International Journal of Chemical Studies

P-ISSN: 2349–8528
E-ISSN: 2321–4902
IJCS 2019; 7(4): 980-986

Received: 01-05-2019
Accepted: 05-06-2019

S Velmani
Department of Agricultural Entomology, Agricultural College and Research Institute, Tamil Nadu Agricultural University (TNAU), Madurai, Tamil Nadu, India

M Shanthi
Department of Agricultural Entomology, Agricultural College and Research Institute, Tamil Nadu Agricultural University (TNAU), Madurai, Tamil Nadu, India

C Chinniah
Department of Agricultural Entomology, Agricultural College and Research Institute, Tamil Nadu Agricultural University (TNAU), Madurai, Tamil Nadu, India

S Vellaikumar
Department of Biotechnology, Agricultural College and Research Institute, Tamil Nadu Agricultural University (TNAU), Madurai, Tamil Nadu, India

Correspondence
M Shanthi
Department of Agricultural Entomology, Agricultural College and Research Institute, Tamil Nadu Agricultural University (TNAU), Madurai, Tamil Nadu, India

Antimetabolic effect on *Spodoptera litura* due to acute feeding of *Adenanthera pavonina* proteinase inhibitor

S Velmani, M Shanthi, C Chinniah and S Vellaikumar

Abstract
Antimetabolic effect of purified proteinase inhibitor (F_{30-60}) isolated from *Adenanthera pavonina* (ApPI F_{30-60}) was evaluated on *Spodoptera litura* larva, by feeding five days old larva on the ApPI F_{30-60}contaminated diet at 0.25, 0.50 and 1.00% for acute period (5 days) and later fed with untreated diet. The parameters of growth and development of *S. litura* were closely monitored throughout its life period. The results indicated that the ApPI 1.0% had significant influence on the reduction of *S. litura* larval weight(41.58%) pupal and adult weight (38.42 and 44.42%, respectively), prolonged the larval and pupal developmental period by 7.2 days and 3.0 days, respectively reduced the adult life span by 4.5 days. Only 60 per cent of the treated larva were pupated, 30 per cent of them were malformed as pupal-adult intermediates. Hence only 30 per cent were emerged as adult but they did not lay eggs. Acute feeding of ApPI 1% significantly interfered in the growth and development of *S. litura*. Hence, it is concluded that purified *A. pavonina* proteinase inhibitor (ApPI) is a potential and promising inhibitor of both chymotrypsin and trypsin in *S. litura*, which can be effectively exploited as bio insecticidal tool against this notorious polyphagous pest on a range of field and horticultural crops.

Keywords: *Adenanthera pavonina*, *Spodoptera litura*, proteinase inhibitor, antimetabolic effect

Introduction
Protease inhibitors (PIs) are one class of plant defense proteins against insect pest infestation. Proteinase inhibitors derived from the plants are ability to inactivate proteases of animals and microbial origin while rarely, inhibiting endogenous digestive enzymes of insects' herbivore. These defensive proteinase inhibitors were identified, isolated and characterized from many plant families like Leguminosae, Solanaceae, Gramineae etc. (Richardson, 1991) [27]. The insecticidal property of many of these isolated proteinase inhibitors were studied both *in vitro* and *in vivo* conditions against several insect pests including the most terrible polyphagous pests like *Helicoverpa armigera* (Hub.), *Spodoptera litura* Fab., etc. (Macedo *et al.*, 2010; Ghodke *et al.*, 2013; Riseh *et al.*, 2014; Gandreddi *et al.*, 2015) [20, 13, 28, 12].

S. litura commonly known as tobacco cut worm is a devastating polyphagous pest of around 120 host plants including important agricultural and horticultural crops. The damage potential of this pest varies from 12 to 100 per cent, depending upon the crop and season. The control of this pest mainly depends on chemical insecticides, which leads to the development of resistant population against insecticides (Armes *et al.*, 1997; Kranthi *et al.*, 2002; Ahmad *et al.*, 2007) [2, 18, 1] and high usage of insecticides causing serious environmental issues.

So there is a need to look for an alternate pest control tactics with environmental friendly nature. Insect growth and development mainly depends on the protein availability (Boggs, 2009; Lee, 2010) [5, 19]. The dietary proteins ingested by the insects mainly in the polypeptide form. It should be converted to simple amino acids for efficient utilization. These protease enzymes also have some other important role in insects other than protein digestion like embryonic development, zymogen activation etc. (Raikhel and Dhadialla, 1992) [26]. So, targeting these protease enzymes using plant proteinase inhibitors is one of the best pest control method for *S. litura* by ecofriendly nature.

