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THE USE OF ALGAL EXTRACTS IN THE CONTROL OF FUNGI IN PLANTS

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ABSTRACT

This study aimed to observe and test the efficacy of a natural, more sustainable, and less harmful fungicide using algae extracts combined with aqueous solutions, such as distilled water as the control group, 70% alcohol in the first analysis, and hexane in the second. The research investigated the antifungal potential of seaweed extracts collected from rocky shores of Indaiá Beach in the municipality of Bertioga and Porchat Island in the municipality of São Vicente, using nine species. The algae were processed with distilled water, 70% alcohol, and hexane and applied to materials contaminated by fungi such as *Sclerotinia sclerotiorum* and *Penicillium italicum*. The extracts were tested on various fruits and breads, with daily application for seven days. The first analysis, using distilled water as a control group, resulted in a significant increase in the fungus *Sclerotinia sclerotiorum* on tomatoes and the appearance of leachate, while contamination in oranges and bananas remained stable. The second analysis, using 70% alcohol, resulted in dehydration of the materials used, resulting in a reduction in fungi, especially on tomatoes. The third analysis, using hexane, showed a reduction in fungi on bread and strawberries, with the loss of color indicating possible fungal death. The algal extracts demonstrated efficacy as potential fungicidal action, varying according to the solvent used. Distilled water demonstrated the least efficacy, allowing the growth of *Sclerotinia sclerotiorum*, while 70% alcohol and hexane were more effective in containing the fungi. Therefore, algal extracts, especially those containing 70% alcohol and hexane, can be explored in future research for fungal control in various contexts.

Keywords: algae,Baixada Santista, fungi.

INTRODUCTION

This study used algal extracts to control fungi in plants. For this study, different groups of algae were used to verify their fungicidal action and their likely large-scale application. Marine macroalgae are sources of metabolites and bioactive compounds belonging to the classes of lectins, terpenes, phenolic compounds, and sulfated polysaccharides[1].These organisms are used for various purposes, including biostimulants, fertilizers, and pest and pathogen control. Some polysaccharides have important applications in the pharmaceutical, food, and biotechnology industries, among other uses[2].In recent years, some countries have been using algal species in agriculture as biostimulants and fertilizers, which have demonstrated increased plant resistance to diseases caused by microorganisms. However, the use of macroalgae in this sector is still limited, as their use in the natural agricultural products trade is low[3]. The use of agricultural pesticides has caused significant harm to both the environment and those who handle them. This doesn't even take into account the fact that many pests become resistant to these pesticides, forcing farmers to increase the pesticide dosage or switch to a more effective, but often more toxic, pesticide. This causes soil and water contamination and degradation, reduces biodiversity, and leads to ecological imbalance, leading to unsustainability. This study tested the efficacy of a natural fungicide that is more sustainable, less harmful to the environment, and offers a more accessible alternative for treating phytopathogens. It is well known that fungicides extracted from plants have been used for centuries. Norrie and Keathley[4] demonstrated the effectiveness of the algae *Ascophyllum nodosum* in reducing fungal diseases. Due to the rich chemical composition of medicinal plants containing microbiocidal active ingredients, they are potential sources of molecules that can be used to defend plants against phytopathogens[5]. The great advantage of using plant extracts is their rapid degradation in the environment and low toxicity. The experiments in this study were conducted in vitro. The experimental trials were conducted at the Darwin Laboratory of the Paulista University (UNIP), Santos-Rangel campus, to study and analyze the expected and actual results, verifying whether the macroalgae were truly effective in their fungicidal action. This was based on the fact that they are rich sources of metabolites and bioactive compounds[6].

MATERIALS AND METHODS

Seaweed samples were collected from two locations: the rocky shore of Indaiá Beach in Bertioga and the rocky shore of Porchat Island in São Vicente. During sampling, spatulas were used to scrape the substrate to remove benthic seaweed from the supra-, mid-, and infralittoral regions and store it in glass or plastic bottles. Sampling was carried out at low tide, and was verified in advance using the tide table provided by the Brazilian Navy. The samples were collected in the coastal zone of the systems, where the studied seaweeds are commonly fixed, fully or partially submerged. These environments are considered algae concentrators. Nine seaweed species and their respective masses were used: *Bostrychia radicans* (120.1 mg), *Bostrychia scorpioides* (12.46 g), *Grateloupia gibbesii* (3.19 g), *Lyngbya majuscula* (2.69 g), *Padinagymnospora*

(1.77 g), *Ulva clathrata* (246 g), *Ulva fasciata* (10 g), *Ulva flexuosa* (11.33 g), and *Ulva lactuca* (30.40 g). The materials were fixed and preserved immediately after sampling, still in the field, in glass or plastic vials to avoid sample contamination. After arriving at the laboratory, the samples were stored in a freezer at -17 to -250°C if not processed immediately.

