Biocidal activity of two marine green algal extracts against third instar nymph of *Dysdercus cingulatus* (Fab.) (Hemiptera: Pyrrhocoridae)

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ABSTRACT

Seaweeds are the extraordinary sustainable resources in marine ecosystem which have been used traditionally as source of food, feed and medicine. The biological effects of thallium Hexane (HE), Chloroform (CH), Methanol (ME) and Water (AQ) extract of *Ulva fasciata* Delile (UF) and *Ulva lactuca* Linnaeus (UL) were tested against *Dysdercus cingulatus* (Fab.) third instar nymphs at different concentrations (100, 200, 400, 600 and 800 ppm). Tested green algae caused dose dependent mortality. The ME of UF (LC_{50}= 313.59 ppm) and UL (LC_{50}= 399.27 ppm) shows more nymphicidal activity at 96 hrs. It is concluded that methanol extract of both UF and UL possesses nymphicidal, antiovipositional activity, reduced fecundity, hatchability, adult longevity and relative growth rate. However, more detailed studies are essential before recommending them for pest management programme.

Key words: Cotton pest management, *Dysdercus cingulatus*, nymphicidal activity, ovipositional activity, seaweeds, *Ulva fasciata*, *Ulva lactuca*

INTRODUCTION

In recent years, yield of cotton has become static rather it is declining due to the infestation of insect pests and diseases. Nearly 162 species of insect pests cause low yield of cotton production. The sucking pests of cotton includes cotton stainer, jassids, aphids, white flies and thrips (Uthamasamy *et al*., 2004).The red cotton bug or cotton stainer *Dysdercus cingulatus* (Fab.) (Hemiptera: Pyrrhocoridae) is considered as a serious pest of cotton (Waterhouse, 1998; Tanu Sharma *et al*., 2010). It infests the cotton from young stage still harvest. This pest causes serious damage by sucking the developing cotton bolls and ripe cotton seeds and transmitting fungi that develops in the immature lint and seeds (Fuseini and Kumar, 1975; Yasuda, 1992). *Dysdercus cingulatus* is difficult to control by insecticidal application because they are highly mobile and have many alternative wild hosts belonging to Malvaceae (Iwata, 1975; Kohno and Ngan, 2004).The marine algae are the renewable living resources which are rich source of structurally important novel and biologically active metabolites. Marine algae have been shown to have bactericidal (Febles *et al*., 1995; Del *et al*., 2001; Ely*et al*., 2004 and Cordeiro*et al*., 2006), fungicidal (Rajesh *et al*., 2011) and insecticidal activities (Biju *et al.*, 2004; Cetin*et al*., 2010; Sahayaraj and Kalidas, 2011; Sahayaraj and Mary Jeeva, 2012). Further more, seaweed extracts offer a novel approach to pest management (Manilal *et al*., 2009; Rajesh *et al*., 2011; Sahayaraj and Kalidas, 2011; Sahayaraj and Mary Jeeva, 2012).*Ulva fasciata* Delile is a marine macroalga, which grows abundantly in the coastal waters of South India.*Ulva fasciata* and *Ulva lactuca* have been reported to possess antioxidant and antibacterial properties (Beach *et al*., 1995; Rouxel *et al*., 2001). The previous reports do not show much information regarding insecticidal activity of seaweed extracts against *D. cingulatus.* The present reports deals with the bioefficacy of *U. fasciata* and *U. lactuca* on biological insecticidal, fecundity, hatchability, adult longevity and relative growth rate (RGR) of *D. cingulatus.*

MATERIALS AND METHODS

Collection, extraction and preparation of seaweed extract

The algae *Ulva fasciata* and *Ulva lactuca* were collected by hand picking method from the submerged marine rocks at Idinthakarai (N 08Ú10' 32.3"; E 077' 44'31.3") Kuthenkuzhi (N 08'10'32.3"; E 077'46'58.4") and Tuticorin (N 08'46'32.1"; E 078'11'56.5"), Tamil Nadu, India from June to December, 2010. The seaweeds were washed thoroughly thrice with tap water and once with sterile distilled water to remove salt, sand and epiphytes. Shade dried for two weeks and partially powered using domestic blender (Preethi XL-7, Maya Appliances (P) Ltd. Madras). For extraction, 500 gm of powdered algal material was extracted by Soxhlation method using polar (methanol- ME, water- AQ) and non-polar (chloroform- CH, hexane- HE)

solvents. The extracts were concentrated under reduced pressure by dessicator, collected in air tight glass vials (9.4 cm) and stored in the refrigerator (LG, India) for further use.

