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Research Article

Insect Lipase Activity as a Useful Indicator of Entomoremediation of Oil Contamination

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Abstract

Anthropogenic releasing of oil into the environment is the main source of environmental pollution. Oil contamination is considered as one of hazardous materials to ecosystem components. The severity of damage may reach to all organisms through food chain and food web. So, the demand for creative and eco-friendly way is vital to decontaminate the deleterious effect of oil contamination. This study focuses on using lipase activity and oxidative stress parameters of black soldier fly larva (BSFL) as an indication of eco-friendly biodegradation tool of oil contamination. The results showed that, the insect lipase activity was significantly increased along time treatment (0-48h). Besides that, the lipid peroxidation concentration as a deleterious effect of oil contamination was decrease in the cuticle homogenates than the fat body. Also, the correlation between application time and lipase activity or lipid peroxides was strong to moderate positive correlation. These results emphasized the ability of using insect lipase activity and oxidative stress parameters as lipid peroxidation amount as a valuable indicator of entomoremediation of oil contamination.

Keywords: *Hermetia illucens*; Oil Contamination; Lipase; Lipid Peroxidation; Entomoremediation

Highlights

- Insect lipase activity allows a potential biomonitoring of oil degradation.
- A highly significant correlation with time post treatment was confirmed in the cuticle and fat body homogenates of BSFL.
- The oil pollution causes comparable adverse effects in living organisms.
- The using of BSFL as an entomoremediation agent of oil contamination was confirmed.

Introduction

Oil contamination is considered as the main problem to environment [1,2]. It can enter marine and terrestrial environmental components through different natural deposits [3]. Also, human activities in the production, transportation and storage of petroleum are another route of oil contamination [4]. Besides that, the accidental spills, misuse of oil products and improper management of oil waste fractions can act as a source of serious pollution [5]. This environmental pollution leads to deleterious effect on all living organisms in association with climate change [6]. So, the demand for eco-friendly and cost-effective methodology for pollutants decontamination is rising nowadays to save our planet [7]. As the results of hydrophobic characters of hydrocarbons, water-insoluble and more resilient to degradation; crude oil causes pollution of drinking water decreases water and air quality, soil fertility [8]. In addition to, petroleum hydrocarbons can block the soil pores which may decrease soil aeration and water permeability, resulting in toxicological and ecological effects on plants [9].

There are a lot of methodologies using for monitoring and controlling oil contamination such as physicochemical and biological treatment [10,11]. The physicochemical methodologies include incineration, thermal deposition, land filling, solvent extraction and cement kiln [12]. Otherwise, biological treatment includes the using of fungus, bacteria, or insects in the treatment of oil contamination. Also, bioremediation behaves as eco-friendly and most effective tool of remediation of oil contamination as a result of living organisms' sensitivity to environmental stressors [13].

In this context, there were a lot of studies concluded that many techniques can be used for the removal of heavy metals pollutants. The using of cation exchanger- sodium dodecyl sulfate acryl amide for the removal of Pb^{+2} from an aqueous solution [14]. Also, adsorption-desorption isotherms can be used to examine the bioavailability of Zn in calcareous soil [15]. Another study revealed the use of acetonitrile stannic selenite composite as ion exchange material for the removal of heavy metals [16]. Although ion exchange methodology is used for removal heavy metals to parts per billion, it considered as expensive, pH sensitive, not specific methodology. Besides that, activated carbon or zirconium oxide composite was used for the adsorption of cadmium ions from the aqueous medium [17]. These methodologies have some limitations including damage of membranes, production of contaminated sludge, inefficient removal of contaminants, expensive, and extensive use of energy [18]. So, the demand for using bioremediation technology is growing up as a result of traditional methods inadequately.

Bioremediation is an efficient and eco-friendly methodology to treat petroleum-contaminated soil [19]. It is characterized by limited cost required and non-toxic production. Also, there are an approach to combine two different living organisms in the bioremediation process such as the combination between plant and rhizosphere bacteria. This synergistic effect can be used to overcome the decreasing of phytoremediation and bioaugmentation alone [20,21]. The using of insects as a biomonitoring agent of environmental pollutants has a great attention nowadays as many features especially belongs to wide availability, cost efficiency, eco-friendly, no ethical consideration, large numbers of individuals per female [22-28]. One of these insect species is black soldier fly (BSF). It occurs in the tropic and temperate regions around the world. The larvae of BSF have been used as alternate protein production, organic waste management and bioremediation of pollutants [29].

