Microbial consortia effects on the yields of water spinach in milkfish aquaponics system

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Abstract. Aquaponics is a technology combining fish, plants, and microorganisms into one system. In order to better understanding concerning the role of microorganism upon plant growth, this research was focused on the effect of microbial consortia inoculation on the yield of hydroponically water spinach (Ipomea aquatica Forssk) integrated with milk fish (Chanos chanos). The plant growth quality were determined to assess the efficacy of the beneficial microorganism. The experimental design was randomized blocks with five treatments which corresponded to the microbial consortia inoculation containing species of Lactobacillus, species of Bacillus, species of Nitrosomonas and Nitrobacter and combination of Lactobacillus casei and Saccharomyces cerevisae with three replicates. The result indicated that the water spinach with microbial consortia inoculation higher growth and yields than that of the uninoculated control significantly. However, it was not significantly different on plant yields from the species composition of microbial consortia. The results support the hypothesis that the use of microbial consortia can increase the yields efficiency of aquaponically water spinach. In interesting implication of result findings was that in the aquaponics system, the milk fish can be coupled with the water spinach growing system since its benefits were not dependent upon continuous recirculation between the plant and fish system but also its living biotic community.

Keywords: Aquaponics, microbial consortia, water spinach, milk fish

1. Introduction
Milkfish (Chanos chanos) is one of the most important species in Asian countries, including Indonesia. The fish is the most extensively cultivated fish in outdoor-based systems in coastal ponds and therefore have to cope with daily and/or seasonally changing environmental conditions. Milkfish is a source of food with high quality of protein, vitamins and minerals such as Omega-3 and also easily digested and
Absorbed by the body. In Banten province, milkfish is usually processed into traditional culinary, Sate Bandeng. Production of milkfish aquaculture in Banten in 2015 reached 10,638 tons [1].

Good water quality must be maintained at all times for milkfish to maintain optimal growth conditions and health of the fish. Accumulation of fish waste as an organic waste and toxic compounds is a significant problem inherent in aquaculture. Solid wastes consisted of faeces, and uneaten food can increase BOD (biochemical oxygen demand) and ammonia toxicity when it decomposes. Nitrite is a naturally occurring intermediate product of the nitrification process. The nitrate ion (NO$_3^-$) is the most oxidized form of nitrogen in nature and is relatively non-toxic to fishes [2]. Microorganisms, in general, are a crucial component in aquaponics systems because they enable the transformation of rough organic matter into molecules absorbable by the plants [3]. Microorganisms also clean the water from the accumulation of compounds such as ammonia which, when reaching high concentration levels, can become toxic to the fish. If the bacteria communities are not healthy and balanced, the whole aquaponics system can crash.

Usually milkfish are traditionally cultured in shallow brackish water pond. Nowadays it is a supporting technology that integrated in aquaculture, therefore milk fish can be cultured together with vegetable in aquaponics system. Networks connection in aquaponics systems act as a symbiotic relationship, in which plants grow by utilizing nutrients released by fish [4] and plants acts as bio filter, remove the metabolites present in the water, which are harmful to the fish, allowing their development [5] before it is returned, cleaned back into the fish tank. The systems can be sustainable to be applied since the system with less labour, less land, and fewer chemicals, and takes up a fraction of the water usage and has the potential for higher yields of produce and protein.

Many types of plants can grow successfully in aquaponics systems. It is not only leafy green vegetable and herb crops could be grown, but a wide variety of fruiting crops, beans, and flowers can be grown effectively. Among them, water spinach, a semi-aquatic, tropical plant grown as a vegetable for its tender shoots and leaves can be cultivated in aquaponics system. A research proved that water spinach could be used as new sources of natural antioxidants as well as other bioactive compounds of human health benefits [6]. Water spinach grown on the wild environment can be harmful to the health since this plant is susceptible to aquatic pollution, water carrying pathogens, and various chemicals such as pesticides. Therefore the aquaponics system will provide a safe environment for growing water spinach. Aquaponics is suitable for limited land and water because it produces about three to six times the vegetables [7] and uses about 1% of the freshwater used by traditional aquaculture [8]. A small scale aquaponics can be used as a solution to the need for an inexpensive, nutritious and reliable food source for a family that has the capacity to provide a full meal both protein and vegetables with low inputs [9].

