The impact of L-DOPA on insect-based waste management tool

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ABSTRACT

The increase in population leads to a rise in consumption and, consequently, a higher generation of wastes. As a result, managing solid wastes has become a major environmental challenge for governments and communities. To tackle this issue, various techniques have been developed worldwide. Insect-based bioconversion is a promising alternative to traditional waste management. The black soldier fly *Hermetia illucens* is widely used for this purpose. However, organic wastes may contain contaminants, such as phenolics, especially L-DOPA, that could impact the bioconversion efficiency by imposing physiological costs. Therefore, the current study aimed to assess the impact of L-DOPA on *H. illucens* larvae by exposing them to different concentrations of L-DOPA (0-150 mg/mL). The fifth instar *H. illucens* larvae's gut tissues exhibited a variety of oxidative stress parameters such as oxidants (superoxide anion radical ($O_2^{\bullet-}$)), antioxidants (2,2-diphenyl-1-picrylhydrazyl (DPPH), phosphomolybdenum ability, reducing power ability, or total antioxidant assay), oxidative damage products (protein carbonyls), and detoxification enzymes (α-esterase, acetylcholine esterase (AChE)) were measured. The highest levels of $O_2^{\bullet-}$, protein carbonyls, GR, PPO, DPPH, phosphomolybdenum, total antioxidant ability, reducing power, α-esterase, and AChE occurred at 150 mg/mL L-DOPA treatment with the fold of 0.7, 0.6, 2, 0.6, 1.3, 3.9, 0.2, 18.8, 2.8, 0.6, respectively, compared to control levels. Moreover, all oxidative stress parameters and detoxification enzymes were significantly affected by L-DOPA except APOX activity. The results emphasized the toxicity of L-DOPA on *H. illucens*.

Keywords: Oxidative stress; Black soldier Fly; L-DOPA; Phenolic compounds; Detoxification enzymes; DPPH.

INTRODUCTION

The management of solid waste remains a significant socioeconomic and governance issue, particularly in urban regions that are tackling with rapid population growth and the subsequent increase in waste production (Abubakar *et al*., 2022; Badwai *et al*., 2023). The management of solid wastes is a complex task owing to the large variety of wastes generated. Various methodologies have been developed to overcome this issue, including landfills, combustion, waste-to-energy, pyrolysis, or other solid materials (Abdelfattah *et al*., 2021). In recent years, insect-based bioconversion has attracted significant attention due to its economic viability and long-term sustainability, as well as its potential to mitigate adverse effects on humans and the ecosystem (Salomone *et al*., 2017). Insects such as *Hermetia illucens* (black soldier fly), *Gryllidae* (crickets), and *Tenebrio molitor* (mealworms) effectively convert organic

waste into protein-rich biomass and fertilizer (Fowles and Nansen, 2019). The black soldier fly is capable of consuming various types of food waste, such as fruit and vegetable scraps, meat, dairy products, and even compostable packaging. Through their feeding process, they transform the waste into nutrient-rich materials that can be utilized as fertilizer and insect biomass that is rich in protein and can serve as animal feed or be processed into food products for human consumption (Siddiqui *et al*., 2022).

Phenolics are endogenously synthesized in certain plants such as velvet bean (*Mucuna pruriens*), fava bean (*Vicia faba*), and jack bean (*Canavalia ensiformis*) (Ayerdi Gotor and Marraccini 2022) and sometimes used in cooking. If these beans are not properly prepared or cooked, they can contain high levels of L-DOPA, which can lead to contamination of food waste (Dahouda *et al*., 2009; Etemadi *et al*., 2018). In addition, plant phenolic compounds are released to contribute to the plant defense mechanisms against abiotic and biotic stresses (Mbaveng *et al*., 2014). L-DOPA is also can be present in organic waste if the waste includes discarded medications. L-DOPA can be produced from the amino acid tyrosine, which is present in many plant proteins (Soares *et al*., 2014). DOPA auto-oxidation increases cytotoxicity by generating reactive oxygen species (ROS) such as hydroperoxyl (HO_2), hydroxyl (OH^-), superoxide anion ($O_2^{\cdot-}$), and hydrogen peroxide (H_2O_2) (Bolton *et al*., 2000; O'Brien, 1991). When these reactions occur in large numbers, extensive cellular damage can be caused (Abdelfattah, 2020; Abdelfattah *et al*., 2017; Renault *et al*., 2016; Yousef *et al*., 2017). Thus, the more antioxidant defenses available, the less damage occurs as free radicals preferentially react with these molecules, resulting in non-toxic byproducts (Kelly, 2003; Yousef *et al*., 2019).

