

# The Efficacy of Natural Antimicrobial Compounds in Antifouling and Combatting Biofilm Formation

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## I. Abstract

This study investigates the efficacy of natural antimicrobial compounds – tea tree essential oil (TTO), clove essential oil (CO), and cinnamon essential oil (CinO) – as environmentally friendly alternatives to chemical antifouling agents in mitigating biofouling on stainless-steel surfaces in marine environments. Biofouling, caused by biofilm accumulation, leads to microbiologically induced corrosion (MIC), posing significant economic challenges with maintenance costs for the marine industry. An experiment was conducted by submerging stainless-steel cylinders coated with TTO, CO, CinO, a chemical agent (falling under benzyl-C12-16-alkyldimethyl ammonium chlorides), or no coating (control) in beakers containing water and moss specimens. After 74 hours, the change in mass and visual growth of biofilm were evaluated. The control group exhibited the highest biofilm accumulation, with a mass increase of 0.9g and a pronounced biofouling covering ~90% of the surface area. The chemical agent coating effectively prevented biofouling, with a minimal 0.1g mass change and <5% surface coverage. Among the natural oils, TTO and CinO showed the most promising antifouling properties, with a 0.3g and 0.5g mass increase and ~60% and ~40% surface coverage respectively. CO exhibited moderate effects, with a mass increase of 0.6g, and ~80% surface coverage. These findings suggest natural antimicrobial compounds, particularly TTO and CinO with their major component terpinen-4-ol and trans-cinnamaldehyde, can serve as viable eco-friendly alternatives to chemical antifouling agents. However, long-term durability assessments and larger-scale studies are crucial for real-world marine applications, considering potential variations and the need for enhanced efficacy.

## II. Acknowledgements

I extend my gratitude for the successful completion of this research, acknowledging my supervisor, Ms. Grace Goodwin's, valuable guidance in steering me towards antifouling from the broad concept of the marine industry; her insight was crucial in shaping the project's direction. The entirety of the study, including the discovery of the problem and research gap, the novel idea of utilizing natural oils in the marine antifouling context, the selection of the specific natural oils and the experimental design, was meticulously crafted by my efforts. I appreciate Ms. Grace Goodwin's support in promptly acquiring the necessary equipment according to my specifications. While her involvement focused on

the initial redirection and equipment acquisition, the project's success demonstrates my dedicated efforts and scientific commitment.

## **1. Introduction**

### **1.1 Context**

The marine industry plays a pivotal role in shaping the economic landscape of Norway, alongside those of diverse nationalities and geographical background including those from Asia, North America and Europe. Encompassing sectors such as shipping, offshore oil and gas, fisheries, and marine biotechnology, this major industries faces significant challenges in regards to microbiologically induced corrosion (MIC) of offshore steel structures and ship hulls.

### **1.2 Problem**

Biofilms are complex layers of microbial cells, usually either bacteria or fungi. These biofilms form through the transition of microorganisms from a free floating planktonic state to a sessile state as they attach to surfaces such as ship hulls, pipe walls, and other metallic offshore structures (Muzamil Ahmad Rather, Gupta and Mandal, 2021). MIC, caused by biofouling – involving the undesirable accumulation of biofilms – results from microbial activities that directly or indirectly contribute to corrosion. Microorganisms in biofilms secrete extracellular polymeric substances (EPS), embedding themselves in a stable structure that is highly resistant to antimicrobial treatments (Ibid). These biofilms create localized environments that promote metal degradation and corrosion through chemical changes such as acid production and by directly using metals as electron donors (Jia et al., 2019); this further leads to the deterioration of these offshore surfaces posing several economic maintenance costs and safety hazards (Francolini et al., 2017; Makhoul and Botello, 2018).

In an effort to mitigate the deleterious effects MIC and biofouling, the global marine industry has developed several chemical antifouling paints and coatings, intended to be applied onto offshore steel structures and ship hulls. These chemical substances are compounds of several biocides, with Tributyltin (TBT), Chlorothalonil, and Dichlofluanid being some of the most common among many others (Amara et al., 2018); however, these biocides hinder oxygen and water exchange, imposing significant consequences on marine life and non-targeted species such as various fish while also polluting and exacerbating water quality (Ibid; Ytreberg et al., 2021).

