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Lethal Effects of Red Macroalgal Extracts against Tobacco Cutworm, *Spodoptera litura* Fabricius

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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Original Research Article

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ABSTRACT

Aims: The purpose of the present paper was to study the toxicity of aqueous and methanol Red macro algal extracts against the cosmopolitan pest, Tobacco cutworm: *Spodoptera litura* Fabricius.
Place and Duration of Study: Department of Agricultural Entomology, Tamil Nadu Agricultural University, Coimbatore, Tamil Nadu.
Methodology: The bioassay was carried by treating aqueous and methanol extracts of three different Red macroalgae at 0.7, 1.00, 3.00, 5.00, 7.00 and 10.00 per cent concentration with three replications of ten larvae per treatment and the mortality data was recorded every 24 hours up to adult emergence.
Results: The aqueous and methanol extracts of three different Red macroalgae *viz., Acanthophora spicifera*, *Gracilaria corticata* and *Jania rubens* were used to find out their lethal effects against tobacco cutworm*, Spodoptera litura* under laboratory condition. The larval mortalities were observed for both extracts. Percentages of mortalities, after 96 hours of exposure, increased at the highest concentration of 10 per cent of *A. spicifera*, *G. corticata* and *J. rubens,* extracts. Respectively, 60.00, 46.66 and 26.66 per cent of larvae mortalities were observed in aqueous extracts and 40.00, 26.66 and 23.33 per cent of lethality were recorded in methanol extracts. The pre-pupal and pupal mortalities were recorded in all the concentrations showing malformations during the growth. The

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pupation and the adult emergence were also affected with the highest inhibition by the extracts of *A. spicifera*.

Conclusion: The Red macroalgae possess an insecticidal and growth inhibitory potential which can be exploited for eco-friendly management of *Spodoptera litura.* Further studies should be carried out to purify the active biomolecules of the tested extracts and to evaluate the effect of Red macroalgae on other lepidopteran larvae.

Keywords: Red algae; Acanthophora spicifera; Gracilaria corticata; Jania rubens; Spodoptera litura, ecofriendly management.

1. INTRODUCTION

Tobacco cutworm, *Spodoptera litura* Fabricius is a polyphagous pest of cosmopolitan distribution and defoliate the crops of economic importance *viz.,* groundnut, pulses, castor, cotton, chilli, tobacco *etc*., [1,2]. Synthetic pesticides used for the pest management invoke insect resistance, pest resurgence; and cause serious threats to the environment [3,4]. To reduce the impact of the synthetic chemical insecticides, use of bioactive molecules is promising. The plant derived pesticides are environmentally safe and lower the risk of insect resistance and insect resurgence [5]. There are numerous plants having the insecticide potential with one such alternative is marine macroalgae. The marine macroalgae, commonly known as seaweeds are one of the dominant creatures that occupy all zones of marine ecosystems. There are three groups of macroalgae, Red algae that belong to the phylum Rhodophyta, Brown algae that belongs to Ochrophyta and Green algae that belongs to Chlorophyta. They are not only the primary producers of marine ecosystem but also the potential sources of bioactive compounds of agricultural importance. Macroalgae have inherently developed defense mechanism with novel bioactive molecules and have amazing capability to survive in the marine environment. Numerous secondary metabolites such as sesquiterpenes, diterpenes, monoterpenoids, and steroids are present in seaweeds which showed an insecticidal [6-11]; nematicidal [12,13]; and antimicrobial properties [14,15]. With this background, the present study was carried out to explore the lethal effects of aqueous and methanol extracts of Red macroalgae *viz., Acanthophora spicifera, Gracilaria corticata* and *Jania rubens* against *Spodoptera litura*.

2. MATERIALS AND METHODS

2.1 Insect Culture

Larvae of *Spodoptera litura* were collected from the castor plant in Department of Oil seeds, Tamil Nadu Agricultural University, Coimbatore district (11°1'N, 76°55'E) and reared with castor leaves as natural diet under laboratory conditions. They were reared up to pupation and allowed to hatch. The emerged healthy adults were released into an oviposition cage and were provided with fresh *Nerium oleander* L leaves for egg laying with petiole immersed in water to prevent the drying of leaves. The adult insects are fed with 10% honey solution fortified with multivitamins to enhance the oviposition rate. The newly emerged F_1 generation larvae were utilized for the experiment.

