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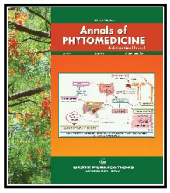

Original Article : Open Access

Exploring the antifungal potential of seaweed extract from *Sargassum cristaefolium* for twister blight management in onion

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Abstract

Seaweed extracts from *Sargassum cristaefolium* at 10% concentrations, effectively inhibited the mycelial growth of *Colletotrichum gloeosporioides*. According to the available literatures, the GC-MS analysis identified various compounds in these extracts that had a antifungal, antibacterial and antioxidant properties as per previous report. In both pot culture trials and field settings, the application of seaweed extracts *via* bulb treatment, soil drench, and foliar spray resulted in a notable decrease in the occurrence of twister blight disease. Among the treatments, treatment three, involving bulb treatment with *S. cristaefolium* at a 10% concentration, soil drench with the same seaweed at a 10% concentration and foliar application of *S. cristaefolium* at a 10% concentration, demonstrated a remarkable 69.39% reduction in twister blight, showcasing efficacy comparable to biocontrol agents and chemical fungicides. In pot culture conditions, increased levels of peroxidase, polyphenol oxidase, and phenylalanine ammonia lyase were observed, suggesting their involvement in enhancing resistance against disease. Histopathological examinations further revealed reduced tissue damage in treated plants. Additionally, protein content in both leaves and bulbs exhibited an increase in treated plants. This comprehensive study not only underscores the potential of seaweed extracts as effective biostimulants for disease management but also highlights their positive influence on overall plant health and productivity.

1. Introduction

Onion (*Allium cepa* L.) holds immense commercial importance globally, deriving its name from the Latin word for "large pearl," owing to its shape and nutritional qualities (Shigyo and Kik, 2008). India, as the second-largest onion producer, witnesses significant onion cultivation, particularly in regions such as Virudhunagar, Trichy, Namakkal, Perambalur, Dindigul, Madurai, and Erode in Tamil Nadu (Muthukumar *et al*., 2022). Despite its agricultural prominence, onion productivity confronts various challenges from infectious agents like fungi, bacteria, viruses, and nematodes, with *C. gloeosporioides* causing substantial yield losses due to twister blight disease (Gyempeh *et al*., 2015).

The history of twister blight disease traces back to its identification in 1969 near Zaria, north Nigeria, with subsequent reports in Maharashtra's Lonand area, followed by outbreaks in Nashik, Pune, and Karnataka in India during the 1980s (Ebenebe, 1980; Qadri and Srivastava, 1985; Qadri, 1988; Singh *et al*., 1994). Recent occurrences of twister blight disease have severely impacted onion cultivation in Tamil Nadu's major onion-producing regions, resulting in significant yield reductions ranging from 50% to 100% (Schwartz and Mohan, 1995; Reecha *et al*., 2022).

In recent years, agricultural yields have faced significant challenges due to a myriad of biotic and abiotic factors. While synthetic agrochemicals have been utilized to address these concerns, their overuse, especially fertilizers and pesticides, has resulted in significant consequences for the environment, humans, and biodiversity. As the global population continues to grow, the escalating reliance on these agrochemicals to boost crop yields raises concerns about its detrimental impact on both the environment and human well-being. This has sparked an increasing interest in investigating alternative, eco-friendly methods to improve crop yield, human well-being, and agricultural efficiency (Gupta *et al*., 2021; Rupinder Kaur *et al*., 2023; Santhosha and Dinesh Mohan, 2023; Seetharamu *et al*., 2023).

Seaweeds, encompassing green (Chlorophyceae), brown (Phaeophyceae), and red algae (Rhodophyceae), present a versatile and flexible biomass utilized in various sectors, including human nutrition, food supplements, animal husbandry, agriculture, renewable energy, skincare, and pharmaceuticals (Anis *et al*., 2017) and antiviral properties are produced by a various algae species (Alam *et al.,* 2021) and also bioactive compounds are abundant in seaweeds (Vijayalakshmi *et al.,* 2022).

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The application of marine algae extracts has demonstrated increased foliar resistance to specific pathogens in pepper and effective control of diseases such as early blight in tomato and onion twister blight (Ambika and Sujatha, 2015). Histopathological examinations have played a crucial role in understanding the infection processes of plant pathogens, providing valuable insights into their strategies (Beltrán *et al*., 2004). In case of onion cultivation, a crop of global significance, challenges arise from infectious agents like *C. gloeosporioides*, causing twister blight disease and substantial yield reductions (Gyempah *et al*., 2015).

Based on this background information, the objectives were framed to manage onion twister disease using seaweeds. This holistic methodology seeks to elucidate how selected seaweeds influence the peroxidase (PO), polyphenol oxidase (PPO), and phenylalanine ammonia-lyase (PAL) activities in onion plants. Understanding these mechanisms is crucial for unlocking the full potential of seaweed based biostimulants in agriculture. The ultimate goal is to contribute to sustainable disease management and improved plant growth, benefiting onion growing farmers.

2. Materials and Methods

2.1 Isolation of onion twister pathogen

Twister blight infected onion samples were collected during field surveys and utilized for pathogen isolation under laboratory conditions. The pathogen was cultured on potato dextrose agar (PDA) medium in Petri dishes using tissue segmentation techniques for isolation. Pure cultures of *C. gloeosporioides* were obtained through the single hyphal tip method. The pathogen's identity as *C. gloeosporioides* was validated using the accession number QR485177 obtained from the National Center for Biotechnology Information (NCBI).

2.2 Collection of seaweeds

Live and healthy specimens of seaweeds, *viz., S. cristaefolium*, *Kappaphycus alvarezii*, *Gracilaria edulis, Caulorpa racemosa* and *Ulva lactuca* were collected from Mandapam coast, Tamil Nadu, India and washed in tap water. To remove any macroscopic epiphytes and extraneous matter and rinsed in distilled water to remove excess salt on the surface. The specimens were spread on blotting paper and shade-dried for 10 days at room temperature. The shade-dried seaweed samples were powdered using mixer grinder and sieved through 0.8 mm sieve plate. The powdered samples were kept in polythene bags, sealed properly and stored at room temperature until further studies.

Wet seaweed **Dry seaweed**

Figure 1: Sea weed *S. crisetifolium* used in the study.

2.3 Preparation of different solvent extracts of promising seaweeds

The collected seaweed material was dried at 40°C for 24 h for removing moisture from the material to create a powdered form. After drying, the material was powdered, presumably by crushing or grinding it into a fine powder. The 100 ml of respective solvent (methanol, hexane, ethyl acetate and acetone) was added to 20 g of the powdered material. This mixture was then kept overnight with intermittent stirring. After the overnight soaking, the mixture was subjected to rotary evaporation at 40°C and 45 rpm. Rotary evaporation was a technique used to separate solvents from compounds based on their different boiling points. The solvent was evaporated to leave behind the liquid seaweed extract, containing the extracted compounds or

nutrients from the original material. From the collected liquid seaweed extract, different concentrations were prepared from stock solution and used for further study (Ambika and Sujatha, 2015).

2.4 *In vitro* screening of different solvents of promising seaweed extract against the *C. gloeosporioides*

The PDA medium was used to assess the inhibitory effect of the different solvent extracts of collected seaweeds and bioinoculants against mycelial growth of *C. gloeosporioides* as described by Dennis and Webster (1971). In each plate, two wells (5 mm in diameter) were made 4 cm apart. One well was inoculated with a disc (5 mm) of *C. gloeosporioides* (4-day-old culture) in the center of the plate and opposite well was inoculated with each of different solvent extracts of seaweeds. The plates inoculated only with *C. gloeosporioides* served as a control. All inoculated plates were incubated at 25 ± 2°C for 6 to 10 days. All plates were examined and the linear growth of the pathogen was measured. The growing culture was observed visually and microscopically for evidence of a reduction of mycelial growth. After four days of incubation, mycelial growth of the pathogen and inhibition zone were measured in the solvent extract/bioinoculants plates, as well as in control plates and per cent inhibition (PI) of mycelial growth was calculated using the formula suggested by Pandey *et al*. (2000).

