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Larvicidal and insect growth regulator activity of a brown algal seaweed, *Sargassum wightii* (Greville) against rice leaf folder, *Cnaphalocrocis medinalis* Guenee

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Abstract
Comparative evaluation of larvicidal and insect growth regulator activity of aqueous extracts and solvent extracts (acetone) of brown algal seaweed, *Sargassum wightii* Greville against rice leaf folder, *Cnaphalocrocis medinalis* Guenee (Lepidoptera: Crambidae) was conducted in the Seaweed Laboratory, Department of Entomology, Faculty of Agriculture, Annamalai University, India during 2021-2022. *Sargassum wightii* was collected from the inter tidal and deep sea of Rameswaram coastal ecosystem of Tamil Nadu, India. The aqueous extracts and acetone solvent extracts of the seaweed @ 2, 4, 6, 8 and 10 per cent concentrations were tested for their toxicity against Rice leaf folder and their bioactivity was compared with a standard check neem leaf extract (3%) and untreated control whereas the solvent extract treatments were compared with an additional treatment i.e., one per cent acetone solvent treatment for comparison. Solvent and aqueous extracts @ 8 and 10 per cent demonstrated the higher order of larval mortality with 46.66 and 33.33 per cent larval mortality at 72 hours respectively and were on par with neem leaf extract (3%) influence. The larval to adult conversion ratio of 1: 0.46 in solvent extracts and 1:0.53 in aqueous extracts at 8 and 10 percent were on par with neem leaf extract (3%) whereas in solvent control and untreated control, the ratio was 1:1.00.

Keywords: *Sargassum wightii*, aqueous and solvent extracts, larvicidal activity, IGR activity, *Cnaphalocrocis medinalis*

Introduction
Rice as a staple food for more than half of the world's population occupied ~162 million hectares of land under cultivation with a production of 755 million tonnes worldwide (Shaheen *et al*., 2022) [15]. India is the world's largest rice producer (124.36 million tonnes) from 43.76 million hectares with the productivity of 2717 kg/ha (Agricultural Statistics at a Glance, 2021). Many biotic stresses constrained the rice cultivation, of which insect pests played a major role in imparting critical damage to the crop in tillering stage. Rice leaf folder *Cnaphalocrocis medinalis* Guenee (Lepidoptera: Crambidae) has been categorized as a major pest of rice in many South Asian countries (Rizwan *et al*., 2021) [14]. Leaf folder attacked the crop from nursery to harvest, wherein their peak infestation was noticed during vegetative to reproductive phase of the crop (Litsinger *et al*., 2011) [11]. The larvae have fastened the longitudinal margins of the leaf together to make a leaf fold and being inside feed on the leaves by scrapping the chlorophyll. The scrapped leaves turn out to membranous, white and later withered. The yield reduction due to their damage was reported to be from 10-15 per cent (Kannan *et al*., 2016) [6, 7]. Chemical insecticides are currently the most effective and frequently used method for controlling rice leaf folders, but they have caused unintended consequences *viz*., insecticide resistance, pest resurgence, residual toxicity and other problems including food safety issues, pollution, destabilization of agricultural ecosystems and reduction in natural biodiversity (Uhl and Bruhl, 2019) [16]. The problems of insecticides lead to employing alternative methods including natural resources wherein seaweeds as an abundant natural resource in oceans and seas all over the world, have been shown to produce several secondary metabolites with a wide range of biological functions have piqued the interest of researchers. During the life cycle of seaweeds, an array of bioactive substances was released with many biological functions. Seaweeds from different localities have been explored for a wide range of biological activities, e.g. antibacterial, antiviral, antifungal, and antialgal activities (Perez *et al*., 2016) [13]. In this context, the insecticidal and IGR activity of a brown algal seaweed (*Sargassum wightii* Greville) against the rice leaf folder was addressed, and the findings were recorded and displayed.

Materials and Methods
Seaweed Collection
Sargassum wightii, a brown algal seaweed, was hand-picked from deep-sea regions of the coast of Rameswaram, Tamil Nadu in collaboration with Aqua agri Processing Pvt Limited, Manamadurai, India. Collected algae were washed in seawater to remove salt, sand, and epiphytes, then it was washed three times with running tap water. The dried seaweeds were stored at room temperature under dry circumstances after being washed and shade dried for a fortnight.

