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Biologically active toxin from macroalgae *Chaetomorpha antennina* Bory, against the lepidopteran *Spodoptera litura* Fab. and evaluation of toxicity to earthworm, *Eudrilus eugeniae* Kinb

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Abstract

Background: Seaweeds harbour a wide array of bioactive compounds shown to be effective in support of sustainable agricultural practices. The green seaweed *Chaetomorpha antennina* found in abundance in coastal areas of India has been reported with various bioactivities. Owing to the requirement of alternative and economical natural pest control method to be applied in sustainable agronomic strategies, the current study attempts to evaluate the efficacy of chemical toxins from *C. antennina*, as insecticidal agents, by inspecting their effects on the physiology, biochemistry, immune system, and histology of one of the most important insect pests of agricultural crops in the Asian tropics, the polyphagous lepidopteran *Spodoptera litura*.

Results: The active fraction 5 isolated from *C. antennina* using methanol extraction produced significant mortality rates of *S. litura* among all the other fractions obtained. GC–MS analysis revealed the presence of various pesticide compounds. The toxin compounds (active fraction 5) were found to negatively influence the pest's immune system performance at sub-lethal concentrations (LC₅₀ 38.73 and LC₉₀ 53.60 ppm), affecting insect development, reducing the haemocyte count (69.24%) and reduced the activity of major defence enzyme phenoloxidase decreased post-treatments. Digestive phosphatase enzymes, acid phosphatase, ACP, alkaline phosphatase, ALP, and ATPase were demodulated by 37.5, 39, and 23.9% compared with untreated. Increase in detoxification enzymes coupled with mid-gut collapse are indicative of the toxicity of the compounds. Earthworms exposed to seaweed compounds displayed no debarring effects.

Conclusion: Extracted seaweed compounds produced significant lethal effect on the insect larvae, affecting the immune as well as digestive systems of the pest. However, no such toxicity was observed in earthworms treated with the seaweed fraction supporting their environmentally benign nature. Since the insect immune system is responsible

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for the development of resurgence against pesticides, suppression of immunological activities by seaweed toxins indicate the long-term applicability of these compounds as prospective pesticides. The results support the potential of chemicals from *C. antennina* for biopesticide development to manage economically important agricultural pests.

Keywords: Algae, Bioactive, Metabolites, Seaweed, Toxicity, Insecticide, Herbivore

Background

Marine ecosystem organisms harbour a wide array of bioactive compounds. Among these are a group of macroalgae referred to collectively as seaweeds, which are a collection of marine plants, existing along the world's coastlines. The marine algae are a rich source of a wide variety of bioactive compounds, that have been developed into biofuels, medicines, fertilizers, plant growth stimulants, and biopesticides to name a few [1–5] and are considered an under-utilized ocean resource. Additionally, several seaweed compounds have been shown to induce toxicity and alter the growth, behaviour, development, and metabolism of several insect pests [2, 6–9].

Among the important benefits from agricultural industries is the capacity to provide sustainable production of food, feeds, and fibres to meet the needs of an increasing global population [2, 10–13]. Changes in climate will also alter crop losses caused by environmental factors, pests, and pathogens [13, 14]. The need for crop treatments, fertilizers, herbicides, insecticides, and fungicides are predicted to intensify on agricultural land throughout the world, resulting in growing research on alternative treatments [12, 14–16]. Alternative strategies for control of pests and pathogens may also reduce the rapid development of insecticide resistance to overused, or improperly used chemical insecticides [17]. In addition to offering an environmentally benign and safe means of pest control, biopesticides could also surpass the development of pest resurgence as well as propose a pronounced aptitude in regulating yield loss deprived of conceding the quality of the produce [18].

Increased use of Integrated Pest Management, IPM, strategies also benefit with incorporation of emerging molecular treatments that have greater safety for beneficial insects and humans over heavy reliance on chemical pesticides and herbicides [19]. Some of these technologies include: exogenous applied or plant expressed RNA interference [20–22], along with antisense oligonucleotides [23, 24] and other gene editing technologies like CRISPR [25–28] all of which can greatly support IPM strategies. Increased use of several sustainable strategies will produce more effective, eco-friendly agronomic strategies, while confronting escalating issues of food insecurity [11].

Tobacco cut worm, *Spodoptera litura* Fab. is among the major polyphagous pests, that severely limits production

[29]. A major problem is that insect pest around the world have developed resistance to a large number of insecticides [30]. Insects have a well-developed immune system encompassing humoral and cellular defences. The insect immune system is mainly governed by the activities of phenoloxidase enzyme as well as haemocytes that are acute against invaders and toxins [31]. Any compound that can interfere with these defences will severely impair the insect biological fitness. Insect detoxification enzymes also play a key role in the development of resistance against insecticides. A reduction in these enzyme levels will result in a reduction of insecticide resistance levels [32]. The insect gut enzymes, acid and alkaline phosphatases, along with ATPase play a crucial role in the hydroxylation of phosphor monoesters. Also, these enzymes in conjunction with the gut neurons moderate the organic molecule [33, 34]. Any oscillations in these enzyme actions can upset the gut physiology, resulting in a significant decrease of the insect biological fitness [35].

Finding novel, eco-friendly, effective insecticides is essential to agriculture, leading to the global bio-prospecting for natural chemical alternatives from unique habitats and organisms, such as in seaweeds (macroalgae) [2, 5, 7, 8]. The green seaweed *Chaetomorpha antennina* (Bory) Kützinger, is a green alga in the Family: Chlorophyceae [36] that have chemicals with bioactivities that can be applied to agricultural problems as biostimulants, bactericides, viricides, fungicides, insecticides [1, 2, 4, 33, 37–39], and repellents. To evaluate the efficacy of the chemical toxins from *C. antennina*, as insecticidal agents, we examined the effects on the haematology, biochemistry, and physiology of the lepidopteran pest, *S. litura*.

Materials and methods

Insect culture

Spodoptera litura larvae were obtained from stock culture reared on castor leaves in cages (Biopesticide and Environmental Toxicology Laboratory, SPKCEES, Manonmaniam Sundaranar University, Tamil Nadu; 28 ± 2°C 85% RH, 12:12 LD).

Seaweed collection, identification, and extract preparation

The seaweed, *C. antennina*, were collected from the rocks of the coastal regions of Colachel beach, Kanyakumari (8°14' 5168" N and 77° 14' 35.209" E) during low tide in August (2018) and processed, labelled, and transported

to the lab in sealed bags. The seaweed was washed thoroughly in tap water 5 times, 10 min each prior to microscopic analysis (Nikon H600L, Japan; 40×). Their organoleptic features were also recorded. The algae were then spread on blotting paper to remove excess water; shade dried (6–7 days). The completely dried sample was pulverised and extracted with methanol (Soxhlet apparatus) for 24 h (1:20 w/v), concentrated in rotary vacuum evaporator (13.36% yield extract powder), and stored at 4 °C. Preliminary mortality feeding bioassays on early fifth-instar *S. litura* larvae were performed with the crude methanol extract (CA-M), which was dried and resuspended in aqueous DMSO, (1 mg/ml. Sigma-Aldrich, Inc.), with chromatographic analyses on the methanol extracts.

Active fraction isolation

Active compounds from CA-M were isolated as fractions using column chromatography; pore size 60–120, hexane:ethyl acetate gradient (90:10, 80:20, 70:30, 60.5:30.5, and 60:40), desiccated then stored (4°C). Each gradient was resuspended in aqueous 0.1% DMSO for use in feeding bioassays to determine mortality as mentioned.

GCMS characterisation of *C. antennina* fraction F5 (CAF-5)

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).

Bioassays

Mortality bioassay

Larvicidal assays against *S. litura* larvae (second, third, fourth, and fifth instars; 20 larvae per treatment—25, 50, 75, and 100 ppm of active fraction prepared in 0.1% DMSO) were performed with active fraction 5, using a leaf-dip assay. Castor leaves were dipped in 10 ml of active fraction 5 at various concentrations for 20 s and air dried before being placed within sealed petri dishes for each individual dilution [32]. The larvae were introduced to the petri dishes and observed for mortality. Leaves treated with 0.1% DMSO served as the control. Mortality was recorded till 48 h and percentage mortality was calculated using Abbott's formula [40]. The mean lethal concentration was calculated using Probit's analysis and carried forward for further treatments

[41]. Cypermethrin (9 ppm) was prepared by dissolving 0.009 mg of cypermethrin (Rallis India Limited) in 100 ml of distilled water (CM).

Haemolymph collection

Ten *S. litura* larvae in the early fifth-instar (25 days after hatching) stage were chosen for the study. The larvae were weighed and haemolymph (10 µl for THC and 8 µl for PO enzyme assay) was drawn by piercing the last proleg (fine needle of a Hamilton syringe-10 µl SGE), 24 h post-exposure to sub-lethal concentration of the active fraction by the leaf-dip assay procedure mentioned above in mortality assay [32]. As a lethal concentration would kill the larvae, sub-lethal concentration (LC₅₀) was used to study the inhibition effect of active fraction 5 on the insect immune system.

