EFFECTS OF IRGAROL-1051 ON FATTY ACID PROFILE OF SOLITARY CORALS, *Fungia fungites* AFTER SHORT TERM EXPOSURE

(Kesan Irgarol-1051 terhadap Profil Asid Lemak Karang Solitari, *Fungia fungites* selepas Pendedahan Jangka Pendek)

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Abstract

The short term exposure (4 days) was done to *Fungia fungites* using different concentration of Irgarol to establish the health effects of this species by measuring their fatty acid composition. It was done by comparing fatty acid composition of the fresh, control and exposed groups. The results indicated that, fresh and control samples of *F. fungites* were not significant different (P >0.05) and dominated by Saturated Fatty Acids (SAFA) followed by Polyunsaturated Fatty Acids (PUFA) and the Monounsaturated Fatty Acids (MUFA). However, trends of other tested groups (20, 100 and 500 µg/L) were significant different (P <0.05), with species suffered more as the dose of chemicals increased. For example, α-Linolenic acid (C18:3ω3) was not detected in *F. fungites* which exposed in this herbicide at 100 and 500 µg/L whereas C20:5ω3 was not detected in all exposed groups. These ecotoxicological data proved that Irgarol may affect the fatty acids composition of coral reef organisms even if exposed at the low levels of this biocide.

Keywords: Irgarol, booster biocides, fatty acids composition, *Fungia fungites*, coral reef organisms

Abstrak

Pendedahan jangka pendek (4 hari) telah dilakukan ke atas *Fungia fungites* menggunakan kepekatan Irgarol yang berbeza untuk membangunkan kesan kesihatan spesies tersebut dengan mengambilkira komposisi asid lemak mereka. Ianya telah dilakukan dengan membandingkan komposisi asid lemak bagi kumpulan segar, kawalan dan yang telah didedahkan. Keputusan menunjukkan bahawa, sampel segar dan kawalan *F. fungites* tidak berbeza secara bererti (P >0.05) dan didominasi olah Asid Lemak Tepu (SAFA) diikuti oleh Asid Lemak Tidak Tepu Poli (PUFA) dan Asid Lemak Tidak Tepu Mono (MUFA). Walaubagaimanapun, corak bagi kumpulan lain yang diuji (20, 100 and 500 µg/L) adalah berbeza secara bererti (P <0.05), dengan spesies mengalami sengsara yang lebih apabila dos bahan kima ditingkatkan. Misalnya asid lemak α-Linolenat (C18:3ω3) tidak dikesan dalam *F. fungites* yang terdedah dengan racun ini pada 100 and 500 µg/L manakala C20:5ω3 tidak dikesan dalam semua kumpulan yang terdedah. Data ekotoksikologi ini membuktikan bahawa Irgarol berkemungkinan menjejaskan komposisi asid lemak organisma terumbu karang sungguhpun jika terdedah pada tahap yang rendah oleh biosid ini.

Kata kunci: Irgarol, biosid perangsang, komposisi asid lemak, *Fungia fungites*, organisma terumbu karang

Introduction

Irgarol-1051 is one among the commonly used alternative antifouling chemical after the prohibition of tri-butyltin (TBT) by International Maritime Organization (IMO) [1, 2]. It was reported that, Irgarol-1051 is very effective in prevention of attachments of biofoulings in recreational boats and yachts [3, 4]. In addition, the Irgarol is considered as a photosystem II inhibitor, which prevents photosynthesis and block conversion of excitation energy into chemical energy [5]. It has been broadly detected in aquatic ecosystems [6–10]. The significant concentrations of Irgarol have been also reported in coastal sediments [11–13]. Numbers of studies have demonstrated the impacts of Irgarol on marine organisms such as corals. For example, Owen et al. [7] reported that 60 ng/L of Irgarol reduced ^{14}C incorporation in the *Madracis mirabilis*, while 63 ng/L reduced net photosynthesis of intact corals. Moreover, 50 ng/L reduced photosynthetic efficiency (F0/Fm0) of *Seriatopora hystrix* [5]. It is also revealed that 1000 ng/L of Irgarol significantly reduced the photosynthesis rate of *G. fascicularis* and 100 ng/L reduced the settlement of coral larvae [4, 10]. The concerns on toxicity nature of Irgarol-1051 to marine habitats lead some countries such as Denmark, UK and Sweden to restrict the use of Irgarol as an antifouling paint to the boats under 25 m [14]. Studies on detection and distribution have reported the level of Irgarol 1015 in the coastal waters of Asian countries, for example, Singapore [15] and Malaysia [2], however, very little is known about its impact to corals in this region. To our knowledge only the study by Ali et al. [16] has reported the effects of Irgarol to the fatty acid composition of Asian Sea-bass (*Lates calcarifer*) from Malaysia. This study therefore presents the ecotoxicological data on the impact of Irgarol-1051 in the *F. fungites* by measuring fatty acid composition after exposed at different doses for four days in laboratory.