Mass Culturing of Rice Leaf Folder

Under greenhouse conditions (25°C and 60% RH), *C. medinalis* was kept alive on Taichung Native-1 (TN1) plants. Adult moths collected from the field were introduced (10 pairs) for oviposition on TN1 plants (20 to 25 days old) kept inside a screen house. Adults were given honey solution (20%) rinsed in a cotton swab soaked as a source of nourishment. The potted TN1 plants inhabit the adults for oviposition and the laid eggs were separated and retained for continued development after a three-day pre-oviposition period. The neonates, third instar and newly emerged adult moths from this stock culture were utilized for the experiments (Javvaji *et al.*, 2021) [5].

Preparation of the Aqueous Extract

The dried seaweed was broken into small pieces and were made in to fine powder using a blender. Dried seaweed powder @ 2, 4, 6, 8 and 10g was mixed with 100 ml sterile distilled water to make an aqueous extract of 2, 4, 6, 8 and 10 per cent and kept for incubation overnight without any disturbance. The extract was filtered using filter paper and the filtrate was stored in amber colour bottle and stored at 4^{O} C. The filtrate as stock solution was used for the bioassay experiments (Kannan and Bharath Kumar, 2016) [6, 7].

Preparation of Solvent Extract

The finely dusted *S. wightii* was weighed at 2, 4, 6, 8 and 10g and then they were homogenized in 100 ml solvent (acetone) separately and digested for 12 hours at room temperature until being filtered. The filtered extracts as stock solution was refrigerated at 4°C in an amber color bottle and utilized for bioassay experiments (Kombiah and Sahayaraj, 2012) [9].

Poison Food Bioassay

The fresh rice leaves were cut into pieces of about five cm in length and soaked in one per cent Tween 20 solution for five minutes to remove the wax layer. The leaf bits were treated with respective concentrations of aqueous and solvent extracts. The assessment of seaweed aqueous extract's efficiency against Rice leaf folder was laid statistically under completely randomized design with seven treatments and were replicated thrice by adopting the procedures described by Gomez and Gomez (1984) [3]. A standard check (neem leaf extract 3%) and untreated control (water control) were maintained to compare the performance of the seaweed extracts on the larva. In the solvent extract's efficiency testing, an additional treatment i.e., one per cent acetone solvent treatment was included for the comparison of solvent's influence on the test insect. The treated leaf bits were air-dried and were placed on moist filter paper in glass Petri dishes (five-leaf bits per Petri plate). Four hours pre-starved third instar larva (homogeneous population) were placed in each Petri plate and allowed to feed on the treated leaf bits for 24 hours after which subsequent feeding (if needed) was provided with untreated leaf bits. The observations on larval mortality was at 24, 48 and 72 hours of exposure was made and then the experiment was continued to assess the insect growth regulator activity of the seaweed treatments (pupation, pupal malformation, adult emergence and adult malformation). The data acquired during the experiment was pooled and were subjected to statistical analysis and presented.

Result and Discussion

The results obtained from the comparative evaluation of larvicidal and insect growth regulator activity of aqueous extract and acetone solvent extracts of *S. wightii* on *C. medinalis* revealed that the larval death was noticed after 24 hours of treatment after which a gradual increase in larval mortality was observed as per dose-response relationship up to 72 hours wherein the larval mortality was in the range of 0.00 to 46.66 per cent. The highest larval mortality was observed in 10 per cent concentration of aqueous (20.00%) and solvent extracts (40.00%) respectively wherein no mortality was exerted by two per cent aqueous extract and untreated control after 24 hours. The highest seaweed treatment (10 per cent concentration of acetone extract) effect and standard check (neem leaf extract 3%) effect on larval mortality (40.00%) were statistically on par with each other (Fig. 1). The data recorded after 48 hours displayed that the aqueous extracts of 2 and 4 per cent concentrations have demonstrated the least level of larval mortality (6.66%) wherein the maximum impact (33.33%) was exerted by 10 per cent concentration and neem leaf extract (3%). The acetone extract at the same time influenced at a greater level with 46.66 per cent mortality at 10 per cent concentration and the 13.33 per cent mortality at 2 per cent concentration. The other treatment of acetone extracts (8%) showed lesser level of mortality *i.e.*, 40.00 per cent which were greater than the impact of aqueous extract at the same concentration and time (Fig. 2). The larval mortality ascertained at 72 hours of exposure displayed that the concentrations 8, 10 and neem leaf extract (3%) of aqueous extract exhibited similar rate of mortality (33.33%) whereas at the same concentrations of solvent extract all the three concentrations (8, 10 and neem leaf extract 3%) have demonstrated the highest value of 46.66 per cent mortality (Fig. 3). In both the experiments, pre pupal mortality was observed in higher concentrations of 8, 10 per cent and neem leaf extract (3%). The maximum pupation was observed at the lower concentration (2%) of aqueous (93.33%) and solvent extracts (80%), respectively. The minimum pupation was recorded (60 and 46.66 per cent) at 8, 10 per cent and neem leaf extract (3%) in the both aqueous and solvent extracts. Pupal malformation data described that the aqueous extract concentrations (6-10%) have exhibited similar level (6.66%) and were on par with the standard check's influence whereas none of the solvent extract treatments showed pupal malformation. The maximum adult emergence was recorded at the lower concentration

