Journal of Physics:
Conference Series

PAPER • **OPEN ACCESS**

Impact of blended used cooking oil-kitchen waste on the bio-recycler, black soldier Fly larvae

To cite this article: Eman Alaaeldin Abdelfattah 2025 *J. Phys.: Conf. Ser.* **3051** 012004

Impact of blended used cooking oil-kitchen waste on the bio-recycler, black soldier Fly larvae

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Abstract. As populations grow, the quantity of organic waste increases dramatically. This organic waste includes agricultural waste, kitchen waste, and used cooking oil (UCO). The improper management of organic waste leads to serious environmental concerns. This study investigates the biochemical impact of mixing kitchen waste with UCO mixing on Black Soldier Fly (BSF) larvae, is reflected as promising implement for the recycling process of organic waste. The paper evaluated oxidative stress responses in 5^{th} instar BSF larvae reared on control food, kitchen waste (KW), and KW mixed with UCO. Lipid peroxidation, superoxide anion radical ($O_2\bullet^-$), superoxide dismutase (SOD) activity, and antiradical activity (DPPH) were measured in larval guts to evaluate the consequence of UCO exposure on the larval oxidative stress system. The outcomes revealed that the elevated levels of reactive oxygen species (ROS), oxidative stress damage (OSD), and antioxidant enzymes and compounds in the treated groups (KW and KW+UCO) compared to other experimental treatments. This research contributes to understanding the potential risks of UCO mixing within BSF-based organic waste management systems therefore pushing the development of sustainable waste treatment strategies.

Keywords: Biomass management, Organic waste management, Bioconversion, DPPH, SOD, Oxidative stress

1. Introduction

The growing quantity of organic waste leads to a serious environmental impact in case of improper management is applied. One of the most significant wastes in municipal solid waste management systems is kitchen waste. As it can struggle with the recycling process of other solid waste compositions, and decrease the economic values of recycling, increasing the deleterious effect on environment. This waste stream, composed of discarded food scraps, uneaten portions, and expired products, contributes significantly to landfill volumes, leading to the generation of methane. Furthermore, the resources invested in producing, processing, and transporting this food waste are wasted, resulting in serious impact on environment [1]. Used cooking oil (UCO is a common by-product of food preparation in both households and commercial kitchens. Improper management of UCO can cause a deleterious effect to the environment and human health [2 and 3]. Water and food particles are also present in UCO. The amount of water and food particles in UCO will vary depending on the cooking method used and the type of food cooked. For example, deep-fried foods will contribute more water and food particles to UCO than foods that are pan-fried. The improper management practices of UCO include collection percentages rate, dumping with solid waste, pouring into wastewater, or simply straining and reusing for cooking in small kitchens. Such unsystematic removal of used cooking oil results in water and soil pollution and contributes to global warming. The uncontrolled disposal of UCO into the trash can cause deleterious impacts on the environment as well as economic loss. The recycling of organic waste is becoming an interesting topic nowadays using several innovative technologies, as biological process [4].

One of these biological processes is industrial insect technology approaches, which have become very popular. Also, it is considered as a sustainable and innovative protein source, especially in the food security problems. The production of eco-friendly products using direct or indirect technical processes have had key attention in recent years especially in the focus of waste management. Some insects have been verified as an effective model for viable management of organic waste as Hermetia illucens and Tenebrio molitor. The black soldier fly is a useful tool for managing food waste due to the wide variety of organic wastes. The parameters used to evaluate the bio-recyclers include ethical consideration, short lifespan, and high biomass conversion ratio. Also, black soldier fly larvae have a positive impact regarding economic sustainability, ecological footprints, and overall efficiency; as a result, they have gained popularity as a model for the management of food waste all over the world. Organic wastes are mostly mixed with UCO [5]. The research and studies of UCO impact on oxidative stress parameters of the bio-recyclers need more investigation.

In this study, the oxidative stress reactions of the gut tissues of H. illucens after treatment of mixed cake of UCO-kitchen waste were assessed. The reactions include the concentration of lipid peroxidation, superoxide anion radical, SOD activity, and DPPH was analyzed.

