International Journal of Entomology Research
www.entomologyjournals.com
ISSN: 2455-4758
Received: 22-11-2023, Accepted: 08-12-2023, Published: 25-12-2023
Volume 8, Issue 12, 2023, Page No. 72-76

Evaluation of larvicidal activity and interaction effects of brown seaweed, *Turbinaria ornata* (Turner) J Agardh with Neem and Pungam Leaf Extracts Against *Cnephalocorcis medinalis* (Guenee) (Lepidoptera: Crambidae)

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Abstract
The study investigates the larvicidal potential of *Turbinaria ornata*, neem, and pungam leaf extracts against *Cnephalocorcis medinalis* larvae. The study focused on determining the lethal concentration and interactions between the extracts, under a Completely Randomized Design with a no-choice leaf dip method at the Department of Entomology, Faculty of Agriculture, Annamalai University, in laboratory conditions with three replications per treatment. In preliminary screening, it was observed that all extracts caused 100% mortality at maximum concentrations, while untreated controls showed no mortality. Probit analysis was used to determine the LC_{20} and LC_{25} doses for *T. ornata*, which were found to be 2.52% and 3.01%, respectively. Meanwhile, neem and pungam leaf extracts showed LC_5, LC_{15}, and LC_{25} concentrations of 0.4%, 0.7%, and 1%, and 1%, 1.5%, and 2%, respectively. To test the binary mixtures (1:1), twelve combinations were tested by combining LC_{20} and LC_{25} doses of *T. ornata* with LC_5, LC_{15}, and LC_{25} doses of NLE and PLE. Among the binary mixtures tested, *T.o* (3%) + NLE (1%) exhibited the highest mortality (60.00%) with a pupa-to-adult conversion ratio of 1:0.71, indicating an additive effect. While antagonistic interaction was observed in *T.o* (2.5%) + PLE (1%) with co-toxicity factor of -24.98. Overall, the study highlights the potential of using these natural extracts as potential agents in integrated pest management strategies against *C. medinalis* larvae.

Keywords: *Turbinaria ornata*, larvicidal activity, *Cnaphalocrosis medinalis*, binary mixtures, neem and pungam leaf extracts.

Introduction
The rice leaf folder, *C. medinalis* (Guenee) (Insecta: Lepidoptera: Crambidae) is a serious pest of rice (*Oryza sativa* L), causing significant damage to foliage (Sulagitti *et al.*, 2017) [17] that leads to drastic yield losses up to 80 per cent in susceptible crops (Padmavathi *et al.*, 2013) [15]. The insect undergoes four stages in its life cycle: egg, larva, pupa, and adult. The larval stage causes significant damage to crops (Liang *et al.*, 2021) [10]. A single larva has the potential to deteriorate numerous rice leaves by twisting and scrapping the chlorophyll (Cheah *et al.*, 2022) [2], with cumulative effects that reduce photosynthesis and crop yield. The extensive planting of high-yielding varieties along with alterations in farming techniques leads to significant outbreaks of leaf folders. Farmers depend on chemical inputs (fertilizers, weedicides, insecticides) for crop production and protection. The indiscriminate use of these pesticides leads to undue problems including insecticide resistance, residue deposition and pest resurgence (Fenner *et al.*, 2013) [6]. To address these challenges in agriculture, a range of alternatives have been employed. Marine algal seaweeds suit better as they are easily available, bio-degradable, and synthesize many secondary metabolites that show broad-spectrum bio-activity against insect pests (Deepa *et al.*, 2016) [3]. In this context, the toxicity and insect growth regulator potential of *Turbinaria ornata* (Turner) J. Agardh, a brown algal seaweed, have been evaluated against the Rice leaf folder, *C. medinalis* under *in vitro* conditions.

Materials and methods
Rearing of *C. medinalis*
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. For the initial establishment of culture, the field-collected pupa 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 cultured in a greenhouse-maintained rice plant and grown-up third instar larvae were used for experiments.

Collection of seaweeds
The brown algal seaweed, *T. ornata*, was collected from the coastal regions of Mandapam (Latitude 9°16'17.7"N and Longitude 79°07'55.7"E) in Rameswaram and Hare Island (Latitude 8°46'29.3"N and Longitude 78°11'51.8"E) in Tuticorin, Tamil Nadu, India. The handpicking method was used to collect the seaweeds during the early morning hours. The collected seaweeds underwent a cleansing process using tap water to eliminate impurities, followed by swabbing with a blotting sheet to remove excess moisture. The seaweeds were then air-dried in the shade for two weeks and stored in an airtight container (Kannan and Bharathkumar, 2016) [9].

