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Evaluation of nymphicidal and ovicidal effect of a seaweed, *Padina pavonica* (Linn.) (Phaeophyceae) on cotton pest, *Dysdercus cingulatus* (Fab.)

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Impact of brown seaweed, *Padina pavonica* (Linn.) chloroform, and benzene extracts were evaluated against an economically important cotton pest, *Dysdercus cingulatus* (Fab.) (Pyrrhocoridae). The result reveled that between the two solvents, benzene extracts of *P. pavonica* caused more nymphal mortality. It reduce *D. cingulatus* egg hatchability than chloroform extract. Benzene extract also reduced the survival rate of *D. cingulatus* eggs from 53.33 to 0.00 per cent for 0.025 to 0.4% concentration of *P. pavonica* benzene extract respectively. *P. pavonica* extracts also significantly reduced the total body protein (22 to 39%) and DNA (27 to 30%) content. Presence of saponin, steroids and phenolic compounds in the extract might be the reason for these activities. It is concluded that *P. pavonica* possess both nymphicidal and ovicidal activity. It can be utilized for the management of sucking pests of cotton and other crops.

[Keywords: Seaweed, Cotton pest, Nymphicide, Ovicide, Macro molecule]

Introduction

Dysdercus cingulatus (Fab.) is a serious pest of cotton and distributed all the cotton growing region of India[1-3]. It is difficult to control by insecticide because it is highly mobile, Polyphagous[4] and Polymorphic[5] pest of many Malvaceae crops. Terrestrial plants like *Catharanthus roseus* G. Don, *Parathenium hysterophorus* Don and *Nephroleps* extracts were have insecticidal activity against red cotton bug[6-8]. Moreover, neem based pesticide like neem gold[9] also shows nymphicidal activity against this pest. Ovicidal activity of *Pedalium murex* (Linn.)[10] on *D. cingulatus* was reported earlier.

Extracts of the red alga *Polcamium cartilagineum,* and *P. violaceum* from California exhibited insecticidal activity against tobacco horn worm[11]. Insecticidal activity of two halogenated monoterpenes, isolated from the red alga, *Plocamimum cartilagimeum*, and two derivatives (dibromomortensene and dihydromertensene) also showed insecticidal activity on tomato moth, *Tuta absolute* and green bug, *Schizaphis graminum*[12]. Studies also reveal that some *Padina* species (Phaeophyceae) showed antibacterial, antifungal, phytotoxic and insecticidal activities[13]. However, information about the insecticidal activity of *Padina Pavonica* (Linn.) was not available in the literature. Present study was conducted to evaluate the impact of chloroform and benzene extracts of *P. pavonica* on nymphal and egg mortality, nymphal development, morphogenesis and total body protein and DNA content of *D. cingulatus*. In addition, preliminary photochemical screening of the seaweed was also recorded.

Material and Methods

The seaweed, *P. pavonica* was collected from Thoothukudi, and Kanyakumari districts coasts of Tamilnadu, India. To remove salts and micro algae, *P. pavonica* leaves were washed thrice with fresh water and once with distilled water. Then shade dried for two weeks continuously and dried seaweed was partially powdered using domestic blender. 100 mg of partially powder weed (PPW) was packed in Soxhlet apparatus and refluxed with chloroform 800 ml (60-80^{o}C), benzene 800 ml (50-60^{o}C) for 12 hours each continuously. The extracts were dried over sodium sulphate, solvents received under reduced pressure and stored at 20^{o}C until further use. Chloroform and benzene extracts were labeled as PPCE and PPBE respectively. Five concentrations each (0.1, 0.2, 0.4, 0.8 and 1.6% for chloroform), (0.025, 0.05, 0.1, 0.2 and 0.4% for benzene), were prepared using respective solvents and used for this experiments. The qualitative photochemical screening of *P. pavonica* was carried out using standard procedures[14,15].

Nymphs and adult of *D. cingulatus* were collected from cotton fields of Tirunelveli district, and Theni district, Tamilnadu, India. They were maintained under laboratory conditions (28 ± 2°C, 70 –75% RH, 11L and 13D hrs photoperiod) in plastic container (300 ml capacity) on water soaked cotton seeds. The laboratory emerged third instar nymphs and laboratory laid eggs were used for the experiments.

Uniform sized third instar *D. cingulatus* were randomly selected from the stock culture and three insects were placed in the plastic container (360 ml capacity). Ten cotton seeds were soaked in different concentration of the extracts separately for overnight and provided to the test insects. Insects were allowed to feed *P. pavonica* extract soaked cotton seeds for 96 hours continuously. Twenty replications (n=60) were maintained for each concentrations. Mortality was corrected using Abbott's formula[16] if any mortality was recorded in the control category. Then the data was subjected to probit analysis[17]. After 96 hrs, alive nymphs were provided with water soaked cotton seeds till their death.

