**Toxicity of Red Macro algae, *Laurencia obtusa* against Growth and Development of *Spodoptera litura* (Noctuidae: Lepidoptera)**

**Abstract**

The present study evaluates the insecticidal efficacy of Red macroalgae, *Laurencia obtusa* against the lepidopteran pest, *Spodoptera litura* under laboratory conditions. The macroalga was harvested in Hare Island, Tuticorin and extracted with three solvents, hexane, dichloromethane and methanol. The extracts were used to treat castor leaf discs with different concentrations (0.7% to 10%) and second instar larvae of *S. litura* were exposed to the treated discs and the larval mortality and growth regulatory effects were determined. The findings showed that all three extracts had a dose-dependent toxic effect with methanol extract having the highest mortality (80.00% at 10% concentration) and the lowest LC 50 value (3.32%), followed by dichloromethane and hexane. Moreover, methanol and dichloromethane extracts had a considerable inhibitory effect on larval development, pupation and adult emergence, and caused deformed adults, which is a strong indicator of Insect Growth Regulatory (IGR) activity. These results indicate that *L. obtusa* methanol extract contains strong bioactive compounds that can manage *S. litura* and interfere with its life cycle. The research identifies the possible use of *L. obtusa* as a source of marine algal biopesticides as an alternative to synthetic insecticides, which is safer to the environment.

**Keywords:** *Laurencia obtusa,* Red macroalgae, *Spodoptera litura*, Larvicidal activity, Insect Growth Regulators, Marine Biopesticide.

**1. Introduction**

*Spodoptera litura* (Fabricius) (Noctuidae: Lepidoptera) represents a polyphagous pest that destroys more than 120 plant species, including commercially vital agricultural and horticultural crops (Kaur *et al.*, 2017). The developmental stages of the *S. litura* lead to serious damage to plant leaves, which results in reduced agricultural yield and diminished product quality. Management of *S. litura* primarily depends on synthetic chemical insecticides, yet this approach produces pest resistance and intensifies pest numbers along with harming natural enemies and other environmental factors (Gerwick and Sparks, 2014).

The search for environmentally friendly pest management strategies has become more intensive during recent years. Currently, the researchers focus on botanical and marine-derived biopesticides because they show effectiveness against targeted pests while being eco-friendly to both biota and ecosystems. The natural compounds derived from botanicals decompose rapidly and reduce pesticide residues in food and the environment. Marine macroalgae contain multiple bioactive compounds such as alkaloids, terpenoids, phenolics, flavonoids and sulfated polysaccharides that demonstrate insecticidal effects together with antifungal, antimicrobial and antioxidant properties.

*Laurencia obtusa,* which belongs to the Rhodophyta macroalgae, produces multiple secondary metabolites, including halogenated sesquiterpenes, acetogenins, polyphenols and other halogenated compounds, that are unique to marine red macroalgae (Kladi *et al.,* 2006; Wang *et al.,* 2013). Multiple studies confirm that these compounds demonstrate strong biological activities because they disrupt essential insect physiological functions and biochemical operations such as enzyme inhibition, hormonal imbalance, neurotoxicity and oxidative stress induction. These metabolites are known to act as feeding deterrents, oviposition inhibitors and growth regulators, which affect multiple life stages of insects. Research on the insecticidal properties of *L. obtusa* extracts against agricultural pests remains scarce, especially when focused on lepidopteran defoliators like *S. litura*.

The research investigates the toxic effects of *L. obtusa* solvent extracts on the *S. litura* population under laboratory-based testing. The research findings will help create sustainable marine algal biopesticides for effective pest management of this important agricultural pest.

**2. Materials and Methods**

**2.1. Collection of macroalgae**

The south-east coast of India features abundant macroalgal diversity in locations such as Tuticorin (Parthiban and Anantharaman, 2018), Rameswaram (Bhagyaraj and Kunchithapatham, 2016), Ramanathapuram (Yuvaraj and Paul, 2017), Tiruchendur (Selvaraj and Selvaraj, 1997), and Kanyakumari (Jenisha and Merina, 2016).

