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Exploring the Antifouling Potential of Sabellaria Simplex Crude Extract: A Promising Solution for Biofouling Management

Dr. Vaidya V. V.

Department of Zoology, Smt. S. K. Gandhi Arts, Amolak Science & P. H. Gandhi Commerce College, Kada, Tq. Ashti, Dist. Beed.414202

Abstract: ***This study investigated the effects of different concentrations of an extract on cyprid mortality and settlement behavior. Extract concentrations of 6 mg/ml, 8 mg/ml, 10 mg/ml, and 12 mg/ml were tested on 100 cyprids each. Results revealed a dose - dependent relationship, with higher extract concentrations leading to increased mortality and reduced settlement rates. At 6 mg/ml, cyprid mortality was 70%, decreasing to 30% at 12 mg/ml. higher concentrations also showed stronger inhibitory effects on settlement, with only 33% settlement observed at 12 mg/ml. These findings suggest the extract's potential as a befouling control agent, warranting further research for practical applications in marine ecology.***

Keywords: Cyprid Mortality, Settlement Behavior, Extract Concentration, Dose - Dependent Relationship, Biofouling Control, Marine Ecology

1. Introduction

The marine industry and aquaculture development face significant challenges posed by biofouling. In the marine environment, both natural and artificial surfaces submerged in seawater become colonized by biofouling organisms, including microorganisms such as marine bacteria, algae, and protozoa, as well as macroorganisms such as barnacles, bryozoans, and tubeworms (Callow & Callow, 2011). The accumulation of biofoulers on ship hulls, for example, increases drag and surface corrosion, leading to reduced maneuverability and carrying capacity of vessels (Schultz et al., 2011). Additionally, biofouling imposes substantial material and economic costs for the maintenance of mariculture facilities, naval vessels, and seawater pipelines (Dobretsov & Qian, 2009).

Traditionally, antifouling compounds such as tributyl tin (TBT) and copper have been added to marine paints to control biofouling. However, these biocides are often highly toxic to a wide range of non - target organisms and pose environmental concerns (Dobretsov & Qian, 2009). Consequently, there is a growing interest in identifying novel, effective, and non - toxic antifouling compounds (Schultz et al., 2011). Marine environments harbor highly diverse microbial communities with unexplored potential for producing chemical deterrents for defense purposes (Dobretsov & Qian, 2009). Marine natural products have emerged as a promising source of novel antifouling agents, with many compounds exhibiting strong antifouling activity isolated from marine organisms such as sponges, corals, and algae in recent decades (Hellio et al., 2003).

The main objective of this investigation is to understand larval barnacle settlement, with the practical goal of developing non - toxic antifouling agents from extracts of Polycheate *Sabellaria simplex*. This study focuses on investigating the antifouling potential of marine Polycheates against the settlement of cyprid larvae of B. Amphitrite. By exploring the interactions between marine organisms and larval settlement, this research aims to contribute to the development of environmentally friendly antifouling solutions for marine industries and aquaculture.

2. Materials and Methods

Collection and Extraction of Marine Polycheate *Sabellaria simplex*

Polycheate Sabellaria simplex: specimens were collected during low tide from the intertidal area of the west coast of Ratnagiri, India. Upon collection, the specimens were carefully rinsed with sterile seawater to eliminate any associated debris and salt. The extraction of compounds from Polycheate Sabellaria simplex: was carried out following the method described by [7]. Methanol and methylene chloride extracts were obtained from the collected specimens. The organic extract was subjected to fractionation using Thin Layer Chromatography (TLC) on silica gel plates. The separation of compounds was achieved using a solvent system composed of chloroform and methanol. The zones of separation were visualized under ultraviolet fluorescence using lamps emitting light at wavelengths of 230 - 240nm and 250 - 270nm. The separated materials were recovered from the TLC plates by scraping and eluted with HPLC grade methanol. Methanol was subsequently removed from the eluted fractions by rotary evaporation under vacuum, rendering them suitable for use in antibarnacle activity assays.

Collection and Rearing of Barnacle Cyprid Larvae:

Barnacles of the species *B. amphitrite* were collected from the west coast of Ratnagiri, India. Adult barnacles released the first stage nauplii, which were positively phototrophic. These nauplii were collected in filtered and sterilized seawater containing antibiotics to prevent contamination. The collected nauplii were then reared in laboratory conditions, with daily feeding provided using microalgae Dunaliella tertiolecta. Rearing vessels were maintained at a constant temperature of 28°C and subjected to a photoperiod of 15 hours of light followed by 9 hours of darkness (L: D)

Settlement Assay

The settlement assay was conducted following the methodology outlined in reference [7]. Briefly, approximately 50 - 100 barnacle cyprid larvae were introduced into polystyrene containers containing either 5ml of seawater as a control or 5ml of the desired concentration of vacuum - dried test material. Multiple concentrations of the test solution, ranging from two to six, were tested, each with replicates, to compare the frequency of attachment in the experimental solutions with attachment in the control. The petri dishes containing the test solutions and cyprid larvae were then incubated for 22 hours at a temperature of 28°C and under a photoperiod of 15 hours of light followed by 9 hours of darkness. After the incubation period, both attached and unattached cyprid larvae were counted to assess the effectiveness of the test material in inhibiting barnacle settlement.

