Variation in the gut hydrolytic enzymes of legume pod borer,
Maruca vitrata (Fabricius) feeding on different pulses

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Maruca vitrata (Fabricius), commonly known as legume pod borer, is a serious pest in pulses affecting the yield. Liberal use of chemical insecticides to control has resulted in diverse array of insect detoxification enzymes produced by the pest to metabolize these toxic chemicals and develop resistance. Here, we studied the gut hydrolytic enzymes of M. vitrata up on its field exposure to different insecticides in order to have an idea about level of adaptation. Among the larval samples collected from different pulses, the maximum protein content of 547.14 mg was recorded per g of gut sample of legume pod borer, M. vitrata collected from green gram. Total trypsin activity was insignificant in gut samples of larvae collected from different samples. The total chymotrypsin and aminopeptidase activities were found maximum in cowpea of 0.5655 and 0.1184 mM/mL/min/g, respectively. Whereas, the maximum GST activity in lablab populations of M. vitrata (2.3335 mM/mL/min/g). The specific activity of trypsin was maximum in Maruca larval populations from lablab (0.0050 nM/mL/min/mg of protein) and red gram or pigeonpea (0.0049 nM/mL/min/mg of protein) and chymotrypsin activity in cowpea (0.0098 nM/mL/min/mg of protein). The larval samples collected from lablab (0.0052 nM/mL/min/mg of protein) and cowpea (0.0037 nM/mL/min/mg of protein) showed the maximum specific activity of aminopeptidase. Maximum specific GST activity of 0.0325 and 0.0294 nM/mL/min/mg of protein was recorded in larvae collected from lablab and red gram, respectively. Larval samples from black gram ranked the last with respect to the activity of gut enzymes.

Keywords: Aminopeptidase, Black gram, Chymotrypsin, Cowpea, Green gram, GST, Lablab, Pesticide resistance, Pigeonpea, Red gram, Trypsin

Pulses have a unique position in sustainable crop production as they provide highly nutritive food from seeds and keep the soil alive and productive from root nodules. Though, India has the distinction of being the world’s largest producer of pulses, average productivity is low because they are mostly grown as dry land crops and also affected by many abiotic and biotic stresses. The infestation of legume pod borer, Maruca vitrata (Fabricius) syn. Maruca testulalis (Geyer) (Lepidoptera: Crambidae) is a highly challengeable menace and has become an important biotic constraint in increasing the production and productivity of pulses irrespective of agroecological zones. It attained a major pest status of cowpea, pigeonpea, lablab, green gram, black gram and common bean from the tropics to the temperate zone all over the world1. The larvae web the leaves and inflorescence and feed inside on flowers, flower buds and pods. The grain yield losses are estimated to be 10-80% in various crops2. The concealed feeding of this pest complicates control by pesticides and difficulty in penetration to feeding sites by natural enemies. Hence, there is an increased demand for some other effective management tactics along with the present practices that are sustainable and eco-friendly.

In order to manage the problem of insect pests, chemical insecticides are liberally sprayed on crops which led to problems like toxic residues, environmental disharmony and development of resistance3. Metabolic detoxification of insecticides by enzymes is a principal mechanism that contributes to the development of insecticide resistance4. Cytochrome 450 monoxygenases (P450) constitute the largest and most functionally diverse class of insect detoxification enzymes5. Herbivorous insects use detoxification enzymes, including cytochrome P450 monoxygenases, glutathione S-transferases, and cholinesterases, to metabolize otherwise deleterious plant secondary metabolites and toxic chemicals. Though Maruca vitrata is a serious pest highly specific to legumes, knowledge on its entire hydrolytic enzyme profile is lacking.
In the present study, we observed different gut enzymes of *M. vitrata* up on its field exposure to different insecticides and tried to understand its degree of adaption to pesticides sprayed on legumes.

