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Effect of pectolinaringenin, a flavonoid from *Clerodendrum phlomidis*, on total protein, glutathione S-transferase and esterase activities of *Earias vittella* and *Helicoverpa armigera*

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Abstract Pectolinaringenin was isolated from chloroform extract of *Clerodendrum phlomidis* L. and was evaluated at 12.5 to 100 ppm concentrations for its effects on total protein, esterase and glutathione S-transferase (GST) activities of *Earias vittella* and *Helicoverpa armigera*. At 100 ppm, the compound reduced total protein content by 55.75% and 53.01% over control with IC_{50} values of 74.37 and 212.31 ppm in *E. vittella* and *H. armigera,* respectively. At 100 ppm it also reduced GST and esterase enzyme activities in *E. vittella* by 37.53% and 43.09% over control, with IC_{50} values of 133.00 and 111.76 ppm, respectively. It also reduced GST and esterase activities in *H. armigera* by 43.14% and 47.421% over control with IC_{50} values of 114.38 and 98.78 ppm, respectively. Data were analyzed for their normality using the Shapiro-Wilk test and Levene's statistics to determine the significant deviation. Pectolinaringenin could be used for the management of agricultural pests.

Keywords Agricultural pests · Detoxifying enzymes · Inhibitory concentration · Isolated compound

Introduction

Plant-derived substances like tannins, phenolics, gossypol, some saponins, and proteinase inhibitors have the potential to interfere directly with an animal's ability to digest dietary protein, and thus reduce the efficiency of utilization of the dietary protein (Birk & Peri 1980; Singleton 1981; Whitaker 1981; Zucker 1983). Flavonoids isolated from *Circium* and *Cardus* species exhibited a broad spectrum of biological activities such as enzyme inhibitors (α-glucosidase and α-amylase), larval growth inhibitor, etc., against insect pests; these have been reported by many researchers (Elliger *et al.* 1980; Hedin & Waage 1986; Kim *et al.* 2000).

Researchers around the world have reported the activity of plant-derived substances on protein content and enzyme activities of different insects. Khalaf (1998) reported that volatile oils of *Cymbopogon citratus* and *Rosmarinus officinalis* induced biochemical disturbances and decreased protein content in *Musca stabulans*. *Melia azedarach* methanol extract was studied for its effect on protein content of *Spodoptera littoralis* and *Agrotis ypsilon;* it was observed that at 100 ppm, the hemolymph concentration of both the insects was reduced after 6 days of treatment (Schmidt *et al*. 1998). Boreddy *et al.* (2000) evaluated the effect of *Annona squamosa* seed extracts on *S. litura* for its total quantitative and qualitative protein contents; they observed that it reduced the protein content in all stages of the insects. Azadirachtin-rich commercial insecticides were evaluated against 4th instar larvae of *H. armigera* and it was found that all the treatments reduced the protein level (Neoliya *et al.* 2007). Rharrabe *et al.* (2007) investigated the effects of 20-hydroxyecdysone on protein content of *Plodia interpunctella;* the protein content was reduced when the larvae consumed treated diet compared with control. Glutathione S-transferases (GSTs) are the multifunctional group of active enzymes

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with detoxification functions (Wood *et al.* 1986, 1990). Esterases constitute a widely distributed family of enzymes that involve hydrolysis of carboxylester, amide, and thioester bonds in a variety of compounds. These enzymes have the ability to detoxify insecticides (Conyers *et al.* 1998). GST and esterases are important enzymes involved in the metabolism of a broad range of foreign and endogenous compounds in insects (Francis *et al.* 2001). The important function of GST is detoxification, conjugating reduced glutathione with a large number of electrophilic metabolites derived from a variety of xenobiotics, including carcinogens, toxins and drugs (Zanden *et al.* 2004). Cho *et al.* (1995) suggested that esterase and mixed function oxidase play an important role in the detoxification of organophosphorus and pyrethroid insecticides in *S. litura*.

