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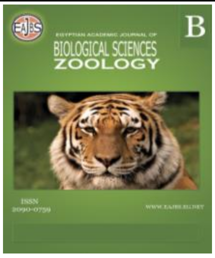

Ameliorative Effects of *Spirulina platensis* and *Ulva sp.* on Biochemical Aspects and Residues of Lambda-Cyhalothrin in the Water of Nile Tilapia (*Oreochromis niloticus*)

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ABSTRACT

This study was designed to assess the ameliorative effects of *Spirulina platensis* (SP) and *Ulva sp.* (US), as feed additives into a fish commercial feed, for reducing pesticide lambda cyhalothrin-residues in water and fish muscles. In the present experiment, the concentration tested was 2.325 µg/l as 1/10 of the determined LC_{50} - 96 hours was used for 28 days. The experimental design represents 6 groups: group 1 was control (cont.), group 2 was treatment with lambda-cyhalothrin (LCT) and two algae species with two concentrations each (5% and 10% of diet + lambda-cyhalothrin) are the other four groups. After the experimental period, serum biochemical tests (Glucose, Total Protein, Albumin, Globulin, AST, ALT, Urea, Creatinine and lipid profile) were determined. All biochemical analysis showed significantly varied in-between treatment except urea levels, while, these parameters were in normal rang in fish groups fed by both concentrations of SP and US. The residue levels of lambda-cyhalothrin in water samples were decrease in 10% concentration compared with 5% concentration in both algae. SP and US with two concentrations can efficiently remove lambda-cyhalothrin residues in fish muscles. Water quality (temperature, dissolved oxygen and pH) were no significant differences between all treatments from the beginning till the end of the experimental period.

Conclusion: *Spirulina platensis* and *Ulva sp* have a big role in the reduction in the effect of lambda-cyhalothrin on fish and the improvement of water quality.

INTRODUCTION

Foreign organic chemicals are continually overloading the aquatic environment. Pesticides are the most important organic trace pollutants extensively used in agriculture and in aquatic areas, having adverse effects on biological life, including the ingestion of affected fish by humans. This makes aquatic pollution a target problem that attracts many researchers worldwide (Firat *et al.*, 2011). In essence, lambda-cyhalothrin [alpha-Cyano-3-phenoxybenzyl 3-(2-chloro- 3,3,3-trifluoropropenyl)-2,2-dimethylcyclopropanecarboxylate; which has 1:1 reaction mixture of the (Z)-(1R,3R), (S) ester and (Z)-(1S,3S), (R) ester] is one of the most successful pyrethroids on the market in the wide range (Kumar *et al.,* 2009). Lambda-cyhalothrin (LCT), is a non-systemic insecticide (a synthetic pyrethroid). It acts on the nervous system of the insects, by disrupting the function of neurons by interaction with the sodium channel. LCT has been used in potatoes, vegetables, cotton, and other crops to control a wide spectrum of insect pests, such as aphids, Colorado beetles, thrips, lepidopteran larvae, coleopteran larvae and adults, etc., (MacBean, 2012).

In Extension Toxicology Network, lambda-cyhalothrin is categorized as a restricted-use pesticide for its toxicity to fish and has been shown to be extremely toxic to marine invertebrates and fishes. It could influence the composition and abundance of fish as they find their way to aquatic environments via runoff (Kumar *et al.,* 2010).

