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Research Article

Potential Use of Algae and Seaweed Extracts as Protection Against Peach Leaf Curl

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Plant pathogenic fungi are a major cause of plant diseases, contributing significantly to the global decline in food production. Their proliferation results in substantial crop losses, fruit and vegetable deterioration, reduced food accessibility, and billions of dollars in economic losses annually. While synthetic chemical fungicides are commonly used to combat fungal diseases, their environmental impact is concerning. Marine algae provide a sustainable alternative, offering diverse compounds with industrial applications and presenting an eco-friendly solution. *Taphrina deformans* is responsible for peach leaf curl, a widespread disease in peach trees that can severely impact crop productivity and tree lifespan. Seaweeds and algae, encountering various biological interactions and extreme abiotic conditions, have evolved defense mechanisms, including the production of biologically active substances. In our study, we investigated the efficacy of water extracts from six algae (*Nannochloropsis* sp., *Tetraselmis chuii*, *Chaetoceros muelleri*, *Thalassiosira weissflogii*, *Tisochrysis lutea*, *Chlorella vulgaris*), four seaweeds (*Palmaria palmata*, *Chondrus crispus*, *Ascophyllum nodosum*), and one cyanobacterium (*Arthrospira platensis*) at two different concentrations (1% and 3%) in suppressing the outbreak of peach leaf curl disease *in vivo* on peach trees. The spray was applied twice in spring just before budbreak. Algae and seaweed extracts showed significant suppression of peach leaf curl outbreaks on peach trees compared to controls. Following the application of water extract of *Chlorella vulgaris* at both concentrations, the lowest incidences of symptomatic disease expression were observed (1.00 ±0.00% and 0.25 ±0.50%). Algae and seaweed extracts demonstrate potential in controlling leaf curl disease in peach trees.

Keywords: antifungal activity, peach leaf curl, seaweed, algae, *Taphrina deformans*

1 Introduction

Peach leaf curl stands as a critical threat to peach production, leading to substantial economic losses. At the heart of this ailment lies *Taphrina deformans*, a fungus recognized as the primary culprit behind peach leaf curl (Cissé et al., 2013). This fungal species, *T. deformans*, garners considerable attention in the scientific community due to its global impact as a plant pathogen, making it the most extensively researched species within its genus (Ogawa et al., 1995). Notably, all documented cultivars of both peach and nectarine are vulnerable to its infectious grasp. Among the hallmark symptoms of this affliction, leaves undergo severe distortion and adopt a reddish hue, signifying the gravity of the infection (Kern & Naef-Roth, 1975). It was determined that meteorological conditions during early April, particularly the average temperature, amount of precipitation, and frequency of rainy days, significantly influenced the occurrence of the disease (Kiss et al., 2022).

As the global population continues to grow, the demand for higher agricultural yields has made pesticides and agrochemicals indispensable components of worldwide farming practices over the last century. At the same time, in recent decades, agrochemical residues have entered the environment, leading to contamination of terrestrial and aquatic ecosystems and thus to profound impacts on wildlife in all ecological regions (Carvalho, 2017; Cruz de Carvalho et al., 2020; Duarte et al., 2021; Gee et al., 2013; Köhler & Triebskorn, 2013). Furthermore, pesticides exhibit a propensity for environmental persistence, enduring for extended durations within soils and/or sediments, and eventually leaching into aquatic

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environments. This culminates in their accumulation across trophic webs, inflicting severe toxic effects at the population level (Köhler & Triebskorn, 2013). Substitutes for synthetic pesticides must prioritize safety for both humans and the environment while also adhering to principles of sustainability (Asimakis et al., 2022).

