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Effect of *Cladophora glomerata* Extract Against Some Fungal Pathogens

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Abstract: This study examines the impact of imported *Cladophora* green growth from the Caspian Sea on tomato infection causing parasites, *Curvularia manamgodae* and *Fusarium solani,* leading to most foodborne illnesses. The study found that at a concentration of 5% alcoholic extract, *Fusarium solani* and *Curvularia manamgodae* were reduced from 14 to 0, with the highest productivity against these parasitic microbes at a concentration of 10%, with an inhibition percentage of 74.4% and 61.5%, respectively. The study found that at different concentrations of alcoholic extract, normal provinces measurements and inhibition percentages were observed for both genus. At 20% concentration, 100% inhibition percentage and zero inhibition loss were observed. Water extract at 5% and 10% concentrations resulted in 50 mm inhibition loss and 50% inhibition percentage. The study found that at a 10% concentration, *Fusarium solani* and *Curvularia manamgodae* showed inhibition losses of 10 and 30 mm, respectively, while at a 20% concentration, they showed 100% inhibition. The study found that a 20% alcoholic extract was most effective in inhibiting *Fusarium solani and Curvularia manamgodae* for 3-6 working days at 28°C. The Gas Chromatography-Mass Spectrophotometry technique analysed the alcoholic and watery extract of *Cladophora glomerata*, revealing the presence of phytochemical compounds like Hexadecane and Octadecane, which have strong inhibitory effects on organisms.

Key words: *Cladophora, Curvularia manamgodae, Fusarium solani*, gas-chromatography,

Introduction

The majority of real fungi are filamentous and branching, and they are spore-forming, non-chlorophytic, creatures. Saprophytes make up the majority of the more than 100,000 species of fungus. On the other hand, more than 20,000 different types of fungus are parasites that harm plants and crops (Imperial College, 2012). Specific fungal species have the ability to parasitise one or more types of plants, resulting in fungal infections that can show a wide range of disease symptoms (Almeida et al., 2019). Necrotrophic organisms are ones that cause necrosis in the plant's afflicted area or even destroy tissue; they can also get nourishment from decomposing leaves. Organisms that can utilise both live and dead plant tissues as substrates (biotrophs initially, later necrotrophs) make up the third type, known as hemibiotrophic organisms (Divon & Fluhr, 2007). A few pathogenic fungi linked to plant diseases include *Fusarium solani*, the most important soil-borne fungal pathogen that cause damping off and root rot diseases in a variety of crop and vegetable plants, such as peas, beans and *Curvularia sp.* belongs to Pleosporaceae, Pleosporales and Ascomycota species, including endophytes, saprobes, and diseases, are found in Curvularia (Manamgoda et al., 2015). This genus includes pathogenic species of humans, animals, and plants, as well as phytopathogenic species, which

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affect both people and plants (Carter & Boudreaux, 2004). Certain Curvularia species exhibit significant biological activities, including antibacterial, antifungal, antioxidant, cytotoxic, and phytotoxic properties, as they can create secondary metabolites. These properties may make them useful in medicine and agriculture (Khiralla et al., 2019). Microbial contamination is the main cause of foodborne illnesses, but marine plants, animals, and microorganisms have developed sustainable solutions with high biological activity over the past decade (Šimat et al., 2020). In the near future, the need to develop environmentally friendly biological preservatives as an alternative to chemicals has become a priority (Gowda et al., 2020).

Many bioactive compounds found in macro-algae, including proteins, lipids, vitamins, enzymes, sterols, and pigments, are significant in the pharmaceutical industry (Ak & Cirik, 2017). Antifungal proteins and peptides may shield food and crops against fungus infections and shield humans from fungal illnesses (Gowda et al., 2020). The bioactive substances with antibacterial, antifungal, antiviral, and antioxidant qualities include lutein, oleic acid, palmitoleic acid and vitamins (Ak & Cirik, 2017).

