

Evaluation of Baja Peninsula seaweeds against plant pathogens

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Abstract

The widespread use of synthetic pesticides to control pests has generated serious consequences on the environment and human health. Currently, efforts focused on the search for new pesticides with less impact on the environment have been doubled. Marine algae synthesize compounds with activity against various agricultural pests (insects, nematodes, and fungi); however, marine pesticides are an underdeveloped alternative. This represents an opportunity to explore new sources of compounds. The objective of this work is to evaluate the antimicrobial, nematocidal and insecticidal activity of seaweed extracts against pathogens of agricultural importance. Therefore, seaweeds were collected from different locations at the Baja California peninsula, Mexico, and ethanolic extracts were obtained. The antibacterial and antifungal activity against five phytopathogenic strains and *Fusarium oxysporum* was evaluated by disc diffusion on agar. Nematocidal activity was assessed by egg hatching inhibition on *Meloidogyne incognita* and insecticidal activity against maize weevil *Sitophilus zeamais*. Regarding the antibacterial potential, *Laurencia johnstonii*, *Asparagopsis taxiformis* and *Dictyota dichotoma* showed higher inhibition against all the strains. *Padina concrescens* exhibited the highest percentage of egg hatching inhibition against *M. incognita* (59%) followed by *L. johnstonii* (48%). All the seaweeds cause mortality of *S. zeamais*. However, the highest insecticidal activity was found on *L. johnstonii* (71.9%). Red and brown seaweeds showed in general higher content of total phenolic compounds. Red and brown seaweed showed great potential for the control of phytopathogens through appropriate agricultural practices. However, further research is necessary to identify the active compounds.

1. Introduction

Pests cause more than 40% annually loss on economically important crops production. Plant diseases cause more than \$220 billion of damage and invasive insects cause around \$70 billion (FAO, 2019). Furthermore, between 30–40% of the produced food is lost during post-harvest storage, processing and transportation facilities (Gustavsson et al., 2011). All these factors have a significant impact on food security, including food availability, economic and physical access to it, and therefore its utilization. Thus, it is necessary to manage or prevent the development of infectious diseases at all stages of crop production (Nazarov et al., 2020).

Bacteria and fungi are major plant pathogens that cause significant damage to plants, leading to reduced germination, plant length, yield and productivity, adverse effects on soil health and post-harvest rotting of fresh fruits and vegetables (Tewari & Sharma, 2019; Kwon-Ndung et al., 2022; Vicente et al., 2023). Plant-parasitic nematodes alter normal root growth and function, leading to nutrient deficiencies and crop losses of around 12.3% worldwide. One of the most economically important pathogens is the genus *Meloidogyne*. The damage it causes to the plants can lead to secondary infections by pathogenic microorganisms, increasing crop loss (Sikandar et al 2020; Mendoza-de Gives, 2022).

Post-harvest insect pests such as the maize weevil *Sitophilus zeamais* can cause significant damage to the quantity and quality of stored cereals, with huge economic implications as the cereals are ready for consumption and had already been grown (agricultural process) and harvested. Losses in stored grain have been estimated to be as high as 60% (Arrahman et al., 2022; Odjo et al., 2022).

To control these pathogens synthetic agrochemicals with antimicrobial properties are applied. However, the extensive application of these chemical compounds causes many environmental and toxicological risks to human health (Devi et al., 2022). In a more sustainable approach, alternative methods have been explored to improve food production, and to ensure the quality and environmental safety (Vicente et al., 2021). In recent years, the use of seaweeds in sustainable agriculture has increased due to the numerous benefits that improve crop productivity and

stress resilience: fertilizers, biostimulants, root promoters, germination enhancers, phytoelicitors, resistance inducers to biotic and abiotic stress, antibacterial and antifungi (Ali et al., 2021; Shukla et al., 2021; Deolu-Ajayi et al., 2022; Parab & Shankhadarwar, 2022).

Seaweeds are a rich source of bioactive metabolites with remarkable chemical diversity that can promote plant defense against pathogens which results in higher biomass yield and quality (Pan et al., 2019; Jiménez et al., 2011). Therefore, the use of seaweed extracts has potential benefits in the prevention and management of pathogens in crops of economic importance. Despite the numerous benefits associated, research into plant disease management from marine sources is still underdeveloped. This provides the opportunity to identify new sources of active compounds and strategies for sustainable agriculture. Thus, the objective of this work is to evaluate the antibacterial and antifungal potential of seaweed extracts found in Baja California peninsula, Mexico.

2. Materials & Methods

2.1 Seaweed recollection and extract preparation.

Sixteen seaweed species were hand-collected during low tide from different localities in México (Table 1). Taxonomic identification was assessed by morphological characters and confirmed by Dr. Juan Manuel Lopez-Vivas. The fresh material was washed with running tap water, air-dried and ground to 40 mesh size. The dried seaweed was extracted by maceration with ethanol and concentrated in a rotary evaporator (Buchi II) at less than 40°C, under diminished pressure. The extracts were stored at 4°C until further use.

Table 1
Collection localities of seaweeds in Baja California Sur, México.

