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RESEARCH ARTICLE

Possibility to use fermented water hyacinth as a substrate supplement by black soldier fly (*Hermetia illucens*)

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Abstract

The black soldier fly is known as an efficient decomposer of various organic wastes and can be incorporated as a food additive into livestock and aquaculture diets. Water hyacinth (WH) is a rapidly spreading invasive plant that poses environmental challenges. Furthermore, WH has been utilized as a nutritional supplement for various animals and may be used as a dietary additive for BSFL. However, its efficacy is still being investigated. To date, no research has looked into the use of fermented WH and its impact on the BSF life cycle. This study aims to evaluate the possibility of using fermented water hyacinth as a rearing substrate for BSFL. The experiment was conducted using five rearing substrates: a control diet, a fermented water hyacinth diet, and three different ratios of mixtures between them. Our results revealed that biochemical components were analyzed statistically by Principal components analysis (PCA) to elucidate a possible correlation between the biochemical parameters of rearing substrate and the biochemical components of black soldier fly prepupae (BSFPP) and black soldier fly pupae (BSFP). Hence, it is needed to find out unconventional diet formulations for BSF rearing that do not compete with human energy sources and protein requirements while being eco-friendly and affordable economically.

Keywords: Black Soldier Fly, Principal component analysis, Waste management, heavy metals, sustainable protein

1. *Introduction*

The black soldier fly (BSF), *Hermetia illucens* (*L.*) (Diptera: Stratiomyidae), is a non-vector disease insect whose larvae are insatiable consumers of a variety of organic material, ranging from agricultural residues to culinary waste, animal remains, and manure (Nguyen *et al.*, 2015). Recently, BSF has a near cosmopolitan distribution in the tropical and temperate regions (Kaya *et al.*, 2021). BSFL mature quickly (approximately 21 days) making them better protein sources than mealworms and crickets (Oonincx *et al.*, 2019). Processed larvae are abundant in protein (41–44%) and lipid (15–49%), making them suitable for animal feed and biodiesel production (Suryati *et al.*, 2023). Furthermore, BSF prepupae are high in nutrients (42% CP, and 35% fat) and can partially substitute soybean meal in livestock, poultry and aquaculture (Barragan-Fonseca *et al.*, 2017); meanwhile, larvae inclusion in diets formulation is dependent on their nutritional composition that is directly related to what they feed on (Cammack and Tomberlin, 2017). While BSFL have been regarded as powerful bioconverters, their diet contains possible harmful substances. These contaminants might involve herbicides, heavy metals, medicines, and microbiological infections. As a result, it is critical to limit these risks and ensure the safety of the finished products obtained from BSF. Water hyacinth (WH) (*Eichhornia crassipes Mart*.) is a perennial, free-floating weed, belonging to Pontederiaceae. It reproduces both asexually and sexually producing seeds, which are fertile for greater than 20 years and hence, can multiply its total mass across 8–10 days (Hailu *et al.*, 2020). WH is well known for heavy metals accumulating property by their root system from contaminated water (Singh and Kalamdhad, 2012). Conventional controlling techniques, comprising mechanical, chemical, and biological controls,

