Journal of Scientific Research and Reports

Volume 30, Issue 9, Page 411-421, 2024; Article no.JSRR.122579
ISSN: 2320-0227

Antifungal Efficacy of *Kappaphycus alvarezii* and Other Macro-algae against *Alternaria solani* Induced Early Blight in Tomato (*Solanum lycopersicum* L.)

Elanselvi. A [a], Mahalakshmi. P [a*], Thamizh Iniyan. A [b], Harish. S [a], Kavitha M [c], Santhana Krishnan V.P [d] and Muthukumar. G [a]

[a] Department of Plant Pathology, Tamil Nadu Agricultural University, Coimbatore -641003, Tamil Nadu, India.
[b] Department of Genetics and Plant Breeding, Tamil Nadu Agricultural University, Coimbatore -641003, Tamil Nadu, India.
[c] Department of Vegetable Science, Tamil Nadu Agricultural University, Coimbatore - 641003, Tamil Nadu, India.
[d] Department of Medicinal and aromatic crops, Tamil Nadu Agricultural University, Coimbatore -641003, Tamil Nadu, India.

Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

Article Information

DOI: https://doi.org/10.9734/jsrr/2024/v30i92364

Open Peer Review History:
This journal follows the Advanced Open Peer Review policy. Identity of the Reviewers, Editor(s) and additional Reviewers, peer review comments, different versions of the manuscript, comments of the editors, etc are available here: https://www.sdiarticle5.com/review-history/122579

Original Research Article

Received: 22/06/2024
Accepted: 28/08/2024
Published: 01/09/2024

**Corresponding author: E-mail: mahalakshmi.p@tnau.ac.in;*

Cite as: *A, Elanselvi., Mahalakshmi. P, Thamizh Iniyan. A, Harish. S, Kavitha M, Santhana Krishnan V.P, and Muthukumar. G. 2024. "Antifungal Efficacy of Kappaphycus Alvarezii and Other Macro-Algae Against Alternaria Solani Induced Early Blight in Tomato (Solanum Lycopersicum L.)". Journal of Scientific Research and Reports 30 (9):411-21. https://doi.org/10.9734/jsrr/2024/v30i92364.*

ABSTRACT

Aims: To evaluate the antifungal activity of various seaweed extracts against *A. solani* under in vitro conditions.

Methodology: The pathogen *A.solani* was isolated and its pathogenicity confirmed through inoculation on tomato plants. Molecular characterization was performed using PCR amplification and sequencing of the Internal Transcribed Spacer (ITS) region. Seaweed species, including *Sargassum wightii*, *Kappaphycus alvarezii*, *Gracilaria edulis*, *Caulerpa racemosa*, and *Ulva lactuca*, were collected, processed, and extracted using various solvents. The antifungal activity of these seaweed extracts was assessed using the Poisoned Food Technique, measuring the inhibition of *A. solani* growth.

Results: Among the seaweed extracts tested, *Kappaphycus alvarezii* demonstrated the highest antifungal activity, with a maximum mycelial growth inhibition of 76% at a 5% concentration. *Sargassum wightii* also showed significant antifungal properties, with a 71.10% reduction in mycelial growth.

Conclusion: The study reveals the promising potential of seaweed extracts, particularly *Kappaphycus alvarezii* and *Sargassum wightii*, as natural antifungal agents against *A. solani*. These findings suggest that seaweed-based bio-stimulants could serve as effective, eco-friendly alternatives to chemical fungicides in managing early blight disease in tomatoes are in tomato crops and highlights the potential of seaweed extracts as a natural antifungal agent.

Keywords: Antifungal activity; early blight disease; seaweeds; tomato; organic solvents.

1.INTRODUCTION

A member of the Solanaceae family, the tomato (*Lycopersicon esculentum* Mill) is one of the world's most widely grown and highly profitable vegetables. Tomato is the second most consumed vegetable after potatoes [1]. Because of their many colours, shapes, sizes, and tastes, tomatoes are enjoyed in many situations and can be eaten raw or cooked [2]. It is cultivated because of its edible fruits, which may be eaten raw or cooked and are rich in minerals and vitamins A, B, and C. Plant disease caused by bacteria, fungi, and viruses can severely affect tomato crops, which leads to reduced fruit yield and quality. Early blight of tomato caused by *Alternaria solani* is one of the main diseases that affects tomatoes resulting in major yield losses. It infects many parts of the plant, like leaves, roots, seeds and destroys up to 79% of plants in worldwide [3]. Fungal diseases are often controlled with chemical fungicides, but these chemicals are hazardous to the environment, harm beneficial microorganisms and make plants resistant to the fungicides. The development of alternative control strategies, such as the application of bio-stimulants like seaweed extracts, is increasingly essential for the efficient management of diseases. Recent years have observed an increase in interest for macro- algae due to their antifungal, antibacterial, antiviral, antioxidant, anti-inflammatory, cytotoxic, and antimitotic properties [4]. According to reference [5], seaweeds are rich in antifungal compounds such phenolic compounds and terpenes, that promote plant growth and productivity by inhibiting the growth of diseases. Therefore, our present study the use of seaweed products as a natural and eco-friendly way of combating *A. solani* under *invitro* conditions.

