Repellent activity of *Caulerpa scalpelliformis* extracts and its formulations against *Spodoptera litura* and *Dysdercus cingulatus* (Fab.)

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ABSTRACT

Botanical pesticides have pesticidal or ovicidal or ovipositional or antifeedant or repellent activities. The development of resistance to existing conventional synthetic pesticides and the increasing public concern over environmental pollution and health hazards created by synthetic pesticides, generate a great need for new types of pest management agent's advantage with higher activity against the target pests, and lower impact on humans and environmental quality. By keeping this idea in our mind, experiments were carried out to formulate and to determine the repellency of thallus extract and emulsifiable concentrations (EC) of *Caulerpa scalpelliformis* (R.Br.) Web. V. Bosse against third stadium *Spodoptera litura* larvae and *Dysdercus cingulatus* nymphs by Y-shape olfactometer. Obtained results indicated that the formulated sea weed extract showed more repellent property than crude extract in dose dependent manner. Crude chloroform extract highly repel *S. litura* (API = -1.00 at 150 min after exposure) than *D. cingulatus* (API = -1.00 at 180 min after exposure) than methanol and hexane extract of *C. scalpelliformis*. All the prepared EC formulations (EC 0.2, 0.4, 0.6, 0.8 and 1.0%) having good physically stable and were highly effective against *S. litura* and *D. cingulatus* respectively. Hence, *C. scalpelliformis*-based formulation can be utilized for the management of agricultural pests like *S. litura* and *D. cingulatus.*

Key words: *Caulerpa scalpelliformis, Dysdercus cingulatus*, formulation, repellent activity, seaweed, *Spodoptera litura*

INTRODUCTION

The widespread use of chemical pesticides has resulted in health hazards to human beings and domestic animals, development of pesticide resistance by pest, pest outbreak and also adverse environmental effects by harming beneficial organisms like natural enemies and pollinators (Sahayaraj, 2011). Mankind has been using naturally occurring compounds of biological substances (Anjan Bhattacharyya *et al.,* 2009), which is considered as a part of ecofriendly management methods in Integrated Pest Management (IPM) approach (Ganga Visalakshy and Krisnamoorthy, 2009). However, marine organisms have not been integrated in IPM. Marine algae are widely spread throughout the coastal areas around many continents and have pesticidal value (Sahayaraj and Kalidas, 2011; Rajesh *et al.,* 2011; Sahayaraj and Mary Jeeva, 2012). Moreover, drifted algae are considered as a biowaste, and this can be utilized in agriculture production purpose.

Tobacco cutworm, *Spodoptera litura* (Fab.) (Lepidoptera: Noctuidae) is polyphagous crop pests, has emerged as a serious and dominant pest on many agricultural crops causing enormous losses (Deshmukhe *et al.,* 2010) and distributed throughout south and eastern world tropical infesting 112 species belonging to 44 families (Chari and Patel, 1983). In India, it feeds on 74 species of cultivated crops and some wide plants (Hummelbrunner and Isman, 2001; Ranga Rao *et al.,* 2008).

The cotton stainer *Dysdercus cingulatus* (Fab.), commonly known as red cotton bug causes serious damage by feeding on developing bolls and ripe cotton seeds (Natarajan and Rajendren, 2005). It is distributed all over the cotton producing regions of India (Sahayaraj and Illayaraja, 2008). In India cotton production is about 295 million bales (H" 480 1b bales) during 2009-2010 against 113.9 million bales in the rest of the world. Also, India has the largest area under cotton cultivation (10.31 million ha) and yield was 486 kg ha^{-1} during 2009-2010 (Cotcorp, 2010). Due to hazards associated with the increased use of synthetic pesticides the use of biopesticides especially from marine algae has gained considerable attention on the ecofriendly approaches for the management of insect pest.