Algae have become significant organisms for the biological purification of wastewater since they are able to accumulate plant nutrients, heavy metals, pesticides, organic and inorganic toxic substances and radioactive matters in their cells/bodies. *Spirulina platensis* is a photosynthetic, filamentous, and blue-green micro-algae. It is generally regarded as a rich source of vitamins, essential amino acids, minerals, essential fatty acids (γ-linolenic acid), and antioxidant pigments such as carotenoids and phycocyanin (Jaime-Ceballos *et al*., 2006). *Spirulina platensis* has been speculated to be associated with modulation of the host immune system (Hironobu *et al*., 2006). Dried algae improve growth and physiological response to stress and diseases in several species of fish. In addition, it is effective as an immune-modulator (Takeuchi *et al*., 2002). Spirulina contains several active pigments, notably β-carotene and phycocyanin, which have anti-oxidant and anti-inflammatory activities (Wu *et al*., 2016). Also, the chlorophyll content of spirulina also acts as a cleansing agent and a detoxifying phyto-nutrient. Macro-algae, commonly known as seaweeds, are fast-growing organisms that resemble plants. Due to their varying intrinsic characteristics, seaweeds are used in ecosystem balancing in mitigating eutrophication for nutrient management or as bioremediation (Kim *et al*., 2017). The genus Ulva, popularly known as sea-lettuce, is one of the most common and abundant green macro-algae throughout the world (Lahaye and Robic, 2007). Ghouneim *et al*., (2014) concluded that the dead biomass of green alga *Ulva sp*. could be used as efficient, low cost and very important to the health of the ecosystem.

The present study was carried out in order to investigate:

1 - Effects of lambda-cyhalothrin on some biomarkers of fish and water quality.

2 – Determination of lambda-cyhalothrin residues in water and fish muscles.

3 - Quantify the effect of some algae (*Spirulina and Ulva*) on the elimination of lambda-cyhalothrin and the improvement of some biochemical constituents in exposed fish.

MATERIALS AND METHODS

Pesticide Selected:

Lambda-cyhalothrin (Effect Power 5% EC) formulation by the Mammalian and Aquatic Toxicology Department, Central Agricultural of Pesticides Laboratory (CAPL), Agricultural Research Centre (ARC), Dokki, Giza, Egypt.

Collection of Marine Algae Samples:

Green macro-algae, *Ulva sp*. samples were collected along the north coast of Egypt, Alexandria region. The samples were picked up by hands and immediately washed with seawater to remove the foreign particles, sand particles and epiphytes as much as possible. Then it was kept in an icebox, containing frozen gel cold packs and immediately transported to the laboratory. On arrival, plants were washed thoroughly using tap water to remove the salt on the surface of the plants, then thoroughly cleaned out to eliminate the other species of seaweeds as well as adhering epibiota (zooplankton and bivalves) to the algal surface, sediments and detritus, then washed again with fresh water next rinsed in distilled water to remove any existing mineral particles. After that, the algal fronds were individually blotted on a paper towel to remove excess water then dried in the drying oven at 60 ºC for 48 h then kept refrigerated until extraction was carried out (Reham *et al*., 2019).

Cultivation and Production of Spirulina:

Spirulina platensis was obtained from the phytoplankton lab belonging to the limnology department - Central Laboratory for Aquaculture Research - Agriculture Research Center. The culture was routinely maintained in modified Aiba and Ogawa, (1977) liquid medium. Spirulina production and in-door cultivation were done using vermicompost (from fish sludge). The culture medium used was 100 g of dry vermicompost. The vermicompost was suspended in 10 l of aerated tap water for 2 days before being used. Sodium Metabisulfite (50 mg / 10 l media) was added to prevent microbial contamination. After 24 h, sodium bicarbonate 850 g / 10 l media were added before the beginning of the injection of *S. platensis* (Thepparath *et al*., 2009). The production of *S. platensis* was started by inoculation (10 ml / l) from the *S. platensis* that was cultivated before in Aiba and Ogawa liquid (Aiba and Ogawa, 1977).

Harvesting, Extraction and Processing the Algal Biomass:

In the stationary phase, the algal culture reached maximum growth, in this case, the circulation provided by the pumping system was stopped and the algal cells were harvested by filtrating the carboy using cheesecloth of nylon. The algal cells (*Cyanophyta*) were dried in an oven at 60 ºC and ground in an electric coffee mill (Reham *et al*., 2019).

The Experimental Design:

This work was conducted in the Central Laboratory for Aquaculture Research - Agriculture Research Center – Egypt to investigate the effect of using two algae species (*Spirulina platensis* and *Ulva sp*.) for removing residues of lambda-cyhalothrin pesticide from water and bodies of Tilapia fish *(Oreochromis niloticus)*.

