Impacts of booster biocides (Irgarol-1051 and diuron) on photosynthesis and calcification rate of coral *Galaxea fascicularis*

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ABSTRACT

Recently, Irgarol-1051(2-methylthio-4-tert-butylamino-6-cyclopro-pylamino-s-triazine) and Diuron, N'-(3,4-dichlorophenyl)-N,N-dimethyl-urea (DCMU) are popular and effective alternative antifouling compounds used worldwide. Acute effects of diuron and Irgarol-1051 on photosynthesis and calcification rates of coral *Galaxea fascicularis* have been assessed under controlled laboratory scale for 96h exposure. Photosynthesis rate of the coral *Galaxea fascicularis* was significantly reduced by 6.5% and 75.7 % relative to control when the coral exposed to 1 and 10 µg/L of diuron, respectively. Calcification rate was only significantly impacted at the highest concentration treatment 10 µg/L of diuron, which dropped to 32.7% relative to control. Photosynthesis rate of the exposed corals were dropped by 18% and 121% for 1 and 10 µg/L Irgarol, respectively. Complete inhibition of photosynthesis was observed when corals were exposed to concentration 10 µg/L of Irgarol 1051. The calcification rate was reduced by 98.3% relative to control treatment when the corals exposed to 10 µg/L Irgarol 1051. The findings indicate that the environmental relevance concentrations of diuron and Irgarol pose significant threat on carbon metabolism especially coral photosynthesis rate in ambient coastal waters.

Keywords

Irgarol, diuron, calcification, photosynthesis, corals.

1. INTRODUCTION

Scleractinian corals are mostly found in shallow coastlines of tropical and subtropical areas. Degrading of coral reefs in East Asia has been partly contributed by anthropogenic activities including pollution [1]. Herbicides including diuron enter in the aquatic ecosystem via leaching, run-off, spray drift or from antifouling paint together with Irgarol 1051 [2,3]. Notable contamination of herbicides has been reported in coral reefs around Great Barrier Reef, Australia [4]. Recently, it has been revealed that diuron has been widely detected in aquatic ecosystems around coral reef areas in Okinawa Island [5; 6]. There is convincing evidence that the ambient concentrations of new antifouling herbicides diuron and Irgarol are relevant to those that can cause deleterious effects to corals [7;8], sea grass [9] and marine algae [10;11].

Both diuron and Irgarol are recent common being used in antifouling paint after replacement of tributyl-tin (TBT). They are effective on preventing the growth of algae. PSII herbicides including diuron and Irgarol could affect algal symbionts (zooxanthellae) or/and host [12]. Zooxanthellae supply the host with photosynthetic product and their photosynthesis enhances the calcification in the coral skeleton [13]. PS II herbicides inhibit photosynthesis and block conversion of excitation energy in to chemical energy [12]. Previous studies have reported the effects of the herbicides on photosynthesis efficiency of isolate dinoflagellates [14; 15], coral tissue [14], delay in growth of juvenile coral [8]; early life history stage of coral [16], metabolism of coral `gross primary production rate, effective quatum yield, gross primary production rate , gross primary production to respiration ratio [17]

Despite of scientific evidence on high frequency of detection of Irgarol and diuron and their associated eco-toxicological behavior in coral reefs, there are very little data found in literature on their effects on carbon metabolism on corals. The aim of this study was to evaluate the acute effects of diuron and Irgarol on calcification rate and photosynthesis rate of coral *Galaxea fascicularis* after exposure for 96 h in the controlled laboratory scale.

2. Materials and method

Preparation of toxicants

Irgarol 1051

A stock solution of 1000mg/L Irgarol 1051 (Ciba Specialty Chemicals Corporation, Tokyo) was prepared using acetone. All solutions used for exposure exposure were obtained after dilution of the stock solution. 1mg/L of Irgarol in filtered seawater was obtained by diluting 1000 times and mixed over night to get homogeneous solution. Various doses (1 and, 10 µg/L) were made by the similar procedures. A carrier control treatment was prepared with a second stock solution containing acetone the same volume found in Irgarol solution.