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have displayed efficiency but have some limitations as they are cost and require long time. so we need to recycle WH via using it as an inexpensive source of animal feed (Namasivayam *et al*., 2023). The abundance of heavy metals in the substrate inhibited larval growth, resulting in considerably decreased post-trial larval mass and the conversion rate of feed. Based on these results, the researchers highlighted the necessity of monitoring contaminants, particularly Cd and Pb, in both the substrates and feed containing BSFL is utilized in livestock feed to guarantee feed and food safety across the value chain (Purschke et al., 2017). Small-scale recycling of waste with BSFL has already been attempted with various of organic by-products, including rice straw, grains, foodstuffs, and faecal sludge and manure. The higher the bioconversion process rate, the more efficiently BSFL transform organic matter into valuable products (Siddiqui *et al*., 2022). The bioconversion rate of BSFL refers to the total quantity of biomass (protein and lipids) that may be generated by a given amount of organic waste. BSFL are a source of many useful products, one of these products is frass that can be used as a soil fertilizer or amendment (Klammsteiner *et al*., 2020). Several factors can affect the development rate and end product of BSF inclusion (van Rozen *et al*., 2023). These characteristics include the types of diets adopted., heavy metals contamination, the environmental conditions, and the larval density (Cheng *et al*., 2017). Many investigations indicate that E. crassipes can be used as dietary for animals, particularly ruminants, as an essential feed supplement, thereby reducing its negative environmental effects. It provides sufficient protein, xanthophylls, carotenes, unsaturated fats, carbohydrates, and vital minerals like calcium, phosphorus, and iron (Fanta *et al*., 2024). There were only a few papers describing the meat output and quality attributes of fast-growing broilers feeding on water hyacinth-based diets with variable components and nutrient content globally (Al-Aboudi and Hamodi, 2023). The strategy of this study was to estimate the possibility of BSFL rearing on tested substrates using fermented WH (FWH) as a supplement. This study aims to cultivate BSFL utilizing FWH- incorporated diets as a more sustainable and affordable source than traditional diets helping to assess good nutritional composition of resulting BSF biomass, and evaluate heavy metal bioaccumulation risks.

2. Materials and Methods.

2.1. Insect rearing and colony maintenance

BSF lifecycle include four stages in its growth cycle: egg, larva (with prepupa as the last larval instar), pupa, and adult. BSF eggs were acquired from a commercial insect rearing company in Alexandria, Egypt. Breeding culture of BSF was established and maintained for three successive generations in the Insect Laboratory, Department of Zoology, Faculty of Science, South Valley University, Egypt. In a laboratory incubator, the colony was cultivated at 27 ± 2 °C, 70 ± 5% RH, and 14:10 (L:D). Stock larvae were fed a moisture-retaining diet in enamel pans (30-35 cm in diameter and 8-10 cm deep). According to May (1961), daily monitoring was conducted till all larvae reached the prepupal stage or died. Larval change of integument color from white to black is taken as an indicator for prepupae identification .Pupae were maintained in transparent plastic cups lined with straw litter.

Figure 1: BSF lifecycle stages A: eggs, B: preimaginal stages (larvae, prepupae and pupae), C: adult.

Figure 2: BSF preimaginal stages A: larva, B: prepupa, C: pupa.

2.2. Experimental design

Totally, 2500 starter larvae were used in this experiment, then 500 larvae for each treatment with five replicates, and a set of hundred larvae 5–6 days post hatching were assigned per replicate in transparent plastic container (23 cm × 15 cm × 10 cm) with a net lid kept at a constant temperature of 26 ± 2 °C in a laboratory incubator. The larvae of each replicate were supplied with 10 g of substrate every 48 h, till all larvae had turned into prepupae and thereafter to pupae by monitoring their integument color change. The 5th instar larvae, prepupae and pupae were recognized and collected for further biochemical analysis.

2.3. Water hyacinth fermentation

Water hyacinth whole plants were harvested from the main stream of River Nile at Al-Tramash town, Qena, Egypt. According to El-Sayed (2003), fresh leaves were separated from leaf stalks and roots and were washed under current water many times to eliminate of dirts. They were sliced into tiny pieces (1-2 cm) and were dried till its moisture reduced to about 50% at room temperature. Fermented water hyacinth was processed as the following method: By continual mixing, orthophosphoric acid and 5% sugar cane molasses were added to each kg of water hyacinth. The mixture was stored in an incubator at 35-37°C for 55 days, in plastic, air tight bags. After that, the WH compost was ready to use and stored in plastic bags at 4 °C for further use.

2.4. Rearing substrates

Five experimental larval meals were utilized in this study alongside common dipteran feed (composed of 50% corn flour meal and 50% wheat bran, saturated with water at a portion of 1:1 by volume) as the control meal (T1; C diet). The meals were categorized as following: T2; M 1:1 (C diet 50 % + 50 % fermented water hyacinth), T3; M 1:2 (33.33 % C diet + 66.66 % fermented water hyacinth), T4; M 1:3 (25 % C diet + 75 % fermented water hyacinth), and T5; FWH (100% fermented water hyacinth).

