American Journal of Agricultural and Biological Sciences

Original Research Paper

Oxidative Stress Response of Black Soldier Flies Fed a Mixed Cake of Coffee and Fruit Waste

Eman Alaaeldin Abdelfattah

Department of Entomology, Faculty of Science, Cairo University, Egypt

Article history
Received: 10-07-2024
Revised: 07-10-2024
Accepted: 11-10-2024

Email: eman.alaaeldein@gmail.com

Abstract: The worldwide elevation in population and consumption has exacerbated the issue of sustainable agricultural waste management, mainly for fruit and coffee waste. The black soldier fly (*Hermetia illucens*) is gaining responsiveness as a valued bioconversion agent for waste management, accomplished by producing multiple derived products. This study investigates the influence of a mixed cake composed of Coffee Grounds (CW) and Fruit Waste (FW) on the oxidative stress levels in fifth-instar black soldier fly larvae. The results showed an elevation pattern in the insect's crude protein and superoxide anion radicals in those fed the FW-CW mixed cake, with a fold increase of +9x and +1.1x, respectively. Lipid peroxidation, a marker of oxidative damage, decreased significantly in the insects fed FW-CW mixed cake and FW only compared to the control group, with reductions of -17 and -14%, respectively. The outcomes will give a clear understanding of the mechanistic actions of *H. illucens* in the recycling of mixed waste streams without causing excessive oxidative stress. This knowledge will have significant implications for BSF-derived industries, such as animal feed, compost, and biodiesel production.

Keywords: Organic Waste, *Hermetia illucens*, Antioxidants, Oxidative Iomarker, Biochemical Assessment, Insects Derived Products

Introduction

As the global population increases and our consumption of natural resources exaggerates, the issue of managing agricultural by-products sustainably becomes progressively critical. Among the many by-products generated every year, fruit waste and coffee waste deserve particular attention. Globally, food waste has a direct effect on food security issues. The Food and Agriculture Organization (FAO) estimates that a staggering 931 million tons, or 17% of all food available for consumption, ends up wasted each year. This waste occurs at various stages, households discard roughly 10%, while food service providers and retailers contribute 5% and 2%, respectively (Kamel and El Bilali, 2022). In Egypt, about 50, 40, 20, and 30 %of vegetables and fruits, fish, coffee waste, and milk and wheat, respectively are wasted every year.

Previous studies have shown that inadequate removal of coffee grounds can cause a serious environmental risk as a result of high tannin and caffeine content (Kotsou *et al*., 2023). The spent Coffee Grounds (CW) are rich in bioactive compounds, particularly polyphenols. Additionally, CW contains various phenolic compounds like neochlorogenic and chlorogenic acids. These compounds have antilipidemic, anticancer, antidiabetic, antiviral, and antipyretic effects. Also, coffee by-products can be used as a main component in poultry feed. This versatility highlights the potential of CW in promoting a circular economy by finding new ways to utilize this abundant waste stream (Franca and Oliveira, 2009; Kaza *et al*., 2018). The inappropriate management of CW can lead to several environmental problems including green gas emissions, and water and soil pollution, which lead to an increase in the costs for waste management and environmental remediation, loss of biodiversity due to polluted water and disrupted ecosystems, reduced soil fertility, impacting agricultural productivity, contribute to climate change and spread of diseases through pest infestations (Chanakya and De Alwis, 2004). Briefly, improper management of coffee waste is a significant environmental concern. By implementing proper disposal and recycling methods, we can minimize these negative impacts.

Previous literature investigated that insects have a key role in environmental management (Abdelfattah *et al*., 2022). One investigated area involves the use of insects as biomarkers. By analyzing the insects' biochemical

makeup, such as stress levels or enzyme activity, research can improve valuable insights into the health of the surrounding environment. The biomarkers act as living sensors, reflecting the impact of pollutants, climate change, and other environmental changes (Renault *et al*., 2016; Abdelfattah, 2022). Also, insects are representative of enormous potential as bioconversion agents (Abdelfattah and El-Bassiony, 2022; Alaaeldin Abdelfattah and Renault, 2023). Their unique biological processes allow them to transform organic waste streams into valuable resources. The larvae of BSF can powerfully convert food scraps and agricultural byproducts into nutrient-rich compost or even insect protein for animal feed. This bioconversion capability offers a sustainable solution to waste management problems while creating valuable products including biodiesel, bioplastic, and organic compost (Mahmoud *et al*., 2022). So, the assessment study of improper management of coffee waste should be done. Additionally, evaluating the impact of coffee waste on the oxidative stress of converter agents is mandated to ensure the quality of products.

Oxidative stress, a circumstance caused by an unevenness between the Reactive Oxygen Species (ROS) concentration and the antioxidant defenses of insects, can have deleterious effects on the health and survival of living organisms (Felton and Summers, 1995; Abdelfattah *et al*., 2022). ROS are highly reactive molecules, such as hydrogen peroxide, superoxide radicals, and hydroxyl radicals, that can cause damage to cellular components, including DNA, proteins, and lipids (Renault *et al*., 2016). Insect biochemical assessments have been employed as valuable biomarkers to monitor oxidative stress levels and evaluate the impact of various environmental factors (Abdelfattah and El-Bassiony, 2022).

This study investigates the influence of mixed coffee and fruit waste on the black soldier fly. Also, this study explores how this coffee waste impacts the fly's oxidative stress levels, a key indicator of its overall health. By understanding the physiological effects of this mixed waste diet, we can gain valuable insights into the suitability of this approach for large-scale black soldier fly rearing and its potential implications for the insect's wellbeing. To achieve these objectives, the biochemical parameters were evaluated in the 5th instar larvae of *H. illucens* as well as the (i) Concentration of ROS (H_2O_2), (ii) Antioxidant amounts (SOD activity and concentration of ascorbic acid), (iii) Non-enzymatic antioxidants amount (DPPH and antioxidant ability) and (iv) Lipid peroxides concentration and (v) Crude protein concentration.