Materials and Methods

Sample collection

Fresh corals (*F. fungites*) were taken from Bidong Island, Terengganu Malaysia. The corals were acclimatized for 2 weeks in well aerated holding polyethylene tanks (500 L), containing natural seawater with a salinity of 30 ppt, under a natural photoperiod 12:12 hours (light: dark) cycle. The water in the tank was passed through a 1-mm filter, treated with UV-sterilized and refilled daily. The coral was not fed at all.

Chemical

A stock solution of 1000 mg/L Irgarol was prepared by using acetone and the working concentrations were made up by spiked the required concentrations to the sea water.

Experimental procedures

F. fungites were acclimatized for two weeks in the laboratory before the acute exposure tests to Irgarol for 96 hours. Water quality characteristics were measured daily: dissolved oxygen (DO) 7.2 ± 0.5 mg/L, and pH 7.69 ± 0.2, but the temperature was automatically controlled to 27 °C using cooling chiller throughout the whole experiment duration. After the two weeks acclimatization period, corals were transferred into different experimental tanks containing three litres of water and exposed to test concentrations of 20, 100, and 500 μg/L of Irgarol in three replicates for 96 hours. The control and acetone (check-up tanks) were kept for the sake of comparison. After four days (96 hours), the tissue of the coral were removed by using water pick and taken to the laboratory for fatty acid analysis.

Analytical procedure

Tissues of *F. fungites* coral were taken as samples for fatty acid composition analysis. The one step method [17] was used in this experiment to combine extraction and esterification processes using a single tube. Three replicates of each tissue samples (200 – 300 mg) were mixed with 4 mL of hexane and 1 mL of internal standard solution in a 50 mL centrifuge tube. After adding 2mL of 14% BF_3 in methanol and a magnetic stirring bar, the head space of tube was flushed with nitrogen gas and then closed tightly with a Teflon-lined screw-cap. The capped tube was heated on a hot plate at 100 °C for 120 min under continuous stirring. After cooling to room temperature, 1 mL of hexane was added followed by 2 mL of distilled water. The tube was then shaken vigorously for 1 min and centrifuged for 3 min at 2500 rpm (650 ×g). Of the two phases which formed, the upper phase was hexane layer containing the FAMEs. Finally, approximately 1 – 2 mL of the hexane layer was transferred using a Pasteur pipette into a clean sample vial to be injected into the GC-FID for FAME analysis.

Fatty acid concentrations (C_{FA}, mg/g of dry sample) were calculated by comparing the peak area of fatty acid in the sample with the peak area of internal standard as equation (1) follows:

$$C_{FA} = {A_S}/{A_{IS}} \times {C_{IS}}/{W_S} \tag{1}$$

where A_S = peak area of fatty acid in the sample in chromatogram, A_{IS} = peak area of internal standard in chromatogram, C_{IS} = concentration of internal standard (mg) and W_S = weight of sample (g). Qualitatively (as a percentage), composition of individual fatty acids were calculated by comparing the peak area of each fatty acid with the total peak area of all fatty acids in the sample.

Gas chromatography conditions

The FAMEs were separated and quantified using a gas chromatograph (GC 14-B Shimadzu) equipped with flame ionization detector. Separation was performed with an FFAP-polar capillary column (30 m × 0.32 mm internal diameter, 0.25 μm film thickness). Purified hydrogen was used as a carrier gas. After injection at 60 °C, the oven temperature was raised to 150 °C at a rate 40 °C min^{-1}, then to 230 °C at 3 °C min^{-1}, and finally held constant for 30 min. The flame ionization was held at 240 °C. FAME peaks were identified by comparing their retention times with those of authentic standards (Supelco Inc.). Fatty acids were designated as an n:pωx, where n is the number of carbon atoms in the aliphatic chain, p is the number of double bonds and x is the position of the first double bond from the terminal methyl group. The analytical precision for samples was generally <5 % for total amounts and major components of FAMEs.

