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# Inhibition of pathogen and induction of resistance against early blight: *Alternaria solani* Sorauer in tomato plants (*Solanum lycopersicum* L.) through external application of compounds from seaweed *Chaetomorpha antennina* Bory

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## Abstract

**Background** The indiscriminate application of pesticides has depleted soil fertility. Marine algae are regarded as excellent sources of bioactive compounds that can stimulate plant growth and also enhance their resistance against biotic and abiotic stressors. Compounds derived from the marine algae *Chaetomorpha antennina* (Cladophoraceae) were analysed for their biocontrol efficiency against the tomato disease pathogen *Alternaria solani* as an alternate technique to preserve agricultural output sustainability.

**Results** The active fraction from the methanol extract of *C. antennina* (CA-F8) proved effective against the pathogen in vitro by affecting *A. solani*'s spore germination (92.13%) along with their mycelial growth thereby decreasing their dry weight (44.71%). GC–MS examination revealed the presence of fatty acids (defence pathway activators), terpenes (antimicrobial compound) and antioxidants (deactivators of pathogen enzymes). Seaweed compounds also elicited polyphenol oxidase (PPO) (78.8%) and peroxidase (PO) (54.6%) synthesis, along with stimulating the accumulation of foliar phenols (67.5%) that are components of jasmonic acid defense pathway, higher compared to control plants. Salicylic acid was detected only in treated plants, thereby offering efficient disease control.

**Conclusions** By triggering natural systemic defences, salicylate (SA) and jasmonate (JA) mediated pathways; the study demonstrates the elicitor potentials of chemicals from *C. antennina* that was efficient in controlling early blight (EB) disease. In addition to providing a different method for crop protection to reduce or replace the demand for chemical pesticides, this study reveals unique projections.

**Keywords** Early blight, Mycelium inhibition, Disease control, Defense enzymes, Foliar phenol

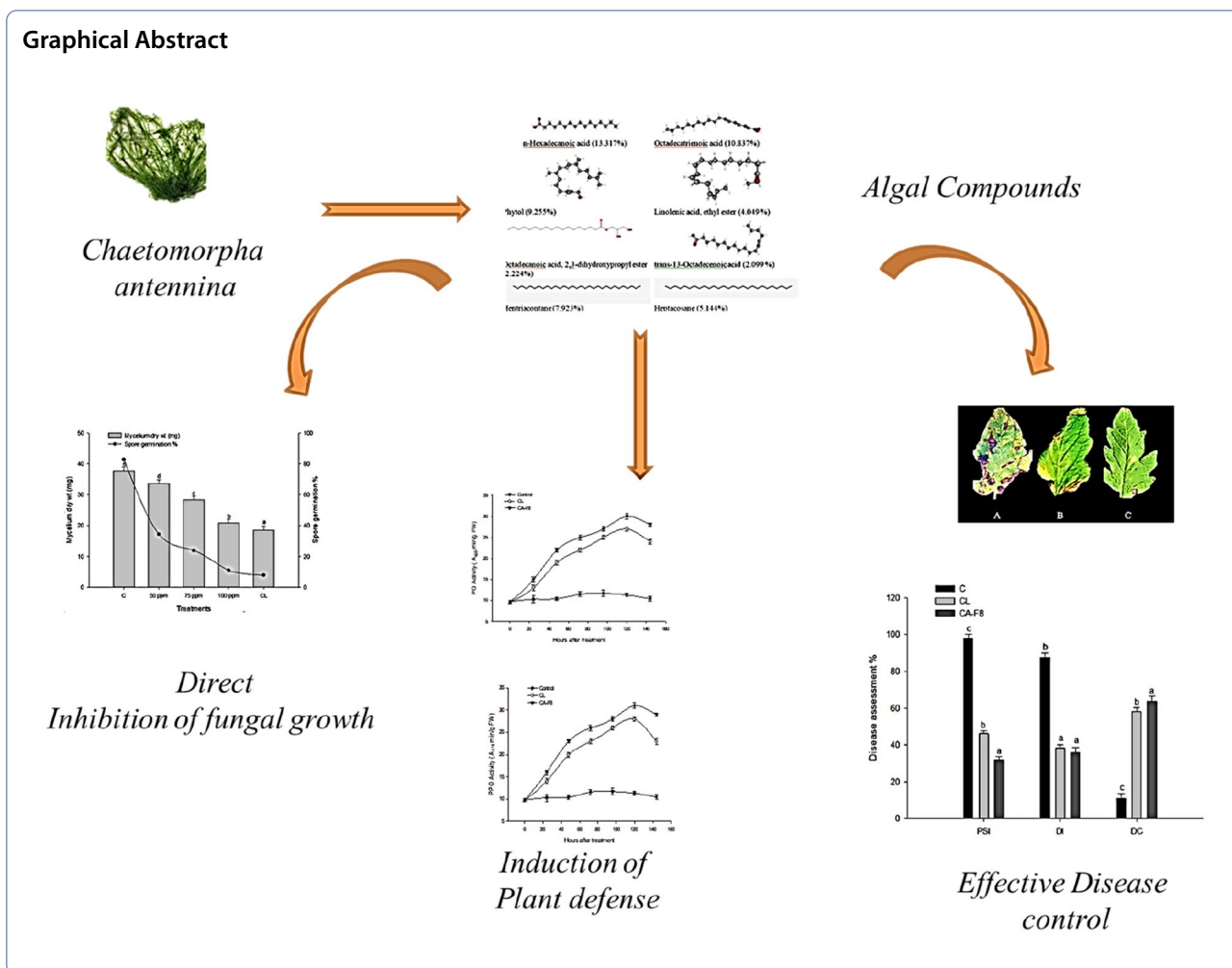
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**Background**

Agriculture is the most important activity that mankind has devised for its survival and propagation on our planet [1]. The predicted global population will entail a major decision to enhance food production to meet the increased demand. There are other agricultural challenges that must be addressed to produce the additional food. This necessitates the use of sustainable agriculture techniques [2]. Various approaches that are often used in ecological agriculture besides food systems to improve soil health, reduce water consumption, and reduce pollution. Organic insecticides and fertilisers are gradually displacing inorganic chemicals, which are costly and harmful to the environment [3]. As a result, improving farming techniques to include the use of organic mediators is critical to ensure the long-term viability of agriculture and, as a result, the ecosystem [4].

Tomatoes are a worldwide important crop with a high nutritional value. The world produced 186.821 million metric tonnes of tomatoes on 5,051,983 hectares in 2020,

achieving an average yield of 37.1 metric tonnes/hectare (mT/ha) [5]. The tomato plant, on the other hand, is susceptible to a variety of illnesses and insect pests that can cause extensive damage and reduce productivity. Integrated pest management (IPM), which entails the use of crop rotation strategies, the cultivation of disease-free cultivars, and fungicides, has been shown to be successful among several disease control methods [6]. Alternative disease control procedures have been enforced due to environmental pollution and health risks caused by chemical treatments [7].

