PLANT SCIENCE TODAY
Vol 12(sp3): 01–10
https://doi.org/10.14719/pst.8833

eISSN 2348-1900

RESEARCH ARTICLE

Padina gymnospora: A promising phyto elicitor for managing cluster bean anthracnose

Suthin Raj Thankaraj[1], Bhakiyathu Saliha Basheer Ahmed[1], Ebenezer Babu Rajan Rajaian[1]*, Shoba Devadhasan[1], Muthukumar Arjunan[2], Sathiya Aravindan Venkatesan[2] & Ann Suji Hudson[3]

[1]Agricultural Research Station, Tamil Nadu Agricultural University, Kovilpatti 628 501, Tamil Nadu, India
[2]Department of Plant Pathology, Annamalai University, Chidambaram 608 002, Tamil Nadu, India
[3]Centre for Advance Studies in Marine Biology, Annamalai University, Parangipettai 608 502, Tamil Nadu, India

*Correspondence email - ebenezer.babu18@gmail.com

Received: 12 April 2024; Accepted: 14 June 2025; Available online: Version 1.0: 07 September 2025

Cite this article: Suthin RT, Bhakiyathu SBA, Ebenezer BRR, Shoba D, Muthukumar A, Sathiya AV, Ann SH. *Padina gymnospora*: A promising phyto elicitor for managing cluster bean anthracnose. Plant Science Today. 2025;12(sp3):01–10. https:/doi.org/10.14719/pst.8833

Abstract

Cluster bean (*Cyamopsis tetragonoloba*) is a vital leguminous crop cultivated worldwide, valued for its diverse values in culinary and medicinal uses. Despite the economic significance, the crop faces substantial yield losses due to anthracnose, a fungal disease in cluster bean incited by *Colletotrichum lindemuthianum*. This study assesses the efficacy of different seaweed extracts in suppressing fungal growth and controlling cluster bean anthracnose under *in vitro* conditions. Field experiment was conducted using randomised block design (RBD) in Annamalai Nagar, Cuddalore District, Tamil Nadu, involving eight treatments, including an untreated control group. Among the tested extracts, *P. gymnospora* (brown seaweed) exhibited the highest inhibition rates, ranging from 85.31 % to 86.54 % at 30 % concentration. Comprehensive field trials were conducted, wherein *P. gymnospora* was applied as foliar spray at 30 % concentration at 50, 70 and 90 days after planting. This application strategy substantially reduced anthracnose incidence by 30.16 %, 49.58 % and 64.45 % on 60th, 80th and 100th days, respectively. These findings strongly suggest that *P. gymnospora* is a potent bio agent that can effectively manage cluster bean anthracnose. This research investigation highlights the potential of seaweeds in enhancing crop yield and reducing the dependency on synthetic chemicals. The Gas Chromatography-Mass Spectrometry (GC-MS) results identified nine phytochemical compounds were in *P. gymnospora*, among which, 1,2-Benzenedicarboxylic Acid, Diethyl Ester and Naphthalene might be responsible for the suppression of the growth of *C. lindemuthianum.* The study also facilitates scope for the further exploitation of the seaweed based biopesticides for managing other fungal pathogens, supporting the establishment of cropping systems designed for long term resilience and sustainability.

Keywords: cluster bean; *Colletotrichum lindemuthianum*; GC-MS; *Padina gymnospora*; seaweed extracts

Introduction

Cluster bean (*Cyamopsis tetragonoloba*) constitutes an essential vegetable crop, playing a pivotal role in both the global economy and nutritional standards. India being one of the leading producers, confronts considerable obstacles in cluster bean cultivation attributable to biotic and abiotic stressors. Notably, anthracnose, incited by the fungus *Colletotrichum lindemuthianum*, presents a significant challenge, resulting in severe fruit decay and potential yield reductions of up to 50 % in affected locales (1). This pathogen is widespread across all the cluster bean-cultivating states in India, imposing notable economic and agricultural havocs (2). Customarily, the control of anthracnose has highly relied on chemical fungicides. Nevertheless, the incessant application of these chemical substances poses the severe concerns regarding human health, environmental sustainability and the development of pathogen resistance. Taking this into account, green solutions such as biopesticides and biological control organisms have started to stand out. Among these, seaweed extracts have proven to be a promising solution due to their eco-friendly nature and minimal impact on the environment (3).

Seaweed extracts are rich in bioactive constituents, comprising polysaccharides, polyphenols and essential minerals, which stimulate plant growth and induce systemic resistance and exhibit antimicrobial properties against a wide range of plant pathogens (4, 5). Prior investigations have established that these extracts elevate germination of seeds, improve plant health and effectively manage plant pathogens, making them a sustainable and safer alternative to chemical pesticides. Despite these promising properties, only a limited number of studies have directly compared the effectiveness of seaweed extracts with conventional fungicides in controlled environments, specifically against *C. lindemuthianum.* This main aim of the research investigation is to evaluate the antimicrobial effectiveness of various seaweed extracts against *C. lindemuthianum*. Furthermore, it investigates their performance both *in vitro* and *in vivo*, providing comprehensive

insights into the capability of seaweed as a viable option for anthracnose management. Additionally, research was conducted on the various seaweed extracts used and their potential for promoting cluster bean growth. The study also included GC-MS chemical profiling of *Padina gymnospora* and an analysis of the binding affinity of its volatile compounds to the target protein of *C. lindemuthianum*.

Materials and Methods

In vitro studies

Collection of seaweed samples and preparation of seaweed extracts

Seaweeds were sourced from the intertidal zone during low tide using a random sampling method. The samples were preserved in labelled polythene bags using both wet and dry methods (6), shade-dried in the laboratory for seven days and identified at the Centre for Advanced Studies in Marine Biology, Annamalai University. Fifteen seaweeds, comprising five each of brown, red and green species, were used for the study. Seaweeds were washed with freshwater to remove impurities, dried at 40 °C, stored in a cool, dry environment and ground into fine powder. For water filtrate preparation, 100 g of powder was boiled in 100 mL of distilled water for one hour, filtered through muslin cloth and adjusted to 100 mL. Alternatively, 100 g of powder was soaked in 100 mL distilled water for two days and filtered to obtain 100 % Solvent Water Concentrate (SWC) (6). For Soxhlet extraction, 20 g of powder was packed in a porous cellulose thimble (25 × 80 mm) and placed in a Soxhlet extractor with 250 mL of water in a round-bottom flask. The setup was attached to a condenser and the solvent was heated, evaporated, condensed and siphoned back to the reservoir in cycles for six hours (8). Extracts were cooled, transferred to clean containers, stored at 4 °C and diluted to required concentrations for use.

In vitro efficacy of seaweed extracts

Poison food assay was conducted with sterilised Potato Dextrose Agar (PDA) medium. Seaweed extract was added to the growth medium at concentrations of 10 mL, 20 mL and 30 mL in 70 mL, 80 mL and 90 mL of medium, respectively. A control treatment without seaweed extract was also maintained. Plates were inoculated at the centre with a 10-day-old culture disc (9 mm) of *C. lindemuthianum* and incubated at 28 ± 2 °C for 10 days. Colony diameters were measured and the per cent inhibition over control was calculated using the formula:

$$\text{Per cent Inhibition} = \frac{(T_1 - T_2) \times 100}{T_1} \quad \text{(Eqn 1)}$$

where T_1 is colony diameter in control plates and T_2 is colony diameter in treated plates.