2.2 Collection of Macroalgae

The three species of Red macroalgae, *Acanthophora spicifera* (M.Vahl) Boergesen, *Gracilaria corticata* (J. Agardh) J. Agardh and *Jania rubens* (L.) J.V. Lamouroux were collected from Hare Island located at Lat 9^{o}12'0'' N and Long 79^{o}4'48''E which is the part of Gulf of Mannar region, Tuticorin, Tamil Nadu, India during May and October 2019. The algal thalli were collected by hand picking method, washed to remove salts and debris, dried under shade and stored in sealed container at room temperature for further studies**.** The collected algal specimens were identified and authenticated by the Botanical Survey of India, South Regional Centre, Coimbatore and the specimens were deposited in the Department of Agricultural Entomology, Tamil Nadu Agricultural University, Coimbatore.

2.3 Preparation of Macroalgal Extracts

The dried macroalgae were powdered and sieved to uniform particle size of 0.5 mm. The extraction of bioactive compounds was done with ultrapure water (H_2O) and methanol (MeOH) at the biomass solvent ratio of 1:10 *w/v* by using ultra high Frequency sonicator (SONICS®, sonics and materials, Inc.) at 20kHz for 30 minutes followed by centrifugation (Dynamica, VELOCITY 14R) at 10000 rpm (10380xg) for 10 min. The supernatant was collected and the pellets were re-extracted with fresh solvent to enhance the extraction efficiency. The supernatant was pooled and concentrated under reduced pressure at 35^{o}C and 100 rpm using rotary cum vacuum evaporator (Heidolph, Hei-VAP core) and the crude extracts were stored at -20^{0}C until utilization. The extraction yield of crude extracts is calculated as follows:

Extraction yield (%) = Yield of crude extracts obtained (g)/Amount of raw material used (g) X 100

2.4 Bioassay by leaf dip method

The leaf discs of known size (4 cm diameter) were cut and dipped for 10 seconds in different concentration 0.7, 1.0, 3.0, 5.0, 7.0, 10.00 per cent prepared with aqueous and methanol extract solution along with 0.1% Triton X-100 as sticking agent Water and methanol with 0.1% Triton X were used as control. The leaf discs were dried, placed in Petri plates and offered to 4 hours pre-starved second instar larvae of *Spodoptera litura*. Three replications with 10 larvae per replicate were designed for toxicity studies and the experiment was Completely Randomized. Mortality and growth of larvae were recorded every 24 hours after treatment up to adult emergence. The dose-response curve was constructed and the lethal concentration (LC_{10}, LC_{20} and LC_{50}) of macroalgal extracts was estimated.

2.5 Statistical Analysis

The mortality data were *arc sine* transformed before one-way ANOVA analysis, and means were compared using Tukey's Honestly Significance Difference (HSD) (p=.05) test. Data on mortality were submitted to Probit analysis at 95% confidence level ($P < 0.05$) to obtain dose-response curve. All the statistical procedures were done with IBM SPSS v. 20 statistical software.

3. RESULTS AND DISCUSSION

The Red macroalgae have the potential to act as biopesticide that can be utilized in Integrated Pest Management. The current study involves the mortality bioassays with crude aqueous and methanol extracts of Red macroalgae, *A. spicifera, G. corticata* and *J. rubens* that showed significant toxicities against second instar larvae of *S. litura* at different concentrations. Previous reports indicated that members of Rhodophyta have provided an assortment of insecticidal compounds potential for pest management use in agriculture [16]. The extraction yield of crude extracts of A. *spicifera, G. corticata* and *J. rubens* were 9.6, 6.2, 3.2 and 5.1,3.2, 2.9 per cent for aqueous and methanol extracts respectively (Table 1).