2. MATERIALS AND METHODS

2.1 Survey on Disease Incidence of Early Blight of Tomato

Early blight-infected leaf samples have been collected from major tomato-growing regions of TamilNadu Coimbatore, Dharmapuri, Krishnagiri, Theni, Salem, and Madurai. The purified cultures have been preserved on PDA slants and used for further study. The percent disease incidence was determined using Vidhyaksekaran's method [6].

$$\text{PDI } \% = \frac{\text{No of Plants Infected}}{\text{Total number Plants observed}} \text{x } 100$$

2.2 Symptoms

According to reference [7], the disease's initial manifestations were small, black necrotic lesions on the older leaves that eventually spread upward. As the lesions develop, they usually become larger and resemble target boards with concentric rings often surrounded by a yellow zone and the whole plant defoliates. Lesions on

young tomato seedlings can entirely girdle the stem, resulting in "collar rot," which can cause reduced plant vigor or death [8] (Fig.2).

2.3 Isolation of Early Blight Pathogen

The infected plant samples were taken from major tomato-growing districts of Tamil Nadu, based on the symptoms caused by early blight. The affected specimens will be used to isolate the pathogen. A sterile scalpel will be used to cut the diseased tissue from the infected plant, along with some healthy tissue, into small pieces (5 mm diameter). The plant exhibiting typical symptoms will then be rinsed three times with sterilized water and disinfected with 1% sodium hypochlorite solution for a minute. The infected samples will be placed aseptically on potato dextrose agar (PDA) medium, and the plates will be incubated at room temperature in the invert position. After seven days of incubation, the fungal growth transferred aseptically to PDA slants and purified following the Single Spore Technique [9].

2.4 Pathogenicity Test

Earthen pots of 30 cm diameter were filled with sterilized potting mixture and placed in glass house. The potting mixture consists of red soil + sand + FYM (2:1:1) was sterilized in an autoclave at 121 °C at 15 psi for 2 hrs for two consecutive days. For testing the pathogenicity, the tomato cultivar PKM 1 was used. Three tomato seeds per pot were planted in the PKM1 variety. The pathogenicity of eight distinct *A. solani* isolates (TA-1, TA-2, TA-3, TA-4, TA-5, TA-6, TA-7, and TA-8) was examined under potculture condition. Thirty to forty-five days after seeding, the tomato plants were sprayed with a spore solution that contained $2.5x10^6$ spores/ml. The plants started to show symptoms of early blight. The inoculated plants were re-isolated if the symptoms were similar to those from the infected field. The re-isolated cultures were again inoculated to PKM-1 cultivar and observed the characteristics symptoms. Control pots are sprayed only with sterile distilled water [10].

2.5 Molecular Characterisation

2.5.1 PCR amplification of the Internal Transcribed Spacer (ITS) region of the rDNA

CTAB method was used to extract the DNA after the pathogenic isolates had been cultured in potato dextrose broth for 15 days. Amplification of ITS regions in isolates was carried out by using a universal primer ITS1 and ITS4. PCR was performed with a reaction volume of 10 µl and the reaction cycle consisted of 60 seconds at 94 °C for denaturation, 45 seconds at 53 °C for annealing, and 90 sec at 72 °C for extension [11]. ITS1 (5'-TCC GTA GGT GAA CCT GCG G-3') ITS4 (5'- TCC TCC GCT TAT TGA TAT GC-3').

