Impact of Organic Waste Digestion on Sustainability of Tilapia - Basil Decoupled Aquaponic System

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ABSTRACT
The current study was designed to evaluate the impact of using digested insect frass (DIF) as an external organic fertilizer on the sustainability of the tilapia- basil decoupled aquaponic system (DAS). The experiment consisted of two treatments; in the first treatment the digested tilapia sludge (DTS) was used solely as a nutrient source for the hydroponic unit, while in the second treatment supplementation of DIF as an external organic fertilizer (DTS+DIF) was added to the first protocol. The results showed that the concentration of K was higher in the DIF solution compared to the DTS solution. In contrast, Ca was higher in DTS solution in comparison with the DIF solution. No significant differences were detected in all the tilapia’s growth parameters between both treatments. Compared to DTS treatments (0.82kg/ m²), DTS+DIF treatment (0.98kg/ m²) recorded the highest biomass of basil. Moreover, the highest chlorophyll content (SPAD) was recorded in DTS+DIF treatment compared to DTS treatment. DTS+DIF treatment produced the highest diameter of the main stem, thickness of fiber strands, phloem and xylem tissues (+16.6, +15.7, +29.3 and +40.65 ±% to DTS, respectively). On the contrary, DTS treatment had a higher vessel diameter than DTS+DIF treatment. To sum it up, it could be concluded that using digested insect frass as an external organic fertilizer may influence the succession of tilapia - basil decoupled aquaponic system.

INTRODUCTION
Aquaponics is a rapidly growing field within the realm of sustainable food production (Zhang et al., 2020). The traditional of coupled aquaponics system (CAS) can be defined as one loop connection between fish tank and hydroponic unit. One disadvantage of the CAS is the requirement of different distinct environment for fish, bacteria and plants (Rodgers et al., 2022). There is new generation of aquaponic system with multi-loops between the fish tanks and hydroponic units, namely: decoupled aquaponic system (DAS) (Goddek et al., 2016). The advantages of DAS include: recycling the fish sludge to nutrients solution for plants and increasing the ratio between the plant culture and fish farming areas simultaneously (Rodgers et al., 2022b; Aslanidou et al., 2023).
During the aerobic digestion in DAS, the mineral is released in nutrient solution to increase the availability of nutrients for plant uptake in the hydroponic unit. However, DAS faces a great challenge; shortage in potassium, calcium, magnesium, and iron levels (Rakocy et al., 2003; Endut et al., 2016).

Insect "frass" is one of the organic fertilizer sources that is anticipated to become more readily available as a result of the sustainable growth of insect farming which serves as a feed source for cattle and aquatic animals (Romano et al., 2022). Frass is the insect’s excrement (insect poop) that includes chitin and a variety of nutrients which is necessary for plant growth (Fischer et al., 2021). Additionally, previous studies have demonstrated that the frass from black soldier fly (Hermetia illucens) larvae (BSFL) produces comparable plant growth compared with inorganic fertilizers (Klammsteiner et al., 2020; Chirere et al., 2021; Romano et al., 2022).

The tilapia and basil are one of the most successful model in DAS (Anderson et al., 2017; Espinosa-Moya et al., 2018). There is a need to support the traditional DAS with additional organic nutrient solution in order to avoid the plant mineral deficiency and enhance the tilapia welfare. Aerobic mineralization is a fermentation process carried out by a diverse group of heterotrophic organisms, which leads to a breakdown of organic wastes and release of macro and micro minerals (Khiari et al., 2019).

The objective of this study was to evaluate the impact of using digested insect frass as an external organic fertilizer on the sustainability of the tilapia- basil decoupled aquaponic system.

**MATERIALS AND METHODS**

This study was conducted during the period from October to late December 2022 (12 weeks) at the Fish Nutrition Laboratory (FNL), Department of Animal Production, Faculty of Agriculture, Cairo University, Giza, Egypt.

