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Metabolic Response of the Two Marine Unicellular Algae *Chlorella salina* and *Dunaliella bardawil* to Toxicity of the Antifouling Agent Irgarol 1051

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Abstract

Toxic pollutants are metabolic poisons that can seriously injure or destroy the photosynthetic organisms upon which the food chain depends. Since microalgae play a key role in marine ecosystems, marine microalgae are proposed as excellent bio-indicators of pollution due to their high sensitivity, which can give warning of the toxic effects of chemicals sooner than any other species. The aim of this work concentrated on the effect of different concentrations of the antifouling biocide (Irgarol 1051) on growth and chlorophylls content (as an essential metabolite) of the two marine unicellular green algae *Chlorella salina* and *Dunaliella bardawil* that usually used in fish feeding. The growth of the wall-less *Dunaliella bardawil* was more sensitive to Irgarol 1051 than the walled cells *Chlorella salina*, although the concentrations used were greatly different. The product of photosynthesis in the two algal species greatly affected since in the presence of Irgarol 1051, a serious destructive effect was observed. The cell wall appeared to play a significant role in protecting the organism against toxicity of the antifouling agent either by adsorption or degradation. The strength of toxicity depends mainly on the concentration of the antifouling agent, the length of culturing period and the type of organism tested.

Keywords

Antifouling Biocide, Irgaol 1051, *Chlorella salina*, *Dunaliella bardawil*

1. Introduction

The term biofouling is commonly employed to distinguish the undesirable ac-

cumulation of microorganisms such as bacteria, fungi and microalgae, plants and invertebrates on any artificial surfaces submerged in seawater [1]. Vessel bottoms not protected by anti-fouling systems may gather 150 kg of fouling per square meter in less than six months of being at sea. On a very large crude arriver with 40,000 square meter underwater areas, this would add up to 6000 tones of fouling. Just a small amount of fouling can lead to an increase of fuel consumption of up to 40%, and possibly as much as 50%, since the resistance to movement will be increased. A clean ship can sail faster and with less energy [2]. The use of coatings containing antifouling compounds on vessel hulls inhibits the settlement of marine organisms [3]. So, the purpose of using of antifouling coatings is to prevent growth of fouling organisms and to maintain the fractional resistance as low as possible for a maximum period.

However, antifouling of boats and ships is not a new concept. In the ancient periods, the civilization specially the navy people used different methods to prevent fouling. By the advent of time, the antifouling paints began to develop [1]. These paints considered to be the only really successful method in the use of antifouling paints but under a controlled manner. It is generally admitted that the prevention of fouling growth is obtained by the controlled release of bioactive molecules (booster biocides) from paint coatings. In the late 1950s and early 1960s, a new formulation using tributyltin (TBT) proved to be excellent in the prevention of fouling. By time another compounds were introduce to restrict the use of TBT, these compounds have been termed (booster biocides). The most used booster biocides were: TBT, diuron, Irgarol 1051, dichlofluanid, chlorothalonil and Sea-Nine 211. The herbicide Irgarol was introduced after prohibition of using TBT as antifouling agent in 2008 [2].

The sensitivity and response of microalgae to booster biocides compounds varies from species to species, however very little information is available on the uptake of Irgarol and degradation by microalgae. Herbicide Irgarol 1051 (2-methylthio-4-terbulylamino-6-cyclopropylamino-s-triazine) is now widely distributed through European coastal waters. Irgarol 1051 has also been showed to be very toxic to growth of fresh water and marine microalgae [4] [5]. Occurrence of Irgarol has been widely reported in coastal waters of many countries [6] [7] [8] [9]. Irgarol 1051 was firstly reported as an aquatic contaminant since 1993 in the Mediterranean [10]. Diuron and Irgarol 1051 are widely used antifouling booster herbicides to control the growth of redundant algae on submerged structures. They pose serious threats to the marine ecosystem especially on the non-target algal species which is of serious environmental concern [11]. Algal cells are able to produce specific molecules or to increase specific enzyme activities in response to stress caused by the presence of substances that are toxic to them [12]. Therefore, with the appropriate tools, these organisms can be used in the design of better "early warning systems" of pollution in the environment, Marine microalgae are proposed as excellent bio-indicators of pollution due to their high sensitivity, which can give warning of the toxic effects of chemicals

sooner than any other species [13]. Since microalgae play a key role in marine ecosystems, they are considered potentially useful for quick and sensitive toxicity bioassays. The aim of this work concentrated on the effect of different concentrations of the biocide (Irgarol 1051) on growth and chlorophyll content (as an essential metabolite) of the two marine unicellular green algae *Chlorella salina* and *Dunaliella bardawil* that usually used in fish feeding.

2. Materials and Methods

The biological materials chosen in this paper were the axenic unicellular green algae, *Dunaliella bardawil* and *Chlorella salina* obtained from Botany Department, Faculty of Science, Alexandria University. The basal medium for *Chlorella salina* and *Dunaliella bardawil* was used in this work described by [14] and pH was adjusted at 7.5 for both organisms. The axenic cultures of *Chlorella salina* and *Dunaliell bardawil* were grown each in 50 ml of the selected media with or without (control) a known concentration of Irgarol 1051. Each flask inoculated with a known number of cells in 250 ml Erlenmeyer Pyrex-glass flasks under controlled laboratory conditions (temperature at 28˚C ± 3˚C and light at 80 μmol·mol·m^{-2}·S^{-1}) in a culturing chamber. This temperature was chosen depending on the results of [15] who observed among others insignificant variation in the growth rate of the halotolerant *Dunaliella* and *Chlorella* species in the range of temperature corresponding to 26˚C and 32˚C respectively. Culture experiments were conducted under a regime of 16 hour light/8 hour dark.

