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Potential control of invasive species of orange cup coral *Tubastrea coccinea* Lesson, 1829 using a synthetic natural compound

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Abstract

Non-toxic defence against marine biofouling species including invasive species is urgently required. The effect of a synthetic natural compound "1-hydroxy-2-O-acyl-sn-glycero-3-phosphocholine" was tested against larvae of the invasive orange cup coral *T. coccinea* Lesson, 1829. The larvae were placed in 24-well microtiter plates immediately after their release and subjected to the compound at concentrations of 0.5, 5, 10, 50, and 100 µg mL^{-1} and three treatments (copper sulfate, solvents, and seawater). Larval mortality ranged from 35% (100 µg mL^{-1}) to 3% (5 µg mL^{-1}), and their average of lethal concentration (LC50) was 142.2 µg mL^{-1}. The results of this study show that compound is a potential option to be applied in the management and control of T. coccinea on artificial structures.

Keywords: *T. coccinea*, 1-hydroxy-2-O-acyl-sn-glycero-3-phosphocholine, Bioinvasion control, Management.

Marine biofouling is the undesirable growth of attached organisms on submerged surfaces (Callow and Callow, 2011), considered one of the major vectors of introduction of non-indigenous marine species (NIMS) worldwide (Mineur et al., 2012). The globalization of maritime transport has played a key role in the spreading of marine species beyond their native ranges, dispersing them as fouling on hulls of vessels (Seebens et al. 2016). These NIMS are considered an ecological problem because the introduction of these species can affect the integrity of native communities and change ecosystem biodiversity (Montefalcone et al., 2015). NIMS can out-compete local species for space and food (Blackburn et al. 2014), as well

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as alter the normal functioning of ecosystems (Vilà et al., 2009).

The scleractinian cup orange coral *T. coccinea* Lesson, 1829 is a native species from the Pacific Ocean (Cairns, 2000). However, this azooxanthellate coral has been considered a cosmopolitan species due to its worldwide introduction through biofouling on oil rigs and ship hulls (Fenner and Banks 2004). This coral species is a major invasive species along the Brazilian coast (Creed et al., 2021), having been identified as one of the priority invasive species targeted by National Plans for Prevention, Control, and Monitoring (MMA, 2018). *T. coccinea* features reproductive strategies that facilitate rapid colonization and expansion into new environments. These include brooded larvae (Glynn et al. 2008), high rates of recruitment (Costa et al. 2014), the potential for occasional long-distance dispersal through polyp clustering (Mizrahi, 2014), and a low predation rate (Moreira and Creed, 2012) due to allelopathic compounds (Lages et al., 2012). A few studies have proposed strategies to control the species (e.g., Moreira et al., 2014; Mantelatto et al., 2015; Altvater et al., 2017; Creed et al., 2019; Creed et.al. 2021).

Traditional antifouling paint is of limited efficiency in controlling exotic species (Minchin et al., 2006). Thus, new antifouling agents against invasive species, such as the orange cup coral, are urgently needed. The synthetic compound “1-hydroxy-2-O-acil-sn-glycero-3-phosphocholine” (Batista, 2012) is analogous to the glycerophospholipids found in soybean lecithin, which showed efficacy against biofouling in laboratory and field tests (Batista et al., 2015). This compound has been patented (US 8657943-B2, 2014). This study aimed to investigate the effectiveness of “1-hydroxy-2-O-acyl-sn-glycero-3-phosphocholine” in controlling *T. coccinea*. We conducted experiments under controlled laboratory conditions to evaluate the effect of the compound on the mortality of *T. coccinea* larvae.

The chemicals copper sulfate (Reagen, Brazil), methanol (Merck, Brazil), and chloroform (TEDIA; Brazil) used in this study were analytical grade. Seawater was obtained from an uncontaminated area (23º00’04.77’’S; 42º00’24.42’’W) and filtered using a cellulose acetate filter (0.45 µm, Millipore). Filtered seawater was used to dilute chemical solutions and as the seawater negative control. The solvent control was prepared by mixing in chloroform, methanol, and LC-MS water (2.0 ml: 2.0 ml: 2.0 ml). These solvents were used because of studies demonstrating that chloroform and methanol are more effective for the extraction of lipids (Bligh and Dyer, 1959). Seawater and copper sulfate solution were used as negative and positive controls, respectively. The positive control was included because copper is the main component of antifouling paints. Copper sulfate was prepared by diluting 100 mg in 1 liter of seawater. This stock solution served as the basis for the preparation of solutions with concentrations of 0.5, 5, 10, 50, and 100 µg mL^{-1}. The antifouling compound 1-hydroxy-2-o--acyl-sn-glycero-3-phosphocholine was synthesized as reported previously (Batista, 2012). The compound was then dissolved in a 1:1:1 mixture of chloroform, methanol, and LC-MS water. Five concentrations (0.5, 5, 10, 50, and 100 µg mL^{-1}) of this solution were prepared for the experiment.