2.5 GC-MS analysis of methanol extract of *S. cristaefolium*

GC-MS analysis was carried out utilizing an Agilent GC-MC-5975C instrument equipped with a triple-axis detector and an auto sampler. The GC column utilized was a fused silica capillary column measuring 30 m in length, 0.25 mm in diameter, with a film thickness of 0.25 mm. Helium served as the carrier gas at a flow rate of 1.51 ml per minute for the initial minute. The mass spectrometer operated in electron ionization (EI) mode at 70 eV and scanned a range from 40 to 700 mass-to-charge ratio (m/z). The sample underwent injection with a split ratio of 1:10 and an injected volume of 1 µl. The injector temperature was maintained at 250°C, while the oven temperature began at 70°C for 3 min before ramping up to 250°C at a rate of 14°C per min, resulting in a total run time of 41 min. Peak identification of crude seaweed extracts was conducted by comparing retention times with standards, and the obtained mass spectra were compared with those available in NIST libraries (NIST 11-Mass Spectral Library, 2011 version) with an acceptance criterion of a match above a critical factor of 80%, as per the criteria established by Musharraf *et al*. (2012).

2.6 Evaluating the efficacy of promising seaweed extract against onion twister blight under controlled and open field conditions

An experimental trial was performed at the Department of Plant Pathology, Agricultural College and Research Institute, Madurai, to assess the effectiveness of seaweed extracts against the pathogen *C. gloeosporioides* in potted onion cultures and field condition at orchard. The study was carried out in a controlled environment within a glasshouse using Co (On) 5 onion. The growth medium used consisted of a sterilized mixture of sand, red soil and farmyard manure (FYM) in a 1:1:1 ratio. The pathogen was cultivated in a sand maize medium and subsequently introduced into the earthen pots at a ratio of 1:20 (w/w), with some pots serving as controls without pathogen inoculation. To test the impact of biostimulants; namely, *S. cristaefolium* and *K. alvarezii*, onion bulbs were treated, and spray was performed at 25, 40, and 60 days after sowing (DAS). The recommended commercial biocontrol formulation, *viz.*, *Trichoderma asperellum* TV1, *Bacillus subtilis* Bbv 57 and the recommended chemicals fungicide Mancozeb also compared with the seaweeds. Each treatment was replicated three times using a completely randomized design (CRD) for pot culture and randomized block design (RBD) for open field condition.

The treatment details were as follows:

T_1	Bulb Treatment (BT) with *S. cristaefolium* @ 3% + Soil Drenching (SD) with *S. cristaefolium* @ 3% + FA of *S. cristaefolium* @ 3%;
T_2	BT with *S. cristaefolium* @ 5% + SD with *S. cristaefolium* @ 5% + Foliar application (FA) of *S. cristaefolium* @ 5%;
T_3	BT with *S. cristaefolium* @ 10% + SD with *S. cristaefolium* @ 10% + FA of *S. cristaefolium* @ 10%;
T_4	BT with *K. alvarezii* @ 3% + SD with *K. alvarezii* @ 3% + FA of *K. alvarezii* @ 3%;
T_5	BT with *K. alvarezii* @ 5% + SD with *K. alvarezii* @ 5% + FA of *K. alvarezii* @ 5%;
T_6	BT with *K. alvarezii* @ 10% + SD with *K. alvarezii* @ 10% + FA of *K. alvarezii* @ 10%;
T_7	BT with *T. asperellum* @ 4 g/kg of bulb + SD with *T. asperellum* @ 2.5 kg/ha + FA of *T. asperellum* @ 0.5%;
T_8	BT with *B. subtilis* @ 10 g/kg of bulb + SD with *B. subtilis* @ 2.5 kg/ha + FA of *B. subtilis* @ 0.5%;
T_9	BT with Mancozeb @ 2 g/kg of bulb + SD with Mancozeb @ 2.5 g/liter of water + FA with Mancozeb @ 0.25%;
T_{10}	Control

2.7 Induction of defense mechanism

Assays were conducted to analyze the activities of peroxidase (PO), polyphenol oxidase (PPO), and phenylalanine ammonia lyase (PAL) enzymes in onion leaves from various treatments, following established methods as outlined by Hammerschmidt *et al*. (1982), Mayer *et al*. (1966), and Dickerson *et al*. (1984). Additionally, protein concentrations were determined using the Bradford method, with bovine serum albumin serving as a standard (Bradford, 1976).

2.8 Histopathological studies and microscopic examination

For histology, three onion leaf samples from infected, infected treated, and control plants were collected, and the experiment was carried out at the Central Aquaculture Pathology Laboratory in Mayiladuthurai, Tamil Nadu, India.

Scanning electron microscopy (SEM) was employed to assess the antagonistic effect of *S. cristaefolium* against *C. gloeosporioides* (OCMK-3), both before and after seaweed treatment. The samples

underwent preparation through fixation and dehydration methods. Subsequently, examination of the samples was conducted using a field emission electron microscope at the Centre for Agricultural Nanotechnology, TNAU, Coimbatore.

3. Results

3.1 *In vitro* screening of different solvents of promising seaweed extract @ 10 % against the *C. gloeosporioides*

The primary objective of this study was to assess the effectiveness of seaweed extracts specifically biostimulants in combating the pathogen causing the twister blight of onion. In this study, the different solvents, *viz.*, methanol, ethyl acetate, hexane and acetone were used to collect the extract from five distinct seaweeds *viz., S. cristaefolium*, *K. alvarezii*, *G. edulis, C. racemosa* and *U. lactuca*. Among the promising seaweed extracts examined, *S. cristaefolium* stood out by displaying an unparalleled and remarkable suppression of mycelial growth of *C. gloeosporioides*. Among the different solvents extracts, the methanol extract of *S. cristafolium* significantly inhibited the mycelial growth (2.43 cm) which recorded the maximum growth inhibition of 73.00 per cent reduction over control, followed by methanol extract of *K. alvarezii* which exhibited the maximum inhibition of mycelial growth of 59.22 per cent reduction over control (Figure 2; Table 1). The ethyl acetate extract of *U. lactuca* showed the least inhibition, recording 11.11% reduction over control and still exhibited some degree of antifungal activity.

