The Pharma Innovation Journal 2023; 12(7): 1241-1248

www.ThePharmaJournal.com

The Pharma Innovation

ISSN (E): 2277-7695
ISSN (P): 2349-8242
NAAS Rating: 5.23
TPI 2023; 12(7): 1241-1248

www.thepharmajournal.com
Received: 01-04-2023
Accepted: 06-05-2023

P Jeevitha
PG Scholar, Department of Plant Pathology, Agricultural College and Research Institute, Agricultural University, Madurai, Tamil Nadu, India

Dr. P Mahalakshmi
Assistant Professor, Department of Plant Pathology, Agricultural University, Coimbatore, Tamil Nadu, India

Dr. I Yesu Raja
Professor, Department of Plant Pathology, Agricultural College and Research Institute, Agricultural University, Madurai, Tamil Nadu, India

Dr. K Sujatha
Professor and Head, Department of Seed Science and Technology, Agricultural College and Research Institute, Agricultural University, Tamil Nadu, India

Dr. ML Mini
Associate Professor (Biochemistry), Department of Biotechnology, Agricultural College and Research Institute, Tamil Nadu Agricultural University, Tamil Nadu, India

M Ayyandurai
Ph.D Scholar, Department of Plant Pathology, Agricultural College and Research Institute, Madurai. Tamil Nadu Agricultural University, Tamil Nadu, India

Corresponding Author:
P Mahalakshmi
Assistant Professor, Department of Plant Pathology, Agricultural University, Coimbatore, Tamil Nadu, India

Unleashing the antifungal power of seaweeds against *Colletotrichum gloeosporioides* (OCMK-3) in onion production: An *in vitro* study on combatting pathogen growth

P Jeevitha, Dr. P Mahalakshmi, Dr. I Yesu Raja, Dr. K Sujatha, Dr. ML Mini and M Ayyandurai

Abstract
This study aimed to investigate the occurrence and severity of twister blight disease in onion crops in selected villages of Tamil Nadu, India. The disease incidence ranged from 33.45% to 86.96% across the surveyed villages, with the highest incidence recorded in Kalvelipatti village of Madurai district (86.96%), followed by Kesampatti village in Madurai district (79.89%), and the lowest incidence in Pottikampatti village of Dindigul district (33.45%). The isolated pathogen exhibited characteristics such as non-septate mycelium, bullet or cylindrical-shaped spores, and the formation of pale orange-colored acervuli above the mycelial growth. Pathogenicity tests were conducted on ten different isolates of *Colletotrichum gloeosporioides*, with OCMK-3 demonstrating the highest virulence (97.75% disease incidence) followed by OCDP-2 (90.77%). Furthermore, seaweed extracts were prepared from collected seaweeds using different solvents (methanol, ethyl acetate and hexane) and evaluated for their antifungal activity against the pathogen. The agar well method was employed for *in vitro* screening, and *Sargassum cristaefolium* exhibited the highest inhibition of mycelial growth (73.00%) under aseptic conditions. This study provides valuable insights into the prevalence of twister blight disease in onion crops and highlights the potential of seaweed extracts as a natural antifungal agent.

Keywords: Onion, twister blight disease, seaweeds, organic solvents, antifungal activity

Introduction

Onion (*Allium cepa* L.) is a highly popular spice known for its rich source of secondary metabolites and phytochemicals, particularly flavonoids like quercetin and kaempferol, earning it the title of "Queen of the kitchen" (Dorant *et al*., 1994) [6]. India holds the position as the second-largest producer of onions, with a production of 22.07 million metric tons and productivity of 16.78 tons per hectare (Kumawat & Raheman, 2022) [15]. However, onion productivity faces significant challenges due to various infectious agents, including fungi, bacteria, viruses, and nematodes (Jain *et al*., 2014) [12]. Among the major foliar diseases affecting onions, onion twister caused by *Colletotrichum gloeosporioides* is particularly devastating in the onion-growing regions of Tamil Nadu. Symptoms of onion twister include leaf twisting, yellowing, sunken lesions on leaf sheaths, and the presence of acervuli on the crop's neck region (Reecha *et al*., 2022) [20]. This disease can cause substantial yield losses ranging from 50% to 100%. While chemical fungicides are commonly used to manage the disease, their use poses risks of environmental pollution, phytotoxicity, development of fungicide resistance in plants, and harm to non-targeted beneficial microorganisms (Bharathi *et al*., 2015) [3]. Therefore, the development of alternative control strategies, such as the use of bio-stimulants like seaweed extracts, has become critically important in disease management.