The efficacy of seaweed extracts was further validated through an agar well assay and disc diffusion assay. In the disc diffusion assay, PDA plates containing 15 mL medium were seeded with a 9 mm disc of *C. lindemuthianum* culture. Then, sterile filter paper discs (10 mm) were dipped in seaweed extracts three different concentrations of 10 %, 20 % and 30 % and kept at the peripheral corners of the plates. Control discs dipped in sterile distilled water were also maintained. Plates were incubated at 28 ± 2 °C for 10 days and the zone of inhibition around the treated discs were measured. In the agar well assay, standard wells of 9 mm were created in PDA plates and 100 µL of seaweed extracts at the three concentrations were added to the wells. Then, the PDA plates were inoculated with the culture of *C. lindemuthianum* and under the same incubation conditions as before, zones of inhibition surrounding the wells were measured, thereby further validating the efficacy of the seaweed extracts against the fungus. In all these assays, three replicates of each treatment were maintained.

GC-MS Analysis of *P. gymnospora* extract

The GC-MS analysis was carried out with *P. gymnospora* extract. The preparation of methanolic extracts through the process of solvent extraction and filtration ensured that phytochemicals of differential polarities were recovered. The gas chromatograph separated the individual compounds based on their retention time and identification of their molecular structure was done by the mass spectrometer, comparing the fragmentation patterns with established libraries. The compounds were docked using the software Molegro Viewer and Auto Dock Vina to find the recognition, successful binding and establishment of *P. gymnospora* with the target protein of the pathogen.

In vivo studies

Seed germination and seedling vigour (Rolled towel method)

The impact of seaweed extracts on cluster bean seed germination and seedling vigour was investigated using the rolled towel method. Cluster bean seeds were surface-sterilized and then treated with varying concentrations of *P. gymnospora* seaweed extract (1, 2 and 3 %, respectively). After being rolled and sandwiched between damp germination sheets, treated seeds were incubated for seven days at 25 °C in a germination chamber. Numerous metrics, including vigour index, shoot length, root length and germination percentage, have been measured and contrasted with untreated controls.

Pot culture experiment

The seeds of cluster bean were sown in pots filled with sterilized soil mixture comprising red soil, sand and farmyard manure in the ratio of 2:1:1. The seeds were treated with *P. gymnospora* extract at two different concentrations, namely 1 %, 2 % and 3 %. The treatments consisted of soil drenching at the root zone and foliar sprays at 50, 70 and 90 days after sowing (DAS). Controls consisted of untreated plants. The treatments' effectiveness was measured by recording various growth parameters like plant height, the number of branches and pod yield per plant on 60th, 80thand 100th DAS. Disease suppression was measured by observing anthracnose incidence and severity caused by *C. lindemuthianum.* Statistical Package for Social Sciences (SPSS) software (version 25.0, IBM Corp., Armonk, NY) was used to statistically analyse the data for Analysis of Variance (ANOVA). Duncan's Multiple Range Test (DMRT) was used to assess if there were significant differences between treatment means at p=0.05.

Field trials

Cluster bean seedlings were transplanted at 21 DAS when they had developed 3-4 true leaves, ensured optimal establishment while minimized transplant shock. Growth parameters were

recorded at 30, 50 and 70 days after transplanting, while disease incidence and severity were assessed weekly starting from first disease appearance 45 days after transplanting (DAT) until harvest. Final yield parameters were recorded at harvest maturity (90 days after transplanting) when the pods had reached their physiological maturity. Analysis of variance (ANOVA) was carried out using SPSS software (version 25.0, IBM Corp., Armonk, NY). Duncan's Multiple Range Test (DMRT) was used to identify significant differences between treatment means at p=0.05.

Results

Efficacy of seaweed extracts - *In vitro* studies

The antimycotic properties of fifteen species of seaweed including five brown algae, six red algae and four green algae were tested against *C. lindemuthianum* at three concentrations (10 %, 20 % and 30 % w/v) and the results were presented in (Tables 1, 2) and (Fig. 1, 2). In the poison food assay, *P. gymnospora* showed the best antimycotic activity with an 86.50 % inhibition of mycelial growth at 30 % concentration at 15 days post inoculation, followed by *Caulerpa racemosa* (74.43 %) and *Ulva lactuca* (73.28 %). The inhibitory effect was concentration dependent, with the maximum fungal growth inhibition at 30 % concentration ($p < 0.05$). The organisms exhibited greatest activity in the disc diffusion and agar well assays with *P. gymnospora* producing zones of inhibition of 83.11 % and 84.52 %, respectively, at 30 % concentration at 15 days post inoculation (Fig. 3-5). The results confirmed the potent antimycotic activity of the species *P. gymnospora* and *C. racemosa*, while *Acanthophora spicifera* showed least inhibition ranging from 46.10 % to 47.45 %. The rolled towel method using *P. gymnospora* extract demonstrated exceptional growth promotion, achieving 96.50 % germination (Table 3)

Efficacy of seaweed extracts - *In vivo* studies

P. gymnospora macroalgae extract demonstrated exceptional growth promotion a mean shoot length of 9.80 cm, a root length of 7.60 cm and a vigour index of 1720.00, outperforming other seaweed extracts (Table 3). In pot trials, cluster bean plants treated with 3 % *P. gymnospora* extract showed significantly improved growth and yield parameters, with the highest plant with average height of (175 cm), number of branches (7.5) and pod yield (62.1 g/plant) observed with soil application, followed closely by foliar treatment (46.2 cm, 7.2 branches and 60.4 g/plant). Field trials mirrored these trends, 28.1 % disease incidence (76.75 % reduction) yielding the tallest plants with average height of (200.50 cm), the highest number of pods per plant (30.8), the maximum total yield (7.3 kg/plot) and the lowest disease incidence (26.5 % with 79.35 % reduction). Foliar application of 3 % extract also showed strong performance, with 49.3 cm plant height, 29.4 pods per plant, 6.9 kg/plot yield and control plants consistently showed the poorest performance across all metrics, confirming the efficacy of *P. gymnospora* extract, particularly with soil application, in enhancing cluster bean growth, yield and anthracnose disease suppression (Table 4). Disease incidence was lowest with 3 % soil application (25.3 %, with reduction of 79.35 % reduction), slightly higher with 3 % foliar application (27.5 %, 76.75 % reduction) and significantly higher in untreated controls (65.3 %) (Table 5).

Gas chromatography and Molecular Docking analysis

GC-MS analysis of methanolic extract of *P. gymnospora* unmasked the presence of bioactive compounds including naphthalene (22.45 % peak area, RT: 13.82 min), which could interfere with fungal metabolic pathways and 1,2-benzenedicarboxylic acid diethyl ester (18.67 % peak area, RT: 19.45 min), a compound reported for its sporicidal and growth-inhibitory action against fungi (Table 6) (Fig. 6).

