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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Research Article

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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 defence signalling pathways of the plant system. The current study looks at the defense-eliciting potentials of fatty acids from the green seaweed *Chaetomorpha antennina* 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) was successful in eliciting salicylic acid and phenolic compounds biosynthesis along with enzymes peroxidase (PO) and polyphenol oxidase (PPO). The defense enzymes and phenol levels increased post infestation with *S. litura*. CFA was also effective in causing direct mortalities to the larvae (II-V instars). *S. litura* larvae exposed to elicited tomato plants were severely affected physiologically and morphologically, displaying visible aberrations as well as morphogenetic defects such as altered larval-pupal duration and biomass. Reproductive performances of adults were also severely affected. Decrease in food utilization, nutritional indices with a corresponding decrease in phosphatase and gut enzymes affirm feeding deterrence of the larvae, which was endorsed by histological analysis of midgut cell disruption of exposed larvae. Detoxification enzyme levels of exposed larvae denote the inability of larval immune system to evade harmfulness of CFA.

Conclusion

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

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 exist 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 in order 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]. Detailed studies on the induction of defensive resistance in plants employing a variety of biological inducers are presented. Plant biostimulants not only affect physiological processes to improve crop productivity, but they also improve nutrient absorption, which optimises fertilizer consumption and utilisation [6].

Algae have been applied as soil fertilizers in coastal areas, and beneficial benefits of applying seaweed extracts as exfoliar application on plants have been documented. Thus, management of diverse plants by the usage of compounds/extracts from several marinealgae have attributed to the betterment of agricultural produces from germination until consumption [7]. Fatty acids, proteins, minerals, polysaccharides, pigments, plant growth hormones, phenols, along with many other bio-active substances are elated from algal biomass through liquid extraction. Fatty acids (FA) account for a significant percent (> 20%) of their total composition [8]. 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 host immune system to anti-infection actions [9].

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 [10]. Elicitor compounds function as indicator molecules, whose detection at the cell-surface as well as consequent transmission activates plant defence genes [11]. As a result, innumerable defense complexes are synthesised, particularly structural proteins that support cell wall and also several enzymes intricate in the inhibition of invading mechanisms opted by the pests, through intricate activation of plant defense pathways (salicylic acid (SA), jasmonic acid (JA), and ethylene [12].

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 [13]. 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. 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 [14].

The green seaweed *Chaetomorpha antennina* (Bory) Kützing is a green alga contains compounds with wide-array of bio-activities that can be used as PBs, microbicides, insecticides, and repellents [15]. It has been stated that the seaweed possess significant concentration of fatty acids. With the plant growth promoting and salinity stress alleviating potentials as well as insecticidal activity of the seaweed already documented, 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.

Materials and methodology

2.1. Collection and extraction of seaweed

Methanol extract of *C. antennina*, collected, processed and used in previous trials was used for this investigation. The extraction yielded 4.02 g of crude extract powder [5].

2.2. 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 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) and the fraction with significant mortality rate was carried forward for further studies, CFA (Chanthini et al., 2021).

2.3. The insect pest – S. litura

S. litura culture was maintained in the laboratory, where it was fed castor leaves (*Ricinus communis*), was used for this study [15].

2.4. 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].

2.5. Mortality bioassay (MB)

MB against larvae, II-V instars (20 /treatment) were performed with CFA (25, 50, 75 and 100 ppm), sprayed on castor leaves, maintained at 27 ± 2°C with 80% RH. Leaves were treated with DMSO-$(CH_3)_2SO$

(0.1%) and 9 ppm cypermethrin (CM) respectively serve as comparison controls. MB was calculated by timely observation [15, 17].

2.6. Experimental setup

For elicitation assay, 3 mL of CFA was dissolved in 1L of sterile distilled water, from which 20 µl was injected into the intermodal region (above fourth leaf) of 45 days old tomato seedlings propagated in greenhouse conditions (1 seedling/pot). Post 48h of injection, the elicitation effect of CFA was assayed by dissection the leaves above the injected area [18].

In plants propagated in similar conditions, seedlings in 5 leaflet phase were infested with pre-weighed 10-II instar *S. litura* larvae per treatment and transferred to respective metal cages immediately. 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; T4 – *S. litura* + 20µl 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.

2.7. Effect of CFA on eliciting plant defense

Salicylic acid (SA) determination in leaves 24 hours post elicitation was done using HPLC unit from Agilent Technologies LC 8A [18]. 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) [19]. The activities of PR proteins, PO and 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. Peroxidase (PO) was estimated by pyrogallol based method of Hammerschmidt et al. [20] and polyphenol oxidase (PPO) by Mayer and Harel [21], observing absorbance at 420 and 490 nm spectrophotometrically. Enzyme levels were expressed in min/gFW.

2.8. Insecticidal effect of CFA elicited tomato plants on S. litura

2.8.1. 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 [22]. 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 [23].

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

2.8.2. 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 comparative consumption (RCR) as well as growth rate (RGR), along with their digestibility (AD) [24]. 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 [25].

2.8.3. Enzyme extracts preparation

The supernatant of treated and control IV instar larva ground in phosphate buffer was used for enzyme assays [26].

2.8.4. Estimation of phosphatase enzyme activities

The estimation of acid (ACP) and alkaline (ALP) phosphatases was estimated by using 0.02Mphosphate buffer substrate (pH 7.2) as substrate and correspondingly measuring abdorbances 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 [27]. 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 [28].