Haematological parameter assay

Total haemocyte count (THC) was determined by preparing the haemolymph as described [42]. The drawn haemolymph was immediately added to anticoagulant (5 µl of EDTA + 5 µL of glycerol) which was drawn using a WBC pipette (till 0.5 mark), diluted with Tauber–Yeager fluid (to reach till 11 mark), and shaken well. Post-discard of first three drops, a drop of diluted haemolymph was placed on the Neubauer haemocytometer and counted (4 and 1 central, 1 mm² squares), under the microscope (40 ×, Nikon Eclipse DS-RI2Y-TV55, Japan). THC/mm³ was determined using the formula

$$\text{THC/mm}^3 = \frac{X \times \text{dilution factor} \times \text{depth factor}}{\text{Number of squares counted}}$$

(X—total number of cells counted; dilution factor—20; Depth factor—10).

Phenoloxidase activity (PO) assay

PO was estimated, and expressed as PO units by following the method of Karthi et al. [32]. The drawn haemolymph (8 µl) was added to phosphate buffer saline (PBS, 400 µl ice-cold—pH 7.4) and vortexed; from which 100 µl was added to equal volume of L–Dopa solution (20 mM) and incubated (25 °C/30 min), following which absorbance was read at 475 nm in a spectrophotometer (Spectrum Tek, ST2700,).

Enzymatic profile

Preparation of enzyme extract

The larvae (early fifth instar) treated with active fraction 5 (25 ppm) was used for enzyme assays (24 h post-treatment). The treated larvae were anaesthetized, and then, their digestive system was dissected out followed by treatment with ice-cold insect Ringer's solution. The

guts were individually weighed and homogenised (300 μ l of ice-cold citrate–phosphate buffer, pH 6.8; 3 min, at 4 °C). The homogenate was resuspended in the same buffer to produce 1 ml volume. The samples were centrifuged (500 \times g for 1 min). The supernatant was used as the enzyme source [42].

Estimation of enzymes

Digestive enzymes such as ACP and ALP, and ATPase were estimated [43]. The levels of catalase, CAT [44], superoxide dismutase, SOD [45], and peroxidase, POX were determined [46–48].

Histology

Histological effects of active fraction on the mid-gut tissues of *S. litura* (fifth instar) treated with 25 ppm active fraction 5 were studied by comparing with untreated control. The mid-gut was dissected and fixed (Bouin's Solution; O/N; Sigma-Aldrich). Fixed tissues were dehydrated in ascending grades of alcohol, viz., 50, 60, 70, 80, 90, and 100%, every 2 h, and then transitioned into paraffin. Then were placed in a warming oven to prepare blocks. Sections were produced using a microtome (Cryostat Microtome AB-92-02, India). Sections were dewaxed by dipping into 100% Xylene, 5 min. The blocks were stained (Delafield's haematoxylin) and counterstained (Eosin), and dried. The sections were observed and photographed under phase contrast microscope (Nikon eclipse SMZ1270, Japan).

Earthworm toxicity: contact filter paper test

The effect of active compounds, active fraction 5, on non-target organism, earthworm, *Eudrilus eugeniae* Kinb was performed using the Contact Filter Paper Test [40]. The earthworms were maintained in culture in the laboratory (28.9 \pm 0.36 °C, Food source: crop residues amended with cattle dung) which were used for bioassay. The test was carried out by moistening the filter papers (cut to 9 cm disc, suitable for petri plate) with 50 ppm active fraction 5 along with 9 ppm cypermethrin and incubated (dark at 20 \pm 1 °C for 48 h). The experiment was replicated five times and the percentage of mortality was calculated using Abbott's formula [40].

Statistical analysis

Each experiment was replicated five times. The mortality rates obtained were expressed as the mean of five replicates and data were normalised by applying arcsine-square root transformation. The lethal concentrations (LC₅₀ and LC₉₀) were calculated using Probit analysis. Analysis of variance (ANOVA) was used to identify the

difference in percentage of mortality and was fitted with linear regression using Minitab®17 for lethal concentration. Tukey–Kramer HSD test was used to determine the differences between the treatments ($P \leq 0.05$). Sigma plot 11 was used for graphs.

Results

Seaweed identification

Seaweed species were confirmed based on visible taxonomic key features, micro and macroscopically [Guiry W. In Algae Base. Accessed August 2020]. Organoleptic features such as fishy odour, light green colour, salty taste, holdfast base, and smooth texture were also characteristic traits of *C. antennina*.

GC–MS analysis of active fraction *C. antennina* fraction-5

Among the 11 compounds in *C. antennina* fraction-5, methyl esters of chloroacetic acid were abundant such as dichloroacetaldehyde, methyl chloroacetaldehyde, and methylene Chloride (Table 1). The presence of carboxylic acid derivative, Cyclohexan-1, 4, 5-triol-3, 1, 1-carboxylic acid was also observed. Compounds such as cis-2-Chlorovinylacetate, trichloromethane, and chloro-methanesulfonyl chloride were also present. A heterocyclic aromatic organic compound (2, 6-lutidine 3, 5-dichloro-4-dodecylthion), cyclo-alkane (Cyclobutanol), and a chloro-ethyl-ester of butyric acid were also present in the active fraction (Table 1).

Mortality bioassay of active fraction 5 against *S. litura*

Active fraction 5 exhibited pronounced larvicidal activity against third, fourth, and fifth-instar larvae. Although the mortality rate increased with treatment concentrations, they decreased with increasing age of instars. The greatest mortality was observed in third-instar larvae, 96.7% at 100 ppm treatment concentration of active fraction 5 ($F_{5,24} = 49.63$; $P < 0.0001$). The fourth instar being comparatively less susceptible to the treatment concentrations 50, 75, and 100 ppm exhibiting 45.74, 70.06, and 95.9% mortality rates ($F_{5,24} = 41.88$; $P < 0.0001$), with that of fifth instar that resulted only in 45.4, 69.29, and 95.6% mortality rates' treatment concentrations 50, 75, and 100 ppm ($F_{5,24} = 33.96$; $P < 0.0001$) (Fig. 1). The lethal concentration, LC₅₀ and LC₉₀ for third-instar larvae was observed at 24.76 and 77.02 ppm respectively. The respective LC₅₀ and LC₉₀ values for fourth- and fifth-instar larvae were detected at 38.73, 127.35 ppm and at 53.60 and 158 ppm (Fig. 2). At the sub-lethal concentration, 158 ppm, active fraction 5 substantially affected the development of early *S. litura* larvae by producing uncharacteristic or striped pupae and adults (Fig. 3).

Table 1 Compounds present in active fraction identified through GCMS

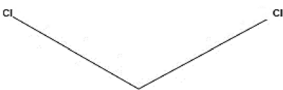
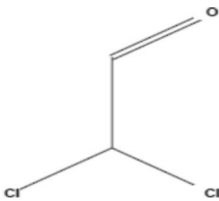

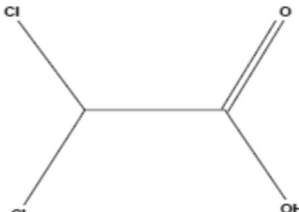
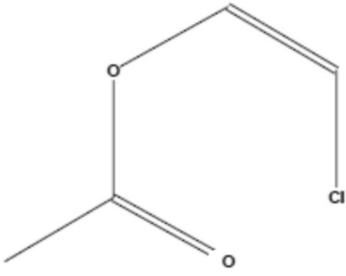
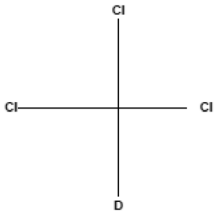
RT	Compound	MW	Chemical formula and structure
1	Methylene chloride	84	CH_2Cl_2 
2	Dichloroacetaldehyde	112	$\text{C}_2\text{H}_2\text{OCl}_2$ 
3	Methyl chloroacetaldehyde	108	$\text{C}_3\text{H}_5\text{O}_2\text{Cl}$ 
4	Dichloroacetic acid	128	$\text{C}_2\text{H}_2\text{O}_2\text{Cl}_2$ 
5	Cis-2-Chlorovinylacetate	120	$\text{C}_4\text{H}_5\text{O}_2\text{Cl}$ 
6	Trichloromethane	119	CCl_3D 

Table 1 (continued)

