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Safe and sustainable propagation of insulin plant (*Costus igneus)* through a recirculating aquaponic system using Nile Tilapia (*Oreochromis niloticus*) and Duckweed *(Lemna perpusilla*)

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Abstract The study introduced an innovative approach to cultivate *Costus igneus* using aquaponic system, ensuring both sustainable cultivation and safe consumption of this valuable medicinal herb. The study reported the growth of Insulin plant in an aquaponic system containing Tilapia, Duckweed and Mollies. Two identical aquaponic systems were constructed within a greenhouse, each comprising a fish tank, a tank housing molly fish and duckweed, and a growing bed. These systems were stocked with 8 kg of Nile tilapia and 1 kg of duckweed each, with one of the systems additionally containing 24 molly fish. Both systems housed 75 Insulin plants. Environmental conditions within and outside the greenhouse, as well as water quality parameters, were closely monitored. The system with mollies maintained a pH level between 7.6-7.8, total dissolved solids (TDS) of 296-314 ppm, and temperatures between 28.7°C to 29.6°C. Results indicated that the presence of mollies in the aquaponic system led to slightly faster growth and improved yields of Nile tilapia, as well as higher duckweed yields. Water analysis revealed that the molly-containing system exhibited higher levels of essential nutrients such as nitrogen, potassium, and phosphorus, resulting in increased Insulin plant fresh weight. The molly fish's presence proved beneficial in breaking down fish waste and facilitating nutrient conversion, particularly nitrogen, for plant utilization.

Keywords: Environmental parameter, Water quality parameter, Aquaponics

Introduction

The global prevalence of diabetes is on the rise, with a worrisome projection of a more than 50% increase in diabetes-related deaths over the next decade, as reported by the World Health Organization in 2006. In the Philippines, there has been a significant upsurge in diabetes cases. A survey conducted in

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2008 revealed that one out of every five Filipinos was affected by diabetes, solidifying the country's position among the top 15 nations with high diabetes incidence. Amidst this growing health concern, the efficacy of synthetic drugs has been waning, and contraindications have been mounting. Consequently, an increasing number of individuals grappling with diabetes are turning to alternative and traditional medicine, particularly herbal remedies.

Costus igneus commonly known as insulin plant has gained popularity due to its ability to cure diabetes. Originally introduced in India as a medicinal plant, it was utilized by the indigenous people of the Kolli hills to practically cure diabetes, as reported by Hedge *et al.* in 2014. The powdered leaves of *C. igneus* have demonstrated the ability to bring blood sugar levels back to normal, as indicated by Shetty *et al*. in 2010. Diabetic individuals consume one leaf daily to keep their blood glucose in check (Devi and Urooj, 2008).

As the demand for natural remedies for diabetes management increases, there is a need to develop sustainable cultivation methods for *C. igneus* to ensure a consistent and high-quality supply of this medicinal plant. Traditional methods of cultivating *C. igneus* often involve the use soil as growing medium. However, application of synthetic fertilizers and manmade wastes contaminate the soil. These contaminants degrade the environment, reduce the plant's medicinal properties, and pose health risks to consumers. To address these concerns and meet the growing demand for this medicinal herb, alternative and sustainable cultivation methods need to be explored.

Aquaponics, a soilless cultivation method, integrates the growth of fish and plants through aquaculture and hydroponic systems. The major componnets of aquaponic system are fish, plants and bacteria. The fish constitutes the aquacultural element of the system, and their excretions provide nutrients for the plants Shu, 2014). The nutrient produced in the fish tank is pumped and utilized to fertigate the plants in the growing bed (Rakocy, 2007). The plants do not just consume the nutrients provided by the fish; it filters the water as it absorbs the nitrates that can be toxic to the fish before it is recirculated back to the fish tank (Monnet *et al.,* 2002). It helps cleanse the water as they consume these nutrients, creating a harmonious ecosystem where aquacultural crops thrive (Roe and Midmore, 2008). This continuous nutrient cycle prevents depletion and reduces environmental discharge, as explained by Rakocy *et al.* in 2003. The bacteria also play a major role in the cycling of nutrients in an aquaponics system. They convert the wastes into forms that can be absorbed by the plants, a process called nitrification (Shu, 2014).

The study aimed to develop a safe and sustainable method for propagating insulin plant through a recirculating aquaponics system. It specifically determined the effect of environmental and water quality parameters, as well as the presence of molly on the growth performance of the plant in the aquaponic system.