The most damaging disease of tomato plants is EB, which is instigated by the necrotrophic fungus *Alternaria solani* commonly known as early blight disease, and results in a 79% yield loss [8]. During epidemics, disease control measures mostly entail the use of chemical fungicides, which are both costly and ineffectual. The lack of EB resistant cultivar variety adds to the difficulty of developing an efficient disease control strategy [9]. Plant secondary metabolites that can operate as

growth regulators, giving crops with improved nutrient absorption capacities, antibiotics, and the generation of induced systemic resistance are being studied for usage as biofertilizers, biostimulants, and biopesticides in this environment [10, 11]. Defensive role of plant's secondary metabolites have been confirmed by in vitro examining of plants for which expression of secondary metabolites was modified by modern techniques [12, 13]. Formation of secondary metabolites is the result of millions of years of plant's interaction with pathogens and it is considered that more than 100,000 metabolites are known to be involved in plant defense system, so the situation is still not clear [14, 15].

Oceans have traditionally been used as a valuable resource for providing commercially vital commodities. Countless marine organisms have yielded a variety of bioactive subordinate metabolites, which have been identified and described [16]. They have a repository of chemicals when it comes to modifying soil qualities. They are also notable for producing a diverse range of physiologically active biocidal compounds that are effective against phytopathogens. In terms of plant growth promotion and elicitation, phytohormones and other bigger compounds found in seaweeds, such as polysaccharides, are physiologically active [17]. Plants treated with seaweed extracts produce more pathogenesis-related proteins (PRPs) that aid in the protection against several pests and pathogens in a more effective manner [18].

A very wide and significant group of marine green algae are found in the Cladophoraceae family to which *Chaetomorpha antennina* (Bory) Kützinger, which belongs to. Numerous bioactive substances, including flavonoids, triterpenoids, alkaloids, coumarins, quinones, and saponins are present in seaweed, which is what gives it its distinctive properties [18]. Flavonoids are significant phytochemicals as they possess antioxidant and free radical scavenging properties. They are well-recognized for having biological properties such as being insecticidal, antibacterial, antioxidant, antiparasitic, and antifouling [19]. In addition, they contain a lot of phenolic chemicals, which are thought to be the reason for their resistance to a variety of microbial illnesses and insect infestations [18]. Pigments (carotenoids), fatty acids, sterols, terpenes, polysaccharides, phenolic compounds, proteins, and peptides are examples of chemical families. have antibacterial efficacy against a variety of phytopathogens, including *Alternaria cucumerinum*, *Alternaria solani*, *Didymella applanata*, *Fusarium oxysporum*, and *Botrytis cinerea* [20].

The seaweed *C. antennina* has been identified to possess compounds with previously proved elicitor potentials from other species. Hence, the current study was carried out to shed a light on potential of

the seaweed compounds to elicit both non-specific and specific immuno-stimulatory mechanisms in response to early blight (EB) disease.

## Materials and methods

### Seaweed collection and extract preparation

The seaweed, *C. antennina*, was obtained during low tide in August (2018) from the rocks of the coastal areas of Colachel beach), Kanyakumari (8°14' 51.68'' N and 77° 14' 35.209'' E). *C. antennina* collected and processed for extraction. The gathered seaweed was properly washed in tap water numerous times, weared, and stretched out to get rid of surplus water before being shade dried (7 days) [18]. Fully dry sample was ground up and 500 g of the ground powder was extracted with methanol, a solvent with high polarity, for 24 h using a Soxhlet system, then concentrated in a rotary vacuum evaporator [18]. Crude extract powder (4.02 g) which was kept at 4 °C until use, were acquired from the extraction (CA-M).

### The phytopathogen-*A. solani*

*A. solani* cultures (NAIMCC-F-00112) were purchased from National Bureau of Agriculturally Important Microorganisms (NBAIM). This pathogenic fungal culture was maintained in Malt Extract Agar (MEA) medium and incubated at the laboratory condition at 25 ± 3 °C. After 48 h, hypha tips were excised then transmitted on to potato dextrose agar (PDA) medium (stored at 24–26 °C) [21].

### Active fraction isolation and characterization (CA-F8)

After preliminary antimicrobial activity analysis, CA-M was carried forward for column chromatography. The silica gel column (60–120 pore size) (Merck kGaA, Darmstadt, Germany) was used to separate the methanol extract. The column was washed with a gradient of hexane: ethyl acetate at ratios of 90:10, 80:20, 70:30, 60.5:30.5, and 60:40. The result was a series of fractions that were labelled as F1, F2, and so on. All of the produced fractions were subjected to a preliminary antifungal assay (disc-diffusion assay) and the active fraction CA-F8 was recorded with the highest antifungal activity was selected and carried forward for further analysis and characterisation investigations [21].

### Characterization of CA-F8

#### Gas chromatography–mass spectroscopy (GC–MS)

GC of active fraction was carried out at Oven: Initial temp 60 °C for 2.80 min, ramp 10 °C/min to 300 °C (holding time for 6 min, Inj A auto = 260 °C, He—carrier gas, split was 10:1). Using the National Institute of Standards and Technology's database, the mass spectrum

of the GC–MS (PerkinElmer, India) was interpreted to identify the molecular weight, molecular formula, and structural characteristics of the compounds in the active fraction F8 [18].

#### Fourier transform infrared spectroscopy (FT-IR)

FT-IR spectra were recorded on a Perkin Elmer Spectrum One equipped with an ATR–FTIR unit. A few milligrams of CA-F8 sample were placed in the head of Attenuated Total Reflectance of the FT-IR unit (Perkin Elmer, India). Spectrum software was used to gather and analyse the spectra.

#### Antifungal assay–disc-diffusion method

The antifungal assay was performed using CA F8 fraction using disc-diffusion method at different concentrations, chosen after a preliminary assay using range of CA-F8 concentration (20–100) and omitting those with insignificant differences in zones of inhibition (Table 1) [21]. The active fraction was desiccated in rotary vacuum evaporator and the powder (50 µg) was dissolved in 5% DMSO. The solution was loaded onto sterile paper discs (Himedia), dried and placed on Mueller Hinton Agar plates swabbed with *A. solani* spores ( $10^4$  spores/ml) and incubated at room temperature. Discs loaded with 5% DMSO served as control. Inhibition zones were recorded after 24 h. Broth-dilution method was used to determine the Minimum inhibitory (MIC) and fungicidal concentration of the CA-F8 fraction [22].

#### Effect of CA-F8 on mycelium dry weight

A shake flask culture of *A. solani* was carried out in 250-ml flasks containing 50 ml of the medium incubated at room temperature, 130 rpm for 7 days. After 7 days, the fungus' mycelium was extracted and filtered to remove it from the culture medium. The mycelial pellet underwent many rounds of distilled water washing before being dried (70 °C overnight). The following formula was used to determine the fungus' dry weight: [23]  $DW = (\text{weight of filter paper} + \text{mycelium}) - (\text{weight of filter paper})$  where DW = Dry weight.

#### Spore germination assay

Different concentrations of CA-F8 (50, 75 and 100 ppm) were added to sterilized cavity slide. Spore suspension

of *A. solani* ( $10^4$  spores/ml) was added to the cavity and mixed well. Slides were then placed sterile petri dish and incubated for  $28 \pm 2$  °C for 2 days. They were directly placed under the microscope for observation. Slides added with sterile distilled water served as control. Percentage of spore germination was calculated using the following formula [24]:

$$\text{Percentage of spore germination} = \frac{\text{Number of spores germinated}}{\text{Total number of spores}} \times 100$$

#### Test for elicitation of tomato plants

The elicitor properties of active fraction CA-F8 was studied on 45-day-old tomato seedlings propagated in greenhouse conditions following the protocol published earlier [25]. The CA-F8 (3 g) was dissolved in 1 L of sterile distilled water. The elicitation assay was performed by the injection of 20 µl CA-F8 in internodal region of tomato seedlings. Post 48 h of injection, the elicitation effect of CA-F8 was assayed by dissection the leaves above the injected area. Plants injected with sterile distilled water served as control.

