Toxicity of Chlorophyllin against *Lymnaea acuminata* at Different Wavelengths of Visible Light

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Abstract: Fasciolosis is a water and food-borne disease caused by liver fluke *Fasciola hepatica* and *F. gigantica*. This disease is widespread in different parts of the world. Snail Lymnaeidae and Planorbidae is the intermediate host of these flukes. Snail population management is a good tool to control fasciolosis because gastropods represent the weakest link in the life-cycle of trematode. Chlorophyll can be extracted from any green plant. Chlorophyllin was prepared from spinach in 100% ethanol by using different types of chemicals. The chlorophyll obtained from spinach was transformed into water-soluble chlorophyllin. In the present paper toxicity of chlorophyllin against snail *Lymnaea acuminata* was time and concentration dependent. The toxicity of extracted and pure chlorophyllin at continuous 4 h exposure of sunlight was highest (4 h, LC_{50} 331.01 mg/l and 2.60 mg/l, respectively) than discontinuous exposure of sunlight up to 8 h (8 h, LC_{50} 357.04 mg/l and 4.94 mg/l, respectively). Toxicity of extracted chlorophyllin was noted in the presence of different monochromatic visible light. The highest toxicity in yellow light (96 h, LC_{50} 392.77 mg/l) and lowest in green light (96 h, LC_{50} 833.02 mg/l) was obtained. Chlorophyllin in combination with solar radiation or different wavelength of monochromatic visible light may become a latent remedy against the snail *L. acuminata*. It was demonstrated that chlorophyllin was more toxic in sunlight. Chlorophyllin is ecologically safe and more economical than synthetic molluscicides which have the potential to control the incidence of fasciolosis in developing countries.

Keywords: Fasciolosis, *Fasciola gigantica*, *Lymnaea acuminata*, Chlorophyllin, Monochromatic Light

INTRODUCTION

Fasciolosis is one of the most debilitating zoonotic diseases. Fresh water snail *Lymnaea acuminata* is the vector of liver flukes *Fasciola gigantica*, which cause endemic fasciolosis in cattle population of Northern parts of India (Singh & Agarwal 1981). About 94% buffaloes slaughtered in Gorakhpur, U.P., India, are infected with *F. gigantica* (Singh & Agarwal 1981). No continent is free from fasciolosis (Soliman 2008). The epidemiology of human fasciolosis has changed in recent years and significantly increased in last two decades (Mas-Coma *et al.* 2005, 2009). Snail control is one of the best methods of choice to eliminate fasciolosis. Molluscicіding is still in the centre of efforts to control fasciolosis. Synthetic molluscicides have been widely used for the effective control of carrier snails (Agarwal & Singh 1988; Singh *et al.* 2010). Now it has been realized that synthetic molluscicides are toxic to non-target animals and have a long term detrimental effect on aquatic environment (Shafer *et al.* 2005; Upadhyay & Singh 2011). The molluscicides of plant origin are gaining importance than their synthetic counterpart (Marston & Hostettmann 1985; Singh *et al.* 1996). The derivates of chlorophyll is a very promising substance for the snail control. Chlorophyllin can be easily extracted from any green plants. It is very economical and biodegradable. Earlier, molluscicidal activity of chlorophyllin has been reported against *L. stagnalis, Biomphalaria spp.* and *Physa marmorata* (Mahmoud *et al.* 2013). Previously, it has been reported that chlorophyllin is a potent larvicide (Wohllebe *et al.* 2009). Recently, it has been reported by Singh & Singh (2015) that combination of monochromatic visible light with chlorophyllin has effective larvicidal activity against *F. gigantica*. Visible light spectrum initiates the orientation and locomotion of exposed snails towards light source (Sakakibara *et al.* 2005). It was also noted that snail *L. acuminata* monitor the intensity variation of visible light (Tripathi *et al.* 2013, 2014). The aim of this present study is to evaluate the photo-toxicodynamic activity of extracted/pure chlorophyllin against fresh water snail *Lymnaea acuminata* in sunlight and in different wavelengths of monochromatic visible light.

MATERIALS AND METHODS

Pure Compound

Chlorophyllin was purchased from Sigma Chemical Co. USA.