In recent years, macroalgae have garnered attention for their diverse biological activities, including antifungal, antibacterial, antiviral, antioxidant, anti-inflammatory, cytotoxic, and antimitotic properties (Demirel *et al*., 2012) [5]. Seaweeds are rich sources of antifungal compounds such as phenolic compounds and terpenes, which inhibit the growth of pathogens and enhance plant growth and productivity (Rathore *et al*., 2009) [19]. Therefore, exploring the potential of seaweed extracts as a natural and eco-friendly solution to combat *Colletotrichum gloeosporioides* and improve onion crop health and productivity is of great interest. This research focuses on exploring the antifungal efficacy of various seaweeds against the onion twister blight pathogen.

The seaweed that performs the best will be further investigated for its potential application in future plant disease management. This study provides a valuable platform for managing emerging plant diseases through seaweeds for upcoming researches.

Materials and Methods

Survey to assess the incidence of twister blight pathogen

A comprehensive survey was conducted to assess the disease incidence in major onion-growing areas of Tamil Nadu from January to March 2023. The surveyed districts included Madurai, Dindigul, Namakkal, Virudhunagar and Perambalur. Disease incidence was calculated using the formula developed by Vidhyaksekaran (2004) [23]:

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

Symptoms of *Colletotrichum gloeosporioides* on onion

Onion twister blight is a disease that primarily occurs during the rainy season, leading to a high incidence in onion-growing regions. The initial symptom is the appearance of small whitish lesions near the leaf sheaths. These lesions gradually develop into elliptical-shaped dark spots, causing cell death and reducing photosynthetic activity. Prominent lesions contain acervuli, which later turn brown to black. Infected plants exhibit black acervuli, loss of turgidity, brittleness, and ultimately rotting, resulting in the curling and twisting of leaves. Additionally, pale oval sunken lesions can be observed on the leaf sheaths (Gyempeh *et al*., 2015) [9]. In severe cases, the lesions can spread to the bulb, leading to bulb rot, decreased quality, and ultimately plant death (Ebenebe 1980, Sikirou *et al*., 2011) [7, 21] (Fig.1).

Isolation of Twister blight Pathogen

To isolate the pathogen responsible for onion twister blight, plant samples showing typical symptoms of the disease were collected from onion-growing areas. The infected plant samples were washed under running water, air-dried, and then cut into small pieces (5 mm diameter), including both diseased tissue and some healthy tissue, using a sterile scalpel. The infected samples were disinfected by immersing them in a 1% sodium hypochlorite solution for 30 seconds, followed by rinsing three times with sterilized water. After removing excess water by placing the samples on sterile tissue paper, they were aseptically placed on potato dextrose agar (PDA) medium supplemented with a pinch of streptomycin. The plates were incubated at 27 °C for 10 to 12 days in an inverted position to prevent contamination (Neevana *et al*., 2020) [17]. The fungus was then purified using the single hyphal tip method, and the culture was maintained at -4 °C for further experiments.

Pathogenicity Test

For the pathogenicity studies, the Co (On) 5 onion cultivar was chosen. Six onion bulbs were planted in each pot. Ten different isolates of *Colletotrichum gloeosporioides* (OCKM-1, OCMP-2, OCMK-3, OCDS-4, OCDP-5, OCDD-6, OCDT-7, OCNV-8, OCVP-9, and OCPK-10) were tested for their pathogenicity under *In vivo* conditions. The onion plants were sprayed with a spore suspension containing a concentration of 2.5x106 spores/ml, 35-45 days after sowing. The plants started to exhibit signs of twister blight symptoms. If the symptoms observed in the inoculated plants matched those from the infected field, they were re-isolated. The re-isolated cultures exhibited the same characteristics as the initially isolated pathogen. As a control, pots were sprayed only with sterile distilled water (Gyempeh *et al*., 2015; Ebenebe, 1980) [9, 7].

Collection of Seaweeds

Seaweeds from various species were handpicked from the deep-water areas along the coastlines of Rameswaram in Tamil Nadu. The collected seaweed samples were carefully washed with fresh water followed by distilled water to eliminate any debris or impurities. Subsequently, the seaweed samples were gently blotted to remove excess moisture and then subjected to shade drying for a period of two to three weeks at room temperature. After the drying process, the seaweed samples were stored under dry conditions in an environmental chamber at an average temperature ranging from 28 °C to 37 °C (Kannan and Bharathkumar, 2016) [13] and they were subsequently powdered. Different solvents such as methanol, ethyl acetate, and hexane were used to prepare extracts from the powdered seaweeds to evaluate their antifungal activity.

Preparation of Seaweed Extract through Soxhlet Apparatus

To prepare the seaweed extract, 20 g of partially blended seaweed powder was loaded into a Soxhlet apparatus. These a weed powder packed with cellulose thimble paper, which was refluxed for 12 hours using various solvents such as ethyl acetate, methanol, and hexane with 150 ml of each solvent. The extracted solvent was then filtered through what man No.1 filter paper to remove any impurities. The collected solution was subjected to evaporation using a rotary evaporator at a temperature of 40 °C and an RPM of 45 until the solvent completely evaporated (Bhosle *et al*., 1975) [4]. The final extract was diluted with the respective solvents and stored at -4 °C for further use (Kombiah and Sahayaraj, 2012) [14].

