Toxicity of *Caulerpa scalpelliformis* selected fractions with fatty acids on *Porthesia scintillans*

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**ABSTRACT**

*Porthesia scintillans*, a serious pest threat to major crops. Here, we evaluated the toxicity of *Caulerpa scalpelliformis* fractions (CSF1, CSF2, and CSF3) against *P. scintillans*. Fraction CSF1 attracted *P. scintillans* larvae whereas CSF2 and CSF3 showed repellency. *P. scintillans* showed significant higher insecticidal activity when fed with CSF2 fraction sprayed castor leaves. Similar observation was recorded in standards, vijayneem and monocrotophos. The GC–MS analysis of CSF2 and CSF3 described hexadecanoic acid, octadecenoic acid, and octadecatrienoic acid which might promote insecticidal activity. Hence, fatty acid based *C. scalpelliformis* extract preparations may be used for safe and eco-friendly agricultural pest.

**Introduction**

*Porthesia scintillans* Walker (Lymantriidae: Lepidoptera) (=*Euproctis scintillans* Walker) is a polyphagous pest of many cultivable crops (Subba et al. 1974, Yuanfu 1989, Zeya et al. 2000, Hill 2008, Chen et al. 2010, Xi-sheng et al. 2010). In order to mitigate the serious threats of this pests, varied pesticides are been popularized (Thapinta and Hudak 2000, Chen et al. 2010, Panuwet et al. 2012, Praneetvatakul et al. 2013). Yet there consequence and long-term risk posed are forever more evident. Contrarily, the pest resurgence and resistance is attained due to extensive or prolonged use of pesticides (Suthamma and Roland 2014). Thereof careful administration has become pivotal and has made it necessary to find more effective and healthier alternative.

Plants are the richest source of renewable active chemicals and alternative products (Koul and Dhaliwal 2000, Regnault-Roger et al. 2005). To control early stage larvae of *P. scintillans*, neem (*Azadirachta indica* A.Juss.) seed kernel extract (NSKE) or neem oil is employed, some plant extracts (Chockalingam and Sundari 1988) were used to manage *P. scintillans*.

Seaweeds are rich source of diverse secondary metabolites that are currently exploited among marine plant resources. They are classified into three major taxa that include Phaeophyceae, Rhodophyceae, and Chlorophyceae based upon their pigments presented (Yu et al. 2014). Superior than terrestrial plants seaweeds are found to possess various bioactive compounds, which are employed in industrial products, food, and medical (FAO 2004, Smit 2004, Cardozo et al. 2007, Cotas et al. 2021, Priyadarshini et al. 2021).

The genus *Caulerpa*, a green algae (Chlorophyta) is represented by nearly 60 species. Several reports are already available on the metabolic activities of various other species of *Caulerpa*. *Caulerpa scalpelliformis* (R.Brown ex Turner) is been claimed to possess pesticidal activity (Kombiah and Sahayaraj 2012, Sahayaraj et al. 2012, Asharaja and Sahayaraj 2013). In addition, seaweed fatty acids (FAs) had been demonstrated for their plant growth promotion (Kalaiawan et al. 2012), fungicidal and cytotoxic (Shahnaz and Shameel 2006) activities. However, so far no one has utilized marine macroalgae in the management practice of *P. scintillans* and it is not available in the literature. *Bombyx mori* is considered as a model organism in the field of basic and applied entomology. Previously, impact of marine algae *Turbinaria conoides* (Kumari et al. 2011), *Caulerpa serrulata*, *Gracilaria edulis*, and *Ulva fasciata* (Kumari et al. 2017) extracts that were utilized to manage silkworm diseases suggested them as eco-friendly disinfectants. Henceforward, considering the
lacuna of Caulerpa extracts against P. scintillans, the proposed project is aimed to study the following objectives, (1) extracting and eluting specific bioactive fractions of C. scalpelliformis using column chromatography, (2) qualitative, UV–visible spectrophotometric and identifications by FT-IR and GC–MS analysis of column chromatographic fractions of C. scalpelliformis, (3) evaluate the impact of C. scalpelliformis column chromatographic fractions against P. scintillans, and (4) to record the impact of C. scalpelliformis against model insect B. mori.