Materials and Methods

Rearing

Black soldier fly (*Hermetia illucens*) adults were propagated in a colony established at the Entomology Department, Faculty of Science, Cairo University, Egypt. These flies were reared in cages (30×30×40 cm) under controlled conditions: 60% Relative Humidity (RH), a 14:10 light-dark cycle (L: D), and a temperature of 35°C. For the experiment, pools of 450 offspring larvae were collected from this colony and placed in separate plastic boxes (15×30×20 cm) under previous controlled conditions, except being kept in complete darkness.

Experimental Design

To achieve the reliability of the research hypothesis, the insect food, including Fruit Waste (FW) and Coffee Waste (CW) was collected from catering services inside the Cairo University Campus and provided to the insects. The fifth developmental larval stage was divided into three groups of 150 each (Fig. 1). The first group acted as a control group, the second one fed on the FW, and the third one fed on the mixed cake of FW and CW, with the ratio of 2:1 respectively, according to the guidance of Mahmoud *et al*., (2022) and matched with the real field applications. All groups were kept in the same conditions of insect rearing as mentioned before. After that, the larval gut tissues were collected at 4°C and stored at -20°C for further analysis as detailed below.

Biomarkers of Oxidative Stress

Hydrogen peroxide (H_2O_2) concentration in tissue samples was measured using a spectrophotometric method based on Junglee *et al*. (2014) using potassium phosphate buffer (pH 7.0), Trichloroacetic Acid (TCA) and Potassium Iodide (KI). The absorbance was measured at 240 nm.

While the lipid peroxidation was done along with the Hermes-Lima *et al*. (1995) methodology. The reaction mixes $FeSO_4$, H_2SO_4, and xylenol orange. After the mixture was in darkness, the wavelength was used at 580 nm once and reused after a 1 h incubation period with 10 µL of 0.5 mM cumene hydroperoxides. The difference in absorbance was calculated.

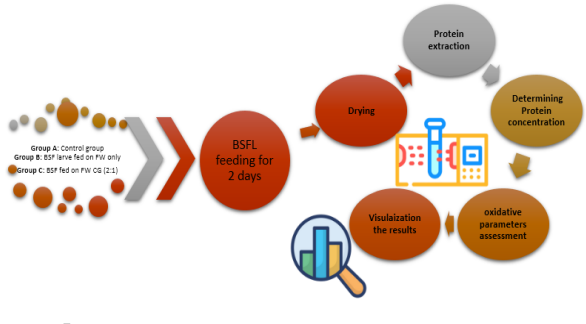


Fig. 1: Experimental design setup

DOI: 10.3844/ajabssp.2024.37.46

Assessment of Antioxidant Capacity

The total antioxidant capacity of experimental tissues was evaluated in the guidance of Prieto *et al*. (1999) method. The reaction containing ammonium molybdate, sulfuric acid and sodium phosphate. After incubation for 90 min at 95°C, the absorbance was assessed at 695 nm.

The antioxidant activity of the experimental samples was measured using the DPPH (α, α-diphenyl-β-picrylhydrazyl) assay, as described by Blois (1958). The wavelength was measured at 525 nm (Contreras-Guzmán and Strong, 1982).

Enzymatic antioxidants as SOD activity was assessed under the guidance of Misra and Fridovich (1972). The reaction included sodium carbonate buffer, EDTA, and epinephrine and was assessed at 480 nm. Protein concentration of samples was assessed using Bradford (1976). All experiments were performed in triplicate for better accuracy.

Statistical Analyses

Statistical analyses were accomplished with Microsoft Excel and SPSS Statistics. The parametric analysis with one-way ANOVA, *Tukey B* Post Hock tests was done, represented as median and standard deviations. Advanced statistical techniques were employed to empathize the effect of coffee waste on the oxidative status of BSF larval.

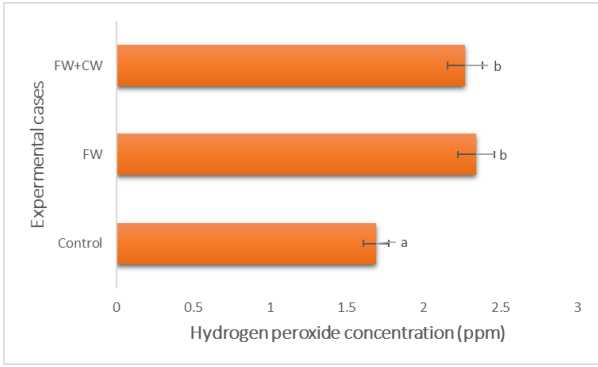
Results

The study emphasizes the influence of various treatments (Control, FW, and FW+CW) on antioxidant and enzyme activity of the 5th instar developmental stages of *H. illucens*. The results disclose interesting patterns across the measured parameters, providing valuable insights into the treatments' influence.

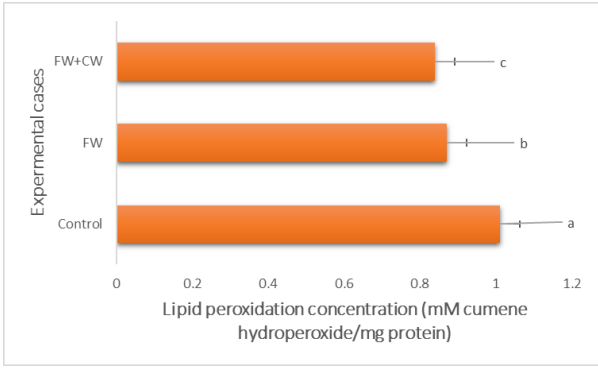
The hydrogen peroxide (H_2O_2) concentration displayed a different trend (Fig. 2A). Here, the FW group showed the highest activity (2.34) compared to control (1.69) and FW+CW (2.27). The significant decrease of the H_2O_2 concentration in the FW and CW mixed cake group is relevant to control one (p-value <0.05). The reference group demonstrated the highest LP activity compared to FW and FW+CW groups (Fig. 2B). The lower LP activity was observed in FW and FW+CW groups with the fold of -0.14 and -0.17-x fold (p-value <0.05).