In this study, on treatment with different concentrations *viz.,* 0.7, 1.00, 3.00, 5.00, 7.00 and 10.00 per cent of aqueous and methanol extracts of Red algae, the larval mortality had occurred in all the concentrations used in a dose dependent manner with a highest mortality of 60.00 and 40.00 per cent at 10 per cent concentration and the least larval mortality of 26.66 and 10.00 per cent at 1.00 per cent concentration in aqueous and methanol extracts of *A. spicifera* at the end of 96 hours of exposure to insects. It is followed by *G. corticata* with the maximum mortality of 46.66 and 26.66 per cent and minimum of 10.00 per cent mortality in aqueous and methanol extracts respectively. Among the used red macroalgae, *J. rubens* exhibited the least mortality at the highest concentration *i.e.,* 26.66 and 23.33 per cent larval mortality and a low mortality of 3.33 per cent at the lowest used concentration. The lowest percentage of mortality was noticed at 1.0 per cent concentration in all the used macroalgal extracts (Figs 1 and 2). The lethal concentration (LC_{10}, LC_{20} and LC_{50}) of *Acanthophora, Gracilaria* and *Jania* were observed at percentages of 8.323, 11.542 and 25.100 for aqueous and 16.404, 33.246 and 26.923 per cent for methanol extracts, respectively and the data were provided in Table 2. After 96 hours of treatment, the pupation had initiated with the occurrence of pre-pupal mortality of 13.33 per cent in both aqueous and methanol extracts of *A. spicifera* and *J. rubens* whereas the morality of 20 and 6.66 per cent in aqueous and methanol extracts of *G. corticata* respectively (Fig 3). The least pupation had occurred in *A. spicifera i.e.,* 30.00 and 46.66 per cent followed by *G. corticata i.e.,* 33.33 and 66.66 per cent and *J. rubens i.e.,* 60 and 63.33 per cent in aqueous and methanol extracts respectively. Some of the pupae were failed to emerge as adults with pupal mortality of 13.33 and 16.66 per cent in *A. spicifera* followed by 13.33 and 20.00 per cent in *G. corticata* and 23.33 and 10.00 per cent in *J. rubens* for aqueous and methanol respectively (Fig 4). The Red macroalgal extracts have the potential to inhibit the growth of *S. litura*, with the least larval to adult conversion ratio of 1:0.55 and 1:0.42 *A.*

spicifera followed by *G. corticata* with the ratio of 1:0.20 and 1:0.46 and *J. rubens* with the ratio of 1: 0.36 and 1:0.53 respectively for aqueous and methanol extracts and it represented in Fig 5.

Table 1. Extraction yield of Red macroalgae

Macroalgae	Extraction yield (%)	
	Aqueous	Methanol
Acanthophora spicifera	9.6 ± 0.09[d]	5.1 ± 0.19[d]
Gracilaria corticata	6.2 ± 0.13[e]	3.2 ± 0.14[e]
Jania rubens	3.2 ± 0.05[f]	2.9 ± 0.06[f]

All values are mean ± SD for triplicate experiments (n=3). Different letters within the same column indicate significant difference between the values (p=.05). Extraction yield (%) calculated as g of dried extract/10 g DW