Firstly, determination of 96 hours-LC_{50}, was carried by five concentrations were taken for each test and 10 fish (*O. niloticus)* were introduced in aquarium of 80 l capacity, and percentage of mortality was recorded during 96 hours. According to the equation of Behreus and Karbeur (1953), the 96 hours-LC_{50} of lambda-cyhalothrin was 23.25 µg/l.

In the current experiment, the concentration tested was 1/10 LC_{50} of lambda cyhalothrin (2.325 µg/l) for sub-lethal exposures (28 days)**.** Twelve aquaria represent six treatments each of two replicates were used. A total of 120 fish, fingerlings of about 27 g initial average weights were randomly allotted in the twelve experimental aquaria with 10 fish / aquarium. Six groups are defined by the experimental design as follows: Group 1 was control (received artificial diets only), group 2 was treatment with lambda cyhalothrin (LCT), group 3 was LCT + 5% *Ulva sp*, group 4 was LCT +10% *Ulva sp* , group 5 was LCT + 5% *S. platensis* and group 6 was LCT +10% *S. platensis*. During the experimental period, all fish were fed a commercial diet (2 mm, 30% protein, floating pellets) from Alleraqua Egypt at (09:00 and 14:00 h) at a rate of 2.5 % of tilapia biomass.

Water Quality:

Water temperature (T ºC) and dissolved oxygen (D.O mg / l) were measured by using an oxygen meter (WPA 20 Scientific Instrument), while pH was determined by using a glass electrode pH-meter (Digital Mini-pH Meter, model 55, Fisher Scientific, USA), the previous water quality parameters were measured once a week, according to APHA (1992).

Blood Biochemistry:

The blood of the live fish specimens which was collected without anticoagulants was left to be coagulated in clean and dry centrifuge tubes and then centrifuged for 15 minutes at 5000 rpm. The blood serum was separated, labelled and kept at -20 ºC in a deep freezer for biochemical investigations.

Serum glucose concentration was measured by using kits as described by Trinder (1969). The most widely used method of measuring serum protein is the biuret reaction. So the total serum protein was determined according to Henry (1974). The albumin content in the serum of fish was determined by the colorimetric method of Young, (2000). The total globulin fraction is generally determined by subtracting the serum albumin protein content from the total serum protein content (Busher, 1990):

Serum globulin content = Serum total protein – Serum albumin

Transaminases, aspartate aminotransferase (AST) and alanine aminotransferase (ALT) activities were determined calorimetrically according to Reitman and Frankel (1957) method. Serum urea and creatinine levels were measured according to young (2001) and Henry (1974) respectively.

Lipid profile, triglycerides according to Stein, (1987) and cholesterol according to Ellefson and Caraway (1976) were assayed. HDL cholesterol measured using a colorimetric determination, according to the national cholesterol education program working group on lipoprotein measurement (Warnick and Wood, 1995), and LDL cholesterol according to Friedewald *et al*., 1972

LDL-C (mg/dl) = Total cholesterol – HDL-C – (triglycerides/5),

Extraction, Clean Up and Residue Determination:

For lambda-cyhalothrin residue (LCT) analysis in water, (100 ml) were taken and transferred to a flask (250 ml) with a Teflon-lined screw cap. Lambda-cyhalothrin was extracted from the water samples by adding hexane (35 ml) to each sampling flask and shacked for a half-hour on a flat-bed shaker. The phases were separated and the water layer discarded. The hexane extract was transferred to a clean tube and evaporated under a stream of air (water-bath at 40 ◦C), and the residue re-dissolved in1ml of acetonitrile before HPLC analysis (Leistra *et al.,* 2003).

Extraction was conducted by adding 10 ml acetonitrile to 5 g well-homogenized fish muscles, tubes were closed and vigorously shaken by hand for 1 min then vortexed. To ensure separation and partitioning, a salt mixture of 4 g of anhydrous magnesium sulphate and 1 g of sodium chloride was added. The tubes were re-closed, vigorously shaken by hand for 1 min, vortexed and then centrifuged for 5 min at 3500g. Clean-up was carried out by transferring 1 ml of the acetonitrile phase into 15 ml centrifuge tubes containing 25 mg C^{18} and 150 mg anhydrous magnesium sulphate, the tube was vortexed for 1 min and then centrifuged for 5 min at 3500g. The supernatants were filtered using a 0.2 µm PTFE filter (Millipore, Billerica. MA) in glass vials to be injected in HPLC (Anastassiades and Lehotay 2003).

