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CHANGES OF FATTY ACID COMPOSITION IN SCLERACTINIAN CORAL, *Galaxea fascicularis* (LINNAEUS, 1767) BY ACUTE EXPOSURE OF IRGAROL-1051

(Perubahan Komposisi Asid Lemak dalam Karang Scleractinia, *Galaxea fascicularis* (LINNAEUS, 1976) oleh Pededahan Akut Irgarol-1051)

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Abstract

Antifouling biocide such as Irgarol 1051 has been widely used as a replacement of tributyl tin (TBT). In Malaysia, we reported the level of Irgarol in coastal water up to 2021ng/L. This raises concern because high dosage of chemical pollutant in the seawater can affect the marine organisms. This study therefore, examined the effect of Irgarol 1051 on fatty acids composition of reef building coral *Galaxea fascicularis*, collected in Pulau Bidong, Malaysia. The corals were exposed to different doses of Irgarol 1051 under short term exposure (96 hrs) and the fatty acid compositions of the coral tissues were determined using the gas chromatography technique. The findings revealed no clear different (p >0.05) among untreated samples (fresh and control) and both were dominated by polyunsaturated fatty acids (PUFA), followed by saturated fatty acids (SAFA) and monounsaturated fatty acids (MUFA). In contrast, the treated samples of *G. fascicularis* (20, 100 and 500 µg/L) were significant different (p <0.05) where both SAFA and PUFA were significantly lowered than untreated samples especially at the samples exposed to higher dose of Irgarol 1051 (100 and 500 µg/L). As the level of dose increased, SAFA such as 16:0 and unsaturated fatty acid from ω3 and ω6 series were largely affected by the toxicology effect of the Irgarol 1051. The results indicate that Irgarol 1051 significantly affecting the health of the corals even at the lowest dose of Irgarol 1051 applied in this study. It is suggested that the antifouling biocide may have implication on metabolisms of the corals.

Keywords: booster biocides, antifouling chemicals, fatty acids, hard coral, coral reefs

Abstrak

Biosid anti-kotoran seperti Irgarol 1051 telah digunakan secara meluas sebagai pengganti kepada tributil tin (TBT). Di Malaysia, kami telah melaporkan tahap Irgarol di perairan pantai mencapai setinggi 2021ng/L. Ini menimbulkan kebimbangan kerana dos bahan pencemar kimia yang tinggi dalam air laut boleh menjejaskan organisma marin. Oleh itu, kajian ini mengkaji kesan Irgarol

1051 pada komposisi asid lemak di dalam karang keras *Galaxea fascicularis*. Pendedahan jangka pendek (96 jam) telah dilakukan ke atas spesis karang ini dengan menggunakan kepekatan Irgarol yang berbeza dan komposisi asid lemak didalam tisu karang ditentukan dengan menggunakan teknik gas kromatografi. Hasil kajian mendapati tiada perubahan yang ketara pada karang yang tidak terdedah dengan Irgarol (sampel segar dan kawalan) dan kedua-duanya mempunyai kandungan asid lemak yang didominasi oleh asid lemak poli tidak tepu (PUFA), diikuti dengan asid lemak tepu (SAFA) serta asid lemak mono tidak tepu (MUFA). Sebaliknya, terdapat perbezaan yang ketara antara sampel karang yang tededah dengan kepekatan berbeza Irgarol (20, 100 and 500 µg/L) dimana komposisi SAFA dan PUFA lebih rendah berbanding sampel segar dan kawalan, terutamanya pada sampel yang terdedah pada kepekatan Irgarol 1051 yang tinggi. SAFA seperti 16:0 dan asid lemak tidak tepu dari kumpulan ω3 dan ω6 adalah antara asid lemak yang sangat terkesan terhadap pendedahan pada bahan kimia ini. Dapatan kajian juga menunjukkan, Irgarol 1051 sangat mempengaruhi kesihatan karang walaupun hanya terdedah pada dos yang rendah. Ini menunjukkan bahawa terdapat implikasi pada metabolisma karang apabila terdedah kepada bahan kimia ini.