Approximately 30 colonies of *T. coccinea* were collected by SCUBA divers at Porcos Island in Arraial do Cabo, southeastern Brazil (23º57’58’’S, 41º59’23’W). Colonies were removed from the substrate using a hammer and immediately placed in individual plastic bags and stored in cooling boxes. Maintenance of the colonies in laboratory was conducted according to Oliveira (2016). After the acclimation period, the specimens were transferred to an aquarium with seawater regulated to a temperature of 22 °C and photoperiod of 12:12. Larvae were spontaneously released under these after approximately one week of maintenance. Released larvae were collected using a Pasteur pipette and kept in an aeration beaker under the same temperature and light conditions as the colonies. After 24 hours of acclimation, the larvae were used in the experiments.

The larvae were placed in 24-multiwell plates (Ciencor) containing 2 ml of the compound solutions at concentrations of 0.5 µg mL^{-1}, 5 µg mL^{-1}, 10 µg mL^{-1}, 50 µg mL^{-1}, and 100 µg mL^{-1}, as well as two controls (copper sulfate and solvents). Four replicates were used per treatment, and seven larvae were added to each well. The same amount of replicates was used in the seawater control. Mortality and survival rates of larvae were verified after 24 hours using a stereoscopic microscope (Zeiss, model KL 1500 LCD). Mortality was defined by the change of color and/or disintegration of larvae. Three independent bioassays were performed.

A non-parametric Kruskal-Wallis test was applied to verify the differences between average values in each treatment; results were considered significant for p-values below 0.05. Probit analysis was used to calculate the 50% lethal concentration (LC_{50}) of the tested organisms (Finney, 1971). All tests were performed using the software Statistica 7.0.

The reactions of approximately 1,344 larvae were analyzed over all experiments. Figure 1 shows the mortality induced by the controls and synthetic compound. The highest mortality rates were found at 100 and 50 µg mL^{-1} of the compound (35% and 15%, respectively), followed by copper at the same concentrations (100 and 50 µg mL^{-1}) (50% and 35%, respectively). The lowest mortality rate (≈ 2%) was observed in the negative control at 0.5 µg mL^{-1} (Figure 1).

Larval mortality varied significantly between treatments (H=168,1726; $p<0.01$) and was significantly higher (Kruskal-Wallis, $p<0.05$) in the positive control ($CuSo_4$) at concentrations of 100 and 50 µg mL^{-1} compared to the negative control (seawater) and all solvent treatments. Mortality was also significantly higher (Kruskal-Wallis; H=168,1726; $p<0.05$) at 100 µg mL^{-1} of the compound, compared to the solvent and seawater (negative control). In contrast, mortality was significantly lower at the concentration of 100 µg mL^{-1} of the compound compared to the concentration of 0.5 µg mL^{-1} of copper sulfate (Kruskal-Wallis; H=168,1726; $p<0.05$). All other concentrations of the compound did not show significant differences compared to copper sulfate (100, 50, 10, and 5 µg mL^{-1}) (Kruskal-Wallis; H=168,1726; $p>0.05$). No significant difference was observed between concentrations of solvents and seawater (Kruskal-Wallis; H=168,1726; $p>0.05$). The LC_{50} of the compound was 142.2 µg mL^{-1} for *T. coccinea* larvae.

Marine invasive species are a major problem for the government, environmental managers, and researchers (Hulme, 2006) who seek efficient

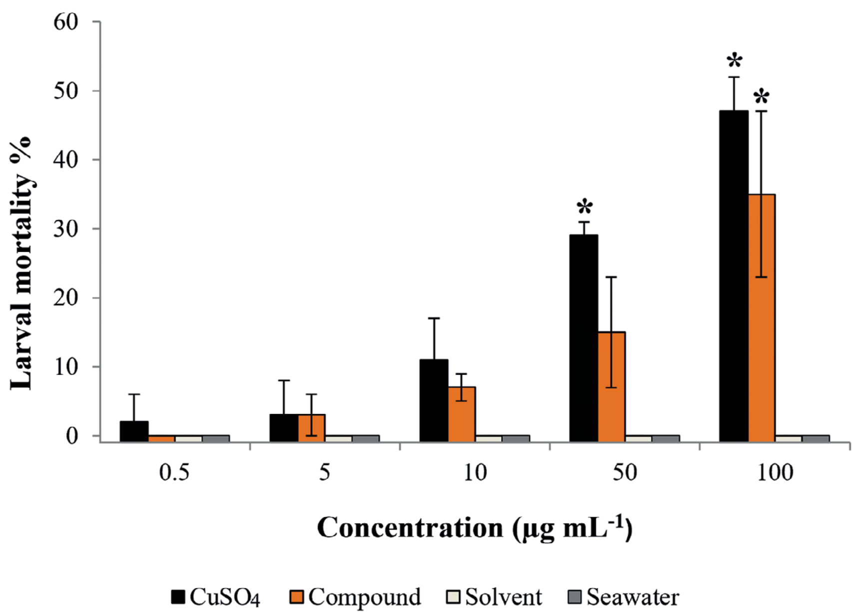

Figure 1. Mean percentage (%) (± standard deviation) of T. coccinea larval mortality after 24 hours of exposure to four concentrations of the following treatments: synthetic compound ("1-hydroxy-2-O-acil-snglycero- 3-phosphocholine"), copper sulfate (positive control), solvents (methanol and chloroform). Seawater was used as a negative control. Data were revived from three independent bioassays with four replicates per treatment. The asterisk (*) indicates treatments with statistically significant differences in relation to the negative control (Kruskal-Wallis, $p<0.05$).