Insects have various antioxidant responses, both enzymatic and non-enzymatic, which can cooperate to defend against dietary and endogenous oxidants (Abdelfattah and Renault, 2022; Nassar *et al*., 2020; Yousef *et al*., 2017). Insect antioxidant enzymes include ascorbate peroxidase (APOx), polyphenol oxidase (PPO), catalase (CAT), glutathione-s-transferase (G-S-T), superoxide dismutase (SOD), and glutathione reductase (GR). Besides that, the non-enzymatic antioxidants include reduced glutathione, acrobat, α-tocopherols carotenes, 2, 2 diphenyl-1-picylhydrazyl (DPPH), phosphomolbdeum ability, reducing power ability, and total antioxidant ability (Blois, 1958; Oyaizu, 1986; Felton and Summers, 1995; Prieto *et al*., 1999; Renault *et al*., 2016; Abdelfattah *et al*., 2021). In addition, the various oxidative stressors can affect the activity of acetylcholine esterase (AChE) (Prezenská *et al*., 2019) and the activity of detoxification enzymes such as carboxyl esterase including α- and β-esterase (Moustafa *et al*., 2021).

So, Insects can be used as biomarkers to detect toxicity from environmental pollutants or oxidative stressors. Additionally, they can assess the side effects of overuse of toxins or pollutants (Ahmad, 1995; Abdelfattah *et al*., 2017; Yousef *et al*., 2019; Abdelfattah *et al*., 2021). Black soldier fly larvae are effective for long-term organic waste treatment in the sense that they can help to both reduce organic waste and transform it into beneficial products such as biofuel, animal feed, and fertilizers (Mutafela, 2015). Therefore, this study investigates the impact of toxicity of different L-DOPA concentrations (0, 50, 100, and 150 mg/mL) on ROS concentration, oxidative stress damage amount, detoxification enzymes activities, non-enzymatic and enzymatic antioxidants levels in 5th instar *H. illucens* larvae's gut tissues.

MATERIALS AND METHODS
Samples preparation:

Colony of 100 adult black soldier insects (*Hermetia illucens*) was established under controlled conditions (37°C, 60% RH, 12:12 light-dark cycle), then larvae from this colony were raised in the dark and exposed to different concentrations of L-DOPA (0, 50, 100, or 150 mg/mL) by immersing their food in L-DOPA solutions for 24 hours. A control group received distilled water. Gut tissues from 50 insects per experimental group were homogenized and stored at -20°C for subsequent analysis. The experiment was replicated three times.

To assess oxidative stress, the production of superoxide anion radicals ($O_2 \bullet -$) was measured using Chen and Li's colorimetric method (2001). This involved monitoring the rate of adrenochrome formation from epinephrine in the presence of NADPH.

Protein carbonyl content, an indicator of oxidative damage, was determined using a modified version of Levine *et al*. method (1990) which involves derivatizing protein carbonyls with 2,4-dinitrophenylhydrazine (DNPH) and measuring the absorbance at 366 nm.

The antioxidant and detoxification enzyme activities of the 5th instar larvae's gut tissues were assessed using various biochemical assays. GR activity was measured using the method of Carlberg and Mannervik (1985) by monitoring the increase in absorbance at 420 nm due to the reduction of DTNB by GSH. While, APOX activity was determined using Asada's method (1984) by measuring the decrease in absorbance at 290 nm due to the oxidation of ascorbic acid by H_2O_2. PPO activity was measured using the method of Kumar and Khan (1982) by monitoring the formation of purpurogallin at 495 nm. GPX activity was measured using the method of Mazhoudi *et al*. (1997) by monitoring the oxidation of guaiacol at 470 nm. The antioxidant capacity was assessed using the DPPH method (Blois, 1958), phosphomolybdenum method (Prieto *et al*., 1999), and reducing power assay (Oyaizu, 1986).

Statistical analysis:

It was done using Kruskal-Wallis H (P<0.05) to examine how different L-DOPA concentrations affected the levels of oxidative stress markers in the guts of fifth-instar *H. illucens*. IBM SPSS Statistics for Windows (Version 17.0; Armonk, NY: IBM Corp.) was used for all statistical analyses.

RESULTS AND DISCUSSION

Oxidative stress parameters are indicators of the balance between reactive oxygen species (ROS) production and elimination in biological systems. ROS molecules can damage cellular components including DNA, proteins, and lipids. But also play important roles in signalling and regulation. Therefore, oxidative stress parameters can reflect the physiological state and adaptive responses to various environmental factors (Pizzino *et al*., 2017). By measuring and analyzing oxidative stress parameters, we can gain insights into the mechanisms underlying the interaction process of these parameters and how they affect the survival, growth, performance, and evolution of organisms. This is especially relevant for scientists dealing with phenomena of adaptation, toxicology, evolution, and bioremediation, as they can use oxidative stress parameters as tools to assess the effects of environmental stressors on organisms and to evaluate their potential for adaptation and resilience. In recent decades, studies on the environment, human impact, and ecosystem pressure have become increasingly important (Abdelfattah *et al*., 2021). The effect of various oxidative stressors on the biochemical parameters of the organisms should be assessed. Studying the mechanisms of oxidative stress response and adaptation is possible using the model organisms, for example *Schistocerca gregaria* (Renault *et al*., 2016); *Aiolopus thalasinus* (Abdelfattah, 2020; Abdelfattah *et al*., 2017; Yousef *et al*., 2019, 2017); and *Hermetia illucens* (Nassar *et al*., 2020).

