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Studies on Methanolic Extract of Brown Algal Seaweed *Liagora ceranoides* J.V. Lamouroux from Southern Coast of Tamilnadu: *In vitro* Anti-Insect Properties and Phytochemicals

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Abstract

Red algal seaweed *Liagora ceranoides* collected from Rameswaram coast, Tamilnadu, India was investigated for its repellent, antifeedant, larvicidal, insect growth regulatory activities on *Spodoptera litura* during 2016-18. Larvicidal action of methanol extract of sea weed was observed from first day of treatment and exhibited gradual increase in mortality up to fifth day. The seaweed's methanol extracts @200 µl/L concentration resulted 80.00 per cent larval mortality and the survived larvae got pupated. No repellent and antifeedant activity was observed among the treatments. Adult emergence was not observed at 200 µl/L concentration with a pupal: adult conversion ratio of 1:0.00 whereas in control and solvent control the ratio was 1:1.00. Twenty- three phytochemicals were identified in *L. ceranoides* using GC-MS detection system (Gas Chromatography-Mass Spectrometry) respectively and few of them may be responsible for ant-insect properties.

Keywords: Red algal seaweed; *Liagora ceranoides; Spodoptera litura*; Larvicidal action; Phytochemicals

Introduction

Harmful marine algae are widely spread throughout the coastal areas around many continents and have insecticidal value [1]. *Liagora ceranoides* J.V. Lamouroux (Liagoraceae: Rhodophyta) is a macroscopic marine red algal seaweed which is heavily calcified, pinkish in color and ramiform with cylindrical and dichotomous branches [2]. *Spodoptera litura* (Fab.) is a polyphagous pest of cosmopolitan distribution which comes out as a serious and dominant pest in agricultural and horticultural crops [3]. Indiscriminate use of synthetic pesticides caused serious ill effects to the precious mother earth, offers pest resistance and harming beneficial organisms viz., predators, parasitoids and pollinators [4]. Hence, switching over to eco-friendly insect pest management considered to be a better alternative. Among different alternatives, seaweeds offer a non-toxic way to manage the pests. Hence, the present study dealt with testing the bio-efficacy of methanol extracts of *L. ceranoides* against growth and development of *S. litura*.

Materials and Methods

Collection of seaweeds

Seaweed belonging to Rhodophyta, Red algae, *Liagora ceranoides* was collected from Rameswaram coast, Tamil Nadu, India. The seaweed collected by hand picking method was immediately washed with fresh sea water and then carefully washed thoroughly three times with fresh water to remove the excess salt, sand and epiphytes to drain off the water, the algae were wiped with a blotting sheet and air dried under shade for a fortnight and stored [1]. The sea weeds were identified at Parangipettai, Faculty of Marine Biology, Annamalai University, Tamilnadu, India.

Mass culturing of *Spodoptera litura*

Spodoptera litura was mass cultured on natural diet castor leaves under laboratory conditions in sterilized plastic buckets and the required amount of third instar larvae was used for the bio-assay experiments.

Preparation of seaweed solvent extract

Fifteen gram of partially powdered red algal seaweed *L. ceranoides* was packed and loaded in Soxhlet apparatus (GI-1706 A) individually and refluxed with Methanol for 12 hours continuously. The extracted solvents were evaporated in a hot plate. The final extract was elucidated with methanol and used for the evaluation experiments. The extracts were stored at -20°C [5].

Methodology

Prepared *L. ceranoides* solvent extracts were treated to the uniform aged third instar larva @1, 10, 30, 50, 100, 200 µl/L with solvent and water control. The methanol extracts were tested against a homogenous culture (third instar larva) of *Spodoptera litura* using leaf dip bio-assay. To compare the performance of solvent effect on the larva, a control was maintained along with an absolute control, replicated thrice under completely randomized block design. Data on repellency, antifeedant, larval mortality, pupation, pupal malformation, adult malformation and adult emergence were recorded, and the data were statistically analysed and the results were presented [6].

GC-MS analysis

Preparation of extract: The seaweed was shade dried and powdered with the help of electric blender. The sample was extracted with ethanol and 2 µl of this solution was employed for GC-MS analysis.