**Materials and Methods**

Laboratory experiments were conducted at Molecular Ecology laboratory, Department of Plant Biotechnology, Tamil Nadu Agricultural University, Coimbatore, to work out variation in the gut enzymes of *M. vitrata* larval populations collected from different pulses during 2012 and 2013. Single 5th instar larvae of *M. vitrata* collected from redgram, greengram, blackgram, cowpea and lablab were chilled on ice and dissected in cold 0.1 M Tris-HCl buffer (pH 8.6) to isolate larval midgut tissue. In order to prepare midgut samples, the entire midgut was cleared from other attached tissues without detaching the midgut from the dissected larva, and then the midgut was detached from the larva by holding the anterior and posterior ends of the midgut with forceps to avoid leakage of gut contents from the midgut lumen. The dissected tissues were weighed after removal of the excess buffer. The isolated tissue from each midgut was individually homogenized and suspended in 0.1M Tris-HCl buffer (pH 8.6) to a final volume of 400 µL to estimate the activity of midgut enzymes viz., trypsin, chymotrypsin and aminopeptidase. Similar methodology was followed for estimating the activity of glutathione S transferase (GST) enzyme with 0.1 M potassium phosphate buffer (pH 6.5). The samples were centrifuged at 10000 rpm for 10 min at 4°C. The supernatant of each homogenate was transferred to an individual eppendorf tube and stored at -20°C until enzyme assay. The protein quantity of the midgut homogenate was determined by the Bradford method using BSA (bovine serum albumin) as standard.

**Preparation of protein standard**

Protein concentration of extracts was determined by the Bradford method, using bovine serum albumin as a standard protein. BSA 1000 ppm standard solution was prepared by dissolving 50 mg of BSA in 50 mL distilled water. Ten mL of 1000 ppm solution was dissolved in 500 mL of distilled water and used as stock solution. A series of working standards viz., 0.2, 0.4, 0.6, 0.8 and 1.0 mL were prepared and volume made to 1.0 mL in all test tubes using distilled water. Simultaneously, a blank was run using 1.0 mL of distilled water. The intensity of blue colour was read at 560 nm in spectrophotometer (Systronics UV VIS Spectrophotometer 117) after an incubation time of 10 to 30 min. OD (Optical Density) values were plotted against concentration and a standard graph was prepared to quantify the amount of proteins. This standard graph was used to estimate the protein concentration in the midgut samples of *M. vitrata*. After determining the protein concentration, homogenate was used for measurement of enzyme activity using specific substrate.

**Chemicals**

The substrates for measuring specific activity of gut enzymes of *M. vitrata* such as N-α-benzoyl-DL-arginine-p-nitroanilide (BAPNA), N-succinyl-(Ala) 2-Pro-Phe-p-nitroanilide (SAAPFpNA), leucine-p-nitroanilide (LpNA), 1-chloro-2,4-dinitrobenzene (CDNB) and glutathione reduced were purchased from M/s. Aldrich Chemicals, USA.

**Trypsin activity assay**

BAPNA was used as substrate to measure the trypsin activity. The universal buffer (Tris HCl) of pH 8.6 was used in assaying trypsin. About 50 mg of BAPNA diluted in 1 mL of DMSO (dimethyl sulfoxide) served as stock solution. The working BAPNA solution was prepared with the buffer to obtain a final concentration of 0.5 mg/mL (by adding 0.01 mL of stock solution in 0.99 mL of Tris HCl buffer). Activity of trypsin was determined by addition of 100 µL of enzyme homogenate, 100 µL of substrate (diluted BAPNA) and final volume of 3mL was made by adding 2.8 mL of Tris HCl buffer. The solution was allowed to develop pale yellow colour after incubation of 5 to 10 min and OD measured using a spectrophotometer at 15 s interval for 5 min. Absorbance was measured at 405 nm and 37°C. Specific trypsin activity was computed based on the extinction coefficient (8.8 mM$^{-1}$ cm$^{-1}$) for N-α-benzoyl-DL-arginine-p-nitroanilide.