The relative levels of $O_2^{\cdot-}$ and protein carbonyls amount in the 5th instars of *H. illucens* treated with different concentrations of L-DOPA are shown in Figure (1). The insects exposed to the highest concentration of L-DOPA exhibited a significant rise in $O_2^{\cdot-}$ and protein carbonyls levels compared to those from the control group (Fig. 1A&B). This increase was significantly correlated with the L-DOPA concentration (Table 1). The $O_2^{\cdot-}$ production rate in the gut tissues was significantly difference at 50 mg/mL L-DOPA concentration than control insects. However, there were no significant differences observed in insects treated with 100 mg/mL L-DOPA from control (Fig. 1A). The level of protein carbonyls in the gut tissues significantly increased, with the highest value observed in insects treated with 150 mg/mL L-DOPA. (Fig. 1B).

The levels of antioxidant enzyme activities in 5th instar *H. illucens* are shown in Figure (2 A-D). The 5th instar *H. illucens* larvae treated with 150 mg/mL L-DOPA had the highest activity of GR and PPO (Fig. 2A & C). APOX activity didn't reveal significant differences among tested different L-DOPA concentration samples (Fig. 2B). However, GPx activity in individuals treated with 100 mg/mL L-DOPA showed a significant increase among all samples (Fig. 2D). Also, the levels of (DPPH) inhibition showed a significant elevation with respect to control insects. Moreover, the highest value was observed in insects treated with 100 mg/mL L-DOPA (Fig. 3A). A significant effect was especially observed in the case of the insects treated with 150 mg/mL L-DOPA, level of phosphomolybdic antioxidant capacity increased over 5-fold in the gut tissues of 5th instar *H. illucens* (Fig. 3B). The Reduction power ability in insect's gut tissues treated with 50 and 100 mg/mL L-DOPA were significantly lower compared to the control (Fig. 3C). The levels of the total antioxidant ability in the gut tissues revealed a gradually increase compared to individuals from the control group. Levels of the total antioxidant ability increased over 10-fold in insects treated with 150 mg/mL L-DOPA (Fig. 3D).

Also, the results showed a significant elevation of detoxification enzymes after treated BSFL with different concentrations of L-DOPA (Fig. 4). The α-esterase and AChE enzymes activities were significantly up-regulation than constitutive levels in the insect gut tissues treated with 150 mg/mL L-DOPA form all examined samples. Moreover, no significant differences were observed in other concentrations (Fig. 2A & B). Moreover, individuals treated with 50 mg/mL L-DOPA didn't reveal significant differences from the control sample (Fig. 2B).

The correlation coefficient results of oxidative stress parameters ($O_2^{\cdot-}$, protein carbonyl amount, GR, PPO, GPX, PPO, GPx, DPPH, phosphomolybdic antioxidant, reducing power, total antioxidant ability α-esterase, and acetylcholine esterase) showed significant positive correlation with the effect of different L-DOPA concentration except APOX which show negative correlation (Table 1). Besides that, the prediction linear equation showed a significant positive chi-square value along L-DOPA concentration in all oxidative stress parameters (Table 1).

The biochemical effects of a specific oxidative stressor of L-DOPA on the insect *H. illucens* treated with different concentrations of L-DOPA (0-150 mg/mL) were studied and presented in Figures (1-4) and (Table 1). The oxidative stressor, L-DOPA, can interact with O_2 to form $O_2^{\cdot-}$ which can convert to reactive particles like hydroxyl radical and hydrogen peroxide. All of them can cause oxidative stress in cellular models (Komarov *et al.*, 2006). Besides that, the study of Dekant *et al.* (2021) suggested that the quinone -N-acetyl-cysteine complex can be oxidized using a catalyzing enzyme, cytochrome-p-450 to produce quinone/hydroquinone redox couple, which finally conjugated with GSH. In the present results, it was