Preparation of Neem and Pungam Leaf powder

The collected neem (*Azadirachta indica*) and pungam (*Pongamia pinnata*) leaves were washed three times with sterile distilled water to remove dust and other residues. It is then shade-dried for three weeks, blended into a fine powder and sieved through a 40-mesh sieve and used for solvent extraction.

Solvent extraction through ultrasonication

Acetone and ultrapure water at the ratio of 1:1v/v were used as key solvents and the fine powder of *T. ornata*, neem and pungam leaf powder was dissolved individually in the respective solvents at the ratio of 1:10 w/v and subjected to ultrasonic waves at the frequency of 20kHz (65% amplitude) for 45 minutes. It is then centrifuged at 6000 rpm for 15 minutes and concentrated using a rotary evaporator at 55°C (Ummat *et al.*, 2020) [18] and freeze-dried.

Preparation of stock solution

For bioassay studies, a 10 % stock solution (Iqbal *et al.*, 2015) [8] was prepared by diluting crude extract of macroalgae, neem and pungam leaf in respective solvents (Acetone) separately and stored at 4°C for further studies.

Bioassay

The various concentrations of *T. ornata* extract, viz., 2, 4, 6, 8, 10, and 20 per cent, Neem leaf extract (NLE), viz., 0.5, 1, 1.5, 2, 2.5, 3, 3.5 and 4 per cent and Pungam leaf (PLE), viz., 1, 1.5, 2, 2.5, 3, 3.5, 4, 4.5 and 5 per cent in addition to an absolute control and a standard check were evaluated against third instar larva of *C. medinalis* under no-choice leaf dip method (Yang *et al.*, 2018) [19]. Surface sterilized rice leaves were cut into pieces (5 cm long), and the wax layer was removed and treated with 0.1% Triton X-100 (non-ionic detergent) and then washed with distilled water. 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. The mortality and IGR activity of seaweed extracts were calculated at different time intervals starting with 12 hours after treatment (HAT) up to adult emergence. The mortality percentage was calculated using,

$$\text{Mortality (\%)} = \frac{\text{Number of larvae dead in the treatment}}{\text{Total number of larvae released in the treatment}} \text{ X } 100$$

The calculated mortality per centage was corrected using the Abbott formula (Abbott, 1925) [1].

The lethal concentrations have arrived for individual leaf extracts using Probit analysis performed in SAS 9.4 (SAS Institute, Inc., Cary, NC, USA) (Finney, 1971) [7].

Binary combination of *T. ornata* (*T.o*) with Neem (NLE) and Pungam Leaf Extracts (PLE)

For screening the combined efficacy, the lethal concentrations of *T. ornata* extract, viz., LC_{20} and LC_{25} have been combined with sublethal concentrations of neem and pungam leaf extract, viz., $LC_{5,}$ LC_{15} and LC_{25} as binary combinations. Each component in the mixture developed individually and then combined in equal proportions (1:1).

The co-toxicity factor was derived using the following equation (Norris *et al.*, 2019) [12]

$$\text{Co-toxicity factor} \quad \frac{\text{Observed Mortality} - \text{Expected mortality}}{\text{Expected mortality}} \text{ X } 100$$

In which, Observed mortality was the lethal effect observed experimentally in the binary and ternary combinations. Whereas, Expected mortality represents the additive sum of the lethal effect of individual compounds in the combinations. The co-toxicity and additive response were determined based on the values,

$>$ 20 = synergistic mixtures, −20 to +20 = additive mixtures, $<$ −20 = Antagonistic mixtures

