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Systemic defense induced by fatty acid compounds from marine macroalgae, *Chaetomorpha antennina* in tomato (*Solanum lycopersicum*) plants alters the susceptibility of the polyphagous agricultural pest, *Spodoptera litura* Fab

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Abstract

Background Seaweeds contain a widespread range of fatty acids (FA), and several of them have potential bioactivity. FAs are dynamic members of all biota, as well as being acknowledged for their critical function in initiating phytohormone interactions and acting as important participants in many defense signalling pathways of the plant system. The current study looks at the defense-eliciting potentials of fatty acids from the green seaweed *Chaetomorpha antennina* (Bory) Kützing and their impact on the polyphagous insect pest *Spodoptera litura* (Fab).

Results The seaweed was detected with 19 fatty acids, with larger proportion of hexa and octadecanoic and linoleic acids. The algal fatty acid compounds (CFA) were successful in eliciting salicylic acid and phenolic compounds biosynthesis along with defense enzymes peroxidase (PO) and polyphenol oxidase (PPO). CFA enhanced the synthesis of defense enzymes, PO and PPO and phenols, post infestation with *S. litura* (> 50%) compared to control plants exposed to the pest. CFA was also effective in causing direct mortalities (96–98%) to the larvae (II-V instars). *S. litura* larvae exposed to elicited tomato plants displayed physiological incursions that extended larval-pupal duration to 26–28 days, preventing both morphogenetic transitions as well as affecting their morphology, that lead to the emergence of adults with malformed wings, legs. As a consequence, the fecundity was reduced by 60% affecting the reproductive performances of second-generation adults. The consumption rate (RCR) of larvae exposed to CFA was decreased by 84%, depicting feeding deterrence. These larvae were also observed with > 50% reduction in the levels of phosphatase enzyme secretion, bringing down larval growth rate from 0.58 to 0.34 mg/day. Histological analysis of exposed larvae displayed midgut cell disruption.

Conclusion Hence, the study finally confirms the elicitor potentials of fatty acid compounds from *C. antennina*, by inducing natural systemic defenses. This investigation unlocks novel forecasts besides delivering an unconventional method for crop protection to moderate or interchange the solicitation of chemical pesticides.

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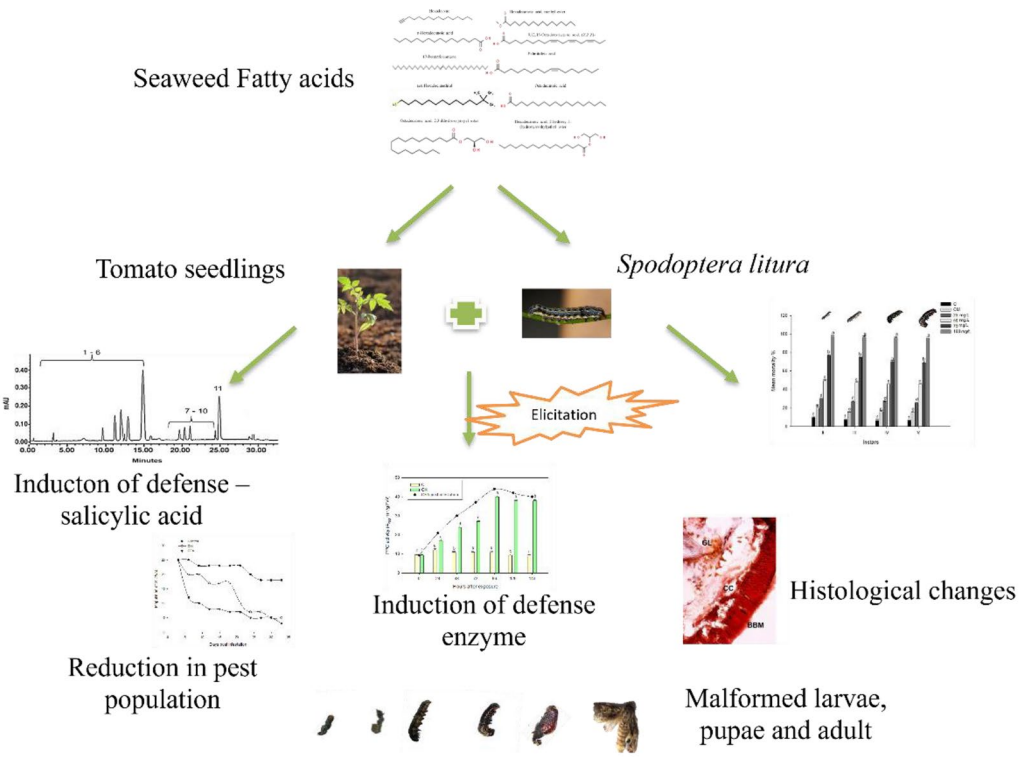
Keywords *C. antennina*, Elicitor, Salicylic acid, Feeding deterrence, Jasmonic acid, Morphogenesis, Mortality, Midgut cell disruption.

Graphical Abstract



Background

Agriculture is continually confronted with restrictions, such as abiotic stresses, which considerably inhibit crop production, as the global population expands in tandem with the environmental magnitudes of climate change [1]. Plants are imperilled to a variety of stresses in their environment. In the past, synthetic pesticides as well as fertilizers have been practically necessary for boosting crop production and the management of plant diseases. Because of their detrimental effects inclusive of hazards to health of humans and the environment, the widespread usage of these chemical compounds exists as a matter of global apprehension [2]. Additionally, the development of disease resistance is mostly brought about by the disproportionate usage of chemical produces. In this scenario, it seems essential to promote environmentally friendly alternatives to lessen the usage of synthetic substances in agriculture and, consequently lessening their negative environmental impacts [3].

There has been a rising interest in integrated agricultural management methods that encourage the use of alternative practises such as adopting traditional cultural practices and the use of organic goods in recent decades [4]. Plant-based biostimulants (PBs) have attracted a lot of research in attempt to boost quantitative and qualitative quotients of agricultural produces along with offering an enhanced rate of protection [5]. Plant biostimulants not only affect physiological processes to improve crop productivity, but they also improve nutrient absorption, which optimizes fertilizer consumption and utilisation, along with induction of defensive resistance in plants employing a variety of biological inducers [6].

Seaweeds have been applied as soil fertilizers in coastal areas, and beneficial benefits of applying seaweed extracts as exfoliar application on plants have been documented [7]. Thus, management of diverse plants by the usage of compounds/extracts from several marine algae has attributed to the betterment of agricultural produces from germination until consumption [7–10]. Fatty acids,

proteins, minerals, polysaccharides, pigments, plant growth hormones, phenols, along with many other bioactive substances are elated from algal biomass through liquid extraction. Fatty acids (FA) account for a significant percent (> 20%) of their total composition [11]. Because of their antibacterial and antioxidant capabilities, seaweeds contain significant levels of which are compounds of great importance in the agricultural industry as biostimulants. They are also said to have immunological features that range from nonspecific activation of the plant host immune system [12].

Marine algal compounds operate as stimulators of plant defense retorts due to their actions as plant protectants. However, their method of action is not well known, limiting their use as useful products [13]. The seaweed *Chaetomorpha antennina* (Bory) Kützing is a green alga contains compounds with wide-array of bio-activities that can be used as PBs, microbicides, and insecticides [4, 5, 14]. It has been stated that the seaweed possesses significant concentration of fatty acids. Both plant growth promoting and salinity stress alleviating potentials as well as insecticidal activity of the seaweed have been already documented. Elicitor compounds function as indicator molecules, whose detection at the cell-surface as well as consequent transmission activates plant defense genes [15]. Many defense complexes are thus produced, including structural proteins that support the cell wall and a number of enzymes involved in the complex activation of plant defense pathways (salicylic acid (SA), jasmonic acid (JA), and ethylene). These mechanisms cumulatively inhibit the invasive mechanisms adopted by the pests [16].

Since crop domestication and selection for higher yield and quality may affect the crop's defensive capacity, increasing dependency on artificial crop protection some plant defense features may be absent or inadequately expressed in domesticated plants as a result of selection for other beneficial qualities [17]. Given that tomatoes have been one of the most important crops to be domesticated since time immemorial, and are currently the second most important vegetable crop grown in the world, they are widely used as a model for investigating plant development and pest resistance mechanisms due to their relatively short reproductive cycle, availability of a fully sequenced genome, and a variety of mutants that allow investigation of individual plant characteristics [18].

Tomato is a globally important crop with a high nutritional value. However, the tomato plant is susceptible to several diseases and insect pests that cause significant destruction and so reduce productivity. Integrated pest management (IPM), which includes crop rotation practises, the cultivation of disease-free cultivars, and the use of fungicides, has been shown to be successful among several disease control approaches [19]. However, environmental contamination besides the healthiness risks instigated by these applications has necessitated the development of alternate disease control strategies. *Spodoptera litura* Fab is a severe and polyphagous pest of several economically important crops, with a strong migratory capacity and a global distribution [20]. With a vast host specificity of over 389 host plants (and rising), *S. litura* travels in massive groups, transferring on host plants one by one, causing considerable commercial harm mostly by producing osmotic imbalance as well as oxidative stress. *S. litura* has also developed resistance to a diversity of widely available chemical pesticides [21].

Henceforth in view of finding an alternative and effective environmentally friendly pest control strategy, the current study aims to evaluate the possibilities of their fatty acids to elicit plant defense and analyse the effect on the fitness of *S. litura* in an environmentally controlled greenhouse assay. Detailed objectives of the study include the extraction of fatty acids from *C. antennina* and to test their potentials to elicit defense system of tomato plants both non-specifically and specifically against the infestation of polyphagous pest, *S. litura*. Additionally, the direct effect of the fatty acids on larval growth and development along with physiological as well as metabolical parameters were also studied.

Materials and methodology

Collection and extraction of seaweed

Methanol extract of *C. antennina*, collected from the rocks of the coastal areas of Colachel beach), Kanyakumari (8°14′ 5168′′ N and 77° 14′ 35.209′′ E), processed and used in previous trials by Chanthini et al. was used for this investigation [5]. The extraction yielded 4.02 g of crude extract powder.

Fatty acid extraction

The extract was carried forward to chromatographic separation using petroleum ether (80–40%) based solvent system with combinations of acetic acid, methanol, and ethyl acetate (20–60%). The fractions (I–VII) were subjected to spectrophotometric assessment after processing the extracts with toluene and chloroform (3:2 *v*/*v*) through a series of wavelengths (670–880 nm, Spec- trum Tek, ST2700) [16]. The fraction that displayed absorbance in the nm range was carried forward for preliminary mortality bioassay (MB, *S. litura* II instar larvae) [22]. The fraction III displayed significant mortality rate against *S. litura* II instar larvae and was carried forward for further studies, CFA.