Experimental Animal

The fresh water snail *Lymnaea acuminata* (2.35±0.30 cm in length) were collected locally from ponds, lakes and low lying submerged fields in Gorakhpur and used as the test animals. The collected snails were acclimatized for 72 h in laboratory condition. The experimental animals were kept in a glass aquarium containing dechlorinated tap water at room temperature (22-25ºC). The pH, dissolved oxygen, free carbon dioxide and bicarbonate alkalinity were 7.1-7.3, 6.5-7.3 mg/l, 5.2-6.3 mg/l and 102-105 mg/l, respectively. Dead animals were removed immediately to prevent the water from being contaminated by decaying tissue.

Preparation of Extracted Chlorophyllin

Preparation of chlorophyllin was done according to the method of Wohllebe *et al.* (2012). Chlorophyll was isolated from spinach using 100% ethanol (for about 2 h at 55ºC). Then, $CaCO_3$ (about 1mg/gm plant material) was added as a buffer, it prevent the transformation of chlorophyll into pheophytin. Before adding benzene the extract was irradiated with solar radiation for 1-2 h. The extract was filtered and 50 ml benzene was added. After addition of benzene the mixture was well shaked as a result the chlorophyll moved into the lipophilic benzene phase. The two phases were separated in separatory funnel and about 1.0 ml methanolic KOH was added to 50 ml of the benzene phase. Upon agitation the chlorophyll came into contact with the methanolic KOH and was transformed into water-soluble chlorophyllin. (This process occurs due to the breakage of the ester bond between the chlorophyllin and the phytol tail by saponification). After separation of the methanolic KOH phase and the benzene phase most of the chlorophyllin was found in KOH phase. The extract was stored in a dark flask at room temperature. However, only fresh chemicals were used in the course of these experiments.

Design of Photo Toxicity Experiments

Light experiment was set up according to the method of Tripathi *et al.* (2013). Xenon arc lamp (500W) was used as visible light source. Spectral response from 400 nm to 650 nm, were produced with the help of the interference color filters. Exposure of monochromatic light at different wavelengths and fix intensity (500 Wm^{-2}) was used to study their effects on snail's mortality.

A glass aquarium of 70 cm diameter and 15 cm height was filled with 3 litres of dechlorinated tap water. Ten snails *L. acuminata* were placed in each aquarium. Then, all the ten snails were exposed to different concentrations of extracted chlorophyllin and monochromatic visible light. Each treatment of extracted chlorophyllin and monochromatic visible light was replicated 6 times. Experiment was done at room temperature of 22-25ºC.

Toxicity Experiment

Toxicity experiments were done according to the method of Singh & Agarwal (1984). A total of ten snails were placed in a glass aquarium containing 3 litres of dechlorinated tap water. In all three sets (Treated/Control) of experiment ten snails were used. First set of experiment snails were treated with extracted chlorophyllin (600 mg/l, 700 mg/l, 800 mg/l, 900 mg/l) in laboratory condition (no exposure of sunlight or monochromatic visible light). Mortality of snails in laboratory condition was recorded at 24 h up to 96 h. In second set of experiment the extracted and pure chlorophyllin (200 mg/l, 300 mg/l, 500 mg/l, 700 mg/l and 2 mg/l, 4 mg/l, 6 mg/l, 8 mg/l) was continuously exposed for 4 h in sunlight (10:00A.M. to 2:00P.M.). Mortality was recorded after every 1 h interval up to 4 h, and in another experiment the extracted and pure chlorophyllin (200 mg/l, 300 mg/l, 400 mg/l, 500 mg/l and 3 mg/l, 5 mg/l, 7 mg/l, 9 mg/l) was discontinuously exposed for 8 h in sunlight and break of 1 h was given after each 2 h exposure of sunlight up to 8 h. Mortality was recorded after every 1 h interval up to 8 h. During break period the animals were placed under laboratory condition for 1 h. The third set of experiment was set up to observe the mortality of extracted chlorophyllin (300 mg/l, 500 mg/l, 700 mg/l, 900 mg/l) treated snails in the presence of different wavelength of monochromatic visible lights in place of sunlight, while other controlled variables were held constant. Mortality of snails in laboratory condition was recorded at 24 h up to 96 h. For set first, set second and set third experiments, three types of control groups I, II, and III were

also set up. In control group I no treatment of chlorophyllin was given in laboratory condition. In control group II snails were only exposed to sunlight without any treatment of chlorophyllin. In control group III snails were only exposed to monochromatic visible lights in laboratory condition without chlorophyllin treatment. Each treatment (Treated/Control) was replicated 6 times. Dead animals were removed from the aquarium immediately to avoid any contamination of water. Snail mortality was established by the contraction of the body within the shell and absence of response to a needle probe was taken as evidence of death.