The using of insect oxidative stress parameters in addition to lipase activity is considered as a novel application to measure the bioremediation ability As environmental pollutants lead to production of reactive oxygen species (ROS). The oxidative damage to lipids, proteins and nucleic acids as results of ROS production can be used as an indicator of decontamination ability [30-33]. Therefore, the aim of this study was to assess the activity of lipase enzyme and lipid peroxidation of BSFL along time course (0, 12, 24, 48 h) of oil contamination treatment, comparing to control group. This phenomenon can be used to answer some questions as: Is black soldier fly larvae can decontaminate oil pollution effect? Are the biochemical parameters can be used as an eco-friendly, cost reduction, and effective tool for bioremediation concept?

Materials and Methods

Experimental sample

The larval 5th instar of black solider fly, *Hermetia illucens* were obtained from colony at Entomology Department, Faculty of Science, Cairo University. About 10 Kg Organic waste was obtained from household source and was mixed with 1 L used oil. Insects were fed on contaminated oil source for 5 days then were forced to stop feeding after 0, 12, 24, 48 h post treatment. For each experimental group, 50 insects pool of 5th instars of *H. illucens* were dissected to isolate tissues for further analysis and were stored at -20°C until use.

Biochemical analysis

The activity of lipase enzyme was determined spectrophotometrically according to the method of Abd-Elhakeem., *et al*. [34]. Briefly, after homogenization of samples in tris HCl buffer (0.1 M; pH=7.0) containing Triton X-100 (0.1% (w:v)), 2.4 mL of 165 mM phenyl acetate was added to 0.1 ml sample and incubated at 40˚C for 10 min. The absorbance was measured at 750 nm. Lipase activity was expressed as OD/mg protein/min.

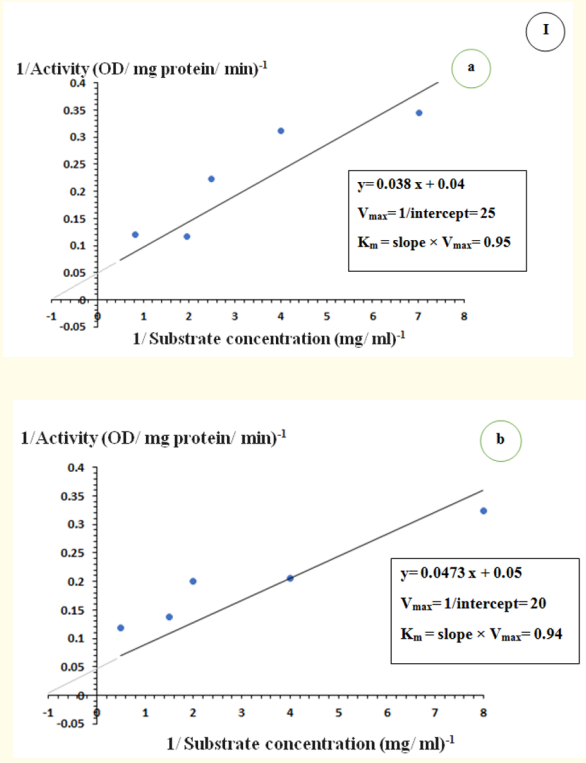
The lipid peroxides concentration was measured according to Hermes-Lima., *et al*. [35]. Experimental tissues were isolated in phosphate buffer (pH = 7.0), and homogenized in ice-cold methanol (1:5, w/v). After homogenization (mortar, 10 strokes/30 seconds), the samples were centrifuged at 2000 *g* for 10 min at 4˚C. A 5 mL aliquot of the supernatant was used for the assay. The following components were sequentially added to the samples (200 µL of supernatant): 400 µL of 1 mM $FeSO_4$, 200 µL of 0.25 M H_2SO_4, and 200 µL of 1 mM xylenol orange. Samples were then incubated under dark conditions at room temperature for 3 h. The absorbance was measured at 580 nm. Then, 10 µL of 0.5 mM cumene hydroperoxides (as an internal standard) was added to each sample, and the samples were maintained at room temperature for 1 h before the absorbance was re-measured at 580 nm. The change in absorbance due to addition of internal standard was calculated. Lipid peroxides concentration was expressed as mM cumene hydroperoxides/mg protein. The total protein concentration of samples was determined using spectrophotometer according to the method of Bradford [36].

