RESEARCH ARTICLE

Comparative Toxicity of Aqueous and Methanolic Extracts of Brown Macroalgae against Tobacco Cutworm, *Spodoptera litura* Fabricius

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ABSTRACT

The present study aimed to find the toxicity of aqueous and methanol extracts of three different brown macroalgae *viz., Padina tetrastromatica, Sargassum wightii* and *Turbinaria conoides* against tobacco cutworm*, Spodoptera litura*. Larval mortality was noticed in both the extracts at all the concentrations tested in a dose-dependent increase after 96 h of exposure with 73.33, 56.66, and 50.00 per cent in aqueous extract; and 50.00, 46.66 and 43.33 per cent in methanol extracts at 10 % concentration in *S. wightii, T. conoides and P. tetrastromatica,* respectively. Pre-pupal and pupal mortality were recorded in all the concentrations showing malformations during the growth. Pupation and adult emergence were also affected by the extracts of *S. wightii*. From the results, it is surmised that brown macroalgae possess insecticidal potential and growth inhibitory properties which can be exploited for eco-friendly management of *Spodoptera litura* and possibly other lepidopteran larvae as well.

Keywords: *Brown macroalgae; Padina tetrastromatica; Sargassum wightii; Turbinaria conoides; Spodoptera litura; Eco-friendly management*

INTRODUCTION

Tobacco cutworm, *Spodoptera litura* Fabricius (Lepidoptera: Noctuidae), is a polyphagous pest of cosmopolitan distribution that feeds on more than 150 different host plants in tropical and subtropical regions (Gong *et al.*, 2014; Selinrani *et al.,* 2016). It has caused pernicious destruction to many important field and vegetable crops, such as pulses, cotton, chilli, and cabbage (Kaur *et al*., 2016 and Kiran *et al.,* 2016). Pest management with chemical insecticides and their indiscriminate use exhibits insecticide resistance and negative consequences on the environment and human well-being. Hence, the search for an eco-friendly alternative is always on the go. In this search, the plants with rich biochemicals that offer potential phytochemical molecules having insecticidal properties were explored. The plant-derived insecticidal molecules could surpass the development of insect resistance, non-toxic, and eco-friendly due to rapid degradation, thus regulating yield loss and improving the quality of produce (Rattan, 2010; Ahmad *et al.,* 2013 and Lengai *et al*., 2020). Of several options of plants, the ocean had a magnificent potential to meet all kinds of human needs, which were furnished in ancient mythology. Marine organisms are a great reservoir of novel bioactive compounds with unique structures having a broad range of biological properties, which may not be present in natural terrestrial organisms. There is a massive disparity in salinity, temperature, pressure, and biological habitats in the ocean environment. Several insecticidal compounds have been extricated from marine flora and fauna, especially from micro and macroalgae, corals, sponges, and annelids. Among the marine resources, seaweeds or marine macroalgae are the outstanding troops of organism which occupies all the zones of the marine ecosystem *viz*., intertidal, and subtidal zones on hard substrata and from shallow to deep-sea producing a diverse range of novel bioactive compounds that can be exploited in agriculture as plant bio-stimulants, fertilizers, and biopesticides (Hamed *et al*., 2018). Macroalgae have a high chemical diversity with protective

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functions against phytophage (Machado *et al.,* 2014). A recent review recognized nearly 3,200 natural products from marine macroalgae, representing 13 % of compounds reported from marine organisms (Leal *et al.,* 2013). Currently, there are over 1,200 compounds reported from brown algae (Phaeophyceae) and sulfated polysaccharides that have antimicrobial and insecticidal properties (Paulert *et al*., 2009) and Gonzalez-Castro, 2019). The Brown algal seaweeds such as *Sargassum tenerrimum, Padina pavonica, Lobophora variegate* and *Dictyota dichotoma* (Sahayaraj and Kalidas, 2011; Sahayaraj and Mary Jeeva, 2012); the Red algal seaweeds like *Acanthophora spicifera*, *Amphiroa anceps*, *A. fragilissima*, *Gracilaria corticata, G. edulis, Liagora ceranoides* and *Laurencia pinnata* (Fukuzawa and Masamune, 1981; Dharanipriya *et al.,* 2020 and Gowthish and Kannan, 2019); and Green algal seaweeds such as *Chaetomorpha antennina, Caulerpa scalpelliformis* (Chanthini *et al.,* 2021 and Sahayaraj *et al*., 2021) are reported to affect the insect species due to the presence of terpenoids, steroids, phenols, flavonoids alkaloids, steroids, coumarin, quinine, etc., In order to elucidate the detrimental effect of the different Brown macro-algal extract, *viz., P. tetrastromatica, S. wightii* and *T. conoides* against the larvae of *S. litura*, the present study was conducted to reveal their toxicity and growth inhibitory property.