Seaweed	Extract code	Locality	Latitude, Longitude
Green seaweed			
<i>Caulerpa racemosa</i>	22 – 04	Calerita, BCS	24°21'03"N- 110°17'07" W
<i>Caulerpa sertularioides</i>	22 – 03	Calerita, BCS	24°21'03"N- 110°17'07" W
<i>Codium amplivesiculatum</i>	23 – 11	La ventana, BCS	24°03'24.5"N- 109°59'15.3"W
<i>Halimeda discoidea</i>	22 – 02	Calerita, BCS	24°21'03"N- 110°17'07" W
<i>Ulva</i> sp.	23 – 08	Calerita, BCS	24°21'03"N- 110°17'07" W
Brown seaweed			
<i>Dictyota dichotoma</i>	23 – 06	Califin, BCS	24°16'13"N- 110° 37'01"W
<i>Ecklonia arborea</i>	23 – 12	La Bocana, BCS	26°47'25.5"N- 113°42'58.1"W
<i>Egregia menziesii</i>	23 – 16	La Bocana, BC **	31°32'06.82"N- 116°39'43.99"W
<i>Macrocystis pyryfera</i>	23 – 15	La Bocana, BC **	31°32'06.82"N- 116°39'43.99"W
<i>Padina concretsens</i>	23 – 09	Califin, BCS	24°16'13"N- 110°37'01"W
<i>Sargassum horridum</i>	23 – 07	Califin, BCS	24°16'13"N- 110°37'01"W
<i>Sargassum lapazeum</i>	23 – 13	El Sauzoso, BCS	24°18'55" N, 110°38'32"W
Red seaweed			
<i>Acanthophora spicifera</i>	22 – 01	El Sauzoso, BCS	24°18' 55"N- 110°38'32"W
<i>Asparagopsis taxiformis</i>	23 – 14	La ventana, BCS	24°03'24.5"N- 109°59'15.3"W
<i>Laurencia johnstonii</i>	23 – 05	Calerita, BCS	24°21'03"N- 110°17'07"W
<i>Laurencia johnstonii</i>	23 – 10	Califin, BCS	24°16' 13"N- 110° 37'01"W

** samples collected by Baja Kelp.

2.2 Antibacterial assay by disk diffusion method.

Antibacterial activity was evaluated against five phytopathogenic strains: *Clavibacter michiganensis*, *Ralstonia solanacearum*, *Xanthomonas campestris*, *Pseudomonas syringae* & *Pseudoxanthomonas*. The bacterial strains were provided by Laboratory of Microbiology of Instituto Politécnico Nacional. All the bacteria were grown on Tryptic Soy Agar (TSA, BD Bioxon) and incubated at 35°C for 24 h.

The agar disk diffusion method was used in the antibacterial assay. Sterile paper disks (Whatman, 6 mm) were loaded with seaweed stock solution ($10 \text{ mg} \cdot \text{mL}^{-1}$) to achieve a final concentration of 2 mg per disk after several applications. Impregnated disks were placed on agar plates previously inoculated with 100 μL a bacterial strain suspension adjusted to 0.5 McFarland units ($\sim 1.5 \times 10^8 \text{ CFU} \cdot \text{mL}^{-1}$). The plates were incubated at 35°C for 24 h. After incubation, the growth inhibition zones were measured. All assays were performed in duplicate.

2.3 Antifungal activity by disk diffusion method.

The pathogenic strain *Fusarium oxysporum* was provided by the fungal collection of Phytopathology Laboratory of Autonomous University de Baja California Sur (UABCS). The fungus was cultured on potato dextrose agar (PDA, BD Bioxon) at 28°C for seven days. For the assay, the inoculum suspension was obtained with cotton swabs from spores of colonies grown on PDA and adjusted to 0.5 McFarland units. Sterile paper disks loaded with 2 mg of seaweed extract were placed on preinoculated PDA plates. The plates were incubated at 28°C for seven days. After incubation, the inhibition of fungal growth appeared around the paper disk. All assays were performed in duplicate.

2.4 Nematicidal activity against root-knot nematode *Meloidogyne incognita* by egg hatch inhibition assay.

M. incognita eggs were obtained from infected *Solanum melongena* roots. Galled roots were cut in small segments and washed with sodium hypochlorite solution (0.5%). The eggs were extracted by centrifugation-flotation method with sucrose solution (40%). The total number of eggs was counted under microscope and adjusted to approximately 200 eggs per milliliter of water. Assays were conducted in sterile 24-well tissue culture plate (Costar), each well contained 1 ml of nematode egg suspension and 1 ml of seaweed extract (10 mg·mL⁻¹). Four replicates were evaluated by extract. Distilled water was considered as control. Plates were covered and incubated at 28°C for one week. Percentage of egg hatch inhibition was assessed after seven days of incubation with Eq. (1).

$$\text{Egghatchinginhibition (\%)} = \frac{C-T}{C} * 100 \text{ Eq. (1)}$$

where C is the total number of hatched eggs in control, and T is the number of hatched eggs in the seaweed treatment. Statistical analysis of nematicidal activity was achieved using one-way ANOVA and mean comparison by Tukey with $\alpha = 0.05$ for significant differences.

2.5 Insecticidal activity against maize weevil *Sitophilus zeamais*.

The maize weevil colony of *S. zeamais* was provided by Integrated Pests Management Laboratory of UABCS. For each assay, 20 female and male adults of *S. zeamais* were placed in petri dishes covered with sterile paper disks (Whatman, 8 cm) previously impregnated with 10 mg of seaweed extract. Four replicates were evaluated for each seaweed. Distilled water and ethanol were considered as controls. Mortality was assessed after five days of incubation in darkness at 28 ± 2 °C. Statistical analysis of insecticidal activity was achieved using one-way ANOVA and mean comparison by Tukey with $\alpha = 0.05$ for significant differences.

2.6 Determination of total phenolic content on microplate.

The determination of total phenolic content (TPC) of seaweed extracts was carried out by Folin-Ciocalteu reagent according to Zhang et al., 2006 method with minor modifications. Specifically, 20 μ L of each extract was mixed with 100 μ L of Folin-Ciocalteu (2N, Sigma aldrich) followed by the addition of 80 μ L of aqueous Na₂CO₃ (7.5%). The microplates were incubated in darkness at room temperature for 2 hours. Absorbance was measured at 620 nm with a spectrophotometer reader (Multiskan FC, Thermo Scientific). For each extract four replicates were assessed. Gallic acid was used as standard reference. TPC was expressed as mg gallic acid equivalents per gram of dry extract (mg GAE/g). Statistical analysis of amount of phenolic content was achieved using one-way ANOVA and mean comparison by Tukey with $\alpha = 0.05$ for significant differences.