The category of algae/seaweed encompasses a diverse array of photosynthetic organisms predominantly inhabiting freshwater and marine environments, with some species also capable of thriving in terrestrial habitats, either independently or through symbiotic associations with other organisms (Gärtner et al., 2021; Specht et al., 2017). Algae (microalgae) and seaweed (macroalgae) are being increasingly explored as a reservoir of bioactive compounds for various applications, including their potential use in biopesticides (Duarte et al., 2021). Over the past decades, there has been a significant surge in the discovery of metabolites with biological activities derived from macroalgae and microalgae. The vast array of phytochemicals produced by marine algae encompasses those exhibiting antifungal and antibacterial properties (O'Keeffe, 2019). Brown seaweeds generally exhibit stronger antifungal activity compared to red algae due to their abundance of lipophilic compounds, including fatty acids and phenolic compounds. Laminarin, a polysaccharide found in brown seaweeds, triggers plant defense responses and can stimulate immunity mechanisms when applied to plants. Other brown algae polysaccharides like fucoidans and alginates also contribute to plant defense. However, amino acids, peptides, and proteins do not play a significant role in plant disease suppression (Hanaa et al., 2008; Holdt & Kraan, 2011; Trouvelot et al., 2014). Microalgae utilize light energy and inorganic nutrients, including carbon dioxide, nitrogen, and phosphorus, to biosynthesize bioactive compounds containing therapeutic properties such as lipids, proteins, carbohydrates, pigments, and polymers. Moreover, they have the capability to generate various chemical compounds with diverse biological activities, including polyunsaturated fatty acids (PUFA), carotenoids, proteins, polysaccharides, vitamins, and sterols. Numerous bioactive compounds derived from microalgae exhibit a range of biological activity (Abo-State et al., 2015; Amaro et al., 2011; Correa et al., 2011; Vehapi et al., 2020).

The aim of our work was to test aqueous extracts at different concentrations (1% and 3%) of 6 microalgae, 3 macroalgae and one cyanobacterium in *in vivo* tests on peach trees as a prevention against the peach leaf curl disease-causing fungus *Taphrina deformans*.

2 Material and Methods

2.1 Algae and Seaweeds

Tested microalgae *Nannochloropsis* sp., *Tetraselmis chuii*, *Chaetoceros muelleri*, *Thalassiosira weissflogii*, *Tisochrysis lutea* were purchased in dried, powdered form from the Belgian company Proviron (Fig. 1). Proviron seaweeds are produced under the most strictly controlled conditions and continuously checked for the presence of pathogens (HACCP and FCA certified by SGS (FCA certificate BE 01/1522. GF).

The macroalgae *Palmaria palmata* (Irish origin), *Chondrus crispus* (Canadian origin), belonging to the red macroalgae group, and *Ascophyllum nodosum* (Canadian origin), a brown seaweed, were purchased in powder form from the German company Biotiva. The seaweed comes from controlled organic cultivation and is gently, organically processed in a factory in Bavaria from controlled organic cultivation (DE-ÖKO-005).