Pest collection and rearing

Nymphs and adults of *D. cingulatus* were collected from cotton fields of Tirunelveli districts, Tamil Nadu, India. The collected pests were maintained in the insectory under laboratory conditions (temperature 28 ± 2°C, 70 ± 5 % RH and a photoperiod of 11L: 13D hrs) in transparent plastic containers (8cm height × 6.5cm diameter) containing a layer of sterile coarse sand (4cm thick). Insects were fed with its natural host cotton seeds and also cotton- seed- based artificial diet (Sahayaraj *et al.*, 2011). Insects were maintained at least for 2 generations. The laboratory emerged 6-12 hrs old third stadium *D. cingulatus* were used for this experiment.

Nymphicidal activity bioassay

Bioassay studies were carried out using uniform sized (24.7±0.4 mg weight), 6-12 hrs old third stadium *D. cingulatus* which was selected randomly from the stock culture. Five insects were placed in a transparent plastic container (8 cm height × 6.5 cm diameter). Different concentrations of *U. fasciata* and *U. lactuca* extracts [100, 200, 400, 600 and 800ppm (4mg extract in 5ml diet- 800 ppm)] mixed in artificial diet (AD). Experimental animals were allowed to feed the AD for 96 hrs continuously. 200µL AD was pour into the small cotton ball and provided to the insects. The food was changed every day. Six replications were maintained for each concentration. Mortality was recorded every 24 hrs, till 96 hrs. The mortality was corrected using Abbott's formula (Abbott, 1925), if more than 10% mortality was observed in control category. The corrected mortality data was subjected to probit analysis (Finney, 1971) to find out the LC_{50}, Chi square, df and p values. After 96 hrs, live nymphs were provided with water soaked cotton seeds till their death.

Parameters recorded

The nymphal weight of *D. cingulatus* was recorded during the experimental period (0, 24, 48, 72 and 96 hrs). The relative growth rate (RGR) (Isikiber and Copland, 2002) of *D. cingulatus* was calculated at 24, 48, 72 and 96 hrs by the following formula: RGR = (Fwt - Iwt)/ [(Fwt + Iwt)/2] x D. Where, Fwt = final weight of the insect, Iwt = initial weight of the insect and D = experimental days after the emergence, the sex ratio, male longevity, female longevity, copulation time, fecundity, hatching percentage and incubation period were recorded for all the live insects.

Statistical analysis

Data of male longevity, female longevity, copulation time, fecundity, hatchability and incubation period were subjected to paired sample't' test, the significance was expressed at 5% level. Data were analyzed by using SPSS software (11.5 versions).

RESULTS

Invariably, *Ulva* extracts caused dose dependent mortality, among the four solvents; methanol extract of *Ulva* caused more mortality than other extracts (Figure 1a, 1b). The methanol extract of UF (LC_{50}= 313.59 ppm) and UL (LC_{50}= 399.27ppm) showed potent nymphicidal activity (81.48% and 70.37% respectively) at 96 hrs compared to other extracts (Table 1). During the experimental time the weight of the insect was significantly decreased when the concentration was increased in HE (t=2.5; df= 5; p=0.057), CH (t=9.07; df=5; p=0.000) and ME(t=15.31; df=5; p=0.000) and UL-CH (t=22.55; df=5; p=0.000), ME (t=18.20; df=5; p=0.000). But in water extract of both UF and UL, the weight was increased as control (Table

Figure 1. Impact of *U. fasciata* (a) and *U. lactuca* (b) hexane, chloroform, methanol and water extracts (ppm) on the total nymphal corrected mortality (%) of *D. cingulatus* third instar nymph at 96 hrs after the exposure