The study investigated the anti-fungi properties of *Cladophora glomerata* against *Fusarium solani* and *Curvularia manamgodae*, two tomato-pathogenic fungi, from tomato samples from three Baghdad sites. The results were analysed using a one-way ANOVA program to compare means of multiple groups.

Materials and Methods

Culture Media (PDA)

The manufacturer's instructions involve dissolving 39 grams of PDA in distilled water, lowering pH to 5.5, autoclaving the medium for 20 minutes, and placing the antibiotic (chloramphenicol) in sterile culture medium at 10,000 µg/ml to stop bacteria growth, allowing fungi to be separated and identified.

Samples Collection for Isolation of Fungal Pathogens

Tomato crops from different areas of Baghdad were found to contain fungi. Samples were surface sterilised with 0.6% sodium hypochlorite solution, washed twice with sterile water, dried, and cultured on PDA plates treated with chloramphenicol. The plates were incubated at 28°C for three to six days. After that, each fungal colony was sub-cultured on PDA in order to be further characterised and taxonomically identified (Samson et al., 2010).

Identification of Fungal Pathogen

Taxonomic keys were used to identify and classify isolated fungi based on their morphological characteristics, including colony advantages and microscopic examination of conidiophores, conidia, and conidia arrangement (Raper & Fennell, 1965; Simmons, 1967), to determine each isolated fungus species' proportion of occurrence and frequency of isolation:

$$\%\ \text{Occurence of species} = \frac{\text{colonies number of species}}{\text{total number of species colonies}}$$

$$\%\ \text{Frequency of species} = \frac{\text{Number of species appearance in the sample}}{\text{total number of species appearance}}$$

Algal Isolates

In June 2022, *Cladophora* sp. algae were collected from the Caspian Sea in Iran. They were washed, cleaned, and ground into a powder before being air-dried at room temperature from 25°C to 30°C on absorbent paper.

Algal extracts-Alcoholic hot extract

According to Soxhelet, an alcoholic hot extract was prepared (Petchsomrit et al., 2023). The *Cladophora* extract was extracted by supercritical fluid extraction, and the instability of the emulsions was not significantly impacted by further additions. This method extracted a dried powder of alga substance using ethanol alcohol. Ethanol has desirable advantages, including a low toxicity, and good operational security. Thus, in this study solid–liquid extractions were performed under equilibrium conditions in which the variables evaluated were temperature (ranging from 25 to 30 °C).

Algal Extracts- Hot Water

The preparation of algal extract by dried, powdered *Cladophora* sp. After 15 minutes at 121°C in an autoclave and extracted using distilled water. Insoluble components were extracted from the extracts using centrifugation for 30 minutes at 4°C. Following filtration, the residue was lyophilised and kept at –20°C after the solvent was evaporated in a vacuum condenser (Dwaish et al., 2018).

The Inhibition Percentage of mycelia growth in every case was calculated through this formula:

A = mycelial, biomass of fungi/dry weight in control

B = mycelial, biomass of fungi/dry weight in controlled in many test, and concentrations (Mondali et al., 2009).

Evaluation of Some of the Active Compounds in the Algal Extracts by Gas Chromatography-Mass Spectrophotometry

.Using accepted techniques, it was determined whether groups of active compounds were present in the algal extracts (Petchsomrit et al., 2023). Agilent Technologies (SHIMADZU, Japan) provided a high-temperature column for GC-MS spectrophotometric analysis.

The initial column temperature is fixed at 100°C, while the injector and detector temperatures are maintained at 280°C. After inserting a 5 µL tester volume into the column, split (1:10) mode is activated. The oven temperature is raised to 225°C in the next minute at a ramp ratio of 12.5°C/min (hold duration, 4 min). The oven temperature was raised to 300°C with a 5-minute hold period at a ramp level of 7.5°C/min. Mass spectra were obtained and processed using both Agilent GC-Mass, with the helium transporter gas set to a low level of 17.5 mL/min. After a 5-minute hold period, the oven's temperature was increased to 300°C at a ramp rate of 7.5°C/min. Using an Agilent GC-Mass, mass spectra were acquired and analysed, then the helium transporter gas was adjusted to a low level of 17.5 mL/min.