However many plants have proteinase inhibitors, insects have developed an ability to overcome these inhibitors if it is from their host plants (Waghmare and Kamble, 2018) [37]. So, it is necessary to find the promising proteinase inhibitor from the non-host plant of the targeting insects. One of the promising proteinase inhibitors with high trypsin and

Chymotrypsin inhibitory units was isolated and characterized from the seeds of red lucky seed, *Adenanthera pavonina* L. (ApPI) and its insecticidal effect was tested on some insects (Sasaki *et al.*, 2015; de Souza *et al.*, 2016; Chandrashekharaiah *et al.*, 2017) [29, 8, 7]. This ApPI is acting as the non-host plant proteinase inhibitor for *S. litura.* Even though there are many studies expressing the effect of purified inhibitor on some insects, its effect on the growth and development of dangerous polyphagous pest *S. litura* is very minimum. Hence, this project was taken up to evaluate the antimetabolic effect of *Adenanthera pavonina* proteinase inhibitor on *S. litura.*

Materials and Methods

The seeds of *A. pavonina* were collected from Agricultural College and Research Institute, Madurai. Dialysis tube (LA 393-10 MT having 12,000-14,000 molecular weight cut off) from M/s. Hi-media chemicals and other chemicals used for analysis were analytical grade (AR), purchased through TNAU rate contract.

Isolation and purification of proteinase inhibitor from *A. pavonina*

The target proteinase inhibitor (ApPI) was isolated and purified from the seeds of *A. pavonina* in the NADP sponsored Central Instrumentation Laboratory, Agricultural College and Research Institute, Madurai, as detailed below. Crude protein extract was prepared from 1kg of seed powder (30 mesh size) in ice cold condition. The seed powder was defatted and depigmentated using acetone (1:3 (w/v) ratio) and n-hexane (1:2 (w/v) ratio), respectively and filtered through Whatman No. 1 filter paper, using Buchner funnel under vacuum and dried in 4˚C for overnight. The defatted powder was mixed with extraction buffer, 0.01M sodium phosphate buffer containing 0.15M NaCl pH 7.2 (at 1:10 ratio) and stirred for 6 hours in magnetic stirrer (REMI – 2MLH), in ice cold condition, after adding 1% Poly Vinyl Pyrrolidone (PVP). The resulting suspension was centrifuged (Dynamica velocity 14 R) at 10,000 rpm for 30 minutes (4˚C), and the supernatant was filtered through two to three layers of cheese cloth, in order to obtain the crude extract (Maggo *et al*., 1999) [22].The protein in the crude extract was partially purified by ammonium sulfate precipitation (at the range of 0 - 90%) and fraction 30-60 (F_{30-60}) was taken for this study and it was dialyzed, against the extraction buffer, in order to obtain inhibitor rich fraction, which was lyophilized (SCANVAC COOL SAFE 55-4)and stored at –20˚C, until further study.

Mass culturing of test insect

The initial culture of the test insect, *S. litura*, egg mass was purchased from National Bureau of Agricultural Insect Resource (NBAIR), Bangalore and the accession number was NBAII-MP-NOC-02. The mass culturing was done using the semi-synthetic diet, as per the NBAII recommendation, in the Insectary, Department of Agricultural Entomology, Agricultural College and Research Institute, Madurai. The rearing tray and other materials used for rearing purpose was disinfected with 0.1% formaldehyde solution. The diet composition is given below.

Fraction 'A' of the diet was mixed thoroughly in a blender with 390 ml of water for two minutes. Another 390 ml water was added with fraction 'B' and boiled. Then the Fractions 'A' and 'B' was mixed in the blender for one minute. Finally, fraction 'C' were added to the admixture of 'A' and 'B' and mixed for one minute. At the end 1ml of 10% formaldehyde solution were added and thoroughly mixed. Then the diet was poured into the pre sterilized container. It was stored in refrigerator condition upto 15 days.

The larvae emerged from the egg mass was released using camel hair brush into the container having semi-synthetic diet. Initially, the larvae were reared in bulk, third instar onwards they were transferred to the sterilized multi cavity tray. Every day the diet was changed with clean tray containing fresh food. When the larvae attained the pre pupal stage, they were transferred to the tray containing sawdust for pupation. Adult emerged from the pupa were transferred to the oviposition cage (56.5 x 60 x 56.5cm) and 10% honey solution containing 2-3 drops of vitamin E solution, was given as adult feed. The castor leaves were used as substrate for collection of egg mass. The egg mass was used for bioassay (90%) and for mass culturing (10%).

Table: Composition of semi-synthetic diet of *S. litura*

Item	Quantity
Fraction A	
Chickpea (Kabuligram) flour	105 g
Methyl para- hydroxyl benzoate	2 g
Sorbic acid	1 g
Streptomycin sulphate	0.25 g
Fraction B	
Agar-agar	12.75 g
Fraction C	
Yeast	40 g
Ascorbic acid	3.25 g
Multivitamin	2 capsules
Vitamin E	1 gm
Formaldehyde 10%	2 ml
Distilled water	780 ml

In vivo evaluation of ApPI F30-60 on *S. litura*

Antimetabolic effect of *A. pavonina* proteinase inhibitor (ApPI – F_{30-60}) was evaluated on *S. litura*, by feeding the five days larva with inhibitor treated artificial diet, in comparison with untreated diet. Purified inhibitor isolated from ammonium sulphate saturation F_{30-60} was lyophilized and incorporated through diet at different concentration *viz*., 0.25%, 0.5% and 1%.The inhibitor was added with the ingredients of fraction A, then the fraction B was added after cooing to 60ºC and then fraction C was added as per normal diet preparation and diet without inhibitor served as control.