Materials and methods

Preparation of vijayneem, monocrotophos

A neem-based commercialized product vijayneem (Madras Fertilizer Ltd., Chennai, India) was procured from local pesticide company from Palayamkottai used as positive control. The experimental study was performed using the field recommended concentration (0.03%) with water. Another commercial synthetic insecticide monocrotophos (monocrotophos 36% SL, Anucron insecticide) was used as standard. This commercial product was purchased from local pesticide company at Palayamkottai. This experiment was done using field recommended concentration of (0.5%).

Collection, extraction, and characterization

Caulerpa scalpelliformis were collected and dried from Gulf of Mannar, Ramanathapuram District of Tamil Nadu. Debris and sands were removed using tap water as well as distilled water twice each time. They were dried under shade for two weeks and then partially grounded with domestic mixer. For the extraction of secondary metabolites, powdered algal material (250 g) was extracted through soxhlation method using methanol under hot continuous extraction (24 h) (40–50°C) in a Soxhlet apparatus. After removal, the residual solvent (10 ml) was evaporated and dried in vacuum dissector. Thus, concentrated extracts were collected in air tight glass vials (9.4 cm) refrigerated (LG, Noida, India) until further use. Later, preparative chromatographic methods were done to isolate the desired bioactive compounds of C. scalpelliformis from crude extract (hexane + chloroform + methanol at 1:1:1 ratio v/v).

Column chromatography

The vertical glass tube of 3 cm outer diameter and 73 cm height was used for the separation through a column apparatus and the silica gel was supported with a sintered glass disk. In order to prevent air bubbles, the column was filled with petroleum ether of 50 ml volume. Column packing was done with adsorbent silica gel (250 g) of mesh sized 60–120 (Merck, Specialties Private Ltd, Mumbai, India) mixed with petroleum ether (500 ml) to a height of 45 cm. To equilibrate the silica gel (30°C), petroleum ether was passed into the pre-running solvent through a solvent reservoir that is connected to the column top. To the silica gel (250 g), a 2.5 g of crude extract was mixed with petroleum ether (500 ml). This mixture was coated on over the silica column. Initial eluting solvent was 100% petroleum ether whose polarity was increased at 80:20, 60:40, 40:60, and 20:80 ratios till next solvent of 100% toluene, chloroform, acetone, ethyl acetate, methanol, and acetic acid utilized in the same sequence. Under gravitational flow, the columns were eluted in marked test tubes (15 ml capacity) at 10 ml/10 min until the solutes in column appear to be more. Simultaneously, the eluted fractions were monitored by thin layer chromatography (TLC) as stationary phase, pre-coated with aluminum sheets of silica gel (20 × 20 cm, silica gel 60 F254, Merck, Specialties Private Ltd, Mumbai, India) and as mobile phase hexane, ethyl acetate, and methanol (6:3:1 and 3:6:1), chloroform and methanol (9:1) and, chloroform, ethyl acetate, and methanol (8:1:1) ratios were used for the separation of column chromatographic fractions such as F1 (48–112 elutions), F2 (176–330 elutions), and F3 (683–759 elutions) were designated as C. scalpelliformis fraction 1 (CSF1), CSF2, and CSF3, respectively. The qualitative phytochemical analysis of these fractions was performed using standard procedures of Harborne (1984).

