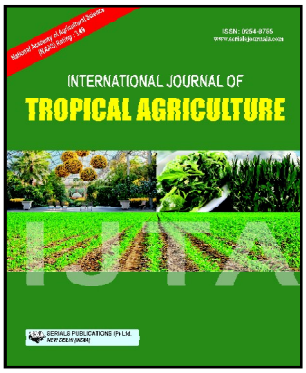

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Insecticidal and Insect growth regulator activity of a red algal seaweed *Gracilaria edulis* (S.G.Gmelin) P. C. Silva against Tobacco caterpillar *Spodoptera litura* Fabricius (Lepidoptera: Noctuidae)

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Abstract: Seaweeds are the unexploited natural resources widely distributed in the marine environment. They have constituted with major bioactive compounds which works in many different ways against various group of organism including, fungi, bacteria, nematodes, mites and insects. This study was focused towards exploring the insecticidal and Insect Growth Regulator activity of a red algal seaweed (*Gracilaria edulis* (S.G.Gmelin) P.C.Silva) against different life stages of *Spodoptera litura* (Fab.) under controlled conditions in Department of Entomology, Faculty of Agriculture, Annamalai University during 2016-18. The marine algae collected from Gulf of Mannar, Tuticorin, Tamil Nadu, India has been used for the toxicity studies. *Gracilaria edulis* methanol solvent extract obtained from Soxhlet extraction process was screened for their toxicity and IGR activity against *S. litura* at different concentrations *viz*., 0.5, 1.0, 2.0, 4.0, 6.0, 8.0 and 10 per cent under free choice test with three replications per treatment in Completely Randomized Design. Data on larval mortality, pre-pupal mortality, pupation, pupal malformation and adult emergence was collected accordingly and the data were analysed statistically and presented. Among the different concentrations tested, 10.00 per cent showed the maximum larvicidal activity (53.33%), pre-pupal mortality (26.66%), pupal malformation (20.00%) and the pupal to adult conversation ratio (1:0.00) followed by the lowest concentration (0.5 per cent) of the treatment was exerted (20.00%), (6.66%), (6.66%) and (1: 0.66) respectively. The minimum pupation (20.00%) and adult emergence (0.00%) were recorded in the 10.00 per cent concentration whereas it was highest at 0.5 per cent (73.33%) and (66.66) after 120 hours of treatment. Toxic effect of the seaweed extract was observed in the test insect as larval mortality, pre-pupal mortality and pupal malformation.

Key words: Insecticide, Red marine algae, *Gracilaria edulis, Spodoptera litura.*

Farming as an important occupation in Asian countries especially in India almost providing livelihood to nearly 65 per cent of the population. In Agriculture, insects are one of the destructive enemies of farmers known to cause severe loss to crops that resulted in yield reduction. Tobacco leaf caterpillar, *Spodoptera litura* (Fab.) favoured wide diversity of hosts (Ahmad *et al.*, 2013) nearly 120 host plants all over world. It has changed as major damage causing pest in agricultural field on commonly cultivable crops *viz*., Tobacco, Bhendi, Chilli, Cotton, Groundnut, Castor, Pulses etc. (Arain *et al.*, 2018).

Over use of inorganic insecticides in farming land directed the scientists towards eco-friendly alternatives such as naturally available bio-rationales for bio-intensive pest management (Mall *et al.*, 2018). Seaweeds contains plenty of bioactive compounds which plays various biological activities (Tuney, 2006). Seaweeds shown to have bactericidal (Soleimani *et al.*, 2018); fungicidal (Abbassy *et al.,* 2014) and insecticidal activities (Sahayaraj and Mary Jeeva, 2012; Xin Yu *et. al.*, 2015; Kannan and Bharathkumar, 2016 and Ishii *et al.*, 2018). This article described the *Gracilaria edulis* methanol solvent extract toxicity on the *Spodoptera litura*.

MATERIALS AND METHODS

Collection of seaweeds

The red algal seaweed (*Gracilaria edulis*) was collected from Gulf of Mannar, Tuticorin, Tamil Nadu, India using hand picking method and immediately washed with fresh sea water and again thoroughly washed three times with tap water to remove the excess salt, sand and epiphytes. The algae were wiped with a blotting sheet (to drain away the moisture) and air-dried under shade for a fortnight for complete drying to safe storage (Kannan and Bharathkumar, 2016). The seaweeds were identified at CAS Marine Biology, Annamalai University.