1. **Experimental design**

   The present experiment consisted of two treatments; in the first treatment the digested tilapia sludge (DTS) was used solely as a nutrient source for hydroponic unit. While in the second treatment, the same protocol of the first treatment was applied plus the supplementation of DIF as an external organic fertilizer (DTS+DIF).

2. **Decoupled aquaponic system**

   The current study was conducted in six identical aquaponic units under a greenhouse. Each unit consisted of a sump tank, deep water system (DWS), sedimentation tank, biological filter, and fish tank. The size of the fish tank was 700L and it was made from fiberglass. The sedimentation tank (47cm in diameter and 90cm in height) received the discharge of the fish tank effluent. The biological filter (45cm in diameter, 70cm in height) was then loaded with 10L of commercial plastic media (Kaldnes media K1).
3. Digestion of tilapia sludge and insect frass

The solid wastes were weekly collected from the sedimentation tank and placed in the digester under the aerobic conditions process. Starch was used as a carbon source, and the pH was maintained below 7 in the first week by phosphoric acid to provide a suitable environment for bacterial activity until the fermenter reached maturity in the sixth week. The aeration was weekly disconnected for an hour from the digester to deposit solid waste.

The insect frass (IF) was collected from the Fish Nutrition Laboratory (FNL), Department of Animal Production, Faculty of Agriculture, Cairo University, Egypt. IF was dried, then placed in a mesh bag and placed in a mixture of water in the aerobic digester with a ratio of 2.5g of IF to 1L of fish water. The soluble fertilizer from DTS and IF tanks were weekly harvested and added at the rate of 10L for hydroponics units’ treatments.

Aerobic digester was used as a tool for releasing the minerals from the tilapia sludge and insect frass. Each digester was mainly injected by air into the sludge and insect frass water with air blowers connected to diffusers and propellers. Air injection also ensures a proper mixing of the sludge. Both digesters were 45cm in diameter and 100cm high with an operating volume of 150L.

4. Plant germination

The seeds of sweet basil (Ocimum basilicum) were obtained from the Fish Nutrition Laboratory (FNL). The foam trays with 209 holes were employed in the seed culture process. Vermiculite and peat moss were combined in a 2:1 ratio to serve as the culture process’ medium. To guarantee a decent rate of germination, three to five sweet basil seeds were inserted into each hole, and then covered with another tray of foam, sprayed with water, wrapped in plastic, and let to sit for three days. The trays were kept wet for twenty-three days until the plants got three leaves. Afterward, they were transported to the hydroponic system. Finally, the basils were harvested after 3 months.

5. Experimental fish

The Red Nile tilapia (Oreochromis niloticus) adults were stocked in one cubic meter polyethylene tanks. The fish were received from an Egyptian commercial farmer in the Baltim Kafr El-Sheikh Governorate. Ninety fish with an average weight of 32.43± 0.19g were randomly stocked into six independent DAS (700L fiberglass tanks). Commercial floating feed was hand-fed daily at a rate of 3%. The proximate composition of the feed was 8.2% moisture, 8.6% ash, 3.5% crude fiber, 4.1% crude fat and 30% crude protein.
6. Measurements

6.1 Fish performance parameters

1) Weight gain (%) = \( W_2 - W_1 \)  \hspace{1cm} (1)

Where, \( W_1 \) is the initial weight of the Nile tilapia (g), while \( W_2 \) is the final weight of the Nile tilapia (g).

2) Feed conversion ratio (FCR) = Total weight of dry feed given(g)/ Total wet weight gain (g) \hspace{1cm} (Gicha et al., 2019).

3) Specific growth rate (SGR) (%/day) = \( \frac{\ln W_2 - \ln W_1}{T_2 - T_1} \times 100 \) \hspace{1cm} (3)

Where, \( W_2 \) is the final body weight (g), \( W_1 \) is the initial body weight (g), and \( (T_2 - T_1) \) is the experimental period per day \hspace{1cm} (Kpundeh et al., 2015).

4) Feed efficiency (FE) = \( \frac{\text{final body weight gain (g)}}{\text{total feed consumed (g)}} \) \hspace{1cm} (De Verdal et al., 2017).