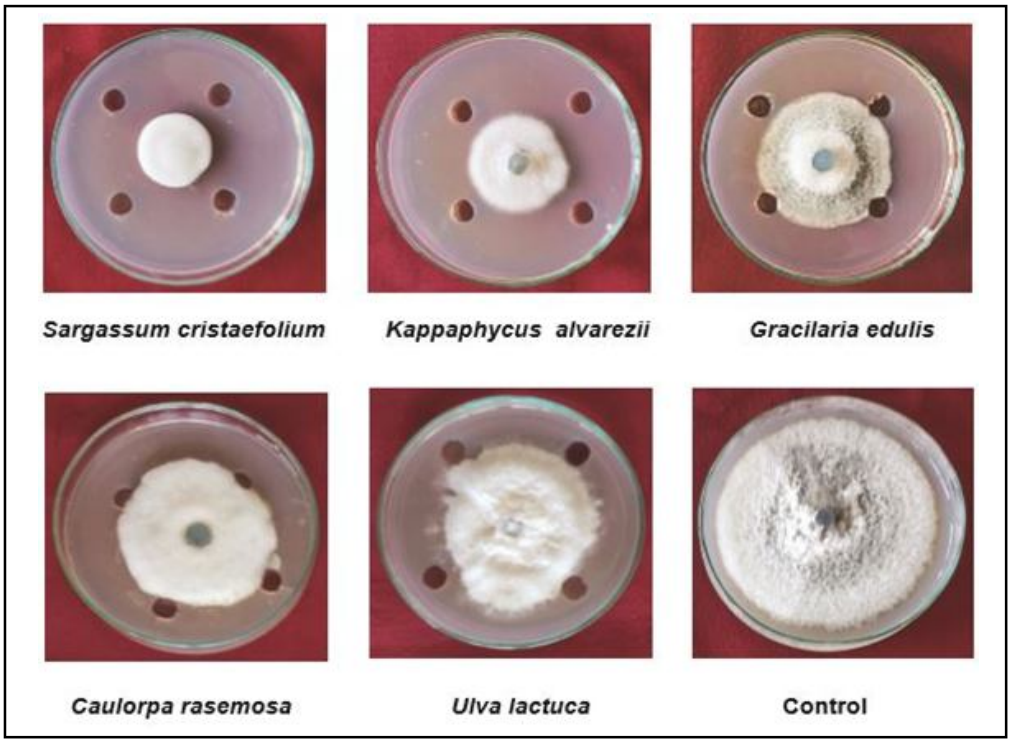


Figure 2: Methanol extracts of different seaweeds at 10% concentration.

Table 1: *In vitro* screening of different solvents of promising seaweeds extract against the *C. gloeosporioides*

Different seaweeds	Methanol extract		Ethyl acetate extract		Hexane extract		Acetone extract	
	Radial growth *(cm)	PIOC (%)	Radial growth *(cm)	PIOC (%)	Radial growth *(cm)	PIOC (%)	Radial growth *(cm)	PIOC (%)
S. cristaefolium	**2.43**^{a}	**73.00**^{a}	**3.50**^{a}	60.81^{a}	3.20^{a}	64.13^{a}	5.10^{a}	43.33^{a}
K. alvarezii	3.67^{b}	59.22^{b}	4.90^{b}	45.50^{b}	4.86^{b}	46.00^{b}	6.81^{b}	24.33^{b}
G.edulis	5.40^{c}	40.00^{c}	6.58^{c}	26.80^{c}	6.12^{bc}	32.00^{bc}	7.10^{c}	21.11^{c}
C. racemose	5.71^{d}	36.56^{d}	6.74^{d}	25.11^{d}	6.53^{d}	27.40^{d}	7.41^{d}	17.66^{d}
U. lactuca	7.56^{e}	16.00^{e}	8.01^{e}	11.11^{e}	7.92^{e}	12.66^{e}	7.90^{e}	12.22^{e}
Control	9.00	0	9.00	0	9.00	0	9.00	0
CD (p=0.05%)	**0.38**	**5.65**	**0.36**	**6.54**	**0.23**	**5.50**	**0.43**	**4.67**

*Mean of three replications.

Figures followed by same alphabets are not significantly different.

3.3 GC-MS Analysis of *S. cristaefolium* secondary metabolites

The GC-MS analysis of the crude extract from *S. cristaefolium* provided valuable insights into its chemical composition. A total of 25 compounds were identified, highlighting the wide variety of volatile compounds and secondary metabolites present in the extract (Figure 3; Table 2). Notable compounds included squalene, ethanone, and phytol, each contributing to the observed antifungal properties. Squalene, with a peak area of 6.33% at a retention time of 25.49 min, and ethanone, exhibiting 4.90% peak area at 19.319 min, were particularly noteworthy. Conversely, phytol, observed at a retention time of 17.91 min with a peak area of 0.54%, contributed to the complex chemical profile of the extract.

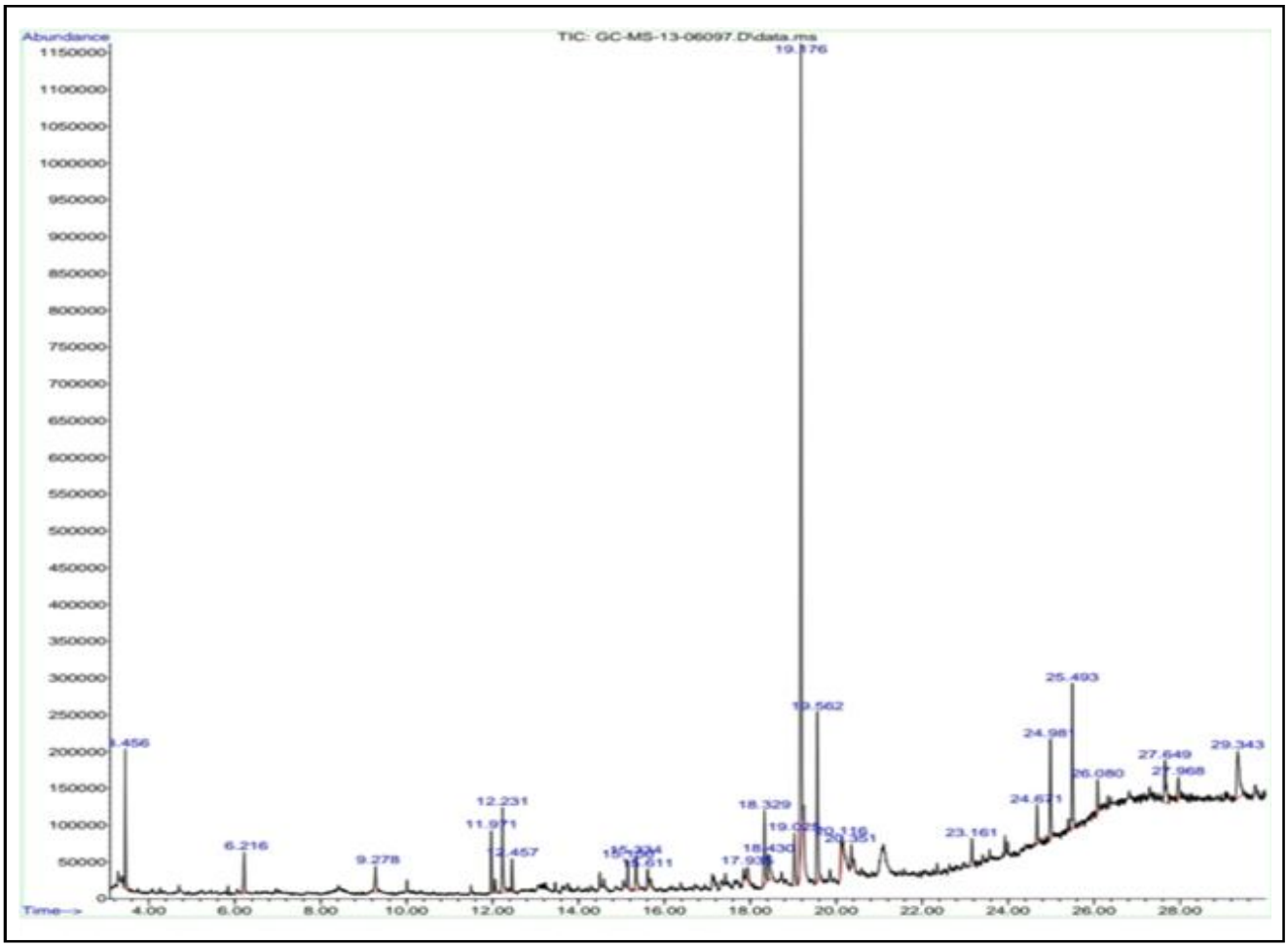


Figure 3: GC-MS chromatogram of *S. cristaefolium* secondary metabolites.