### **1.3 Inquiry**

Thus as an alternative, certain natural essential oils containing antimicrobial compounds propose solutions to preventing MIC. Tea tree – *Melaleuca alternifolia* – essential oil (TTO), Clove –

*Eugenia caryophyllata* – essential oil (CO), and Cinnamon – *Cinnamomum cassia* – essential oil (CinO) propose alternative antimicrobial compound solutions to preventing MIC and biofouling.

This paper will investigate the efficacy of these essential oils (EOs) in comparison to a chemical agent in preventing the growth of biofilms. It endeavours to provide valuable insights into alternative environmentally friendly and natural solutions promoting sustainability for marine life and improved water quality, while also overcoming the posed economic hardships and safety hazards associated with MIC.

## **2. Motivation**

Marine biofouling is a significant environmental and economic concern, particularly in the context of stainless-steel structures used in marine settings. Traditional antifouling methods often rely on synthetic chemicals, which while effective, have been shown to cause substantial harm to marine ecosystems and water quality (Amara et al., 2018; Ytreberg et al., 2021). The leaching of toxic substances from antifouling paints and coatings into the ocean disrupts marine life, affecting biodiversity and the health of marine organisms (Ibid). These toxic substances can accumulate in the food chain, posing risks to higher trophic levels, including humans. In light of these detrimental effects, there is a pressing need for environmentally friendly alternatives that can mitigate biofouling without compromising marine life nor water quality. This study is motivated by the urgent requirement to find sustainable antifouling solutions that can protect marine environments while maintaining the integrity and functionality of marine infrastructure.

## **3. Research Gap**

Despite the well-documented adverse effects of conventional antifouling methods, there has been minimal practical experimentation and application of natural antifouling alternatives in marine environments. While laboratory studies have highlighted the potential antimicrobial properties of various essential oils, such as TTO, CO, and CinO, their effectiveness in real-world marine settings remains largely unexplored. Current literature lacks comprehensive field studies that evaluate the practical application, effectiveness, and environmental impact of these natural compounds on biofouling in marine environments. This research aims to bridge this gap by conducting practical experiments to assess the efficacy of TTO, CO, and CinO in preventing biofouling on stainless-steel surfaces in marine conditions, thereby contributing to the development of sustainable antifouling strategies, alongside improving water quality and marine life welfare.

## 4. Method

### 4.1 Hypothesis

This experiment hypothesises that the application of natural antimicrobial compounds, specifically TTO, CO and CinO, can effectively mitigate biofouling on stainless-steel surfaces in marine settings. These three EOs are theorised to be effective because of their ability to disrupt bacterial cell structures and inhibit growth; TTO, composed of various antimicrobial components – monoterpenes and related alcohols – primarily comprised of terpinene-4-ol – disrupts the bacterial cell membrane, leading to cell death being (Hammer, Carson and Riley, 2012); CO, rich in antimicrobial components such as eugenol, disrupts bacterial cells membranes and denatures proteins, leading to cell death (Núñez and Mhc de Aquino, 2012); and CinO, containing the potent antimicrobial compound trans-cinnamaldehyde, disrupts bacterial cell walls and membranes, inhibiting bacterial growth and preventing biofilm formation (Yassine El Atki et al., 2019). By extrapolating this knowledge to marine settings, we expect a consequent decline in biofouling on stainless-steel components.

The experiment aims to encapsulate a stainless-steel cylinder within a laboratory-grade beaker filled with water, ensuring complete submersion of the steel in a controlled aquatic environment. Additionally, each beaker will host a specimen of moss as a representative organism for biofilm and bacterial growth. The variation in antifouling coatings applied to the stainless-steel cylinder serves as the independent variable. Through systematic manipulation of these coatings, the study will investigate and quantify the development of biofilms, and bacteria on the steel surfaces. This approach facilitates the comparison of the efficacy of different natural antifouling coatings in inhibiting undesired growth on stainless-steel surfaces within the established experimental conditions.