3. Results

3.1 Antibacterial and antifungal activity of seaweeds

Seaweeds extracts showed antibacterial activity against some phytopathogenic bacteria strains. Red seaweeds *Asparagopsis taxiformis* and *Laurencia johnstonii* showed strong activity against all strains (≥ 12 mm). *Dictyota dichotoma* was the most active extract of brown algae (≥ 6.5 mm), followed by the giant kelp *Macrocystis pyrifera*. The *Sargassum* genus, exhibited no activity within the first 24 hours of incubation. Among the green algae, only *Ulva* sp. extract showed moderate activity against two bacterial strains (8.0 mm).

Table 2
Zones of bacterial growth inhibition (mm) of seaweeds after 24 hours of incubation.

Seaweed extract	<i>Ralstonia solanacearum</i>	<i>Clavibacter michiganensis</i>	<i>Xanthomonas campestris</i>	<i>Pseudomonas syringae</i>	<i>Pseudoxanthomonas</i>
Green seaweed					
<i>Caulerpa racemosa</i>	ND	ND	ND	ND	ND
<i>Caulerpa sertularioides</i>	ND	ND	ND	ND	ND
<i>Codium amplivesiculatum</i>	ND	ND	ND	ND	ND
<i>Halimeda discoidea</i>	ND	ND	ND	ND	ND
<i>Ulva</i> sp.	ND	ND	ND	8.0	8.0
Brown seaweed					
<i>Dictyota dichotoma</i>	6.5	10	8.5	9.5	9.5
<i>Ecklonia arborea</i>	ND	ND	ND	ND	8.0
<i>Egregia menziesii</i>	ND	8.0	ND	8.0	9.5
<i>Macrocystis pyrifera</i>	8.5	8.5	ND	9.0	8.5
<i>Padina conchescens</i>	ND	ND	ND	8.0	ND
<i>Sargassum horridum</i>	ND	ND	ND	ND	ND
<i>Sargassum lapazeanum</i>	ND	ND	ND	ND	ND
Red seaweed					
<i>Acanthophora spicifera</i>	ND	ND	ND	ND	8.5
<i>Asparagopsis taxiformis</i>	14	15	15	16	19
<i>Laurencia johnstonii</i> *	14	15	14	18	16
<i>Laurencia johnstonii</i> **	13	12	13	17	19

* Sample collected in Calerita (extract code: 23 – 05), ** sample collected in Califin (extract code: 23 – 10). ND = non detected.

The most susceptible strain to macroalgae extracts was *Pseudoxanthomonas* (Fig. 1). Nine of the sixteen extracts showed inhibition. Specifically, extracts 23 – 14 of *A. taxiformis* and 23 – 10 of *L. johnstonii* showed a zone of inhibition diameter of 19 mm, followed by extract 23 – 05 of *L. johnstonii* with a diameter of 16 mm. *L. johnstonii* extract 23 – 05 from a different locality had slightly lower activity against all strains tested. This suggests a strong correlation between chemical composition and biological activity.

Only nine extracts showed antifungal activity against *Fusarium oxysporum* (Table 3). Significant inhibition was observed in all extracts from red seaweeds and the brown algae *Dictyota dichotoma*. Our results suggest that red and brown seaweeds have the potential to be a source of compounds with antimicrobial properties against plant pathogens.

Table 3
Antifungal activity of seaweed extracts against *Fusarium oxysporum* after seven days of incubation.

Seaweed extract	<i>Fusarium oxysporum</i>
Green seaweed	
<i>Caulerpa racemosa</i>	+
<i>Caulerpa sertularioides</i>	ND
<i>Codium amplivesiculatum</i>	ND
<i>Halimeda discoidea</i>	+
<i>Ulva sp.</i>	ND
Brown seaweed	
<i>Dictyota dichotoma</i>	++
<i>Ecklonia arborea</i>	ND
<i>Egregia menziesii</i>	+
<i>Macrocystis pyrifera</i>	+
<i>Padina concrescens</i>	ND
<i>Sargassum horridum</i>	ND
<i>Sargassum lapazeanum</i>	ND
Red seaweed	
<i>Acanthophora spicifera</i>	+
<i>Asparagopsis taxiformis</i>	++
<i>Laurencia johnstonii</i> *	++
<i>Laurencia johnstonii</i> **	++

* extract number 23 – 05, sample collected in Calerita, ** extract number 23 – 10, sample collected in Calfin. ND = non detected, + = moderate activity, ++ = strong activity.

3.2 Insecticidal activity of seaweed extracts

Mortality of the maize weevil *Sitophilus zeamais* was assessed after five days of exposure. All the seaweed extracts showed insecticidal activity against adult *S. zeamais* (Table 4). The highest insecticidal activity was observed in *Laurencia johnstonii* collected at Calerita (72%), followed by *L. johnstonii* collected at a different location (52%). *Caulerpa racemosa* and *Asparagopsis taxiformis* also exhibited strong activity (44% and 40% respectively). The rest of the extracts had moderate activity.

Table 4
Insecticidal activity of seaweeds.