Experiment was conducted to test the acute toxicity of the $ApPIF_{30-60}$ on *S. litura*. The acute toxicity was evaluated by feeding the contaminated diet to five days old larva for five days, later were fed with untreated diet. Five days old *S. litura* larva 15 numbers were used for each treatment. Each replication consisted of three larva and totally five replications were maintained. The experiment was conducted under Completely Randomized Design (CRD).

Daily larval weight (Till all the larvae in control pupate), developmental period of the larva, pupal weight, pupal period, adult weight, adult life span and fecundity were recorded. Per cent pupation and adult emergence was calculated based on the total larvae used in the treatment. Percent oviposition was estimated based on the following formula (Singh *et al.*, 2014) [32].

$$\text{Oviposition}(\%) = \frac{\text{Number of eggs laid per female in treated}}{\text{Number of eggs laid per female in control}} \text{ x } 100$$

Using the above data, the growth indices *viz*., Larval-pupal index, Pupal weight index, Adult weight index (Deshmukh *et al.*, 1977) [9], Adult emergence index (Tripathi *et al.*, 1982) [35], Development index (Prasad and Bhattacharya, 1975) [24], Larval growth index (Sharma *et al.*, 1982) [30], Howe's growth index (Howe, 1971) [15], Suitability index and Antibiosis index (Dhillon *et al.*, 2005) [10] were estimated.

$$\text{Larval pupal index} = \frac{\text{Average larval period on control diet (days)} + \text{Average pupal period on control diet (days)}}{\text{Average larval period on treated diet (days)} + \text{Average pupal period on treated diet (days)}}$$

$$\text{Pupal weight index} = \frac{\text{Average pupal weight on treated diet (mg)}}{\text{Average pupal weight on control diet (mg)}}$$

$$\text{Adult weight index} = \frac{\text{Average adult weight on treated diet (mg)}}{\text{Average adult weight on control diet (mg)}}$$

$$\text{Adult emergence index} = \frac{\text{Average emergence (\%) on treated diet}}{\text{Average emergence (\%) on control diet}}$$

$$\text{Developmnetal index} = \frac{\text{Average developmental period on treated diet (days)}}{\text{Average developmental period on control diet (days)}}$$

$$\text{Larval growth index} = \frac{\text{Pupation (\%)}}{\text{Average larval period (days)}}$$

$$\text{Howe's growth index} = \frac{\text{Log adult emergence (\%)}}{\text{Average developmental period (days)}}$$

$$\text{Suitability index} = \frac{\text{Sum of all indices on treated diet}}{7}$$

$$\text{Antibiosis index} = \text{Larval pupal index} + \text{Pupal weight index} + \text{Adult weight index} + \text{Adult emergence index} + \text{Devolpmental index} + \text{Larval growth index} + \text{Howe's growth index}$$

Results and Discussion

The results of the study on the anti-metabolic effect of purified ApPI-F_{30-60} on *S. litura* through acute feeding are presented in Table 1 to 5. The ApPI F30-60 inhibited the growth and development of larvae at all stages when compared to control.

Table 1: Impact of purified ApPI-F_{30-60} on larval weight of *S. litura* when fed on treated diet for five days from 5th day