Table 2: GC-MS analysis for secondary metabolites produced by *S. cristaefolium*

S. No.	Name of the compound	Peak Area (%)	Retention Time	MW (g/mole)	Molecular formula	Specific role	References
1	Methanamine	4.30	3.456	31.05	CH_3NH_2	Antibacterial activity	Spinu *et al.* (2016)
2	Dodecane	1.57	9.278	170.33	$C_{12}H_{26}$	Antimicrobial activity	Ortansa *et al.* (2020)
3	2-Naphthalenemethanol	1.91	15.334	158.20	$C_{11}H_{10}O$	Antifungal activity	Carrillo *et al.* (2023)
4	Silane	3.56	18.329	32.11	H_4Si	Antifungal activity	Oldertrøen *et al.* (2017)
5	Tridecanoic acid	1.98	18.430	214.34	$C_{13}H_{26}O$	Antimicrobial activity	Chowdhury *et al.* (2021)
6	Methaqualone	2.74	19.025	250.30	$C_{16}H_{14}N_2O.$	Antibacterial activity	Du *et al.* (2021)
7	6-Octadecenoic acid	1.18	20.351	282.46	$C_{18}H_{34}O$	Antimicrobial properties	Chelliah *et al.* (2017)
8	Eicosane	1.59	23.161	282	$C_{20}H_{42}$	Antifungal, antibacterial, antitumor and cytotoxic effects	Hsouna *et al.* (2011)

9	1,4-Benzenedicarboxylic acid	4.41	24.981	166.02	$C_8H_6O_4$	Antibacterial and Antifungal activities	Guo *et al.* (2022)
10	Squalene	6.33	25.493	410.71	$C_{30}H_{50}$	Antimicrobial, antioxidant, antistatic and anticarcinogenic.	Chenniappan *et al.* (2020)
11	hexamethyl-Cyclotrisiloxane	1.56	26.080	222.46	$C_6H_{18}O_3Si_3$	Antibacterial activity	Bhuyar *et al.* (2020)
12	2,4-dimethyl- Benzo[h] quinolone	3.37	27.649	207.27	$C_{15}H_{13}N$	Antibacterial activity	Sánchez*et al.* (2022)
13	Silicic acid	1.32	27.968	96.113	H_4O_4Si	Antibacterial activity	Kalaivani *et al.* (2023)
14	Phenol	1.48	17.918	94.113	C_6H_6O	Antibacterial activity	Maddox *et al.* (2010)
15	n-Hexadecanoic acid	2.71	18.472	256.424	$C_{16}H_{32}O$	Antibacterial, antifungal and anti-inflammatory	Krishnaveni *et al.* (2014)
16	Ethanone	4.90	19.319	196.24	$C_{14}H_{12}O$	Antibacterial activity	Ferdosi *et al.* (2021)
17	Phytol	0.54	19.848	296.53	$C_{20}H_{40}O$	Antibacterial	Pejin *et al.* (2014)
18	Octadecanoic acid	1.35	20.334	284.47	$C_{18}H_{36}O_2$	Antibacterial activity	Pejin *et al.* (2014)
19	3-Cyclopenten-1-one	2.10	21.072	82.10	C_5H_6O	Antifungal activity	Soliman *et al.* (2022)
20	Stigmasterol	1.93	29.343	412.69	$C_{29}H_{48}O$	Antimicrobial activity	Mailafiya *et al.* (2018)

3.4. Evaluating seaweed extract efficacy against onion twister blight

In the pot culture experiment, all treatments exhibited significant reductions in disease incidence compared to the control (Figure 4). Treatment T3, utilizing *S. cristaefolium* extract, stood out with a remarkable 69.39% reduction in disease incidence, comparable to the chemical check Mancozeb (T9), which showed a 71.73% disease reduction. These results underscore the efficacy of *S. cristaefolium* extracts in managing onion twister blight, suggesting their potential as alternatives to traditional chemical treatments.

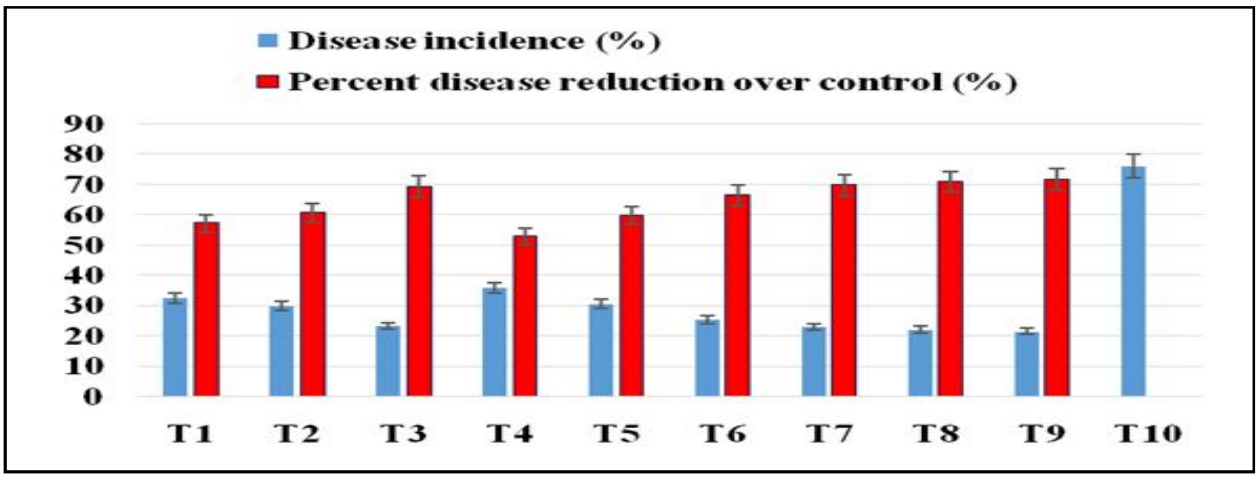


Figure 4: Evaluating the efficacy of promising seaweed extract against onion twister blight under pot culture conditions.

3.5 Induction of defense mechanisms in onion plants

Upon challenge inoculation with *C. gloeosporioides*, biochemical changes in treated onion plants were meticulously examined. Activities of peroxidase (PO), polyphenol oxidase (PPO), and phenylalanine ammonia-lyase (PAL) were evaluated over a period of 0 to 7 days after inoculation. Treated plants demonstrated significant increases in enzyme activities compared to the control group. Notably, Treatment T3 exhibited remarkable induction of PO activity on the 7th day after inoculation, with a change in absorbance of 0.910 /min/ g of leaf tissue (Figure 5). Similarly, T3 displayed the highest PPO activity at 1.371 changes in absorbance /min/g of leaf tissue on the 7th day, followed closely by T6 at 1.368 (Figure 6). PAL activity was also substantially enhanced in treated plants, with T3 exhibiting the highest activity on the 7th day after inoculation, doubling from 0.557 to 0.847 µm of transcinnamic acid/ min/ g of leaf tissue (Figure 7). These findings suggest that seaweed extracts induce defense mechanisms in onion plants, potentially contributing to enhanced disease resistance.