Statistical analysis

Statistical analysis was performed using IBM SPSS Statistics for Windows (Version 17.0. Armonk, NY: IBM Corp.) and Microsoft Excel. Non-parametric tests were carried out using the k independent Kruskal–Wallis test. These non-parametric tests were assessed on the lipase activity and lipid peroxidation concentration are expressed using median and quartile deviation (25th and 75th percentiles: P25 and P75). Correlations between the time of incubation and the lipase activity and lipid peroxidation concentration were performed based on Pearson's regression analysis using multiple regression models. However, the kinetics parameters of lipase enzymes were determined and drawn using Microsoft Excel functions.

Results

The results showed the kinetics parameters of lipase enzymes for naïve samples in figure 1. It revealed that most significant highest value of lipase enzymes activity at 2 mg/mL substrate and at 15% enzyme concentration and therefore the V_{max} of lipase enzyme equal to 25 in cuticle samples and 20 in fat body experimental samples with a chi square value equal to -0.121 and -8.01 in cuticle and fat body experimental tissues samples (Figure 1).



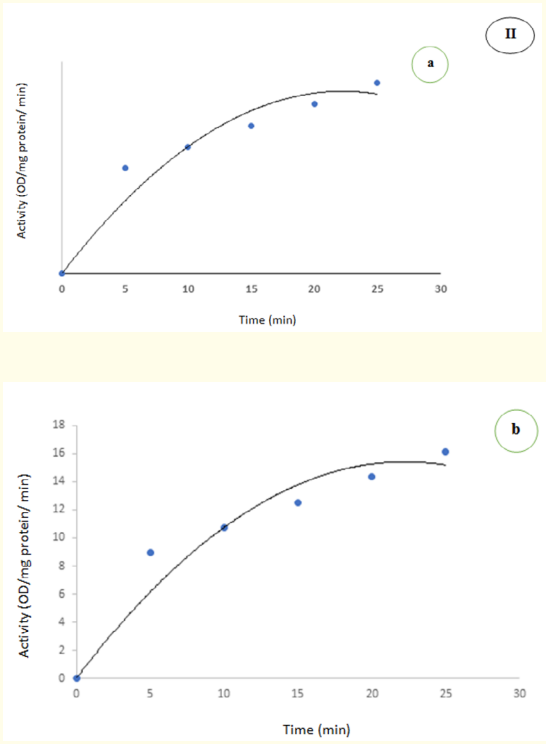
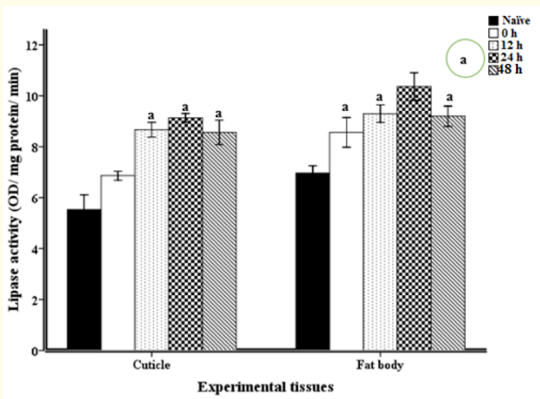


Figure 1: Kinetics of lipase enzymes (I) different substrate concentration, using Linear Weaver-Burk plot, to determine Vmax and Km of the degradation enzyme of lipid (II) different enzyme concentration, to determine the optimal concentration, inside experimental tissues (a) cuticle (b) fat body of naïve 5th instars of black solider fly (BSFL) *Hermetia illucens*, which expressed as OD/mg protein/min.