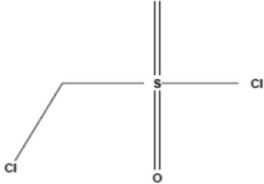
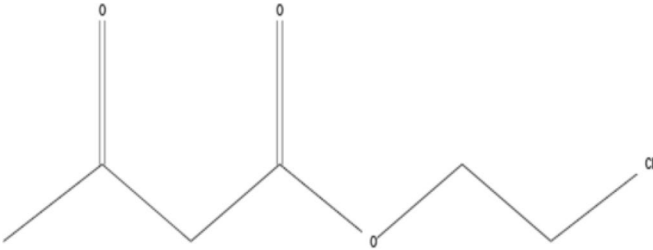
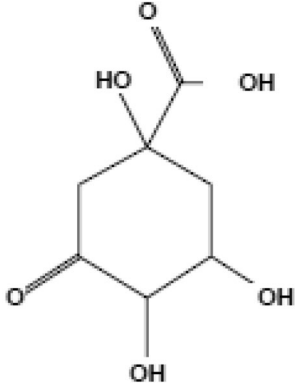
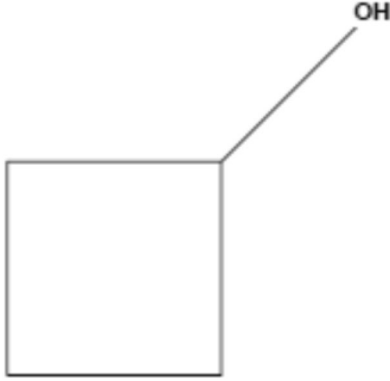
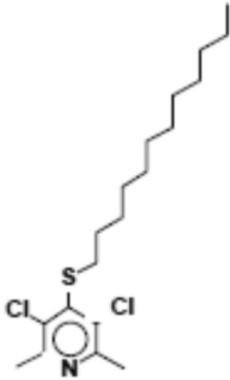
RT	Compound	MW	Chemical formula and structure
7	Chloro-methanesulfonyl chloride	148	$\text{CH}_2\text{O}_2\text{Cl}_2\text{S}$ 
8	Butanoic acid, 2-chloro-3-oxo-, ethyl ester	164	$\text{C}_6\text{H}_9\text{O}_3\text{Cl}$ 
9	Cyclohexan-1,4,5-triol-3,1,1-carboxylic acid	190	$\text{C}_7\text{H}_{10}\text{O}_6$ 
10	Cyclobutanol	72	$\text{C}_4\text{H}_8\text{O}$ 

Table 1 (continued)

RT	Compound	MW	Chemical formula and structure
11	2,6-Lutidine 3,5-Dichlo-4-Dodecylthion	375	C ₁₉ H ₃₁ NCl ₂ S 

Effects of sub-lethal concentration of active fraction 5 and cypermethrin, CM on early 5th instar larvae
Changes in haematology

THC The natural change in THC in controls across the four time points: 24 h, 48 h, 72 h, and 96 h post-treatment showed lower levels at 24 and 48 h, with an increase of THC at 72 h, with a slight decrease at 96 h (Fig. 4).

However, the larvae treated with active fraction 5 showed no significant change in concentration of THC

over time (24 to 96 h), but THC concentrations were significantly lower than those in control.

CM (cypermethrin)-treated larvae showed no significant difference from the active fraction 5-treated larvae at 24 and 48 h, in THC, but produced significantly lower concentrations at 72 h (21.4%) and at 96 h post-treatment (17.6%) ($F_{2,12}=6.86$; $P \leq 0.01$). The reduced THC of CM-treated larvae remained low at both the 72 and 96 h time points and were not significantly different ($F_{2,12}=6.86$; $P \leq 0.816$). Both treatments active fraction 5 and CM,

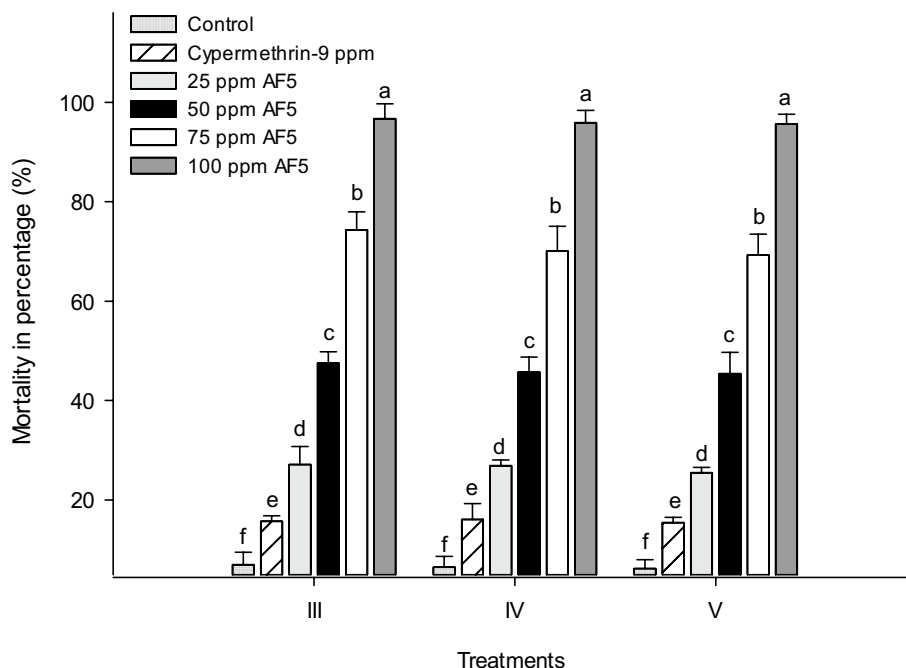


Fig. 1 Effect of active fraction 5 (AF5) on third-, fourth-, and fifth-instar *S. litura* larvae. Mean (\pm SEM) followed by the same letter in individual larval instar in bars indicates no significant difference ($P < 0.05$) in a Tukey's test

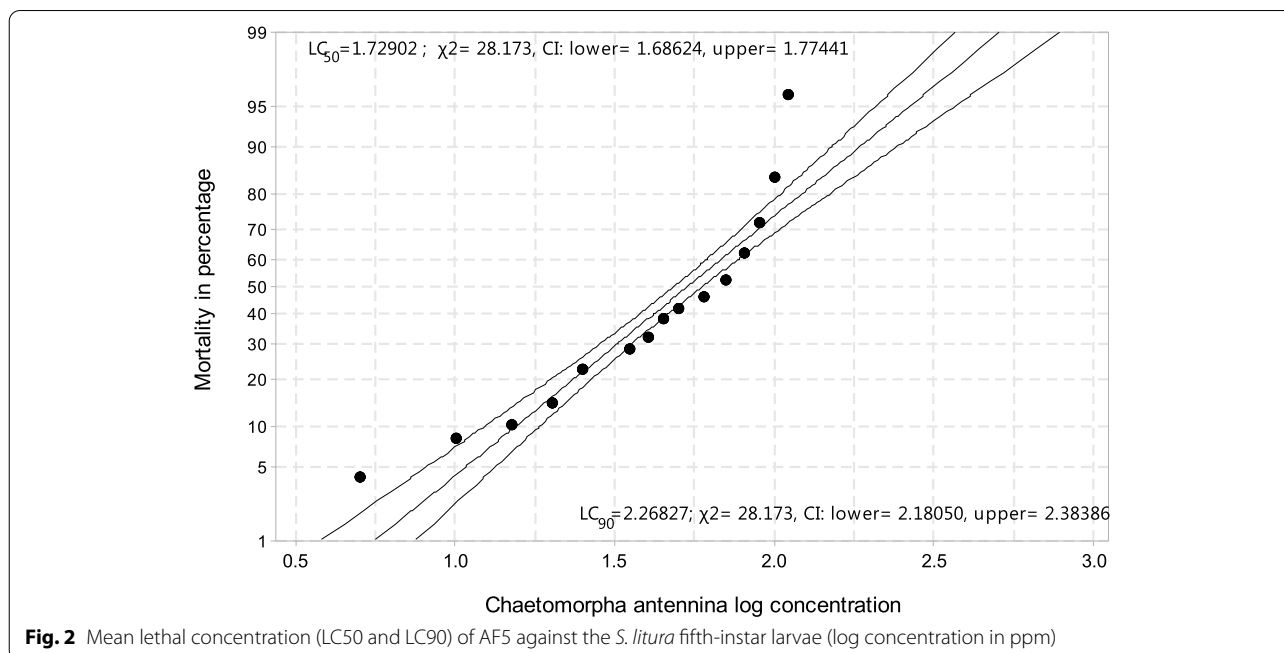


Fig. 2 Mean lethal concentration (LC50 and LC90) of AF5 against the *S. litura* fifth-instar larvae (log concentration in ppm)

resulted in significantly lower THC concentrations across time points, with greater reduction of THC at 72 h and 96 h, being 60.15 and 60.54% ($F_{2,12}=133.6; P \leq 0.0001$) compared with control. The greatest reduction was produced in CM-treated larvae at 72 and 96 h; 75.709 and 69.24% ($P > 0.05$) in CM-treated larvae, respectively (Fig. 4).

Phenoloxidase activity (PO)

The PO activity was significantly reduced by CM treatment, 0.62 from 1.27 PO units ($F_{2,12}=6.37; P \leq 0.001$). While, active fraction 5 decreased PO activity by 25% compared to control ($F_{2,12}=6.37; P \leq 0.209$) (Fig. 5A).

Catalase activity (CAT)

There was an increase in CAT activity from 5.04 to 6.18 μmoles in Active fraction 5 treated and to 6.85 μmoles of H₂O₂ decomposed/min/mg protein, being significantly increased in CM-treated larvae over control ($F_{2,12}=26.63; P \leq 0.001$) (Fig. 5B).