MATRIALS AND METHODS

Insect culture

The larvae of *Spodoptera litura* were collected from the castor plants in the Department of Oil Seeds, Tamil Nadu Agricultural University, Coimbatore district (11°1'N, 76°55'E) and reared on castor leaves as a natural diet under laboratory conditions. They were reared up to pupation and the pupae were kept in the Petri dishes *in-vitro* condition at 28 ± 2 °C temperature, 47% relative humidity with 11 hours of light and 13 hours of dark period. Emerged healthy adults were released into an oviposition chamber and the fresh *Nerium oleander* L. leaves were kept as ovipositional substrate with petiole immersed in water to prevent drying of leaves. Adult insects were fed with 10 % honey solution fortified with vitamin mixture to enhance the oviposition. The newly emerged F_1 generation larvae were utilized for the experiment.

Macro algal material and study area

The brown macroalgae were procured from Hare Island, located at Latitude 9°12'0'' N and Longitude 79°4'48''E, which is the part of the Gulf of Mannar Region, Tuticorin, Tamil Nadu, along the South-Eastern coast of the Indian subcontinent between May and October, 2019. The three species of macroalgae, *Padina tetrastromatica* Hauck, *Sargassum wightii* Greville, and *Turbinaria conoides* (J. Agardh) Kutzing, according to Guiry and Guiry (2011) were collected by handpicking method in morning hours at low tides where the water moves away from the shore. The algal thalli were cleansed to detach the leftover salts, sand particles, epiphytes, and the entire thalli were dried under shade and stocked in a sealed container at room temperature for further studies. The algal specimens were identified and authenticated by the Botanical Survey of India, South Regional Centre, Coimbatore, Tamil Nadu, and the specimens were deposited in the Department of Agricultural Entomology, Tamil Nadu Agricultural University, Coimbatore.

Preparation of crude solvent extracts from macroalgae

The macroalgal biomass was pulverized and sieved to a uniform particle size of 0.5 mm. The solid/liquid extraction was performed using extraction solvent ultrapure water (H_2O) and methanol (MeOH) at the biomass solvent ratio of 1:10 *w/v* and subjected to sonication using an ultra-high-frequency sonicator (SONICS®, Sonics and Materials, Inc.) at 20kHz and power output of 1500 W for 30 minutes. They were centrifuged (Dynamica, VELOCITY 14R) at 10000 rpm (10380xg) for 10 min and the resulting pellets were re-extracted with the same amount of fresh solvent twice to maximize the extraction (Chew *et al.,* 2008). Thus, the extracts were concentrated using a rotary cum vacuum evaporator (Heidolph, Hei-VAP core) under reduced pressure at 35°C and 100 rpm. The extracts were stored at -20°C until used. To obtain the extraction yield, the extracts were freeze-dried using a lyophilizer (Kambic, L10 - 5PLT) at -60°C, 0.015 kPa and the lyophilized extracts were weighed.

Concentration-mortality bioassay

The crude extracts prepared with six concentrations 0.7, 1.0, 3.0, 5.0, 7.0, 10.0 % in an aqueous and methanol solutions with 0.1% Triton X-100 as a sticking agent were used to evaluate the toxicity of each macroalgal extract against *S. litura* larvae, constructed concentration - mortality curves and estimated the lethal concentrations (LC_{10}, LC_{20} and LC_{50}). Water and methanol with 0.1% Triton X were used as control. Feeding toxicity was assayed using a castor leaf disc with known size (4 cm diameter) dipped with gentle agitation in the respective concentrations for 10 seconds and dried. The larvae were placed inside the Petri dishes and the algal extract-treated leaves were provided for feeding. Three replicates with 10 larvae of each were used in each concentration in a Completely Randomized Design. The mortality due to direct toxicity/growth regulatory activity on the larvae was recorded every 24 hours after treatment up to adult emergence.