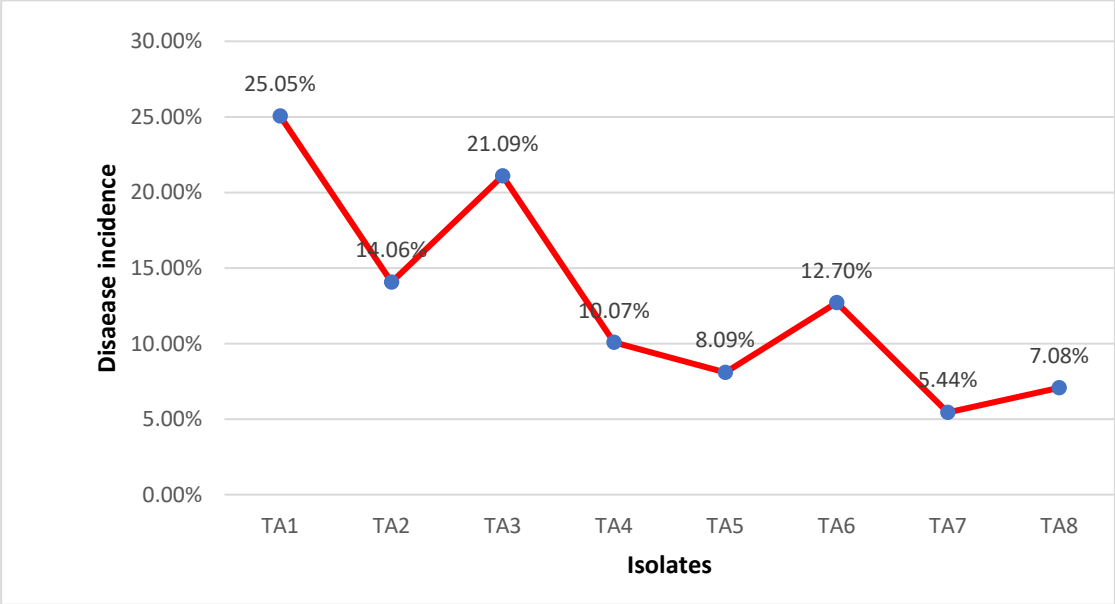

Fig. 1. Occurence of early blight from Tamil Nadu

Fig. 2. Symptoms of early blight

2.5.2 Agarose gel electrophoresis

Based on the procedure outlined by reference [12], agarose gel electrophoresis was carried out to assess the DNA's purity and to separate the products of the polymerase chain reaction. The gel was photographed and analysed using an ultraviolet transilluminator. The PCR product sizes were evaluated by comparing them to a standard 1kb ladder (Bangalore Genei Pvt. Ltd., Bangalore, India).

2.6 Collection of Seaweeds

Different kinds of seaweed were carefully chosen from the deep-water regions of Rameswaram, Tamil Nadu. To remove any debris or contaminants, the seaweed samples were carefully cleaned twice: once with fresh water and once with distilled water. The seaweed samples were then allowed to air dry for two or three weeks under shade after being carefully wiped to eliminate any remaining moisture. Following the process of drying, the seaweed samples were ground into a powder and kept in an room temperature with dry conditions and an average temperature between 28 and 37 degrees Celsius [13]. The seaweed extracts were made using a variety of solvents, including hexane, ethyl acetate, and methanol to assess their antifungal activity.

2.7 Preparation of Seaweed Extract through Soxhlet Apparatus

Twenty grams of partly mixed seaweed powder were put into a Soxhlet instrument to make the seaweed extract. This seaweed powder was encased in cellulose thimble paper and refluxed for 12 hours with 150 milliliters of the solvents *viz.,* ethyl acetate, methanol, acetone and hexane. After extraction, the solvent was filtered using Whatman No. 1 filter paper to get rid of any impurities. A rotary evaporator operating at 40 °C and 45 revolutions per minute was used to evaporate the collected solution until all of the solvent had evaporated [14]. After being diluted with the appropriate solvents, the final extract was kept for future use at -4 °C [15].

2.8 Antifungal Activity of Seaweeds against *A. solani*

2.8.1 (Poisson food Technique)

The poissoned food technique was used to test the antifungal properties of seaweed extracts. Different seaweed extracts (1%, 3%, and 5%) were incorporated into potato dextrose agar medium, which was then autoclaved and transferred into sterile petriplates. Using a sterile cork borer, discs of 5 mm in diameter were cut from the periphery of an *A. solani* culture which was 5 days old. The discs were then aseptically placed onto PDA plates poisoned with seaweed extracts. As a control, the medium was used without adding the extract. Following 48, 72, and 96 hours of incubation, the colony diameter of the infected plates was measured and recorded at 25 °C. For every treatment, three plates per replication were maintained. The average colony diameter was measured after the experiment was conducted twice. The percentage inhibition (PI) of mycelial growth was calculated using the formula suggested by Pandey et al. [16].

$$PI = Dc – Dt / Dc \times 100$$

Where,

PI = Percent Inhibition
C= Average diameter of fungal growth (cm) in control,
T= Average diameter of fungal growth (cm) in treatment.

