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Using of Black Solider Fly Larvae as A Biodegradation Tool of Oil Contamination of Organic Waste

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Abstract

This study focusses on using black soldier fly, *Hermetia illucens* larvae (BSFL) as a biodegradation tool of oil contamination of organic waste. The results showed that, the 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. The tested samples revealed a positive correlation (from moderate to strong relationship) in both tissues cuticle and fat body in both experimental tests lipase enzyme activity and lipid peroxidation concentration. Lipid peroxidation concentration were significantly lower in homogenates of cuticle samples than fat body of 5^{th} instar flesh fly larvae *H. illucens* in naïve and polluted samples.

Keywords: Hermetia Illucens, Oil Contamination, Lipase, Lipid Peroxidation.

Introduction

In the tropic and temperate regions of the world, the black soldier fly (BSF) is distributed throughout. The larvae of BSF have been proposed as a means to address two global issues: alternate protein production and organic waste management [1]. Functional homeostasis which is termed oxidative stress affected by various chemicals, physical and physiological stressors and it is characterized by enhanced production of reactive oxygen species (ROS) with the simultaneous impairment of their scavenging systems. The oxidative damage to lipids, proteins and nucleic acids and thus the function of the cells, organs or the whole organism may be seriously disrupted, leading to death is resulted from increased concentrations of ROS [2] Hydrogen peroxide, H2O2 mostly produces ROS as a steady intermediate among all kinds of stressors, sever damage of proteins or DNA, as well initiate lipid peroxidation are resulted from high concentrations of H2O2 [3, 4, 5]. Therefore, in this study the activity of lipase enzyme was determined and lipid perioxidation of BSFL were studied, with the aim of using of related Dipteran insects as a biodegradation tool of oil contamination of organic waste.

Materials and Methods

The larval 5th instar of black solider fly, *Hermetia illucens* were obtained from colony at Entomology Department, Faculty of Science, Cairo University, organic waste which obtained from household within Giza government, Egypt and was mixed with 6.78 mg/L waste oil contamination. Insects were divided into 10 groups (which divided to 2 sets of different tissues fat body and cuticle) each set of same tissues divided into 5 subgroups naïve, 0, 12, 24, 48 h post incubation organic waste contaminated with waste oil. For each experimental group, 50 insects pool of 5th instar of *H. illucens* were dissected to isolate tissues for further analysis and were stored at −20 °C until use.

The activity of lipase enzyme was determined spectrophotometrically according to the method of [6] 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 165mM 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.

