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RESEARCH

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Seaweed extracts as biological control of aflatoxins produced by *Aspergillus parasiticus* and *Aspergillus flavus*

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Abstract

Background Mycotoxins are secondary metabolites made by a variety of molds and fungi. They contaminate a lot of food products and local crops during pre- and post-harvesting under favorable conditions like high temperature and moisture. *Aspergillus* species are the most common fungi that contaminate food and produce biochemicals known as mycotoxins. Aflatoxins (AFB1, AFB2, AFG1, and AFG2) are the major mycotoxins produced by *A. flavus* and *A. parasiticus* that harm animal and human health. These fungi are controlled by chemical fungicides, but these are harmful to the environment. The aim of this study was to determine whether the aflatoxigenic fungi can be exterminated only by marine algal extracts or not.

Results The findings showed that the tested seaweed extracts inhibited fungal growth and aflatoxins production to varying degrees. The maximum antifungal activity was recorded in *Halimeda opuntia* extract against *A. parasiticus*-24 and *A. flavus*-18 and *Turbunaria decurrens* extract against *A. flavus*-18 (with an inhibition percentage of 77.78%), followed by *Jania rubens* extract against *A. parasiticus*-16 with inhibition percentage 75.88% compared to the control. Aqueous extract of *H. opuntia* effectively eliminated aflatoxins (B1, B2, G1, and G2) in *A. parasiticus*-16 and *A. parasiticus*-24. *T. decurrens* extract could detoxify 100% of aflatoxins in three isolates of *A. parasiticus*. *J. rubens* extract eliminated aflatoxins in *A. parasiticus*-15 and *A. parasiticus*-16 compared to their normal production using high-performance liquid chromatography.

Conclusions According to this study, the macroalgal species with numerous distinctive antifungal properties constituents significantly inhibited the growth and production of aflatoxin in *A. parasiticus* and *A. flavus* isolates. The findings supported the use of macroalgae as a biological control agent against fungi and their toxins.

Keywords *Aspergillus flavus*, Aflatoxins, Detoxification, Seaweed extracts, HPLC

Background

Aflatoxins are terminology means mycotoxins produced by the fungus, *Aspergillus flavus* that have a significant health risk due to their identification as human carcinogens (Loi et al. 2020). Aflatoxins have been found in members of three *Aspergillus* genus sections: *Flavi* (B- and G-type aflatoxins), *Ochraceorosei* (aflatoxins B1 and B2), and *Nidulantes* (formerly *Emericella* genus; aflatoxin B1) (Pildain et al. 2008). *A. flavus* and *A. parasiticus* are the two species of section Flavi which produce aflatoxins (AFB and AFG) under a variety of environmental conditions. Heat cannot decompose aflatoxins while processing agricultural products (Akinola et al. 2019). The toxic consequences of aflatoxicosis, also, known as liver cancer, hepatotoxicity, immune system depression, and impaired growth in both humans and animals (IARC 2012).

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Occurrence of aflatoxins in food and feed has led to both human and animal health risks and economic losses, especially for exporting countries. There are several ways to control aflatoxins that fall into two categories: (1) mold contamination and growth prevention; and (2) detoxification of contaminated products (El-Shanshoury et al. 2022). Similarly, AFS contamination can be avoided through various strategies, generally classified in two categories: pre- and post-harvest controls (Daou et al. 2021). Before harvesting, control techniques are generally carried out through proper field management (such as crop rotation, irrigation, and soil cultivation), employment of biological, natural, chemical treatments, Development of AF-resistant crop types and application of fungal and bacterial inhibitors. Biological, chemical and natural irradiations were used in post-harvest control strategies to improve drying and storage conditions (Agriopoulou et al. 2020).

Seaweeds are visible macroalgae that grow on rocks and along the coast, and can be found in a variety of aquatic habitats (Rashad and El-Chaghaby 2020). Marine macroalgae are divided into three groups based on their pigment contents and morphological and anatomical characteristics: green algae (Chlorophyaceae), brown algae (Phaeophyaceae), and red algae (Rhodophyaceae) (Manzelat et al. 2018). Seaweed contains various secondary metabolites that have been identified as possible bioactive compounds, such as active antimicrobial molecules. Many studies have found bioactive compounds with antifungal activity in the crude extracts of various Chlorophyta, Phaeophyta and Rhodophyta species, including proteins, carbohydrates, fatty acids (FAs), polyunsaturated fatty acids (PUFAs), antioxidants, amines, amides, and pigments (El-Sheekh et al. 2020). These antimicrobial substances work by disrupting the cytoplasmic membrane's structure, function, or integrity, as well as by destroying enzymes and inhibiting protein synthesis (Swain et al. 2017). The antimicrobial activity depends on the type of algae and the solvent used (Radhika et al. 2012). Algal extracts are natural antimicrobial agents that are nutritionally safe and easily degradable (Charway et al. 2018).

Synthetic chemical fungicides are used extensively to fight fungal diseases, although these ingredients are harmful to the environment. As one of the richest marine sources of compounds with diverse bioactivities, seaweeds provide an environmentally friendly alternative in the search for various compounds with industrial applications (Vicente et al. 2021).

Decontamination/detoxification processes help recover mycotoxin-contaminated goods to obtain such low levels. The ideal decontamination method should be simple to use, cost effective, and avoid the creation of toxic compounds, the parent mycotoxin reforming, or modifications to the food's nutritional and palatable properties. As a result, several researchers developed methods to detoxify aflatoxins in food and feedstuffs using physical, chemical and biological methods (Nazhand et al. 2020).

The present study aimed to explore the antifungal activity of macroalgal extracts from *Halimeda opuntia*, *Turbunaria decurrens* and *Jania ruben* against four isolates of *Aspergillus flavi* group (AP15, AP16, AP24, and AF18) and its effect on aflatoxins (AFB1, AFB2, AFG1 and AFG2) produced by the four studied fungi.

Methods

Tested fungi

The fungi used in this investigation were three isolates of *A. parasiticus* (AP15, AP16 and AP24) and one isolate of *A. flavus* (AF18). These samples were isolated from wheat flour. Dilution-Plate method of quantitative fungal determination was described by Moubasher et al. (1972). For isolating and purifying fungi, Czapek's agar medium was used at 28 °C. El-Shahir (2021) reported that the isolates AP15, AP16, AP24, and AF18 are the most aflatoxigenic, so they were chosen for the current study.