The macroalga *Laurencia obtusa* was collected from Hare Island in Tuticorin, Tamil Nadu, which is part of the Gulf of Mannar region, located at 9°12'0'' N latitude and 79°4'48'' E longitude. The collection was carried out during the morning and evening hours when the water receded from the shore. The algae were hand-picked, thoroughly washed to remove salt, sand particles, and small attached organisms, then shade-dried and stored in airtight containers at room temperature for further use.

**2.2. Preparation of macroalgal extracts**

The dried macroalga were ground into a fine powder using a blender and sieved to get 0.5 mm size particles. The solvents *viz.,* Hexane, Dichloromethane and Methanol were choosen based on polarity. The powder was mixed with each of the solvent in the ratio of 1:10 (*w/v*). The mixture was then subjected to sonicator with the frequency range of 20 kHz, 1500 W for 30 minutes which is feasible to break the algal cell and releasing the bioactive compounds. The mixture was centrifuged and the supernatant was collected, and the solid part was extracted again twice with fresh solvent to get more extract. All the extracts were combined and concentrated using a rotary vacuum evaporator at 35°C. The final thick extracts were freeze-dried and weighed to find the yield. A 10% stock solution was prepared by mixing the extract with the same solvent, which was used for bioassay tests.

**2.3. Test insect culturing and bioassay**

*Spodoptera litura* was reared in the laboratory on castor leaves. For the experiment, second instar larvae from the third generation were used. To test the toxicity of *L. obtusa*, the leaf dip method was followed using a no-choice feeding setup.

Crude extracts were prepared using three solvents: Hexane, Dichloromethane, and Methanol. Six different concentrations (0.7%, 1.0%, 3.0%, 5.0%, 7.0%, and 10.0%) were made using these solvents. To help the extracts stick to the leaves, 0.1% Triton X-100 was added. Castor leaf discs (4 cm in diameter) were dipped in each concentration for 30 seconds. The discs were then placed on filter paper to dry in the shade. After drying, the treated leaves were kept in Petri dishes. Larvae that had been starved for 4 hours were placed in each Petri dish and allowed to feed only on the treated leaves.

Each concentration had three replications with 10 larvae in each. A Completely Randomized Design (CRD) was used for the experiment. Mortality and other effects on larval growth were observed from 24 hours after treatment until adult emergence. The percentage of mortality was calculated as per the methods of Duraipandiyan *et al.* (2011).

Mortality (%) = Number of insects dead

 Total number of insects released × 100

**3. Results and Discussion**

The insecticidal potentiality of the solvent extracts of *Laurencia obtusa* (Hexane, Dichloromethane and Methanol) was assessed on the growth and development of *Spodoptera litura* under laboratory conditions. The percentage of mortality and insect growth regulatory (IGR) activities was evaluated after 72 hours of treatment and in the further developmental stages.

**3.1. Larval Mortality of *Spodoptera litura***

The three solvent extracts of *L. obtusa* exhibited dose-dependent larvicidal efficacy against *S. litura*. The methanol extract was the most toxic followed by dichloromethane and hexane extracts. At 72 hours post treatment (HAT), methanol extract induced 80.00 per cent mortality at 10 per cent concentration, whereas dichloromethane and hexane extract induced 43.33 and 30.00 per cent mortality, respectively at the same concentration (Fig. 1). The lethal concentration LC 50 values also reinforced the same trend with methanol extract showing the lowest LC50 of 3.32 per cent indicating the higher potency, followed by 20 per cent for dichloromethane and 251 per cent for hexane, which demonstrated limited efficacy of the latter.