SOD activity

There was a significant increase in the SOD activity in both the active fraction 5 and CM treatments compared to control. SOD activity increased from 0.08 in control larvae, to 0.14 in CM-treated, and 0.16 unit/mg/min protein in active fraction 5 ($F_{2,12}=36.67; P \leq 0.0001$). Active

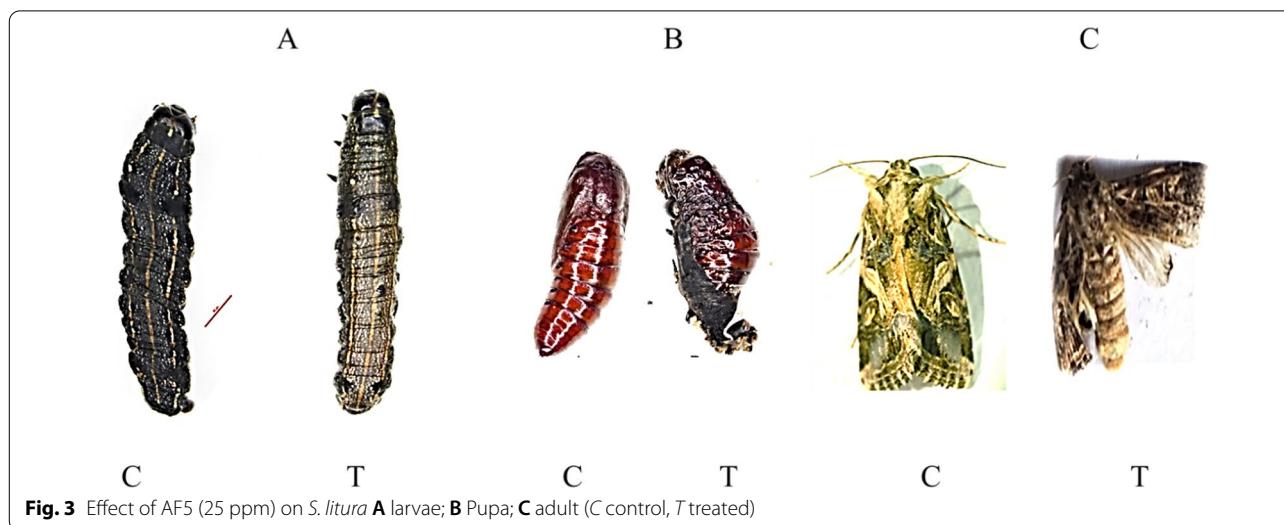


Fig. 3 Effect of AF5 (25 ppm) on *S. litura* A larvae; B Pupa; C adult (C control, T treated)

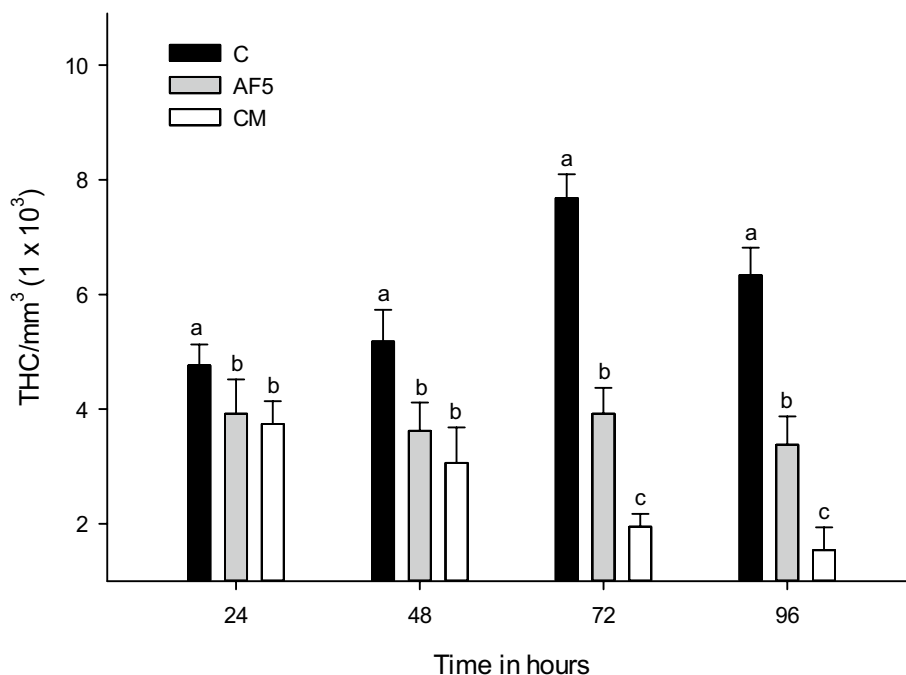


Fig. 4 Effect of AF5 and cypermethrin, CM, sub-lethal treatments on concentration of haematology (THC) in fifth-instar larvae. Mean (\pm SEM) of 20 larvae. Within each time point, the same letter indicates no significant difference ($P < 0.05$) in a Tukey's test (C control, CM cypermethrin, AF5 active fraction 5)

fraction 5 treatment increased SOD activity by 49.63% (Fig. 5C).

POX activity

POX activity significantly increased in treated larvae exposed to seaweed compounds containing active fraction 5 and CM ($P \leq 0.05$). The POX activity increased over control, from 0.62 to 0.724 and 0.88 unit/mg/min protein for active fraction 5 and CM, respectively ($F_{2,12} = 36.76$; $P \leq 0.0001$) (Fig. 5D).

Digestive enzymes

The digestive enzymes ACP, ALP, and ATPase were significantly reduced by both treatments, providing significantly greater reduction on exposure to active fraction 5. The active fraction 5 compounds significantly reduced digestive enzymes ACP, ALP, and ATPase by 37.47 ($F_{2,12} = 28.34$; $P \leq 0.001$), 39 ($F_{2,12} = 21.5$; $P \leq 0.001$) and 23.9% ($F_{2,12} = 104.79$; $P \leq 0.001$) compared with control (Fig. 6).

The CM-treated larvae showed the greatest significant reduction of all enzymes reducing the ACP activity to 14.18 from 21.86 unit/mg/min protein in control. However, the active fraction 5-treated larvae produced a lower significant change in ACP ($F_{2,12} = 28.34$; $P \leq 0.007$). The levels of the enzymes ALP ($F_{2,12} = 21.5$; $P \leq 0.008$) and ATPase ($F_{2,12} = 19.75$; $P \leq 0.0001$) in larvae were

significantly different from control, and between treatments active fraction 5 and CM.

Histology

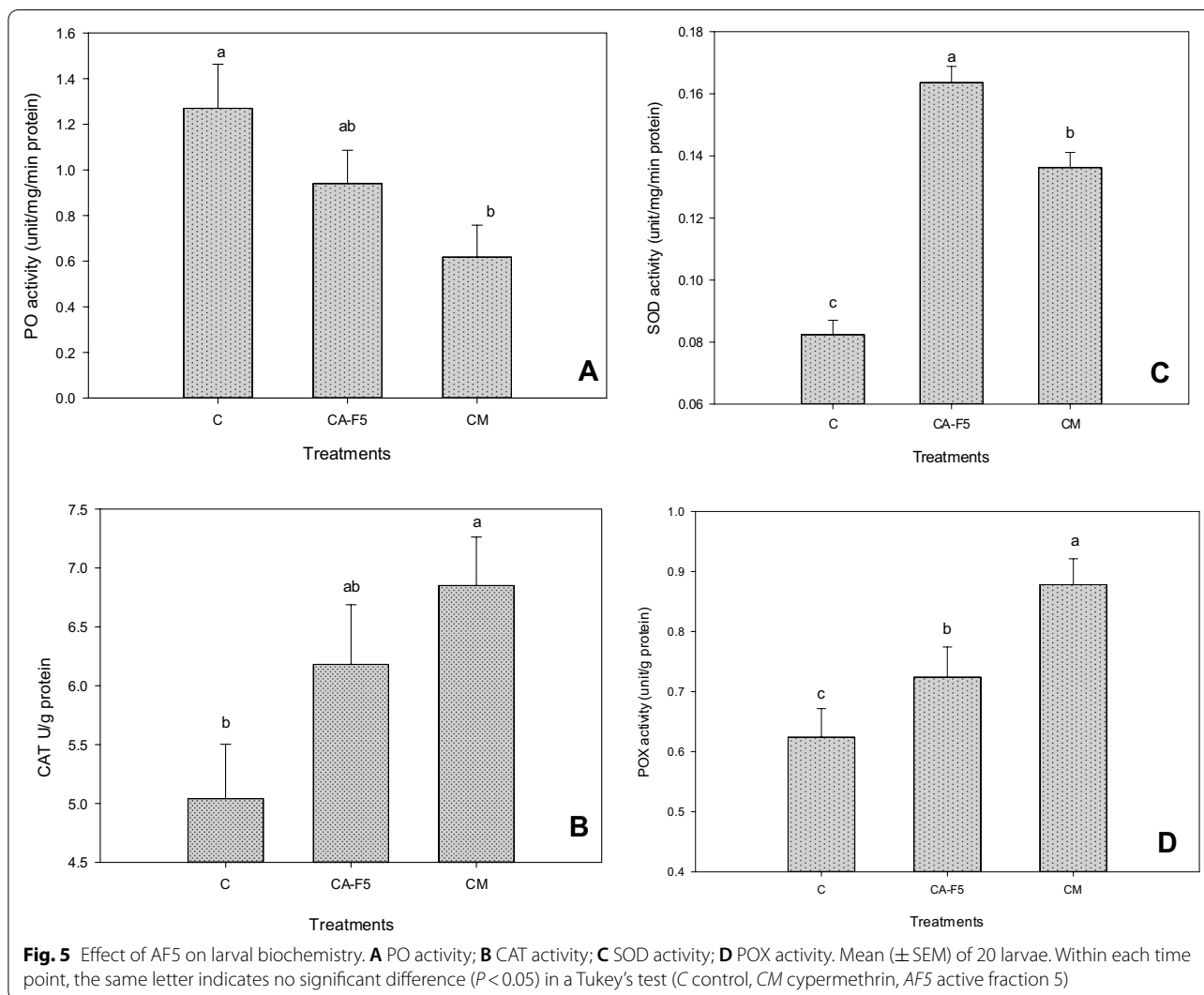
The mid-gut histology of the treated larvae was severely affected displaying impaired cell organelles, abridged brush-border membrane, and leaky columnar cells, laterally with cellular component mixing as a consequence of shattered membranes (Fig. 7B).