Statistical Analyses

Every experiment was organised using a fully randomised block design. To examine variations among treatments, a one-way ANOVA analysis is used to compare means of more than two groups, The SAS (SAS Institute Inc.) analytical system's general linear model option (SAS, 1996) was employed in the ANOVA. Mean separation was determined using Duncan's multiple range tests at $P < 0.05$.

Results and Discussion

Samples Collection for the Isolation of Fungal Pathogens

Fungi were isolated from tomato fields from different locations in Baghdad city (Al-Mahmoudiyah, Alwa Al-Rasheed and Abu Ghraib). The samples were initially subjected for surface sterilisation with 0.6 % sodium hypochlorite solution for 2 min and rinsed twice with sterilised distilled water. Samples were dried with sterile filter paper, cultured on (PDA) plates supplemented with antibiotic chloramphenicol at 3 pieces from samples per plate in triplicates and incubated at 28°C for 3-6 days. Each fungal colony obtained was then sub cultured on PDA for subsequent characterisation and taxonomic identification (Samson et al., 2010).

Characterisation of the Pathogenic Fungi

Samples of tomatoes suspected of having *F. solani* and *C. manamgodae* infections were collected from three locations. Table 1 lists the number of samples that were obtained from each site out of all the samples that were discovered. The outcome of the week-long incubation procedure at 28°C was the isolation of fungi, which were then purified and categorised under the protocol.

The second site of Baghdad, Alwa Al-Rasheed, which is situated southwest of Baghdad, is distinguished in the table by the quantity of fungi that have been isolated from it. This is a result of the tomatoes' lengthy storage and transportation times, as they were primarily imported (Diana Escamilla et al., 2019), and the variations in climate between the cultivation and sale locations. Based on the information presented in the above table, it was discovered that a variety of fungi were connected to samples taken from plants that were affected (Figure 1), Because for a variety of reasons, including the variations in the circumstances surrounding their presence from one habitat to another, none of those fungi have the capacity to germinate once again. Numerous studies have confirmed this, citing the pesticides used on farms to battle this fungus as well as the presence of strong rivals from the surrounding fungi (Ananya et al., 2018).

Test the Effect of Alga Extracts

At concentrations of 5% and 10% (v/v) from the extract and for both types of fungal, respectively (74.4% and 61.5%), the inhibition loss reached 8.2 mm. While at the concentration of 15%, the average colonies diameters

Table 1: Showed the numbers of tomatoes samples having *F. solani* and *C. manamgodae* were collected from three locations

Sample site	*Number of samples*	*Number of fungi colony*	*Fusarium occurrence*	*Fusarium frequency*	*Curvularia occurrence*	*Curvularia frequency*
Al Mahmoudiyah	211	342	67	20%	80	24%
Alwa Al-Rasheed	166	377	81	22%	72	19%
Abu Ghraib	89	189	45	24%	11	6%