Results and Discussion

Fatty acid composition

The fatty acid results were calculated and presented as mean ± standard deviation (Table 1). Significant differences among the mean value of treatments were treated by One-way Analysis of Variance (ANOVA) followed by Least Significant Different (L.S.D). Two-way Analysis of Variance was used to support one way Analysis of Variance to get the F-probability interactions between chemicals, concentrations and / or species. Duncan's multiple range test ($P < 0.05$) were calculated.

Fatty acid composition data for acute exposure of the *F. fungites* in Irgarol are presented in Table 1. The corals were dominated by SAFA 27.4 ± 6.17 (54.3 %), 23.8 ± 5.04 (49 %) and 23.2 ± 6.33 (50.6 %) mg/g dry weight, followed by PUFA 20.5 ± 5.48 (40.6 %), 23.2 ± 6.67 (47.8 %) and 20.9 ± 6.31 (45.8 %) mg/g dry weight and last is MUFA 2.25 ± 1.14 (5.06 %), 1.15 ± 0.69 (3.21 %) and 1.65 ± 0.74 (3.61 %) mg/g dry weight of fresh, control and acetone samples respectively.

In this study, *F. fungites* samples exposed with 20, 100 and 500 μg/L Irgarol have the same trend as fresh, control and acetone samples of SAFA >PUFA >MUFA (Figure 1). It was noted that SAFA, PUFA and MUFA are reduced as the dose of Irgarol increased in tested species. It is also interesting to note that in *F. fungites* (Figure 1), MUFA groups contributed less in the total FA among all exposed groups. The behavior of some groups of corals organisms to show different trends as in fresh and control samples can be justified by the fact that the activities in these groups were affected not only by the chemicals but also the ability of the individual organism to fight the toxic behavior of the used chemical.

Saturated fatty acids (SAFA)

The *F. fungites* are relatively enriched in Saturated Fatty Acids (SAFA) and the total SAFA are not significant different (P >0.05) between fresh and control groups, however the other groups and individual SAFA were significant difference ($P < 0.05$) in this species (Table 1). Palmitic acid (C16:0) was found dominant in all SAFAs of the *F. fungites* (Table 1) and showed a significant difference ($P < 0.05$) between the samples. SAFA is pronounced more in average in *F. Fungites* (Figure 1). It was also reported that palmitic acid was the predominant in SAFA group as found in freshwater channel catfish (*Ictalurus punctatus*) (19.2 %) [18], and in freshwater rainbow trout (*Oncorhynchus mykiss*) (21.3 %) [19]. The fresh water species domination on SAFA fatty acids is also reported in the study conducted by Jabeen and Chaundhry [20].

Table 1. Fatty acid composition (mg/g) dry weight of tissue samples of *Fungia fungites* (mushroom) after 96 hours acute exposure test. Values are means ± standard deviation (SD) for n=3.