5) Protein efficiency ratio (PER) = \( \frac{\text{body weight gain (g)}}{\text{protein consumed (g)}} \) \hspace{1cm} (Haidar et al., 2018).

6.2 Morphological characteristics of basil vegetative growth and anatomical sections

A random sample of 30 basils in each treatment was taken to determine the plant fresh and dry weights (kg/m\(^2\)), stem diameter (mm) and chlorophyll content in the leaves.

At the age of 12 weeks, specimens were collected during the final growth season of 2023. The specimens were immersed in FAA (10 ml formalin, 5 ml glacial acetic acid, and 85 ml 70% ethyl alcohol) for a minimum of 48 hours. The selected materials were washed in 50% ethyl alcohol, dehydrated in a normal butyl alcohol series, embedded in paraffin wax of melting point 56°C, sectioned to a thickness of 20 microns, double stained with crystal violet-erythrosine, cleared in xylene and mounted in Canada balsam according to the method of Nassar and El-Sahhar (1998).

7. Mineral determination by ICP-MS in tilapia sludge digestion and insect frass digestion

The extracts were filtered using disposable of 0.2m PTFE (Polytetrafluoroethylene) syringe filters (DISMIC-25HP, Advantec, Tokyo, Japan). The metal concentrations of these extracts were ascertained by inductively coupled plasma-mass spectroscopy (ICP-MS) (iCAP, Thermo, Germany). Certified reference materials (Merck, Germany) were used in the analyses. Metals were found within the approved bounds. The average and relative standard deviation were determined using the Qtegra program \hspace{1cm} (Lambers et al.,...
8. Statistical analysis

Statistical Package for the Social Sciences (SPSS) Statistics 18.0 was used for all statistical calculations. To find out if there were significant changes between the treatments, the data were examined using the independent-samples t-test. The data were shown as the mean ±SEM (standard error of means), with $P<0.05$ selected as the significance level (Faridah et al., 2016). Figures were performed using Excel program.

RESULTS AND DISCUSSION

1. Minerals in the digested tilapia sludge and insect frass

The concentration of macro and micro elements in the tilapia sludge and insect frass solution after mineralization process were shown in Fig. (1). It was noted that the concentration of K was higher in the DIF solution compared to DTS solution. However, Ca was higher in DTS solution in contrast with DIF solution. Whereas, the concentrations of Zn and Cu were higher in DIF solution compared to DTS solution.

![Fig. 1. Macro (a) and micro (b) minerals levels in the tilapia sludge and insect frass](image)

The aforementioned results indicate that the digested tilapia sludge solution may be considered as a rich source of macro and micro elements. In the current study, K, Ca and Mg levels in DTS solution were in the range of 63.44, 238 and 22.5mg/ L, respectively. This result disagrees with that of Goddek et al. (2018), who recorded the same minerals in the digested tilapia sludge solution with different levels (K, Ca and Mg level were 43,
120 and 48mg/ L, respectively). The same difference in the previous study was noticed in micro-elements of the DTS solution. The levels of Cu and Zn were 1.25 and 2.27mg/ L, respectively, higher than the obtained results of Goddek et al. (2018), where Cu and Zn levels were 0.02 and 0.05mg/ L, respectively. The difference in minerals levels may be attributed to the difference in minerals availability in the feed.

Starch was used as a carbon source in our study to supply the substrates for heterotrophic bacteria. The maturity stage in the current study began in the reactor from the sixth week in the tilapia sludge reactor, compared to the third week in insect frass reactor. The aerobic digestion process is preferable to the anaerobic one since it has a high conversion rate in a short period of time (Jang et al., 2014).

Even though the proportion of elements in the study contradicts with other different literature (Tan et al., 2021; Wang, 2022; Romano et al., 2023), the current study revealed a high level of macro and micro elements, such as K, Mg, Zn and Cu in DIF solution after mineralization process.