UV visible spectral analysis

The column chromatographic fractions such as CSF1, C. scalpelliformis treated fractions 2 (CSF2), and CSF3 were dissolved in petroleum + toluene (2:3 v/v); toluene and toluene + chloroform (3:2 v/v), respectively. These samples were examined in the UV–spectrophotometer (Shimadzu, Mumbai, India) at various nm (870–880, 680–700, 660–670, 600–610, and 410–420) to find out λmax. Caulerpa scalpelliformis fractions like CSF2 and CSF3 were subjected to FT-IR (Perkin-Elmer Spectrum RX I FT-IR system, Waltham, MA) and GC–MS (Shimadzu QP5000, Kyoto, Japan) analyses.

Collections and nurturing of pest

Different life stage of P. scintillans was collected from castor plantations at St. Xavier’s College premises
Life stages of *P. scintillans* were reared up to pupation and these pupae were kept in the plastic container (height 7.4 cm × diameter 15.5 cm) at room temperature (29 ± 2 °C), relative humidity 47% and photoperiod of 11 L and 13.0 D hours. The emerged healthy adults were released into an insect oviposition cage (height 43 cm × diameter 35 cm) for egg laying. As an ovipositional substrate petioles of fresh castor leaves were immersed in 25 ml bottle to maintain its turgidity and provided in the cage. To enhance its oviposition, adult insects are fed with 10% sucrose fortified vitamin (1%) mixture. The newly hatched larvae were maintained in plastic container (height 5.6 cm × diameter 4.2 cm) and utilized for the experiment.

**Host plant preference tests**

*P. scintillans* is a polyphagous pestiferous insect and needs to record its selective individual host plants with a choice-test. This choice-test involves five varied treatments including six replications of each treatment and an untreated control. The experiment includes food sources such as pomegranate (*Punica granatum*), mango (*Mangifera indica*), castor (*Ricinus communis*), cotton (*Gossypium hirsutum*), and rose (*Rosa centifolia*) leaves. The experiment was conducted in autoclavable Petri-plates (diameter 110 mm × height 25 mm). Leaves were cut into 1 × 1 cm, and placed inside the petri-plates maintaining equal distance between two leaf. In order to find out feeding preference, two newly emerged five-days old and 10-days old larvae of *P. scintillans* were released in a petri dishes covered with a glass lid. The assay consisted of six replicates and was carried out in the laboratory conditions as mentioned above. Number of larvae first selecting their host plant was recorded. Similarly, second and their selection was also recorded. In addition, time taken for selection and time spent by an insect in each host plant was also observed.

**Insecticidal bioassay**

Various concentrations of the *C. scalpelliformis* extract fractions (1000 μl, 800 μl, 400 μl, and 200 μl), mixed with 10 μl of Teepol and 100 ml of distilled water are used for the test. Castor leaf discs of 3 × 3 cm were dipped in the above-mentioned concentrations for 5 min separately, then it was shade dried again for 5 min and placed over filter paper over a plastic tray and used for the insecticidal bioassay. Six uniform sized third instar 12 h pre-starved and pre-weighed larvae were released to visible plastic containers of 5.6 cm × 4.2 cm for feeding. After 24 h, number of dead and live larvae was weighed for each treatment separately using monopan balance (Dona 160D Balance, Hyderabad, India) with 0.1 mg accuracy. Live larvae from both control and experiment were fed with fresh castor leaves next day. Again numbers of live larvae, dead, and moulting of the larvae were recorded after 24 h. Similar procedure was followed for 94 h continuously. The diet was changed every day. Mortality was recorded up to 11th day (pre-pupation day).

\[
\text{Corrected percent mortality (CPM)} = \frac{\% \text{ kill in treated} - \% \text{ kill in control}}{100 - \% \text{ kill in control}} \times 100
\]

Using Abbott correction formula for natural mortality, the corrected mortality of insect was calculated in untreated control (Abbott 1925) and it was subjected to Probit analysis using SPSS (Finney 1971, SPSS V0.20, Chicago, IL) in statistical analysis. Fiducial limits (LC30, LC50, and LC 90), regression coefficient, chi-square value, and regression equation were recorded. Then, live larvae were maintained till pupation and emerged adults were maintained in a chamber (43.7 cm height × 35.0 cm diameter) where petioles of fresh castor leaves were immersed in 25 ml bottle to maintain turgidity of leaves as ovipositional substrate. Adult feed consist of 10% sucrose solution to enhance the oviposition.