Molecular docking experiments found that naphthalene has a 3.76 Å binding affinity to the fungal target protein chitin deacetylase (PDB ID: 2IW0), indicating possible disease suppression efficacy. The 3D Docking view (Fig. 7.) showed the chemical fitting into the target protein's active site and interacting with crucial amino acid residues. Green shading emphasizes hydrophobic and van der Waals interactions, which keep the molecule in the binding pocket, whereas dashed lines reflect hydrogen bonds (H-bonds), which are essential for polar interactions that increase binding affinity. The comprehensive interaction diagram (Fig. 8) showed specific interaction details, such as hydrophobic interactions

Table 1. Seaweeds against *Colletotrichum lindemuthianum* under *in vitro* using poison food

Sl. No.	Seaweed extract	Poison food technique					
		Mycelial growth(mm)*			Inhibition over control (%)		
		10 %	20 %	30 %	10 %	20 %	30 %
1.	*Padina gymnospora*	38.95^{a}	36.01^{a}	12.15^{a}	56.72 (48.91)	59.99 (50.72)	86.50 (68.98)
2.	*Caulerpa racemosa*	55.88^{ef}	52.45^{fg}	30.76^{g}	37.91 (37.93)	41.72 (40.23)	65.81 (54.20)
3.	*Ulva lactuca*	52.42^{def}	49.20^{fg}	29.45^{fg}	41.75 (40.19)	45.33 (42.27)	66.72 (54.80)
4.	*Helimeda gracilis*	55.85^{fg}	53.71^{g}	35.10^{h}	38.06 (37.79)	40.87 (39.67)	60.99 (51.14)
5.	*Caulerpa peltata*	60.42^{h}	58.18^{hi}	38.11^{i}	32.86 (34.85)	35.47 (36.56)	57.65 (49.42)
6.	*Sargassum wightii*	49.15^{cd}	47.10^{de}	27.81^{ef}	45.39 (42.34)	47.66 (43.73)	69.10 (56.21)
7.	*Liagora ceranoides*	56.11^{gh}	53.92^{h}	34.21^{i}	37.66 (37.72)	40.55 (39.56)	62.98 (52.48)
8.	*Hypnea panosa*	43.75^{b}	41.51^{bc}	23.01^{b}	51.39 (45.82)	53.87 (47.11)	74.43 (59.71)
9.	*Ulva reticulata*	46.13^{bc}	42.85^{cd}	24.05^{cd}	48.74 (43.61)	52.83 (46.33)	73.28 (59.02)
10.	*Hydroclathrus clathratus*	41.48^{b}	39.22^{b}	23.32^{c}	53.91 (47.19)	56.52 (48.61)	73.83 (59.33)
11.	*Chnoospora implexa*	61.42^{ij}	59.10^{ij}	40.58^{j}	31.75 (34.22)	33.89 (35.52)	54.91 (47.81)
12.	*Dictyota bartyrensiana*	52.87^{cde}	50.89^{ef}	30.67^{ef}	40.14 (39.31)	42.90 (40.84)	65.92 (54.31)
13.	*Jania rubens*	45.18^{cd}	43.54^{de}	25.24^{de}	49.80 (44.85)	51.62 (45.97)	71.96 (58.08)
14.	*Acanthophora spicifera*	59.16^{gh}	56.11^{h}	37.02^{i}	34.27 (35.81)	36.85 (37.31)	59.98 (50.72)
15.	*Gracilaria corticata*	53.87^{de}	51.77^{ef}	34.12^{fg}	40.15 (39.35)	42.55 (40.65)	62.08 (52.00)
	Control	90.00^{j}	90.00^{j}	90.00^{k}	-	-	
	CD Values	5.24	4.98	3.76	4.31	4.07	5.13

*Mean of three replications, Data in parameters are arc sin transformed values,

Values in the column followed by common letters do not differ significantly by DMRT (P=0.05)

Table 2. Efficacy of different seaweed extracts against Colletotrichum lindemuthianum under in vitro conditions using disc diffusion assay and agar well method

	Disc diffusion assay						Agar well assay					
	Inhibition zone											
Sl.No.	Mycelial growth(mm)*			PROC			Mycelial growth(mm)*			PROC		
	10 %	20 %	30 %	10 %	20 %	30 %	10 %	20 %	30 %	10 %	20 %	30 %
1.	50.86^{a}	41.53^{a}	15.20^{a}	43.49 (41.26)	53.8 (47.21)	83.11 (65.73)	49.59^{a}	41.39^{a}	13.93^{a}	44.90 (42.07)	54.01 (47.30)	84.52 (66.83)
2.	69.84^{ef}	65.79^{f}	43.27^{g}	22.40 (28.25)	26.9 (31.24)	51.92 (46.10)	68.56ef	64.52^{f}	42.00^{h}	23.82 (29.21)	28.31 (32.15)	53.33 (46.91)
3.	65.79^{def}	62.43^{ef}	39.92^{fg}	26.90 (31.24)	30.6 (33.60)	55.64 (48.24)	64.52^{def}	61.16^{ef}	38.64^{gh}	28.31 (32.15)	32.04 (34.48)	57.07 (49.06)
4.	70.54^{fg}	66.94^{f}	43.93^{h}	21.62 (27.70)	25.6 (30.41)	51.19 (45.68)	69.26^{fg}	65.67^{f}	42.77^{i}	23.04 (28.69)	27.03 (31.32)	52.48 (46.42)
5.	75.87^{hi}	70.48^{gh}	48.37^{i}	15.70 (23.34)	21.6 (27.75)	46.26 (42.86)	74.59^{hi}	69.21^{gh}	47.21^{j}	17.12 (24.45)	23.10 (28.73)	47.54 (43.59)
6.	63.79^{cd}	59.02^{cd}	36.82^{de}	29.12 (32.65)	34.4 (35.93)	59.09 (50.24)	62.51^{cd}	57.74^{cd}	35.66^{def}	30.54 (33.54)	35.84 (36.77)	60.38 (50.99)
7.	71.01^{gh}	67.76^{g}	44.60^{i}	21.10 (27.35)	24.7 (29.81)	50.44 (45.25)	69.74^{gh}	66.48^{g}	43.32^{j}	22.51 (28.32)	26.13 (30.74)	51.87 (46.07)
8.	56.59^{b}	52.03^{bc}	30.30^{b}	37.12 (37.54)	42.1 (40.51)	66.33 (54.53)	55.32^{b}	50.75^{bc}	29.03^{b}	38.53 (38.37)	43.61 (41.33)	67.74 (55.39)
9.	59.13^{bc}	53.30^{bcd}	32.44^{cd}	34.30 (35.85)	40.7 (39.69)	63.96 (53.10)	57.86^{bc}	52.03^{bcd}	31.17^{cd}	35.71 (36.70)	42.19 (40.51)	65.37 (53.96)
10.	53.85^{b}	49.13^{b}	30.96^{c}	40.17 (39.33)	45.4 (42.37)	65.60 (54.09)	52.58^{b}	47.85^{b}	29.81^{c}	41.58 (40.15)	46.83 (43.18)	66.88 (54.87)
11.	78.35^{i}	71.81^{h}	51.21^{j}	12.94 (21.09)	20.2 (26.71)	43.10 (41.04)	77.07^{ij}	70.53^{h}	49.93^{k}	14.37 (22.28)	21.63 (27.71)	44.52 (41.85)
12.	67.90^{de}	62.36^{de}	41.15^{ef}	24.56 (29.71)	30.7 (33.65)	54.28 (47.45)	66.73^{de}	61.09^{de}	39.87^{efg}	25.86 (30.56)	32.12 (34.52)	55.70 (48.27)
13.	59.71^{cd}	53.49^{cd}	32.71^{de}	33.66 (35.47)	40.5 (39.57)	63.66 (52.92)	58.43^{cd}	52.21^{cd}	31.43^{de}	35.08 (36.31)	41.99 (40.39)	65.08 (53.78)
14.	74.24^{h}	68.22^{g}	46.24^{i}	17.51 (24.74)	24.2 (29.47)	48.62(44.21)	72.97^{h}	66.94^{g}	44.97^{j}	18.92 (25.78)	25.62 (30.41)	50.03 (45.02)
15.	68.22^{def}	60.86^{ef}	41.99^{efg}	24.20 (29.47)	32.3 (34.68)	53.3(46.92)	67.03^{de}	59.58^{ef}	40.71^{fgh}	25.52 (30.34)	33.80 (35.55)	54.77 (47.73)
	90.00^{j}	90.00^{i}	90.00^{k}	-	-	-	90.00^{j}	90.00^{i}	90.00^{l}	-	-	-
CD	2.64	2.48	2.77	2.71	2.95	2.67	2.39	2.67	2.60	2.60	2.58	2.74

