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Evaluation of some seaweed extracts from Baja Peninsula, Mexico, against plant pathogens.

Evaluación de extractos de algunas algas marinas de la península de Baja California, México, contra patógenos en plantas.

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ABSTRACT

Background. The widespread use of synthetic pesticides to control pests has generated serious consequences on the environment and human health. Currently, efforts focused on searching for new pesticides with less environmental impact have been doubled. Marine algae synthesize chemical compounds with biological activity, antibacterial and antifungal, and recent studies on brown seaweeds have reported activity against some agricultural pests, insects, and nematodes. However, marine pesticides are an underdeveloped alternative. This represents an opportunity to explore new sources of active compounds. **Goals.** Evaluate the antibacterial, antifungal, nematicidal, and insecticidal activity of seaweed extracts against pathogens of agricultural importance. **Methods.** 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. The nematicidal activity was assessed by egg hatching inhibition on *Meloidogyne incognita* and insecticidal activity against maize weevil *Sitophilus zeamais.* Additionally, the total phenolic content of the seaweed extracts was assessed. **Results.** Regarding the antibacterial potential, *Laurencia johnstonii*, *Asparagopsis taxiformis,* and *Dictyota dichotoma* showed the highest inhibition against all the phytopathogenic strains and the fungus *F. oxysporum*. Regarding egg hatching inhibition against nematode *M. incognita*, the extract of *Padina concrescens* exhibited the highest percentage of inhibition (59 %) followed by *L. johnstonii* (48 %). All the seaweeds cause the mortality of the insect *S. zeamais*. However, the highest insecticidal activity was identified on *L. johnstonii* (71.9%). In general, red and brown seaweeds showed a higher content of total phenolic compounds. **Conclusions.** This study showed that species of red and brown seaweeds evaluated have a great potential for controlling the phytopathogens evaluated. However, further research is necessary to identify the active compounds and established lethal doses.

Keywords: antibacterial, ethanolic extracts, insecticide, *Laurencia johnstonii*, nematicidal.

RESUMEN

Antecedentes. El uso indiscriminado de plaguicidas sintéticos para el control de plagas ha generado consecuencias graves en el ambiente y en la slaud humana. Recientemente, se han duplicado los esfuerzos enfocados en la búsqueda de nuevos pesticidas con menor impacto ambiental. Las algas marinas sintetizan compuestos activos con actividad biológica: antibacteriana y antifúngica. Estudios recientes con algas pardas han identficiado actividad contra algunas plagas agrícolas: insectos y nematodos. Sin embargo, los plaguicidas marinos aún son un alternativa poco desarrollada. Esto representa una oportunidad para explorar nuevas fuentes de compuestos activos. **Objetivos.** Evaluar la actividad antibacteriana, antifúngica, nematicida e insecticida de extractos de algas marinas contra patógenos de importencia agrícola. **Métodos.** Se recolectaron algas marinas de distintas localidades en la Peninsula de Baja California, México. Se obtuvieron extractos etanólicos. La actividad antibacteriana contra cinco cepas fitopatógenas y la antifúngica para *Fusarium oxysporum* se evaluaron por el método de difusión en agar. La actividad nematicida se evaluó por la inhibición de eclosión de huevos del nemátodo *Meloidogyne incognita* y la actividad insecticida contra el gorgojo del maíz *Sitophilus zeamais.* Adicionalmente, se cuantifico el contenido fenólico de los extractos.

Resultados. *Laurencia johnstonii*, *Asparagopsis taxiformis* y *Dictyota dichotoma* mostraron la mayor inhibición contra todas las cepas fitopatógenas y contra el hongo *F. oxysporum*. En el ensayo de inhibición de la eclosión contra el nematodo *M. incognita* el extracto de *Padina concrescens* mostró la mayor inhibicón (59%), seguido del de *L. johnstonii* (48 %). Todos los extractos evaluados causaron mortalidad al insecto *S. zeamais*. Sin embargo, la mayor actividad insecticida fue identificada en *L. johnstonii* (71.9%). Las algas rojas y pardas mostraron el mayor contenido de compuestos fenólicos. **Conclusiones.** Este estudio suguiere que las especies de algas rojas y pardas evaluadas tienen potencial para el control de los fitopatógenos evaluados. Sin embargo, es necesario continuar con las investigaciones para identificar los compuestos activos y establecer dosis letales.