2.8.2 Different concentrations of promising seaweeds extract against the *A.solani*

The different concentrations of methanol extract of *S. wightii* and *K. alvarezii* were prepared from the stock solution (1%, 3% and 5%) by the poisson food method.

2.9 Statistical Analysis

To evaluate the mean differences among the treatments, an analysis of variance (ANOVA) was conducted and Duncan's Multiple Range Test at a significance level of 5% was employed [17].

3. RESULTS

3.1 Survey

Roving method of survey conducted in major tomato growing areas of Tamil Nadu cultivating tomato. Kongu Thirupathi, Viraliyur, Kambainallur, Vadakarai, Krishnagiri, Dharmapuri, Salem, Perambalur, Madurai and kinnathukadavu are the places surveyed. Samples were taken from plants having early blight symptoms. The results showed that the percentages of disease occurrence varied from 5.44% to 25.05%. Kongu Thirupathi village in Coimbatore showed the highest disease incidence of 25.05%, followed by shoolagiri village in Krishnagiri district, which showed 21.09%. However, the lowest disease incidence percentage of 5.44 % was recorded in Salem district (Table 1 and Fig.1).

3.2 Isolation of Tomato Early Blight

The isolated pathogen cultures in the current investigation began initially white but eventually developed to a greyish-white color with cottony growth on the PDA medium. The single hyphal tip approach was used to purify the cultures in order to maintain pure cultures. The disease took around 10 to 12 days to cover the entire 9 cm plate. As shown in Fig. 3, the pure cultures were frequently sub-cultured and kept in the refrigerator to ensure the purity of the isolate. Mycelium are septate, branched and have dark color, whereas spores are elongated, muriform, beaked, and septate. The margin of colonies was either uneven or smooth. These results were consistent with the initial observations of the genus Alternaria infecting crops published [18]. Colony traits of *Alternaria* exhibit color variations, ranging from grey to ashy grey, whitish grey,blackish grey and mycelial growth was observed flat or elevated, colony texture varied from velvety to rough, and colonies were seen to be smooth or uneven [19].

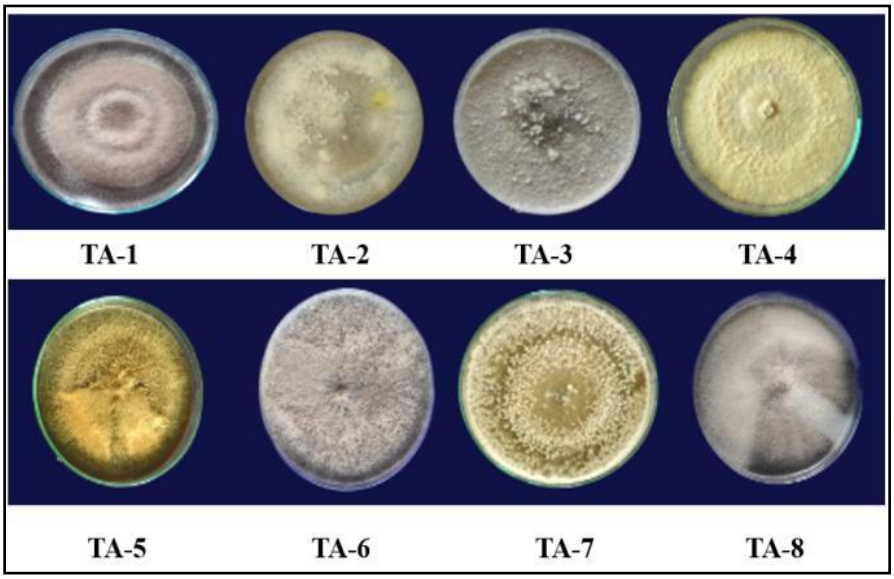

Fig. 3. Different isolates of *A. solani*

Table 1. Disease incidence of early blight disease in major tomato growing areas of Tamil Nadu

S.No.	Place of collection	District	Isolates	Stage of the crop	Geo coordinates		Early blight incidence (%)
					Latitude	Longitude	
1.	Kongu Thirupathi	Coimbatore	TA1	Flowering and early fruit formation	11.0017262º	76.8140771º	25.05[a]
2.	Viraliyur		TA2	Flowering and early fruit formation	11.0102502º	76.8021622º	14.06[c]
3.	Shoolagiri	Krishnagiri	TA3	Flowering stage	10.8172022º	76.9843839º	21.09[b]
4.	Kambainaallur	Dharmapuri	TA4	Vegetative stage	12.218872º	78.320145º	10.07[e]
5.	Puliyur		TA5	Flowering stage	12.219437º	78.322255º	8.09[f]
6.	Aviyur	Madurai	TA6	Early fruit formation stage	9.91311º	77.98699º	12.70[d]
7.	Sikkampatti	Salem	TA7	Flowering and early fruit formation stage	11.563942º	78.148933º	5.44[g]
8.	Koneripalayam	Perambalur	TA8	Vegetative stage	11.26138º	78.858461º	7.08[h]
SEd =							0.0857
CD (0.05) =							0.1817