Values in the column followed by common letters do not differ significantly by DMRT (P=0.05); *Mean of three replications, Data in parameters are arc sin transformed values .PROC Per cent Reduction Over Control

Fig. 1. Axenic culture of *Colletotrichum lindemuthianum* (Front side).

Fig. 2.Axenic culture of Colletotrichum lindemuthianum (Back side).

Fig. 3. Poison food assay of *P. gymnospora* against *Colletotrichum lindemuthianum at*15 DAI.

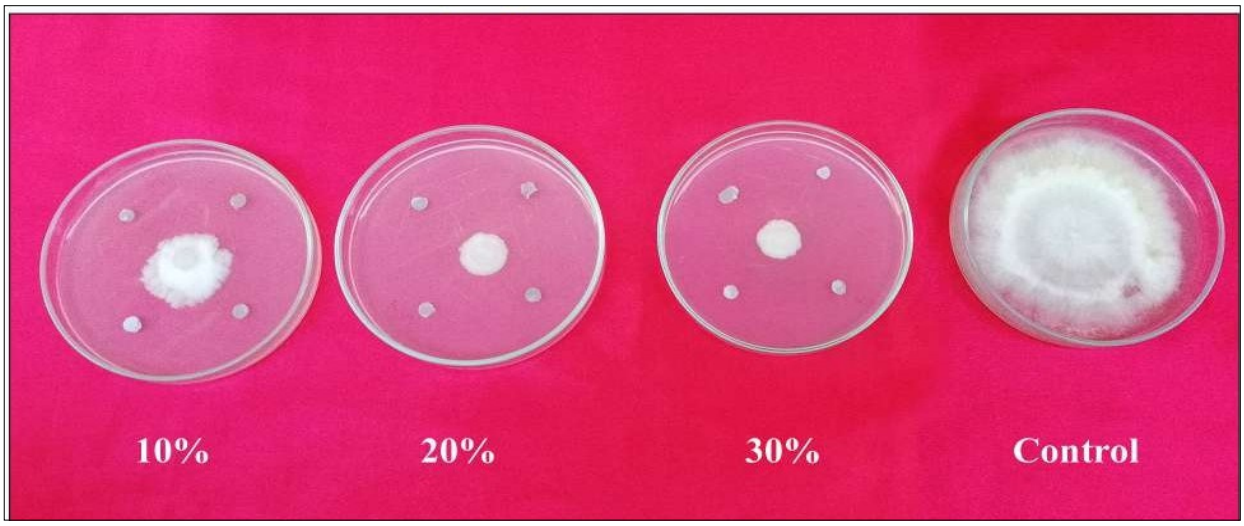

Fig. 4. Disc diffusion assay of *P. gymnospora* against *Colletotrichum lindemuthianumat*15 DAI.

Fig. 5. Agar well assay of *P. gymnospora* against *Colletotrichum lindemuthianum at*15 DAI.

Table 3. Effect of different seaweed extract on growth promotion of cluster bean by rolled towel method

Name of the Seaweed Extract	Germination (%)	Shoot Length (cm)	Root Length (cm)	Vigour Index
Padina gymnospora	96.50 (82.57) ^{a}	9.80^{a}	7.60^{a}	1720.00^{a}
Caulerpa racemosa	95.00 (78.80) ^{ab}	9.60^{ab}	7.50^{ab}	1650.00^{ab}
Ulva lactuca	90.50 (73.00) ^{b}	9.50^{ab}	7.30^{abc}	1550.00^{b}
Helimeda gracilis	88.50 (70.30) ^{bc}	9.40^{ab}	7.20^{abc}	1500.00^{bc}
Caulerpa peltata	86.00 (68.00) ^{cd}	9.10^{bc}	6.90^{bcd}	1410.00^{cd}
Sargassum wightii	87.00 (69.00) ^{bc}	9.30^{bc}	7.10^{bcd}	1465.00^{bc}
Liagora ceranoides	89.00 (71.00) ^{b}	9.60^{ab}	7.40^{abc}	1540.00^{b}
Hypnea panosa	87.50 (69.20) ^{bc}	9.20^{bc}	7.10^{bcd}	1450.00^{bc}
Ulva reticulata	94.80 (78.70) ^{ab}	9.70^{a}	7.60^{a}	1370.00^{ab}
Hydroclathrus clathratus	82.50 (65.40) ^{de}	8.80^{cd}	6.60^{cd}	1310.00^{de}
Chnoospora implexa	82.30 (65.30) ^{de}	8.70^{cd}	6.50^{de}	1300.00^{de}
Dictyota bartyrensiana	79.50 (63.00) ^{ef}	8.30^{de}	6.10^{de}	1180.00^{ef}
Jania rubens	81.00 (64.20) ^{de}	8.50^{cd}	6.30^{de}	1220.00^{de}
Acanthophora spicifera	78.00 (61.90) ^{ef}	8.20^{e}	6.00^{e}	1120.00^{ef}
Gracilaria corticata	83.50 (66.00) ^{cd}	9.00^{bc}	7.00^{bcd}	1380.00^{cd}
Carbendazim	95.50 (80.00) ^{ab}	9.70^{a}	7.55^{a}	1680.00^{ab}
Control	59.00 (50.20) ^{f}	4.90^{f}	3.70^{f}	530.00^{f}