2.8.5. Estimation of gut enzyme activities

Dinitrosalicyclic acid (DNS) based method was used for the estimation of amylase activity by spectrophotometric estimation at 550 nm [29]. 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 [30]. Protease activities were estimated with BSA as substrate and spectrophotometric estimation of reaction mixture at 600 nm [31].

2.8.6. 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 the methods of Habig et al.[32], Pradeepa et al. [33] and Govindappa et al. [34] (Spectrum Tek, ST 2700).

2.8.7. Histological analysis

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

2.8.8. 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 [35].

2.9. Statistical analysis

All experiments were performed in replications of five. Treatment effects on insect bioassay, enzyme activities and PRP estimations were analysed 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 eight fractions with absorbance in wavelength range (420–880 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.

Table 1
Fatty acid compounds identified through GC-MS

	RT	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

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 ppm. The mortality rates of *S. litura* larvae treated with 100 ppm 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 ppm ($F_{5, 24}$ = 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/mg 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 hour after treatment. The phenolic compound levels in CM treated seedlings continued to ascent linearly after 24 hours, reaching a high of 3.67 g GAE/mgFW. The levels then continued to fall, remaining steady for two 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 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). The larvae reared on untreated plants displayed a steady increase in biomass reaching a maximum weight gain of 480.51mg in 28 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 was affected in both the treatments and differed significantly with control (P<0.005). Post 24h 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 72h, 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% 72h 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 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 0.34 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.

Table 2
Effect of elicitor treatments on feeding and nutritional of *S. litura* larvae.. Means (± SE standard error) followed by the same letters above bars indicate no significant difference ($P \leq 0.05$) according to a Tukey test.

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]

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 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).

Table 3
Effect of elicitor treatments on enzyme activities of *S. litura* larvae.. Means (± SE standard error) followed by the same letters above bars indicate no significant difference ($P \leq 0.05$) according to a Tukey test.

	Enzymes (µmol/mg/h^{-1})	C	CM	CFA
Phosphatase activities	ACP	16.16 ± 1.23^{a}	14 ± 1.25^{b}	7.74 ± 1.45^{c}
	ALP	23.28 ± 1.12^{a}	22.6 ± 1.34^{b}	12.37 ± 1.49^{c}
	ATPase	85.01 ± 1.62^{a}	80.19 ± 1.45^{b}	51.4 ± 1.52^{c}
	LDH	27.97 ± 1.64^{a}	26.15 ± 1.62^{b}	16.01 ± 1.74c
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 ± 0.052^{c}
	CarE	0.48 ± 0.045^{c}	0.6 ± 0.019 ^{b}	0.76 ± 0.09 a

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 hours 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 also displayed a minor increase in PO levels till 96 hours, 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 infection 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 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 24h and maintained a hike up to 48 h in control plants and 120h 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 hours in control leaves (Fig. 8B). The algal compounds induced PPO secretion to 31.7 µg/min/g after 120h 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 24h 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 120h. 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 disease incidence and *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 [36]. 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 [37]. 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 analysed in this study.

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. [38]. 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 [40]. Seaweeds have a high fatty acid (FA) variety, and many of them have potential bioactivity [40]. 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 [41].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 [42]. 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 [43]. 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 [44].

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 the SA signalling pathway was induced by the application of algal polysaccharides, laminarin and carrageenans [45]. El Modafar et al. also proved ulvan compounds of *Ulva lactuca* stimulated SA-dependent systemic acquired resistance in tomato seedlings [18].

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 [46]. 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 [40]. 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* [47].

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 [48], *Momordica charantia* secondary metabolites [49] and *Citrullus colocynthis* [50]. 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 [51]. 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 [22]. 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* [49]. 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 recognised 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 effected the development and physiology of rice leaf folder larvae treated with a biopesticidal combination [54] and *Dysoxylum* triterpenes [22].

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 [37]. 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 [55] and azadirachtin [22]. 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 [37]. ATPase reduction was caused due to the cease of metabolism as a consequence of either food indigestibility or absence of food intake [56]. LDH enzyme plays a vital role in carbohydrate metabolism, are also indicators of chemical stress [22]. 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 were observed in neem limonoids treated rice leaf folder larvae [22]and *S. litura* larvae treated with and *C. colocynthis* extracts [50].

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 were 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 [51].

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]. Application of MeSA has stimulated the production of phenolic compounds [60]. Jasmonate application on tomato plants were found to stimulate the production of defensive proteins that negatively influenced herbivores [61].There exists a linear relationship between phenolic compound concentration and antimicrobial potentials [19]. Consequently, the allegation of phenol accumulation in plant defense has also been proved [62].

Plant mediated interactions amongst pathogens besides arthropod herbivores can ensure significant concerns for individual pests as well as their population dynamics. 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 [61].

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* [63]. 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* [64]. 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.

A similar increase in PO and PPO activities were 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 PRPs post infestation in elicited plants were also observed in tomato plants treated with commercial elicitors, commercial elicitors benzothiadiazole and methyl jasmonate post infestation with green peach aphid, *Myzus persicae* [65]. 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 120h, 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 [65]. 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 the probable instigation of defense genes typically connected with pathogen attack [61].

Conclusion

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 *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 in order to turn scientific discoveries on seaweed and microalgae into practical solutions for industry.

Declarations

Ethics approval and consent to participate

Not Applicable

Consent for publication

The authors offer consent for publication.

Availability of data and materials

Data will be available on request

Competing interests

The authors declare no conflict of interest.