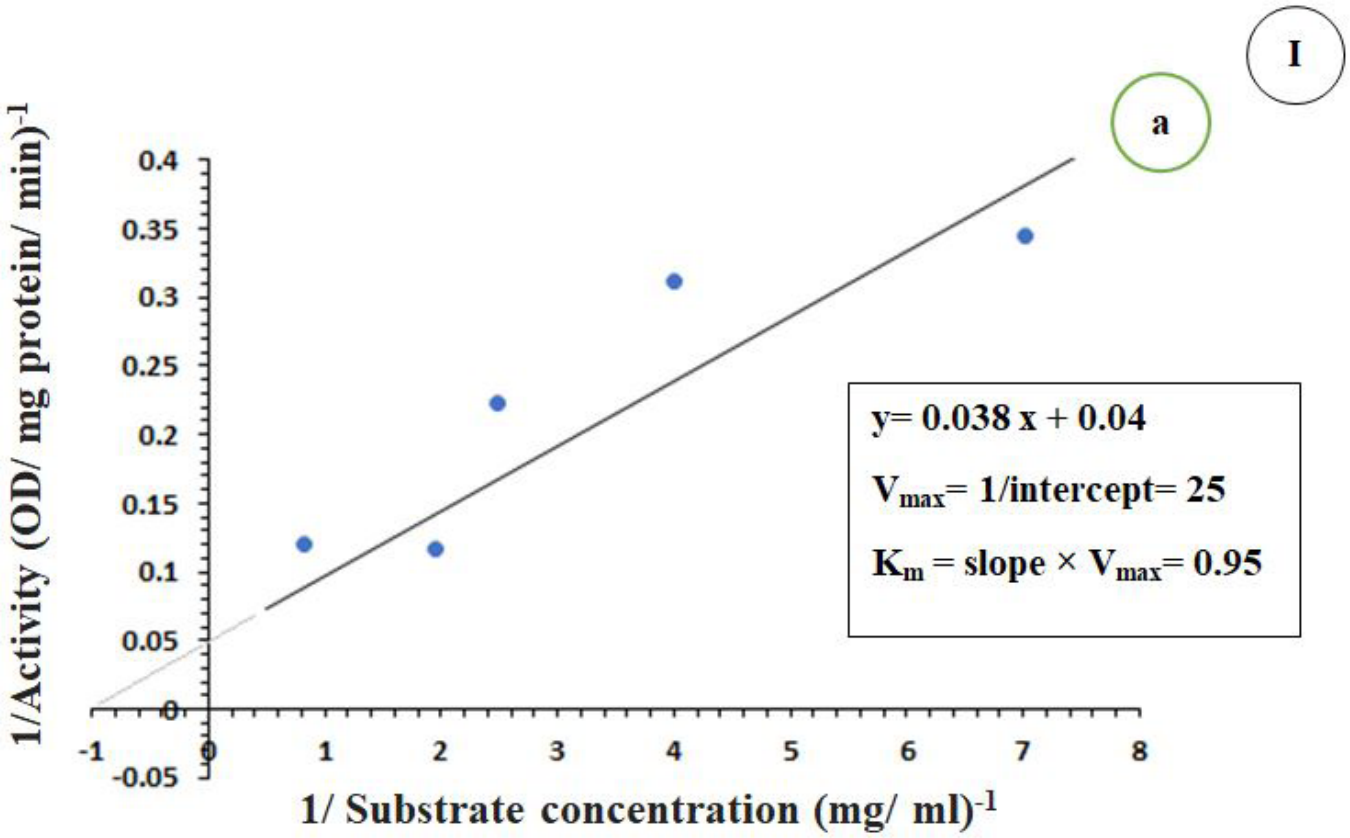
The lipid peroxides concentration was measured according to [7]. Experimental tissues were isolated in phosphate buffer (pH 7.0), and homogenized in ice-cold methanol (1:5, w/v). After homogenization (mortar, 10 strokes/30 seconds), the samples were centrifuged at 2000 *g* for 10 min at 4 ˚C. A 5 mL 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 spectrophotometrically according to the method of [8].

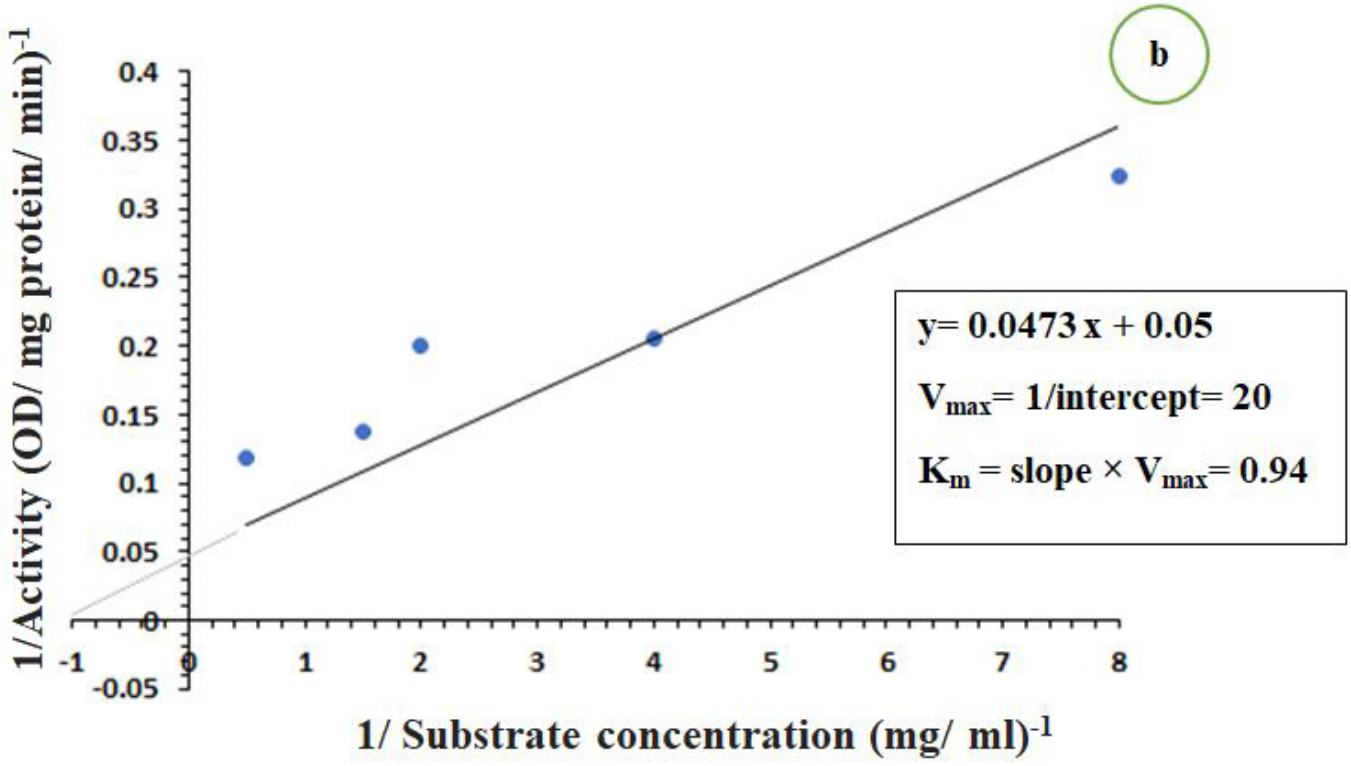
Statistical analysis was performed using IBM SPSS Statistics for Windows (Version 17.0. Armonk, NY: IBM Corp.). Non-parametric tests were carried out using the k independent Kruskal–Wallis test. These non-parametric tests were assessed on the lipase activity and lipid peroxidation concentration are expressed 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.

