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The efficacy of *Sargassum cristaefolium* seaweeds from manora coast as potential pesticides activity against *Spodoptera litura* fab (Lepidoptera: Noctuidae)

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Abstract
The present study was conducted to assess the larvicidal and insect growth regulatory activities of methanol extract of *Sargassum cristaefolium* against *Spodoptera litura Fabricius*. The efficacy of brown algal seaweed collected from the Manora coastal landscape against the polyphagous pest Spodoptera litura was tested in the laboratory. Among the various treatments subjected, the methanol extract of the seaweed with the lowest feeding rate at greater concentrations showed potential antifeedant activity. The larvicidal content was observed initially from 48 hours and steadily increased up to 72 hours, with a larval death rate of up to 75.98percent when compared to control. The larvae remained pupated with a minimal pupal deformity. At 15% concentration, the pupation to adult conversion ratio was 1:0.27, whereas the conversion ratio in control was 1:1.01. The insecticidal activity of the test brown algae was confirmed by these findings.

Keywords: *sargassum cristaefolium*, *spodoptera litura*, antifeedant, larvicidal, methanol

Introduction

Spodoptera litura (Fab.) is an important polyphagous and damage-causing pest in agricultural crops such as tobacco, cotton, groundnut, castor, and pulses. It has also caused significant damage to economically vital crops in India, China, and Japan, with crop losses ranging from 10% to 30% in major crops (Kamaraj *et al*., 2008; Ahmad *et al*., 2013), and still poses to be a threat of major concern. In the past, crop insect pests were mostly controlled using costly and poisonous chemical pesticides that were particularly hazardous to natural enemies and other invertebrates, and in turn had an adverse effect in humans.

The aquatic environment contains almost half of the world's biodiversity and is a huge source of novel chemicals. Seaweeds and marine algae are potentially abundant sources of highly bioactive secondary metabolites that might be used to produce novel pharmacological medicines (Chanda *et al*., 2010) [3]. Seaweeds are a form of primordial plant that grows abundantly in the shallow waters of the sea, estuaries, and backwaters. They thrive anywhere rocky, coral, or other appropriate substrates that are accessible for attachment. Seaweeds have been utilized as food, fodder, fertilizer, and a source of medical medications since ancient times.

Recently, scientists all around the globe have focused on the potentiality of marine species as alternate sources for isolating new metabolites with intriguing biological and pharmacological features (Faulkner, 2002) [5] such naturally occurring resource is marine algal seaweeds, which provide a unique method in insect pest management and have been shown to contain numerous secondary metabolites. The current study aims to investigate the antifeedant, larvicidal and insect growth regulatory (IGR) activities of a macro algal seaweed solvent extract (Methanol) of Sargassum cristaefolium against S. litura.

Materials and Methods

Collection of seaweeds

The seaweed samples were collected from the Manora coast (Latitude: 10.2644º N and Longitude: 79.2874º E) Thanjavur district, Tamilnadu, India. During the month of November 2021, the live and healthy macro algal sample was taken by handpicking at a depth of 1-2m and promptly cleaned with fresh seawater before being carefully washed three times with tap water to eliminate excess salt, sand, and epiphytes. The algae were cleaned off with a blotting sheet and air-dried in the shade for a fortnight to ensure safe storage.

Table 1: Scientific classification Sargassum Cristaefolium

Kingdom	**Seaweedae**
Phylum	Ochrophyta
Class	Phaeophyceae
Order	Fucales
Family	Sargassaceae
Genus	Sargassum
Species	Cristaefolium

Massculturing of Tobaccocaterpillar, *Spodoptera litura*

The *Spodoptera litura* egg masses collected from groundnut fields at Peravurani village in Thanjavur district of Tamil Nadu were allowed to hatch on fresh castor leaves kept in Plastic trays. The neonates were fed with tender castor leaves for two days before being limited to fifty larvae per tray and spread in numerous trays with castor leaves and cultured up to the final instar. S. litura pupa were put in an ovipositional cage (40 x 25 x 25 cm) with Nerium twigs in conical flasks to enhance egg-laying. The newly emerging adult moths (five pairs) were fed a 10% honey solution and allowed to mate and deposit eggs. The females' egg masses were moved to trays containing fresh tender castor leaves, and the culturing cycle was repeated. In the present work, the bioassay experiment was performed with uniformly aged third instar larvae from the culture.