The herbicide triazine (Irgarol 1051) was purchased from Fluka company. The stock solution 1000 mg of the standard Irgarol was prepared in acetone and stored in dark at 4˚C. 0.1 ml of stock solution mixed with 100 ml of sterilized distilled water. The mixture is stirred by a magnetic stirrer for half an hour. Dilution of this stock solution was mixed with the medium. Different concentrations were prepared and added each to one liter medium. The different concentrations used were 0.25, 0.5 and 0.75 μg/L for *Chlorella salina* and 0.012, 0.025 and 0.05 μg/L for *Dunaliella bardawil*. These concentrations were chosen after long term of several experiments.

Growth measurement: The growth of the investigated algae was determined every couple days by cell count and growth rate, cell count using the hemacytometer. The growth rate (number of division/day) was calculated by using the formula proposed by [16]: $R = (3.322/(t_2 - t_1) \times (\log N_2/N_1)$, where: 3.322 = growth constant., t_1 = time at the beginning of the experiment., t_2 = time at the end of the experiment., N_1 = Number of cells/ml culture at t_1., N_2 = Number of cells/ml culture at t_2.

Chlorophylls estimation: The spectrophotometer method is the simplest method for estimating chlorophyll "a" and "b" according to the equation of [17]: Chlorophyll "a" (mg·l^{-1}) = 11.93 E_{664} − 1.93 E_{647}, Chlorophyll "b" (mg·l^{-1}) = 20.36 E_{647} − 5.50 E_{664}.

3. Result and Discussion

Part (I): In this part primarily experiments were done in order to select the suitable concentrations that could be used for growth of the two marine unicellular green algae *Chlorella salina* and *Dunaliella bardawil*. The results obtained were nearly endless. The two organisms were firstly cultured under similar concentrations of Irgarol 1051. The results obtained cleared that both organisms died after two days of culturing under all the concentrations used (300, 250, 200, 150 μg/L). The detrimental effects were clearer for *Dunaliella bardawil* than for *Chlorella salina*. So, the foregoing experiments were conducted under different separate concentrations for *Chlorella salina* and for *Dunaliella bardawil*. Table 1 & Table 2 represent the effect of different concentrations of Irgarol 1051 (μg/L) on growth of *Chlorella salina* and *Dunaliella bardawil* respectively. The first experiment was conducted at concentrations 100.0, 75.0, 50.0 and 25.0 μg/L for *Chlorella*

Table 1. Effect of different concentrations of Irgarol 1051 (μg/L) on growth (cell number × 10^6/ml) of *Chlorella salina*.

Time (*Day*)	First experiment					Second experiment					Third experiment			
	Control	100	75.0	50.0	25.0	Control	10.0	7.5	5.0	2.5	Control	0.25	0.50	0.75
	No. of cells	*No. of cells*	*No. of cells*	*No. of cells*	*No. of cells*	*No. of cells*	*No. of cells*	*No. of cells*	*No. of cells*	*No. of cells*	*No. of cells*	*No. of cells*	*No. of cells*	*No. of cells*
0	1.20	1.20	1.20	1.20	1.20	2.25	2.25	2.25	2.25	2.25	1.00	1.00	1.00	1.00
2	2.01	0.247	0.512	0.916	1.129	3.14	2.01	2.270	2.61	2.72	1.10	1.03	1.03	1.02
4	2.51	0.124	0.134	0.210	0.233	4.00	2.07	2.207	2.50	2.52	1.23	1.11	1.04	1.03
6	4.25	---	---	---	---	6.25	0.694	0.780	1.25	1.77	1.45	1.26	1.19	1.06
8	4.50	---	---	---	---	6.07	---	---	---	---	1.74	1.46	1.25	1.07
10	4.62	---	---	---	---	6.12	---	---	---	---	1.80	1.48	1.28	0.94
12	4.81	---	---	---	---	6.43	---	---	---	---	1.81	1.25	1.08	0.54
14	4.20	---	---	---	---	6.02	---	---	---	---	1.41	1.19	1.01	0.30

Table 2. Effect of different concentrations of Irgarol 1051 (μg/L) on growth (cell number × 10^6/ml)) of *Dunaliella bardawil*.

Time (*Day*)	First experiment					Second experiment					Third experiment			
	Control	10.0	5.0	2.50	1.25	Control	1.0	0.5	0.25	0.125	Control	0.012	0.025	0.050
	No. of cells	*No. of cells*	*No. of cells*	*No. of cells*	*No. of cells*	*No. of cells*	*No. of cells*	*No. of cells*	*No. of cells*	*No. of cells*	*No. of cells*	*No. of cells*	*No. of cells*	*No. of cells*
0	1.10	1.10	1.10	1.10	1.10	1.75	1.75	1.75	1.75	1.75	0.70	0.70	0.70	0.70
2	2.00	0.78	1.01	1.30	1.50	2.41	1.02	1.52	1.74	1.92	0.80	0.80	0.71	0.70
4	2.50	0.76	1.01	1.025	1.025	3.00	2.05	2.41	2.71	2.80	1.11	1.02	0.80	0.76
6	3.75	---	---	1.01	1.025	4.5	1.03	2.37	2.80	2.91	1.91	1.70	1.00	0.89
8	4.50	---	---	---	---	3.81	---	---	---	---	3.42	3.15	1.08	1.02
10	4.52	---	---	---	---	4.11	---	---	---	---	4.45	3.20	1.09	1.01
12	4.54	---	---	---	---	4.20	---	---	---	---	3.46	3.28	1.09	0.95
14	4.25	---	---	---	---	3.81	---	---	---	---	2.30	1.45	1.02	0.06

salina, and under concentrations 10.0, 5.0, 2.5 and 1.25 μg/L for *Dunaliella bardawil* the results cleared that both organisms suffered greatly and died nearly at the 6th day of culturing. In the second experiment the following concentrations were examined (10.0, 7.5, 5.0 and 2.5 μg/L) for *Chlorella salina* and at concentrations (1.0, 0.5, 0.25 and 0.125 μg/L) for *Dunaliella bardawil*. The results cleared that a sudden drop in growth of both organisms was recorded at the 8th day of culturing, the organisms appeared pale in color and dropped at bottom of the flasks. So, we tried to pass through these huge results as a trial to get the rialable concentration of Irgarol 1051 that could be experimented.

