al., 2001; Iijima et al., 2006; Mandoulakani et al., 2017) and is an important source of aromatic and flavouring in food, industrial, and pharmaceutical product (Charles & Simon, 1990). The essential oil content in the plant depends on the development state of synthesising tissue and the metabolic process. The seaweed fertilizers applied to the *O. basilicum* are rich in polysaccharides, proteins, polyunsaturated fatty acids, pigments, polyphenols, minerals, and plant growth hormones. Thus, it might be able to increase cellular metabolism and positively affect the plant's conditions, such as root elongation and root formation.

Furthermore, the positive influence of cell metabolisms also improves bud and cell division to give better vegetative growth and increase the number of glands. On top of that, the hormones found in the fertilizers will also interfere with the growth stimulation, increasing photosynthesis's effectiveness. The increasing photosynthesis will protect the chlorophyll from degradation and enhance its content in the leaves. Thus, *CAD* and *PAL* genes were upregulated to produce secondary metabolites such as essential oil (Jamali et al., 2014; Tawfeeq et al., 2016). The expression of the *CAD* gene in the positive group is significantly higher than in the control group. The differences between the groups might be because of

the different seaweed fertilizers used. The genetic expression in the plants can be inferred by the surrounding conditions such as temperature, water conditions, light intensity, plant growth hormone, and the fertilizer applied (Lobo, 2008). The commercial seaweed extract fertilizer is rich in amino acids, cytokinins, mannitol, auxin, and vitamins. Thus, these factors might be why the expression level for the *CAD* gene in the positive group is higher than in the experimental group.

Expression of *CPR* correlates with the biosynthesis of aromatic and flavonoid metabolism in the *O. basilicum*. Therefore, upregulated expression of *CPR* might increase the production of the flavour of the *O. basilicum*, thereby increasing its value of it (Ayabe & Akashi, 2006). Besides, *CPR* is also proposed to be associated with ursolic and oleanolic acid production (Misra et al., 2017).

Besides, supplementation with the fertilizers leads to upregulation in the *CPR* gene. The effects of fertilizer on hydroxyl radical generated in the DNA resulted in strand breakage of DNA and caused a significant biological effect such as carcinogenesis, mutagenesis, and cytotoxicity. To counterpart the effect of hydroxyl radical thus, the flavonoid is generated. Flavonoids are well-known strong superoxide radical oxygen scavengers and singlet oxygen quenchers widely used as the therapeutic agent (Sorata et al., 1984). Besides, flavonoids also reacted with peroxy radicals, which take part in the termination of a chain reaction during the autooxidation of polyunsaturated fatty acids (Torel et al., 1986). Thus, the fertilizers do upregulate not only the flavonoids content but also phenolics content and vitamins in the plants (Osuagwu & Edeoga, 2013; Salama et al., 2015).

Mycotoxins and Heavy Metals in *O. basilicum*

Food safeness of *O. basilicum* was evaluated by determining mycotoxin and heavy metals contents. There was no mycotoxin aflatoxin (B_1, B_2, G_1, G_2), deoxynivalenol (DON), zearalenone, T-2 toxin, fumonisin (B_1, B_2), ochratoxin A, and heavy metals (arsenic, cadmium, lead, and mercury) detected in *O. basilicum* grown in the aquaponics system. The results for mycotoxins and heavy metals in *O. basilicum* are shown in Table 8.

Table 8
Detection of mycotoxins and heavy metals in O. basilicum

Test parameters	Unit	Reading
Aflatoxin (B_1, B_2, G_1, G_2)	ppb	ND (< 0.10)
Deoxynivalenol (DON)	ppb	ND (< 5.00)
Zearalenone	ppb	ND (< 10.00)

Table 8 *(Continue)*

Test parameters	Unit	Reading
T-2 toxin	ppb	ND (< 5.00)
Fumonisin (B_1, B_2)	ppb	ND (< 50.00)
Ochratoxin A	ppb	ND (< 0.50)
Arsenic	ppm	ND (< 0.01)
Cadmium	ppm	ND (< 0.10)
Lead	ppm	ND (< 0.10)
Mercury	ppm	ND (< 0.01)

Note. Values = Mean ± standard deviation; ND = Not detected

As the *O. basilicum* will be served as food thus, the safeness of *O. basilicum* to be consumed has become a concern. Therefore, this study investigated the common food-related mycotoxins and heavy metals in *O. basilicum*. In Table 8, all the mycotoxins and heavy metals parameters were not detected in the *O. basilicum*. The tested mycotoxins and heavy metals were aflatoxin (B_1, B_2, G_1, G_2), deoxynivalenol (DON), zearalenone, T-2 toxin, fumonisins (B_1, B_2), ochratoxin A, arsenic, cadmium, lead, and mercury. Mycotoxins are secondary metabolites produced by microfungi and bacteria, which are pathogenic to humans and plants (Gallo, 2001; Bennett & Klich, 2003). Exposure to mycotoxins can result in cancers, kidney toxicity, and immune suppression; thus, growing a mycotoxins-free plant is important.

The application of heavy metals contaminated fertilizer and water to the plants has raised food safety concerns in public. Food contaminated with arsenic, cadmium, lead, and mercury is dangerous and can harm human health even in low concentrations. Arsenic poisoning might bring complications in body organ systems such as renal, respiratory, and immune systems (Mohammed Abdul et al., 2015). Besides, the cadmium that humans consume will be retained and accumulated in the kidney (proximal tubular cells), which is toxic to humans (Bernard, 2008). Furthermore, mercury poisoning can lead to a nephritic syndrome in severe cases with hematuria and anuria (Bjørklund et al., 2017; Park & Zheng, 2012). Lastly, lead is the most hazardous and cumulative environmental pollutant through exposure to air, water, and food sources. Accumulating lead in the body could lead to human poisoning and death (Assi et al., 2016; Patra et al., 2011). Thus, it is important to ensure that the crops grown in agriculture are free from heavy metals. Aquaponics supplemented with biowaste from seaweed as biofertilizer could be a better design for agriculture because all the water parameters in the aquaponics system are under monitoring.

CONCLUSION

The *K. alvarezii* biofertilizer was rich in phosphorus, potassium, calcium, sulfur, magnesium, and chloride, which is suitable for growing *O. basilicum* in the aquaponics system. In the aquaponics system, nitrifying and heterotrophic bacteria are abundant, which are needed to convert the food waste into nutrients needed for the plant to grow. The *O. basilicum* supplemented with *K. alvarezii* biofertilizer has a significantly higher growth rate compared to the negative control. Furthermore, *CAD*, *PAL*, and *CPR* genes were found to upregulated express, which the genes were correlated to essential oil, and the development of *O. basilicum*. *Ocimum basilicum* grows in the aquaponics system and is supplemented with *K. alvarezii* biofertilizer is free from mycotoxin and heavy metals thus it is safe to be consumed as food. Therefore, *K. alvarezii* biofertilizer could be a potential biofertilizer that can assist in plant growth.

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