Collection of seaweeds and extraction preparation

The green alga, *H. opuntia*, brown algae *T. decurrens,* and red alga, *J. rubens* were collected in May 2019 from the Hurghada city (27° 17′ 03″ N, 33° 46′ 21″ E), Red Sea coast of Egypt and identified according to Aleem (1993). The macroalgal samples were collected and stored in an ice box in sterile polyethylene bags until they could be prepared and analyzed in the laboratory (Patra et al. 2008). The algal samples were spread out on plates, dried in the shade and then blended in an electric blender (El-Sheekh et al. 2020). A total of 10 g dried algae were dissolved in 100 ml distilled water (10% conc.) for 48 h and filtered using Whatman filter paper no.1 and stored at 4 °C until needed.

Antifungal activity of marine macroalgae extracts

The antifungal activities of aqueous extracts of *H. opuntia*, *T. decurrens,* and *J. rubens* were determined against four isolates of *A. flavi* group (AP15, AP16, AP24 and AF18) using agar plate diffusion method (growth inhibition assay method), according to Guo et al. (2007). Fungal spores were grown in Petri dishes with Potato Dextrose Agar (PDA). After seven days of incubation at 28 °C, an aliquot of the algal extracts (1 ml) was added to sterile melting PDA medium aseptically and individually. Control plates were prepared without algal extracts and plates with sodium benzoate 0.1% as a preservative substance (each was carried out in triplicate). The fungal inocula were placed onto the center of agar surface after

the plates had cooled. All Petri plates were incubated at 28 °C for 10 days. The dry weight of mycelia was measured every two- days according to (Sudini et al. 2015). During the 10th-day incubation period, the colony diameter was measured every 24 h. The antifungal activities were measured as the mean colony diameter in cm for growth inhibition. The percentage of inhibition for each fungus strain was determined once the mycelia in the control plate had grown to the edge of the Petri dish. The data were shown as the mean, standard deviation (± SD) of three replicates. The following formula was used to obtain the inhibition percentage (Harlapur et al. 2007).

$$\text{Inhibition}(\%) = (R - r)/R \times 100$$

where *R*: the fungal radial growth on the control plate. *r*: the fungal radial growth on the plate treated with algal extracts.

Quantification of aflatoxins by high-performance liquid chromatography (HPLC) after detoxification with algal extracts

The four *A. flavi* group isolated from wheat flour was grown on Czapek's liquid medium, with some modifications than Samson et al. (2002). A total of 100 ml of the liquid medium was added to each 250 ml sterile Erlenmeyer flask. Following sterilization, each flask was inoculated with three agar discs made from seven-day old cultures on yeast extract sucrose (YES) agar plates and 10 ml of an aqueous solution of the three marine algae. The flasks were then incubated at 28°C on the shaker at 125 rpm, for 10 days. The dry weight of the mycelia was determined after incubation by filtering the cultural filtrate through Whatman No.1 filter paper. A total of 100 ml of cultural filtrate was extracted with an equal volume of chloroform for 24 h. at 20°C with shaking at (160 rpm). After being dried over anhydrous sodium sulfate, the chloroform extract was distilled to near dryness. The residue was diluted to 1 ml with chloroform. HPLC analysis was carried out, using an Agilent 1260 series. The column ZORBAX Eclipse Plus C18 (4.6×250 mm, 5 µm) was used. The mobile phase was water: methanol: acetonitrile (6:3:1) with a flow rate of 1ml/min. The injection volume was 20 µl. The FLD was adjusted with an excitation/emission wavelength of 360/450 nm. The column temperature was maintained at 40 °C.

Statistical analysis

Data were statistically analyzed using the one-way analysis of variance test in the program SPSS (version 20) to determine the degree of significance.

Results

Antifungal activity

The active constituents of the seaweeds *H.opuntia*, *T. decurrens*, and *J. rubens,* extracted with water demonstrated antifungal against three isolates of *A. parasiticus* (AP15, AP16, and AP24) and one isolate of *A. flavus* (AF18) (Fig. 1). The results revealed that the three extracts effectively suppressed the growth of *A. parasiticus* and *A. flavus* with variable potency. The inhibition percentage of algal extracts against tested fungi ranged between 40.37 and 77.78% than the control (Table 1). Maximum antifungal activity was noted at the treatment of *H. opuntia* extract against *A. parasiticus*-24 and *A. flavus*-18, and *T. decurrens* extract against *A. flavus*-18, the mean colony diameter reached 2.0 cm after 10 days of incubation with an inhibition percentage of (77.78%) compared to the control. It is followed by *J. rubens* extract (2.17 and 2.33 cm) against *A. parasiticus*-16 and *A. flavus*-18 with an inhibition percentage of (75.88 and 74.11%) compared to the control, respectively, whereas *T. decurrens* extract had the lowest antifungal activity (5.37 cm) against *A. parasiticus*-15 with an inhibition percentage of (40.33%) than the control, followed by *H. opuntia* extract against the fungus, *A. parasiticus*-16 (4.0 cm, colony diameter) with inhibition percentage (55.56%) than the control. In the case of sodium benzoate 0.1%, the mean colony diameter reached (0.77 cm) after 10 days of incubation with an inhibition percentage of (91.44%) than the control against *A. parasiticus*-15, while the lowest antifungal activity of sodium benzoate occurred against *A. parasiticus*-24 (1.13 cm, colony diameter) with an inhibition percentage (87.44%) than the control (Table 1).

The findings revealed that the extracts of *H. opuntia*, *T. decurrens*, and *J. rubens* significantly decreased the mycelial growth of aflatoxigenic fungi than the control. The mycelial dry weight of the four tested aflatoxigenic fungi was less than the control by (33.87 to 65.45%). *H. opuntia* extract had the highest reduction in mycelial dry weight (65.45% of control) against *A. parasiticus*-16. Treatment with *T. decurrens* extract inhibited mycelial dry weight of aflatoxigenic fungi (AP15, AP16, AP24, and AF18) with efficacy ranging from 41.53 to 54.54% of the control. In case of *J. rubens* extract treatment, mycelial growth of tested fungi was reduced by (33.87–49.09%) of the control (Table 2).