Palabras clave: antibacteriano, extractos etanólicos, insecticida, *Laurencia johnstonii*, nematicida.

INTRODUCTION

The precise quantification of global food losses and waste remains a significant challenge due to the absence of harmonized global estimates, as well as the lack of recent data (Gatto & Chepeliev, 2024). The most recent estimates indicate that pests cause more than 40% of the annual loss in economically important crop production and 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 (Sarker *et al.*, 2024; Gustavsson *et al.*, 2011).

All these factors have a significant impact on food security, including food availability, economic and physical access, and therefore the use. 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 these organisms' 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* (Motschulsky & V.de, 1855), can cause significant damage to the quantity and quality of stored cereals. This has huge economic implications as the cereals are ready for consumption and have 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). Alternative methods have been explored to improve food production, and to ensure 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 antifungal (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, resulting 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, 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, this work aims to evaluate the antibacterial, antifungal, nematicidal and insecticidal activity of seaweed extracts found in Baja California peninsula, Mexico.

MATERIALS AND METHODS

Seaweed recollection and extract preparation. Sixteen seaweed species were collected during low tide from different localities in México (table 1, fig. 1). In particular, the red seaweed *Laurencia johnstonii* was collected from two distinct localities: Calerita and Califin (table 1). Taxonomic identification was assessed by morphological characters (Abbott & Hollenberg, 1976) and confirmed by a taxonomist. The fresh material was washed with fresh water, air-dried and ground to 40 mesh size. The dried seaweed was treated after maceration with ethanol and in a rotary evaporator (Buchi II) at less than 40ºC, under reduced pressure, the extract was concentrated. The extracts were stored at 4ºC until further use.

Antibacterial assay by disk diffusion method. Antibacterial activity was evaluated against five phytopathogenic strains: *Clavibacter michiganensis* (Smith, 1910) Davis *et al.*, 1984, *Ralstonia solanacearum* (Smith, 1896) Yabuuchi *et al.,* 1996, *Xanthomonas campestris* (Pammel, 1885) Dowson, 1939, *Pseudomonas syringae* van Hall, 1902, *Pseudoxanthomonas sp*. Finkmann *et al.*, 2000.The Laboratory of Microbiology of the Instituto Politécnico Nacional provided the bacterial strains. All the bacteria were grown on Tryptic Soy Agar (TSA, BD Bioxon) and incubated at 35ºC for 24 h.

The agar disk diffusion method was used in the antibacterial assay. Sterile paper disks (Whatman, 6 mm) were loaded with seaweed extract stock solution (10 mg•mL^{-1}) to achieve a final concentration of 2 mg per disk. Impregnated disks were placed on agar plates previously inoculated with 100 **µ**L a bacterial strain suspension adjusted to 0.5 McFarland units (~1.5x10^{8} CFU•mL^{-1}). The plates were incubated at 35ºC for 24 h. The growth inhibition zones were measured. All assays were performed in triplicate.

Antifungal activity by disk diffusion method. The pathogenic strain *Fusarium oxysporum* Schlechtendal was provided by the fungal collection of Phytopathology Laboratory at the Universidad Autónoma de Baja California Sur (UABCS). The fungus was cultured on potato dextrose agar (PDA, BD Bioxon) at 28ºC 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 pre-inoculated PDA plates. The plates were incubated at 28ºC for seven days. The inhibition of fungal growth appeared around the paper disk. All assays were performed in triplicate.

Nematicidal activity against root-knot nematode *Meloidogyne incognita* by egg hatch inhibition assay. *M. incognita* eggs were obtained from infected *Solanum melongena* Linnaeus roots. Galled roots were cut into small segments and washed with sodium hypochlorite solution (0.5%). The eggs were extracted using the centrifugation-flotation method with a sucrose solution (40%). The total number of eggs was counted under a microscope and adjusted to approximately 200 eggs per mL of water. Assays were conducted in a 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 a control. Plates were covered and incubated at 28**ºC for one week.** The percentage of egg hatch inhibition was assessed after seven days of incubation with Eq. (1).

$$\text{Egg hatching inhibition (\%)} = \frac{(C-T)}{C} * 100 \qquad \text{Eq. (1)}$$

C is the total number of hatched eggs in control, and T is the number of hatched eggs in the seaweed treatment.