Residues of lambda-cyhalothrin were analyzed with high-performance liquid chromatography (Agilent HPLC1260) using a UV-detector set at the wavelength 260 nm. Column Eclipse XDB-C^{18} (5 um, 4.6*250 mm) was used and the mobile phase was methanol/water (90:10, v/v) at flow rate: 1ml/min. These conditions resulted in good separations and high sensitivity was obtained.

Recovery Assay:

To estimate the efficacy of the used extraction, clean-up and a final determination procedure, recovery assay was using untreated fish and water. Three samples from fish and water were spiked with known concentrations of the pure lambda-cyhalothrin standard solution. Extraction, clean-up as well as procedures of final determination were performed as described earlier. To evaluate the accuracy and precision of the method, blank samples were analyzed under the same chromatographic conditions. Recovery percentage was calculated by the following equation: % Recovery = [(µg) found/(µg) added] × 100.To ensure linearity five levels of standard solution were injected, triple injection.

Statistical Analysis:

The data obtained from the biochemical analysis of various groups are represented as Mean ± Standard error (M ± SE) in tables. The significance of the difference between the groups was calculated by two - way analysis of variance (ANOVA) followed by Duncan's test at P ≤ 0.05 using the SPSS - PC computer software package version 24. Means in the same column having the same superscript letters are not significantly different (P ≤ 0.05).

RESULTS

Water Quality:

Data obtained in Table (1) revealed that, there were no major variations in water quality between all treatments from the start until the end of the experimental period (Temperature, dissolved oxygen and pH).

Growth:

The initial average weight of fish in this experiment was 27 g, while the final average weight of fish was (32.1, 26.3, 33.4, 35.9, 32.7 and 36.5 g), respectively in all treatments. This result indicated that the highest final average weight fish was in treatments with 10% *Spirulina* and 10% *Ulva*, while the lowest weight was in treatment with LCT.

Table 1: Water quality parameters after exposure lambda-cyhalothrin alone and mixed with *Spirulina platensis* and *Ulva sp* for 28 days.

Quality / Treatments		Temperature (T °C)	Dissolved Oxygen (mg / l)	pH
Cont.		21.7 ± 0.255	5.43 ± 0.109	7.2 ± 0.038
LCT		21.8 ± 0.268	5.45 ± 0.112	7.1 ± 0.032
LCT + US	5 %	21.7 ± 0.205	5.53 ± 0.205	7.2 ± 0.070
	10 %	21.7 ± 0.320	5.55 ± 0.112	7.2 ± 0.092
LCT + SP	5 %	21.7 ± 0.148	5.63 ± 0.148	7.2 ± 0.158
	10 %	21.7 ± 0.212	5.60 ± 0.071	7.2 ± 0.053

Biochemical Blood Parameters:

After the experimental period, blood samples were collected from all groups to measure some biochemical parameters serum results were shown in Table (2). Its highest serum protein value (25.82 g/dl) was realized in control, however the lowest serum protein value (16.85 g/dl). Serum protein consists of Albumin and Globulin, they were highly significant between treatment control group to have height value from Globulin (25.46 g/dl) and high value from albumin showed in fish grope fed by feed contain *Spirulina* 5%. the lowest value from albumin/globulin ratio was at fish grope was feed by 5% *Ulva sp* However fish group was treated by pesticide and control feed have the highest value 0.034 g/dl.