Also, the results showed that, lipase enzyme activity and lipid peroxidation concentration were significantly lower in homogenates of cuticle samples than fat body of 5th instars larvae *H. illucens* in naïve and polluted samples (Figure 2). Besides that, the lipase activity in the cuticle tissue of 5th instars BSFL showed a fluctuation pattern at 12, 24, and 48 hours post treatment. Unlikely, the fat body samples were significantly increased in lipase enzyme activity at 24 h post oil waste treated, with respect to control and naïve insects' samples (Figure 2a). Indeed the highest values of Lipid peroxidation concentration were found in 24 h post treatment in both cuticle and fat body experimental tissues (Figure 2b), however, the concentration of lipid peroxidation decline post 24 hours treatment with the fold of 0.12 than 24 h in both cuticle and fat body samples (Figure 2b). In the 48 hours post treatment case, the insect

lipase activity showed no significance difference between 12, 24, 48 h and 0, 12,48 h post treatment in cuticle and fat body tissues, respectively (P > 0.05) (Figure 2a). However, the lipid peroxidation in cuticle and fat body insects showed a significant decrease than 24 h post treatment (Figure 2b).



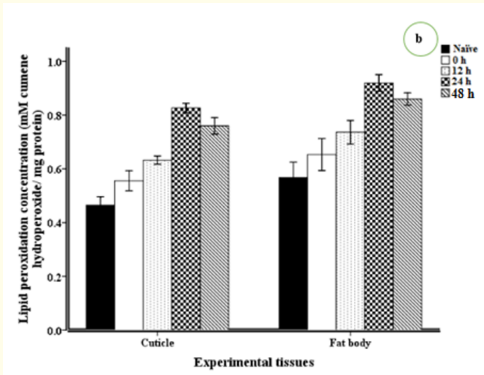


Figure 2: Lipase enzyme activity assay (a), which expressed as (OD/mg protein/min) and Lipid peroxidation concentration (b), which expressed as (mM cumene hydroperoxide/mg protein) of naïve, and treated insect with waste oil at different time of incubation (0, 12, 24, and 48 hours), experimental tissues obtained from cuticle and fat body homogenates of 5th instars of *Hermetia illucens*.

Additionally, the results emphasized that Pearson's correlation coefficient among time post treatment and lipase enzyme activity or lipid peroxidation concentration were positive strong to moderate correlation in both cuticle and fat body homogenates of 5th instars of *Hermetia illucens* (Table 1).

Treatment	Experimental tissues	r	Equation	Type of equation	R^2
Lipase enzyme	Cuticle	0.702*	$y= -0.004x^2 + 0.19x +6.8$	Polynomial	0.93
	Fat body	0.455	$y=-0.003x^2 + 0.14x + 8.4$	Polynomial	0.63
Lipid peroxidation concentration	Cuticle	0.838**	$y=-0.003x^2 + 0.015x + 0.53$	Polynomial	0.82
	Fat body	0.831**	$y=-0.0002x^2 +0.015x +63$	Polynomial	0.80

Table 1: Pearson's correlation coefficient among time post treatment and lipase enzyme activity or lipid peroxidation concentration (which expressed as OD/mg protein/min, and mM cumene hydroperoxide/mg protein), samples obtained from cuticle and fat body homogenates of 5th instars of *Hermetia illucens* treated with contaminated oil waste.

Discussion

In the present work, the biochemical parameters including lipase activity and oxidative stress parameters were used to assess the impacts of oil decontamination ability using insect. Black soldier fly larvae, *Hermetia illucens*, were treated with oil waste pollutants and the biochemical results of both treated and naïve insects were compared with each other. However, entomoremediation considered as a new area, there were a little of studies discuss this essential topic [37-42]. In the quoted works, some studies covered the xenochemicals detoxification by insects through studying specialized enzymes or using insect's gut bacteria. The main difference between this study and quoted works is the using of source of polluted. In this study, the pollutant source is oil waste, while the others used polyethylene, polystyrene, heavy metals, hydrocarbons, and wastewater pollutants. Almost these pollutants source can disrupt the redox hemostasis in cases of within or exceed normal permitted levels and cause macromolecules damage as lipid peroxidation, protein carbonylation and even DNA damage to all surrounding living organisms and even through food chain [22-24,46-48].