Diuron

Stock solutions of diuron (Sigma-Aldric, Germany) were prepared in filtered seawater using acetone (PCB and pesticide analysis grade) to improve dissolution. Another stock solution containing only acetone in filtered seawater was prepared for control treatment.

Coral incubation experiments

A colony of the coral Galaxea fasicularis was collected from the shallow zones in front of the Tropical Biosphere Research Center (TBRC) of the University of the Ryukyus, located at Sesoko Island (127°25'E26°39'N) with permission from the Okinawa Prefectural Government (# 17-04). The colony was then transported in a bucket with approximately 5L of seawater and then transferred immediately in to open circuit fresh seawater aquaria exposed to sunlight through black mesh roofs. The coral colony was tagged and cut into 1.5-2.0cm pieces being anchored in PVC tubes on acryl resin screws. The fragments were acclimatized for ~4 weeks in the aquarium before being brought to the laboratory for the experiment.

This laboratory study was conducted in a continuous flow seawater aquarium (15 x 30 x 20 cm^3). Seawater temperature (27 °C) and was carefully controlled by Chiller (GXC-200, China) while light intensity was controlled by a metal halide lamp (Neo Beam Light 24W, KAMIHATA, Japan) which provided illumination at the coral surface at a Photosynthetic Available Radiation (PAR) of 300 µmol m -2 s - 1 during a 12:12-h light/dark cycle , respectively.

Six replicates *Galaxea fascicularis* were used for each treatment. Each coral was incubated in an acryl chamber (0.18 L) for duration of 2 h. Water circulation was stopped during the incubation period. The seawater samples were collected at the beginning and at the end of incubation. pH of seawater were recorded in-situ using pH Meter (Orion 290 A+, Japan). Total alkalinity (AT) was measured using an Auto Titration System (TIM 860 Radiometer, France) within 10 days after sampling. The accuracy of alkalinity measurements was 0.1 %

Changes in inorganic carbon production (IP) and organic carbon production (OP) were then determined by the alkalinity and total inorganic carbon depletion method [18; 19; 20] as follows;

$IP = ½\ \Delta AT.\rho.V/\Delta t.A$…… (i)

$OP = \Delta Ct.\ \rho.\ V/\Delta t.A - IP$…….. (ii)

$Ct = [CO_2*(aq)] + [HCO^{3-}] + [CO3^{2-}]$…….. (iii)

IP= inorganic production, OP=organic production, ΔTA=alkalinity change, ρ= density of seawater,

ΔT= incubation time, A=surface area of coral, V=volume of sea water used for coral incubation, Ct= total inorganic carbon.

Data Analysis

Statistical analyses were performed using SPSS 11, test for significant difference were performed between individual treatments. Difference between doses (treatments) was tested significance by one way analysis of variance (ANOVA), with an alpha of 0.05.

3. Results

3.1 Effects of Diuron on photosynthesis rate

Photosynthesis was significantly reduced (ANOVA p <0.05) when the coral exposed to 10 µg/L. Diuron concentration of 10 µg/L caused rapid decrease of photosynthesis for 96 hrs exposure (Fig. 1). The results show that 1 µg/L reduced the photosynthesis for 6.5 % relative to control but not significant (ANOVA p >0.05), while the photosynthesis was inhibited by 75.7 % relative to control when corals was exposed to 10 µg/L.

3.2 Effects of Diuron on calcification rate

Diuron had a significant impact on calcification rate only at the highest concentration of diuron (10 µg/L) (ANOVA p <0.05) (Fig. 1). After 96 h exposure, no significant reduction of calcification rate was observed at the treatment of 1µg/L of diuron (p>0.05). The calcification rate was dropped to 32.7 % less than control