From the given data in Table (2), the blood glucose content of the studied fish group varied significantly between all treatments. Spirulina treatment has the highest value 32.28 it represented in Spirulina 5% but Spirulina 10% have the lowest result 21.61. Alanine amino transaminase (ALT) is a transaminase enzyme previously was found in blood and various body tissues especially the liver. In the present studies, it was found that, the lowest ALT level (26.09 U/l) in the tilapia's serum was reported in 10% *Ulva sp,* but the highest one (34.63 U/l) informed in group Spirulina 10%. These differences statistically were significant. The Aspartate aminotransferase (AST) levels in the present studies were ranged from 15.14 U/l as the lowest value recorded in Nile tilapia reared in 5% Ulva sp and 134.57 U/l (the highest one) was recorded in the fish group was treated by pesticide and control feed. Statistically, these serum variations treatment were significantly liver function represent in AST or ALT. The AST/ALT ratio which is the ratio between the concentrations of (AST) and (ALT) in the fish blood is commonly used as an indicator of liver damage and consequently fish health. So, AST/ALT ratio was calculated for fish reared in different treatment averages were 1.186, 5.888, 0.732, 1.773, 1.451 and 1.634 respectively.

Blood urea nitrogen (BUN) is a chemical waste product produced when protein is broken down during metabolism in the liver and passed out through kidneys and gills. So, blood urea level is a common blood test, it reveals information about how the fish kidneys and liver are working. In the Nile tilapia of the current studies, blood urea level was insignificantly varied in-between the treatment. Blood creatinine level is also very important for the detection of fish kidneys and liver impairment as it is also a waste product of protein metabolism. The values of blood creatinine of the present cultivated Nile tilapia repeated the former blood urea nitrogen picture. Its level was significantly varied in-between treatment. The control showed a high value of 4.97 but the lowest value of 2.87 in the Ulva 5% treatment.

Table 2: Biochemical Blood parameters after exposure lambda-cyhalothrin alone and mixed with *Spirulina platensis* and *Ulva sp*. for 28 days.

Treatments / Parameters	Cont.	LCT	LCT + US		LCT + SP	
			5 %	10 %	5 %	10 %
Glucose (mg / dl)	23.512^{cd} ± 1.172	25.795^{bc} ± 2.548	29.435^{ab} ± 1.065	24.017^{c} ± 0.537	32.285^{a} ± 2.831	21.611^{d} ± 1.453
T. protein (g / dl)	25.823^{a} ± 1.068	19.684^{b} ± 1.994	25.234^{a} ± 1.270	17.760^{b} ± 0.6898	16.851^{b} ± 1.377	24.969^{a} ± 0.832
Albumin (g / dl)	0.3665^{c} ± 0.0218	0.6130^{ab} ± 0.1138	0.3763^{c} ± 0.1047	0.3688^{c} ± 0.0684	0.6260^{a} ± 0.0593	0.5753^{b} ± 0.0501
Globulin (g / dl)	25.457^{a} ± 1.086	19.071^{b} ± 2.054	24.608^{a} ± 1.363	17.391^{b} ± 0.7050	18.975^{b} ± 2.080	24.393^{a} ± 0.825
Alb./Glob. ratio	0.021^{NS}	0.034	0.016	0.018	0.030	0.027
AST (U / l)	37.188^{d} ± 2.117	134.57^{a} ± 17.82	15.141^{e} ± 1.571	47.445^{c} ± 2.307	53.256^{b} ± 1.341	55.971^{b} ± 1.944
ALT (U / l)	33.400^{ab} ± 1.917	31.348^{b} ± 2.429	29.959^{c} ± 1.733	26.095^{d} ± 0.700	32.602^{ab} ± 2.037	34.627^{a} ± 1.090
AST/ALT ratio	1.186^{bc}	5.888^{a}	0.732^{c}	1.773^{b}	1.451^{b}	1.634^{b}
Urea (mg / dl)	6.2729^{NS} ± 0.634	5.4315 ± 0.457	6.0455 ± 0.395	5.5455 ± 0.911	6.1590 ± 0.316	5.7728 ± 0.861
Creatinine (mg / dl)	4.9718^{a} ± 0.209	3.8652^{b} ± 0.603	2.872^{c} ± 0.626	4.688^{ab} ± 0.237	4.2268^{ab} ± 0.816	3.844^{b} ± 0.813
Cholesterol (mg / dl)	35.724^{d} ± 1.659	49.318^{c} ± 2.199	50.423^{c} ± 0.927	56.233^{b} ± 1.605	76.463^{a} ± 1.529	58.699^{b} ± 2.159
Triglyceride (mg / dl)	50.834^{a} ± 3.690	34.997^{b} ± 3.491	14.973^{c} ± 1,468	14.080^{c} ± 0.951	15.390^{c} ± 1.264	16.847^{c} ± 0.708
HDL (mg / dl)	30.025^{a} ± 2.103	16.526^{d} ± 0.452	26.349^{b} ± 1.576	22.450^{c} ± 1.679	30.051^{a} ± 1.530	30.087^{a} ± 0.772
LDL (mg / dl)	69.313^{a} ± 0.041	47.793^{c} ± 3.051	60.721^{b} ± 2.208	31.524^{e} ± 1.678	35.334^{d} ± 2.176	31.643^{e} ± 2.052

