Egyptian Journal of Aquatic Biology & Fisheries
Zoology Department, Faculty of Science,
Ain Shams University, Cairo, Egypt.
ISSN 1110 – 6131
Vol. 28(1): 185 – 200 (2024)
www.ejabf.journals.ekb.eg

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

Eslam Fawzy[1] *, Reda Hassanien Emam Hassanien [1], Gamal Mansour [1], Ashraf Suloma[2]

[1]Agricultural Engineering Department, Faculty of Agriculture, Cairo University, Giza, 12613, Egypt
[2]Fish Nutrition Laboratory (FNL), Animal Production Department, Faculty of Agriculture, Cairo University, Giza, 12613, Egypt
***Corresponding Author:** aslamfawzy20@agr.cu.edu.eg

ARTICLE INFO

Article History:
Received: Dec. 18, 2023
Accepted: Jan. 7, 2024
Online: Jan. 17, 2024

Keywords:
Tilapia,
Basil,
Aerobic digestion,
Sludge,
Insect frass,
Sustainable,
Decoupled aquaponic

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/ m^2), DTS+DIF treatment (0.98kg/ m^2) 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 mulit-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 (%) = $W_2 - W_1$ (1)

Where, W1 is the initial weight of the Nile tilapia (g), while W2 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) (**Gichana *et al*., 2019**). (2)

3) Specific growth rate (SGR) (%/day) = $\frac{\ln W_2 - \ln W_1}{T_2 - T_1} \times 100$ (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 **(Kpundeh *et al.*, 2015)**.

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

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

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 (10ml formalin, 5ml glacial acetic acid, and 85ml 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^{o}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 **(Lambers *et al.*,**

2008; APHA, 2017) at the Research Laboratories Complex, Faculty of Agriculture, Cairo University, Giza, Egypt.

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.

