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Screening of insecticidal activity of brown macroalgal extracts against *Dysdercus cingulatus* (Fab.) (Hemiptera: Pyrrhocoridae)

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ABSTRACT

The marine algae, popularly known as seaweeds are one of the most important marine resources of the world. The biocidal activity of hexane (HE), chloroform (CH), methanol (ME) and water (WA) extracts (100, 200, 400, 600 and 800 ppm) of brown algae (Ochrophyta), *Sargassum wightii* (Greville ex J. Agardh) (SW) and *Padina pavonica* (Linn.) Thivy (PP) was assessed against *Dysdercus cingulatus* (Fab.). GC-MS results revealed that *S. wightii* showed the presence of stigmastan-6, 22-dien, 3, 5-dedihydro- (71.34%) whereas *P. pavonica* showed hexadecanoic acid, methyl ester (43.26%). The chloroform extract of *S. wightii* caused more nymphal mortality at 96 hrs (LC_{50}= 631.8 ppm) than *P. pavonica* (LC_{50}= 1062.5 ppm). Further, column chromatographic fractions of *S. wightii* (F164 - F323) (LC_{50}= 175.2 ppm) and *P. pavonica* (F800 - F965) (LC_{50}= 292.7 ppm) showed more nymphal mortality. After 96 hrs, live insects were maintained with normal food and life parameters like adult longevity, mating period, fecundity, hatchability and incubation period were recorded. In *S. wightii* chloroform extract, male longevity and water extracts male longevity (df= 5, 27; F= 8.177; p= 0.005); female longevity (df=5, 24; F= 6.838; p=0.005). Mating period was highly prolonged by the water extracts. Fecundity and hatchability were highly reduced at 800 ppm both by chloroform and methanol extracts. In *P. pavonica*, mating period was highly prolonged by the water extract; fecundity and hatchability were highly reduced by hexane and the incubation period was slightly increased by extracts. Hence, these algal extracts can be used as biocide in cotton pest management.

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INTRODUCTION

Macroscopic marine algae, popularly known as 'seaweeds', are one of the important living resources of the ocean. They are found attached to the bottom in relatively shallow coastal waters. Algae contain rich and largely entrapped sources of a vast assortment of biologically active substances (Daoudi *et al*., 2001; Huang and Lee, 2005; Gouveia *et al*., 2008; Ghosh *et al*., 2009; Seenivasan *et al*., 2010; Chojnacka *et al*., 2012; Munirasu *et al*., 2013). Chemical pesticides have played a significant role in increasing the agricultural production and also in the protection of crops from damage caused by insect pests. It has been estimated that hardly 0.1% of the agrochemical used in crop protection reach the target pests and the remaining 99.9% enter into the environment (Mancini *et al*., 2005; Remor *et al*., 2009; Sharma *et al*., 2012; Goulson, 2013; Abang *et al*., 2013), human beings (Wesseling *et al*., 2001; Konradsen *et al*., 2003; Gupta, 2004; Shrestha *et al*., 2010; Hossain *et al*., 2010; Gulati *et al*., 2010; Tholkappian and Rajendran, 2011; Simon Mburu *et al*., 2013; Chaturvedi *et al*., 2013), domestic animals (Carrea, 2002; Natala and Ochoje, 2009; Chaturvedi *et al*., 2013) and wild animals (Brakes and Smith, 2005; Forson and Storfer, 2006; Kohler and Triebskorn, 2013).

Botanical insecticides (Isman, 1994; Prakash *et al*., 2008; Dadang *et al*., 2009; Mansour *et al*., 2011; Kabiri *et al*., 2012; Abbad and Besheli, 2013; Li, 2013) are ecofriendly and environmentally safer alternative methods for crop protection. The evaluation of plant extracts for their deleterious effects on insects is one of the approaches used for the search of novel botanical insecticides (Isman, 1995). Marine algae are the renewable living

resources which are a rich source of structurally important novel and biologically active secondary metabolites. Marine algae have been shown to have insecticidal activities (Cetin *et* al., 2010; Sahayaraj and Kalidas, 2011; Sahayaraj and Mary Jeeva, 2012; Asha *et al*., 2012; Sahayaraj *et al*., 2012; Syed Ali *et al*., 2013; Bantoto and Danilo Dy, 2013). Furthermore, seaweed extracts offer a novel approach in pest management (Manilal *et al*., 2009; Rajesh *et al*., 2011; Sahayaraj and Kalidas, 2011; Sahayaraj and Mary Jeeva, 2012; Asha *et al*., 2012). Further, they are used as a source of human and animal feed, as well as for fertilizer and herbicide (Manilal *et al*., 2009).