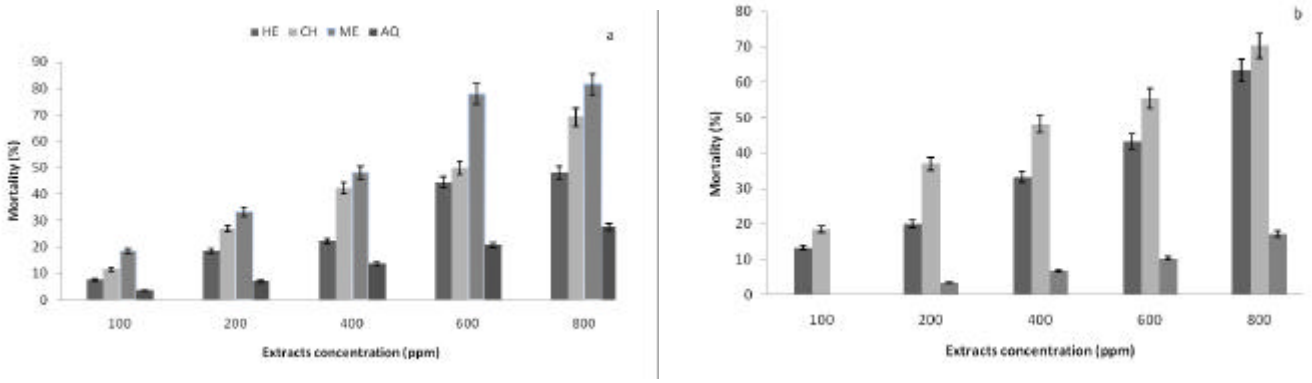


Table 1. Impact of *U. fasciata* and *U. lactuca* hexane, chloroform, methanol and water extracts (ppm) on LC_{50} values and fiducidal limits, chi square parameters of *D. cingulatus* third instar nymphs exposed after 96 hrs.

Extract	LC_{30}	LC_{50}	LC_{90}	Chi Square	df	p
Ulva fasciata						
HE	400.91	875.13	5896.64	4.131	3	0.248
CH	244.87	493.96	2744.54	2.798	3	0.424
ME	173.65	313.59	1329.46	7.182	3	0.066
AQ	954.09	2282.47	19238.67	0.233	3	0.972
Ulva lactuca						
CH	294.22	643.61	4359.48	4.911	3	0.178
ME	170.89	399.27	3176.53	2.224	3	0.527
AQ	1456.28	2938.49	16338.05	1.488	3	0.685

2 and 3). The relative growth rate (RGR) was relatively low in HE, CH and ME extract of both UF and UL. Whereas, in water extract has slightly increased their growth rate (Fig. 2a, 2b).

Males lived longer than the females both in experimental and control categories. Shorter male longevity was observed significantly (t=2.88; df=5; p=0.102) in UF hexane extract at 100 ppm. During the adult life time of the male and female control category spent one fifth of their life time for mating. The time was prolonged twice when 600 ppm algal seaweed extracts mixed diet was provided. In 200 ppm category, male biased sex ratio was reduced from 0.46 to 0.12; 0.42 to 0.20; 0.44 to 0.33 for hexane, chloroform and methanol extract of *U. fasciata* respectively. Similar impact was also observed for *U. lactuca* treatments. When, the seaweed concentration increased, fecundity UF and hatchability of UF and UL, respectively decreased and minimum fecundity was observed in methanol extract of UF at 200 ppm (Table 4). In UL, low fecundity was observed in methanol extract at 400 ppm. Incubation period lasted for 3.5-5.5 days in higher concentrations of both seaweeds.