The original substrate given to the BSFL affects the nutritional uptake of DIF (Zarantoniello et al., 2023) and its potential efficacy as an organic fertilizer. It was reported that the nitrogen-rich substrates typically resulted in an increase of nitrogen levels in the frass (Fischer & Romano, 2021).

### 2. Fish growth and RAS performance

Table (1) shows the growth parameters of the tilapia under experimental conditions. No significant differences were detected in all growth parameters between DTS and DTS+DIF treatments.

**Table. 1. Fish growth parameters of the experimental treatments (mean ±SEM)**

<table>
<thead>
<tr>
<th>Variable</th>
<th>DTS</th>
<th>DTS+DIF</th>
<th>T value</th>
<th>P -value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Initial body weight (g)</td>
<td>32.43± 0.19</td>
<td>32.50± 0.27</td>
<td>0.18</td>
<td>0.48</td>
</tr>
<tr>
<td>Final body weight (g)</td>
<td>82.46± 3.33</td>
<td>82.81± 0.99</td>
<td>0.10</td>
<td>0.08</td>
</tr>
<tr>
<td>Weight gain (g)</td>
<td>48.18± 2.23</td>
<td>49.56± 0.69</td>
<td>0.59</td>
<td>0.11</td>
</tr>
<tr>
<td>Feed conversion ratio (%)</td>
<td>1.32± 0.03</td>
<td>1.18± 0.02</td>
<td>3.56</td>
<td>0.29</td>
</tr>
<tr>
<td>Specific growth rate (%)</td>
<td>0.98± 0.008</td>
<td>1.01± 0.004</td>
<td>3.68</td>
<td>0.33</td>
</tr>
<tr>
<td>Feed efficiency</td>
<td>0.43± 0.006</td>
<td>0.46± 0.003</td>
<td>3.70</td>
<td>0.36</td>
</tr>
<tr>
<td>Protein efficiency ratio</td>
<td>1.44± 0.022</td>
<td>1.53± 0.011</td>
<td>3.70</td>
<td>0.39</td>
</tr>
<tr>
<td>Survival rate (%)</td>
<td>99.95±0.025</td>
<td>99.98±0.013</td>
<td>1.08</td>
<td>0.20</td>
</tr>
</tbody>
</table>

Means in the same row with different superscripts are significantly different (P< 0.05).

All the recorded growth parameters were in the optimum range according to previous studies (Moustakas et al., 2004; Timmons & Ebeling, 2013; Rakocy et al., 2016). Results indicate that the conditions in both RAS treatments were similar. Removing the
tilapia sludge weekly from both treatments may lead to a reduction in TSS and influence the water quality in RAS. Hence, the DAS can be considered as a successful system to improve the welfare of the fish. Furthermore, under DAS system condition, fish in tanks were not affected by the external fertilizer supplementation in hydroponic unit.

3. Morphological characters of vegetative growth

Figs. (2, 3) illustrate the influence of using IF as an external organic fertilizer mineral level in basil biomass (kg/m²), and stem diameter (mm) under both treatments' condition. It is worth mentioning that, DTS+DIF treatment recorded higher basil biomass and stem diameter compared to DTS treatments.

![Fig. 2. The effect of experimental treatments on basil weight](image1)

![Fig. 3. The impact of experiment treatments on the basil stem diameter](image2)

The current study revealed that the growth performance of basil was affected by IF supplementation. After 4 weeks, the harvest fresh biomass in the current study ranged
from 0.82 to 0.984 kg/m². **Rakocy et al. (2004)** reported that the fresh biomass yield of basil production was approximately 1.8 kg/m² in 12 weeks period. This may be due to the high amount of Ca, K, Mg and the other essential micro-nutrients availability in DIF. Moreover, K additions are particularly helpful in encouraging growth in plants bearing fruits and vegetables (**Hager et al., 2021**). Consequently, DIF is rich in macro and micro nutrients, chitin and plant growth regulators which cause faster and better plant growth with higher marketable yield (**Fischer et al., 2021**).