Cotton is the most economically important natural fiber material in the world. One of the major obstacles hindering cotton cultivation is insect pest infestations. India has the largest area under cotton cultivation. Exports are expected to reach 7.5 million 170 kg bales (5.8 million 480 lb bales/1.3 mmt), down from the increased 2012/13 estimate of 9.0 million 170 kg bales (GAIN Report: 2013-2014). 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 (Amin and Gergis, 2006; Ozyigit *et al*., 2007; Singh and Singh, 2007; Minfal, 2008).

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 a serious pest of cotton, which infests cotton in all the cotton growing regions of India (Tanu Sharma *et al*., 2010). The cotton stainers *D. cingulatus* causes serious damage by feeding on developing cotton bolls and ripe cotton seeds and transmitting fungi that develops on the immature lint and seeds (Yasuda, 1992; Sontakke *et al*., 2013). Moreover, the red cotton bug introduces fungi, *Nematospora gossypii* (S. F. Ashby and W. Nowell) (Evemotheciaceae) into bolls causing red staining of the lint, besides depositing excreta, which make the seeds unfit for sowing (Sundaramurthy and Chitra, 1992; Vasantharaj David and Kumaraswami, 1996; Anonymous, 2002; Radhika and Reddy, 2007; Dhaka and Pareek, 2007; Ashfaq *et al*., 2011). *Dysdercus cingulatus* are 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).

Several algal crude extracts such as *Caulerpa scalpelliformis* (Rajesh *et al*., 2011; Kombiah and Sahayaraj, 2012), *Padina pavonica* (Sahayaraj and Kalidas, 2011), *Sargassum tenerrimum* (Sahayaraj and Mary Jeeva, 2012), *U. fasciata* (Asha *et al*., 2012; Sahayaraj *et al*., 2012) and *U. lactuca* (Asha *et al*., 2012; Sahayaraj *et al*., 2012) showed insecticidal activity against *Dysdercus* spp. The previous reports does not show much information regarding insecticidal activity of *Sargassum wightii* extracts against *D. cingulatus.*The present reports deal with the bioefficacy of selected macroalgae crude, olumn chromatographic fractions and their life traits against *D. cingulatus* nymphs.

MATERIALS AND METHODS

Collection and extractions of seaweeds

The selected seaweeds were collected by hand picking method from the submerged marine rocks at four southern districts of Tamil Nadu from July 2009 to June 2010. The seaweeds were collected during low tide in the intertidal and sub-tidal regions where the vegetation was discontinuous and occurring in patches. Moreover, drifted algae were also collected using disposable latex gloves in glass bottles and polythene bags. After collection, the seaweeds were washed thoroughly thrice with tap water and once with sterile distilled water to remove salt, sand and epiphytes. Fresh samples were preserved in 4% formalin. The voucher specimens and herbarium sheets were prepared and deposited in Crop Protection Research Centre, St. Xavier's College (Autonomous), Palayamkottai. The macroalgae were identified by Dr. K. Eswaran, Scientist in-charge at Central Salt and Marine Algal Research Station (CSMARS), Mandapam, Tamil Nadu, India.

The latitude and longitudes of the study areas were recorded using GPS- map 76 (GARMAN). Cleaned seaweeds were shade dried for two weeks, partially powdered using domestic blender (Preethi XL-7, Maya appliances (P) Ltd, Madras) and used for the

experiments. The powdered algal material was extracted by soxhlation and cold percolation method using polar (chloroform- CH, methanol- ME, water- WA) and non- polar solvent (hexane (HE).

Pest collection and rearing

Nymphs and adults of *D. cingulatus* were collected from cotton fields of Tirunelveli districts, Tamil Nadu, India. The collected insects 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 their natural host cotton seeds and also cotton- seed- based artificial diet (Sahayaraj *et al.*, 2011). Before the experimentations, 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 nymphs of *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 [100, 200, 400, 600 and 800 ppm (4mg extract in 5mL diet- 800 ppm)] of the seaweed extracts were prepared and used for the oral toxicity test. In oral toxicity, 10 mg of small cotton ball was soaked in respective extracts in artificial diet + 0.05% Tween 80 was provided to the insects and allowed to feed the same for 96 hrs continuously and was changed every day. In control category, the animals were fed with diet devoid of extract. Six replications were maintained for each concentration. Mortality was recorded for every 24 hrs up to 96 hrs. In addition, the column chromatography fractions of PP (F 14-75; 85-275; 800-965) and SW (F 164-323) were tested against third stadium of *D. cingulatus* with different concentrations (100, 200, 300, 400 and 500 ppm) by oral toxicity bioassay alone.