Results and discussion

The results revealed that, the 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).

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 instar flesh fly larvae H. illucens in naïve and polluted samples. In 5th instar flesh fly larvae treated with contaminated organic waste with oil for 12, 24, 36 hours incubation in cuticle experimental tissues of lipase enzymes. Fat body samples were significantly increase in lipase enzyme activity at 24 h post oil waste treated, with control and naïve samples of insect larvae (Figure 2a) The highest values of Lipid peroxidation concentration were found in 24 h post treatment in both cuticle and fat body experimental tissues (Figure 2b), then the concentration of lipid peroxidation decline around 24 hours' treatment.


I
a
1/Activity (OD/ mg protein/ min)-1
0.4
0.35
0.3
0.25
0.2
0.15
0.1
0.05
0
-0.05
-1
0
1
2
3
4
5
6
7
8
1/ Substrate concentration (mg/ ml)-1
y= 0.038 x + 0.04
Vmax= 1/intercept= 25
Km = slope × Vmax= 0.95


b
1/Activity (OD/ mg protein/ min)-1
0.4
0.35
0.3
0.25
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0.15
0.1
0.05
0
-0.05
-1
0
1
2
3
4
5
6
7
8
1/ Substrate concentration (mg/ ml)-1
y= 0.0473 x + 0.05
Vmax= 1/intercept= 20
Km = slope × Vmax= 0.94