2.5. Biochemical assay

Homogenates (100 mg) of fresh weight from each diet and BSF stages (whole early prepupae and early pupae: each harvested within 9 h) were frozen in liquid nitrogen, crushing to powder with a pestle and mortar and then ice-cold phosphate buffer, pH 7 (10% w/v) was added. Whole homogenates were centrifuged for 20 min, at 15000 rpm (4 °C) and the supernatants have been extracted and kept at –20 °C for the subsequent chemical assays. In this way, for each analyzed parameter, at least four replicates were measured for each experimental treatment. Each replicate was a pool of three individuals randomly selected. Measurements were expressed in mg/mg fresh diet and mg/individual (prepupa or pupa). Total protein, total lipid, and total antioxidants were determined in samples (diets and BSF stages) spectrophotometrically (T60 UV–VIS spectrophotometer) using assay kits (Biodiagnostic and research reagents, Egypt). Total protein, lipid, and antioxidant contents were measured at 550, 545 and 505 nm, respectively. According to Singh and Sinha (1977), total carbohydrate content was assayed using anthrone reagent and glucose as standards. Total free amino acids (FAA) content was estimated spectrophotometrically at 570 nm by ninhydrin method (Rosen, 1957) using glycine as standard.

2.6. Heavy metals determination

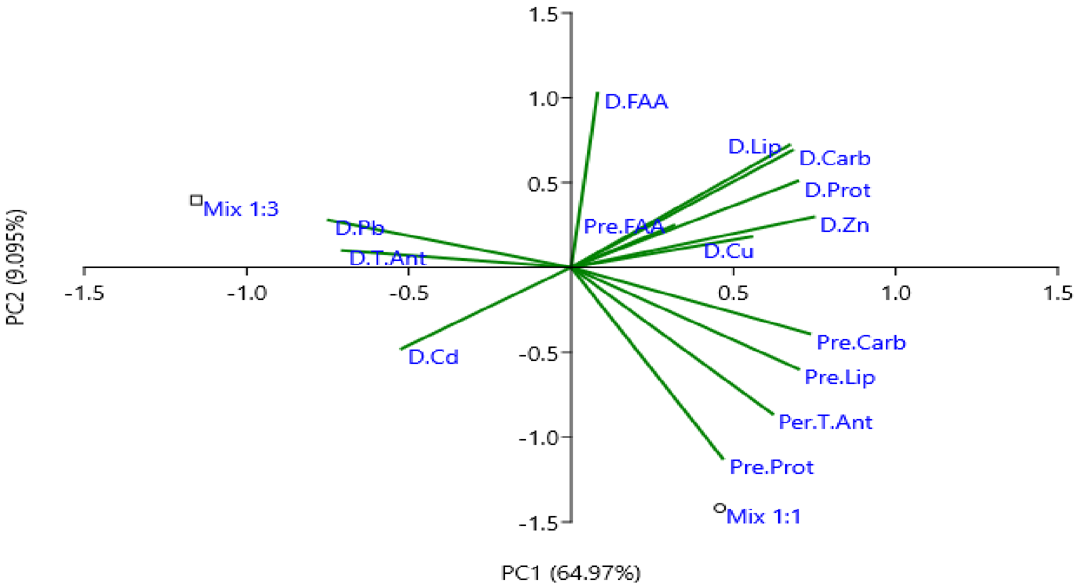
Concentrations of heavy metals (µg/g) were measured in diet samples and BSF pupae by using the Inductively Coupled Plasma Emission Spectrometer (iCAP 6200) at Faculty of Agriculture, Central Lab, Assiut university. According to Jackson (1974), (Cu), (Cd), (Pb), and (Zn) were measured at wavelengths of 228.802, 324.754, 220.353, and 213.856 nm, respectively. The precision of the heavy metals concentration was validated by repeating every sample four times.

