Journal of Entomology and Zoology Studies

Available online at www.entomoljournal.com

E-ISSN: 2320-7078
P-ISSN: 2349-6800
www.entomoljournal.com
JEZS 2021; 9(1): 1473-1477

Received: 01-11-2020
Accepted: 03-12-2020

T Manoramya
Department of Agricultural Entomology, Agricultural College & Research Institute (AC&RI), Tamil Nadu Agricultural University (TNAU), Madurai, Tamil Nadu, India

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

M Murugan
Department of Agricultural Entomology, TNAU, Coimbatore, Tamil Nadu, India

E Kokiladevi
Department of Plant Biotechnology, Centre for Plant Molecular Biology and Biotechnology, TNAU, Coimbatore, Tamil Nadu, India

ML Mini
Department of Biotechnology, Centre of Innovation, AC&RI, TNAU, Madurai, Tamil Nadu, India

Corresponding Author:
T Manoramya
Department of Agricultural Entomology, Agricultural College & Research Institute (AC&RI), Tamil Nadu Agricultural University (TNAU), Madurai, Tamil Nadu, India

Acute feeding effects of *Erythrina variegata* proteinase inhibitor and *Momordica balsamina* proteinase inhibitor on the growth and development of *Spodoptera frugiperda*

T Manoramya, M Shanthi, M Murugan, E Kokiladevi and ML Mini

DOI: https://doi.org/10.22271/j.ento.2021.v9.i1u.8343

Abstract
Proteinase inhibitors (PIs) are widely distributed plant defense proteins against herbivores. Exploiting these proteins in insect control tactics is one of the environmental friendly methods. In this context the present study was undertaken to study the effect of partially purified proteinase inhibitors from Indian coral tree, *Erythrina variegata* (EvPI) and balsam apple, *Momordica balsamina* (MbPI) on growth and development of fall armyworm, *Spodoptera frugiperda*. The three day old larvae were fed with maize leaves treated with EvPI (1, 3 and 6 %) and MbPI (1, 3 and 6 %) and EvPI 6% + MbPI 6% for three days (acute feeding) and untreated leaves until pupation. After feeding, the growth parameters like larval weight, pupal weight, adult weight, larval and adult period were not influenced upon treatments. However, malformations were observed in these treatments and it ranged from 10 to 20 %. There was pupal mortality (12.5 %) in MbPI 6% and combination treatment and adult emergence was reduced upto 12.5 % in these treatments. These results suggest that these treatments did not have great influence on growth and development when fed for short period. Hence, the PIs from *E. variegata* and *M. balsamina* shall be used in development of transgenic plants, as transgenic plant will provide opportunities for the larva to continuously feed on thePIs which will affect the growth and development of fall armyworm.

Keywords: Proteinase inhibitor, *Erythrina variegata*, *Momordica balsamina*, growth and development, acute feeding, *Spodoptera frugiperda*

Introduction
Fall armyworm (FAW), *Spodoptera frugiperda* is an emerging invasive insect pest in India that has been reported with a wide range of host plants (353 host plants) [13]. Even though FAW feeds on many host plants, its damage on Poaceae is more accountable. The grain yield loss caused by this pest in maize was 34% in Brazil, amounting US$ 400 million [10]. With the native of subtropical America, this devastating insect pest's first invasion was found in Africa during 2016 [4], later it crossed the borders of many countries. As the tropical climate and host plant availability in African and Asian subcontinents provided a conducive condition for its growth and development, FAW started to produce more generation per season and is becoming a big menace in these areas [2]. As the negative impacts like insecticide resistance development among the target insects and environmental health hazards of chemical insecticides usage has warranted the development of environmental friendly pest control tactics and many research works on various aspects of eco friendly pest management approaches are in progress. One among them is the use of proteinase inhibitors, a natural defender in plants against herbivores. As these inhibitors affect the protein digestion in insect gut by blocking protease activity, the growth and development of insect get reduced drastically [17]. There are four major class of proteinase inhibitors in nature based on the type of protease they inhibit namely serine, cysteine, aspartic and metallo-carboxipeptidase inhibitors. Among them, serine proteinase inhibitors were wide spread in nature [8, 12]. Also, serine proteases (trypsin and chymotrypsin) are the major proteolytic enzymes expressed in the gut of Lepidopterans [16]. In our earlier study, the seeds of 38 plants from different families were screened for trypsin and chymotrypsin inhibitory activity and found that the presence of trypsin and chymotrypsin inhibitory units were high in *M. balsamina* and *E. variegata* [11]. Hence, the present study was undertaken to evaluate the effect of serine proteinase inhibitor from *E. variegata* (EvPI) and *M. balsamina* (MbPI) against *S. frugiperda*.