Figure 1 Powder samples of algae and seaweed

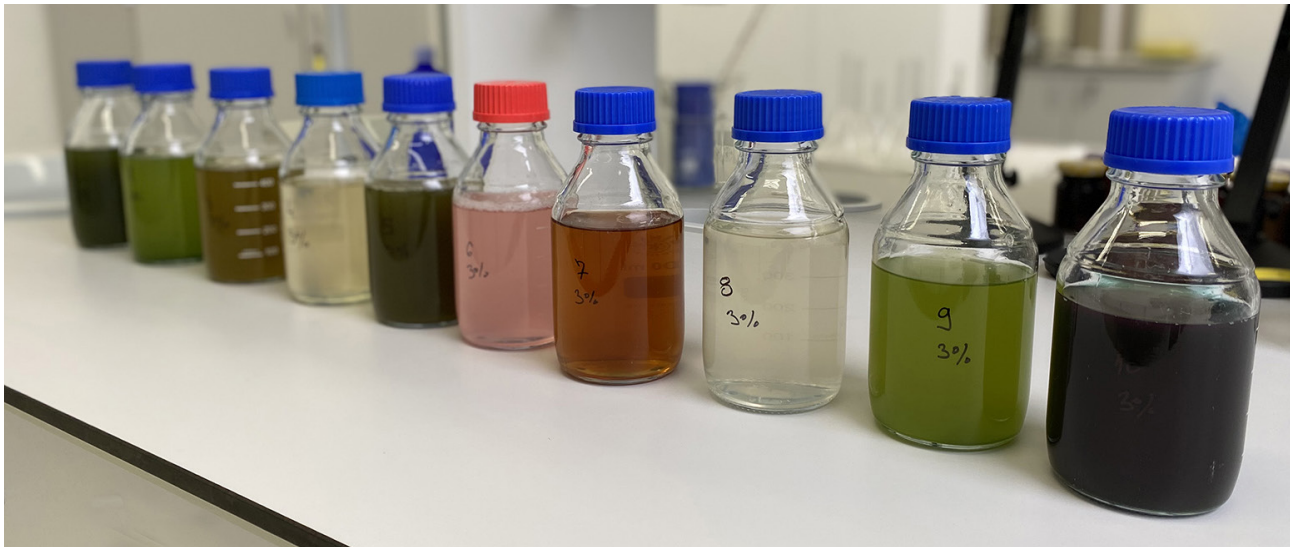


Figure 2 Water extracts of algae and seaweed used as sprays on peach trees

The microalgae *Chlorella vulgaris* (origin China) and the cyanobacteria *Arthrospira platensis* (origin China) in powder form were purchased from the Slovenian company FutuNatura from certified organic production SI-EKO-002 agriculture outside the EU.

All samples were purchased in 2022, stored in a dry environment, in the dark at laboratory temperature (±20 °C), and hermetically sealed.

2.2 Extract Preparation

The algae water extract was prepared in two different concentrations (1% and 3%). Powdered dried algae was weighed 1g and 3g and poured with distilled water to a volume of 1 L into a glass bottle (Fig. 2). Each alga and each concentration was prepared in two replicates (two 1 L bottles of each algae and each concentration). The extracts thus prepared were macerated in the dark for 24 hours with stirring. At the end of the time, the extract was filtered through Whatman no. 2 filter paper and used for spraying fruit trees on the same day.

2.3 Fruit Trees

The peach trees are located in Nové Zámky (Poľnohospodár Nové Zámky a.s.). The peach varieties used for the research were redhaven. The peach orchard was not treated with any chemical preparations before application of extracts (Fig. 3).

2.4 Application of Sprays

Algae extracts were applied to peach trees in two replications, in the spring just before budburst (28. 2. 2023) and (14. 3. 2023), and evaluation of leaf curl infection was 19. 4. 2023. Application was made uniformly using hand sprayers with a spray nozzle from a distance of 20 cm from the buds (Fig. 4). Each spray lasted 5–10 seconds and 1 L of extract was applied to each tree. Each concentration from each algae/seaweed was applied to two different trees. Control peach trees were not treated in any way.



Figure 3 Peach orchard in Nové Zámky called Poľnohospodár



Figure 4 Spray application using hand sprayers with spray nozzle

2.5 Evaluation and Statistical Evaluation

The efficacy of the extracts to suppress the occurrence of leaf curl disease of peach was expressed as a percentage of leaf infestation. A random sample of 2x100 shoots on each tree was identified and observed for disease symptoms (Fig. 5). The mean and standard deviation were calculated from two trios of 2x100 shoots and the percentage incidence of leaf curl disease of peach was calculated. Shoots that were not treated with any spray were used as control. Statistically significant differences were also calculated using One-way ANOVA, followed by the Tukey's HSD test at $p \leq 0.05$ significance, was performed using online Astatsa Anova One Way.

3 Results and Discussion

Leaf curling on unsprayed trees can become dangerous. Year after year, it can destroy the first leaves and most of the crop, gradually weakening the tree until it dies. Nevertheless, fungicide sprays have been a relatively simple way to combat leaf curl for nearly a century. Growers could use varieties and compounds made from plants, algae or seaweed as methods to combat leaf curl. Growers have shown interest in using algae and seaweed extracts to prevent leaf curl in peach (Shukla et al., 2021), although few studies have been conducted in this area.



Figure 5 Symptomatic expression of peach leaf curl disease

Although the relative resistance of locally grown stone fruit cultivars to leaf curl has not been thoroughly investigated, it is known that most cultivars grown for export are susceptible to some degree (Rodríguez-Verástegui et al., 2022). The objective of this study was to determine how well algae and seaweed and algae extracts work in preventing leaf curl in peach. Leaf curl has been successfully suppressed by several fungicides. Further research is needed on possible applications of algae and seaweed extracts as well as resistant or tolerant peach varieties. There is much more to stone fruit breeding than developing resistance to leaf curl (Agarwal et al., 2021).