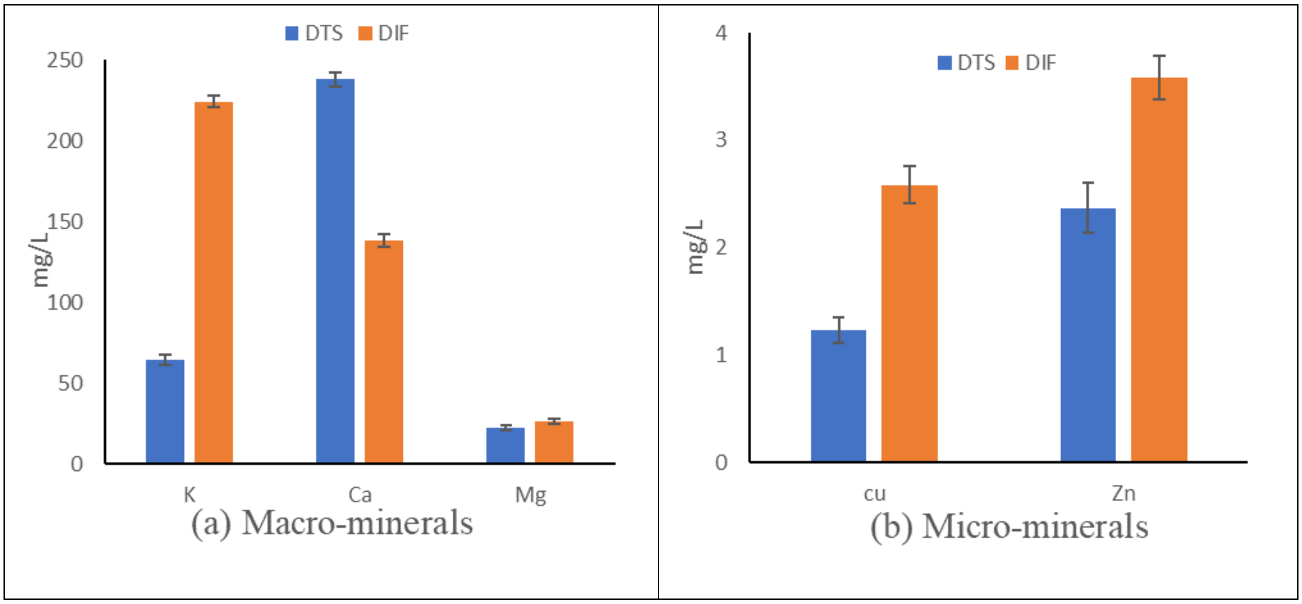

Fig. 1. Macro (a) and micro (b) minerals levels in the tilapia sludge and insect frass digestion

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 ang 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)

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

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/m^2), 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.

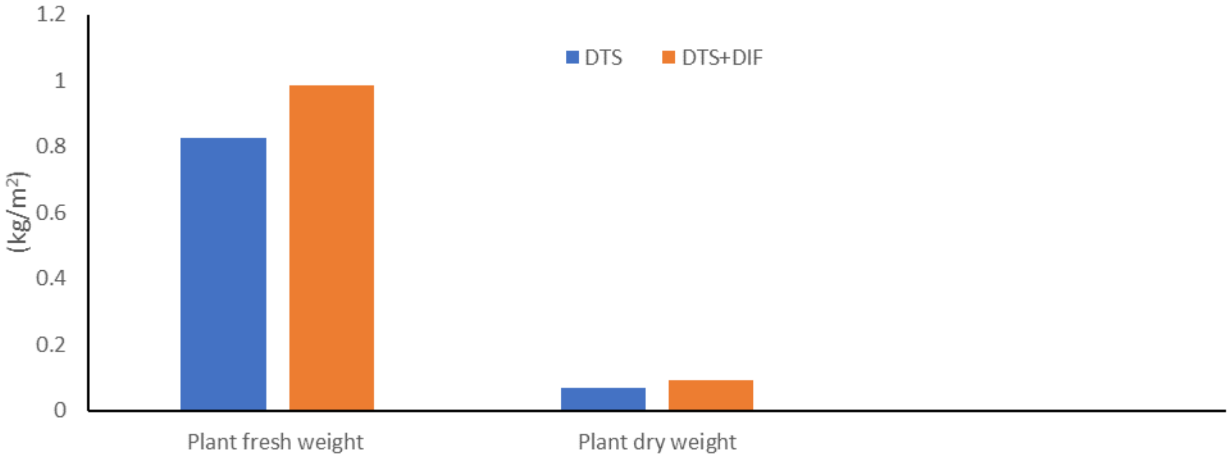

Fig. 2. The effect of experimental treatments on basil weight

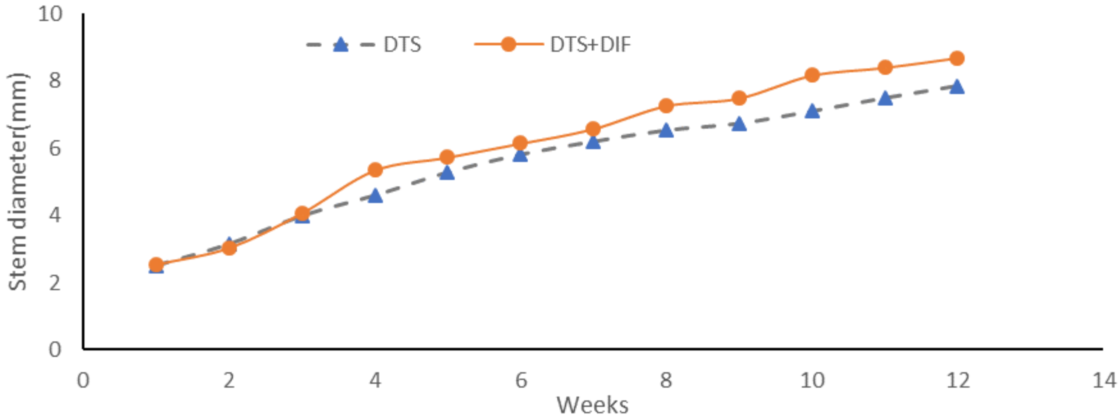

Fig. 3. The impact of experiment treatments on the basil stem diameter

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 0.984kg/ m^2. **Rakocy *et al.* (2004)** reported that the fresh biomass yield of basil production was approximately 1.8 kg/m^2 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.