#### Identification and quantification of salicylic acid (SA)

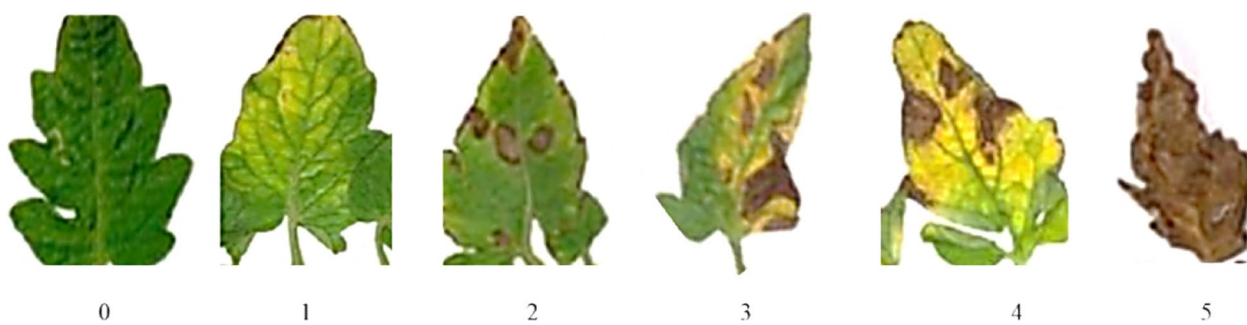
SA content in leaves was characterized by HPLC by co-injection and by comparing their chromatographic and spectral characteristics to salicylic acid standard (Merck). SA was determined by HPLC 24 h after elicitation and expressed in equivalent salicylic standard. HPLC analysis was carried out in Agilent Technologies LC 8A with C18 column (250 mm×4.0 mm, 5 µm). Samples were eluted in a 40 min run time (acetonitrile and aqueous acetic acid, 1 ml/min flow rate). SA was identified by comparison with SA standard [26].

#### Extraction and quantification of phenolic compounds

The phenolic assay was performed for 5 day post-elicitation assay. Fresh leaves (100 g) were crushed in 5 mL of 80% aqueous methanol in the presence of liquid nitrogen. The solution was then filtered, and centrifuged at 15000 g for 10 min. The supernatant was used for spectrophotometric assay by adding 75 µL of supernatant with 100 µL of Folin phenol reagent. The mixture was thoroughly stirred, followed by the addition of 200 µL of  $\text{Na}_2\text{CO}_3$ . The mixture was then incubated in dark (30 min at 37 °C). The absorbance values were read at 760 nm and phenolic acid content was expressed in gallic acid equivalents (µg GAE/mgFW). Phenolic acids were also quantified by HPLC using the procedure described

**Table 1** Treatment concentrations—CAF8 extracts

Treatments (ppm)	Crude extract (µl)	5% DMSO (µl)
50	50	950
75	75	925
100	100	900



**Fig. 1** EB disease rating scale

above. The Phenolic acids were expressed in equivalent chlorogenic standards [27].

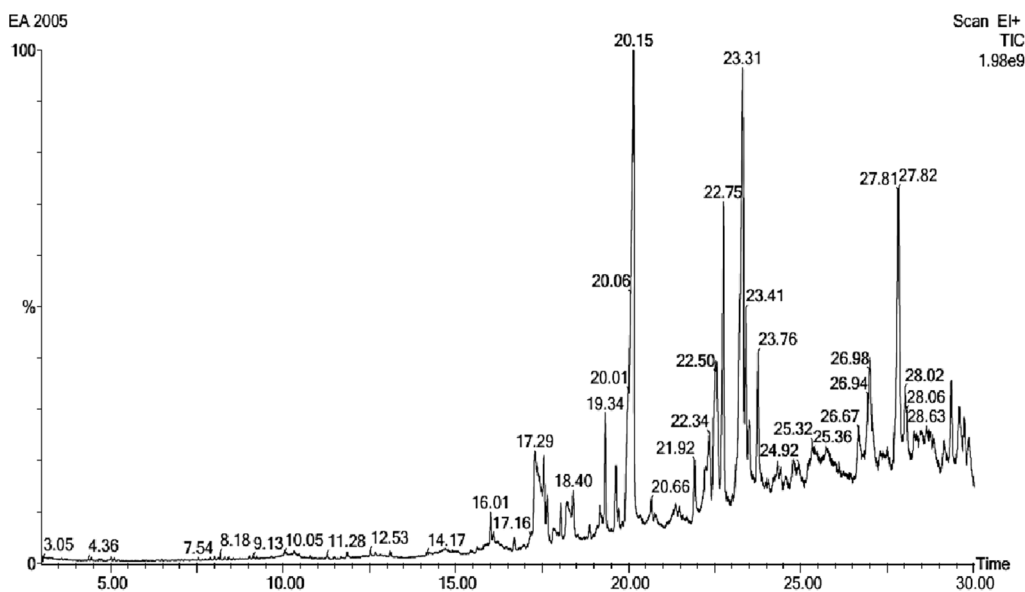
Fresh leaves (1 g) were crushed in 5 mL of 80% aqueous methanol. The solution was then filtered, and centrifuged at 10,000 g for 10 min. The supernatant was collected and quantified using HPLC. HPLC analysis was carried out in Agilent Technologies LC 8A with C18 column (250 mm × 4.0 mm, 5 μm). Samples were eluted in a 40 min run time with a solvent system consisting of acetonitrile and aqueous acetic acid following a gradient of 5–37% of acetonitrile with 1 ml/min flow rate. Phenolic acids were expressed in equivalent chlorogenic acid [28].

#### Disease assessment

Surface sterilized tomato seeds (PKM 1) were propagated to seedlings (2/3 leaf stage) and transplanted to pots containing sterile medium (red soil: cow dung: vermiculate

at 2:1:1, w/w/w), 1 seedling/pot. *A. solani* spore ( $10^4$  spores/ml) suspension was sprayed on to 45-day-old tomato plants. The CA-F8 (3 g) was dissolved in 1L of sterile distilled water. Chlorothalonil, 10 ppm (CL, 1897–45-6, Sigma Aldrich), was prepared by adding 10 mg of CL in 1 L of distilled water. The elicitation assay was performed by the injection of 20 μl CA-F8 and 10 ppm CL in internodal region of tomato seedlings. Post 48 h of injection, the elicitation effect of CA-F8 was assayed by dissection the leaves above the injected area. Seedlings injected with sterile distilled water served as control.

The symptoms were monitored and recorded for 4 weeks. The disease intensity was documented on 0–5 scale (Fig. 2) with corresponding variables as listed in below [29]. Disease incidence (DI) and percent severity index (PSI) was calculated for leaves and shoots [18]. Five replications were maintained per treatment:



**Fig. 2** GC-MS spectrum of CA-F8



$$\text{Disease Incidence} = \frac{\text{Number of diseased plants}}{\text{Total number of plants inspected}} \times 100$$

Percent severity Index

$$= \frac{\text{Sum of individual disease ratings}}{\text{No. of leaves observed} \times \text{Maximum rating}} \times 100$$

where maximum rating is 5, which is the highest numerical rating on the disease scale (Fig. 1).