Quantification of aflatoxins by HPLC after detoxification by using marine macroalgae extracts

According to El-Shahir (2021), *A. parasiticus*-15 was the highest isolate in the production of aflatoxin G2 (79.50 µg/l), and it also produced aflatoxins B1 and B2 at

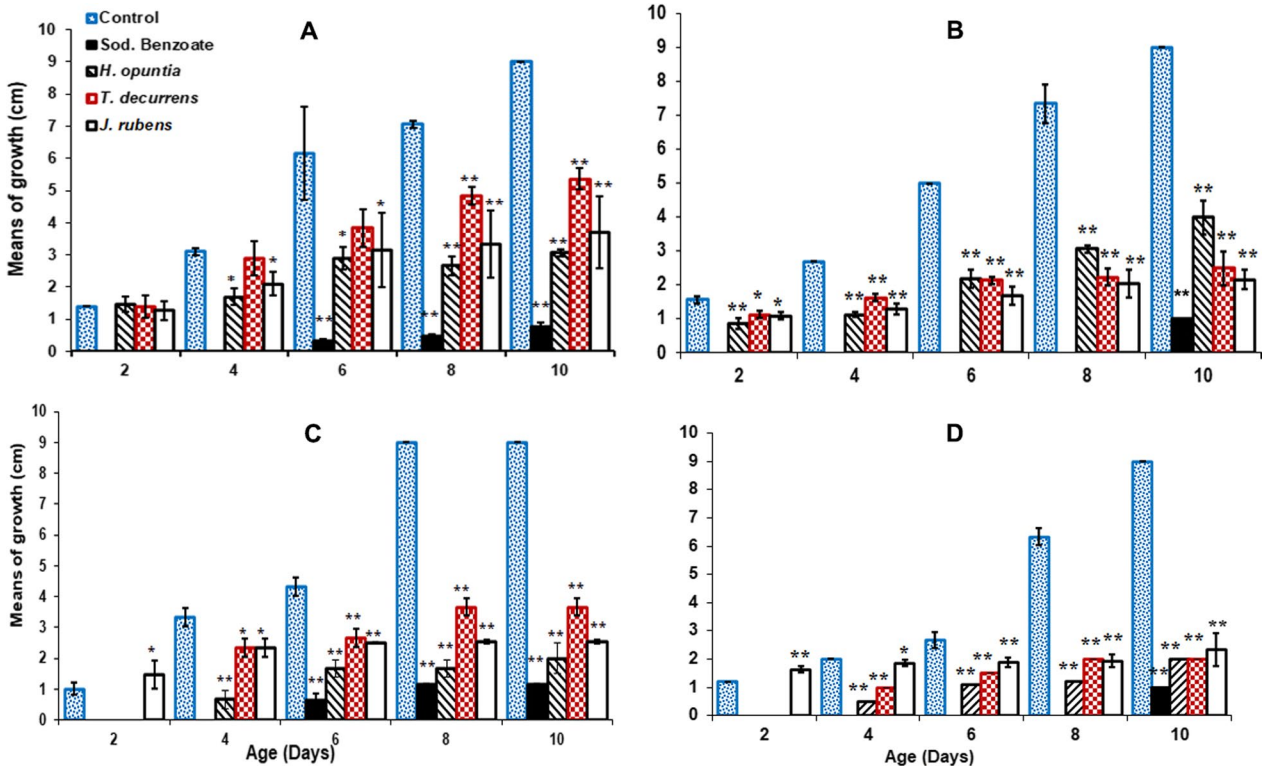

Fig. 1 Antifungal activity of seaweed extracts; *Halimeda opuntia*, *Turbunaria decurrens* and *Jania rubens* against *Aspergillus parasiticus* (AP15 **(A)**, AP16 **(B)**, and AP24 **(C)**) and *Aspergillus flavus*18 (AF18, **D**). Values are the mean of three replicates ± standard deviations (SD). * Significant differences at $p < 0.05$ ** Highly significant differences from control at $p < 0.05$

Table 1 Inhibition rate (%) of *Aspergillus parasiticus* (AP15, AP16, and AP24) and *Aspergillus flavus*18 (AF18) by (0.1%) sodium benzoate, *Halimeda opuntia*, *Turbunaria decurrens,* and *Jania rubens* extracts

Fungi/ Extracts	Sod. benzoate	*Halimeda opuntia*	*Turbunaria decurrens*	*Jania rubens*
A. parasiticus-15	91.44	65.88	40.33	58.89
A. parasiticus-16	88.89	55.56	72.22	75.88
A. parasiticus-24	87.44	77.78	59.22	71.89
A. flavus-18	88.89	77.78	77.78	74.11

rates of 16.29 µg/l and 17.52 µg/l, respectively. The fungus, *A. parasiticus*-16 produced aflatoxins B1, B2, and G2 at rates of 55.07, 9.54, and 15.42 µg/l, respectively. The fungus, *A. parasiticus*-24 was the most productive isolate in terms of aflatoxin B1 production (9229.34 µg/l) and aflatoxin B2 production (5.67 µg/l). The fungus, *A. flavus*-18 produced the most aflatoxin B2 (152.66 µg/l), followed by aflatoxin B1 (24.54 µg/l) and aflatoxin G2 (8.08 µg/l) (Table 3 and Fig. 2). So that, in the present study, aqueous solutions of *H. opuntia*, *T. decurrens* and *J. rubens* were used to detoxify the four aflatoxigenic isolates mentioned above and to determine the amounts of aflatoxins and the dry weight of the mycelia.

Table 2 Dry weight of mycelia for *Aspergillus parasiticus* (AP15, AP16, and AP24) and *Aspergillus flavus*18 (AF18) after detoxification with *Halimeda opuntia*, *Turbunaria decurrens* and *Jania rubens* extracts

Fungal species	Means of mycelial dry weight (g)						
	Halimeda opuntia		*Turbunaria decurrens*		*Jania rubens*		Control
	g	% of control	*g*	% of control	*g*	% of control	*g*
A. parasiticus-15	0.35 ± 0.05*	53.84	0.27 ± 0.01*	41.53	0.28 ± 0.02*	43.07	0.65 ± 0.03
A. parasiticus-16	0.36 ± 0.05*	65.45	0.30 ± 0.04*	54.54	0.27 ± 0.01*	49.09	0.55 ± 0.02
A. parasiticus- 24	0.36 ± 0.02*	58.06	0.28 ± 0.03*	45.16	0.21 ± 0.02*	33.87	0.62 ± 0.02
A. flavus-18	0.37 ± 0.05*	46.83	0.40 ± 0.03*	50.63	0.35 ± 0.03*	44.30	0.79 ± 0.03

* Significant differences from control at $p < 0.05$

Table 3 Aflatoxins concentration (µg/l) in *Aspergillus parasiticus* (AP15, AP16, and AP24) and *Aspergillus flavus*-18 (AF18) cultural extracts by HPLC analysis

Fungi	AFB1	AFB2	AFG2
A. parasiticus-15	16.296	17.525	79.507
A. parasiticus-16	55.076	9.547	15.428
A. parasiticus-24	9229.343	5.671	–
A. flavus-18	24.543	152.668	8.082