Table 2. *In vitro* screening of different solvents of different seaweed extracts against *A.solani* (TA-1)

Different seaweeds	1%		3%		5%	
	Radial growth*(cm)	PIOC (%)	Radial growth*(cm)	PIOC (%)	Radial growth*(cm)	PIOC (%)
Sargassum wightii	7.13	20.00[b]	5.10	43.33[b]	2.66	71.10[b]
Kappaphycus alvarezii	6.76	25.50[a]	4.20	53.33[a]	2.16	76.00[a]
Ulva lactuca	7.40	17.70[c]	5.46	39.33[c]	3.16	65.5[c]
Caulerpa racemosa	7.50	16.66[d]	5.50	38.88[d]	3.43	62.22[d]
Gracilaria edulis	8.03	10.70[e]	6.23	30.77[e]	5.05	44.4[e]
Control	9.00	0	9.00	0	9.00	0
CD(P=0.05%)	0.269	0.433	0.289	0.417	0.257	0.269

**Mean of three replications*

3.3 Pathogenicity

The pure culture of *A. solani* was obtained by single spore isolation method and sub culture was used for the pathogenicity experiment by following Koch's postulate. The pathogenicity experiment was carried by pre-inoculation with spore suspension and homogenized mycelial bits of *A. solani* on foliage of 30 days old plants of PKM 1 cultivar of tomato. After inoculation, the symptoms appeared on inoculated leaves as brown,oval or circular necrotic spots with concentric rings surrounded by a border of yellow host tissue shown in (Fig.4).The fungus was re-isolated and purified culture from these artificially infected leaves was similar to that of original culture. Thus, pathogenicity on tomato early blight was confirmed.

Among the isolates, TA-1 demonstrated the highest virulence, resulting in a disease incidence of 96.14%. It was closely followed by TA-4, which exhibited a disease incidence of 90.12%. On the other hand, isolate TA-8 displayed the lowest disease incidence, with a rate of 35.65%. Based on these findings, it was determined that the TA-1 isolate of *A.solani* was the most virulent among the tested isolates, warranting further investigation (Fig.5).

3.4 Molecular Characterization of Pathogen (*Alternaria solani*)

To identify *Alternaria* spp. by sequencing, Polymerase Chain Reaction was performed for eight isolates using universal primers such as ITS1 and ITS4. The amplified ITS-DNA sequence was run in Agarose gel and ITS-DNA sequence of 560 bp was viewed using gel documentation system shown in (Fig.6). Further, the amplified ITS PCR product was sequenced by Sanger sequencing. The ITS sequences of the pathogen were BLAST searched at NCBI database and the ITS sequences of pathogen showed 98% homology to that of *A. solani* and obtained sequences were entered into GenBank database to get accession number (PP724524). The results resembled the findings of the reference [20], who observed that all Alternaria isolate obtained 560 bp bands with their ITS primers. While the *A. macrospora* specific primers (Am) and the *A. alternata* species-specific primers (Aa) consistently amplified at 442 bp and 320 bp, respectively, for all the isolates, the genus-specific primers amplified several bands for every isolate. The identified *Alternaria spp.* population exhibited a high level of genetic homogeneity, according to sequence alignments of the ITS (570 bp) and TEF1 (470 bp) [21].

3.5 *In vitro* Screening of Seaweeds against the pathogen *A. solani*

The present study aimed to investigate the antifungal activity of seaweed extracts, specifically bio-stimulants, against the tomato early blight pathogen *A. solani*. Different solvent extracts, namely methanol, ethyl acetate, and hexane were obtained from five seaweed species, including *Sargassum wightii, Kappaphycus alvarezii, Gracilaria edulis, Caulerpa racemosa* and *Ulva lactuca* (Fig.7). These extracts were then analysed for their antifungal potential. Notably, the results revealed a novel finding in the field of natural antifungals.