The bhendi fruit borer, *Earias vittella* Fab. (Lepidoptera: Noctuidae), is a serious pest causing more than 80% damage to okra (Radake & Undirwade 1981; Srinivasan & Gowder 1960). It reduced the seeds by 16.47% per fruit (Sinha *et al.* 1978). *Helicoverpa armigera* Hübner (Lepidoptera: Noctuidae) is a major pest of many crops worldwide (Fakrudin *et al.* 2004; Jallow *et al.* 2004) and it is also a polyphagous pest causing severe loss to economically important crops (Subramanian & Mohankumar 2006). *H. armigera* alone damages more than 80 species of crop plants. In India, it caused a yield loss of 158 million US$ during 1996–1997 and about 54% of the total insecticides was used for the protection of the cotton crop for this serious pest (Jalali *et al.* 2004; Jayaraj 2003). Hence, an attempt has been made to study the effect of pectolinaringenin (Fig. 1), a flavonoid isolated from chloroform extract of *Clerodendrum phlomidis* L. leaves, on total protein and esterase and GST enzymes activities in two lepidopteran pests, *E. vittella* and *H. armigera*.

Materials and methods

Collection, extraction, fractionation and isolation of pectolinaringenin Collection of plant materials, extraction of crude extracts, identification of active crude extract, fractionation and isolation of the compound pectolinaringenin have been described in our earlier reports (Muthu *et al.* 2012a,b).

Rearing of insects *Earias vittella* larvae were collected from Thandalam village near Thirupporur, Kancheepuram district, Tamil Nadu. They were reared until pupation in glass jars (21 cm x 15 cm) and fed with bhendi fruits (*Abelmoschus esculentus* L.) under laboratory conditions (27 ± 2°C and 75 ± 5% r.h.). After pupation, the pupae (cocoon) were collected and kept in different glass jars covered with white muslin cloth. After emergence of the adults (8–10 days), they were fed with 10% honey solution absorbed in cotton swabs inside 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, and in the neonate stage, the larvae were fed with tender leaves of bhendi; after that they were fed with bhendi fruit. A laboratory-reared culture was used for the experiment.

Helicoverpa armigera Larvae were collected from a farmer's field in Melakondayar village in the Tiruvallur district of Tamil Nadu and the collected larvae were reared individually in a plastic container (vials) and fed regularly with bhendi fruit until the larvae became pupae under the laboratory conditions. Sterilized soil was provided for pupation. After pupation, the pupae were collected from soil and placed inside a cage for emergence of adults. Cotton soaked with 10% honey solution mixed with a few drops of

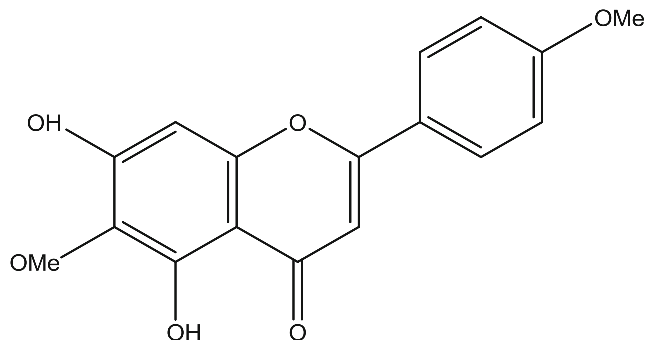

Fig. 1 Structure of pectolinaringenin (5,7-dihydroxy- 4′,6-dimethoxy-flavone) isolated from *Clerodendrum phlomidis* leaves

multivitamins was provided for adult feeding to increase fecundity. A potted cotton plant was kept inside the adult emergence cage for egg laying (Baskar & Ignacimuthu 2012). After hatching, the larvae were collected from the cage and fed with a standard artificial diet as recommended by Koul *et al.* (1997) and were used for the experiment.

Treatments Toxic effects of pectolinaringenin on protein content and detoxifying enzymes in the midgut and hemolymph of 3rd instar larvae of *E. vittella* and *H. armigera* were studied after 24 h of oral treatment using a fruit disc of *A. esculentus* for *E. vittella* and a cotton leaf disc for *H. armigera* under no-choice conditions. Fresh bhendi fruit discs (10 mm thickness) for *E. vittella* and fresh cotton leaves (4 cm diam) punched with a cork borer for *H. armigera* were used. The tested materials – bhendi fruit discs and cotton leaves – were dipped individually in pectolinaringenin at 12.5, 25, 50 and 100 ppm concentrations. Leaf discs and fruit discs dipped in acetone + Tween 80, used to dissolve the compound, were used as negative control. For comparative analysis, a reference control – azadirachtin (purity 40.86%), was used. Ten replicates were maintained for each concentration of the tested compound, reference compound and control.

Protein The total protein content in the hemolymph and midgut of *E. vittella* and *H. armigera* was estimated according to Bradford's method using bovine serum albumin as the standard (Bradford 1976).