Finally, the 3rd experiment was conducted at concentrations (0.75, 0.50 and 0.25 μg/L) for *Chlorella salina*, while for *Dunaliella bardawil* concentrations (0.050, 0.025 and 0.012 μg/L) were tested. Under the effect of these concentrations the two tested organisms remained alive but with different rates of growth. Consequently in this work these concentrations were chosen for the two tested organisms. The results cleared also that, the effective concentration (EC50) of Irgarol for *Chlorella salina* was recorded nearly in concentration 0.5 μg/L at the 8th day, while for *Dunaliella bardawil*, the effective concentration was recorded at 0.025 μg/L. This means that, *Dunaliella bardawil* is more sensitive to Irgarol than *Chlorella salina*.

Part (II) was concerned with the effect of different concentrations that have been chosen from part (I) of the antifouling agent Irgarol 1051 on growth and chlorophylls content as an essential metabolite of *Chlorella salina* and *Dunaliella bardawil* cells. The growth parameters were measured and calculated bi-daily while the chlorophylls content was conducted every 4 days. **Table 3** & **Table 4** show the growth parameters of *Chlorella salina* and *Dunaliella bardawil* which affected by the different concentrations of Irgarol 1051. The data obtained cleared that, suppression of algal growth under the effect of the different tested concentrations of Irgarol may be due to the increasing of toxicity of this biocide. **Figure 1** and **Figure 2** show the effect of different concentrations of Irgarol 1051 on growth of *Chlorella salina* and *Dunaliella bardawil* cultured for 14 days. The same results were also obtained by [18] [19] [20], [13] found that new molecular tools are capable of detecting cell-stress after short exposure times, and provide rapid information about cellular status. Compared to control, the rate of growth increased gradually till the 8th day of culturing where it reached at maximum in both organisms but with different values, that is cleared in **Figures 3-6**. However [21] found that Irgarol 1051 inhibit growth of *Dunaliella tertiolecta* at concentration higher than 0.8 μg/l and at concentration 3.0 μg/l, the compound killed almost all the cells. [22] revealed that, sensitivity and response of microalgae to booster biocides varies from species to species, size of the cell wall composition, consequently some species appeared to be resistant to booster biocides and posses the ability to accumulate and/or degradate these compounds. The same results were also obtained in our work. The growth of the wall-less *Dunaliella bardawil* was more sensitive to Irgarol than the walled cells *Chlorella salina*,

Table 3. Effect of different concentrations of Irgarol 1051 (0.25, 0.50 and 0.75 µg/l) on growth of *Chlorella salina* cultured for 14 days.

Time (Days)	Control			0.25 µg/l			0.50 µg/l			0.75 µg/l			F (p)	LSD
	Cell No ×10^6 cells/ml	Growth rate	% of increasing (+) or decreasing (−) in Growth rate	Cell No ×10^6 cells/ml	Growth rate	% of increasing (+) or decreasing (−) in Growth rate	Cell No ×10^6 cells/ml	Growth rate	% of increasing (+) or decreasing (−) in Growth rate	Cell No ×10^6 cells/ml	Growth rate	% of increasing (+) or decreasing (−) in Growth rate	0.000 (1.000)	0.002
0	1.00 ± 0.01^a	----	----	1.00 ± 0.01^a	----	----	1.00 ± 0.01^a	----	----	1.00 ± 0.01^a	----	----	52.340** (<0.001)	0.003
2	1.10 ± 0.03^a	0.069	10.00	1.03 ± 0.02^b	0.021	3.00	1.03 ± 0.04^c	0.021	3.00	1.02 ± 0.03^d	0.014	2.00	1472.308** (<0.001)	0.003
4	1.23 ± 0.03^a	0.081	11.82	1.11^a ± 0.027	0.054	7.77	1.04 ± 0.01^c	0.007	0.97	1.03 ± 0.01^d	0.007	0.98	5103.000** (<0.001)	0.002
6	1.45 ± 0.02^a	0.119	17.89	1.26 ± 0.03^b	0.091	13.51	1.19 ± 0.0^c	0.097	14.42	1.06 ± 0.01^d	0.021	2.91	3530.429** (<0.001)	0.003
8	1.74 ± 0.01^a	0.132	20.00	1.46 ± 0.14^b	0.106	15.87	1.25 ± 0.01	0.036	5.04	1.07 ± 0.02^d	0.007	0.94	147406.88** (<0.001)	0.003
10	1.80 ± 0.03^a	0.025	3.45	1.48 ± 0.03^b	0.010	1.37	1.28 ± 0.03^b	0.017	2.40	0.94 ± 0.02^d	---	−12.15	204049.09** (<0.001)	0.003
12	1.81 ± 0.04^a	0.004	0.56	1.25 ± 0.05^a	---	−15.54	1.08 ± 0.05^c	---	−15.63	0.54 ± 0.03^e	---	−42.55	415865.85** (<0.001)	0.003
14	1.41 ± 0.06^a	---	−22.10	1.19 ± 0.07^b	---	−4.80	1.01 ± 0.02^d	---	−6.48	0.30 ± 0.05^e	---	−44.44	456256.74** (<0.001)	0.003

F (p): F-test (ANOVA) and its significance between groups. LSD: Least significant difference at 0.05. *: Statistically significant at $p \leq 0.05$. **: Statistically significant at $p \leq 0.01$. Different subscribts are significant. Data are expressed in mean ± SD.

Table 4. Effect of different concentrations of Irgarol 1051 (0.012, 0.025 and 0.050 µg/l) on growth of *Dunaliella bardawil* cultured for 14 days.