4. Chlorophyll content

Fig. (4) displays the chlorophyll contents in the basil plant leaves. It was noticed that DTS+DIF treatment recorded higher SPAD level than DTS treatment.

![Figure 4. Basil chlorophyll content during the experiment period](image)

The leaf chlorophyll content was higher in DTS+DIF treatment (32 SPAD) than DTS treatment (29.5 SPAD). **Pasch et al. (2021)** reported that the chlorophyll content in the basil plant leaves was 32 SPAD. The higher SPAD in DTS+DIF treatment may be assigned to the supplemented organic fertilizer which increased the nitrogen contents in the basil leaves. **Ruiz-Espinoza et al. (2010)** postulated that the plant's photosynthetic efficiency could increase upon the increase in the nitrogen content in basil.

The magnesium concentration was high in both treatments due to the high magnesium level in the tilapia sludge and insect frass digester. **Hawkesford et al. (2012)** mentioned that the chlorophyll molecule's core atom is magnesium due to its link to the emergence of lower leaf interveinal chlorosis under magnesium deficiency stress.
5. Anatomy of the main stem

DTS+DIF treatment had diameter higher main stem, thickness of fiber strands, phloem tissue and xylem tissue compared to the DTS treatment (16.6, 15.7, 29.3 and 40.65%, ±% to DTS, respectively), as shown in Table (2) and Figs (5, 6). On the contrary, DTS treatment had a higher vessel diameter than DTS+DIF treatment.

Table 2. Measurements in micro-meter (µm) of certain histological features in transverse sections through the lower portion of the main stem of basil plant after 12 weeks

<table>
<thead>
<tr>
<th>Histological character</th>
<th>Treatments</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>DTS</td>
</tr>
<tr>
<td>Stem diameter</td>
<td>3272.3</td>
</tr>
<tr>
<td>Cortex thickness</td>
<td>241</td>
</tr>
<tr>
<td>Fiber strands thickness</td>
<td>70</td>
</tr>
<tr>
<td>Phloem tissue thickness</td>
<td>82</td>
</tr>
<tr>
<td>Xylem tissue thickness</td>
<td>1013</td>
</tr>
<tr>
<td>Vessel diameter</td>
<td>39</td>
</tr>
<tr>
<td>Pith diameter</td>
<td>1305</td>
</tr>
</tbody>
</table>
Fig. 5. Transverse sections through lower portion of the main stem of basil plant (a- DTS treatment & b- DTS+DIF treatment).
The obtained results indicated an increasing in the most stem measurement for DTS+DIF treatment compared to DTS treatment, which may be explained by the high levels and availability of macro and micro-minerals in DTS+DIF solution. It is worth noting that, the macronutrient K is needed by plants for a number of functions including photosynthesis, osmoregulation, enzyme activation, and the production of proteins, carbohydrates and nucleic acids (Demidchik, 2014). It was noticed that, DTS+DIF treatment recorded high level of K which may lead to an increase in the stem diameter (Attia et al., 2022). Liu et al. (2021) reported that basil plants could modify the amount
of produced lignin under availability of K by producing more lignin in certain tissues engaged in conduction (xylem) or support tissues since lignification is critical for the structural integrity of plant cell walls and needed for plant development. Datnoff et al. (2007) elucidated that the increased thickness of the epidermal cell wall caused by K may possibly be the source of the resistance to infections.

**CONCLUSION**

Using the insect frass digestion as an external organic fertilizer may influence the succession of decoupled aquaponic system. Consequently, the insect frass appears to be a promising organic fertilizer for supporting both basil growth and the tilapia welfare. However, further research must be conducted by changing insect frass substrate to achieve a rich and balance insect frass digestion in nutrients for development at a next generation of decoupled aquaponic system.

**ETHICAL STATEMENT**

The Cairo University Institutional Animal Care and Use Committee (CU-IACUC) strictly recommended and approved this work. Aquatic animal care and use of ethics committee (CU-IACUC, Code: Cu- II-F-22-22).

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