2. Materials and methods

2.1. Experimental design

For subsequent experimental procedures, 5th larval offspring were collected from the established colony and transferred to individual plastic containers under conditions 24 D, RH: 60%, Temp. 37˚C [6]. Then divided into three groups of 150 each as the following Figure 1. After 2 days post treatment, these larvae were dissected to isolate the gut tissues. The samples freeze at -20°C for further analysis as mentioned below.

2.3. Biomarkers of oxidative stress and antioxidant response

The oxidative stress markers including $O_2^{\bullet-}$ production rate, and lipid peroxidation of samples were assessed in the guidance of the method of Chen and Li [7], and Hermes-Lima et al. [8] respectively. While the non-enzymatic antioxidant activity as the α,α-diphenyl-β-picrylhydrazyl (DPPH) was determined according to Blois [9], enzymatic antioxidant SOD activity was measured according to Misra and Fridovich [10], and the total protein concentration was assessed according to the method of Bradford [11]. Triplicate replications were performed for all experiments.

2.5 Statistical analyses

Statistical analyses were achieved with SPSS Statistics. The parametric analysis was done using one-way ANOVA, *Tukey B* Post Hock tests. Data were written as median and standard deviations.

3. Result

Oxidative parameters including oxidative damage and antioxidant enzymes were assessed in the experimental samples through the different feeding conditions. The results presented that the Superoxide anion radicals ($O_2^{\bullet-}$) levels were slightly higher in kitchen waste, and mixed KW with UCO group compared to the control group with the fold of 1.1, and 1.0-x fold respectively (MS=0.13, F=13, *df*= 2, *p value*<0.05). (Figure 2a). As well as the lipid peroxidation concentration, the results revealed a significant elevation in kitchen waste group compared to the control group with 3.2x fold. Remarkably, the UCO mixed group showed a slightly lower lipid peroxides concentration compared to kitchen waste only group (MS=0.28, F=451, *df*= 2, p value<0.05; Figure 2b). The marked increase in lipid peroxides concentration in kitchen waste group compared to the control group.

For the non-enzymatic antioxidant compounds, the DPPH assay can measure the free radical scavenging activity, the results showed a significant increase in the UCO-KW mixed group (70%) than kitchen waste group only (40%) (MS=541, F=7.41, *df*= 2, *p value*<0.05). These antioxidants act as a key role in mitigating free radical damage during normal physiological processes. This finding suggests that UCO exposure might have stimulated the larvae to enhance their antioxidant defenses. The breakdown of organic matter in kitchen waste might generate free radicals that overcome the larvae's essential antioxidant capacity. Regarding the enzymatic antioxidants response, the results revealed that SOD activity was elevated in kitchen waste cluster relevant to the control group and the UCO mixed group with fold 1.11, 1.02-x fold, respectively (MS=0.15, F=19, *df*= 2, p value<0.05, Figure 3b). The observed increase in SOD activity in kitchen waste assembly compared to the control suggests that BSF larvae may be upregulating their antioxidant defenses in response to the oxidative stress induced by digesting kitchen waste.

4. Discussion

The oxidative stress responses in bio-recycler of organic waste, black soldier fly larvae, reared on UCO-KW mixed cake were investigated to evaluate the biochemical impact of UCO on Black Soldier Fly (BSF) larvae. Larval gut tissues were analyzed for assessing the oxidative markers including lipid peroxidation, $O_2^{\bullet-}$ levels, DPPH and SOD.

The significant increase in $O_2^{\bullet-}$ levels in kitchen waste group suggests that oxidative stress can prompt by the digestion of kitchen waste. This might be due to the natural breakdown processes within the waste generating reactive oxygen species (ROS) as by-products [6]. Low to moderate exposure to stressors can sometimes trigger a deleterious response, where organisms enhance their antioxidant defenes to mitigate the stress. In this case, the presence of UCO in kitchen waste might have induced a mild side effect, leading to a slight upregulation of antioxidant mechanisms and lower $O_2^{\bullet-}$ levels.