Earthworm toxicity

Earthworms showed no significant mortality when treated with 50 ppm active fraction 5, displaying mortality rates lower than control ($P > 0.005$). *E. eugeniae* exposed to 9 ppm cypermethrin resulted in significant mortality rate of 58% ($F_{3,16} = 40.2$; $P \leq 0.001$). The earthworms treated with chemical pesticide displayed external morphological changes such as (a) degeneration, (b) coiling, (c) anomalous clitellar swelling, and (d) tail constriction and dehydration (Fig. 8).

Discussion

Development of environmentally friendly, highly effective chemical control is imperative. Currently, about 2500 marine natural bioactive compounds have been isolated from seaweeds of sub-tropical and tropical populations [7–9]. The bioinsecticidal and microbicidal activities



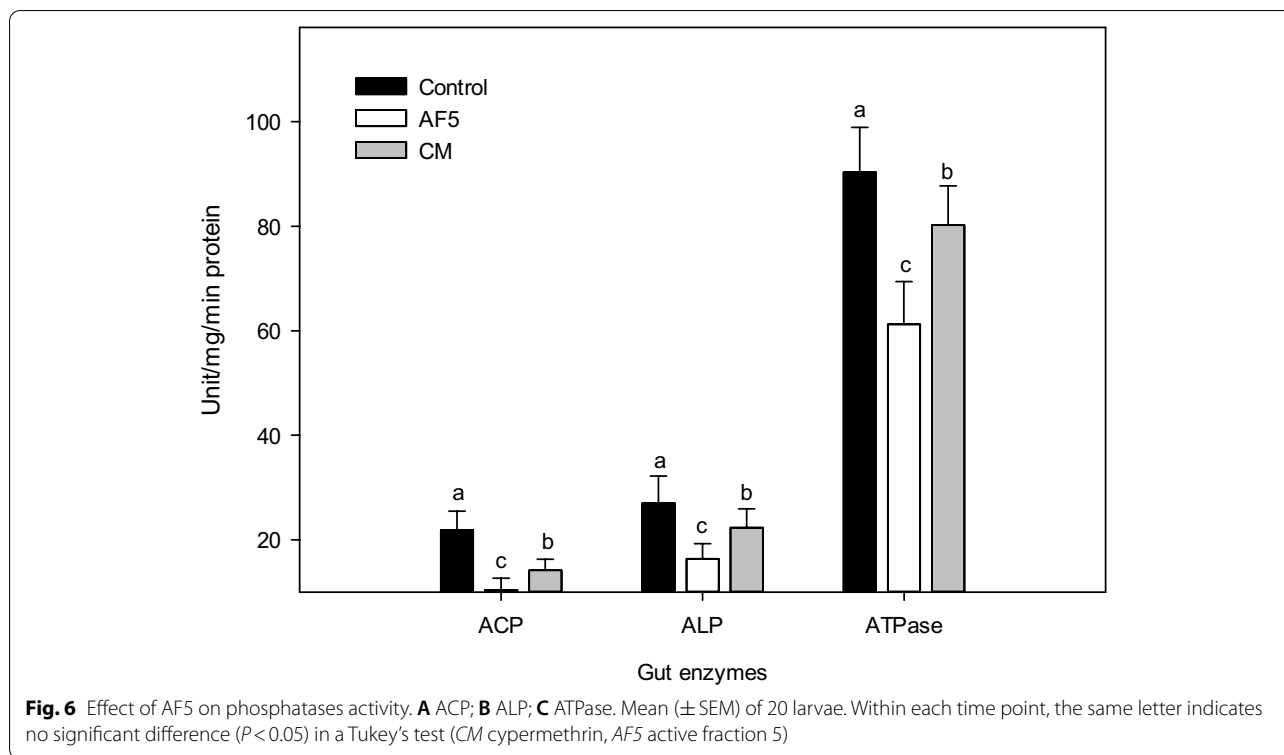
of marine macroalgae bioactive compounds have been reviewed by Hamed et al. [2]. Many of these natural bioactive compounds are important in maintaining marine ecosystems [1, 15], providing pathogen protection, growth stimulants, and support of beneficial microbiomes [49–51].

Exposing the insect pest to a mix of active compounds rather than a single compound would probably delay the development of pesticide resistance [37, 52, 53]. The current study showed that bioactive compounds from the seaweed *C. antennina* were able to significantly affect the haematology, biochemistry, and histology of *S. litura* at sub-lethal concentrations.

The preliminary mortality bioassays of crude methanol extract of *C. antennina* showed significant toxicity against *S. litura* larvae. Then, the extract was subjected to column chromatography, and the fraction active

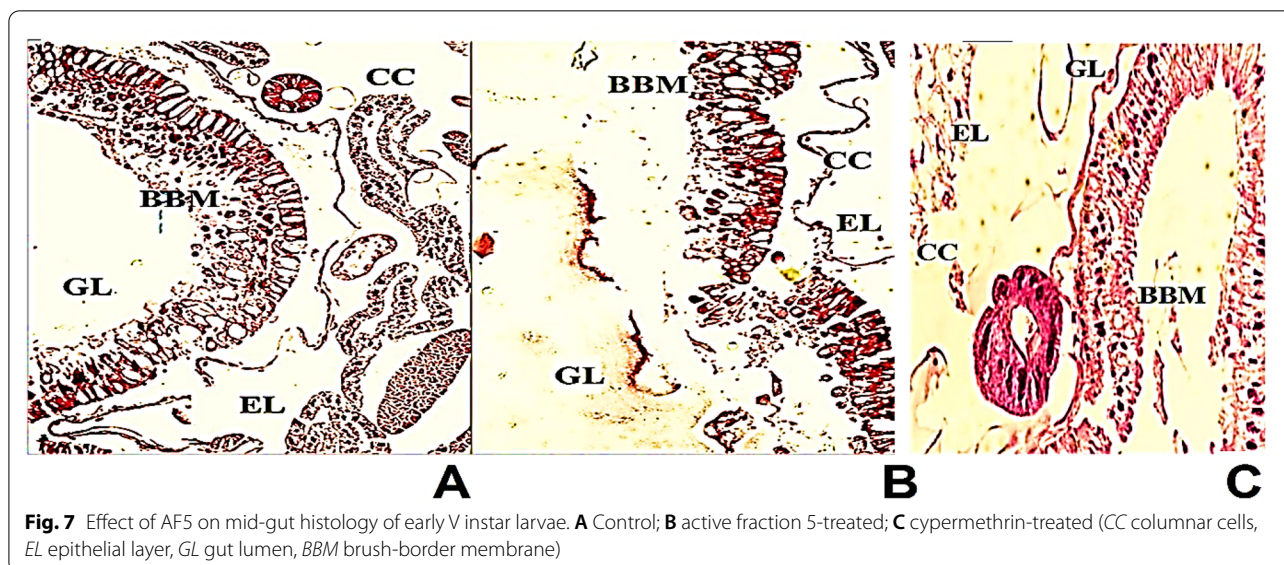
fraction 5 was identified to cause the greatest mortality rate against third-, fourth-, and fifth-instar larvae at concentrations of 25, 50, 75, and 100 ppm. Earlier instars were more susceptible to treatments, displaying increased mortality rate, which is due to their increased feeding and metabolic rates resulting in increased consumption and assimilation of toxins.