Concentration of purified ApPI F_{30-60}	Mean fresh weight of the larvae (mg)														
	D5	D6	D7	D8	D9	D10	D11	D12	D13	D14	D15	D16	D17	D18	D19
0.25 %	2.66±0.72 (1.61)^{a}	5.7± 1.51 (2.36)^{b}	8.84±0.36 (2.97)^{c}	11.74± 3.54 (3.41)^{b}	27.35± 6.72 (5.19)^{b}	41.39± 7.31 (6.37)^{bc}	46.78±12.73 (6.76)^{b}	109.33±19.97 (10.30)^{bc}	158.37±38.57 (12.39)^{b}	241.96±17.32 (15.54)^{bc}	337.11±91.36 (18.18)^{bc}	426.03±64.27 (22.81)^{b}	525.29±90.35 (23.81)^{b}	601.59±99.14 (21.48)^{b}	684.07±71.03 (26.15)^{b}
0.5%	2.44±0.44 (1.55)^{a}	4.71±0.77 (2.36)^{b}	5.72±0.45 (2.38)^{b}	11.69± 1.24 (3.39)^{b}	21.12± 3.67 (4.58)^{b}	38.47± 5.99 (6.18)^{b}	45.97±6.93 (6.73)^{b}	89.46±17.12 (9.42)^{ab}	145.28±16.73 (12.03)^{b}	155.30±34.52 (12.39)^{ab}	273.61±57.10 (16.46)^{ab}	401.73±31.87 (23.26)^{b}	550.20±96.33 (22.81)^{b}	532.84±98.79 (22.96)^{ab}	560.75±101.54 (23.55)^{ab}
1%	2.63±0.51 (1.61)^{a}	2.87±0.60 (1.68)^{a}	2.97±0.54 (1.63)^{a}	7.29±1.19 (2.69)^{a}	11.25± 2.66 (3.33)^{a}	15.15± 3.25 (3.87)^{a}	29.60±6.12 (5.41)^{a}	68.91±23.74 (8.20)^{a}	112.94±30.56 (10.55)^{a}	152.05±54.48 (12.05)^{a}	253.10±73.90 (15.77)^{a}	332.80±52.49 (21.47)^{a}	462.45±55.88 (21.47)^{a}	481.11±77.40 (21.87)^{a}	541.15±98.52 (23.07)^{a}
Control	2.23±0.26 (1.49)^{a}	7.77±0.50 (2.78)^{c}	12.15± 1.50 (3.47)^{d}	16.92± 2.10 (4.10)^{c}	36.37± 2.73 (6.02)^{c}	51.95± 4.26 (7.20)^{c}	71.54±11.42 (8.43)^{d}	125.70±13.45 (11.19)^{c}	179.61±18.84 (13.38)^{c}	306.18±23.10 (17.48)^{c}	376.13±31.67 (19.37)^{c}	531.76±44.90 (26.26)^{c}	721.56±95.48 (26.81)^{c}	813.63±50.49 (28.51)^{c}	985.92±132.39 (31.39)^{c}
Mean	2.49	5.26	7.42	11.91	24.02	36.74	48.47	98.35	149.05	213.87	309.99	423.08	564.88	607.29	692.97
S.Ed	0.1070	0.1280	0.0828	0.1974	0.2926	0.3969	0.5181	0.8732	0.9862	1.0700	1.4721	1.1200	1.4233	1.2585	1.2845

Mean values of five replications are represented as mean ± standard deviation; figures in the parentheses are square root transformed values. The mean followed by the same letter are not significantly different from each other, DMRT ($P \leq 0.05$).SEd: Standard Error of the difference, D: day

Table 2: Impact of purified ApPI-F_{30-60} on pupal and adult weight of *S. litura* when fed on treated diet for five days from 5th day

Concentration of purified ApPI F_{30-60}	Pupal weight*# (mg) (n=10)	Adult weight*# (mg) (n=5)
0.25%	312.00 ± 4.57 (2.49)^{c}	175.30 ± 6.24 (2.24)^{c}
0.50%	270.93 ± 13..02 (2.43)^{b}	157.78 ± 8.14 (2.19)^{b}
1.00%	224.70 ± 13.03 (2.35)^{a}	123.70 ± 6.97 (2.09)^{a}
Control	364.90 ± 5.03 (2.56)^{d}	222.60 ± 8.89 (2.34)^{d}
Mean	293.13	169.84
SEd	0.0107	0.0129

*Mean values of five replications are represented as mean ± standard deviation
#Figures in the parentheses are log transformed values.
The mean followed by the same letter are not significantly different from each other, DMRT ($P \leq 0.05$). SEd: Standard Error of the difference.

Table 3: Impact of purified ApPI-F_{30-60} on developmental period (days) of *S. litura* when fed on treated diet for five days from 5th day

Concentration of purified ApPI F_{30-60}	Average larval period (days)*# (n=10)	Average pupal period (days)*# (n=5)	Average adult longevity(days)*# (n=5)
0.25%	20.5 ± 1.00 (4.52)^{c}	6.80 ± 0.83 (2.60)^{bc}	5.25 ± 1.48 (2.25)^{cd}
0.50%	22.8 ± 0.44 (4.77)^{b}	7.80 ± 1.30 (2.78)^{ab}	4.40 ± 2.30 (2.04)^{bc}
1.00%	24.3 ± 0.44 (4.92)^{a}	8.60 ± 1.14 (2.92)^{a}	3.00 ± 1.30 (1.64)^{a}
Control	17.1 ± 0.96 (4.13)^{d}	5.60 ± 0.54 (2.36)^{c}	7.50 ± 1.14 (2.71)^{d}
Mean	21.175	7.20	5.03
SEd	0.0547	0.1159	0.2400

*Mean values of five replications are represented as mean ± standard deviation
#Figures in the parentheses are square root transformed values.
The mean followed by the same letter are not significantly different from each other, DMRT ($P \leq 0.05$). SEd: Standard Error of the difference.