Glutathione S-transferase GST activity was studied according to the method of Oppenoorth (1979). Enzyme activity was determined using chlorodinitrobenzene (CDNB) as a substrate. The reaction mixture contained 10 μl of diluted enzyme solution (the stock solution was diluted tenfold with 0.1 M, pH 7.6, sodium phosphate buffer), 90 μl of 0.1 M sodium phosphate buffer, 100 μl of 1.2 mM CDNB and 100 μl of 6 mM GSH. Optical density at 340 nm was recorded at intervals of 10 s for 5 min. Controls without the enzyme always accompanied each assay. Enzyme activity was expressed as mOD/sec/mg protein at 27°C, and the specific activity was calculated (Huang & Han 2007).

Esterase General esterase activity was determined using α-naphthyl acetate as the substrate following the procedure of Han *et al.* (1998). Fifty microliters of 0.1 M sodium phosphate buffer (pH 7.6) and 200 μl mixture solution containing 0.3 mM eserine, 10 mM α-naphthyl acetate solution and 4 mM fast blue RR salt were added into each sample. The reaction was initiated by addition of 20 μl of diluted enzyme solution (the stock solution was diluted tenfold with 0.1 M, pH 7.6, sodium phosphate buffer). Optical density at 450 nm was recorded at intervals of 30 s for 10 min. Controls without the enzyme always accompanied each assay. Enzyme activity was expressed as mOD/sec/mg protein at 27°C, and the specific activity was calculated (Huang & Han 2007).

Statistical analysis One-way ANOVA was used to analyze all the data. Significant differences between treatments were determined using Tukey's HST multiple range tests ($P \leq 0.05$). Homogeneity of Variances (Levene Statistic) was used to determine significant deviation. Analyses were performed with the original data. Distribution of the data exhibited significant deviations from normality (Shapiro-Wilk test). IC_{50} and IC_{90} values were calculated using probit analysis (Finney 1971). Statistical package SPSS version 11.5 was used for statistical analysis.

Results

Protein The effects of pectolinaringenin at 100, 50, 25 and 12.5 ppm concentrations on total protein content of *E. vittella* and *H. armigera* are presented in Table 1. Pectolinaringenin reduced the protein content of *E. vittella* and *H. armigera* in a dose-dependent manner. Maximum reductions of 174.98 mg ml^{-1} (55.75%) and 210.01 mg ml^{-1} (53.01%) of total protein content over control were recorded against *E. vittella* and *H. armigera*, at 100 ppm concentration. Total protein reduction was statistically significant at all the concentrations. Effective IC_{50} and IC_{90} values for total protein content were 74.37, 83.33 and 212.31, 186.15 ppm for *E. vittella* and *H. armigera,* respectively (Table 2). The chi-square values were significant for both the insects.

Enzyme activity The effect of different concentrations of pectolinaringenin on GST and esterase enzyme activities was studied against the larvae of *E. vittella* (Table 3). Compared with control, the isolated compound, pectolinaringenin, reduced GST and esterase enzyme activities; the activities were 0.146 and 0.143 OD/5

Table 1 Effect of pectolinaringenin on total protein content (mg ml^{-1}) and percent reduction in *Earias vittella* and *Helicoverpa armigera*

Treatment	Concentration (ppm)	Total protein (mg ml^{-1}) *E. vittella*	% Reduction	Total protein (mg ml^{-1}) *H. armigera*	% Reduction
Pectolinaringenin	12.5	252.64 ± 5.15^{e}	19.48 ± 2.18^{a}	350.84 ± 4.23^{g}	11.41 ± 1.49^{a}
	25	192.85 ± 4.38^{c}	38.54 ± 1.67^{c}	296.65 ± 5.27^{e}	25.10 ± 1.6^{c}
	50	164.91 ± 3.82^{b}	47.45 ± 1.23^{d}	216.9 ± 4.32^{c}	45.23 ± 1.50^{e}
	100	138.88 ± 4.74^{a}	55.75 ± 1.22^{e}	186.09 ± 4.02^{b}	53.01 ± 1.02^{f}
Azadirachtin^{z}	12.5	258.84 ± 2.82^{e}	17.51 ± 1.68^{a}	329.04 ± 2.83^{f}	16.92 ± 1.43^{b}
	25	213.65 ± 2.77^{d}	31.92 ± 1.17^{b}	272.25 ± 4.96^{d}	31.25 ± 1.72^{d}
	50	173.50 ± 4.09^{b}	44.72 ± 0.94^{d}	188.10 ± 3.78^{b}	52.50 ± 1.33^{f}
	100	130.08 ± 3.82^{a}	58.55 ± 4.04^{e}	154.28 ± 4.31^{a}	61.05 ± 0.99^{g}
Control	-	313.86 ± 3.92^{f}	-	396.11 ± 4.16^{h}	-
Homogeneity ^{y}		00	00	0.861	0.756