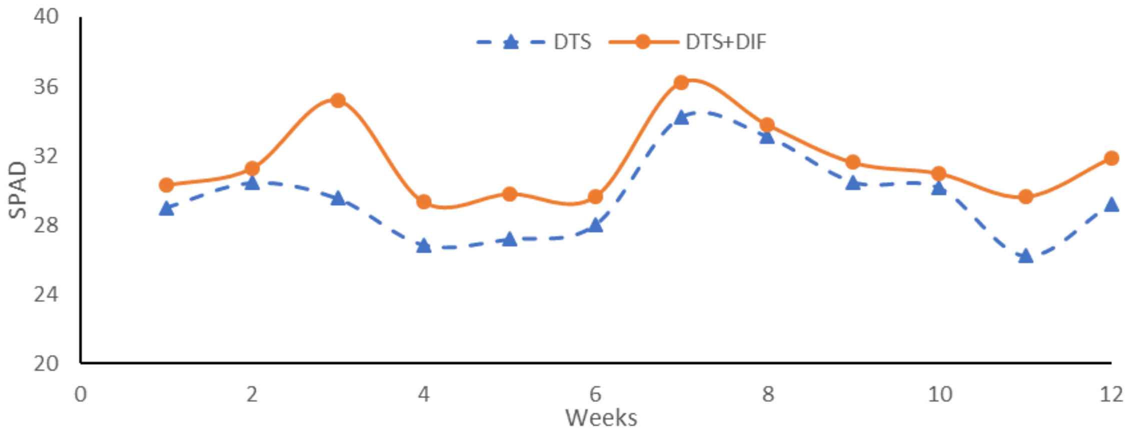

Fig. 4. Basil chlorophyll content during the experiment period

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

Histological character	Treatments		
	DTS	**DTS+DIF**	**±% to DTS**
Stem diameter	3272.3	3818	+16.6
Cortex thickness	241	301.040	+24
Fiber strands thickness	70	81	+15.7
Phloem tissue thickness	82	106.04	+29.3
Xylem tissue thickness	1013	1424.831	+40.65
Vessel diameter	39	31.048	-20.3
Pith diameter	1305	1606.621	+23.1