Table 4: Impact of purified ApPI-F_{30-60}on pupation (%), adult emergence (%), fecundity of *S. litura* when fed on treated diet for five days from 5th day

Concentration of purified ApPI F_{30-60}	Mortality of larva (%)	Larval-pupal intermediates (%)	Pupation (%)	Pupal-adult malformation (%)	Adult emergence (%)	Fecundity (mean) (n=3)	Oviposition (%)
0.25%	20	-	80	10	80	128	50.39
0.50%	26.67	6.67	80	20	75	59	23.22
1.00%	33.34	13.33	60	30	60	NL	-
Control	-	-	100	-	100	254	-
Mean	26.67	10	80	20	78.75	-	-

NL: not laid.

Table 5: Impact of purified ApPI-F_{30-60} on growth index of *S. litura* when fed on treated diet for five days from 5th day

Concentration of purified ApPI F_{30-60}	Larval Growth index	Larval-Pupal index	Pupal Weight index	Adult Emergence index	Adult Weight index	Howe's Growth index	Developmental index	Suitability index	Antibiosis index
0.25%	3.9024	0.8315	0.8550	0.80	0.7875	0.0512	1.0575	1.1836	8.2852
0.50%	3.5088	0.7418	0.7425	0.75	0.7088	0.0480	1.1122	1.0874	7.6121
1.00%	2.4691	0.7281	0.6158	0.60	0.5557	0.0418	1.2109	0.8888	6.2214
Control	5.4054	-	-	-	-	0.0569	-	-	-
Mean	3.8214	0.7578	0.7378	0.72	0.6840	0.0495	1.1269	1.0533	7.3729

Impact of ApPI F_{30-60} on larval weight gain

When the larvae were fed on artificial diet without the proteinase inhibitor, the larval weight was ranged from 2.23 to 985.92 mg, at different stages. The larval weight was minimum when the larvae were fed with ApPI 1.00% concentration, and the weight gain of larvae was slow from 2.63 to 541.15 mg, it was not comparable with untreated control. While comparing the larval weight of untreated and ApPI F_{30-60} 1% treated larva on 19th day (the last day of larval stage in untreated), there was 45.11 per cent reduction in larval weight gain. The weight of the larvae exposed to ApPI 1% and ApPI 0.50% were statistically on par during day 12, 14, 15, 18 and 19.Likewise, the weight of larvae fed on ApPI at 0.25% and 0.50% was statistically similar from day 8 till 19th day. Generally, the data showed that treatment with ApPI1.00% concentration cause retardation of the larval growth throughout the duration of larva (Table 1).

These results were agreement with several studies done by various scientists. Larval weight of Maducasexta was reduced due to the treatment of inhibitor in the diet (Shukle and Murdock, 1983) [31]. The lower weights can be observed with the higher concentration of SBTI (0.5 %), and also the effect influenced on treated diet upto day 12, with 0.2 and 0.5 per cent concentration (McManus and Burgess, 1995) [23].Comparable result was reported on *H. zea* and *S. exigua* due to action of soybean trypsin inhibitor (Broadway and Duffey, 1986), on *S. litura* by bitter gourd proteinase inhibitor (Telang *et al.*, 2003) [34], Kunitz inhibitor on the larvae of *C. maculatus* (Macedo *et al.*, 2004) [21] and soybean trypsin inhibitor on larvae of *S. litura* (Dorrah, 2004) [11].

Impact of ApPI F_{30-60} on pupal and adult weight gain

With regard to pupal and adult weight of *S. litura*, there was significant difference among the various treatments studied. Among the different treatments the ApPI 1.00% contaminated diet showed best results with reduced pupal weight of 224.70 mg followed by the ApPI 0.50% contaminated diet with the average pupal weight of 270.93 mg. While comparing the control (364.90 mg), even the lowest concentration of ApPI 0.25% contaminated diet also showed significant difference

(312.00 mg). Reduction in pupal weight in ApPI 1% treated diet over control was amounting to 38.42 per cent.

In adult weight, there was pronounced different among the all the treatments. The treatment with higher concentration of ApPI1.00%, reduced the adult weight drastically (123.70 mg) followed by the next concentration ApPI 0.50% with decreased weight of 157.78 mg. Whereas, the adult weight in control was 222.60 mg. The per cent reduction in adult weight over control was 44.42, 29.11 and 21.25 per cent in ApPI 1%, 0.5% and 0.25% treated diet. The results revealed that ApPI1.00% concentration was considered as the best treatment in reducing the adult weight (Table 2).

Telang *et al.* (2003) [34] reported that the pupal weight of *S. litura* was reduced, with the increase in the concentration of the BGPI inhibitor, in a dose dependent manner. The pupal weight was reduced in *H. armigera* with the higher concentration of chick pea inhibitor (Kansal *et al.*, 2008) [17]. Significant reduction of pupal weight was observed at higher concentrations of subabul trypsin inhibitor in *S. litura* (Vasudev and Sohal, 2015) [36]. The report of Rahbé *et al.* (2003) [25] the cystatin from rice seeds, showed reduction in fecundity and weight of the adult in *Myzus persicae*.