**Chymotrypsin activity assay**

SAAPFpNA was used as substrate to measure activities of chymotrypsins. The universal buffer (Tris...
HCl) of pH 8.6 was used in assaying chymotrypsins. SAAPFpNA solution was prepared by diluting 50 mg/mL stock (in DMSO) with the buffer to obtain a final concentration of 0.5 mg/mL (by adding 0.01 mL of stock solution in 0.99 mL of Tris HCl buffer). Activity of chymotrypsin was determined by addition of 100 µL of enzyme homogenate, 100 µL of substrate (diluted SAAPFpNA) and final volume of 3 mL was made by adding 2.8 mL of Tris HCl buffer. The colour of the solution was changed to pale yellow and absorbance was measured in a spectrophotometer (@ 405 nm and 37°C) at 15 s interval for 5 min. The specific activity of chymotrypsin calculation was performed based on the extinction coefficient (14 mM⁻¹ cm⁻¹) for N-succinyl-(ala) 2-pro-phe-nitroanilide.

Aminopeptidase N (APN) activity assays
The APN activity was measured using LpNA as a chromogenic substrate. About 0.8 mM of LpNA was prepared in 0.1M of Tris HCl. In a clean test tube, 0.99 mL of freshly prepared LpNA was added followed by 1.89 mL of pre warmed (37°C) 0.1M Tris-HCl buffer (pH 8.0) and 0.12 mL of gut homogenate was added in. The enzymatic reaction was immediately monitored for the optical absorbance increase at wave-length 405 nm at 37°C for 15 min. One unit of the enzyme activity was defined as the amount of enzyme that hydrolyzed one μ mol of substrate to chromogenic product per min. Specific APN activity were calculated based on extinction coefficient (9.9 mM⁻¹ cm⁻¹) for p-nitroanilide.

GST activity assay
The total glutathione s-transferases (GST) activity was measured using CDNB and Glutathione Reduced as substrate. Glutathione-S-transferases activities were determined spectrophotometrically with some modifications. Potassium phosphate buffer (0.1M) of pH 6.5 was used in assaying GST. About 0.1M glutathione reduced and 0.1M CDNB substrate was prepared in pure ethanol and distilled water respectively. Activity of GST was determined by addition of 0.1 mL of glutathione reduced, 0.1 mL CDNB and 0.1 mL gut homogenate solution to final volume 3mL. The enzymatic reaction was immediately monitored for the optical absorbance increase at wave length 340 nm at 37°C for 5 min at 30 s interval in the spectrophotometer. Specific GST activity was calculated using the extinction coefficient of the product formed (9.6 mM⁻¹ cm⁻¹) and was expressed as n moles of CDNB conjugate per minute.

Analysis
Protein concentration was calibrated from the OD values and expressed in terms of mg per gram of dissected gut weight. General activity of enzymes was worked out without considering the concentration of proteins and expressed in terms of milli moles per mL of homogenized gut extract per min in a gram of gut sample. Whereas, enzyme specific activity was computed after including protein concentration in the larval gut sample and expressed in terms of nano moles per mL (nm/mL) of homogenized gut extract per min in a milli gram of protein. The formulae used for the calculations are here under,

Protein concentration (mg/g) = \( \frac{X \times B}{A \times C} \times 1000 \)

where, X is the concentration of protein in the mid gut homogenate (µg); A, the volume of samples in the cuvet for measuring OD (mL); B, final volume of supernatant prepared (mL); and C, initial sample weight (mg). In order to obtain volume in terms of mg/g the constant ‘1000’ was included.

General activity of enzymes (mMol/mL/min) = \( \frac{D \times 60 \times B}{A \times I \times W} \times 1000 \)

where, D is the mean of OD difference (nm), 60 - to get result per min (s); B, volume of buffer used (mL); A, volume of homogenate taken (mL); I, absorbance interval (s); and W, mean weight of gut sample (g). In order to obtain the result per gram, the constant ‘1000’ was included.

Specific activity of enzymes (mMol/mL/min/mg of protein) = \( \frac{D \times A \times T \times P}{B \times C \times S} \times 1000 \)

where, D is the mean of OD difference (nm); A, volume of sample taken in cuvet (mL); C, extinction coefficient of enzyme; B, volume of substrate taken (mL); T, incubation time (min); and P, protein content (mg/g). In order to obtain the result per gram, the constant ‘1000’ was included.