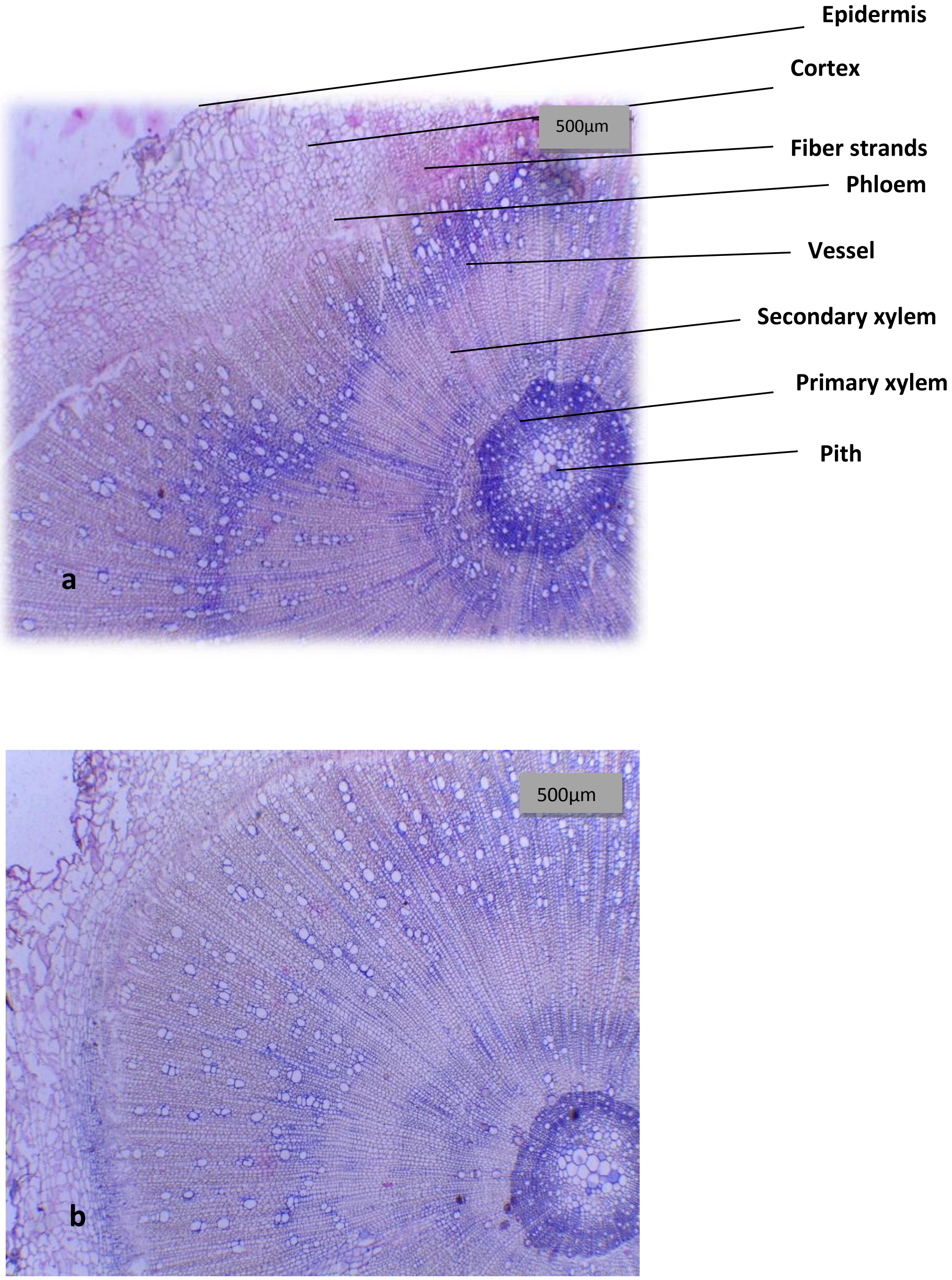

Fig. 5. Transverse sections through lower portion of the main stem of basil plant (**a-** DTS treatment & **b-** DTS+DIF treatment).