The upward trend of mortality in all extracts and concentrations show that *L. obtusa* has strong bioactive compounds, particularly in methanol-soluble fractions. The higher toxicity of the methanolic extract may be attributed to its ability to extract polar secondary metabolites such as phenolics, flavonoids, and sulfated polysaccharides that could interfere with insect metabolism. It has been established in previous reports that *Laurencia* spp. contains halogenated compounds and cyclic ethers that have insecticidal activity. As an example, laurepinnacin and isolaurepinnacin of *L. pinnata* (El Sayed *et al*., 1997 and Fukuzawa and Masamune, 1981), deoxyprepacifenol and (Z)-laureatin of *L. nipponica* were shown to be toxic to mosquito larvae *C. pipiens* (Abou elnaga *et al*., 2011). In a similar way, *L. papillosa* yielded a C15-acetogenin, (12E)-cis-maneonene-E, that was active against *Tribolium confusum* (Abou-Elnaga *et al*., 2011) and *Culex pipiens* larvae. Moreover, *L. dendroidea* was found to induce larval mortality in *Aedes aegypti* through elatol, obtusol and laureatin (Salvador-Neto *et al.,* 2016). Consistent with the previous reports, the current study reaffirms the prospect of *L. obtusa* as a source of insecticidal compounds of marine origin, whereby methanol extract had the best larvicidal effect on *S. litura.*

**HE - Hexane; DCM - Dichloromethane and ME - Methanol**

**Fig 1 Larvicidal activity of *Laurencia obtusa* on *Spodoptera litura***

**3.2. Growth Inhibitory effects and Developmental Disruptions**

There was a significant influence on the growth and development of *S. litura* larvae by extracts. With higher concentrations, a progressive increase in pre-pupal mortality and inhibition of pupation and adult emergence was observed. Methanol extract at 7 and 10 per cent concentration totally inhibited adult emergence, which showed that it has a strong insect growth regulatory (IGR) effect.

The IGR activity was also significant in hexane and dichloromethane extracts with the hexane extract reducing the emergence of adults to 0.00% (10.0%) compared to the control emergence of 60.00% (0.7%). Likewise, dichloromethane extract led to 0.00% adult emergence at 5% concentration and above. Treated groups produced malformed adults indicating that the extracts disrupt hormonal control or cuticle development during metamorphosis. The ratios of the malformation also reduced as the concentrations increased pointing to more developmental toxicity.

The index of IGR (larval: adult ratio) was 1:1.0 in the control and 1:0.0 in the higher concentration of all the extracts with methanol extract being the most effective in interfering with the metamorphosis.

Methanol extract was invariably the most efficacious in all the three solvents with respect to mortality and developmental inhibition. This indicates that methanol is more effective in extracting the active compounds of *L. obtusa* probably because it can dissolve a broad spectrum of polar bioactive compounds. Dichloromethane which is semi-polar exhibited intermediate activity whereas the non-polar extract of hexane exhibited relatively low toxicity.

The results of the present study concur with the findings of earlier researchers that red macroalgae such as *L. obtusa* contain high levels of halogenated compounds, sesquiterpenes, and phenolics that have neurotoxic and IGR activities against insect pests (Manilal *et al.,* 2009).

**PPE - Pre-Pupal Mortality; AE - Adult Emergence; HE - Hexane; DCM - Dichloromethane and ME - Methanol**

**Fig 2 Insect Growth Regulatory activity of *Laurencia obtusa* on *Spodoptera litura***

**4. Implications for Pest Management**

The paper shows promise of *L. obtusa* extracts as a prospective bioinsecticide, particularly the methanol extract, in the control of *S. litura*, a notorious lepidopteran pest. The high larvicidal and growth-inhibitory activities indicate that *L. obtusa* has the potential to be developed as a botanical replacement of synthetic insecticides. Nevertheless, additional research efforts should be devoted to isolate and characterize the individual compounds that mediate the observed bioactivities as well as assess their performance at the field level and non-target safety.

**References**

Abou-Elnaga, Z. S., Alarif, W. M., & Al-Lihaibi, S. S. (2011). New larvicidal acetogenin from the red alga *Laurencia papillosa*. *Clean – Soil, Air, Water, 39*(8), 787 - 794.

Bhagyaraj, I., & Kunchithapatham, V. R. (2016). Diversity and distribution of seaweeds in the shores and water lagoons of Chennai and Rameswaram. *Indian Journal of Science and Technology, 9*(45), 1 - 6.

