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Bioefficacy of Pectolinaringenin from *Clerodendrum phlomidis* Linn. F. against *Anopheles stephensi* and Bhendi Fruit Borer, *Earias vittella* fab.

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ABSTRACT

Larvicidal activity of pectolinaringenin from Clerodendrum phlomidis *was evaluated against* Anopheles stephensi *and antifeedant, larvicidal and growth inhibitory activities were evaluated against* Earias vittella. *Pectolinaringenin exhibited larvicidal activity of 100 and 98.24% against 2nd and 4th instar larvae of* Anopheles stephensi *at 5ppm concentration. It exhibited LC_{50} values of 0.35 and 0.55 ppm for 2nd and 4th instar larvae, respectively. At 100 ppm concentration, pectolinaringenin exhibited maximum antifeedant activity of 74.00% and larvicidal activity of 89.98%. The LC_{50} values were 36.2 and 10.23 ppm for antifeedant and larvicidal, respectively. The compound completely prevented the adult emergence at 50 and 100 ppm concentrations. This is the first report of pectolinaringenin from* C. phlomidis *evaluated against* An. stephensi *and* E. vittella. *The results suggested that the pectolinaringenin from* C. philomidis *could be used to develop a new botanical formulation to manage vector mosquitoes and agricultural pests.*

Key words: *Anopheles stephensi,* adult emergence, Fraction, flavonoid, Effective concentration

INTRODUCTION

Insect control has been a challenging task for human race since the beginning of agriculture. By the application of chemical pesticides, particularly chlorinated hydrocarbons, pest problem was controlled to a certain level and it was thought that the pest problem was solved. But soon it was realized that the pests developed resistance to these chemicals; besides, they increase the cost of application, leave toxic residues and pollute the environment (Ahmed et al. 1981; Ali and Rizvi 2008). Over-application of these pesticides increase the evolution of insect pests, destroys natural enemies, turns existing harmless species into pests, affects other non-target organisms and contaminates food and feed (WCS 1980). Bami (1997) reported that hardly 0.1% of the agrochemicals reached the target pests and the remaining 99.9% entered the environment and affected the non-target organisms. Pentachlorophenol (PCP) containing chemical insecticides inhibit symbiotic nitrogen fixation, which lead to reduce the crop yields and affect seed germination (Fox et al. 2007). Hence, a search for alternate techniques for the

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management of insect pests is needed.

Several plants have been described as unique species due to their medicinal properties, which have been used for veterinary, human health care and crop protection (Ved and Goraya 2008). Plant-based pesticides are useful for the consumers and agriculturists due to economic considerations and potential health benefits and also for their naturalness (Isman 2008). Plants produce wide range of secondary metabolites such as alkaloids, flavonoids, phenols, etc. These phytochemicals are known to protect the plants from the attack of insect-pests (Ahmad 2007).

There are several reports describing many compounds isolated from different plants with varied biological properties against various pests. Napal et al. (2009) isolated flavonoids from *Flourensia oolepis* Blake (Asteraceae) and screened for antifeedant activity against *Epilachna paenulata* (Germar, 1824), *Xanthogaleruca luteola* (Müller, 1766) and *Spodoptera frugiperda* (J.E. Smith, 1797). Lakshmi et al. (2010) screened six flavonoids for adulticidal activity against filarial parasite *Brugia malayi* (S.L. Brug, 1927). Thymol and 1,8-Cineole from essential oils showed antifeedant activity at least concentrations against *Chilo partellus* (Swinhoe, 1885) (Singh et al. 2011). Antifeedant, larvicidal and growth inhibitory activities of ononitol monohydrate isolated from *Senna tora* (L.) Roxb. (syn. *Cassia tora*) against *Helicoverpa armigera* (Hübner, 1809) and *Spodoptera litura* (Fabricius, 1775) were reported by Baskar and Ignacimuthu (2012). Baskar et al. (2014) observed that triterpenoid, friedelin isolated from hexane extract of *Azima tetracantha* (syn. *Monetia barlerioides* L'Her.) (Salvadoraceae) leaves exhibited biological activity against *H. armigera* and *S. litura*, but did not show any ecotoxicological effect against *Cyprinus carpio* (Linnaeus, 1758). *Allium macrostemon* (Amaryllidaceae) *Bunge* and its major compounds, dimethyl trisulfide and methyl propyl disulfide exhibited strong larvicidal activity against *Aedes albopictus* (Skuse, 1894) (Liu et al. 2014). Mudalungu et al. (2013) reported that *p*henanthrene carboxylic acid derivatives, Hexyl-9, 10-dihydroxydec-5-enoate and Methylheneicosane ester derivatives from *Fagaropsis angolensis* (Engl.) Dale (Rutaceae) showed larvicidal activity against *An. gambiae* (Giles 1902).