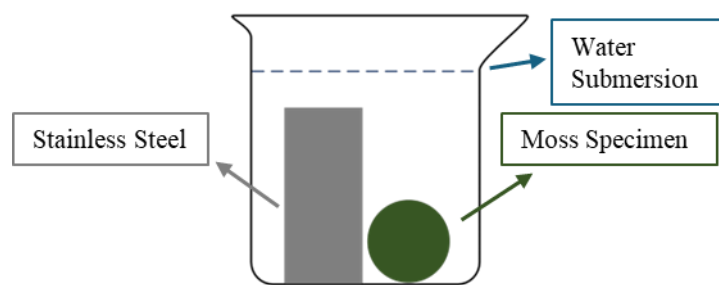


Figure 1: Graphic Illustrating the Experimental Design Setup

### 4.2 Independent Variable

The independent variable for this experiment is the range of antifouling coatings applied to the stainless-steel metal pieces. There will be five test values representing distinct coating conditions: the first test value involves no coating as a control group; the second features a CinO; the third utilizes CO; the fourth incorporates TTO coating; and the fifth employs a chemical agent as an additional

comparative measure. This systematic variation in coatings aims to evaluate and compare their efficacy in mitigating biofouling on stainless-steel surfaces in marine settings.

Among various natural oils, TTO was chosen for this study due to its major component Terpinen-4-ol. This monoterpene showcases antibacterial and antibiofilm activities against various bacteria, especially against *Staphylococcus aureus*, including methicillin-resistant strains (MRSA), found in clinical environments (Cheng et al., 2021). Noting that *Bacillus* species, a common bacteria found in marine biofilm, shares similarities with *S. aureus* in terms of being gram-positive bacteria, it's reasonable to infer that TTO's efficacy against *S. aureus* may extend to *Bacillus* species commonly found in marine environments (Beleneva, Skriptsova and Svetashev, 2017; Omeed Sizar, Leslie and Unakal, 2023). Additionally, while direct evidence on marine biofilm bacteria is lacking, the broad-spectrum antibacterial and antibiofilm effects of Terpinen-4-ol against gram-positive bacteria like *S. aureus* suggest its potential effectiveness against other marine biofilm bacteria, enhancing its suitability for antifouling applications.

Clove oil was selected for this study due to its primary bioactive compound, eugenol, which demonstrates robust antibacterial and antibiofilm activities against a diverse array of bacteria (Marchese et al., 2017; Núñez and Mhc de Aquino, 2012). Eugenol's mechanism of action involves disrupting bacterial cell membranes, leading to leakage of cellular contents and impairment of essential cellular processes such as protein and DNA synthesis (Ulanowska and Olas, 2021). Its efficacy against gram-negative bacteria like *Escherichia coli* and *Pseudomonas aeruginosa* suggests potential effectiveness against related marine biofilm bacteria such as *Pseudoalteromonas* species (Beleneva, Skriptsova and Svetashev, 2017; Mahmoud, El-Sherbiny and Moghannem, 2023). Additionally, eugenol's inhibition of biofilm formation and disruption of mature biofilms make it a promising candidate for combating marine biofilm-associated bacteria (Mahmoud, El-Sherbiny and Moghannem, 2023).

Cinnamon oil was selected for this study due to its primary bioactive compound, trans-cinnamaldehyde, which demonstrates potent antibacterial activity against a wide range of bacteria through multiple mechanisms (Usai and Di Sotto, 2023). Trans-cinnamaldehyde disrupts bacterial cell membranes, leading to leakage of cellular contents and impairment of essential cellular processes like respiration and cell division (Ibid). Additionally, it induces oxidative stress by generating reactive oxygen species, further damaging bacterial cells (Ibid). Its inhibition of various cellular processes, including cell division and efflux pumps, along with its ability to disrupt biofilm formation, makes it a promising candidate for combating marine biofilm-associated bacteria (Ibid).

A benzyl-C12-16-alkyldimethyl ammonium chlorides (ADBAC/BKC) was chosen as the antifouling chemical for its potent antibacterial properties, disrupting cell membranes and inhibiting

essential cellular processes, including protein synthesis and biofilm formation (European Chemicals Agency, 2021; Merchel, and Tagkopoulos, 2019). While direct evidence on marine biofilm bacteria is lacking, its broad-spectrum mechanisms suggest effectiveness against common species like *Pseudoalteromonas*, *Vibrio*, and *Bacillus* (Beleneva, Skriptsova and Svetashev, 2017).

### 4.3 Dependent Variable

The dependent variable (DV) measuring the efficacy of the EOs will involve both quantitative and qualitative measures. Quantitatively, the change in mass (grams) of the stainless-steel will be determined by measuring its initial and post-bacterial growth masses, allowing for the calculation of the change in mass. Qualitatively, the visible growth of biofilms and surface area coverage by bacteria will be compared by assigning each coating a qualitative rating between 0-0.5 and through taking qualitative notes. The addition of a control group and a chemical agent facilitates meaningful comparisons and enhances the overall interpretability and significance of the results.