eradication and control. In Brazil, for example, the sun coral is considered a pest (Moreira et al. 2014), causing impacts at ecological, social, and economic levels (Creed et al. 2017). The present study tested, for the first time, a synthetic natural compound as a solution for cup orange coral control. The compound "1-hydroxy-2-O-acyl-sn-glycero-3--phosphocholine" showed toxicity to *T.coccinea* larvae similar to that of copper sulfate. Copper is an efficient biocide commonly used to prevent marine biofouling (ICOMIA, 2006). However, its negative effects on native corals are well documented, including coral infertility (Negri and Heyward, 2001). Commercial biocides (e.g., Irgarol, Sea-Nine, Zinc pyrithione) are efficient in controlling biofouling but are also highly toxic to various native coral species (Bao et al., 2011). Accordingly, Irgarol has been banned in Bermuda, for example, due to its acute toxicity to coral reefs (Carbery et al., 2006). As a potential biocide, the synthetic compound also requires testing on various non-target organisms to assess the ecological impact.

The effects of crude extract of sun coral larvae have been reported. For example, Mizrahi (2014) demonstrated that crude extract of the coral *Carijoa riisei* was toxic to *T.coccinea* larvae within 48 hours of exposure. However, as concentrations were not reported, a comparison to the data derived herein is not possible. Oliveira (2016) also showed that crude extracts of the sponge *Darwinella* cf. *oxeata*, zoanthid *Palythoa caribaeorum*, and *Tubastrea tagusensis* can inhibit larval settlement and have a lethal effect on *T. coccinea* larvae. However, compounds from these crude extracts have not yet been isolated and identified (Mizrahi, 2014; Oliveira, 2016).

Compared to candidate marine antifouling products, the synthetic compound has certain advantages for industrial application. For example, a large number of organisms is required to manufacture the biocide based on natural products, compromising production viability (Qian et al. 2010). It is noteworthy that Brazil is the world's largest producer of soybeans, with about 135,409 million tons produced (EMBRAPA, 2020). Furthermore, lecithin can also be extracted from soy production waste, and it is estimated that every hectare produced generates 3-4 tons of waste (Guimarães et al. 2015). As such, the abundance of raw material favors the production of compost. The synthetic compound responded similarly to copper sulfate (100-10 µg mL^{-1}), although it did not show a significant difference, indicating that more experiments with a larger sample number and new concentrations are needed.

The government and environmental agencies seek practical solutions to minimize ecological and economic losses from the proliferation of invasive species (Olenin et al., 2007). Some current studies propose alternatives to control and manage *Tubastraea* spp. populations in Brazil, including the application of different salinities (Moreira et al., 2014) and temperatures (Mizrahi, 2008). Chemical control using sodium hypochlorite (Altvater et al., 2017) and acetic acid (Creed et al., 2019) has been also performed. Mantellato et al., (2015) proposed an alternative method of elimination of *T. coccinea* using plastic wraps and raffia leaves, which induce the mortality of colonies due to the lack of oxygen, food, and/or physical wear. To date, manual removal has been the sole control method used in protected areas in Brazil, where approximately 231,000 colonies have already been removed (Creed et al. 2021). However, the effectiveness of mechanical removal is controversial, given the lack of access to certain complex areas (IBAMA, 2017) and the possible generation of new polyps from detached fragments of sun coral during the process (Luz et al., 2018).

In comparison, the synthetic compound "1-hydroxy-2-O-acyl-sn-glycero-3-phosphocholine" can be easily applied, as it is incorporated into a paint from which it leaches out. The synthesis of this compound is inexpensive (US 8657943-B2, 2014), and its raw material is biodegradable soybean lecithin (Gao et al. 2020), which can cost as little as 1/10 as much as the oxides used in traditional antifouling paints (Batista, 2012). In a next step, experiments should be conducted with native corals and others species to evaluate the potential for harmful effects of the compound on the environment.

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AUTHOR CONTRIBUTIONS

R.D.S.C.: Conceptualization, investigation, writing - original draft;

D.B.: Formal Analysis, software, writing - review & editing;

W.R.B., M.H.B.N., C.C.L., R.S.B.L., E.F.B., T.S.N.: Methodology, investigation & review;

S.D: Writing - review & editing;

RC: Writing - review & editing, resources, supervision.

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