found that $O_2^{\bullet-}$ production rate increases as the concentration of L-DOPA increases (Fig. 1A). These results support the findings of Krishnan and Sehnal (2006), who demonstrated that exposure of *Spodoptera littoralis* larvae to high concentrations of tannic acid led to an increase in $O_2^{\bullet-}$ production rate compared to the control group. This suggests that black solid fly larvae (BSFL) can increase their oxidative metabolism and stress response to cope with the toxic effects of L-DOPA. Additionally, A lot of accepted literature assumed that protein carbonyl amounts can be used as an oxidative stress marker (Renault *et al*., 2016; Suzuki *et al*., 2010). So, the significant increase in protein carbonyls amount, as shown in Figure (1B), also corresponds with Yousef *et al*. (2019) who found the increase amount of protein carbonyls in 5th instars nymph gut of *A. thalassinus* as a result of increase concentration of environmental pollutant. These findings suggest that Reactive Oxygen Species (ROS) exist over a wide range and can potentially impact biomolecules (Augustin and Partridge, 2009; Even *et al*., 2012; Abdelfattah, 2016; Renault *et al*., 2016). According to the concentration-series measurements, damage was detected at a concentration of 50 mg/mL L-DOPA after 24 hours. This suggests that L-DOPA affects gut tissues quickly after feeding. The negative effects persisted up to 150 mg/mL, as shown by sustained elevation of $O_2^{\bullet-}$ and protein carbonyls, while antioxidant enzyme activity (GR, APOX, PPO, or GPx) notably increased. Non-enzymatic antioxidants like DPPH, phosphomolybdium, reducing power, and total antioxidant ability also increased. Detoxification enzyme activity, such as α-esterase and AChE, also increased. Furthermore, the oxidation of amino acids and cleavage of polypeptide chains can lead to protein carbonyl formation (Costa *et al*., 2007).

Several proteins are considered a catalyst of Pivotal biochemical events, implying that any modifications or alterations may result in biological system damage (Quiney *et al*., 2011). The homeostasis of oxidative status can be restored through integrated antioxidant systems (Birben *et al*., 2012). From the results obtained, the increase of L-DOPA concentration led to an increase in GR enzyme activity significantly, as shown in Figure (2A). These results are in line with the finding of Renault *et al*. (2016), who consummated that GR activity increased with time post-injection with L-DOPA or Ferrous ion on the 5th instars of the desert locust *Schistocerca gregaria*. The enhanced GR activity suggests that they have a high capacity to cope with L-DOPA-induced oxidative stress and to prevent cellular damage. Moreover, GR is involved in the recycling of glutathione, which is another key antioxidant molecule. Thus, the increased GR activity in BSFL may also indicate an increased glutathione turnover and L-DOPA elimination. These findings imply that black soldier fly larvae (BSFL) have a robust antioxidant defense system that enables them to tolerate and detoxify L-DOPA. Also, in the present study results, APOX enzyme activity showed no significant difference as a result of increasing L-DOPA concentration, as shown in Figure (2B). These findings may explain the ability of BSFL to detoxify the H_2O_2 levels. It was previously approved that GPx and APOx antioxidant enzymes can catalyze the ROS detoxification reaction only at a low concentration of H_2O_2, unlike catalase enzymes (Krishnan and Sehnal, 2006), Particularly considering the presupposition of the enhanced capacity of midgut tissues to manage ingested nutrients, pro-oxidants, and ROS (Renault *et al*., 2016). However, the non-significant difference in APOX activity didn't occur in the finding of Yousef *et al*. (2019), who concluded that APOX enzyme activity in 5th instars nymphs of *Aiolopus thalassinus* significantly increases as the concentration of environmental pollutant increases. On the other hand, PPO enzyme activity from this study showed a fluctuated response

with different concentrations of L-DOPA; it increased significantly with 50 mg/mL L-DOPA, then decreased when the concentration of L-DOPA raised to 100 mg/mL, and finally, the PPO activity increased with 150 mg/mL L-DOPA as showed in Figure (2C). These findings are coordinated with those of Yousef *et al.* (2019) who found that when *A. thalassinus* exposure to low concentration of environmental pollutant, the PPO enzyme activity reduce comparing to activity at site with normal environmental pollutants percentage, then at site with high environmental pollutants, the activity of PPO enzyme increase significantly. The current results also showed a significant change in GRx enzyme activity with a change in L-DOPA concentration; it shows that the activity increases gradually until it reaches a maximum at 100 mg/mL L-DOPA, then it falls at 150 mg/mL L-DOPA as shown in Figure (2D), These results agree with those found by Renault *et al.* (2016) who showed that GRx enzyme activity of *S. gregaria* starts to increase significantly with time when injected with L-DOPA, then activity decrease remarkably after 24 hrs. As a result, the fluctuating pattern of antioxidant enzyme response may render the stress progression in parallel to repair the detected damages. For example, the oxidized protein removal process may not be enough to restore redox homeostasis. Therefore, this fluctuating pattern of these results indicates that the interactions between macromolecules, L-DOPA, and the substances that result from their breakdown are not simple but rather dynamic and variable.