The marked increase in lipid peroxides concentration in kitchen waste group compared to the control strongly suggests that the digestion of kitchen waste induces oxidative stress in BSF larvae. This is likely due to the generation of ROS during the breakdown of organic matter in the waste [12]. The lower concentration of lipid peroxidation observed in the UCO mixed group related to kitchen waste only may be due to the addition of UCO to kitchen waste might dilute the overall concentration of freely oxidizable substrates in the diet. This could lead to a reduction in ROS generation and consequently lower lipid peroxidation levels [13]. According to literature, the metabolic pathways of insects lead to oxygen utilization and comes with a potential downside including, the production of ROS, also known as free radicals can cause cellular damage. Insect can promote the oxidative stress through a complex mechanism of antioxidants system [14]. For example, antioxidant enzymes can prevent the ROS formation, and facilitate its removal through production of antioxidants to restore the balance between oxidants and antioxidants [6 and 15-16].

The significant increase level of DPPH inhibition activity was observed in the UCO-KW mixed group comparing with the control. Suggesting that exposure to UCO can stimulate the antioxidant defenes mechanism of larvae. Besides that, some used cooking oils might retain antioxidant compounds from their original state (e.g., vegetable oils rich in phenolic compounds). These compounds could contribute directly to the higher DPPH inhibition activity observed in the UCO mixed group [18]. In addition to, the breakdown of organic matter in kitchen waste might generate free radicals that overcome the larvae's integral antioxidant capacity. [6 and 19]. The significant increase in SOD activity in kitchen waste group relevant the control suggests that BSF larvae may be upregulating their antioxidant defenes in response

Journal of Physics: Conference Series **3051** (2025) 012004 doi:10.1088/1742-6596/3051/1/012004

to the oxidative stress induced by digesting kitchen waste. This finding aligns with the expected response of organisms to neutralize the damaging effects of reactive oxygen species (ROS) generated during food breakdown [6, 14-19]. The generalized linear model (GLM) showed in Table 1 indicates there is significant difference between different parameters as $O_2{\bullet}^-$, lipid peroxidation, DPPH, and SOD. The GLM analysis revealed statistically significant effects ($p < 0.001$) of treatment on all the measured oxidative stress markers in BSF larvae [19].

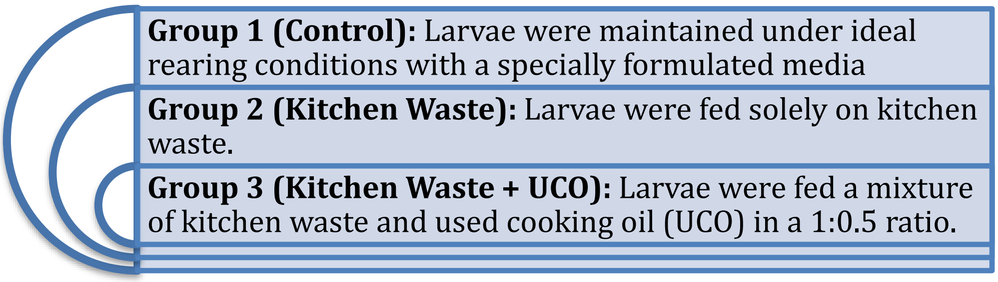

Figure 1. *Grouping of the experimental samples (including 50 larvae for each replicate)*