Freshly laid *D. cingulatus* eggs were collected from the laboratory culture for the experiment. Whatmann no.1 filter paper was placed at the bottom of the Petri dishes (9cm diameter) and placed 10 eggs over the paper. *P.pavonica* extracts were sprayed over the egg (2ml/10 eggs/Petri plate). During spraying eggs were rotated and see that all parts should be adequately contacted with the extract. The control category was sprayed with water alone. Six replications (n=60) were maintained for each concentration and control categories.

Immediately after the death, insects were kept in hot air oven at 50ºC for three to four hours for protein estimation. Five gram of the dried sample was grinded well using mortar and pastel with 1 ml of phosphate buffer (pH 7.2). Centrifuge the sample at 1000 rpm about 15 minutes, collect the supernatants and utilized for the estimation of total body protein[18,19] using BSA as standard. Pipette out 0.1, 0.2, 1.0 ml of the standard protein solution into a series of test tubes. The volume of the standard was making up to 1ml by adding distilled water. A test tube with 1 ml of distilled water was served as blank. Add five ml of protein reagent to each test tube including the blank. Mix well and allow standing for 10 minutes. Similarly test insect sample was also treated and allow them for incubation between five to 10 minutes. Finally the absorbance was red at 595 nm against blank as check. The optical density was compared with the standard graph to estimate protein quantity and expressed in mg/100 mg dried sample. For DNA extraction, insect stored in –20º C was used. DNA was successfully extracted and quantified by Sambrook[19] method.

Results

When PPBE was treated with *D. cingulatus* egg, the survival rate was gradually diminished from lower concentration to higher concentration (0, 16.6, 36.6, 40.0, 53.3 and 90 percentage for 0.4, 0.2, 0.1, 0.05, 0.025 per cent concentrations and control respectively). However, irrespective of PPCE, eggs precede their embryogenesis up to third day, and then it was stopped.

First and second instar nymphs of *D. cingulatus* seldom present on the floor of the cotton field. Then they dispersed in to the cotton plant and start to damage the plant. Hence we selected third instars *D. cingulatus* nymphs for toxicity evaluation. PPBE was highly toxic (LC_{50}=0.004%) than chloroform extract (LC_{50}=0.039%). Similar trend was also recorded for LC_{30} and LC_{90} (Table 1).

Table 1—Impact of *Padina pavonica* extracts on regression equation, LC_{50} values and fiducial limits parameters of *D. cingulatus*

Exposure Time (in hour)	Regression Equation	LC_{30}	LC_{50}	LC_{90}	Variance	Chi-square
		Chloroform extract				
24	Y = 5.349 x+ 6.46	1.1754	1.387	1.6364	0.0013	0.00
48	Y = 3.419 x+ 1.85	0.8392	1.011	1.2184	0.0017	26.05
72	Y = 2.293 x+ 0.89	0.4815	0.621	0.8009	0.0032	20.72
96	Y = 1.728 x+ 3.00	0.0359	0.039	0.045	0.0123	0.0941
		Benzene extract				
24	Y = 1.083 x + 3.36	0.1649	0.330	0.6605	0.0237	10.22
48	Y = 0.754 x + 4.71	0.0130	0.024	0.0760	0.3830	31.62
72	Y = 1.096 x + 4.75	0.0180	0.017	0.018	0.0611	15.29
96	Y = 1.063 x + 5.40	0.0030	0.004	0.0084	0.3220	1.85

Total body protein of *D. cingulatus* was significantly reduced by PPCE (20.4mg/100mg) and PPBE (16.1mg/100mg) ($P<0.05$) compared to the control (26.4 mg/100mg). Similar impact was also observed in total body DNA content (3.84µg/µL and 4.02µg/µL for chloroform, benzene respectively) of *D. cingulatus* were as in control the total body DNA content was 5.52µg/µL.

The preliminary phytochemical analyses of *P. pavonica* chloroform and benzene extracts revealed that steroids, saponins and phenolic compound were present in both the solvent extracts. Whereas triteriphenoids, alkaloids, tannin and flavonoids were absent. Reducing sugar and xanthoprotein were absent in PPCE extract and carbohydrate was absent in PPBE. The qualitative test was done for identifying different components in the plant extracts by using[14,15] methods.