2.7. Statistical analysis

SPSS version 26 (IBM Corporation, NY, USA) was used to analyze data (Mean ± SE). Statistical significance among treatments was evaluated by One-way ANOVA analysis then post hoc as Duncan analysis test was done. Repeated ANOVA was used to test a statistically significant difference among stages within each treatment. The association among biochemical parameters in rearing substrates and in BSFL was detected by the principal components analysis (PCA) via the software PAST v.4.07b.

3. Results

The PCA presented in Figure (3A) explained 74.065% of the total observed variance [first axis (PC1): 64.97% and second axis (PC2): 9.095%]. Biplot of PCA (PC1) on prepupal biochemical parameters and diet biochemical parameters revealed a direct correlation between prepupal protein, lipid, carbohydrate, total antioxidant activity, and FAA and diet protein, lipid, carbohydrate, FAA, Cu and Zn. Also, diet total antioxidants are associated with diet Cd and Pb. Contrary, prepupal parameters (protein, lipid, carbohydrate, total antioxidant activity and FAA) presented a negative association with diet total antioxidant activity, Cd and Pb. Investigated diet biochemical parameters (protein, carbohydrate, lipid, total antioxidants, and FAA) showed strong influence on prepupal body composition where D.carb and D.Lip were highly correlated and remarkably close together that hardly overlapped. A dendrogram of hierarchical cluster analysis was able to partition the studied diets into four clusters. The first cluster grouped C diet, the second cluster grouped M1:1 and M 1:3 and the third cluster grouped M 1:2 and the fourth cluster grouped FWH (Fig. 3B). This cluster analysis revealed that mixed diets enhance nutritional composition of prepupal stage than FWH diet which was the worst one.



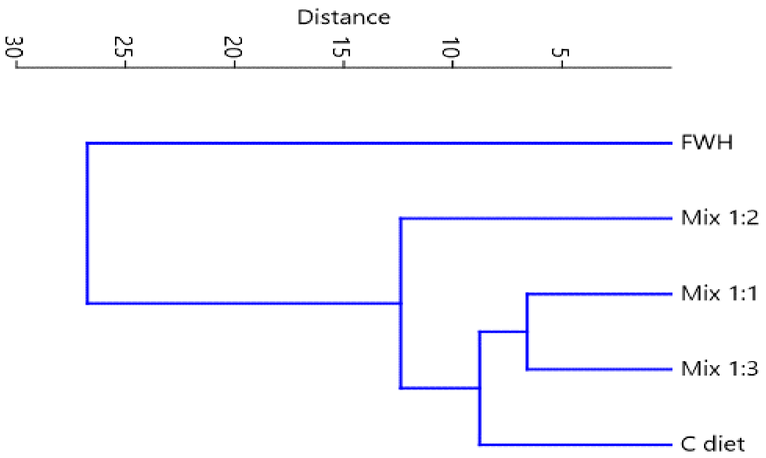


Figure 3: (A) Biplot of PCA on prepupal biochemical parameters and diet biochemical parameters, and (B) a dendrogram of hierarchical cluster analysis of the studied diets. C, Control; Carb., Carbohydrate; Cd, Cadmium ; Cu, Copper; D, diet; FAA, Free amino acids; FWH, Fermented water hyacinth; L, larval; Pb, Lead; Prot., Protein; T.Ant., Total antioxidant activity and Zn, Zinc.