(2%) of aqueous (93.33%) and solvent extracts (80%), respectively. The minimum adult emergence was observed (53.33 and 46.66 per cent) at 8, 10 per cent and neem leaf extract (3%) in the both aqueous and solvent extracts (Fig. 4). Considering the treatment effect on tested insects it was obvious that, in control the larval to adult conversion ratio was 1:1.00 whereas in solvent extracts of seaweed treatment of 8, 10 percent concentration and neem leaf extract (3%) it was 1:0.46 and 1:0.53 in aqueous extracts of same concentration. The treatment 8, 10 percent concentration and neem leaf extract (3%) was similar and comparable with each other (Table 1). The present findings revealed that higher concentrations of seaweed extracts (aqueous and solvent) at 8, 10 percent and neem leaf extract (3%) performed better against *C. medinalis* with little differences. Similar studies by Anandhan and Sornakumari (2011) [2] methanol extract of *Gracilaria crassa* and *Hypnea valentia* showed the larvicidal efficiency on *Aedes aegypti* and *Lobophora variegata* against *A. aegypti* and *Culex quinquefasciatus* (Manilal *et al.,* 2011). Crude chloroform extract and emulsifiable concentrate (EC) of seaweed *Caulerpa scalpelliformis* repelled *S. litura* and *Dysdercus cingulatus* in a dose dependent manner (Kombiah and Sahayaraj, 2012) [9]. Methanol extracts from different marine algae *Caulerpa racemosa, C. scalpelliformis, U. fasciata, Padina tetrastromatica, Stoechospermum polypodioides, Sargassum wightii, Cheilosporum spectabile* and *Gracillaria edulis* were effective on the root knot nematode (Karthick *et al*., 2014) [8]. Further reports like, *Chaetomorpha antennina* demonstrating larvicidal and insect growth regulator activity against *S. litura* (Kannan and Bharathkumar, 2016) [6]; *S. cristaefolium* methanol extract's efficiency against *Spodoptera litura* (Gowthish and Kannan, 2018) [4] and *Sargassum wightii* acetone extract's efficiency against *Spodoptera litura* (Niroja and Kannan, 2020) [12] have resembled the present investigations results and confirmed the efficiency of *S. wightii* in inducing larvicidal and insect growth regulator activity.