There is lack of consistency in recording of mortality data. The experiment was designed to observe the effect of extracted chlorophyllin in laboratory condition (Table 2). Thereafter, it was noted that is there is any effect of continuous exposure of sunlight or interrupted exposure of sunlight (interruption of 1 h in laboratory condition or not) (Table 3-4)? In (table 3-4) the whole sunlight was used. Then, another experiment was set up to observe any effect of different monochromatic visible light on extracted chlorophyllin toxicity against *L. acuminata* (Table 5). By doing all these types of experiment it was concluded that continuous exposure of sunlight cause more toxicity against chlorophyllin treated snails than laboratory condition exposure. Interruption of light exposure also affects toxicity of chlorophyllin which is assigned that use of chlorophyllin in cloudy environment will certainly affect the chlorophyllin activity against snails. Toxicity observed in different spectral band also indicates that variation in wavelength of visible light has a significant effect on the toxicity of chlorophyllin. Longer wavelength range red and yellow was more effective against chlorophyllin treated snails.

The Lethal concentration values, lower and upper confidence limits (LCL, UCL), slope values, t-ratio, g-values and heterogeneity factors were calculated by using POLO computer software (Robertson *et al.* 2007). The regression co-efficient between exposure time and different values of LC_{50} was determined (Sokal & Rohlf 1996).

RESULTS

The molluscicidal activity of extracted/pure chlorophyllin was tested at different time of exposure to various light spectra and chlorophyllin concentration against host snail *L. acuminata* (Table 1). A

significant ($p<0.05$) negative regression was observed between the exposure time and the LC_{50} of the treatments (Table 2-5). The toxicity of extracted chlorophyllin at 96 h LC_{50} was 666.56mg/l in laboratory condition (Table 2). Continuous 4 h exposure of sunlight in experimental aquarium containing chlorophyllin caused significant mortality in snails. Toxicity of extracted and pure chlorophyllin at continuous 4 h exposure of sunlight (4 h, LC_{50} 331.01 mg/l and 2.60 mg/l, respectively) was more than break of 1 h after each 2 h exposure of sunlight up to 8 h (8 h, LC_{50} 357.04 mg/l and 4.94 mg/l), respectively (Table 3-4). Toxicity experiment was conducted in laboratory condition with extracted chlorophyllin in the presence of monochromatic visible light. The highest toxicity in yellow light (96 h, LC_{50} 392.77 mg/l) and lowest in green light (96 h, LC_{50} 833.02 mg/l) was observed (Table 5). There was no mortality in control group I, II, and III. It was observed that both extracted/pure chlorophyllin was more toxic in sunlight than laboratory condition.

The steep slope value indicates that a small increase in the concentration of molluscicide caused higher mortality. The t-ratio value greater than 1.96 indicates that the regression is significant ($p < 0.05$). The heterogeneity factor value less than 1.0 denotes that in the replicate test of random samples; the concentration response is limited and thus the model fits the data adequately. The index of significance of the potency estimation g indicates that the value of the mean is within the limit at all probability levels (90, 95 and 99 respectively) since it is less than 0.5 (Table 2-5).

DISCUSSION

Chlorophyllin, product of chlorophyll is a very effective photodynamic substance in control of host *L. acuminata.* Recently, the toxicity of chlorophyllin in different wavelengths of visible light against redia and cercaria larvae of *Fasciola gigantica* has been reported by Singh & Singh (2015). The larvicidal efficiency of chlorophyllin was demonstrated by Wohllebe *et al.* (2009) from spinach against mosquito larvae (*Chaoborus crystallinus*). Even at low concentrations, chlorophyllin was able to kill mosquito larvae and other small animals within a few hours in sunlight (Wohllebe *et al.* 2009). Chlorophyllin was also able to kill

the protozoan parasite *Ichthyophthirius multifiliis* (Fouquet) of fresh water fish species (Wohllebe *et al.* 2012). Erzinger *et al.* (2011) also noted that mosquito larvae were killed by the chlorophyllin.