Kata kunci: biosid penggalak, bahan kimia anti-kotoran, asid lemak, karang keras, terumbu karang

Introduction

Booster biocide such as Irgarol 1051 is categorized as priority hazardous substance by the European commission [1] but is used in worldwide as a means to control the fouling on boats and ships. The research to date has tended to report a contamination by alternative biocides Irgarol 1051 in coastal water of Malaysian peninsular [2] as well as in sediments and mussels around Malaysian coast [3]. Despite their increased use worldwide, far too little attention has been paid to the fate, toxicity and persistence of this booster biocide Irgarol as well as their potential risks on marine ecosystems [4]. The toxicity of this booster biocide on marine organisms, especially those from tropical and subtropical regions are still largely unknown, as most available and relevant toxicity data were generated with temperate species [5]. As a consequence, coral reefs around the world are facing a number of threats of the toxic chemical's inputs from terrestrial such as domestic activities, agricultural, and industrial [6, 7]. For example, in Japan, it was reported that, development activities such as road building, farming, and modification of drainage systems have increased runoff of red soil in coral reefs which have negative impact to coral reef ecosystems [4, 8]. Toxic chemical substances are contributing to the destruction of coral reef ecosystems. Research by Knutson et al. [9] revealed a potential effect of Irgarol 1051 on coral larvae which significantly reduce the larvae settlement even at low dose of Irgarol 1051 (100ng/l). However, in Malaysia most of the pollutant's studies have been focused on heavy metals either in ports, marinas and islands; but attention has not been paid to the distribution and impacts of antifouling chemicals (*e.g.,* Irgarol) in this region. Recently, we reported the effects of Irgarol on fatty acid of Asian sea-bass, *Lates calcarifer* in Malaysia [10] but still there is a significant gap on research information regarding to the toxicity of new antifouling chemicals such as Irgarol in relation with the health of coral reef organisms worldwide.

Comparatively few studies have dealt with the effects of new antifouling Irgarol in particular on different coral reef organism in aquatic systems. Furthermore, the studies that have dealt with the effects of Irgarol on lake or river bacteria, have seldom examined these effects on the composition of these communities [11] or have analysed the whole microbial communities or the whole plankton [12] without testing the direct effects of these pollutants on marine organisms using fatty acid as an indicator. Hence, the direct effects of Irgarol and many other pesticides on natural aquatic organisms are poorly known. Understanding the effects of Irgarol and other pesticides on coral reef is of interest, given that the alteration of marine communities might affect their ecosystem function. Therefore, this study was carried out to determine the fatty acid profile of fresh sample (control) and compared with those short term exposed *Galaxea fascicularis* in different doses of Irgarol to realize environmental causing damages to these vital marine natural resources.

Materials and Methods

Experimental design

Fresh corals (*Galaxea fascicularis*) were taken from Pulau Bidong, Terengganu, Malaysia (5°37′N 103°4′E). 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 h:12 h (light: dark) cycle. The water in the tank was passed through a 1-mm filter, treated with UV-sterilized and refilled daily. For the purpose of the experiment, the coral was not fed at all.

A stock solution of 1000 mg/l Irgarol (Sigma-Aldrich) was prepared by using acetone and the working concentrations were made up by spiking the required concentrations to the sea water (volume= 3000 mL). *Galaxea fascicularis* were acclimatized for two weeks in the laboratory before the acute exposure tests to Irgarol for 96 hrs [13] as shown in Figure 1. 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 ^{0}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 0 (T1), 20 µg/L (T2), 100 µg/L (T3), and 500 µg/L (T4) of Irgarol in three replicates for 96h. Samples in control the control tank was kept for the sake of comparison. After four days (96hrs), the tissue of the coral was removed by using water pick and taken to the laboratory for fatty acid analysis.