Figure 1

Figure2

During sample collection, spatulas were used to remove seaweed, which was stored in glass or plastic jars. The organic materials used in the study were bananas, oranges, sliced bread, strawberries, and tomatoes. In the control group, after extraction, distilled water was used at the following concentrations to clean the samples: *Bostrychia radicans* – 0.1201 g/L, *Ulva clathrata* – 0.2460 g/L, *Ulva flexuosa* – 0.0780 g/L, and *Ulva lactuca* – 1.0066 g/L. The samples were then properly sorted and macerated using a mortar and pestle. The resulting maceration was diluted in 1 L of distilled water to obtain an algal extract for application to the fungal materials. The algae were infused in boiling water in a closed container. To evaluate their potential antifungal potential, the extracts were placed in spray bottles and stored in the refrigerator. 2.5 mL of the extract was applied daily for seven days to the following materials: bananas, oranges, sliced bread, and tomatoes contaminated with the fungi *Sclerotiniasclerotiorum* and *Penicillium italicum*. After application, the materials were stored in Styrofoam trays covered with PVC film. For the initial analysis of the algae's antifungal potential, the algae extracts were mixed with 70% alcohol in the following proportions: *Grateloupia gibbesii* (3.19 g to 63.80 mL), *Bostrychiascorpioides* (12.46 g to 249.20 mL), *Ulva fasciata* (10 g to 100 mL), and *Ulvaflexuosa* (5 g to 14.87 mL). The extracts were placed on a Thelga® magnetic stirrer at 90 rpm to homogenize the extract. They were then filtered to remove algal residue. They were then placed in spray bottles and stored in the refrigerator. To observe their antifungal activity, 1.0 mL of the extract was applied once daily for seven days to the following materials: bananas, oranges, sliced bread, and tomatoes contaminated with the fungi *Sclerotinia sclerotiorum* and *Penicillium italicum*. After application, the materials were stored in Styrofoam trays covered with PVC film. In the second analysis, the algal extract was mixed

with hexane in the following proportions: *Lyngbia majuscula* – 2.69 g per 350 mL; *Padina gymnospora* – 1.77 g per 189 mL; *Ulva flexuosa* – 6.25 g per 200 mL and *Ulva lactuca* – 29.39 g per 274 mL. They were placed on a Thelga® brand heated magnetic stirrer at an average temperature of 52°C for one hour at 90 rpm to extract the mixture, then transferred to sprayers. To analyze the antifungal potential, 1.0 mL of the mixture was applied once daily for seven days to the following materials: sliced bread and strawberries contaminated with *Penicillium italicum* and *Botrytis cinerea*, respectively.

RESULTS

In the control group, after applying the compound extracts with distilled water at a rate of 2.5 mL daily for 7 days to the fungal materials used for the tests, a significant increase in the fungus *Sclerotinia sclerotiorum* was observed on the tomato on the third day, along with the formation of a large amount of leachate (a product of bacterial and fungal decomposition). The fungi appeared to stabilize in sliced bread, bananas, and oranges using the extracts of *Ulva clathrata* and *Ulva lactuca* combined with distilled water. After the distilled water extracts were discontinued, fungal growth occurred on the test materials, and the fungal materials were subsequently discarded. In the first analysis, performed with 70% alcohol after applying 1.0 mL daily for 7 days to the fungal materials used for the tests, it was observed that the materials absorbed the daily extracts, causing them to become dehydrated due to the 70% alcohol. No increase in fungi was observed, but there was a decrease in the fungus, particularly in tomatoes. The first analysis showed an increase in *Sclerotinia sclerotiorum* and the appearance of a large amount of leachate. In the second analysis, conducted with hexane after applying 1.0 mL daily for 7 days to the fungal materials used for the tests, a decrease in fungi was observed. It was also found that with the application of the algal extracts with hexane, the fungi present on the bread and strawberries turned brown, indicating possible death. There was no dehydration, as observed in the first analysis with 70% alcohol.