The significance in variation of protein concentration and general and specific enzyme activities were analysed after suitable transformations. Analysis of variance was used to test the significance differences in the mean of general and specific enzyme activities with different hosts. Multiple comparisons test by Tukeys HSD (Honestly Significant Difference) were performed using statistical package.
for social sciences (SPSS) software version 16 in order to find the significance of differences between the treatments. Data was considered to be significantly different within the treatments if the $F$–value obtained was higher than the critical $F$–value at a probability level of 0.05. Critical differences (CD) between HSD values were calculated by subtracting subsequent values of the averages and comparing with the calculated CD at $P <0.05$.

### Results

#### Mid gut protein content of *M. vitrata* larvae from different hosts

The maximum protein content of 547.14 mg was recorded per g of gut sample of *M. vitrata* collected from green gram followed by 514.74 and 508.33 mg per g in *Maruca* mid gut samples of red gram (pigeonpea) and cowpea, respectively (Table 1). The minimum protein content (363.97 mg/g) was recorded in *Maruca* larva samples collected from lablab. The concentration of protein in insect system varied with time and food on which it establishes its life.

#### General activity of gut enzymes of *M. vitrata*

Total trypsin activity was found to be insignificant in gut samples of *M. vitrata* larvae collected from different samples (Table 2). The maximum trypsin activity was in larvae from red gram (0.3330 mM/mL/min/g) followed by larval samples from cowpea (0.2059 mM/mL/min/g) and green gram (0.1948 mM/mL/min/g) as against 0.0956 mM/mL/min/g of gut sample from black gram.

The significant highest chymotrypsin activity of 0.5655 mM/mL/min/g was registered in *Maruca* gut samples from cowpea (Table 2). Whereas, in larval samples collected from green gram, red gram and black gram it was 0.2255, 0.2105 and 0.1996 mM/mL/min/g when compared to 0.1271 mM/mL/min/g in *M. vitrata* larvae from lablab.

As the general activity of chymotrypsin, the significant maximum aminopeptidase activity was noted in cowpea larval populations of *M. vitrata* (0.1184 mM/mL/min/g) followed by 0.1370 mM/mL/min/g in lablab larval samples which were on par with each other. In other larval samples it ranged from 0.0105 to 0.0776 mM/mL/min/g in green gram and black gram, respectively.

The significant peak general activity of glutathione s-transferases (GST) was recorded in lablab population of *M. vitrata* (2.3335 mM/mL/min/g), followed by 2.0788, 1.3720 and 0.9372 mM/mL/min/g in larval samples collected from red gram, green gram and cowpea, respectively when compared to 0.4813 mM/mL/min/g in black gram population.

#### Specific activity of gut enzymes of *M. vitrata*

The specific activity of trypsin was maximum in *Maruca* larval populations from lablab (0.0050 nM/mL/min/mg of protein) and red gram (0.0049 nM/mL/min/mg of protein) and red gram (0.0049 nM/mL/min/mg of protein).

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**Table 1 — Midgut protein concentration (mg/g) of larvae of *Maruca vitrata* collected from different pulse hosts**

<table>
<thead>
<tr>
<th>Crop</th>
<th>Protein content (mg/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Red gram</td>
<td>514.74* (22.70)</td>
</tr>
<tr>
<td>Green gram</td>
<td>547.14* (23.40)</td>
</tr>
<tr>
<td>Black gram</td>
<td>452.39* (21.28)</td>
</tr>
<tr>
<td>Cowpea</td>
<td>508.33* (22.56)</td>
</tr>
<tr>
<td>Lablab</td>
<td>363.97* (19.09)</td>
</tr>
</tbody>
</table>

*Mean value of five replications per enzyme per host and each replication consist of five larvae.