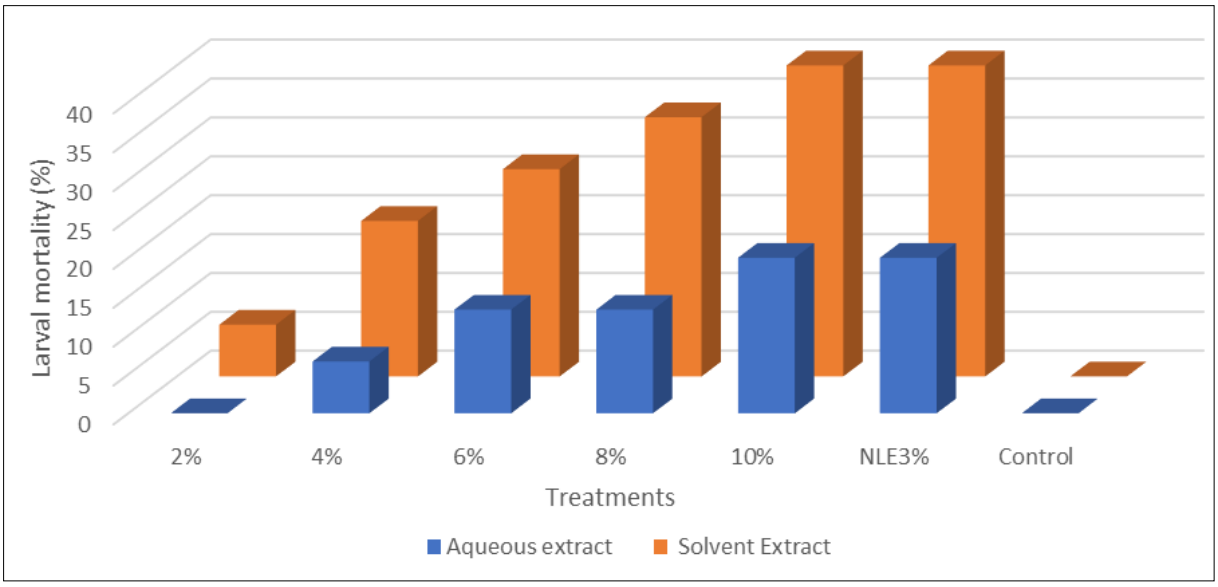

Fig 1: Comparative toxicity of aqueous and solvent extract of *S. wightii* on Rice leaf folder at 24 hours of treatment

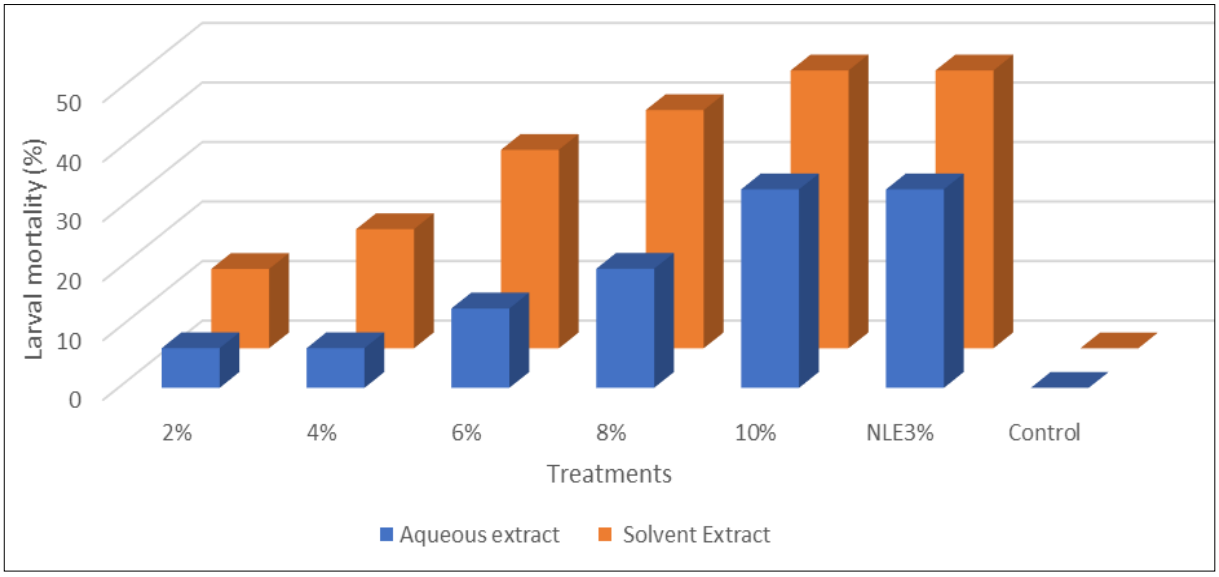

Fig 2: Comparative toxicity of aqueous and solvent extract of *S. wightii* on Rice leaf folder at 48 hours of treatment