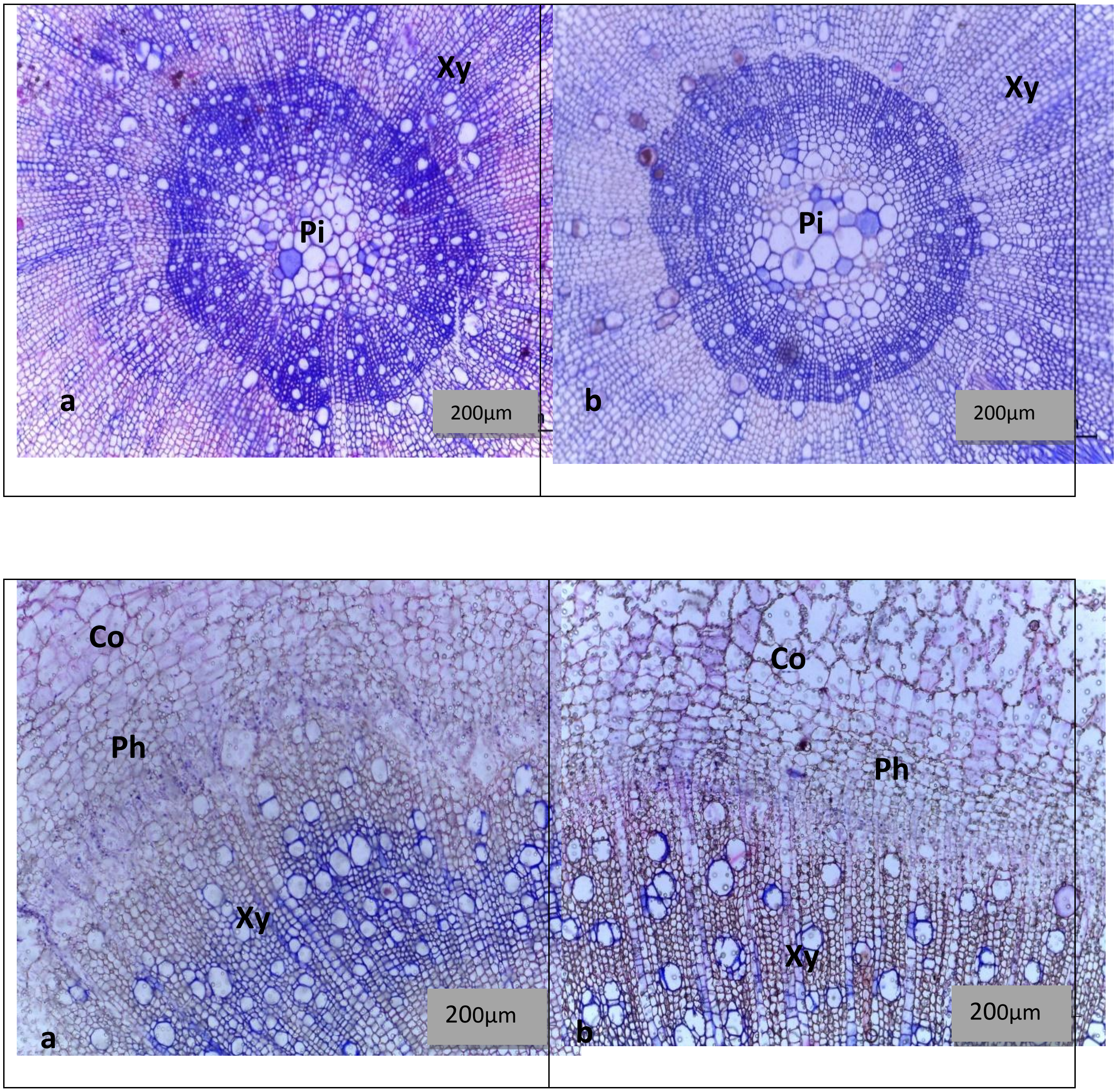

Fig. 6. Transverse sections through lower portion of the main stem of basil plant. **a-** DTS treatment and **b-** DTS+DIF treatment. (Ep: Epidermis, Co: Cortex tissue, Ph: Phloem tissue, Xy: Xylem tissue, Pi: pith tissue)

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).

REFERENCES

Anderson, T.S.; Martini, M.R., De Villiers, D. and Timmons, M.B. (2017). Growth and tissue elemental composition response of Butterhead lettuce (*Lactuca sativa*, *cv. Flandria*) to hydroponic conditions at different pH and alkalinity. Horticulturae, 3(3): 41. https://doi.org/10.3390/horticulturae3030041

Aslanidou, M.; Elvanidi, A.; Mourantian, A.; Levizou, E.; Mente, E. and Katsoulas, N. (2023). Nutrients Use Efficiency in Coupled and Decoupled Aquaponic Systems. Horticulturae, 9(10): 1077. https://doi.org/10.3390/horticulturae910107