Lipid profile is a combination of various blood tests, which is performed to measure the levels of 4 types of lipids in fish blood as total cholesterol, low-density lipoprotein cholesterol (LDL cholesterol), high-density lipoprotein cholesterol (HDL cholesterol), triglycerides. The control group showed the highest value in triglycerides, HDL and LDL (50.83, 30.03 and 69.31), respectively but showed the lowest average Total cholesterol 35.72. The second treatment (control feed with pesticide) has the lowest value in HDL 16.53 but, Ulva 10% showed the lowest value in Triglycerides 14.08 and LDL 31.52. Fish group spirulina 5% showed high value in Total cholesterol (76.46). Total cholesterol, Triglycerides, HDL cholesterol and LDL cholesterol levels were significantly varied in-between treatments.

Residues Analysis:

The obtained recovery percentages ranged from 89% to 95% for both fish and water. Linearity was determined by constructing a calibration curve obtained from mean standards triple injections. The correlation coefficient was found to be 0.999 which is satisfactory Fig (1). The data detected that lambda-cyhalothrin residues in fish samples were non detected (ND) so, the two algae (*Spirulina platensis and Ulva sp.*) with two concentrations (5% and 10%) can efficiently remove lambda-cyhalothrin residues from all fish muscles (Table 3).

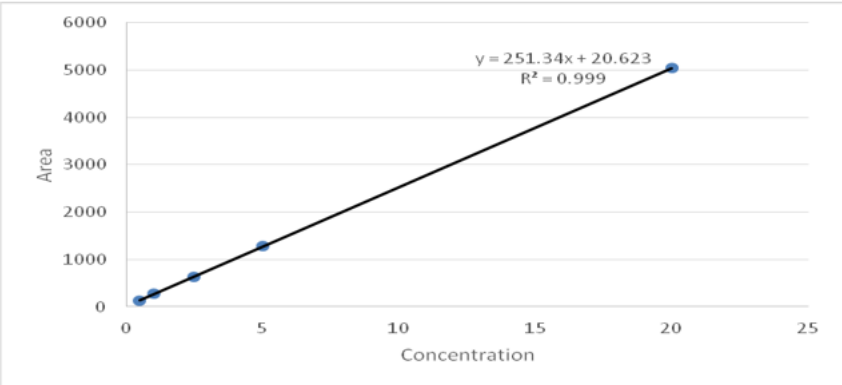

Fig. 1: Calibration curve for five standard solution concentrations.

Table 3: Residual concentrations of lambda-cyhalothrin in water and fish muscle samples after exposure to 2.325 µg / l for 28 days**.**

Treatment		Water		Muscle	
		Residues (ppp)	RSD (%)	Residues (ppp)	RSD (%)
Control		-	-	-	-
LCT		0.3	6.67	0.08	3.95
US	5 %	0.1	6.39	ND	ND
	10 %	0.01	2.49	ND	ND
SP	5 %	0.09	6.39	ND	ND
	10 %	0.01	3.3	ND	ND

Results of lambda-cyhalothrin residues in water samples were given in Table (3). Water samples noted that a bio-sorbent of *S. platensis* and *Ulva sp.* had great potential to remove *lambda-cyhalothrin* from aqueous media comparing with the residue levels in the exposed group with lambda-cyhalothrin alone (from 0.3 to 0.01 ppp). Therefore, the decrease of lambda-cyhalothrin levels in the high concentration of SP and UL (0.01 ppp) was great than the low concentration in both algae (0.09, 0.1 ppp). Precision has been determined as the relative standard deviation (% RSD), which is the ratio between standard deviation and average concentration found. Relative standard deviation ranged from 3.3 and 6.67% which is satisfactory (ECDGHFS 2015).