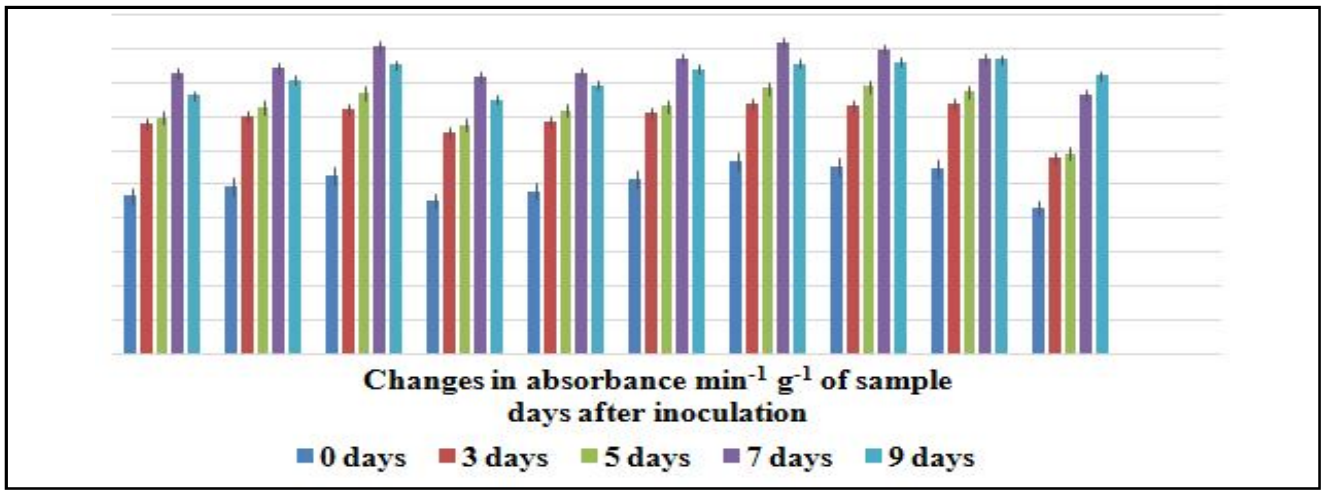


Figure 5: Induction of peroxidase (PO) activity in treated onion challenged with *C. gloeosporioides*.

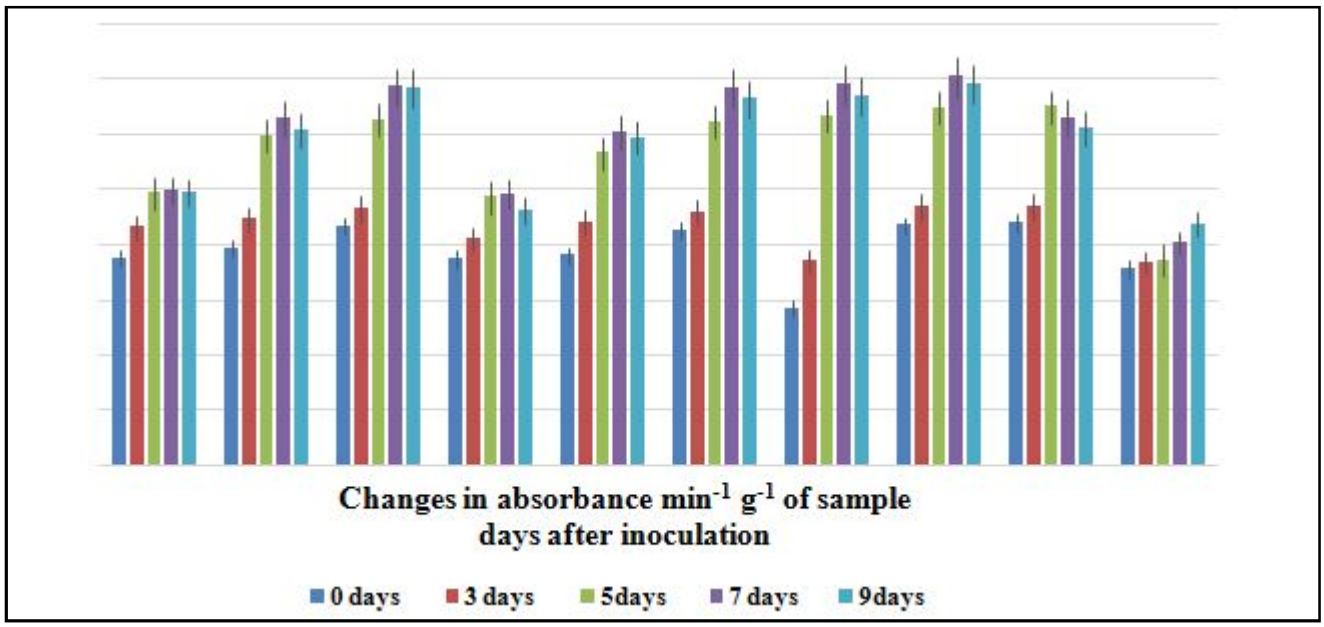


Figure 6: Induction of polyphenol oxidase (PPO) activity in treated onion challenged with *C. gloeosporioides.*

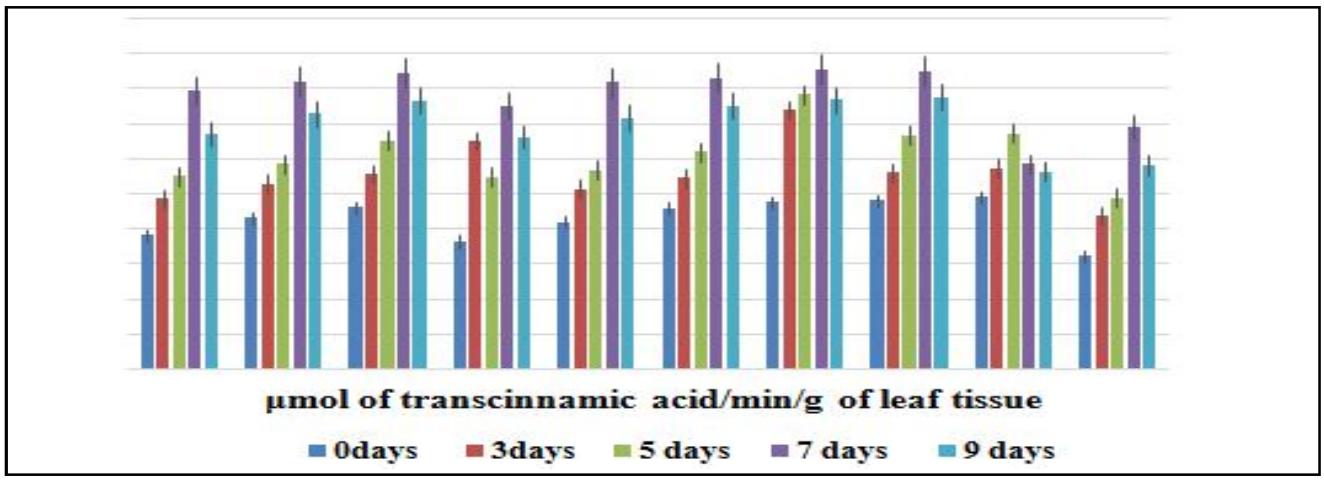


Figure 7: Induction of phenylalanine ammonia lyase (PAL) activity in treated onion challenged with *C. gloeosporioides.*

The analysis of leaf and bulb protein content in treated onion plants revealed variations across different treatments. The leaf protein content ranged from 0.28 to 0.44 mg/g, with Treatment 3 (T3) exhibiting the highest leaf protein content of 0.41 mg/g. Treatments 7 (T7), 8 (T8), and 9 (T9) also showed comparable leaf protein content to T3. Conversely, Treatment 4 (T4) and the control group exhibited lower leaf protein content, with values of 0.29 mg/g and 0.28 mg/g, respectively. Similarly, bulb protein content varied from 0.85 to 1.20 mg/g among the different treatments. Treatments 7, 8, 9, and 3 displayed the highest bulb protein content, with values of 1.20 mg/g, 1.18 mg/g, 1.12 mg/g, and 1.09 mg/g, respectively (Table 3).