**Life trait observations**

During the experiment, larval periods, percent of pupae emerged, number of male and female emerged and adult longevity was recorded. From the data, pupation rate, sex ratio was calculated as follows:

\[
\text{Pupation rate(%) = number of pupae/ total number of larvae} \times 100
\]

\[
\text{Sex ratios (male : female) = number of females appeared from a group/ total number of adults appeared}
\]

Additionally, deformed adults, pupae and larvae were observed if any. They were preserved in 70% alcohol for micro-photographic purpose.

**Feeding inhibition bioassay**

Feeding inhibition (FI) assays were performed with castor leaves in a no-choice test. Using cork borer
discs (12.5 cm²) were hit out of the leaves. The treating castor leaves were dipped with test solution in a concentration range of 0.02, 0.04, 0.06, 0.08, and 0.10% (w/v) for 30 s. The test solution contained water (w/v) with 1% emulsifier (w/v) of the total volume set. Control was served by the water + emulsifier (1% w/v) solution. In order to maintain the castor leaf turgidity, a moistened filter paper was kept at bottom of a petri dish (9.5 cm in diameter, 1.5 cm height). Four fifth instar pre-starved (6-h) larvae were let out individually into petri dish to feed on the disc for 24 h. Then, next three-days untreated castor leaves were provided. Unconsumed food and fecal particles were carefully removed after every 24 h and weight accurately. Per replication each larva was tested and 10 times replication was done for each test concentration. Similarly, control (without any plant extract treated), vijayneem (0.03) and monocrotophos (0.05%) categories were maintained for comparisons. In comparison to control its feeding values were measured. The data were translated to FI using the formula of Bomford and Isman (1996).

In another experiment, pre-weighed mulberry leaf discs of 7 × 7 cm were dipped in various concentrations of the C. scalpelliformis fractions CSF1, CSF2, CSF3 and provided to the pre-starved fourth instar silkworm larvae. There were three larvae maintained in transparent plastic containers (5.6 cm × 4.2 cm) for each concentration with 10 replications. In addition, control category (without any plant extract treated), vijayneem (0.03) and monocrotophos (0.05%) categories were maintained for comparisons. In comparison to control its feeding values were measured. The data were translated to FI using the formula of Bomford and Isman (1996).

Results

Distribution of Porthesia scintillans

We recorded various stages of larvae and egg masses in P. scintillans in P. granatum, M. indica, R. communis, G. hirsutum, and R. centifolia at Tirunelveli and Thoothukudi districts of Tamil Nadu.

Target food plant species preference

Plant food preference of both five-days old and 10-days old P. scintillans larvae is presented in Figure 1. Results revealed that 40% of five-days old and 36.7% of 10-days old larvae of P. scintillans highly preferred R. communis than the other plant species tested. Based on the pest preference over the plant species, the test fractions of C. scalpelliformis were applied into the R. communis leaves for further examinations against P. scintillans.

Figure 1. Food plant preference of Porthesia scintillans larvae (5 and 10-days old) and their mean preference (%) against five different food plants (n = 10).

