



Larvicidal and insect growth regulator activity of brown algal seaweed *Turbinaria ornata* (Turner) J. Agardh against rice leaf folder *Cnaphalocrocis medinalis* (Guenee) (Lepidoptera: Pyralidae)

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Abstract

Seaweeds are widely occurring natural resources in marine ecosystem which possess various bioactive compounds which were reported to active against many insects of crops. The bio-efficacy of aqueous, solvent and fortified brown algal seaweed extracts of *Turbinaria ornata* at different concentrations (2, 4, 6, 8, 10 and 20%) along with standard check Neem leaf extract (NLE 3%) were tested (free choice test) against Rice leaf folder *Cnaphalocrocis medinalis* Guenee (leaf dip method) in laboratory conditions in Department of Entomology, Faculty of Agriculture, Annamalai University during 2021-2022 under Completely Randomized Design with three replications per treatment. Treated leaves after shade drying were fed to the test insect and based on their feeding and response data on larval mortality, pupation, pupal malformation and adult emergence was recorded and the data were analyzed statistically and presented. Compared to aqueous and solvent extracts, fortified seaweed solvent extracts with Neem leaf extract (3%) showed higher level of larvicidal action in which *T. ornata* (20%) + Neem leaf extract 3% recorded the highest larval mortality (56.66%), lowest pupation (43.33%), highest pupal malformation (20.00%) and the lowest adult emergence (26.66%) wherein the pupa to adult conversation ratio of 1:0.67 was lower when compared to standard check.

Keywords: brown marine algae, *Turbinaria ornata*, larvicidal, insect growth regulator activity, *Cnaphalocrocis medinalis*

Introduction

The rice leaf folder *Cnaphalocrocis medinalis* (Guenee) (Insecta: Lepidoptera: Pyralidae) as a predominant foliage feeder of rice (*Oryza sativa* L) (Sulagitti *et al.*, 2017) [14] has reported causing drastic yield loss to the crop ranged between 63 and 80 per cent in susceptible crops (Kumari and Prasad, 2022) [8]. It is a holometabolous insect undergoing four developmental stages in its life cycle such as egg, larva, pupa and adult in which larval stage causes considerable damage to the crop (Chen *et al.*, 2022) [4]. A single larva can damage a number of rice leaves by folding and scrapping the chlorophyll (Cheah *et al.*, 2022) [3], with cumulative effects that reduce photosynthesis and thus lead to a reduction in yield. The widespread cultivation of high yielding rice varieties and the accompanying changes in agronomic practices lead to severe infestations and economic yield loss due to leaf folder. Farmers depend on chemical inputs (fertilizers, weedicides, insecticides) for crop production and protection but used them indiscriminately which leads to undue problems including insecticide resistance, residue deposition and pest resurgence. Several alternatives have been utilized in Agriculture to overcome these hazards and marine algal seaweeds suits better as they are easily available, biodegradable and synthesize many secondary metabolites which showed broad-spectrum bio-activity against insect pests (Deepa *et al.*, 2016) [5]. In this context, a potential brown algal seaweed *Turbinaria ornata* (Turner) J. Agardh have been tested for their toxicity and insect growth regulator activity against Rice leaf folder *C. medinalis*.

Materials and Methods

Rearing of Rice Leaf Folder

Rice leaf folder *C. medinalis* culture was maintained on TN 1 (Taichung Native 1) rice variety (Susceptible cultivar) under greenhouse conditions at Annamalai University, Chidambaram. TN1 seedlings were raised in cement pots covered with nylon mesh and irrigated regularly. The field-collected larva were introduced into the pots in the greenhouse for the initial establishment of culture and pupated larva was kept in an oviposition cage containing rice seedlings to facilitate adult emergence. The adult moth was provided with a 5 per cent honey solution and allowed for mating. After egg-laying, the leaf portion with eggs was clipped off and kept in a petri dish with moist filter paper and were cultured in green house maintained rice plant and grown-up third instar larvae were used for experiments.

Collection of Seaweeds

The brown algal seaweed (*Turbinaria ornata*) was collected from the coastal region of Rameshwaram, Tamil Nadu, India in association with Aquagri Processing Pvt. Ltd., Manamadurai, Tamilnadu using the hand-picking method. The collected seaweeds were washed with tap water to remove debris and swabbed with a blotting sheet to take away excess moisture. It is shade dried for a fortnight for complete drying and stored in an airtight container (Kannan and Bharathkumar, 2016) [7]. The seaweeds were identified at CAS Marine biology, Annamalai University.

Preparation of Extracts

Aqueous Extract

T. ornata aqueous extract was prepared by soaking 100g of seaweed powder in 200 ml of sterile distilled water. The setup was allowed to stand for overnight and filtered using filter paper. The filtrates were stored at room temperature. The aqueous extract of concentrations such as 2, 4, 6, 8, 10 and 20 percent was prepared and used for bioassay (Mohan *et al.*, 2014) [10].