### 4.4 Control Variables

The implementation of control variables (CVs) in this experiment serves to enhance the validity of the study, ensuring methodological rigour and minimizing potential confounding factors, thus facilitating the generation of impartial and precise results.

**Table 1:** Control Variables

Control Variables	Explanations
Stainless-Steel Metal	The goal is to consistently utilize Stainless-Steel in each test, selected for its prevalence in offshore structures and ship hulls, as well as its inherent anti-rust properties – as rust may hinder measuring the growth of biofilm (Løvland, 1993). While efforts are made to maintain consistent volume, surface area, and shape of the steel, the utilization of recycled metals – emphasizing recycling practices – may introduce challenges in precisely matching sizes. Nevertheless, the anticipated minor variations in size are not expected to significantly impact the study's results due to the primary focus on the effects of antifouling coatings on biofilm and bacterial growth.
Water Volume	A standardized volume of 150 ml will be poured into each beaker, thereby ensuring complete submersion of each moss specimen and stainless-steel metal, promoting impartial experimental conditions
Water Temperature	As different bacteria's and fungi's propensity to form biofilms exhibits variation based on distinct water temperatures, maintaining a constant water

	temperature ensures minimal confounding disturbances in the validity of the data (Qi Wei, 2020).
Beaker Size and Material	Maintaining uniformity in the size and material of the laboratory-grade beakers used to encapsulate the stainless-steel cylinders helps control for any potential impact of beaker characteristics on the experimental outcomes.
Moss Specimen and Type	The moss specimens were systematically sourced from identical locations within the same lake (Stokka Vannet), ensuring that any observed differences in biofilm or bacterial growth can be attributed to the variation in antifouling coatings rather than differences in moss characteristics. The quantity of moss will be impartially placed in each beaker.
External Environment (Temperature and Lighting Conditions)	Given the predominant preference of moss for shaded and dimly lit environments, the experimental procedures were executed in low-light conditions (Pike, 2013).
Time of Exposure	Ensuring that the stainless-steel cylinders are exposed to the water and moss for the same duration in each experimental condition, eradicates the impact of exposure times on the observed outcomes.

## 4.5 Methodology

### 4.5.1 Equipment List

- 750ml of water
- 5 cylindrical pieces of stainless-steel
- 70 grams of moss
- 10 ml of [Cinnamon Essential Oil](#)
- 10 of [Clove Essential Oil](#)
- 10 ml of [Tea Tree Essential Oil](#)
- 10 ml of a [Chemical Antifouling agent](#)

*For multiple trials, multiples of the quantities above would be necessary.*

- Five 250 ml laboratory grade beakers
- Thermometer: To measure temperature of water (CV)
- Precise Scale: To measure change in mass (DV)

- Safety Gear and personal protective equipment (PPE): Gloves, lab coat, safety goggles, mask, tissues or paper towels, and closed toe shoes.

#### 4.5.2 Safety Measures

To ensure a safe conduction of the experiment, possible *safety hazards and measures* must be considered.

**Table 2:** Safety Measures

Safety Measures	Explanations
Chemical Exposure	The manipulation of antifouling coatings introduces potential hazards related to skin and respiratory irritation. Precautions encompass the utilization of PPE, comprising gloves and masks. Furthermore, the experimental procedures mandate execution within well-ventilated environments to minimize exposure risks expected to significantly impact the study's results due to the primary focus on the effects of antifouling coatings on biofilm and bacterial growth.
Glass Beakers	Submersion of stainless-steel in glass beakers pose hazards of the beakers breaking. Adequate safety measures involve careful handling of the beakers.
Water Spillage	Handling chemicals and water pose disturbances in the process. Safety measures involve ensuring proper containment structures to prevent water spillage and careful handling of liquids.

#### 4.5.3 Environmental and Ethical Considerations

To address *environmental and ethical considerations*, the potential ecological impacts and ethical implications of using natural antifouling agents must be evaluated.

**Table 3:** Environmental and ethical considerations

Environmental and ethical considerations	Explanations
Organism Well-Being and Utilization	The use of moss as a surrogate organism instead of algae is necessitated by adverse weather conditions affecting the availability of algae. While the primary objective of antifouling measures is the eradication of bacterial and fungal growth, a judicious application of moss in minimal quantities is employed to mitigate potential adverse effects and minimize ecological impact.