Time (Days)	Control			0.012 µg/l			0.025 µg/l			0.050 µg/l			F (p)	LSD
	Cell No ×10^6 cells/ml	Growth rate	% of increasing (+) or decreasing (−) in Growth rate	Cell No ×10^6 cells/ml	Growth rate	% of increasing (+) or decreasing (−) in Growth rate	Cell No ×10^6 cells/ml	Growth rate	% of increasing (+) or decreasing (−) in Growth rate	Cell No × 10^6 cells/ml	Growth rate	% of increasing (+) or decreasing (−) in Growth rate	0.000 (1.000)	0.003
0	0.70 ± 0.002^a	----	----	0.70 ± 0.002^a	----	----	0.70 ± 0.002^a	----	----	0.70 ± 0.002^a	----	----	37.059* (<0.001)	0.003
2	0.80 ± 0.002^a	0.096	14.29	0.80 ± 0.001^a	0.096	14.29	0.71 ± 0.003^a	0.010	1.43	0.70 ± 0.004^c	----	----	812.857** (<0.001)	0.004
4	1.11 ± 0.007^a	0.236	38.75	1.02 ± 0.02^a	0.175	27.50	0.80 ± 0.01^b	0.086	12.68	0.76 ± 0.013^c	0.059	8.57	15207.000** (<0.001)	0.003
6	1.91 ± 0.03^a	0.392	72.07	1.70 ± 0.004^b	0.369	66.67	1.00 ± 0.004^b	0.161	25.00	0.89 ± 0.005^c	0.114	17.11	31197.000** (<0.001)	0.005
8	3.42 ± 0.02^a	0.420	79.06	3.15 ± 0.006	0.445	85.29	1.08 ± 0.008^b	0.056	8.00	1.02 ± 0.008	0.098	14.61	57015.000** (<0.001)	0.004
10	4.45 ± 0.002^a	0.190	30.12	3.20 ± 0.004^b	0.011	1.59	1.070.04^b	---	−0.93	1.01 ± 0.013^d	----	−0.98	225033.191** (<0.001)	0.004
12	4.46 ± 0.02^a	0.002	0.23	3.28 ± 0.007^b	0.003	0.31	1.06 ± 0.015^c	----	−0.94	0.95 ± 0.019^c	----		370898.571** (<0.001)	0.004
14	2.30 ± 0.04^a	---	−48.43	1.45 ± 0.01^b	----	−54.83	1.02 ± 0.005^c	----	−3.77	0.06 ± 0.011^d	----	−93.68	262496.000** (<0.001)	0.005

F (p): F-test (ANOVA) and its significance between groups. LSD: Least significant difference at 0.05. *: Statistically significant at $p \leq 0.05$. **: Statistically significant at $p \leq 0.01$. Different subscribts are significant. Data are expressed in mean ± SD.

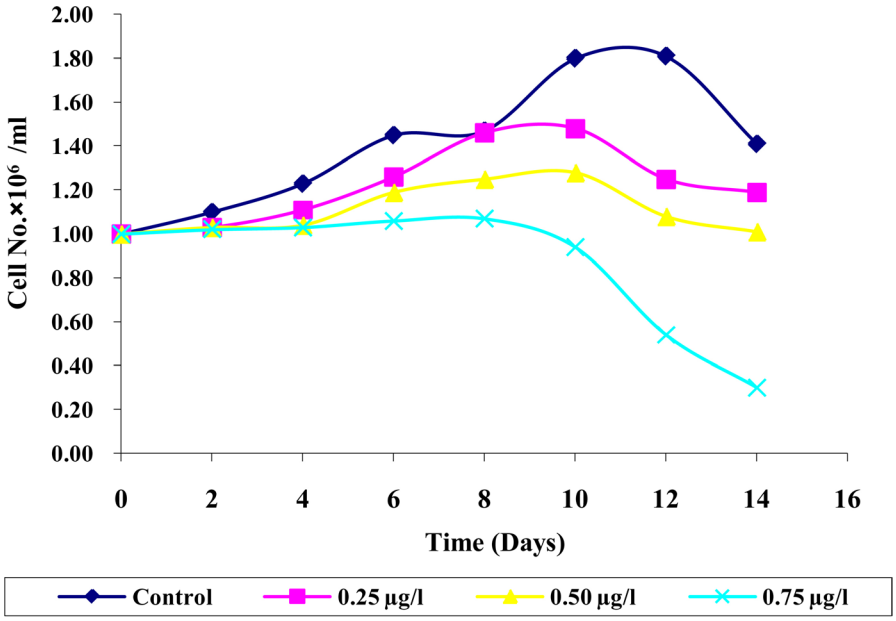

Figure 1. Effect of different concentrations of Irgarol 1051 (0.25, 0.50 and 0.75 µg/l) on growth of *Chlorella salina* cultured for 14 days.

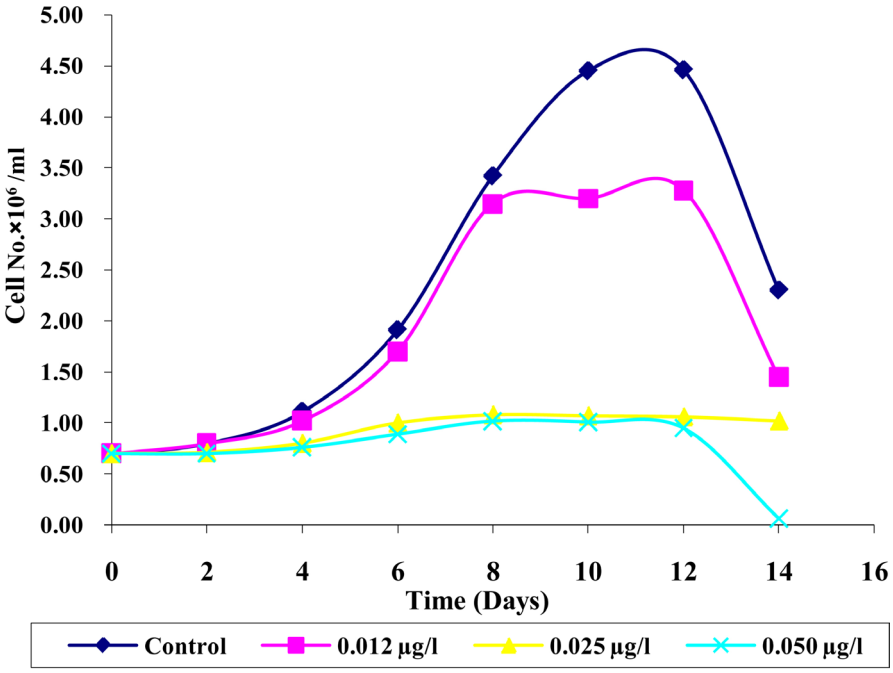

Figure 2. Effect of different concentrations of Irgarol 1051 (0.012, 0.025 and 0.050 µg/l) on growth of *Dunaliella bardawil* cultured for 14 days.