Materials and methods

The study employed completely randomized design (CRD to evaluate the growth performance of the insulin plant in two aquaponics systems. Treatment 1 (T_1), consisted of a system containing molly while treatment 2 (T_2) was a system without molly. Each treatment was replicated three times. The plants were cultivated in three growing beds for each system. The plant samples were selected randomly from each of the growing beds.

The study was carried out at the Hydroponics and Aquaponics Demonstration Farm and Experiment Station, located at the College of Engineering in Central Luzon State University, Science City of Muñoz, Nueva Ecija, Philippines, from March to June 2018. This study compared the growth of *C. igneus* in two systemswith molly and without molly.

Two aquaponics systems were built within a 4m x 3m x 3.5m greenhouse. Each system comprised a Tilapia fish tank, a duckweed and molly tank, and growing beds. The fish tank measured 74 cm x 96 cm x 137 cm with a total volume of 0.97 m3, while the duckweed and molly tank measured 30 cm x 100 cm x 96 cm with a total volume of 0.288 m3.

Insulin plant stem cuttings were rooted in plastic bottles filled with a medium of carbonized rice hull, rice hull, and sand in a 1:1:1 ratio. These cuttings were cultivated inside the greenhouse and regularly watered. Prior to stocking of fish and planting, the systems were filled with tap water and operated for one week to check for leaks and establish bacterial and algal populations. Subsequently, Nile tilapia was stocked in the fish tanks based on a density lower than 17 kg/m3, as recommended by Lennard (2012). The fish were fed with commercial pelletized feed containing 32% protein, supplemented with duckweed twice a day. After seven days, molly fish were introduced into one of the systems, fed with pre-starter pelletized feed and consuming unconsumed fish feed waste. Additionally, 1 kilogram of duckweed was added to each system.

A month after initiating insulin plant growth in plastic bottles, the rooted plants were transplanted into the growing beds. Twenty-five rooted insulin plants were planted at 20 cm off-set spacing in each growing bed. Environmental parameters inside and outside the greenhouse such as air temperature and relative humidity were monitored and recorded everyday. Water quality parameters such as temperature, pH, total dissolved solid and dissolved oxygen were measured and recorded daily. Samples of culture water from the two systems were analysed to determine nitrite, nitrate, potassium and phosphorous content.

The data on the growth and yield of the Insulin plant and duckweed were gathered during the first and second harvest while data on the growth and yield of the Tilapia and Molly were gathered after the second harvest. Growth parameters such as number of leaves and height of the plant were gathered every week. Fresh weight and dry weight of the insulin plant determined the yield of the plant. Collected data on the number of leaves, height, fresh weight, and oven dry weight were recorded, tabulated and represented graphically. Statistical analysis was performed using single-factor Analysis of Variance (ANOVA).

Gain in weight of tilapia was computed by subtracting the initial weight from the previous weight. While the growth rate was computed by dividing the gain weight by the number of days of culture. Feed conversion ratio was computed by dividing the feed intake over the average daily gain weight. The yield of the golden molly was determined by subtracting its initial number from its final number. While yield of duckweed was determined by subtracting its initial weight from its final weight.