**Table 2 — General activity of midgut enzymes of *Maruca vitrata* collected from various hosts**

<table>
<thead>
<tr>
<th>Crop</th>
<th>Trypsin (mM/mL/min/g)</th>
<th>Chymotrypsin (mM/mL/min/g)</th>
<th>Aminopeptidase (mM/mL/min/g)</th>
<th>GST (mM/mL/min/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Redgram</td>
<td>0.3330* (0.9127)</td>
<td>0.2105* (0.8429)</td>
<td>0.0120* (0.7156)</td>
<td>2.0788* (1.6059)</td>
</tr>
<tr>
<td>Greengram</td>
<td>0.1948* (0.8335)</td>
<td>0.2255* (0.8518)</td>
<td>0.0105* (0.7145)</td>
<td>1.3720* (1.3682)</td>
</tr>
<tr>
<td>Blackgram</td>
<td>0.0956* (0.771)</td>
<td>0.1996* (0.8364)</td>
<td>0.0776* (0.7600)</td>
<td>0.4813* (0.9906)</td>
</tr>
<tr>
<td>Cowpea</td>
<td>0.2059* (0.840)</td>
<td>0.5655* (1.0322)</td>
<td>0.1184* (0.7864)</td>
<td>0.9372* (1.1988)</td>
</tr>
<tr>
<td>Lablab</td>
<td>0.1786* (0.823)</td>
<td>0.1271* (0.7919)</td>
<td>0.1370* (0.7981)</td>
<td>2.3335* (1.6833)</td>
</tr>
</tbody>
</table>

*Mean value of five replications per enzyme per host and each replication consist of five larvae.

*Figures in the parentheses are √X + 0.5 transformed values. [Means with common superscripts are not significantly different from each other ($P<0.05$) for various treatments (as indicated by Tukeys HSD)].
protein) which was found to be on par with each other and superior to all other populations. This was followed by 0.0033 and 0.0025 nM/mL/min/mg of protein in larval populations of cowpea and green gram as against minimum level in black gram populations (0.0017 nM/mL/min/mg of protein) as shown in Table 3.

The significant maximum chymotrypsin activity of 0.0098 nM/mL/min/mg of protein was registered in cowpea populations of M. vitrata. Larval populations of red gram, black gram, green gram and lablab showed a specific chymotrypsin activity of 0.0055, 0.0049, 0.0043 and 0.0035 nM/mL/min/mg of protein, respectively. The ratio of specific activity of trypsin and chymotrypsin was found to be highest (1.4286) on lablab larval populations and in other hosts studied it ranged from 0.3367 to 0.8909 indicating more activity of chymotrypsin than trypsin.

The maximum aminopeptidase (APN) activity was observed in lablab (0.0052 nM/mL/min/mg of protein) and cowpea (0.0037 nM/mL/min/mg of protein) populations of M. vitrata and they were on par with each other. In other host populations, the specific activity ranged from 0.0002 nM/mL/min/mg of protein in green gram and 0.0052 nM/mL/min/mg of protein in black gram.

The maximum specific GST activity of 0.0325 and 0.0294 nM/mL/min/mg of protein was recorded in larval populations collected from lablab and red gram, respectively which were on par with each other and superior than other larval populations. This showed the peak GST activity in lablab and red gram populations. Larval populations of cowpea recorded 0.0198 nM/mL/min/mg of protein followed by minimum levels of 0.0099 and 0.0042 nM/mL/min/mg of protein in black gram and green gram respectively. The present findings clearly revealed that the Maruca larval populations from lablab showed invariably maximum activity of all four mid gut enzymes followed by pigeonpea and cowpea and green gram populations. The black gram populations of M. vitrata showed least values of all analysed gut enzymes.

### Discussion

According to earlier research findings, it is clear that continuous application of synthetic pesticides and Bt products impose high selection pressure on many target insects resulted in evolving resistance in some insects through production of detoxifying enzymes like chimotrypsin, aminopeptidase and other protease inhibitors\(^{14}\). Present study clearly revealed the presence of different detoxifying enzymes from the field collected M. vitrata larvae exposed to pesticides. Generally, lepidopteran larvae reported to have at least four classes of aminopeptidases in their midgut ranged about 90 to 200 kDa\(^{15}\).