D. cingulatus nymphs took 16.6 days to attain in to adult. It was insignificant ($P<0.05$) at 0.1 and 0.2 percent PPCE treated seed feed *D. cingulatus.* Other three concentrations of PPCE, *D. cingulatus* developed only up to fourth instars and their nymphal period was significantly ($P<0.05$) prolonged both in 0.8 and 1.6 percent concentrations (Table 2). In PPBE, *D. cingulatus* developed up to fourth instar except at 0.4 percent concentration of *P. pavonica*. All the concentrations extends the nymphal period significantly ($P<0.05$) except at 0.025 percent PPBE (Table 2).

Discussion

Impact of chloroform and benzene extracts of *Padina pavanica* were evaluated against *D. cingulatus* nymphs and eggs. Red alga *Plocamimum cartilagineum*, caused 91% mortality after 48 hrs exposure[12]. However, this study revealed that benzene (86%) and chloroform (96%) extracts caused more than 85 per cent mortality at 72 hrs and 48 hrs after the exposure respectively. Similarly *Osmundae pinnatifida* showed low insecticidal activity[20]. Brown algae of the family Dictyotaceae produce a new diferpene dictyo crenulol, which possesses insecticidal activity against tomato moth *Tuta absoluta* [21]. In addition to the defoliators, sucking pest like *Aphis fabae* was also controlled by *Plocamium cartilagineum*[22,23]. Present study revealed that in addition to the nymphicidal activity, *P. pavonica* either reduced or increase the nymphal developmental period. This is showed that *P.pavonica* extracts interphere with physiology of *D. cingulatus.*

D. cingulatus eggs are elliptical, creamy white in colour with a thick chorion. During embryogenesis creamy white colour changes to yellow on early development (fourth day) and to orange on fifth day or on nearly sixth day. Application of PPCE & PPBE of *P. pavonica* elicited a dreadful impact on *D. cingulatus* embryogenesis. All the eggs were changed their colour from creamy white to yellow and then shrinked completely. It showed that all the tested concentration benzene extract of *P. pavonica* showed higher ovicidal activity. However, chloroform extracts also showed ovicidal activity in dose dependent manner. This might be due to the degrees of morphogenetic malformation in the recipient pest embryo was found to be dose dependent and also showed the anti-juvenile hormonal compounds present in the *P. pavonica* chloroform extract to stop

Table 2—Chloroform and benzene extracts of *Padina pavonica* on the nymphal developmental period (in days) of *D.cingulatus*

Solvents	Concentration	Up to fourth instar	Up to fifth instar	Up to Adult
Chloroform	Controls	6.62 ± 0.58	11.60 ± 0.7	16.62 ± 0.6
	0.1	-	-	16.83 ± 0.3 ^{NS}
	0.2	-	-	16.91 ± 0.1 ^{NS}
	0.4	-	11.71 ± 0.3 ^{NS}	-
	0.8	-	12.80 ± 0.1*	-
	1.6	-	13.60 ± 0.3*	-
Benzene	0.025	-	11.80 ± 0.2*	-
	0.05	-	12.32 ± 0.1 *	-
	0.1	-	3.13 ± 0.5 *	-
	0.2	-	14.32 ± 0.4*	-
	0.4	7.81 ± 0.7*	-	-

NS - indicates note significant ; * - show significant at 5% level.

the embryonic development of *D. cingulatus*. The effects were higher when the benzene extract was used in high concentrations (0.05 to 0.4%) and was brought into contact with the embryo in very early as reported in *D. cingulatus* by[24] and the authors reported that juvenile hormone is essential for the normal development of *D. cingulatus*. Bioactive principles present in *P. pavonica*, blocked either at germ bund stage, or at blastokinesis stage. Similar impact was also reported by in *D. cingulatus* when exposed to root extracts of *Pedalium murex*[10].

Proteins are an integral part of the cuticle and play an important role in metamorphosis and insect growth. Our study shows that *P. pavonica* extracts significantly reduced the total body protein. Previously it was reported that seed extracts of *Annona*[25], leaves extracts of *Lantana wightiana, Premna tomentosa* and *Synedrella nodiflora*[26] reduced the total body protein content of the tested insect. The plant extracts usually reduce the macromolecular content and they were fed along with the natural food of the host[27]. *Porteresia coarctata* Takeoka leaf extract at different concentration showed significant reduction in protein and DNA content in the fat body and midgut tissues[28].

Benzene extract caused mortality, arrest the nymphal development, highly reduce total body protein content whereas chloroform extract interfere with the embryonic development and reduce whole body DNA content (30.43%). Both extracts can be used in pest management mainly sucking type of mouth parts.

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