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Disposal of Experiment Materials	Materials will be properly disposed, minimizing any environmental impact associated with the disposal of experimental materials, including antifouling coatings.
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#### **4.5.4 Procedure**

##### Safety:

1. Ensure all personnel wear appropriate PPE, including gloves, lab coats, and safety goggles.

##### Preparation:

2. Label five beakers as follows: "Control Group," "Cinnamon Oil," "Clove Oil," "Tea Tree Oil," and "Chemical Agent."

##### Setup: For each beaker, perform the following steps:

3. Get the first beaker and a stainless-steel cylinder.
4. Record the initial mass of the stainless-steel cylinder.
5. Apply the designated coating to the stainless-steel cylinder.
6. Insert the coated stainless cylinder into the corresponding labelled beaker.

##### Measurements and Additions:

7. Measure and record approximately 13 grams of moss before placing it in the beaker.
8. Measure and record the temperature of 150 ml of water.
9. Pour the measured water into the beaker

##### Positioning:

10. Position the beaker in a shaded area.

##### Documentation and Monitoring:

11. Document the time to establish a reference point for monitoring the duration of each experimental trial.
12. Repeat steps 3-11 for each of the remaining four beakers.

##### Observation Period:

13. Allow a waiting period of 48 hours or more – endeavour to maintain a consistent duration throughout the tests.

##### Final Measurements:

14. Carefully retrieve the first stainless-steel piece and weigh it using a scale.
15. Record the final mass.

16. Repeat steps 14-15 for the other four stainless-steel cylinders

Cleanup:

17. Clean and dispose of materials with consideration for the environment.

**6. Results**

After conducting the experiment across a duration of approximately 74 hours, the results acquired are as follows:



*Figure 2: Images of Biofilm Growth on Stainless-Steel with No Coating (Control group)*



*Figure 3: Images of Biofilm Growth on Stainless-Steel with CinO Coating*



*Figure 4: Images of Biofilm Growth on Stainless-Steel with CO Coating*



Figure 5: Images of Biofilm Growth on Stainless-Steel with TTO Coating



Figure 6: Images of Biofilm Growth on Stainless-Steel with Chemical Agent Coating

**Table 4:** Comparative Analysis of the impact of Stainless-Steel Coatings on Biofilm Growth – Assessing Changes in Mass and Qualitative Visual Impact

Coating	Initial Mass of Stainless-Steel (g)	Final Mass of Stainless-Steel (g)	Change in Mass of Stainless-Steel (g)	Qualitative Ratings (0.0-0.5)	Qualitative Notes
Control Group	282.2	283.1	0.9	0.5	Displays the highest biofilm accumulation and pronounced biofouling, covering the largest surface area (~90% based on visual observation) relative to the total (Figure 2).
CinO	336.1	336.6	0.5	0.2	Indicates notable biofilm accumulation and biofouling, with certain areas remaining unaffected (~40% surface coverage based on visual observation) and showing resistance to biofilm growth (Figure 3).
CO	332.4	333.2	0.6	0.4	Exhibits notable biofilm accumulation and biofouling (~80% surface coverage based

					on visual observation), except for the bottom of the cylinder remaining unaffected and showing resistance to biofilm growth (Figure 4).
TTO	313.6	313.9	0.3	0.3	Displays notable biofilm accumulation and biofouling (~60% surface coverage based on visual observation), with only a few areas resisting biofilm growth (Figure 5).
Chemical Agent	503.2	503.3	0.1	0.0	Presents negligible biofilm accumulation (<5% surface coverage based on visual observation), signifying a robust antifouling performance and effective protection (Figure 6).