Impact of ApPI F_{30-60} on mortality and malformations

In acute feeding, the larval mortality was 20.0, 26.67 and 33.34 per cent, due to ApPI 0.25, 0.50 and 1.0% treated diet, respectively. Similarly, there were larval-pupal intermediates in the 0.50 and 1.00% concentration due to acute exposure (6.67 and 13.33%, respectively) while; the control did not exhibit any larval mortality and malformations (Table 3). Pupal-adult malformation was observed (20%) when the larvae were fed with ApPI in five days.

These findings are supported the evidences given below. The adults were malformed when fed with the bitter gourd PI treated diet in *S. litura* (Telang *et al.*, 2003) [34]. The malformations were due to lack of protein during metamorphosis, as some proteins are necessary at this stage. Ten to twenty percent of malformations in both pupal and adult stages were observed in *H. armigera*. In *H. armigera*, the over stress on gut and starvation of insects led to secrete comparatively higher amount of new proteinases, led to reduction in growth and mortality (Babu *et al.*, 2012) [3].

Pupation was also affected by the ApPI, when treated in the larval stage. Only 60 per cent of the larvae pupate when the larvae were fed with the higher concentration of ApPI (1%). And also observed few pupal malformations in acute feeding. There was 30 per cent pupal-adult malformation in 1.00% concentration followed by 0.50 and 0.25% which showed 20 and 10 per cent, respectively due to acute feeding.

In the present study, the adult emergence was reduced to 30 per cent in acute feeding in higher concentration (1%) of ApPI followed by the 0.5% and 0.25% concentration, while in control 100 per cent adult emergence was recorded Where as in treatments, the emerged adults were not healthy and showed malformations. Even when they emerged normally, they did not able to lay eggs. Whereas in the 0.50% and 0.25% concentration exhibited 80 and 75 per cent adult emergence and the reduction in fecundity over control was 50.39 and 23.22 per cent, respectively, in acute feeding experiment (Table 4).

These results are supported by the findings of Kansal *et al.* (2008) [17] and Macedo *et al.* (2010) [20]. When chickpea PI was fed along with diet to the larvae of *H. armigera*, there was reduction in the adult emergence (Kansal *et al.*, 2008) [17]. When *E. kuehniella* fed with ApTI treated diet at 1.0 per cent, there was only 28 per cent adult emergence, which decreased about 62 per cent of survival, but there was increase in survival with decrease in the concentration (Macedo *et al.*, 2010) [20].

Impact of ApPI F_{30-60} on growth indices

The larval growth index was more in the control (5.4054), which reveals that the food was preferably accepted by the larva to complete its larval stage, whereas it was not acceptable in the treatment of 1.00% concentration (2.4691) and 0.50% concentration (3.5088) treatments (Table 5). It shows that the larval growth was affected when treated diet was fed to *S. litura* larva. At ApPI at 1.00% concentration, they exhibited the lowest larval-pupal index (0.7281), adult emergence index (0.60) and Howe's growth index (0.0418). All indices recorded were very low in the 1.00% concentration followed by the 0.50 and 0.25% concentrations.

The data exposed that the ApPI 1.00% treated diet was not acceptable or suitable for the growth and development of *S. litura*. Whereas, larvae fed on the lower concentration such as 0.50 and 0.25% concentration of treated diet had negative influence on the pupa and adult weight index when compared to control. The highest developmental index revealed that it affected the duration of all the life stages. The suitability index was low (0.888), when fed on ApPI 1% treated diets, it indicates that they did not support the growth and development of larvae; it was substantiated by the lowest antibiotic index.

Same results obtained by few others while studying the proteinase inhibitors. The decreased pupal weight of inhibitor fed larvae showed decreased reproduction capacity, because, a direct relationship was found between reproductive potential and weight of the adult (Tammaru *et al.*, 1996) [33]. It was observed in winged bean proteinase inhibitor on fecundity of *H. armigera* (Gupta *et al.*, 2002) [14]. The adults emerged from inhibitor fed diet showed reduced fertility and fecundity, the eggs in control was 790 eggs per female, whereas it was 260 eggs in bitter gourd inhibitor fed diet of *S. litura* (Telang *et al.*, 2003) [34]. Babu and Subrahmanyam (2010) [4] reported that *in vivo* study of AsPIs (*Acacia Senegal* proteinase inhibitors) against the larvae of *H. armigera*, the AsPI caused reduction in larval growth and development and in adult stage it also affected the egg laying capacity. This negative effect was also observed in *Brassica oleracea* partially purified proteinase inhibitor on *S. litura* (Vasudev and Sohal, 2015) [36].The eggs laid by female was 593.73 eggs in 200 µg per ml, whereas it was 939.20 in control. Proteins plays major role in every stage and every metabolism, any change in uptake of protein at larval stage, it directly affected fecundity in higher concentration proteinase inhibitor (Vasudev and Sohal, 2015) [36]. Jadhav *et al.* (2016) [16] stated that *C. partellus* fecundity was decreased by 50 to 80%, when they are treated with Can PIs contaminated diet.