In the present investigation, the repellent activity of algal seaweed, *Caulerpa scalpelliformis* extracts and its formulations was evaluated against insect pests such as, *S. litura* and *D. cingulatus.*

Table 1. Material safety data sheet for *Caulerpa scalpelliformis* extract based emulsifiable concentration

Identity/appearance (colour)	Greenish yellow
Odour	Mild odour
Type of formulation	EC (Emulsifiable concentration)
Content of active ingredient(s)	*Caulerpa scalpelliformis* thallus chloroform extract
Adjuvant	Teepol
Solvent (Carrier material)	Acetone (99%) CH_3 $COCH_3$, M = 58.08g/mol
Water content/ Moisture	There was no formation of turbidity, solid or oily matter. Thus it proved that it was stable even under hot and cold conditions
a) Heat stability b) Cold stability	
Viscosity	Non-viscous liquid
Storage stability	Stable at room temperature
Flammability	Flammable
pH	6.0-6.5
Emulsion stability	No turbidity or No creaming layer or No sedimentation while 2 ml of the same dissolved in 100 ml water in measuring cylinder
Flow ability	Freely flowable

MATERIALS AND METHODS

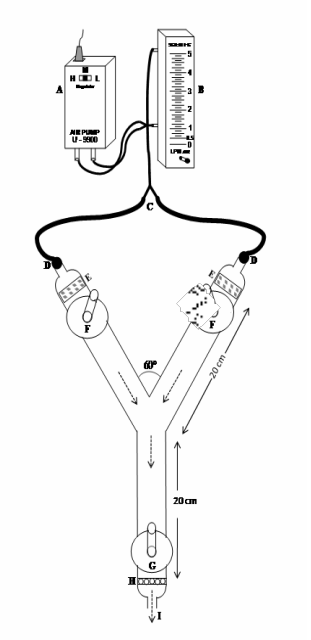
Collection and extraction of seaweeds

The algae, *Caulerpa scalpelliformis* was collected by hand picking method from the submerged marine rocks at Idinthakarai, Tirunelveli coast of Tamil Nadu, India from June 2009 to 2010. They were washed thoroughly thrice with tap water and once with sterile distilled water to remove salt, sand and epiphytes. Then shade-dried for two weeks and partially powdered using domestic blender. 100 gm of partially powdered seaweed was packed in Soxhlet apparatus and refluxed with hexane (30° C– 40° C), chloroform (30° C – 40° C) and methanol (40°C-50°C) individually for 24 hrs continuously. Extraction solvent was evaporated and dried over Sodium Sulphate in dessicator under vacuum. The crude extracts were stored at -20° C until further use.

Preparation of *C. scalpelliformis* based Emulsifiable Concentration (EC)

Five concentrations (0.2, 0.4, 0.6, 0.8 and 1 %) of EC formulations were prepared using acetone as solvent and teepol as emulsifier and epichlorohydrin (1mL) as stabilizer. For preparing 100 ml of 1.0% EC, 1 gram of extract was mixed with 90.5 ml of acetone and 7.5 ml of teepol in 250 mL conical flask. The solution was mixed well in a rotary shaker (Remi, Mumbai) for 30 minutes at room temperature. The mixture was filtered using Whatman No. 1 filter paper. Finally, 1 mL epichlorohydrin was added to the filtrate and mixed well. Similarly other EC's such as 0.2, 0.4, 0.6, and 0.8% were prepared.

Figure 1. Y-shape olfactometer used for the assessment of repellent activity of pests analysis. A) Aerator, B) Airflow meter, C) Rubber tube, D) Air inlet, E) Activated charcoal filter chamber, F) Sample chamber, G) Insect releasing chamber, H) Sieve glass filter, I) Air outlet, ⇢) Direction of airflow



Physical stability tests of EC formulations

For spontaneity test, the EC formulations were added slowly drop by drop in a beaker containing 200 mL of well and tap water separately and the spontaneity property was observed while mixing with a glass rod. For emulsion stability test, exactly 2 mL of each EC formulation was taken in a 10 ml capacity beaker and 100 ml of standard hard water (SHW) was added slowly using a dropping funnel with continuous stirring. The milky white emulsion formed was transferred to a 100ml measuring cylinder and kept undisturbed for an hour. For heat stability test, 50 mL of EC taken in a 100 mL bottle with airtight lid was placed in an incubator maintained at 55°C for 24 hrs. Then it was allowed to cool at room temperature. Similarly for cold stability, about 50 ml of the EC was taken in a 100 ml glass vial, closed airtight with a lid and cooled to 10°C by placing in ice cold water for 1 hr. The above tests were conducted using water of varying hardness such as distilled water (DW), well water and pond water. All the above tests were compared with the commercially available EC formulations of neem oil (Vijay neem, Fortune Bio-Tech Ltd, Hyderabad). All the physical stability parameters were tested at zero day, thirty days, sixty days and ninety days after their preparation using the methodology of Meenakshisundaram (1991).