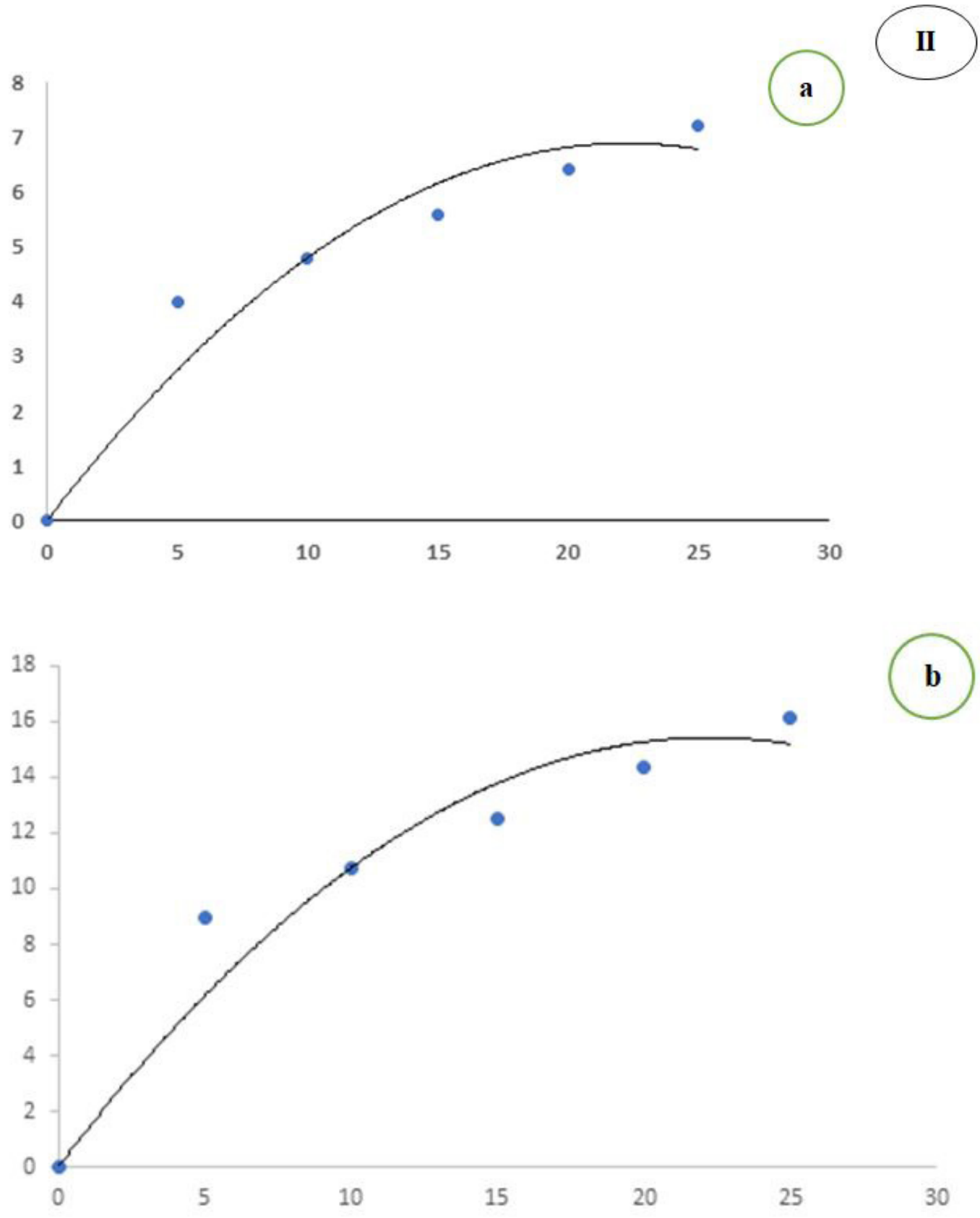

Figure 1: Kinetics of lipase enzymes (I) different substrate concentration, using Linear Weaver-Burk plot, to determine Vmax and Km of the degradation enzyme of lipid (II) different enzyme concentration, to determine the optimal concentration, inside experimental tissues (a) cuticle (b) fat body of naïve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