DISCUSSION

There were no significant differences between all treatments from the beginning till the end of the experimental period. The temperature has been found to be inversely proportional to the aquatic toxicity and bioavailability of pyrethroids (Werner & Moran 2008). In fact, the increase of toxicity of pyrethroids with decreasing temperature has been used to implicate pyrethroids as the source of toxicity in environmental samples (Phillips *et al*., 2004). The inverse relationship between temperature and pyrethroid toxicity is likely due to the increased sensitivity of an organism's sodium channels at low temperatures (Narahashi *et al*., 1998). The effect of pH on sorption was examined by conducting experiments over a range of four different pH values: 2, 4, 6, or 9, the results showed that, over this range, pH did not have a significant impact (Ali and Baugh 2003).

Blood parameters are a useful tool for the detection of fish health and nutritional status as well as other physiological disturbances in intensively cultured fish (Satheeshkumar *et al.*, 2012). Changes in the biochemical blood profile indicate alterations in metabolism and physiological processes of the organism, resulting from the effects of various pollutants. This makes it possible to study the effect mechanisms of these pollutants (Luskova *et al.*, 2002). The blood glucose level of fish may be a factor modulating stress response and highly dependent on feeding status. Jiang *et al.,* (2017) stated that the increased glucose concentrations may cause hyperglycemia due to the reaction of the hormone affected by stress. Such a raise may be due to the increased reaction of stressed fish to gluconeogenesis to meet their additional energy requirement. Madibana *et al.*, (2017) informed no differences in Serum protein, albumin and globulin parameters in Nile tilapia fed with *Ulva lactuca*-holding diets and those fed the control diet. In general, Ulva supplementation in commercial diets did not cause any physiological disturbances to the experimental fish, but in the present study total protein and globulin were lower than control, nonetheless albumin level was higher in the fish group fed by spirulina 5%. The decreased protein content might be attributed to the destruction or necrosis of cells and consequent impairment in protein synthesis. Claimed early that, the amount of protein is dependent on the rate of protein synthesis, and /or on the rate of its degradation, also it may be affected due to impaired incorporation of amino acids into polypeptide chains (Ram *et al*., 2003).

AST and ALT enzymes activity in blood is used as a stress indicator. Significant variations in the actions of these enzymes in blood plasma show tissue impairment caused by stress. A high level of AST enzymes in the second treatment may be referred to the hepato-cellular damage or cellular degradation in the liver, spleen, or muscles (Kaoud *et al*., 2011). Awed *et al*., (2020) reported that *S. platensis* affects serum by decreasing AST and ALT levels, but in the present study, liver enzyme level was near as in control except for the second treatment. The measure of AST and ALT enzymes, in serum, is related to oxidation of glucose, while reduction of protein concentration may be linked to produce the energy request to overcome the stress for fish survival (Ganeshwade *et al*., 2012). Otherwise, the levels of serum creatinine may be induced by the kidney's glomerulus insufficiency and/or increased muscle protein catabolism as illustrated by Hadi *et al*., (2009). Otherwise, the creatinine level has been usually used to diagnose impaired kidney function. Marginal, creatinine levels tended to be higher in fish fed Ulva-containing diets (Madibana *et al.*, 2017), as was detected in the present study especially with treatment 10% Ulva.

Total cholesterol is defined as the sum of HDL and LDL. The body makes some triglycerides which come from the food and the body uses carbohydrate calories for immediate energy and the left-over calories are turned into triglycerides and stored in fat cells for later use (Kulkarni and Bedjargi, 2016). In the present study, higher cholesterol concentration spotted in all treatments against control, especially *spirulina sp* treatment then

Ulva sp this is contrary to results of Madibana *et al*., (2017), who reported that the lower cholesterol level was observed in fish fed 50, 100, 150 and 200 g seaweed Ulva meal/kg commercial feed.