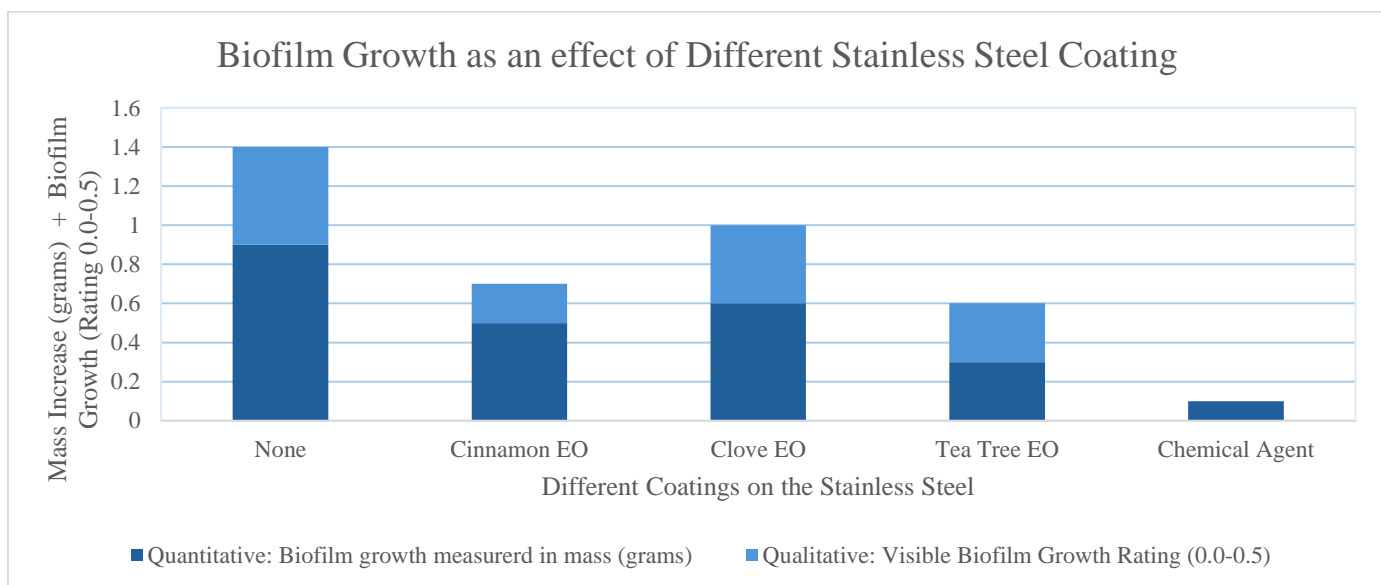


Figure 7: Change in Mass as an indicator of Biofilm Growth resulting from Different Stainless-Steel Coatings

**Table 5:** Comparative Analysis of the Stainless-Steel Coatings on Biofilm Growth – Summarising Inconsistent Control Variables

Coating	Initial Water Temperature (°C)	Mass of Moss (grams)	External Environment (Temperature and Lighting Conditions)	Time of Exposure
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Control Group	21.9	12.6	Location A: Home Setup <ul style="list-style-type: none"> <li>• Shaded and Dimly lit</li> <li>• Roughly room temperature</li> </ul>	54 hours and 41 minutes
CinO	21.5	13.3	Location B: School Laboratory: <ul style="list-style-type: none"> <li>• Shaded and Dimly lit</li> <li>• Roughly room temperature</li> </ul>	74 hours and 6 minutes
CO	21.1	12.9	Location A: Home Setup: <ul style="list-style-type: none"> <li>• Shaded and Dimly lit</li> <li>• Roughly room temperature</li> </ul>	54 hours and 30 minutes
TTO	21.3	11.4	Location B: School Laboratory <ul style="list-style-type: none"> <li>• Shaded and Dimly lit</li> <li>• Roughly room temperature</li> </ul>	73 hours and 58 minutes
Chemical Agent	21.4	14.5	Location A: Home Setup <ul style="list-style-type: none"> <li>• Shaded and Dimly lit</li> <li>• Roughly room temperature</li> </ul>	54 hours and 16 minutes

## 7. Limitations

While these results provide valuable insights into the effectiveness of various coatings on stainless-steel, it is essential to acknowledge certain limitations that could have influenced the observed outcomes.

Firstly, variations in testing durations across different coatings (seen in Table 5) potentially introduce variability in the observed results. However, the two coatings – TTO and CinO – tested for the longest duration (73 hours 58 minutes and 74 hours 6 minutes, respectively) appeared to be the most effective natural EOs, countering concerns about the impact of varied testing durations on the overall assessment of coating performance. This observation is rationalized by the understanding that the active compounds in TTO and CinO, such as terpinen-4-ol and trans-cinnamaldehyde, are known to act rapidly in disrupting bacterial cell membranes and inhibiting cellular processes crucial for biofilm formation (Cheng et al., 2021; Usai and Di Sotto, 2023). The longer exposure time does not necessarily enhance their antimicrobial efficacy but instead subjects them to more rigorous conditions by allowing extended biofilm and bacterial growth, which the oils had to withstand over the prolonged duration.