Values represent mean ± SD of five replicates

Within columns, values followed by the same letter do not differ significantly at $P \leq 0.05$

^{z} Purity 40.86%

^{y} Significant deviation for homogeneity is > 0.05%

min/larva, respectively, at 100 ppm concentration; the percentages of reduction over control were 37.53 and 43.09, respectively. The compound reduced the enzyme activity in a dose-dependent manner. A statistically significant reduction was observed at all the concentrations for both the enzymes. The effective concentration of pectolinaringenin to reduce GST and esterase activities is presented in Table 4. The IC_{50} and IC_{90} values for GST and esterase activity of *E. vittella* were 133.00, 111.76 ppm and 318.81, 262.96 ppm, respectively. The chi-square value for GST showed significant activity.

The compound reduced GST and esterase activities in *H. armigera*; the activities were 0.184 and 0.122 OD/5 min/larva, respectively, at 100 ppm concentration; the percentages of reduction over control were 43.14 and 47.42, respectively. The reduction of the enzyme activity by the compound was in a concentration-dependent manner. Statistically significant reduction over control was observed at all the concentrations (Table 5). Effective inhibitory concentrations of pectolinaringenin for GST and esterase activities of *H. armigera* are presented in Table 6. The IC_{50} and IC_{90} values for GST and esterase activities of *H. armigera* were 114.38, 98.78 and 272.06, 272.42 ppm, respectively.

Discussion

The present study revealed that the larvae treated with pectolinaringenin showed reduced total protein content in a dose-dependent manner against both the pests. It may be due to the interaction of the compound with the

Table 2 Inhibitory concentration of pectolinaringenin on total protein content in *Earias vittella* and *Helicoverpa armigera*

Treatment	IC_{50}	Fiducial limit		IC_{90}	Fiducial limit		$\chi^{2\ z}$
E. vittellla							
Pectolinaringenin	74.37	63.54	90.12	212.31	174.05	281.61	41.08^{z}
Azadirachtin^{y}	74.49	68.73	81.40	185.32	167.13	209.61	26.65
H. armigera							
Pectolinaringenin	83.33	72.79	98.64	186.15	157.02	235.10	54.82^{z}
Azadirachtin^{y}	67.37	59.00	78.02	168.62	144.33	207.52	48.60^{z}

^{z} Chi-square values are significant at 0.05%

^{y} Purity 40.86%

Table 3 Effect of different concentrations of pectolinaringenin on glutathione S-transferase and esterase enzyme activities in *Earias vittella*

Treatment	Concentration (ppm)	GST (OD/5 min/larva)	% Reduction	Esterase (OD/5 min/larva)	% Reduction
Pectolinaringenin	12.5	0.205 ± 0.002[e]	12.34 ± 1.96[b]	0.215 ± 0.002[e]	14.66 ± 1.77[a]
	25	0.167 ± 0.002[d]	28.47 ± 1.99[c]	0.187 ± 0.012[d]	25.81 ± 4.25[b]
	50	0.154 ± 0.002[c]	34.18 ± 2.14[d]	0.162 ± 0.005[c]	35.70 ± 2.44[c]
	100	0.146 ± 0.003[b]	37.53 ± 1.02[de]	0.143 ± 0.003[b]	43.09 ± 1.39[d]
Azadirachtin[z]	12.5	0.216 ± 0.005[f]	7.81 ± 2.39[a]	0.211 ± 0.007[e]	16.28 ± 2.33[a]
	25	0.164 ± 0.003[d]	29.93 ± 1.52[c]	0.176 ± 0.003[d]	30.07 ± 1.16[b]
	50	0.148 ± 0.004[bc]	36.67 ± 2.25[d]	0.151 ± 0.003[bc]	40.06 ± 1.76[cd]
	100	0.137 ± 0.002[a]	41.35 ± 2.08[e]	0.129 ± 0.005[a]	48.65 ± 1.65[e]
Control	-	0.234 ± 0.004[g]		0.252 ± 0.002[f]	
Homogeneity [y]		0.361	0.865	0.228	0.423