It is concluded that when the *S. litura* were fed to five days old larvae with ApPI F_{30-60} treated diet at 1% concentration for five days, it reduced the weight gain in larval, pupal and adult stage, prolonged the larval and pupal period, reduced adult life span, no fecundity and affected the growth indices.

References

1. Ahmad M, Arif MI, Ahmad M. Occurrence of insecticide resistance in field populations of *Spodoptera litura* (Lepidoptera: Noctuidae) in Pakistan. Crop Protection. 2007; 26(6):809-17.

2. Armes NJ, Wightman JA, Jadhav DR, Ranga Rao GV. Status of insecticide resistance in *Spodoptera litura* in Andhra Pradesh, India. Pesticide Science. 1997; 50(3):240-8.
3. Babu SR, Subrahmanyam B, Santha IM. *In vivo* and *in vitro* effect of *Acacia nilotica* seed proteinase inhibitors on *Helicoverpa armigera* (Hübner) larvae. Journal of biosciences. 2012; 37(2):269-76.
4. Babu SR, Subrahmanyam B. Bio-potency of serine proteinase inhibitors from *Acacia senegal* seeds on digestive proteinases, larval growth and development of *Helicoverpa armigera* (Hübner). Pesticide Biochemistry and Physiology. 2010; 98(3):349-58.
5. Boggs CL. Understanding insect life histories and senescence through a resource allocation lens. Functional Ecology. 2009; 23(1):27-37.
6. Broadway RM, Duffey SS. Plant proteinase inhibitors: mechanism of action and effect on the growth and digestive physiology of larval *Heliothis zea* and *Spodoptera exigua*. Journal of Insect Physiology. 1986; 32(10):827-33.
7. Chandrashekharaiah KS, Shashank A, Bharadwaj R, Raju NG, Swamy NR. Characterization of Protease Inhibitors from the Seeds of *Adenanthera pavonina*. International Journal of Biotechnology and Biochemistry. 2017; 13(4):361-70.
8. de Souza DD, Brandão-Costa RM, Albuquerque WW, Porto AL. Partial purification and characterization of a trypsin inhibitor isolated from *Adenanthera pavonina* L. seeds. South African journal of botany. 2016; 104:30-4.
9. Deshmukh PD, Rathore YS, Bhattacharya AK. Studies on growth and development of *Diacrisia obliqua* Walker (Lepidoptera: Arctiidae) on sixteen plant species 1. Zeitschriftfür Angewandte Entomologie. 1977; 84(1-4):431-5.
10. Dhillon MK, Sharma HC, Reddy BV, Singh R, Naresh JS, Kai Z. Relative susceptibility of different male-sterile cytoplasms in sorghum to shoot fly, Atherigona soccata. Euphytica. 2005; 144(3):275-83.
11. Dorrah MA. Effect of soybean trypsin inhibitor on digestive proteases and growth of larval *Spodoptera littoralis* (Boisd.). Efflatounia. 2004; 4:23-30.
12. Gandreddi VS, Kappala VR, Zaveri K, Patnala K. Evaluating the role of a trypsin inhibitor from soap nut (*Sapindus trifoliatus* L. Var. Emarginatus) seeds against larval gut proteases, its purification and characterization. BMC biochemistry. 2015; 16(1):23.
13. Ghodke AB, Chavan SG, Sonawane BV, Bharose AA. Isolation and *in vitro* identification of proteinase inhibitors from soybean seeds inhibiting *Helicoverpa* gut proteases. Journal of plant interactions. 2013; 8(2):170-8.
14. Gupta GP, Ranjekar PK, Birah A, Rani S, Gupta V, Sainani MN. Effective inhibition of *Helicoverpa armigera* by non-host plant proteinase inhibitors. ICAR News. 2002; 8:1-2.
15. Howe RW. A parameter for expressing the suitability of an environment for insect development. Journal of Stored Products Research. 1971; 7(1):63-5.
16. Jadhav AR, War AR, Nikam AN, Adhav AS, Gupta VS, Sharma HC, *et al*. *Capsicum annuum* proteinase inhibitor ingestion negatively impacts the growth of sorghum pest *Chilo partellus* and promotes differential protease expression. Biochemistry and biophysics reports. 2016; 8:302-9.
17. Kansal R, Kumar M, Kuhar K, Gupta RN, Subrahmanyam B, Koundal KR, *et al*. Purification and characterization of trypsin inhibitor from *Cicer arietinum* L. and its efficacy against *Helicoverpa armigera*. Brazilian Journal of Plant Physiology. 2008; 20(4):313-22.
18. Kranthi KR, Jadhav DR, Kranthi S, Wanjari RR, Ali SS, Russell DA. Insecticide resistance in five major insect pests of cotton in India. Crop Protection. 2002; 21(6):449-60.
19. Lee KP. Sex-specific differences in nutrient regulation in a capital breeding caterpillar, *Spodoptera litura* (Fabricius). Journal of insect physiology. 2010; 56(11):1685-95.
20. Macedo ML, Durigan RA, da Silva DS, Marangoni S, Freire MD, Parra JR. *Adenanthera pavonina* trypsin inhibitor retard growth of *Anagasta kuehniella* (Lepidoptera: Pyralidae). Archives of Insect Biochemistry and Physiology: Published in Collaboration with the Entomological Society of America. 2010; 73(4):213-31.
21. Macedo ML, Sá CM, Freire MD, Parra JR. A Kunitz-type inhibitor of coleopteran proteases, isolated from *Adenanthera pavonina* L. seeds and its effect on *Callosobruchus maculatus*. Journal of agricultural and food chemistry. 2004; 52(9):2533-40.
22. Maggo S, Malhotra SP, Dhawan K, Singh R. Purification and characterization of protease inhibitor from rice bean (*Vigna umbellata* T.) seeds. Journal of plant biochemistry and biotechnology. 1999; 8(1):61-4.
23. McManus MT, Burgess EP. Effects of the soybean (Kunitz) trypsin inhibitor on growth and digestive proteases of larvae of *Spodoptera litura*. Journal of Insect Physiology. 1995; 41(9):731-8.
24. Prasad J, Bhattacharya AK. Growth and development of *Spodoptera littoralis* (Boisd.) (Lepidoptera, Noctuidae) on several plants 1. Zeitschriftfür Angewandte Entomologie. 1975; 79(1-4):34-48.
25. Rahbé Y, Deraison C, Bonadé-Bottino M, Girard C, Nardon C, Jouanin L. Effects of the cysteine protease inhibitor oryzacystatin (OC-I) on different aphids and reduced performance of *Myzus persicae* on OC-I expressing transgenic oilseed rape. Plant science. 2003; 164(4):441-50.
26. Raikhel AS, Dhadialla TS. Accumulation of yolk proteins in insect oocytes. Annual review of entomology. 1992; 37(1):217-51.
27. Richardson M. Seed storage proteins: the enzyme inhibitors. Amino acids, proteins and nucleic acids, 1991.
28. Riseh Saberi N, Ghadamyari M, Moetamedinia B, Aghaali N, Hosseinnaveh V. Effect of Inhibitors from Plant Seeds on Digestive Proteolytic Activities in Larvae of the Date Palm Fruit Stalk Borer, *Oryctes elegans* Prell (Coleoptera: Scarabaeidae). Journal of Agricultural Science and Technology. 2014; 16:981-992.
29. Sasaki DY, Jacobowski AC, de Souza AP, Cardoso MH, Franco OL, Macedo ML. Effects of proteinase inhibitor from *Adenanthera pavonina* seeds on short-and long term larval development of *Aedes aegypti*. Biochimie. 2015; 112:172-86.
30. Sharma HG, Aarwal RA, Singh M. Effect of some antibiotic compounds in cotton on post-embryonic development of spotted bollworm (*Earias vittella* F.) and the mechanism of resistance in *Gossypium arboreum*. Proceedings: Animal Sciences. 1982; 91(1):67-77.