The previous studies showed limited data on how L-DOPA affects oxidative stress indicators such as DPPH, Phosphomolybdeum capacity, total antioxidant capacity, and reducing power. Therefore, the findings of this study were compared with other studies that used different treatments and organisms. The study of Duan *et al.* (2021) showed a strong correlation between L-DOPA content and

DPPH of young and old leaves of the fava bean with values of 0.826 and 0.984, respectively. These findings occurred in this study, as the elevation percentage of DPPH was obvious with increasing the L-DOPA concentration (Fig. 3A). Also, there was a strong correlation coefficient among L-DOPA concentration and DPPH values (Table 1). Although the study of Blois (1958) recommended using DPPH analysis as a sensitive, precise, low sample concentration and low-priced biochemical analysis, yet according to Prieto *et al.* (1999) research, the phosphomolybdenum antioxidant method is a reliable way to assess the total antioxidant capacity of insect tissue extracts and determine vitamin E levels. This method also helps understand the structure-activity relationships of antioxidants and analyse changes in plasma antioxidant activity related to oxidative stress. Additionally, the phosphor-molybdenum method is cost-effective and straightforward, making it a useful alternative for measuring antioxidant levels. However, the correlation coefficient factor in the present study showed a strong significant correlation in both DPPH and phosphomolybdeum along L-DOPA concentration (Table 1). This may be used to validate the ability to use insects as a source of antioxidants. The reducing ability of the present study showed a fluctuation pattern along concentration gradients (Fig. 3C). This finding is similar to the results of Howden & Kilby. (1961), which discussed the age variation on the patterns of reducing ability in the hemolymph of *Schistocerca gregaria*. Similarly, to the DPPH results pattern, the total antioxidant ability pattern showed a gradual increase along concentration gradients (Fig. 3D). The study of Rodriguez-Martin *et al.* (2001) has proposed the toxicity of L-DOPA, including neurotoxicity and neurotropism. The elevation levels fluctuation of the non-enzymatic antioxidants in the current study were similar to the explanation of Vatassery *et al.* (2006) study. The cell line PC-12 cells can enhance the neuroprotective effects of

L-DOPA in the case of antioxidants, such as glutathione or ascorbate, and the other bioactive molecules present. However, Diwan et al. (2012) study showed a weak positive correlation between plant phenolics compounds and DPPH, phosphomolybdeum, or reducing power, with the values of 0.433, 0.221, or 0.163, respectively. However, the current results showed a strong positive correlation among DPPH and phosphomolybdeum, unlike, reducing power with the values of 0.86, 0.82, or 0.26, respectively (Table 1). It is believed that the transfer of hydrogen and electrons from antioxidants to DPPH radicals may account for these results. Additionally, the occurrence of Mo (VI) complex is observed in both the DPPH and phosphomolybdenum assay methods. The transfer reactions are dependent on the antioxidant's structure and occur at varying redox potentials. Polyphenols can be detected using DPPH radical scavenging assays, while the phosphomolybdenum method is typically used to detect antioxidants like phenolics, a-tocopherol,

and carotenoids. (Diwan et al., 2012; Prieto et al., 1999).

Acetylcholinesterase (AchE) is an important detoxification enzyme for insects (Zhang et al., 2011). AChEs are generally hydrolyzing both ester and amide bonds of xenobiotics (Jokanovi, 2001). Allelochemicals, organophosphate insecticides, and the metabolic activation products of carcinogenic compounds can all alter AChE activity (Radwan et al., 2024). As shown in the present results, the roles of insect α-esterase and AChE in mediating metabolic detoxification processes and oxidative stress responses were mostly positively correlated with the different L-DOPA concentrations (Table 1). Also, the gradual increase of the α-esterase and AChE activity occurred (Fig. 4 A & B). The current research aligns with Li et al. (2016) findings, which indicate that *Tenebrio molitor* larvae exhibit significantly increased levels of both α- and β-esterase activity after being infected with entomopathogenic nematodes (EPNs). These substances can act as xenobiotic toxins. However, Dubovskiy et al. (2011) concluded that esterase enzyme activity increased in the hemolymph of *Galleria mellonella* larvae treated with a sublethal dose of nickel.

Table 1. Effect of different L-DOPA concentration (0, 50, 100, and 150 mg/mL) on antioxidant enzymes activity, esterase, carboxylesterase, oxidants concentration and antioxidants ability in the gut tissues of 5th instar *Hermetia illucens*.

