Research Paper :

Influence of different hosts on induction of midgut glutathione S-transferase in *Helicoverpa armigera* (Hubner)

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International Journal of Plant Protection (October, 2010), Vol. 3 No. 2 : 342-347

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SUMMARY

Effect of different hosts *viz.,* cotton, pigeonpea and chickpea of *H.armigera* on the induction of gut detoxifying enzymes and its effect on insecticide metabolism was studied in the present investigation. Glutathione S-transferase was found to be induced in higher amount in the gut of *Helicoverpa armigera* when reared on chickpea as compared to the other studied hosts. Midgut proteins were also found to be influenced by these hosts. Toxicity levels of different insecticides were studied against *H.armigera* reared on different hosts. The variability in toxicity was observed among strains *i.e.H.armigera* reared on different hosts. Strain reared on chickpea showed tolerance against indoxacarb, spinosad and emamectin benzoate, whereas, strain reared on pigeonpea showed higher LC_{50} for lambdacyhalothrin. Cotton fed larvae was found to be comparatively susceptible. Different hosts were found to induce GST and protein in mid gut, which intern reflect in terms of tolerance against insecticides.

Key words : Detoxifying enzymes, Glutathione S-transferase *Helicoverpa armigera*, Induction, Protein, Toxicity

Accepted : *September, 2010*

Helicoverpa armigera (Hubner) (Lepidoptera: Noctuidae), is well known as cotton bollworm, gram caterpillar, pod borer or American bollworm. It is highly polyphagous pest with broad spectrum of host families including important agricultural crops such as cotton, maize, chickpea, pigeonpea, sorghum, sunflower, soybean, groundnut etc, (Fitt,1989).

Insect feeding exhibits a host-range that is manifested by the different plants on which they usually are found. When the host plant changes, insect changes its metabolism to adopt the same, so as to allow it to metabolize the new primary and secondary compounds of new plants. Host plants induce the biochemical constituents in insect through feeding on them also affect the susceptibility of pest to particular insecticides. These hosts plants induced detoxifying enzymes are responsible for detoxification of xenobiotics and chemical insecticides (Yu, 1982). The detoxifying enzymes of insect include mostly Glutathione S-transferase (GST) responsible for insecticide resistance (Yang *et al.*, 2001). Plant species differ in the degree to which they stimulate the biochemical defense of insects. Therefore, research on insect host plant interactions may yield information of considerable value in the development of insect pest management programmes, where insecticides are an integral part of the programme (Berry *et al.*, 1980). Host plants found to affect the expression of resistance to chemicals in lepidopteran pest like *Platynota idaeusalis* tufted apple bud moth larval populations (Dominguez Gilly and McPheron, 2000).

The present investigation was carried out to know the induced mid gut GST and protein in midgut of *H.armigera* feeding on different hosts and its effect on the toxicity of new insecticide molecules.

MATERIALS AND METHODS

H.armigera was collected from field and reared in laboratory for homogenization on artificial diet (Armes *et al.*, 1992). There after larvae were reared for one generation on different hosts like cotton, pigeonpea and chickpea along with artificial diet. Commercially available formulations of indoxacarb, spinosad, emamectine benzoate, and lamdacyhalothrin were used in the present study for log dose probit (LDP) assay against 3rd or early 4th instar of *H.armigera.*

All chemicals used for enzyme and protein assay were purchased from Himedia Laboratories Ltd., Mumbai, India.

Laboratory rearing of *H.armigera:*

Field collected *H.armigera* was reared on artificial diet for 3-4 generations to develop homogenized population and escape field

contamination. Last instar larvae were allowed to pupate in sterilized sand. Moths emerged from pupal chamber were subjected to mating chamber with 1male:1female ratio. Adult diet was given to moth. Egg laying was taken on muslin cloth. Neonates hatching from this cloth were taken on artificial diet

Rearing of *H.armigera* on hosts:

Neonates from homogenous population were reared for one generation on plant parts of different host plants, *viz.,* squares and tender bolls of cotton, flowers and pods of pigeonpea, leaves and pods of chickpea. In next generation, neonate reared on same host till they reached to third instar. Bioassays were carried out with this population to determine the LC_{50} value of insecticides and effect of different hosts on susceptibility to insecticides in *H. armigera.* These all rearing done under laboratory condition of 27±2^{0}C and 75±5% RH with 13:11 hrs light: dark period.