Materials and Methods

The laboratory study was conducted to evaluate the effect of acute feeding of partially purified serine proteinase inhibitors isolated from seeds of *E. variegata* and *M. balsamina* through acute feeding on growth and development of *S. frugiperda* during 2019 in the laboratory at the Department of Agricultural Entomology, Agricultural College and Research Institute, Madurai.

S. frugiperda mass culture

Laboratory culture of *S. frugiperda* were maintained under room temperature (32°C and 70% RH) with the photo period of 14h:10h. Newly hatched early instar larvae were placed in plastic boxes (8.5x5.5x2.25 cm) and fed with tender maize leaves (20 nos./box). From 4th instar, the larvae were reared individuallyusing maize leaves to avoid cannibalism. When they reached the pre-pural stage, they were collected and kept for adult emergence in a cage. The adults were fed with 10 per cent honey solution and maize leaves were provided as substrate for egg laying. The uniform second instar larvae were taken from this culture and used for the laboratory bioassay.

Extraction and purification of Proteinase inhibitors

Proteinase inhibitors were partially purified from one kg dry seeds of *E. variegata* and *M. balsamina* [7]. The matured dried seeds were ground, depigmented and defatted. Crude protein from defatted flour was extracted using 0.01 M phosphate buffer (pH 7.2). The proteins in crude extracts were partially purified by Ammonium sulphate precipitation, during this process the unwanted proteins were removed and the protein of interest was concentrated by precipitation and finally three fractions were collected and further dialysed [6]. After dialysis, trypsin and chymotrypsin inhibitory units were estimated. The fractions containing high inhibitory units were independently pooled and lyophilized in SCANVAC COOL SAFE 55-4 lyophilizer.

Bioassay on effect of PI on growth and development of *S. frugiperda*

Lyophilized PI powders from *E. variegata* (EvPI) and *M. balsamina* (MbPI) were used for this bioassay. Different concentrations of EvPI and MbPI *viz.*, 1 %, 3 % and 6 % and a combination of EvPI and MbPI (6% + 6%) were prepared by dissolving in water (w/v) and Tween 20 (0.01%) was added as a wetting agent just prior to leaf treatment. The maize leaves were treated with PI by leaf dip method and air dried before use. The *S. frugiperda* larvae of 3 d old (II instar) were transferred individually into 70 mm Petri plates, to avoid cannibalism, and fed with PI treated maize leaves for three days (acute feeding period), and then the untreated leaves were provided as feed until pupation, to test the effect of PI on growth and development of larva. For each treatment, three replications were maintained at the rate of three larvae per replication. Observations were made on the weight of larvae (daily), pupae and adults, duration of each life stage and malformations, if any.

Data Analysis

Experiment was conducted at completely randomized design (CRD). Data were subjected to square root transformation and were statistically analyzed using SPSS for windows (version 16) software to carryout ANOVA. Grouping of data were done by using Duncan's Multiple Range Test (DMRT)[5].