although the concentrations used were greatly different. [23] found that, irgarol concentrations in estuaries and coastal ecosystems can reach levels representing environmental risk to populations of micro-algae. The stress effect of booster biocides on growth of algae may be due to the metals found in this compound which cause inhibition of normal cell division [24]. Also, [25] speculated that, inhibition of cell division and cessation of new daughter cells could be due to

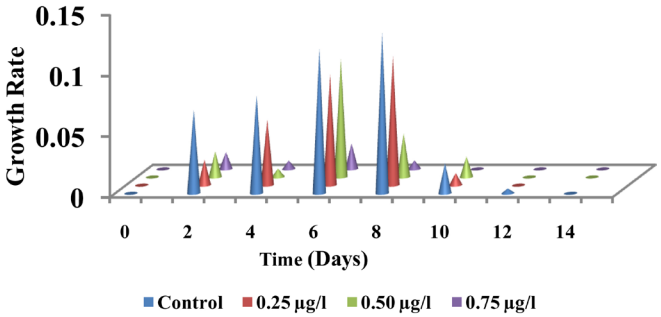

Figure 3. Effect of different concentrations of Irgarol 1051 (0.25, 0.50 and 0.75 µg/l) on growth rates of *Chlorella salina* cultured for 14 days.

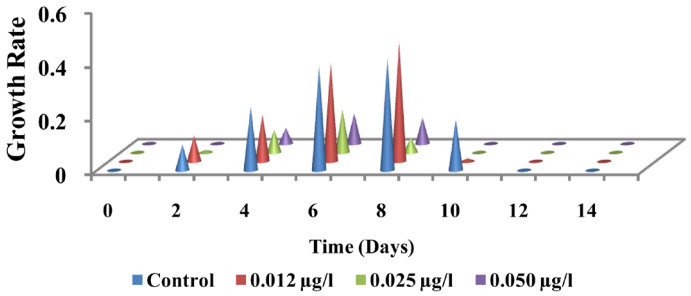

Figure 4. Effect of different concentrations of Irgarol 1051 (0.012, 0.025 and 0.050 µg/l) on growth rates of *Dunaliella bardawil* cultured for 14 days.

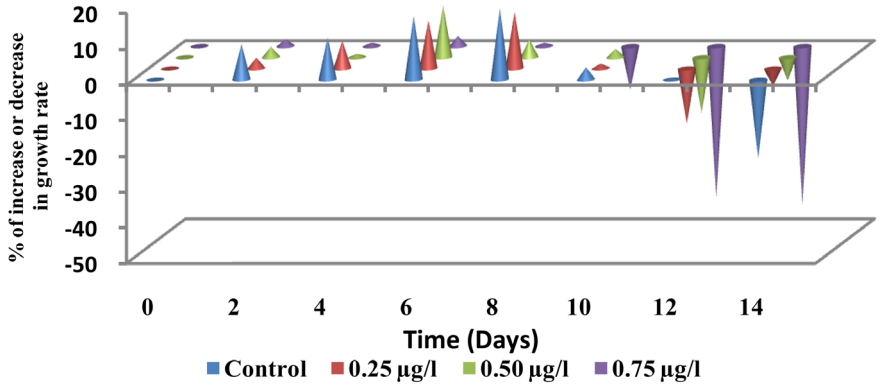

Figure 5. Effect of different concentrations of Irgarol 1051 (0.25, 0.50 and 0.75 µg/l) on % of increasing and decreasing in growth rate of *Chlorella salina* cultured for 14 days.

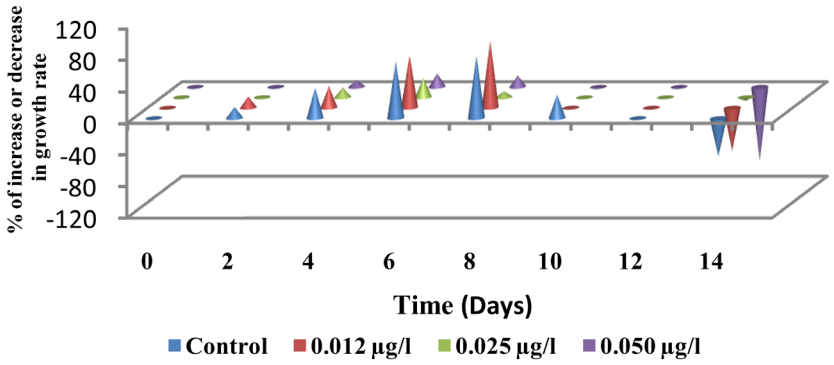

Figure 6. Effect of different concentrations of Irgarol 1051 (0.012, 0.025 and 0.050 µg/l) on % of increasing and decreasing in growth rate of *Dunaliella bardawi* cultured for 14 days.

binding the metals to sulfhydryle groups which are important in regulation cell division. This is in agreement with studies of [26] who suggested that at low concentrations of some antifouling agent, cultures of some algae included *Dunaliella tertiolecta* were killed within 2 days. [27] reported that, low concentrations of the tested antifouling agent caused reduction in growth of *Nannochloropsis oculata*. This coincide with results obtained for growth of *Spirulina* [28] [29]. [30] found that, irgarol 1051 was more toxic than diuron and caused growth inhibition assay with the marine algae *Skeletonema pseudocostatum*.