Secondly, discrepancies in the experimental environments, where the Control Group, CO, and the Chemical Agent were tested at my home setup (Location A: roughly 21°C and dimly lit), while

CinO and TTO were tested at the school laboratory (Location B: roughly 21°C and dimly lit), encompassing potential variations in temperature and humidity, may have exerted a modest influence on the observed outcomes. To mitigate this limitation, precise quantification of environmental factors, such as temperature and humidity, should be implemented in future studies to account for potential confounding effects on bacterial growth rates and biofilm formation.

Finally, potential human errors during the coating application process might have contributed to inconsistencies in the data. This could explain instances, such as specific areas in Figure 3 demonstrating successful resistance to biofouling, while other regions did not, and the bottom of Figure 4 exhibiting resistance – assuming that the oil may have unevenly distributed, possibly running down the sides and collecting at the bottom due to application irregularities. To address this limitation, a standardized application protocol should be developed and implemented – perhaps using an additive to enhance adhesion – ensuring consistent and uniform coating coverage on the stainless-steel surfaces.

## **8. Discussion**

### **8.1 TTO, CO, CinO and Chemical Agent Efficacy**

As clearly and effectively visualized by Figure 7, the Chemical Agent seems the most effective in resisting biofouling and biofilm growth. As illustrated in Figure 6, the minimal biofilm growth observed on the stainless-steel suggests that the recorded 0.1 gram increase may be attributed to factors such as residual water or potential rounding imprecision in the weighing scale. Among the natural EOs tested as antifouling coatings, TTO emerges as the most effective, followed by CinO and CO in decreasing order of efficacy in regards to the increase in mass.

Given the control group's evident full biofilm growth (refer to Figure 2) and an increase in mass of 0.9 grams (Table 4), it is reasonable to infer that the application of natural oils demonstrated efficacy in specific regions. This is further supported by the absence of biofilm in Figures 3, 4, and 5, as well as the noticeable distinction in change of mass depicted in Figure 7. The limited efficacy observed with certain natural EOs such as CO, as elaborated in the Limitations section, is attributed to potential human errors; however, the effectiveness of the chemical agent and other EOs suggests a possibility that the oil may not have adhered to the stainless-steel as intended.

Qualitatively, the Stainless-Steel coated with CinO exhibited more unaffected areas compared to the one coated with TTO (~40% and ~60% surface area coverage respectively as stated in Table 4). However, quantitatively, TTO demonstrated less antifouling than CinO (0.3g and 0.5g increase as stated in Table 4). One factor contributing to the minimal change in mass for TTO could be the difference in mass of the moss employed. Nonetheless, these two natural EOs stand out as the most effective coatings, alongside the chemical agent.

## **8.2 Environmental Impact and Sustainability**

In evaluating the impacts of the three most effective coatings – TTO, CinO, and the Chemical Agent – on marine life, our examination centres on several critical dimensions. While TTO may exhibit lower antifouling efficacy when compared to conventional chemical agents like TBT, it proposes a reduced toxic impact on non-target marine species, thereby offering a more environmentally sustainable approach (Kumar et al., 2018). Similarly, CinO demonstrates an environmentally friendly impact on marine life (Peres et al., 2014); however, contrastingly, the chemical antifouling agents predominantly incorporate biocides, rendering them ecologically hazardous and posing risks to both ecosystems and non-targeted organisms (Francolini et al., 2017; Makhlouf and Botello, 2018).

## **8.3 Economic Feasibility and Cost Comparison of Natural Oils in Antifouling**

Although these natural EOs such as TTO, CO and CinO show promise as alternatives to conventional chemical antifouling agents due to their perceived environmental advantages, their comparatively higher initial costs raise pertinent questions regarding their economic viability contrasting to traditional options like Tributyltin (TBT), Chlorothalonil, and Dichlofluanid.