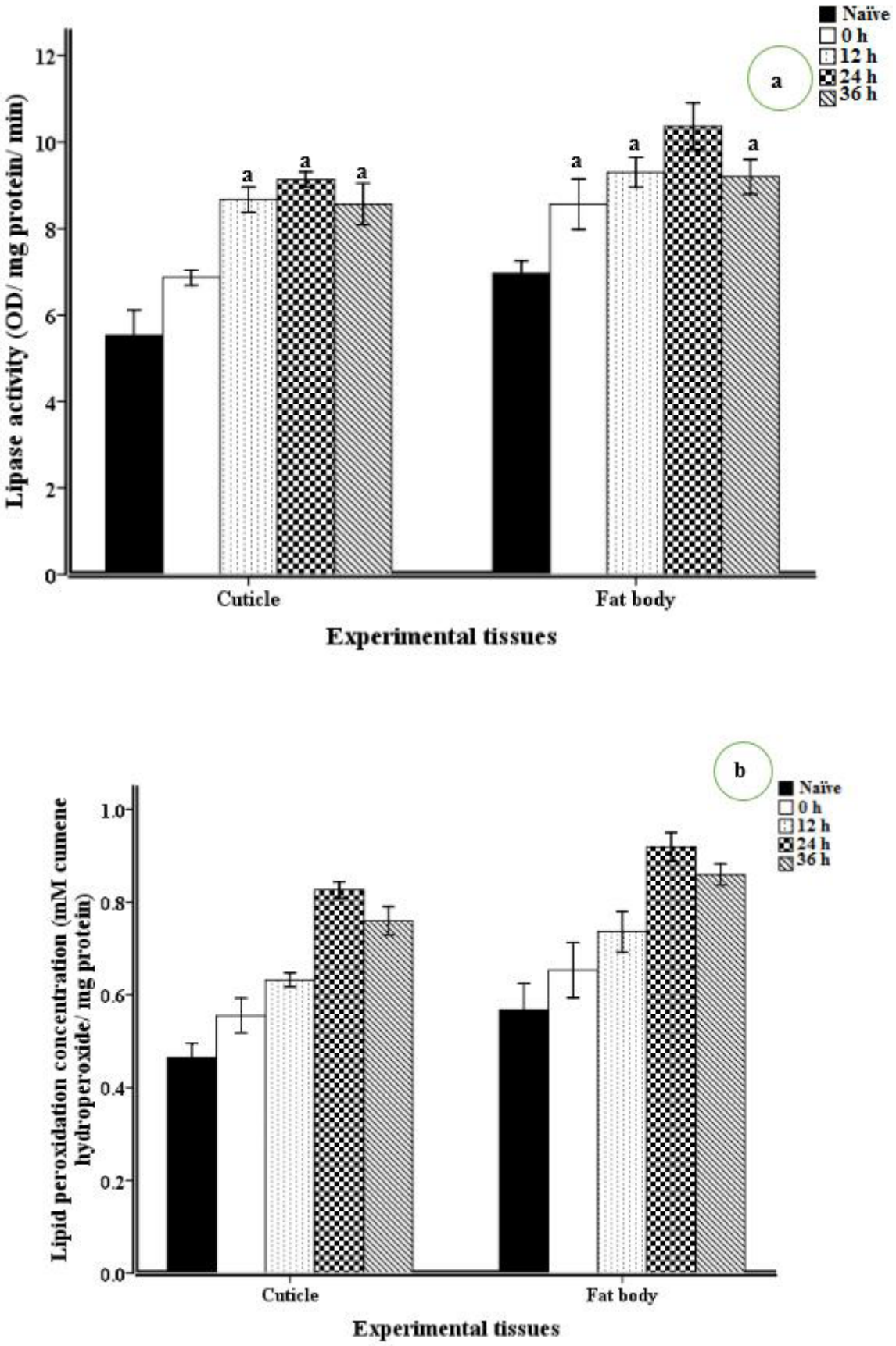

Figure 2: Lipase enzyme activity assay (a), which expressed as (OD/ mg protein/ min) and Lipid peroxidation concentration (b), which expressed as (mM cumene hydroperoxide/ mg protein) of naïve, and as insect fed on organic waste contaminated with waste oil with different time of incubation (0, 12, 24, and 36 hours), experimental tissues obtained from cuticle and fat body homogenates of 5th instar of Hermetia illucens.

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 Lipase enzyme activity and 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 instar of Hermetia illucens fed on organic waste contaminated with oil waste and incubated for different time of incubation (0, 12, 24, and 36 hours) post treated

[9] mentioned that lipids as energy sources are breakdown by lipase which is classified as serine hydrolyses. These enzymes have key role in the physiological process like lipid metabolism and transport, regulation of plasma membrane lipid and cell signal transduction.

Assessment of the overall relationship among incubation time post treatment Organic waste contaminated with oil waste (0, 12, 24, and 36 h) and mean activity of Lipase enzyme activity and lipid peroxidation concentration in cuticle and fat body homogenates samples of 5th instar BSF larvae H. illucens were performed. The tested samples revealed a positive correlation (from moderate to strong relationship) in both experimental tissues cuticle and fat body in both experimental tests lipase enzyme activity and lipid peroxidation concentration. (Table 1).

[10] stated that cellular toxicity like lipid peroxidation is resulted from reactive oxygen species (ROS) which disrupts membrane fluidity and the degradation products led to initiate cellular apoptosis. Higher levels of both lipid peroxidation and protein oxidation at 12 and 24h post-Btk infection of *Ades caspius* larval mosquitoes compared to those of control ones are observed by [11].

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