Growth of *Momordica charantia* (bitter gourd) amended by *Chanos chanos* (bangus) and *Oreochromis niloticus* (tilapia) fish offal fertilizer

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Abstract

Inorganic fertilizers have been widely used in enhancing the growth of crops. However, excessive use of inorganic fertilizers can have adverse environmental effects. Thus, there is a need to find alternative ways and one is by using fish offal from aquaculture by-products as fertilizers. Fish offal are organic fertilizers known to contain a healthy balance of significant plant nutrients. But only few studies were done on plant growth effects using fish offal from different fish species. Here, we compared the number of leaves, area, and growth rate of *M. charantia*, an important medicinal vegetable crop, in soil amended with fish offal of *C. chanos* and *O. niloticus* at 7.5% (v/v) concentration. Results showed that there is no significant difference on the growth of *M. charantia* between the fish offal of *C. chanos* and *O. niloticus*. Therefore, fish offal fertilizers from either fish species enhanced the growth of *M. charantia* plant providing a more cost-efficient and environment-friendly way of growing *M. charantia*.

Introduction. - Inorganic fertilizers have been the staple way of enhancing soil fertility because they are quick-release fertilizers, making the nutrients immediately available for the crop. However, inorganic fertilizers can cause leaching and excess algal growth before the nutrients are actually taken up by the plant, consequently damaging the aquatic ecosystem [1]. Therefore, there is a need to find alternative sources of fertilizer, and one potential source is the fish offal that comes from culturing fish.

Organic fertilizers improve soil fertility, soil structure, water retention capacity, physical and chemical properties, soil pH, microbial activity, and crop yield [2]. Organic fertilizers are also environment-friendly as stated by Akande et. al (2004) in Ahmad et al. [2]. Agricultural wastes, livestock wastes (manure), domestic and industrial wastes (compost), and fishery/aquaculture wastes like fish offals are commonly used organic fertilizers [2, 3].

Soil fertilization using fish waste compost was reported to cause an increase in leaf yield of *L. sativa L.* It caused a significant increase of nitrogen, phosphorus, potassium, sodium, calcium, and magnesium in leaves of the plant [4]. Also, fermented fish waste was found to enrich the soil nutrients required for plant growth and favorably influenced the conducting functions of xylem and phloem vessels [5]. Results of the study conducted by Lema and Degebassa [1] has shown that fertilizers made from fish offal can provide readily absorbed nutrients required for growth and yield production of tomato and onion [1]. Different fish offals have been tested for their properties in enhancing the growth of plants. In addition, the liquid fish silage of *Nemipterus japonicus*, at 5.0%-10.0% has a great effect on the growth of *Brassica rapa subsp. chinensis* in terms of its height, leaf number, leaf area, and fresh weight [3]. Therefore, fish offals help plants grow efficiently by providing the nutrients that are needed for growth such as nitrogen, phosphorus, and potassium.

The test plant, *Momordica charantia L* belongs to the family of Cucurbitaceae and is commonly known as Ampalaya in the Philippines. It is a widespread vegetable crop grown in Asia and in other parts of the world. It is usually grown as an annual crop, but it can also be considered as a perennial crop in mild and frost-free winter areas. *M. charantia* is best sown from October to February as it is its ideal planting season because cool weather is better for production [6]. These conditions are required for the germination of *M. charantia* seeds. *M. charantia* has been suggested to be an economically important crop as it has shown dietary advantages as the immature fruit contains vitamin A and vitamin C [7] [6]. *M. charantia* was chosen as a test plant because it can be cultivated throughout the year. *M. charantia* has been widely utilized in traditional medicine for many treatments due to the phytochemicals present in the herb that have been identified to exhibit medicinal activities such as antibiotic and antidiabetic which makes it one of the most nutritious plants [8]. It was noticed that the vegetative growth and herbage yield of *M. charantia* was significantly enhanced by the application of different organic fertilizers [8].

Fish offal fertilizers are organic fertilizers made from by-products of the fish industry which are known to contain significant quantities of nitrogen as well as a healthy balance of all 18 nutrients such as

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amino acids which can be significant for crops growth. According to the study of Lema and Degebassa [1], it was known that plants rapidly respond to and grow vigorously when they are regularly fertilized with fish fertilizers. Also, fish offal such as heads, guts, and fins are suitable for agricultural use since it has high contents of nutrients, such as N, P, and Ca [9]. Because of this, composting initiatives using fish waste have been carried out in various parts of the world in search of alternative and viable techniques for transforming fish waste into useful agricultural products [10]. A study conducted by Widyastuti [11] has shown that applying fish waste fertilizer of fermented *C. chanos* has a significant effect on the growth of plants. A study also showed that fish wastes of *O. niloticus* boosted the production of *Solanum lycopersicum L.*, and *Allium cepa* [1]. Therefore, fish offals of *C. chanos* and *O. niloticus* are excellent sources of nutrition for soils and plants.