Statistical analysis

The data collected were subjected to statistical procedures and analyzed by using IBM SPSS v. 20 statistical programs. The concentration mortality data were subjected to Probit analysis to obtain a dose-response curve with SPSS software. The mortality data were *arc sine* transformed before one-way ANOVA, and a Tukey's Honestly Significance Difference (HSD) ($p<0.05$) test was applied for comparison of means.

RESULTS AND DISCUSSION

The natural bioactive compounds present in marine macroalgae are important in protecting themselves from biotic stress. Those compounds also provide insect and pathogen protection, act as bio-stimulants, and harbor many beneficial microbiomes. The bio-active compounds isolated from marine macroalgae have bio-pesticidal potential, including insecticidal, anti-pathogenic, and nematicidal activities, as reviewed by Hamed *et al*. (2018). The current study involves the mortality bioassays with crude aqueous and methanol extracts of brown macroalgae, *P. tetrastromatica, S. wightii* and *T. conoides* that showed significant toxicities against second instar larvae of *S. litura* at different concentrations. The extraction yield of crude extracts of *P. tetrastromatica, S. wightii* and *T. conoides* were 11.8, 12.5 and 24.5; and 9.4, 10.2, and 19.6 per cent for aqueous and methanol extracts, respectively (Table 1). These extracts were leaf dipped with respective concentrations and exposed to second instar larvae of *S. litura* and the mortality data are presented in Fig 1 and 2. In all the extracts, the larval mortality increases in a dose-dependent manner at different time periods, in concurrence with Kandal and Khetagoundar (2013) in different macroalgae. Among the tested brown algae in this study, the aqueous extract of *Sargassum* showed maximum mortality (73.33%) followed by *Turbinaria* and *Padina* extracts (56.66 and 50.00%) at 10 % concentration after 96 hours of exposure. A similar trend was observed for methanol extracts too with the mortality of 50.00, 46.66 and 43.33 per cent for *Sargassum, Turbinaria,* and *Padina,* respectively. In an earlier report, the different brown macro-algal species of *Sargassum viz., S. asperifolium, S. dentifolium* and *S. linifolium* exhibited insecticidal activities against lepidopteran pests *viz., Spodoptera littoralis* and *Spodoptera frugiperda* (Matloub *et al*., 2012). The aqueous extract of *Sargassum vulgar* prolonged the larval duration and caused larval mortality of second instar larvae of *S. littoralis* (Rashwan and Hammad, 2020). Among the two solvents, aqueous extract excelled the methanol extracts. The lethal concentration (LC_{10}, LC_{20}, and LC_{50}) values were given in Table 2. The LC_{50} of *Padina, Sargassum,* and *Turbinaria* were 14.73, 4.96 and 9.74 per cent for aqueous and 20.36, 13.61, and 13.11 per cent for methanol extracts, respectively. There observed the blocking of larva to pupal transformation *i.e*., pre-pupal mortality was recorded in all the concentrations tested (Fig 3). This shows that the active principles of brown macroalgae possess insect growth regulatory activity. *S. wightii* exhibited 10.00 and 20.00 per cent pre-pupal mortality out of living larvae, followed by *P. tetrastromatica* (16.66 and 20.00 %) and *T. conoides* (20.00 and 16.66 %) for aqueous and methanol extracts, respectively. These abnormalities are due to the hormonal imbalance during metamorphosis (Lee *et al.,* 2015). Regarding the total pupation, the least pupation of 16.66 and 30.00 per cent for *Sargassum*, 23.33 and 36.66 per cent for *Turbinaria,* and 33.33 and 36.66 per cent for *Padina* had been noticed for aqueous and methanol extracts, respectively (Fig 4). The larval mortality was observed in the highest concentration and the lower concentration is adequate for the deformities in the pupal stages (Ahmed and Arif, 2007). The adult emergence was very low at the highest concentration (Fig 5). The adult emergence recorded with aqueous and methanol extracts were 6.66 and 13.33 per cent for *Sargassum* with the larvae to adult conversion ratio of 1:0.06 and 1: 0.13, 6.66 and 20.00 per cent for *Turbinaria* with the larvae to adult conversion ratio of 1: 0.06 and 1: 0.20 and 20.00 per cent for both the extracts, while, *Padina* had 1: 0.20. Earlier reports with benzene extracts of Brown algae, *P. pavonica* (Sahayaraj and Kalidas, 2011) and chloroform extracts of *S. wightii* (Asharaja and Sahayaraj, 2013) showed nymphal morality and disrupt egg hatchability of red cotton bug, *Dysdercus cingulatus.*