The insect pest–S. litura

S. litura larvae (II instar) were collected from fields around Azhwarkurichi, South Tamil Nadu, India (8°47′22.8"N 77°23′02.4"E) and maintained in cages (10×10×7 cm) the laboratory (Biopesticide and Environmental Toxicology Laboratory, SPKCEES, Manonmaniam Sundaranar University, Tamil Nadu; 28 ± 2℃ 85% RH, 12:12 LD), fed with castor leaves (*Ricinus communis*) for two generations. Larvae emerged out of the eggs of second-generation adults were used for this study [15].

GCMS

CFA GC–MS analysis was performed following the similar temperature (transfer—230 °C and source − 220 °C, Helium gas) and flow conditions used in previous study [15]. GC–MS analysis of C. antennina fraction-5 was performed (Oven: initial temp 60 °C for 2.8 min, ramp 10 °C/min to 300 °C, holding time for 6 min, Inj A auto = 260 °C, Volume applied used 0 µl, split was 10:1, carrier gas used was helium (He), solvent delay was 2.00 min. The transfer temperature was 230 °C and source temperature was 220 °C. The scan was done at 40–600 Da, column 30.0 m×250 µm) and interpreted against a library of standards (National Institute Standard and Technology).

Mortality bioassay (MB)

MB against larvae, II-V instars (20 /treatment; 2 h prestarved) were performed with CFA (25, 50, 75 and 100 mg/L), sprayed on castor leaves, maintained at 27 ± 2 °C with 80% RH. Leaves were treated with DMSO-$(CH_3)_2SO$ (0.1%) and 9 mg/L cypermethrin (CM), respectively, serve as comparison controls. MB was calculated by timely observation [15, 22].

Experimental setup

For elicitation assay, 3 mL of CFA added with 0.1% DMSO was dissolved in 1 L of sterile distilled water, from which 20 µl was injected into the inter modal region (above fourth leaf) of 45 days old tomato seedlings propagated in greenhouse conditions (1 seedling/pot; 25×13 cm- 2 L). Similarly, 0.1% cypermethrin was dissolved in DMSO (0.1%) and injected into tomato plants as same way as CFA. Post 48 h of injection, the elicitation effect of CFA was assayed by dissection the leaves above the injected area [23].

In plants propagated in similar conditions, seedlings in 5 leaflet phases were infested with pre-weighed 10-II instar *S. litura* larvae (3.78 ± 0.19 mg/larvae) per treatment and transferred to respective metal cages immediately (50×30 cm). After 2-day acclimatization period, the plants were treated so as to result in treatment sets, T1–control; T2–*S. litura;* T3–*S. litura* + 0.1% cypermethrin; T –*S. litura* + 20 µl CFA. The treatments were replicated five times.

Plants were examined every 4 days until day 18 by performing bioassays by choosing five larvae from each test per treatment set post-infestation. Leaves (randomly chosen–1 from each replicate) were used for estimation of phenol and defense enzymes both pre and post infestation with *S. litura*.

Effect of CFA on eliciting plant defense

Salicylic acid (SA) determination in leaves 24 h post elicitation was done using HPLC unit from Agilent Technologies LC 8A [24]. 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. Phenol levels in leaves of post elicited plants (0–5 days) were estimated by crushing leaves from each treatment in methanol using liquid nitrogen and spectrophotometrically quantifying phenol levels using folin phenol reagent at 760 nm (µg GAE/mgFW) [24]. The activities of pathogenesis-related (PR) proteins, Peroxidase (PO) and polyphenol oxidase (PPO) were determined pre and post insect introduction. 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. PO was estimated by pyrogallol based method of Hammerschmidt et al. [25] and PPO by Mayer and Harel [26], observing absorbance at 420 and 490 nm spectrophotometrically. Enzyme levels were expressed in min/gFW.

Insecticidal effect of CFA elicited tomato plants on S. litura

The insecticidal effect of CFA on *S. litura* larvae reared on elicited tomato plants mentioned in experimental setup (Sect. "Experimental setup") was determined by the following experiments.

Developmental assay

Developmental studies were carried by observing the II instar *S. litura* larvae left in the plant cages daily for any deaths, larval and pupa weight; larva and pupa duration, deformities throughout and adult longevity for both the sexes [27]. Larval-pupal duration was measured by counting the number of days from hatching to pupation and pupation to adult emergence, respectively. The effect of elicitor treatments on population and hatchability of *S. litura* was determined by counting the number of surviving larvae till the life cycle is complete [28].

$$\text{Hatching rate (\%)} = \frac{\text{Number of hatching eggs}}{\text{Total number of eggs}} \times 100$$

Food utilization, consumption and nutritional indices

The effect of treatments on the feeding as well as nutritional indexes on the pest's IV instars larvae (5/treatment) were estimated by determining the Relative consumption Rate (RCR) as well as Relative growth rate (RGR), along with their Approximate digestibility (AD) [29].

$$\mathrm{RCR} = \frac{\text{Dry weight of food eaten}}{\text{Duration of feeding (days)}} \times \text{Mean dry weight of the larva during the feeding period}$$

$$\mathrm{RGR} = \frac{\text{Dry weight gain of larva during the period}}{\text{Duration of feeding (days)}} \times \text{Mean dry weight of the larva during the feeding period}$$

$$AD = 100 \times \frac{\text{Dry weight of food eaten} - \text{dry weight of feces produced}}{\text{Dry weight of food eaten}}$$

Also, food conversion efficiencies, ECI and ECD was also determined by estimating the rate of food consumed and larval dry weight pre and post feeding [30].

$$\mathrm{ECI} = 100 \times \frac{\text{Dry weight gain of larva}}{\text{Dry weight of food eaten}}$$

$$\mathrm{ECD} = 100 \times \frac{\text{Dry weight gain of larva}}{\text{Dry weight of food eaten - dry weight of faeces produced}}$$

Enzyme extracts preparation

Treated and control IV instar larvae were sacrificed by exposing them to liquid nitrogen, homogenized on ice in homogenization buffer (0.1 M phosphate buffer, pH 7.2), centrifuged at 4 °C, 10,000 g for 15 min. The supernatant of ground in phosphate buffer (pH 7.2) was used for enzyme assays [31].

Estimation of phosphatase enzyme activities

The estimation of acid (ACP) and alkaline (ALP) phosphatases was estimated using 0.02 M phosphate buffer substrate (pH 7.2) as substrate and correspondingly measuring absorbances at 310 and 320 nm. Using TCA precipitation and ANSA reagent adenosine triphosphatase (ATPase) enzyme levels were estimated spectrophotometrically by reading absorbance at 640 nm [32]. Lactate dehydrogenase (LDH) activity in enzyme source was calculated by the reaction with NAD + substrate, followed by spectrophotometric estimation of 2, 4- dinitrophenyl hydrazine and 0.4N NaOH addition to reaction mixture at 440 nm [33].

Estimation of gut enzyme activities

Dinitrosalicyclic acid (DNS)-based method was used for the estimation of amylase activity by spectrophotometric estimation at 550 nm [34]. Lipase activity was estimated using olive oil emulsion followed by titration with 0.05 N NaOH; reaction terminated by development of permanent pink color due to addition of phenolphthalein indicator [35]. Protease activities were estimated with BSA as substrate and spectrophotometric estimation of reaction mixture at 600 nm [36].

Estimation of antioxidant enzyme activities

The activities of Glutathione S-transferases (GST) as well as Cytochrome p450 (Cp-450) and Carboxylesterase (CarE) were spectrophotometrically estimated by following the methods of Habig et al. [37], Pradeepa et al. [38] and Govindappa et al. [39] (Spectrum Tek, ST 2700).

Histological analysis

Eosin and Delafield's haematoxylin method of staining and observation method was used to visualize the effects of FA exposure to IV instar larvae [15].

Population study

The population study of *S. litura* on treated plants was calculated by the comparison of larvae number present at the commencement and end of the trials from the experimental setup (Sect. "Experimental setup") [40].

Statistical analysis

All experiments were performed in replications of five, in a completely randomized trial method. Treatment effects on insect bioassay, enzyme activities and PRP estimations were analyzed by one-way ANOVA, and means were associated (Tukey's-family error test ($P<0.05$), Minitab®17). Prior to doing statistical analysis, the data from the aforementioned studies were arcsine transformed.

Results

Fatty acid compounds–GC–MS analysis

A total of seven fractions with absorbance in wavelength range (670–880 nm), three fractions (F_d, F_e and F_f) was carried forward for preliminary bioassay with *S. litura* (II instar larvae). Amongst them, fraction, F_e was detected with higher mortality rate (97.63%) was taken for characterization assay using GC–MS. The analysis of the fraction (F_e, CFA) revealed the presence of 19 fatty acid compounds (Table 1, Fig. 1). Among them, Hexadecanoic and Octadecatrienoic acids, were detected in higher quantities (28.32 and 23.05%). Linolenic acid, 2-hydroxy-1-(hydroxymethyl)ethyl (8.231%), 17-Pentatriacontene (5.76%) and Octadecanoic acid, 2,3-dihydroxypropyl ester (4.73%) were also detected in significant quantities.

Insecticidal bioassay

CFA was observed with higher mortality rate against II instar larvae of *S. litura*, 97.734% ($F_{3, 16}=39.82$; $P<0.005$) at 100 mg/L. The mortality rates of *S. litura* larvae treated with 100 mg/L CFA decreased with increasing instars (III, IV and V; $P>0.005$). Mortality rates increased in a dose dependent manner, causing 30.3% mortality against II instar larvae at 25 mg/L ($F_{5, 24}=52.18$; $P<0.0001$). CFA was able to increase the mortality rates by 84.54 and 93.8% compared with cypermethrin and control ($F_{5, 24}=33.96$; $P<0.0001$) among V instar larvae, respectively. The active compounds were relatively effective against II and III instar larvae than cypermethrin causing 84.3($F_{5, 24}=48.63$; $P<0.0001$) and 83.9% ($F_{5, 24}=41.88$; $P<0.0001$) mortality rates (Fig. 2).

Induction of foliar phenols

HPLC analysis of CFA elicited leaf was detected with the presence of salicylic acid, 3.5 µg/g FW (SA) and flavonoids along with phenolic compounds that was not detected in control as well as plants elicited with CM (Fig. 3). HPLC analysis of leaves of CFA-treated seedlings revealed the presence of higher amounts of Hydroxycinnamic derivatives, flavonoids and hydroxybenzoic acids, 24 h post treatment. Foliar phenolic accumulation was assessed for both elicitor treatments and compared to control during a 5-day period (Fig. 4).