It has already been studied that fish offal from *C. chanos* and *O. niloticus* are effective fertilizers in improving the growth of plants, but the comparative effectiveness of these fish offal fertilizers in a specific concentration on the growth of a plant has not been determined yet.

Studies have only looked at the comparison of the effects of fish offal fertilizer to inorganic fertilizer and few studies have been done on comparing the effects of fish offal fertilizer sourced from the culture of different fish species. Our research will therefore compare the effects of fish offal fertilizers from *C. chanos* and *O. niloticus* in a specific concentration on the growth of *M. charantia*. This research will also test if *C. chanos* will have a more significant impact on the plant growth of *M. charantia* than the fish offal of *O. niloticus*. *M. charantia* will be used as the test plant of the research as it is native to the Philippines which is one of the parameters of the study. The research will also be exploring the amount of concentration that will be given to the test plant which is 7.5%. The research specifically aims to determine which fish offal fertilizer in a specific concentration is a better alternative to inorganic fertilizers for the optimal and maximum growth of a plant like *M. charantia*.

The study chose the fish species *C. chanos* and *O. niloticus* because they are the second and first most important farmed fish in the Philippines respectively [12]. The significance of this study is to reduce the use of inorganic fertilizers which are harmful to the environment. This study was chosen to be able to propose a better fish offal as an alternative fertilizer to inorganic fertilizer.

This study aims to determine the comparative growth of *Momordica charantia* amended with the fish offals of *Chanos chanos* and the fish offals of *Oreochromis niloticus* in a specific concentration. effects of *C. chanos* and *O. niloticus* fish offals as fertilizers on the growth of *M. charantia* in a specific concentration. It specifically aims to:

(i) To count the number of fully expanded leaves of *M. charantia* after the introduction of the treatment groups, *C. chanos* (B), *O. niloticus* (T), and the negative control group (C), tap water, on the 21st day after planting to determine the average number of fully expanded leaves;

(ii) To measure the height of *M. charantia* from the ground level to the leaf base of the highest fully expanded leaf after the introduction of the treatment groups, B, T, and C, on the 5th, 9th, 13th, 17th, and 21st day after planting to calculate growth rate in cm/day.

(iii) To determine the largest fully expanded leaf per sample per treatment per replicate of *M. charantia* to calculate the leaf area in cm^2; and

(iv) To determine the difference in the growth of *M. charantia* when compared among treatment groups (B, T, and C) and within treatment groups.

Methods. - The experimental study was conducted for a period of 21 days to compare the effects of *C. chanos* and *O. niloticus* fish offals as fertilizers on the growth of *M. charantia* at 7.5% v/v concentration. The fish offals of *C. chanos* and the fish offals of *O. niloticus* were acquired from the local fish markets. The offals were then boiled, settled overnight, and strained using a muslin cloth. Then, the strained fish offals were placed evenly in aluminum pans for sun-drying up to a period of 11 days. The dried fish offals were ground using a mortar and pestle and were added and mixed with previously homogenized soil. After which, twenty seeds of *M. charantia* were planted and ten seeds were randomly chosen for the measurement of plant height, the number of leaves, and leaf area. One-way ANOVA was used for the statistical analysis.

Experimental set-up. The study utilized three blocks with three pots per block in each of the researchers' backyards. The placement of the samples was determined using the randomized complete sampling block design (RCBD) and through an electronic random generator.

Twenty seeds were planted in each pot. Then, the researchers conducted a fishbowl random sampling to identify ten seeds from the total number of seeds sprouted in each pot to be used as test plants for the data analysis. Researchers' backyards were chosen as the study sites because they were the places that were most suitable in the work unit's field of study, which is agriculture. Backyard is the place where the plants can get enough sunlight and is the place that is not frequently disturbed by people. A plant house with the dimension of 421.16 cm x 343.35 cm x 200 cm was built with garden nets in the backyards of the researchers for the plants to grow and be protected against pests and insects. A soil analysis test using the jar test method was carried out by the researchers. The results of the soil analysis indicated that the researchers obtained silt loam soil, sandy loam soil, and pure loam soil.

Materials and Equipment. For the preparation of fish offal fertilizers, *C. chanos* and *O. niloticu*s, were obtained from local markets. Distilled water, aluminum foil, and aluminum pans were obtained from grocery stores and mortar and pestle from local hardware stores. Muslin cloth was bought from a

local textile store and cooking barrels were obtained from the respective homes of the researchers.