The experimental set up contained the following set of treatments: T1—control; T2—*A. solani*; T3—CA-F8; T4—*A. solani*+0.1% Chlorothalonil; T5—*A. solani*+CA—F8.

#### Estimation of pathogenesis-related proteins (PRPs)

The activities of PR proteins, PO and PPO were determined. The leaves of the test plants were isolated at different time intervals post inoculation, 0, 2, 4, 24, 48, 72, 96, 120 and 144 h. The leaves were homogenized in a pre-chilled mortar pestle in 4 ml of potassium acetate buffer (0.05 M; pH 5). The homogenate was further processed for enzyme assays.

#### Peroxidase (PO)

The enzyme extract (100  $\mu$ l) was added with 1.5 ml of pyrogallol (0.05 M). To initiate the reaction, 100  $\mu$ l of hydrogen peroxide (1%) (v/v) was added to the sample cuvette and the absorbance was read at 420 nm. The enzyme activity was expressed as change in absorbance min/g of fresh tissue [30].

#### Polyphenol oxidase (PPO)

PPO activity was determined according to Mayer et al. [19] by adding the enzyme extract (200  $\mu$ l), sodium phosphate buffer (pH 6.5, 1.5 ml, 0.1 M) and catechol (200  $\mu$ l–0.01 M) and the enzyme activity was expressed as changes in absorbance of reaction mixture at 490 nm min/g of fresh tissue [31].

#### Statistical analysis

Distilled water was used as control and chlorothalonil, CL (10 ppm–0.01 mg/ml) as positive control in all experiments. All the tests were replicated five times and were analyzed by Analysis of Variance (one-way ANOVA), and treatment means were compared by Tukey's-family error test ( $P < 0.05$ ) using Minitab<sup>®</sup>17 software package. The data on above said experiments were arcsine transformed before undergoing statistical analysis. Sigma plot 11<sup>®</sup> was used to draw the graphs.

## Results

### Characterization of CA-F8

#### Gas chromatography

GC–MS analysis of active fraction CA-F8 exposed the existence of 39 compounds (Additional file 1: Table S1). The compounds included fatty acids (47.145%), alkanes (16.265%), terpenoids (11.724%), sterols (3.324%) and alcohols (2.036%) (Fig. 2). Among these compounds, n-Hexadecanoic acid was attained a major peak area (13.317%), followed by 9, 12, 15-Octadecatrienoic acid, (Z,Z,Z)—(10.837%), Phytol (9.255%), Hentriacotane (7.923%), Heptacosane (5.144%), Linolenic acid, 2-hydroxy-1-(hydroxymethyl)ethyl ester (Z,Z,Z)—Linolenic acid, ethyl ester (4.049%), Octadecanoic acid, 2,3-dihydroxypropyl ester (2.224%) and trans-13-Octadecenoic acid (2.099%) (Fig. 3).

#### FT-IR

The FT-IR analysis of CA-F8 displayed seven major peaks (Fig. 4). The peaks were formed corresponding to the functional groups present in the CA-F8 (Table 2).

Fourier transform infrared spectroscopy.

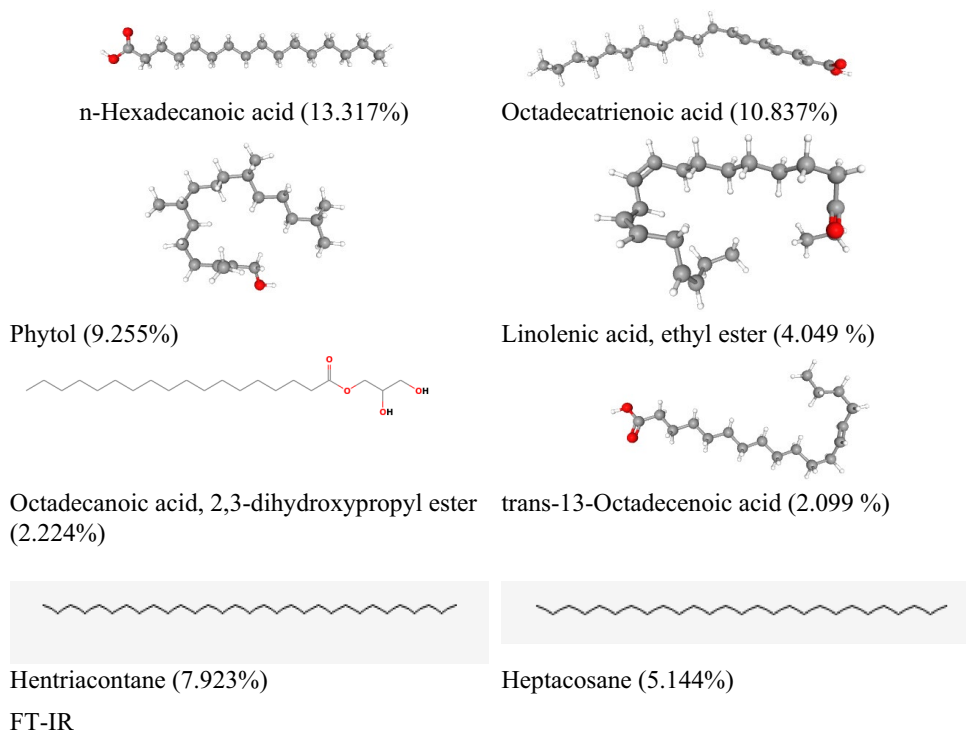
The FT-IR analysis of CA-F8 displayed seven major peaks (Fig. 4). The peaks were formed corresponding to the functional groups present in the CA-F8 (Table 2).

#### Disc-diffusion assay

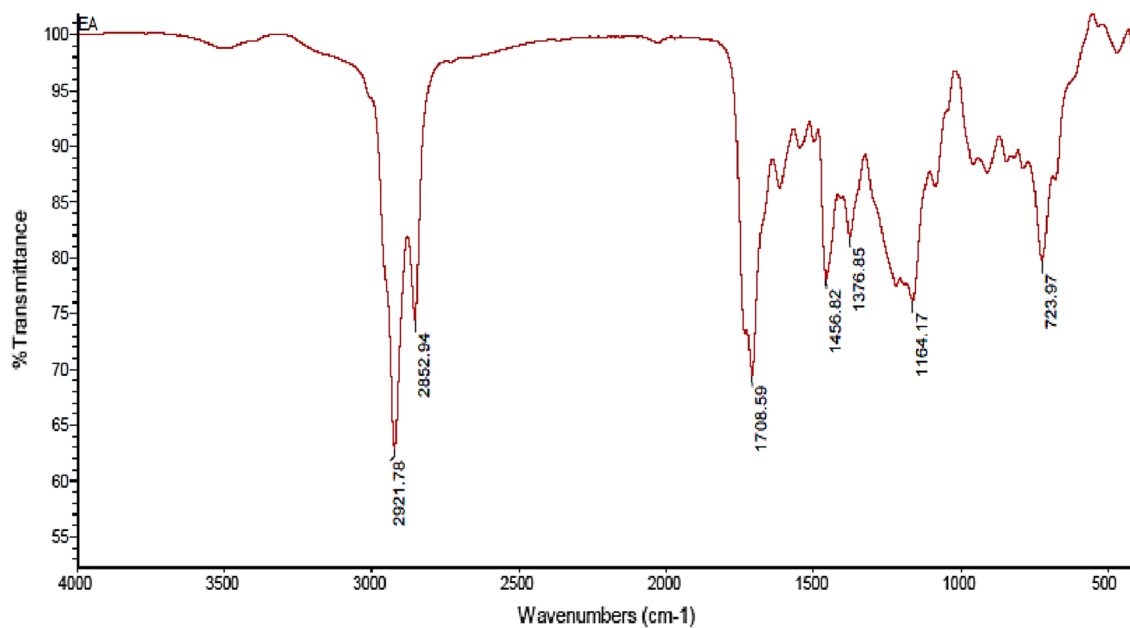
The antifungal activity of crude methanolic extract of *C. antennina* displayed a zone of inhibition in dose-dependent manner (Fig. 5). Highest inhibition zone was observed in disc with 100 ppm treatment concentration, 14.5 mm, followed by 75 and 50 ppm, with zones of 13.82 and 12.4 mm ( $F_{4, 20} = 25.84$ ;  $P < 0.003$ ). The fungicide, CL (0.1%) displayed a zone of 14.81 mm, which was not significantly different with the inhibition zone produced by CA-F8 100% ( $P \leq 0.005$ ). The extracts were able to inhibit the fungal growth even at lower concentration (MIC 3.27  $\mu$ g/ml). The extracts displayed a significant fungicidal activity, with an estimated minimum fungicidal concentration (MFC) of 12.5  $\mu$ g/ml.