After 96 hrs, healthy individuals were maintained till their death using artificial diet as described by Sahayaraj *et al*. (2011). The life parameters like adult longevity, copulation period, fecundity, hatchability, incubation period and relative growth rate were recorded.

Preparation of Column fraction

Preparative chromatography was used to isolate the bioactive chemical compounds from *S. wightii* and *P. pavonica* in the crude extract (hexane + chloroform + methanol at 1:1:1 ratio w/w) crude extract. The column apparatus used for the separation was a vertical glass tube (73 cm height and 3 cm outer diameter) with a sintered glass disk to support the silica gel. The column was filled with 50 mL of petroleum ether initially to prevent the air bubbles. Then the column was packed using adsorbent silica gel (250g) (60-120mesh, Merck, Mumbai) mixed with petroleum ether (500 mL) into the tube to obtain a height of 45 cm. The solvent reservoir (300 mL capacity) was connected to the top of the column and the same petroleum ether was passed through the column for pre- running of solvent to stabilize and equilibrate the silica gel at room temperature (30^{o}C). A 25 g of crude extract was mixed with silica gel (250 g) using petroleum ether (500 mL). The mixture was layered on top of the silica column. The eluting solvent initially with 100% petroleum ether and the polarity was increased at a 80:20, 60:40, 40:60, 20:80 ratio until a 100% of next solvent: Toluene → chloroform → acetone → ethyl acetate → methanol → acetic acid. The column was eluted at 10 mL/ 10 min in marked test tubes (15 mL capacity) under gravitational flow until it appears to be more solute in the column. The fractions eluted were monitored simultaneously by pre-coated analytic thin layer chromatography (TLC) aluminum sheets of Silica gel (20×20 cm, Silica gel 60 F_{254}, Merck, Mumbai) as stationary phase and chloroform and methanol (9:1), chloroform, ethyl acetate and methanol (8:1:1), hexane, ethyl acetate and methanol (6:3:1 and 3:6:1), toluene, acetone and formic acid (7:6:1) ratio as mobile phase were used for the separation of compounds from column chromatographic fractions. The TLC spots were observed under Iodine chamber. Eluents with similar Rf values were pooled together considering as a single eluent. Solvent was evaporated from the pooled eluents, allowed to dry and weighed.

Table 1. Impact of *Padina pavonica* and *Sargassum wightii* seaweeds hexane, chloroform, methanol and water extracts and their fractions against *D. cingulatus* third instar nymphs and its probit analysis data

Solvent	LC_{30}	LC_{50}	LC_{90}	Regression Coefficient	Chi Square	Reqression equation
Padina pavonica						
Hexane	473.1	1326.4	16478.3	1.2	2.0	Y = -6.5240+ 5.6654X
Chloroform	354.3	1062.5	15556.7	1.1	2.4	Y = -6.3369+ 5.5852X
Methanol	448.2	1553.4	32388.3	1.0	3.7	Y = -5.7560+ 4.8501X
Water	1150.0	2486.3	16363.7	1.5	3.2	Y = -5.8687+ 4.9201X
F 14-75	132.3	292.7	2036.2	1.5	0.4	Y =-5.9118+ 6.6150X
F 85-275	180.9	335.5	1517.5	1.9	2.2	Y =-7.8952+ 7.6700X
F 800-965	66.2	141.7	910.6	1.6	0.1	Y =-6.3756+ 6.3073X
Sargassum wightii						
Hexane	591.8	1439.1	12624.9	1.3	1.1	Y = -6.8715+ 5.9219X
Chloroform	311.8	631.8	3549.5	1.7	1.4	Y = -8.6046+ 8.1391X
Methanol	420.4	954.4	7080.2	1.5	7.3	Y = -7.6071+ 6.8403X
Water	2089.9	4520.8	29789.7	1.5	3.4	Y = -4.5923+ 3.6805X
F 164-323	104.2	175.2	623.9	2.3	1.8	Y =-9.6189+ 10.2850X

Statistical analysis

The mortality data, male longevity, female longevity, copulation period, fecundity, hatchability, incubation period data were subjected to one way Analysis of Variance (ANOVA); the mean values compared by Tukey test ($P<0.05$) and 'p' values arrived at to assess the statistical significance of values less than 0.05 were considered as significant using statistical package SPSS (20.0 version).