Seaweed extract	Mortality on <i>Sitophilus zeamais</i> (% of control)
Green seaweed	
<i>Caulerpa racemosa</i>	44 ± 5.5 bc
<i>Caulerpa sertularioides</i>	22 ± 5.1 f
<i>Codium amplivesiculatum</i>	38 ± 3.1 bcde
<i>Halimeda discoidea</i>	36 ± 6.3 bcdef
<i>Ulva</i> sp.	25 ± 6.0 ef
Brown seaweed	
<i>Dictyota dichotoma</i>	29 ± 5.3 def
<i>Ecklonia arborea</i>	29 ± 6.5 def
<i>Egregia menziesii</i>	36 ± 5.4 bcdef
<i>Macrocystis pyrifera</i>	33 ± 5.8 cdef
<i>Padina concrescens</i>	36 ± 5.8 bcdef
<i>Sargassum horridum</i>	35 ± 7.0 cdef
<i>Sargassum lapazeanum</i>	22 ± 6.6 f
Red seaweed	
<i>Acanthophora spicifera</i>	34 ± 4.6 cdef
<i>Asparagopsis taxiformis</i>	40 ± 5.7 bcd
<i>Laurencia johnstonii</i> *	72 ± 5.8 a
<i>Laurencia johnstonii</i> **	52 ± 6.7 b
* Sample collected in Calerita (extract code: 23 – 05), ** sample collected in Calfin (extract code: 23 – 10). Values represent mean ± standard deviation. Different letters represent statistical difference (p ≤ 0.05, n = 4)	

The inhibition of egg hatching of *Meloidogyne incognita* was assessed after seven days (Fig. 2). The brown seaweed *Padina concrescens* exhibited the highest percentage of inhibition (59%) followed by the two extracts of *Laurencia johnstonii* (48% and 42%) and *Sargassum horridum* (43%). *Asparagopsis taxiformis*, *Sargassum lapazeanum* and

Ecklonia arborea showed moderate nematocidal activity (28%, 25% and 22%, respectively). No activity was observed in *Dictyota dichotoma* extract.

3.3 Total phenolic contents

The total phenolic content (TPC) of the seaweed extracts ranged from 1.81 to 32.5 mg of gallic acid equivalents per gram of extract (mg GAE /g) (Table 5). Among all the seaweeds the extract of the green algae *C. amplivesiculatum* had the highest TPC (32.5 ± 0.56 mg GAE /g) followed by the red seaweeds *A. taxiformis* (22.8 ± 2.08 mg GAE /g) and *L. johnstonii* ** (19.1 ± 1.44 mg GAE /g), and the brown algae, *E. arborea* (20.6 ± 2.48 mg GAE /g). However, red and brown seaweeds tested in this experiment showed, in average, higher amounts of TPC.

Table 5
Total phenolic content (TPC) of seaweed extracts.

Seaweed extract	TPC (mg GAE/g)
Green seaweed	
<i>Caulerpa racemosa</i>	5.05 ± 0.41 gh
<i>Caulerpa sertularioides</i>	6.04 ± 0.22 fg
<i>Codium amplivesiculatum</i>	32.5 ± 0.56 a
<i>Halimeda discoidea</i>	2.72 ± 0.39 gh
<i>Ulva</i> sp.	1.81 ± 0.59 h
Brown seaweed	
<i>Dictyota dichotoma</i>	4.89 ± 0.30 gh
<i>Ecklonia arborea</i>	20.6 ± 2.48 b
<i>Egregia menziesii</i>	8.85 ± 2.11 ef
<i>Macrocystis pyrifera</i>	6.36 ± 2.23 fg
<i>Padina conchrescens</i>	15.5 ± 1.71 cd
<i>Sargassum horridum</i>	3.72 ± 0.41 gh
<i>Sargassum lapazeanum</i>	16.9 ± 1.81 c
Red seaweed	
<i>Acanthophora spicifera</i>	2.13 ± 0.99 h
<i>Asparagopsis taxiformis</i>	22.8 ± 2.08 b
<i>Laurencia johnstonii</i> *	12.0 ± 2.00 de
<i>Laurencia johnstonii</i> **	19.1 ± 1.44 bc
*extract number 23 – 05 sample collected in Calerita, ** extract number 23 – 10 sample collected in Calfin. Values represent mean $\pm$ standard deviation. Different letters represent statistical difference ($p \leq 0.05$, $n = 4$)	

4. Discussion

Seaweeds have potential for pest control. In particular, *Laurencia johnstonii* and *Asparagopsis taxiformis* showed the highest antibacterial and antifungal activities against all phytopathogenic strains tested. Our study agrees with earlier studies indicating that red and brown algae exhibited higher antimicrobial activity as compared to green algae (Lakhdar et al., 2015). These is related to the presence of phenolic compounds (Negara et al., 2021) and possibly to halogenated terpenoids in red species as well (Kasanah et al., 2015).

Red algae are primary producers of active halogenated terpenes with antibacterial properties. For example, the sesquiterpene elatol is one of the major compounds found in *Laurencia* species and has shown multiple activities against several human pathogenic bacteria (Kasanah et al., 2015). Previous studies have also investigated the antibacterial activity of *Asparagopsis* sp. against human and aquaculture pathogens, and GC/MS analysis revealed that the active fraction was a mixture of fatty acids and volatile compounds (Manilal et al., 2009, Genovese et al., 2012).

All the seaweed extracts at a concentration of $10 \text{ mg} \cdot \text{mL}^{-1}$ exhibited insecticidal activity against the maize weevil *Sitophilus zeamais*. *L. johnstonii* showed the highest insecticidal activity. However, notable differences in bioactivity were identified between sample collection sites. *L. johnstonii* from Calerita showed higher insecticidal activity (71.9%) than *L. johnstonii* collected from Califin (51.5%). Salvador-Neto et al., (2016) found differences in the larvicidal activity of extract of *Laurencia dendroidea* from two collection sites. Although GC/MS analysis revealed the same major compounds, the differences in bioactivity may be attributed to a synergistic effect between the compounds produced by the macroalgae. The variation type and amount metabolites produced by the same seaweed at two different locations is attributed to the environmental conditions present at each place (Gaubert et al., 2019). This could be the reason why the two *L. johnstonii* collected in this investigation showed different bioactivities. On the other hand, the insecticidal activity of *Laurencia* sp. Has also been assessed against termites and mosquitoes. The main constituents of the extracts are brominated sesquiterpenes such as laurinterol (González-Castro et al., 2024; Ishii et al., 2017), obtusol (Salvador-Neto et al., 2016) and elatol (Bianco et al., 2013). Therefore, the presence of multiple halogenated terpenes in the ethanolic extracts of *Laurencia johnstonii* may be responsible for their biological activities. However, the isolation and identification of the active compounds require further research.