Aqueous extract of *H. opuntia* efficiently eliminated aflatoxins B1, B2, G1, and G2 in *A. parasiticus*-16 and *A. parasiticus*-24. In the fungus, *A. parasiticus*-15, the aflatoxins B2 and G2 were not found but the aflatoxins B1 and G1 were found at low concentrations (0.45 and 0.26 ng/ml, respectively, Table 4). Aflatoxin B1 was detected at low concentrations in *A. flavus*-18 (1.18 ng/ml, Table 4). *T. decurrens* extract could detoxify 100% of aflatoxin in three isolates: *A. parasiticus*-15, *A. parasiticus*-16 and *A. parasiticus*-24, except for *A. flavus*-18, where aflatoxins B1 and B2 were detected at low concentrations (1.99 and 0.34 ng/ml, respectively) and detoxification occurred by 100% for aflatoxins G1 and G2 (Table 4 and Fig. 3). *J. rubens* extract treatment removed aflatoxins from two isolates, *A. parasiticus*-15 and *A. parasiticus*-16, except for aflatoxin G2 produced by 0.14 ng/ml in *A. parasiticus*-16. In the case of *A. flavus*-18, J. *rubens* extract detoxified 100% of aflatoxins G1 and G2, but aflatoxins B1 and B2 were produced in the least quantifies, 3.6 and 0.1%, respectively, compared to their normal production (Fig. 3). Additionally, *J. rubens* extract was able to eliminate aflatoxins G1 and G2 from *A. parasiticus*-24 completely. The production of aflatoxins B1 and B2 decreased to 0.45 and 0.11 ng/ml, respectively (Table 4).

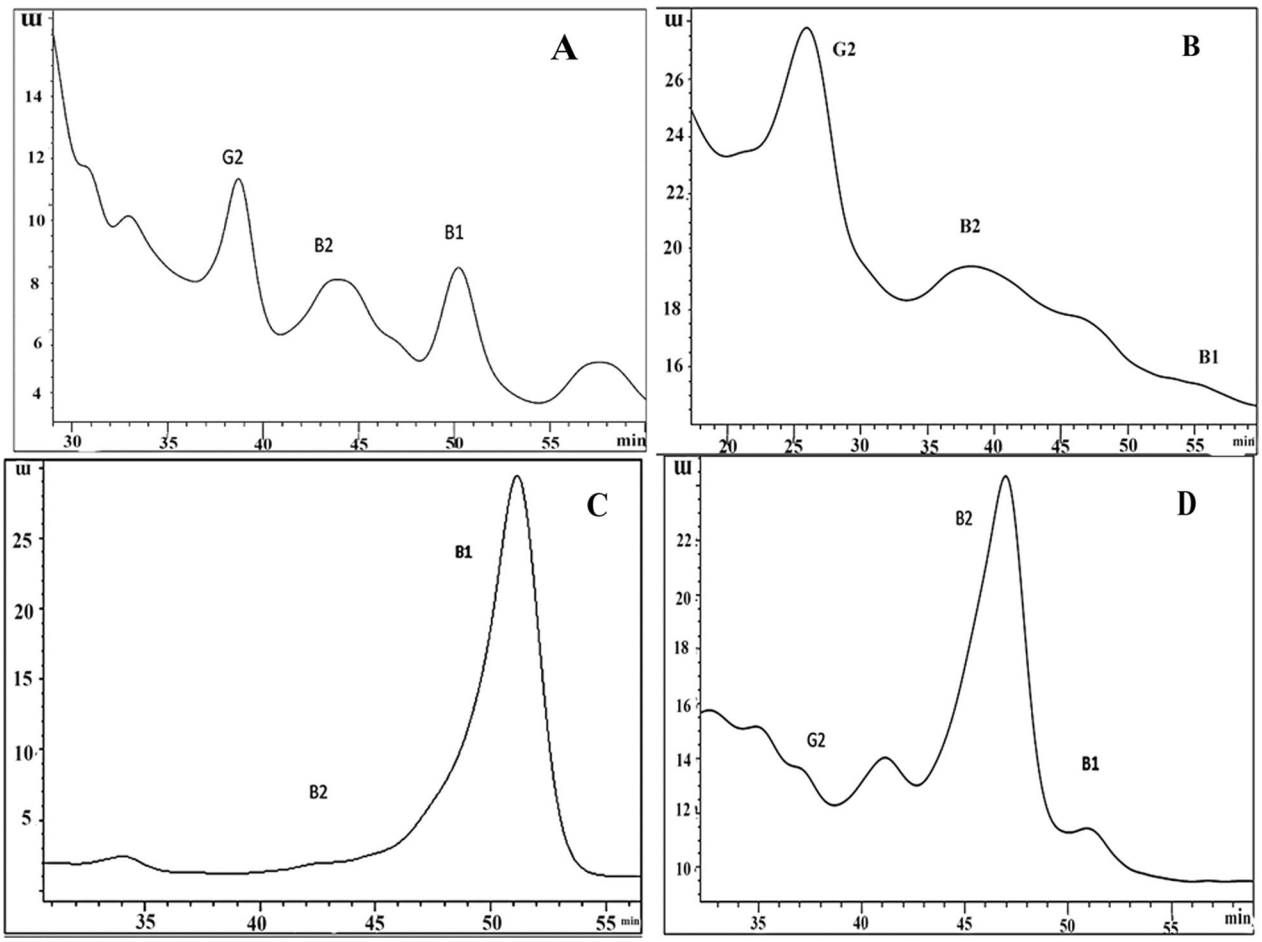

Fig. 2 HPLC analysis of aflatoxins: **A** chromatograms obtained for a mixed aflatoxins standard (AFB1, AFB2, and AFG2), **B** cultural extract of *A. parasiticus*-15, **C** cultural extract of *A. parasiticus*-24, and **D** cultural extract of *A. flavus*-18. The horizontal axes represent time (min.) while the vertical axes represent wave length (ɯ) (El-Shahir 2021)

Table 4 Quantification of aflatoxins (B1, B2, G1 and G2) by HPLC after detoxification with *Halimeda opuntia*, *Turbunaria decurrens* and *Jania rubens* extracts

Fungi	B1	B2	G1	G2
Concentration of aflatoxins (ng/ml) with *Halimeda opuntia*				
A. parasiticus-15	0.45	ND	0.26	ND
A. parasiticus-16	ND	ND	ND	ND
A. parasiticus- 24	ND	ND	ND	ND
A. flavus-18	1.18	ND	ND	ND
Concentration of aflatoxins (ng/ml) with *Turbunaria decurrens*				
A. parasiticus-15	ND	ND	ND	ND
A. parasiticus-16	ND	ND	ND	ND
A. parasiticus- 24	ND	ND	ND	ND
A. flavus-18	1.99	0.34	ND	ND
Concentration of aflatoxins (ng/ml) with *Jania rubens*				
A. parasiticus-15	ND	ND	ND	ND
A. parasiticus-16	ND	ND	ND	0.14
A. parasiticus- 24	0.45	0.11	ND	ND
A. flavus-18	0.89	0.17	ND	ND

ND not detected

Discussion

Mycotoxins, which are produced by a variety of toxigenic, are harmful to humans and cause economic loss (Ouf et al. 2015). Aflatoxins (AFs) are one of the highly toxic secondary metabolites derivatives from polyketides produced by fungal species like *A. flavus*, and *A. parasiticus*, the two principal aflatoxin-producing *Aspergillus* species, as a result of contamination of food, fruits, and vegetables (Altomare et al. 2021). The limited use of chemical preservatives, their susceptibility, toxicity, and microbial resistance, as well as the negative effects on human health, increases the demand for natural, healthy, safer, and potentially effective antifungal agents. Thus, the antimicrobial activities of algal extracts can be used to treat fungal infections and as natural preservatives to ensure healthy and safe food.