The total antioxidant capacity was demonstrated of 5th larval insects in Fig. (3A). The results revealed that the group of FW-CW mixed cake had significant increase than FW group. Also, the DPPH (2,2-diphenyl-1-picrylhydrazyl assay results shown that the larvae fed on the mixed cake of FW-CW displayed the highest activity for DPPH with the fold of 1.6-x fold, with respect to control group (Fig. 3B).

The insect fed on a mixed cake of FW and CW exhibited the highest SOD activity compared to FW only group, with respect to the control group (Fig. 3C). Also, the FW+CW group showed a slight increase over FW, the Control treatment maintained the lowest SOD activity. The increased levels in the FW group and FW-CW mixed group were significantly different 0.9 and 1.1-x fold with respect to the control group (p value <0.05).

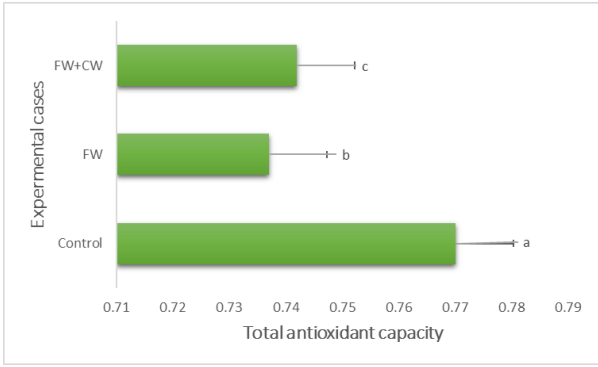


(a)



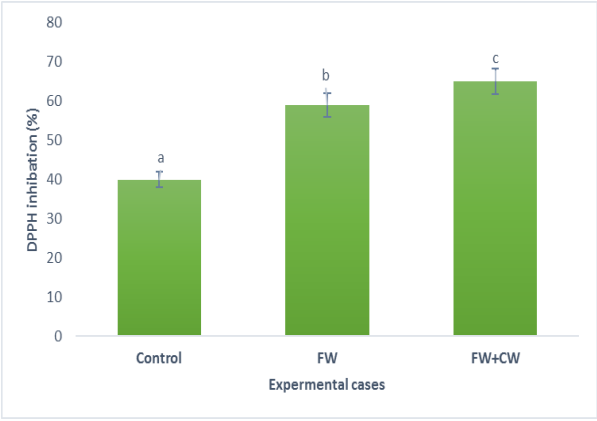
(b)

Fig. 2: Effect of additive addition of coffee waste to fruit waste on the concentration of hydrogen peroxide (H_2O_2); (A) and Lipid Peroxidation (LP); (B) of black soldier fly larvae (*Hermetia illucens*). Median values marked with different letters are significantly different among algae additive addition (one-way ANOVA, Tukey B Post Hock test)

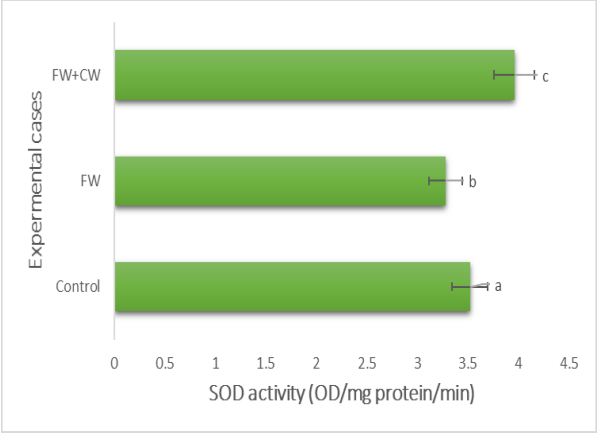


(a)

DOI: 10.3844/ajabssp.2024.37.46



(b)



(c)

Fig. 3: Effect of additive addition of coffee waste to fruit waste on the concentration of total Antioxidant Capacity (AC) (A); α, α-diphenyl-β-picrylhydrazyl (DPPH) (B); and the activity of Superoxide Dismutase (SOD) (C) of black soldier fly larvae (*Hermetia illucens*). Median

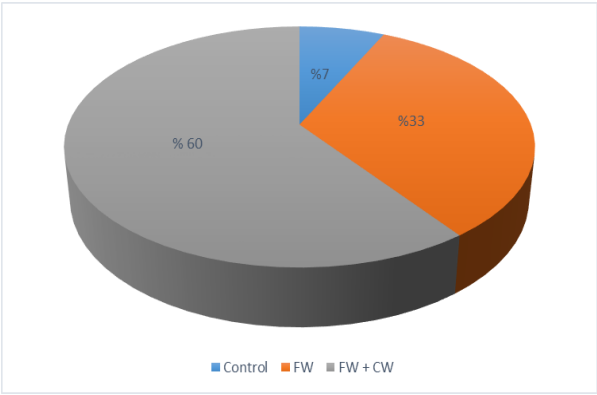


Fig. 4: Pie chart shows a comparison of protein production yield (%) from black soldier fly larvae (*Hermetia illucens*) in three feeding scenarios, control group, fruit waste, and fruit-coffee mixed cake

The analysis also exposed variations in crude protein content across the treatments. FW displayed the highest content compared to the control group (Fig. 4). Overall, this study provides a valuable snapshot of how different treatments can modulate antioxidant and enzyme activity within a sample. The results suggest that FW-CW treatment might offer enhanced protection against oxidative stress by elevating H_2O_2 breakdown and free radical scavenging capacity, while potentially mitigating lipid peroxidation.