Duraipandiyan, V., Ignacimuthu, S., & Paulraj, M. G. (2011). Antifeedant and larvicidal activities of Rhein isolated from the flowers of *Cassia fistula* L. *Saudi Journal of Biological Sciences, 18*(2), 129 - 133.

El Sayed, K. A., Dunbar, D. C., Perry, T. L., Wilkins, S. P., & Hamann, M. T. (1997). Marine natural products as prototype insecticidal agents. *Journal of Agricultural and Food Chemistry, 45*(7), 2735 - 2739.

Fukuzawa, A., & Masamune, T. (1981). Laurepinnacin and isolaurepinnacin: New acetylenic cyclic ethers from the marine red alga *Laurencia pinnata* Yamada. *Tetrahedron Letters, 22*(40), 4081 - 4084.

Gerwick, B. C., & Sparks, T. C. (2014). Natural products for pest control: An analysis of their role, value and future. *Pest Management Science, 70*(8), 1169 - 1185.

Jenisha, S. R., & Merina, R. M. (2016). Studies on the distribution of seaweeds in three different sites of Kanyakumari coast. *Indo Asian Journal of Multidisciplinary Research, 2*(2), 539 - 544.

Kaur, M., Kumar, R., Upendrabhai, D. P., Singh, I. P., & Kaur, S. (2017). Impact of sesquiterpenes from *Inula racemosa* (Asteraceae) on growth, development and nutrition of *Spodoptera litura* (Lepidoptera: Noctuidae). *Pest Management Science, 73*(5), 1031 - 1038.

Kladi, M., Xenaki, H., Vagias, C., Papazafiri, P., & Roussis, V. (2006). New cytotoxic sesquiterpenes from the red algae *Laurencia obtusa* and *Laurencia microcladia*. *Tetrahedron, 62*(1), 182 - 189.

Manilal, A., Sujith, S., Kiran, G. S., Selvin, J., Shakir, C., Gandhimathi, R., & Panikkar, M. V. N. (2009). Biopotentials of seaweeds collected from southwest coast of India. *Journal of Marine Science and Technology, 17*(1), 67 - 73.

Parthiban, C., & Anantharaman, P. (2018). Diversity and biomass of drift seaweeds from the Tuticorin coast, India. *Indian Journal of Geo Marine Sciences, 19*, 72 - 86.

Salvador-Neto, O., Gomes, S. A., Soares, A. R., Machado, F. L. S., Samuels, R. I., da Fonseca, R. N., Souza-Menezes, J., Moraes, J. L. C., Campos, E., Mury, F. B., & Silva, J. R. (2016). Larvicidal potential of biological activities of laurinterol from *Laurencia nidifica*: The halogenated sesquiterpene (+)-obtusol, isolated from the alga *Laurencia dendroidea* J. Agardh (Ceramiales: Rhodomelaceae), against the dengue vector mosquito *Aedes aegypti* (Linnaeus) (Diptera: Culicidae). *Marine Drugs, 14*(1), 20.

Selvaraj, R., & Selvaraj, R. (1997). Distribution and diversity of seaweeds in Tiruchendur and Idianthakarai. *Seaweed Research and Utilisation, 19*(1&2), 115 - 123.

Wang, B. G., Gloer, J. B., Ji, N. Y., & Zhao, J. C. (2013). Halogenated organic molecules of Rhodomelaceae origin: Chemistry and biology. *Chemical Reviews, 113*(5), 3632 - 3685.

Watanabe, K., Umeda, K., & Miyakado, M. (1989). Isolation and identification of three insecticidal principles from the red alga *Laurencia nipponica* Yamada. *Agricultural and Biological Chemistry, 53*(10), 2513 - 2515.

Yuvaraj, P., & Paul, J. P. J. (2017). Analgesic activity of *Dictyopteris australis* (Sonder) Askenasy (brown seaweed) from Pamban, Ramanathapuram District, Tamil Nadu, India. *Indo American Journal of Pharmaceutical Sciences, 4*(8), 2534 - 2537.