Values represent mean ± SD of five replicates

Within columns, values followed by a common letter do not differ significantly at $P \leq 0.05$

[z] Purity 40.86%

[y] Significant deviation for homogeneity is > 0.05%

hormones which are related to protein synthesis (Sieber & Rembold 1983). Senthilkumar & Murugan (1999) reported that azadirachtin at 2 ppm concentration reduced the total protein to 54.26 μg g^{-1} in *H. armigera*. Pectolinaringenin reduced the feeding and utilization of digested food. Diet containing pectolinaringenin reduced 55.75% of the total protein content against *E. vittella* and 53.2% in *H. armigera* at 100 ppm concentration and the reduction was dose-dependent. The present finding is in agreement with the earlier results of Rharrabe *et al.* (2007), who studied the effects of 20-hydroxyecdysone isolated from *Plypodium vulgare* on protein of *Plodia interpunctella* larvae at 50, 100, 200 and 500 ppm concentrations; they found that 20-hydroxyecdysone reduced the protein content (in a dose-dependent manner) when the larvae consumed the treated diet. Neoliya *et al.* (2007) evaluated the effect of azadirachtin from *A. indica* against the larvae of *H. armigera* and observed a significant reduction in protein. Limonoids, deacetylnimbin, 17-hydroxyazadiradione, gedunin, salannin and deacetyl-gedunin from *A. indica* were studied against ovary protein of *H. armigera* (Jayabalan & Murugan 1997). They found that all the compounds reduced the protein concentration

Enzyme activity In the present investigation, pectolinaringenin-treated larvae showed a reduction in GST and esterase activities. The compound reduced GST activity by 37.61% and esterase activity by 43.25% over control against *E. vittella*. In the case of *H. armigera*, the same pectolinaringenin reduced the GST and esterase activities by 43.21% and 47.41%, respectively, over control. The reduction was in a dose-dependent manner in both the pests. The present

Table 4 Inhibitory concentration (ppm) of pectolinaringenin for glutathione S-transferase and esterase enzyme activities in *Earias vittella*

Treatment	IC_{50}	Fiducial limit		IC_{90}	Fiducial limit		$\chi^{2\ z}$
GST							
Pectolinaringenin	133.00	105.91	194.53	318.81	239.16	510.06	42.39[z]
Azadirachtin[y]	111.14	88.86	161.93	253.19	190.39	411.70	80.11[z]
Esterase							
Pectolinaringenin	111.76	99.56	129.28	262.96	226.41	317.92	26.66
Azadirachtin [y]	94.96	85.67	107.56	236.09	206.03	279.79	27.80

[z] Chi-square values are significant at 0.05%

[y] Purity 40.86%

Table 5 Effect of pectolinaringenin on glutathione S-transferase and esterase enzyme activities in *Helicoverpa armigera*

Treatment	Concentration (ppm)	GST (OD/5 min/larva)	% Reduction	Esterase (OD/5 min/larva)	% Reduction
Pectolinaringenin	12.5	0.275 ± 0.008[g]	15.10 ± 3.06[a]	0.185 ± 0.005[e]	20.07 ± 3.72[a]
	25	0.235 ± 0.004[e]	27.33 ± 2.07[b]	0.155 ± 0.002[d]	33.00 ± 2.89[b]
	50	0.215 ± 0.002[cd]	33.64 ± 0.66[cd]	0.136 ± 0.002[c]	41.39 ± 1.35[c]
	100	0.184 ± 0.004[b]	43.14 ± 1.32[e]	0.122 ± 0.005[b]	47.42 ± 1.40[d]
Azadirachtin[z]	12.5	0.264 ± 0.007[f]	18.45 ± 2.18[a]	0.179 ± 0.004[e]	22.86 ± 2.61[a]
	25	0.222 ± 0.005[d]	31.41 ± 1.71[c]	0.145 ± 0.003[c]	37.40 ± 2.68[bc]
	50	0.206 ± 0.003[c]	36.41 ± 0.96[d]	0.122 ± 0.003[b]	47.43 ± 1.51[d]
	100	0.169 ± 0.003[a]	47.70 ± 1.47[f]	0.108 ± 0.008[a]	53.60 ± 2.31[e]
Control	-	0.324 ± 0.003[h]		0.232 ± 0.007[f]	
Homogeneity[y]		0.389	0.143	0.052	0.450

Values represent mean ± SD of five replicates

Within columns, values followed by a common letter do not differ significantly at $P \leq 0.05$