Anopheles stephensi (Liston, 1901) is a causative agent for malaria in urban India. It is predominantly present in China, Afghanistan, Iran, Iraq, India, Bangladesh and Pakistan. Malaria is a major health issue all over the world. Nearly 50% of the global population (3.4 billion) is facing the risk of malaria. About 207 million malaria cases were recorded in 2012 with estimated malarial deaths of 0.673 million. Africa alone recorded 90% of malarial deaths in 2012 and most of them were children below five years of age. Efficient preventive and control measures since 2000 have reduced malarial death to 42% (WHO 2014). Chemical pesticides are mostly unable to kill/control the mosquitoes, which have developed resistance against several chemical pesticides (which also cause severe environmental problems). Due to these reasons, studies are being made to develop natural products to control mosquitoes. Different solvent extracts from *Justicia adhatoda* L. (syn. *Adhatoda vasica*) (Acanthaceae), *Annona squamosa* L, (Annonaceae), *Senna auriculata* (L.) Roxb. (syn. *Cassia auriculata*) (Fabaceae), *Hydrocotyle javanica* Thunb. (Apiaceae), *Solanum torvum* SW. (Solanaceae), *Chrysanthemum indicum* L. (Asteraceae), *Tridax procumbens* L. (Asteraceae) and *Vitex negundo* L. (Lamiaceae) have shown larvicidal activity against *An. stephensi* (Kamaraj et al. 2010; 2011). Govindarajan (2011) reported that *Eclipta alba* L. (Asteraceae), *Cardiospermum halicacabum* L. (Sapindaceae) and *Andrographis paniculata* (Burm.f.) Wall. ex Nees (Acanthaceae) exhibited larvicidal activity against *An. stephensi*. The spotted boll worm, *Earias vittella* (Fabricius, 1794) is a notorious noctuid pest belonging to the order Lepidoptera and causes serious losses with 50–60% reduction in cotton yield (Khan and Rao 1960; Sohi 1964). It also causes damage to the seedlings (14.4%), buds (34–51%), flowers (3.2% flowers) and bolls (3.2–69.0%) resulting nearly 20% loss in cotton seed yield (Patel 1949; Kaushik et al. 1969). It damages 79–97% of the loculi of cotton, *Gossypium* (Malvaceae) (Sidhu and Sandhu 1977). *E. vittella* alone is reported to cause 57.1% fruit infestation and 54.04% net yield loss in okra (Chaudhary and Dadheech 1989).

Traditionally, *Clerodendrum phlomidis* Linn F. (Lamiaceae) leaf extract is used to treat skin diseases in livestock (Ramji 1992). *C. phlomidis* is used as an astringent and also to treat gonorrhea (Rani et al. 1999; Murugesan et al. 2001). *C. phlomidis* (*C. multiflorum*) has been used as botanical pesticide to control aphids and red hairy caterpillar, *Amsacta albistriga* (Walker, 1865)

(Upadhyay et al. 2002; Singh and Saratchandra 2005). Leaf extracts of this plant were used as grain protectant (Charpot 1998) and to control *Heliothis* sp. (Ghanch 1998). The present study was aimed at evaluating the mosquito larvicidal, antifeedant, larvicidal and growth inhibitory activities of pectolinaringenin from the chloroform extract of *Clerodendrum phlomidis* Linn. F. (Lamiaceae) against *An. stephensi* and *Earias vittella*.