The quantity of phenolic compounds in untreated leaves was substantially lower and did not change significantly throughout ($P>0.005$). However, phenolic levels in treated seedlings increased rapidly 1 h after treatment. The phenolic compound levels in CM-treated seedlings continued to ascent linearly after 24 h, reaching a high of 3.67 g GAE/mgFW. The

Table 1 Fatty acid compounds identified through GC–MS

	Retention time	Compound	Peak area (%)	Molecular formula	Molecular weight
1	16.014	Tetradecanoic acid	1.145	$C_{14}H_{28}O_2$	256
2	17.554	Hexadecyne	2.88	$C_{16}H_{30}$	221
3	19.335	Hexadecanoic acid, methyl ester	3.135	$C_{18}H_{36}O_2$	284
4	19.64	Palmitoleic acid	1.078	$C_{16}H_{30}O_2$	254.41
5	20.151	n-Hexadecanoic acid	28.32	$C_{16}H_{32}O_2$	256
6	21.916	Hexadecanol, 2-methyl-	1.67	$C_{17}H_{36}O$	256.4
7	23.407	trans-13-Octadecenoic acid	3.964	$C_{19}H_{36}O_2$	296
8	23.312	9,12,15-Octadecatrienoic acid, (Z,Z,Z)-	23.05	$C_{18}H_{30}O_2$	278
9	23.497	9,12-Octadecadienoic acid (Z,Z)-	2.48	$C_{18}H_{32}O_2$	280
10	23.757	Octadecanoic acid	3.79	$C_{18}H_{36}O_2$	284.4
11	24.777	Z-5-Methyl-6-heneicosen-11-one	1.159	$C_{22}H_{42}O$	322.5
13	25.323	17-Pentatriacontene	5.76	$C_{35}H_{70}$	490
14	25.723	Dodecane, 5,8-diethyl-	3.411	$C_{16}H_{34}$	226
15	27.003	Linolenic acid, 2-hydroxy-1-(hydroxymethyl)ethyl	8.231	$C_{20}H_{34}O_2$	306
16	28.019	Octadecanoic acid, 2,3-dihydroxypropyl ester	4.73	$C_{21}H_{45}BO_7$	420.39
17	29.139	tert-Hexadecanethiol	1.31	$C_{16}H_{34}S$	258
18	29.354	Hexadecanoic acid, 2-hydroxy-1-	2.437	$C_{19}H_{38}O_4$	330.5
19	29.719	Docosanoic acid, methyl ester	1.45	$C_{23}H_{46}O_2$	354

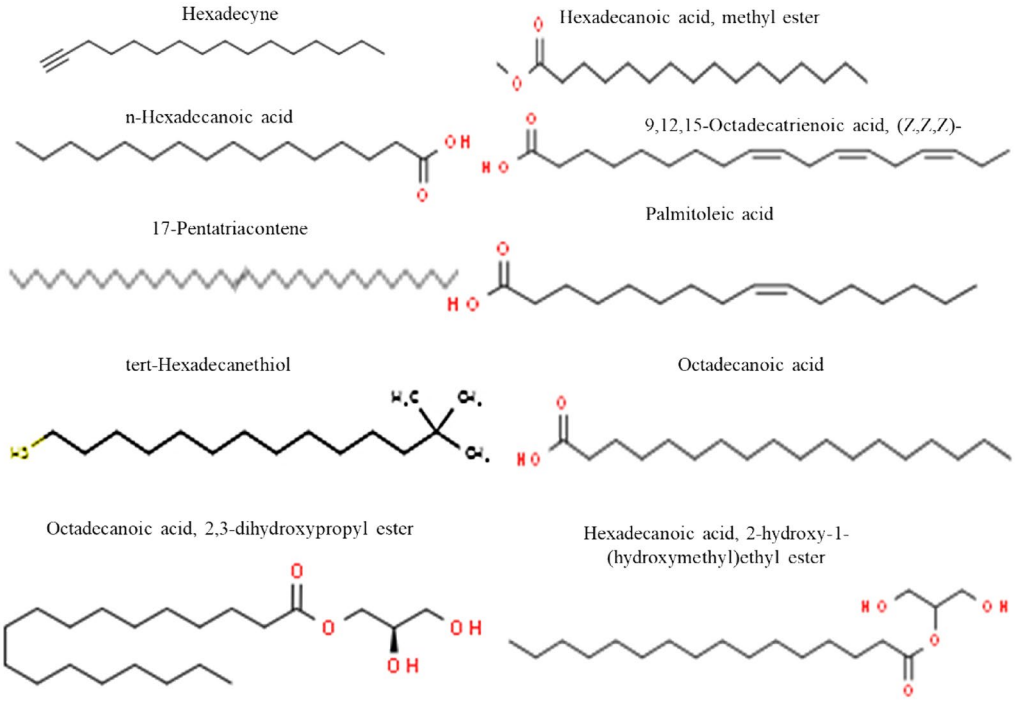


Fig. 1 Fatty acid compounds of *C. antennina*

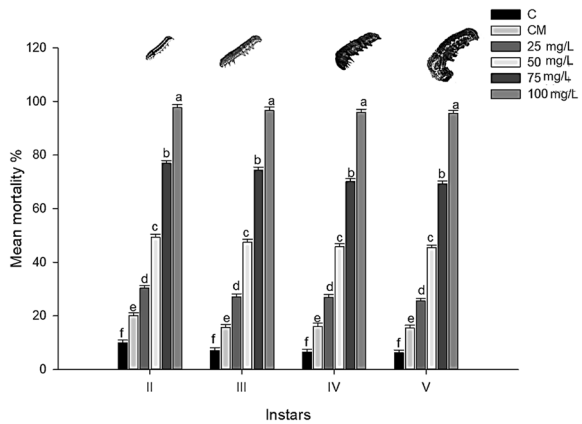


Fig. 2 Percentage mortality of *S. litura* after treatment with CFA. Means (± (SE) standard error) followed by the same letters above bars indicate no significant difference ($P \leq 0.05$) according to Tukey test

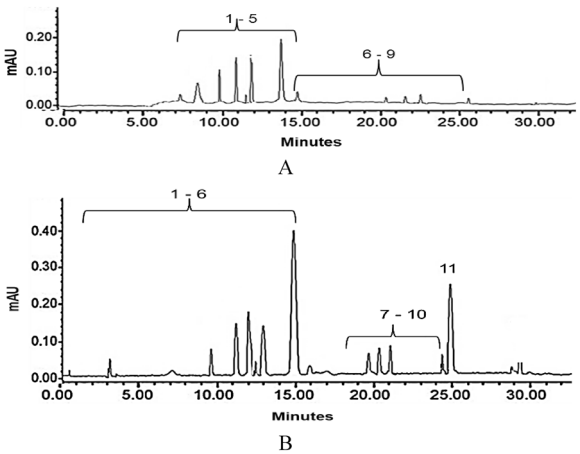


Fig. 3 HPLC chromatogram of plants **A** Control **B** CFA treated (Hydroxycinnamic derivatives (1, 2, 3, 4, 5, and 9), Salicylic acid (10), flavonoids (6 and 8), hydroxybenzoic acids (7, 11)

levels then continued to fall, remaining steady for 2 days (days 2 and 3), exhibiting 3.3 g GAE/mgFW. On the following days, the values dropped to 3.01 and 2.85 g GAE/mgFW ($F_{5,\ 24} = 20.23$; $P < 0.0001$). Similarly, phenolic levels in algal compound treated seedlings grew rapidly on day 1, but were 14% lower than in CM-treated seedlings ($F_{5,\ 24} = 42.32$; $P < 0.004$). The levels increased steadily until day 3, reaching a high of 4 g GAE/mgFW and remaining constant until day 4, when they began to fall by roughly 5%, reaching 3.8 GAE/mgFW on day 5 ($F_{5,\ 24} = 12.5$; $P < 0.0001$). Nonetheless, the levels were substantially greater than in cypermethrin-treated leaves ($P < 0.005$).

Morphogenesis

Insect larvae fed on CFA elicited plants displayed abnormalities in developmental processes such as

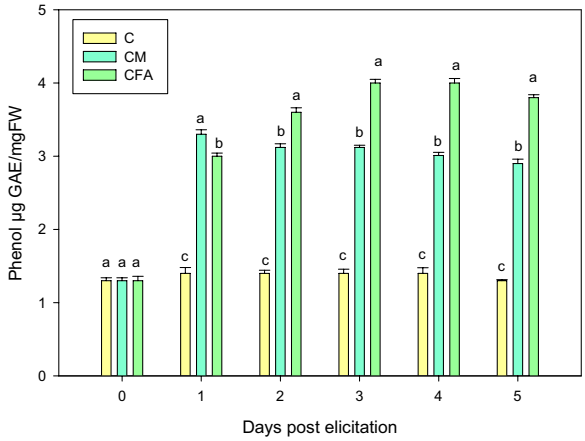


Fig. 4 Accumulation of leaf phenol in response to elicitor treatments, CM and CFA. Mean (± SEM) followed by the same letter in an individual experiment indicate no significant difference ($P < 0.05$) in a Tukeys test

of larval and pupal duration (days), biomass (mg), adult longevity (days), fecundity and morphological deformities. The abnormalities differed significantly in treated larvae compared with control and CM ($P < 0.005$). The larval and pupal duration was severely affected by elicitor treatments. The larval duration on control plants extended to 18 days (Fig. 5A). By the time there was almost complete infestation of tomato plants. The duration of larvae reared on elicited plants was extended to 20 and 28 days by treatments of cypermethrin and CFA, respectively ($F_{5,\ 24} = 15.38$; $P < 0.0001$). However, the pupal duration was drastically reduced due to both elicitor treatments as 4 and 1 day(s) by CM and CFA respectively, from 5 days in control ($F_{5,\ 24} = 17.03$; $P < 0.0001$). After one day of pupation, the pupae began to shrink and develop black coloration, considering it non-viable. The larvae reared on untreated plants displayed a steady increase in biomass reaching a maximum weight gain of 480.51 mg in 18 days which was significantly different with treated one ($F_{4,\ 20} = 22.31$, $P < 0.0001$).