GC condition and identification of compounds: The seaweed powder was extracted with ethanol and 2 µl of this solution was employed for GC-MS analysis. The GC-MS analysis was carried out with 30 m × 0.25 mm ID × 0.25 µm df with column BR-5 MS (5% Diphenyl/95% Dimethyl poly siloxane). The instrument was set to an initial temperature of 110°C and maintained at this temperature for 3.50 minutes. At the end of this period, the oven temperature was rose up to 280°C, at the rate of an increase of 50 C/min, and maintained for 12 min. Injection port temperature was ensured as 280°C. The ionisation voltage was 70 eV. Mass spectral scan range was set at 50-500 (m/z) amu. Using computer searches on a NIST Ver-11 MS data library and comparing the spectrum obtained through GC-MS, compounds present in the seaweed sample were identified with their compound name, chemical formula and molecular weight [7].

Results and Discussion

The results revealed that the larval mortality initiated from day one and increased up to 5th day. Among the sea weed treatments, maximum mortality (80.00%) was exhibited at 200 µl/L concentration followed by 100 and 50 µl/L concentration (73.33 and 66.66%). No larval mortality was observed in solvent control and control in the same period of observation (Table 1).

Treatment µl/L	Percent larval mortality after					% Pupation*	% Pupal mortality*	% Pupal Malformation*	% Adult emergence*	Pupal to adult conversion ratio
	Day 1	Day 2	Day 3	Day 4	Day 5					
1	0.00^{d}	13.33^{d}	26.66^{d}	33.33^{d}	46.66^{e}	53.33^{e}	0.00^{b}	0.00^{c}	53.33^{e}	01:01.0
	-2.866	-21.337	-31.071	-35.252	-43.088	-46.914	-2.866	-2.866	-46.914	
10	0.00^{d}	13.33^{d}	33.33^{c}	33.33^{d}	46.66^{e}	53.33^{e}	0.00^{b}	0.00^{c}	53.33^{e}	01:01.0
	-2.866	-21.337	-35.252	-35.252	-43.088	-46.914	-2.866	-2.866	-46.914	
30	13.33^{c}	20.00^{c}	33.33^{c}	46.66^{c}	53.33^{d}	46.66^{d}	0.00^{b}	6.66^{b}	40.00^{d}	01:00.8
	-21.337	-26.566	-35.252	-43.088	-46.914	-43.088	-2.866	-14.759	-39.233	
50	13.33^{c}	33.33^{b}	46.66^{b}	46.66^{c}	66.66^{c}	33.33^{c}	6.66^{a}	6.66^{b}	20.00^{c}	01:00.6
	-21.337	-35.252	-43.088	-43.088	-54.751	-35.252	-14.759	-14.759	-26.566	
100	20.00^{b}	33.33^{b}	46.66^{b}	53.33^{b}	73.33^{b}	26.66^{b}	6.66^{a}	6.66^{b}	13.33^{b}	01:00.5
	-26.566	-35.252	-43.088	-46.914	-58.931	-31.071	-14.759	-14.759	-21.337	
	33.33^{a}	40.00^{a}	53.33^{a}	66.66^{a}	80.00^{a}	20.00^{a}	6.66^{a}	13.33^{a}	0.00^{a}	01:00.0
200	-35.252	-39.233	-46.914	-54.751	-63.437	-26.566	-14.759	-21.337	-2.866	
Solvent control	0.00^{d}	0.00^{e}	0.00^{e}	0.00^{e}	0.00^{f}	100.00^{f}	0.00^{b}	0.00^{c}	100.00^{f}	01:01.0
	-2.866	-2.866	-2.866	-2.866	-2.866	-87.137	-2.866	-2.866	-87.137	
Control	0.00^{d}	0.00^{e}	0.00^{e}	0.00*e*	0.00^{f}	100.00^{f}	0.00^{b}	0.00^{c}	100.00^{f}	01:01.0
	-2.866	-2.866	-2.866	-2.866	-2.866	-87.137	-2.866	-2.866	-87.137	
SEd	1.1455	1.254	1.2219	1.2111	1.1103	1.1103	1.4775	1.4845	1.3521	
CD (p=0.5)	2.4284	2.6383	2.5903	2.5674	2.3538	23,538	3.1322	3.1469	2.8663	

Each values are the mean of three replicates; values enclosed in parantheses are arc sine transformed values; means followed by common alphabet are not significantly different at 5% level by LSD.

Table 1: Influence of red algal seaweed*Liagora ceranoides* methanol extract on the developmental stages of*Spodoptera litura* .