Regarding nematicidal activity, the brown seaweed *Padina concrescens* showed the highest egg hatching inhibition of *M. incognita* (59%), followed by red seaweed *Laurencia johnstonii* (48%) and *Sargassum horridum* (43%). Previous experiments have shown that aqueous and methanolic extracts of brown and red seaweeds are more effective than those of green macroalgae on nematode egg hatching inhibition and nematicidal activity (Khan et al., 2015; Veronico & Melillo, 2021). Even though the seaweeds studied, in the investigation mentioned before, were collected in very different location compared to the seaweeds collected for this experiment, our results indicate the same tendency. Also, another investigation demonstrated that *Laurencia nidifica* aqueous extract significantly reduced hatchability; however, the aqueous extract was used directly at a concentration of 5–15%, without knowing the amounts of solutes present in it (El-Deen & Issa, 2016). Interestingly, some seaweeds showed negative percentage of egg hatching inhibition which means that these extracts promoted hatching rather than inhibiting. It is well known that some metabolites act as nematode egg hatching stimulants, these metabolites are normally present in root exudates and play a crucial role during nematode infestation (Sikder & Vestergård, 2020). Therefore, some metabolites produced by seaweeds could act as analogues of this metabolites known as hatching factors.

In general, the total phenolic content of red and brown seaweeds algal division was identified in this study, even though Tenorio-Rodriguez et al. (2017) reported that brown seaweeds shown the highest amount of phenolic compounds, followed by red algae and, at last, green algae. Previous studies have reported that a *A. taxiformis*

extract contains 85.8 ± 4.5 mg GAE/g. The extract was obtained with a mixture of methanol/water, which provides a higher yield of phenolic compounds than ethanol solvent. Furthermore, the dried seaweed was collected in a locality on Tunisia (Ktari et al., 2022). In a separate study, a total phenolic content of 1.71 ± 0.13 mg GAE /g was reported from a dried seaweed collected on Madeira Island off the African coast (Nunes et al., 2018). Several studies support that phenolic content of crude extracts shows a spatial variability. Thus, the differences observed in our results can be attributed to the geographical location (Tanniou et al., 2013).

5. Conclusions

Food security is a major concern in many countries worldwide. Therefore, the control of pathogens is essential to maintain crop production and food availability. Seaweeds provide a safer alternative for the control of agricultural pests. In particular, red seaweeds *Asparagopsis taxiformis* and *Laurencia johnstonii* have shown great potential for developing biopesticides. However, further research is necessary to determine their chemical composition. Writing – original draft

Declarations

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Competing interest

The authors declare that they have no competing interests or personal relationships that could have appeared to influence the work reported in this paper.

Availability of data and material

The data generated during and/or analyzed during the current study are available from the corresponding author upon request.

Code availability

Not applicable

Ethical Approval

Not applicable

Authors contribution

Pablo Misael Arce-Amezquita: Writing – review & editing, Validation, Supervision, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization, Project Administration. **Ana Laura González-Castro:** Writing – original draft, Visualization, Validation, Methodology, Investigation, Data curation, Conceptualization.

Maurilia Rojas-Contreras: Methodology, Investigation, Resources. **Mirella Romero-Bastidas:** Methodology, Investigation, Resources. **Ruth Noemí Águila-Ramírez:** Methodology, Investigation, Resources. **Carlos Rangel-Dávalos:** Methodology, Investigation, Resources.