Statistical analyses

Food preference was calculated as mentioned below:

Food preference(%) :
\[
\text{total number of insect preferred a specific plant/ no of insect in the experimental arena \times 100}
\]

Corrected mortality, fiducial limits and LC₃₀, LC₅₀, and LC₉₀ were calculated as mentioned above. Feeding inhibition, mortality, and life traits data were initially subjected to log-transformed to homogenize the variances, before analyzing the data using SPSS Statistics version 20 software subjected to SPSS (version 20, Chicago, IL).
Biological traits like larval period, pupal period, pupation ration, adult emergence, adult longevity, and sex ratio were recorded for *P. scintillans*. Females (1.0–1.5 cm) are larger than the males (0.8–1.0 cm). Male and female moths lived only for 2–3 and 3–5 days, respectively. Females laid their eggs in a patch (60–83 eggs/female) during afternoon time. The eggs are yellow and covered with yellow colored hairs. It is a kind of protective mechanism and also parental care by *P. scintillans*. During the course of embryogenesis, egg color changed from yellow to dark green and the incubation period was lost for 3–4 days. The larval hatching percentage ranged between 90 and 95%.

The newly hatched larvae dispersed and moved to various localities of a leaf. Young larvae fed the young leaves more rigorously. When it consumed castor, its fecal pellet was black in color. The body is yellow whereas the head is dark brown color. Two to three types of hairs were noticed in the early as well as late larval instars of *P. scintillans*. Second instar larvae having pink colored head with five prominent abdominal segments. In second instar larvae, the hairs are very long when compared to other life stages. These five segments have pink colored oval shaped spots at the dorsal side. Pre-pupal stage is prominent, during this stage, the larvae did not feed anything then it undergoes pupation. The cocoon has two distinct layer namely outer rough layer and inner smooth layer. Rough layer consists of enormous number of hairs which are prevailed in the fifth larval stage of *P. scintillans*. It is brown in color and the inner smooth layer is white in color and does not have any hairs. The study recommends utilizing this cocoon for various biological purposes.

**Preliminary phytochemistry**

Preliminary phytochemical screening revealed the presence of FA derivative steroids in CSF2 and CSF3 and phenolic compounds, terpenoids and cardiac glycosides in CSF1. The UV–visible spectral analyses of *C. scalpelliformis* fractions are presented in Table S1. The $\lambda_{\text{max}}$ for CSF1, CSF2, and CSF3 was 413.50, 410.50, and 666.50 nm, respectively. As CSF2 and CSF3 were observed for more repellent and pesticidal activity, these two fractions were subjected for FT-IR and GC–MS analyses.

**FT-IR**

FT-IR results of *C. scalpelliformis* column chromatographic fractions are presented in Table 1. The second and third fraction CSF2 and CSF3 observed with different frequencies (cm$^{-1}$) in the range of 1110–1600 reveals the presence of lipids, 1603.93 for amid I band (protein), 2960–2816 shows proteins and lipids, and 1217.74 in CSF3 shows carbohydrates.

**GC–MS identification**

GC–MS analysis of CSF2 and CSF3 is presented in Table 2. These fractions consisted of high levels of either C$_{17}$ compound hexadecanoic (85.60% in CSF2), C$_{28}$ compound octadecenoic acid (59.83% in CSF3), and octadecatrienoic acid (12.92%). These two scrutinized fractions were considered for further mortality assays as they possessed FA derivatives.

**Repellence activity and feeding inhibition**

*C. scalpelliformis* fraction 1 showed attraction activity whereas CSF2 and CSF3 showed repellent activity (Table S2) against *P. scintillans* larvae. This is also expressed as FI property of *C. scalpelliformis* fractions (CSF1, CSF2, and CSF3). Feeding inhibition of vijay-neem and monocrotophos was very low when compared to CSF1, CSF2, and CSF3 (Figure 2). Significantly high ($p < .01$) FI activity was observed with CSF2 than CSF3 and CSF1. All fractions exhibited linearity in FI as the concentrations raised.

| Table 1. FT-IR analyses of *Caulerpa scalpelliformis* fractions of column chromatography. |
|-----------------------------------------------|-----------------------------------------------|
| **CSF2** | **CSF3** |
| Frequency (cm$^{-1}$) | Functional group | Frequency (cm$^{-1}$) | Functional group |
| 1459.37 | C=O (narrow) | 692.78 | C-Cl (broad) |
| 1494.63 | – | 725.81 | C-H (narrow) |
| 1603.96 | C=O (medium) | 1030.01 | C-F (medium) |
| 1802.10 | C=O (medium) | 1080.78 | C-N (medium) |
| 1857.27 | – | 1217.74 | C-N (medium) |
| 2919.16 | O-H (broad) | 1494.71 | C=O (medium) |
| 3026.75 | – | 2919.76 | C-H (broad) |