Advanced statistical analysis was done to ensure the understanding of the effect of the addition of CW to FW on the oxidative parameters of bioconverter and biomarker insects. Cluster analysis with Dendrogram visualization was analyzed in Fig. (5) as sections of oxidants and oxidative damage parameters for BSFL fed on a control diet, fruit waste only, and mixed FW-CW, respectively. The results showed that the control group has a long arm and so different than FW only mixed cake FW-CW group. The general Linear Model (GLM) is shown in Table (1). This statistical approach likely served to identify significant factors affecting the larval oxidative status. The GLM could account for the effects of algae addition and other potential variables, helping to isolate the specific contribution of algae to protein quality. The results revealed that there was a significant difference between the three feeding scenarios for all oxidative stress parameters and crude protein concentration, except for the corrected model and F-value for total antioxidant capacity and superoxide dismutase activity. Artificial Neural Networks (ANNs) were demonstrated in Fig. (6). This powerful computational tool may have been used to model the complex relationships between various factors, such as coffee waste inclusion level, oxidative stress parameter markers, and environmental factors. Radar analysis was demonstrated in (Fig. 7). This method likely provided a visual representation of multiple variables related to the oxidative parameters of the larval stage. By comparing the radar plots for larvae fed with and without coffee, the results demonstrated that the addition of coffee into the BSFL diet has a significant increase in the level of antioxidants levels.

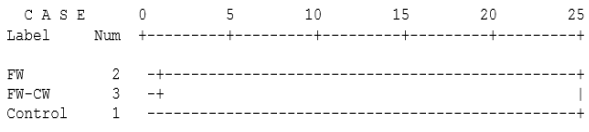


Fig. 5: Dendrogram of the cluster analysis (using Ward's Method) applied for oxidative stress parameters of *Hermetia illucens* larval as a result of three feeding scenarios, control group, fruit waste, and fruit-coffee mixed cake

DOI: 10.3844/ajabssp.2024.37.46

Discussion

This study investigated the influence of various fed stuff (Control, FW, and FW+CW) on biochemical analysis including the non-enzymatic antioxidants and antioxidant enzymes activity within fifth-instar *H. illucens* larvae. The findings of the study conclude that the mixed cake of coffee grounds and fruit waste can have significant consequences for insect health in the form of oxidative response (Bi and Felton, 1995; Felton and Summers, 1995). The changes in the balance between the antioxidants and oxidants can lead to oxidative damage (Renault *et al*., 2016; Abdelfattah and El-Bassiony, 2022). Disproportionate oxidative damage can lead to a range of harmful effects in insects (Chmiel *et al*., 2020; Foster *et al*., 2024). By mitigating oxidative stress, the mixed waste diet used in this study may contribute to the overall health and resilience of black soldier fly larvae.

The mixing between coffee and fruit waste can serve as a feed source for BSF larvae and offer several sustainability benefits including waste reduction by diverting organic waste from landfills (Da Silva and Hesselberg, 2020). Also, the mixed waste contains valuable nutrients that can be assimilated by the larvae, promoting nutrient cycling and reducing the need for external inputs (Čičková *et al*., 2015). Besides that, insect farming, including BSF rearing, can contribute to carbon sequestration by converting organic matter into valuable products (Liu *et al*., 2022; Mahmoud *et al*., 2022).

Oxidative Damage and Reactive Oxygen Species Concentration

The observed patterns across measured parameters, particularly hydrogen peroxide (H_2O_2) concentration and Lipid Peroxidation (LP) activity, offer valuable insights into the treatments' impact on the flies' oxidative stress response. Briefly, the FW group displayed the highest H_2O_2 activity matched with the Control and FW+CW groups. This finding might be linked to the breakdown pathways within the FW treatment. Increased H_2O_2 production could be a consequence of enhanced metabolic activity or specific enzymatic processes stimulated by the FW components (Kaur *et al*., 2014; El-Zohri *et al*., 2020).

Also, the results showed significantly lower LP activity observed in both FW and FW+CW groups compared to the Control. Lipid peroxidation is a marker of oxidative damage within cells. Its reduction in the FW and FW+CW groups suggests a potential protective effect against cellular damage in the larvae fed these treatments. This observation aligns with the concept that the FW and FW+CW treatments might be promoting the larvae's antioxidant defenses, potentially mitigating the detrimental effects of free radicals. The Control group exhibited the highest initial LP concentration, while FW displayed the highest H_2O_2 activity. These apparently opposing findings highlight the complex interplay between various antioxidant pathways and the potential for compensatory mechanisms (Paes *et al*., 2001; Bednářová *et al*., 2013).

Enzymatic and Non-Enzymatic Amount

Previous literature has established that insects can exhibit varying responses to different dietary components. These variations can significantly influence the larvae's physiology and susceptibility to oxidative stress (Abdelfattah *et al*., 2022). The current study aligns with these findings by demonstrating an elevation in the total antioxidant capacity and free radical scavenging capacity of larvae fed the FW-CW mixed cake in accordance with the FW and control one (Fig. 3A-B). This observation suggests that the mixed diet potentially enhanced the larvae's antioxidant defense mechanisms, making them better well-found to decontaminating oxidative stress. Previous research suggested that certain dietary components, potentially present in the FW-CW mixture, can scavenge the free radicals in insects (Alaaeldin Abdelfattah and Renault, 2023). For instance, studies have shown that bioactive compounds like phenolic acids and flavonoids, commonly found in fruits, can act as antioxidants and enhance the host's antioxidant defense system (Renault *et al*., 2016). Coffee grounds, on the other hand, might contribute beneficial antioxidants like chlorogenic acid (Janissen and Huynh, 2018; Hue *et al*., 2020; Kotsou *et al*., 2023).