Scientific classification spodopteralitura

Table 2

Kingdom	Animalia
Phylum	Arthropoda
Class	Insecta
Order	Lepidoptera
Superfamily	Noctuoidea
Family	Noctuidae
Genus	*Spodoptera*
Species	*S. litura*

Preparation of solvent extract

The *Sargassum cristaefolium* was shade dried. *Sargassum cristaefolium* was then partly powdered, and the powdered seaweed was wrapped in a cotton cloth (30g each) and individually loaded in Soxhlet apparatus (GI-1706 A-Biocoction) and constantly refluxed with 300ml of methanol solvent for 24 hours. The solvent extract was then transferred to a 500ml beaker and evaporated on a hot plate. The extracted crude was clarified again with 100 ml of solvent and utilized in the assessment assays. The extract was kept at -200 ° C. (Kombiah and Sahayaraj, 2012) [2, 11, 14].

Bioassay-Leafdisc method (Freechoice)

By dissolving the crude extracts in methanol, the standard concentration (40%) was obtained. The castor leaf discs were subjected to the following treatment regimen: - 0.1, 1.00, 3.00, 5.00, 7.00, 9.00, 11.00, and 15% solvent extract with one absolute control (methanol) and standard check. The experiment used a totally randomized block design with ten treatments spread over three replications. Fresh castor leaf discs with a diameter of four cm were immersed in the various treatments (for five minutes) in accordance with the treatment schedule. The treated leaf discs were air-dried and maintained separately in Petri plates (five-leaf discs per Petri plate) with the necessary moisture (wet filter paper). The antifeedant action was studied using the methods outlined below. Under laboratory circumstances, the test insects were allowed to feed on treated leaves (free choice method) for up to 24 hours, and the percent antifeedant activity was estimated by measuring the percent protection of leaf area with graph paper or a planimeter.

Percent Antifeedant activity = Percent protection in treated leaf - Percent protection in control. 100 - Percent protection in control.

Results and Discussion

Around the world, seaweeds are employed in agriculture, nutrition, and medications (Kolanjinathan *et al*., 2014; Pati *et al*., 2016; Stranska-Zachariasova *et al*., 2017) [10]. However, the economic application of seaweeds for pest control remained restricted. As a result, the purpose of this study was to investigate the insecticidal and antifeedant action of crude methanol extracts of *S. cristaefolium* seaweeds against *Spodoptera litura*.

Based on the findings, data on antifeedant, larval mortality, and Insect Growth Regulator (IGR) activity of the methanol extract of the brown algal seaweed *S. cristaefolium* were gathered. The antifeedant action was seen in all treatments, with the highest activity at 15% concentration (60.79 percent) and the lowest activity at 0.1 percent concentration (31.25 percent). The larval mortality and growth regulator activities investigated by the methanol solvent extract of S. cristaefolium revealed that the highest mortality rate was demonstrated by the maximum concentration of 15% (75.98percent), followed by the 11%concentration (64.31 percent) compared to other treatments and controls (Table 2). Spodoptera litura mortality continued up to 72 hours, after which the living larvae converted to the pupal stage. With increasing concentration, there was a progressive rise in larval mortality. Several researchers have discovered that brown algal seaweeds have insecticidal properties. Zubia *et al*. (2008) [16] discovered that brown algae contain a unique form of tannin called "phlorotannins," and that the larvicidal activity of brown algae may be attributable to phenolics, terpenoids, or unsaturated fatty acids (Schnitzler *et al*., 2001) [15]. The pupal transformation data collected throughout the experiment indicated that the highest pupation (81.12 percent) occurred at the lowest concentration (0.1%) and the lowest pupation (26.82 % at 15 percent) (Fig. 2). The pupal deformity was varied, with the maximum (21.00 percent) observed at a concentration of 5, 7,9,11, and 15%, followed by 0.1, 1, and 3% concentrations with 14.25 percent pupal abnormality. The pupal to adult conversion rate was lowest (1:0.27) at the greatest seaweed dosage (15%) and highest (1:0.93) at the lowest seaweed concentration, whereas it was 1:105 in the control.

Sargassum dentifolium may have insecticidal properties against the various life stages of Spodoptera litura. The aqueous extract of Hypnea musciformis proved effective against *Plutella xyllostella* larvae, pupae, lifespan, and fecundity (Roni *et al*., 2015) [13]. *Spodoptera litura* has been found to be resistant to the insecticides *Sargassum cristaefolium* (Gowthish and Kannan, 2018) [6], *Acanthopora spicifera* (Dharanipriya and Kannan, 2018), and *Liagora ceranoides* (Kannan and Dharani Priya, 2019) [9].