Antifungal Activity of Seaweeds against *C. gloeosporioides* (Agar Well Diffusion Method)

The antifungal activity of the promising seaweeds against *C. gloeosporioides* was evaluated through the agar well diffusion method. The pathogen was placed at the center of the agar plate, and wells were made at four corners of the plate. These wells were filled with different solvents at a concentration of 10% and tested against *Colletotrichum gloeosporioides* under in vitro conditions. The inoculated plates were incubated at 25 ± 2 °C for 10-12 days. After 12 days of treatment application, the growth of the pathogen was measured. The percentage inhibition (PI) of mycelial growth was calculated using the formula suggested by Pandey *et al*. (1996) [18].

$$\text{PI} = \frac{\text{C} - \text{T}}{\text{C}} \text{ x } 100$$

Where,

PI = Percent Inhibition

C= Average diameter of fungal growth (cm) in control,

T= Average diameter of fungal growth (cm) in treatment

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 (Gomez and Gomez, 1984) [8].

Result and Discussion

Survey

The results of the survey conducted in several onion-growing villages of Tamil Nadu using the roving survey method are presented in this section. The survey included villages such as Kesampatti, Panangudi, Kalvelipatti, Sikkamanaickenpatti, Pottikampatti, Dindigul, Toppampatti, Vaiyappamalai, Paranakutti, and Keelakkari. Samples were collected from infected plants displaying symptoms of twister blight disease. The findings revealed a range of disease incidence percentages, varying from 33.45% to 86.96%. Among the surveyed villages, Kalvelipatti village in Madurai district exhibited the highest disease incidence percentage of 86.96%, followed by Kesampatti village in Madurai district with 79.89% disease incidence. On the other hand, Pottikampatti village in Dindigul district had the lowest disease incidence percentage, recording 33.45% (Table 1).

These results are consistent with the research conducted by Reecha *et al*. (2022) [20], who reported that the highest incidence of onion twister blight disease was observed in Rayarpatty village in Virudhunagar district, with a rate of 76.67%. Conversely, the lowest disease incidence was found in Allathur village in Madurai district, with a rate of 23.33%. Similarly, in 2022, Manthesha *et al.* documented a disease severity range from 0 to 77.65% in the Kalyan-Karnataka regions.

Isolation of Twister blight pathogen

In the present study, the isolated pathogen cultures initially displayed a white colour, which gradually transformed into a greyish-white shade with cottony growth on the PDA medium. To maintain pure cultures, the cultures were purified using the hyphal tip method. It took approximately 10-12 days for the pathogen to completely cover the 9 cm plate. The mycelium of the pathogen appeared non-septate, and it produced bullet or cylindrical-shaped hyaline spores. Upon completing the growth on the entire plate, pale orange-colored acervuli were observed above the mycelial growth. To ensure the purity of the isolates, the pure cultures were stored in the refrigerator and subculture regularly, as depicted in Fig 2. Similar observations were reported by Sikirou *et al.* (2011) [21], where the cultures exhibited a transition from white to grey mycelia, eventually turning to a dark brown color. The conidia of the pathogen were described as hyaline, unicellular, non-septate, and cylindrical with rounded ends. Furthermore, than *et al*. (2008) [22] documented that *C. gloeosporioides* displayed greyish-white colonies with a dark grey to black colour on the backside of the plates. These findings provide a detailed characterization of the pathogen cultures, including their growth patterns, spore morphology, and colony appearance. The similarities observed with previous studies reinforce the consistency and reliability of the observations made in the current investigation.

Pathogenicity Test

In order to assess the pathogenicity of the ten isolates of *C. gloeosporioides*, a pot culture experiment was conducted. Among these isolates, OCMK-3 demonstrated the highest virulence, resulting in a disease incidence of 97.75%. It was closely followed by OCDP-2, which exhibited a disease incidence of 90.77%. On the other hand, isolate OCPK-10 displayed the lowest disease incidence, with a rate of 45.78%. Based on these findings, it was determined that the OCMK-3 isolate of *C. gloeosporioides* was the most virulent among the tested isolates, warranting further investigation (Fig 3 & 4). Infected plants exhibited several characteristic symptoms, including twisting, curling, yellowing, and abnormal elongation of the neck, ultimately resulting in plant death. The pathogenicity was evaluated through both foliar and soil inoculation methods, as documented by Gyemph *et al.* 2015 [9]. Additionally, the pathogenicity of onion twister disease was confirmed using the spray inoculation method in onion seedlings, as reported by Hill *et al.*, 2008 [10]. These results highlight the varying virulence levels among different isolates of *C. gloeosporioides* and emphasize the significant impact of the pathogen on the development of onion twister disease. The symptoms observed in infected plants further support the pathogenicity of the identified isolates, aligning with previous studies conducted in this field.