Mass culturing of Tobacco caterpillar, *Spodoptera litura*

Spodoptera litura egg masses were collected from black gram field at Vallampadugai village, Cuddalore district of Tamil Nadu. The field-collected egg masses were allowed to hatch under laboratory condition (27±2°C) temperature and (RH 76±5%). Newly hatched larvae were reared on fresh castor leaves (*Ricinus communis* L.) on regular basis up to the pre-pupal stage. For pupation, sterilized (surface sterilized with formalin 1%) plastic trays filled with soil was provided. After pupation, the pupae were collected from soil and placed inside the oviposition chambers (40 x 25 x 25 cm) in a Petri plate. After adult emergence, the moths were collected and five pairs were allowed inside the oviposition chamber for mating and subsequent oviposition. Emerged adults were fed with 10% honey solution and were allowed for egg laying on Nerium leaves (petiole immersed in conical flask with water) kept inside oviposition cage. The laid eggs were collected and sterilized with 0.02% Sodium hypochlorite, then eggs were allowed to dry and placed in a sterilized plastic tray covered with a muslin cloth. After hatching, the neonates were fed with fresh tender castor leaves and were reared up to third instar stage in plastic trays. The uniform aged third instar larvae were used for bioassay experiment.

Preparation of solvent extracts

Thirty gram of partially powdered red algal seaweed *G. edulis* was packed and loaded in Soxhlet apparatus (GI-1706 A-Biocoction) individually and refluxed with solvent @ 300ml of methanol for 24 hours continuously. The extracted solvent was were evaporated in a hot plate and the final extract was elucidated with corresponding solvent and used for the evaluation experiments. The extracts were stored at -20^{0}C (Kombiah and Sahayaraj, 2012).

Bioassay - Leaf disc method (Free choice)

The stock solution of crude extract (10%) was prepared by dissolving in solvents. The solvent

extracts at different concentrations: 0.5, 1.00, 2.00, 4.00, 6.00, 8.00 and 10.00 per cent along with and absolute control and standard check were evaluated against homogenous population (third instar) of *S. litura* larva using leaf dip assay method. Surface sterilized Castor leaf discs (5cm dia) were dipped in the solvent extract concentrations for ten minutes and shade dried. The treated leaves after drying were placed inside the Petri dishes (five leaf discs per Petri plate) separately and provided with required moisture using wet filter paper. Four hours pre-starved third instar homogenous larva were introduced in each Petri plate according to the treatment schedule and allowed to feed on the treated leaves. The experiment was laid under completely randomized design with nine treatments under three replications. Data on larval mortality, pupation, pre-pupal mortality, pupal malformation, adult emergence were collected and the means were pooled and analysed statistically the presented.

RESULTS AND DISCUSSION

Data on larval mortality, pre-pupal mortality, pupation, pupal malformation and pupal to adult conversion ratio by the methanol solvent extract of red algal seaweed *G. edulis* exhibited the following results:-

The influence of methanol solvent extracts of *G. edulis* on larvicidal action was observed in 24 hours interval and the larval dead was occurred only after 48 hours of treatment and gradually increased up to 120 hours and the per cent morality was recorded between 0.00 and 53.33 per cent. The larval mortality was exhibited at the highest level 53.33 per cent in the 10 per cent concentration. Among the different concentrations, the minimum larval mortality was noticed in 0.5 percent concentration, but the effect differ with concentrations and no larval mortality was observed in absolute and solvent control treatments (Table 1).

All the concentrations of methanol solvent extract of *G. edulis* had shown to bereavement of pre-pupal stage of test insects where the highest pre-pupal mortality (26.66%) was recorded at maximum test concentration 10 per cent in the treatment. Wherein the least dose of the treatment 0.5 per cent revealed (6.66%) lowest pre-pupal mortality and there is no death in absolute and solvent control treatments (Fig. 1).