Value in parentheses indicates proposed functional group for the specific frequency. (–) unassigned functional group.
Mortality

The castor leaf treated with *C. scalpelliformis* extract fractions (CSF1, CSF2, and CSF3) fed to *P. scintillans* showed dose dependent mortality (Figure 3). *P. scintillans* mortality was significantly higher in fraction-CSF2 (df = 3,80, \( F = 9.198, p < .01 \)) (LC\(_{50} = 0.810\)) than CSF3 (df = 3,48, \( F = 10.981, p = .05 \)) (LC\(_{50} = 0.859\)) and insufficient and least in CSF1 (df = 3,62, \( F = 0.173, p = .914 \)) (LC\(_{50} = 0.910\)). CSF3 exhibited more than 60% of mortality most of the days test while CSF1 revealed lesser mortality of below 60%. Similar observation was recorded in vijayneem (df = 2,93, \( F = 0.0362, p = .697 \)) and monocrotophos (df = 3,92, \( F = 0.664, p = .719 \)) treated larvae.

Impact on pupation rate adult longevity

The castor leaf treated with different fractions (CSF1, CSF2, and CSF3) of *C. scalpelliformis* and vijayneem was given four days exposure to *P. scintillans* in order to observe its pupation rate and adult longevity (Table 3). The *C. scalpelliformis* treated fractions 2 (CSF2) exhibited lower pupation rate at 0.10% (19.6 ± 0.4%) and higher pupation rate by CSF1 at 0.02% (45.1 ± 0.3%). Monocrotophos also showed lowest pupation rates of 11.0 ± 0.8%. Similarly, adult longevity was recorded in a depleting trend to almost half as the concentrations increased, when compared with the longevity of control in all the fractions. Also the vijayneem treated samples exhibited a similar form of reduction rates, stating that the extracted fractions are well efficient and are in pace to standards vijayneem and monocrotophos (Table 3).

Morphogenesis

Vijayneem treatment reduced the larval size by 10–20% and monocrotophos treatment reduced the

| Fractions | Name of the compound | Common name | RT | MF | MW | Peak area (%) |
|-----------|----------------------|-------------|----|----|----|---------------|
| CSF2      | Hexadecanoic acid, methyl ester | Palmitic acid | 12.67 | C17H34O2 | 270 | 85.60 |
|           | Cholesta-8,24-dien-3-ol, 4 methyl- [3a, 4x]- | | 32.43 | C28H46O | 398 | 8.49 |
|           | 11,14,17-Eicosatrienoic acid , methyl ester | | | | |
|           | Eicosanoic acid, methyl ester | | | | |
|           | 9,12-Octadecadienoic acid, methyl ester (E,E) | | | | |
|           | Cyclopentaneundecanoic acid, methyl ester | | | | |
| CSF3      | 9-Octadecenoic acid, 12-(acetoxy)-, methyl ester, [R-(Z)]- | | | | |
|           | Dodecane, 5,8-diethyl- | | 5.54 | C16H34 | 226 | 7.87 |
|           | Benzhydrazide, 4-hydroxy-N2-(4,5,6,7-tetrahydro-5-hydroxiamino benzofuran-4-ylideno)- | | 8.23 | C13H11N5 O4 | 301 | 11.80 |
|           | 9,12,15-Octadecatrienoic acid, 2-phenyl-1,3-dioxan-5-yl ester | | | | |

Table 2. GC–MS spectrum of *Caulerpa scalpelliformis* fractions of column chromatography (CSF2 and CSF3).
larval size by 3–6%. In C. scalpelliformis treated, we observed morphological abnormalities in its body which leads to quick mortality.