Figure (4A) illustrated the PCA that explained 74.6% of the total observed variance [first axis (PC1; Y axis): 63.22% and second axis (PC2; X axis): 11.38%]. Biplot of PCA (PC1) on pupal biochemical parameters and diet biochemical parameters revealed a direct correlation between pupal nutritional parameters (protein, lipid, carbohydrate, total antioxidant activity, Cu and Zn) and diet nutritional parameters (protein, lipid, carbohydrate, FAA, Cu and Zn). Also, pupal FAA, Cd and Pb are associated with diet total antioxidant, Cd and Pb. Contrary, pupal parameters (protein, lipid, carbohydrate, total antioxidant activity, Cu and Zn) presented a negative association with pupal FAA, diet total antioxidant activity, Cd and Pb. Furthermore, pupal parameters (protein, and total antioxidant) and diet parameters (carbohydrate, and lipid) were grouped together, indicating a strong positive correlation among them. Also, pupal Pb, and diet total antioxidant and Pb were highly correlated. Investigated diet biochemical parameters (protein, carbohydrate, FAA, lipid, Cu and Zn) showed strong influence on pupal body composition. A dendrogram of hierarchical cluster analysis was able to partition the studied diets into four clusters. The first cluster grouped C diet, the second cluster grouped M 1:3 and the third cluster grouped M 1:2 and M 1:1 and FWH the fourth cluster grouped (Fig. 4B). This cluster analysis revealed that mixed diets come between C diet and FWH which mean they are preferable for BSF prepupal rearing than C diet as they low cost and more sustainable and preferable than FWH as they high in their nutritional composition.