In vitro* Screening of Seaweeds against the *C. gloeosporioides

The present study aimed to investigate the antifungal activity of seaweed extracts, specifically bio-stimulants, against the onion twister blight pathogen *C. gloeosporioides*. Different solvent extracts, namely methanol, ethyl acetate, and hexane were obtained from five seaweed species, including *Sargassum cristaefolium*, *Kappaphycus alvarezii*, *Gracilaria edulis*, *Caulerpa racemosa*, and *Ulva lactuca*. These extracts were then analyzed for their antifungal potential.

Notably, the results revealed a novel finding in the field of natural antifungals. Among the tested seaweed extracts, *Sargassum cristaefolium* exhibited an unprecedented and remarkable reduction in mycelial growth, with a size of only 2.43 cm under aseptic conditions. This dramatic inhibition translated to a maximum percent reduction over the control, reaching an impressive 73.00%. This significant antifungal activity of *Sargassum cristaefolium* showcases its untapped potential as a potent source of natural compounds for combating onion twister blight. Furthermore, *Kappaphycus alvarezii* demonstrated a noteworthy second-highest percent reduction in mycelial growth, with a size of 3.67 cm and a reduction of 59.22%. This finding highlights the promising antifungal properties of *Kappaphycus alvarezii*, which can potentially contribute to the development of alternative strategies to manage onion twister blight. Interestingly, *Ulva lactuca*, although exhibiting a relatively lower percent reduction over the control at 16.00%, still displayed some degree of antifungal activity. This suggests the presence of unique bioactive compounds within *Ulva lactuca* 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 *C. gloeosporioides*. The remarkable inhibitory effect of *Sargassum cristaefolium* and the promising results obtained from *Kappaphycus alvarezii* 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 onion twister blight disease.

Table 1: Disease incidence of twister blight disease in major onion growing areas of Tamil Nadu

S. No	Place of collection	District	Isolates	Stage of the crop	Soil type	Geo coordinates		Twister blight incidence (%)
						Latitude	Longitude	
1.	Kesampatti	Madurai	OCKM-1	Bulb development stage	Red sandy loam	10.148527°	78.291008°	79.63[b]
2.	Panangudi		OCMP-2	Vegetative stage	Red sandy loam	9.958487°	78.394531°	42.18[de]
3.	Kalvelipatti		OCMK-3	Bulb development stage	Black clay	10.060441°	78.037228°	85.47[a]
4.	Sikkamanaickenpatti	Dindigul	OCDS-4	Vegetative stage	Red soil	10.562403°	77.691732°	38.87[def]
5.	Pottikampatti		OCDP-5	Bulb development stage	Red soil	10.561435°	77.692198°	31.86
6.	Dindigul		OCDD-6	Bulb development stage	Red sandy loam	10.232926°	77.860624°	66.47[b]
7.	Toppampatti		OCDT-7	Vegetative stage	sandy loam	10.264458°	77.916226°	58.55[c]
8.	Vaiyappamalai	Namakkal	OCNV-8	Bulb development stage	Red loam	11.416676°	78.082885°	43.55[d]
9.	Parnakuti	Virudhunagar	OCVP-9	Vegetative stage	Black clay	9.636782°	78.233926°	35.38[f]
10.	Keelakkarai	Perambalur	OCPK-10	Bulb development stage	Red sandy loam	11.264011°	78.859698°	52.46[c]
CD (*P*=0.05%)								7.180

Table 2: *In vitro* screening of different solvents of different seaweed extract against the *C. gloeosporioides* (OCMK-3)

Different seaweeds	Methanol extract		Ethyl acetate		Hexane	
	Radial growth *(cm)	PIOC (%)	Radial growth*(cm)	PIOC (%)	Radial growth *(cm)	PIOC (%)
Sargassum cristaefolium	2.43	73.00[a]	3.50	60.81[a]	3.20	64.13[a]
Kappaphycus alvarezii	3.67	59.22[b]	4.90	45.50[b]	4.86	46.00[b]
Gracilaria edulis	5.40	40.00[c]	6.58	26.80[c]	6.12	32.00[bc]
Caulerpa racemose	5.71	36.56[d]	6.74	25.11[d]	6.53	27.40[d]
Ulva lactuca	7.56	16.00[e]	8.01	11.11[e]	7.92	12.66[e]
Control	9.00	0	9.00	0	9.00	0
CD (*P*=0.05%)	1.18	5.65	0.36	6.54	0.23	5.50