Results and Discussion

Effect of EvPI and MbPI on larval weight of *S. frugiperda*

When FAW larvae were fed with PI treated leaves only for 3 days from day 3 to 5after hatching, there was no significant difference observed in larval weight until pupation (Table 1). The larval weight gain in treated larva ranged from 358.69 to 406.23 mg and in untreated larva, it was 393.49 mg. It may be due to the larval adaptations through enzyme loss by production of more digestive enzymes/ PI insensitive enzymes. [3] found that when the larvae of *S. frugiperda* were fed on artificial di*et al*ong with Kunitz trypsin inhibitor from *Entada acacifolia* (EATI), the midgut trypsin and chymotrypsin activity was increased upto 40 and 30 per cent respectively. They also found that PI insensitive trypsin and chymotrypsin genes i.e. *SfTry5*, *SfTry7* and *SfChy5* were transcribed during the study. When the larvae of *S. exigua* were fed with potato proteinase inhibitor II (PI2) transgened tobacco leaves chronically (from late second instar to final instar), the larval growth was not affected, which may be due to the production of PI insensitive trypsin enzymes in the larval gut [9]. Whereas in our study, when EvPI and MbPI were fed chronically to *S. frugiperda*, there was significant reduction in larval weight compared to control (unpublished data).

Effect of EvPI and MbPI on pupal and adult weight of *S. frugiperda*

The pupal weight of *S. frugiperda* ranged from 185.12 to 191.77 mg. In case of adult weight,it was 94.54 to 104.78 mg (Table 2).Similar to the larval weight of *S. frugiperda*, there was no prominent difference in the pupal and adult weight. When *S. frugiperda* fed EATI in a chronic manner, there was no difference in pupal weight, adult emergence and life-cycle [3]. The same results were observed when the *S. frugiperda* larvae fed transgenic maize plants expressed with soybean proteinase inhibitor genes [14]. While doing transcriptome and quantitative RT-PCR of the midguts of 6th instar *S. frugiperda* after ingestion of soybean proteinase inhibitors (SPI) rich diet for 48 h, the upregulation of constitutive protease genes and production of SPI insensitive proteases were observed [1]. Hence, this may be the reason for non-significant difference between treatments in the developmental stages of *S. frugiperda*. While, in our study, the *S. frugiperda* larvae feeding with EvPI and MbPI chronically, reduction in pupal and adult weight was observed (unpublished data).

Effect of EvPI and MbPI on developmental periods of *S. frugiperda*

The developmental time like larval, pupal periodsand adult life span of *S. frugiperda* were not differed due to the treatments and it was ranged from 13 to 13.31 days, 8.0 to 8.8 days and 11.3 to 12.0 days respectively (Table 3).While, the chronic feeding of EvPI and MbPI extended the larval period of *S. frugiperda* at higher concentrations (unpublished data). When the larvae of *S. frugiperda* fed on artificial diet incorporated with kunitz-type inhibitor of *Platypodium elegans* seeds (PeTI), the larval period was extended, but there was no difference in pupal and adult period [15].

Partially purified EvPI and MbPI on malformities of *S. frugiperda*

During the study period, larval and adult malformations were observed in*S. frugiperda* when fed on partially purified EvPI and MbPI (Plate 1). There was no malformation in pupal

stage, but there was mortality of pupae. There was 10 % larval malformation in combination treatment (EvPI 6% + MbPI 6%), 10 % pupal-adult malformation in 3% and 6% MbPI, 20 % adult malformation in EvPI 6% and 10 % in EvPI 3% and MbPI 1%. Pupal mortality was observed as 12.5 % in MbPI 6 % and in combination treatment and adult emergence was reduced upto 12.5 percentage in these treatments (Table 4). This may be because of the energy loss during the adaptive process in larval period like over expression of proteolytic enzymes and production of PI insensitive enzymes. As extra energy and nutrients exploited in the enzyme production in larval stage, there may be less availability of nutrients for pupal and adult development and this might have led to malformations.

From the present study it was concluded that, partially purified proteinase inhibitors from *E. variegata* and *M. balsamina* did not inflict significant effect on the growth and development of *S. frugiperda* when fed only for short period (3 days). This may be due to the development of adaptive mechanisms in larvae to overcome enzyme loss. However slight malformations were observed in treated larvae. Hence the PIs from *E. variegata* and *M. balsamina* shall be used for the development of transgenic plants.