MATERIAL AND METHODS

Plant collection, crude extraction, fractionation and isolation of pectolinaringenin

Extraction, fractionation and isolation of pectolinaringenin from *C. phlomidis* were similar as reported by Muthu et al. *(*2012).

Rearing of vector mosquitoes

Larvae of *An. stephensi* were collected from various stagnant water bodies in Chennai, India. They were cultured in the laboratory for many generations and were maintained at 27±2°C, 75–85% RH and photoperiod of 14:10h (light/dark) continuously in the laboratory. Larvae were fed with finely ground dog biscuit (nutritional contents are proteins, fats, sodium, carbohydrates and fatty acids) and yeast extract in the ratio of 3:1. The water was changed daily to avoid scum formation. Pupae were transferred from the trays to a plastic cup containing tap water and placed inside the cage (30x30x30 cm dimension) for adult emergence. The emerged adults were reared in the respective cages. The adult colony was provided with 10% sucrose solution and it was periodically blood-fed on restrained rats. After three days, ovitrap was kept in the cages for egg laying and the eggs were collected and transferred to enamel trays. Newly hatched second and fourth instar larvae were used for the experiment.

Rearing of *Earias vittella*

Earias vittella larvae were collected from Thandalam village near Thirupporur, Kancheepuram district, Tamil Nadu. They were reared on glass jars (21 cm x 15 cm) fed with bhendi fruits up to pupation in the laboratory condition (27±2°C and 75±5% relative humidity). After pupation, the pupae (cocoon) were collected and kept in different glass jars covered with white muslin cloth. After the emergence of the adults (8-10 days), they were fed with 10% honey solution absorbed in cotton swabs inside the glass jars. Muslin cloth was provided as an oviposition substrate. The eggs laid were kept in a glass jar covered with muslin cloth for hatching. After hatching, the larvae were fed with tender leaves of bhendi (*Abelmoschus esculentus*) in the neonate stage after that they were fed with bhendi fruit.

Mosquito larvicidal activity

Bioassay was done using the method of World Health Organization (2014) with second and fourth instar larvae of *An. stephensi*. Pectolinaringenin was studied at different concentration of 0.5, 1.0, 2.5 and 5 ppm. A minimum of twenty larvae per replicate and five replicates were maintained (total N= 100). The experiments with 249 mL dechlorinated tap water and 1/0 ml of the required concentration of test solution was dissolved in water with emulsifier (0.1% Tween 80).Tween80 was used as a negative control. Mortality was observed after 24 h of treatments.

Antifeedant activity

Pectolinaringenin was evaluated for their antifeedant activity against *E. vittella* using fruit disc no choice method. Fresh bhendi fruit discs of 10 mm thickness were used for this study. The bhendi fruit discs were dipped individually in 100, 50, 25 and 12.5 ppm concentrations. The fruit discs dipped in acetone + Tween 80 were used as negative control since it was used to dissolve the compound. In each plastic Petri dish, a wet filter paper was placed to avoid early drying of the test materials and three 3rd instar larvae were introduced into each Petri dish containing five discs of bhendi fruit. Five replicates were maintained for each treatment with 15 larvae per replicate (total n=75). Progressive consumption of the fruit discs consumed by *E. vittella* larvae was observed. After 24 h, the fruit discs were weighed using Metler digital balance and the difference between the initial and final weights was calculated. Real consumption was calculated as follows:

Weight loss due to desiccation (D) = initial weight – final weight

Real consumption = initial weight – (final weight + D)

The experiment was conducted at laboratory condition (27 ± 2°C) with 14:10h light and dark photoperiod and 75 ± 5% relative humidity.