#### Mycelium dry weight (DW)

The CA-F8 extracts affected the growth of *A. solani* in terms of DW and inhibition of spore germination in a dose-dependent manner (Fig. 6). The algal compounds reduced the DW in all treatments that significantly differed with each other and control ( $P \leq 0.005$ ). The DW decreased from 37.82 mg to 33.64, 28.365 and 20.91 mg



**Fig. 3** Major bioactive compounds of CA-F8



**Fig. 4** FT-IR spectrum of CA-F8

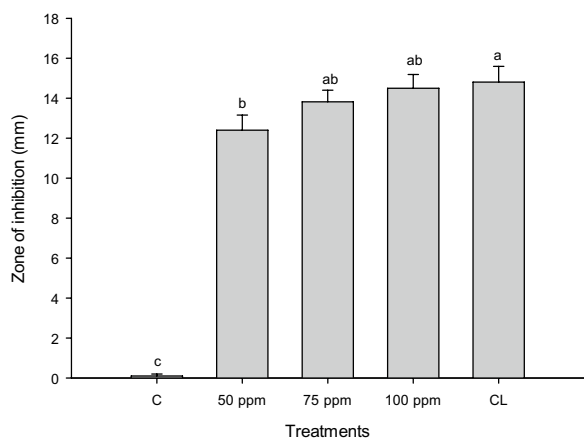
in 50, 75 and 100 ppm treatment concentrations ( $F_{4, 20}=23.11$ ;  $P<0.0001$ ). However, CL reduced the dry weight by 50.89% compared to control ( $F_{4, 20}=23.11$ ;  $P<0.0001$ ).

#### Spore germination

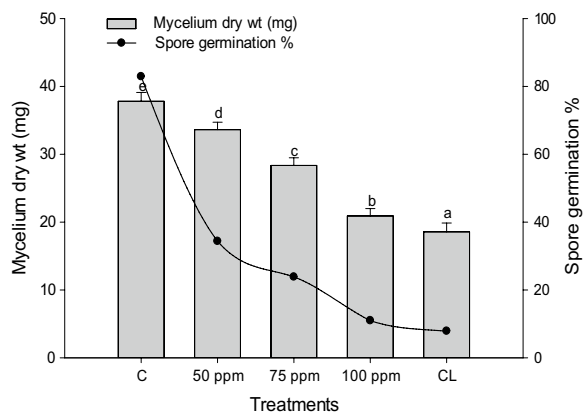
A similar rate of spore germination inhibition was also observed with all the treatment concentrations. The spore germination was inhibited significantly to 34.37,

**Table 2** FT-IR spectra of CAF8

	Absorption (cm <sup>-1</sup> )	Appearance	Group	Compound class
1	723.97	Strong	C=C bending	Alkene
2	1164.17	Strong	C-O	Ester
3	1376.85	Medium	O-H bending	Phenol
4	1456.82	Medium	C-H bending	Alkane
5	1708.59	Strong	C=O stretching	Aliphatic ketone
6	2852.94	Medium	C-H stretching	Alkane
7	2921.78	Medium	C-H stretching	Alkane



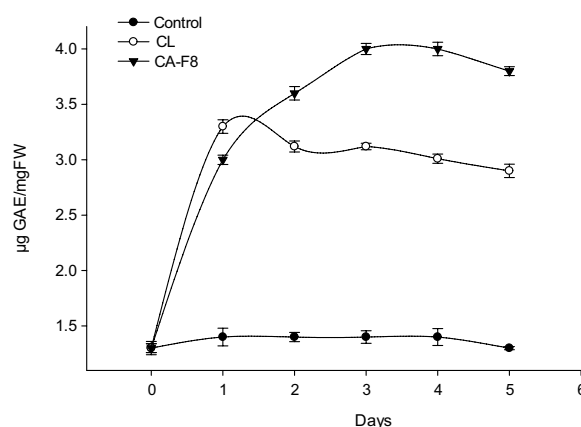
**Fig. 5** Zone of inhibition (mm) of fungal growth by CA-F8 extracts



**Fig. 6** Effect of CA-F8 on *A. solani* mycelium dry weight and spore germination

23.847, 10.96 and 7.87% at 50, 75 and 100 ppm treatment concentrations as well as CL ( $F_{4, 20}=21.09$ ;  $P<0.0001$ ) (Fig. 6).

Mean ( $\pm$ SEM) followed by the bars indicate no significant difference ( $p < 0.05$ ) in a Tukey's test.



**Fig. 7** Accumulation of phenolic compounds in response to elicitor treatments, CL and CA-F8

**Elicitor assays**

**Accumulation of foliar compounds in response to elicitor treatment**

HPLC analysis of leaves treated with CA-F8 revealed the presence of SA in significant levels. However, SA was not detected in untreated and CL leaves. The amount of SA present in CA-F8-treated leaves 3.5 µg/mg FW. HPLC analysis of leaves of CA-FA-treated seedlings revealed the presence of higher amounts of Hydroxycinnamic derivatives, flavonoids and hydroxybenzoic acids, 24 h post treatment (Additional file 1: Fig. S1).

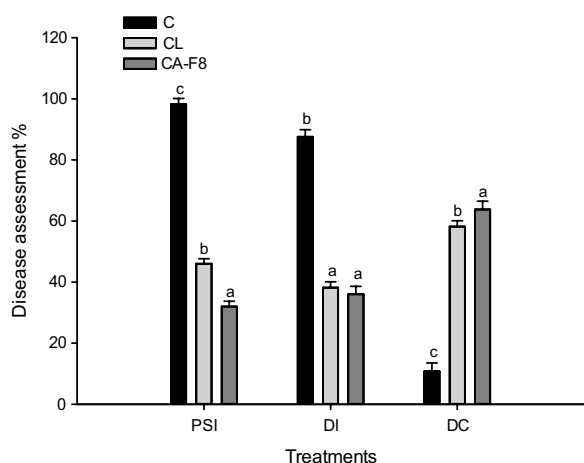
Foliar phenolic accumulation was estimated for 5 days intended for both the elicitor treatments and compared with control. The levels of phenolic compounds in untreated leaves were detected in relatively lower quantities and were not significantly different throughout ( $P \geq 0.05$ ).