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Author contributions

Conceptualization and methodology design was done by KMPC, SSN. Investigation was carried out by KMPC, PKR 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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Figures

Figure 1.

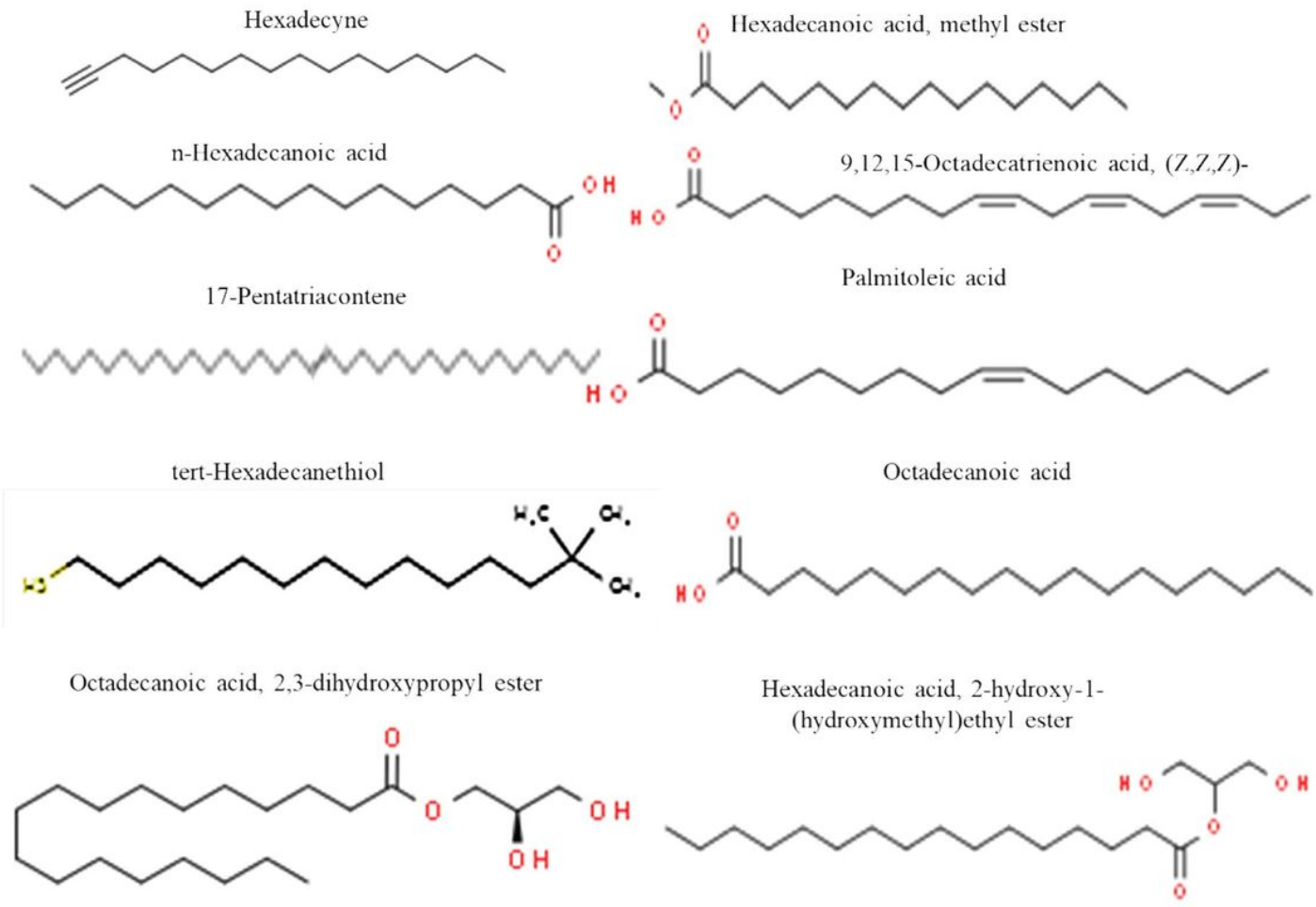

Figure 1

Fatty acid compounds of C. antennina

Figure 2.