This study examined the patterns of Superoxide Dismutase (SOD) activity in response to different treatments (Control, FW and FW+CW). The findings suggested a complex interplay between treatments and the larvae's antioxidant response. The results showed the highest SOD activity in the control group aligns with previous studies suggesting that organisms possess inherent antioxidant defense mechanisms (Dixit *et al*., 2017). This base level of SOD activity might be critical for maintaining cellular homeostasis and mitigating oxidative stress under normal conditions. The SOD elevation activity detected in the FW and FW+CW groups compared to the Control is particularly interesting (Fig. 3C). This finding suggests that the treatments might have activated an upregulation of the larvae's antioxidant defense system. This could be a response to potential stressors present in the FW or FW+CW diets, such as free radicals produced during the interruption of these waste materials (Priftis *et al*., 2018). Also, the FW group displayed SOD activity elevation compared to the control one, the FW+CW group showed a slightly higher elevation. This observation proposes that the mixture of FW and CW in the mixed diet might have exerted a stronger stimulatory effect on SOD activity compared to FW alone (Alaaeldin Abdelfattah and Renault, 2023).

Crude Protein Concentration

The observed increase in crude protein content (Fig. 4), particularly the FW group with respect to control, is reliable with existing literature. Studies have reported that fruit waste is considered rich in protein and other essential element sources for insects (Jucker *et al*., 2017; Vuong *et al*., 2017). This aligns with our observations and suggests that FW could serve as a valuable protein supplement for insect diets (Alaaeldin Abdelfattah and Renault, 2023).

The study by Kotsou *et al.* (2023) recommended that using Spent Coffee Grounds (SCG) can act as a sustainable feed additive for mealworm larvae (*Tenebrio molitor*). The results showed that larvae-fed diets with increasing SCG content (10 and 25%) showed significant improvements in their nutritional profile. Crude protein content increased by a substantial 45.26%, while vitamins A and C content skyrocketed by over 800 and 80%, respectively. Also, the larvae also showed a rise in polyphenols, known for their antioxidant properties, with the 25% SCG diet yielding the highest increase. Besides that, the oil extracted from larvae fed SCG diets exhibited enhanced nutritional value and better resistance to oxidation. Overall, this research suggests that SCG can be a valuable and sustainable feed additive for mealworms, significantly boosting their nutritional content and potentially offering health benefits through increased antioxidant properties (Kotsou *et al*., 2023).

The BSF is a favorable insect for integrated waste management and protein production. These flies don't bite or transmit disease and their larvae can efficiently break down organic waste. BSF larvae themselves are a nutritious protein source for animals and the process of raising them can create jobs and reduce reliance on land for traditional feed production. Studies have shown that BSFL can thrive on various waste materials, like manure and maize straw, while also altering the composition of the waste product. Research is ongoing to better understand the nutritional content of BSFL and how factors like diet and development stage affect their protein and fat profiles. Large-scale studies are also investigating optimal conditions for BSFL waste processing, including factors like manure type and larval density (Abdelfattah *et al*., 2022; Siddiqui *et al*., 2024).

The Cruz *et al*. (2014) study examined the impact of adding spent espresso coffee grounds to lettuce during cultivation. They found that fresh coffee grounds, in increasing amounts, significantly boosted the lettuce's overall antioxidant capacity and specific antioxidant compounds like carotenoids, particularly violaxanthin. Additionally, chlorophyll content effectively distinguished the coffee-treated lettuce from the control group, while vitamin E levels remained unchanged. These findings suggest that fresh coffee acts as a valued cradle of valuable bioactive complexes, demonstrably enhancing the overall antioxidant profile and quality of the cultivated lettuce.

In conclusion, this study demonstrates that FW and FW+CW treatments can modulate antioxidant activity and oxidative stress markers. The detected elevation in crude protein content and the potential for enhanced protection against oxidative stress (Hutabarat and Mangindaan 2024; Siddiqui *et al*., 2024).

Advanced Statistical Analysis

Cluster analysis and Dendrogram visualization (Fig. 5) effectively segregated the three dietary groups (control, FW, and FW-CW) based on their oxidative stress profiles. The distinct clustering of the control group highlights the significant differences in oxidative parameters compared to larvae-fed waste-based diets (FW and FW-CW).

General Linear Model (GLM) analysis (Table 1) served as a robust statistical tool to identify factors influencing larval oxidative status. The significant differences observed across all oxidative stress markers and crude protein concentration between the three feeding scenarios solidify the impact of diet composition. However, the lack of significant differences in the corrected model and F-value for Total Antioxidant Capacity (TAC) and Superoxide Dismutase (SOD) activity warrants further investigation. This might indicate a more complex interplay between diet and these specific antioxidant mechanisms.

Artificial Neural Networks (ANNs) (Fig. 6), a powerful computational approach, were likely employed to model the intricate relationships between various factors. This could include the level of coffee waste inclusion, oxidative stress markers, and potentially environmental factors. By building a comprehensive model, ANNs can provide valuable insights into how these factors interact and influence the overall oxidative status of the larvae.

Radar analysis (Fig. 7) served as a valuable visual tool to represent the multifaceted nature of oxidative stress parameters. By comparing the radar plots of larvae fed with and without coffee waste, a clear increase in antioxidant levels is evident. This visually reinforces the findings of elevated free radical scavenging capacity observed in the FW-CW group.