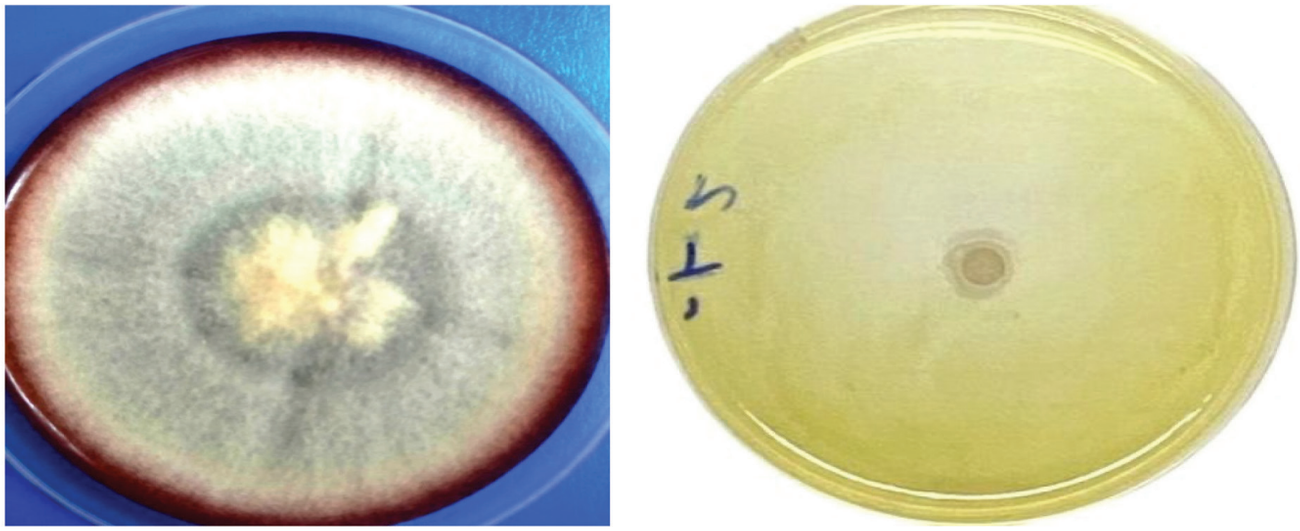

Figure 1: The effect of ethanolic hot extracts of 20% concentration on *C. glomerata*, against *F. solani.*

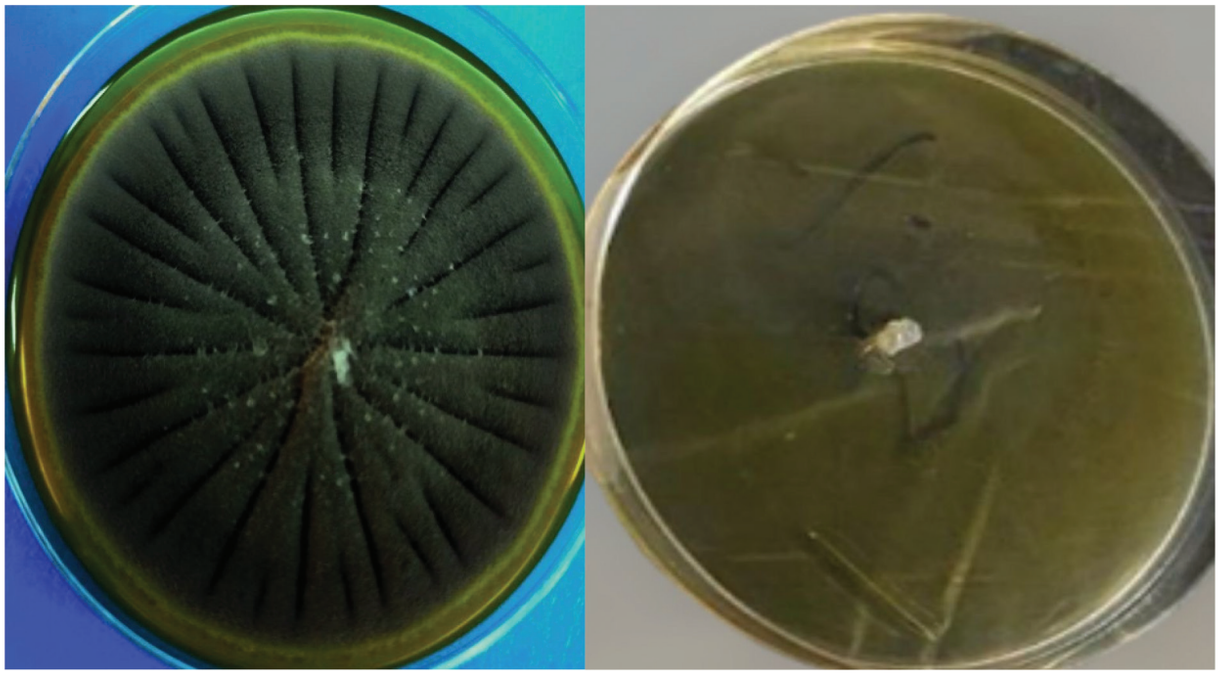

Figure 2: The effect of ethanolic hot extracts of 20% concentration on *the C. glomerata* against *C. manamgodae*.