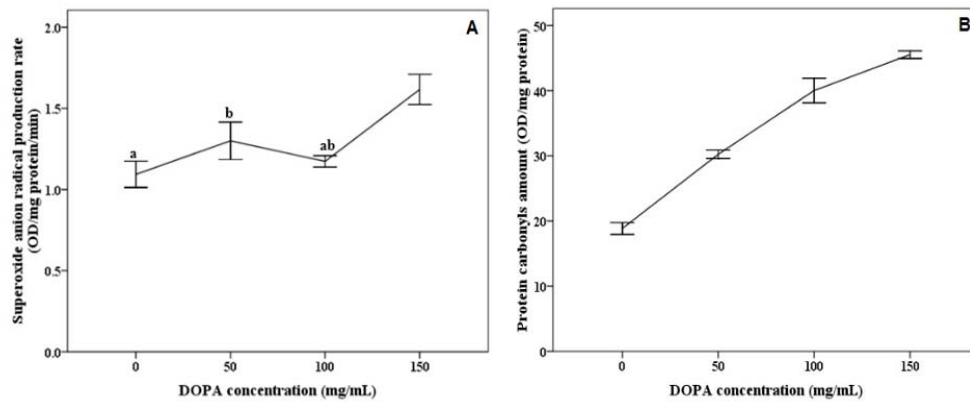|	Item	r	Equation	R^2	Equation Type
Antioxidant enzyme activity	GR	0.882**	Y=0.0385 X+3.4	R^2=0.7	
	APOX	-0.28	Y=-0.0038 X+35.7	R^2=0.043	
	PPO	0.805**	Y=0.0141 X+2.94	R^2=0.64	
	GPx	0.381	Y=0.0042 X+0.32	R^2=0.14	
Enzymes	Esterase	0.834**	Y=0.0197X+0.6913	R^2=0.69	
	Carboxylesterase	0.941**	Y=0.0127 X+2.29	R^2=0.885	
Oxidants concentration	Protein carbonyl amount	0.986**	Y=0.179 X+20.18	R^2=0.97	Linear
	$O_2^{\cdot-}$ production rate	0.774**	Y=0.009 X+1.079	R^2=0.59	
Antioxidant concentration	DPPH	0.860**	Y=0.293 X+52.4	R^2=0.74	
	Phosphomolybdeum antioxidant	0.821**	Y=0.379 X+11.53	R^2=0.673	
	Reducing power	0.296	Y=0.0381 X+45.05	R^2=0.087	
	Total antioxidant ability	0.961**	Y=0.697 X+2.7	R^2=0.92	

Fig. 1. Oxidants concentration as a result of oxidative stress expressed as superoxide anion radical production rate (A) and protein carbonyls amount (B) in the gut tissues of 5th instar *Hermetia illucens* treated with (10:1(w:v); organic waste: L-DOPA); of different L-DOPA concentration (0 (control sample), 50, 100, and 150 mg/mL) per 50 larvae. The values measured after 12 hours post treatment. Values shown as median ± P75 (*N=3*). Bars marked with same small letters indicates no significant difference using *k independent Kruskal-Wallis H (P>0.05)* between L-DOPA concentration.
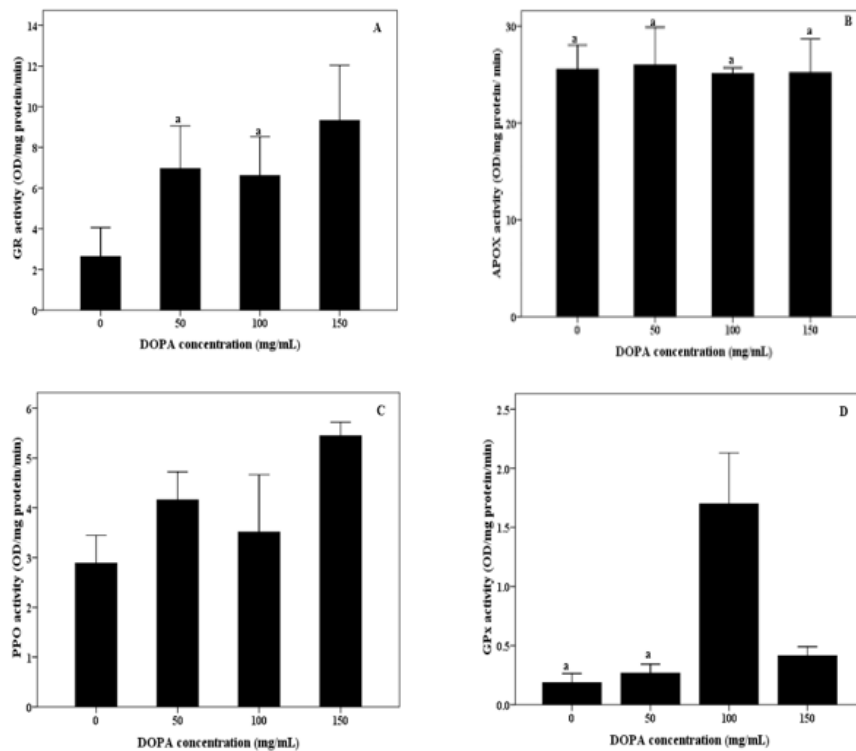
Fig. 2. Activity of antioxidant enzymes (glutathione reductase (GR) (A); ascorbate peroxidase (APOX) (B); polyphenol oxidase (PPO) (C); and glutathione peroxidase (GPx) (D)) in the gut tissues of 5th instar *Hermetia illucens* treated with different L-DOPA concentration (0 (control sample), 50, 100, and 150 mg/mL). Values represented as median ± P75 (N=3). Bars marked with same small letters indicates no significant difference using *k independent Kruskal-Wallis H (P>0.05)* between L-DOPA concentration.