*Mean for three replications

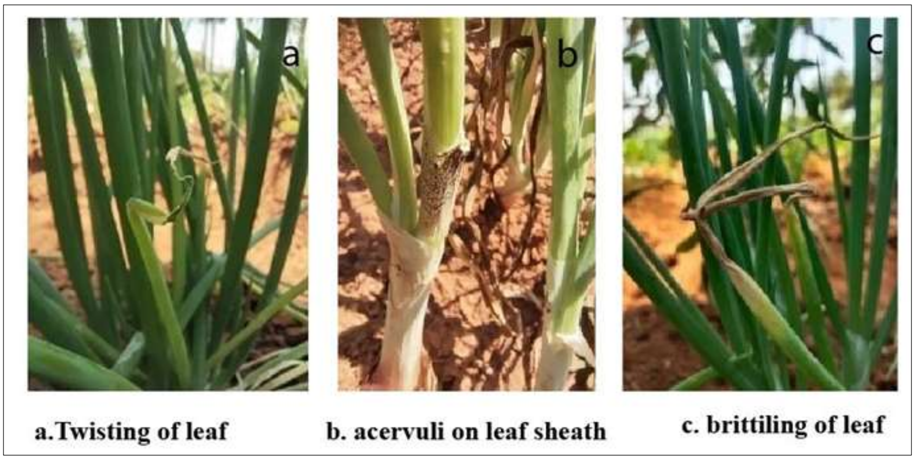

Fig 1: Symptoms of Onion twister

Fig 2: Different isolates of *Colletotrichum gloeosporioides*

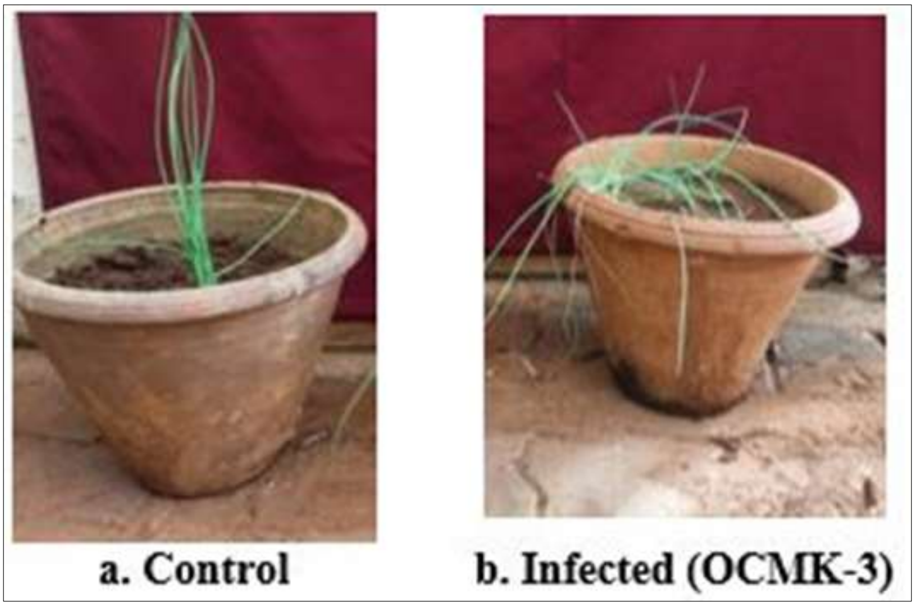

Fig 3: Pathogenicity test

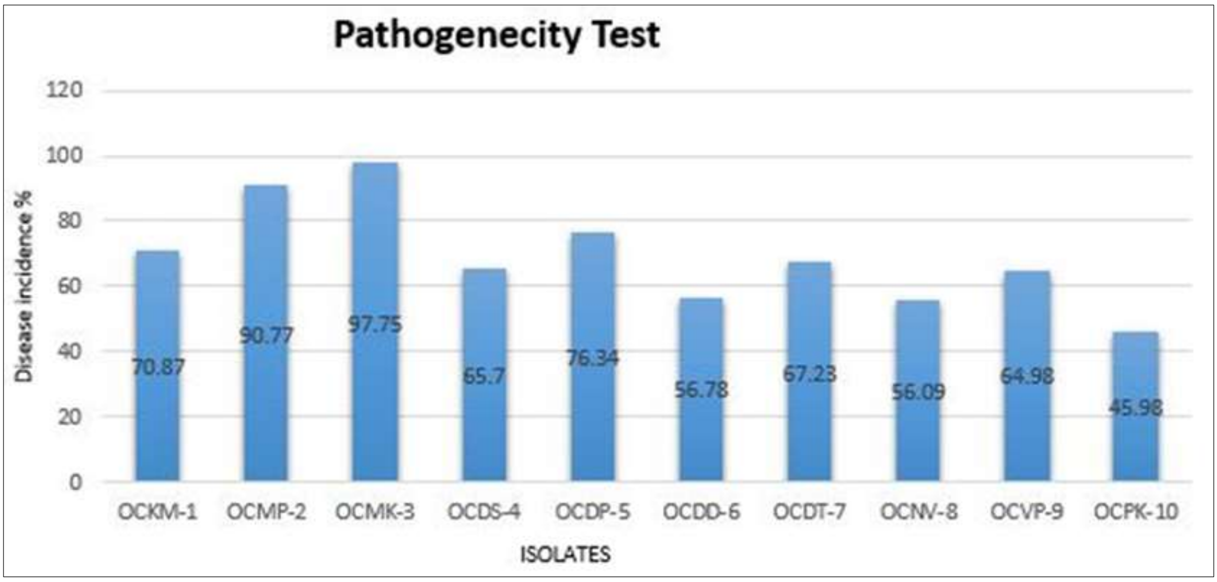

Fig 4: Virulence of different isolates of *Colletotrichum gloeosporioides*