RESULTS

Nymphicidal activity

Chloroform extract of *S. wightii* (59.3%) (df= 7,40; F= 60.76; P= 0.005) caused more nymphal mortality at 96 hrs than *P. pavonica* (50.0%) (df= 7,40; F= 60.76; P= 0.003) (Figure 1) against third instar nymphs of *D. cingulatus.* Based upon the LC_{50} values, it was concluded that *S.wightii* (LC_{50}= 631.8 ppm) chloroform extract was considered as the best nymphicidal algae than *P. pavonica* (LC_{50}= 1062.5 ppm) (Table 1). In column chromatographic fractions, *S. wightii* fraction (F164-323) showed higher nymphicidal activity (86.7%) (df= 5,24; F= 11.82; P= 0.004) (LC_{50} = 175.2 ppm) than *P. pavonica* (F800-965) (63.3%) (df= 5,24; F= 11.82; P= 0.001) (LC_{50} = 292.7 ppm) fractions (Table 1).

Life traits

Adult longevity

In *S. wightii*, shorter male longevity was observed in chloroform (df=5,30; F= 47.618; p=0.005) and water extract (df= 5,27; F= 8.177; p= 0.005) but the female longevity was highly shortened by chloroform extract (df= 5,24; F= 6.838; p= 0.005) (Table 2). In *P. pavonica*, shorter male longevity (df= 5,36; F= 1.372; p= 0.005) and female longevity (df= 5,17; F= 3.230; p= 0.031) were observed in chloroform extract (Table 3).

Copulation period

In *S. wightii* the copulation period was more prolonged in the water extracts (df= 5, 24; F= 8.645; p= 0.005) than in hexane (df= 5, 31; F= 6.409; p=0.005), chloroform (df= 5, 28; F= 10.189; p= 0.005) and methanol (df= 5,30; F= 6.807; p= 0.005) extracts (Table 3). In *P. pavonica*, copulation period was more prolonged by the water extract (df= 5, 24; F= 5.058; p= 0.003) than in chloroform (df= 5, 17; F= 9.449; p= 0.005) and methanol (df= 5, 22; F= 5.428; p= 0.002) extracts (Table 4).

Fecundity

In the case of *S. wightii*, both chloroform (df= 5,28; F= 30.691; p= 0.005) and methanol (df= 5,28; F= 47.450; p= 0.005) extracts reduced the fecundity

Table 2. Effect of *Sargassum wightii* hexane, chloroform, methanol and water extracts on male (ML) and female (FL) adult longevity (days), mating period (MP) (days), fecundity (FE) (number of eggs/female), hatchability (HA) (%) and incubation period (IP) (days) of *D. cingulatus*