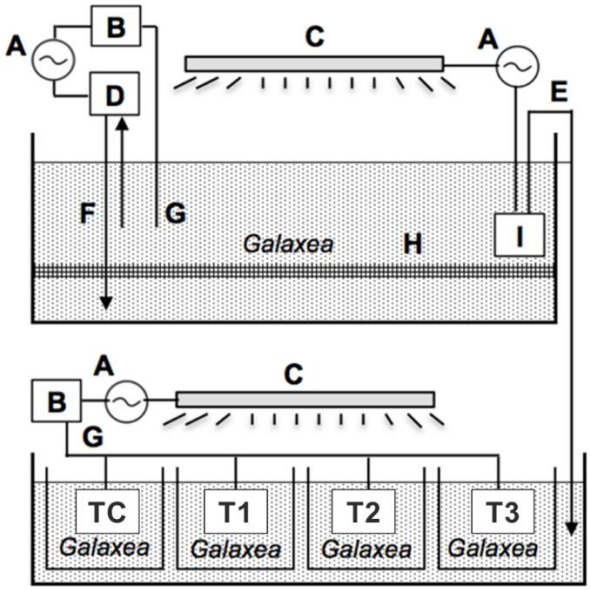

Figure 1. Schematic diagram of acclimation tank (top) and experiment tanks (bottom) during exposure experiment of Irgarol on the corals *Galaxea fascicularis*. A, power supply; B, aeration compressor; C, lamp; D, water chiller; E, outflow water; F, inflow and outflow chiller water; G, aeration tubes; H, filter; I, water pump. T1 to T3 are Irgarol test concentrations of 20 µg/L, 100 µg/L, and 500 µg/L, respectively while TC as control.

Sample preparation

Fatty acid methyl ester extraction

Tissues of *Galaxea fascicularis* were taken as samples for fatty acid composition analysis. The one step method [14] was used in this experiment to combine extraction and esterification processes in a single tube. Three replicates of each tissue samples (200-300 mg) were mixed with 4 mL of hexane and 1 mL (1 mg/mL) of internal standard (analytical standard of nonadecanoic acid, C19:0, Sigma-Aldrich) solution in a 50 mL centrifuge tube. After adding 2 mL 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. Of the two phases which formed, the upper phase was hexane layer containing the FAMEs. Finally, ~1–2 ml of the hexane layer was transferred using a Pasteur pipette into a clean sample vial prior to for injection for the GC-FID FAME analysis. Fatty acid concentrations (CFA, 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 follows:

$$C_{FA} = {}^{A_S}/_{A_{IS}} \times {}^{C_{IS}}/_{W_S} \quad (1)$$

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 was 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 chromatography equipped with flame ionization detector, GC-FID (GC = Agilent Technologies 6890N, FID = Agilent 5973 MSD). Separation was performed with an FFAP-polar capillary column (30 m×0.32 mm internal diameter, 0.25 μm film thickness) in split-less mode. Hydrogen was used as a carrier gas. The inlet temperature is 250 °C. 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.

Statistical analysis

Differences among the mean value of fatty acid compositions (ΣSAFA, ΣMUFA, ΣPUFA and fatty acid components) were examined by Generalize Linear Model (GLM) which involves the Multivariate Analysis of Variance (MANOVA). The null hypotheses of 'no difference between treatments' were tested and significant levels less than 0.05 (p <0.05) indicated significant differences for the test treatment. Any significance differences between the treatment samples were further tested via multiple comparison using Bonferroni post-hoc test. Prior to the analysis, data were tested for homogeneity of variances (Leven's test) and the normality of the data (Shapiro–Wilk's test). Statistical analysis was performed using IBM SPSS statistic software.

Results and Discussion

Major fatty acid composition

There was a significant different on the composition of ΣSAFA [$F_{(4,10)} = 39.965$, $p < 0.001$], ΣMUFA [$F_{(4,10)} = 81.811$, $p < 0.001$] and ΣPUFA [$F_{(4,10)} = 30.449$, $p < 0.001$] among the treatment samples. As shown in Figure 2, lower composition of ΣSAFA, ΣMUFA and ΣPUFA were found in the samples exposed with Irgarol 1051. Pairwise test revealed no clear different ($p > 0.05$) between fresh, control and T1 in ΣSAFA composition but significantly different (p <0.05) with the samples exposed to 100 μg/L (T2) and 500 μg/L (T3) Irgarol 1051. Both ΣMUFA and ΣPUFA were not significantly differ ($p > 0.05$) between fresh and control but the composition in the samples exposed to Irgarol 1051 do not follow the trend. Higher composition of ΣMUFA was found in T1 and T2 with palmitoleic acid (16:1) highly contribute to the MUFA composition while ΣPUFA significantly lowered as compared to fresh and control samples.