Briefly, the multi-pronged statistical approach adopted in this study provides a comprehensive understanding of how incorporating coffee waste into the BSFL diet modulates the larvae's oxidative status. The observed increase in protein content, enhanced antioxidant activity, and reduced lipid peroxidation suggest a promising avenue for sustainable waste management while promoting insect production. Future research should delve deeper into the underlying mechanisms responsible for the non-significant effects on TAC and SOD activity, further solidifying the applicability of coffee waste as a viable BSFL-rearing substrate.

DOI: 10.3844/ajabssp.2024.37.46

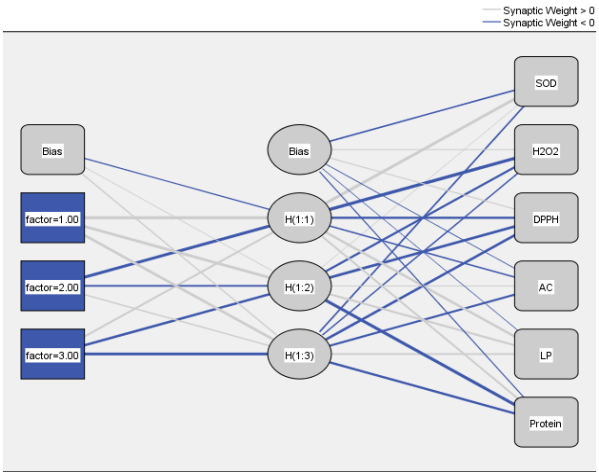


Fig. 6: An Artificial Neural Network with three layers, three inputs, three bias terms, and six outputs applied for oxidative stress parameters of *Hermetia illucens* larval as a result of three feeding scenarios, control group, fruit waste, and fruit-coffee mixed cake

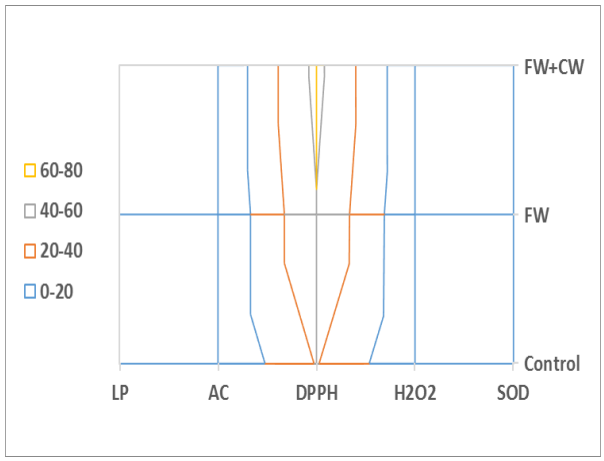


Fig. 7: Radar analysis applied for oxidative stress parameters of *Hermetia illucens* larval as a result of three feeding scenarios, control group, fruit waste, and fruit-coffee mixed cake

Table 1: General Linear Model to analyze the corrected model, intercept, treatment of addition of coffee waste to fruit waste for feeding *Hermetia illucens* larval on the oxidative stress parameters and protein concentration of the BSFL

Parameters	Source	Type III sum of squares	df	Mean square	F	p-value
H_2O_2	Corrected model	2.05	2	1.02	25	<0.001
	Intercept	42	1	42	1066	<0.001
	F	2.05	2	1.02	25	<0.001
LP	Corrected model	0.04	2	0.023	129	<0.001
	Intercept	7.27	1	7.27	40905	<0.001
	F	0.04	2	0.02	129	<0.001
AC	Corrected model	0.001	2	0.001	1.01	>0.05
	Intercept	5	1	5	8617	<0.001
	F	0.001	2	0.001	1.01	>0.05
DPPA	Corrected model	972	2	486	875	<0.001
	Intercept	26569	1	26569	47824	<0.001
	F	972	2	486	875	<0.001
SOD	Corrected model	0.10	2	0.05	1.15	>0.05
	Intercept	106	1	106	2463	<0.001
	F	0.10	2	0.05	1.15	>0.05
CP	Corrected model	4.45	2	2.22	247	<0.001
	Intercept	10.10	1	10.01	1122	<0.001
	F	4.45	2	2.23	247	<0.001

Conclusion

This study investigated the impact of various treatments (Control, FW, and FW+CW) on the non-enzymatic antioxidants and antioxidant enzymes activity of 5th instar *H. illucens* larvae. The results highlight the optimistic effects of incorporating coffee waste into the BSF diet, indicating its potential to increase the inclusive health and resilience of black soldier fly larvae. This research contributes to a better understanding of the nutritional and physiological benefits of using mixed waste streams as a sustainable

feed source for insect rearing. Consequently, the production of insect-derived products, such as biodiesel, animal feed and compost, can be enhanced.

Acknowledgment

The author gratefully acknowledges the dedicated work of the "Industrial Insect Technology Approaches" research group.

Funding Information

The present study was funded by Cairo University (projects and research grants) and Academy of research and technology, ASRT (projects grants) for "Industrial Entomology approaches projects and Industrial biomass technology" projects.

Ethics

The author applied the best practices for insect rearing and handling, ensuring optimal environmental conditions and minimizing any potential distress. No specific ethical approvals were required for this study.

References

Abdelfattah, E. A. (2022). Effect of vitamin B12 on the oxidative stress of Sitophilus oryzae under different time course. In *African Journal of Biological Sciences* (Vol. 4, Issue 1, pp. 82–93).