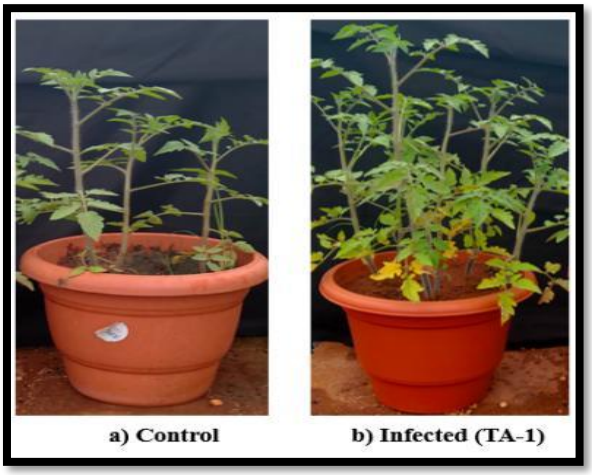

Fig. 4. Pathogenicity test under glass house experiment

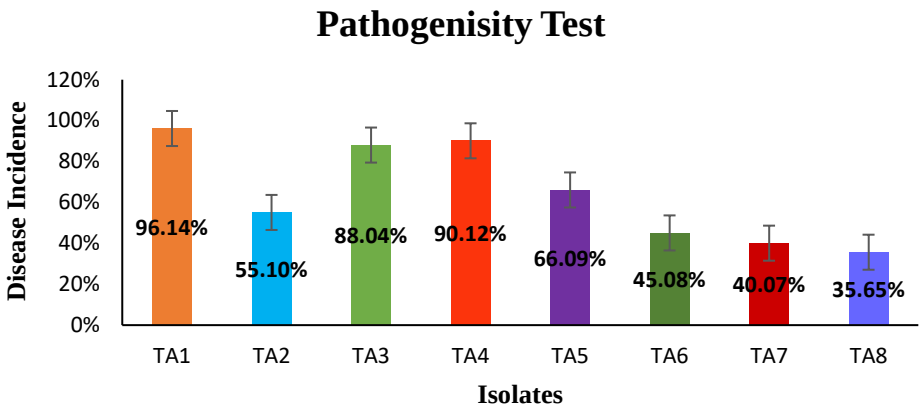

Fig. 5. Evaluating the virulence of different isolates of *A.solani*

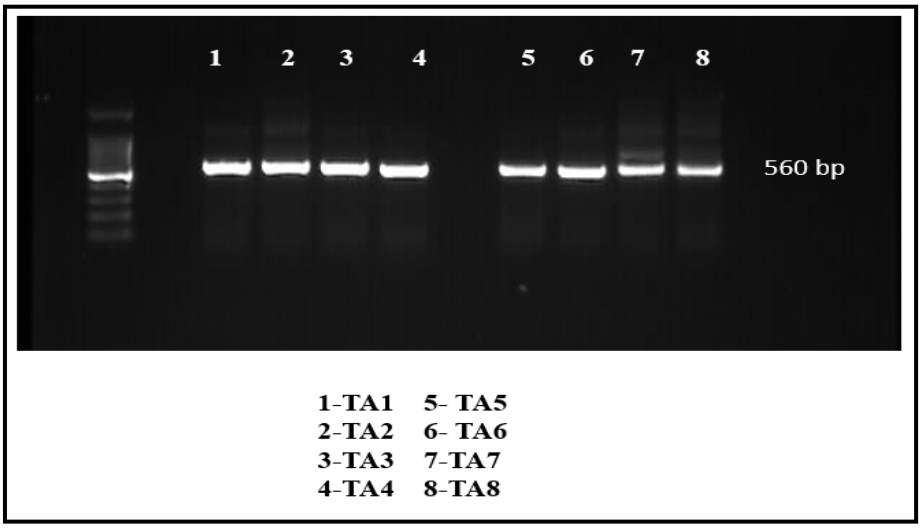

Fig. 6. Gel documentation of various isolates of *A.solani*

Fig. 7. Collection of seaweeds

Among the tested solvents the methonalic extract showed the highest reduction percetange compared to others. Among the different seaweed extracts, *Kappaphycus alvarezii* exhibited an unprecedented and remarkable reduction in mycelial growth, with a size of only 2.61 cm under aseptic conditions. This dramatic inhibition translated to a maximum percent reduction over the control, reaching an impressive 76.00%. This significant antifungal activity of *Kappaphycus alvarezii* showcases its untapped potential as a potent source of natural compounds for combating early blight of tomato. Furthermore, *Sargassum wightii* demonstrated a noteworthy second-highest percent reduction in mycelial growth, with a size of 2.66 cm and a reduction of 71.10%. This finding highlights the promising antifungal properties of *Kappaphycus alvarezii*, which can potentially contribute to the development of alternative strategies to manage tomato early blight. The *Gracilaria edulis*, although exhibits lower percent reduction over the control at 44%, still displayed some degree of antifungal activity (Table 2, Fig. 8, Fig. 9 and Fig. 10). This suggests the presence of unique bioactive compounds within that may contribute to the overall antifungal potential of seaweed extracts. Overall, the novel findings of this study shed light on the previously unexplored antifungal activity of seaweed extracts against *A. solani*. The remarkable inhibitory effect of *Kappaphycus alvarezii* and the promising results obtained from *Sargassum wightii* emphasize the potential of these seaweed species as valuable sources of natural antifungal compounds. These findings open new avenues for further research and the development of eco-friendly alternatives for managing early blight of tomato.