In the present study, the aqueous extracts of three seaweed species, *H. opuntia*, *J. rubens*, and *T. decurrens*, demonstrated fungicidal activities against aflatoxigenic fungi, *A. parasiticus* (AP15, AP16, and AP24) and *A. flavus*-18, with varied inhibitory effects depending on algal species. The highest inhibition percentage (77.78%) was recorded in *H. opuntia* against *A. parasiticus*-24 and *A. flavus*-18 and *T. decurrens* extract against *A. flavus*-18, while *T. decurrens* extract showed the lowest inhibition percentage (40.33%) against *A. parasiticus*-15. Obtained results agreed with Krishnamoorthi and Sivakumar (2019), who recommended the seaweed *Ulva lactuca L.* as an antifungal agent in preparing eco-friendly disinfectants against *A. flavus*, the inhibition percentage reached (44.7%) lesser than the control.

Jania rubens extract showed the greatest reduction in mycelial dry weight (33.87% of control) against *A. parasiticus*-24, followed by *T. decurrens* extract (41.53% of control) against *A. parasiticus*-15. These results agree with that of Ambika and Sujatha (2014) who studied the efficacy of algal water extracts of *Gracilaria edulis* and *Sargassum myricocystm* against mycelial growth *Colletotrichum falcatum*. They found that the reduction percentage was 8.14% over control. This low reduction percentage disagrees with our result, because they used a low concentration of aqueous seaweed extracts (5%) in their study. Similar results were obtained by Indira et al. (2013) who revealed that water extract of *H. tuna* had antifungal activity against *A. flavus*, another eight fungal species and ten bacterial strains. These results confirmed the prospective use of *H. tuna* as a source of antimicrobial compounds.

Seaweeds (marine algae) have a rich source of various active secondary metabolites with diverse biological functions that are used by microorganisms for defense against herbivores and pathogens, such as polyphenols, terpenes, alkaloids and acetogenins (Saleh and Al-Mariri 2018). Green, brown, and red algae have been found to contain composites with cytostatic, antiviral, antifungal, antihelminthic, and antibacterial properties (Ahmed et al. 2020). Also, Khan et al. (2017) confirmed that the inhibition percentage of the aqueous extract of *J. adhaereus* reached 78% against *F. oxysporum*. Begum et al. (2016) obtained different results after 6 days of incubation with *T. conoides* extract; no mycelial growth (100% inhibition) was detected by 15 and 20% *T. conoides* extract-treated

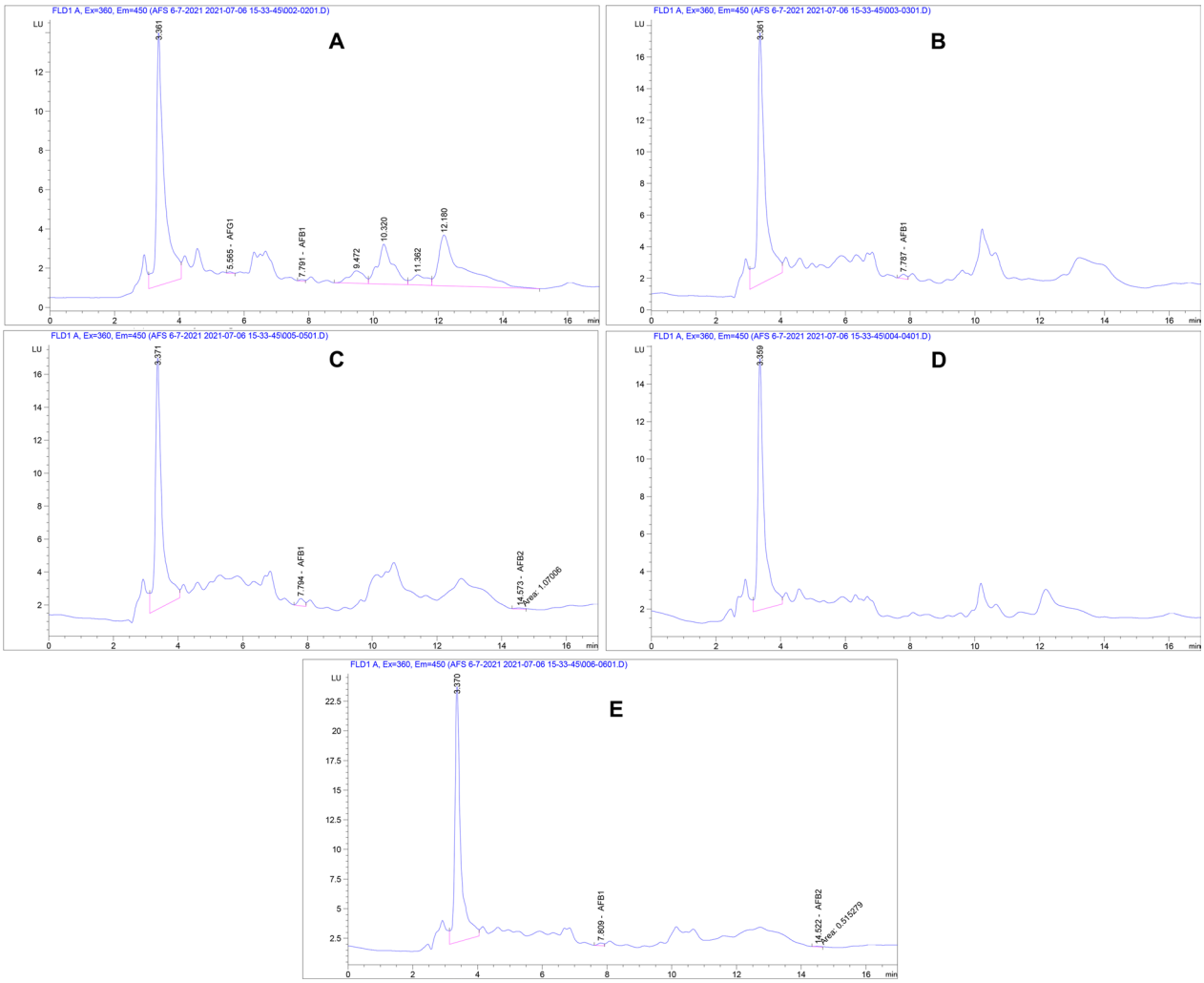

Fig. 3 HPLC chart for detoxification of aflatoxigenic fungi *A. parasiticus*-15 (**A**) and *A. flavus*-18 (**B**) by *Halimeda opuntia* extract, *A. flavus*-18 (**C**) by *Turbunaria decurren* extract, *A. parasiticus*- 24 by *T. decurrens* extract (**D**), and *A. flavus*-18 by *Jania rubens* extract (**E**). The horizontal axes represent time (min.) while the vertical axes represent wave length (ɯ)