Table 1
G. edulis* methanol solvent extract on the larval mortality of *S.litura

	Larval mortality (%)										
Treatment	T_1 *(0.5%)*	T_2 *(1%)*	T_3 *(2%)*	T_4 *(4%)*	T_5 *(6%)*	T_6 *(8%)*	T_7 *(10%)*	*Absolute Control*	*Solvent control*	*SEd*	*CD (p = 0.05)*
48hrs	6.66 (9.046)	6.66 (9.046)	6.66 (9.046)	13.33 (17.80)	13.33 (17.80)	20.00 (26.56)	20.00 (26.56)	0.00 (0.286)	0.00 (0.286)	11.146	24.28
72hrs	6.66 (9.046)	13.33 (17.80)	13.33 (17.80)	20.00 (26.56)	20.00 (26.56)	26.66 (30.78)	33.33 (35.01)	0.00 (0.286)	0.00 (0.286)	7.803	17.00
96hrs	13.33 (17.80)	20.00 (26.56)	20.00 (26.56)	26.66 (30.78)	26.66 (30.78)	33.33 (35.01)	46.66 (43.07)	0.00 (0.286)	0.00 (0.286)	6.769	14.74
120hrs	20.00 (26.56)	26.66 (30.78)	33.33 (35.01)	33.33 (35.01)	40.00 (39.23)	46.66 (43.07)	53.33 (43.07)	0.00 (0.286)	0.00 (0.286)	4.733	10.31

Means are value of three replications; values in brackets are arcsine transformed values

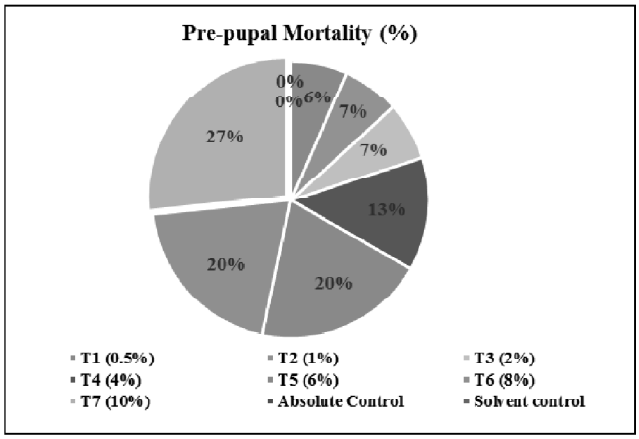

Figure 1: *G. edulis* methanol solvent extract on the Pre - pupal mortality of *S.litura*

The left over larvae from both larval mortality and pre-pupal mortality test were allowed to pupate and the data confirmed that the lower most pupation (20.00%) was exhibited at 10 per cent concentration and the uppermost pupation (73.33%) was evidenced in 0.5 per cent concentration respectively wherein the solvent and absolute control exhibited 100 per cent pupation in the experiment (Fig. 2).

The toxic effect of methanol solvent extracts of *G. edulis* was observed as pupal malformation and the deformed pupae were not converted in to adults. All the concentrations shown pupal malformation except both solvent and absolute control. The pupal malformation data were observed in all the concentration ranges from 6.66 to 20.00 per cent wherein the highest concentration 10 per cent exerted the maximum malformation (20.00%) and as it was least (6.66%) in 0.5 per cent respectively (Fig. 3).

The adult emergence per cent was reduced due to toxic effect exposed in the larval stage, pre-pupal stage and pupal stage of the test insect. Among the different concentrations were tested 10 per cent concentration displayed (0.00%) adult emergence and there was no treated larvae changed in to adults as it demonstrated that pupal to adult conversion ratio as 1:0.00 wherein it was noted in lowest concentration 0.5 per cent of the treatment was (66.66%) with pupal to conversion ratio 1:0.66 respectively followed by (100%) adult emergence was recorded with 1:1.00 pupal to adult conversion ratio in the solvent and absolute control (Fig. 4) and (Table 2.)