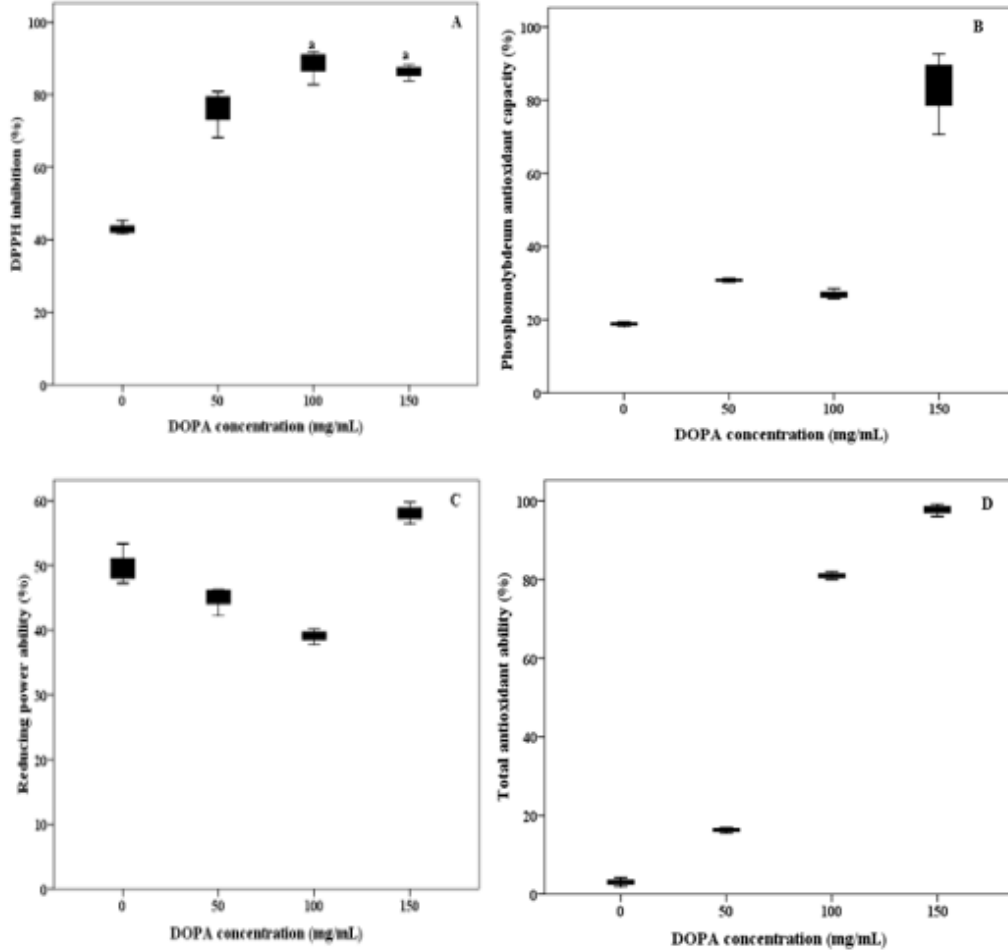**The impact of L-DOPA on insect-based waste management tool**

Fig. 3. Antioxidants ability expressed as 1,1-diphenyl-2-picrylhydrazyl (DPPH) inhibition (A), phosphomolybdeum antioxidant capacity (B), Reduction power ability (C) and total antioxidant ability (D) in the gut tissues of 5th instar *Hermetia illucens* treated with (10:1(w:v); organic waste: L-DOPA); of different L-DOPA concentration (0 (control sample), 50, 100, and 150 mg/mL) per 50 larvae. The values measured after 12 hours post treatment. Values shown as median ± P75 (*N=3*). Bars marked with same small letters indicates no significant difference using *k independent Kruskal-Wallis H (P>0.05)* between L-DOPA concentration.
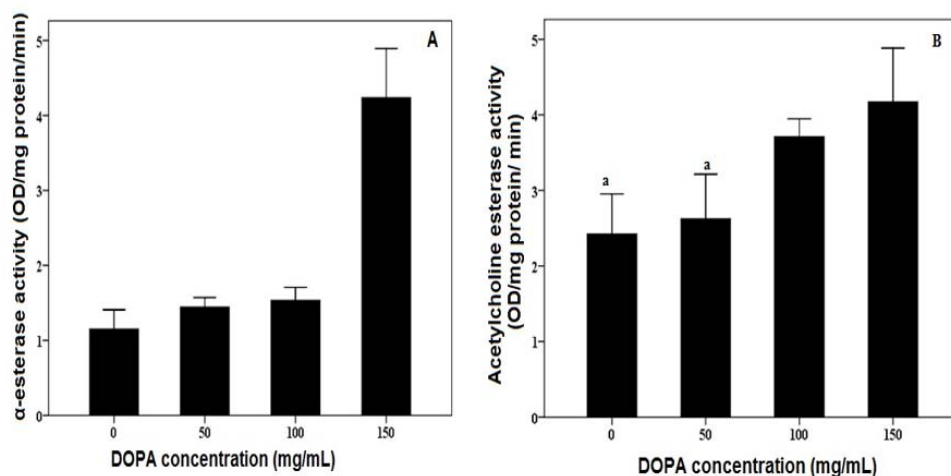
Fig. 4. Activity of detoxification enzymes α-esterase (A) and Acetylcholine esterase (B) enzymes in the gut tissues of 5th instars *Hermetia illucens* treated with different L-DOPA concentration (0 (control sample), 50, 100, and 150 mg/mL). Values represented as median ± P75 (N=3). Values shown as median ± P75 (*N=3*). Bars marked with same small letters indicates no significant difference using *k independent Kruskal-Wallis H (P>0.05)* between L-DOPA concentration.