Bioassay of *H.armigera* against insecticides:

3rd-4th instar larval population was reared on different hosts used for bioassay using leaf dip method under laboratory conditions. About one square cm. leaf disc of cotton leaf dipped in insecticide solution, allowed to air dry and 7-8 hrs starved larvae were released on that treated leaf disc in multicellular rearing tray. Five concentrations were taken with 10 larvae for each treatment with three replications. Control was also taken without insecticide. All the bioassays were carried out at 25±2^{0}C and 60-80% RH. Moribund larvae not responding to probing were considered as dead. Observations were recorded at 24, 48 and 72 hrs after treatment.

The median lethal concentration (LC_{50}) for the insecticides was worked out by using Indostat software, Hydrabad, India which is based on principles given by Finney (1977) and corrected mortality was calculated by using Abott's formula (1925).

Resistance ratio:

The resistance intensity of a population or a strain of insects to a particular insecticide is frequently quoted as the resistance ratio (RR), sometimes also called as resistance factor (RF) which was calculated by the formula :

$$\mathbf{RR = \frac{LC_{50}\ of\ host\ reared\ strain}{LC_{50}\ of\ diet\ reared\ strain}}$$

Enzyme preparation from midgut of *H.armigera:*

Randomly twenty larvae of late third or early fourth instar, *H. armigera* weighing 300-350 mg approximately selected from F_1 of different hosts reared populations, *viz.,* cotton, pigeonpea, chickpea and diet. Larvae were starved for six to eight hours and chilled in refrigerator prior to dissection. The larvae were pinned dorsally at head and anal region in wax plate and dissected out with the help of sterilized dissecting needle in ice-cold sodium phosphate buffer (0.1 M, pH 7.0) containing KCl (1.15%). Midguts were isolated with removing adhered fat bodies. About 10 midguts were placed in glass homogenization tubes containing 1 ml SPB (0.1mM pH 7.0 containing 0.1 mM EDTA, PTU and PMSF each). The homogenate thus obtained was centrifuged at 10,000 rpm for 15 minutes at 4^{0}C in high-speed refrigerated centrifuge (Eppendrorf, Germany). Solid debris and cellular material was discarded. The resultant supernatant obtained was stored at –20^{0}C and used for quantitative and qualitative analysis of protein and enzymes.

Quantification detoxifying enzymes and protein:

GST quantification was carried out by method described by Kao *et al*. (1989). 50 mM 1-chloro-2, 4-dinitrobenzene (CDNB) and reduced glutathione (GSH) were used for this assay. Absorbance taken at 340 nM on UV spectrophotometer (U 2001 Hitachi, Japan). Calculation was done with the following formula:

$$\mathbf{CDNB\text{-}GSH\ conjugate\ (\mu M\ mg\ protein^{-1}\ min^{-1}) = \frac{Abs\ (increase\ in\ 5\ min) \times 3 \times 1000}{9.6^* \times 5 \times mg\ of\ protein}}$$

*9.6 mM/cm – extinction coefficient for CDNB-GSH conjugate

Protein concentration of insect homogenate was determined by Bradford method (1976) by using Bovine Serum Albumin (BSA) as a standard protein to construct the standard curve. 5 µl enzyme and 5 X Bradford reagent was used with three replications. Absorbance was taken at 600nM on microplate reader (Metertech model å960, USA)

Qualitative studies for protein and detoxifying enzymes:

12.5 % SDS PAGE was performed according to Laemmeli (1970). Samples run along with standard protein marker (MBI Fermentas) for 4-5 hours. Gel was incubated in 15 % trichloro acetic acid solution for half hour and then gel stained with commassie brilliant blue G-250 for 4-5 hours. Dark blue bands were observed on gel documentation system (Alpha Innotech, USA).

10 % Nondenaturing Polyacrylamide Gel

Electrophoresis (PAGE) was performed in Hoefer SE600 slab gel unit (Hoefer, San Fransisco, CA) for glutathione s-transferase (GST). Gel was incubated for 20 minute in destaining solution of 0.1 M SPB (pH 6.5), containing 5.0 mM GSH and 1 mM each of CDNB and NBT in dark. Then gel transferred into tris HCl buffer, 9.6 pH with 4 mM phenozine methosulfate (PMS) for 10 min intermittent shaking (Kranthi, 2005).