Solvent Extract

The powdered *T. ornata* was individually weighed as 2, 4, 6, 8, 10 and 20g and soaked in 100ml of Acetone and kept for 12 hours incubation at room temperature. The filtered extract was stored and used in experiments. In the enhanced solvent extraction, Neem Leaf Extract 3% was added along with seaweed powder into the solvent and respective concentration was made and used for bioassay.

Bioassay

The aqueous, solvent and enhanced solvent extracts of brown algal seaweed at various concentrations: 2, 4, 6, 8, 10 and 20 percent along with absolute control and a standard check was tested against third instar larva of *C. medinalis* using no choice leaf dip method. Surface sterilized rice leaves were cut into pieces (5cm in length) and the wax layer was removed by using a hairbrush and it is treated with extracts prepared. The treated leaves after drying were placed inside the Petri dish (5 leaves per Petri dish) and filter paper with adequate moisture was provided to avoid early drying of test materials. Four hours pre-starved third instar larva was released into each Petri dish (5 larvae per Petri dish) and allowed to feed on treated leaves for 24 hours. The experiments were laid under a completely randomized design with eight treatments and three replications. Data on larval mortality, pupation, pre-pupal mortality, pupal malformation, adult emergence were collected and the means were pooled and analyzed statistically presented.

Result and Discussion

The observation of larval mortality and Insect growth regulator activity of aqueous, solvent and enhanced solvent extracts of brown algal seaweed, *T. ornata* against *C. medinalis* shows the following results. The efficacy of aqueous extracts on leaf folder larva was observed at regular intervals wherein the larval mortality was detected from 24hours after treatment and found to be constantly increasing up to 96hours. The larval mortality percentage ranged between 0.00 and 53.33 percent. Among different concentrations, the higher level of mortality was recorded in the standard check (NLE 3 per cent - 53.33%) followed by 20 per cent concentration (33.33%) and the least mortality was noticed in 2 per cent concentration (13.33%). No larval death occurred in untreated control and effectiveness varied with different concentrations (Fig. 2). In the case of IGR activity, pupation rate was recorded as minimum in 20 per cent concentration (66.66%) next to standard check (Fig. 3). Due to the toxic effect of *T. ornata*, pupal malformation was observed in higher concentrations ranging between 6.67 to 13.33 per cent (Fig. 4). The adult emergence rate was reduced to 20 per cent at standard check (Fig. 5) whereas malformation in adults was observed due to growth regulator activity of seaweed and a higher larva to pupal

conversion rate was recorded at 2 per cent concentration (1:0.92) (Table 1).

In solvent extracts, the larval mortality ranged from 6.67 to 46.67 per cent wherein the highest impact was demonstrated by standard check (53.33%) pursued by 20 per cent concentration (46.66%) at 96 hours after treatment (Fig. 2). Due to insect growth regulator properties, the pupation rate was lessened to 53.33 per cent at 20 percent concentration next to the standard check (Fig. 3) and utmost pupal malformation was recorded at 8 per cent concentration (20.00%) which was on par with 6 per cent concentration (20.00%) (Fig. 4). The solvent and untreated check exhibited cent per cent adult emergence (Fig. 5) wherein malformation in adult was recorded in 6, 8, 10, 20 per cent and standard check respectively. The pupa to the adult conversion ratio was worked out to ascertain the adult emergence rate from the treatment check wherein the lowest range was observed in 20 per cent concentration (1:0.57) (Table 1) which was on par with the standard check.

The influence of fortified solvent extract of *T. ornata* with neem on larvicidal action was recorded in 24 hour intervals in which *T. ornata* (20% + NLE 3%) perceived a high mortality rate (56.66%). Due to the combined action of neem leaf extract, effective larvicidal action was observed in all other treatments. In the case of IGR activity, the data showed that maximum pupal formation was exhibited by 2 per cent concentration combined with NLE 3 per cent (Fig. 3). Due to the synergistic effect, pupal malformation was observed in all the treatments in which *T. ornata* 20 per cent + NLE 3 per cent recorded higher level (20.00%) of malformation (Fig. 4). The standard check (NLE 3%) demonstrated lower rate of pupal to adult conversion ratio (1:0.57) followed by *T. ornata* (20% + NLE 3%) (1:0.61) which was on par with *T. ornata* (10% + NLE 3%) (1:0.61) respectively (Table 1).