CONCLUSION

This study investigated the impact of L-DOPA, a common phenolic compound found in organic waste, on the black soldier fly (*H. illucens*), a key species in insect-based waste management. The current findings demonstrate that exposure to L-DOPA induces significant oxidative stress in the larvae, as evidenced by increased levels of reactive oxygen species (ROS) and oxidative damage to proteins. In response to this stress, the larvae exhibited a robust antioxidant defense system, upregulating various antioxidant enzymes, such as glutathione reductase (GR) and polyphenol oxidase (PPO), and increasing the levels of non-enzymatic antioxidants. Additionally, the larvae activated detoxification enzymes, including α-esterase and acetylcholine esterase (AChE), to mitigate the toxic effects of L-DOPA. These results highlight the resilience of *H. illucens* larvae to environmental contaminants and their potential to biodegrade organic waste, even in the presence of challenging compounds like L-DOPA. However, prolonged exposure to high concentrations of L-DOPA may compromise the health and performance of the larvae, potentially affecting their ability to efficiently convert waste into valuable products. Therefore, it is crucial to consider the potential presence of contaminants in organic waste feedstock's and optimize feeding strategies to minimize adverse effects on the insect population. Further research is needed to explore the long-term consequences of L-DOPA exposure on the development, reproduction, and overall suitability of *H. illucens*

Ethical approval and consent to participate.

There are no experiments involving humans or animals that necessitate ethical approval in this article.

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تأثير **L-DOPA** على إدارة المخلفات باستخدام أدوات قائمة على الحشرات

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المستخلص

كلما زادت الزيادة السكانية زادت معها معدل توليد المخلفات. نتيجة لذلك، أصبح إدارة المخلفات الصلبة تحديًا بيئيًا رئيسيًا للحكومات والمجتمعات. لمواجهة هذه المشكلة، تم تطوير تقنيات مختلفة في جميع أنحاء العالم قائم على التدوير الحيوي فعلي سبيل المثال تم استخدام الحشرات كبديل واعد لإدارة المخلفات العضوية. ولكن مع ظهور خلط المخلفات بأنواعها قد تحتوي المخلفات العضوية على ملوثات، مثل الفينولات، وخاصة L-DOPA، والتي يمكن أن تؤثر على كفاءة التحويل الحيوي من خلال فرض تكاليف فسيولوجية. لذلك، هدفت هذه الدراسة إلى تقييم تأثير L-DOPA على يرقات ذبابة الجندي الأسود *Hermetia illucens* من خلال تعريضها لتركيزات مختلفة من L-DOPA (150-0 ملغ / مل). أظهرت النتائج أن التعرض الى L-DOPA يسبب إجهادًا أكسديًا كبيرًا في اليرقات، كما يتضح من زيادة مستويات الأكسجين التفاعلي (ROS) والتلف التأكسدي للبروتينات. واستجابةً لهذا الإجهاد، أظهرت اليرقات نظام دفاع أكسدة قويًا، حيث زادت من نشاط العديد من إنزيمات مضادات الأكسدة، مثل غلوتاثيون ريدكتيز (GR) وبوليفينول أوكسيديز(PPO) ، وزيادة مستويات مضادات الأكسدة غير الإنزيمية. بالإضافة إلى ذلك، نشطت اليرقات إنزيمات إزالة السموم، بما في ذلك α-esteraseوacetylcholine esterase (AChE)، لتخفيف الآثار السامة لـ L-DOPA. تُسلط هذه النتائج الضوء على مرونة يرقات *H. illucens* في مواجهة الملوثات البيئية وقدرتها على تحليل النفايات العضوية بيولوجيًا، حتى في وجود مركبات صعبة مثل L-DOPA. ومع ذلك، فإن التعرض المطول لتركيزات عالية من L-DOPA قد يضر بصحة وأداء اليرقات، مما قد يؤثر على قدرتها على تحويل النفايات بكفاءة إلى منتجات قيمة. لذلك، من المهم مراعاة وجود ملوثات محتملة في المواد الخام للنفايات العضوية وتحسين استراتيجيات التغذية لتقليل الآثار الضارة على تعداد الحشرات. يلزم إجراء المزيد من الأبحاث لاستكشاف العواقب طويلة المدى لتعرض L-DOPA على نمو وتكاثر ولياقة *H. illucens* بشكل عام.

الكلمات المفتاحية : إنزيمات إزالة السموم، الإجهاد التأكسدي، ذبابة الجندي الأسود، تدوير المخلفات العضوية، المركبات الفينولية.