The vulnerability of early instars to toxins was also observed in *S. litura* larvae treated with andrographolide [54]. Similarly marine algae have been proved as repositories of various bioactive compounds with broad spectrum agricultural applications [1, 2, 7]. A likely biocidal activity of acetone extract of *C. antennina* was reported by Kannan and Bharathkumar [55] who proved that the seaweed extract had a profound effect on the larval survivability, pupal duration, as well as emergence of *S. litura*. Additional reports on bioactivities of *C. antennina*



encompass antiviral [56], antifungal, as well as cytotoxic effects in the form of crude extracts [57]. Seaweeds are reported to possess insecticidal compounds such as chloroacetic acid, a main component of pesticide formulation such as chlorpyrifos, insecticide-dimethoate, and herbicides benazoline besides methyl b-naphthoxyacetate [57]. The compound 2, 6-lutidine 3, 5-dichloro-4-dodecylthio—present in seaweeds—has been found to be the active component of medicinal plants *Andrographis echinoides* [58] and *Ficus arnottiana* [59]. Cyclobutanol

is a recognised microbial herbicide *Streptomyces rochei* antimetabolite [60] and also a major pesticide ingredient OECD [61]. Butanoic acid (or) butyric acid is an active component of plant hormone, IBB—a pesticide compound [62]. Also, cyclobutanol, is registered as an active ingredient in a malathion pheromone traps used in cotton boll weevils' control [63]. These toxic compounds could be are regarded as safe due to their target specificity and biological origin [54].



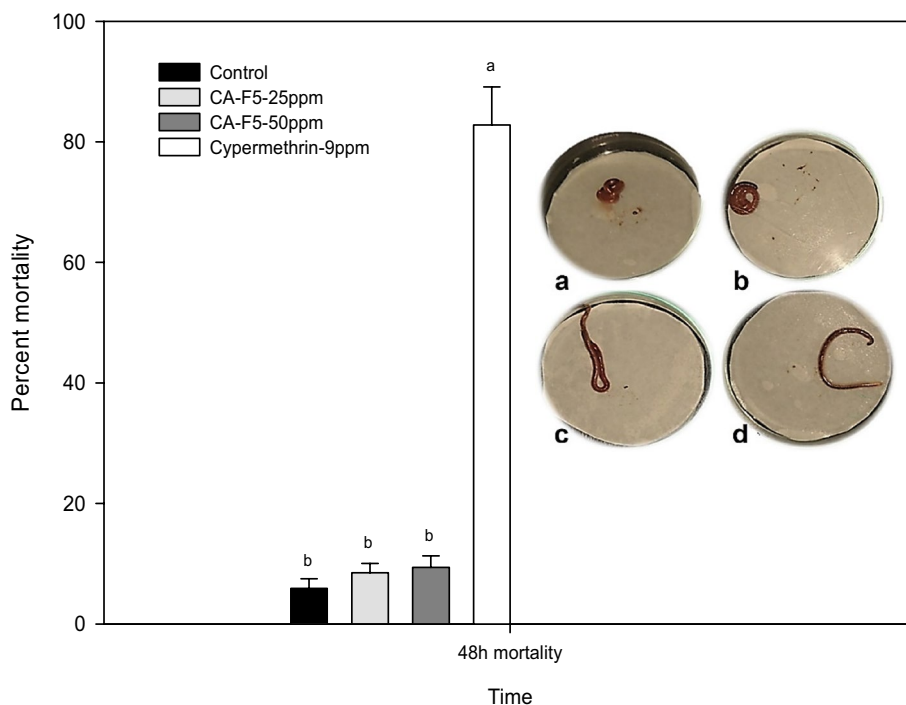


Fig. 8 Percentage mortality of earthworm in artificial 1 soil test (AST) post-treatment with chemical pesticide cypermethrin and AF5. Cypermethrin treatments were significantly different from AF5 treatments when compared to other treatments. In the AF5 treatments and control, there was no significant difference between. Means (\pm SE) standard error ($P \leq 0.05$), Tukey's test. B. Earthworm toxicity test—pesticide treated (a) 9 ppm cypermethrin; (b) AF5—50 ppm; (c) Control

The developmental defects of *S. litura* larvae treated with sub-lethal concentration of active fraction 5 resulted in deformed pupae and adults. The treated pupae were dark, deformed, and unable to emerge into adults. The debarred adults had deformed wings. This morphological anomaly was also observed in *S. litura* treated with methanolic extract of brown seaweeds, *Sargassum cristaefolium* [5] and *Liagora ceranoides* [8]. A significant decline in THC in active fraction 5-treated larvae may be due to cytotoxic effects and a reduction in the number of defensive haemolytic cells. An analogous THC decrease in insect pest treated with pesticide compounds has also been reported [7, 32, 57, 58]. PO is an important component of innate immune system in insects that is activated in response to the presence of foreign agents [64, 65]. Reduction in PO in the haemolymph of active fraction 5-treated larvae might indicate the ability of algal compounds to deregulate the insect immune system.

An increase in the levels of detoxification enzymes—SOD, CAT, and POX—indicates an increase in oxidative stress stimulation in the insect body confirming the toxicity of the studied bio compounds. Larvae treated with active fraction 5 also showed an increase in antioxidant enzyme levels. A similar increase in the antioxidant

enzymes was observed in *S. littorals* treated with pro-oxidant allelochemicals [66].

Phosphomonoesters are hydrolysed by acid phosphatases in acid conditions and alkaline phosphatases in alkaline conditions by transphosphorylation reactions [45]. These activities are recorded in higher rates in the insect mid-gut [61]. ATPases are another group of enzymes crucial for glucose, amino acids, and other organic molecule transport within the insect body [35, 67]. Any modulation in their activity will affect the insect gut physiology [33, 65]. The activities of ACP, ALP, and ATPase were influenced by the active compounds present in the seaweed extract. The reductions in the enzyme levels in insect gut are often associated with pesticide exposure [63]. A similar decrease in ACP–ALP enzyme activities was observed in *Cnaphalocrocis medinalis* and *S. litura* larvae treated with azadirachtin [68]. Reduced ACP–ALP enzyme activities correlate with low energy levels caused by the disruption of metabolite transport. A similar effect on ATPase enzymatic activity was detected in *C. medinalis* larvae treated with a biopesticide formulation that contained neem seed kernel, *Vitex negundo* extracts and *Bt* toxin [69]. ATPase reduction was caused by decreases in metabolism influencing food indigestibility or cessation of feeding [68, 70, 71]. The effect of active

fraction 5 was more significant in the insect mid-gut as the assimilation and nutrient absorption takes place in this region. The mid-gut histological analysis of treated larvae showed a disrupted brush-border membrane, owing to the interruption in metabolite or ion transport caused by the active compounds [72]. This might have triggered a series of consecutive cellular events, such as cell disruption, oozing out of cellular components and eventually disabling the insect feeding activity [69, 70]. This caused an enlargement of the mid-gut region of the treated larvae. Similar aberrations were observed in *Helicoverpa armigera* larvae treated with *Thevetia neriifolia* stem extract [71], as well as *S. litura* larvae treated with andrographolide [29, 49, 56, 63], and *Anticarsia gemmatilis* treated with squamocin [73].

Eudrilus eugeniae, earthworms, are used as bioindicators for assessments of environmental ecotoxicology [52, 74]. In vitro evaluation of active fraction 5 against *E. eugeniae* did not show any toxicological effects. Instead, the worms grew up healthy, displaying a relatively low mortality rate compared with the control. The positive effects of seaweed extracts on earthworm were reported previously by [75], who studied the effects of seaweed extracts of *Caulerpa scalpelliformis*, *Cheilosporum spectabile*, and *Sargassum wightii* on *E. eugeniae*. On the other hand, cypermethrin was highly toxic to these worms. The negative effects of synthetic pesticides on earthworms have been widely reported [30, 74, 76, 77].

Conclusion

Algae are an under-utilized resource of active compounds that have wide applications in agriculture and human health. The macroalgae examined produced an active fraction with insecticidal activity that significantly reduced larval *S. litura* survival inducing haematological, morphological, physiological, and biochemical changes. The present research adds to the increasing amount of data on marine macroalgae use in agriculture as effective biopesticides and plant protectants, as environmentally friendly and safer alternatives. Increasing interest, research, and production of products from marine macroalgae and other plants continues to provide evidence of their importance as microbicides, nematocides, insecticides, and biofertilizers. Advances in improving the effectiveness of macroalgae bioactive compounds will incorporate improvements in formulation that will open a new epoch in the biopesticide development sector against insect pests, across all agricultural sectors.

Abbreviations

SPKCEES: Sri Paramakalyani Centre for Excellence in Environmental Sciences; RH: Relative humidity; LD: Light:dark; CA-M: *C. antennina* crude methanol extract; AF5: Active fraction 5; DMSO: Dimethyl sulfoxide; CM: Cypermethrin;

THC: Total haemocyte count; PO: Phenoloxidase; CAT: Catalase; SOD: Superoxide dismutase; POX: Peroxidase; ACP: Acid phosphatase; ALP: Alkaline phosphatase; ATPase: Adenosine triphosphatase; O/N: Overnight.

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Authors' contributions

K.M-PC. and S.S-N designed the experiments; K.M.P.C., S.K., V.S-R, R.R., and H.S. conducted the experiments; S.S.-N., A.H.M., D.M.E. A.G. M.S.A, M.S.E., and W.B.H analysed the data, and wrote the manuscript. All authors reviewed, read, and approved the final manuscript.

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All available data are shown in the figures and tables.