Observed pupation was minimum in 200 µl/L concentration (20.00%) and maximum in 1 µl/L (53.33%) in *L. ceranoides* with cent percent pupation in control. Pupal malformation was 13.33 per cent at 200 µl/L concentration. The pupal mortality and pupal malformation was 6.66 and 13.33 per cent at 200 µl/L concentration which was higher than the other treatments and control. Cinnamomum camphora oil extracts are evaluated against *S. litura* and reduction in feeding, pupation and adult emergence at 2.5 and 2.0 mg/larva concentration have been observed [8]. There was no adult emergence at 200 µl/L concentration i.e., the pupal to adult conversion ratio was 1:0.00 whereas complete emergence was noticed at 1 µl/L concentration (1:1.00) in both the seaweeds. The results obtained in this study are in accordance with the reports wherein they have observed that the methanol extract of *Ulva fasciata* and *Chaetomorpha*

antennina (green algal seaweed) have showed increased larval mortality and growth inhibitor activity against *S. litura*. Methanol extract of *Thevetia nerifolia* leaves at 2.5 per cent concentration demonstrate 53.8 and 29.6 per cent larval mortality and pupation of *S. litura* respectively [6]. The pupation of the insect have been reduced significantly in all the concentrations and fractions of hexane, chloroform, ethyl acetate and methanol fractions [9].

In this study, it has been observed that the sea weed used for evaluating their potential against test insect was well supported by many evidences from other plants, green algae and red algae against many insects as follows.

Methanolic extract of *Gracilaria crassa* and *Hypnea Valentia* demonstrate higher level larval mortality of *A. aegypti* [10]. The chloroform extract of *Calotropis procera* exhibits the best larvicidal activity against *S. litura* [11]. Crude chloroform extract and emulsifiable concentrate (EC) of seaweed *Caulerpa scalpelliformis* repel *S. litura* and *Dysdercus cingulatus* in dose dependent manner [5]. Methanol extracts from different marine algae *Caulerpa racemosa*, *C. scalpelliformis*, *U. fasciata*, *Padina tetrastromatica*, *Stoechospermum polypodioides*, *Sargassum wightii*, *Cheilosporum spectabile* and *Gracillaria edulis* are effective on the root knot nematode (*Melodogyne javanica*) [12].

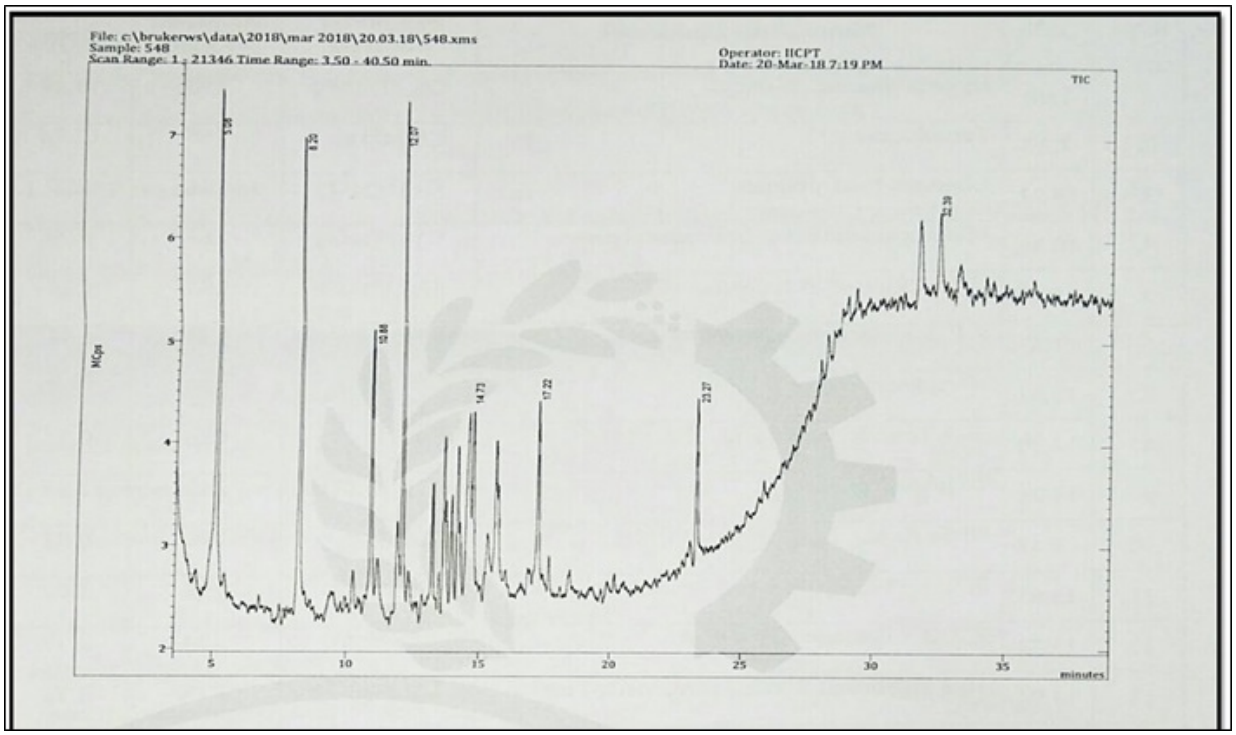

Figure 1: GC-MS chromatogram of *Liagora ceranoides*.