of Fusarium and *Curvularia* were (0, 0 mm) and the percentage of Inhibition were 100% for both types of fungal. Besides, at the concentrations of 20% the same percentage of inhibition as 100% for both types of fungal, the average diameters of fungi were zero. As shown in Figure 2, the effect of ethanolic hot extracts on *C. glomerata* with concentration of 20% against *C. manamgodae* for 3-6 days.

The average diameters of the fungal colonies *Fusarium* and *Curvularia* at concentrations of 5% and 10% of the aqueous extract were 50 and 50 mm, respectively, with corresponding inhibition % of 50 and 50%. At concentrations of 10%, the fungal colonies' diameters were 10 and 30 mm, with corresponding of inhibition of 88.9%, and 66.9%, respectively, and 100% for the fungi at 20% concentration, as shown in Table 2.

Table 2: Effect of alcoholic and aqueous extracts of *C. glomerata* on fungal growth (mm)

Extract concentration	*Fungal growth (mm)*			
	Alcoholic extract		*Aqueous extracts*	
	Fusarium	*Curvularia*	*Fusarium*	*Curvularia*
5	14	20	50	50
10	8	2	10	30
15	0	0	3	14
20	0	0	0	0
Cont.-	90	85	90	90
L.S.D.	0.05	4.12		

Substantial variations between the several *Cladophora* extracts were shown by the statistical analysis at the 0.05 probability level. Since the fungus were the ones most impacted by *Cladophora* extracts, the alcoholic extract ranked highest in terms of inhibitory activity, followed by the aqueous extract.

The chemicals from the *Cladophora* extracts may have contributed to the alcoholic extract's advantage over the aqueous extract in inhibiting the growth of fungi and poisonous materials including glycosides, tannins, flavonoids, and resins (Mittal & Gupta,2010). Depending on the type of microorganism and the type of extract, different levels of inhibition are seen by the extract, according to the data. Due to the high concentration of active chemicals present in the extract, the inhibitory efficiency was enhanced by increasing the concentration of the extract. This indicates that the effect depends on the type and strength of the extract, in addition to the type of mushroom used. The fungicidal activity of the extract can be attributed to a variety of chemical components, including phenols, triterpenoids, and flavonoids, which have an impact on the development and metabolism of the fungus. Our findings align with those of (Dwaish and Maarb, 2018), who identified similar compounds in algae, particularly green algae. Therefore, it is essential to consider expanding algae production due to its capacity to inhibit the growth of fungi that cause economic damage to crops

Gas-Mass Chromatography Method for Active Compound Detection

An effective technique for separating and identifying erratic organic substances from a mixture of non-organic chemicals is gas chromatography (GC-MASS) (Duan et al., 2020). Moreover, their composition, concentration, compounds, free hydroxyl group amides, and alkaloids may have an activating or inhibitory influence on microbial development (Yu et al., 2009). The extract includes many peaks and consists of at least a mixture of more than ten major components compounds, these compounds constitute 96.68% of the area of the extract, while the remaining percentage was neglected because the area of each compound is very teeny, and some of them did not correspond to the standard data compounds stored in the machine system. In the alcoholic extract of *Cladophora,* the main compounds are hexadecane (55%), octadecyl (22%), and acetic acid, (1,1- dimethyl ethyl) 5.57%) as shown in Table 3.