4. Chlorophylls Content

It is clear that, at the 8th day of culturing, in which the organism reached its maximum value of growth, chlorophyll a and b content greatly affected by the tested concentrations of Irgarol 1051. For *Chlorella salina*, the ratio between control: 0.25: 0.50: 0.75 μg/L Irgarol 1051 for chlorophyll a reached 1: 0.76: 0.35: 0.34 respectively. On the other hand for chlorophyll b, these ratios reached 1: 0.75: 0.49: 0.26 respectively. A glimpse at these results it could be concluded that, total chlorophylls content after 8 days of culturing, the ratio between control and the three different concentrations of Irgarol 1051 reached 1: 0.76: 0.39: 0.32 respectively. This means that, total chlorophylls at concentrations 0.5 and 0.75 μg/L Irgarol 1051 greatly affected at these two concentrations, while at concentration 0.25 μg/L Irgarol 1051, the content of total chlorophylls slightly affected.

It is clear also from the data recorded in **Table 5** that, the percent of decrease of total chlorophylls differs according to the concentrations used. The gradual decrease in the total chlorophylls reached its maximum value at concentration 0.75 μg/L Irgarol 1051 and at the 8th and 16th day of culturing. The percent of decrease reached 67.69 and 83.45% respectively compared to control. Owing to the release of various herbicides in to the environment and the transfer in to aquatic ecosystems, periphytic communities are mainly exposed to a mixture of PSII inhibitors [31] [32]. Herbicides with this specific cellular target,such as atrazine,terbutryn,diuron and isoproturon, are known to affect benthic microalgae at low concentrations[33] [34]. [35] who found that EC50 of Irgarol 1051 on *Chlorella vulgaris* was 0.52 μg/l affected PSII.

In correlation with these results it is clear that, the toxic effect of Irgarol 1051 in *Dunaliella bardawil* is more prominent than that in case of *Chlorella salina*. This could be observed from the results obtained in **Table 6** that at 8, 12 and 16 days of culturing, the content of chlorophyll a decreased gradually and reached its minimum value at the 16th day of culturing. The ratio of total chlorophylls at control and at the three different concentrations used at the 8th day reached 1:0.77:0.48:0.25 while at the end of the experiment (16 days) these ratios reached 1:0.73:0.49:0.23 for concentrations 0.012, 0.025 and 0.050 μg/L Irgarol 1051 respectively. This means that, the content of total chlorophylls in *Dunaliella bardawil* greatly affected even at the 8th day of culturing where the organism reached its maximum rate of growth.

Chlorophyll "a" content in *Dunaliella bardawil,* was lower than those reported [36] for *Dunaliella salina*. However, chlorophyll "a" and "b" content in both organisms greatly affected under the stress effect of the tested concentrations of Irgarol. The normal ratio between the concentration of chlorophyll "a" and "b" (3:1) greatly differed under the stress effect of Irgarol. The results cleared also that, the percent of decrease in the total chlorophylls differed according to the concentrations used, period of culturing and type of the organism, this is cleared in **Figure 7** and **Figure 8**. These results are in harmony with those obtained by [37], represented that, the photosynthetic health of *tetraselmis suecica* is reduced by 50% with Irgarol 1051 present at concentration between 0.14 and 1.39 µg/l then growth of the alga is stopped completely. [38] [39] represented that,

Table 5. Effect of different concentrations of Irgarol 1051 (0.25, 0.50 and 0.75 µg/l) on chlorophylls content (mg/l) of *Chlorella salina* cultured for 16 days.

Time (Days)	Parameter	Control	Irgarol 1051 concentrations (µg/l)			F (p)	LSD
			0.25 µg/l	0.50 µg/l	0.75 µg/l		
0	Chl.a	0.582 ± 0.002^{a}	0.582 ± 0.002^{a}	0.582 ± 0.002^{a}	0.582 ± 0.002^{a}	1111.304** (<0.001)	0.003
	Chl.b	0.210 ± 0.001^{a}	0.210 ± 0.001^{a}	0.210 ± 0.001^{a}	0.210 ± 0.001^{a}	147.692** (<0.001)	0.002
	Total	0.792	0.792	0.792	0.792		
2	Chl.a	0.730 ± 0.004^{a}	0.632 ± 0.02^{b}	0.591 ± 0.001^{d}	0.632 ± 0.003^{c}	12477.465** (<0.001)	0.004
	Chl.b	0.230 ± 0.001^{a}	0.271 ± 0.004^{b}	0.243 ± 0.004^{d}	0.201 ± 0.002^{c}	804.000**	0.003
	Total	0.960	0.903	0.834	0.833		
4	Chl.a	1.150 ± 0.008^{a}	0.950 ± 0.013^{b}	0.941 ± 0.02^{d}	0.662 ± 0.003^{d}	22491.304** (<0.001)	0.004
	Chl.b	0.540 ± 0.008^{a}	0.480 ± 0.006^{b}	0.302 ± 0.001^{d}	0.294 ± 0.010^{d}	1760.000** (<0.001)	0.002
	Total	1.690	1.430	1.243	0.956		
8	Chl.a	4.280 ± 0.004^{a}	3.260 ± 0.011^{b}	1.510 ± 0.04^{d}	1.473 ± 0.006^{d}	12261.818** (<0.001)	0.004
	Chl.b	1.520 ± 0.015^{a}	1.140 ± 0.007^{b}	0.742 ± 0.004^{d}	0.401 ± 0.004^{d}	348.333** (<0.001)	0.003
	Total	5.800	4.400	2.252	1.874		
12	Chl.a	3.220 ± 0.003^{a}	2.640 ± 0.019^{b}	1.424 ± 0.006^{c}	0.840 ± 0.005^{d}	8030.182** (<0.001)	0.003
	Chl.b	1.400 ± 0.005^{a}	0.850 ± 0.004^{b}	0.561 ± 0.03^{c}	0.360 ± 0.007^{d}	3450.000** (<0.001)	0.002
	Total	4.620	3.490	1.985	1.200		
16	Chl.a	3.350 ± 0.021^{a}	2.140 ± 0.02^{b}	1.020 ± 0.02^{d}	0.650 ± 0.004^{c}	51107.027** (<0.001)	0.004
	Chl.b	1.220 ± 0.004^{a}	0.810 ± 0.01^{b}	0.570 ± 0.008^{d}	0.310 ± 0.005^{c}	17730.000** (<0.001)	0.002
	Total	4.570	2.950	1.590	0.960		

F (p): F-test (ANOVA) and its significance between groups. LSD: Least significant difference at 0.05. *: Statistically significant at $p \leq 0.05$. **: Statistically significant at $p \leq 0.01$. Different subscribts are significant. Data are expressed in mean ± SD.