For planting, *M. charantia* seeds were obtained from Pacifica Agrivet Supplies. Watering cans and pots were bought from local agricultural and hardware stores while soil and tap water were obtained from the researchers' houses.

For measurements, vernier calipers and kitchen scales were bought from local hardware stores.

Data Gathering and Analysis. The data gathering was done for 45 days and involved the preparation of the fish offal fertilizers, planting of *M. charantia* seeds, and the measurements of the identified parameters such as the number of leaves, growth rate, and leaf area.

Preparation of fish offal fertilizers. One kg of *C. chanos* and *O. niloticus* fish offals, specifically the fins, liver, intestine, heart, kidney, and stomach, were separated and used as fertilizer following the method of Lema & Deghebassa [1]. The identified fish offals were then cut into smaller pieces. After that, 222 ml of distilled water was boiled until it reached a temperature of 100 degrees Celsius, and the cut fish offals were added. The fish offals were cooked for 17 minutes and were stirred every three minutes [13]. The resulting mixture was left overnight to allow the solids to settle. After 12 hours, the liquid components were separated using a muslin cloth and disposed. The solid components were sundried for 11 hours every day from six in the morning until four in the afternoon for 12 days. After every drying process, the aluminum foil pan was covered with an aluminum foil sheet and stored at room temperature [1]. The dried fish offal were crushed using a mortar and pestle to obtain an amorphous and flaky fertilizer.

Incorporation of the dried fish offal fertilizers. The dried fish offals, measured at five parts, were directly mixed with 67 parts of soil to achieve a 7.5% volume-to-volume concentration [14] in the sack using a trowel and placed in a pot [15].

Planting of Momordica charantia seeds. Twenty seeds of *M. charantia* were sown evenly in each pot with the dimensions of 44.45 cm x 20.32 cm x 14.00 cm. The pots in each block were placed five cm apart and each block separated with a 30 cm distance. The seeds were sown one cm deep and placed four cm apart in length and 6.5 cm apart in width for each seed. For watering, *M. charantia* were watered at around four to five in the afternoon everyday using one L of tap water in each pot [6].

Measurements. The number of leaves of *M. charantia* were manually gathered by counting the total number of fully expanded leaves at the end of the experiment at Day 21.

The height of *M. charantia*, was measured using a vernier caliper from the ground level to the leaf base of the highest fully expanded leaf and was manually recorded by the researchers on the 5th, 9th, 13th, 17th, and 21st day after planting for computations.

The growth rate of *M. charantia* was determined using the following formula,

$$\left(\frac{final\ height - initial\ height}{n}\right)$$

where n is the number of days between the final day and the initial day of determination [16].

The leaf area of *M. charantia* was computed using the Counting Grid Squares (CGS) method, using the formula: Leaf Area = (NGS × OGA), where NGS is the number of grid squares inside the leaf outline and OGA is the area of a single square grid [17]. The data was gathered after the 21st day of planting for computations.

Data Analysis. The number of leaves, growth rate and leaf area were computed by the researchers using their respective formulas. Standard deviation was also calculated based on the number of replicate samples. The statistical analysis of the study was done using the Jamovi Version 2.2.2.0 software. One-way analysis of variance (ANOVA) was used to compare the means and the least significant difference (LSD) was used to separate the means at significance level $p<0.05$. A normality test was also performed to ensure that the data, specifically for the leaf area, was normally distributed despite its small sample size.

Safety Procedure. Personal protective equipment (PPE) such as gardening gloves and aprons were used in order to protect the researchers while performing the experiments when working with hazards.

Proper waste disposal was practiced. Excess fish offals were properly identified before they were disposed of in the food wastes bin.

Results and Discussion. - This section is divided into 3 components. The first component shows the effect of the application of the dried fish offals of *C. chanos* and *O. niloticus* and the non-fertilized control on the number of leaves of the *M. charantia* plant. The second component shows the effect of the application of fish offals on the leaf area of the *M. charantia* plant. Lastly, the third component compares the effect of the *C. chanos* and *O. niloticus* dried fish offals as fertilizers and the control group on the growth rate of the *M. charantia* plant.