The biomasses of the larvae were influenced by both the treatments. In cypermethrin treatments, the biomass of the larvae displayed a steady increase parallel with control, yet weighing significantly lesser than that of control ($F_{4,\ 20} = 22.31$, $P < 0.0001$). However, the larval biomass was deliberately reduced due to the effect of CFA (Fig. 5B). The biomass increased steadily, in par with control and cypermethrin treatments, yet displaying significantly lower biomass ($P < 0.005$). The algal compounds increased the biomass until day 18, reaching only 130.45 mg, that was 72.85% lesser than control ($F_{4,\ 20} = 13.93$, $P < 0.0001$). Further decrease in biomass was observed on day 20 that declined to 125.15 mg, still 62.07% lesser than that of cypermethrin treatments ($F_{4,\ 20} = 18.08$; $P < 0.0001$).

The elicitor treatments by CFA affected the biomass of pupa, reducing their weight by 8 and 60.78% ($F_{2,\ 12} = 28.08$; $P < 0.003$) (Fig. 5C). The longevity of adults was also dramatically reduced due to elicitor treatments (Fig. 5D). However, the duration of adults in cypermethrin treatments did not vary significantly with control ($P > 0.005$). The seaweed compounds intensely reduced the male and female longevity by 27.5 ($F_{2,\ 12} = 12.48$; $P < 0.001$) and 31.11% ($F_{2,\ 12} = 10.16$; $P < 0.001$). The males were more susceptible to treatments than females ($F_{2,\ 12} = 28.14$; $P < 0.001$).

Reproductive behaviour

The treatments vividly reduced the fecundity of *S. litura*, bringing down the rates to 18.83 ($F_{2,\ 12} = 35.18$; $P < 0.001$) and 58.91% ($F_{2,\ 12} = 23.72$; $P < 0.0001$) in cypermethrin and CFA elicited plants, respectively (Fig. 5E). The hatchability rates were affected in both the treatments and differed significantly with control ($P < 0.005$). Post 24 h after fecundity, the hatching rate of eggs laid on control plants reached 88% which rose to 95 and 97% post 48 and 72 h ($F_{2,\ 12} = 45.01$; $P < 0.005$). Conversely, post 48 and 72 h, the hatching rates did not indicate a significant variation ($P > 0.005$). Adult hatching rates emerged out of cypermethrin and CFA-treated plants increased from 58 to 70 and 75% ($F_{2,\ 12} = 17.36$; $P < 0.0001$) and 7, 12 and 20% with time ($F_{2,\ 12} = 15.6$; $P < 0.0001$). Cypermethrin treatments reduced the overall hatching rate by 22.68% 72 h post fecundity ($F_{2,\ 12} = 48.31$; $P < 0.004$). Moreover, the final hatching rate was further decreased in eggs laid by adult in CFA-treated plants by 79.38% ($F_{2,\ 12} = 14.7$; $P < 0.0001$) (Fig. 5F).

S. litura population and food utilization

The population of *S. litura* in control plants exhibited 72% survivability rate at the end of the experiment. The population of *S. litura* decreased to 20% in treatments with cypermethrin and only 12% of them survived at the end of 33 days in larvae exposed to plants elicited with CFA, after which the larvae developed into adults (Fig. 6). Among the survived per cent of larvae, pupae and adults, many were deformed (Fig. 7). Though the larvae metamorphosed, they either resulted in defective pupae or the emerged adults were observed with deformities.

The IV instar larvae feeding manifestations were vividly affected by both the elicitor treatments (Table 2). The AD of IV instar larvae significantly increased in CFA treatment from 52.34 to 64.45% ($F_{2,\ 12} = 32.85$; $P < 0.004$). Also, the AD of IV instar larvae of CM-treated plants increased only by 4.57%, which was still significantly

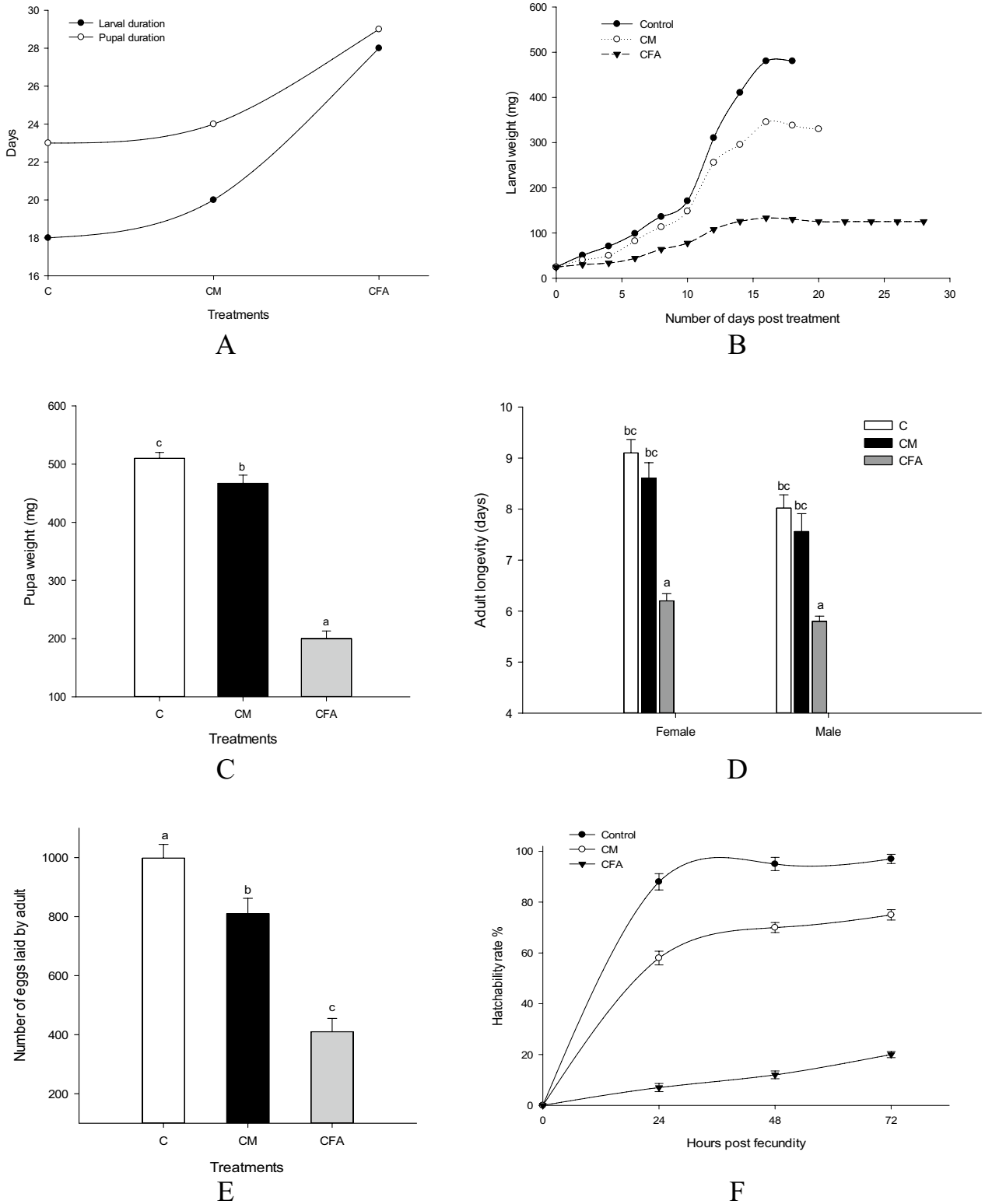


Fig. 5 Effect of elicitor treatments on **A** Larval-pupal duration (days); **B** Larval weight (mg); **C** Pupal weight (mg); **D** Adult longevity (days); **E** Fecundity (%); **F** Hatchability (%)

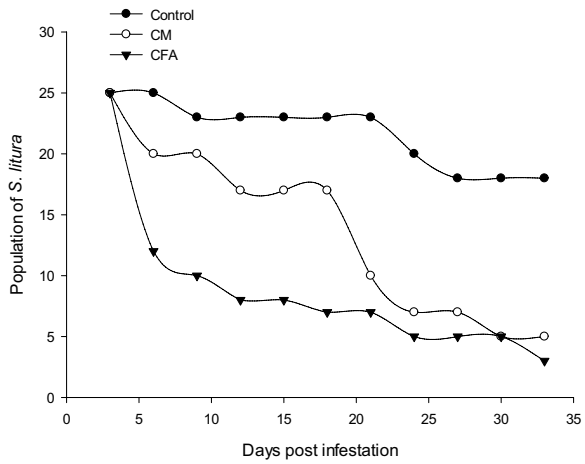


Fig. 6 Effect of elicitor treatments on population of *S. litura*

different compared with control ($F_{2,\ 12}=49.48$; $P<0.005$). In contrast, the ECI and ECD rates decreased in CM and CFA treatments from 26.57 and 50.94% to 25.03, 19.9% ($F_{2,\ 12}=29.62$; $P<0.005$) as well as 47.63 and 31.12 ($F_{2,\ 12}=20.6$; $P<0.0001$), respectively. A similar decrease in RGR and RCR were also observed in IV instar larvae in both the treatments and significantly differed with control (Table 2). The RGR and RCR values decreased from 0.58 and 2.21 mg/mg/day to 0.54 and 0.38 mg/mg/day ($F_{2,\ 12}=27.58$; $P<0.005$) and 2.18 and 1.94 mg/mg/day ($F_{2,\ 12}=14.39$; $P<0.002$), respectively.