Table 1: Impact of partially purified $EvPI_{0-60}$ and $MbPI_{30-90}$ on larval weight of *S. frugiperda* due to acute feeding

Treatment	*Mean fresh weight of the larvae (mg)								
	Fed with treated leaf			Fed with untreated leaf					
	D3	D4	D5	D6	D7	D8	D9	D10	D11
EvPI 1%	0.4±0.06 (0.63)	1.2±0.07 (1.09)	3.8±0.02 (1.95)	9.7±0.81 (3.11)	29.1±1.45 (5.39)	76.3±4.16 (8.73)	134.1±3.39 (11.58)	257.5±14.44 (16.05)	396.7±29.50 (19.92)
EvPI 3%	0.4±0.03 (0.66)	1.1±0.07 (1.07)	3.6±0.17 (1.89)	9.6±0.62 (3.10)	29.3±1.25 (5.41)	79.0±7.15 (8.89)	145.1±7.65 (12.05)	256.9±6.49 (16.03)	406.7±42.84 (20.17)
EvPI 6%	0.5±0.05 (0.69)	1.1±0.10 (1.06)	3.9±0.83 (1.98)	9.2±1.05 (3.03)	28.3±1.91 (5.32)	68.7±3.06 (8.29)	130.5±19.98 (11.42)	251.0±12.12 (15.84)	359.2±51.48 (18.95)
MbPI 1%	0.5±0.02 (0.67)	1.1±0.09 (1.06)	4.2±0.54 (2.05)	9.9±0.89 (3.15)	26.9±1.75 (5.19)	78.1±5.32 (8.84)	141.7±8.30 (11.90)	262.0±28.15 (16.19)	396.8±71.87 (19.92)
MbPI 3%	0.5±0.02 (0.69)	1.2±0.07 (1.08)	3.5±0.13 (1.88)	10.2±0.87 (3.19)	29.4±3.79 (5.43)	83.1±16.11 (9.12)	135.5±14.92 (11.64)	234.6±21.60 (15.32)	386.0±9.62 (19.65)
MbPI 6%	0.5±0.03 (0.68)	1.2±0.03 (1.08)	4.1±0.29 (2.02)	9.3±1.16 (3.04)	29.5±3.46 (5.43)	75.8±7.66 (8.71)	140.5±8.95 (11.85)	248.9±9.00 (15.78)	389.8±16.80 (19.74)
EvPI 6%+ MbPI 6% (1:1)	0.5±0.05 (0.67)	1.2±0.04 (1.09)	4.2±0.27 (2.04)	9.4±1.05 (3.07)	29.9±2.25 (5.47)	78.8±1.97 (8.88)	140.3±5.80 (11.85)	247.4±8.66 (15.73)	383.5±23.17 (19.58)
Untreated check	0.5±0.03 (0.71)	1.1±0.10 (1.06)	3.9±0.62 (1.98)	10.0±0.84 (3.16)	29.8±2.00 (5.46)	78.1±5.94 (8.84)	131.0±11.76 (11.44)	245.1±14.79 (15.65)	394.0±23.00 (18.6)
Mean	0.5	1.2	3.9	9.6	29.0	77.3	137.3	250.4	389.1
SEd	NS (0.03)	NS (0.03)	NS (0.07)	NS (0.13)	NS (0.18)	NS (0.36)	NS (0.38)	NS (0.43)	NS (0.85)
*Mean values of three replications are represented as mean ± standard deviation; Figures in the parentheses are square root transformed values;D3- Day 3 (age of the larva), and so on;SEd: Standard Error of the difference; NS: Non-significant.									