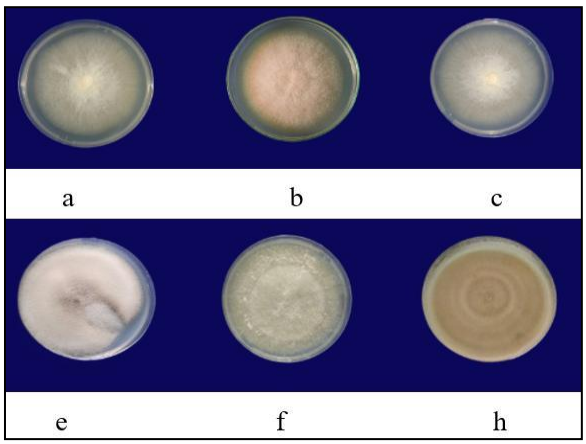

Fig. 8. Methanol extract of different seaweeds @ 1%
(a. Sargassum wightii, b. Kappaphycus alvarezii, c. Ulva lactuca, d. Caulerpa racemosa, e. Gracilaria edulis, f. Control)

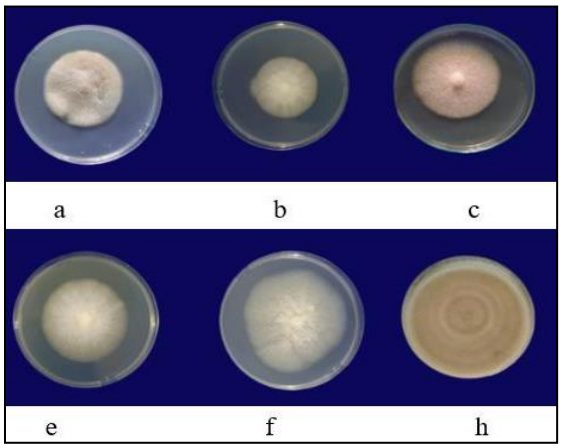

Fig. 9. Methanol extract of different seaweeds @ 3%
(a. Sargassum wightii, b. Kappaphycus alvarezii, c. Ulva lactuca, d. Caulerpa racemosa, e. Gracilaria edulis, f. Control)

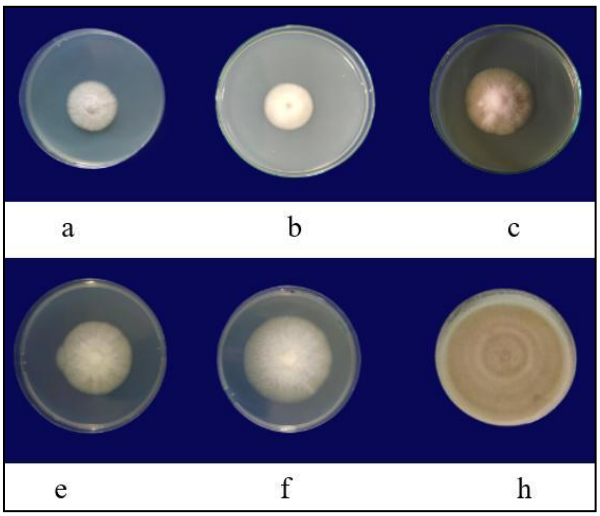

Fig. 10. Methanol extract of different seaweeds @ 5%
(a. Sargassum wightii, b. Kappaphycus alvarezii, c. Ulva lactuca, d. Caulerpa racemosa, e. Gracilaria edulis, f. Control)