Table 6. Effect of different concentrations of Irgarol 1051 (0.050, 0.025 and 0.012 µg/l) on chlorophylls content (mg/l) of *Dunaliella bardawil* cultured for 16 days.

Time (Days)	Parameter	Control	Irgarol 1051 concentrations (µg/l)			F (p)	LSD
			0.012 µg/l	0.025 µg/l	0.050 µg/l		
0	Chl.a	0.520 ± 0.002^{a}	0.520 ± 0.002^{a}	0.520 ± 0.002^{a}	0.520 ± 0.002^{a}	17582.143** (<0.001)	0.002
	Chl.b	0.135 ± 0.001^{a}	0.135 ± 0.001^{a}	0.135 ± 0.001^{a}	0.135 ± 0.001^{a}	2107.059** (<0.001)	0.002
	Total	0.655	0.655	0.655	0.655		
2	Chl.a	0.680 ± 0.002^{a}	0.540 ± 0.003^{b}	0.406 ± 0.004^{b}	0.210 ± 0.004^{d}	60551.661** (<0.001)	0.003
	Chl.b	0.220 ± 0.003^{a}	0.270 ± 0.003^{b}	0.201 ± 0.003^{b}	0.190 ± 0.002^{d}	4008.571** (<0.001)	0.004
	Total	0.900	0.810	0.607	0.300		
4	Chl.a	1.590 ± 0004^{a}	0.860 ± 0.001^{b}	0.450 ± 0.003^{c}	0.305 ± 0.004^{d}	44392.258** (<0.001)	0.004
	Chl.b	0.540 ± 0.001^{a}	0.300 ± 0.02^{b}	0.220 ± 0.002^{c}	0.210 ± 0.001^{d}	1703.529** (<0.001)	0.003
	Total	2.130	1.160	0.670	0.515		
8	Chl.a	1.520 ± 0.004^{a}	1.110 ± 0.001^{b}	0.550 ± 0.003^{c}	0.330 ± 0.006^{d}	29707.619** (<0.001)	0.004
	Chl.b	0.640 ± 0002^{a}	0.560 ± 0.004^{b}	0.480 ± 0.004^{c}	0.210 ± 0.007^{d}	7388.571** (<0.001)	0.002
	Total	2.160	1.670	1.03	0.540		
12	Chl.a	1.460 ± 0.004^{a}	1.180 ± 0.004^{b}	0.5160 ± 0.002^{c}	0.240 ± 0.002^{d}	26656.579** (<0.001)	0.004
	Chl.b	0.640 ± 0.003^{a}	0.416 ± 0.002^{b}	0.480 ± 0.003^{c}	0.250 ± 0.004^{d}	2062.500** (<0.001)	0.003
	Total	2.100	1.590	0.990	0.470		
16	Chl.a	1.420 ± 0.001^{a}	0.850 ± 0.004^{b}	0.501 ± 0.00.^{c}	0.210 ± 0.001^{d}	12054.684** (<0.001)	0.004
	Chl.b	0.530 ± 0.002^{a}	0.580 ± 0.001^{b}	0.460 ± 0.004^{cc}	0.230 ± 0.001^{d}	305.294** (<0.001)	0.003
	Total	1.950	1.430	0.961	0.440		

F (p): F-test (ANOVA) and its significance between groups. LSD: Least significant difference at 0.05. *: Statistically significant at $p \leq 0.05$. **: Statistically significant at $p \leq 0.01$. Different subscribts are significant. Data are expressed in mean ± SD.

reduction in chlorophyll content with reduced growth rate is due to decrease in photosynthetic rate. [40] [41] [42] regarded that, the antifouling boostering agent Irgarol 1051 is a strong inhibitor of photo system II (PSII) with high efficiency (toxicity) toward algae.

5. Conclusions

The effect of different concentrations of Irgarol 1051 on the two tested organisms