Among larval samples the maximum trypsin activity was recorded in larvae collected from red gram (0.3330 mM/mL/min/g) followed by cowpea (0.2059 mM/mL/min/g) and green gram (0.1948 mM/mL/min/g). The highest chymotrypsin activity (0.5655 mM/mL/min/g) was registered in Maruca gut samples from cowpea when compared to 0.1271 nM/mL/min/g in M. vitrata larvae from lablab. In the mid gut extracts of locusts, two novel chymotrypsins viz., CTR 1 and CTR 2 and were found to be hydrolyzed by N-terminally extended substrate, SAAPFpNA\(^{16}\). Serine proteases and metallo-
proteinases were already found to be the dominant active proteases in the digestive system of lepidopteran larvae. Similarly, the maximum activity of chymotrypsin and trypsin were also reported in the gut extracts of larvae of *H. virescens* and fig tree skeletoniser moth, *Choreutis nemorana* and *M. vitrata* exposed to different insecticides. Midgut proteinases in *Conogethes punctiferalis* revealed that trypsin and elastase like chymotrypsin as prominent digestive proteinases. Dietary experiments conducted on larvae of *Ephesia kuehniella* with 0.25% labramin in the diet also showed a reduced growth and development due to elevated levels of total trypsin activity (19%) in midgut and fecal matter. These indicated that trypsin and chymotrypsins were dominant digestive enzymes and play an important role in *M. vitrata* when feeding on different pulses. In the present study, among five pulses, larval samples from cowpea (0.1184 mM/mL/min/g) and lablab (0.1370 mM/mL/min/g) recorded highest activity of aminopeptidase. Similarly, in *C. punctiferalis* also protein digestion was predominantly influenced by leucine aminopeptidases in larval samples collected from cardamom.

The peak general activity of glutathione s-transferases (GST) was recorded in lablab populations of *M. vitrata* (2.3335 mM/mL/min/g) than that fed on other pulses. These results are in line with earlier reports that the increased GST enzyme activity in green peach aphid, *Myzus persicae* upon ingestion of isothiocyanates. Similarly, studies on swallowtail butterflies, *Papilio* spp. showed that the generalist feeders possess a broader array of P₄₅₀ for detoxification.

The highest specific activity of trypsin (0.0050 nM/mL/min/mg of protein) and amino- peptidase (APN) (0.0052 nM/mL/min/mg of protein) was recorded in *Maruca* larval populations from lablab, whereas the chymotrypsin activity was maximum in cowpea populations (0.0098 nM/mL/min/mg of protein). The present findings are in consonance with the similar earlier reports of other lepidopterans. In *Anticarsia gemmatalis*, a basal trypsin-like activity of 0.217 mmol p-nitroaniline min⁻¹ mg protein⁻¹ was recorded. Protease inhibitors isolated from few wild cultivars of pigeonpea showed 10 to 50 fold and 3 to 9 fold higher activity against midgut trypsin-like proteinases of *Achaea janata* and *Spodoptera litura*, respectively when compared to bovine pancreatic trypsin. Similarly, purified Bowman–Birk proteinase inhibitors from red gram (RgPI) and black gram (BgPI) showed inhibitory activity against midgut trypsin and chymotrypsin like proteinases of *A. janata*, *H. armigera* and *S. litura*. It is also reported that trypsin activity was maximum in alkaline pH (>8.5) and optimal pH for its activity in the gut of fig tree skeletoniser, *Choreutis nemorana* larvae was around 11.0. Similarly, in other lepidopterans viz., *S. littoralis*, *Conogethes punctiferalis* and *Mamestra brassicae*, the optimum pH was found between 10 to 11. Capsicum annuum proteinase inhibitor (CanPI7) is a multidomain potato type II inhibitor in transgenic tomato lines showed increased protease inhibitor activity, antibiosis and delayed growth rate against *H. armigera*. In *H. armigera*, trypsin like protease from gut was purified to 37 fold with 22% yield and its molecular weight was found to be 18.8 kDa. Kansal *et al.* (2008a) reported that 95% inhibition of larval gut proteinase of *Helicoverpa armigera* by the trypsin inhibitor present in seed extract of soybean. Soybean Kunitz type trypsin inhibitor (SBTI) and Soybean Bowman–Birk type trypsin-chymotrypsin inhibitor (SBBI) have been shown to reduce larval weights of *H. armigera* in artificial diet. Similarly, trypsin inhibitors from *Cicer arietinum* seeds also contain insecticidal potential against *Helicoverpa armigera*. Soybean protease inhibitor reported to inhibit gut proteinase i.e. trypsin activity in *S. litura* at 100 μg/mL with increase in exposure intervals. Reduction of trypsin activity was also recorded in *Eurygasta integriceps* at lower doses of SBTI.