The GC-MS-MS studies of *L. ceranoides* has shown many phytochemicals which contribute to various biological activity. The active principles, with their retention time (RT), molecular formula, molecular weight (MW), concentration (peak area%) and the molecular structure are presented in Table 2. The GC-MS Chromatogram of the number of peaks of the compounds detected was shown in Figure 1. The total number of compounds identi ied in ethanol extract of *L. ceranoides* was 13 [13-16].

S. No.	RT	Name of the compound	Molecular formula	Molecular weight	Peak area
1.	5.08	Hydroxyl amine, O-decyl-	$C_{10}H_{23}NO$	173	15.21
2	8.20	Tetradecane	$C_{14}H_{30}$	198	12.74
3	10.21	Limonen- 6-ol, pivalate	$C_{15}H_{24}O_2$	236	1.17
4.	10.88	Methoxyaceticacid, 2-tetradecyl ester	$C_{17}H_{34}O_3$	286	6.94
5.	11.14	cis-1-Chloro-9-octadecene	$C_{18}H_{35}Cl$	286	1.23
6.	11.87	Z,E-3,13-Octadecadien-1-ol	$C_{18}H_{34}O$	266	2.34
7.	12.07	Nonadecane	$C_{19}H_{40}$	268	11.90

8.	12.30	E-9-Tetradecenoic acid	$C_{14}H_{26}O_2$	226	0.31
9.	13.21	2-Hexadecanol	$C_{16}H_{34}O$	242	2.36
10.	13.47	Oleic acid	$C_{18}H_{34}0_2$	282	0.82
11.	13.63	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	$C_{20}H_{40}O$	296	3.69
12.	13.72	Z,Z-2,5-Pentadecadien-1-ol	$C_{15}H_{28}O$	224	2.57
13.	13.94	10- Heptadecen-8-ynoic acid, methyl ester (E)-	$C_{18}H_{30}O_2$	278	2.52
14.	14.16	17- Octadecynoic acid	$C_{18}H_{32}O_2$	280	3.47
15.	14.24	7-Heptadecene, 17-chloro-	$C_{17}H_{33}Cl$	272	1.67
16.	14.56	Albuterol	$C_{13}H_{21}NO_3$	239	8.35
17.	14.73	Methyl tetradecanoate	$C_{15}H_{30}O_2$	242	4.76
18.	15.29	Z-8-Methyl-9-tetradecenoic acid	$C_{15}H_{28}O_2$	240	2.18
19.	15.61	Hexadecanoic acid, ethyl ester	$C_{18}H_{36}O_2$	284	3.34
20.	15.68	4-Methyldocosane	$C_{23}H_{48}$	324	1.59
21.	17.22	12- Methyl-E,E-2,13-octadien-1-ol	$C_{19}H_{36}O$	280	5.01
22.	23.27	1-aminononadecane, N-trifluoroacetyl-	$C_{19}H_{40}F_3NO$	379	3.56
23.	32.39	Desoximetasone	$C_{22}H_{29}FO_4$	376	2.27

Table 2: Phytochemical in red algal seaweed *Liagora ceranoides* (Based on GC-MS analysis).

Conclusion

The volatile oils of *L. ceranoides* displayy insecticidal activity on stored product pests viz., *Oryzeaphilus mercator*, *Tribolium castaneum* due to the presence of principle bioactive compounds viz., Ethyl cinnamate, Tetradecane, Pseudoionone and methyl tetradeconate. Oleic acid is responsible for the mosquito repellent activity. Nonadecane act as a pheromone and this attracts the parasitoid of the host insect. Benzyldiethyl- (2,6-xylylcarbamoylmethyl)-ammonium benzoate called denatonium benzoate present in *G. corticata* has been used as toxic bait for pestiferous German wasp (*Vespula germanica*). The toxic effect of *L. ceranoides* on *S. litura* may be due to the presence of any one or more than one phytochemical identi ied and its characterization in future would pave for many insights in developing a potential biopesticide for future.

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