Attia, H.; Rebah, F.; Ouhibi, C., Saleh, M.A.; Althobaiti, A.T.; Alamer, K.H.; Ben Nasri, M. and Lachaâl, M. (2022). Effect of Potassium Deficiency on Physiological Responses and Anatomical Structure of Basil, *Ocimum basilicum* L. Biology, 11(11): 1557. https://doi.org/10.3390/biology11111557

Chirere, T.E.S.; Khalil, S and Lalander, C. (2021). Fertiliser effect on Swiss chard of black soldier fly larvae-frass compost made from food waste and faeces. J. Insects as Food and Feed, 7(4): 457–469. https://doi.org/10.3920/JIFF2020.0120

Datnoff, L.E.; Elmer, W.H. and Huber, D.M. (2007). Mineral nutrition and plant disease. American Phytopathological Society (APS Press).

De Verdal, H.; Mekkawy, W.; Lind, C.E.; Vandeputte, M.; Chatain, B. and Benzie, J.A.H. (2017). Measuring individual feed efficiency and its correlations with performance traits in Nile tilapia, *Oreochromis niloticus*. Aquaculture, 468: 489–495. https://doi.org/10.1016/j.aquaculture.2016.11.015

Demidchik, V. (2014). Mechanisms and physiological roles of K+ efflux from root cells. Journal of plant physiology, 171(9): 696–707. . https://doi.org/10.1016/j.jplph.2014.01.015.

Endut, A.; Lananan, F.; Abdul Hamid, S.H.; Jusoh, A. and Wan Nik, W.N. (2016). Balancing of nutrient uptake by water spinach (*Ipomoea aquatica*) and mustard green (*Brassica juncea*) with nutrient production by African catfish (*Clarias gariepinus*) in scaling aquaponic recirculation system. Desalination and Water Treatment, 57(60): 29531–29540.https://doi.org/10.1080/19443994.2016.1184593

Espinosa-Moya, A.; Álvarez-González, A.; Albertos-Alpuche, P.; Guzmán-Mendoza, R. and Martínez-Yáñez, R. (2018). Growth and development of herbaceous plants in aquaponic systems. Acta Universitaria, 28(2): 1–8. https://doi.org/10.15174/au.2018.1387

Faridah, N.; Yoshizaki, G.; Nuryati, S. and Setiawati, M. (2016). Growth, survival, and body composition of transgenic common carp Cyprinus carpio 3rd generation expressing tilapia growth hormone *cDNA. HAYATI*. Journal of biosciences, 23(3): 150-154. https://doi.org/10.1016/j.hjb.2016.12.002

Fischer, H., Romano, N. (2021). Fruit, vegetable, and starch mixtures on the nutritional quality of black soldier fly (*Hermetia illucens*) larvae and resulting frass. Journal of Insects as Food and Feed, 7(3): 319-327.https://doi.org/10.3920/JIFF2020.0100

Fischer, H.; Romano, N.; Jones, J.; Howe, J.; Renukdas, N., and Sinha, A.K. (2021). Comparing water quality/bacterial composition and productivity of largemouth bass Micropterus salmoides juveniles in a recirculating aquaculture system versus aquaponics as well as plant growth/mineral composition with or without media. Aquaculture 538: 736554. https://doi.org/10.1016/j.aquaculture.2021.736554

Gichana, Z.; Liti, D., Wakibia, J.; Ogello, E.; Drexler, S.; Meulenbroek, P. and Waidbacher, H. (2019). Efficiency of pumpkin (*Cucurbita pepo*), sweet wormwood (*Artemisia annua*) and amaranth (*Amaranthus dubius*) in removing nutrients from a smallscale recirculating aquaponic system. Aquaculture International, 27(6): 1767–1786. https://doi.org/10.1007/s10499-019-00442