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Consent for publication

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Competing interests

The authors declare that he has no competing interests.

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References

- Chanthini KM, Senthil-Nathan S, Stanley-Raja V, Thanigaivel A, Karthi S, Sivanesh H, Sundar NS, Palanikani R, Soranam R. *Chaetomorpha antennina* (Bory) Kützinger derived seaweed liquid fertilizers as prospective bio-stimulant for *Lycopersicon esculentum* (Mill). *Biocatal Agric Biotechnol*. 2019;20: 101190.
- Hamed SM, Abdel-Rhman AA, Abdel-Raouf N, Ibraheem IBM. Role of marine macroalgae in plant protection and improvement for sustainable agriculture technology. *Beni-Suef Univ J Appl Sci*. 2018;7:104–10.
- Senthil-Nathan S. A review of biopesticides and their mode of action against insect pests. In: *Environmental sustainability*. New Delhi: Springer; 2015. p. 49–63.
- Martins RM, Fernanda N, Guimarães VBS, da Silva AF, Colepicolo P, de Pereira CMP, Lund RG. Macroalgae extracts from Antarctica have antimicrobial and anticancer potential. *Front Microbiol*. 2018;9:412.
- Milledge JJ, Nielsen BV, Bailey D. High-value products from macroalgae: the potential uses of the invasive brown seaweed *Sargassum muticum*. *Rev Environ Sci Biotechnol*. 2016;15:67–88.

6. Ali SMY, Ravikumar S, Beula JM. Mosquito larvicidal activity of seaweeds extracts against *Anopheles stephensi*, *Aedes aegypti* and *Culex quinquefasciatus*. *Asian Pac J Trop Dis*. 2013;3:196–201.
7. Gowthish K, Kannan R. Pesticidal potentials of some red algal seaweeds from Tuticorin coast against the tobacco cutworm *Spodoptera litura* Fab. (Lepidoptera: Noctuidae). *Int J Sci Technol Res*. 2019;8(10):502–6.
8. Kannan R, Priya ND. Studies on methanolic extract of brown algal seaweed *Liagora ceranoides* JV Lamouroux from southern coast of Tamilnadu. In vitro anti-insect properties and phytochemicals. *Nat Prod Res*. 2019;7:1.
9. Yu KX, Wong CL, Ahmad R, Jantan I. Larvicidal activity, inhibition effect on development, histopathological alteration and morphological aberration induced by seaweed extracts in *Aedes aegypti* (Diptera: Culicidae). *Asian Pac J Trop Med*. 2015;8(12):1006–12.
10. Brookes G, Barfoot P. Global income and production impacts of using GM crop technology 1996–2014. *GM Crops Food*. 2016;7:38–77.
11. Maarten E, Florian S. Global demand for food is rising. Can we meet it? 2016. <https://hbr.org/2016/04/global-demand-for-food-is-rising-can-we-meet-it>. Accessed 9 Sep 2019.
12. Sinisterra-Hunter X, Hunter WB. Towards a holistic integrated pest management: lessons learned from plant–insect mechanisms in the field. In: *The biology of plant–insect interactions: a compendium for the plant biotechnologist*. 2018; 236:204–26.
13. De Pinto A, Cenacchi N, Kwon HY, Koo J, Dunston S. Climate smart agriculture and global food-crop production. *PLoS ONE*. 2020;15: e0231764.
14. Emani C, Hunter W. Insect resistance. In: *Genomics and breeding for climate-resilient crops*. Springer, Berlin, Heidelberg; 2013. pp 315–332.
15. Chanthini K, Kumar CS, Kingsley SJ. Antifungal activity of seaweed extracts against phytopathogen *Alternaria solani*. *J Acad Indus Res*. 2012;1(2):86–90.
16. Kumar S, Kaushik G, Dar MA, Nimesh S, Lopez-Chuken UJ, Villarreal-Chiu JF. Microbial degradation of organophosphate pesticides: a review. *Pedosphere*. 2018;28(2):190–208.
17. Senthil-Nathan S. A review of resistance mechanisms of synthetic insecticides and botanicals, phytochemicals and essential oils as alternative larvicidal agents against mosquitoes. *Front Physiol*. 2020;10:1591.
18. Kumar KK, Sridhar J, Murali-Baskaran RK, Senthil-Nathan S, Kaushal P, Dara SK. Arthurs S Microbial biopesticides for insect pest management in India: current status and future prospects. *J Invertebr Pathol*. 2019;16:74–81.
19. Dara SK. The new integrated pest management paradigm for the modern age. *J Integr Pest Manag*. 2019;10(1):12.
20. Burand JP, Hunter WB. RNAi: future in insect management. *J Invertebr Pathol*. 2013;112:568–74.
21. Scott JG, Michel K, Bartholomay LC, Siegfried BD, Hunter WB, Smagghe G, Zhu KY, Douglas AE. Towards the elements of successful insect RNAi. *Insect Physiol*. 2013;59(12):1212–21.
22. Fletcher SJ, Reeves PT, Hoang BT, Mitter N. A perspective on RNAi-based biopesticides. *Front Plant Sci*. 2020;11:51.
23. Hunter WB, Sinisterra-Hunter XH. Emerging RNA suppression technologies to protect citrus trees from citrus greening disease bacteria. In: *Advances in insect physiology*. Academic Press; 2018. p. 163–197.
24. Sandoval Mojica AF, Altman S, Hunter WB, Pelz-Stelinski KS. Peptide conjugated morpholinos for management of the Huanglongbing pathosystem. *Pest Manag Sci*. 2020. <https://doi.org/10.1002/ps.5877>.
25. Chen K, Rios JJ, Pérez-Gálvez A, Roca M. Comprehensive chlorophyll composition in the main edible seaweeds. *Food Chem*. 2017;228:625–33.
26. Garczynski SF, Martin JA, Griset M, Willett LS, Cooper WR, Swisher KD, Unruh TR. CRISPR/Cas9 Editing of the codling moth (Lepidoptera: Tortricidae) CpmOR1 gene affects egg production and viability. *J Econ Entomol*. 2017;110(4):1847–55. <https://doi.org/10.1093/jeet/tox166>.
27. Sun D, Guo Z, Liu Y, Zhang Y. Progress and prospects of CRISPR/Cas systems in insects and other arthropods. *Front Physiol*. 2017;8:608.
28. Taning CN, Van Eynde B, Yu N, Ma S, Smagghe G. CRISPR/Cas9 in insects: applications, best practices and biosafety concerns. *J Insect Physiol*. 2017;98:245–57.
29. Dinesh-Kumar A, Srimaan E, Chellappandian M, Vasantha-Srinivasan P, Karthi S, Thanigaivel A, Ponsankar A, Chanthini KM, Shyam-Sundar N, Annamalai M, Kalaivani K. Target and non-target response of *Swietenia Mahagoni* Jacq. chemical constituents against tobacco cutworm *Spodoptera litura* Fab. and earthworm *Eudrilus eugeniae* Kinb. *Chemosphere*. 2018;199:35–43.
30. Vasantha-Srinivasan P, Senthil-Nathan S, Thanigaivel A, Edwin ES, Ponsankar A, Selin-Rani S, Pradeepa V, Sakthi-Bhagavathy M, Kalaivani K, Hunter WB, Durairaj V. Developmental response of *Spodoptera litura* Fab. to treatments of crude volatile oil from *Piper betle* L. and evaluation of toxicity to earthworm, *Eudrilus eugeniae* Kinb. *Chemosphere*. 2016;155:336–47.
31. Dhivya K, Vengateswari G, Arunthirumeni M, Karthi S, Senthil-Nathan S, Shivakumar MS. Bioprospecting of *Prosopis juliflora* (Sw.) DC seed pod extract effect on antioxidant and immune system of *Spodoptera litura* (Lepidoptera: Noctuidae). *Physiol Mol Plant Pathol*. 2018;101:45–53.
32. Karthi S, Vaideki K, Shivakumar MS, Ponsankar A, Thanigaivel A, Chellappandian M, Vasantha-Srinivasan P, Muthu-Pandian CK, Hunter WB, Senthil-Nathan S. Effect of *Aspergillus flavus* on the mortality and activity of antioxidant enzymes of *Spodoptera litura* Fab. (Lepidoptera: Noctuidae) larvae. *Pestic Biochem Physiol*. 2018;149:54–60.
33. Horie B. The alkaline phosphatase in the midgut of silkworm, *Bombyx morio* L. *Bull Seric Exp Strn*. 1958;15(4):275–89.
34. Shahriari M, Sahbzadeh N, Zibae A, Khani A, Senthil-Nathan S. Metabolic response of *Ephesia kuehniella* Zeller (Lepidoptera: Pyralidae) to essential oil of Ajwain and thymol. *Toxin Rev*. 2017;36(3):204–9.
35. Azuma M, Takeda S, Yamamoto H, Endo Y, Eguchi M. Goblet cell alkaline phosphatase in silkworm midgut epithelium: its entity and role as an ATPase. *J Exp Zool*. 1991;258(3):294–302.
36. Guiry W. World-wide electronic publication. In: Guiry MD, Guiry GM, editors. *Algae base*. Galway: National University of Ireland; 2020.
37. Asha A, Rathil JM, Patric Raja D, Sahayaraj K. Bicial activity of two marine green algal extracts against third instar nymph of *Dysdercus cingulatus* (Fab.) (Hemiptera: Pyrrhocoridae). *J Biopest*. 2015;5(Suppl):129–34.
38. Oliveira AFCS, Teixeira RR, Oliveira AS, Souza APM, Silva ML, Paula SO. Potential antivirals: natural products targeting replication enzymes of dengue and chikungunya viruses. *Molecules*. 2017;22:505.
39. Gonzalez-Castro AL, Muñoz M. Repellent activity of marine macroalgae extracts against *Diaphorina citri*. *J Chem Biol Phys Sci*. 2014;4(5):27–30.
40. Abbott WS. A method of computing the effectiveness of an insecticide. *J Econ Entomol*. 1925;18(2):265–7.
41. Finney DJ. Probit analysis. 3rd ed. London: Cambridge University Press; 1971.
42. Jones JC. Current concepts concerning insect hemocytes. *Am Zool*. 1962;2:209–46.
43. Applebaum SW. The action pattern and physiological role of *Tenebrio* larval amylase. *J Insect Physiol*. 1964;10(6):897–906.
44. Bessey OA, Lowky OH, Brock MJ. A method for the rapid determination of alkaline phosphatase with five cubic millimeters of serum. *Biol Chem*. 1946;164:321–9.
45. Fiske CH, Subbarow Y. The colorimetric determination of phosphorus. *J Biol Chem*. 1925;66(2):375–400.
46. Luck H. Catalase estimation. In: *Methods in enzyme analysis*. London: Academic Press; 1971.
47. Marklund S, Marklund G. Involvement of the superoxide anion radical in the autoxidation of pyrogallol and a convenient assay for superoxide dismutase. *Eur J Biochem*. 1974;47(3):469–74.
48. Reddy KP, Subhani SM, Khan PA, Kumar KB. Effect of light and benzyladenine on dark-treated growing rice (*Oryza sativa*) leaves II. Changes in peroxidase activity. *Plant Cell Physiol*. 1985;26(6):987–94.
49. OECD. Guideline for testing of chemicals. Earthworm acute toxicity. In: OECD, ed. OECD, Paris; 1984.
50. Faulkner DJ. Marine natural products. *Nat Prod Rep*. 2001;18(1):1R-49R.
51. Hay ME, Paul VJ, Lewis SM, Gustafson K, Tucker J, Trindell RN. Can tropical seaweeds reduce herbivory by growing at night? Diel patterns of growth, nitrogen content, herbivory, and chemical versus morphological defenses. *Oecologia*. 1988;75(2):233–45.
52. Rattan RS. Mechanism of action of insecticidal secondary metabolites of plant origin. *J Crop Prot*. 2010;29(9):913–20.
53. Edwin E, Vasantha-Srinivasan P, Senthil-Nathan S, Thanigaivel A, Ponsankar A, Selin-Rani S, Kalaivani K, Hunter WB, Durairaj V, Al-Dhabi NA. Effect of andrographolide on phosphatases activity and cytotoxicity against *Spodoptera litura*. *Invertebr Surviv J*. 2016;13(1):153–63.
54. Ullmann F, Gerhartz W, Yamamoto YS, Campbell FT, Pfefferkorn R, Rounsaive JF. Ullmann's encyclopedia of industrial chemistry. Weinheim: VCH publishers; 1985.