Results

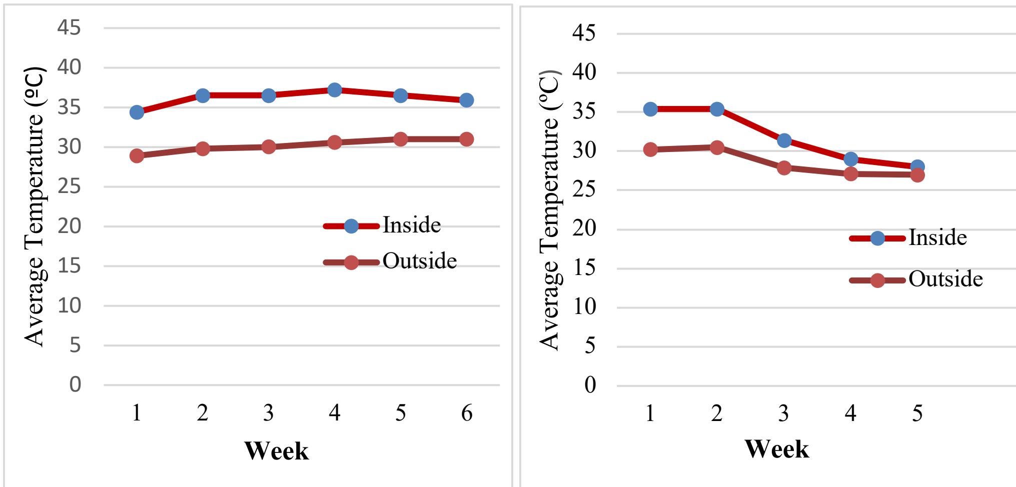
Air temperature

The weekly average temperature inside and outside the greenhouse is shown in Figure 1. During the first harvest, lowest average temperature inside the greenhouse was recorded in the first week at 34.4 °C, while the highest average temperature was recorded in the fourth week, reaching 37.2 °C. While, outside the greenhouse the lowest average temperature was recorded in the first week at 28.9 °C and the highest in the fifth week at 31 °C.

During the second harvest, the temperature inside and outside the greenhouse dropped. The greenhouse recorded its lowest temperature in the fifth week, averaging 27 °C, and the highest temperature in the second week, reaching 35.4 °C. While the outside temperature reached its lowest point in the fifth week at 27.1 °C and peaked in the first week at 30.2 °C.

Relative humidity

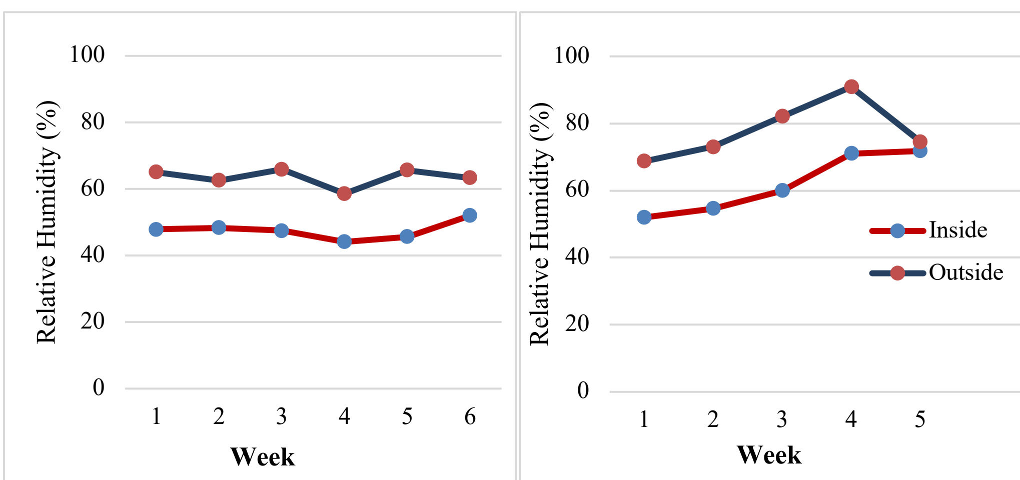
The relative humidity inside and outside the greenhouse is shown in Figure 2. During the first harvest, the lowest average relative humidity inside the greenhouse was recorded in the fourth week at 44.1% and the highest was recorded in the sixth week reaching 52%. While, outside the greenhouse, the lowest relative humidity was recorded in the fourth week at 58.6% and the highest was recored in the third week at 65.9%.



FIRST HARVEST **SECOND HARVEST**

Figure 1. Weekly average temperature inside and oustside the greenhouse during first and second harvests

During the second harvest, the greenhouse recorded its lowest average relative humidity in the first week at 52% and the highest relative humidity in fifth week reaching 74.6%. While the outside relative humidity reached its lowest point in the fifth week at 27% and peaked in the second week at 30.51%.



FIRST HARVEST **SECOND HARVEST**

Figure 2. Weekly average relative humidity inside and outside the greenhouse during first and second harvests

Water quality parameters

The average pH levels of the systems during the first and second harvests are shown in Figure 3. Throughout the first harvest, the pH level in the system without mollies was slightly higher than the system with mollies, with averages of 7.7 and 7.6, respectively. Meanwhile, during the second harvest, average pH levels slightly increased to 7.7 in the system with mollies and 7.8 in the other system.

The average TDS levels of the systems during the first and second harvests are shown in Figure 4. TDS level throughout the first harvest in system with mollies is slightly higher than the other system with averages of 304 ppm and 296 ppm respectively. These values increased throughout the second harvest with an average of 330 ppm in the system with mollies and 314 ppm in the other system.