Goddek, S.; Delaide, B.P.L.; Joyce, A.; Wuertz, S.; Jijakli, M.H.; Gross, A.; Eding, E.H.; Bläser, I.; Reuter, M. and Keizer, L.C.P. (2018). Nutrient mineralization and organic matter reduction performance of RAS-based sludge in sequential UASB-EGSB reactors. Aquacultural engineering, 83: 10-19. https://doi.org/10.1016/j.aquaeng.2018.07.003

Goddek, S.; Espinal, C.A.; Delaide, B.; Jijakli, M.H.; Schmautz, Z.; Wuertz, S. and Keesman, K.J. (2016). Navigating towards decoupled aquaponic systems: A system dynamics design approach. Water, 8(7): 303. https://doi.org/10.3390/w8070303

Hager, J.; Bright, L.A.; Dusci, J. and Tidwell, J. (2021). Aquaponics Production Manual. A Practical Handbook for Growers-Aquaponics Production Manual. Kentucky State University, Kentucky, USA.

Haidar, M.N.; Bleeker, S.; Heinsbroek, L.T.N. and Schrama, J.W. (2018). Effect of constant digestible protein intake and varying digestible energy levels on energy and protein utilization in Nile tilapia. Aquaculture 489: 28–35. https://doi.org/10.1016/j.aquaculture. 2017.12.035

Jang, H.M.; Cho, H.U.; Park, S.K.; Ha, J.H. and Park, J.M. (2014). Influence of thermophilic aerobic digestion as a sludge pre-treatment and solids retention time of mesophilic anaerobic digestion on the methane production, sludge digestion and microbial communities in a sequential digestion process. Water research, 48: 1-14. https://doi.org/10.1016/j.watres.2013.06.041

Khiari, Z.; Kaluthota, S. and Savidov, N. (2019). Aerobic bioconversion of aquaculture solid waste into liquid fertilizer: Effects of bioprocess parameters on kinetics of nitrogen mineralization. Aquaculture 500: 492–499. https://doi.org/10.1016/j.aquaculture .2018.10.059

Klammsteiner, T.; Turan, V.; Fernandez-Delgado Juarez, M.; Oberegger, S. and Insam, H. (2020). Suitability of black soldier fly frass as soil amendment and implication for organic waste hygienization. Agronomy, 10(10): 1578. https://doi.org/10.3390/agronomy10101578

Kpundeh, M.D.; Qiang, J.; He, J.; Yang, H. and Xu, P. (2015). Effects of dietary protein levels on growth performance and haemato-immunological parameters of juvenile genetically improved farmed tilapia (GIFT), *Oreochromis niloticus*. Aquaculture international, 23: 1189-1201. DOI 10.1007/s10499-014-9876-1

Liu, Y.; Yin, Q.; Dai, B.; Wang, K.; Lu, L.; Qaseem, M.F.; Wang, J.; Li, H. and Wu, A.-M. (2021). The key physiology and molecular responses to potassium deficiency in Neolamarckia cadamba. Industrial Crops and Products, 162: 113260. https://doi.org/10.1016/j.indcrop.2021.113260

Moustakas, C.T.; Watanabe, W.O. and Copeland, K.A. (2004). Combined effects of photoperiod and salinity on growth, survival, and osmoregulatory ability of larval southern flounder Paralichthys lethostigma. Aquaculture, 229(1-4): 159-179. https://doi.org/10.1016/S0044-8486(03)00366-1

Nassar, M.A. and El-Sahhar, K.F. (1998). Botanical preparations and microscopy (Microtechnique). Acad. Bookshop, Dokki, Giza, Egypt 219pp.