Results of the present study indicate that the chlorophyllin extracted from the spinach is potent molluscicide. Chlorophyllin toxicity against *L. acuminata* was time and concentration dependent, by the negative regression between exposure period and LC_{50} values of the different treatments. The time-dependent toxic effect of tested plant products may be due to the uptake of active compound by the snails, which progressively accumulated in the body with an increase time of exposure period. It is also possible that the active compound could change into more toxic forms in the aquarium water or in the snail's body due to the sunlight. Four hours of continuous exposure of sunlight treatment was more effective as compared to the intermittent sunlight treatment of 2 hours exposure followed by one hour break. In sunlight solubilized chlorophyllin transferred its excitation energy to oxygen, which produce singlet oxygen and other reactive oxygen species (ROS), which have the potential to kill the vector organism (Hader *et al*. 2002; Tominaga *et al.* 2004).

In another set of toxicity test, extracted chlorophyllin treatments were given in combination of various wavelength of monochromatic visible light at fix intensity (500 Wm^{-2}). Table 5 shows that yellow light spectrum recorded the highest toxicity of extracted chlorophyllin against *L. acuminata* (96 h, LC_{50} 392.77 mg/l) than other wavelength of light. Higher toxicity of extracted chlorophyllin was also noted in other monochromatic visible light than extracted chlorophyllin treatment in laboratory condition (96 h, LC_{50} 666.56 mg/l) without any exposure of monochromatic visible light. Chlorophyllin is a photodynamic substance (Wohllebe *et al.* 2009). Consequently, toxicity of the chlorophyllin was higher in sunlight or in monochromatic visible light than in laboratory condition. All the monochromatic visible lights have sufficient energy which can elicit the response of photodynamic product of chlorophyllin. Absorbed photon produces reactive oxygen species (ROS). Even though toxicity of chlorophyllin in laboratory condition is also noted against *L. acuminata*, exposure of sunlight and monochromatic light of any wavelength caused comparatively higher mortality of snails within a few hours. It was also reported that the retina of snails contains only one type of photo pigment rhodopsin; they can differentiate only gradation of light intensity but not the color of light (Chernorizov & Sokolov 2010). Earlier, it has been reported that *L. stagnalis*'s eye has two types of ocular photoreceptors and 3 types of statocyst hair cells (Sakakibara *et al.* 2005).

Type-A photoreceptor had more spectral sensitivity between 480 nm and 500 nm. Type-T photoreceptor had a much broader spectral sensitivity between 450 nm and 600 nm. At low intensity Type-A photoreceptor and at higher intensity Type-T photoreceptor are more sensitive. Probably, it seems that treatment of extracted chlorophyllin in exposure of same intensity of visible monochromatic light; there is a significant variation in their toxicity at evident from different LC_{50} of chlorophyllin. Obviously, it also indicates that variation in wavelength of light has significant effect on mortality, as evident from higher toxicity of extracted chlorophyllin in yellow light.

CONCLUSION

In conclusion, the present study demonstrated that the treatment of photodynamically active chlorophyllin in solar light or in different wavelength of visible light has significant toxicity effects on vector snail *Lymnaea acuminata*. This is an investigative research work by means of plant extracts to control snail population. Chlorophyllin is a photodynamic product of chlorophyll and chlorophyll is present in all green plants. Therefore, the production of chlorophyllin is inexpensive, easy and environmentally safe and sound. It is a promising approach to control water-borne diseases. This molluscicide might be a valuable, ecologically safe tool against vector snails which has the potential to replace the synthetic molluscicides and control the incidence of fasciolosis in developing countries. For proper utilization of chlorophyllin as molluscicides, further studies are required to elucidate the mechanism of action in snail body.

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Table 1: Concentration of extracted and pure Chlorophyllin used in toxicity experiment against *L. acuminata*.

Experimental design	Chemical	Concentration (mg/l)
Continuous exposure of sunlight	Ext Chl	200, 300, 500, 700
	Pure Chl	2, 4, 6, 8
Discontinuous exposure of sunlight	Ext Chl	200, 300, 400, 500
	Pure Chl	3, 5, 7, 9
Different spectra of light	Ext Chl	300, 500, 700, 900
Laboratory condition	Ext Chl	600, 700, 800, 900

Abbreviation: Ext Chl- Extracted chlorophyllin, Pure Chl- Pure Chlorophyllin

Table 2: Toxicity of extracted chlorophyllin in laboratory condition against *L. acuminata*.