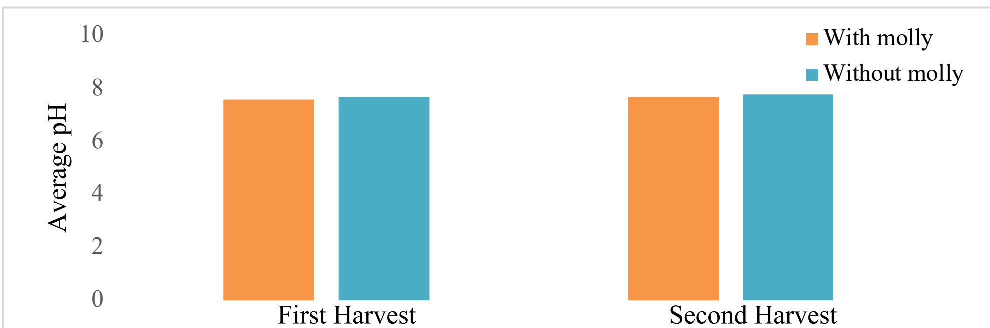


Figure 3. The average pH of the two systems during first and second harvests

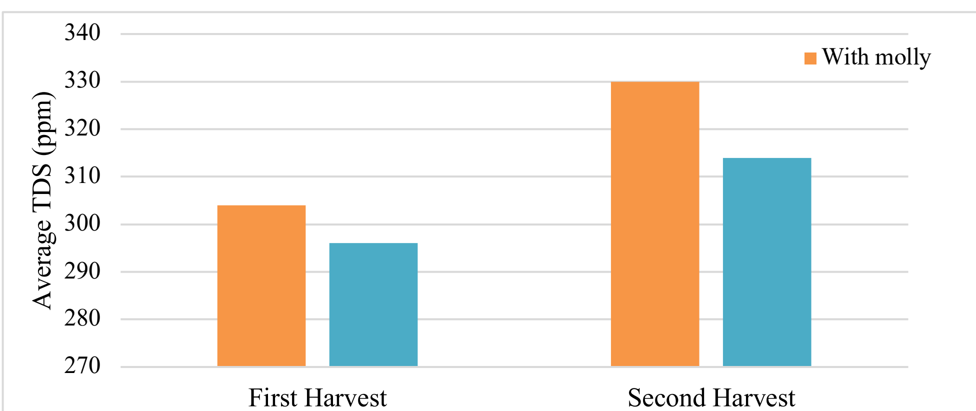


Figure 4. The average TDS of the two systems during first and second harvests

The average water temperature inside during the first and second harvest are shown in Figure 5. Throughout the first harvest, the temperature in the system with mollies ranged from 28.19 °C to 30.94 °C with an average of 29.6 °C. This is slightly higher than the temperature of the other system which ranged from 28. °C to 29.4 °C. While during the second harvest, the temperature in both systems was the same which ranged from 27 °C to 30 °C with an average of 29 °C.

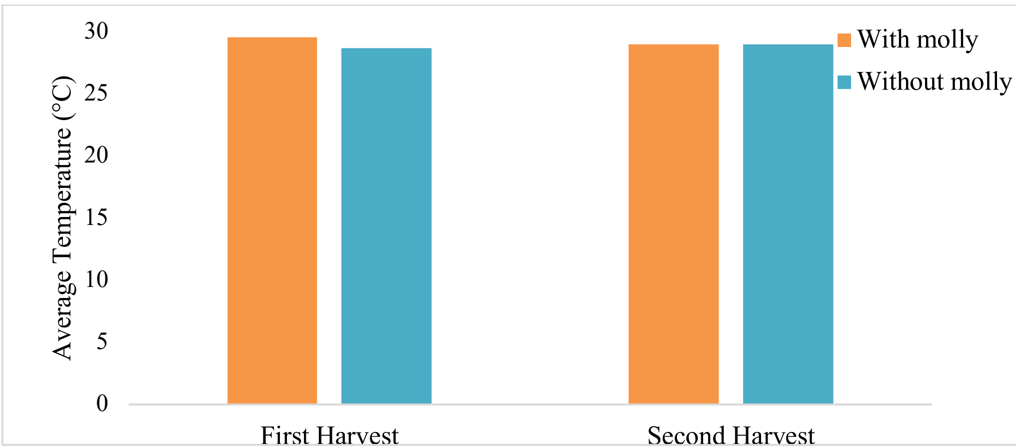


Figure 5. The average water temperature of the two systems during first and second harvests

Water analysis showed that the amount of nitrogen, phosphorus and potassium (NPK) in both systems are presented in Table 1. The system with mollies contained higher levels of nitrogen, phosphorus, and potassium, registering values of 12 mg/L for nitrogen, 9.4 mg/L for phosphorus, and 12 mg/L for potassium. In contrast, the system without mollies recorded lower levels, specifically 9.3 mg/L for nitrogen, 8.8 mg/L for phosphorus and 11 mg/L for potassium.