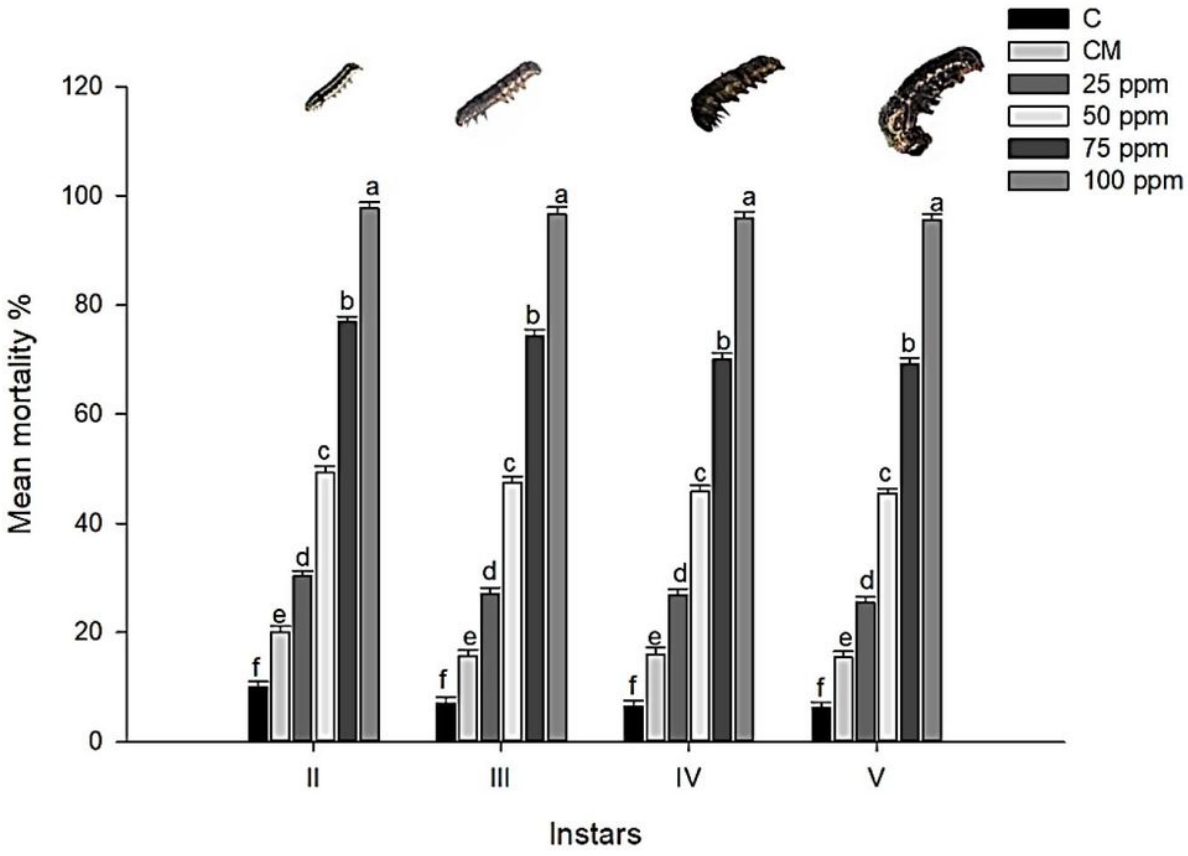

Figure 2

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≤0.05) according to Tukey test.

Figure 3.

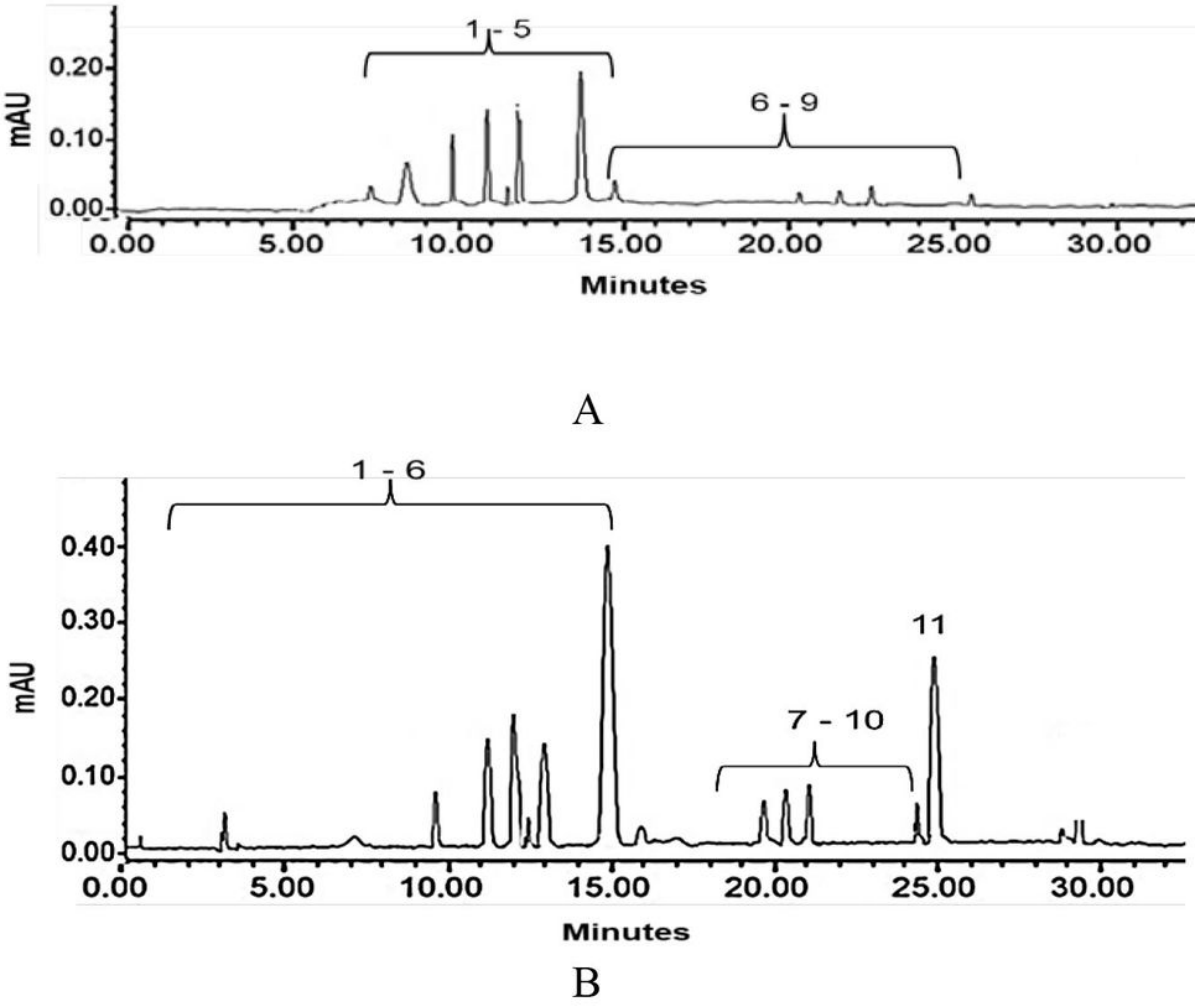

Figure 3

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

Figure 4.