Result and discussion

The preliminary screening results of *T. ornata*, neem and pungam leaf extract for determining lethal concentrations against *C. medinalis* show that 100 per cent mortality occurs in all utmost concentrations. In contrast, no mortality rate was observed in untreated controls. The macroalgae (*T.o*), neem (NLE) and pungam leaf extract (PLE) caused *C. medinalis* larval death with respective LC_{50} values of 6.41, 2.01 and 3.01 per cent (Table 1, 2). The LC_{20} and LC_{25} values of *T. ornata* extract estimated through probit analysis were 2.52 and 3.01% against third-instar larvae of *C. medinalis* (Table 1). For neem (NLE) and pungam (PLE) extracts, the estimated $LC_{5,}$ LC_{15} and LC_{25} values were 0.3 and 1.04, 1.52, 1.98 per cent (Table 2), respectively. These LC_{20} (2.5%) and LC_{25} (3%) concentrations of macroalgal extract were used to prepare binary mixtures with LC_5 (0.4%), LC_{15} (0.7%) and LC_{25} (1%) concentrations of NLE and also with LC_5 (1%), LC_{15} (1.5%) and LC_{25} (2%) concentrations of PLE for testing synergistic, additive and antagonistic interactions.

Table 1: Larvicidal activity of *T. ornata* solvent extract against *C. medinalis*

Name of the extract	LC_{20} (%) 95% Fiducial limit	LC_{25} (%) 95% Fiducial limit	LC_{50} (%) 95% Fiducial limit	LC_{90} (%) 95% Fiducial limit	Chi square	R^2	SD (σ)
Solvent extract of *T. ornata* (Acetone)	2.52 (1.78 – 3.65)	3.01 (2.14 – 4.38)	6.41 (4.48 – 9.16)	26.06 (18.22 – 37.27)	0.947	0.879	0.452

Abbreviation; SD (σ) - Standard Deviation, R^2 - Regression equation

Table 2: Larvicidal activity of Neem (NLE) and Pungam (PLE) extracts against *C. medinalis*

Name of the extract	LC_5 (%) 95% Fiducial limit	LC_{15} (%) 95% Fiducial limit	LC_{25} (%) 95% Fiducial limit	LC_{50} (%) 95% Fiducial limit	LC_{90} (%) 95% Fiducial limit	Chi square	R^2	SD (σ)
Neem Leaf extract (Acetone)	0.37 (0.285 – 0.505)	0.70 (0.52 – 0.93)	1.01 (0.76 – 1.35)	2.01 (1.51 – 2.68)	7.39 (5.55 – 9.85)	0.975	0.888	0.420
Pungam Leaf extract (Acetone)	1.04 (0.87 – 1.25)	1.52 (1.29 – 1.85)	1.98 (1.63 – 2.33)	3.01 (2.51 – 3.59)	6.84 (5.71 – 8.17)	0.998	0.946	0.270

Abbreviation; SD (σ) - Standard Deviation, R^2 - Regression equation

Binary mixtures of all three extracts were evaluated for larvicidal activity individually at their lethal concentrations and were mixed in a 1:1 proportion. Fifteen larvae were tested per dose in three replicates. Two experiments were conducted for each binary mix. The expected mortality was estimated as the total of the mortality observed when the lethal doses were tested individually. The values are the mean of two experiments.

The first set of binary combinations consisting of *T. ornata* (LC_{20}) with LC_5, LC_{15} and LC_{25} of neem and pungam leaf extracts shows the larval mortality ranges from 20.00 to 53.33 per cent at 96 hours after treatment. Among the six binary mixtures, the utmost death rate of larvae (53.33%) was recorded at *T.o* (2.5%) + NLE (1%) with pupa to adult conversion ratio of 1:0.71 followed by *T.o* (2.5%) + PLE (2%) with mortality rate of 40.00 and pupa to adult conversion ratio of 1:0.88, respectively (Table 3). All three binary combinations of *T. ornata* with neem leaf extract show an additive effect on comparing observed mortality (26.66, 33.33 and 53.33 per cent) with expected mortality (26.66, 33.33, 46.66 per cent). In the case of *T. ornata* conjugated with pungam leaf extract, *T. o* (2.5%) + PLE (1%) recorded the antagonistic interaction with the co-toxicity factor of -24.98 (CTF = < −20) with the least larval mortality rate (20.00). In comparison, *T. o* (2.5%) + PLE (1.5%) and *T. o* (2.5%) + PLE (2%) delivered additive interactions (Table 5), respectively.