References

9. References

1. Ali, O., Ramsubhag, A., & Jayaraman, J. (2021). Biostimulant properties of seaweed extracts in plants: Implications towards sustainable crop production. *Plants*, *10*(3), 531. <https://doi.org/10.3390/plants10030531>
2. Arrahman, A., Mirsam, H., Djaenuddin, N., Pakki, S., Saenong, M. S., & Sebayang, A. (2022). An in-depth study on *Sitophilus zeamais* Motsch (Coleoptera: Curculionidae) pests on corn plants. In *IOP Conference Series: Earth and Environmental Science* (Vol. 1107, No. 1, p. 012060). IOP Publishing.
3. Bianco EM, Pires L, Santos GKN, Dutra KA, Reis TNV, Vasconcelos E, Cocentino A, Navarro D (2013) Larvicidal activity of seaweeds from northeastern Brazil and of a halogenated sesquiterpene against the dengue mosquito (*Aedes aegypti*). *Ind Crops Prod* 43:270–275.
4. Deolu-Ajayi, A. O., van der Meer, I. M., Van der Werf, A., & Karlova, R. (2022). The power of seaweeds as plant biostimulants to boost crop production under abiotic stress. *Plant, Cell & Environment*, *45*(9), 2537–2553.
5. Devi, P.I., Manjula, M. & Bhavani, R. V. (2022). Agrochemicals, Environment, and Human Health. *Annual Review of Environment and Resources*, *47*(1):399–421.
6. El-Deen, A. H. N., & Issa, A. A. (2016). Nematicidal properties of some algal aqueous extracts against root-knot nematode, *Meloidogyne incognita* in vitro. *Egypt J Agron*, *15*(1), 67–78.
7. Food and Agriculture Organization of the United Nations. (2019). New standards to curb the global spread of plant pests and diseases.
8. Gaubert, J., Payri, C.E., Vieira, C., Solanki, H. & Thomas, O.P. (2019) High metabolic variation for seaweeds in response to environmental changes: a case study of the brown algae *Lobophora* in coral reefs. *Sci Rep* 9, 993.
9. Genovese, G., Faggio, C., Gugliandolo, C., Torre, A., Spano, A., Morabito, M., & Maugeri, T. L. (2012). In vitro evaluation of antibacterial activity of *Asparagopsis taxiformis* from the Straits of Messina against pathogens relevant in aquaculture. *Marine environmental research*, *73*, 1–6.
10. González-Castro AL, Torres-Estrada JL, Muñoz-Ochoa M. (2024) Larvicidal and oviposition deterrent activity of sesquiterpenes from the red seaweed *Laurencia johnstonii* against *Aedes aegypti*. *J Appl Phycol*, 1–6.
11. Gustavsson, J., Cederberg, C., Sonesson, U., Van Otterdijk, R., & Meybeck, A. (2011). Global food losses and food waste. Rome: Food and Agriculture Organization of the United Nations (FAO).
12. Ishii T, Nagamine T, Ngunyen BCQ, Tawata S (2017) Insecticidal and repellent activities of laurinterol from the Okinawan red alga *Laurencia nidifica*. *Rec Nat Prod* 11:63–68.
13. Jiménez, E., Dorta, F., Medina, C., Ramírez, A., Ramírez, I., & Peña-Cortés, H. (2011). Anti-phytopathogenic activities of macro-algae extracts. *Marine drugs*, *9*(5), 739–756. <https://doi.org/10.3390/md9050739>
14. Kasanah, N., Triyanto, T., Seto, D. S., Amelia, W., & Isnansetyo, A. (2015). Antibacterial compounds from red seaweeds (Rhodophyta). *Indones J of Chem*, *15*(2), 201–209.
15. Khan, S. A., Abid, M., & Hussain, F. (2015). Nematicidal activity of seaweeds against *Meloidogyne javanica*. *Pakistan Journal of Nematology*, *33*(2), 195–203.
16. Ktari, L., Ismail, A., Selmi, H. *et al.* (2022). Biological potential of the alien red alga *Asparagopsis taxiformis* and characterization of its culturable associated bacteria. *J Appl Phycol* *34*, 2769–2782.

17. Kwon-Ndung, E.H., Terna, T.P., Goler, E.E., Obande, G. (2022). Post-harvest assessment of infectious fruit rot on selected fruits in Lafia, Nasarawa State Nigeria. *Journal of Plant Science and Phytopathology*, 6:154–160.
18. Lakhdar, F., Boujaber, N., Oumaskour K., Assobhei, O., & Etahiri, S. (2015). Inhibitive activity of 17 marine algae from the coast of El Jadida-Morocco against *Erwinia chrysanthemi*. *International Journal of Pharmacy and Pharmaceutical Sciences*, 7(10),376–380.
19. Manilal, A., Sujith, S., Kiran, G. S., Selvin, J., Shakir, C., Gandhimathi, R., & Lipton, A. P. (2009). Antimicrobial potential and seasonality of red algae collected from the southwest coast of India tested against shrimp, human and phytopathogens. *Annals of Microbiology*, 59, 207–219.
20. Mendoza-de Gives, P. (2022). Soil-Borne nematodes: Impact in agriculture and livestock and sustainable strategies of prevention and control with special reference to the use of nematode natural enemies. *Pathogens*, 11(6), 640.
21. Nazarov P. A., Baleev, D. N., Ivanova, M. I., Sokolova, L. M., & Karakozova, M. V. (2020). Infectious Plant Diseases: Etiology, Current Status, Problems and Prospects in Plant Protection. *Acta Naturae*, 12(3):46–59. <https://doi.org/10.32607%2Factanaturae.11026>
22. Negara, B. F. S. P., Sohn, J. H., Kim, J. S., & Choi, J. S. (2021). Antifungal and larvicidal activities of phlorotannins from brown seaweeds. *Marine Drugs*, 19(4), 223.
23. Nunes, N., Valente, S., Ferraz, S., Barreto, M. C., & De Carvalho, M. P. (2018). Nutraceutical potential of *Asparagopsis taxiformis* (Delile) Trevisan extracts and assessment of a downstream purification strategy. *Heliyon*, 4(11).
24. Odjo, S.; Bongianino, N.; González Regalado, J.; Cabrera Soto, M.L.; Palacios-Rojas, N.; Burgueño, J.; Verhulst, N. (2022). Effect of Storage Technologies on Postharvest Insect Pest Control and Seed Germination in Mexican Maize Landraces. *Insects*, 13,878.
25. Pan, S., Jeevanandam, J., & Danquah, M. K. (2019). Benefits of algal extracts in sustainable agriculture. En Hallmann, A., Rampelotto, P. (Eds.). *Grand Challenges in Algae Biotechnology* (pp.501–534). Springer, Cham. https://doi.org/10.1007/978-3-030-25233-5_14
26. Parab, A., & Shankhadarwar, S. (2022). Growth enhancement of agricultural crops using seaweed liquid fertilizer. *Plant Science Today*, 9(2), 322–330.
27. Salvador-Neto O, Azevedo-Gomes S, Ribeiro-Soares A, Silva-Machado FL, Samuels RI, Nunes na Fonseca R, Souza-Menezes J, Cunha-Moares JL, Campos E, Borges-Mury F, Silva JR (2016) Larvicidal potential of the halogenated sesquiterpene (+)-obtusol, isolated from the alga *Laurencia dendroidea* J. Agardh (Ceramiales: Rhodomelaceae), against the dengue vector mosquito *Aedes aegypti* (Linnaeus) (Diptera:Culicidae). *Mar Drugs* 14:1–14.
28. Shukla, P. S., Borza, T., Critchley, A. T., Prithiviraj, B. (2021). Seaweed-Based Compounds and Products as Biostimulants for Sustainable Protection against Plant Pathogens. *Marine Drugs*, 19(59):1–32. <https://doi.org/10.3390/md19020059>
29. Sikder, M. M., & Vestergård, M. (2020). Impacts of root metabolites on soil nematodes. *Frontiers in plant science*, 10, 1792.
30. Sikandar, A., Zhang, M.Y., Wang, Y.Y., Zhu, X.F., Liu, X.Y., Fan, H.Y., Xuan, Y.H., Chen, L.J & Duan, Y. X. (2020). *Meloidogyne incognita* (root-knot nematode) a risk to agriculture. *Applied Ecology & Environmental Research*, 18(1).
31. Tanniou, A., Vandanjon, L., Incera, M. Serrano-Leon, E., Husa, V., Le Grans, J... Stiger-Pouvreau, V. (2013). Assessment of the spatial variability of phenolic contents and associated bioactivities in the invasive alga