*Mean of three replications, Data in parameters are arc sin transformed values, Values in the column followed by common letters do not differ significantly by DMRT (P=0.05)

Table 4. Assessment of cluster bean growth enhancement by various growth extract using rolled towel method

Pot culture trial							
Treatment	Avg plant height in (cm)	Branches/pot @			Avg yield (g/plant) recorded	Avg disease incidence	Avg disease reduction recorded
		60 DAS	80 DAS	100 DAS			
P. gymnospora (3 % soil)	175[a]	5.8[a]	6.7[a]	7.5[a]	62.1[a]	25.3	79.35
P. gymnospora (3 % foliar)	168[ab]	5.6[a]	6.5[a]	7.2[a]	60.4[a]	27.5	76.75
P. gymnospora (2 % soil)	160[b]	5.2[b]	6.3[b]	6.8[b]	56.3[b]	29.2	73.55
P. gymnospora (2 % foliar)	152[c]	5.0[b]	6.0[b]	6.3[b]	54.0[b]	33.6	67.55
Control (No treatment)	120[d]	4.0[c]	4.8[c]	5.2[c]	45.6[c]	65.3	-
CD (P=0.05)	8.0	0.3	0.3	0.5	5.0	3.0	

*Mean of three replications,, Data in parameters are arc sin transformed values,, Values in the column followed by common letters do not differ significantly by DMRT(P=0.05)

Table 5. Effect of *P. gymnospora* on cluster bean anthracnose incidence under field condition

Field trials					
Treatment	Plant height in (cm)	Pods/no./plant	Avg. yield (kg/plot)	Avg. disease incidence %	Avg. disease reduction %
P. gymnospora (3 % soil)	200.5[a]	30.8[a]	7.3[a]	25.3	79.35
P. gymnospora (3 % foliar)	190.8[b]	29.4[a]	6.9[a]	27.5	76.75
P. gymnospora (2 % soil)	180.3[c]	27.5[b]	6.1[b]	29.2	73.55
P. gymnospora (2 % foliar)	170.6[d]	26.1[b]	5.8[b]	33.6	67.55
Control (No treatment)	140.2[e]	22.3[c]	4.6[c]	65.3	-
CD (P=0.05)	7.5	1.8	0.5	3.2	

*Mean of three replications, Data in parameters are arc sin transformed values, Values in the column followed by common letters do not differ significantly by DMRT (P=0.05)

Table 6. Compounds identified in the *Padina gymnospora*

Sl. No	RT (min)	Name of the compound	Molecular formula	Molecular weight	Peak area %
1	5.27	Naphthalene	$C_{10}H_8$	128	35.47
2	10.04	Docosane	$C_{22}H_{46}$	310	14.02
3	11.36	1,2 – Benzenedicarboxylicacid,diethylester	$C_{12}H_{14}O_4$	222	21.96
4	12.84	Docosanoicacid,methylester	$C_{23}H_{46}O_2$	354	2.18
5	13.15	6-Hydroxy-4,4,7a-trimethyl-5,6,7,7 a-tetrahydrobenzofuran-2(4H)-one	$C_{11}H_{16}O_3$	196	3.57
6	14.16	Z, E-3,13-Octadecadien-1-ol	$C_{18}H_{34}O$	266	2.47
7	14.24	11,14-Eicosadienoicacid, methylester	$C_{21}H_{38}O_2$	322	6.66
8	14.68	Ethanol,2-(9,12-octadecadienyloxy),(Z,Z)-	$C_{20}H_{38}O_2$	310	2.33
9	15.21	Hexadecanoicacid, methylester	$C_{17}H_{34}O_2$	270	11.34

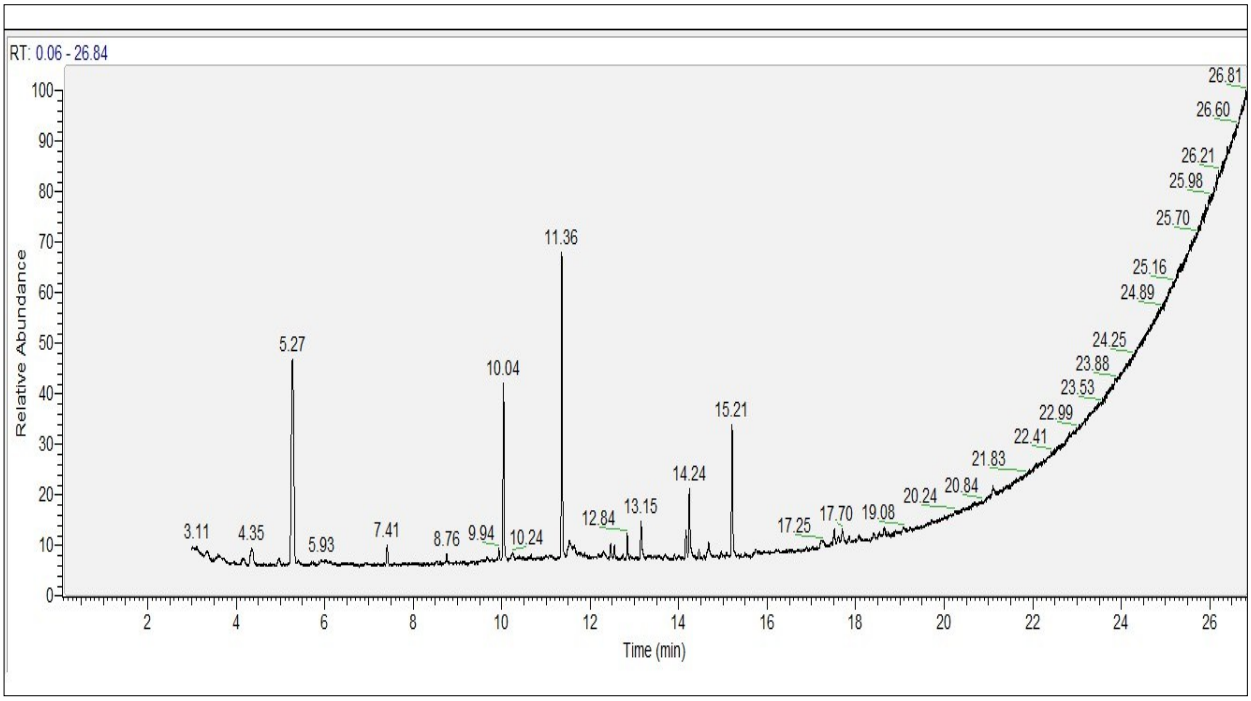

Fig. 6. Chromatogram of *P. gymnospora* GC-MS analysis.