Table 1. Extraction yield of Brown macroalgae

Macro algae	Extraction yield (%)	
	Aqueous	Methanol
Padina tetrastromatica	11.8 ± 0.01[c]	9.4 ± 0.23[c]
Sargassum wightii	12.5 ± 0.50[c]	10.2 ± 0.43[b]
Turbinaria conoides	24.5 ± 0.30[a]	19.6 ± 0.22[a]

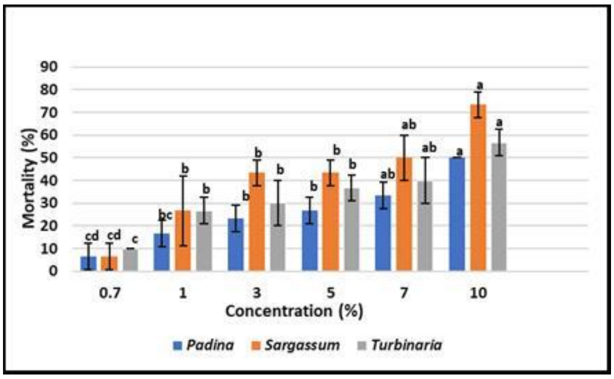

Fig. 1. Larval mortality of *Spodoptera litura* on treatments with aqueous extracts of different brown algae. Treatment means (± SEM) with different letters show significant differences by Tukey's Honestly Significant Difference Test at p<0.05 level

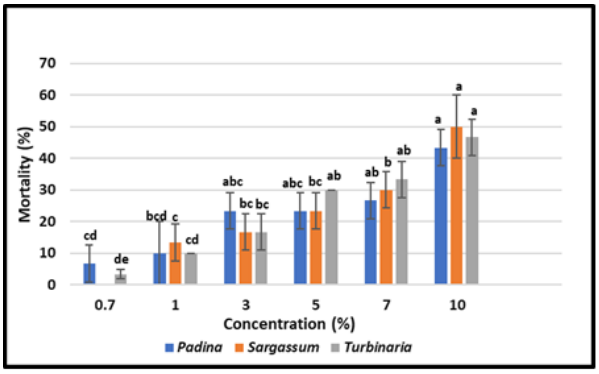

Fig. 2. Larval mortality of *Spodoptera litura* on treatments with methanol extracts of different brown algae. Treatment means (± SEM) with different letters show significant differences by Tukey's Honestly Significant Difference test at p<0.05 level

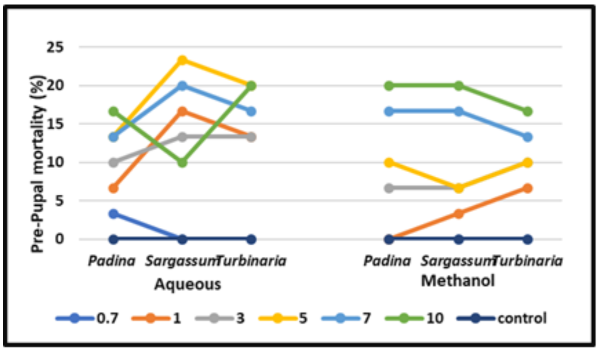

Fig. 3. Pre-pupal mortality of *Spodoptera litura* on treatments with aqueous and methanol extracts of different brown algae

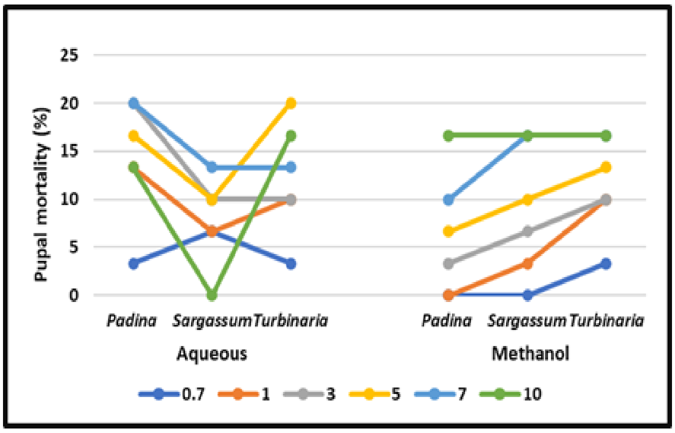

Fig. 4. Pupal mortality of *Spodoptera litura* on treatments with aqueous and methanol extracts of different brown algae