Sargassum muticum sampled along its European range from Norway to Portugal. J Appl Phycol 26, 1215–1230. <https://doi.org/10.1007/s10811-013-0198-x>

32. Tenorio-Rodriguez, P.A., Murillo-Álvarez, J.I., Campa-Cordova, Á.I. Angulo, C. (2017). Antioxidant screening and phenolic content of ethanol extracts of selected Baja California Peninsula macroalgae. J Food Sci Technol 54, 422–429. <https://doi.org/10.1007/s13197-016-2478-3>
33. Tewari, S. & Sharma, S. (2019). Molecular Techniques for Diagnosis of Bacterial Plant Pathogens. En D. Surajit & R. D. Hirak (Eds.). Microbial Diversity in the Genomic Era (pp. 481–497). Academic Press. <https://doi.org/10.1016/B978-0-12-814849-5.00027-7>
34. Veronico, P., & Melillo, M. T. (2021). Marine organisms for the sustainable management of plant parasitic nematodes. Plants, 10(2), 369.
35. Vicente, T. F. L., Lemos, M.F. L., Félix, R., Valentão, P. & Félix, C. (2021). Marine Macroalgae, a Source of Natural Inhibitors of Fungal Phytopathogens. Journal of Fungi, 7(1006):1–25. <https://doi.org/10.3390/jof7121006>
36. Vicente, T. F. L.; Félix, C., Félix, R., Valentão, P., & Lemos, M. F. L. (2023). Seaweed as a Natural Source against Phytopathogenic Bacteria. Marine Drugs, 21(1):23. <https://doi.org/10.3390/md21010023>
37. Zhang, Q., Zhang, J., Shen, J., Silva, A., Dennis, D.A., & Barrow, C.J. (2006). A simple 96-well microplate method for estimation of total polyphenol content in seaweeds. Journal of applied phycology, 18(3–5), 445–450.