Table 2. Effect of *U. fasciata* hexane, chloroform, methanol and water extract on whole body wet weight (mg) of *D. cingulatus* nymphs at different time intervals

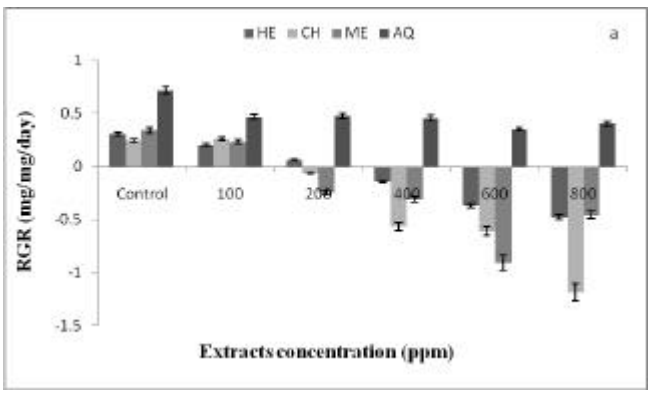
Conc (ppm)	Weight of the nymphs (mg)				
	00	24 hrs	48 hrs	72 hrs	96 hrs
Hexane					
000	25.1±0.8	25.4±0.8	26.3±0.7	27.0±0.7	27.2±0.9
100	25.5±0.5	25.6±0.6	26.1±0.5	26.3±0.5	26.8±0.4
200	25.6±0.4	25.7±0.4	25.8±0.3	27.2±0.8	27.3±0.7
400	27.7±0.8	27.2±0.7	27.2±0.6	26.9±0.5	26.7±0.4
600	25.5±1.5	24.9±1.5	24.7±1.2	24.5±1.4	23.2±1.2*
800	26.5±0.8	25.7±0.7	25.6±0.6	25.4±0.3	23.5±0.9
Chloroform					
000	25.7±1.2	25.8±1.2	26.5±1.0	26.9±0.9	27.3±0.9
100	25.7±0.4	25.9±0.4	26.4±0.3	27.1±0.1	27.4±0.2
200	26.7±0.6	27.1±0.6	27.5±0.7	26.9±0.7	26.3±0.7
400	26.6±0.6	26.4±0.6	26.2±0.6	24.9±0.3	23.1±0.5*
600	27.6±0.8	27.4±0.8	26.5±0.6	24.7±0.8	23.7±0.8*
800	27.6±0.8	27.3±0.8	25.6±0.7	23.7±0.6	20.5±0.6*
Methanol					
000	25.5±0.3	25.7±0.3	26.2±0.3	26.9±0.3	27.8±0.2
100	25.7±0.2	25.9±0.2	26.3±0.2	26.8±0.2	27.3±0.2*
200	24.8±0.2	24.7±0.2	24.6±0.1	24.3±0.1	23.4±0.3*
400	25.9±0.2	25.5±0.2	25.0±0.2	24.7±0.2	24.0±0.1*
600	25.9±0.3	24.9±0.6	24.2±0.5	23.0±0.3	20.6±0.4*
800	25.9±0.2	23.5±0.5	21.3±0.4	19.4±0.2	16.3±0.7*
Water					
000	25.0±0.3	25.3±0.3	26.8±0.2	27.9±0.1	29.9±0.5
100	25.6±0.4	26.1±0.4	26.7±0.3	27.6±0.3	28.7±0.4
200	25.5±0.3	26.0±0.2	26.8±0.2	27.8±0.2	28.7±0.2
400	25.2±0.3	25.6±0.3	26.8±0.3	27.6±0.4	28.2±0.2*
600	25.7±0.2	25.9±0.2	26.6±0.2	27.3±0.2	28.1±0.2
800	25.4±0.2	25.9±0.3	26.6±0.3	27.1±0.3	28.2±0.2*

*-indicates significance at 5% level with Paired t test

DISCUSSION

Sahayaraj and Kalidas (2011) reported that the seaweed *Padina pavonica* caused nymphicidal and ovicidal effect on cotton pest *D. cingulatus*. Our results revealed that the algal extract mixed with artificial diet enter into the alimentary canal while feeding and caused mortality. The methanol extract of *Ulva fasciata* and *Ulva lactuca* caused highest nymphal mortality against *D. cingulatus.* The red algae, *Laurencia nipponica* showed strong insecticidal activity against *Culex pipens pallens* larvae as reported by Watanabe *et al*. (1989) and El Sayed *et al*. (1997). Cetin *et al*. (2010) reported that acetone extract of *Caulerpa scalpelliformis* (thalli) showed