Mean ( $\pm$ SEM) followed by the same letter in an individual experiment indicate no significant difference ( $P < 0.05$ ) in a Tukey's test.

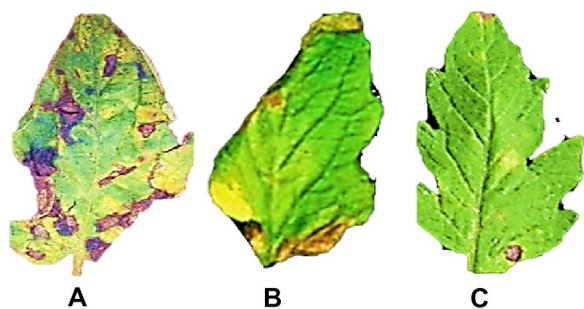
However, the phenolic levels in treated seedlings displayed rapid increase in 1 h post treatments. In CL-treated seedlings, the phenolic compound intensities began to rise in linear way for 24 h, displaying a maximum of 3.3 µg GAE/mgFW. After which, the levels began to decline, remaining constant for 2 days (days 2 and 3), displaying 3.12 µg GAE/mgFW. The values further decreased to 3.01 and 2.9 µg GAE/mgFW on the next consecutive days ( $F_{5, 24}=19.8$ ;  $P<0.0001$ ) (Fig. 7).

Similarly, the levels of phenolics in algal compound-treated seedlings increased rapidly on day 1, which was 14% lower than that observed in CL-treated seedlings ( $F_{5, 24}=39.5$ ;  $P<0.004$ ). The levels unveiled a stable upsurge until day 3 reaching a maximum of 4 µg GAE/mgFW and remained constant on day 4 also, after which the levels





**Fig. 8** Effect of elicitor treatments on EB disease—Assessment of Percentage Severity Index (PSI), Disease Incidence (DI) and Disease Control (DC)



**Fig. 9** Infected leaves isolated from plants **A**—Control; **B**—0.1% cypermethrin-treated; **C**—CA-F8-treated

started to decline about 5%, reaching 3.8 GAE/mgFW on day 5 ( $F_{5, 24}=15.17$ ;  $P<0.0001$ ). Yet the levels were significantly higher compared to CL-treated leaves ( $P<0.005$ ).

#### Effect of elicitor treatments on Early Blight disease

CA-F8 treatments reduced the incidence of EB disease (Fig. 8). Tomato seedlings treated with CA-F8 prior to *A. solani* infection consistently reduced the disease incidence by 58.904% ( $F_{2, 12}=33.84$ ;  $P<0.0001$ ).

Mean ( $\pm$ SEM) followed by the same letter in an individual experiment indicate no significant difference ( $P<0.05$ ) in a Tukeys test.

Consequently, the algal treatments reduced the severity index to 67.41% ( $F_{2, 12}=29.017$ ;  $P<0.0001$ ). Thus, the algal extract was able to offer effective disease control, 63.8% ( $F_{2, 12}=58.79$ ;  $P<0.0001$ ). Treatment of seedlings with CL also offered a similar disease control effect, 58.2% ( $F_{2, 12}=58.79$ ;  $P<0.0001$ ), also reducing the incidence (38.2%;  $F_{2, 12}=33.84$ ;  $P<0.0001$ ) and severity (46%;  $F_{2, 12}=29.017$ ;  $P<0.0001$ ) of EB disease (Fig. 9).

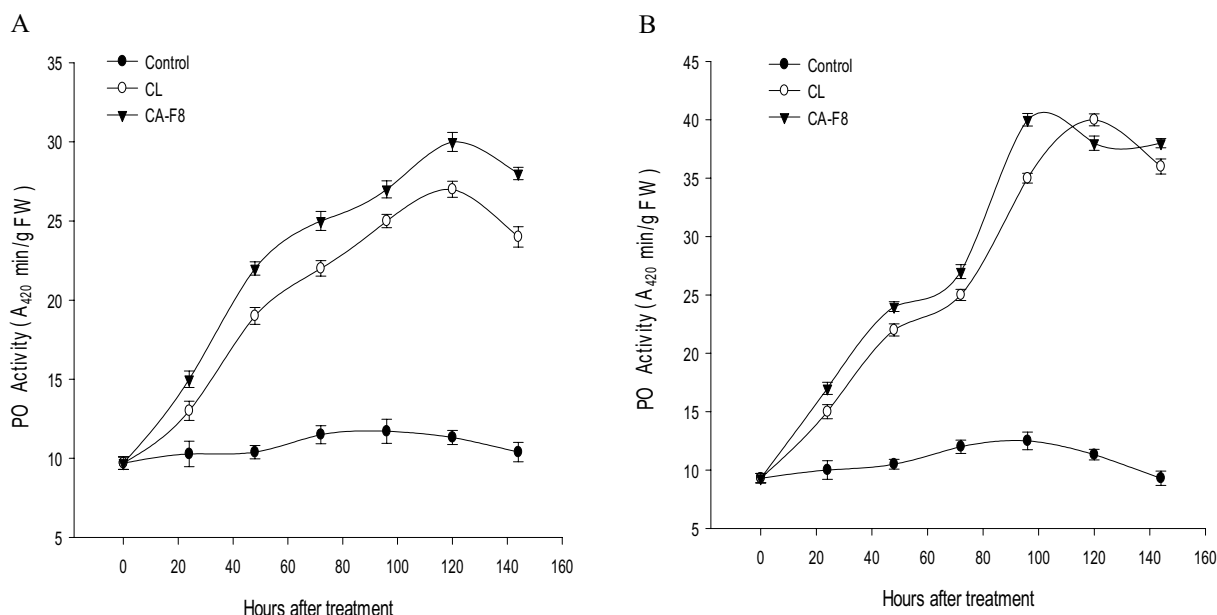
#### Effect of elicitor treatments on plant pathogenesis-related proteins pre- and post-infection

The PO and PPO levels were estimated till 144 h pre and post infection and infestation. The levels of both the enzymes were significantly different compared with control and also pre and post inoculations ( $P<0.005$ ).