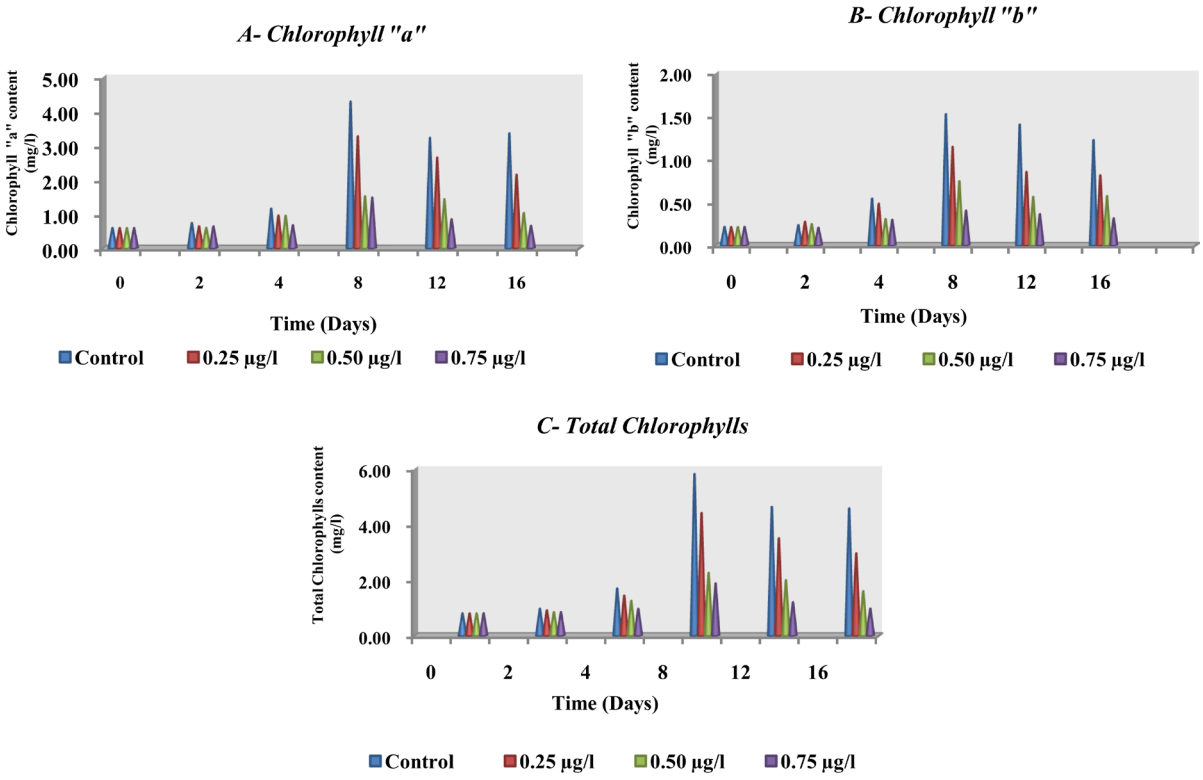

Figure 7. Effect of different concentrations of Irgarol 1051 (0.75, 0.50 and 0.25 µg/l) on chlorophylls content (mg/l) of *Chlorella salina* cultured for 16 days.

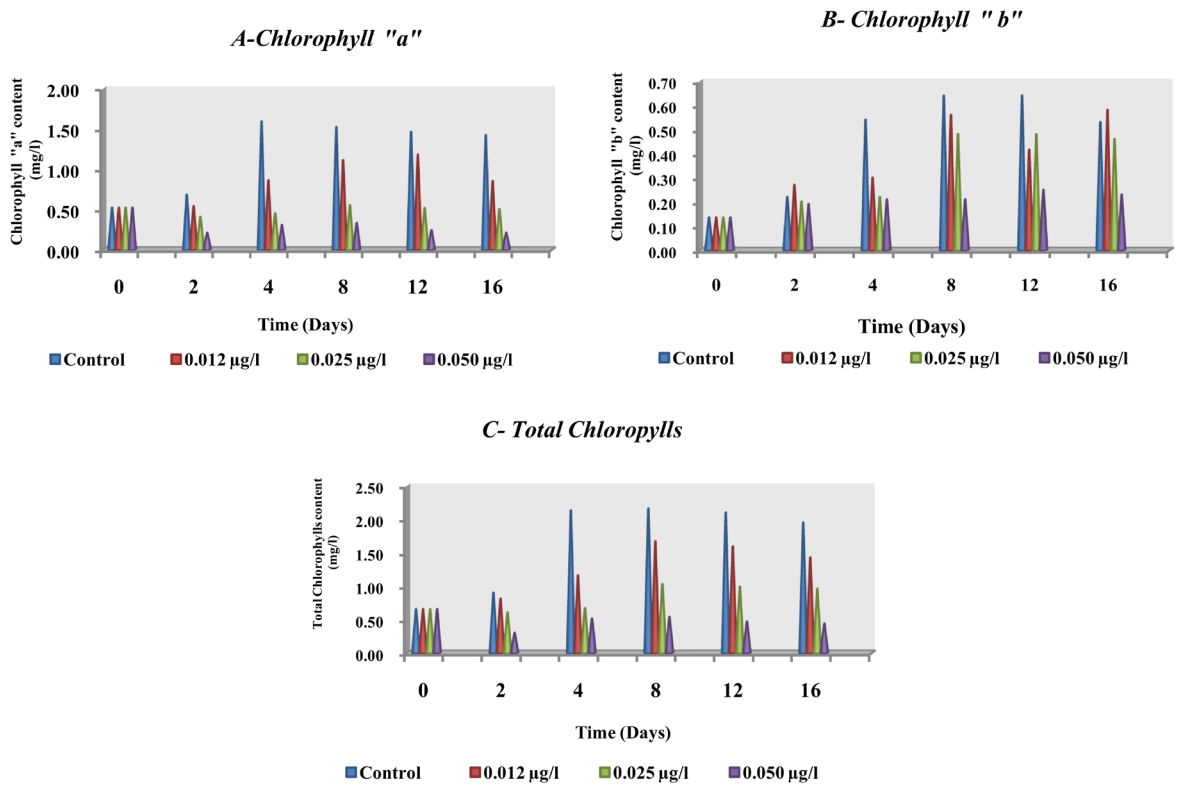

Figure 8. Effect of different concentrations of Irgarol 1051 (0.050, 0.025 and 0.012 µg/l) on chlorophylls content (mg/l) of *Dunaliella bardawil* cultured for 16 days.

Chlorella salina and *Dunaliella bardawil* caused suppression of algal growth which may be due to the increasing of toxicity of this biocide. Chlorophylls a and b content in *Dunaliella bardawil* cleared that, the toxic effect of Irgarol 1051 is clearer than in case of *Chlorella salina* and prove that the strength of toxicity depends mainly on the concentration of the antifouling agent, the length of culturing period and the type of organism tested.

This work clears that water polluted by the booster biocide Irgarol 1051 which leached out from the antifouling paints will cause high pollution rate in marine environment which leads to cause weak in growth, damage or death of the several phytoplankton species that considered to be the essential base of fish food chain. There is a need to develop a non-toxic control of fouling growth to prevent damage to marine ecosystems due to pollution arising from antifouling compounds.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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