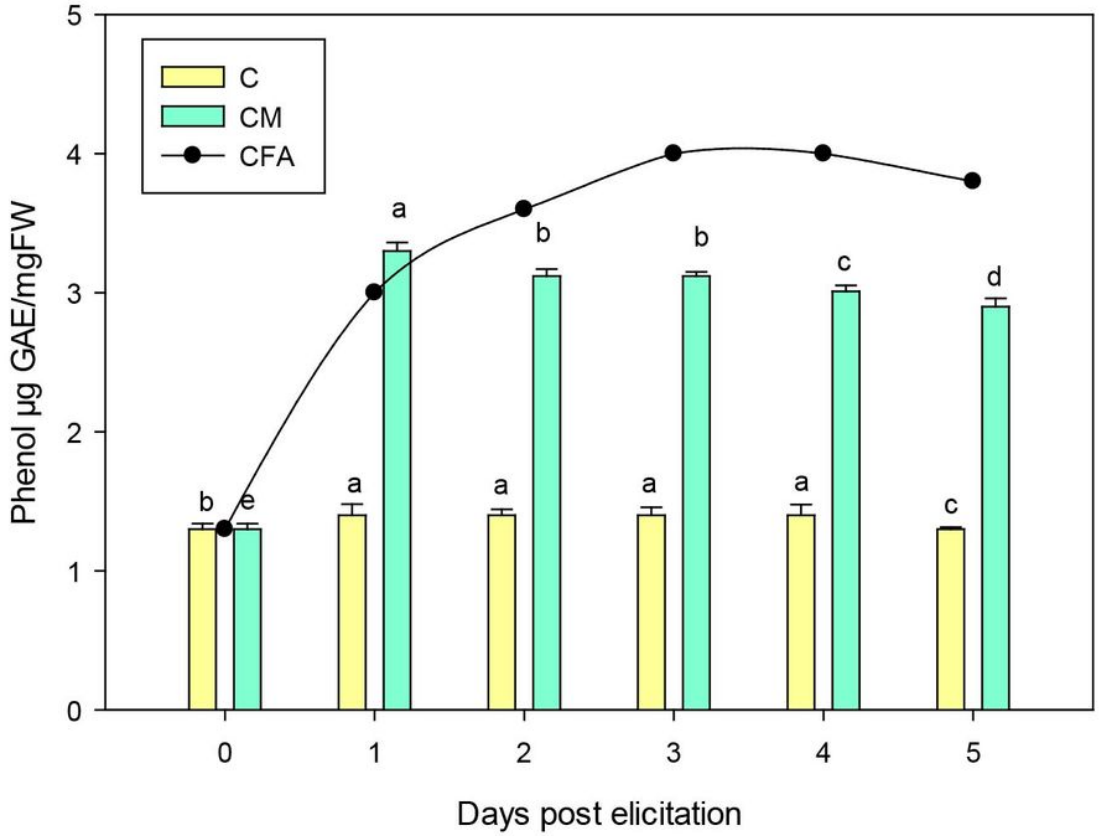

Figure 4

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.

Figure 5.