Abdelfattah, E. A., & El-Bassiony, G. M. (2022). Impact of malathion toxicity on the oxidative stress parameters of the black soldier fly *Hermetia illucens* (Linnaeus, 1758) (Diptera: Stratiomyidae). In *Scientific Reports* (Vol. 12, Issue 1, p. 4583). https://doi.org/10.1038/s41598-022-08564-8

Abdelfattah, E. A., Bassiony, D., Nagah, A., Fawzy, M. A., Hussein, M. Y., Ibrahim, H. M., Ibrahim, N. Y., Ashry, H., Aboelhassan, A. M., Mahmoud, A. H., & Abd El-Monem, D. H. (2022). Toxicity Effect of Cu2+ Contaminated Kitchen and Vegetable Wastes on Oxidative Stress Response of Black Soldier Fly Larvae, *Hermetia Illucens*. In *Journal of Biotechnology Research* (Issue 81, pp. 7–25). https://doi.org/10.32861/jbr.81.7.25

Alaaeldin Abdelfattah, E., & Renault, D. (2023). Does the presence of heavy metal and catechol contaminants in organic waste challenge the physiological performance of the bioconverter *Hermetia illucens*? In *Journal of Insect Physiology* (Vol. 144, p. 104469). https://doi.org/10.1016/j.jinsphys.2022.104469

Bednářová, A., Krishnan, N., Cheng, I., Večeřa, J., Lee, H., & Kodrík, D. (2013). Adipokinetic hormone counteracts oxidative stress elicited in insects by hydrogen peroxide: *in vivo* and *in vitro* study. *Physiological Entomology*, *38*(1), 54–62. https://doi.org/10.1111/phen.12008

Bi, J. L., & Felton, G. W. (1995). Foliar oxidative stress and insect herbivory: Primary compounds, secondary metabolites and reactive oxygen species as components of induced resistance. *Journal of Chemical Ecology*, *21*(10), 1511–1530. https://doi.org/10.1007/bf02035149

Blois, M. S. (1958). Antioxidant Determinations by the Use of a Stable Free Radical. *Nature*, *181*(4617), 1199–1200. https://doi.org/10.1038/1811199a0

Bradford, M. (1976). A Rapid and Sensitive Method for the Quantitation of Microgram Quantities of Protein Utilizing the Principle of Protein-Dye Binding. In *Analytical Biochemistry* (Vol. 72, Issues 1–2, pp. 248–254). https://doi.org/10.1006/abio.1976.9999

Chanakya, H. N., & De Alwis, A. A. P. (2004). Environmental Issues and Management in Primary Coffee Processing. *Process Safety and Environmental Protection*, *82*(4), 291–300. https://doi.org/10.1205/095758204323162319

Chmiel, J. A., Daisley, B. A., Pitek, A. P., Thompson, G. J., & Reid, G. (2020). Understanding the Effects of Sublethal Pesticide Exposure on Honey Bees: A Role for Probiotics as Mediators of Environmental Stress. *Frontiers in Ecology and Evolution*, *8*, 22. https://doi.org/10.3389/fevo.2020.00022

Čičková, H., Newton, G. L., Lacy, R. C., & Kozánek, M. (2015). The use of fly larvae for organic waste treatment. *Waste Management*, *35*, 68–80. https://doi.org/10.1016/j.wasman.2014.09.026

Contreras-Guzmán, E. S., & Strong III, F. C. (1982). Determination of tocopherols (vitamin E) by reduction of cupric ion. *Journal of the Association of Official Analytical Chemists*, *65*(5), 1215-1221.

Cruz, R., Gomes, T., Ferreira, A., Mendes, E., Baptista, P., Cunha, S., Pereira, J. A., Ramalhosa, E., & Casal, S. (2014). Antioxidant activity and bioactive compounds of lettuce improved by espresso coffee residues. *Food Chemistry*, *145*, 95–101. https://doi.org/10.1016/j.foodchem.2013.08.038

Da Silva, G. D. P., & Hesselberg, T. (2020). A Review of the Use of Black Soldier Fly Larvae, *Hermetia illucens* (Diptera: Stratiomyidae), to Compost Organic Waste in Tropical Regions. *Neotropical Entomology*, *49*(2), 151–162. https://doi.org/10.1007/s13744-019-00719-z

Dixit, G., Praveen, A., Tripathi, T., Yadav, V. K., & Verma, P. C. (2017). Herbivore-responsive cotton phenolics and their impact on insect performance and biochemistry. *Journal of Asia-Pacific Entomology*, *20*(2), 341–351. https://doi.org/10.1016/j.aspen.2017.02.002

El-Zohri, M., O. Bafeel, S., & Al-Zahrani, W. (2020). Differential oxidative and biochemical responses of tomato and maize leaves to Spodoptera exigua herbivory. *Pakistan Journal of Botany*, *52*(4). https://doi.org/10.30848/pjb2020-4(23)

Felton, G. W., & Summers, C. B. (1995). Antioxidant systems in insects. *Archives of Insect Biochemistry and Physiology*, *29*(2), 187–197. https://doi.org/10.1002/arch.940290208

Foster, L. J., Tsvetkov, N., & McAfee, A. (2024). Mechanisms of Pathogen and Pesticide Resistance in Honey Bees. *Physiology*, *39*(4), 193–207. https://doi.org/10.1152/physiol.00033.2023

Franca, A. S., & Oliveira, L. S. (2009). Chapter 8: Coffee processing solid wastes: current uses and future perspectives. *Agricultural Wastes*, *9*, 155–189.