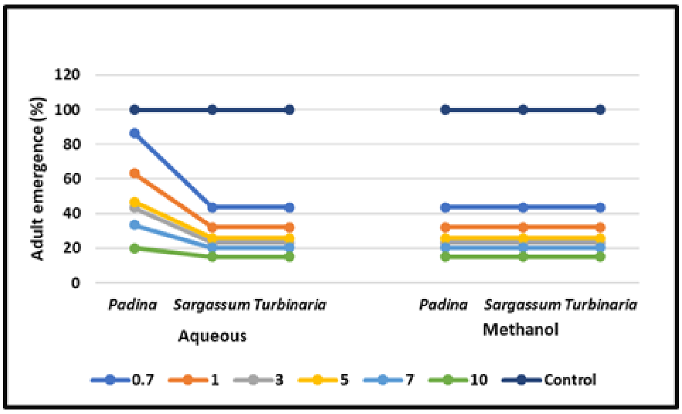

Fig. 5. Adult emergence of *Spodoptera litura* on treatments with aqueous and methanol extracts of different brown algae

Table 2. Lethal concentration of Brown algal extracts after 96 hours of exposure

Macroalgae	Extracts	No. of insects	LC	EV (%)	95 % CI (%)	Slope ± SE	χ2 Value
Padina	Aqueous	30	LC_{10}	0.808	0.140 - 1.548	1.016 ± 0.267	1.993
		30	LC_{20}	2.189	0.932 - 3.486		
		30	LC_{50}	14.736	8.032 -72.258		
	Methanol	30	LC_{10}	1.084	0.199 - 1.991	1.006 ± 0.280	1.200
		30	LC_{20}	2.967	1.426 - 4.892		
		30	LC_{50}	20.367	10.032 -168.76		
Sargassum	Aqueous	30	LC_{10}	0.525	0.156 - 0.952	1.314 ± 0.252	5.021
		30	LC_{20}	0.936	0.512 - 1.744		
		30	LC_{50}	4.963	3.523 - 7.866		
	Methanol	30	LC_{10}	1.566	0.609 - 2.445	1.364 ± 0.310	5.259
		30	LC_{20}	3.290	1.990 - 4.763		
		30	LC_{50}	13.619	8.472 - 38.634		
Turbinaria	Aqueous	30	LC_{10}	0.582	0.029 - 0.905	0.911 ± 0.246	2.848
		30	LC_{20}	1.161	0.283 - 2.044		
		30	LC_{50}	9.747	5.597 - 37.925		
	Methanol	30	LC_{10}	1.410	0.516 - 20247	1.323 ± 0.300	0.931
		30	LC_{20}	3.031	1.774 - 4.406		
		30	LC_{50}	13.113	8.156 - 36.834		

LC - Lethal Concentrations; EV - Estimated Value, CI - Confidence Interval, S.E. - Standard Error; χ2 -Chi-square. The chi square value refers to the goodness of fit at $p>0.05$.

CONCLUSION

The brown macroalgal extracts of *P. tetrastromatica, S. wightii* and *T. conoides* using aqueous and methanol extracts showed potential for insecticidal activity through contact/ feeding toxicity supported with growth regulatory activity against *S. litura* in this study. The LC_{50} values of both aqueous and methanol extracts of the brown algae suggest that it can be improved by using the insecticidal compounds/ principles through nano-formulation/ controlled release formulation, thereby full potential of the brown algae can be realized. Hence, brown macroalgae would be a befitting alternative to synthetic pesticides on the grounds of green pesticides for organic farming.

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Ethics statement

No specific permits were required for the described field studies because no human or animal subjects were involved in this research.

Originality and plagiarism

The authors assure that the research work submitted here is original and not subjected to any plagiarized content.

Consent for publication

All the authors agreed to publish the content.

Competing interests

There was no conflict of interest in the publication of this content

Data availability

All the data of this manuscript are included in the MS. No separate external data source is required. If anything is required from the MS, certainly, this will be extended by communicating with the corresponding author through official mail at dharanipriya1123@gmail.com

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