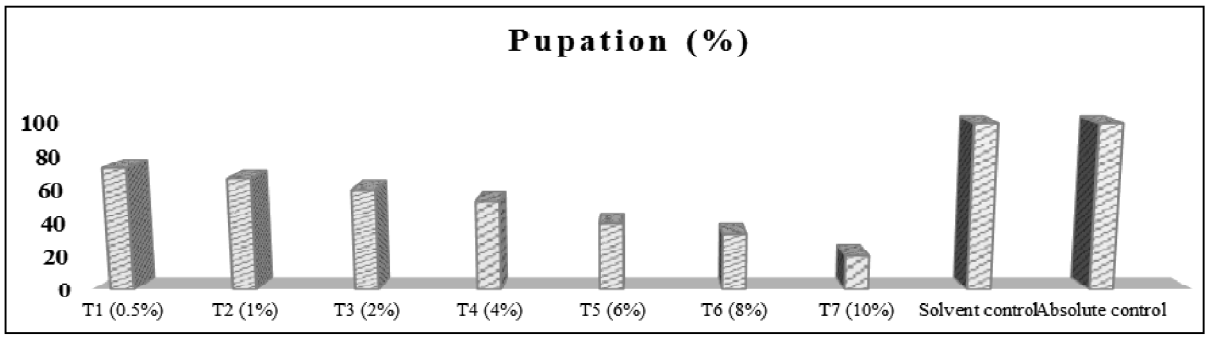

Figure 2: Effect of *G. edulis* methanol solvent extract on the pupation of *S.litura*

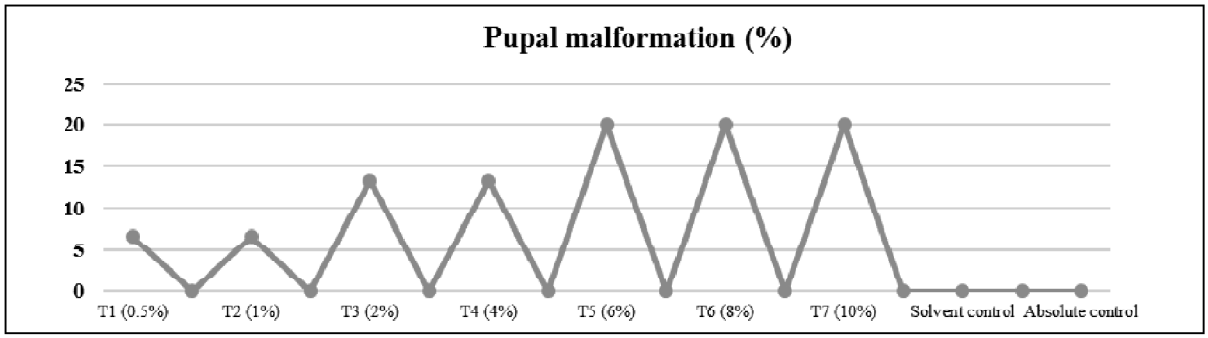

Figure 3: Effect of *G. edulis* methanol solvent extract on pupal malformation of *S.litura*

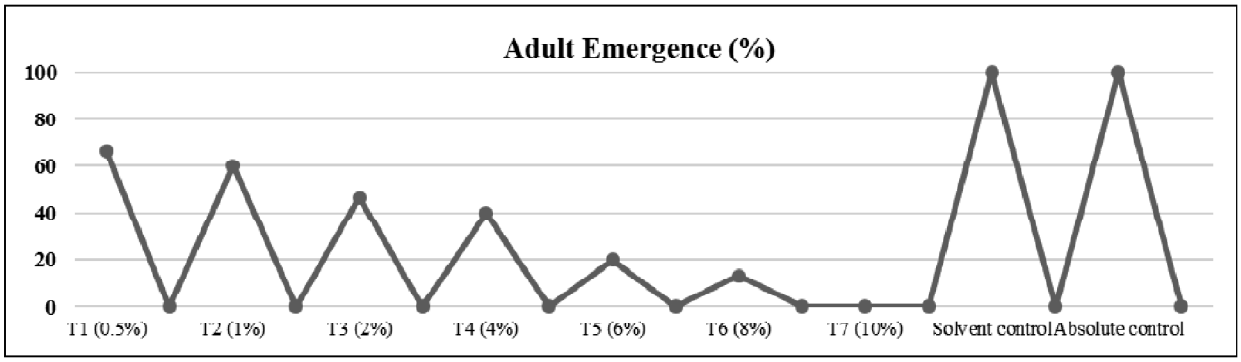

Figure 4: Effect of *G. edulis* methanol solvent extracton the adult emergence of *S. litura*

Table 2
Effect of *G. edulis* methanol solvent extract on pupal to adult conversion ratio