Table 3: Determination of leaf and bulb protein in onion challenged with *S. cristaefolium* against *C. gloeosporioides*

T. No.	Treatments	Leaf protein (mg/g FW)	Bulb protein (mg/g FW)
T 1	BT with *S. cristaefolium* @ 3% + SD with *S. cristaefolium* @ 3% + FA of *S. cristaefolium* @ 3%	0.30	0.92
T 2	BT with *S. cristaefolium* @ 5% + SD with *S. cristaefolium* @ 5% + FA of *S. cristaefolium* @ 5 %	0.35	0.96
T 3	BT with *S. cristaefolium* @10% + SD with *S. cristaefolium* @ 10% + FA of *S. cristaefolium* @ 10%	0.41	1.09
T 4	BT with *K. alvarezii* @ 3% + SD with *K. alvarezii* @ 3% + FA of *K. alvarezii* @3%	0.29	0.89
T 5	BT with *K. alvarezii* @ 5% + SD with *K. alvarezii* @ 5% + FA of *K. alvarezii* @ 5%	0.31	0.94
T 6	BT with *K. alvarezii*@10% + SD with *K. alvarezii* @ 10% + FA of *K. alvarezii* @10%	0.39	0.98
T 7	BT with *T. asperellum* @ 4 g/kg of bulb + SD with *T. asperellum* @ 2.5 kg/ha + FA of *T.asperellum* @ 0.5%.	0.43	1.12
T 8	BT with *B. subtilis* @ 10 g/kg of bulb + SD with *B. subtilis* @2.5 kg/ha + FA of *B. subtilis* @ 0.5%	0.44	1.18
T 9	BT with Mancozeb @ 2 g/kg of bulb + SD with Mancozeb @ 2.5 g/litre of water + FS with Mancozeb @ 0.25%	0.43	1.20
T 10	Control	0.28	0.85

3.6. Histopathological studies and microscopic examination

Microtome sections of onion leaf tissues provided visual insights into tissue responses to infection. Infected leaf tissues exhibited structural damage, including collapsed xylem and phloem vessels, along with the presence of brown-colored mycelium within plant cells. In contrast, *S. cristaefolium*-treated plants displayed reduced tissue damage, with a regular arrangement of xylem and phloem observed (Figure 8). These histopathological findings support the potential of *S. cristaefolium* extract in mitigating pathological effects and maintaining tissue integrity.

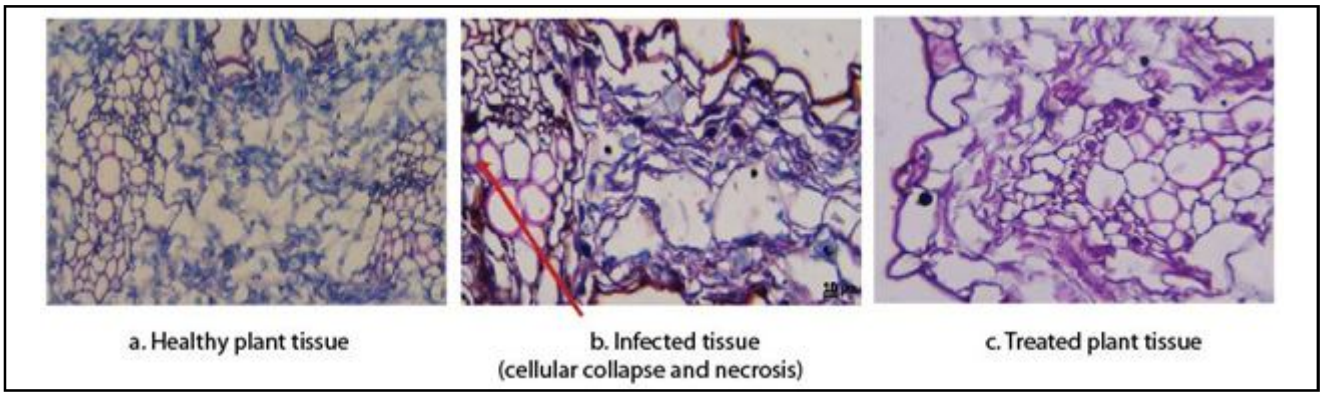


Figure 8: Histopathological studies. a. Healthy plant tissues, b. Infected plant tissues lead to the collapse of cells and necrosis, c. Treated plant tissues maintain cellular health, with a regular arrangement of xylem and phloem.

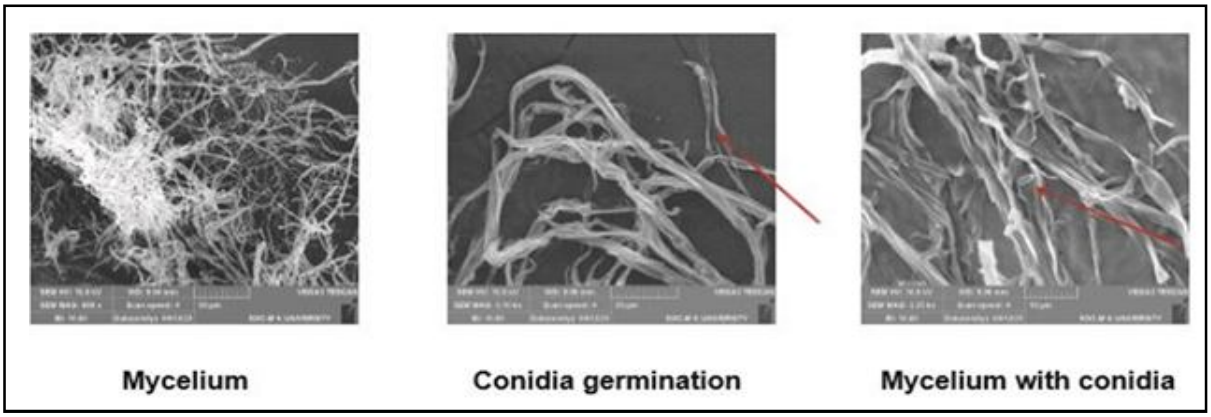
3.7 Study on the effect of promising seaweed extract on mycelial growth of *C. gloeosporioides* through scanning electron microscope

The study observed an antagonistic impact of *S. cristaefolium* against *C. gloeosporioides* (OCMK-3) using scanning electron microscope (SEM). The mycelium growth was affected, leading to its collapse and the absence of conidia production. Additionally, the pathogen growth was visibly damaged by the production of the antifungal properties generated by the seaweed. Before Seaweed Treatment, under the scanning electron microscope (SEM), the mycelial characteristics of a virulent isolate of *C. gloeosporioides* (OCMK-3) were analyzed. The results revealed that the mycelium exhibited branching, while the conidia appeared cylindrical in shape with rounded ends (Figure 9a).