Insecticidal activity against maize weevil *Sitophilus zeamais*. The maize weevil colony of *S. zeamais* was provided by the 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 used as controls. Mortality was assessed after five days of incubation in darkness at 28 ± 2 ºC.

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

Seaweed	Extract code	Locality	Latitude, Longitude
Green seaweed			
Caulerpa racemosa (Forsskål) J. Agardh	22-04	Calerita, BCS	24°21'03"N- 110°17'07"W
Caulerpa sertularioides (S. G. Gmelin) M. Howe	22-03	Calerita, BCS	24°21'03"N- 110°17'07"W
Codium amplivesiculatum Setchell & N. L. Gardner	23-11	La ventana, BCS	24°03'24.5"N- 109°59'15.3"W
Halimeda discoidea Decaisne	22-02	Calerita, BCS	24°21'03"N- 110°17'07"W
Ulva sp.	23-08	Calerita, BCS	24°21'03"N- 110°17'07"W
Brown seaweed			
Dictyota dichotoma (Hudson) J. V. Lamouroux	23-06	Califin, BCS	24º16'13"N- 110º 37'01" W
Eisenia arborea Areschoug	23-12	La Bocana, BCS	26°47'25.5"N- 113°42'58.1"W
Egregia menziesii (Turner) Areschoug	23-16	La Bocana, BC	31º32'06.8"N- 116º39'43.9"W
Macrocystis pyrifera (Linnaeus) C. Agardh	23-15	La Bocana, BC	31º32'06.8"N- 116º39'43.9"W
Padina concrescens Thivy	*2*3-09	Califin, BCS	24º16'13"N- 110º37'01"W
Sargassum horridum Setchell & N. L. Gardner	23-07	Califin, BCS	24º16'13"N- 110º37'01"W
Sargassum lapazeanum Setchell & N. L. Gardner	23-13	El Sauzoso, BCS	24º18'55" N- 110º38'32"W
Red seaweed			
Acanthophora spicifera (M. Vahl) Børgesen	22-01	El Sauzoso, BCS	24º18'55"N- 110º38'32"W
Asparagopsis taxiformis (Delile) Trevisan	23-14	La ventana, BCS	24°03'24.5"N- 109°59'15.3"W
Laurencia johnstonii * Setchell & N. L. Gardner	23-05	Calerita, BCS	24°21'03"N- 110°17'07"W
Laurencia johnstonii **	23-10	Califin, BCS	24º16' 13"N- 110º 37'01"W

* Sample collected in Calerita, ** sample collected in Califin.