Fatty Acids	Fresh	Control	Acetone	Irgarol		
				20µg/L	100µg/L	500µg/L
SAFA						
C14:0	2.05[a] ± 0.58	1.57[b] ± 0.08	1.40[b] ± 0.16	1.41[b] ± 0.08	1.58[b] ± 0.13	1.60[b] ± 0.13
C16:0	15.9[a] ± 1.39	13.2[bc] ± 1.53	15.2[ab] ± 1.77	14.1[ab] ± 1.64	11.4[c] ± 1.20	6.25[d] ± 0.61
C18:0	5.27[a] ± 0.34	5.15[a] ± 0.18	3.84[ab] ± 0.44	4.39[a] ± 1.01	3.69[ab] ± 0.47	4.46[a] ± 0.97
C20:0	4.21[a] ± 0.15	3.89[ab] ± 0.61	2.76[c] ± 0.11	ND	3.44[bc] ± 0.31	ND
MUFA						
C16:1	2.55[a] ± 0.45	1.55[c] ± 0.18	1.65[bc] ± 0.08	1.78[bc] ± 0.13	1.41[c] ± 0.61	1.56[bc] ± 0.07
C17:1	ND	ND	ND	ND	ND	ND
C18:1ω9	ND	ND	ND	ND	ND	ND
C20:1	ND	ND	ND	ND	ND	ND
PUFA						
C18:3ω6	3.03[bc] ± 0.12	3.30[b] ± 0.22	2.28[c] ± 0.19	2.29[c] ± 0.29	6.15[a] ± 0.25	2.84[bc] ± 1.05
C18:3ω3	13.7[bc] ± 1.07	16.4[a] ± 0.76	15.1[ab] ± 0.86	11.7[d] ± 1.04	0.00	0.00
C20:3ω3	1.24[cd] ± 0.30	2.04[a] ± 0.14	1.06[d] ± 0.07	1.45[bcd] ± 0.19	1.01[d] ± 0.11	2.29[a] ± 0.26
C20:5ω3	2.54[a] ± 0.08	1.46[b] ± 0.25	2.55[a] ± 0.23	ND	ND	ND
C22:6ω3	ND	ND	ND	ND	ND	ND
ΣSAFA	27.4[a] ± 6.17	23.8[ab] ± 5.04	23.2[ab] ± 6.33	19.9[b] ± 6.39	20.1[b] ± 4.37	12.3[cd] ± 2.85
ΣMUFA	2.55[a] ± 1.14	1.55[c] ± 0.69	1.65[bc] ± 0.74	1.78[bc] ± 0.80	1.41[c] ± 0.80	1.56[bc] ± 0.70
ΣPUFA	20.5[ab] ± 5.48	23.2[a] ± 6.67	20.9[ab] ± 6.31	15.4[c] ± 4.92	7.2[d] ± 2.67	5.1[d] ± 1.43
ΣFA	50.4[a] ± 5.04	48.5[a] ± 5.18	45.8[ab] ± 5.18	37.1[b] ± 4.58	28.7[c] ± 3.30	19.0[d] ± 2.02

SAFA: Saturated fatty acids; MUFA: Monounsaturated fatty acids; PUFA: Polyunsaturated fatty acids; Means within rows followed by the same superscript(s) are not significant different (P >0.05); ND – Not detected
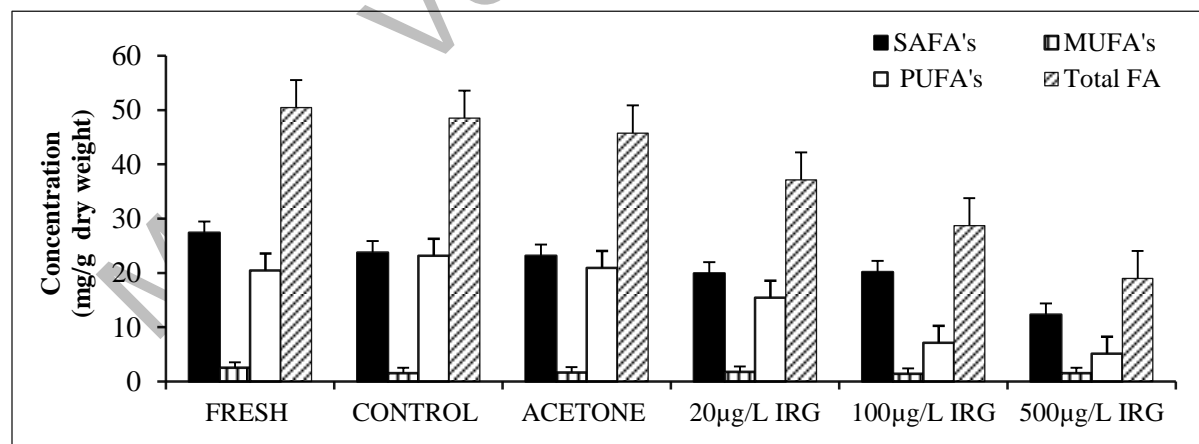

Figure 1. Fatty acid concentration of different classes and total fatty acid (mg/g dry weight) from tissues of Fungia fungites after exposed at different concentrations of Irgarol. Values are mean ± S.D, n = 3.