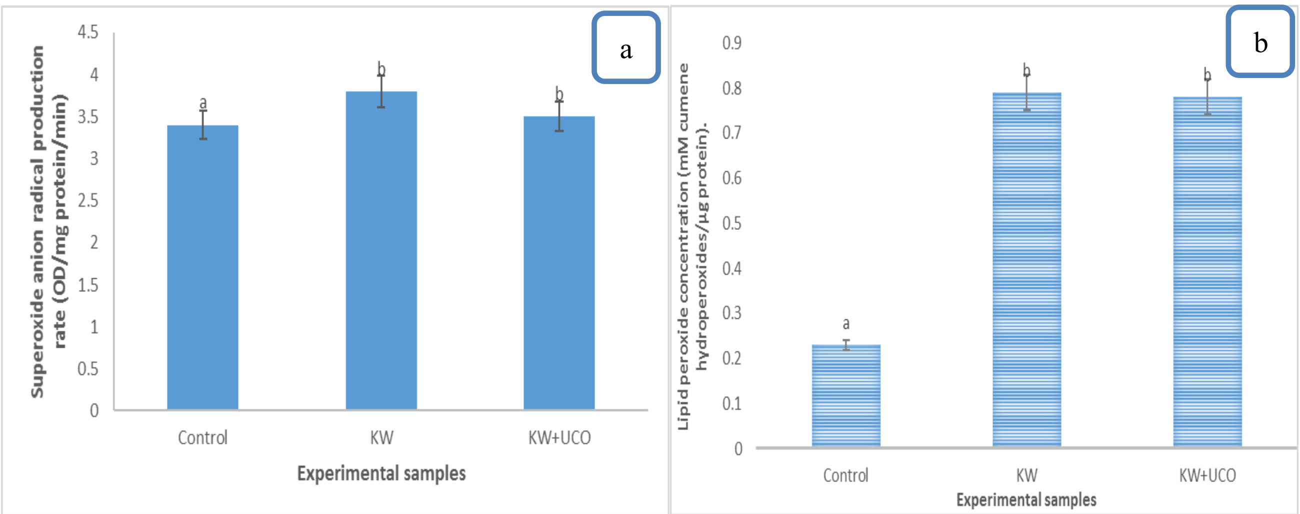

Figure 2. Oxidative stress parameters, superoxide anion radical ($O_2{\bullet}^-$) (a), and lipid peroxides (b) concentration assessed in the gut tissues of *Hermetia illucens* (5th instars), supplied with different feed, (control mash, kitchen wastes (KW), and mixed cake of kitchen waste with used cooking oil (UCO)).

Values are expressed as median and standard deviations. Median values with different small letters represent significant differences among control and the different experimental samples, KE, KW mixed with UCO (one-way ANOVA, *Tukey B* Post Hock tests, $p < 0.05$).

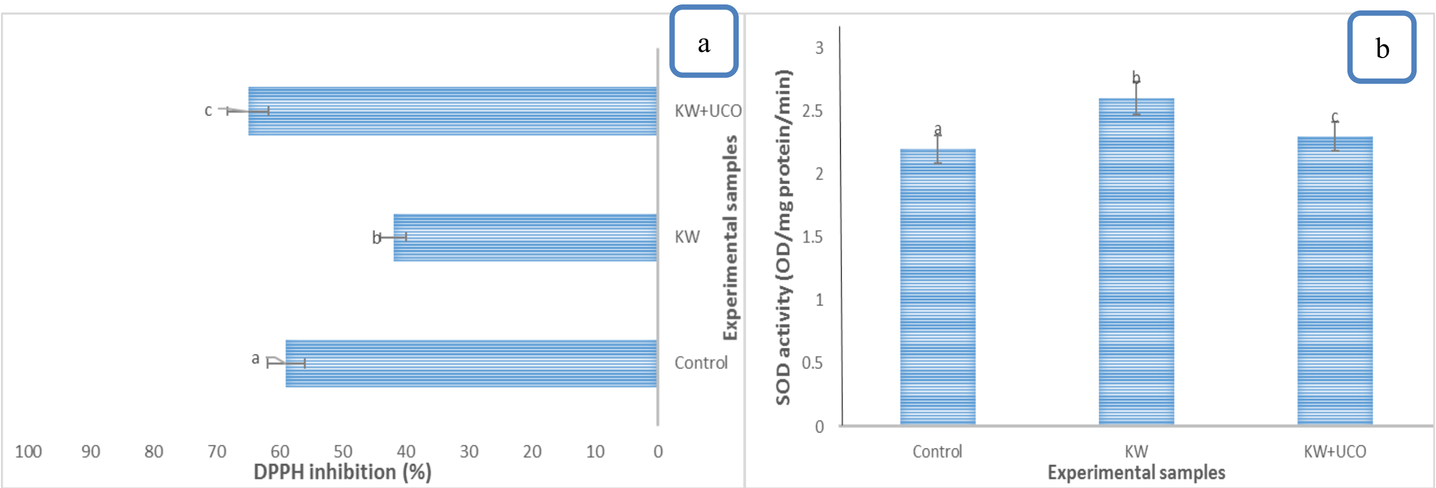

Figure 3. Non-enzymatic, α,α-diphenyl-β-picrylhydrazyl (DPPH) antioxidant inhibition activity (a), and enzymatic superoxide dismutase (SOD) (b) assessed in the gut tissues of *Hermetia illucens* (5^{th} instars), supplied with different feed, (control mash, kitchen wastes (KW), and mixed cake of kitchen waste with used cooking oil (UCO)).