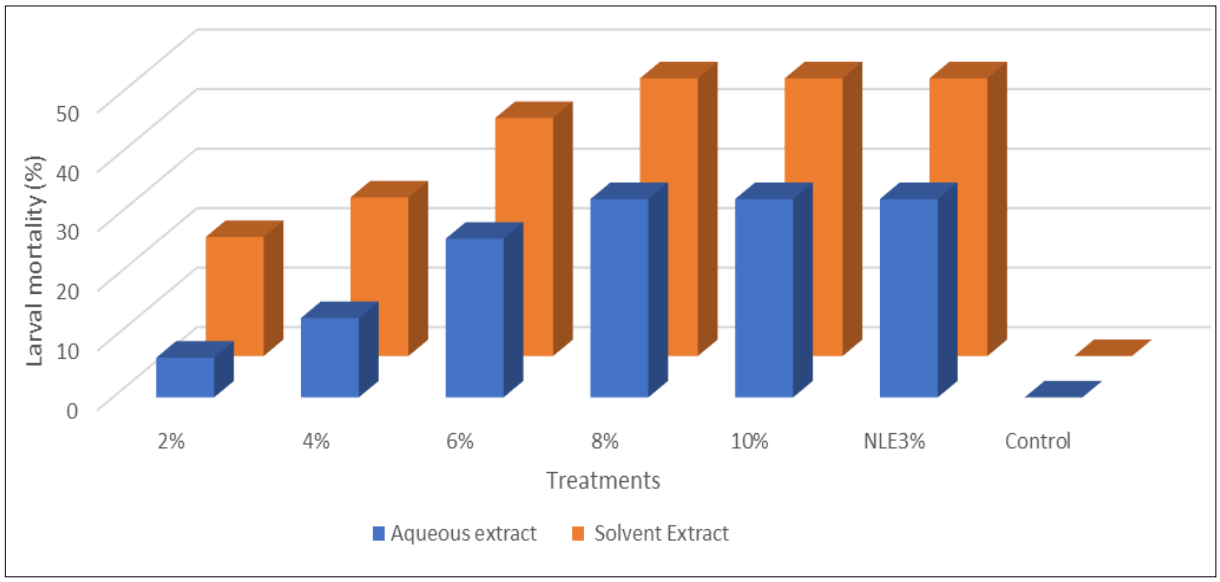

Fig 3: Comparative toxicity of aqueous and solvent extract of *S. wightii* on Rice leaf folder at 72 hours of treatment

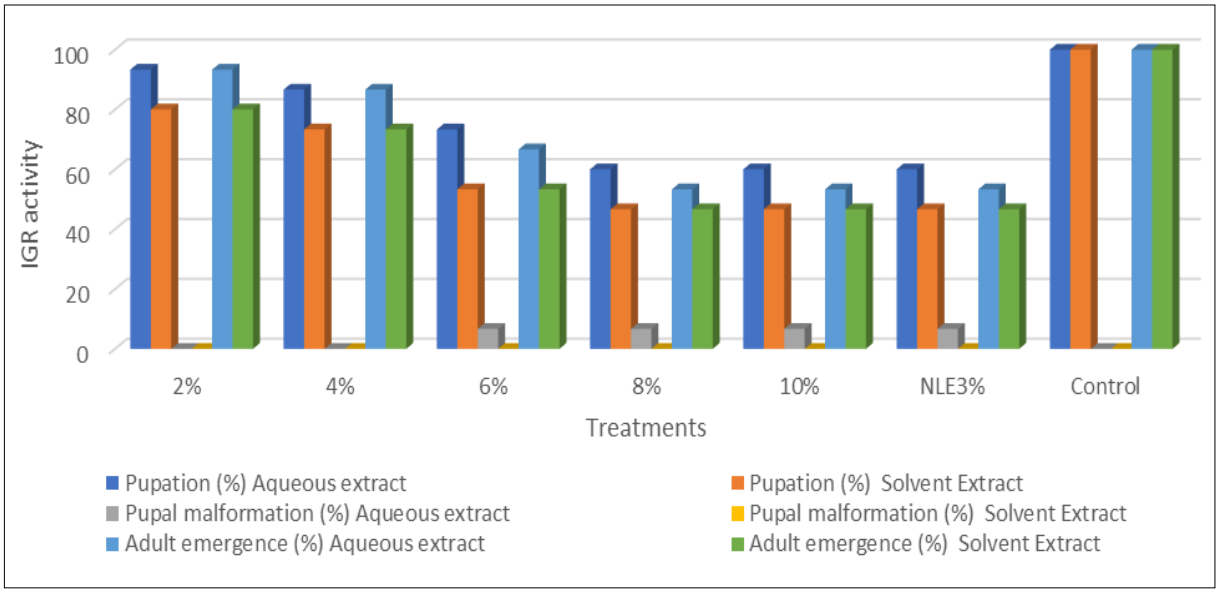

Fig 4: Comparative IGR activity of aqueous and solvent extract of *S. wightii* on Rice leaf folder

Table 1: Comparative evaluation of larval to Adult conversion ratio of aqueous and solvent extract of *S.wightii* on rice leaf folder

Treatment	Larval: Adult conversion ratio	
	Aqueous Extract	Solvent Extract
2%	1:0.93	1:0.80
4%	1:0.86	1:0.73
6%	1:0.66	1:0.53
8%	1:0.53	1:0.46
10%	1:0.53	1:0.46
NLE3%	1:0.53	1:0.46
Control	1:1	1:1
Solvent control	-	1:1

Conclusion

The present study to evaluate the aqueous and solvent extracts of *S. wightii* against rice leaf folder have demonstrated that both extracts influenced larvicidal and IGR activity on the rice leaf folder wherein the effect of solvent extracts were greater compared to aqueous extracts and its efficacy has to be enhanced with organic inputs for better leaf folder management.

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