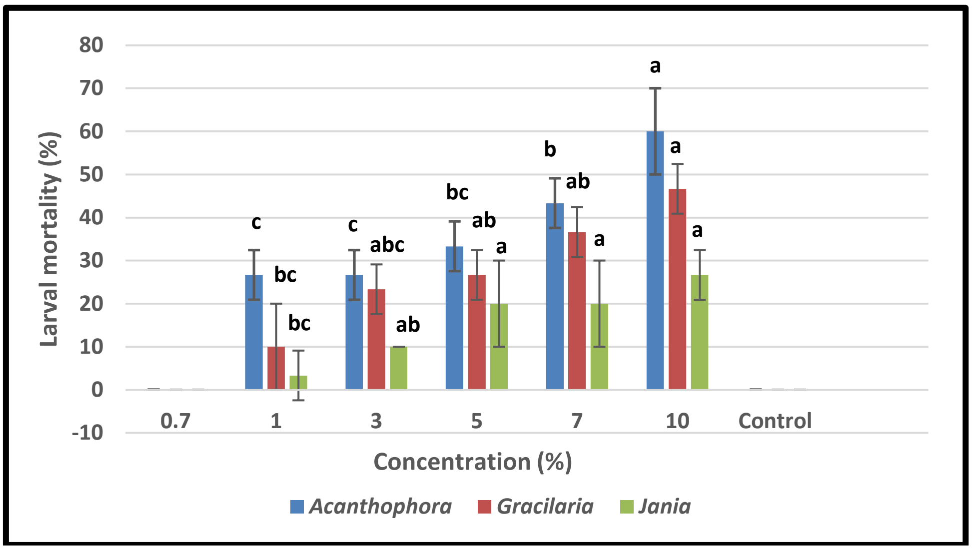

Fig. 1. Larval mortality of *Spodoptera litura* on treatments with aqueous extracts of different Red algae. Treatment means (± SEM) with different letters show significant differences by Tukey's Honestly Significant Difference Test at p=.05 level

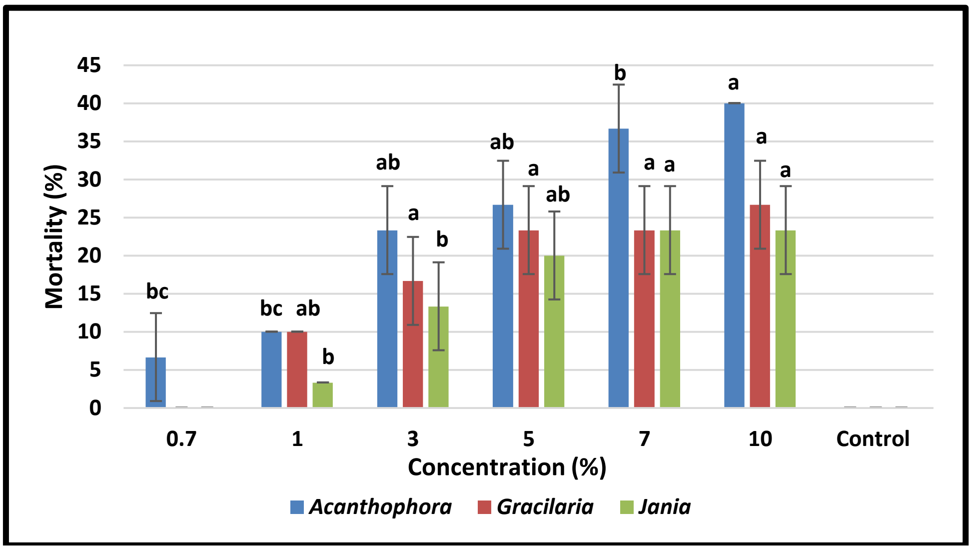

Fig. 2. Larval mortality of *Spodoptera litura* on treatments with methanol extracts of different Red algae. Treatment means (± SEM) with different letters show significant differences by Tukey's Honestly Significant Difference test at p=.05 level

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

Macroalgae	Extracts	Lethal concentration	Estimated value	Confidence interval	Slope ± SE	χ2 Value
Acanthopora	**Aqueous**	LC_{10}	0.976	0.000 - 2.058	1.243 ± 0.266	9.410
		LC_{20}	2.751	0.000 - 3.936		
		LC_{50}	8.323	3.708 - 42.30		
	Methanol	LC_{10}	1.029	0.221 - 1.858	1.066 ± 0.278	0.221
		LC_{20}	2.662	1.287 - 4.190		
		LC_{50}	16.404	8.857 - 84.199		
Gracilaria	**Aqueous**	LC_{10}	1.527	0.631 - 2.347	1.459 ± 0.314	2.409
		LC_{20}	3.058	1.864 - 4.299		
		LC_{50}	11.542	7.631 -26.583		
	Methanol	LC_{10}	1.804	0.385 - 3.114	1.013 ± 0.315	2.634
		LC_{20}	4.904	2.762 - 10.537		
		LC_{50}	33.246	13.672 - 949.680		
Jania	**Aqueous**	LC_{10}	3.100	1.348 - 4.654	1.411 ± 0.400	0.980
		LC_{20}	6.356	4.194 - 11.972		
		LC_{50}	25.100	12.900 - 207.944		
	Methanol	LC_{10}	2.832	1.114 - 4.357	1.310 ± 0.380	1.469
		LC_{20}	6.136	3.946 - 12.060		
		LC_{50}	26.923	13.169 - 284.871		