Collection and rearing of pests

Different larval stages of *S. litura* and nymphs, adults of *D. cingulatus* were collected from castor and cotton fields of Tirunelveli district, Tamil Nadu, India. The collected insects were maintained in the insectory under laboratory conditions (28±2° C; 70-75% RH; 11L and 13D Hrs photoperiod). Larvae of *S. litura* were maintained with castor leaves, whereas *D. cingulatus* were maintained with cotton seeds in plastic containers (22 cm height, 8 cm diameter) containing a layer of sterile moist coarse sand (4 cm height). Pests were maintained for a generation. During second generation, the laboratory emerged third instar larvae and nymphs were used for the present experiment.

Repellent activity bioassay

Bioassay studies were carried out using uniform sized 3 hr starved third stadium *S. litura* (17.8±0.62 mg) and *D. cingulatus* (24.7±0.4 mg) selected randomly from the stock culture. The repellent bioassay was conducted in a customized Y- shaped glass olfactometer (Figure 1) (2.5 cm internal diameter, 20 cm stem length, 20 cm arms length). The olfactometer was clamped on to a tripod in a horizontal position. An activated charcoal (Sigma) filtered air stream (Universal Lab Product) (200 mL min^{-1}) was supplied to each arm of the olfactometer by using an electric pump (Boy U, U-9900, China). Each air stream then passed through a glass chamber (4 × 8 cm) containing test material (a piece of 7.065 cm^3 filter paper dosed with 100 µl of EC formulation).

The crude extracts and its emulsifiable concentrations (EC's) (0.2, 0.4, 0.6, 0.8 and 1.0 EC) of *C. scalpelliformis* was impregnated with Whatman No. 1 filter paper (3 cm diameter) and placed into the test chamber of Y- shape olfactometer and at the other end a filter paper with respective solvents

Table 2. Assessment of physical stability tests of EC formulations of *Caulerpa scalpelliformis* chloroform extract

Storage time (days)	Formulation	Spontaneity/ emulsion stability tests	Test for hard water stability	Test for heat/cold stability	P^H	Maximum absorbance
0	CSE	yellowish white emulsion	no flocculation, turbidity, solid or oily matter	no formation of turbidity, solid or oily matter	6.0 - 6.5	0.289
	NOE	Milky white emulsion	no flocculation, turbidity, solid or oily matter	no formation of turbidity, solid or oily matter	7.0	0.135
30	CSE	yellowish white emulsion	no flocculation, turbidity, solid or oily matter	no formation of turbidity, solid or oily matter	6.0 - 6.5	0.282
	NOE	Milky white emulsion	no flocculation, turbidity, solid or oily matter	no formation of turbidity, solid or oily matter	7.0	0.137
60	CSE	yellowish white emulsion	no flocculation, turbidity, solid or oily matter	no formation of turbidity, solid or oily matter	6.0 - 6.5	0.285
	NOE	Milky white emulsion	no flocculation, turbidity, solid or oily matter	no formation of turbidity, solid or oily matter	7.0	0.135
90	CSE	yellowish white emulsion	no flocculation, turbidity, solid or oily matter	no formation of turbidity, solid or oily matter	6.0 - 6.5	0.292
	NOE	Milky white emulsion	no flocculation, turbidity, solid or oily matter	no formation of turbidity, solid or oily matter	7.0	0.134

CSE - *Caulerpa scalpelliformis* chloroform extract based EC formulation
NOE - Neem oil Extract (Commercially available Vijay Neem)

Figure 2. Repellent activity of *S. litura* third instar caterpillars and *D. cingulatus* third instar nymphs exposed at different concentrations (%) of *C. scalpelliformis* hexane (HE), methanol (ME), chloroform (CH) and crude extract