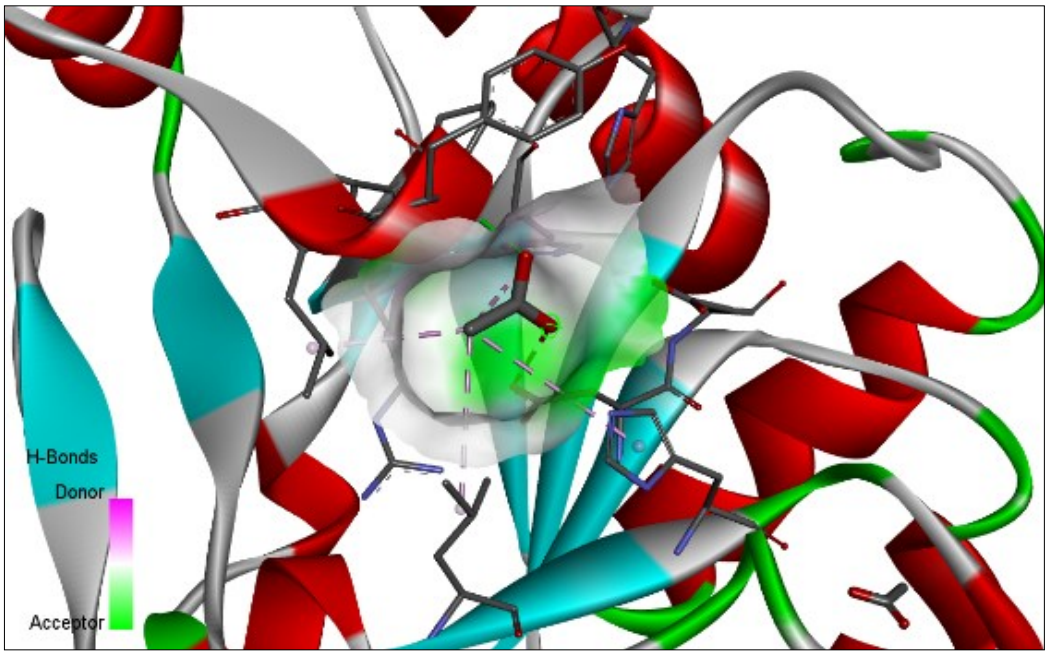

Fig. 7. 3D Docking view of *P. gymnospora* with *Colletotrichum lindemuthianum* target protein (PDB ID: 2IW0).

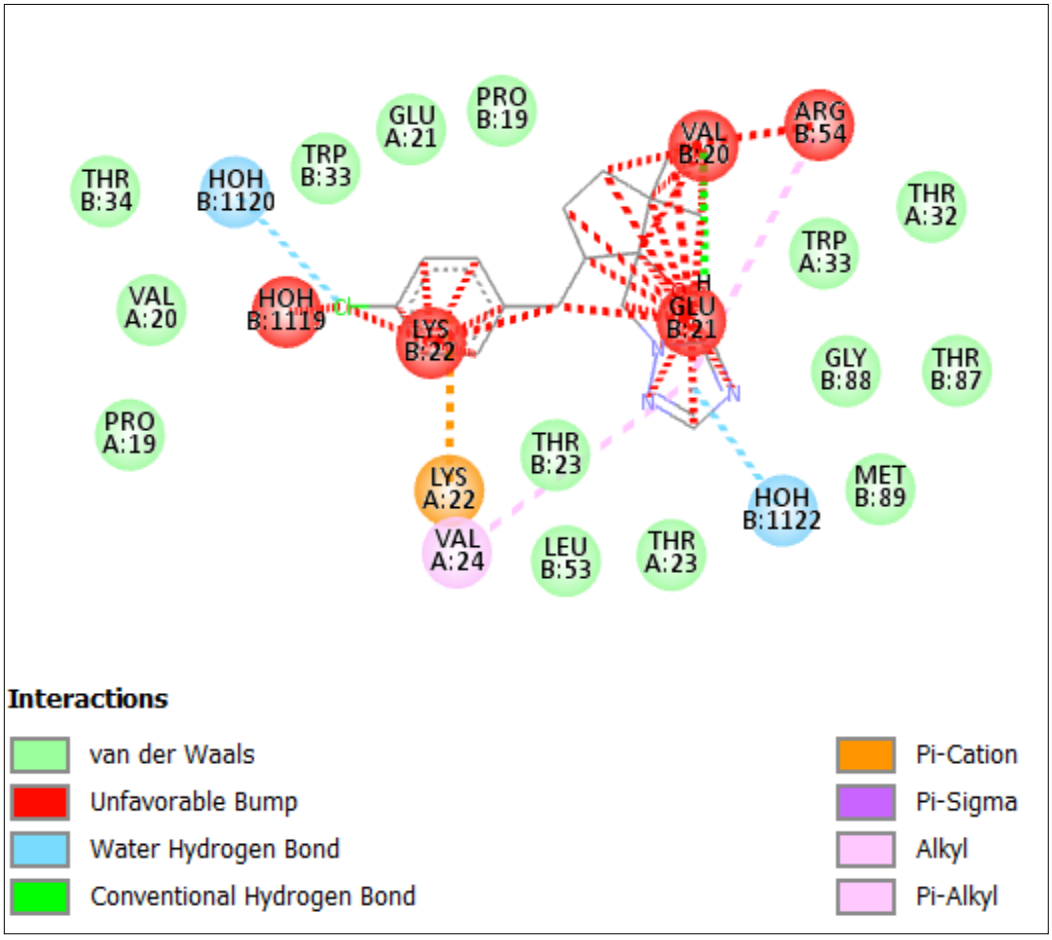

Fig. 8. Comprehensive interaction diagram of *P. gymnospora* with *C. lindemuthianum*.

from residues like VAL, PRO and MET, conventional H-bonds represented by green lines and π-cation and π-alkyl interactions from residues like ARG and GLU, which contributed to the compound's orientation and stability. Red patches in the diagram represented steric hindrance, indicating areas where binding efficiency may be slightly lowered due to spatial conflicts.

Discussion

The present investigation highlights the anti-mycotic properties of seaweed extracts *in vitro* against *C. lindemuthianum* causing cluster bean anthracnose. *P. gymnospora* showed maximum antimycotic activity out of the 15 screened seaweed species under all the *in vitro* methods. At a 30 % concentration of *P. gymnospora*, the poison food assay resulted in 86.50 % inhibition of mycelial growth; this was closely followed by that of *Caulerpa racemosa* and *Ulva lactuca*

with 74.43 % and 73.28 %, respectively. This was further validated by the clear zones of inhibition reflected in both the disc diffusion and agar well diffusion assays, where *P. gymnospora* reflected maximum inhibition percentage values of 83.11 % and 84.52 %, respectively, at a 30 % concentration. Therefore, the clear dose-dependent response reflected in all the *in vitro* assays demonstrated the efficacy of the tested seaweed species at higher concentrations ($p < 0.05$).