Larval enzymatic profile

The elicitor treatments decreased the levels of phosphatase enzyme activities of larvae in fourth instar, differing significantly from each other and with control (Table 3). The ACP levels decreased from 16.16 to 14 and 7.74 µmol/mg/h^{-1} in CM and CFA treatments ($F_{2,\ 12}=19.83$; $P<0.0001$). The CM treatments reduced the

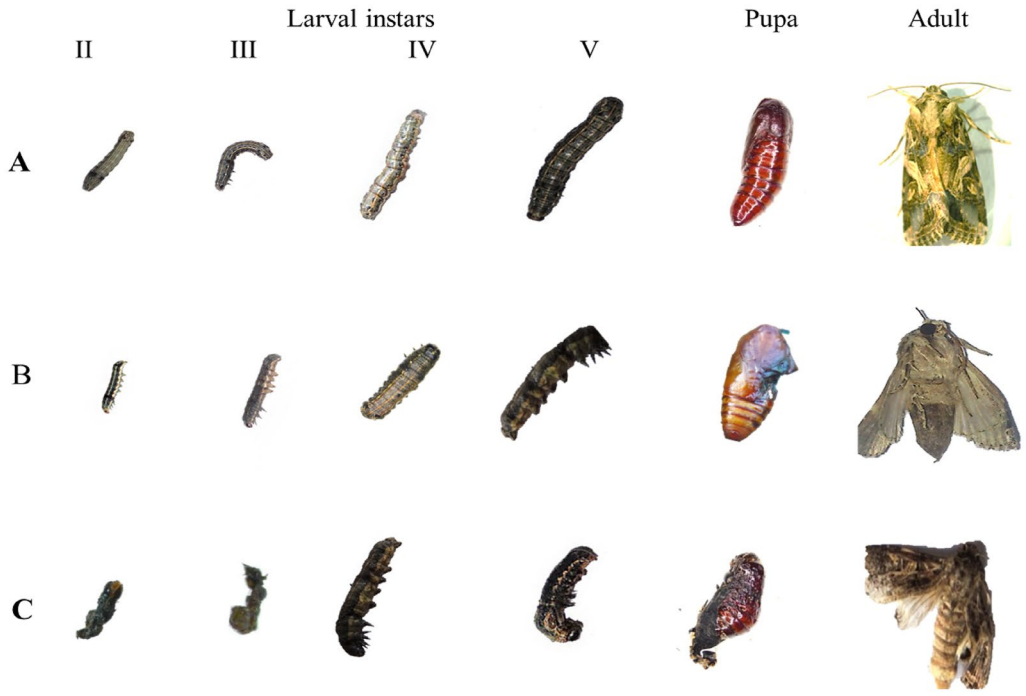


Fig. 7 Effect of elicitor treatments on the development of *S. litura*; **A** –Control; **B**–CM treated; **C**–CFA treated

Table 2 Effect of elicitor treatments on feeding and nutritional of *S. litura* larvae

Treatments	RGR (mg/mg/day)	RCR (mg/mg/day)	ECI %	ECD %	AD %
C	0.58 ± 0.003^{a}	2.21 ± 0.04^{a}	26.57 ± 1.6^{a}	50.94 ± 1.23^{a}	52.34 ± 0.8^{c}
CM	0.54 ± 0.003^{b}	2.18 ± 0.057^{b}	25.03 ± 1.3^{b}	47.63 ± 1.25^{b}	54.83 ± 0.91^{b}
CFA	0.38 ± 0.0039^{c}	1.94 ± 0.036^{c}	19.9 ± 1.15^{c}	31.12 ± 1.1^{c}	64.45 ± 0.79^{a}

Means (± SE standard error) followed by the same letters above bars indicate no significant difference ($P\leq0.05$) according to a Tukey test

Table 3 Effect of elicitor treatments on enzyme activities of *S. litura* larvae

	Enzymes (µmol/mg/h^{-1})	C	CM	CFA
Phosphatase activities	ACP	16.16 ± 1.2^{a}	14 ± 1.25^{b}	7.74 ± 1.45^{c}
	ALP	23.28 ± 1.1^{a}	22.6 ± 1.34^{b}	12.37 ± 1.49^{c}
	ATPase	85.01 ± 1.6^{a}	80.19 ± 1.45^{b}	51.4 ± 1.52^{c}
	LDH	27.97 ± 1.6^{a}	26.15 ± 1.62^{b}	16.01 ± 1.74^{c}
Gut enzymes	Amylase	7.2 ± 1.75^{a}	5.81 ± 1.16^{a}	3.01 ± 1.3^{b}
	Lipase	1.6 ± 0.086^{a}	1.4 ± 0.012^{b}	1 ± 1.01^{c}
	Protease	19 ± 0.9^{a}	15.29 ± 1.6^{b}	6.52 ± 1.75^{c}
Detoxifying enzyme	GST	0.5 ± 0.06^{c}	0.74 ± 0.07^{b}	1.12 ± 0.04^{a}
	Cp-450	1.04 ± 0.034^{a}	1 ± 0.031^{b}	0.65 ± 0.05^{c}
	CarE	0.48 ± 0.045^{c}	0.6 ± 0.019^{b}	0.76 ± 0.09^{a}

Means (± SE standard error) followed by the same letters above bars indicate no significant difference ($P \leq 0.05$) according to a Tukey test

levels of ALP, ATPase and LDH by 2.92, 5.66 and 6.5% compared with control ($F_{2, 12} = 39.53$; $P < 0.004$). The algal compounds profoundly reduced the of ALP, ATPase and LDH activities by 46.86, 39.53 and 42.76% compared with control ($F_{2, 12} = 13.9$; $P < 0.0001$).

The activities of gut enzymes of larvae reduced drastically exposed on elicited tomato plants, by CM and CFA (Table 3). The amylase activity was reduced by 19.3 and 58.19% compared with control ($F_{2, 12} = 17.86$; $P < 0.0001$). A similar decrease in lipase and protease levels in tomato plants elicited by CM and CFA were also observed by a reduction of 12.5, 37.5% ($F_{2, 12} = 13.17$; $P < 0.0001$) as well as 19.52 and 65.68% ($F_{2, 12} = 11.94$; $P < 0.0001$), respectively. The activities of detoxifying enzymes were increased in of larvae (IV) exposed to elicited plants (Table 3). The levels of GST, C p-450 and CarE increased from 0.5, 1.04 and 0.48 to 0.74, 1 and 0.6 µmol/mg/h^{-1} in CM treatments ($F_{2, 12} = 38.53$; $P < 0.005$). The algal compounds increased the detoxifying enzyme activities of larvae to 1.12, 0.65 and 0.76 from 0.5, 1.04 and 0.48 µmol/mg/h^{-1}, respectively ($F_{2, 12} = 22.7$; $P < 0.0001$). Cp-450 enzyme quantities were not found to vary with that of control in CM treatments ($F_{2, 12} = 73.86$; $P > 0.005$).

Plant defense enzyme activities

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

Control seedlings also displayed a minor increase in PO levels till 96 h, yet the levels post 48 h were not expressively unlike ($P > 0.005$). PO levels of CFA-treated seedlings displayed similar enzyme kinetics to those treated with CM. An immediate hike of 54.6% in PO levels after infestation was observed ($F_{5, 24} = 12.41$; $P < 0.0001$). The levels increased 76.75%, but decreased after 96 h ($F_{5, 24} = 28.8$; $P < 0.0001$). However, the final

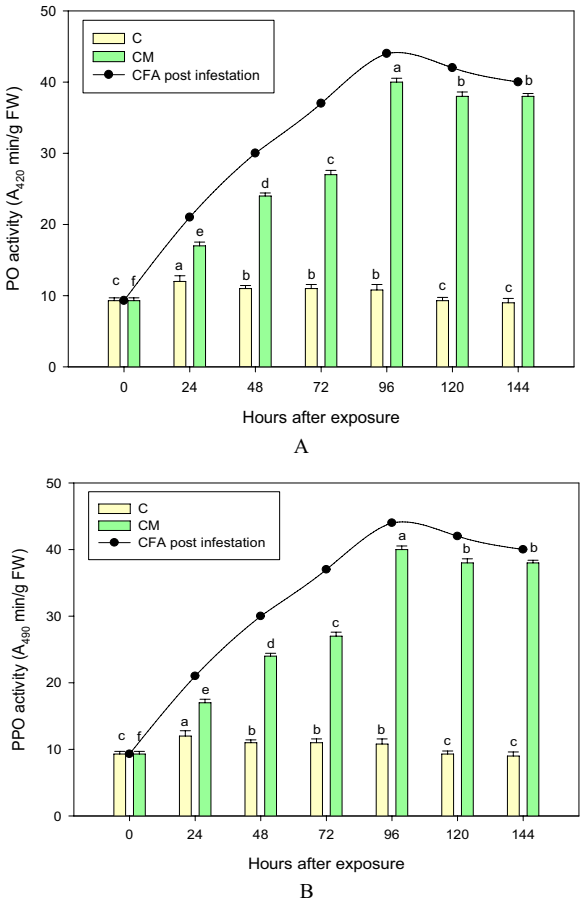


Fig. 8 Estimation of **A** peroxidase (PO) and **B** Polyphenoloxidase (PPO) activities after elicitor treatments

level was 75 and 5.5% higher compared with inoculated control and cypermethrin-treated seedlings ($F_{5, 24} = 42.04$; $P < 0.0001$). There were distinctive variations in plant PO levels among infested and un-infested control plants. The enzyme activity almost doubled

24 h and maintained a hike up to 48 h in control plants and 120 h in both the elicitor treatments ($F_{5,\ 24}=16.03$; $P<0.005$). After 120 h, PO activity decreased gradually, yet the levels were much higher in elicitor-treated plants compared with control ($F_{5,\ 24}=26.3$; $P<0.005$) (Fig. 8A). PPO activities amplified expressively and remained in higher amounts till 96 h in control leaves (Fig. 8B). The algal compounds induced PPO secretion to 31.7 µg/min/g after 120 h after which the levels decreased by 9.3% ($F_{5,\ 24}=41.3$; $P<0.005$). An increase in PPO levels was found with *S. litura* infestation post elicitor treatments. The levels doubled post 24 h reaching the maximum at 120 h in both treatments (Fig. 8B). However, the decrease in PPO levels after 120 h was 77.6 and 80.19% higher than control ($F_{5,\ 24}=12.37$; $P<0.004$). The PO and PPO levels were induced in maximum amounts by algal compounds at 120 h. While, PO and PPO levels were minimal in untreated plants, *S. litura* infestation further decreased these enzyme activities ($F_{5,\ 24}=52.16$; $P<0.005$). Plants elicited with CM, displayed similar induction in PO levels after *S. litura* infestation ($P>0.05$). However, PPO levels were induced more by *S. litura* infestation post elicitation by CM. The algal compounds elicited both the enzyme activities in higher amounts post *S. litura* infestation.

Larval midgut histology

The IV instar larvae reared on the elicited tomato plants were taken for histological analysis. The histology of larval midgut displayed a disruption in their columnar cells. The columnar cells were found to be disconnected from the peritrophic membrane, consequently increasing the intercellular spaces. Additional damage to the epithelial layer along with the brush border membrane was observed. However, the midgut of the untreated larvae displayed an undisturbed midgut that visualised an intact layer of columnar cells along with the epithelial lining and brush border membrane (Fig. 9).