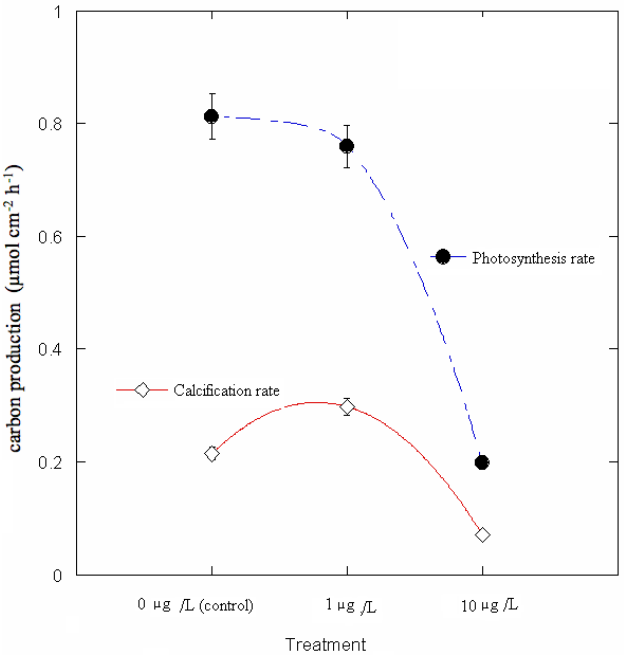

Figure 1.Effect of diuron on photosynthesis rate and calcification rate of coral *Galaxea fascicularis* exposed control, 1 and, 10 µg /L for 96 h.

3.3 Effects of Irgarol 1051 on photosynthesis rate

Photosynthesis rate of *Galaxea fascicularis* was significantly reduced when exposed to both treatments of Irgarol 1051 (1 and 10 µg/L) (ANOVA p <0.05). (Fig.2). In comparison to control corals, the photosynthesis rate of exposed corals were dropped by 18 % and 121 % for 1 and 10 µg/L, respectively. Complete inhibition of photosynthesis was observed when corals were exposed to Irgarol of concentration 10µg/L

3.4 Effects Irgarol 1051 on calcification rate

Significant reduction of calcification was noticed when corals were exposed to the Irgarol treatment of 10 µg/L (ANOVA p <0.05), while the treatment of 1µg/L Irgarol did not show significant effect on calcification rate to the exposed corals (ANOVA $p > 0.05$) (Fig.2). The calcification rate was reduced by 98.3% relative to control treatment, when corals exposed to 10 µg/L Irgarol. Exceptionally, the calcification rate of corals at 1 µg/L exceeded to that of control treatment

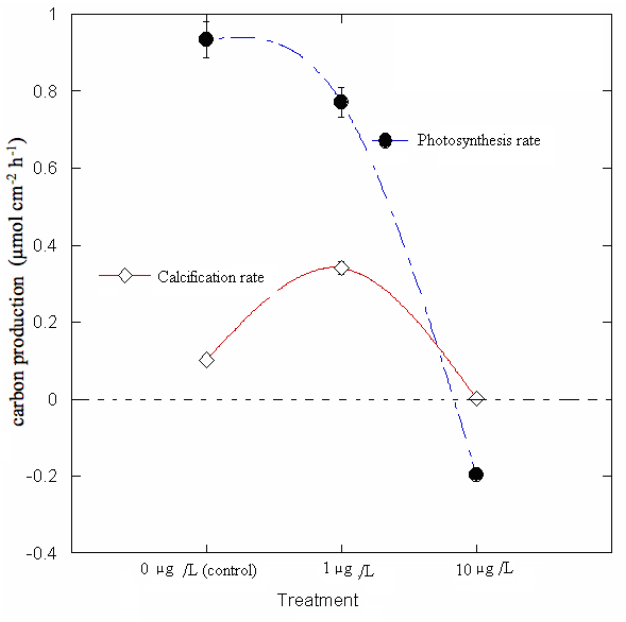

Fig.2. Effect of Irgarol on photosynthesis rate and calcification rate of coral Galaxea fascicularis exposed control, 1 and, 10 µg /L for 96 h.