Antifeedant activity was calculated according to the formula of Bentley et al. (1984):

$$\text{Antifeedant activity} = \frac{\text{Consumption in control - Consumption in treated}}{\text{Consumption in control}} \times 100$$

Larvicidal activity

Larvicidal activity was screened using fruit disc no choice method. Bhendi fruit discs were dipped in varied concentrations as mentioned for antifeedant bioassay. After 24 h of treatment, the larvae were continuously reared on the untreated fresh bhendi fruits. At every 24 h, the diet was changed. Larval mortality was recorded up to 96 h of treatment. The number of larvae, replicates used and laboratory conditions were the same as in antifeedant experiment. Percent mortality was calculated using Abbott's formula (Abbott 1925).

$$\text{Abbott corrected mortality} = \frac{\text{\% mortality in treatment} - \text{\% mortality in control}}{100 - \text{\% mortality in control}} \times 100$$

Adult emergence

The treated larvae were maintained for adult emergence. Adult emergence was calculated by subtracting the number of emerging adults from the total number of pupae.

Statistical analysis

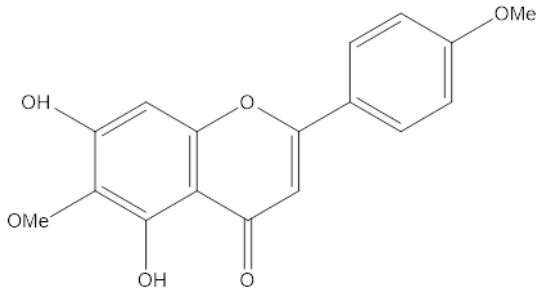
The data for antifeedant and larvicidal activities and adult emergence were analysed using one-way ANOVA. Significant differences between the treatments were determined using Tukey's multiple range test ($P \leq 0.05$). The LC_{50} and LC_{90} values were calculated using probit analysis (Finney 1971).

RESULTS

Isolation and Characterization of the compound

The fraction eluted with the solvent hexane: ethyl acetate (1:1) gave a compound as pale yellow crystal from methanol (melting point 210°C, Lit. mp 210 – 212°C). The yield was 850 mg. It gave positive ferric reaction by giving green colour with alcoholic ferric chloride for phenol. It also was positive for Shinoda test for flavonoid by giving reddish pink colour with mg/HCl. TLC over silica gel G with Chloroform: Ethyl acetate (9:1) as the developing system gave a single spot, pale yellow turning dark yellow, on exposure to ammonia (Rf = 0.35). The purity of the compound was 98.2% checked using HPLC.

Spectral analyses were done using UV, IR, ^{1}HNMR, ^{13}CNMR and EI-MS. The ^{1}HNMR spectrum showed absence of substituent at C-3, H-3 appearing as singlet at δ = 6.56. The lone aromatic proton H-8 appeared as singlet at δ = 6.54. The A_2B_2 system in ring B was shown by two sets of ortho coupled protons at δ 7.85 and 7.03 (J=9.0 Hz) corresponding to H-2′ , H-6′ and H-3′ and H-5′. 4′-OMe appeared as three proton singlet at δ - 3.94 and 6-OMe appeared at 3.90. 7-OH appeared as a broad at singlet δ 9.39 and the chelated 5-OH appeared downfield at δ 12.96. The ^{13}CNMR also confirmed the structure of the compound as Pectolinaringenin in the present investigation. The flavonoid carbonyl appeared slightly downfield at δ = 182.33. This suggested the un-substituted C-3 with 5-OH. The single peak at δ 93.09 was assigned to C-8 and the absence of slightly downfield peak around δ 97.0 found for C-6 in 5,7-oxygenated flavones showed that C-6 was substituted. C-6 OMe appeared at δ 131.08. C-5 attached to chelated OH appeared at δ 152.72. The δ_c values in the ring B corresponded to pectolinaringenin. The EI-MS gave M^+, the molecular ion at m/z 314 corresponded to the molecular formula $C_{17}H_{14}O_6$. The peak at m/z 296 corresponded to $[M\text{-}H_2O]^+$. The above data confirmed the structure as Pectolinaringenin (Fig. 1). The molecular formula is $C_{17}H_{14}O_6$.