Exposure Period	Treatment	LC_{50} mg/L (w/v)	LCL	UCL	Slope value	t-ratio	g-value	Heterogeneity
24h	Ext Chl	882.71	831.01	981.00	7.68±1.45	5.27	0.13	0.18
48h	Ext Chl	795.51	755.58	851.20	7.46±1.35	5.52	0.12	0.27
72h	Ext Chl	728.41	687.15	768.60	7.31±1.32	5.54	0.12	0.26
96h	Ext Chl	666.56	629.89	695.48	9.79±1.44	6.75	0.08	0.48

Six batches of 10 snails were exposed to different concentration. Mortality was determined at 24h to 96h.Concentrations given are the final concentration (w/v) in the glass aquarium water. Abbreviation: Ext Chl- Extracted Chlorophyllin, Pure Chl- Pure Chlorophyllin, LCL- Lower confidence limit, UCL-Upper confidence limit. Significant negative regression ($p < 0.05$) was observed between exposure time and LC_{50} treatments. Ts – testing significant of the regression coefficient- Ext Chl -3.72^{+} to -2.23^{+} and 265.8^{++} to 1268^{++} was observed. $^{+}$Linear regression between X and Y. $^{++}$Non- linear regression between X and Y.

Table 3: Toxicity of chlorophyllin against *L. acuminata* with continuous exposure (10:00 A.M.-02:00P.M.) of sunlight.

Exposure period	Treatment	LC_{50} mg/L(w/v)	LCL	UCL	Slope values	t-ratio	g-value	Heterogeneity
1h	Ext Chl	938.16	715.53	1688.32	2.36±0.50	4.68	0.17	0.28
	Pure Chl	11.23	8.30	22.81	2.03±0.47	4.31	0.20	0.21
2h	Ext Chl	597.05	492.40	827.10	2.18±0.43	5.08	0.14	0.15
	Pure Chl	7.43	5.85	11.76	1.75±0.39	4.40	0.19	0.22
3h	Ext Chl	492.68	408.85	645.86	2.04±0.41	4.94	0.15	0.25
	Pure Chl	4.17	3.38	5.04	2.06±0.38	5.38	0.13	0.31
4h	Ext Chl	331.01	287.98	374.68	3.21±0.44	7.22	0.07	0.48
	Pure Chl	2.60	1.96	3.13	2.43±0.40	6.03	0.10	0.59

Six batches of 10 snails were exposed to different concentration. Mortality was determined at 1h upto 4h. Concentrations given are the final concentration (w/v) in the glass aquarium water. Abbreviation: Ext Chl- Extracted Chlorophyllin, Pure Chl- Pure Chlorophyllin, LCL- Lower confidence limit, UCL-Upper confidence limit. Significant negative regression ($p < 0.05$) was observed between exposure time and LC_{50} of treatments. Ts – testing significant of the regression coefficient- Ext Chl -343.9^{+} to -41.22^{+} and 0.0^{++} to 1973^{++} and Pure Chl -4.47^{+} to -1.35^{+} and 0.0^{++} to 27.00^{++} was observed. $^{+}$Linear regression between X and Y. $^{++}$Non- linear regression between X and Y.

Table 4: Toxicity of chlorophyllin against *Lymnaea acuminata* with discontinuous (1h interval) exposure in sunlight.

Exposure period	Treatment	LC_{50} mg/l(w/v)	Limits		Slope value	t-ratio	g-value	Heterogeneity
			LCL	UCL				
2h	Ext Chl	686.57	562.90	1098.42	4.12±0.93	4.42	0.19	0.94
	Pure Chl	12.19	9.51	22.06	2.64±0.61	4.30	0.20	0.25
4h	Ext Chl	556.74	481.10	733.07	3.90±0.74	5.26	0.13	0.67
	Pure Chl	8.83	7.30	12.87	2.32±0.51	4.53	0.18	0.18
6h	Ext Chl	451.68	403.14	538.18	4.24±0.67	6.27	0.11	0.79
	Pure Chl	6.50	5.60	7.86	2.56±0.49	5.21	0.14	0.31
8h	Ext Chl	357.04	320.26	403.29	4.74±0.6	7.39	0.11	0.58
	Pure Chl	4.94	4.41	5.46	4.02±0.52	7.61	0.06	0.73

Six batches of 10 snails were exposed to different concentration. Mortality was determined at 2h to 8h each 1h interval. Concentrations given are the final concentration (w/v) in the glass aquarium water. Abbreviation: Ext Chl- Extracted Chlorophyllin, Pure Chl- Pure Chlorophyllin, LCL- Lower confidence limit, UCL-Upper confidence limit. Significant negative regression ($p < 0.05$) was observed between exposure time and LC_{50} treatments. Ts – testing significant of the regression coefficient- Ext Chl -66.85^{+} to -42.51^{+} and 0.0^{++} to 1268^{++} and Pure Chl -1.81^{+} to -0.59^{+} and 0.0^{++} to 25.10^{++} was observed. $^{+}$Linear regression between X and Y. $^{++}$Non- linear regression between X and Y.