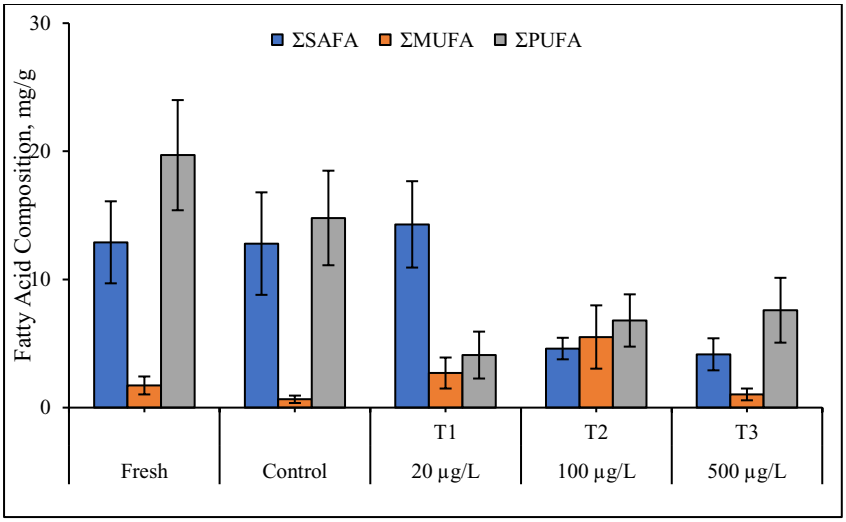

Figure 2. Composition (mg/g) dry weight of SAFA, MUFA, PUFA and total fatty acid in tissue samples of *Galaxea fascicularis* after 96hrs acute exposure test of Irgarol 1051. Values are means with standard deviation (n=3)

Fatty acid components

There was a statistically significant difference in the composition of C14:0 [F (4,10) = 6.101, p = 0.009], C16:0 [F (4,10) = 34.282, p <0.001] and other SAFA [F (4,10) = 46.661, p <0.001] among the treatment samples. Based on post-hoc test, the composition of C16:0 was found significantly lower (p <0.05) in the samples expose to 20 µg/L,100 µg/L and 500 µg/L Irgarol as compared to fresh and control sample, whereby no clear differences (p > 0.05) were found between fresh and control. As shown in Figure 3, the fatty acid compositions of 16:0 was largely affected when the samples were exposed to100 µ/g and 500 µ/g Irgarol. No clear trend was found in the composition of 14:0 and In this study, four PUFA components comprised of omega-6 and omega-3 fatty acids namely γ-Linolenic acid (18:3ω6), α-linolenic acid (18:3ω3 or ALA), eicosatrienoic acid (20:3n3) and eicosapentaenoic acid (20:5ω3 or EPA) were examined. The composition of 18:3ω6 [F (4,10) = 89.997, p < 0.001], 18:3ω3 [F (4,10) = 19.808, p <0.001], 20:3n3 [F (4,10) = 17.364, p <0.001] and 20:5ω3 [F (4,10) = 56.966, p <0.001] were significantly different among the treatment samples. No clear differences (p > 0.05) were found between fresh, and control as indicated by pairwise test but significantly lowered in the samples exposed to 20 µg/L/g ,100 µg/L and 500 µg/L Irgarol (Figure 4).

This study revealed the effect of different concentrations of Irgarol on fatty acid composition in the tissue of *G. fascicularis*. The present findings demonstrate that Irgarol 1051 affecting the health of corals due to changes of fatty acids composition of *G. fascicularis* and may have implications on metabolisms of the corals. Lower fatty acid composition of ΣSAFA and ΣPUFA was observed in the tissue of *G. fascicularis* especially when exposed to high concentration (100 µg/L and 500 µg/L) of Irgarol. Previously, similar trend was also found in a study using Asian sea-bass *Lates calcarifer* where lower ΣSAFA and ΣPUFA was found in the samples exposed to 10%, 30% and 50% of the 96 hrs LC50 = 0.535 mg/ml of triazine herbicides Irgarol 1051 as compared to fresh and control [10]. In contrast, toxicity test of Irgarol 1051 to hard coral *Fungia fungites* revealed that ΣSAFA, ΣPUFA and ΣMUFA were reduced in a as the dose of Irgarol 1051 increased [15]. The inconsistency of fatty acid composition between different species of hard corals can be related to their species-specific and dietary of the species [16, 17].