The result obtained from the above study confirmed the presence of toxicodynamics properties of *T. ornata* that showed a concerned insecticidal action against *C. medinalis* in its various life stages and the result are in accordance with the following studies tested against various insects. The aqueous extract of *Turbinaria conoides* evidenced the higher degree of retardance in the development and inducing high mortality in wrigglers and tumbler of *A. aegypti* with a minimum LC₅₀ value of 18.47 mg/L (Valentina *et al.*, 2015) [15]. Manilal *et al.* (2011) [9] reported the mosquito larvicidal efficiency of methanol extract of brown algal seaweed, *Lobophora variegata* against *Aedes aegypti* and *Culex quinquefasciatus*.

Sahayaraj and Jeeva (2012) [12] reported that various extracts of brown algal seaweed, *Sargassum tenerrimum* showed growth regulator activities such as reduction in adult longevity, decreased egg hatchability and minimal fecundity against *Dysdercus cingulatus*. Similar studies of testing the different concentrations of seaweeds *Padina pavonica* against *D. cingulatus* (Sahayaraj and Kalidas, 2011) [13] showed higher mortality potential. Ali *et al.* (2013) examine the different marine algal seaweed extracts of *Ulva lactuca*, *Caulerpa racemosa*, *Sargassum microystum*, *C. scalpelliformis*, *Gracilaria corticata*, *Turbinaria decurrens*, *T. conoides* and *C. toxifolia* exhibited maximum larval fatality against the tested mosquito larvae. Among the different seaweed extracts tested, *C. racemosa* recorded the highest level of toxicity against *A. aegypti* with reduced efficiency and LC₅₀ values.

In *T. ornata*, a wide range of biological properties such as antibacterial (Alharbi *et al.*, 2020) [1], anti-coagulant, anti-inflammatory (Bhardwaj *et al.*, 2020) and antioxidant properties have been reported. Further, GC- MS analysis of *T. ornata* methanolic crude extract revealed the presence of a mixture of volatile compounds. The phytochemicals reported were 1,2,3,4-Tetrachlorobenzene C₆H₂Cl₄ 215.89, Diethylether, 1,2-Benzenedicarboxylic acid bis(2-methylpropyl) ester, Benzo(k)fluoranthene, Bufencarb-2, Heptachlor, Kresoxim-Methyl, n-Tetradecane, Isopropylisothiocyanate, Di-n-octylphthalate, Vanillylmandelic acid, Tetramethrin-1, Acetamiprid,

Eicosapentaenoic acid (EPA), Heptanal and Humulene epoxide III (Neelamathi and Kannan, 2016) [11] respectively may be responsible for the toxic activities. Deepak *et al.* (2017) [6] investigated the mosquito-larvicidal property of *Turbinaria ornata*-mediated gold nanoparticles (To-AuNPs) and its boiled aqueous extract (To-AE) against the malarial vector *Ades stephensi* and recorded lethal concentration (LC₅₀ and LC₉₀) values of (37.77 and 159.55) in To-AE and (12.79 and 78.70) in To-AuNPs against fourth instar larvae of *A. stephensi*.

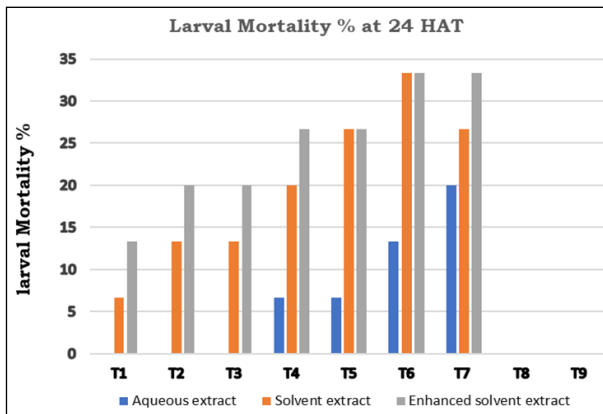


Fig 1: Larvicidal effect of different concentration of *T. ornata* extracts on *C. medinalis* at 24 HAT

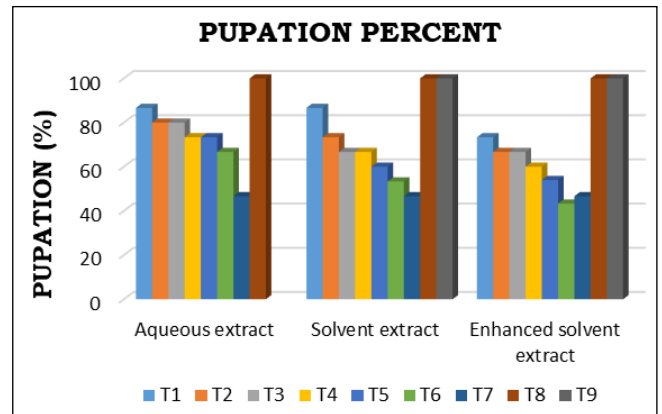


Fig 3: Effect of different concentration of *T. ornata* extracts on Pupation of *C. medinalis*