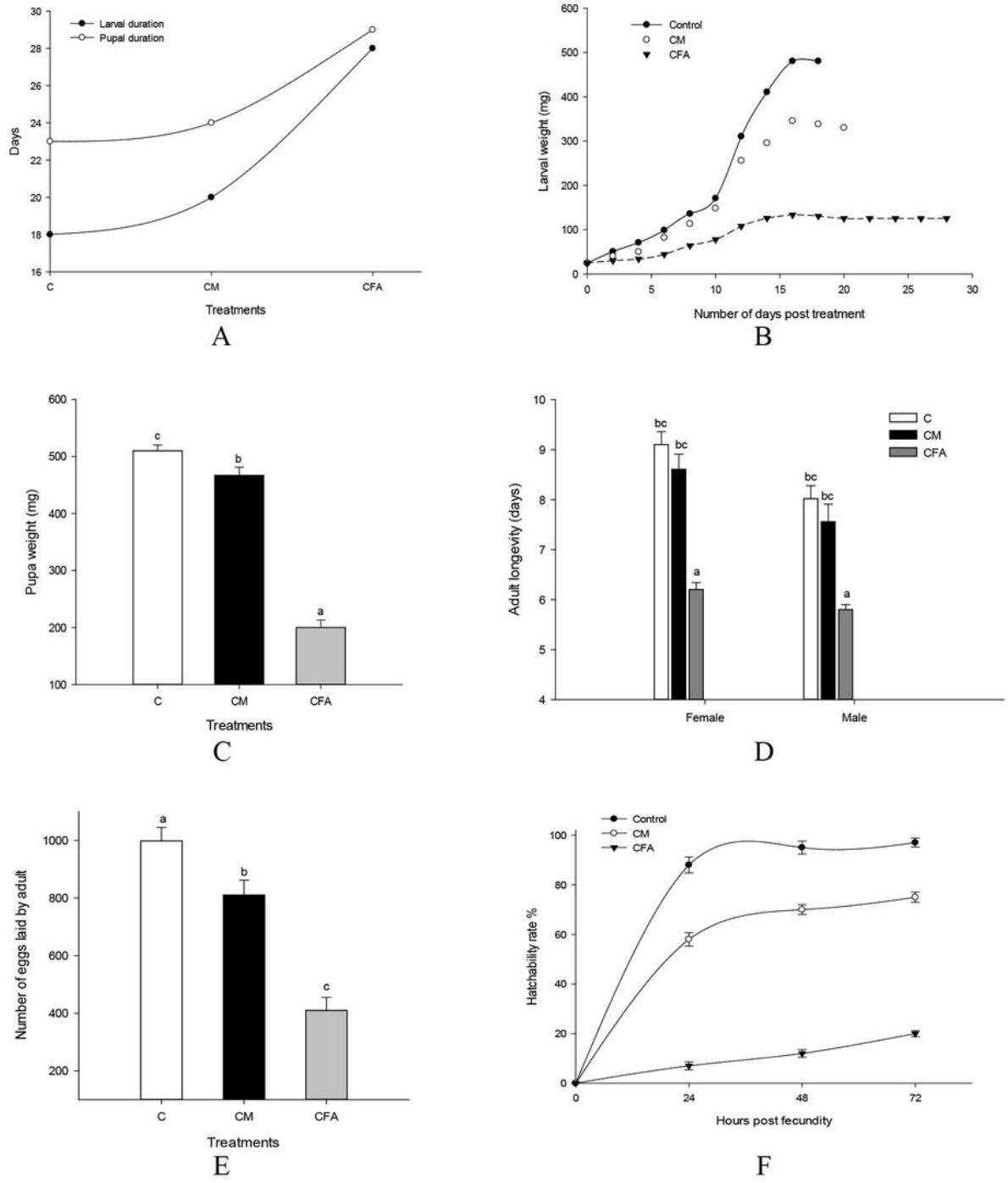

Figure 5

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 (%)

Figure 6.

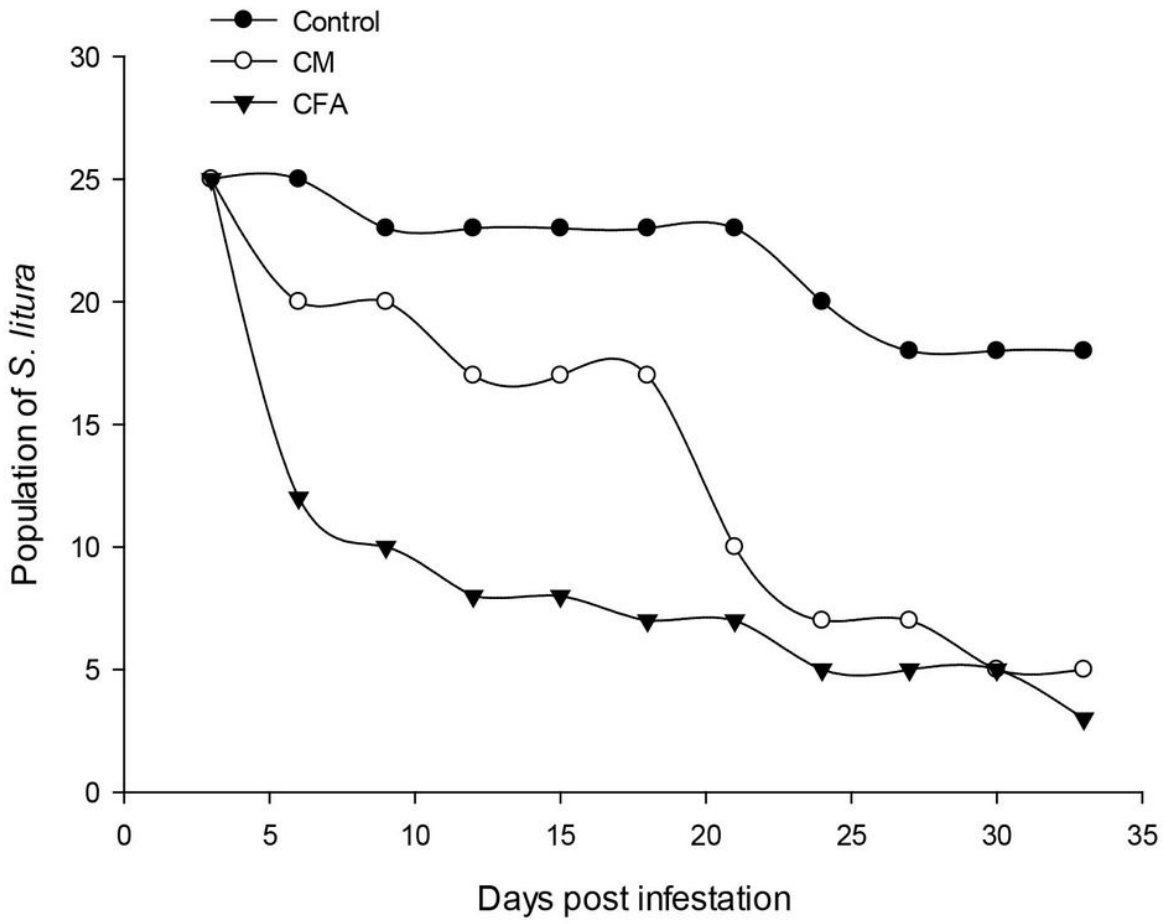

Figure 6

Effect of elicitor treatments on population of S. litura

Figure 7.