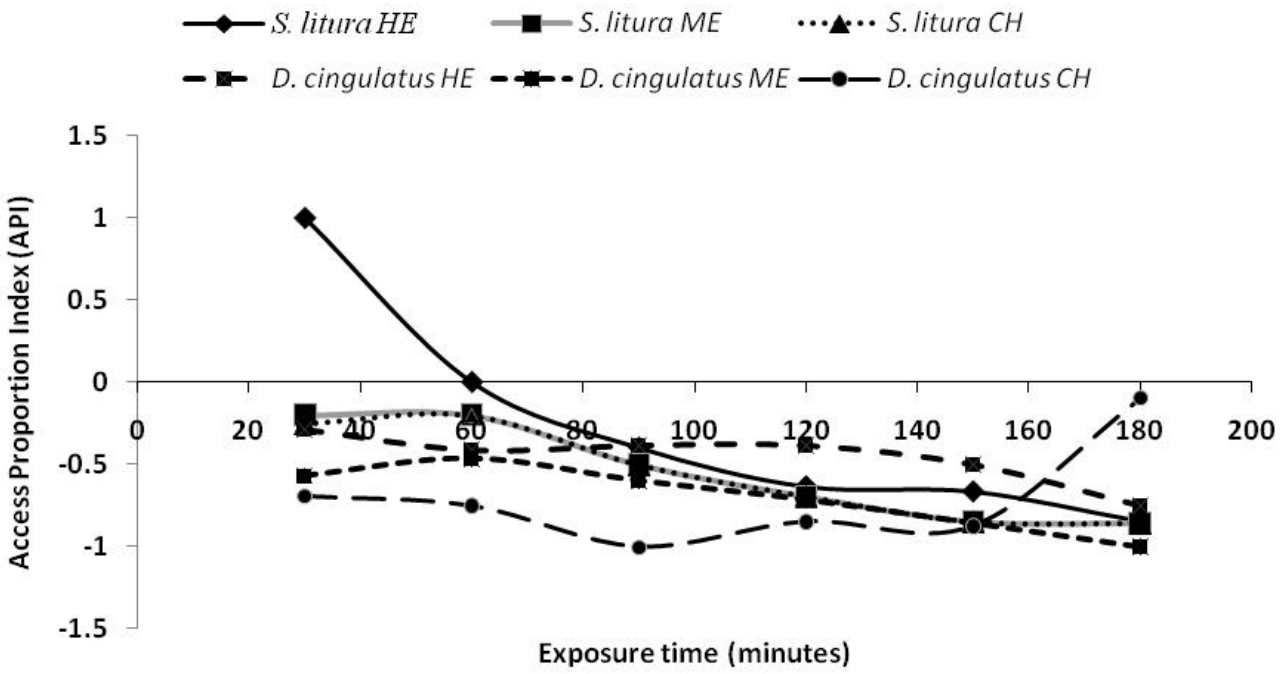
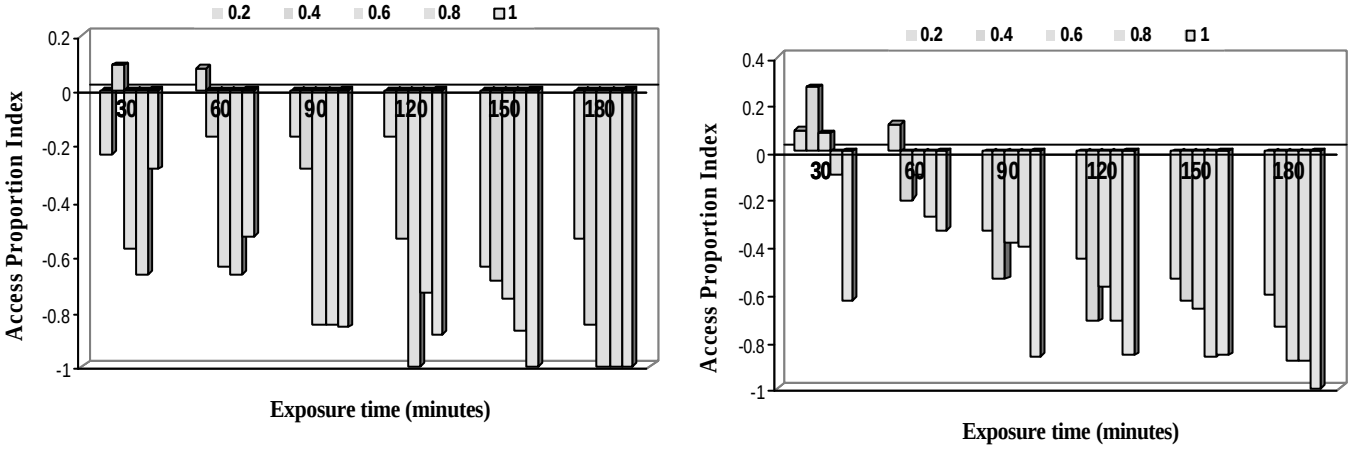