SP and US protect fish from pesticide residues as a result of our study. The protective effects of SP are owed to their content of many active pigments, vitamins notably β-carotene, minerals and C-phycocyanins, which have anti-oxidant and anti-inflammatory activities (Wu *et al.*, 2016). In addition to its content of proteins, carbohydrates, lipids, and essential amino and fatty acids have high nutritive value and potent immune-stimulant (Alvarenga *et al.*, 2011). Since SP is rich in phycocyanin, (the open tetrapyrrole chromophore, phycocyanobilin) so it could link covalently to contaminants (Hirata *et al.*, 2000). Therefore, Adel *et al.*, 2016 and Abdel-Daim *et al.*, 2013 recommended that SP supplementation could be used as a preventive dietary supplement against the toxicity of deltamethrin and other contaminants.

CONCLUSION:

Thus, diets containing the bio-sorbent *Spirulina platensis* and *Ulva sp.* can remove toxicants from aquatic environments and alleviated the toxic effects of contaminants on marine invertebrates and fish. So, in Egyptian aquaculture, spirulina and Ulva biomass should therefore be recommended because of their nutritional composition and bioactive compounds. This may be eco-friendly in the bioremediation of pollutants. Also, we recommended that in further researches using the live biomass of spirulina and Ulva algae to remove pesticides from all water sources to introduce healthy sources for man and fish.

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ARABIC SUMMARY

التأثيرات التحسينيه للاسبيرولينا بلاتنسيس و جنس الأولفا لبعض الخصائص البيوكيميائيه ومتبقيات لمبادا - سيهالوثرين في مياه البلطي النيلي

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تم تصميم هذه الدراسة لتقييم الآثار التحسينية للاسبيرولينا بلاتنسيس- SP (*Spirulina platensis*) - وجنس الأولفا– US (*Ulva sp*) -، كإضافات غذائية لأعلاف الأسماك التجارية، لتقليل مخلفات مبيدات الآفات لمبادا سيهالوثرين - LCT (lambda-cyhalothrin)- في المياه وعضلات الأسماك. في التجربة الحالية، تم إستخدام تركيز 2,325 ميكروجرام/ لتر والذى يمثل 10/1 قيمة التركيز النصفى المميت لـ 50% من الاسماك (LC_{50} - 96 hours) الذى تم تقديره لمدة 28 يومًا. تم تصميم التجربة باستخدام 6 مجموعات من الأسمالك اذ كانت المجموعة الأولى تمثل المجموعة الكنترول، أما المجموعة الثانية تم معاملتها بـمبيد لمبادا سيهالوثرين (LCT) أما المجموعات الأربعة الآخرى تم معاملتها بنوعين من الطحالب بتركيزين لكل منهما (5% و 10% من النظام الغذائي + لمبادا- سيهالوثرين). بعد إنتهاء فترة التجربة (28 يوم) تم تقدير الإختبارات الكيميائية الحيوية في سيرم الأسماك (الجلوكوز، البروتين الكلي، الألبومين، الجلوبيولين، AST، ALT، اليوريا، الكرياتينين وصورة الدهون). أظهرت النتائج تفاوت جميع المعايير البيوكيميائية تفاوتًا معنويًا في فترة المعاملة المعاملة باستثناء مستويات اليوريا لم تتغير معنوياً، بينما كانت هذه المعايير في النطاق الطبيعي في مجموعات الأسماك التي تغذت على تركيزى SP وUS. وكذلك إنخفضت مستويات بقايا المبيد في عينات المياة للمجموعات المغذاة على كلا الطحلبين فى التركيز 10% مقارنة بتركيز 5%. كما أنه المعاملة بكلا التركيزين من الطحلبين SP و US أمكن إزالة بقايا LCT بكفاءة في عضلات الأسماك. أما بالنسبة لمعايير جودة المياة فلم توجد فروق ذات دلالة إحصائية بين جودة المياه (درجة الحرارة، الأكسجين المذاب، pH) بين جميع المعاملات منذ البداية وحتى نهاية فترة التجربة. مما سبق يمكننا أن نخلص إلى أن كلا الطحلبين *Spirulina platensis* و *Ulva sp* لهما دور كبير في الحد من تأثير lambda-cyhalothrin على الأسماك وتحسين جودة المياه.