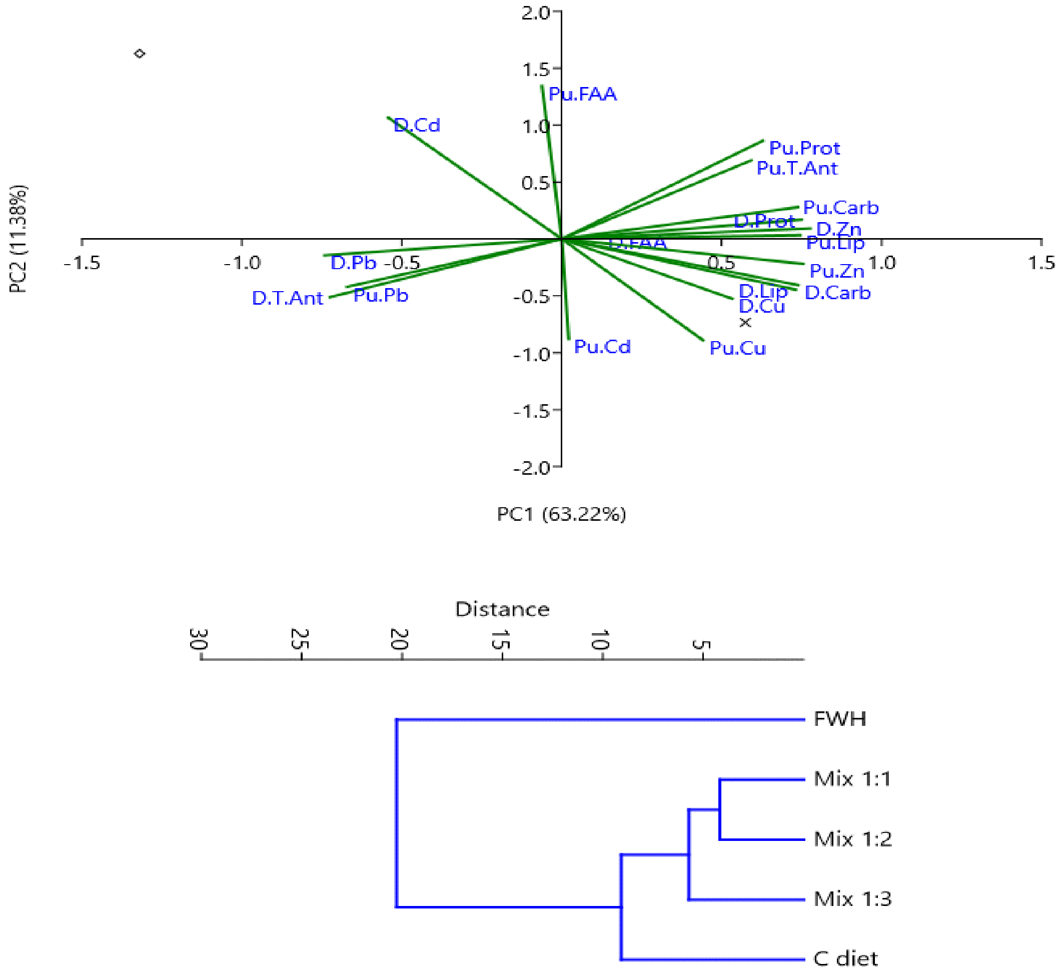


Figure 4: (A) Biplot of PCA on pupal biochemical parameters and diet biochemical parameters, and (B) a dendrogram of hierarchical cluster analysis of the studied diets. C, Control; Carb., Carbohydrate; Cd, Cadmium ; Cu, Copper; D, diet; FAA, Free amino acids; FWH, Fermented water hyacinth; L, larval; Pb, Lead; Prot., Protein; T.Ant., Total antioxidant activity and Zn, Zinc.

4. Discussion

In the present study, our results revealed that BSFL can degrade FWH- incorporated diets with priority to mixed diet than FWH alone this due to water hyacinth has cellulose fibres that can hard degradation but by using fermentation technique helping BSFL to utilize it. Figure (3A and 4A) confirmed a direct relation of nutritional composition of used diet on BSF prepupal and pupal stage growth where where D.carb and D.Lip were highly correlated and remarkably close together that hardly overlapped, this results confirmed by Tomberlin *et al*. (2002) and Gobbi *et al*. (2013) quality and quantity of diet consumed by *Hermetia illucens* larvae had a vital impact on the growth performance, mortality and development time. Also, the same for According to Nguyen et al. (2015) the influence of various rearing substrates (FWH-incorporated substrates) on the dietary requirements of BSF larva. Meneguz *et al*. (2018) observed significant variance in larval development and growth on various substrates such as poultry feed, animal waste, vegetables, and flesh. Variations in growth rate could be explained by the chemical structure of the substrates; however, this was not examined in this experiment because the goal was to elite the most promising substrates for growth. The most ideal substrates from this experiment were designated and will be evaluated again or combined as test substrate to produce BSF larvae in a follow-up experiment.

Contaminated rearing substrates might cause the accumulation of posing chemicals, which is a possible threat to rearing insects. It is vital to consider the potential buildup of heavy metals and poisons when employing insects as animal feed. Consequently, it is critical to report this concern and guarantee the safety of insects utilized for food and feed (Raksasat *et al*., 2020). In our study, determined elements (Cd, Pb, Cu and Zn) were in safe limits and below the initial substrate levels, Cd and Pb were negatively correlated with other nutritional parameters whereas Cu and Zn were positively correlated. The measured heavy metals were in permissible levels for both prepupal and pupal stage. The recommended daily intake of lead and zinc is 20 – 280 μg/day and 15 μg/day, respectively. Significantly higher levels of lead and zinc were recorded in this study. Copper and cadmium were below detection limit. Lead concentration was higher than the other metals.

5. Conclusion

The current study explored the probability of using the bioconversion technology assisted by BSFL to help with the control of water hyacinth through plant treatment via fermentation process. In fact, the mixture at T2; M 1:2 gave the best results for BSF rearing (both prepupae or pupae) on the industrial scale and for the significant decrease. This was due to it provide good nutritional benefits than M1:3 and FWH also, low cost and more sustainable than C diet and M1:1 substrate. At the end of the procedure, larvae produced a valued product. However, further study and innovation are required to guarantee the safety, efficiency, and economic sustainability of BSF-based products for animal and human use.

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Declarations

Author contributions

Conceptualization: [Amer I. Tawfik], [Eman M. Omar]; Methodology: [Eman M. Omar]; Formal analysis and investigation:[Amer I. Tawfik], [Eman M. Omar]; Writing – original draft preparation: [Eman M. Omar]; Writing– review and editing: [Amer I. Tawfik], [Eman M. Omar], [Mohammed Z. Y. Aly], [Khaled S. M. Osman]

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Availability of data and materials

All the data are already presented in the manuscript.

Ethics approval and consent to participate.

All experiments in this study were accepted by the Research Ethics Committee of the Faculty of Science, South Valley University, Qena, Egypt (Code No. 026/11/22).

Consent for publication

Not applicable.

Competing interests

The authors declare no competing interests.

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