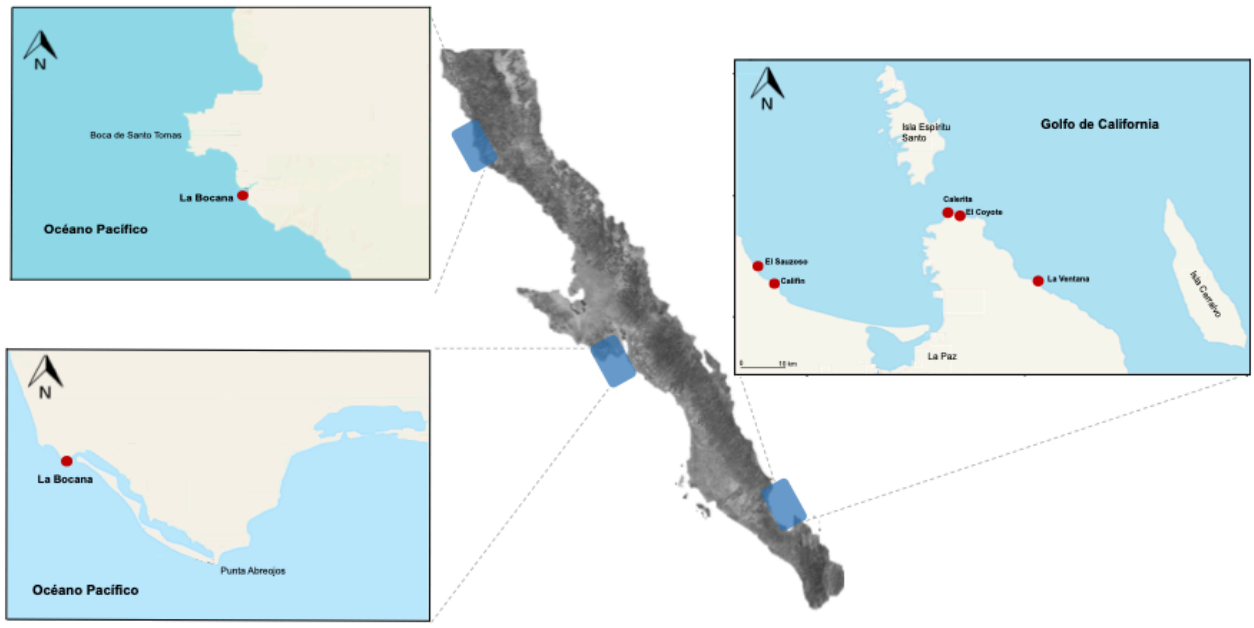

Figure 1. Seaweeds collection sites of Baja California Península.

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 µL of each extract was mixed with 100 µL of Folin-Ciocalteu (2N, Sigma aldrich) followed by the addition of 80 µL of aqueous Na_2CO_3 (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.

Prior to the statistical analyses, all data were tested for normality (Anderson-Darling) and homogeneity of variance (Barlett). No transformations were necessary. One-way ANOVA and mean comparison by Tukey ($\alpha = 0.05$) were performed for nematicidal activity, insecticidal activity and total phenolic content of the seaweed extracts.

RESULTS

Antibacterial and antifungal activity of seaweeds. Seaweeds extracts showed antibacterial activity against some phytopathogenic bacteria strains (table 2). 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).

The most susceptible strain to macroalgae extracts was *Pseudoxanthomonas*. 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 relationship 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 evaluated in this study have the potential to be a source of compounds with antimicrobial properties against plant pathogens.

Insecticidal and nematicidal 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 higher insecticidal activity was observed with the extract from *Laurencia johnstonii* collected at Calerita (72%), followed by *L. jhonstonii* collected at a different location (52%). *Caulerpa racemosa* and *Asparagopsis taxiformis* also exhibited significant activity (44% and 40% respectively). The rest of the extracts had moderate activity.

The inhibition of egg hatching of *Meloidogyne incognita* was assessed after seven days (fig. 2). The brown seaweed *Padina concrescens* exhibited a higher percentage of inhibition (59 %) followed by the two extracts from *Laurencia johnstonii* (48 % and 42 %) and *Sargassum horridum* (43 %). *Asparagopsis taxiformis, Sargassum lapazeanum* and *Eisenia arborea* showed moderate nematicidal activity (28 %, 25 % and 22 %, respectively). No activity was observed with extracts from *Dictyota dichotoma*.

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

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

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

Total phenolic content. 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 higher 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* collected from Califin, BCS (19.1 ± 1.44 mg GAE /g), and the brown algae, *E. arborea* (20.6 ± 2.48 mg GAE /g). In general, red and brown seaweeds showed higher amounts of TPC.

DISCUSSION

Some of the seaweeds studied have the potential for pest control. In particular, *Laurencia johnstonii* and *Asparagopsis taxiformis* showed higher 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 than green algae (Lakhdar *et al.,* 2015). This is related to the presence of phenolic compounds (Negara *et al.,* 2021) and possibly to halogenated terpenoids in red species (Kasanah *et al*., 2015).