Discussion

The predominance of the implication of green revolution practices that were once effective has attained a plateau. These methodologies relied on the use of high yielding disease resistant varieties, modified cultivation practices along with sumptuous application of chemicals for plant growth promotion as well as protection [41]. Considering all the negative impacts posed upon by the application of chemicals, there has emerged an immediate and alternative strategy to maintain the sustainability of agricultural production in a long run [42]. In this aspect, biofertilizers and biopesticides, sourced from natural materials are looked upon as an unconventional approach towards increasing the productivity. They are environmentally benign along with being safe, non-toxic and effective. Since these compounds are complex, the chances of development of resistance among the pest population are also significantly lower [3]. Seaweeds, which are superior to terrestrial plants in terms of bioactive chemicals, are used in culinary, medicinal, and industrial goods. Thus, the discovery of new botanical insecticides based on the different bioactive components of seaweed is critical. Since seaweeds are reported with higher weight/volume proportions of fatty acids, the possibility of testing their potentials as elicitors against the polyphagous pest, *S. litura* was analyzed in this study.

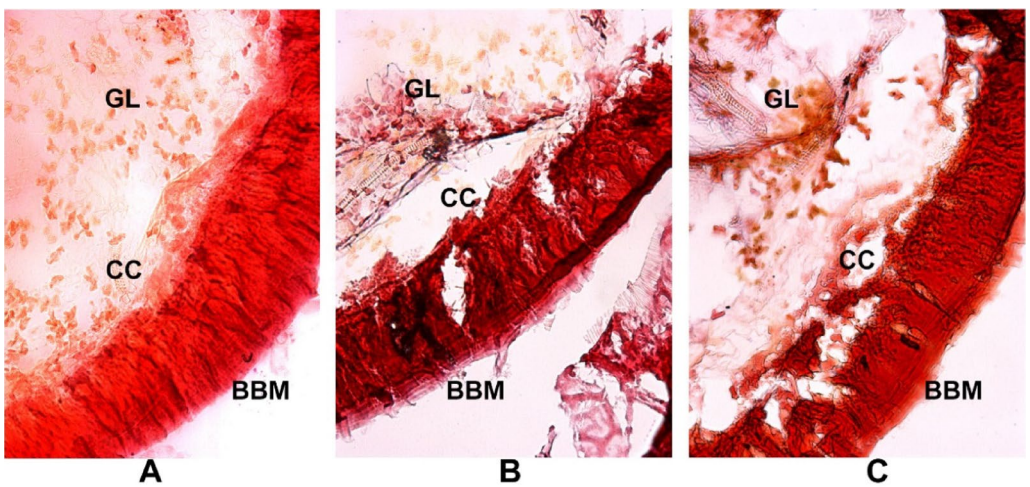


Fig. 9 Histological changes of *S. litura* fed on treated tomato plants; **A**–Control; **B**–cypermethrin treated; **C**–CFA treated. *BBM* Brush border membrane, *GL* gut lumen, *CC* columnar cells

Marine algae are regarded as excellent bases of assorted bioactive-compounds that can stimulate plant growing and also enhance resistance against environmental stressors. Further our experiment results do support the previous findings of Battacharyya et al. [43]. Fatty acids occupy a larger proportion of seaweed chemical composition. They are said to contribute to over 30% dry weight existing either as polyunsaturated or extractable fatty acids in green and brown algae [44]. Seaweeds have a high fatty acid (FA) variety, and many of them have potential bioactivity [45]. FAs are dynamic constituents of all biota, besides recognized 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 [46]. In the current study, *C. antennina* active fraction (CFA) displayed the presence of 19 fatty acids, with Hexadecanoic acid, Octadecatrienoic acid, linolenic acid, pentatriacontene and Octadecanoic acid in significant quantities. Hexa and octa decanoic acids are being reported with direct insecticidal activities. Fatty acids of *Laminaria digitate*, *Undaria pinnatifida* were reported with biocidal activities directly and offering protection by inducing innate defense system of plants in strawberry and lemon trees [47]. This was evident from the presence of salicylic acid and phenolic compounds in leaves of plants elicited with CFA.

The algal compounds induced the activities of PPO and PO, which are components of SA signalling pathways. Algal compounds have also increased the accretion of phenolic compounds in tomato leaves. Phenolic compound accumulation promoted by algal fraction application was significantly higher compared with cypmethrrin applications. Simultaneously, the treatments also offered effective protection against early blight disease and herbivory of *S. litura*. Algal treatments dramatically reduced the number of juvenile *Meloidogyne incognita* root-knot nematodes in soil while enhancing cumulative phenolic as well as antioxidant intensities [48]. The same results were obtained when commercial seaweed products *Ecklonia maxima* and *Ascophyllum nodosum* were used, which inhibited the reproductive as well as behaviour patterns of *M. hapla* and *M. chitwoodi*, that had infected tomato plants [49].

The elicitation test by CFA induced the production of SA which was evident from the HPLC chromatogram. Also, 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 Jaulneau et al. who reported that the SA signalling pathway was induced by the application of algal polysaccharides, laminarin and carrageenans [50]. El Modafar et al. also proved ulvan compounds of *Ulva lactuca* stimulated SA-dependent systemic acquired resistance in tomato seedlings [23].

CFA also exhibited significant mortality rate against the larvae of *S. litura* (Instars II to V). Similar mortality properties of *C. antennina* phenols were demonstrated with a wide pesticidal activity against mosquitoes [51]. The seaweed extracts are more effective against mosquito larvae even at lower concentrations. This was demonstrated by Manilal et al. who showed that the extracts of various green and brown algae had a greater effect on the dipteran larvae, at lower LC_{50} values [45]. Sahayaraj et al. stated the efficacy of seaweed compound, tetradecanoic acid of *Caulerpavera veravalensis* were active against *Dysdercus cingulatus* nymph, a serious cotton pest [16]. It has been demonstrated that saturated FAs are abundant in seaweeds and are capable of killing agriculturally significant pests such as *Sitophilus granarius* [46].

The II instar larvae reared on elicited plants displayed extended larval duration due to reduction in feeding. Consequently, the biomass of these larvae was also very low. A similar larval period extension due to reduced feedant activity was observed in tobacco cut-worm larvae treated with *Aristolochia tagala* extracts [47], *Momordica charantia* secondary metabolites [48] and *Citrullus colocynthis* [49]. The extended larval periods drastically affected the pupae, reducing their biomass, size and duration. The extreme morphological damage to the pupa that metamorphosed from larvae with lower biomass and extended larval duration was also reported by treating *Musca domestica* larvae with parsley and citronella oil [50]. The pupal duration is the base for major nutrient and energy consumption required for the development of a healthy adult as well their fecundity and egg hatching rates [51]. The reduced pupal weight and duration also affected the further development of *S. litura* that extended the adult longevity, female fecundity and egg hatchability. A likely reduction in fecundity of females that emerged out of extended larval periods and lower pupal biomass was reported in *S. litura* larvae treated with leaf extracts of *Momordica charantia* [48]. Delayed metamorphosis moderates post-larval performances. An increase in larval duration increasingly posed a damaging effect on post-larval growth and survivability of *Echinometra* sp [52].

Plant metabolites are lethal to insect herbivores, by interfering with their food consumption and/or utilization. Food consumption is recognized as one of an organism's toxicological endpoints. [53]. The elicitation negatively affected the food consumption and consequently their nutrition. A reduction in the utilization of consumed food resulted in the lower growth rate of the larvae. This directly influenced the

behaviour and physiology of the larvae post ingestion. An analogous effect on dietary utilization decline affected the development and physiology of rice leaf folder larvae treated with a biopesticidal combination [54] and *Dysoxylum* triterpenes [55]. However, an increase in digestibility indicates that the fatty acids, as plant compounds were easily digestible within the larval body, reasoning for higher toxicities. Similar increased AD in larvae treated with goniothalamin and proportionately increasing toxicity was described earlier by Senthil-Nathan et al. [29].

Transphosphorylation processes hydrolyze phosphomonoesters by acid phosphatases in acidic settings and alkaline phosphatases in alkaline conditions. These actions are observed at a greater frequency in the insect midgut, the weakening of which will debar insect survival [56]. Current study reports the reduction in these enzymes, that was reported by previous research indicating the drop-down in such enzymes in insect pests treated with pesticides [56] and azadirachtin [27]. Reduced ACP-ALP enzyme activity correlates with low energy levels induced by metabolite transport disruption. In rice leaf folder larvae subjected to a biopesticide formulation including neem seed kernel and Bt toxin, a similar impact on ATPase enzyme activity was seen [54]. ATPase reduction was caused due to the cease of metabolism as a consequence of either food indigestibility or absence of food intake [57]. LDH enzyme plays a vital role in carbohydrate metabolism, are also indicators of chemical stress [29]. Reduced LDH levels in larvae developed on elicited plants is an indicative of lower carbohydrate metabolism as a result of reduced feedant activities. A likely reduction in LDH levels due to insect toxic allelo-chemicals in plant extracts was observed in neem limonoids-treated rice leaf folder larvae [54]and *S. litura* larvae treated with and *C. colocynthis* extracts [49].

The digestive enzymes such as amylase, lipase and protease also displayed a downfall in larvae due to elicitor treatment. The overall decrease is due to the relative decline in the food consumption of the larvae. Similar decreases in digestive enzymes were observed in *S. littoralis* treated with extracts of *Calotrophis procera* [57]. The potential of insect larvae to detoxify the ingested toxic compounds depends on the efficiency of detoxifying enzymes such as GSTs, cytochrome P-450 and esterases. A reduction in overall activities of these enzymes in the larvae that fed on elicited plants signifies the inability of development of resistance by the insect pests against the defense compounds. A likely decline in the activities of detoxification enzymes was found in the insect larvae of *Hyphantria cunea* treated with *Ginkgo biloba* secondary metabolites [58]. Because larvae digest and absorb nutrients in the larval midgut area, histological study of the larval midgut was performed. The midgut histological study of treated larvae revealed a damaged brush boundary membrane, which might be owing to the active chemicals interfering with metabolite or ion transport. It's possible that this started a chain reaction of cellular processes that finally prevented the insect from eating, such as cell disintegration and the leakage of cellular components. This resulted in enlargement of the treated larvae's midgut area. Similar abnormalities in the midgut of *S. litura* treated with seaweed chemicals have also been documented [15].

Both the 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 [59, 60]. Application of MeSA has stimulated the production of phenolic compounds [61]. Jasmonate application on tomato plants were found to stimulate the production of defensive proteins that negatively influenced herbivores [62]. There exists a linear relationship between phenolic compound concentration and antimicrobial potentials [24]. Consequently, the allegation of phenol accumulation in plant defense has also been proved [63].

Plant-mediated interactions amongst pathogens besides arthropod herbivores can ensure significant concerns for individual pests as well as their population dynamics [64]. These types of interactions are mediated by SA and JA facilitated plant interactive pathways. While herbivore attack stimulates JA pathway, pathogen infection stimulates SA-related defense signalling pathways. PO and PPO activities are components of early response in plants to pathogen infection and insect infestation [65].