Pasch, J.; Appelbaum, S.; Palm, H.W. and Knaus,U. (2021). Growth of Basil(Ocimumbasilicum) in Aeroponics, DRF, and Raft Systems with Effluents of AfricanCatfish(*Clariasgariepinus*)inDecoupledAquaponics(s.s.). *AgriEngineering*, *3*(3): 559-574. https://doi.org/10.3390/agriengineering3030036

Rakocy, J.; Masser, M.P.; Losordo, T. and others (2016). Recirculating aquaculture tank production systems: aquaponics-integrating fish and plant culture. Oklahoma Cooperative Extension Service. http://osufacts.okstate.edu

Rakocy, J.; Shultz, R.; Bailey, D.S. and Thoman, E.S. (2003). Aquaponic production of tilapia and basil: comparing a batch and staggered cropping system. South Pacific Soil. In South Pacific Soilless Culture Conference-SPSCC 648 (pp. 63-69). https://doi.org/10.17660/ActaHortic.2004.648.8

Rakocy, J.E.; Bailey, D.S.; Shultz, R.C. and Thoman, E.S. (2004). Update on tilapia and vegetable production in the UVI aquaponic system, in: New Dimensions on Farmed Tilapia: Proceedings of the Sixth International Symposium on Tilapia in Aquaculture, Held September. pp. 12–16.

Rodgers, D.; Won, E.; Timmons, M.B. and Mattson, N. (2022). Complementary Nutrients in Decoupled Aquaponics Enhance Basil Performance. Horticulturae. https://doi.org/10.3390/horticulturae8020111

Romano, N.; Datta, S.N.; Pande, G.S.J.; Sinha, A.K.; Yamamoto, F.Y.; Beck, B.H. and Webster, C.D. (2023). Dietary inclusions of black soldier fly (Hermetia illucens) larvae frass enhanced production of channel catfish (*Ictalurus punctatus*) juveniles, stevia (*Stevia rebaudiana, and lavender* (*Lavaridula angustifolia*) in an aquaponic system. Aquaculture. 575: 739742. https://doi.org/10.1016/j.aquaculture.2023.739742

Romano, N.; Powell, A.; Islam, S.; Fischer, H.; Renukdas, N.; Sinha, A.K. and Francis, S. (2022). Supplementing aquaponics with black soldier fly (*Hermetia illucens*) larvae frass tea: Effects on the production and composition of sweetpotato slips and sweet banana peppers. Aquaculture, 555: 738160. https://doi.org/10.1016/j.aquaculture.2022.738160

Tan, J.K.N.; Lee, J.T.E.; Chiam, Z.; Song, S.; Arora, S.; Tong, Y.W. and Tan, H.T.W. (2021). Applications of food waste-derived black soldier fly larval frass as incorporated compost, side-dress fertilizer and frass-tea drench for soilless cultivation of leafy vegetables in biochar-based growing media. Waste Management, 130: 155-166. https://doi.org/10.1016/j.wasman.2021.05.025

Timmons, M.B. and Ebeling, J.M. (2013). Recirculating aquaculture 3rd ed. Ithaca, NY.

Wang, L. (2022). mealworm frass and vermicompost tea as novel plant-ready nutrients for indoor hydroponics bok choy production system. (Doctoral dissertation, California State Polytechnic University, Pomona)

Zarantoniello, M.; Chemello, G.; Ratti, S.; Pulido-Rodríguez, L.F.; Daniso, E.; Freddi, L.; Salinetti, P.; Nartea, A.; Bruni, L. and Parisi, G. (2023). Growth and welfare status of giant freshwater prawn (*Macrobrachium rosenbergii*) post-larvae reared in aquaponic systems and fed diets including enriched black soldier fly (*Hermetia illucens*) prepupae meal. Animals 13(4): 715. https://doi.org/10.3390/ani13040715

Zhang, H.; Gao, Y.; Shi, H.; Lee, C.T.; Hashim, H.; Zhang, Z.; Wu, W.M. and Li, C. (2020). Recovery of nutrients from fish sludge in an aquaponic system using biological aerated filters with ceramsite plus lignocellulosic material media. Journal of Cleaner Production, 258: 120886. https://doi.org/10.1016/j.jclepro.2020.120886