2. Materials and Method

2.1 Time and site

This study was conducted at the Department of Biology Education, Faculty of Teacher Training and Education, University of Sultan Ageng Tirtayasa from May-September 2018.

2.2 Aquaponics system preparation

The aquaponics system was designed by placing a container as a fish tank connected to the plant tray with inlet and outlet channels. The inlet channel directly connected to the pump AR 650 filter (Armada), for watering the plant (Figure 1). Seeds of the water spinach var. Sutera was surface sterilized by immersed in a 70% solution of ethanol for 30 seconds, followed by a solution of sodium hypochlorite (2% available chlorine) for 1 minute. Then seeds were rinsed three times with sterilized distillate water, dried overnight. The seed was sown and sprouted in the sowing trays before being transferred to the aquaponics system. Rockwool used as the growing medium for the seedlings. Parameter for water qualities i.e. water temperature (°C), dissolved oxygen (mg L$^{-1}$) pH and ammonia were measured during the study. Observation of temperature; pH; and DO was performed daily at 4 pm, while for ammonia levels were performed every seven days. Ammonia measurement used the reference SNI 06-6989. 30-2005.
2.3 Experimental design

The complete random design and non-factorial were used on a small scale of the aquaponics system. The tested factor was the microbial consortia inoculation containing species of *Lactobacillus* (L), species of *Bacillus* (B), species of *Nitrosonomas* and *Nitrobacter* (N) and combination of *Lactobacillus casei* and *Saccharomyces cerevisiae* (LS), while for the control was aquaponics system without inoculation. Each treatment was conducted by three replicates. Data on the water spinach morphological parameters were collected at 30 days intervals from planting to harvesting date. At harvest, the plant was washed carefully in tap water to remove from the growing media. Plants were then separated into roots, and the fractions weighed and dried at 80°C until constant weight.

2.4. Data analysis

The data obtained in this study were analysed using oneway ANOVA. The significance of the difference among the means will be analysed by Duncan’s multiple ranges. The statistics software was using SPSS version 22.

3. Result and Discussion

3.1. Aquaponics water quality parameters

Microbial inoculation in a milkfish aquaponics system can improve water quality. It can be seen through the water quality parameters (Table 1). The temperature both in controls and by microbial consortia inoculation as the treatment in the range of 27°C-30°C. The pH with a range of 6.0-8.0. DO range was 4.58-7.83 mg/l and 4.18-8.97 mg/l for the treatment and control, respectively.
Ammonia is the main nitrogenous matter. D. Ammonia is the main nitrogenous

consortia inoculation and particularly for those inoculated with resulted in a significant increase of fresh and dry weight of shoots by up to 79 and fresh leaves than in

The effects of microbial consortia inoculation with on the growth of 30

3.2 Performance of water spinach influenced by microbial consortia inoculation

The quality of water in aquaponics systems become the biological limits for milk fish sustainable production. A co-culture organisms such as plants and microorganism affected the welfare conditions through complex interactions between parameters of water qualities. The range of temperature in aquaponics system support the growth of fish and microorganism. The good temperature range for the fish in the tropics is 25-32°C [10]. The milkfish in their natural habitat or pond environment encounter daily or seasonal temperature fluctuations which greatly influence their growth. As [11] reported that milkfish in ponds behave normally at temperatures between 20 to 33°C.

Dissolved oxygen is one of important aquaponics parameter which is required by the milkfish for respiration, water spinach for respiration, health, and strength of their roots and nutrient uptake and microorganism for respiration and nitrification process through the decomposition of organic matter. The first product resulted in ammonia. In addition, the urine and faeces from the aquatic animals can cause high content of ammonia nitrogen and an increase of BOD. Ammonia is the main nitrogenous waste produced by fish via metabolism and is excreted across the gills [12] Ammonia is steadily released into the water through the excretion of the fishes as a product of their metabolism, must be filtered out of the water since higher concentrations of ammonia (commonly between 0.5 and 1 ppm) can kill fish [13]. Although plants can absorb ammonia from the water to some degree, but nitrates are assimilated more easily [14], thereby efficiently reducing the toxicity of the water for fish