Figure 3. Repellent behaviour of *S. litura* larvae (a) and *D. cingulatus* nymphs (b) against *C. scalpelliformis* based formulations



(Hexane, Chloroform and Methanol) and acetone, respectively were inserted. Three third instar larva/nymphs of *S. litura* and *D. cingulatus* were released one after another into the release chamber (Bottom arm). Number of insects found on the treated and untreated filter paper was recorded after 30, 60, 90, 120, 150 and 180 minutes continuously. The experiment was replicated six times with different insects. The olfactometer was washed with sodium hypochloride (0.1%) after each replicate. The insects preferred either control or treated filter paper or neither. If the insects chose neither of the chambers then it was considered that they made no choice. From the observation recorded the Access Proportion Index (API) was performed using the following formula Access Proportion Index = NS-NC/NS+NC, Where NS = Number of insects choosing the sample side and NC = Number of insects choosing the control side. Pest's olfactory behavioral responses were observed in the Y-shape olfactometer itself.

RESULTS AND DISCUSSION

EC formulation physical stability

The prepared EC formulation of *C. scalpelliformis* extracts were easily mixed with water producing yellowish white emulsion instantaneously like commercially available neem oil EC formulation that proves spontaneity property. EC formulation had the emulsion stability since the volume of yellowish white cream formed at the top and sediments at the bottom did not go beyond 2 mL per minute. The results showed that there was no flocculation, turbidity, solid or oily matter when EC formulations were mixed with hard water. The hot and cold stability test also showed that there was no formation of turbidity, solid or oily matter. Thus it was stable even under hot (50°C) and cold (10°C) conditions (Table 2). All the physical stability parameters showed similar results, while it was tested at 0, 30, 60 and 90 days after their as recorded by Vanitha (2010). As reported by Tawfik and El-Sisi (1991), Farghaly *et al*. (2009), Abou-Yousef *et al.* (2010), the reduced values of pH of formulated solutions have more attraction between the extracted particles and surface treated plants. Further more, the decrease in the surface tension of the formulated extracted particle decreases for their spreading and decomposition on the surface of the treated plants.

Repellent activity bioassay

Results of the present study revealed that the formulated sea weed extracts have more repellent property than crude extracts in dose dependent manner. Crude chloroform extract highly repel *S. litura* (API = -1.00 at 150 min after exposure) higher than *D. cingulatus* (API = -1.00 at 180 min after exposure) followed by methanol and hexane extract of *C. scalpelliformis* (Fig. 2). Moreover, 0.6 to 1 EC were highly effective for *S. litura* and *D. cingulatus* (Fig. 3).

Work on the algae for the management of insect pest is very scanty. Previously, it was reported that (Sahayaraj and Kalidas, 2011; Sahayaraj and Mary Jeeva, 2012) seaweed *Padina pavonica* and *Sargassum tenerrimum* crude extracts have ovicidal, ovipositional and insecticidal activity against *D. cingulatus*. In this present study we recommend not only the crude extract but also the formulations of sea weed *C. scalpelliformis* which can be utilized for the management of *S. litura* and *D. cingulatus*. During the observation of repellent activity in the Y- shape olfactometer, we noticed some of the behavioral changes. *D. cingulatus* frequently cleaned their antenna after exposed in the formulation. *S. litura* slowly moves to the opposite direction, the larvae turned into black colour in crude chloroform extract and its formulation. After 180 minutes of exposure, 33.33% of the insects died at 1.0 percent EC. Cetin*et al.* (2010) reported the larvicidal efficacy of the acetone extract of the thalli of *Caulerpa scalpelliformis* against late second to early third instars of *Culex pipiery* at 1200 ppm. Rajesh *et al.* (2011) reported that *C. scalpelliformis* was highly toxic to *D. cingulatus* and *Fusarium oxysporum* f.sp. *vasinfectum.* Hence, *C. scalpelliformis* - based formulation can be utilized for the management of agricultural pest like *S. litura* and *D. cingulatus.*

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