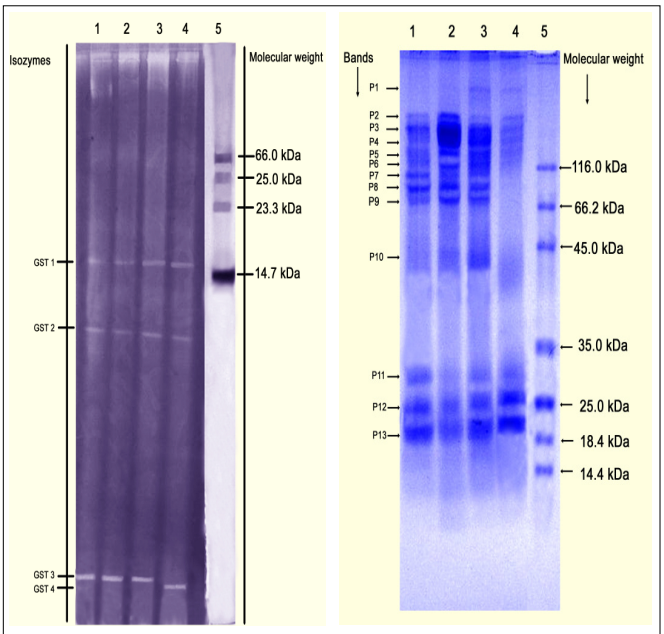
RESULTS AND DISCUSSION

The results obtained from the present investigation as well as relevant discussion have been presented under following heads:

Induction of gut detoxifying enzymes in *H.armigera:*

Table 1 shows the induction of gut detoxifying enzymes in *H.armigera,* reared on different hosts. Higher titer (1.77 µM mg protein^{-1} min^{-1}) of GST was induced in chickpea reared *H. armigera*, which was 2.29 fold higher that *H.armigera* larvae reared on artificial diet. Not much difference was observed in between diet and cotton reared larvae with respect to induction of GST. Pigeonpea fed larvae showed 1.46 µM mg protein^{-1} min^{-1} GST activity with increase of 1.89 fold over diet strain. Significant difference was not observed on when homogenate was electrophoresed on native PAGE and stained for GST activity.

Similarly, induction of midgut protein was also observed by different host plants. Diet reared strain showed higher values of gut protein (98.59 µg gut^{-1}) followed by pigeonpea reared strain (82.26 µg gut^{-1}), chickpea reared strain showed 79.68 µg gut^{-1} protein concentration. Lowest protein content (75.99 µg gut^{-1}) was observed in *H.armigera* reared on cotton plants, among all strains studied in the present investigation (Table 1). In electrophoratic banding pattern, pigeonpea and chickpea fed larvae showed maximum numbers of protein bands, in which pigeonpea has 2 prominent dark bands (P3 and P4) (Fig. 1).



(a) GST isozymes from midgut of *H.armigera*
Lane 1- Cotton strain,
Lane 2- Pigeonpea strain
Lane 3- chickpea strain
Lane 4- Diet strain and
Lane 5- Std, Mol.Wt Marker

(b) Protein bands from midgut of *H.armigera*
Lane 1- Cotton strain,
Lane 2- Pigeonpea strain
Lane 3- chickpea strain,
Lane 4- Diet strain and
Lane 5- Std, Mol.Wt Marker (Fermentas)

Fig. 1 : Electrophoretic profile of GST and soluble proteins from the gut of *H.armigera* reared on different hosts

Toxicity studies:

Table 2 shows the toxicity data of different insecticides in *H.armigera* reared on different hosts. LDP assays showed that chickpea imparted higher resistance ratio of 8.81 in pigeonpea reared strain than artificial diet reared larvae. Chickpea reared strain showed resistance ratio of 4.51 fold and cotton strain found comparatively susceptible among different hosts studied in present investigation.

Cotton strain possesses lower resistance ratio (1.25 fold) against spinosad in comparison with diet strain. Chickpea was found to impart higher resistance (1.98 fold) in *Helicoverpa* followed by pigeonpea (Table 2).

LDP assay of emamectine benzoate in diet reared strain showed lowest (1.92 ppm) LC_{50}. Chickpea reared strain found resistant (2.34 fold) over diet reared strain. Pigeonpea reared strain exhibited resistance of 1.64 fold and cotton strain upto1.26 fold (Table 2).