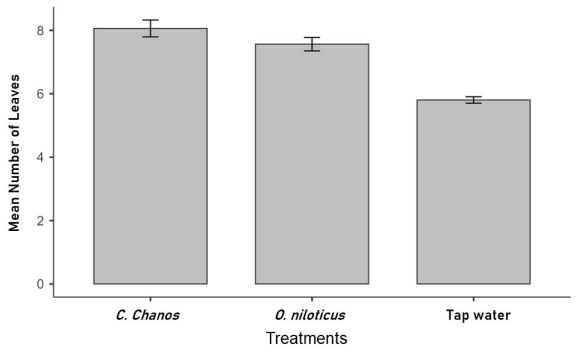

Figure 1. This figure shows the mean number of leaves of *M. charantia* amended by *C. chanos and O. niloticus* offal fertilizer.

Effects on the number of leaves. The mean number of leaves of the treatments fertilized with *C. chanos* offal has the highest mean value among all the treatments at 8.06 as shown in Fig. 1. It is followed by the treatments containing *O. niloticus* which measures an average mean number of 7.57. Lastly, the control group, tap water, has the lowest mean value of 5.81.

The mean number of leaves varied among treatment groups. This is probably due to the difference in the amount of nitrogen (N) present in the soil. According to [15], the amount of available N is directly proportional to the number of leaves to be produced by the plant. The available organic nitrogen present in the fish fertilizer enhanced the number of leaves and branches of the plant [1]. At $p<0.001$, there was a significant difference between the two (2) treatment groups and the control group. This significant difference observed between the two treatment groups and the control group is supported by the study done by Hamaiel et al. [8]. They found that the high nitrogen concentration present in the soil improved the physical condition of the soil, providing enough energy for microbial activity thereby increasing the availability and uptake of nutrients. However, no significant difference was observed between the two (2) treatment groups, *C. chanos*, and *O. niloticus* offal fertilizers indicating that both treatments acted the same in terms of producing leaf numbers of *M. charantia* plant. It is to be noted that only fully expanded leaves were manually counted by the researchers.

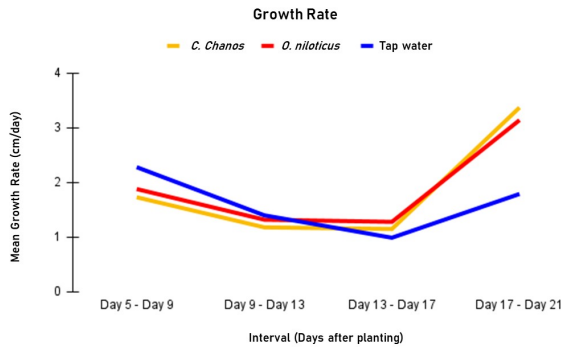

Figure 2. This figure shows the mean growth rate of *M. charantia* amended by *C. chanos* and *O. niloticus* offal fertilizer.

Growth rate of M. charantia. The growth rates based on plant height were measured at different time intervals (Day 5 - Day 9, Day 9 - Day 13, Day 13 - Day 17, and Day 17 - Day 21). Fig. 2 shows the changes in the growth rates per treatment group at different intervals. At Day 5 to Day 9, the control group, tap water, (2.28 cm/day) has the highest mean growth rate as compared to *O. niloticus* (1.88 cm/day) and *C. chanos* (1.73 cm/day). At Day 9 to Day 13, the tap water (1.40 cm/day) still exhibited the highest mean growth rate, followed by the *O. niloticus* (1.32 cm/day) and lastly, the *C. chanos* (1.18 cm/day). At Day 13 to Day 17, *O. niloticus* (1.28 cm/day) had the highest mean growth rate and was followed by the *C. chanos* (1.15 cm/day) while the tap water control measured the lowest growth rate at 0.99 cm/day. Lastly, at Day 17 to Day 21, *C. chanos* (3.37 cm/day) produced the highest mean growth rate as compared to the *O. niloticus* (3.14 cm/day) and tap water (1.79 cm/day). This time interval also recorded the fastest mean growth rate in all the treatments indicating the plants reached the exponential growth stage.

Day 0 to Day 4 measurements were not included in the data gathering as this time point was allocated for the *M. charantia* plants to grow.

Variation in the mean growth rates among treatment groups at different time intervals was observed. It is to be noted that the plant height was used as an indicator for the growth rate parameter. At $p<0.01$, there was a significant difference between the *O. niloticus* treatment group and the control group. This can be attributed to an increase in available organic nitrogen present in the soil as fish offal fertilizers were applied [14]. Furthermore, nitrogen nutrition is directly linked to the development of plants [17]. However, no significant difference was observed between the two (2) treatment groups, *C. chanos* and *O. niloticus*, offal fertilizers, implying that both offal treatments provided similar amounts of nitrogen to the soil.