Previously research reported that bacteria isolated from marine environments could remove certain levels of nitrogen [15, 16]. Strains of Lactobacillus spp. has been used as probiotics and can the removal of up to 400 μM NH₄⁺,NO₂⁻, and NO₃⁻ [17]. According to [18] Bacillus inoculation in aquaponics system can affected nutrient dynamics showed by faster decreases in ammonia concentration and faster increase in nitrite and nitrate concentrations than the control. The two most common species of nitrifying bacteria are Nitrosomonas sp. and Nitrobacter sp. Nitrosomonas sp. Converts ammonia (NH₃) to nitrite (NO₂⁻) while Nitrobacter sp. uses nitrates for their energy source during its conversion to nitrate (NO₃⁻). Nitrogen in nitrate form is absorbed and used as a nutrient by plants

3.2 Performance of water spinach influenced by microbial consortia inoculation

The effects of microbial consortia inoculation with on the growth of 30-day-old water spinach are shown in Table2. Inoculated water spinach produced more leaves, taller plants with more developed and bigger leaves than those without inoculation. There was an increment of number of leaves, shoot height shoot and fresh root weight and dry weight to inoculation over control. The enhancement of plant growth resulted in a significant increase of fresh and dry weight of shoots by up to 79 and 75 %, respectively, particularly for those inoculated with microbial consortia combination of species Lactobacillus casei and Saccharomyces cerevisae. Fresh weight of roots was also increased by 45-53% in all microbial consortia inoculation

### Table 1. Milkfish aquaponics water quality parameters

| Treatments | Parameters | Temperature (°C) | Dissolve Oxygen (mg.L⁻¹) | pH | Ammonia (mg. L⁻¹) |
|------------|------------|------------------|--------------------------|----|------------------|
| C          |            | 27-30            | 4.18-8.97                | 7.8-8.0 | 10.00-5.815 |
| L          |            | 27-30            | 4.58-7.83                | 6.8-7.0 | 10.00-0.553  |
| B          |            | 27-30            | 4.58-7.83                | 6.5-7.0 | 10.00-0.010  |
| N          |            | 27-30            | 4.58-7.83                | 6.2-7.0 | 10.00-0.532  |
| LS         |            | 27-30            | 4.58-7.83                | 6.0-7.0 | 10.00-0.545  |

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Tabel 2. Plant growth characteristics of non-inoculated and inoculated water spinach with microbial consortia inoculation

| Treatments | Number of Leaves | Shoot height (cm) | Shoot Fresh weight (g. L⁻¹) | Root Fresh Weight (g. L⁻¹) | Shoot Dry weight (mg. L⁻¹) |
|------------|-------------------|-------------------|----------------------------|----------------------------|---------------------------|
| Control    | 5ᵃ                | 7.4ᵃ              | 0.370ᵃ                     | 0.013ᵃ                     | 0.02ᵃ                     |
| L          | 7ᵇ                | 10.08ᵇ            | 1.410ᵇ                     | 0.026ᵇ                     | 0.056ᵇ                    |
| B          | 7ᵇ                | 10.31ᵇ            | 1.490ᵇ                     | 0.028ᵇ                     | 0.056ᵇ                    |
| N          | 7ᵇ                | 10.36ᵇ            | 1.703ᵇ                     | 0.026ᵇ                     | 0.066ᵇ                    |
| LS         | 8ᵇ                | 12.25ᵇ            | 1.816ᵇ                     | 0.024ᵇ                     | 0.083ᵇ                    |

Values in a column with similar superscript letter are not significantly different, LSD, P<0.05

Bacillus, Nitrosomonas and Nitrobacter are categorized as PGPR microorganism, act through N₂ fixation, extracting nutrients from the soil, production of various kinds of plant hormones and biological controls, and promotion of plant growth [19, 20, 21]. Lactobacillus sp. and Saccharomyces cerevisiae has been used as probiotics in aquaculture that can help the process decomposition of ammonia as a bioremediation agent for water quality control. Then the ammonia is converted to the nitrate and use for the plant growth. All species used in this research significantly affected water spinach growth compared to the non-inoculated control. The species composition of microbial consortia inoculated to the aquaponic system was not significantly affected by plant yields.

4. Conclusion
The research on milkfish aquaponics system integrating with water spinach has been successfully conducted. The result indicated that the water spinach with microbial consortia inoculation showed higher growth and yields than that of the uninoculated control significantly. However, it was not significantly different on plant yields from the species composition of microbial consortia.

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