Higher accumulation of foliar phenols and increased in the activities of enzymes PO and PPO by SA treatments are reported in *Solanum melongena*, *Brassica juncea* (var. Rlm619) that provided resistance against *Ralstonia solanacearum* [66]. Additionally, increased phenolics associated with amplified PAL activities conferred resistance against fungal phytopathogen, *Fusarium oxysporum* that was exposed with algal polysaccharides [18]. 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* [67]. The ability of algal compounds to offer better resistance compared with chemical pesticide, is attributed to the ability of these compounds to induce additional defense signalling pathways along with that of SA mediated defense responses [68].

A similar increase in PO and PPO activities was also observed with respect to elicitor treatments pre and post *S. litura* infestation. The levels were higher in infested elicited plants compared with that of un-infested. A likely

increase in Pathogenesis-related proteins (PRPs) post infestation in elicited plants was also observed in tomato plants treated with commercial elicitors, commercial elicitors benzothiadiazole and methyl jasmonate post infestation with green peach aphid, *Myzus persicae* [69]. These elicitors also effectively reduced the aphid population and fecundity. The elicitor treatments induced plant PRPs in response to both pathogen and insect attacks. However, at 120 h, the levels of PPO and PO were higher in response to herbivory. Nevertheless, Boughton et al. proved that the antagonistic reactions flanked by JA then SA reliant plant signalling conduits was able to deactivate infestation mechanisms of aphids [70]. Hence higher level of protection was conferred against insect infestation by the activation of both JA and SA pathways that resulted in the production of various secondary metabolites such as lignins, tannins, flavonoids, and enzymes such as PO, PPO, PAL and LOX. Stout et al. also stated that the probable instigation of defense genes typically connected with pathogen attack [71].

Conclusion

Hence, the study confirms the elicitor potentials of compounds from *C. antennina*, by inducing natural systemic defenses 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 *S. litura* pest infestation in tomato seedlings exposed to compounds of *C. antennina*. In addition to providing a different method for crop protection to reduce or replace the demand for chemical pesticides, this study reveals unique projections. Seaweed research is one of the keys to future agricultural progress and is progressing every year along with the processes behind each species and/or extract evolving to be more precise. Despite the fact that organic goods continue to gain market share, a gap has to be filled to turn scientific discoveries on seaweed and microalgae into practical solutions for industry.

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Author contributions
Conceptualization and methodology design was done by KMPC, SSN. Investigation was carried out by KMPC, PKR, BOA and ADA. Data curation and original draft preparation was performed by KMPC and SSN; Writing—review and editing, was done by SSN.

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Availability of data and materials
Data will be available on request.

Declarations

Ethics approval and consent to participate
Not applicable.

Consent for publication
The authors offer consent for publication.

Competing interests
The authors declare no competing interests.

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References

1. Fiodor A, Singh S, Pranaw K. The contrivance of plant growth promoting microbes to mitigate climate change impact in agriculture. Microorganisms. 2021;9(9):1841.
2. Rathi BS, Kumar PS, Vo DV. Critical review on hazardous pollutants in water environment: occurrence, monitoring, fate, removal technologies and risk assessment. Sci Total Environ. 2021;797: 149134.
3. Senthil-Nathan S. A review of biopesticides and their mode of action against insect pests. Environmental sustainability Role of green technologies. 2014. 49–63.
4. Chanthini KM, Senthil-Nathan S, Soranam R, Thanigaivel A, Karthi S, Sreenath Kumar C, Kingsley SJ, Kanagaraj MBR. Bacterial compounds, as biocontrol agent against early blight (*Alternaria* solani) and tobacco cut worm (*Spodoptera litura Fab*) of tomato (*Lycopersicon esculentum Mill*). Arch Phytopathol Plant Prot. 2018;51(13–14):729–53.
5. Chanthini KM, Senthil-Nathan S, Stanley-Raja V, Thanigaivel A, Karthi S, Sivanesh H, Sundar NS, Palanikani R, Soranam R. *Chaetomorpha antennina* (Bory) Kützing derived seaweed liquid fertilizers as prospective biostimulant for *Lycopersicon esculentum* (Mill). Biocatal Agric Biotechnol. 2019;20: 101190.
6. Chanthini KM, Stanley-Raja V, Thanigaivel A, Karthi S, Palanikani R, Shyam Sundar N, Sivanesh H, Soranam R, Senthil-Nathan S. Sustainable agronomic strategies for enhancing the yield and nutritional quality of wild tomato, *Solanum Lycopersicum* (l) var Cerasiforme Mill. Agronomy. 2019;9(6):311.
7. Ali O, Ramsubhag A, Jayaraman J. Biostimulant properties of seaweed extracts in plants: implications towards sustainable crop production. Plants. 2021;10(3):531.
8. Benítez García I, Dueñas Ledezma AK, Martínez Montaño E, Salazar Leyva JA, Carrera E, Osuna RI. Identification and quantification of plant growth regulators and antioxidant compounds in aqueous extracts of *Padina durvillaei* and *Ulva lactuca*. Agronomy. 2020;10(6):866.
9. Spagnuolo D, Russo V, Manghisi A, Di Martino A, Morabito M, Genovese G, Trifilò P. Screening on the presence of plant growth regulators in high biomass forming seaweeds from the Ionian Sea (Mediterranean Sea). Sustainability. 2022;14(7):3914.
10. Stirk WA, Van Staden J. Plant growth regulators in seaweeds: occurrence, regulation and functions In Advances in botanical research 2014. Cambridge: Academic Press; 2014.
11. Górka B, Lipok J, Wieczorek PP. Biologically active organic compounds, especially plant promoters, in algae extracts and their potential application in plant cultivation marine algae extracts: processes, products, and applications. Hoboken: Wiley; 2015.
12. Feng JC, Cai ZL, Zhang XP, Chen YY, Chang XL, Wang XF, Qin CB, Yan X, Ma X, Zhang JX, Nie GX. The effects of oral *Rehmannia glutinosa*