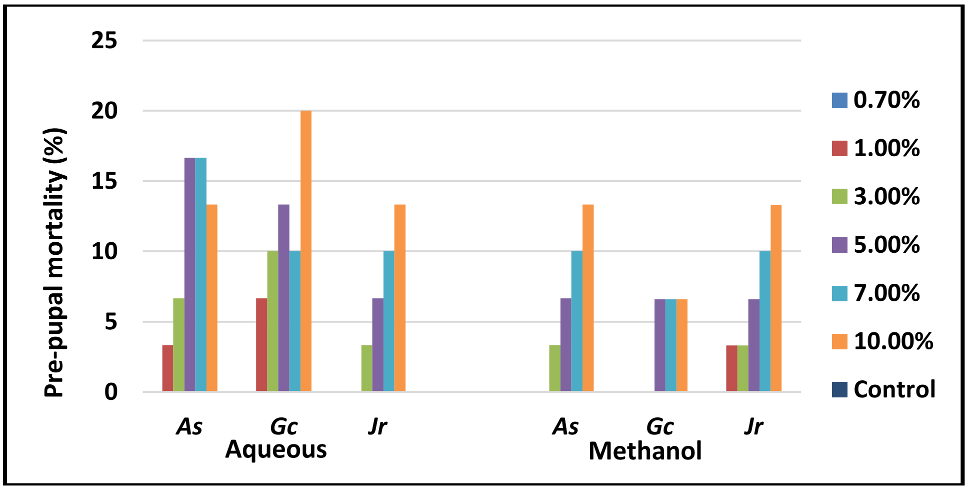

Fig. 3. Pre-pupal mortality of *Spodoptera litura* on treatments with aqueous and methanol extracts of different Red algae

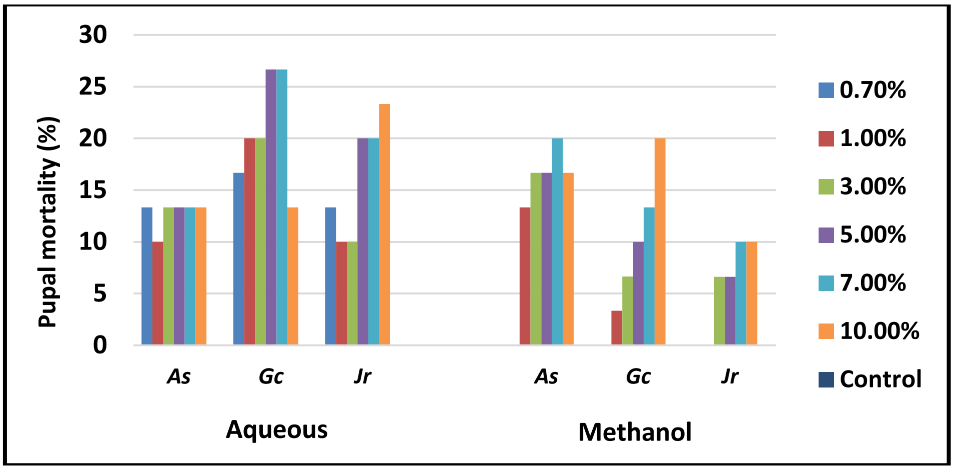

Fig. 4. Pupal mortality of *Spodoptera litura* on treatments with aqueous and methanol extracts of different Red algae