Table 1 : Induction of glutathione S-transferase and protein in gut of *H.armigera* reared on different hosts

Strain	GST		Protein	
	µM mg protein^{-1} min^{-1} (±SE)	Fold increase over diet	µg gut^{-1} (±SE)	Fold increase over diet
Cotton	0.93 (0.01)	1.21	75.99 (0.94)	0.77
Pigeonpea	1.46 (0.05)	1.89	82.26 (0.23)	0.83
Chickpea	1.77 (0.05)	2.29	79.68 (0.45)	0.80
Diet	0.77 (0.05)	-	98.59 (0.36)	-

Pigeonpea fed *H.armigera* larvae reported higher LC_{50} of lambdacyhalothrin about 30.13 ppm. Diet reared population of *H.armigera* showed LC_{50} 10.62 ppm. Chickpea strain showed LC_{50} of 24.55 ppm and cotton fed larvae found comparatively susceptible with 14.2 ppm LC_{50} (Table 2).

During the present investigation pigeonpea was found as good inducer of protein and chickpea was found to be responsible for induction of glutathione s-transeferase in the gut of *H. armigera.* Lower induction of protein and GST in midgut of *H.armigera* was observed by cotton. Higher protein content was observed in diet strain. Followed to diet reared strain, pigeonpea reared strain showed higher titer values for midgut protein. Some plant allelochemicals may induce enzyme activity in the midgut of herbivore, which found to be associated with resistance mechanism. Malarvannan and Subashini (2007) found more protein in haemolymph of *H.armigera* reared on cotton than larvae which were reared on chickpea and pigeonpea. Further, they concluded that may be due to less accumulation of digested constituents in haemolymph.

The qualitative and quantitative nutritional contents occurring naturally in the host plants would have influenced the insect ability to synthesize the different detoxifying enzymes (Brattsten and Wilkinson, 1977; Yu *et al.*, 1979). This reveals that host-pest interaction depends on the levels of cellular constituents and host also influences the same.

Feeding on host plants exhibits the level of resistance in insects. Some plant species and plant allelochemicals are found to increase the tolerance in insect against various insecticides. Similar results were found by Berry *et al.* (1980), Riskallah *et al.* (1986), Brattsten (1988), and Lindorth (1989) who found the effect of host plant allelochemicals on toxicity of different insecticides. Effect of different cotton cultivars and the effect on toxicity of insecticides and insecticide synergists were reported by David *et al.* (1989). Hoque (1991 and 1992) reported the variation of tolerance to insecticides by insect reared on different diets. Similar results which strongly support our findings have been given by Ghodki *et al.* (2009), which showed that chickpea imparts resistance against indoxacarb in *H. armigera*. It was also observed that chickpea fed larvae showing tolerance to indoxacarb, spinosad and emamectin benzoate. Similar kinds of studies were also reported in various other lepidopteran insect pests. Satpute *et al.* (2003) found okra fed *E. vitella* resistant to all tested insecticides.

LDP assay showed that pigeonpea imparted tolerance in *H.armigera.*Same reports were also documented earlier by Loganathan and Gopalan (1985) against pyrethroids and organophosphates. Similar results were obtained by Chavan (2001), Tikar (1999), Undirwade (2002), Tikar (2003) in *H. armigera* against pyrethroids.

Allelochemicals addition in insect diet also imparts tolerance against insecticides, like gossypol and terpenoid (David *et al.*, 1989). Tannin was found to increase the resistance against pyrethroid when added to *H. armigera* diet, similarly, reverse was found to be true for gossypol