Red algae are primary producers of active halogenated terpenes with antibacterial properties. For example, sesquiterpene elatol is one of the main 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 10 mg•mL^{-1} concentration exhibited insecticidal activity against the maize weevil *Sitophilus zeamais*. *Laurencia 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 *Laurencia dendroidea* J. Agardh extract from two collection sites. Although GC/MS analysis revealed the same significant compounds, the differences in bioactivity may be attributed to a synergistic effect between

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

Seaweed	*Fusarium oxysporum*
Caulerpa racemosa	+
Caulerpa sertularioides	ND
Codium amplivesiculatum	ND
Halimeda discoidea	+
Ulva sp.	ND
Dictyota dichotoma	++
Eisenia arborea	ND
Egregia menziesii	+
Macrocystis pyrifera	+
Padina concrescens	ND
Sargassum horridum	ND
Sargassum lapazeanum	ND
Acanthophora spicifera	+
Asparagopsis taxiformis	++
Laurencia johnstonii *	++
Laurencia johnstonii **	++

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

Table 4. Insecticidal activity of seaweeds against the maize weevil *Sitophilus zeamais* after five days of incubation.

Seaweed	Mortality on *Sitophilus zeamais* (%)
Caulerpa racemosa	44 ± 5.5 bc
Caulerpa sertularioides	22 ± 5.1 f
Codium amplivesiculatum	38 ± 3.1 bcde
Halimeda discoidea	36 ± 6.3 bcdef
Ulva sp.	25 ± 6.0 ef
Dictyota dichotoma	29 ± 5.3 def
Eisenia arborea	29 ± 6.5 def
Egregia menziesii	36 ± 5.4 bcdef
Macrocystis pyrifera	33 ± 5.8 cdef
Padina concrescens	36 ± 5.8 bcdef
Sargassum horridum	35 ± 7.0 cdef
Sargassum lapazeanum	22 ± 6.6 f
Acanthophora spicifera	34 ± 4.6 cdef
Asparagopsis taxiformis	40 ± 5.7 bcd
Laurencia johnstonii *	72 ± 5.8 a
Laurencia johnstonii **	52 ± 6.7 b

* 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 ($p \leq 0.05$, $n = 4$)

the compounds produced by the macroalgae. The variation type and amount of metabolites produced by the same seaweed at two different locations is attributed to the environmental conditions present at each place (Gaubert *et al*., 2019). That could be why the two *L. johnstonii* collected in this research 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, 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 research mentioned before were collected in a very different locations compared to the seaweeds collected for our experiment, our results suggest the same tendency. Also, another research demonstrated that *Laurencia nidifica* J. Agardh aqueous extract significantly reduced hatchability; however, the aqueous extract was used directly at a concentration of 5-15 %, without knowing the amounts of