Figue 1 - Pectolinaringenin (5,7-dihydroxy- 4′,6-dimethoxy-flavone).

Mosquito larvicidal activity

Pectolinaringenin exhibited larvicidal activity against *An. Stephensi* at concentration dependent manner. Maximum larvicidal activity of 100 and 98.94% were noticed at 5ppm concentration of pectolinaringenin against 2nd and 4th instar larvae,

respectively. At 2.5 ppm concentration, pectolinaringenin exhibited more than 85% larvicidal activity. It exhibited the LC_{50} values of 0.35 and 0.55 ppm against 2nd and 4th instar larvae, respectively. Significant χ^2 values indicated the heterogeneity of tested population (Table 1).

Table 1 - Larvicidal activity of pectolinaringenin against human vector mosquito *Anopheles stephensi.*

Instars	Concentration (ppm)	% larvicidal activity	LC_{50}	95% confidential interval		LC_{99}	95% confidential interval		χ^2
				Lower	Upper		Lower	Upper	
				Anopheles stephensi					
2nd	0.5	50.52±4.16	0.35	0.11	0.53	3.42	3.00	4.04	34.76*
	1.0	75.21±4.61							
	2.5	92.73±2.98							
	5.0	100.0±0.00							
4th	0.5	43.26±5.37	0.55	0.18	0.82	4.59	3.86	5.71	70.19*
	1.0	67.00±2.81							
	2.5	87.57±3.08							
	5.0	98.94±2.35							

* Chi-square values are significant at P <0.05.

Antifeedant activity

The pectolinaringenin showed maximum antifeedant activity of 74% at 100 ppm concentration, followed by 50 ppm concentration against *E. vittella*. The LC_{50} and LC_{90} values for the pectolinaringenin were 36.20 and 146.86 ppm, respectively (Table 2).

Larvicidal activity

Larvicidal activity of 89.98% was observed against *E. vittella* at 100 ppm concentration, followed by 50 and 25 ppm concentrations. The LC_{50} and LC_{90} values for the compound were 10.23 and 92.67 ppm, respectively. Significant Chi-square values were noticed (Table 2).

Adult emergence

The pectolinaringenin at 100 and 50 ppm concentrations completely prevented the adult emergence of *E. vittella.* At 12.5 and 25 ppm concentrations also it inhibited more than 40% adult emergence of *E. vittella*. When compared to control, all the treatments showed significant reduction of adult emergence of *E. vittella* (Table 2).

Table 2 - Percent antifeedant, larvicidal activities with effective concentration (ppm) and adult emergence of pectolinaringenin against *E. vittella.*

Treatments	Dose (ppm)	Antifeedant	LC_{50}	95% fiducial limit		LC_{90}	95% fiducial limit		χ^2
				Lower	Upper		Lower	Upper	
				Antifeedant (%)					
Pectolinaringenin	12.5	32.53±3.90[b]	36.20	28.72	42.90)	146.86	128.37	174.46	29.40*
	25	48.48±3.50[c]							
	50	62.60±3.35[d]							
	100	74.00±1.58[e]							
Control		3.10±1.82[a]							
				Larvicidal (%)					
Pectolinaringenin	12.5	45.70±2.99[d]	10.23	0.43	17.57	92.67	82.07	107.92	36.7*
	25	62.87±5.43[e]							
	50	77.19±5.53[f]							
	100	89.98±3.91[g]							
				Adult emergence (%)					
Pectolinaringenin	12.5	52.85±3.91[b]							
	25	50.00±7.07[b]							
	50	00±00[a]							
	100	00±00[a]							
Control		92.5±6.84[c]							

Values represent Mean ± SD of five replicates (n=75); Similar alphabets in a column do not differ significantly using Tukey's test (P≤0.05); * Chi-square values are significant at P <0.05.