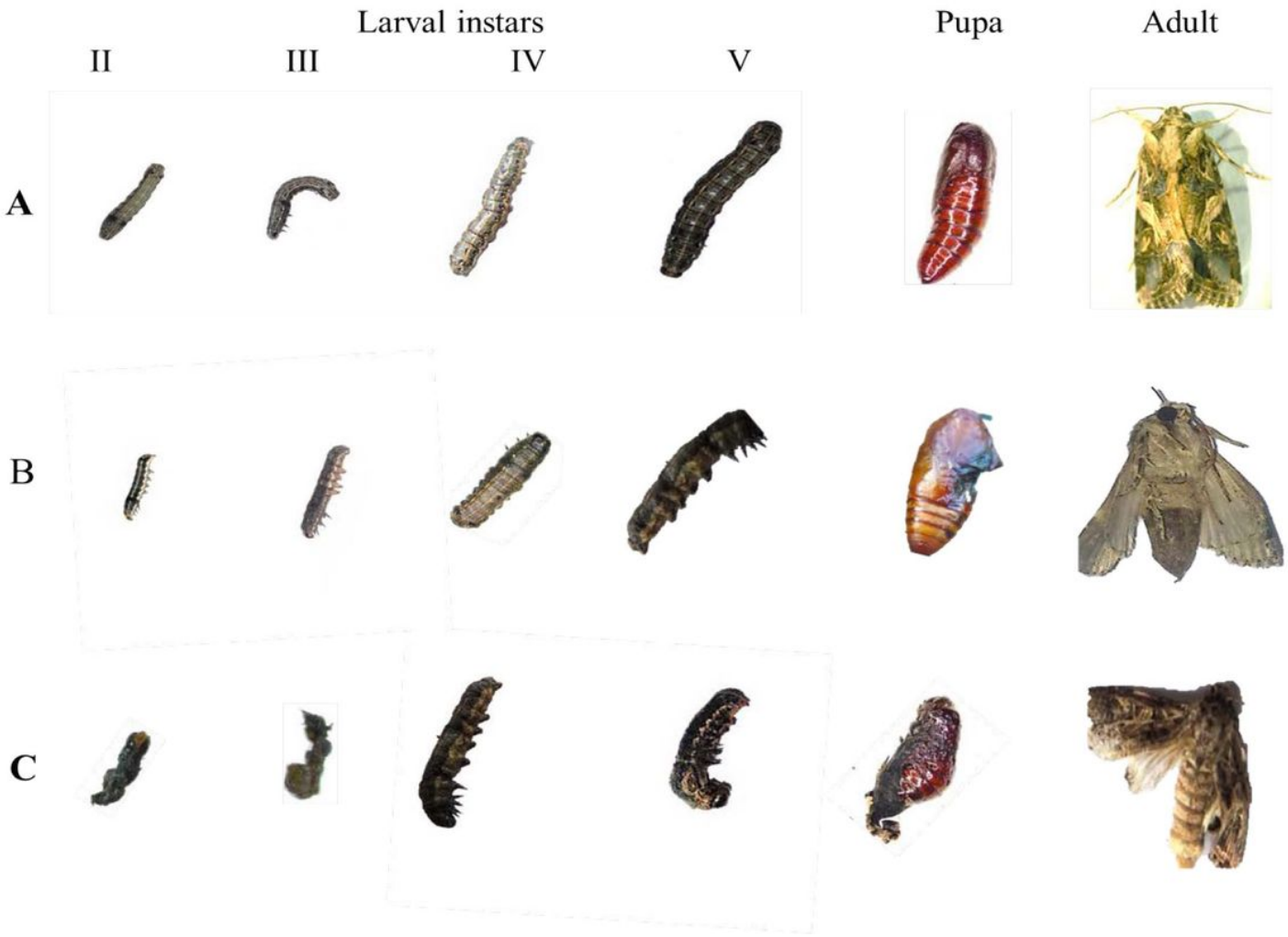

Figure 7

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

Figure 8.