Mortality and feeding inhibition against B. mori

The silkworm larvae fed with C. scalpelliformis fractions, vijayneem, monocrotophos treated mulberry foliage caused larval mortality in a dose-dependent manner. Monocrotophos treated mulberry leaves fed B. mori showed 100% mortality within a day. When compared to C. scalpelliformis fractions, vijayneem showed much higher mortality and FI effect. The mulberry leaves treated with different fractions of C. scalpelliformis showed the higher corrected mortality in CSF1 (df = 3.56, F = 1.519, p = .220), CSF2 (df = 3.56; F = 5.464, p = .002), and in CSF3 (df = 3.56, F = 1.270, p = .293) (Figures S1–S3). At 0.8% of tested concentrations, mortality was observed to begin from day 4 and 3 for CSF1, CSF2 respectively while CSF3 also exhibited mortality from day 3 yet at 0.10% of tested concentrations. All fractions revealed 100% mortality. Feeding inhibition impact of different fractions of C. scalpelliformis is presented in Figure 4. Results revealed that CSF1 has less impact as observed in P. scintillans. However, CSF2 showed more impact than CSF3.

Discussion

In Asia, P. scintillans was observed in China, India, Indonesia, Malaysia, Singapore, Sri Lanka, Thailand, and Vietnam (https://www.cabi.org/isc/datasheet/23364#toreferences). P. scintillans was recorded in mango (Soumya 2019), Oak (Xi-Sheng et al. 2010), apple (Gupta and Tara 2014), castor (Muthukrishnan and Selvan 1993), etc. Previously, the life trait of P. scintillans was studied by Koshiya et al. (1977) using castor and also by Shamila and Pandey (2004) using
Robinia pseudoacacia as food in a briefly. This gave an elaborate view of *P. scintillans* biology can be used for future research and related purposes.

Superior to terrestrial plants seaweeds had been known for its many bioactive compounds that are employed in food, medical, and industrial products. Thus, development of novel botanical insecticide is pivotal from the diverse bioactive compounds of seaweed. Thereby with the objectives of this study novel chromatographic fraction of *C. scalpelliformis* was established. Among the different plant foods presented to phytophagous *P. scintillans*, *R. communis* was of utmost preference. The food utilization differs in relation to odor (Senthamizhselvan and Muthukrishnan 1988). Physical texture, chemical composition, and nutrient levels indicate castor preference over other food plants.

The column chromatographic fractions of *C. scalpelliformis* such as CSF2 and CSF3 revealed FA derivative to be dominant phytochemical analyzed. Further, the FT-IR analysis of fractions revealed functional groups of lipids, carbohydrate, alkenes, alkyl haline, and aromatic. The water extracts of *C. racemosa* displayed several peaks positioned at 660, 1019, 1061, 1389, 1631, 2854, 2924, and 3416 cm\(^{-1}\) (Kathiraven et al. 2015). The GC–MS examinations had established FA such as hexadecanoic acid, octadecanoic acid, and octadecatrienoic acid in fraction CSF2 and CSF3. Previous studies also revealed the presence of FAs in *Caulerpa* species such as *Caulerpa taxifolia* (M. Vahl.) C. Ag. (Iveša et al. 2004), *Caulerpa lentillifera* (Ratana-arporn and Chirapart 2006), *Caulerpa racemosa* (Blazina et al. 2009, Hao et al. 2019). Dodecane, 5,8-diethyl-constituted 7.87% in CSF3 as reported in another green algae *Ulva lactuca* Linn (Babu et al. 2014). 3-Dioxan-5-yl ester (12.92%), 2-phenyl-1 and 9,12,15-octadecatrienoic acid was also reported from *Nelumbo nucifera* seeds (Abdelhamid et al. 2015). Previously, dodecane, 5,8-diethyl- was recorded from the leaf extract of *Lantana camara* (Ashmawy et al. 2018) as observed from CSF3 (7.87%).