Hermes-Lima, M., Willmore, W. G., & Storey, K. B. (1995). Quantification of lipid peroxidation in tissue extracts based on Fe(III)xylenol orange complex formation. *Free Radical Biology and Medicine*, *19*(3), 271–280. https://doi.org/10.1016/0891-5849(95)00020-x

Hue, H. T., Tinh, H. T., Van Bao, N., & Dao, P. T. A. (2020). Screening for antioxidant activity of vegetable and fruit by-products and evaluating the ability of coffee sediment to preserve fish meal. *SN Applied Sciences*, *2*(7), 1282. https://doi.org/10.1007/s42452-020-3030-7

Hutabarat, D. J. C., & Mangindaan, D. (2024). Cultivation of Black Soldier Fly (*Hermetia illucens*) Larvae for the Valorization of Spent Coffee Ground: A Systematic Review and Bibliometric Study. *Agriculture*, *14*(2), 205. https://doi.org/10.3390/agriculture14020205

Janissen, B., & Huynh, T. (2018). Chemical composition and value-adding applications of coffee industry by-products: A review. *Resources, Conservation and Recycling*, *128*, 110–117. https://doi.org/10.1016/j.resconrec.2017.10.001

Jucker, C., Erba, D., Leonardi, M. G., Lupi, D., & Savoldelli, S. (2017). Assessment of Vegetable and Fruit Substrates as Potential Rearing Media for *Hermetia illucens* (Diptera: Stratiomyidae) Larvae. *Environmental Entomology*, *46*(6), 1415–1423. https://doi.org/10.1093/ee/nvx154

Junglee, S., Urban, L., Sallanon, H., & Lopez-Lauri, F. (2014). Optimized Assay for Hydrogen Peroxide Determination in Plant Tissue Using Potassium Iodide. *American Journal of Analytical Chemistry*, *05*(11), 730–736. https://doi.org/10.4236/ajac.2014.511081

Kamel, I. M., & El Bilali, H. (2022). Agri-Food Sustainability and Food Security in Egypt. *Sustainable Agriculture and Food Security*, 443–458. https://doi.org/10.1007/978-3-030-98617-9_25

Kaur, R., Gupta, A. K., & Taggar, G. K. (2014). Role of catalase, H2O2 and phenolics in resistance of pigeonpea towards Helicoverpa armigera (Hubner). *Acta Physiologiae Plantarum*, *36*(6), 1513–1527. https://doi.org/10.1007/s11738-014-1528-6

Kaza, S., Yao, L. C., Bhada-Tata, P., & Van Woerden, F. (2018). *What a Waste 2.0: A Global Snapshot of Solid Waste Management to 2050*. https://doi.org/10.1596/978-1-4648-1329-0

Kotsou, K., Chatzimitakos, T., Athanasiadis, V., Bozinou, E., Athanassiou, C. G., & Lalas, S. I. (2023). Utilization of Spent Coffee Grounds as a Feed Additive for Enhancing the Nutritional Value of Tenebrio molitor Larvae. *Sustainability*, *15*(23), 16224. https://doi.org/10.3390/su152316224

Liu, T., Klammsteiner, T., Dregulo, A. M., Kumar, V., Zhou, Y., Zhang, Z., & Awasthi, M. K. (2022). Black soldier fly larvae for organic manure recycling and its potential for a circular bioeconomy: A review. *Science of the Total Environment*, *833*, 155122. https://doi.org/10.1016/j.scitotenv.2022.155122

Mahmoud, A. H., Hussein, M. Y., Ibrahim, H. M., Hanafy, M. H., Salah, S. M., El-Bassiony, G. M., & Abdelfattah, E. A. (2022). Mixed microalgae-food waste cake for feeding of *Hermetia illucens* larvae in characterizing the produced biodiesel. *Biomass and Bioenergy*, *165*, 106586. https://doi.org/10.1016/j.biombioe.2022.106586

Misra, H. P., & Fridovich, I. (1972). The Role of Superoxide Anion in the Autoxidation of Epinephrine and a Simple Assay for Superoxide Dismutase. *Journal of Biological Chemistry*, *247*(10), 3170–3175. https://doi.org/10.1016/s0021-9258(19)45228-9

Paes, M. C., Oliveira, M. B., & Oliveira, P. L. (2001). Hydrogen peroxide detoxification in the midgut of the blood-sucking insect, *Rhodnius prolixus Archives of Insect Biochemistry and Physiology*, *48*(2), 63–71. https://doi.org/10.1002/arch.1058

Prieto, P., Pineda, M., & Aguilar, M. (1999). Spectrophotometric Quantitation of Antioxidant Capacity through the Formation of a Phosphomolybdenum Complex: Specific Application to the Determination of Vitamin E. *Analytical Biochemistry*, *269*(2), 337–341. https://doi.org/10.1006/abio.1999.4019

Priftis, A., Goutzourelas, N., Halabalaki, M., Ntasi, G., Stagos, D., Amoutzias, G. D., Skaltsounis, L. A., & Kouretas, D. (2018). Effect of polyphenols from coffee and grape on gene expression in myoblasts. *Mechanisms of Ageing and Development*, *172*, 115–122. https://doi.org/10.1016/j.mad.2017.11.015

Renault, D., Dorrah, M. A., Mohamed, A. A., Abdelfattah, E. A., & Bassal, T. T. M. (2016). Assessment of oxidative stress and activities of antioxidant enzymes depicts the negative systemic effect of iron-containing fertilizers and plant phenolic compounds in the desert locust. *Environmental Science and Pollution Research*, *23*(21), 21989–22000. https://doi.org/10.1007/s11356-016-7391-9

Siddiqui, S. A., Harahap, I. A., Osei-Owusu, J., Saikia, T., Wu, Y. S., Fernando, I., Perestrelo, R., & Câmara, J. S. (2024). Bioconversion of organic waste by insects-A comprehensive review. *Process Safety and Environmental Protection*, *187*, 1–25. https://doi.org/10.1016/j.psep.2024.04.122

Vuong, Q. V. (2017). *Utilisation of bioactive compounds from agricultural and food production waste*.