plates. These disparities could be attributable to differences in extract content, seaweed species, and the type of the tested fungi. Several studies assessed the antifungal activities of the seaweeds with different solvents viz., ethyl acetate, methanol, ethanol, acetone, chloroform and water (Ahmed et al. 2020). El-Sheekh et al. (2022) reported that the most abundant phytochemical constituents of the seaweeds extracts were hexadecanoic acid, methyl ester (palmitic acid, methyl ester), octadecanoic acid, methyl ester, 10-octadecenoic acid, methyl ester and 11-octadecenoic acid, methyl ester, and those were found to be responsible for antifungal activity against various fungal genera.

These active phytochemicals and metabolites compounds may be responsible for the high efficacies of seaweed extracts against aflatoxigenic fungi, which are normally accumulated in the lipid layer of the cell membrane and mitochondria (Righini et al. 2019). As a result, the cell's structural integrity was disrupted and the cell became permeable (Belakhdar et al. 2015). Free fatty acids could stimulate peroxidative processes, preventing microbial fatty acid synthesis (Desbois and Smith 2010). Free fatty acids may also interact with microbial cell membranes, resulting in molecular leakage, reduced nutrient absorption or suppression of respiration (Suresh et al. 2014). Phenol derivatives also interrupt cell homeostasis, resulting in cell death and growth inhibition (Devi et al. 2010).

The present study demonstrated the ability of algae to act as an antifungal agent via their stable biologically

active compounds. Additionally, they provide insights into the development of novel antifungal drugs for clinical use or food preservation.

Aflatoxin detoxification using biological agents is divided into two steps: absorption and enzymatic decadence (Jard et al. 2011). Extracellular or intracellular enzymes can also degrade aflatoxins; the end products of such enzymatic degradation are mostly water and CO_2 (Mwakinyali et al. 2019).

Aspergillus is a significant food fungus, causing more spoilage and bio-deterioration than other fungi (Dao and Dantigny 2011). *Aspergillus* is one of the most important genera of mycotoxigenic fungi, with over 40 species known to be capable of producing a wide range of mycotoxins that are harmful to the health of humans and animals that consume them. Aflatoxins are produced by *A. flavus* and *A. parasiticus*, while ochratoxin-A is produced by *A. niger*, *A. ochraceus*, and *A. carbonarius* (Zain 2011). Additionally, according to Drusch and Aumann (2005), mycotoxins can diffuse into food without the presence of mycelial growth. Therefore, the absence of mold does not necessarily mean that food is free of mycotoxins.

In the present study, the algal extracts were used to control the production of aflatoxins from the tested fungi. The highest production of aflatoxins, B1 (9229.34 µg/l), B2 (152.66 µg/l), and G2 (79.50 µg/l) were recorded in *A. parasiticus*-24, *A. flavus*-18, and A. *parasiticus*-15, respectively (El-Shahir 2021). These results agree with Manjunath and Mohana (2018) who stated that aflatoxin production was influenced by the types of strains. *A. flavus* produced fewer aflatoxins than *A. parasiticus*.

Present data showing that all the three macroalgae tested for their efficacy in controlling aflatoxins production were found to be suitable to varying degrees. *H. opuntia* extract completely eliminated aflatoxins (B1, B2, G1, and G2) in *A. parasiticus*-16 and *A. parasiticus*-24, whereas *T. decurrens* extract detoxified 100% of aflatoxins in three *A. parasiticus* isolates. *J. rubens* extract could completely detoxify aflatoxins in *A. parasiticus*-15 and *A. parasiticus*-16, except for aflatoxin G2. The observed decrease in aflatoxin accumulation in cultural media could be attributed to mechanisms activated by algal metabolites. Vanhoutte et al. (2016) found that microorganisms, including algae, can produce enzymes capable of degrading or modifying mycotoxin molecules. Banu et al. (2008) reported that 1.8% of the algal biomass of *Sargassum wightii* successfully removed 100% aflatoxins B1 and entirely prevented the growth of *A. flavus*. *S. platensis* significantly reduced aflatoxin B1 from its initial concentration of 36 ppm to 14 ppm, using the HPTLC method produced by *A. flavus* (Banu et al. 2008). The presence of algal chlorophyll-Chlorophyllin contributes to the ability of algal biomass to control and eliminate toxins produced by *A. flavus*. It is principally a water-soluble mixture of chlorophyll sodium-copper salts formed by saponification of the relatively nonpolar parent compound (Banu et al. 2008). It works as an interceptor, producing tight molecular complexes with planar, aromatic compounds like aflatoxin B1 and a strong hepatocarcinogenic (Breinholt et al. 1995). Chlorophyllin may prevent the absorption of carcinogens, decreasing their bioavailability in target tissues and, as a result, reducing DNA adducts formation and tumor burden (Egner et al. 2003). Microorganisms utilized in biological decontamination may be capable to bind, break down, or convert mycotoxins in specific foods and animal feeding into less hazardous compounds through acetylation, glucosylation, deamination, decarboxylation, or hydrolysis. Biological approaches are less expensive and do not harm the environment because no chemicals are employed. Though, their application can be time-consuming (Ji et al. 2016). Pleadin et al. (2019) confirmed that yeasts and lactic acid bacteria are the most commonly utilized in decontamination because they can minimize mycotoxins by binding them to the cell surface or converting them to less harmful compounds.

Conclusion

Green, brown and red marine macroalgae significantly inhibited the growth and production of aflatoxins (B1, B2, G1, and G2) in *A. parasiticus* and *A. flavus* isolates. The study supported the possibility of macroalgae as a biological control agent against fungi and their aflatoxin. Further research on the active components of effective seaweed to establish as well as the utilization of algal biomass to protect food against fungi and aflatoxins, could lead to a cost-effective and safe method of achieving the community health mandate.