DISCUSSION

The use of seaweed extracts has demonstrated great potential for controlling and containing fungi, with significant variations in results depending on the solvent used for extraction. Seaweed extracts contain bioactive compounds, such as fatty acids, polyphenols, and other secondary metabolites, which have demonstrated antifungal activity in several studies[7]. Exposure of seaweed to extreme environmental conditions, such as high salinity, intense UV radiation, and temperature fluctuations, appears to induce the production of substances with strong antifungal potential. These natural defense mechanisms of seaweed can be harnessed to develop alternative and sustainable solutions for controlling fungal diseases[8]. The choice of solvent for extracting seaweed bioactive compounds has a significant impact on the extract's antifungal efficacy. In the case of tests conducted with distilled water, the results were mixed. Although the extract was effective in preserving tomatoes, bananas, and oranges, preventing the proliferation of the *Sclerotinia sclerotiorum* mold, an increase in leachate formation and mold growth was observed in the tomatoes. This

situation can be attributed to the limited interaction between the bioactive compounds and the fungi, as distilled water may not have been the best choice for extracting more lipophilic or hydrophobic substances present in the algae. On the other hand, the use of 70% alcohol as a solvent yielded positive results. Alcohol, being a nonpolar solvent, has the ability to extract lipophilic compounds, such as terpenes and flavonoids, which demonstrate antimicrobial activity[9]. When applied to the algal extracts, 70% alcohol contributed to food dehydration, preventing mold growth. The difference observed between the use of distilled water and 70% alcohol was notable, especially in tomatoes, where increased leachate formation was evident in the analyses with water, but virtually absent when 70% alcohol was used (Figures 16 and 27). The microbiocidal action of 70% alcohol, proven in several studies, contributes to the inhibition of fungal growth. However, this inhibition is not complete; it occurs primarily on the surfaces to which it was applied[12], allowing the presence of spores that, in turn, did not develop, demonstrating the effectiveness of the extracts with the solvent used, especially in the tests with *Ulva lactuca*. When hexane was used as the solvent, a visible decrease in fungi present on the materials was observed, with a change in the color of the food surfaces, especially bread (Figures 30 and 31). Hexane is a nonpolar solvent that favors the extraction of lipophilic compounds, such as waxes and fatty acids, which may have fungicidal activity[10]. The reduction in fungal growth in hexane-treated foods can be attributed to the action of these compounds, which inhibit spore germination and mycelial development. Furthermore, the observed color change suggests an interaction between the algal extracts and the food compounds, indicating that hexane not only extracted bioactive substances but also affected the food's structure. Although the use of algal extracts as fungicides is still a growing field of research, the results obtained, both in the literature and in this study, demonstrate that these extracts have great potential in inhibiting fungal growth[11]. Furthermore, algal extracts have the advantage of having a low toxicity index for humans and the environment. This means that, unlike many synthetic fungicides, which can leave toxic residues and cause environmental damage, algal extracts are a more sustainable and safer alternative. The use of solvents such as 70% alcohol and hexane, which have proven microbiocidal properties, increases the effectiveness of the extracts, enhancing their antifungal action without causing harm to the environment. The results of this research, combined with previous studies, suggest that algal extracts have significant antifungal potential, especially when combined with appropriate solvents. The use of these extracts, with the advantage of not leaving toxic residues, could represent an important advance in the search for natural and sustainable alternatives for controlling fungi in food and other materials. However, further studies are needed to fully understand the mechanisms of action of algal compounds and to optimize the extraction and application conditions of these extracts. Integrating this knowledge could lead to the development of new products with greater efficiency and lower

environmental impact, meeting the demand for more sustainable solutions.

Based on the three analyses performed, it is possible to conclude that algal extracts have some fungicidal potential, but their effectiveness varies and depends on the solvent used. Distilled water proved to be less effective, allowing the growth of *Sclerotinia sclerotiorum* in some cases, such as tomatoes, considering that humidity is a perfect environment for fungal growth. In contrast, 70% alcohol and hexane proved to be more effective in containing and reducing fungi. 70% alcohol was particularly effective in promoting dehydration of the materials, inhibiting fungal growth, while hexane not only reduced the presence of fungi but also altered the color of the materials, suggesting the death of the microorganisms. Therefore, algal extracts have some potential as antifungal agents, especially when used with solvents such as 70% alcohol and hexane, which have been shown to be more effective in inhibiting and eliminating fungi. These solvents, especially when mixed with algal extracts from *Ulva lactuca* and *Ulva clathrata*, visibly prevented spore development and fungal spread after application to the fungi-infected materials.

These results may guide future, more in-depth research on the use of algal extracts for fungal control in different materials and environments, given that the data presented in this study were the first collected on the algae used and their effectiveness for fungal control.

LIST OF ILLUSTRATIONS

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