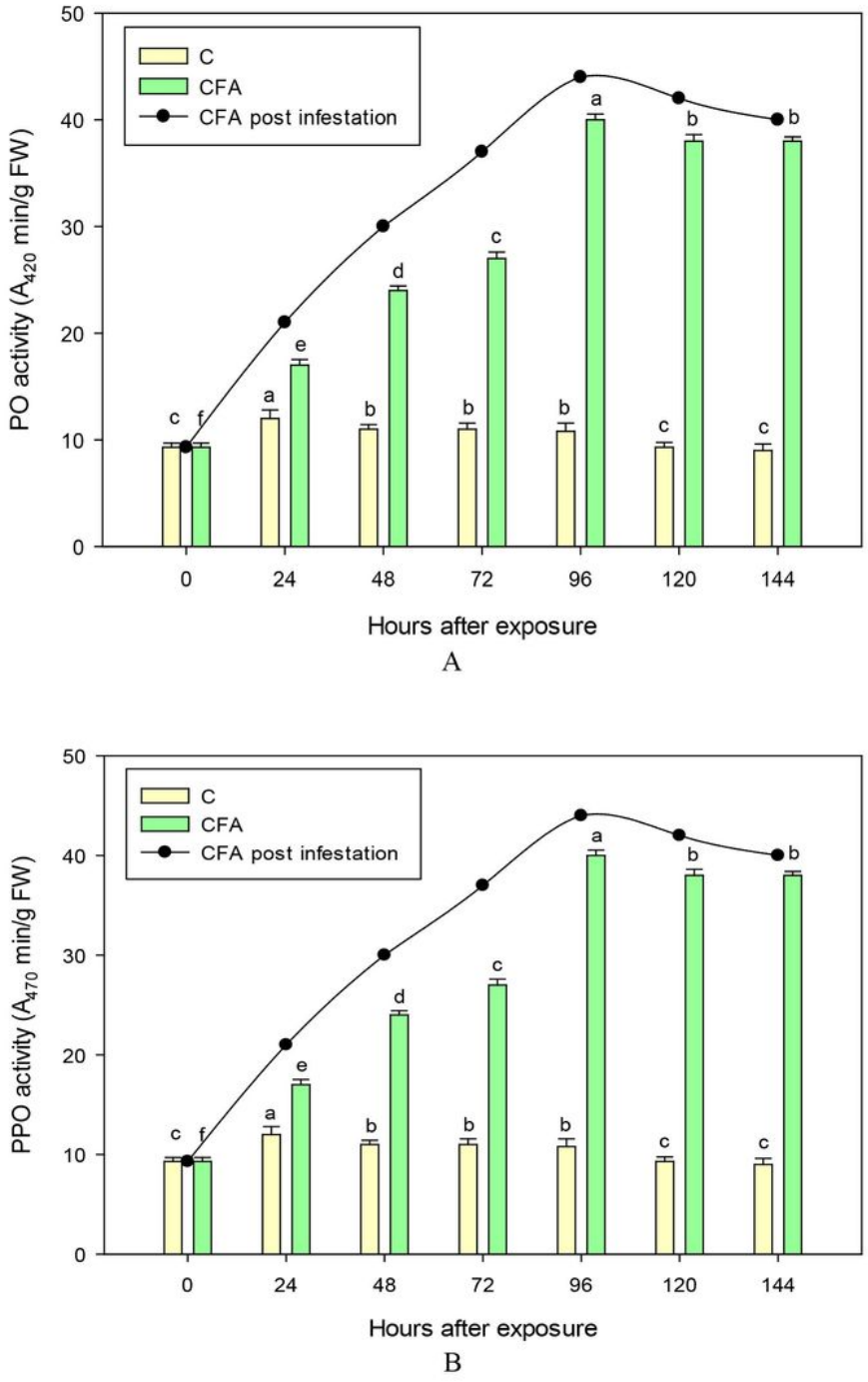

Figure 8

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

Figure 9.

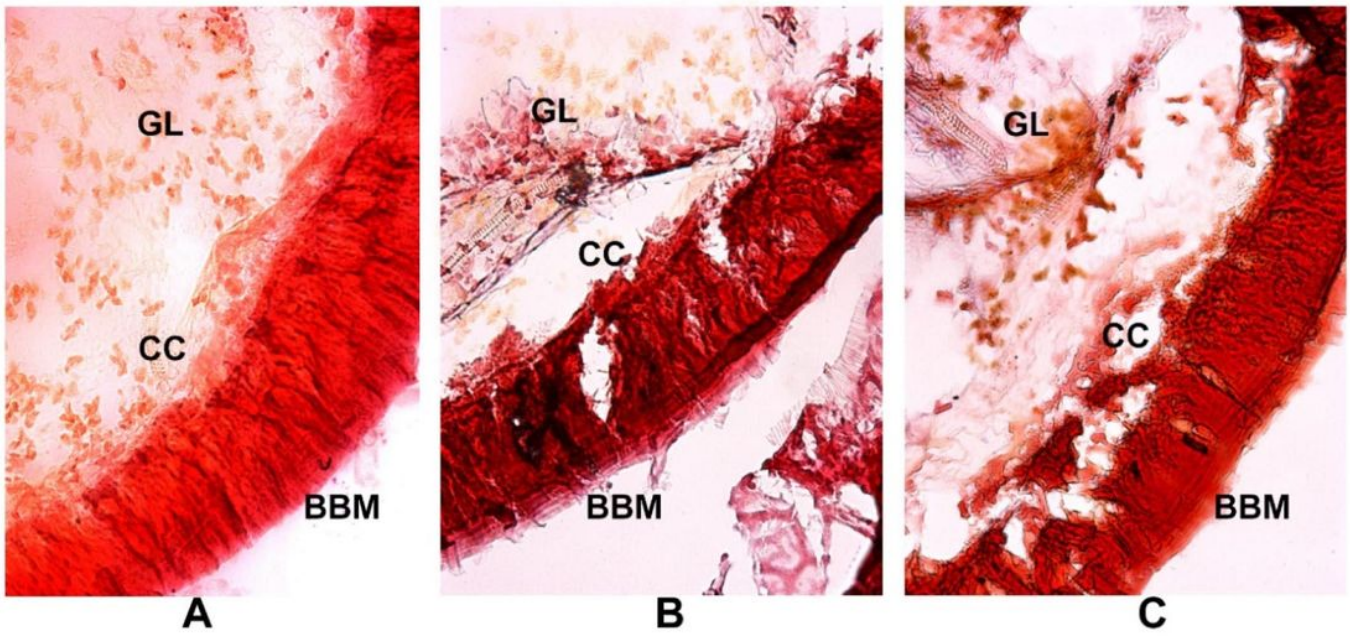

Figure 9

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

Supplementary Files

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- GraphicalAbstract.png