DISCUSSION

In the present study, plant derived pectolinaringenin exhibited 100% larvicidal activity against *An. stephensi*. These findings agreed with those of Senthil Nathan et al. (2005) who reported that natural compounds of azadirachtin, salannin, deacetylgedunin, Gedunin, 17-Hydroxyazadiradione and deacetylnimbin exhibited larvicidal activity against *An. stephensi*. Leaves and seed methanol extract of *Melia azedarach* (Meliaceae) exhibited more than 70% larvicidal activity against 2nd and 4th instar larvae of *An. stephensi* (Senthil Nathan et al. 2006). Piperitenone oxide isolated from the essential oil of *Mentha spicata* L. var. viridis (L.) (Lamiaceae) exhibited larvicidal activity against *An. stephensi* (Tripathi et al. 2004). Mullai et al. (2008) reported that different organic solvent extracts of *Citrullus vulgaris* Schrad. by L. H. Bailey (Cucurbitaceae) exhibited larvicidal activity against *An. stephensi*. Pectolinaringenin exhibited 100% larvicidal activity against *An. stephensi* in the present study. The present study was in agreement with the findings of Medhi et al. (2010) who reported that major constituents of 1,8-cineole (69.46%), γ – Terpinene (15.10%), α –Pinene (5.47%) and Globulol (2%) from *Eucalyptus camaldulensis* Dehnh (Myrtaceae) exhibited larvicidal activity against *An. stephensi*. Flindersine from *Toddalia asiatica* Lam. (Rutaceae) exhibited larvicidal activity against *An. Stephensi* by Duraipandiyan et al. (2014)

The secondary metabolites produced by the plants act as defensives against insects. Different plant extracts with various organic solvents extracted in varied proportions yield secondary plant metabolites, which possess various types of biological activities to protect the plants from insects (Sharma and Bhist 2008). Fraenkal (1959) stated that food preference by the insects is based solely on the presence or absence of secondary plant metabolites. In this study, pectolinaringenin showed promising antifeedant activity against *E. vittella*. The results were in agreement with the findings of Napal et al. (2009) who reported that antifeedant activity of flavonone, pinocembrin isolated from ethanolic extract of *Flourensia oolepis* Blake (Asteraceae) aerial parts exhibited more than 80% antifeedant activity against *E. paenulata, X. luteola* and *S. frugiperda*.

The present results revealed that pectolinaringenin showed promising antifeedant activity. Bioassay-guided fractionation of the extracts of *Nothofagus dombeyi* Mirb. (Oerst.) (Nothofagaceae) and *N. Pumilio* (Poepp. and Endl.) Krasser yielded several triterpenes and flavonoids including 2-O-acetylmaslinic acid, 3-O-acetyl 20,24,25-trihydroxydammarane, and 3,20,24,25-tetrahydroxydammarane. They were screened for antifeedant activity against larvae of *Ctenopsteustis obliquana* (Walker, 1863) and it was noticed that 12-hydroxyoleanolic lactone and pectolinarigenin from *N. dombeyi* and dihydrooroxylin A from *N. pumilio* exhibited significant antifeedant activity (Thoison et al. 2004).

In the present investigation, a flavonoid, pectolinaringenin exhibited good antifeedant activity at 100 and 50 ppm concentration. This finding coincided with the results of Simmonds and Stevenson (2001) who studied four isolated flavonoids from various wild varieties of *Cicer* (Fabaceae), namely judaicin 7-O-glucoside, 2-methoxy-judaicin, judaicin and maackiain against the feeding behaviour and development of *H. armigera.* All the four isoflavonoids showed antifeedant activity against *H. armigera* at 100 ppm concentration. Antifeedant activity of six *neo*-clerodane diterpenoides isolated from *Clerodendrum* species were evaluated against *E. vittella* and *S. litura* by Kumari et al. (2003) and it was observed that all the compounds exhibited more than 65% antifeedant activity against both the pests at 10 µg/cm^2 concentrations.

Simmonds et al. (1990) reported that the antifeedant activity of flavonoids such as chalcones, flavones and flavanones was due to the predominant stimulation of the deterrent neurons in the medial sensillum stylonicum and more than one receptor could be involved. Some flavonoids and flavonoid-containing extracts possess anthelmintic and nematicidal activity, which induce embryonic and larval lethality in *Bursaphelenchus xylophilus* (Steiner & Buhrer, Nickle) and *Caenorhabditis elegans* (Maupas, 1900) (Lee et al. 2008; Kim et al. 2009).