Benzene extract of *Padina pavonica* (Sahayaraj and Kalidas, 2011); methanol extract of green algae *Ulva fasciata* and *U. Lactuca* (Asha *et al*., 2012) [2]; and chloroform extract of *S. weightii* and *P. pavonica* (Asaraja and sahayaraj, 2013) [1] had shown nymphicidal and ovicidal activityin the past. Ismail *et al*. (2020) [7] reported the activity of seaweeds varies depending on the extracting solvent and seaweed type. Understanding the chemical composition of seaweeds would give insight into the structure-activity link (Dias and Moreas, 2014; Yu *et al*, 2014) [4].

According to the findings, the brown algal seaweed *Sargassum cristaefolium* might be employed as an appropriate alternative to existing botanicals and synthetic pesticides in lowering insect population and can be used in bio-intensive pest control of agricultural pests.

Table 3: Anti feed antactivity of *Sargassumcristaefolium* (Methanol extract) against *Spodopteralitura*

Treatment Dose	Anti feedent activity (%)
T1-*Sargassum cristaefolium* (0.1%)	31.25 (34.82)
T2-*Sargassum cristaefolium* (1%)	42.88 (38.70)
T3-*Sargassum cristaefolium* (3%)	50.39 (43.61)
T4-*Sargassum cristaefolium* (5%)	54.98 (47.89)
T5-*Sargassum cristaefolium* (7%)	56.32 (48.22)
T6 - *Sargassum cristaefolium* (9%)	56.89 (48.98)
T7-*Sargassum cristaefolium* (11%)	58.85 (49.92)
T8-*Sargassum cristaefolium* (15%)	60.79 (51.91)
T9- (Solvent control**)**	7.56 (16.92)
T10- (Absolute control)	0.75 (5.35)
SEd	2.987
CD(p=0.01)	5.021

Table 4: Larvicidal effect of brown algal seaweed Sargassum cristaefolium methanol solvent extracton Spodoptera litura

Treatment Dose (%)	Larval mortality% after		
	24hrs	48hrs	72hrs
T1-*Sargassum cristaefolium* (0.1%)	7.12 (9.03)	16.24 (18.70)	22 (27.93)
T2-*Sargassum cristaefolium* (1%)	14.66 (16.51)	21.12 (26.22)	34.89 (35.07)
T3-*Sargassum cristaefolium* (3%)	22.78 (18.16)	31.98 (36.24)	41.79 (39.61)
T4-*Sargassum cristaefolium* (5%)	25.98 (30.92)	41.32 (39.99)	56.99 (47.65)
T5-*Sargassum cristaefolium* (7%)	26.78 (32.90)	43.92 (40.42)	58.23 (48.92)
T6 – *Sargassum cristaefolium* (9%)	28.92 (34.78)	45.02 (42.80)	59.12 (50.13)
T7-*Sargassum cristaefolium* (11%)	34.15 (35.56)	47.90 (43.34)	64.31 (52.66)
T8-*Sargassum cristaefolium* (15%)	38.90 (36.06)	55.82 (45.92)	75.98 (60.43)
T9-(Solventcontrol**)**	0.00 (0.378)	0.00 (0.292)	0.00 (0.292)
T10-(Absolutecontrol)	0.00 (0.292)	0.00 (0.292)	0.00 (0.292)
SEd	9.153	5.750	6.341
CD(p=0.01)	19.212	11.789	12.320