The exemplary antimycotic activity of the seaweed *P. gymnospora* resonated with the previous research findings that affirmed the efficiency of brown seaweed algae against fungal pathogens. *Sargassum myricocystum* reduced the mycelial growth of *C. falcatum* by nearly up to 50 % *in vitro*, while methanol extracts of *Cystoseira myriophylloides* inhibited tomato pathogens effectively (8). Likewise, the chloroform extracts of *Hormophysa cuneiformis* exhibited potent antifungal activity against multiple pathogens with minimum inhibitory concentrations (MICs) comparable to standard antifungal agents (9). The above depicted findings collectively underscored the broad- spectrum potency of brown algae, particularly *P. gymnospora*, as effective bio agents in plant disease management. In conjunction with *P. gymnospora*, green algae such as *C. racemosa* and *U. lactuca* demonstrated notable antimycotic properties, inhibiting fungal growth by over 70 % in the poison food assay. *Kappaphycus alvarezii* achieved up to 76 % inhibition of *Alternaria solani* at 5 % concentration, suggesting its strong efficacy against various *Colletotrichum* species and other foliar pathogens (10). This research investigation was further consolidated by the previous research findings (11), where the *Sargassum muticum* exhibited effective control of *Botrytis cinerea*, in fruit crops whereas *S. myricocystum* inhibited *Alternaria polianthi*, reducing the disease severity and disease incidence in pot culture experiment.

These findings emphasized that antifungal properties of these seaweeds could be further exploited to the greenhouse and field conditions. The superior germination rates observed under the rolled towel method highlighted the extract's efficacy in promoting seedling vigour, as evidenced by a germination percentage of 99.23 % and a vigour index of 1754.39. These observations can be attributed to the bioactive compounds and polysaccharides in *P. gymnospora*, which act as stimulants for early seedling establishment. The results were consistent with the earlier research findings (12), those reported the significant improvements in germination percentage (70 %) and germination index (0.933) in *Capsicum annuum* treated with *P. gymnospora* extract. Furthermore, studies have demonstrated that polysaccharide-enriched extracts of *P. gymnospora* significantly enhanced germination and growth in crops such as tomato and mung bean, reaffirming its role as a potent bio stimulant (13).

The application of *P. gymnospora* extract, particularly at a 3% concentration *via* soil application, significantly improved growth and yield parameters in cluster bean across pot and field trials. In pot trials, plants treated with 3 % v/w of soil achieved the highest average plant height of (175 cm), number of branches (7.5 at 100 DAS) and pod yield (62.1 g/plant), while field trials demonstrated similar trends, with the same treatment yielding the tallest plants with average height of (200.8 cm), the maximum pods/plant (30.8) and maximum total yield (7.3 kg/plot). The previous research investigation corroborated that seaweed extracts minimized productivity losses in tomato plants under salinity stress from 28.7 % to 3.4 %, while enhancing plant vigour and biomass (14). Further studies have highlighted the role of seaweed polysaccharides in enhancing nutrient absorption and strengthening plant resilience under stress, corroborating the significant improvements in growth and productivity of cluster green observed in the present study (15, 16)

The suppression of anthracnose disease caused by *C. lindemuthianum* highlighted the dual functionality of *P. gymnospora* extract. In both pot and field trials, the 3 % soil treatment resulted in the lowest disease incidence (25.3 % in pots and 26.5 % in fields), corresponding to disease reductions of 79.35 %. These results aligned with earlier findings, which demonstrated that *P. gymnospora* contains bioactive compounds with antimicrobial properties (17). The GC-MS analysis conducted in this study identified naphthalene (RT: 13.82 min, peak area: 22.45 %) as a major compound, likely contributing to fungal suppression by inhibiting fungal metabolism and spore germination, as supported by studies. Furthermore, the identification of compounds such as 1, 2-benzenedicarboxylic acid diethyl ester (RT: 19.45 min, peak area: 18.67 %) and docosane (RT: 27.13 min, peak area: 14.33 %) suggested additional protective mechanisms, such as enhanced plant resilience and hydrophobic barrier formation, as suggested by (18). The GC-MS analysis confirmed a diverse phytochemical profile in *P. gymnospora*, with ethyl acetate extracts containing key compounds such as fucosterol (12.45 %) and L-(+)-ascorbic acid 2, 6-dihexadecanoate (8.13 %), which are known for their antioxidant properties (17). The higher polyphenol concentrations in aqueous extracts, as reported in the studies, likely contribute to the observed improvements in plant vigour and disease suppression.

These research findings supported the role of *P. gymnospora* as a natural chemical defense against pathogens and environmental stressors, consistent with earlier studies on seaweed bioactivity. Molecular docking analysis of *P. gymnospora* bioactive compounds against Chitin Deacetylase (CDA), a key virulence factor produced by the fungal pathogen *C. lindemuthianum.* CDA catalyses the conversion of chitin, a structural polysaccharide in fungal cell walls, into chitosan, thereby evading plant immune responses by reducing the recognition of chitin as a Pathogen-Associated Molecular Pattern (PAMP) molecule. The docking studies revealed that naphthalene, a major bioactive compound identified in *P. gymnospora*, effectively binds to the target protein CDA (PDB ID: 2IW0) with a binding affinity of 3.76 Å, indicating a strong interaction. These results suggested that naphthalene may interfere with the enzymatic activity of CDA, thereby compromising the pathogen's ability to evade host defenses and establish infection.

The identification of bioactive molecules in *P. gymnospora* further enhances its potential as a bioagent. Previous studies have shown the broad-spectrum bioactivity of *P. gymnospora* through its antioxidant and antimicrobial properties (18). Molecular docking thus showed that the binding of naphthalene was effective with CDA, in line with previous literature highlighting the antifungal potency of

compounds from seaweed. The molecular docking could be applied to identify lead compounds targeting fungal proteins, including β-tubulin in the fungal pathogen, *Colletotrichum* (19). In the same vein, the binding of naphthalene to CDA in this study reinforces the use of docking studies for the elucidation of molecular mechanisms involved in antifungal activity. The outcome of this investigation combined with previously reported studies provided very strong support for continued studies involving brown seaweed algae species as nature's antifungal agents. Sustainable approaches to the management of diseases of plants could be achieved using such bioagents.

Conclusion

P. gymnospora, a macroalga, showed considerable promise as an antifungal agent against the causative agent of cluster bean anthracnose, *C. lindemuthianum*. From the *in vitro* assays, it was evident that this fungus was potentially inhibited by *P. gymnospora*, especially at a 30 % concentration. Field and pot trials have also pointed out the enormous increase in the growth of the plants, yields and disease suppression, particularly by using the extract as soil application. Its bioactive compounds responsible for antifungal and growth-promoting activity were identified by GC-MS. The present study underlines the use of *P. gymnospora* as an effective substitute to synthetic fungicides in an eco-friendly manner for sustainable agriculture.

Acknowledgements

The authors gratefully acknowledge the Department of Plant Pathology and the Centre for Advanced Studies in Marine Biology, Annamalai University, for providing laboratory and field infrastructure. We thank the Agricultural Research Station, Tamil Nadu Agricultural University, Kovilpatti, for experimental support. Special thanks to technical assistants who contributed to the GC-MS analysis and molecular docking studies. No external funding was received for this work.

Authors' contributions

SR carried out the field experiments, assisted in sample processing and contributed to manuscript preparation. BSBA participated in the GC-MS interpretation and biochemical assay standardization. EBRR conceived the study, coordinated the overall research activities and reviewed the manuscript. SD supported statistical data analysis and literature consolidation. MA supervised the pathogen isolation, maintained cultures and guided in vitro bioassays. SAV conducted the molecular docking, GC-MS profiling and drafted the manuscript. ASH helped in seaweed authentication, extraction preparation and contributed to study design. All authors read and approved the final manuscript.