The isolated compound, pectolinaringenin exhibited maximum larval mortality at 100 ppm. At lower concentration (12.5 ppm), it exhibited more than 50% larval mortality. This finding was in accordance of Kumari et al. (2003) who reported that six *neo*-clerodane diterpenoides isolated from *Clerodendrum* species at 10 µg/cm^2 exhibited 20 and 44% larval mortality against *E. vittella* and *S. litura*, respectively. Strongest

insecticidal activity of isoflavones were from the stems without bark of *Endosamara racemosa* (Roxb.) R. Geesink (syn: *Millettia racemosa*) (Leguminosae) (Kumar 1988).

In the present investigation, pectolinaringenin either completely reduced the adult emergence or larval-pupal intermediate or pupicidal activities were observed. This was in agreement with Rembold and Garcia (1989), who observed that the treated *Rhodnius prolixus* (Stål, 1859) larvae managed to enter the pupal stage even at low concentration of azadirachtin but emerged adults were not able to reproduce, which implied that azadirachtin inhibited ecdysis. Adults developed from the treated larvae were mostly deformed (Sharma and Jalan 1997).

In the present finding, the compound, pectolinaringenin prevented 100% adult emergence at 100 and 50 ppm concentrations. Our findings corroborate with the earlier findings of Abdelgaleil and El-Aswad (2005) who evaluated Methyl 6-hydroxyangolensate and 3,7-dideacetylkhivorin derived from *Khaya ivorensis* A. Chev. (Meliaceae) against *S. littoralis* (Boisduval, 1833) and observed reduction in larval growth, pupation, adult emergence and egg hatchability. Six *neo*-clerodane diterpenoides were isolated from *Clerodendrum* species by Kumari et al. (2003) and observed that all the compounds prolonged the larval and pupal durations of *E. vittella* compared to control. Clerodendrin B was the most promising compound which prolonged the larval and pupal durations and reduced the adult emergence.

Antifeedant activity of pectilonaringenin was maximum against *E. vittella* in a dose-dependent manner. The activity could be due to the functional groups such as hydroxyl, methoxyl and carbonyl present in the compound. The compound had hydroxyl groups at 5^{th} and 7^{th} position in A- ring, which contributed to strong antifeedant activity. The pyran ring had C-4 carbonyl, which was essential for high antifeedant activity. The methoxyl group at C-4′ in the B-ring contributed to mild activity. Phagodeterrent activity of the pectilonaringenin coincided with the results of Ohmura et al. (2000) who reported that the presence of hydroxyl group at 7^{th} position in the A-ring and carbonyl group at C-4 in the pyran ring in naringenin contributed to higher antifeedant activity of subterranean termite, *Coptotermes formosanus* (Shiraki, 1909) than the C-4′ in the B-ring. Medeiros et al. (1994) reported that keto group at C-4 in quercetin, karanjin, chrysoeriol and butein showed strong antifeedant activity and the presence of 7-hydroxyl group in the flavone was responsible for the highest antifeedant activity against *Mythmina unipuncta* (Haworth, 1809). Pectolinaringenin isolated from *Clerodendron siphonenthus* was screened against *Sitophilus oryzae* (Linnaeus, 1763) by Pal et al. (1989) and reported that the compound inhibited the feeding. Kaemperol and quercetin flavonoids having an oxidised C-ring, inhibited feeding in *Scolytus multistriatus* (Marsham, 1802) (Norris 1970).

CONCLUSION

The results showed that pectolinaringenin from *C. phlomidis* chloroform extract exhibited promising mosquito larvicidal, antifeedant and larvicidal activities and reduced the adult emergence of *E. vittella*. Pectolinaringenin could be effectively used to develop a novel pesticidal formulation for disease causing vector mosquitoes and agricultural pest management programme.

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