Conc (ppm)	ML	FL	MP	FE	HA	IP
Hexane						
Control	7.0±0.2^{abcdef}	6.7±0.2^{abcdef}	1.7±0.2^{abcd}	78.6±3.1^{abc}	65.5±0.2^{abc}	3.2±0.2^{abcdef}
100	6.9±0.3^{abcdef}	6.4±0.3^{abcdef}	1.9±0.2^{abcde}	76.1±4.8^{abc}	57.0±6.4^{abc}	3.6±0.2^{abcdef}
200	6.8±0.2^{abcdef}	6.2±0.2^{abcdef}	2.3±0.4^{abcde}	70.4±4.9^{abc}	52.6±5.8^{abc}	3.6±0.3^{abcdef}
400	6.7±0.2^{abcdef}	6.1±0.3^{abcdef}	2.6±0.4^{abcde}	47.6±3.8^{de}	19.4±1.7^{def}	3.7±0.2^{abcdef}
600	6.5±0.3^{abcdef}	5.9±0.2^{abcdef}	3.0±0.2^{bcdef}	35.5±2.5^{def}	15.5±1.7^{def}	3.8±0.3^{abcdef}
800	6.3±0.2^{abcdef}	5.5±0.3^{abcdef}	3.7±0.2^{ef}	19.3±3.8^{ef}	9.7±1.2^{def}	4.3±0.2^{abcdef}
Chloroform						
Control	6.8±0.1^{abcde}	6.4±0.1^{a}	1.3±0.1^{ab}	82.6±4.3^{ab}	69.6±3.9^{ab}	3.0±0.2^{ab}
100	6.5±0.2^{abcde}	6.0±0.2^{b}	1.7±0.2^{abcde}	79.1±2.9^{ab}	52.4±3.0^{ab}	3.4±0.2^{abcde}
200	6.3±0.3^{abcde}	5.5±0.2^{c}	2.0±0.1^{bcdef}	51.2±3.3^{cd}	26.5±4.1^{cde}	3.8±0.2^{bcde}
400	6.2±0.2^{abcde}	5.2±0.2^{d}	2.3±0.1^{bcdef}	42.5±4.3^{cde}	11.3±1.3^{cdef}	3.9±0.2^{bcde}
600	5.8±0.3^{abcde}	5.0±0.2^{e}	2.5±0.3^{bcdef}	27.6±3.3^{def}	6.0±1.2^{cdef}	4.1±0.3^{bcde}
800	5.6±0.5^{f}	4.5±0.5^{f}	2.8±0.3^{cdef}	17.5±1.5^{ef}	0.0±0.0	0.0±0.0
Methanol						
Control	7.0±0.4^{abcdef}	6.2±0.2^{abcdef}	1.6±0.1^{abce}	79.0±2.5^{abc}	59.9±3.4^{ab}	3.4±0.2^{abcde}
100	6.8±0.3^{abcdef}	6.1±0.2^{abcdef}	1.9±0.1^{abcef}	71.0±3.9^{abc}	45.6±3.6^{ab}	4.0±0.3^{abc}
200	6.8±0.2^{abcdef}	5.7±0.3^{abcdef}	2.1±0.2^{abcdef}	67.2±4.3^{abc}	36.3±4.3^{c}	4.4±0.3^{abce}
400	6.5±0.2^{abcdef}	5.4±0.3^{abcdef}	2.3±0.3^{cdef}	48.8±0.3^{de}	17.4±3.6^{def}	4.8±0.3^{ad}
600	6.5±0.4^{abcdef}	5.0±0.3^{abcdef}	2.7±0.2^{abcdef}	33.0±5.2^{def}	9.0±1.5^{def}	5.1±0.2^{ade}
800	6.0±0.5^{abcdef}	4.8±0.3^{abcdef}	2.8±0.3^{bcdef}	14.0±4.0^{ef}	0.0±0.0	0.0±0.0
Water						
Control	7.5±0.2^{abcd}	6.5±0.2^{abcde}	1.6±0.1^{abcd}	84.6±3.7^{a}	72.1±4.9^{ab}	3.7±0.2^{abcde}
100	7.4±0.3^{abcde}	6.2±0.4^{abcde}	1.9±0.2^{abcde}	70.3±4.3^{b}	60.9±3.1^{ab}	3.8±0.1^{abcdef}
200	6.1±0.3^{abcdef}	5.5±0.4^{abcdef}	2.0±0.2^{abcde}	51.6±3.1^{cd}	42.9±4.0^{cd}	3.9±0.1^{abcdef}
400	5.8±0.3^{abcdef}	5.0±0.3^{abcdef}	2.2±0.4^{abcde}	42.0±6.1^{cde}	34.8±3.9^{cde}	4.2±0.2^{abcdef}
600	5.4±0.2^{bcdef}	5.0±0.0^{abcdef}	3.0±0.0^{bcdef}	36.0±0.0^{de}	21.0±0.0^{def}	4.5±0.0^{abcdef}
800	4.5±0.5^{cdef}	3.3±0.3^{cdef}	3.8±0.3^{ef}	17.3±0.3^{f}	6.0±2.1^{ef}	5.5±0.5^{bcdef}

Means followed by the same letters in a column for each solvent separately are not significantly different by DMRT at P=0.05

(Table 3). The hexane extracts of *P. pavonica* highly reduced the fecundity more than chloroform (df= 5,17; F= 29.193; p= 0.005), methanol (df= 5,22; F= 44.873; p= 0.005), and water extracts (Table 3) did.