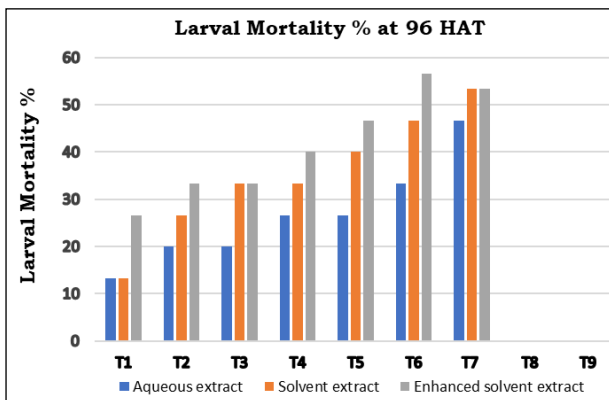


Fig 2: Larvicidal effect of different concentration of *T. ornata* extracts on *C. medinalis* at 96 HAT

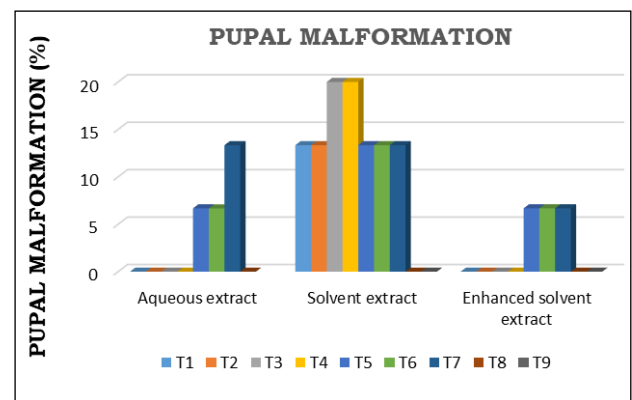


Fig 4: Effect of different concentration of *T. ornata* extracts on Pupal malformation of *C. medinalis*

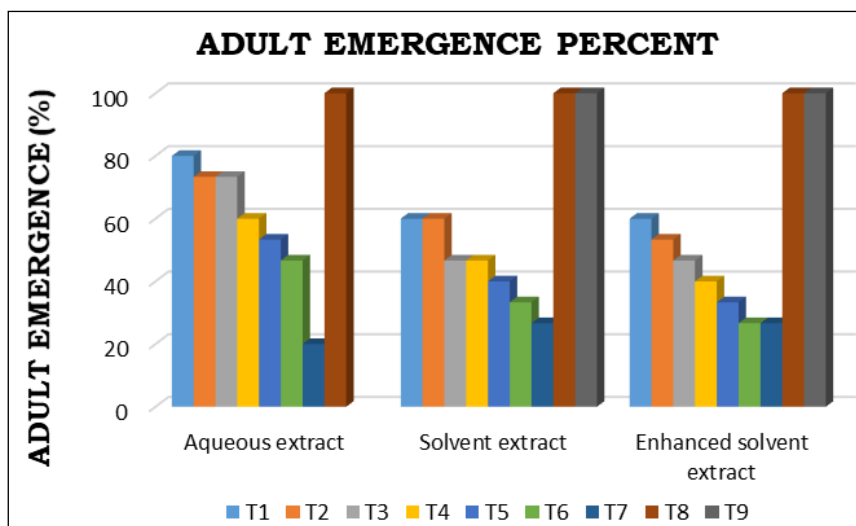


Fig 5: Effect of different concentration of *T. ornata* extracts on Adult emergence of *C. medinalis*

Table 1: The effect of Aqueous, Solvent and Enhanced solvent extracts of *T. ornata* on pupa to adult conversion ratio

| Treatments | T1 (2%) | T2 (4%) | T3 (6%) | T4 (8%) | T5 (10%) | T6 (20%) | Absolute Control | Untreated Control | Solvent Control |
|----------------------------------|---------|---------|---------|---------|----------|----------|------------------|-------------------|-----------------|
| Aqueous Extracts | 1:0.92 | 1:0.91 | 1:0.91 | 1:0.81 | 1:0.72 | 1:0.69 | 1:0.42 | 1:1 | - |
| Solvent Extracts | 1:0.75 | 1:0.75 | 1:0.70 | 1:0.70 | 1:0.66 | 1:0.51 | 1:0.57 | 1:1 | 1:1 |
| Enhanced Solvent Extracts | 1:0.81 | 1:0.79 | 1:0.69 | 1:0.66 | 1:0.61 | 1:0.61 | 1:0.62 | 1:1 | 1:1 |

Conclusion

Based on the above results, it can be concluded that enhanced solvent extract of *Turbinaria ornata* could be used as a better alternative to synthetic chemical pesticides for reducing pest infestation and it can be used in bio-intensive pest management modules.

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