Figures

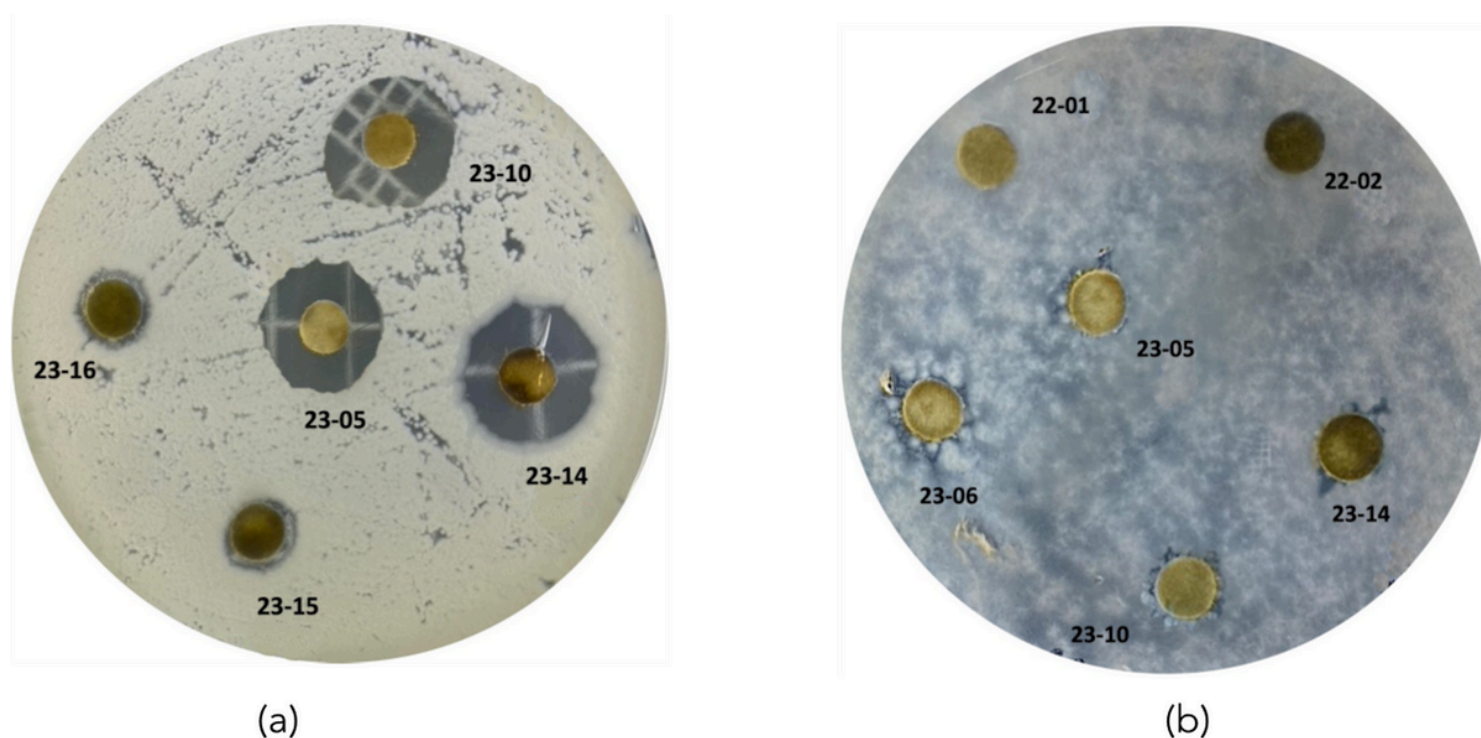


Figure 1

Antimicrobial activity of seaweed extracts on agar plates. (a) Antibacterial activity against *Pseudoxanthomonas* after 24 h of incubation. *L. johnstonii* (extract 23-10 & 23-05), *A. taxiformis* (extract 23-14), *M. pyrifera* (extract 23-15), *E. menziesii* (extract 23-16); (b) Antifungal activity against *F. oxysporum* after 7 days of incubation. *A. spicifera* (extract

22-01), *H. discoidea* (extract 22-02), *A. taxiformis* (extract 23-14), *L. johnstonii* (extract 23-10 & 23-05), *D. dichotoma* (extract 23-06).

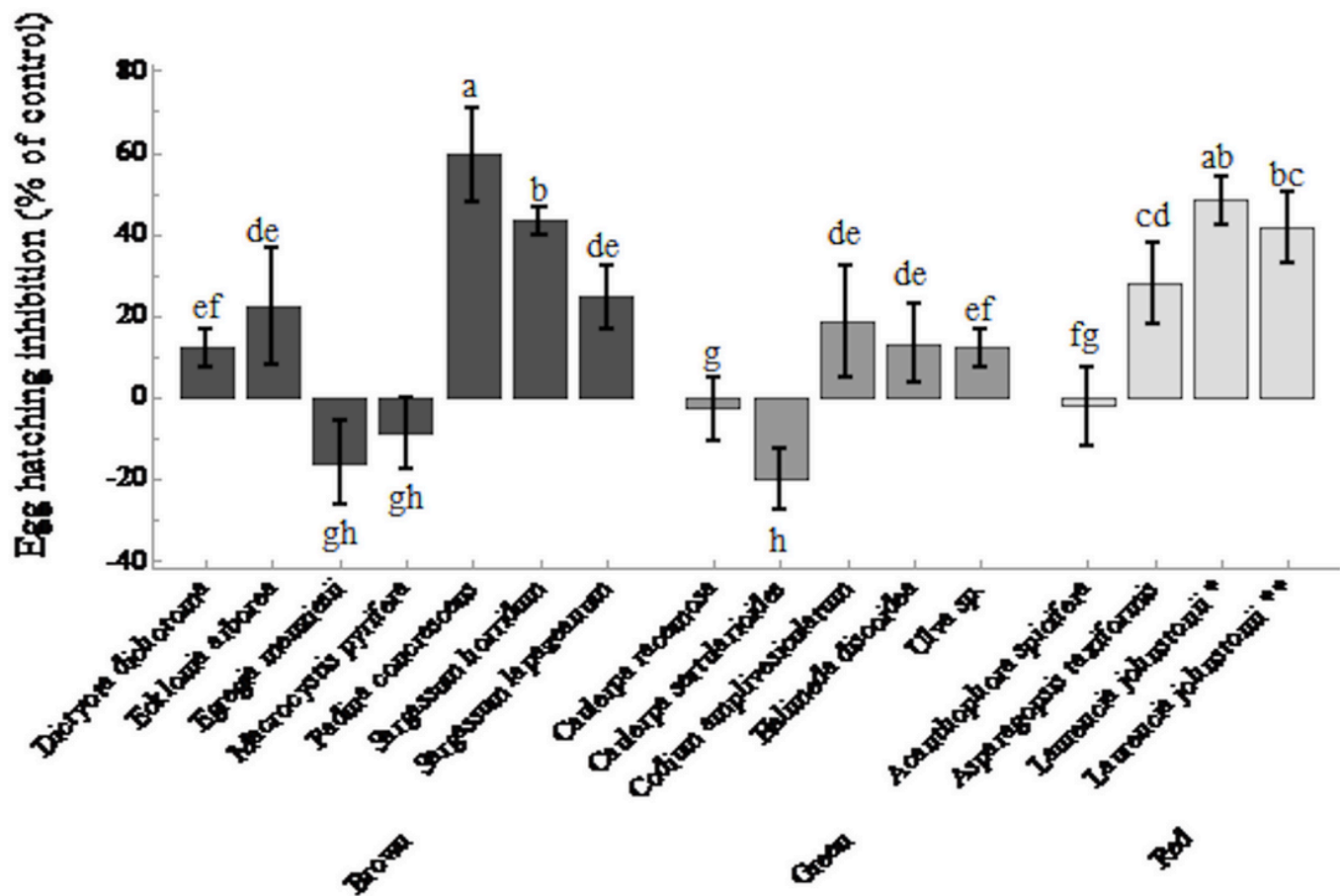


Figure 2

Nematicidal activity in terms of egg hatching inhibition of *Meloidogyne incognita* at day seven. * Sample collected in Calerita (extract code: 23-05), ** sample collected in Califin (extract code: 23-10). Values represent mean ± standard deviation. Different letters represent statistical difference ($p \leq 0.05$, $n = 4$)