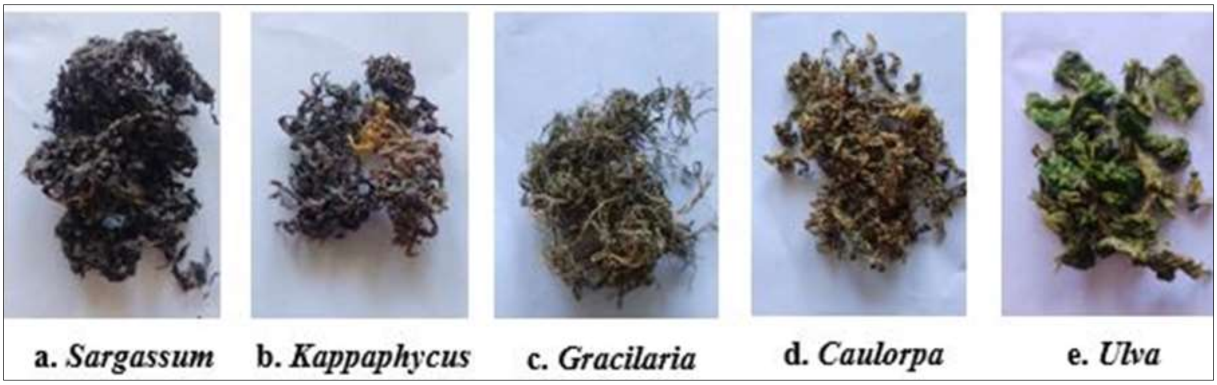

Fig 5: Collection of seaweeds

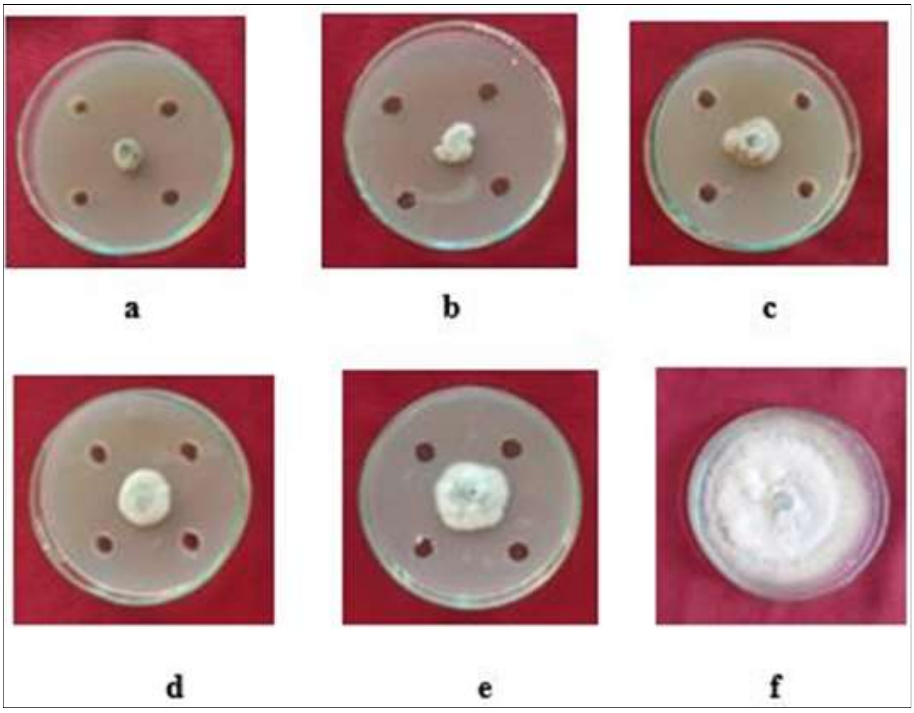

Fig 6: Methanol extract of different seaweeds
a. *Sargassum cristaefolium* b. *Kappaphycus alvarezii* c. *Gracilaria edulis* d. *Caulerpa racemose* e. *Ulva lactuca* f. Control