[z] Purity 40.86%

[y] Significant deviation for homogeneity is >0.05%

findings corroborated the findings of Mukanganyama *et al.* (2003), who investigated the effect of DIMBOA (2,4-dihydroxy-7-methoxy-1,4-benzoxazin-3-one) isolated from *Triticum aestivum* on GST and esterase of *Rhopalosiphum padi* at 0.5, 1, 2 and 4 mM concentrations. The insects fed with treated diet exhibited decreased GST activity *in vitro* and *in vivo* by 33% and 30%, respectively,, and also reduced esterase activity by 50% and 75%, respectively. The effect of cyclodepsipeptidic mycotoxin, destruxin from *Metarhizium anisopliae* on glutathione and oxidized glutathione in larvae of *S. litura* was studied by Sree & Padmaja (2008); they observed that after 24 and 48 h of treatment, the levels of glutathione and oxidized glutathione were decreased in direct proportion to concentration. The larvae treated with Celangulin V isolated from the Chinese plant *Celastrus augulatus* reduced the esterase activity of *M. separata* by 7% (Lu *et al.* 2008). Azadirachtin from *A. indica* inhibited the enzyme activity of acetylcholinesterase in *Nilaparvata lugens* at concentrations of 0.10, 0.25 and 0.50 ppm, in a dose-dependent manner (Senthil Nathan *et al.* 2008).

Sosa *et al.* (2000) investigated 20 flavonoids isolated from Argentina native plants and some others purchased commercially against *T. molitor* larval growth. Quercetin was the most effective growth inhibitor for *T. molitor* larvae and it decreased the GST activity of nymphs of *Triatoma infestans*. Sintim *et al.* (2009) observed that *Sesamum indicum* extract reduced the GST activity of 1st and 2nd instar larvae of *S. litura.*

Thiboldeaux *et al.* (1998) reported the effects of 1,4-naphthoquinones on mid gut glutathione levels of *Actias luna* and *Callosamia* larvae at 0.05% and 0.5% concentrations. After 3 days mid gut total glutathione decreased in *A. luna*. Bullangpoti *et al.* (2007) studied the effect of

Table 6 Inhibitory concentration of pectolinaringenin on glutathione S-transferase and esterase enzyme activities in *Helicoverpa armigera*

Treatment	IC_{50}	Fiducial limit		IC_{90}	Fiducial limit		χ^{2} [z]
GST							
Pectolinaringenin	114.38	101.34	133.47	272.06	232.67	332.36	20.64
Azadirachtin[y]	101.56	90.34	117.64	260.69	223.30	317.75	16.96
Esterase							
Pectolinaringenin	98.78	87.12	115.95	272.42	230.25	339.25	26.35
Azadirachtin[y]	78.25	67.16	94.75	237.37	194.28	314.55	29.88[z]

[z] Chi-square values are significant at 0.05%

[y] Purity 40.86%

magnostin from the fruit extract of *Garcinia mangostana* against the brown planthopper *Nilaparvata lugens*. They observed that magnostin reduced the detoxifying enzyme level of GST by 1.01–3.34-fold. Rambutan's seed extracts from *Nephilium lappaceum* and mangosteen's peel extracts from *Garcinia mangostana* reduced esterase and GST activities on rice weevil (Bullangpoti *et al.* 2004).

Pectolinaringenin significantly reduced the total protein content, esterase and GST enzyme activities of *E. vittella* and *H. armigera* in this study. Renuga & Sahayaraj (2009) reported that plant-derived substances reduced the head protein content of *Spodoptera litura*. Many investigations demonstrated the effect of botanicals on different parameters such as feeding of insects, digestibility of consumed food, conversion efficiency of the ingested food, conversion efficiency of digested food and consumption index (Shekari *et al*. 2008). Inhibitory effects of botanical insecticides on digestive enzymes have been illustrated by Zibaee & Bandani (2010a). Plant-derived substances induced the expression of esterase activity in the early stages of the insects and reduced it in the later stages due to their toxicity (Zibaee & Bandani 2010b).

Conclusion In the present investigation, pectolinaringenin isolated from *C. phlomidis,* reduced the total protein content, esterase and GST enzyme activities. Reductions in protein and enzyme activities affect the growth and survival of *E. vittella* and *H. armigera* larvae. It is concluded that pectolinaringenin could be used to develop an effective botanical insecticidal formulation for the management of important agricultural pests.

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