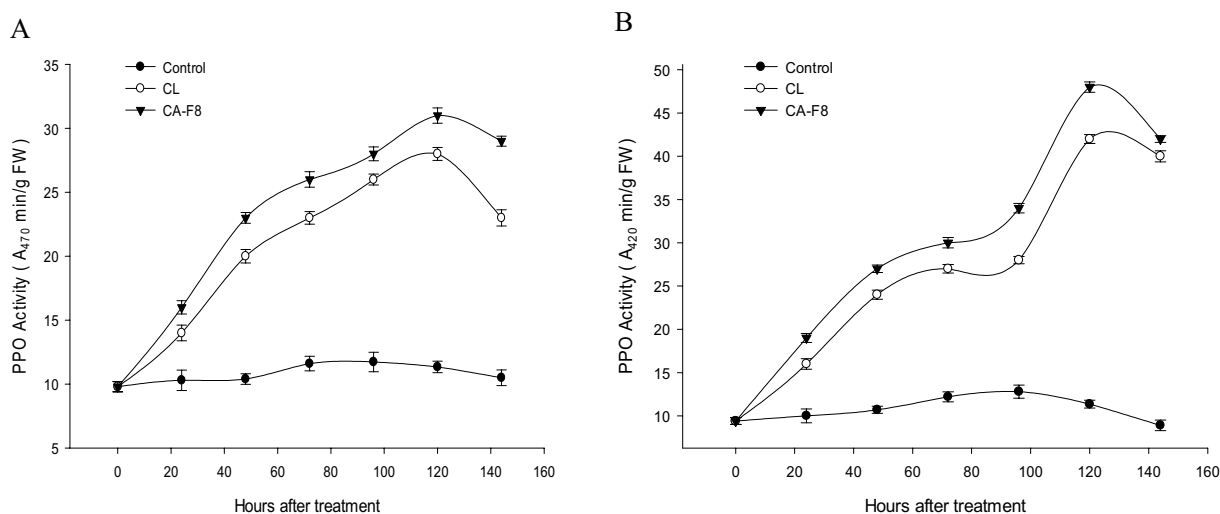
Control seedlings injected with distilled water also displayed a minor increase in PO levels till 96 h, yet the levels post 48 h were not significantly different ( $P>0.005$ ). However, the fungal inoculation increased the PO levels by 6.7% after 96 h ( $F_{5, 24}=47.8$ ;  $P<0.004$ ). Post 96 h, the levels decreased similar to control and declined further, to 9.3  $\mu\text{g}/\text{min}/\text{g}$ , lower than uninoculated control ( $F_{5, 24}=34.8$ ;  $P<0.002$ ). The PO levels in seedlings treated with CL increased linearly till 120 h, reaching a maximum of 27  $\mu\text{g}/\text{min}/\text{g}$ , which was 58% increase compared with un-inoculated control ( $F_{5, 24}=26.03$ ;  $P<0.0005$ ). However, the final PO levels in uninoculated CL level was 56.6% higher compared with control ( $F_{5, 24}=24.12$ ;  $P<0.0001$ ) (Fig. 10A). The PO levels were modified by fungal infection, increasing the PO levels by 15.38% 24 h after infection ( $F_{5, 24}=27.14$ ;  $P<0.003$ ). After a linear increase in PO levels till 120 h, the levels decreased by 10% displaying 36  $\mu\text{g}/\text{min}/\text{g}$  on 144<sup>th</sup> h ( $F_{5, 24}=22.08$ ;  $P<0.0001$ ) (Fig. 10B).

The PO levels of CA-F8-treated seedlings displayed a similar enzyme kinetics to those treated with CL, yet the levels were significantly different before and after fungal infection ( $P<0.005$ ). An immediate hike of 54.6% in PO levels after infection was observed ( $F_{5, 24}=12.41$ ;  $P<0.0001$ ). The kinetics of PO began to rise till 120 h reaching 30.06  $\mu\text{g}/\text{min}/\text{g}$ , which was 67.73% higher compared with un-inoculated control ( $F_{5, 24}=28.8$ ;  $P<0.0001$ ). A likely increase in PO levels inoculated with fungal pathogen was observed (Fig. 11B). The levels increased 76.75%, but decreased after 96 h ( $F_{5, 24}=28.8$ ;  $P<0.0001$ ). However, the final level was 75 and 5.5% higher compared with inoculated control and CL-treated seedlings ( $F_{5, 24}=42.04$ ;  $P<0.0001$ ).

PPO activities increased significantly and remained in higher amounts till 96 h in control leaves. The PPO levels differed between immunized as well as uninoculated control ( $P<0.005$ ). Meanwhile, fungal inoculation steadily increased PPO levels by 36.17% till 96 h ( $F_{5, 24}=37.02$ ;  $P<0.0001$ ) (Fig. 11A). The kinetics of PPO levels in both the treatments differed with control seedlings, reaching the peak at 120 h and declining afterwards ( $P<0.005$ ) (Fig. 11). The PPO levels in CL-treated seedlings reached a maximum of 28.28 ( $F_{5, 24}=23.18$ ;  $P<0.0003$ ) and 41.6  $\mu\text{g}/\text{min}/\text{g}$  ( $F_{5, 24}=29.1$ ;  $P<0.0003$ ) at 120 h in uninoculated and inoculated leaves. The PPO levels increased 54.34 ( $F_{5, 24}=22.42$ ;  $P<0.004$ ) and 73.73% ( $F_{5, 24}=12.37$ ;  $P<0.004$ ) post



**Fig. 10** Estimation of PO activities after elicitor treatments **A** before *A. solani* infection **B** after *A. solani* infection



**Fig. 11** Estimation of PPO activities after elicitor treatments **A** before *A. solani* infection **B** after *A. solani* infection

144 h in CL-treated uninoculated and inoculated leaves, respectively, compared with control (Fig. 11). The algal compounds induced PPO secretion to 31.7  $\mu\text{g}/\text{min}/\text{g}$  after 120 h after which the levels decreased by 9.3% ( $F_{5, 24}=41.3$ ;  $P<0.005$ ). A further hike in PPO levels was induced after fungal infection, reaching 48.46  $\mu\text{g}/\text{min}/\text{g}$  at 120 h. Post 120 h the PPO levels in inoculated seedlings treated with algal compounds was still 78.8 and 5% ( $F_{5, 24}=12.37$ ;  $P<0.004$ ) higher than inoculated control as well as CL-treated seedlings (Fig. 11B).

### Discussion

The seaweed compounds of the active fraction F8 were found to elevate the defense signals of the plants, thus designating themselves as potential biological elicitors. Phyto-pharmacological investigation on the composition and biological activity of active algal fraction showed the existence of various classes of active phytochemicals [32]. The composition was dominated by fatty acids (FA), contributing to 47.669% of peak area. FAs are dynamic constituents of all biota, besides recognised for

their imperative part in triggering the phytohormone interactions, apart from acting as key role players of various defense signalling pathways of the plant system [33]. The fatty acids of macro algae were attributed to the antifungal potentials of *Laminaria digitata*, *Undaria pinnatifida* and *Porphyra umbilicalis* [34]. Considered among the major classes of metabolites produced by marine algae, terpenes offer wide range of antimicrobial activities [35]. Terpenes contributed to the second major compound in the active fraction (6.403%).

While phytol is a renowned antimicrobial compound, the sesquiterpenes are also recognized for their antimicrobial potentials [36]. The phytol concentration was attributed to higher antifungal potentials of *Caulerpa racemosa* against *Ganoderma boninense*, important phytopathogenic fungi [37]. Antioxidant compounds predominantly constitute (29.11%), the active fraction. Algal compounds are acknowledged to show an imperative role alongside innumerable diseases and aging processes [38, 39]. Cornish and Garbary [40] correlated the marine antioxidant activities to their bioactive potentials, such as anti-inflammatory, antimicrobial, cytotoxic and anticancer properties. Most of the compounds are constituents of medicinal plants. Octadecatrienoic acid, 17-Pentatriacontene, hexadecanoic acid, phytol, Heptadecane are components of medicinal plant *Tiliacora acuminata* [41], *Sida cordata* [42] and *Holarrhena antidysentrica*, Wall Flower [43].