Figure 2. Relative growth rate (RGR) (mg/mg/day) of *D. cingulatus* effect of *U. fasciata* (a) and *U. lactuca* (b) hexane, chloroform, methanol and water extracts(ppm) at 96 hrs



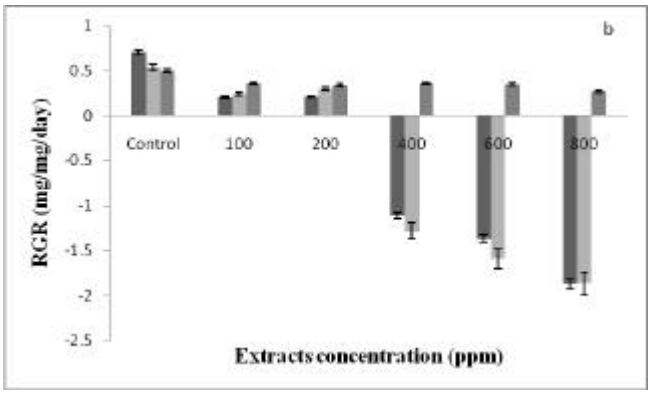


Table 3. Effect of *U. lactuca* chloroform, methanol and water extract on whole body wet weight (mg) of *D. cingulatus* nymphs at different time intervales

Conc (ppm)	Weight of the nymphs (mg)				
	00	24 hrs	48 hrs	72 hrs	96 hrs
Chloroform					
000	24.5±0.3	25.1±0.3	25.4±0.3	26.8±0.3	29.2±0.3
100	25.5±0.2	25.6±0.2	26.0±0.2	26.1±0.2	26.8±0.1*
200	25.3±0.1	25.5±0.1	25.9±0.1	26.3±0.1	26.7±0.1*
400	25.4±0.2	24.9±0.4	24.2±0.4	22.7±0.6	19.2±0.5*
600	24.7±0.2	24.0±0.5	23.4±0.6	20.3±0.5	17.5±0.7*
800	25.5±0.2	24.8±0.2	23.8±0.4	18.9±0.4	15.8±0.5*
Methanol					
000	25.4±0.7	25.8±0.7	26.0±0.7	27.1±0.7	29.1±0.7
100	25.9±0.5	26.1±0.5	26.5±0.5	26.9±0.5	27.6±0.4
200	25.6±0.5	25.7±0.4	26.4±0.3	26.7±0.3	27.6±0.3
400	24.3±0.3	23.3±0.5	22.5±0.5	20.4±0.7	17.6±0.5*
600	25.1±0.4	24.5±0.4	23.1±0.3	19.1±0.2	16.8±0.4*
800	25.4±0.2	24.9±0.2	23.5±0.2	18.9±0.3	15.8±0.3*
Water					
000	25.5±0.3	25.9±0.4	26.5±0.3	27.4±0.2	28.9±0.3
100	25.9±0.4	26.2±0.4	26.5±0.4	27.3±0.4	28.4±0.4
200	25.9±0.4	26.2±0.4	27.0±0.4	27.8±0.4	28.2±0.4
400	25.8±0.2	26.2±0.2	26.6±0.2	27.2±0.2	28.2±0.2
600	25.7±0.2	26.1±0.2	26.4±0.2	27.7±0.1	28.0±0.2*
800	25.8±0.3	26.1±0.2	26.5±0.2	26.9±0.2	27.6±0.1*

*-indicates significance at 5% level with Paired t test

larvicidal activity against late second to early third instars of *Culex pipiery*. Previously, Argandona *et al*. (2000) reported that the red alga *Plocamimum cartilagineum* and *P. violaceum* caused insecticidal activity against tobacco horn worm. Furthermore, brown algae of the family *Dictyotaceae* produce a new diferpene dictyo crenulol, which possesses insecticidal activity against tomato moth *Tuta absoluta* (Soto and San, 2002). Recently, Sahayaraj and Mary Jeeva (2012) reported that the sea weed *Sargassum tenerrimum* extracts and chromatographic fractions caused mortality, reduced the nymphal developmental period, adult longevity, fecundity, total body protein and genomic DNA content of *D. cingulatus*. Similarly our results showed that, the green algal seaweeds *Ulva fasciata* and *Ulva lactuca* reduced the relative growth rate, adult longevity, fecundity and hatchability of *D. cingulatus*. From our results we concluded that *U. fasciata* and *U.lactuca* can be used to mange the red cotton bug *D. cingulatus* as an ecofriendly pest management component.