After seaweed treatment, the study observed an antagonistic impact of *S. cristaefolium* against *C. gloeosporioides* (OCMK-3) using

scanning electron microscopy (SEM). The growth of the mycelium was notably affected, leading to its collapse and the absence of conidia production. Moreover, visible damage to the pathogen growth was observed, likely due to the antifungal properties produced by the seaweed (Figure 9b). Overall, visual observations revealed that seaweed-treated plants exhibited vigorous growth and a healthier appearance compared to the control plant (Figure 10).

a. Observation of *C. gloeosporioides* (OCMK-3) before *S. cristaefolium* treatment



b. Observation of *C. gloeosporioides* (OCMK-3) after *S. cristaefolium* treatment

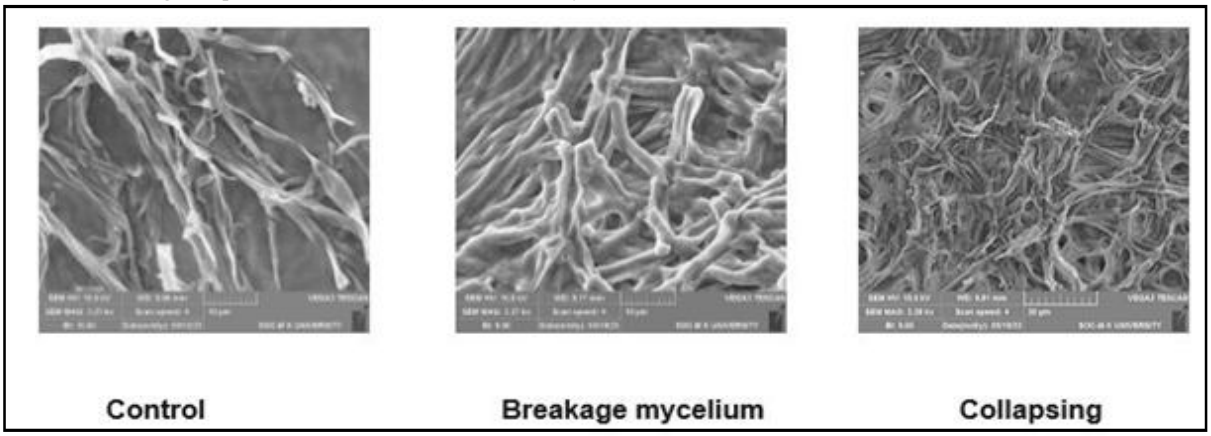


Figure 9: Scanning electron microscopy (SEM) of *C. gloeosporioides* (OCMK-3). a. normal mycelia characteristics, b. breaking and collapsing of mycelium.

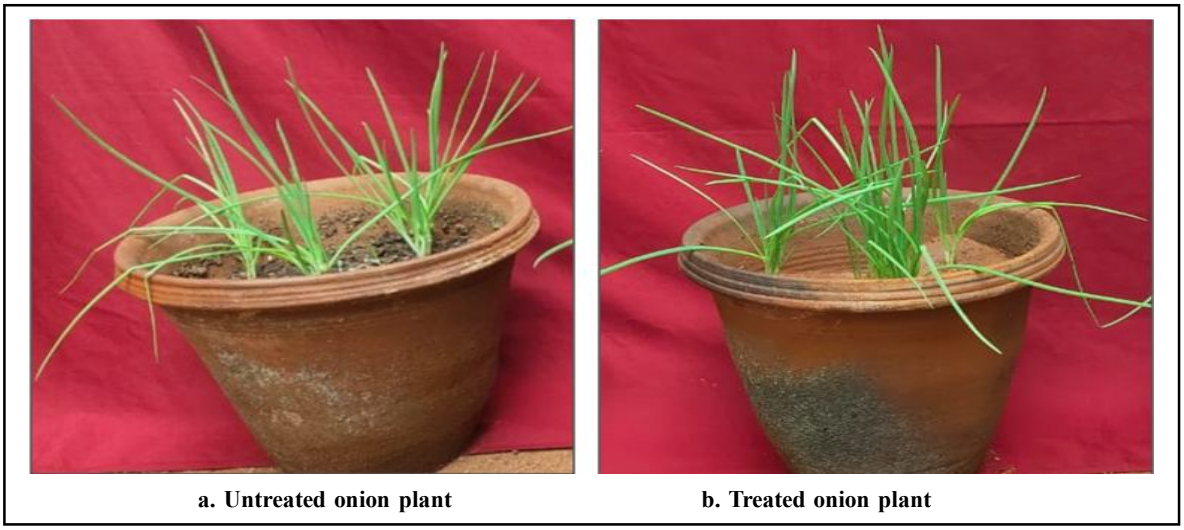


Figure 10: Observation of onion plants treated with *S. cristaefolium* a. Untreated onion plant, b. Seaweed treated onion plant.

3.8 Evaluating efficacy against onion twister blight under field condition

The efficacy of seaweed extracts from *S. cristaefolium* and *K. alvarezii* in reducing twister blight incidence was assessed under field condition. Among the seaweed extracts, *S. cristaefolium* exhibited notable effectiveness in reducing disease incidence. Treatment T3, involving *S. cristaefolium* at a concentration of 10%, demonstrated a significant disease reduction of 67.51% compared to the control (Table 4). This reduction in disease incidence was comparable to the efficacy of the chemical fungicide Mancozeb (T9), which recorded a 69.44% reduction. Additionally, biocontrol agents such as *T. asperellum* (T7) and *B. subtilis* (T8) displayed substantial disease reductions of 68.53% and 67.95%, respectively. These results underscore the potential of *S. cristaefolium* extracts in managing twister blight, aligning with the efficacy of traditional chemical and biological treatments.

Table 4: Evaluating the efficacy of promising seaweed extract against onion twister blight under field conditions

T. No.	Treatments	Disease incidence (%)	Percent disease reduction over control (%)
T 1	BT with *S. cristaefolium* @ 3% + SD with *S. cristaefolium* @ 3% + FA of *S. cristaefolium* @ 3%	31.58 (34.19)	51.16
T 2	BT with *S. cristaefolium* @ 5% + SD with *S. cristaefolium* @ 5% + FA of *S. cristaefolium* @ 5 %	28.57(32.30)	55.82
T 3	BT with *S. cristaefolium* @10% + SD with *S. cristaefolium* @ 10% + FA of *S. cristaefolium* @ 10%	21.01(27.28)	67.51
T 4	BT with *K. alvarezii* @ 3% + SD with *K. alvarezii* @ 3% + FA of *K. alvarezii* @3%	32.89 (34.99)	49.14
T 5	BT with *K. alvarezii* @ 5% + SD with *K. alvarezii* @ 5% + FA of *K. alvarezii* @5%	29.57 (32.93)	54.27
T 6	BT with *K. alvarezii* @10% + SD with *K. alvarezii* @ 10% + FA of *K. alvarezii* @10%	23.67(29.11)	63.39
T 7	BT with *T. asperellum* @ 4 g/kg of bulb + SD with *T. asperellum* @ 2.5 kg/ha + FA of *T. asperellum* @ 0.5%.	20.35(26.81)	68.53
T 8	BT with *B. subtilis* @ 10 g/kg of bulb + SD with *B. subtilis* @2.5 kg/ha + FA of *B. subtilis* @ 0.5%	20.72 (27.28)	67.96
T 9	BT with Mancozeb @ 2 g/kg of bulb + SD with Mancozeb @ 2.5 g/litre of water + FS with Mancozeb @ 0.25%	19.76(26.39)	69.44
T 10	Control	64.67 (53.53)	0.00
CD (p=0.05%)		0.856	

*Mean of three replications.

*Values in the parentheses are arc sine transformed values.