Table 2: Impact of partially purified $EvPI_{0-60}$ and $MbPI_{30-90}$ on larval weight gain, pupal and adult weight of *S. frugiperda* – Acute feeding

Treatment	Larval weight gain over initial weight* (mg)	Pupal weight* #(mg)	Adult weight* #(mg)
EvPI 1%	396.30	185.28 ± 3.9 (13.61)	101.0 ± 17.36 (10.03)
EvPI 3%	406.23	191.77 ± 5.6 (13.85)	96.64 ± 6.28 (9.83)
EvPI 6%	358.69	185.12 ± 2.4 (13.61)	94.54 ± 7.49 (9.72)
MbPI 1%	396.38	184.41 ± 11.4 (13.58)	101.97 ± 14.36 (10.08)
MbPI 3%	385.51	185.62 ± 6.5 (13.62)	103.85 ± 4.21 (10.19)
MbPI 6%	389.37	185.42 ± 4.1 (13.62)	100.67 ± 3.75 (10.03)
EvPI 6%+ MbPI 6% (1:1)	383.00	187.91 ± 4.9 (13.71)	104.78 ± 10.97 (10.23)
Untreated check	393.49	186.58 ± 7.7 (13.66)	94.57 ± 5.80 (9.72)
SEd	-	NS (0.20)	NS (0.41)

*Mean values of three replications are represented as mean ± standard deviation; #Figures in the parentheses are square root transformed values; SEd: Standard Error of the difference;NS: Non-significant.

Table 3: Effect of partially purified EvPI and MbPI on Larval period, pupal period and adult longevity of *S. frugiperda*– Acute feeding

Treatment	Larval period (days)*	Pupal period (days)*	Adult period (days)*
EvPI 1%	13.11±0.19 (3.62)	8.0± 0.0 (2.83)	11.3±1.53 (3.37)
EvPI 3%	13.11±0.19 (3.62)	8.7±0.25 (2.95)	11.7±0.57 (3.42)
EvPI 6%	13.06±0.42 (3.61)	8.8±0.63 (2.96)	11.7±1.15 (3.42)
MbPI 1%	13.31±0.04 (3.65)	8.7±0.33 (2.94)	12.0±1.0 (3.46)
MbPI 3%	13.11±0.19 (3.62)	8.6±0.51 (2.92)	11.7±0.57 (3.42)
MbPI 6%	13.03±0.29 (3.61)	8.3±0.25 (2.88)	11.3±0.56 (3.37)
EvPI 6%+ MbPI 6% (1:1)	13.00±0.00 (3.61)	8.4±0.13 (2.89)	11.7±0.57 (3.42)
Untreated check	13.08±0.14 (3.62)	8.4±0.35 (2.90)	11.3±1.16 (3.37)
Mean	13.10	8.47	11.6
SEd	NS (0.19)	NS (0.30)	NS (0.12)
*Mean values of three replications are represented as mean ± standard deviation; #Figures in the parentheses are square root transformed values; SEd: Standard Error of the difference; NS – Non significant.			

Table 4: Effect of partially purified EvPI and MbPI in causing malformations in different life stages of *S. frugiperda* – Acute feeding

Treatments	Malformation (%) in different life stages				Corrected pupal mortality	Adult emergence percentage
	Larva	Pupal – adult	Adult	Total malformations		
EvPI 1%	Nil	Nil	Nil	Nil	Nil	100
EvPI 3%	Nil	Nil	10	10	Nil	100
EvPI 6%	Nil	Nil	20	20	Nil	100
MbPI 1%	Nil	Nil	10	10	Nil	100
MbPI 3%	Nil	10	Nil	10	Nil	90
MbPI 6%	Nil	10	Nil	10	12.5	87.5
EvPI 6% + MbPI 6% (1:1)	10	Nil	Nil	10	12.5	87.5
Untreated check	Nil	Nil	Nil	Nil	Nil	100
	n = no. of larvae used in the study					

Larval malformation in combination treatment (EvPI 6% + MbPI 6%)