4. DISCUSSION AND CONCLUSION

This study demonstrates the wide range in the occurrence of Alternaria solani-caused tomato early blight in Tamil Nadu, India. The most significant incidence was observed at 25.05% in Kongu Thirupathi, Coimbatore. The pathogen had been identified by its distinctive morphological characteristics, that were in line with earlier studies on *Alternaria spp*. and included a septate, branching mycelium and muriform, beaked spores. The isolated strains were shown to be the cause of the symptoms, according to pathogenicity testing, with the TA-1 isolate exhibiting the maximum virulence at 96.14%. *A. solani* identification was verified by molecular characterisation using ITS sequencing, which revealed 98% similarity with known sequences. The sequence was then added to GenBank (Accession No. PP724524). These findings provide important understandings for the management of early blight disease in tomato and highlight the potential of seaweed extracts as alternative control measures. Further research is needed to explore the active compounds responsible for the antifungal activity and their practical application in disease management strategies.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc) and text-to-image generators have been used during writing or editing of manuscripts.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Quinet M, Angosto T, Yuste-Lisbona FJ, Blanchard-Gros R, Bigot S, Martinez JP, Lutts S. Tomato fruit development and metabolism. Frontiers in plant science. 2019;10:1554.
2. Zörb C, Piepho HP, Zikeli S, Horneburg B. Heritability and variability of quality parameters of tomatoes in outdoor production. Research; 2020.
3. Chaerani R, Voorrips RE. Tomato early blight (Alternaria solani): the pathogen, genetics, and breeding for resistance. Journal of general plant pathology. 2006;72:335-47.
4. Demirel Z, Yildirim ZD, Tuney I, Kesici K, Sukatar A. Biochemical analysis of some brown seaweeds from the Aegean Sea. Botanica Serbica. 2012;36(2):91-5.
5. Rathore SS, Chaudhary DR, Boricha GN, Ghosh A, Bhatt BP, Zodape ST, Patolia JS. Effect of seaweed extract on the growth, yield and nutrient uptake of soybean (*Glycine max*) under rainfed conditions. South African Journal of Botany. 2009;75(2):351-5.
6. Vidhyasekaran P. Concise encyclopedia of plant pathology; 2004.
7. McCarter SM, Jaworski CA, Johnson AW. Soil fumigation effects on early blight of

tomato transplants. Phytopathology. 1976;66(9):1122-4.
8. Bessadat N, Berruyer R, Hamon B, Bataille-Simoneau N, Benichou S, Kihal M, Henni DE, Simoneau P. Alternaria species associated with early blight epidemics on tomato and other Solanaceae crops in northwestern Algeria. European Journal of Plant Pathology. 2017;148:181-97.
9. Tuite J. Plant pathological methods. Fungi and bacteria.
10. Gyempeh N, Offei SK, Cornelius EW, Honger JO. Importance of the onion leaf twister disease in Ghana and the effect of Trichoderma asperellium on the mycelial growth and sporulation of the causal agent. Ghana Journal of Science. 2015;55:51-65.
11. Mohammadi A, Bahramikia S. Molecular identification and genetic variation of Alternaria species isolated from tomatoes using ITS1 sequencing and inter simple sequence repeat methods. Current Medical Mycology. 2019;5(2):1.
12. Sambrook J. Molecular cloning: A laboratory manual, Cold Spring Harbor, NY: Cold Spring Harbor Laboratory Press. 9. (No Title). 1989;14:23.
13. Kannan R, Bharathkumar R. Bio-efficacy of two sea weeds methanol extract on growth and development of Spodoptera litura Fabricius. Annals of Plant Protection Sciences. 2016;24(1): 1-5.
14. Bhosale JW. Physiological analysis of growth. and yield in sesamum *(Sesamum indicum* L.) Genotypes.
15. Kombiah P, Sahayaraj K. Repellent activity of Caulerpa scalpelliformis extracts and its formulations against Spodoptera litura and Dysdercus cingulatus (Fab.). Journal of biopesticides. 2012;5:145.
16. Pandey MC, Sharma JR, Dikshit A. Antifungal evaluation of the essential oil of Cymbopogon pendulus (Nees ex Steud.) Wats. cv. Praman. Flavour and Fragrance Journal. 1996;11(4):257-60.
17. Gomez KA, Gomez AA. Statistical procedures for agricultural research.
18. Barnett HL, Hunter BB. Illustrated genera of imperfect fungi.
19. Pravallika PL, Bhattiprolu SL, Radhika K, Raghavendra M. Effect of Alternaria sesami on Germination and Seedling Growth of Sesame. Indian Journal of Agricultural Research. 2023;57(4).
20. Sampathkumar A, Raghavendra KP. Molecular Identification and Genetic Diversity of Alternaria Isolates Causing Leaf Spot Disease in Cotton from Major Cotton Growing Areas of South Zone of India. Indian Journal of Agricultural Research. 2024;58(3).
21. Le DP, Gregson A. Alternaria leaf spot of cotton seedlings grown in New South Wales, Australia is predominantly associated with Alternaria alternata. Australasian Plant Pathology. 2019;48 (3):209- 216.

Peer-review history:
The peer review history for this paper can be accessed here:
https://www.sdiarticle5.com/review-history/122579