A discernible discrepancy exists in the cost comparison between natural oils and chemical agents. TTO, renowned for its antimicrobial efficacy, commands a premium price ranging from approximately \$200 to \$300 per litre, notably surpassing the pricing of chemical alternatives (Amazon, 2024a; Walmart, 2024c). CO and CinO, though moderately priced at \$100 to \$150 and \$150 to \$200 per litre, respectively, still represent a premium compared to conventional agents like Chlorothalonil, and Dichlofluanid, which generally fall within the \$30 to \$100 per litre range (Amazon 2024b; 2024c; Chemical Book, 2024; Myhometurf, 2024; Walmart, 2024a; 2024b).

Despite the higher initial investment, natural oils offer considerable environmental benefits. They mitigate the toxicity to non-target marine organisms, enhance water quality, and contribute to long-term sustainability objectives (Amara et al., 2018; Ytreberg et al., 2021). In addition, over time, the upfront expenditure on natural oils may be offset by potential savings in environmental remediation and regulatory compliance costs. In contrast, chemical agents, though initially cost-effective, bear significant environmental and ecological drawbacks and are subject to strict regulatory scrutiny, leading to potential extra costs.

## **8.4 Future Research Directions**

To gain a deeper understanding of the natural EOs' efficacy in the field of antifouling, investigating the potential synergistic effects of combining different natural EOs, and thus merging the impacts of compounds like terpinene-4-ol (in TTO), eugenol (in CO), and trans-cinnamaldehyde (in CinO), or incorporating them with other eco-friendly compounds could be an interesting avenue for

future research. Synergistic combinations may enhance the overall antifouling efficacy while maintaining environmental sustainability, potentially overcoming the limitations observed with individual EOs in this study.

The assessment of antifouling coatings extends beyond their initial effectiveness to encompass a crucial consideration – their long-term durability in authentic marine conditions. Although the short-term efficacy of TTO, CinO, and the Chemical Agent is evident in the experimental results, a thorough exploration of their sustained performance under prolonged exposure to harsh marine elements becomes imperative. Elements such as wave action, UV radiation, and fluctuations in water temperature wield significant influence over the stability and efficacy of these coatings over time. Undertaking a comprehensive and protracted study to monitor the coatings' resilience would yield indispensable insights into their durability, offering a nuanced understanding of their practicality and effectiveness in real-world marine applications. Exploring strategies to enhance the coatings' resilience, such as incorporating stabilizing agents could improve their practical applicability.

Furthermore, a more in-depth investigation into the efficacy of these natural EOs as antifouling coatings on a larger scale is warranted. Given the potential for minor inconsistencies and inaccuracies during the application process, a scaled-up experiment becomes crucial to discern the genuine effectiveness of these EOs. The inherent challenges and variations associated with practical applications can be better addressed on a larger scale, offering a more nuanced understanding of their performance and viability in real-world scenarios, including offshore structures and ship hulls.

## **9. Conclusion**

In conclusion, this research provides valuable insights into the efficacy of natural antimicrobial compounds – specifically, Tea Tree Oil (TTO), Cinnamon Oil (CinO), and Clove Oil (CO) – in comparison to a chemical agent for mitigating biofouling and microbial growth on stainless-steel surfaces. While the chemical agent demonstrates superior short-term efficacy, both TTO and CinO emerge as promising natural and environmentally friendly alternatives, exhibiting substantial antifouling capabilities.

However, it is crucial to consider the long-term durability of these coatings in authentic marine environments, alongside potential trade-offs between efficacy and ecological sustainability. Future research directions could explore synergistic combinations of natural compounds or investigate the coatings' performance under different marine conditions on a larger scale to explore the EOs sustainability under varying temperatures, salinities or pH levels.

This research provides valuable insights into the continual pursuit of sustainable and effective antifouling solutions, playing a crucial role in preserving economic interests and marine ecosystems



while improving water quality and enhancing marine life welfare, concurrently mitigating MIC. However, the potential applications and implications of these findings extend beyond the marine industry. The natural antifouling coatings could potentially be applied in other industries or settings where biofilm formation is a concern, such as in medical devices, water treatment systems, or food processing facilities.

Overall, this research represents a significant step towards the development of sustainable and effective antifouling strategies, contributing to the preservation of marine ecosystems and the mitigation of economic losses associated with biofouling and MIC. The results indicate that both TTO and CinO showcase clear short-term efficacy as promising natural and environmentally friendly alternatives for combating biofouling. By addressing the limitations identified and pursuing the suggested future research directions, further advancements in this field can be achieved, ultimately leading to a more sustainable and eco-friendly approach to combating biofouling in marine environments.

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