Peach leaf curl disease outbreak symptoms were monitored after application of 1% algae and seaweed water extract (Table 1). The algae extracts were applied just before peach budburst and then sprayed again two weeks later. One month after application, we identified the incidence on 29.50 ±7.94% of shoots on control trees. After application of *Chlorella vulgaris* microalgae extract, the number of leaves on which disease outbreak occurred was significantly reduced to 1.00 ±0.00%. Similar results were obtained by the microalgae *Tetraselmis chuii* (1.50 ±0.58%) and *Tisochrysis lutea* (1.50 ±1.29%). The most efficient seaweed was *Ascophyllum nodosum* (1.25 ±1.89%), which belongs to the brown algae. The weakest effect was shown for the microalga *Nannochloropsis* sp. (31.25 ±7.27%) and cyanobacteria *Arthrospira platensis* (4.00 ±2.94%). Compared with the control after application of algal and seaweed extracts, the statistical significance was confirmed except for the alga *Nannochloropsis* sp.

Table 1 Number of peach leaves affected by peach leaf curl disease (%) treated with 1% algae extract

Algae/seaweed extract	Infested leaves (%)
***Nannochloropsis* sp.**	31.25^{a} ±7.27
Tetraselmis chuii	1.50^{b} ±0.58
Chaetoceros muelleri	2.50^{b} ±1.00
Thalassiosira weissflogii	2.25^{b} ±1.71
Tisochrysis lutea	1.50^{b} ±1.29
Palmaria palmata	3.50^{b} ±1.29
Ascophyllum nodosum	1.25^{b} ±1.89
Chondrus crispus	3.25^{b} ±2.87
Chlorella vulgaris	1.00^{b} ±0.00
Arthrospira platensis	4.00^{b} ±2.94
Control	29.50^{a} ±7.94

Data are the mean (± SD) of infested leaves out of a total of 200 leaves. Different letters in each column refer to significant differences (Tukey, $p \leq 0.05$)

Research must take into account tree vigour, fruit productivity, and resistance to various pests and diseases (Field et al., 2019). If growers have easy access to information on when leaf curl infection occurs during the growing season, they can increase the effectiveness of fungicide treatments. Research needs to be conducted to determine the best way to provide growers with this information (McLaughlin et al., 2023). Peach leaf curl disease outbreak symptoms were also observed after application of 3% water extract of algae and seaweed (Table 2). As the concentration of the extract increased, the incidence of the disease on the leaves decreased. As with the application of 1% extract, the 3% extract of the microalga *Chlorella vulgaris* showed the strongest antifungal activity with an incidence of peach leaf curl of only 0.25 ±0.50%. Like the previous results, 3% extract of *Tetraselmis chuii* reduced the incidence to 0.75 ±0.50%. Cyanobacteria *Arthrospira platensis* and microalga *Nannochloropsis* sp. also at 3% concentration achieved the lowest efficiency compared to other samples (6.50 ±3.11%; 7.00 ±3.92%). At this concentration, the statistical significance was confirmed for all tested extracts compared to the control.

Table 2 Number of peach leaves affected by peach leaf curl disease (%) treated with 3% algae extract

Algae/seaweed extract	Infested leaves (%)
***Nannochloropsis* sp.**	7.00^{a} ±3.92
Tetraselmis chuii	0.75^{a} ±0.50
Chaetoceros muelleri	1.00^{a} ±0.82
Thalassiosira weissflogii	2.00^{a} ±1.41
Tisochrysis lutea	1.25^{a} ±0.50
Palmaria palmata	2.25^{a} ±2.22
Ascophyllum nodosum	1.25^{a} ±0.96
Chondrus crispus	1.50^{a} ±1.73
Chlorella vulgaris	0.25^{a} ±0.50
Arthrospira platensis	6.50^{a} ±3.11
Control	29.50^{b} ±7.94

Data are the mean (± SD) of infested leaves out of a total of 200 leaves. Different letters in each column refer to significant differences (Tukey, $p \leq 0.05$)