Abbreviations

AP-15	*Aspergillus parasiticus*-15
AP-16	*Aspergillus parasiticus*-16
AP-24	*Aspergillus parasiticus*-24
AF18	*Aspergillus flavus*18
AFB1	Aflatoxin B1
AFB2	Aflatoxin B2
AFG1	Aflatoxin G1
AFG2	Aflatoxin G2
PDA	Potato Dextrose Agar

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AAMF and AAE performed assays and statistical analyses, designed the figures and tables, and wrote the manuscript draft. MME oversaw all experimental setups and assisted in statistical analyses and writing/improving the manuscript. All authors read and approved the final manuscript.

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References

Agriopoulou S, Stamatelopoulou E, Varzakas Th (2020) Advances in occurrence, importance, and mycotoxin control strategies: prevention and detoxification in foods. Foods 9(2):137. https://doi.org/10.3390/foods9020137

Ahmed EA, Al-Kahtani MM, El-Diasty EM, Shaban AA, Saber H, Abbas AM, Diab HM, Alshehri M, Elmansi AA, Hussein MA (2020) Diversity of toxigenic molds and mycotoxins isolated from dairy products: antifungal activity of Egyptian marine algae on *Aspergillus* and *Candida* species. J Pure Appl Microbiol 14(1):215–232. https://doi.org/10.22207/JPAM.14.1.23

Akinola SA, Ateba CN, Mwanza M (2019) Polyphasic assessment of aflatoxin production potential in selected Aspergilli. Artic Toxins 11(12):692–699. https://doi.org/10.3390/toxins11120692

Aleem AA (1993) The marine algae of Alexandria, Egypt. Univ. Alexandria, Alexandria, p 139

Altomare C, Logrieco AF, Gallo A (2021) Mycotoxins and mycotoxigenic fungi: risk and management. A challenge for future global food safety and security. Encycl Mycol 1:64–93

Ambika S, Sujatha K (2014) Antifungal activity of seaweed extract against *Alternaria porri* in onion. In: National Seminar on algae for sustainable agricultural production, Madurai, Tamil Nadu, India, p 87

Banu N, Sridhar S, Muthumary J, Rengasamy R (2008) Algae as biological detoxificant of aflatoxin produced by *Aspergillus flavus* isolated from sunflower seed. Indian J Sci Technol 1(3):1–5. https://doi.org/10.17485/ijst/2008/v1i3/29226

Begum MAJ, Selvaraju P, Vijayakumar A (2016) Evaluation of antifungal activity of seaweed extract (*Turbinaria conoides*) against *Fusarium oxysporum*. J Appl Nat Sci 8(1):60–62. https://doi.org/10.31018/jans.v8i1.747

Belakhdar G, Benjouad A, Abdennebi EH (2015) Determination of some bioactive chemical constituents from Thesium humile Vahl. J Mater Environ Sci 6(10):2778–2783

Breinholt V, Schimerlik M, Dashwood R, Bailey G (1995) Mechanisms of chlorophyllin anticarcinogenesis against aflatoxin B1 complex formation with the carcinogen. Chem Res Toxicol 8(4):506–514

Charway GN, Yenumula P, Kim YM (2018) Marine algae and their potential application as antimicrobial agents. J Food Hyg Saf 33(3):151–156. https://doi.org/10.13103/JFHS.2018.33.3.151

Dao T, Dantigny P (2011) Control of food spoilage fungi by ethanol. Food Control 22(3–4):360–368. https://doi.org/10.1016/j.foodcont.2010.09.019

Daou R, Joubrane K, Maroun RG, Khabbaz LR, Ismail A, El Khoury A (2021) Mycotoxins: factors influencing production and control strategies. AIMS Agric Food 6(1):416–447. https://doi.org/10.3934/agrfood.2021025

Desbois AP, Smith VJ (2010) Antibacterial free fatty acids: activities, mechanisms of action and biotechnological potential. Appl Microbiol Biotechnol 85(6):1629–1642. https://doi.org/10.1007/s00253-009-2355-3

Devi KP, Arif SN, Sakthivel R, Karutha SP (2010) Eugenol (an essential oil of clove) acts as an antibacterial agent against *Salmonella typhi* by disrupting the cellular membrane. J Ethnopharma 130(1):107–115. https://doi.org/10.1016/j.jep.2010.04.025

Drusch S, Aumann J (2005) Mycotoxins in fruits: microbiology, occurrence and changes during fruit processing. Adv Food Nutr Res 50:33–78. https://doi.org/10.1016/S1043-4526(05)50002-0

Egner PA, Muñoz A, Kensler TW (2003) Chemoprevention with chlorophyllin in individuals exposed to dietary aflatoxin. Mut Res 523–524:209–216

El-Shahir AA (2021) Detection of mycobiota and aflatoxigenic fungi in wheat flour from markets in Qena City, Egypt. Egypt J Microbiol 56(1):1–10. https://doi.org/10.21608/ejm.2021.48485.1180

El-Shanshoury AR, Metwally MA, El-Sabbagh SM, Emara HA, Saba HE (2022) Biocontrol of *Aspergillus flavus* producing aflatoxin B1 by *Streptomyces exfoliates*. Egypt J Bot 62(2):457–473

El-Sheekh MM, Mousa SHH, Farghl AAM (2020) Antibacterial efficacy and phytochemical characterization of some marine brown algal extracts from the Red Sea, Egypt. Rom Biotechnol Lett 25(1):1160–1169. https://doi.org/10.25083/rbl/25.1/1160.1169

El-Sheekh MM, Galal HR, Mousa SHH, Farghl AAM (2022) Screening of antifungal activity of bioactive chemical constituents in some brown marine macroalgae from the Red Sea, Egypt. Egypt J Bot 62(3):865–878. https://doi.org/10.21608/EJBO.2022.141379.2008

Guo Z, Xing R, Liu S, Zhong Z, Ji X, Wang L, Li P (2007) The influence of the cationic of quaternized chitosan on antifungal activity. Int J Food Microbiol 18(2):214–217. https://doi.org/10.1016/j.ijfoodmicro.2007.07.003

Harlapur SI, Kulkarni MS, Wali MC, Srikantkulkarni H (2007) Evaluation of plant extracts, bio-agents, and fungicides against *Exserohilum turcicum* causing *Turcicum* leaf blight of Maize. J Agric Sci 20(3):541–544. https://doi.org/10.3390/jof7121006

IARC (International Agency for Research on Cancer) (2012) IARC monographs on the evaluation of carcinogenic risks to humans, volume 100 C. A review of human carcinogens: arsenic, metals, fibres, and dusts. Lyon, France: https://monographs.iarc.fr/wp-content/uploads/2018/06/mono100C.pdf