The algal compounds induced the activities of PO and PPO, which are components of SA signalling pathways. Algal compounds have also increased the accumulation of phenolic compounds in the tomato leaves. The phenolic compound accumulation promoted by algal fraction application was significantly higher compared with CL applications. Simultaneously, the treatments also offered effective fortification against EB disease. The elicitation test by algal fraction induced the production of SA which was evident from the HPLC chromatogram. In addition, SA was not observed in the chromatogram of control leaves. Hence, the algal compounds were able to elicit plant's systemic acquired resistance. Similar induction of SA by algal compounds was proved by Stadnik and Freitas [44] who reported the SA signalling pathway was induced by the application of algal polysaccharides, laminarin and carrageenans. El Modafar et al. also proved oligoulvans, in addition to ulvan and of *Ulva lactuca*-stimulated SA-dependent systemic acquired resistance in tomato seedlings [45].

The algal compounds were very effective against *A. solani*, exhibiting a significant fungicidal activity, even at lower concentrations (12.5 ppm). Major compounds in the algal fraction constituted to antimicrobial compounds (41.299%). Hentriacontane (7.923%) in the algal

fraction is reported with inhibiting the spore germination of many fungal pathogens. Hentriacontane is an important phytoconstituent of Neem leaves, reported with antifungal activities against *Candida albicans* [46]. Terpenes that contribute 11.724% of the fraction possess antifungal potentials [36]. Pinteus et al. [47] also reported the growth inhibition action of methanolic extracts of seaweeds *Asparagopsis armata* and *Sphaerococcus coronopifolius*. The crude extract of *C. antennina* was also reported with antifungal activity against *A. solani* [48].

The algal compounds significantly reduced the DW by 44.711%. The crude extract of *Padina gymnospora* was effective in constricting the dry weight of *Ulocladium botrytis* and also inhibiting their pathogenesis-related enzymes [49]. Seaweed compost tested against damping off tomato pathogens exhibited significant effect on *Pythium ultimum* besides *Rhizoctonia solani*'s mycelial growth [50]. The algal compounds also inhibited the spore germination by 92.13%. Raj et al. [51] declared the efficiency of *Sargassum wightii* in reducing the germination of *R. solani* by 80.4%. They also reported the compounds n-Hexadecanoic acid and 9, 12-Octadecadienoic were responsible for the inhibition of fungal growth.

Elicitor treatments increased foliar phenols, PO and PPO activities significantly higher compared with control. Increased production of phenolic compounds is considered as biomarkers of induced resistance [52]. Application of MeSA has stimulated the production of phenolic compounds in rice [53]. Jasmonate application on tomato plants were found to stimulate the production of defensive proteins that negatively influenced herbivores [54]. There exists a linear relationship between phenolic compound concentration and antimicrobial potentials [49]. Consequently, the allegation of phenol accumulation in plant defense has also been proved [55].

There was a noteworthy upsurge in PO and PPO activities in elicitor-treated seedlings post infection and infestation. *A. solani* exposure to the elicited seedlings increased the multitude of PO and PPO enzymes by 75 and 5.5% as well as 78.8 and 5% higher compared with inoculated control and CL-treated seedlings. An effective disease control (63.8%) was obtained by the treatment with algal compounds. This was attributed to the induction of systemic acquired resistance that was enhanced by the algal compounds. In addition, a similar disease control rate was observed as a result of CL treatments.

Higher accumulation of foliar phenols and increased in the actions of enzymes PO and PPO by SA treatments are reported in *Solanum melongena*, *Brassica juncea* (var. Rlm619) that provided fortification against *Ralstonia solanacearum* [56] and *Alternaria brassicae* [57]. In

addition, increased phenolics associated with amplified PAL activities conferred resistance against *Fusarium oxysporum* f. sp. *lycopersici* that was treated with algal polysaccharides [36]. A similar resistance to fungal pathogens, *Botrytis cinerea* and *Phytophthora infestans* was observed by the treatment of tomato seedlings with algal products from *Sargassum fusiforme* [58]. The ability of algal compounds to offer better resistance compared with chemical pesticide, is accredited to the aptitude of these compounds to induce additional defense signalling pathways along with that of SA mediated defense responses. Plants treated with extracts of *Gelidium serrulatum*, *U. lactuca* and *Sargassum filipendula* have been conveyed to amplify the expression of genes specific to defense signalling pathways, such as jasmonate and salicylate-mediated pathways [59].

Hence, the study confirms the elicitor potentials of compounds from *C. antennina*, by inducing natural systemic defences along with the induction of SA mediated pathways along with several other pathways. This was evident by the magnification of foliar phenolics, PO and PPO accumulation along with effective control of EB disease control in tomato seedlings exposed to compounds of *C. antennina*. This investigation unlocks novel forecasts besides delivering an alternative approach for crop protection to moderate or interchange the solicitation of chemical pesticides.

## Conclusion

Marine algae are regarded as excellent sources of bioactive compounds that can stimulate plant growth and also enhance their resistance against biotic and abiotic stressors. The active fraction of methanol extract of *C. antennina* (CA-F8) was effective against the EB pathogen, *A. solani*. The algal compounds in the active fraction can trigger the phytohormone interactions, apart from acting as key role players of various defense signalling pathways of the plant system in addition to possessing antimicrobial potentials. Predominant quantities of antioxidant compounds facilitate oxidative burst, detoxifying the pathogenesis enzymes produced by the pathogen, further preventing their incidence. The active compounds constricted the mycelial growth along with reducing their weight and inhibiting their spore germination. Elicitation assays performed with CA-F8 provided notable protection to tomato plants against EB disease that could be attributed to the activation of SA and JA mediated defense pathways which as evident by the increase in the synthesis and accumulation of foliar phenols and PRPs (PO and PPO). This investigation unlocks novel forecasts besides delivering an alternative approach for crop

protection to moderate or interchange the solicitation of chemical pesticides.

## Abbreviations

CA	<i>Chaetomorpha antennina</i>
F	Fraction
GC-MS	Gas chromatography–Mass spectroscopy
PPO	Polyphenol oxidase
PO	Peroxidase
SA	Salicylic acid/salicylate
JA	Jasmonic acid/Jasmonate
EB	Early blight
PRPs	Pathogenesis-related proteins
M	Methanol
NBAIM	National Bureau of Agriculturally Important Microorganisms
MEA	Malt Extract Agar
PDA	Potato dextrose agar
FT-IR	Fourier-transform infrared spectroscopy
ATR	Attenuated total reflection
DMSO	Dimethyl sulfoxide
HPLC	High-performance liquid chromatography
FW	Fresh weight
CL	Chlorothalonil
DW	Dry weight

## Supplementary Information

The online version contains supplementary material available at <https://doi.org/10.1186/s40538-023-00484-7>.

**Additional file1: Table S1.** Compounds present in CAF8 identified through GC-MS. **Figure S1.** HPLC chromatogram of plants **A** Control **B** CA-F8-treated (Hydroxycinnamic derivatives (1, 2, 3, 4, 5, and 9), flavonoids (6, 8), hydroxybenzoic acids (7, 10) and AS (acetylsalicylic acid).

## Author contributions

KM-PC and SS-N designed the experiments; KMPC, SK, RR, and HS conducted the experiments; NS-5 and HS performed data curation, KMPC and SS-N, analysed the data, and wrote the manuscript. All authors reviewed, read, and approved the final manuscript.

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## Availability of data and materials

All available data are shown in the figures and tables.

## Declarations

### Consent for publication

All authors agree to publish the work.

### Competing interests

The authors declare no competing interest.

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