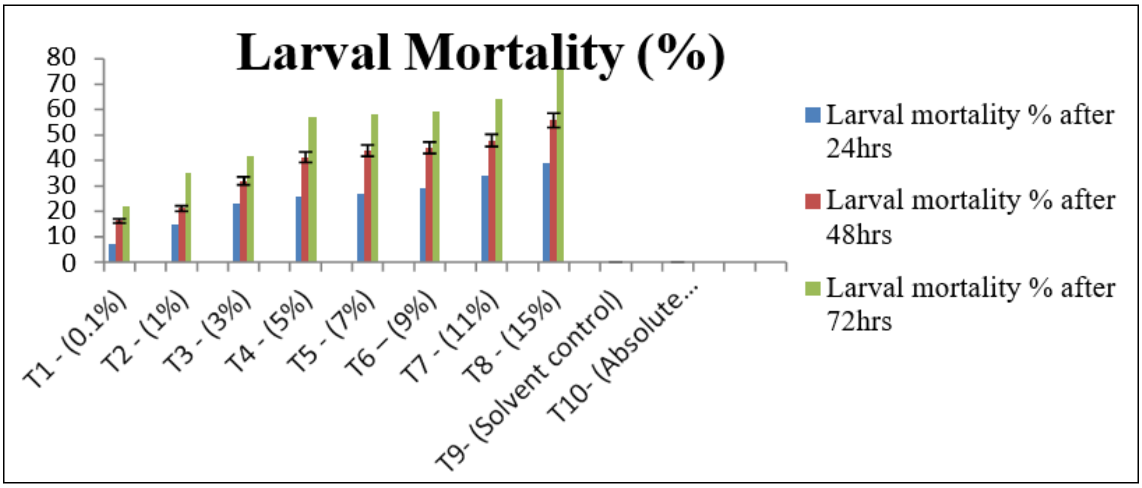

Fig 1: Effect of *Sargassum cristaefolium* methanol extract on the larval mortality of Spodopteralitura

Table 5: Effect of brown algal seaweed S*argassum cristaefolium* methanol solvent extract on IGR activity of *Spodoptera litura*

Treatment Dose (%)	Pupation (%)	Pupalmal formation (%)	Adult emergence (%)	Pupal to Adult conversion ratio
T1 (0.1%)	81.12	14.25	67.90	1: 0.93
	(64.98)	(18.24)	(55.57)	
T2 (1%)	67.31	14.79	54.28	1: 0.89
	(55.14)	(18.22)	(47.34)	
T3 (3%)	61.10	14.83	47.92	1: 0.79
	(52.34)	(18.20)	(43.97)	
T4 (5%)	46.21	21.90	27.15	1: 0.58
	(42.31)	(26.92)	(31.16)	
T5 (7%)	45.32	21.43	26.46	1:0.56
	(41.87)	(26.76)	(30.32)	
T6 (9%)	43.68	21.22	24.98	1:0.53
	(39.21)	(26.12)	(28.76)	
T7 (11%)	38.42	21.14	21.14	1: 0.48
	(37.99)	(25.94)	(26.98)	
T8 (15%)	26.82	21.00	7.69	1: 0.27
	(30.92)	(25.22)	(9.098)	
T9- (Solvent control)	100.00 (81.15)	0.00 (0.360)	100.00 (81.15)	1: 1.05
T10-(Absolute control)	100.00 (90.21)	0.00 (0.360)	100.00 (90.21)	1: 1.05
SEd	8.925	0.6372	8.904	
CD(p=0.01)	16.351	1.2586	16.365	

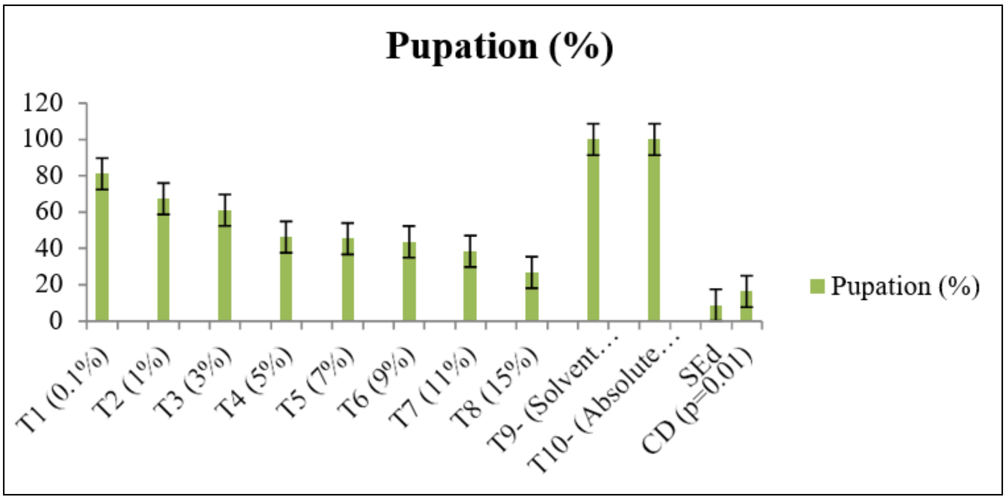

Fig 2: *Sargassum cristaefolium* methanol solvent extracts influence on the Pupation of *Spodoptera litura*