Treatments	T_1 *(0.5%)*	T_2 *(1%)*	T_3 *(2%)*	T_4 *(4%)*	T_5 *(6%)*	T_6 *(8%)*	T_7 *(10%)*	*Absolutecontrol*	*Solventcontrol*
Methanol	1: 0.66	1: 0.60	1: 0.46	1: 0.40	1: 0.20	1: 0.13	**1: 0.00**	1: 1.00	1: 1.00

In the present study, *G. edulis* solvent extracts exhibited larval mortality, reduced pupation, pre-pupal mortality and reduced adult emergence and these results are in accordance with the following studies on the toxicity of seaweeds against different insects conducted by many scientists in India and abroad.

Lavanya *et al.* (2019) reported that green synthesis of silver nanoparticles of *Gracilaria birdiae* at 1000 ppm concentration resulted (99.00%) larvicidal activity against *Aedes aegypti*. Three different seaweeds namely, brown (*Dictyota dichotoma*), green (*Enteromorpha intestinalis*) and red algae (*Acanthopora spicifera*) were tested for their effectiveness using ethanol solvent extract of against *A. aegypti* (Beula *et al.,* 2011).

Recently, Sahayaraj *et al.* (2019) reported that the secondary metabolites presented in various solvent extract of *Caulerpavera veravalensis* shown toxicity to different stages (egg, nymph and adult) of *Dysdercus cingulatus*.

Dayuti (2018) analyzed and concluded that the methanol solvent extract of red algal seaweed *Gracilaria verrucosa* constituting various group of phytochemicals such as alkaloids, saponins and flavonoids which was inhibited the bacterial activity. John and Devi (2014) conducted an experiment for screening eleven different types of phytochemicals using methanol extract of *Gracilaria dura* and among them alkaloids, flavonoids, glycosides, phenolic groups, saponins, tannins and terpenoids were confirmed to be present whereas Anthra-quinones, Catechin, Reducing sugars and Sterols & Steroids were not present.

The human diseases (lymphatic filariasis) transmitting vectors *Culex quinquefasciatus* and *Chironomus circumdatus* have been controlled in both larval and pupal stage using the aqueous extract of *G. edulis* along with silver nanoparticles (Madhiyazhagan *et al.,* 2016). The three group of seaweed such as red, brown and green algae of methanol extract have produced strong larvicidal action against *A. aegypti* and *A. albopictus* (Ahmad *et al.*, 2016). Similarly, 100 per cent larval mortality was reported by ZnO nanoparticles (50 ìg/ml) with green algae *Ulva lactuca* against *Aedes aegypti* (Ishwarya *et al.,* 2017).

The larvicidal action, pre-pupal mortality and the deformed pupa of *S. litura* by the methanol

solvent extract of *G. edulis* may be due to the presence of phytochemicals which yet to be explored. Several studies mentioned below supported our view that the secondary metabolites in seaweeds may attribute for pest reduction and IGR activities

The red algae *Laurencia papillosa* crude extract contains pure secondary metabolite (acetogenin) which act as larvicide against flour beetle larvae *Tribolium confusum* and *Culex pipiens* (Elnaga *el at.,* 2011).

The red algae *L. dendroidea* contains Elatol, a chamigrene-type halogenated sesquiterpene was confirmed the larvicidal activity against *A. aegypti* and they recommended that some marine halogenated sesquiterpenes isolated from red seaweeds, may be used as prototypes for larvicidal agents (Bianco *et al.*, 2012).

The various organic solvent extracts such as hexane, chloroform, ethyl acetate and acetone were screened for phytochemicals of *G. edulis* and *G. verrucosa* among them the methanol extract was confirmed the presence of following bio-active chemicals *viz.*, terpenoids, tannins, cardiac glycosides, steroids, alkaloids, phenolic compounds, coumarins and diterpenoids (Chandrasekaran *et al.*, 2016).

In red algae group the only genus *Gracilaria* is extensively screened for their secondary metabolites (Torres *et al.,* 2019).

CONCLUSION

From the findings it was concluded that the methanol extract of red algal seaweed *Gracilaria edulis* could be used as an ideal alternate to available botanicals and synthetic pesticides in reducing the pest population and it can be used in bio-intensive pest management of crop pests.

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