The main compounds were found in alcoholic crude extracts of algae was *hexadecane*, which is included in the acyclic di terpenes, this component has antimicrobial activity, also *Octadecane* which belongs to the hydrocarbons class, which has many bioactivities such as Transformer oil and Pheromones, the general term for the family of aliphatic hydrocarbons Cn-H2n+2, which represent reactive groups, is Pentadecane. In order to assess the chemical complexity of analytical samples according to their ability for separation and identification, GC-MS is often used. Advances in GC-MS technology have made it easier to apply global metabolomics to study biological processes and biological system disturbances, as well as for quality monitoring and diagnostic applications.

Conclusion

The current research presents findings that demonstrate the significance of *Cladophora algae* imported from the Caspian Sea on tomato disease-causing fungi such as *C. manamgodae and F. solani*. It was determined that the alcoholic extract of *C. glomerata* exhibited superior inhibition of fungal growth compared to the aqueous extract across all concentrations. It was found that increasing the concentration of aqueous and alcoholic extracts (5%, 10%, 15%, and 20%) v/v, leads to a decrease in the number of fungi. The results showed

Table 3: The compounds where identified in ethanol hot extract of *Cladophora glomerata* by using GC-Mass spectrophotometer

No.	*Rt.*	*Area%*	*Name of compound*	*Biological Activity*	*Reference*
1	9.477p	35.33	Methane, sulfinylbis	Anti-bacterial activity	Seguí et al., 2021
2	9.47p7	35.33	Dimethyl sulfoxide	Antimicrobial Effect	Kirkwood et al., 2018
3	49.473	1.40	Tetradecanoic acid	Anti-bacterial activity	Mohadjerani et al., 2016
4	52.050	0.35	2-Pentadecanone	Antioxidant Activities	Zhao et al., 2018
5	54.011	0.46	Nonadecane	Antimicrobial activity	Zhao et al., 2018
6	55.702	48.49	Bis (2-ethylhexyl) phthalate	Cytotoxic activity	Momen et al., 2018
7	55.702	48.49	Phthalic acid,	Antibacterial activity	Rajamanikyam et al., 2017
8	55.702	48.49	1,2-Benzenedicarboxylic acid,	Antimicrobial activity	Shoge et al., 2016
9	56.302	3.92	n-Hexadecanoic acid	Anti-bacterial activity	Mohadjerani et al., 2016
10	60.435	0.69	Octadecane	Anti-bacterial activity	Rouis-Soussi et al., 2014
11	60.435	0.69	Heneicosane	Microbicidal bioactive	Vanitha et al., 2020
12	60.435	0.69	Nonadecane	Antimicrobial activity	Hsouna et al., 2011
13	61.658	1.44	9,12-Octadecadienoic acid	Antimicrobial activity	Rahman et al., 2014
14	61.846	4.06	Oleic Acid	Antibacterial	Awa et al., 2012
15	61.846	4.06	9-Octadecenoic acid	Antibacterial activities	El-Aty et al., 2014
16	66.156	1.92	Methyl ester of Ricin oleic acid	Antibacterial activity	Fitranda & Marfu'ah, 2020
17	68.253	0.58	9-Octadecenamide	Antifungal activity.	Dos Reis et al., 2019

a difference in the degree of inhibition of the extract dependent on the type of the microorganism and the type of extract. The competence of the inhibition was increased to 100% by increasing the concentration of the alcoholic extract to 20% because of increasing the active compounds. This means that the impact depends on the type and concentration of the extract, as well as the genus of fungi, which is being used. This resulted in preventing the growth of fungi including attributed to the *Cladophora* alcoholic and aqueous extracts which contain toxic substances such as resins, flavonoids, tannins and glycosides as previously proven (Mittal and Gupta, 2010). The algal extract contains various active compounds, including hexadecane, diterpene compound with potent antifungal properties, and octadecane, where associated with hydrochlorocarbon compounds, along with numerous alkanes. Cultivating seaweeds is an additional advantage for the large-scale production of potential antifungal products.

Conflict of Interest

The authors declare that there are no conflicts of interest.

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