Table 2 : Toxicity of insecticides in *H.armigera* reared on different hosts

Chemical	Strain	LC_{50} ppm (95 % FL)	LC_{95} ppm	Slope (±SE)	Chi-square	Resistant ratio
Indoxacarb	Cotton	2.45 (1.74-3.45)	17.47	1.92 (0.45)	0.203	2.37
	Pigeonpea	4.65 (3.35-6.44)	28.80	2.07 (0.50)	0.936	4.51
	Chickpea	9.08 (6.61-12.47)	56.60	2.08 (0.50)	0.729	8.81
	Diet	1.03 (0.86-1.24)	4.23	2.68 (0.54)	0.769	-
Spinosad	Cotton	21.24 (15.75-28.64)	132.48	2.06 (0.49)	0.229	1.25
	Pigeonpea	30.66 (23.18-40.57)	170.34	2.20 (0.53)	0.201	1.80
	Chickpea	33.81 (27.20-42.01)	165.07	2.38 (0.49)	1.61	1.9
	Diet	16.99 (12.60-22.91)	105.99	2.06 (0.49)	0.229	-
Emamectin	Cotton	2.42 (1.71-3.44)	18.58	1.86 (0.45)	0.118	1.26
benzoate	Pigeonpea	3.15 (2.40-4.15)	16.72	2.27 (0.54)	0.701	1.64
	Chickpea	4.50 (3.25-6.23)	29.71	2.00 (0.49)	0.608	2.34
	Diet	1.92 (1.41-2.61)	12.06	2.04 (0.55)	1.22	-
Lambdacyhalothrin	Cotton	14.2 (11.01-18.42)	68.73	1.87 (0.45)	2.14	1.34
	Pigeonpea	30.13 (26.65-34.07)	66.0	4.83 (0.97)	0.18	2.83
	Chickpea	24.55 (20.16-29.90)	90.29	2.90 (0.69)	0.36	2.31
	Diet	10.62 (8.36-13.48)	69.09	2.03 (0.45)	3.96	-

(Tikar, 2003). This may be the reason that, why cotton fed larvae showed lower LC_{50} for insecticides.

Interactions of host plant induced GST enzymes in *H. armigera* and insecticide tolerance:

Detoxifying enzyme study was found to play important role in resistance development in *H. armigera*. Induced activity of GST by host plants reported to decrease toxicity of insecticide (Berry *et al.*, 1980).

Tolerance in *H. armigera* to emamectin benzoate, avermectin group of insecticides, was found in chickpea reared strain than pigeonpea and cotton reared strain of *H.armigera*. The involvement of increased activity of GST was reported by Lin *et al.* (2009) in *Tetranychus cinanabarinus* against abamectin, avermectin group of insecticides resistance. Abamectin is closely related chemical with the emamectin. The present results also showed that chickpea imparted tolerance to emamectin followed by pigeonpea, which might be due to induced activity for this induced activity of GST in *H.armigera*. GST involved in abamectin resistance in *B. tabaci* (Wang and Wu,2007). Midgut GST activity suppress the toxicity of emamectin benzoate in *H. armigera.*

Spinosad metabolism involves the action of detoxifying enzymes like GST with variable amounts. In present study, chickpea strain imparted higher tolerance than pigeonpea strain followed by cotton reared strain. Chickpea was found to induce higher activity of GST and less induced activity of gut protein. Spinosad tolerance was found to be associated with increased titer values of GST in *P. xyllostella* (Sudhakar, 2005).

Chavan (2001) and Tikar (1999) reported same results of pyrethroid tolerance in pigeonpea fed larvae. Availability of succession hosts to *H. armigera* leads to spread and maintenance of insecticide resistance (Duraimurugan and Reghupathy, 2005). Lambdacyhalothrin resistance was found to be governed by GST in red mites (Kumaral *et al.*, 2009). Report was also available that chickpea field collected strain of *H.armigera* showed low resistance to cypermethrin (Dhingra *et al.*, 1988).

Thus, the response of insects to insecticides may be greatly modified by the presence and concentration of host plant allelochemicals rather than being an independent phenomenon. This suggests that insects use the same system to defend themselves with induced levels of detoxifying enzymes and protein against dietary poison, insecticides and xenobiotics, allelochemicals.

The present investigation gives insight to mechanism of host-pest interaction with induction of detoxifying enzymes and insecticide susceptibility in *H.armigera.* Insecticide tolerance development is found to be associated with host plant induction of detoxifying enzymes in *H.armigera.* This importance of detoxifying enzyme induction and toxicity alteration in *H.armigera* by different hosts on which they feed, should not be neglected while suggesting pest management strategies. By understanding this mechanism of induction in insects, we can set novel targets for pest management which will be helpful to overcome insecticide tolerance development. Considering the immunity developed by insect against insecticides we need to modify the quantity of insecticides applied in field, as each host plays important role in imparting tolerance against insecticides, which is due to induced levels of detoxifying enzymes in gut of insects.

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