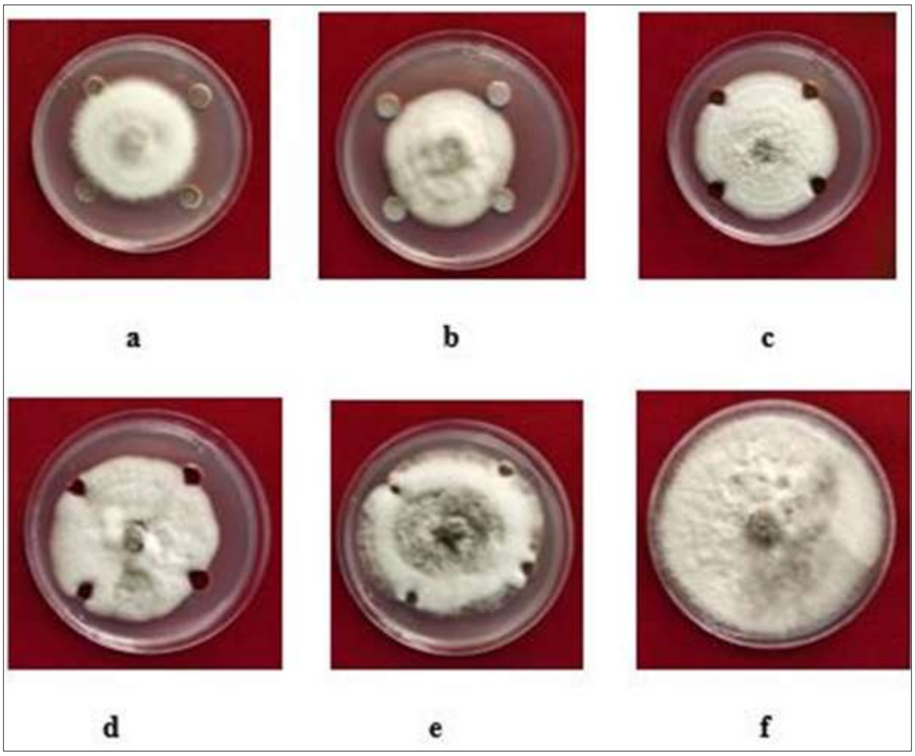

Fig 7: Ethyle acetate extract of different seaweeds
a. *Sargassum cristaefolium* b. *Kappaphycus alvarezii* c. *Gracilaria edulis* d. *Caulerpa racemose* e. *Ulva lactuca* f. Control

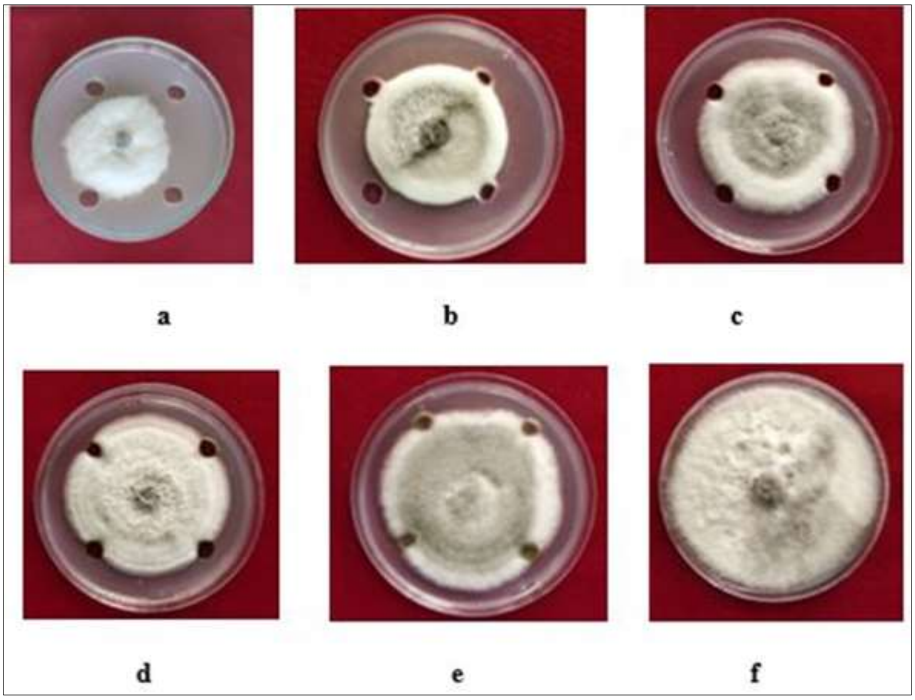

Fig 8: Hexane extract of different seaweeds
a. *Sargassum cristaefolium* b. *Kappaphycus alvarezii* c. *Gracilaria edulis* d. *Caulerpa racemose* e. *Ulva lactuca* f. Control

Conclusion

In conclusion, the survey were conducted in onion-growing regions of Tamil Nadu revealed varying levels of twister blight disease incidence, with Kalvelipatti village in Madurai district having the highest disease incidence percentage. The pathogen *Colletotrichum gloeosporioides* was successfully isolated and characterized from infected plants. Among the tested isolates, OCMK-3 demonstrated the highest virulence. Additionally, seaweed extracts, particularly *Sargassum cristaefolium*, exhibited promising antifungal activity against *C. gloeosporioides*. These findings provide important insights for the management of twister blight disease in onion cultivation 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.