Incubation period

In the case of *S. wightii* chloroform (df= 5,28; F= 22.774; p= 0.005) and methanol extracts (df= 5,30; F= 12.997; p= 0.005) increased the incubation period (Table 3). *Padina pavonica* methanol (df= 5,22; F= 2.694; p= 0.048) and water extract increased the incubation period of *D. cingulatus* (Table 3).

Hatchability

In *S. wightii* hatchability was highly reduced at 800 ppm both in chloroform and methanol extracts (Table 3). In *Padina pavonica*, hatchability was

Table 3. Effect of *Padina pavonica* hexane, chloroform, methanol and water extracts on male (ML) and female (FL) adult longevity (days), mating period (MP) (days), fecundity (FE) (number of eggs/female), hatchability (HA) (%) and incubation period (IP) (days) of *D. cingulatus*

Conc (ppm)	ML	FL	MP	FE	HA	IP
Hexane						
Control	7.6±0.4^{abcdef}	6.5±0.1^{abcdef}	1.9±0.1^{abcdef}	81.3±2.6^{abcd}	68.0±3.5^{ab}	3.4±0.2^{abcdef}
100	7.4±0.2^{abcdef}	6.4±0.2^{abcdef}	2.0±0.2^{abcdef}	79.8±3.7^{abcd}	57.0±3.2^{ab}	3.5±0.3^{abcdef}
200	7.1±0.1^{abcdef}	6.2±0.2^{abcdef}	2.2±0.2^{abcdef}	66.8±3.3^{abcd}	37.0±7.2^{cd}	3.8±0.2^{abcdef}
400	6.9±0.2^{abcdef}	6.0±0.2^{abcdef}	2.5±0.3^{abcdef}	65.5±2.8^{abcd}	33.3±3.8^{cdf}	3.9±0.2^{abcdef}
600	6.8±0.2^{abcdef}	5.5±0.3^{abcdef}	2.6±0.2^{abcdef}	45.3±3.6^{ef}	15.0±2.1^{def}	4.1±0.2^{abcdef}
800	6.7±0.2^{abcdef}	5.0±0.2^{abcdef}	2.8±0.3^{abcdef}	25.0±5.0^{ef}	07.0±1.0^{def}	4.3±0.3^{abcdef}
Chloroform						
Control	7.3±0.2^{abcdef}	5.3±0.3^{a}	1.8±0.3^{a}	85.3±2.3^{a}	72.5±2.4^{a}	3.6±0.1^{abcdef}
100	7.1±0.4^{abcdef}	5.5±0.3^{b}	2.5±0.2^{b}	74.2±2.3^{b}	53.6±2.4^{b}	3.7±0.3^{abcdef}
200	7.0±0.2^{abcdef}	5.8±0.2^{c}	3.2±0.2^{c}	64.5±2.7^{c}	33.5±1.9^{c}	3.9±0.2^{abcdef}
400	6.8±0.3^{abcdef}	6.0±0.0^{d}	3.3±0.2^{d}	52.5±7.5^{d}	16.0±2.0^{d}	4.0±0.5^{abcdef}
600	6.6±0. 1^{abcdef}	6.3±0.2^{e}	3.5±0.0^{e}	43.5±7.5^{e}	10.0±3.0^{e}	4.2±0.5^{abcdef}
800	6.5±0.2^{abcdef}	6.4±0.2^{f}	3.8±2.2^{f}	28.0±0.0^{f}	06.0±0.0^{f}	4.5±0.0^{abcdef}
Methanol						
Control	7.6±0.3^{abdef}	6.3±0.1^{abcdef}	1.8±0.1^{abcde}	87.8±2.0^{a}	74.5±2.0^{a}	3.4±0.2^{abcdef}
100	7.3±0.3^{abcdf}	6.2±0.3^{abcdef}	2.0±0.2^{abcde}	68.5±1.9^{bc}	42.7±5.0^{bc}	3.5±0.3^{abcdef}
200	6.8±0.2^{bcdf}	5.7±0.3^{abcdef}	2.1±0.3^{abcde}	60.8±5.7^{bcd}	38.7±7.2^{bcd}	3.6±0.2^{abcdef}
400	6.5±0.3^{abdef}	5.5±0.3^{abcdef}	2.3±0.2^{abcdef}	45.0±3.6^{cdef}	29.3±4.0^{cdef}	4.0±0.2^{abcdef}
600	6.1±0.1^{aef}	5.1±0.2^{abcdef}	2.5±0.3^{abcdef}	34.7±4.7^{def}	15.0±1.1^{def}	4.3±0.1^{abcdef}
800	5.5±0.5^{abcdef}	4.2±0.5^{abcdef}	3.2±0.2^{def}	27.0±3.0^{def}	09.5±1.5^{def}	4.7±0.2^{abcdef}
Water						
Control	6.8±0.2^{abcdef}	6.0±0.3^{abcd}	2.4±0.2^{abcd}	75.0±5.1^{abc}	60.6±2.5^{abc}	4.0±0.5^{abcdef}
100	6.4±0.1^{abcdef}	5.7±0.3^{abcdef}	2.6±0.3^{abcd}	70.0±4.6^{abc}	51.9±2.0^{abc}	4.6±0.3^{abcdef}
200	6.1±0.3^{abcdef}	5.3±0.0^{abcdef}	2.8±0.3^{abcdef}	66.0±6.4^{abc}	40.8±4.6^{bcd}	5.0±0.3^{abcdef}
400	6.0±0.2^{abcdef}	5.0±0.5^{abcdef}	3.0±0.0^{abcdef}	41.0±1.0^{de}	21.8±5.9^{cdef}	5.2±0.5^{abcdef}
600	5.5±0.0^{abcdef}	4.8±0.0^{bcdef}	3.5±0.0^{cdef}	34.0±0.0^{def}	15.2±0.0^{def}	5.3±0.0^{abcdef}
800	5.0±0.0^{abcdef}	4.5±0.5^{bcdef}	4.0±0.0^{cdef}	22.0±0.0^{ef}	10.1±0.0^{def}	5.6±0.0^{abcdef}