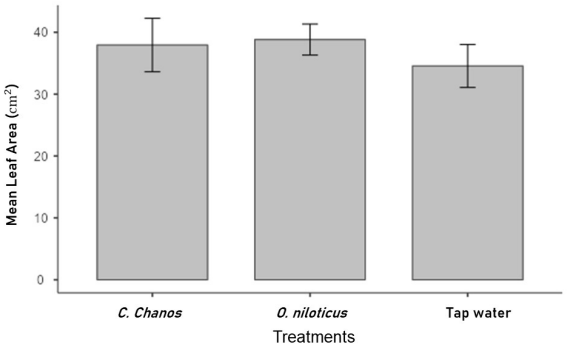

Figure 3. This figure shows the mean leaf area of *M. charantia* amended by *C. chanos and O. niloticus* offal fertilizer.

This figure shows that the leaf area of the treatments containing *C. chanos*, 37.9 cm^2, has the highest mean leaf area. Treatments containing *O. niloticus* measured at 38.8 cm^2 come after. The lowest mean was yielded by the control group, tap water, which was measured at 34.6 cm^2.

Comparison of the leaf area. The area of the largest leaves from each treatment was compared using the CGS method (Fig. 3). *M. charantia* plants grown on *O. niloticus* offal fertilizer were found to have the highest mean leaf area at 38.8 cm^2. Treatments containing *C. chanos* measured at 37.9 cm^2 come after. The lowest mean leaf area was yielded by the control group, tap water, which was measured at 34.6 cm^2.

The mean values of the leaf area among the treatment group varied. The results can be supported by the claim of Rachman and Suwars (1990) which was stated by Karim et. al [15] that the application of nitrogen increases leaf dimensions (length and width of leaf) of the plant. At $p>0.05$, no significant difference was observed between the treatment

groups and the control group. However, the Counting Grid Squares (CGS) method which is a direct and manual method of computing for leaf area was done by the researchers.

The mean values of the leaf area among the treatments varied. These results can be supported by the claim of Rachman and Suwars (1990) mentioned in Karim et. al [15] that the application of nitrogen, in our case the nitrogen-rich fish offal, increases leaf dimensions (length and width of leaf) of the plant. There is no significant difference observed between the treatment groups and the control group ($p<0.05$). However, the Counting Grid Squares (CGS) method, a direct and manual method of measuring leaf area, was the only method employed in this study. Other methods such as the use of ImageJ software may produce more accurate measurements.

Table 1. This table shows the comparison between the effects of *C. chanos*, and *O. niloticus* with the effects of the negative control on the growth rate, leaf number, and number of leaves of the *M. charantia*.

Treatment	Number of leaves	Growth rate	Leaf area
C. chanos	8.06*	1.85	37.9
O. niloticus	7.57*	1.91*	38.8
Tap Water	5.81	1.61	34.6

n.s = no significant differences ($p>0.05$) observed with the control group
* = significant differences ($p<0.05$) observed with the control group

In general, *O. niloticus* fish offal fertilizer has a more significant effect on the growth rate of *M. charantia* when compared to *C. chanos*. For the number of leaves, no significant difference was observed between the two treatment groups. However, compared to the control group, *C. chanos* and *O. niloticus* fish offal fertilizers have significant effect on increasing the number of leaves of *M. charantia*. Lastly, in terms of the leaf area, no significant difference was observed among the two fish offal fertilizers and the control group.

Conclusion. - The One-way ANOVA analysis showed that there is a significant difference on the overall growth parameters of *M. charantia* between the control and the fish offal fertilizers of *C. chanos* and *O. niloticus*. These results indicate the efficacy of fish offal as organic soil fertilizer. However, *C. chanos* and *O. niloticus* showed no significant difference when compared between each other on the overall growth parameters of *M. charantia.* This implies that the application of either fish offal fertilizer enhanced the growth of *M. charantia* plant. Thus, the application of *C. chanos* and *O. niloticus* offal fertilizers would have similar effects on the growth of juvenile *M. charantia* plants. However, further analysis on the use of fish offal as organic fertilizers should be done as phytotoxicity and nitrogen overload may occur.

The results of the study may serve as one of the bases for field trials of *C. chanos* and *O. niloticus* offal fertilizers on ampalaya. Similar effects on the growth of other crops is not certain because of the findings of this study.

Recommendations. - For the number of leaves, the AV-K method utilizing leaf nodes can be used to calculate the total number of leaves. When the plant sample used in the study is a vine, stem length can be measured instead of plant height. Moreover, software such as ImageJ can be used for the measurement of the leaf area for a more precise result. The soil to be used in the study should be tested for nutrient availability. Lastly, the effects of *C. chanos* and *O. niloticus* offal fertilizers on other crops may be known using the methods of this study.

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