The trypsin and chymotrypsin ratio was highest (1.4286) on lablab larval populations than other pulses which indicated their predominant activity. The present results are in consonance with the findings on substrate analysis of mid gut enzymes in red oak borer, *Enaphalodes rufulus* and showed the presence of trypsin (>100 kDa) and chymotrypsin-like activities (>200 kDa) in larval gut extracts. Similar to the maximum activity of APN in *M. vitrata* larvae of present investigation, serine and metalloproteinases and leucyl aminopeptidase were also recorded in crude midgut extract larvae of long horned beetle, *Morimus funereus*. APN activity in lepidopteran larvae has also been attributed to its association with the midgut brush border membranes. This also clearly revealed that these enzymes play an important
role in activity of many entomopathogenic bacteria since they act on insect gut.

The maximum specific GST activity of 0.0325 and 0.0294 nM/mL/min/mg of protein were recorded in larval populations collected from lablab and red gram, respectively and thus showed the peak GST activity in lablab and red gram populations. The present findings are in consonance to Dowd (1990) who measured the relative levels of midgut detoxifying enzymes in last instar larvae of Heliothis zea and Spodoptera frugiperda and recorded the highest production of glutathione transferase conjugation. Generally GST was associated with elimination of toxic residues from insect system and Li et al. (2007) reported that GST enzymes, conjugate with xenobiotics and endogenously activated to reduced glutathione, thereby targeting them for more rapid excretion or degradation of toxic chemicals. In insects, association of GSTs with insecticide resistance to following insecticides such as DDT, spinosad, diazinon and nitenpyram, which target the nervous system and defence towards organophosphates, organochlorines and cyclodiienes. Earlier reports for the GST associated insect resistance to pyrethroid insecticides were available for S. littoralis. Induction of GST by pyrethroids has also been reported for honey bee and S. frugiperda. These reports are clearly revealed that GST enzymes of M. vitrata also may play an important role in detoxification of insecticides.

Until now the chemical method management to combat insect attack is considered to be effective and widely adopted by farming community. However, adverse effects of many synthetic pesticides are evident through available literatures. In addition, development of resistance in target organisms necessitated use of more selective and environmentally acceptable agents in the context of pest management. In order to fulfill the growing needs of human population and also to reduce the harmfulness of synthetic pesticides on environment, it is necessary to seek new, effective and environment-friendly ways of controlling pests. Hence, transgenic approach may find a new tool in insect pest management. The research findings from the present study revealed that the Maruca larval populations from lablab showed invariably maximum activity of all four mid gut enzymes followed by pigeonpea and cowpea and green gram populations. The black gram populations of M. vitrata showed least values of all analyzed gut enzymes.

Conclusion

Present study showed the maximum general activity of trypsin and Glutathione S transferase (GST) in larvae collected from pigeonpea (0.3330 mM/mL/min/g) and lablab (2.3335 mM/mL/min/g) respectively and cowpea populations recorded the highest general activity of chymotrypsin (0.5655 mM/mL/min/g) and aminopeptidase N (0.1184 mM/mL/min/g). Similarly, specific activity of trypsin and GST was highest in larval populations of lablab (0.0050 and 0.0325 nM/mL/min/mg of protein) and pigeonpea (0.0049 and 0.0294 nM/mL/min/mg of protein). The highest specific activity of chymotrypsin (0.0098 nM/mL/min/mg of protein) and aminopeptidase N (0.0052 mM/mL/min/mg of protein) was recorded from cowpea and lablab populations of M. vitrata. These results make better understanding of major detoxifying enzyme profile and developmental flexibility that Maruca vitrata possesses apart from basic knowledge on different gut digestive enzymes of digestion. However, further research is required to extrapolate these results in transgenics through breakdown of these enzymes to interfere with protein digestion in the insect system. Unravelling the molecular mechanisms controlling these adaptive changes in M. vitrata is also crucial for providing leads to effective crop protection.

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