Compliance with ethical standards

Conflict of interest: Authors do not have any conflict of interests to declare.

Ethical issues: None

References

1. Than PP, Jeewon R, Hyde KD, Pongsupasamit S, Mongkolporn O, Taylor PW. Characterization and pathogenicity of *Colletotrichum* species associated with anthracnose on chilli (*Capsicum* spp.) in Thailand. Plant Pathol. 2008;57(3):562-72. https://doi.org/10.1111/j.1365-3059.2007.01782.x
2. Naznin S, Khalequzzaman KM, Khair A. Effect of new fungicides in controlling anthracnose/die back disease of Chilli. Asian J Appl Sci Eng.. 2016);5(1):117-24. https://doi.org/10.18034/ajase.v5i1.71
3. Reddy PY, Jakhar SS, Dahiya OS. Management of fruit rot of chilli caused by *Colletotrichum capsici*. Int J Curr Microbiol Appl Sci. 2019;8(5):523-38.. https://doi.org/10.20546/ijcmas.2019.805.062.
4. Galal HR, Salem WM, Nasr El-Deen F. Biological control of some pathogenic fungi using marine algae. Res J Microbiol. 2011;6:645-57. https://doi.org/10.3923/jm.2011.645.657
5. Jayaraman J, Norrie J, Punja ZK. Commercial extract from the brown seaweed *Ascophyllum nodosum* reduces fungal diseases in greenhouse cucumber. J Appl Phycol. 2011;23(3):353-61. https://doi.org/10.1007/s10811-010-9547-1.
6. Pise NM, Sabale AB. Effect of seaweed concentrates on the growth and biochemical constituents of *Trigonella foenum-graecum* L. Journal Phytol. 2010;2(4):50-6.
7. Thanigaivel S, Thomas J, Chandrasekaran N, Mukherjee A. Preparation of marine algal (seaweed) extracts and quantification of phytocompounds. In: *Aquaculture Microbiology Protocols*. Springer Protocols Handbooks; 2023. p. 135–40. https://doi.org/10.1007/978-1-0716-3032-7_17
8. Redfern J, Kinninmonth M, Burdass D, Verran J. Using soxhlet ethanol extraction to produce and test plant material (essential oils) for their antimicrobial properties. J Microbiol Biol Educ. 2014;15 (1):45-6. https://doi.org/10.1128/jmbe.v15i1.656..
9. Ambika S, Sujatha K. Antifungal activity of aqueous and ethanol extracts of seaweeds against sugarcane red rot pathogen (*Colletotrichum falcatum*). Sci Res Essays. 2015;10(6):232-5.https://doi.org/10.5897/SRE2015.6198
10. Pereira L, Valado A. Harnessing the power of seaweed: Unveiling the potential of marine algae in drug discovery. Explor Drug Sci.. 2023;1 (6):475-96. https://doi.org/10.37349/eds.2023.00032
11. Kavitha M, VP SK. Antifungal efficacy of *Kappaphycus alvarezii* and other macro-algae against *Alternaria solani* induced early blight in tomato (*Solanum lycopersicum* L.). J Sci Res Rep. 2024;30(9):411-21. https://doi.org/10.9734/jsrr/2024/v30i92364
12. Agado VV. Seaweeds manual. 1976;5(2):365.
13. Zafar A, Ali I, Rahayu F. Marine seaweeds (biofertilizer) significance in sustainable agricultural activities: A review. In: Proceedings of IOP Conference Series on Earth and Environmental Science. 2022;974 (1):012080. https://doi.org/10.1088/1755-1315/974/1/012080
14. Toledo E, Félix C, Vicente TF, Augusto A, Félix R, Toledo B, et al. Seaweed extracts to control postharvest phytopathogenic fungi in Rocha Pear. Journal Fungi. 2023;9(2):269. https://doi.org/10.3390/jof9020269
15. Hernández-Herrera RM, Santacruz-Ruvalcaba F, Zañudo-Hernández J, Hernández-Carmona G. Activity of seaweed extracts and polysaccharide-enriched extracts from *Ulva lactuca* and *Padina gymnospora* as growth promoters of tomato and mung bean plants. Journal Appl Phycol. 2016;28:2549-60. https://doi.org/10.1007/s10811-015-0781-4
16. Hernández-Herrera RM, Sánchez-Hernández CV, Palmeros-Suárez PA, Ocampo-Alvarez H, Santacruz-Ruvalcaba F, Meza-Canales ID, et al. Seaweed extract improves growth and productivity of tomato plants under salinity stress. Agronomy. 2022;12(10):2495. https://doi.org/10.3390/agronomy12102495
17. Hernández-Herrera RM, Santacruz-Ruvalcaba F, Ruiz-López MA, Norrie J, Hernández-Carmona G. Effect of liquid seaweed extracts

on growth of tomato seedlings (*Solanum lycopersicum* L.). Journal Appl Phycol. 2014;26:619-28. https://doi.org/10.1007/s10811-013-0078-4

18. Shanmuganathan B, Sheeja Malar D, Sathya S, Pandima Devi K. Antiaggregation potential of *Padina gymnospora* against the toxic Alzheimer's beta-amyloid peptide 25-35 and cholinesterase inhibitory property of its bioactive compounds. PLoS One. 2015;10 (11):e0141708. https://doi.org/10.1371/journal.pone.0141708
19. Cob-Calan NN, Chi-Uluac LA, Ortiz-Chi F, Cerqueda-García D, Navarrete-Vázquez G, Ruiz-Sánchez E, et al. Molecular docking and dynamics simulation of protein β-tubulin and antifungal cyclic lipopeptides. Molecules. 2019;24(18):3387. https://doi.org/10.3390/molecules24183387
20. Praba N, Sumaya S. Study on phytochemical and antioxidant Properties of *Padina gymnospora* and *Ulva lactuca*. Int J Life Sci Pharma Res. 2022;12:155-60. https://doi.org/10.22376/ijpbs/lpr.2022.12.6.L155-160.
21. Mohamed SS, Saber AA. Antifungal potential of the bioactive constituents in extracts of the mostly untapped brown seaweed *Hormophysa cuneiformis* from the Egyptian coastal waters. Egyptian Journal of Botany. 2019;59(3):695-708. https://doi.org/10.21608/ejbo.2019.5516.1225.

Additional information

Peer review: Publisher thanks Sectional Editor and the other anonymous reviewers for their contribution to the peer review of this work.

Reprints & permissions information is available at https://horizonepublishing.com/journals/index.php/PST/open_access_policy

Publisher's Note: Horizon e-Publishing Group remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

Indexing: Plant Science Today, published by Horizon e-Publishing Group, is covered by Scopus, Web of Science, BIOSIS Previews, Clarivate Analytics, NAAS, UGC Care, etc
See https://horizonepublishing.com/journals/index.php/PST/indexing_abstracting

Publisher information: Plant Science Today is published by HORIZON e-Publishing Group with support from Empirion Publishers Private Limited, Thiruvananthapuram, India.