Table 5: Toxicity of chlorophyllin in the presence of different spectra of light against *L. acuminata*

Expo-sure period	Treat-ment	Different spectra of light	LC_{50} mg/L(w/v)	LCL	UCL	Slope values	t-ratio	g-value	Heterogeneity
24h	Ext Chl	Green	1428.47	1060.01	3264.72	2.71±0.69	3.91	0.25	0.37
		Violet	1427.16	1051.04	3338.91	2.55±0.65	3.89	0.25	0.32
		Blue	1090.88	881.95	1721.74	2.72±0.58	4.63	0.17	0.20
		Orange	1009.97	819.65	1567.96	2.47±0.54	4.57	0.18	0.29
		Red	905.52	744.63	1341.32	2.32±0.51	4.51	0.18	0.22
		White	708.74	609.36	883.19	2.55±0.49	5.11	0.14	0.17
		Yellow	619.73	548.31	711.90	3.19±0.51	6.25	0.09	0.23
48h	Ext Chl	Green	1273.84	920.19	3510.62	1.86±0.53	3.51	0.31	0.20
		Violet	1328.08	953.03	3682.09	1.92±0.53	3.56	0.30	0.23
		Blue	864.70	712.37	1269.62	2.23±0.50	4.41	0.19	0.14
		Orange	775.75	663.90	997.24	2.61±0.51	5.12	0.14	0.43
		Red	721.37	620.75	901.36	2.59±0.50	5.17	0.14	0.29
		White	574.21	492.87	673.09	2.62±0.48	5.38	0.13	0.25
		Yellow	533.09	465.93	604.48	3.14±0.49	6.30	0.09	0.35
72h	Ext Chl	Green	1148.51	831.01	3488.41	1.61±0.49	3.23	0.36	0.12
		Violet	1017.82	784.95	2018.16	1.86±0.50	3.72	0.27	0.22
		Blue	764.53	649.07	1000.67	2.43±0.50	4.86	0.16	0.20
		Orange	630.78	541.77	761.17	2.51±0.48	5.15	0.14	0.37
		Red	556.12	470.96	655.76	2.47±0.48	5.13	0.14	0.31
		White	494.64	418.94	568.22	2.77±0.48	5.69	0.11	0.32
		Yellow	468.70	408.55	524.83	3.50±0.50	6.88	0.08	0.41
96h	Ext Chl	Green	833.02	633.91	1927.15	1.41±0.47	3.01	0.42	0.13
		Violet	826.81	670.98	1278.36	1.95±0.48	4.01	0.23	0.30
		Blue	621.33	527.88	758.46	2.35±0.48	4.87	0.16	0.31
		Orange	505.02	421.30	588.74	2.50±0.48	5.21	0.14	0.37
		Red	472.42	403.63	536.33	3.05±0.49	6.17	0.10	0.33
		White	432.66	368.54	488.57	3.32±0.50	6.53	0.09	0.54
		Yellow	392.77	342.63	436.07	4.28±0.56	7.60	0.06	0.60

Six batches of 10 snails were exposed to different concentration. Mortality was determined at every 24h upto 96h.Concentrations given are the final concentration (w/v) in the glass aquarium water. Abbreviation: Ext Chl- Extracted Chlorophyllin, LCL- Lower confidence limit, UCL-Upper confidence limit. Significant negative regression ($p < 0.05$) was observed between exposure time and LC_{50} of treatments. Ts – testing significant of the regression coefficient of Green light -13.31^{+} to -2.62^{+} and 0.0^{++} to 2520^{++}, Violet light -13.73^{+} to -3.86^{+} and 0.0^{++} to 2629^{++} , Blue light -9.46^{+} to -3.10^{+} and 0.0^{++} to 1889^{++} , Orange light -10.12^{+} to -3.71^{+} and 0.0^{++} to 1887^{++} , Red light -9.05^{+} to -3.14^{+} and 0.0^{++} to 1685^{++} , White light -5.89^{+} to -1.67^{+} and 0.0^{++} to 1188^{++} , Yellow light -3.63^{+} to -2.58^{+} and 0.0^{++} to 1018^{++} was observed. $^{+}$Linear regression between X and Y. $^{++}$Non- linear regression between X and Y.