Mono unsaturated fatty acids (MUFA)

The ΣMUFA for *F. fungites* were significant difference in fresh, control, acetone and other exposed concentrations (Table 1). It is observed that MUFA contribution is less in total fatty acids in *F. fungites* (Table 1). This is due to the fact that, only palmitoleic acid (C16:1) was observed in the GC-FID where the rest of MUFA were below the detectable limit. In fact, Ho and Paul [21] found about 8 % only of oleic acid in total fatty acid in Asian sea bass. However, Steiner-Asiedu et al. [22] found that freshwater tilapia (*Tilapia* sp.) had significantly higher oleic acid levels than flat sardine (*Sardinella*) and sea bream (*Dentex*). The level of oleic acid in American freshwater channel catfish flesh was also reported higher; about 50 % [23] compared to less than 1% in sardine and sea mullet [24].

Polyunsaturated fatty acids (ΣPUFA)

The sum of Polyunsaturated Fatty Acids (ΣPUFA) in *F. fungites* was not significantly different among fresh, control and acetone samples ($P > 0.05$). α-Linolenic acid (C18:3ω3) was dominant PUFA in *F. fungites* (66.8 % fresh sample) (see Figures 1 & 2). The rest of the PUFA in this species have less contribution. The higher levels of omega 3 and 6 in this species was not surprising because seawater organisms obtain their omega-3 and 6 FA from oceanic plankton [16, 25] or are fed fishmeal containing these FA [26].

Individual fatty acids were not detected in some exposed groups which differed with fresh and control groups (Figure 2). For example, α-Linolenic acid (C18:3ω3) was not detected in *F. fungites* exposed in this herbicide at 100 and 500 µg/L whereas C20:5ω3 was not detected in all exposed groups (Figure 2). As expected, individual fatty acids in 500 µg/L exposure groups were much affected than the rest of the groups in *F. fungites* (Figure 2). The results show an inverse relationship between the dose of chemical used and activities of the *F. fungites* in the tanks. It is clear that, as the concentration of the Irgarol increased in different exposed tanks there was a decrease in activities of *F. fungites*.
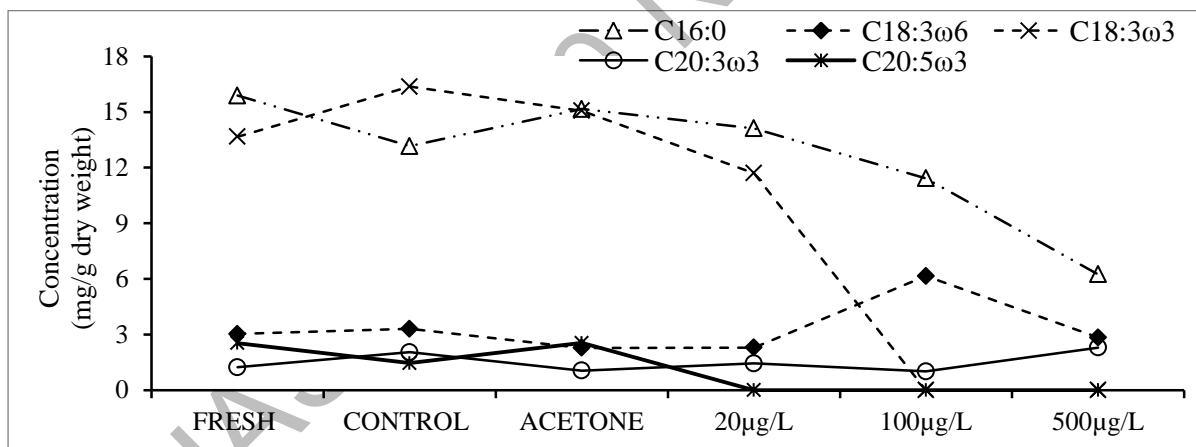

Figure 2. Individual Fatty acid concentrations of *Fungia fungites* exposed at different doses of Irgarol.

Conclusion

This paper explains the toxicological responses of *F. fungites* upon exposure to different concentrations of Irgarol in laboratory experiments. It can be concluded that; acute exposure of *F. fungites* (96 hours) to different concentrations of Irgarol had shown significant impact to the fatty acid composition. Fresh, control and all exposed samples of *F. fungites* were in trend of SAFA >PUFA >MUFA, however the exposed samples suffer more as the dose of Irgarol increased. The results of this study reveal that Irgarol is toxic and reduce the fatty acid composition of the coral even at the low level of exposure (20µg/L) and for a short period of time (96 hours). There is a need of further researches to be conducted in this area of specialization so as to understand more effects of these new antifouling chemicals to marine organisms as well as in coral reef using different species.

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