Pupal - adult malformation in MbPI 6%

Adult malformation in EvPI 6%

Plate 1: Malformations observed during the study

References

1. Brioschi D, Nadalini LD, Bengtson MH, Sogayar MC, Moura DS, Silva-Filho MC. General up regulation of *Spodoptera frugiperda* trypsins and chymotrypsins allows its adaptation to soybean proteinase inhibitor. Insect Biochemistry and Molecular Biology 2007; 37(12):1283-1290.
2. CABI. *Spodoptera frugiperda* (fall armyworm). In: Invasive species compendium. Wallingford, UK: CAB international. 2018. https://www.cabi.org/isc/datasheet/29810.
3. deOliveira CFR, Marangoni S, Macedo MLR. The trypsin inhibitor from *Entada acaciifolia* seeds affects negatively the development of Mediterranean flour moth, *Anagasta kuehniella*. Pesticide Biochemistry and Physiology 2014;108:74-79.
4. Goergen G, Kumar PL, Sankung SB, Togola A, Tamo M. First Report of Outbreaks of the Fall Armyworm *Spodoptera frugiperda* (J E Smith) (Lepidoptera, Noctuidae), a New Alien Invasive Pest in West and Central Africa. PLOS ONE. 2016;11(10):e0165632.
5. Gomez KA, Gomez AA. Statistical procedures for agricultural research: John Wiley & Sons 1984.
6. Green AA, Hughes WL. Methods in Enzymology, Vol. 1. by Colowick, S. P. & Kaplan, N. 0. New York: Academic Press Inc. Hall, JR & Ogston, AG (1956). Biochem. J. 1955;62(401):134.
7. Hajela N, Pande AH, Sharma S, Rao DN, Hajela K. Studies on a double headed protease inhibitor from *Phaseolus mungo*. Journal of Plant Biochemistry and Biotechnology 1999;8:57-60.
8. Haq SK, Khan RH. Characterization of a proteinase inhibitor from *Cajanus cajan* (L.). Journal of Protein Chemistry 2003;22(6):543-554.
9. Jongsma MA, Bakker PL, Peters J, Bosch D, Stiekema WJ. Adaptation of *Spodoptera exigua* larvae to plant proteinase inhibitors by induction of gut proteinase activity insensitive to inhibition. Proceedings of the National Academy of Sciences 1995;92(17):8041-8045.
10. Lima MS, Silva PSL, Oliveira OF, Silva KMB, Freitas FCL. Corn yield response to weed and fall armyworm controls. Planta Daninha. 2010;28(1):103-111.
11. Manoramya T, Shanthi M, Murugan M, Kokiladevi E, Mini ML, Pushpam AK. Occurrence of serine proteinase inhibitors in seeds of some plants. Indian Journal of Entomology 2020;82(3):557-63.
12. Mello GC, Oliva ML, Sumikawa JT, Machado OL, Marangoni S, Novello JC *et al*. Purification and characterization of a new trypsin inhibitor from *Dimorphandra mollis* seeds. Journal of Protein Chemistry 2001;20(8):625-632.
13. Montezano DG, Specht A, Sosa-Gómez DR, Roque-Specht VF, Sousa-Silva JC, Paula-Moraes SV *et al*. Host plants of *Spodoptera frugiperda* (Lepidoptera: Noctuidae) in the Americas. African Entomology 2018;26(2):286-300.

14. Paulillo LCM, Lopes AR, Cristofoletti PT, Parra JRP, Terra WR, Silva-Filho MC. Changes in midgut endopeptidase activity of *Spodoptera frugiperda* (Lepidoptera: Noctuidae) are responsible for adaptation to soybean proteinase inhibitors. Journal of Economic Entomology 2000;93(3):892-896.
15. Ramalho SR, Bezerra CS, deOliveira DGL, Lima LS, Neto SM, deOliveira CFR *et al*. A Novel peptidase Kunitz inhibitor from *Platypodium elegans* seeds is active against *Spodoptera frugiperda* larvae. Journal of Agricultural and Food Chemistry 2017;66(6):1349-1358.
16. Srinivasan A, Giri AP, Gupta VS. Structural and functional diversities in lepidopteran serine proteases. Cellular & molecular biology letters 2005;11(1):132-154.
17. Zhao A, Li Y, Leng C, Wang P, Li Y. Inhibitory effect of protease inhibitors on larval midgut protease activities and the performance of *Plutella xylostella* (Lepidoptera: Plutellidae). Frontiers in Physiology 2019;9:1963.