Means followed by the same letters in a column for each solvent separately are not significantly different by DMRT at P=0.05

highly reduced in hexane (df = 5,27; F = 25.962; p = 0.005) followed by chloroform (df= 5,17; F= 53.433; p= 0.005); methanol (df= 5,22; F= 54.943; p= 0.005) and water (df= 5,24; F= 31.445; p= 0.005) extracts (Table 3).

Morphogenesis : After 96 hrs of treatment, the abnormalities were predominantly recorded at 800 ppm concentration of chloroform extract of *S. wightii* rather than the other extracts. It includes incomplete moulting (b), shrunk abdomen (b), crumbled forewings (c) and hind wings. *D. cingulatus* treated with chloroform extract of *P. pavonica* and *S. wightii* showed delayed and incomplete moulting with deformed wings.

DISCUSSION

Algae synthesize a number of secondary metabolites among them; some of the compounds are recognized as insecticidal molecules. Dose dependent mortality due to plant extracts (Hashim and Devi, 2003) were reported for *D. cingulatus*.

The chloroform extract of *S. wightii* caused more nymphal mortality against third nymphal instars of *D. cingulatus*. This is due to the presence of Stigmastan-6,22-dien, 3,5-dedihydro- (71.34%), which also possesses antioxidant (Prakash *et al*., 2011; Yagi *et al*., 2013; Ojekale *et al*., 2013), antibacterial (Ahmad *et al*., 2012), anti-inflammatory (Harman *et al*., 1980; Geneive *et al*., 2002), antianthiritic (Meechaona *et al*., 2007; Liu *et al*., 2008) and insecticidal activity (Barakat, 2011; Ahmad *et al*., 2012; Ojekale *et al*., 2013). Further these fatty acids were also reported from the other green algae *Enteromorpha prolifera* (Muller) J. Agardh (Zhou *et al*., 2010) and red alga *Laurencia brandenii* (Yamada) (Manilal *et al*., 2011). Previously, Argandona *et al*. (2000) reported that the red alga *Plocamimum cartilagineum* (Linn.) P. S. Dixonand *P. violaceum* (Linn.) P. S. Dixoncopulation showed insecticidal activity against tobacco horn worm *Manduca sexta* (Linn.). Furthermore, brown algae of the family *Dictyotaceae* produce a new diferpene dictyo crenulol, which possesses insecticidal activity against tomato moth *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae) (Soto and San Martin, 2002). Similarly, our results revealed that the brown alga, *S. wightii* and *P. pavonica* chloroform extract caused more mortality against third instar numphs of *D. cingulatus*. This is due to the presence of stigmastan-6, 22-dien, 3, 5-dedihydro-, hexadecanoic acid, methyl ester in *S. wightii* and *P. pavonica* respectively.