Acknowledgement

We would like to express our heartfelt thanks to the Department of Plant Pathology, Agricultural College and Research Institute, Madurai, for their invaluable support, provision of facilities, and financial assistance throughout our research work

References

1. Abera A, Lemessa F, Adunga G. Morphological characteristics of Colletotrichum species associated with mango (*Mangifera indica* L.) in Southwest Ethiopia. Food Science and Quality Management. 2016;48:106-115.
2. Ambika S, Sujatha K. Antifungal activity of aqueous and ethanol extracts of seaweeds against sugarcane red rot pathogen (*Colletotrichum falcatum*). Scientific Research and Essays. 2015;10(6):232-235.
3. Bharathi R, Vivekananthan R, Harish S, Ramanathan A, Samiyappan R. Rhizobacteria-based bio-formulations for the management of fruit rot infection in chillies. Crop Protection. 2015;23(9):835-843.
4. Bhosle NB, Dhargalkar VK, Untawale AG. Effect of seaweed extract on the growth of *Phaseolus vulgaris* L. Indian J Mar. Sc. 1975;4:208-210.
5. 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):91-95.
6. Dorant E, van den Brandt PA, Goldbohm RA. A prospective cohort study on Allium vegetable consumption, garlic supplement use, and the risk of lung carcinoma in The Netherlands. Cancer research. 1994:54(23):6148-6153.
7. Ebenebe AC. Onion twister disease caused by *Glomerella cingulata* in northern Nigeria (No. RESEARCH); c1980.
8. Gomez KA, Gomez AA. Statistical procedures for agricultural research. John Wiley & Sons; c1984.
9. 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.
10. Hill JP. Compendium of Onion and Garlic Diseases. 2 ended. The American Phytopathology Society, St. Paul, MN, IBH Publishing Co. Pvt. Ltd., New Delhi, c2008, p. 163.
11. Jaggi RC. Sulphur levels and sources affecting yield and yield attributes in onion (*Allium cepa*); c2005.
12. Jain G, Kushwah SS, Singh OP, Verma KS. Effect of different doses of nitrogen and sulphur on growth, yield and quality of onion (*Allium cepa*). Indian J. Agr. Sci. 2014;84(11):352-1357.
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. 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.
15. Kumawat L, Raheman H. Mechanization in onion harvesting and its performance: A review and a conceptual design of onion harvester from Indian perspective. Journal of The Institution of Engineers (India): Series A; c2022, p.1-10.
16. Manthesha HD, Kenganal M, Yenjerappa ST, Aswathanarayana DS, Kulkarni V. Management of onion twister disease under field condition. The Pharma Innovation Journal. 2022;11(4):551-555.
17. Naveena P, Kumari PV, Kumar AP, Rani SC. In vitro efficacy of biontrol agents against *Pythium aphanidermatum* (Edson) Fitz. Int. J MIcrobiol. App. Sci. 2020;9(8):2376-2381. http://doi.org/10.20546/ijcmas.2020.908.272.
18. Pandey MC, Sharma JR. Dikshit A. Antifungal evaluation of the essential oil of *Cymbopogonpendulus* (Nees ex Steud.) Wats. cv. Praman. Flavour and Fragrance Journal. 1996;11(4):257-260.
19. 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):351-355.
20. Reecha J, Thiruvudainambi S, Mareeswari P, Oviya R, Vellaikumar S. Morphological characterization of *Colletotrichum gloeosporioides* causing leaf twister blight disease in *Allium cepa* (onion); c2022.
21. Sikirou R, Beed F, Hotègni J, Winter S, Assogba-Komlan F, Reeder R, *et al*. First report of anthracnose caused by *Colletotrichum gloeosporioides* on onion (*Allium cepa*) in Benin. New Dis Rep. 2011;23(7):583-584.
22. Than PP, Prihastuti H, Phoulivong S, Taylor PW, Hyde KD. Chilli anthracnose disease caused by *Colletotrichum* species. Journal of Zhejiang University Science B. 2008;9:764-778.
23. Vidhyasekaran P. Concise encyclopedia of plant pathology. Binghamton, NY: The Haworth Press; c2004.