4. Discussion

Diuron DCMU and Irgarol-1051 inhibit photosynthesis by blocking the electron transport in phosystem II (PSII), eventually cause immediate disruption in the symbiosis between zooxanthellae and host coral [17]. When temporary bound they can disrupt photosynthetic electron flow eventually led to loss of excitation energy at photosynthetic reaction center [14].

Our results show that the photosynthesis rate was gradually decreased when corals were exposed to treatments of 1 and 10 µg/L of Irgarol and diuron. These results are supported by the previous studies by Jones et al., [21] and Owen et al., [15] which also showed that diuron and Irgarol-1051 inhibit the photosynthesis of corals by blocking conversion of excitation energy in to chemical energy [12].

Calcification of corals is controlled by a mutualistic relationship with zooxanthellae. The zooxanthellae utilize the waste product of a host such as CO_2 from respiration, photosynthesis and synchronously donate carbon to the coral [22]. Our results demonstrate that at 10µg/L treatment of both herbicides Irgarol and diuron caused sharp decrease in calcification of Galaxea fascicularis after 96 hrs exposure. This might be associated with the disruption of symbiosis between zooxanthellae and the host coral. PSII compounds (Irgarol and diuron) might also block the energy which may need for trigger calcium uptake from seawater coeleteron of *Galaxea fascicularis* [23]. We suggest that the deterioration of calcification rate might be caused by the blockage of energy by the PSII compounds. Energy is needed for the calcification process to transport ions and for the formation of organic matrix [24].

In comparison, the phytotoxicity of the two herbicides show that; Irgarol > Diuron. The results reveal that when corals exposed to 1µg/L treatment Irgarol the photosynthesis rate was reduced 3 times more than exposure of 1 µg/L treatment Diuron. Additionally, the photosynthesis was reduced more than 90 times when exposed to 10 µg/L of Irgarol compared to 10µg/L of diuron. This suggests that Irgarol is more phytotoxic to coral *Galaxea fascicularis* compared to diuron. Similar trends have been observed in previous studies in coral *S. hystrix* where Irgarol 1051 was 3 times more toxic than Diuron [14].

We have compared the effective concentration for photosynthesis rate for Galaxea fascicularis (1µg/L) of Irgarol and diuron in our study and environmental relevance reported in previous studies in order to predict their fate in coral reef ecosystems. In a recent study done by our group (data not presented in this paper) the maximum concentrations detected around the coral reefs around Okinawa Islands are 0.09 and 0.035µg/L for diuron and Irgarol, respectively. It is suggested that acute effects caused by these herbicides in corals are not likely to happen in Okinawa corals reefs at the present. Contrastingly, remarkable levels up to 1.6 and 4 µg/L of Irgarol have been reported in the natural environments in Hong Kong and Singapore, respectively [25; 26]. High levels of diuron have also been reported in several marine environments such as 3.05µg /L was detected in the Seto inland sea, [27]; 42 µg /L in lagoon water Italy, [28]; 6.74 µg /L estuaries UK, [29], 2 µg /L in Mediterranean coast, Spain, [30], and 1.13 µg /L in Marinas Nerthelands, [31]. The concentrations have exceeded the effect concentrations as demonstrated in our study. These findings suggest that Diuron and Irgarol to be both prevalent to the coastal areas as well have implication on coral photosynthesis rate in natural ambient coastal waters.

5. Conclusions

In this study, we have studied on how the novel antifouling compounds affect calcification rate and photosynthesis rate of coral *Galaxea fascicularis* during short exposure (96h) in the laboratory. The following remarks have been concluded

Both Irgarol 1051 and Diuron of 1 µg/L may be enough to reduce photosynthesis rate of coral for short term exposure but seem not effective for calcification rate

Irgarol 1051 is more phytotoxic to corals compare to diuron.

According to environmental concentrations of Irgarol and Diuron reported in previous studies, it shows that the inhibition of photosynthesis rate of corals is possible to happen in the natural environments.

Further studies for long term exposure are needed for novel antifouling compounds to corals are still needed in order to

formulate appropriate monitoring strategies and guideline levels in coastal uses.

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