Rizvi and Shameel (2004) reported the insecticidal activity of benthic algae belonging to the Chlorophyta, Phaeophyta, and Rhodophyta. They clearly showed that *S. tenerrimum* has insecticidal activity, and this might be due to cytotoxic oxysterol and hydroper 24 cholesterol. Diverse secondary metabolites in many types of seaweed were reported as having defensive action against invertebrates in general (Hay *et al*., 1990) and insects in particular (Rizvi, 2003; Rizvi and Shameel, 2004; Biju *et al.*, 2004). Sahayaraj and Kalidas (2011) reported 85% mortality in chloroform and benzene extracts of *P. pavonica* against *D. cingulatus* nymphs. Similarly *Osmundae pinnatifida* showed insecticidal activity (Rizvi and Shameel, 2003). Hashim and Devi (2003) recorded 1.82 to 2.26 µg of chloroform fraction of *Streblus asper* is essential to cause 50% mortality at 96 hrs. Recent research on insecticidal action of plant materials specially secondary metabolites and essential oils established that they are ecofriendly, biodegradable and species specific (Senthil-Nathan, 2007; Senthil-Nathan *et al*., 2006 a,b and 2008; Rattan, 2010).

Ethanol extracts of fresh leaves and seeds of *T. neriifolia* were tested for juvenomimetic action on red cotton bug, *D. cingulatus*, based on larval mortality, duration of ovipositional period, emergence of malformed adults and reduced fecundity of the *D. cingulatus* (Bai and Koshy, 2004). Sahayaraj and Mary Jeeva (2012) reported that the seaweed *Sargassum tenerrimum* extracts caused more mortality and reduced the nymphal developmental period, adult longevity and fecundity of *D. cingulatus*.

Sontakke *et al*. (2013) noticed that 1 and 1.5% concentrations caused complete fecundity as well as fertility in hexane, chloroform and methanol extracts of *Psoralea corylifolia* against *D. cingulatus*. Similarly our results revealed that the chloroform extract of *S. wightii* and *P. pavonica* and column chromatographic fractions caused more mortality and reduced the fecundity, hatchability, adult longevity and increased nymphal developmental period of *D. cingulatus* as reported by Bai and Koshy, 2004; Sahayaraj and Kalidas, 2011; Sahayaraj and Mary Jeeva, 2012. They reported that *Thevetia neriifolia* (Juss.) (Apocynaceae) extracts caused malformation, reduced fecundity, duration of ovipositional period and increased nymphal period of *D. cingulatus* (Bai and Koshy, 2004), chloroform and benzene extracts of *P. pavonica* reduced *D.cingulatus* egg hatchability, increased nymphal developmental period and interphere with physiology (Sahayaraj and Kalidas, 2011), *S.tenerrimum* extracts and column chromatographic fractions altered life traits of *D. cingulatus* (Sahayaraj and Mary Jeeva (2012). Khan and Qamar (2011) reported that

andalin(flucycloxuron) treated with fifth instars nymphs of *Dysdercus koiengii* treated with different concentrations showed highest mortality, moulting abnormality, nymphal and adult malformation were observed. From our results we concluded that *S. wightii* and *P. pavonica* can be used as an ecofriendly pest management component for red cotton bug *D. cingulatus*.

Moulting disruption, morphological abnormalities and mortality of hemipteran insects treated with macroalgal seaweeds extracts showed a dose dependant response. Pandey and Tiwari (2011) reported that the neem based insecticides caused metamorphic developments, coupling and fecundity. A similar effect was found by Katiyar and Srivastava (1982) in *Callistemon lanceolatus* oil treated nymphs of *D. koiengii* also support present findings. Similarly our results revealed that the chloroform extract of *S. wightii* and *P. pavonica* were more effective than the other extracts. It includes incomplete moulting, shrunk abdomen, crumbled forewings and hind wings. *D. cingulatus* treated with chloroform extract of *P. pavonica* and *S. wightii* showed delayed and incomplete moulting with deformed wings. Thus marine algae can be recommended for use in insect pest management modules for the cotton pest.

The present study once illustrates that the marine alga *S. wightii* and *P. pavonica* are potential ones for the eco-congenial nymphicide development as an alternate to chemical insecticides that are being currently used in cotton pest management programs. This study may provide a useful beginning for the development of biopesticides. The present study highlights that macroalgal bioactives can be operationally used for cotton pest control.

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