31. Shukle RH, Murdock LL. Lipoxygenase trypsin inhibitor, and lectin from soybeans: effects on larval growth of *Manduca sexta* (Lepidoptera: Sphingidae). Environmental Entomology. 1983; 12(3):787-91.
32. Singh NK, Haque M, Singh H, Rath SS, Ghosh S. A comparative study on cypermethrin resistance in *Rhipicephalus* (Boophilus) *microplus* and *Hyalomma anatolicum* from Punjab (India). Ticks and Tick-borne Diseases. 2014; 5(2):90-4.
33. Tammaru T, Kaitaniemi P, Ruohomäki K. Realized fecundity in *Epirrita autumnata* (Lepidotera: Geometridae): Relation to body size and consequences to population dynamics. Oikos. 1996; 77(3):407-16.
34. Telang M, Srinivasan A, Patankar A, Harsulkar A, Joshi V, Damle A, Deshpande V, Sainani M, Ranjekar P, Gupta G, Birah A. Bitter gourd proteinase inhibitors: potential growth inhibitors of *Helicoverpa armigera* and *Spodoptera litura*. Phytochemistry. 2003; 63(6):643-52.
35. Tripathi AK, Bhattacharya AK, Verma SK. Developmental behaviour of *Mythimna separata* (Walker) on some monocotyledonous plants. Indian Journal of Entomology. 1982; 44(4):355-67.
36. Vasudev A, Sohal SK. evaluation of partially purified subabul protease inhibitors as bio insecticidal tool with potential for the control of *Spodoptera litura*. International Journal of Current Research and Review. 2015; 7(18):31.
37. Waghmare S, Kamble L. *Spodoptera* shows adaptation to protease inhibitors. International Journal of Current Research in Life Sciences. 2018; 7(04):1655-7.