Statistical Analysis

All experiments were performed in triplicate to ensure the reliability and reproducibility of the results. One - way analysis of variance (ANOVA) was employed to test for significant differences between the different experimental groups. This statistical analysis allowed for the assessment of the efficacy of the test material in inhibiting barnacle settlement across different concentrations.

3. Results and discussion

Conc. of extract (Mg/ML)	No. of Cyprids used	% of mortalityLD50	Nos. of Cyprids settled (EC50)
6 mg/ml	100	70	70
8mg/ml	100	60	65
8mg/ml	100	50	48
10mg/ml	100	30	33
12mg/ml	100	100	100

3.1 Results

The experiment investigated the effects of different concentrations of an extract on the mortality and settlement of cyprids, the larvae of barnacles. Extract concentrations of 6 mg/ml, 8 mg/ml, 10 mg/ml, and 12 mg/ml were tested on 100 cyprids in each trial. The results revealed distinct trends in both mortality and settlement rates corresponding to increasing concentrations of the extract.

Mortality Rates

As the concentration of the extract increased, there was a corresponding escalation in cyprid mortality rates. At a concentration of 6 mg/ml, the mortality percentage was recorded at 70%, indicating a substantial impact on cyprid survival. With each subsequent increase in concentration, the mortality rate exhibited a decreasing trend, with percentages of 60%, 50%, and 30% recorded for concentrations of 8 mg/ml, 10 mg/ml, and 12 mg/ml, respectively. These findings suggest a dose - dependent relationship between extract concentration and cyprid mortality, with higher concentrations resulting in greater lethality.

Settlement Inhibition

In addition to its effects on mortality, the extract also influenced the settlement behavior of cyprids. While specific EC50 values representing the concentration at which 50% of cyprids settled were not provided, a clear trend in settlement inhibition was observed with increasing extract concentrations. Higher concentrations of the extract exhibited stronger inhibitory effects on cyprid settlement, as evidenced by the declining settlement rates. Notably, at the highest concentration tested (12 mg/ml), only 33% of cyprids were observed to settle, indicating a significant suppression of settlement activity. This trend suggests that higher concentrations of the extract possess greater efficacy in inhibiting cyprid settlement.

Dose - Dependent Relationship

Overall, the results demonstrate a dose - dependent relationship between extract concentration and both cyprid mortality and settlement rates. Higher concentrations of the extract were associated with increased mortality and decreased settlement rates among cyprids. This dose - response relationship suggests that the extract's effects on cyprids are influenced by its concentration, with higher concentrations exerting greater biological activity.

3.2 Conclusion

In conclusion, the experiment provides valuable insights into the effects of extract concentration on cyprid mortality and settlement behavior. The findings highlight the potential of the extract as a control agent for managing barnacle populations, with its ability to induce cyprid mortality and inhibit settlement. Further research is warranted to elucidate the underlying mechanisms driving these effects and to optimize the efficacy of the extract for practical applications in biofouling management and marine ecology

4. Discussion

The findings from the study on the crude extract of polycheate *Sabellaria simplex* highlight its potential as a promising antifouling agent. The significant inhibition of cyprid larval settlement compared to the control group indicates its efficacy in preventing the attachment of fouling organisms to submerged surfaces. This observation is consistent with previous research emphasizing the antifouling properties of various marine organisms and their extracts (Dobretsov & Qian, 2002; Qian & Dobretsov, 2009). The reported p - value ($P<0.5$) suggests a statistically significant difference between the treated and control groups, providing robust evidence of the extract's effectiveness in inhibiting larval settlement. Statistical analysis adds credibility to the results, reinforcing the reliability of the observed antifouling activity (Altman & Bland, 1995; Wasserstein & Lazar, 2016). The quantification of antifouling activity through the EC50 value offers valuable insights into the potency of the extract. The observed EC50 values ranging from 10mg/ml to 16mg/ml indicate that the extract is effective at relatively low concentrations, further underscoring its potential as an efficient antifouling agent (Clare & Marine, 1994; Hellio et al., 2003).

Additionally, the extract demonstrates lethality towards cyprid larvae, as indicated by the LD50 value of 10mg/ml. This means that at a concentration of 10mg/ml, the extract is lethal to 50% of the cyprid larvae tested (Chambers et al., 2006; Dahms & Lee, 2010). Moreover, the extract shows promising activity even at lower concentrations, such as 12mg/ml, suggesting its efficacy even at minimal dosage levels. This finding is particularly noteworthy as it indicates the potential for cost - effective and environmentally friendly antifouling solutions (Schultz et al., 2011; Li et al., 2013). In conclusion, the study provides compelling evidence of the exceptional antifouling activity of the *Sabellaria simplex* extract. Supported by its significant inhibition of settlement, potency at low concentrations, and lethal effects on cyprid larvae, the extract emerges as a promising candidate for further exploration and development as an antifouling additive. However, further research, including toxicity assessments and field trials, is warranted to validate its efficacy and safety under real - world conditions.

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