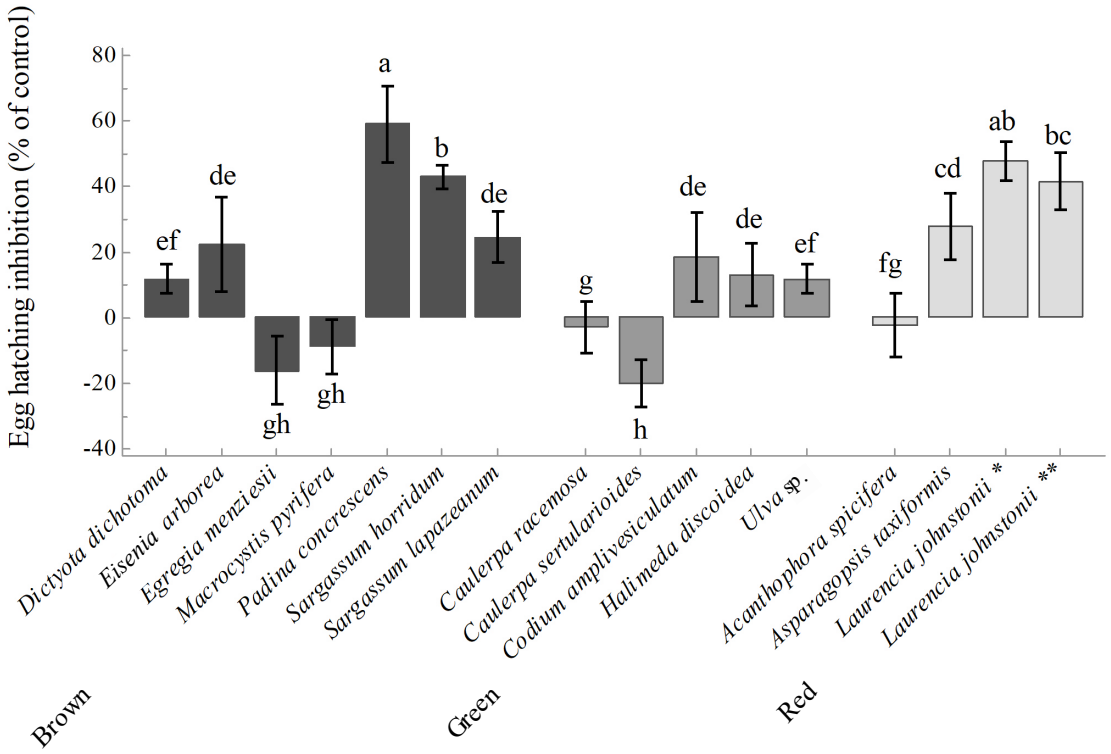

Figure 2. Nematicidal activity regarding egg hatching inhibition of *Meloidogyne incognita* at day seven**.** * Sample collected in Calerita (extract code: 23-05), ** sample collected in Califin (extract code: 23-10). Values represent mean ± standard deviation. Different letters represent statistical difference ($p \leq 0.05$, $n = 4$).

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

Seaweed extract	TPC (mg GAE/g)
Caulerpa racemosa	5.05 ± 0.41 ^{gh}
Caulerpa sertularioides	6.04 ± 0.22 ^{fg}
Codium amplivesiculatum	32.5 ± 0.56 ^{a}
Halimeda discoidea	2.72 ± 0.39 ^{gh}
Ulva sp.	1.81 ± 0.59 ^{h}
Dictyota dichotoma	4.89 ± 0.30 ^{gh}
Eisenia arborea	20.6 ± 2.48 ^{b}
Egregia menziesii	8.85 ± 2.11 ^{ef}
Macrocystis pyrifera	6.36 ± 2.23 ^{fg}
Padina concrescens	15.5 ± 1.71 ^{cd}
Sargassum horridum	3.72 ± 0.41 ^{gh}
Sargassum lapazeanum	16.9 ± 1.81 ^{c}
Acanthophora spicifera	2.13 ± 0.99 ^{h}
Asparagopsis taxiformis	22.8 ± 2.08 ^{b}
Laurencia johnstonii *	12.0 ± 2.00 ^{de}
Laurencia johnstonii **	19.1 ± 1.44 ^{bc}

*extract number 23-05 sample collected in Calerita, ** extract number 23-10 sample collected in Califin. Values represent mean ± standard deviation. Different letters represent statistical difference ($p \leq 0.05$, $n = 4$)

solutes present in it (El-Deen & Issa, 2016). Interestingly, some seaweeds showed a negative percentage of egg-hatching inhibition, which suggests that these extracts promoted hatching rather than inhibiting. It is well known that some metabolites act as nematode egg hatching stimulants which 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 analogs of these metabolites, known as hatching factors.

Some phenolic compounds have shown insecticidal activity against *S. zeamais* (Rodríguez *et al*., 2022). Therefore, the total phenolic content may be associated with the insecticide activity observed in *C. amplivesiculatum* and *A. taxiformis*. Both extracts exhibited a high phenolic content and showed insect mortality rates of around 40%. However, in the case of *L. johnstonii*, the insecticidal activity may be related to the presence of sesquiterpenes in the extract, as previous studies have linked insecticidal activity to brominated sesquiterpenes such as laurinterol (González-Castro *et al*., 2024; Ishii *et al*., 2017). In general, the TPC of the extracts ranged from 1.81 to 32.5 mg of gallic acid equivalents. Several studies support that the phenolic content of crude extracts shows a spatial variability (Tanniou *et al.,* 2013; Van Hees *et al.,* 2017) Thus, the significant differences observed in the phenolic samples of *L. johnstonii* may be attributed to the geographical location. Seaweeds may provide a safer alternative for the control of agricultural pests. In particular, red seaweeds *Asparagopsis taxiformis* and *Laurencia johnstonii* have shown significant potential for developing biopesticides.

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