The FA dominant fractions exhibited repellence activity against *P. scintillans*. *Caulerpa scalpelliformis* extract and its formulation had formerly showed repellent activity against third stadium *Spodoptera litura* larvae and *Dysdercus cingulatus* nymph (Kombiah and Sahayaraj 2012). Fatty acids having C\(_{14}\) to C\(_{24}\) showed feeding (Ramadan et al. 2020), oviposition (Hwang et al. 1984), biting (Ali et al. 2012) deterrence activity to storage pest and mosquitoes. Dodecane, 5,8-diethyl- identified as insect repellent compound (Aanniz et al. 2017). Moreover, CSF2 revealed a potential significant FI activity. Similar observation was also recorded by Cruz-Estrada et al. (2019). According to dos Santos et al. (2019), the negative impacts of extracts on biomass gain through the inhibition of food consumption might be due to FAs. The FA dominant CSF2 displayed greater mortality of *P. scintillans*. It is illustrated that saturated FA, palmitic acid is common in *Caulerpa* sp. (Hao et al. 2019) and also in green seaweed *Ulva rigida* (Gao et al. 2018). Palmitic acid caused only 17.75% mortality in *Sitophilus granarius* (Coleoptera: Curculionidae) in contact toxicity bioassay, but the hexane extracts of *Polytrichastrum formosum* which contain FAs in large quantity showed highest insecticidal activity (70.33%) (Abay et al. 2013). Thus results revealed that highest mortality in CSF2 is due to the combined action of palmitic acid along with other

![Figure 4. Impact of Caulerpa scalpelliformis fractions (SCF1, SCF2, and SCF3) on feeding inhibition (%) Bombyx mori larvae during 4-days observation.](image-url)
phytochemicals observed in the fraction as proposed by Romo-Asunció et al. (2016). Ricinoleic acid is also reported in Caulerpa sp., which showed antioxidant, scavenging, and anti-proliferative activities (Tanna et al. 2018) but insecticidal activity was not reported for ricinoleic acid, hence it is considered as first report. Indeed vijayneem and monocrotophos revealed similar observations stating that the extracted fractions are efficient and are in pace to the standards studied. In India, commercial biopesticides vijayneem has been utilized as positive control against many pestiferous insects along with botanicals (Ezhil Vendan et al. 2010, Muthu et al. 2010, Santhanam and Eligu 2014) as observed. In the instance of B. mori, all the CS fractions illustrated significant mortality. The present findings revealed the pesticide in efficient enough to induce mortality and inhibit larval growth in silkworm. Similar pesticide inhibitory effects such as deformed insect production, delayed molting, complete inhibition at high doses had been observed in many insects species (Schmutterer 1990).

Conclusions

From the present study, it can be concluded that all the tested fractions of Caulerpa scalpelliformis attracted both P. scintillans larvae and adults while higher significant mortality was achieved in the case of CSF1 fraction against P. scintillans, this was also found to be same in the case of treated mulberry leaves when fed to Bombyx mori. A similar observation was obtained in standard pesticides used in this study, thereby stating that the efficiency of the extracted fractions is in pace with the standards that been already commercialized. Furthermore, the treated animals were observed to hold few morphological abnormalities leading to immediate death while the standard pesticides reduced only its larval sizes. The GC–MS spectrum of CSF2 and CSF3 reveals presence of FA or its derivatives (hexadecanoic acid, octadecenoic acid, and octadecatrienoic acid) which might be the compounds that promote botanical insecticidal activity. Thus, the fractions tested had impact over model organism B. mori. Thereupon by examining its propitious results achieved in the present study using C. scalpelliformis, their potential can be further exploited for the control of agricultural pest.

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Disclosure statement

The authors report no conflicts of interest.

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