Lower ΣSAFA is largely associated with the lower composition of palmitic acid (16:0) when the samples exposed to Irgarol 1051 especially at higher doses (100 µ/g and 500 µ/g). Generally, the high content of palmitic acid is commonly found in healthy corals and can reach 50% of the total FA composition [15-17]. Toxicity test

of metal cadmium to marine macroalgae, *Ulva lactuca* revealed that reduction in palmitic acid can be due to an active desaturation process of fatty acid for the production of unsaturated fatty acid such as linoleic (18:2ω6) and linolenic acid (18:3ω6) [18]. However, in the present study, there was no evidence to support the biosynthesis process because some of the PUFAs such as linolenic acid was below the detectable limit.

Among the unsaturated fatty acid, ΣPUFA are largely affected by Irgarol 1051 as compared to ΣMUFA which significantly higher in control and fresh samples. ΣMUFA shows inconsistent trend as compared to fresh and control samples. Perhaps, the MUFA is the intermediate fatty acid in biosynthesis of fatty acid pathway for the conversion from SAFA to PUFA following the contemporary concept [16]. Hence, extensive change in both SAFA and PUFA possibly affecting the inconsistent of ΣMUFA composition. The ΣMUFA having the least composition in fresh and control samples, which is commonly observed in a healthy coral and could remain the low in the samples under stress condition by Irgarol 1051 [10, 15]. In contrast, high composition of ΣMUFA was found in the samples exposed to 100 µ/g of Irgarol 1051 where only palmitoleic acid (16:1) was detected in the GC-FID where the rest of MUFA components were below the detectable limit. High level of ΣMUFA than ΣSAFA possibly due to the active conversion of SAFA to unsaturated fatty acid which leading to high content of ΣMUFA and ΣPUFA in T2 samples [17].

Presently, ΣPUFA was largely affected and significantly lower in all tested samples as compared to fresh and control samples even at lower dosage of Irgarol 1051. It is known that unsaturated fatty acid such as PUFA is highly susceptible to oxidative stress [20]. There is an increasing interest in oxidative stress in the context of chemical stress in marine organisms [21-23]. In corals, cellular mechanism underlying the bleaching is tightly coupled with the accumulation of toxic reactive oxygen species (ROS) which is naturally counterbalance with other SAFA as indicated by the post-hoc test (Figure 3). antioxidant enzymes [24]. A study on acute exposure of Irgarol 1051 on branching coral *Madracis mirabilis* proven that the chemical toxicity greatly enhances the intracellular formation of toxic ROS which compromise by oxidative defence capacity [23]. However, under certain condition, coral will utilize their energy reserve as a barrier or as a repair system against the oxidative stress when the antioxidant enzymes cannot cope with excessive production of ROS [20]. It is well proven that the depletion of PUFA composition after the loss of symbiont can be associated with oxidative stress [20, 25, 26]. Additionally, all PUFA components from the n-3 (18:3ω3, 20:3ω3 and 20:5n3) and n-6 (18:3ω6) were significantly affected by Irgarol 1051. According to Okuyama et al. [27], the long-chain n-3 series such as the EPA (20:5ω3) which has an antioxidative effect are likely to function as a primary protective barrier against the oxidative stress caused by the accumulation of ROS. Among the PUFAs, the 18:3ω6 and 20:5ω3 are known as a fatty acid biomarker for the symbiotic algae [10, 16, 28]. Irgarol is highly toxic and powerful inhibitor to the symbiotic algae in coral because this antifouling chemical is used to prevent the growth of algae [30]. Symbiotic algae provide up to 90% of photosynthetically fixed carbon to corals including fatty acids which is an important nutrient for daily energy requirement of their host [28, 30]. Study on *Seriatopora histrix* [29] and *Acropora tenuis* [31] exposed to Irgarol 1051 revealed that the Irgarol 1051 able to inhibit the PSII that involve with the photosynthesis process in the symbiotic algae. Moreover, it is known that some of the fatty acids especially PUFA were translocated from symbiotic algae to coral [20, 26, 17], hence, lower composition of PUFA in the present samples exposed by Irgarol 1051 is partly associated with the disruption of the photosynthetically fixed carbon production by the symbiotic algae.