55. Kannan R, Bharathkumar R. Effect of green algal sea weed-*Chaetomorpha antennina* (Bory de Saint-Vincent) Kutzing's acetone extract on the growth and development of tobacco caterpillar, *Spodoptera litura* (Noctuidae: Lepidoptera). J Pharmacogn Phytochem. 2019;8(5):1376–8.
56. Benevides NMB, Oliveira SRM, Holanda ML, Melo FR, Freitas ALP, Sampaio AH. Seasonal variations in hemagglutinating activity and chemical composition of two red marine algae *Gracilaria domingensis* and *Gelidium pusillum*. Rev Bras Fisiol Veg. 1999;11:91–5.
57. Gonçalves AG, Ducatti DRB, Grindley TB, Duarte MER, Nosedá MD. ESI-MS differential fragmentation of positional isomers of sulfated oligosaccharides derived from carrageenans and agarans. J Am Soc Mass Spectrom. 2010;21:1404–16.
58. Jeevanantham K, Zahir-Hussain A. Phytochemical screening and GC-MS analysis of *Andrographis echioide*. Eur J Biomed Pharm. 2018; 889–893.
59. Babu A, Anand D, Saravanan P. Phytochemical analysis *Ficus arnottiana* (Miq.) Miq. leaf extract using GC-MS analysis. Int J Res Phytochem Pharmacol Res. 2017;9(6):775–9.
60. Ayer SW, Isaac BG, Luchsinger K, Makkar N, Tran M, Stonard RJ. cis-2-AMINO-1-hydroxycyclobutane-1-acetic acid, a herbicidal antimetabolite produced by *Streptomyces rochei* A13018. J Antibiot. 1991;44(12):1460–2.
61. Elliott M, Janes NF, Pulman DA, Johnson DM. Pesticides. In: office Ep, editor. European Patent Office 0143 593A2 vol. England; 1985.
62. UPA. R.E.D. Facts, Indole-3-Butyric Acid. In: Office of Prevention, Pesticides, H-7508W vol: United States Environmental Protection Agency; 1992.
63. EPA. Boll Weevil Aggregation Pheromone (112401, 112402, 112403, 112404) Fact Sheet. In: Ombudsman BaPPD, ed: Biopesticides and Pollution Prevention Division; 2000.
64. Ali AM, Ibrahim AM. Castor and camphor essential oils alter hemocyte populations and induce biochemical changes in larvae of *Spodoptera littoralis* (Boisduval) (Lepidoptera: Noctuidae). J Asia Pac Entomol. 2018;21(2):631–7.
65. González-Santoyo I, Córdoba-Aguilar A. Phenoloxidase: a key component of the insect immune system. Entomol Exp Appl. 2012;142(1):1–6.
66. Krishnan N, Kodrik D. Antioxidant enzymes in *Spodoptera littoralis* (Boisduval): are they enhanced to protect gut tissues during oxidative stress? J Insect Physiol. 2006;52(1):11–20.
67. Ottaviani E. The importance of studying invertebrate immune-neuroendocrine functions. Invertebr Surviv J. 2013;11(1):1–3.
68. Senthil-Nathan S, Chung PG, Murugan K. Effect of botanical insecticides and bacterial toxins on the gut enzyme of the rice leaf folder *Cnaphalocrocis medinalis*. Phytoparasitica. 2004;32(5):433.
69. Senthil-Nathan S. Physiological and biochemical effect of neem and other Meliaceae plants secondary metabolites against Lepidopteran insects. Front Physiol. 2013;4:359.
70. Senthil-Nathan S, Kalaivani K, Murugan K. Effects of neem limonoids on the malaria vector *Anopheles stephensi* Liston (Diptera: Culicidae). Acta Trop. 2005;96(1):47–55.
71. Zibae I, Bandani AR, Sendi JJ, Talaei-Hassanloei R, Kouchaki B. Effects of *Bacillus thuringiensis* var. *kurstaki* and medicinal plants on *Hyphantria cunea* Drury (Lepidoptera: Arctiidae). Invertebr Surviv J. 2010;7(2):251–61.
72. Mishra M, Gupta KK, Kumar S. Impact of the stem extract of *Thevetia neriiifolia* (Apocynaceae) on food consumption and growth of early fourth instars of *Helicoverpa armigera*. J Biosci. 2015;2:26–35.
73. Fiaz M, Martínez LC, da Silva Coasta M, Cossolin JF, Plata-Rueda A, Gonçalves WG, Sant'Ana AE, Zanuncio JC, Serrão JE. Squamocin induce histological and ultrastructural changes in the midgut cells of *Anticarsia gemmatalis* (Lepidoptera: Noctuidae). Ecotoxicol Environ Saf. 2018;156:1–8.
74. Pelosi C, Barot S, Capowiez Y, Hedde M, Vandebulcke F. Pesticides and earthworms. A review. Agron Sustain Dev. 2014;34:199–228.
75. Karthick N, Selvakumar S, Umamaheswari S. Effect of three different seaweed liquid fertilizers and a chemical liquid fertilizer on the growth and histopathological parameters of *Eudrilus eugeniae* (Haplotaxida: Eudrilidae). Glob J Biosci Biotech. 2013;2:253–9.
76. Wu S, Wu E, Qiu L, Zhong W, Chen J. Effects of phenanthrene on the mortality, growth, and anti-oxidant system of earthworms (*Eisenia fetida*) under laboratory conditions. Chemosphere. 2011;83(4):429–34.
77. Vijayan SR, Santhiyagu P, Ramasamy R, Arivalagan P, Kumar G, Ethiraj K, Ramaswamy BR. Seaweeds: a resource for marine bionanotechnology. Enzyme Microb Technol. 2016;95:45–57.

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