Growth parameters

The average number of leaves of insulin plant during first and second harvests is shown in Table 2. During the first harvest, the average number of leaves of insulin plants in the systems with and without mollies were 28 and 27.7 respectively. While during the second harvest, the average number of leaves in both systems were identical which is 23. Statistical analysis revealed no significant difference in the number of leaves of insulin plant grown in systems with and without mollies during first and second harvests ($p>0.05$).

Table 1. Amount of nitrogen, phosphorus and potassium in the two systems

Systems	Nitrogen (N) (mg/L)	Phosphorous (P) (mg/L)	Potassiun (K) (mg/L)
With mollies	12	9.4	12
Without mollies	9.3	8.8	11

Table 2. Number of leaves of insulin plant during first and second harvests

Systems	Number of leaves	
	First Harvest	**Second Harvest**
With mollies	28	23
Withuot mollies	27.7	23
F-test	ns	ns
P-value	0.96	0.95

ns- not significant at 5% level

The average height of insulin plant in the two systems are shown in Table 3. During the first harvest, the insulin plants in the systems with and without mollies had a higher average height of 32.8 cm and 27.5 cm respectively than during the second harvest with 25.7 and 21.6 respectively. However, statistical analysis revealed no significant difference in the height of insulin plants grown in the two systems ($p>0.05$).

Table 3. Height of insulin plants during first and second harvests

Systems	Plant height (cm)	
	First Harvest	**Second Harvest**
With mollies	32.8	25.7
Withuot mollies	27.5	21.6
	ns	ns
P-value	0.07	0.10

ns- not significant at 5% level

Yield parameter

The average fresh weight of insulin plants during first and second harvests is shown in Table 4. During the two harvests, the fresh weight of insulin plants was higher in the system with mollies with an average of 39.1 and 55.1 g respectively, than in the system without mollies with an average of 37.5 and 39.7 g respectively. Interestingly, the isnsulin plants in both systems weighed higher during the second harvest than during the first harvest. Statistical analysis revealed no significant difference between the fresh weight of insulin plants grown in the two systems during the first harvest. However, during the second harvest, a significant difference between the fresh weight of insulin plants grown in the two systems was revealed (p>0.05).

The average oven dry weight of insulin plants during first and second harvest is shown in table 5. During the two harvests, the oven dry weight of the insulin plants in the system with mollies was slightly higher with an average of 2.1 g and 3.6 g respectively, than in the other systems with an average of 1.8 and 2.5 g respectively. However, statistical anaylsis showed no significant difference between the oven dry weight of insulin plant grown in the two systems (p>0.05).

Table 4. Fresh weight of insulin plants during first and second harvests

Systems	Fresh weight (g)	
	First Harvest	Second Harvest
With mollies	2.1	3.6
Withuot mollies	1.8	2.5
	ns	*
P-value	0.186	0.04

ns-not significant, *-significant at 5% level

Table 5. Oven dry weight of insulin plant during first and second harvests

Systems	Oven dry weight (g)	
	First Harvest	Second Harvest
With mollies	39.1	55.1
Withuot mollies	37.5	39.7
	ns	*
P-value	0.186	0.04

ns-not significant, *-significant at 5% level

Gain in weight and growth rate of Tilapia

Summary of gain weight and growth rate of tilapia is presented in Table 6. In the system with mollies, there were 107 heads of tilapia harvested which weighed 9.55 kg. This number of fish gained a total weight of 1.55 kg at a rate of 18.45 g/day with a feed conversion ratio (FCR) of 0.50. While in the other system, 119 heads of tilapia were harvested weighing 9.2 kg with 14.29g/day growth rate and 0.60 FCR. Individual fish in the system with mollies gained an average weight of 14.48 g at a rate of 0.17 g/day while the fish in the other system gained an average weight of 10.1 g at a rate of 0. 12 g/day.