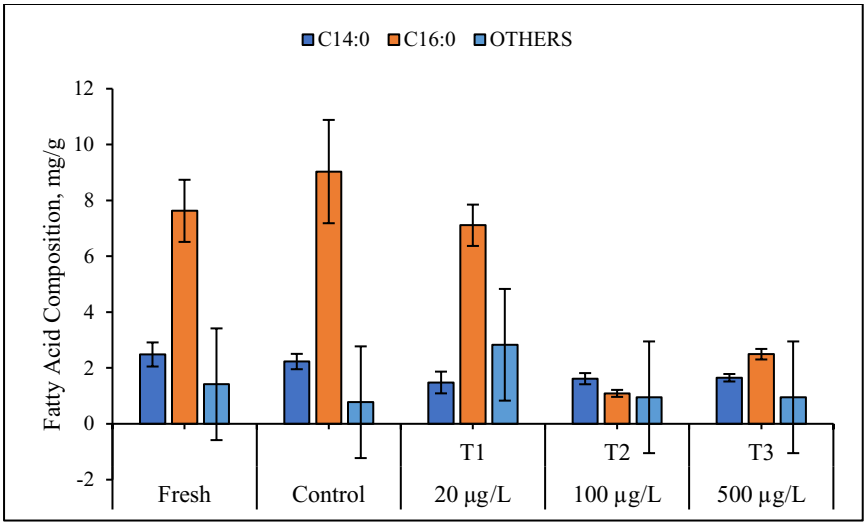

Figure 3. Composition (mg/g) dry weight of SAFA components namely myristic acid (14:0), palmitic acid (16:0) and other components of SAFA (total) in tissue samples of *Galaxea fascicularis* after 96hrs acute exposure test of Irgarol. Values are means with standard deviation (n=3)

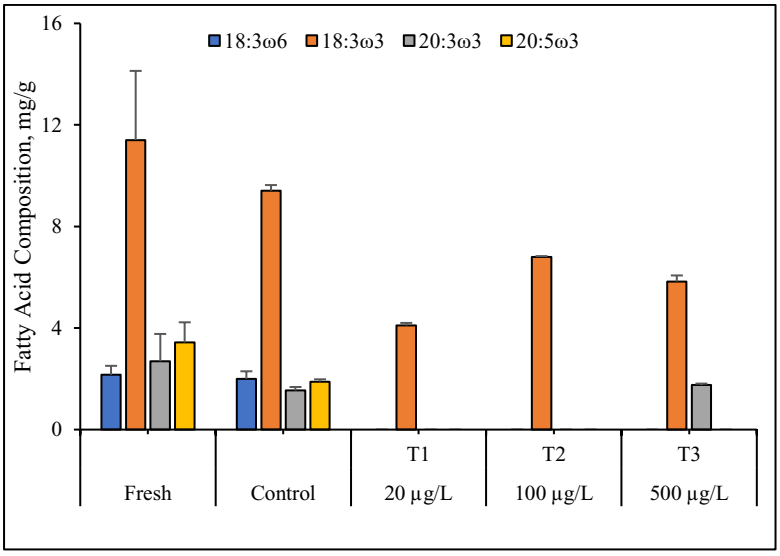

Figure 4. Composition (mg/g) dry weight of PUFA components namely γ-linolenic acid (18:3ω6), α-linolenic acid (18:3ω3), eicosatrienoic acid (20:3n3) and eicosapentaenoic acid (20:5ω3) in tissue samples of *Galaxea fascicularis* after 96hrs acute exposure test of Irgarol. Values are means with standard deviation (n=3)

Conclusion

This paper explains the toxicological responses of *Galaxea fascicularis* upon acute exposure to different concentrations of Irgarol in laboratory experiments. It can be concluded that; acute exposure to *Galaxea fascicularis* (96 hours) to different concentrations of Irgarol 1051 had shown significant impact to the fatty acid compositions of this organism. Moreover, fresh and control samples of *Galaxea fascicularis* were dominated by PUFA followed by SAFA and then MUFA which significantly different trend with the treated samples. It is proven that the Irgarol have affecting the fatty acids composition of the reef-building coral even exposed at the low levels of this biocide. As hard corals are the main reef-builder, further research is required to understand the ecotoxicological effect of Irgarol to their metabolism to comprehend the consequences of this antifouling chemicals to the health of coral reefs ecosystem.

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