Table 3: Evaluation on the toxicity and Insect Growth Regulaory (IGR) activity of binary mixtures (1:1) of T. ornata (LC20) with Neem and Pungam Leaf extracts (LC5, LC15, LC25) on C. medinalis

Treatments	Larval mortality					IGR Activities				
	12 HAT	24 HAT	48 HAT	72 HAT	96 HAT	% Pupation	% Pupal malformation	% Adult emergence	% Adult malformation	Pupa to adult conversion ratio
T_1 –*T. o* (2.5%) + NLE (0.4%)	6.66 (14.95)^d	13.33 (21.40)^d	20.00 (26.55)^d	26.66 (31.07)^d	26.66 (31.07)^e	73.33 (58.91)^e	6.66 (14.95)^c	66.66 (54.74)^e	0.00 (2.02)^b	1:0.90
T_2 –*T. o* (2.5%) + NLE (0.7%)	13.33 (21.40)^c	20.00 (26.55)^c	26.66 (31.07)^c	26.66 (31.07)^d	33.33 (35.25)^d	66.66 (54.73)^d	6.66 (14.95)^c	60.00 (50.77)^d	6.66 (14.95)^a	1:0.90
T_3 –*T. o* (2.5%) + NLE (1%)	20.00 (26.55)^b	26.66 (31.07)^b	33.33 (35.25)^b	40.00 (39.22)^b	53.33 (46.91)^b	46.66 (43.08)^b	13.33 (21.40)^b	33.33 (35.25)^b	6.66 (14.95)^a	1:0.71
T_4 –*T. o* (2.5%) + PLE (1%)	6.66 (14.95)^d	6.66 (14.95)^e	13.33 (21.40)^e	13.33 (21.40)^e	20.00 (26.55)^f	80.00 (63.49)^f	6.66 (14.95)^c	73.33 (58.91)^f	0.00 (2.02)^b	1:0.91
T_5 –*T. o* (2.5%) + PLE (1.5%)	13.33 (21.40)^c	20.00 (26.55)^c	26.66 (31.07)^c	26.66 (31.07)^d	33.33 (35.25)^d	66.66 (54.74)^d	6.66 (14.95)^c	60.00 (50.77)^d	0.00 (2.02)^b	1:0.90
T_6 –*T. o* (2.5%) + PLE (2%)	20.00 (26.55)^b	26.66 (31.07)^b	33.33 (35.25)^b	33.33 (35.25)^c	40.00 (39.22)^c	60.00 (50.77)^c	6.66 (14.95)^c	53.33 (46.91)^c	6.66 (14.95)^a	1:0.88
T_7 – Azadirachtin 1500ppm	33.33 (35.25)^a	40.00 (39.22)^a	53.33 (46.91)^a	60.00 (50.77)^a	66.66 (54.75)^a	33.33 (35.25)^a	20.00 (26.55)^a	13.33 (21.40)^a	6.66 (14.95)^a	1:0.39
T_8 – solvent control	0.00 (2.02)$_e$	0.00 (2.02)^f	0.00 (2.02)^f	0.00 (2.02)^e	0.00 (2.02)^g	100.00 (87.97)^g	0.00 (2.02)^c	100.00 (87.97)^g	0.00 (2.02)^b	1:1
T_9 – untreated control	0.00 (2.02)^e	0.00 (2.02)^f	0.00 (2.02)^f	0.00 (2.02)^e	0.00 (2.02)^g	100.00 (87.97)^g	0.00 (2.02)^c	100.00 (87.97)^g	0.00 (2.02)^b	1:1
CD (p = 0.05)	0.906	1.069	1.344	1.526	1.837	2.191	0.676	1.766	0.402	-
SEd	0.431	0.508	0.639	0.726	0.874	1.043	0.321	0.840	0.191	-

Values are mean three replicates; figures in parentheses are arcsine (x + 0.5) transformed values, mean in column followed by Common letter do not significantly different at 5 per cent level (LSD), HAT- Hour after treatment

On examining the interactions between *T. ornata* (LC_{25}) and LC_5, LC_{15} and LC_{25} of neem and pungam leaf extracts, all six binary mixtures show an additive interaction with the highest mortality rate of 60.00 per cent recorded at *T.o* (3%) + NLE (1%) with a co-toxicity factor of 16.65 (Table 5) and pupa to the adult conversion ratio of 1:0.66. The lowest mortality rate observed at the mixture of *T.o* (3%) with PLE (1%) was 26.66 per cent, respectively. The mortality rate (66.66%) observed at standard check, Azadirachtin 1500 ppm was higher than all twelve binary combinations tested and no mortality was observed in solvent and untreated control (Table 4).