polysaccharide administration on immune responses, antioxidant activity and resistance against *Aeromonas hydrophila* in the common carp. Cyprinus carpio L Front immunol. 2020;11:904.
13. Sanjeewa KA, Kang N, Ahn G, Jee Y, Kim YT, Jeon YJ. Bioactive potentials of sulfated polysaccharides isolated from brown seaweed *Sargassum* spp in related to human health applications: a review. Food Hydrocoll. 2018;81:200–8.
14. Chanthini KM, Senthil-Nathan S, Pavithra GS, Asahel AS, Malarvizhi P, Murugan P, Deva-Andrews A, Sivanesh H, Stanley-Raja V, Ramasubramanian R, Ghaith A. The macroalgal biostimulant improves the functional quality of tomato fruits produced from plants grown under salt stress. Agriculture. 2022;13(1):6.
15. Chanthini KM, Senthil-Nathan S, Stanley-Raja V, Karthi S, Sivanesh H, Ramasubramanian R, Abdel-Megeed A, Maghraby DM, Ghaith A, Alwahibi MS, Elshikh MS. Biologically active toxin from macroalgae *Chaetomorpha antennina* Bory, against the *lepidopteran Spodoptera litura* Fab and evaluation of toxicity to earthworm, *Eudrilus eugeniae* Kinb. Chem Biol Technol Agric. 2021;8:1–5.
16. Sahayaraj K, Ravindran C, Jancy S, Pechidurai G. Toxicity of *Caulerpa scalpelliformis* selected fractions with fatty acids on *Porthesia scintillans*. Toxin Rev. 2022;41(3):880–90.
17. Walling LL. The myriad plant responses to herbivores. J Plant Growth Regul. 2000;19:195–216.
18. Mitchell C, Brennan RM, Graham J, Karley AJ. Plant defense against herbivorous pests: exploiting resistance and tolerance traits for sustainable crop protection. Front Plant Sci. 2016;7: 191972.
19. Jaiswal AK, Mengiste TD, Myers JR, Egel DS, Hoagland LA. Tomato domestication attenuated responsiveness to a beneficial soil microbe for plant growth promotion and induction of systemic resistance to foliar pathogens. Front microbiol. 2020;11: 604566.
20. Mehta MC, Srivastava S, Mall AK, Raghuraman M. Biology pest status and management of armyworm *Spodoptera* litura and Cutworm Agrotis ipsilon (*Noctuidae*: *Lepidoptera*) on sugar beet. Singapore: Springer Nature Singapore; 2022.
21. dos Dora S, Costa DS, Moreno A, Alviano CS, da AntonioSilva JR. Extension of *Solanaceae* food crops shelf life by the use of elicitors and sustainable practices during postharvest phase. Food Bioproc Tech. 2022;15(2):249–74.
22. Abbott WS. A method of computing the effectiveness of an insecticide. J econ Entomol. 1925;18(2):265–7.
23. El Modafar C, Tantaoui A, El Boustani ES. Differential induction of phenylalanine ammonia-lyase activity in date palm roots in response to inoculation with *Fusarium oxysporum* f sp albedinis and to elicitation with fungal wall elicitor. J Plant Physiol. 2001;158(6):715–22.
24. Chanthini K, Kumar CS, Kingsley SJ. Antifungal activity of seaweed extracts against phytopathogen *Alternaria solani*. J Acad Indus Res. 2012;1(2):86–90.
25. Hammerschmidt R, Nuckles EM, Kuć J. Association of enhanced peroxidase activity with induced systemic resistance of cucumber to *Colletotrichum lagenarium*. Physiol Plant Pathol. 1982;20(1):73–82.
26. Mayer AM, Harel E. Polyphenol oxidases in plants. Phytochem. 1979;18(2):193–215.
27. Senthil-Nathan S, Kalaivani K. Combined effects of azadirachtin and nucleopolyhedrovirus (SpltNPV) on *Spodoptera litura Fabricius* (*Lepidoptera*: *Noctuidae*) larvae. Biol control. 2006;39(1):96–104.
28. Xu C, Zhang Z, Cui K, Zhao Y, Han J, Liu F, Mu W. Effects of sublethal concentrations of cyantraniliprole on the development, fecundity and nutritional physiology of the black cutworm *Agrotis ipsilon* (*Lepidoptera*: *Noctuidae*). PLoS ONE. 2016;11(6): e0156555.
29. Senthil-Nathan S, Choi MY, Paik CH, Kalaivani K. The toxicity and physiological effect of goniothalamin, a styryl-pyrone, on the generalist herbivore. Spodoptera exigua Hübner Chemosphere. 2008;72(9):1393–400.
30. Waldbauer GP. The consumption and utilization of food by insects in advances in insect physiology. Cambridge: Academic Press; 1968.
31. Applebaum SW. The action pattern and physiological role of Tenebrio larval amylase. J Insect Physiol. 1964;10(6):897–906.
32. Fiske CH, Subbarow Y. The colorimetric determination of phosphorus. J biol Chem. 1925;66(2):375–400.
33. King J. Practical clinical enzymology. In: King J, editor. Practical clinical enzymology. London: D Van Nostrand Company Ltd; 1965. p. 121–38.
34. Ishaaya I, Swirski E. Invertase and amylase activity in the armoured scales *Chrysomphalus aonidum* and *Aonidiella aurantii*. J Insect Physiol. 1970;16(8):1599–606.
35. Veeraragavan K. A simple and sensitive method for the estimation of microbial lipase activity. Anal Biochem. 1990;186(2):301–5.
36. Snell FD, Snell CT. Colorimetric Methods of Analysis. New York: D Van Norstrand Co Inc; 1949.
37. Habig WH, Pabst MJ, Jakoby WB. Glutathione S-transferases: the first enzymatic step in mercapturic acid formation. J Biol Chem. 1974;249(22):7130–9.
38. Pradeepa V, Senthil-Nathan S, Sathish-Narayanan S, Selin-Rani S, Vasantha-Srinivasan P, Thanigaivel A, Ponsankar A, Edwin ES, Sakthi-Bagavathy M, Kalaivani K, Murugan K. Potential mode of action of a novel plumbagin as a mosquito repellent against the malarial vector *Anopheles stephensi*,(Culicidae: Diptera). Pest Biochem Phys. 2016;134:84–93.
39. Govindappa T, Govardhan L, Jyothy PS, Veerabhadrappa PS. Purification and characterisation of a carboxylesterase from the latex of *Synadenium grantii* Hook, 'f.' J Biosci. 1987;12:71–86.
40. Sivanesh H, Shyam Sundar N, Senthil-Nathan S, Stanley-Raja V, Ramasubramanian R, Karthi S, Chanthini KM, Almoallim HS, Alharbi SA. Toxicity of *Suaeda maritima* (L) against the *Scirpophaga incertulas* (W) and *Xanthomonas oryzae* pv oryzae (Xoo) disease and its non-target effect on earthworm Eisenia fetida Savigny. Toxin Rev. 2022;41(1):143–53.
41. Frenkel O, Jaiswal AK, Elad Y, Lew B, Kammann C, Graber ER. The effect of biochar on plant diseases: what should we learn while designing biochar substrates? J Environ Eng Landsc. 2017;25(2):105–13.
42. Senthil-Nathan S. Physiological and biochemical effect of neem and other Meliaceae plants secondary metabolites against Lepidopteran insects. Front physiol. 2013;4:359.
43. Bhattacharyya C, Banerjee S, Acharya U, Mitra A, Mallick I, Haldar A, Haldar S, Ghosh A, Ghosh A. Evaluation of plant growth promotion properties and induction of antioxidative defense mechanism by tea rhizobacteria of Darjeeling, India. Sci Rep. 2020;10(1):15536.
44. Gosch BJ, Magnusson M, Paul NA, De Nys R. Total lipid and fatty acid composition of seaweeds for the selection of species for oil-based biofuel and bioproducts. Gcb Bioenergy. 2012;4(6):919–30.
45. Manilal A, Sujith S, Sabarathnam B, Kiran GS, Selvin J, Shakir C, Lipton AP. Biological activity of red alga *Laurencia brandenii*. Acta Bot Croat. 2011;70(1):81–90.
46. Nguyen TQ, Sesin V, Kisiala A, Emery RN. Phytohormonal roles in plant responses to heavy metal stress: Implications for using macrophytes in phytoremediation of aquatic ecosystems. Environ Toxicol Chem. 2021;40(1):7–22.
47. Baskar K, Sasikumar S, Muthu C, Kingsley S, Ignacimuthu S. Bioefficacy of *Aristolochia tagala* Cham against *Spodoptera litura* Fab (*Lepidoptera*: *Noctuidae*). Saudi J Biol Sci. 2011;18(1):23–7.
48. Guo Z, Wang G, Zhang M, Liang G, Li Q, Ling B. Evaluation of cytotoxic activity in vitro of charantin a extracted from *Momordica charantia*. Rec Nat Prod. 2018;12(5):416.
49. Ponsankar A, Vasantha-Srinivasan P, Thanigaivel A, Edwin ES, Selin-Rani S, Chellappandian M, Senthil-Nathan S, Kalaivani K, Mahendiran A, Hunter WB, Alessandro RT. Response of *Spodoptera litura Fab* (*Lepidoptera*: *Noctuidae*) larvae to *Citrullus colocynthis* L (*Cucurbitales*: *Cucurbitaceae*) chemical constituents: larval tolerance, food utilization and detoxifying enzyme activities. Physiol Mol Plant Pathol. 2018;101:16–28.
50. Kamel AA, Mohamed MB, El-Dakhly KM. Larvicidal activity and bio-efficacy of some products against larvae of the Housefly, *Musca domestica* (L) (*Diptera*: *muscidae*). J Appl Sci. 2019;19(5):427–33.
51. De Corato U, Salimbeni R, De Pretis A, Avella N, Patruno G. Antifungal activity of crude extracts from brown and red seaweeds by a supercritical carbon dioxide technique against fruit postharvest fungal diseases. Postharvest Biol Technol. 2017;131:16–30.
52. Rahman MA, Yusoff FM, Arshad A, Uehara T. Effects of delayed metamorphosis on larval survival, metamorphosis, and juvenile performance of four closely related species of tropical sea urchins (*Genus Echinometra*). Sci World J. 2014. https://doi.org/10.1155/2014/918028.

53. Hamouda RA, El-Ansary MS. Potential of plant-parasitic nematode control in banana plants by microalgae as a new approach towards resistance Egypt. J. Biol. Pest Control. 2017; 27(2).
54. Senthil-Nathan S, Chung PG, Murugan K. Effect of biopesticides applied separately or together on nutritional indices of the rice leaffolder *Cnaphalocrocis medinalis*. Phytoparasitica. 2005;33:187–95.
55. Senthil-Nathan S, Hisham A, Jayakumar G. Larvicidal and growth inhibition of the malaria vector *Anopheles stephensi* by triterpenes from *Dysoxylum malabaricum* and *Dysoxylum beddomei*. Fitoterapia. 2008;79(2):106–11.
56. Ngala BM, Valdes Y, Dos Santos G, Perry RN, Wesemael WM. Seaweed-based products from *Ecklonia maxima* and *Ascophyllum nodosum* as control agents for the root-knot nematodes *Meloidogyne chitwoodi* and *Meloidogyne hapla* on tomato plants. J Appl Phycol. 2016;28:2073–82.
57. Abdel-Rahman HR, Al-Mozini RN. Antifeedant and toxic activity of some plant extracts against larvae of cotton leafworm *Spodoptera littoralis* (*Lepidoptera*: *Noctuidae*). Pak J Biol Sci. 2007;10(24):4467–72.
58. Pan L, Ren L, Chen F, Feng Y, Luo Y. Antifeedant activity of *Ginkgo biloba* secondary metabolites against *Hyphantria cunea* larvae: mechanisms and applications. PLoS ONE. 2016;11(5): e0155682.
59. Jaulneau V, Lafitte C, Corio-Costet MF, Stadnik MJ, Salamagne S, Briand X, Esquerré-Tugayé MT, Dumas B. An *Ulva armoricana* extract protects plants against three powdery mildew pathogens. Eur J Plant Pathol. 2011;131:393–401.
60. Vimaladevi S, Mahesh A, Dhayanithi BN, Karthikeyan N. Mosquito larvicidal efficacy of phenolic acids of seaweed *Chaetomorpha antennina* (Bory) Kuetz. against *Aedes aegypti*. Biologia. 2012;67:212–6.
61. García-Pastor ME, Giménez MJ, Zapata PJ, Guillén F, Valverde JM, Serrano M, Valero D. Preharvest application of methyl salicylate, acetyl salicylic acid and salicylic acid alleviated disease caused by Botrytis cinerea through stimulation of antioxidant system in table grapes. Int J Food Microbiol. 2020;2(334): 108807.
62. Formenti L, Rasmann S. Mycorrhizal fungi enhance resistance to herbivores in tomato plants with reduced jasmonic acid production. Agronomy. 2019;9(3):131.
63. Wallis CM, Galarneau ER. Phenolic compound induction in plant-microbe and plant-insect interactions: a meta-analysis. Front Plant Sci. 2020;15(11): 580753.
64. Sharma G, Malthankar PA, Mathur V. Insect–plant interactions: a multilayered relationship. Ann Entomol Soc. 2021;114(1):1–6.
65. Appu M, Ramalingam P, Sathiyanarayanan A, Huang J. An overview of plant defense-related enzymes responses to biotic stresses. Plant Gene. 2021;1(27): 100302.
66. Mandal S, Das RK, Mishra S. Differential occurrence of oxidative burst and antioxidative mechanism in compatible and incompatible interactions of *Solanum lycopersicum* and *Ralstonia solanacearum*. Plant Physio Biochem. 2011;49(2):117–23.
67. Sun HX, Tang WC, Chen H, Chen W, Zhang M, Liu X, Zhang GR. Food utilization and growth of cutworm *Spodoptera litura* Fabricius larvae exposed to nickel, and its effect on reproductive potential. Chemosphere. 2013;93(10):2319–26.
68. Asimakis E, Shehata AA, Eisenreich W, Acheuk F, Lasram S, Basiouni S, Emekci M, Ntougias S, Taner G, May-Simera H, Yilmaz M. Algae and their metabolites as potential bio-pesticides. Microorganisms. 2022;10(2):307.
69. Boughton AJ, Hoover K, Felton GW. Impact of chemical elicitor applications on greenhouse tomato plants and population growth of the green peach aphid. Myzus persicae Entomol Exp Appl. 2006;120(3):175–88.
70. Imran M, Abo-Elyousr KA, Mousa MA, Saad MM. Use of Trichoderma culture filtrates as a sustainable approach to mitigate early blight disease of tomato and their influence on plant biomarkers and antioxidants production. Front Plant Sci. 2023. https://doi.org/10.3389/fpls.2023.1192818.
71. Stout MJ. Types and mechanisms of rapidly induced plant resistance to herbivorous arthropods. In: Walters DR, Newton AC, Lyon GD, editors. Induced resistance for plant defense. Singapore: Blackwell Publishing Ltd; 2014. p. 81–105.

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