Indira K, Balakrishnan S, Srinivasan M, Bragadeeswaran S, Balasubramanian T (2013) Evaluation of in vitro antimicrobial property of seaweed (*Halimeda tuna*) from Tuticorin coast, Tamil Nadu, Southeast coast of India. Afr J Biotechnol 12(3):284–289. https://doi.org/10.5897/AJB12.014

Jard G, Liboz T, Mathieu F, Guyonvarc'h A, Lebrihi A (2011) Review of mycotoxin reduction in food and feed: from prevention in the field to detoxification by adsorption or transformation. Food Addit Contam Part A 28(11):1590–1609. https://doi.org/10.1080/19440049.2011.595377

Ji C, Fan Y, Zhao L (2016) Review on biological degradation of mycotoxins. Anim Nutr 2(3):127–133. https://doi.org/10.1016/j.aninu.2016.07.003

Khan SA, Abid M, Hussain F (2017) Antifungal activity of aqueous and methanolic extracts of some seaweeds against common soil-borne plant pathogenic fungi. Pak J Bot 49(3):1211–1216

Krishnamoorthi R, Sivakumar SR (2019) Antifungal activity of seaweed *Ulva lactuca* L. extracted crude protein against pathogenic fungi. Asian J Pharm Clin Res 12(3):393–396. https://doi.org/10.22159/ajpcr.2019.v12i3.30750

Loi M, Paciolla C, Logrieco AF, Mulè G (2020) Plant bioactive compounds in pre- and postharvest management for aflatoxins reduction. Front Microbiol 11:243. https://doi.org/10.3389/fmicb.2020.00243

Manjunath K, Mohana DC (2018) Assessment of contamination levels and characterization of aflatoxin B1 producing *Aspergillus flavus* strain from food and feed samples from local markets of South India. Int J Life Sci 6(1):326–334

Manzelat FS, Mufarrah AM, Hasan BA, Hussain NA, Shuqaiq A, Huraidha A, Qahma A, Birk A (2018) Macroalgae of the Red Sea from Jizan, Saudi Arabia. Phykos 48(1):88–108. https://doi.org/10.13140/RG.2.2.33707.69920

Moubasher AH, El-Naghy MA, Abdel-Hafez SII (1972) Studies on the fungus flora of three grains in Egypt. Mycopathol Mycolo Appl 47(3):261–274

Mwakinyali SE, Ding X, Ming Z, Tong W, Zhang Q, Li P (2019) The recent development of aflatoxin contamination biocontrol in agricultural products. Biol Control 128:31–39. https://doi.org/10.1016/j.biocontrol.2018.09.012

Nazhand A, Durazzo A, Lucarini M, Souto EB, Santini A (2020) Characteristics, occurrence, detection and detoxification of aflatoxins in foods and feeds. Foods 9(5):644. https://doi.org/10.3390/foods9050644

Ouf SA, Basher AH, Mohamed AAH (2015) Inhibitory effect of double atmospheric pressure argon cold plasma on spores and mycotoxin production of *Aspergillus niger* contaminated date palm fruits. J Sci Food Agric 95(15):3204–3210. https://doi.org/10.1002/jsfa.7060

Patra JK, Rath SK, Jena KP, Rathod VK, Thato H (2008) Evaluation of antioxidant and antimicrobial activity of seaweed (*Sargassum* sp.) extract: a study on inhibition of Glutathione-S transferase activity. Turk J Math 32(2):119–125
Pildain MB, Frisvad JC, Vaamonde G, Cabral D, Varga J, Samson RA (2008) Two novel aflatoxin-producing *Aspergillus* species from Argentinean peanuts. Int J Syst Evol Microbiol 58:725–735. https://doi.org/10.1099/ijs.0.65123-0
Pleadin J, Frece J, Markov K (2019) Mycotoxins in food and feed. Adv Food Nutr Res 89:297–345
Radhika D, Veerabahu C, Priya R (2012) Antibacterial activity of some selected seaweeds from Gulf of Mannar Coast, South India. Asian J Pharma Clin Res 5(4):89–90
Rashad S, El-Chaghaby GA (2020) Marine algae in Egypt: distribution, phytochemical composition and biological uses as bioactive resources (a review). Egypt J Aquatic Biol Fish 24(5):147–160. https://doi.org/10.21608/ejabf.2020.103630
Righini D, Zanetti F, Martnez-Force E, Mandrioli M, Gallina Toschi T, Monti A (2019) Shifting sowing of camelina from spring to autumn enhances the oil quality for bio-based applications in response to temperature and seed carbon stock. Ind Crops Prod 137:66–73. https://doi.org/10.1016/j.indcrop.2019.05.009
Saleh B, Al-Mariri A (2018) Antifungal activity of crude seaweed extracts collected from Latakia Coast, Syria. J Fish Aquatic Sci 13(1):49–55. https://doi.org/10.3923/jfas.2018.49.55
Samson R, Hoekstra E, Frisvad J, Filtenborg O (2002) Introduction to food borne fungi, 6th edn. Cen-traalbureau voor Schimmelcultures, Utrecht
Sudini H, Srilakshmi P, Kumar KVK, Njoroge SMC, Osiru M, Seetha A, Waliyar F (2015) Detection of aflatoxigenic *Aspergillus* strains by cultural and molecular methods: a critical review. Afr J Microbiol Res 9(8):484–491. https://doi.org/10.5897/AJMR2014.7309
Suresh A, Praveenkumar R, Thangaraj R, Oscar FL, Baldev E, Dhanasekaran D, Thajuddin N (2014) Microalgal fatty acid methyl ester a new source of bioactive compounds with antimicrobial activity. Asian Pac J Trop Dis 4:S979–S984. https://doi.org/10.1016/S2222-1808(14)60769-6
Swain DL, Singh DE, Horton DE, Mankin JS, Ballard TC, Diffenbaugh NS (2017) Remote linkages to anomalous winter atmospheric ridging over the northeastern Pacific. J Geophys Res Atmos 122(22):12194–12209
Vanhoutte I, Audenaert K, De Gelder L (2016) Biodegradation of mycotoxins: tales from known and unexplored worlds. Front Microbiol 7:561
Vicente TFL, Lemos MFL, Félix R, Valentão P, Félix C (2021) Marine macroalgae, a source of natural inhibitors of fungal phytopathogens. J Fungi 7(12):1006. https://doi.org/10.3390/jof7121006
Zain ME (2011) Impact of mycotoxins on humans and animals. J Saudi Chem Soc 15(2):129–144. https://doi.org/10.1016/j.jscs.2010.06.006

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