Table 4: Evaluation on the toxicity and Insect Growth Regulaory (IGR) activity of binary mixtures (1:1) of *T. ornata* (LC_{25}) with Neem and Pungam Leaf extracts (LC_5, LC_{15}, LC_{25}) on *C. medinalis*

Treatments	Larval Mortality					IGR Activities				
	12 HAT	24 HAT	48 HAT	72 HAT	96 HAT	% Pupation	% Pupal malformation	% Adult emergence	% Adult malformation	Pupa to adult conversion ratio
T_1 –*T. o* (3%) + NLE (0.4%)	6.66 (14.95)^e	13.33 (21.40)^e	26.66 (31.07)^d	33.33 (35.25)^e	33.33 (35.25)^e	66.66 (54.73)^e	6.66 (14.95)^b	60.00 (50.77)^e	0.00 (2.02)^b	1:0.90
T_2 –*T. o* (3%) + NLE (0.7%)	13.33 (21.40)^d	26.66 (31.07)^c	33.33 (35.25)^c	40.00 (39.22)^d	46.66 (43.08)^c	53.33 (46.91)^c	6.66 (14.95)^b	46.66 (43.08)^c	6.66 (14.95)^a	1:0.87
T_3 –*T. o* (3%) + NLE (1%)	26.66 (31.07)^b	33.33 (35.25)^b	46.66 (43.08)^b	53.33 (46.91)^b	60.00 (50.77)^b	40.00 (39.22)^b	13.33 (21.40)^a	26.66 (31.07)^b	6.66 (14.95)^a	1:0.66
T_4 –*T. o* (3%) + PLE (1%)	6.66 (14.95)^e	13.33 (21.40)^e	13.33 (21.40)^e	20.00 (26.55)^f	26.66 (31.07)^f	73.33 (58.95)^f	6.66 (14.95)^b	66.66 (54.73)^f	0.00 (2.02)^b	1:0.90
T_5 –*T. o* (3%) + PLE (1.5%)	13.33 (21.40)^d	20.00 (26.55)^d	26.66 (31.07)^d	33.33 (35.25)^e	40.00 (39.22)^d	60.00 (50.77)^d	6.66 (14.95)^b	53.33 (46.91)^d	6.66 (14.95)^a	1:0.88
T_6 –*T. o* (3%) + PLE (2%)	20.00 (26.55)^c	26.66 (31.08)^c	33.33 (35.25)^c	46.66 (43.08)^c	46.66 (43.08)^c	53.33 (46.91)^c	13.33 (21.40)^a	40.00 (39.22)^c	6.66 (14.95)^a	1:0.75

T_7 – Azadirachtin 1500ppm	33.33 (35.25)[a]	40.00 (39.22)[a]	53.33 (46.91)[a]	60.00 (50.77)[a]	66.66 (54.74)[a]	33.33 (31.07)[a]	13.33 (21.40)[a]	20.00 (26.55)[a]	6.66 (14.95)[a]	1:0.39
T_8 – solvent control	0.00 (2.02)[f]	0.00 (2.02)[f]	0.00 (2.02)[f]	0.00 (2.02)[g]	0.00 (2.02)[g]	100.00 (87.97)[g]	0.00 (2.02)[c]	100.00 (87.97)[g]	0.00 (2.02)[b]	1:1
T_9– untreated control	0.00 (2.02)[f]	0.00 (2.02)[f]	0.00 (2.02)[f]	0.00 (2.02)[g]	0.00 (2.02)[g]	100.00 (87.97)[g]	0.00 (2.02)[c]	100.00 (87.97)[g]	0.00 (2.02)[b]	1:1
CD (p = 0.05)	0.970	1.163	1.498	1.736	2.139	1.862	0.626	1.515	0.409	-
SEd	0.461	0.553	0.712	0.826	1.017	0.886	0.297	0.721	0.194	-

Values are mean three replicates; figures in parentheses are arcsine (x + 0.5) transformed values, mean in column followed by Common letter do not significantly different at 5 per cent level (LSD), HAT- Hour after treatment