Values are expressed as median and standard deviations. Median values with different small letters represent significant differences among control and the different experimental samples, KE, KW mixed with UCO (one-way ANOVA, *Tukey B* Post Hock tests, $p < 0.05$).

Table 1. Generalized Linear Model (GLM) to assess and analyse the oxidative stress parameters including (reactive oxygen species (ROS) as superoxide anion radicals (O_2•⁻), oxidative damage (OD) as lipid peroxidation (LP), enzymatic antioxidants (AO-E) as superoxide dismutase (SOD), non-enzymatic antioxidants (AO-NE) as α, α-diphenyl-β-picrylhydrazyl (DPPH)

Parameters	Source	Wald Chi-Square	*df*	Sig.
ROS ($O_2^{\bullet-}$)	Intercept	17173.500	1	<0.001
	Treatment	39.000	2	<0.001
OD (LP)	Intercept	7298.679	1	<0.001
	Treatment	1354.500	2	<0.001
AO-E (SOD)	Intercept	9813.429	1	<0.001
	Treatment	59.571	2	<0.001
AO-NE (DPPH)	Intercept	629.281	1	<0.001
	Treatment	22.233	2	<0.001

5. Conclusion

This study examined the impact of UCO-kitchen waste mixed cake on the physiological response of BSF larvae. The results investigated that Kitchen waste alone induced oxidative stress in BSF larvae, including $O_2^{\bullet-}$ levels and lipid peroxidation. However, the UCO-KW mixed group displayed slightly lower $O_2^{\bullet-}$ levels and lipid peroxidation compared to kitchen waste only group. The activity of the antioxidant enzyme SOD was slightly higher in kitchen waste group compared to the control and UCO mixed groups. These findings suggested that the dilution effect of UCO, can reduce the concentration of oxidizable substrates (ROS). Also, the potential upregulation of larval antioxidant defenes in response to the oxidative stress from kitchen waste digestion can reduce the oxidative damage. Though, additional research is required to fully comprehend the mechanisms and long-last consequences of UCO exposure on BSF health and performance in waste management applications. Future Considerations can include the following assessment studies (see Figure 4).

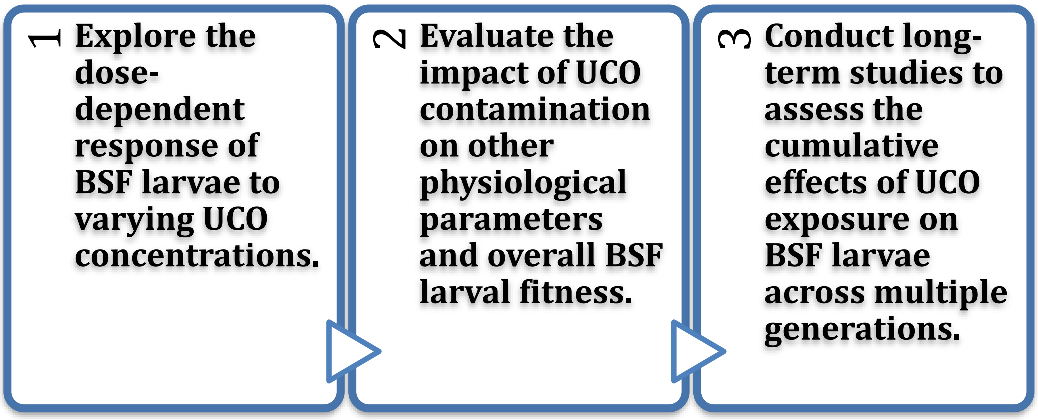

Figure 4. *Future assessment studies for improvement the understanding of UCO metabolism inside BSF*

Funding: The author extends the appreciation to the Ministry of higher Education and Research in Egypt, ASRT for funding this research work through the ASRT project. Besides the funding from Cairo University Egypt

Acknowledgments: The author extends the appreciation to Entomology Department staff members, project of organic waste recycling project and Industrial Entomology approaches project for their help in completion this paper.

Conflicts of Interest: The authors declare no conflict of interest.

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