4. Discussion

4.1 *In vitro* screening of different solvents of promising seaweed extract @ 10% against the *C. gloeosporioides*

This phenomenon was used as a means for *in vitro* screening of bio-stimulants like seaweed extract against *C. gloeosporioides*. The experimental results revealed that among various solvents of promising seaweed extracts, methanol extract of 10 % *S. cristaefolium* showed the least mycelial growth (2.43 cm) of the pathogen under *in vitro* conditions. In contrast, *K. alvarezii* recorded the second-highest reduction in mycelial growth with 3.67 cm and a reduction rate of 59.22 per cent. Among the five seaweed extracts, *S. cristaefolium* exhibited unparalleled and remarkable suppression of mycelial growth of *C. gloeosporioides*, followed by *K. alvarezii*, as recorded by Jeevitha *et al*. (2023). Ambika and Sujatha (2015) reported that *S. myriocystum* extract effectively inhibited the mycelial growth of *Alternaria porri* in onion and *C. gloeosporioides* in sugarcane. Mani *et al*. (2018) found that *K. alvarezii* was resistant to chilli anthracnose caused by *C. gloeosporioides*.

4.2 GC-MS analysis of *S. cristaefolium* secondary metabolites

The comprehensive GC-MS analysis of the elite seaweed extract from *S. cristaefolium* revealed a complex chemical profile, highlighting its potential as a source of bioactive compounds. Notably, hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl) ethyl ester emerged as a predominant compound, suggesting its potential contribution to the observed antifungal activity. This finding corroborates previous research on the efficacy of similar compounds in combating fungal diseases (El-Sheekh *et al*., 2020). Additionally, the presence of phytol underscores the multifaceted benefits of *S. cristaefolium* extracts, given its antimicrobial properties and role as a precursor for essential vitamins (Pejin *et al*., 2014). Further exploration of individual metabolites and their synergistic effects could elucidate the mechanisms underlying the antifungal properties of *S. cristaefolium* extracts.

4.3 Evaluating efficacy against onion twister blight under pot culture

A pot culture study was conducted to assess the efficacy of promising seaweed extracts against *C. gloeosporioides* in onion. The results revealed that all the treatments significantly reduced twister blight incidence. Among all the treatments, T3 (BT with *S. cristaefolium* at 10% + SD with *S. cristaefolium* at 10% and FA of *S. cristaefolium* at 10%) showed 69.39 per cent twister blight disease reduction over the control. These results were consistent with the foliar spray and root drench, respectively at 0.5 and 1 per cent of *Ascophyllum nodosum* extract, effectively reducing disease in greenhouse cucumber plants (Jayaraman *et al*., 2011). Additionally, the report of brown algae extract from *S. myriocystum* at 10 per cent reduced the mycelial growth of *C. gloeosporioides* under *in vitro* conditions (Ambika and Sujatha, 2015).

4.4 Induction of defense mechanisms in onion plants

Biochemical analysis unveiled a robust activation of defense mechanisms in onion plants upon treatment with *S. cristaefolium* extracts. The substantial elevation in peroxidase (PO), polyphenol oxidase (PPO), and phenylalanine ammonia-lyase levels underscores the reinforcement of plant defense responses. These findings are consistent with the concept of induced systemic resistance, where seaweed extracts stimulate plant defenses against pathogens (De Corato *et al*., 2017). The observed elevation in PAL activity further highlights the activation of phenylpropanoid metabolism, which facilitates the synthesis of defense-related compounds. The parallel findings with previous studies support the notion of a multifaceted enhancement of the plant's defense arsenal upon seaweed extract treatment (Jayaraj *et al*., 2008).

The observed variations in leaf and bulb protein content across different treatments suggest the influence of seaweed extracts and other treatments on the nutritional status of onion plants. Treatment 3 consistently showed higher leaf and bulb protein content compared to other treatments, indicating its potential in enhancing protein accumulation in onion tissues. Conversely, Treatment 4 and the control group exhibited comparatively lower protein content, highlighting the importance of effective disease management strategies in maintaining optimal plant nutrition. The findings underscore the multifaceted effects of seaweed extracts on plant physiology, including nutrient uptake and utilization. Additional research is necessary to elucidate the mechanisms driving the observed alterations in protein content and to refine treatment protocols to enhance the nutritional advantages in onion cultivation. Additionally, investigating the impact of protein content on plant growth and disease resistance would provide valuable insights into the overall effectiveness of the treatments in onion production systems.

4.5 Histopathological studies and microscopic examination

Histopathological examination revealed the protective effect of *S. cristaefolium* extracts on onion plant tissues against *C. gloeosporioides* infection. The maintenance of tissue integrity and the absence of pathological damage in treated tissues underscore the potential of seaweed extracts in fortifying plant resilience against fungal pathogens. These observations offer valuable visual evidence of the positive impact of seaweed extract treatment on plant health, emphasizing its potential as a sustainable strategy for disease management.

The SEM analysis provided valuable insights into the structural changes induced in *C. gloeosporioides* (OCMK-3), following treatment with *S. cristaefolium*. The observed collapse of mycelial growth and the absence of conidia production suggest a strong antagonistic effect of the seaweed extract against the pathogen. These findings support the potential of *S. cristaefolium* as an effective agent for controlling fungal infections in plants, highlighting its promising role in integrated disease management strategies. Further investigation into the underlying mechanisms of action and optimization of treatment protocols could enhance the practical application of seaweed-based treatments for disease management in agriculture.

4.6 Evaluating efficacy against onion twister blight under field condition

The findings of the study highlight the effectiveness of *S. cristaefolium* extracts in reducing twister blight incidence, suggesting their potential as alternative or complementary management strategies alongside conventional treatments. Treatment T3, utilizing *S. cristaefolium* at a 10% concentration, exhibited comparable efficacy to chemical fungicides and biocontrol agents, emphasizing the practicality of seaweed extracts in disease management. These findings align with prior research highlighting the antifungal efficacy of seaweed extracts against diverse plant pathogens.

The observed reductions in disease incidence may be attributed to the presence of bioactive compounds in *S. cristaefolium* extracts, which have the potential to either inhibit fungal growth or activate plant defense mechanisms. Further investigations into the specific mechanisms of action and optimization of application methods are warranted to enhance the practicality and efficacy of seaweed extract-based treatments. Overall, the results suggest that *S. cristaefolium* extracts hold promise as effective strategies for twister blight management in onion cultivation, contributing to sustainable and eco-friendly disease management practices.

5. Conclusion

The multifaceted analysis of *S. cristaefolium* seaweed extracts provides compelling evidence of their potential in managing onion twister blight caused by *C. gloeosporioides*. The GC-MS analysis revealed key secondary metabolites, shedding light on the chemical constituents responsible for the observed antifungal activity. Concurrently, the pot culture study demonstrated the practical efficacy of *S. cristaefolium* extracts in reducing disease incidence, highlighting their promise as alternative disease management strategies. Moreover, the induction of defense mechanisms in onion plants treated with *S. cristaefolium* extracts, as evidenced by biochemical changes and enzyme assays, elucidates the intricate ways in which seaweed extracts bolster plant resistance against fungal pathogens. These findings underscore the potential of seaweed extracts to enhance plant resilience and contribute to sustainable agriculture practices. Histopathological studies further complemented these findings, offering a visual representation of the protective effects of *S. cristaefolium* extracts on onion plant tissues. The observed preservation of tissue integrity in treated plants compared to infected tissues provides tangible evidence of the beneficial effects of seaweed extract treatment. Further investigations into the specific modes of action and optimization of application methods will be crucial for maximizing the practicality and integration of seaweed extracts into agricultural practices, thereby enhancing crop health and productivity.

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Author contribution

P. Mahalakshmi: Conceptualization, designed the experiments, interpretation, and writing. P. Jeevitha: Data collection, statistical analysis, and writing. K. Sujatha and Suthin Raj: Conception, designed the experiments. M. Ayyandurai: Data collection, statistical analysis, interpretation. M. Karthikeyan: Writing, interpretation, and data reporting of the study.

Conflict of interest

The authors declare no conflicts of interest relevant to this article.

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