Table 5: Assessment on interactions of binary mixtures of *T. ornata* with Neem (NLE) and Pungam Leaf (PLE) on *C. medinalis*

Binary mixtures (1:1)	Individual mortality of compound i	Individual mortality of compound ii	Expected mortality	Observed mortality	Co-toxicity factor	Interactions
T. o (2.5%) + NLE (0.4%)	20.00	6.66	26.66	26.66	0.00	Additive
T. o (2.5%) + NLE (0.7%)	20.00	13.33	33.33	40.00	20.00	Additive
T. o (2.5%) + NLE (1%)	20.00	26.66	46.66	53.33	14.29	Additive
T. o (2.5%) + PLE (1%)	20.00	6.66	26.66	20.00	-24.98	Antagonistic
T. o (2.5%) + PLE (1.5%)	20.00	13.33	33.33	33.33	0.00	Additive
T. o (2.5%) + PLE (2%)	20.00	20.00	40.00	40.00	0.00	Additive
T. o (3%) + NLE (0.4%)	26.66	6.66	33.33	33.33	0.00	Additive
T. o (3%) + NLE (0.7%)	26.66	13.33	40.00	46.66	16.65	Additive
T. o (3%) + NLE (1%)	26.66	26.66	53.33	60.00	12.5	Additive
T. o (3%) + PLE (1%)	26.66	6.66	33.33	26.66	-20	Additive
T. o (3%) + PLE (1.5%)	26.66	13.33	40.00	40.00	0.00	Additive
T. o (3%) + PLE (2%)	26.66	20.00	46.66	46.66	0.00	Additive

The above results confirm the insecticidal action of macroalgal extract in combination with neem and pungam leaf extracts against *C. medinalis* owing to the presence of bioactive compounds. Rajkumar and Bhavan (2017) [16] carried out the preliminary screening of phytochemical constituents of *T. ornata* through the Harborne method and the results revealed the existence of several active secondary metabolites like steroids, alkaloids, phenols, flavonoids, saponins, tannins, carbohydrates, carboxylic acid, coumarines, xanthoproteins and anthroquinones, respectively. Similarly, Elbrense and Gheda (2021) [5] evaluated the insecticidal and antifeedant activities of seaweed extracts of *Sargassum acinarium*, *T. ornata*, *Petrocladia capillacea* and *Cystoseira myrica* against third and fifth-instar larvae of *Spodoptera littorlais*. Ethanolic extract of *T. ornata* caused the highest mortality (83.33 ± 1.92%) in third-instar larvae of *S. littorlais*. Further, GC-MS analysis of seaweed extracts showed bio-active compounds mainly Diisooctyl phthalate, terpenoids, decane, phenolics and fatty acids which might be responsible for its biopesticide activity, respectively. In addition to that, Deepak *et al.* (2017) [4] investigated the Mosquito-larvicidal efficacy of gold nanoparticles synthesized from the seaweed, *T. ornata* (To-AuNPs) and its boiled aqueous extract (To-AE) was tested against the fourth instar of malarial vector *Anopheles stephensi* and recorded the LC50 and LC90 values of 37.77 and 159.55 µg/ml (To-AE), 12.79 and 78.70 µg/ml (To-AuNPs). Moreover, insecticidal effects of the nanoparticles of brown algal seaweed, *S. muticum* on *Ergolis merione* resulted in significant physiological alterations, including changes in hemolymph protein profiles, variations in hemocyte morphology, and the degradation of midgut structures such as the lumen, basement membrane, and gastric caeca (Moorthi *et al.*, 2015) [11], respectively.

Packiam and Ignacimuthu (2012) [13] tested the antifeedant and growth-regulating activities of PONNEEM, an oil formulation containing neem and pungam (karanj) oils along with individual formulations of neem and pungam oil against fourth-instar larvae of *S. litura* in which PONNEEM recorded the maximum antifeedant activity (88.6%) at 0.6% concentration and also significantly reduced the pupal weight and fecundity. They also reported the synergistic effect of biomolecules such as azadirachtin and karanjin in the PONNEEM formulation, which is compatible with natural enemies (Packim *et al*., 2013), respectively.

Conclusion

Based on above findings, it can be concluded that *T. ornata* extract conjugated with neem and pungam leaf extracts can be utilized as potential agents in pest management strategies against *C. medinalis* larvae.

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