Table 6. The growth rate and feed conversion ratio of Tilapia in thw two systems

	WITH MOLLIES (107 heads)					WITHOUT MOLLIES (119 heads)				
	Initial	Prev.	Gain	Growth rate	FCR	Initial	Prev.	Gain	Growth rate	FCR
NT	8,000	9,550	1,550	**18.45**	**0.50**	8,000	9,200	1,200	**14.29**	**0.65**
IF	74.77	89.25	14.48	**0.17**		67.23	77.3	10.1	**0.12**	

NT- Total number of fish in tank, IF- Individual fish

Gain in number of molly

Summary of yield of molly is presented in Table 7. From an initial number of 24 heads, there was 312 mollies harvested. The number of mollies increased by 28.

Table 7. The yield of molly during the two harvests

Parameter	Initial	Previous	Gain
Number	24	312	288

Discussion

Costus igneus and other medicinal plants are traditionally grown in soil. However, synthetic fertilizers and manmade wastes contaminate the soil. These contaminants are absorbed and accumulated by plants in their parts and may inflict serious consequences on human health (Kulhari *et al*., 2013).

Aquapoincs, a soiless culture is a safe and sustainable method of growing plants. It incorporates the cultivation of both aquacultural crops and plants through an integrated aquaponics system, combining aquaculture with hydroponics for optimal growth and sustainability. The growth of plants in aquaponic system is highly influenced by environmental conditions and water quality parameters. The interplay of these factors influenced the growth and development of the Insulin plant. Temperature is the primary factor that ifluences the growth and development of plants (Hatfield and Pruger, 2015). According to Sace and Fitzzsimmons (2014), a greenhouse should maintain an optimum daylight temperature of 30 to 35 ^{0}C. The environmental temperature should be kept within in this optimum level for fast and successful maturation (Tyson, 2007). (During the first week of first harvest, the greenhouse maintains an optimum air temperature at 34.4 ^{0}C. However, it went beyond the optimum on the fourth week at 37 ^{0}C. While, during the fifth week of the second harvest, the temperature went below the optimum at 27 ^{0}C and went above the optimum level on the second week at 35.4 ^{0}C.

The relative humidity inside the greenhouse affects the transpiration rate of the plant. According to Shamshiri *et al*. (2018), the lower optimal RH for most greenhouse crop is 60% and the upper optimal RH is 85%. During the first harvest, RH inside the greenhouse was below the optimum range at 44.1% to 52%. While during the second harvest, it was within the optimum range at 52% to 71.4%. The pH is another important factor that influences the growth and development of the insulin plant. Most plants grow within a pH range of 5.8 to 6.8 with 6.3 as optimal level (Anderson *et al.,* 1989). When pH values deviate from this, may cause unhealthy growth of plant. At high pH the micronutrients become less available while at low pH they become too available. The system with mollies recorded an average pH level of 7.6 and 7.7 during the first and second harvests respectively. These values exceeded the recommended limits for plants.

Water temperature also greatly influences the health of aquaponic crops. According to Leaffin (2017), the general water temperature in an aquaponics system ranges from 18 to 30 ^{0}C. During the first and second harvests, the system with mollies maintained a temperature within this range at 29.6 ^{0}C and 29 ^{0}C respectively. The growth of plant in an aquaponics system is also greatly influenced by the available nutrients excreted by the aquacultural crops (Pennigton, 2011). The fresh weight of the insulin plant grown in the system with mollies were significantly higher than those that were grown in the other system during the second harvest. This is due to the higher level of NPK in the system. The higher level of NPK in the system with mollies is attributed to the presence of tilapia and mollies. As cited by Shu, (2014), the wastes generated by the fish actually provide the nutrients needed by the plants. The effluent of the the mollies in addition to the wastes excreted by the tilapia generated more nutrients for the plant crop. Furthermore, the mollies helped disintegrate the wastes of fish allowing faster conversion of nutrients like Nitrogen into forms that can be utilized by plants for their growth and development. Also, the established root system of the plant during the second harvest allowed them to absorb more nutrients.

This study benefits people who suffer from diabetes who prefer to use alternative medicine like the Insulin plant. Insulin plant produced through aquaponics system is natural and free from contaminants such as toxic heavy metals. This study also provides basic knowledge on the cultivation of Insulin plant in aquaponic system. Hence, allowing urban households to grow Insulin plant, despite the absence of arable land.

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