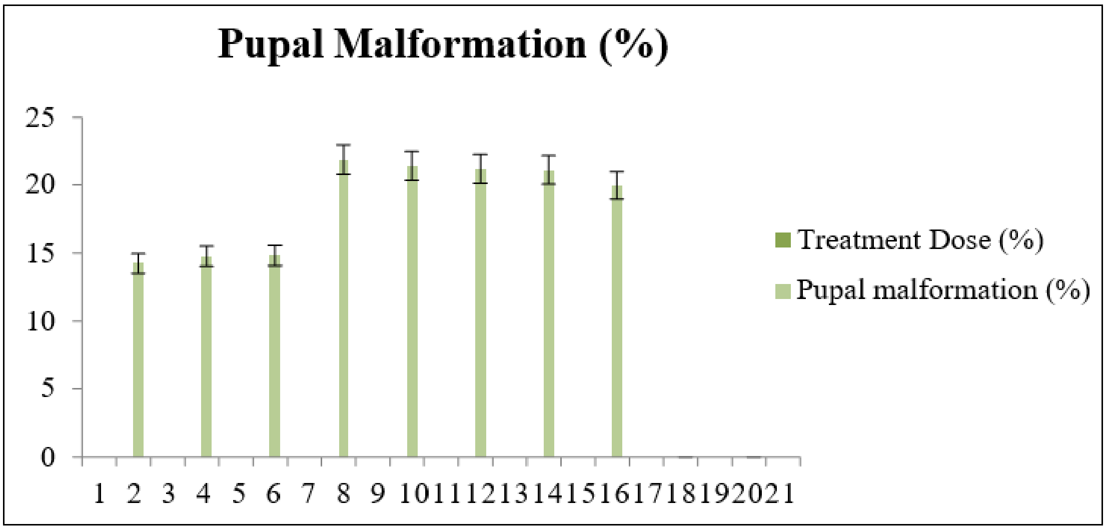

Fig 3: Effect of *Sargassum cristaefolium* methanol extract on the pupal malformation of *Spodopteralitura*

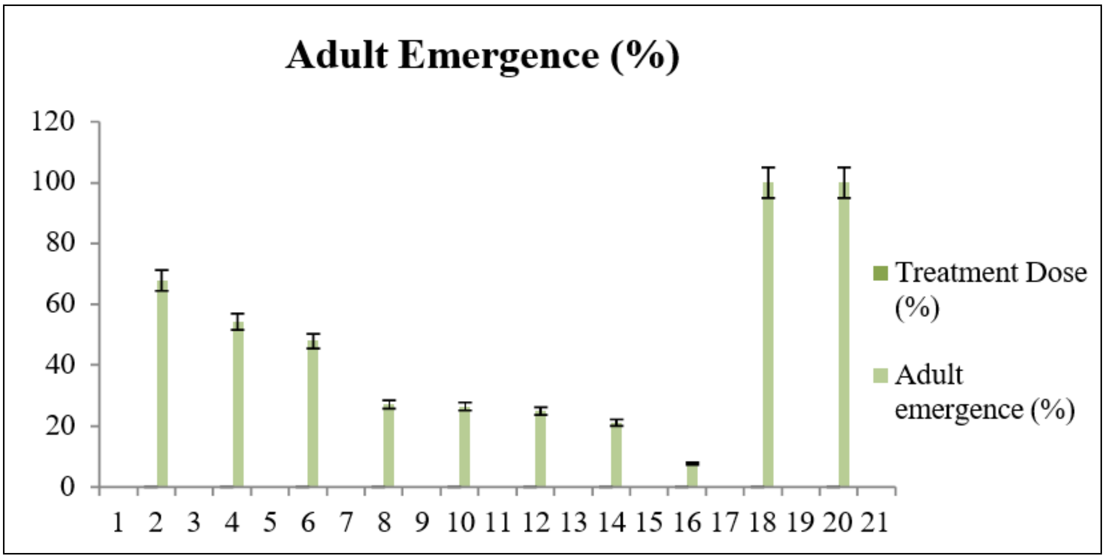

Fig 4: *Sargassum cristaefolium* methanol extract on the adult emergence Of *Spodoptera litura*

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