Currently, there is a growing interest in seaweed and its ingredients due to their rich content of bioactive substances. Seaweeds are known to contain biologically active compounds whose antibacterial and antifungal properties have been extensively recorded. The algae's ability to combat fungal infections might stem from its phenolic compounds like rosmarinic acid and quercetin, alongside substances such as palmitic acid, oleic acid, and other components, whose antifungal properties

have been verified (Pourakbar et al., 2021). There is no information in the available literature on research on whether algal extracts in *in vivo* tests can act as protective sprays on peach trees against the leaf curl disease causing fungus *Taphrina deformans*. However, the antifungal activity has been confirmed by many studies on different crops against several pathogens causing large economic losses. The antifungal efficacy of *Ulva lactuca*, *Chlorella vulgaris*, *Chlorella minutissima*, and *Chlorella protothecoides* was examined against *Aspergillus niger*, *Alternaria alternata*, and *Penicillium expansum* fungi to assess their potential as fungicides. The results revealed that nearly all of the extracts derived from algae species displayed antifungal activity against the selected fungal pathogens. This study underscores the importance of exploring algae's potential for natural fungicide production in pharmaceutical and food industries (Vehapi et al., 2020). The findings regarding the impact of algae extract, applied at rates of 200, 400, 600, 800, and 1,000 µL, on the mycelial growth of four significant plant pathogenic fungi – *Botrytis cinerea*, *Aspergillus niger*, *Penicillium expansum*, and *Pyricularia oryzae* – indicated that the mycelial growth of all four fungi decreased with increasing concentrations of the algae extract (Pourakbar et al., 2021). Carrot plants cultivated in a greenhouse were subjected to foliar spraying with a seaweed extract (0.2%) obtained from *Ascophyllum nodosum* (SW) and subsequently inoculated with fungal pathogens *Alternaria radicina* and *Botrytis cinerea* six hours later. Additional applications of SW were administered 10 and 20 days post-inoculation. The treated plants exhibited significantly reduced disease severity at 10 and 25 days post-inoculation in comparison to control plants treated with water. These findings indicate that *Ascophyllum nodosum* enhances disease resistance in carrots, likely through the induction of defense genes or proteins (Jayaraj et al., 2008). Experiments were conducted to evaluate crude extracts obtained from brown seaweeds including *Sargassum muticum*, *Dictyota bartyrensiana*, *Padina gymnospora*, *Chnoospora implexa* and *Sargassum wightii*. These extracts were tested for their efficacy in controlling rice sheath disease, a widespread problem in India caused by the fungus *Rhizoctonia solani* (Raj et al., 2016).

4 Conclusions

Fungal pathogens are frequently identified as the primary cause of various plant diseases, significantly contributing to the global decline in food production. The proliferation of these fungi results in substantial crop losses, spoilage of fruits and vegetables, reduced food availability, and billions of dollars in economic losses each year. Despite the widespread use of synthetic chemical fungicides to combat fungal diseases, their environmental impact raises concerns. Marine algae offer a sustainable alternative in the search for diverse compounds with industrial applications, presenting an eco-friendly solution. The organism *Taphrina deformans* is responsible for peach leaf curl, a widespread disease affecting peach trees globally. This disease poses a significant threat to both the yield of peach fruits and the longevity of the trees themselves. Seaweeds encounter various biological interactions and extreme abiotic conditions, leading to the evolution of diverse defense mechanisms, including the production of biologically active substances. In our study, we investigated the effectiveness of aqueous extracts from six algae (*Nannochloropsis* sp., *Tetraselmis chuii*, *Chaetoceros muelleri*, *Thalassiosira weissflogii*, *Tisochrysis lutea*, *Chlorella vulgaris*), four seaweeds (*Palmaria palmata*, *Chondrus crispus*, *Ascophyllum nodosum*), and one cyanobacterium (*Arthrospira platensis*) at two concentrations (1% and 3%) in suppressing the outbreak of peach leaf curl disease under *in vivo* conditions on peach trees. The spray application was repeated twice in the spring, just before budbreak. Our findings reveal that extracts from algae and seaweeds significantly reduced disease outbreaks on peach trees compared to controls. The results obtained after applying the aqueous extract of *Chlorella vulgaris*, which demonstrated the lowest incidences of symptomatic disease expression at both concentrations (1.00 ±0.00% and 0.25 ±0.50%, respectively) were particularly notable. This suggests that algae extract holds promise in effectively controlling leaf curl disease in peach trees.

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