Generally, the kinetics parameters in tissues of *H. illucens* from Naïve group were optimized at optimum condition for enzyme and substrate concentration (Figure 1). Also, the results showed that the lipase activity and lipid peroxides concentration of treated insects was significantly higher than in naïve individuals (Figure 2). The obtained results indicated a possible impact of oil waste contamination on the biochemical parameters and even cause macromolecules damage, in form of lipid peroxides, in *H. illucens*. Therefore, the potential use of biochemical analysis and oxidative stress parameters as an indicator of oil contamination entomoremediation [37,41].

Analysis of lipase activity and lipid peroxides concentration parameters (Figure 2) revealed that cuticle tissues of larvae more sensitive than fat body and could be consider as a good responding tissue system. The damage level in this tissue reflected the level of pollution a long hour's post treatment. However, it should be stressed here that lipid damage and activity of lipase varied among analyzed tissue. Surely, the difference is coming from the tissue function and stressing factors. Cells of cuticle have direct contact with all exogenous factors that surrounding individuals in the polluted environment. On the other hand, fat body cells are characterized by storage area and high metabolism rate and extensive consumption of oxygen [43]. Reactive oxygen species (ROS) are generated from natural and anthropogenic source [44]. Nature source occur during cell respiratory, however, anthropogenic source from environmental pollutants [24,26,28]. The oil contamination can increase the production of ROS in the cells, tissues and individuals exposed to them, and therefore can cause oxidative stress [23,25]. This can explain the fluctuation of results along time course post oil contamination treatment. Yang., *et al*. [38] showed that wax worms can degrade polyethylene. Also, Yang., *et al*. [39] reveled that meal worms can degrade polystyrene, which approved the ability of using insect as a biodegradation tool, in form of entomodegradation. While the lipid metabolism or breakdown can be occurred using serine hydrolyses enzyme called lipase. This enzyme has an essential role in the biochemical process like lipid metabolism and transport. Also, it can regulate the cell signal transduction [45].

The relationship between biochemical analysis parameters and time post treatment of oil contamination treatment in the present study has a clear pattern. The positive strong to moderate correlation was obtained in both experimental tissues and biochemical parameters (lipid peroxides concentration and lipase enzyme activity) of *H. illucens* (Table 1). This also were occurred in various previous studies, In Abdelfattah and Renault [46] study, the correlation factors among epinephrine concentration and oxidative stress parameters of *Sarcophaga dux* showed a unique pattern which ensure the potential oxidative stress insect response to all environmental pollutants even within normal levels. Besides that, the study of Abdelfattah [47], tested the different concentrations and application time of vitamin B12 on antioxidant response of *Physiophora alceae*. These results confirmed that correlation factors among the treating insects with different time and concentration of vitamin B12 had a positive correlation. These results indicate the insect ability to reduce oxidative stress factors and use them in biological treatment of drug and plant residues. Additionally, the study of Abdelfattah and Lim [48] approve the ability of using black soldier fly larvae as a possible organic waste recycler. Additionally, BSFL can convert the hazardous effect of un-treated organic waste into insect valuable biomass.

Conclusion

In this study, we have examined the effects of the oil contamination, which is considered as an anthropogenic source of pollution. The oil contamination can be further transferred to the environment, with potential consequences for wildlife. We demonstrated that larvae of the black soldier fly larvae that received the highest oil contamination time exposure concentration were characterized by decreased the lipid peroxides amount regarding to 24 h post treatment, and decreased the activity of lipase enzymes to the value of 0 h post treatment in fat body tissues. Theses results suggesting the ability of BSFL to decontaminate oil pollution and may have had a protective effect against ROS production, as formerly reported from other animal taxa. The changing patterns we measured for lipase and lipid peroxidation levels, combined with former evidence reporting increased oil metabolism in treated tissues (fat body and cuticle), suggested that higher amounts of lipids were go through lipid peroxidation mechanisms. Increased lipase enzyme activity would have helped containing ROS production by restore redox status and even using insect as a bioremediation agent of oil contamination.

Ethical Approval and Consent to Participate

This article does not contain any studies with human participants or animals that require ethical approval.

Consent for Publication

Not applicable.

Availability of Data and Materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Competing Interests

The authors declare that they have no conflict of interest.

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