Previously, it is reported [17,18] that the Red algae, *Plocamium cartilagineum* possess polyhalogenated monoterpenes that have insecticidal and insect feeding deterrence activities against tobacco budworm (*Heliothis virescens*), Tomato moth (*Tuta absoluta*), Southern com rootworm (*Diabrotica undecimpunctata*), European corn borer (*Ostrinia nubilalis*), fall armyworm (*Spodoptera frugiperda*), boll weevil (*Anthonomus grandis*), aster leafhopper (*Macrosteles facifrons*), black bean aphid (*Aphis fabae*), Cereal aphid (*Schizaphis graminum*) and two-spotted spider mite (*Tetranychus urticae*). The members of genus, *Laurencia* have various bioactive metabolites that have an insecticidal potential [19] especially acetylinic sesquiterpene ethers [20]. Researchers compared the effect of crude and active ingredient C_{15} acetogenin from the petroleum ether extracts of red alga *Laurencia papillosa* on grub of confused flour beetle, *Tribolium confusum* and found to inhibit their growth [21]. The Red algae, *Liagora ceranoides* demonstrated the insecticidal activity towards *Oryzeaphilus mercator* and *Tribolium castaneum* [22]. Lectins are first identified from the marine algae [23]. The purified lectins from marine red alga, *Gracilaria ornata* showed higher level of mortality to cowpea weevil, *C. maculatus* when incorporated in artificial seeds due to the inhibition of digestion and absorption of food [24]. The repellent action of red flour beetle, *Tribolium castaneum* was observed at 4 per cent concentration of *G. edulis* with acetone, ethyl acetate and chloroform extracts treatments [25].

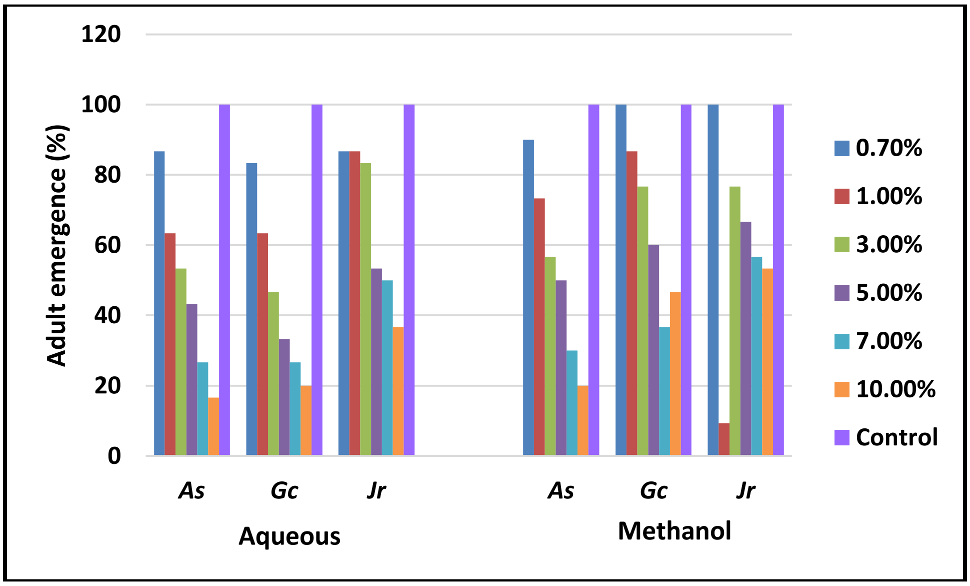

Fig. 5. Adult emergence of *Spodoptera litura* on treatments with aqueous and methanol extracts of different Red algae

4. CONCLUSION

The Red macroalgal extracts of *A. spicifera, G. corticata* and *J. rubens* using aqueous and methanol extracts showed potential insecticidal activity against *S. litura*. The LC_{50} values of both aqueous and methanol extracts of the Red algae suggests that it can be improved by using the bioactive purified molecules of the tested extracts. Hence, Red macroalgae would be a befitting alternative to synthetic pesticides on the grounds of green pesticides for organic farming.

DISCLAIMER

The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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