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Table 4. Effect of *U. fasciata* and *U. lactuca* extract on adult longevity (days), copulation time (days), fecundity (eggs / female), hatchability (%) and incubation period (days) of *D. cingulatus*

Conc (ppm)	*Ulva fasciata*					
	ML	FL	CT	FE	HA	IP
Hexane						
Control	8.2±0.2	6.5±0.2	1.6±0.1	84.6±3.7	72.1±4.9	3.7±0.1
100	7.4±0.3	6.2±0.4	1.7±0.2	72.3±4.3	64.9±3.1	3.8±0.1
200	6.1±0.3 *	5.5±0.4	1.7±0.2	56.6±3.1 *	44.9±4.0*	3.9±0.1
400	5.8±0.3 *	5.0±0.3 *	2.2±0.4	41.0± 2.1	24.8±3.9	4.2±0.2
600	5.4±0.2 *	5.0±0.0	3.0±0.0	30.0±0.0	20.0±0.0	4.5±0.0
800	4.5±0.5 *	0	0	0	0	0
Chloroform						
Control	8.3±0.2	7.6±0.2	1.4±0.2	87.7±3.9	73.8±2.1	3.5±0.2
100	7.4±0.2 *	6.6±0.4	1.9±0.1	75.3±3.1	45.5±2.8*	3.5±0.2 *
200	6.5±0.4	5.3±0.4	2.0±0.0	48.0±4.4	39.4±3.8*	3.7±0.3
400	5.9±0.4*	4.8±0.8	2.2±0.3	36.5± 1.7	27.6±1.4	4.3±0.3
600	5.4±0.4 *	5.0±0.0	3.0±0.0	21.0±0.0	23.8±0.0	4.5±0.0
800	4.3±0.3	0	0	0	0	0
Methanol						
Control	8.3±0.2	7.6±0.2	1.8±0.1	85.5±2.7	76.0±1.4	3.6±0.2
100	7.4±0.7	5.8±0.4	1.7±0.3	60.0±1.2 *	44.9±3.5*	3.8±0.2 *
200	6.6±0.4	5.8±0.3	2.5±0.5	17.0±0.0	9.6±0.5	4.0±0.5
400	4.3±0.4	0	0	0	0	0
600	0	0	0	0	0	0
800	0	0	0	0	0	0
***Ulva lactuca* - Chloroform**						
Control	8.6±0.3	7.6±0.3	1.3±0.2	82.8±0.2	71.5±1.9	3.7±0.2
100	7.2±0.4 *	6.1±0.3 *	1.4±0.2	74.2± 0.8	55.6±1.5	3.3±0.2
200	6.6±0.5 *	5.8±0.8	1.5±0.5	54.5± 1.7	42.0±1.2	4.0±0.0
400	5.8±0.4 *	4.8±0.8	2.0±0.0	38.5± 1.9	37.2±2.8	4.3±0.3
600	5.2±0.2	4.5±0.0	2.5±0.0	33.0±0.0	21.1±0.0	5.0±0.0
800	4.0±0.0	0	0	0	0	0
Methanol						
Control	8.1±0.4	6.7±0.3	1.6±0.2	81.4±3.7	76.9±1.6	3.7±0.2
100	6.6±0.5	5.4±0.4 *	1.5±0.3	75.0±4.7	58.5±4.2	4.1±0.1
200	5.6±0.3 *	5.3±0.3	1.5±0.5	35.5±1.5 *	22.9±2.2	4.8±0.3
400	5.2±0.2 *	4.5±0.0	2.0±0.0	26.0±4.0	11.8±0.0	5.5±0.0
600	3.8±0.8	0	0	0	0	0
800	0	0	0	0	0	0

ML – Male longevity; FL – Female longevity; CT – Copulation time; FE – Fecundity; HA – hatchability; IP – Incubation period; *-indicates significance at 5% level with Paired t test

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