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Role of Glutathione-S-Transferase in Imparting Resistance in DBM Against Rynaxypyr

KEYWORDS

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ABSTRACT *Present investigation was undertaken to to know the biochemical mechanism of rynaxypyr resistance in P. xylostella. DBM was selected against rynaxypyr upto seven generations. Selection process resulted in about 34.88 folds resistance to that of homogeneous susceptible F5 generation. Biochemical studies observed increased Glutathione-s-transferase activity by 2.385 fold in resistant strain as compared to susceptible strain. Higher levels of enzymes shown positive correlation with rynaxypyr resistance. Native PAGE demonstrated considerable difference in isozymes pattern.*

INTRODUCTION :

Plutella xylostella commonly called as Diamondback moth is a serious pest of cruciferous crops like cabbage, cauliflower, knoll khol, radish etc. It is one of the most widely distributed insect in the world being reported from more than eight countries (Mohan and Gujar, 2003). The diamondback moth was first recorded in India in 1914 infesting cruciferous vegetables (Fletcher, 1914). The larva of this insect feeds on the foliage of cruciferous plant from the seedling stage to harvest and greatly reduce the quality and yield of produce. The first incidence of diamondback moth resistance in India was reported against DDT (Verma and Sandhu, 1968).

Glutathione-S-transferase is a family of multifunctional isozymes found in all eukaryotes, one of the major functions of GST is to catalyse xenobiotics, including pesticides, resulting in elimination of toxic compounds. In insects it has major role for neutralizing or detoxifying the insecticides (Renuka et al., 2003).

Rynaxypyr controls insect pest through a new mode of action. It activates Rynodine receptors (RyRs) Rynodine receptors are the distinct class of ligand gated calcium channels controlling the release of calcium from intracellular stores. Rynaxypyr, the anthranilic diamide, developed by Dupont Co. Ltd. act on these rynodine receptor (Settele et al., 2008).

MATERIAL AND METHODS:

Rearing of insect

The insects were reared on mustard seedlings , methods used to rear larvae and adults were essentially as described by Liu and Sun (1984). Continuous colonies of Plutella xylostella from different geographical locations were reared in the laboratory under controlled conditions of temperature 25°C ± 2°C, 75 ± 5 per cent relative humidity and photoperiod of 13 hrs light : 11 hrs dark. Mustard seeds were soaked for 12-24 hrs in water and then sown in plastic cups with soil rite/ peat moss and coir peat (20-25 seeds/cup).The seedlings were placed in mating chamber for oviposition once in two days . The adults were provided with liquid adult diet. After hatching, the neonate larvae mine into the mustard seedlings. Subsequently, the larvae were transferred to fresh seedling.

Selection procedure

Leaf dip method of bioassay as described by Tabashnik et al. (1987) was adopted in the present studies. Cabbage leaves were first washed with distilled water containing 0.1 per cent Triton X-100 and dried for about 1 hrs. Cabbage leaf disc (5 cm diameter) were cut and then dipped in a test solution for 10 seconds. The leaf disc was placed for about 2 minutes over a blotting paper in a tray to drain excess solution at room temperature. Ten third instar larvae (5 days old) were released on each disc in an individual petriplate where in blotting paper was placed at the bottom. Three replications were used for each concentration. The bioassay were conducted at room temperature. Similarly ten larvae were released on cabbage leaf disc dipped in water only, which was treated as control.

Preparation of enzyme

The third instar larvae (weighing 3.0 - 4.0 mg approximately were separated and starved for 7-8 hours to remove all digested food particles. Whole larvae were homogenized using mortar and pestle in sodium phosphate buffer (PB) (100 mM, pH 6.5), containing 0.1 mM of EDTA, PTU and PMSF each, insects were chilled in refrigerator before homogenization. The homogenate thus obtained was centrifuged at 10,000 rpm for 15 minutes at 4°C in high speed refrigerated centrifuge, solid debris and cellular material was discarded. The resultant post mitochondrial supernatant obtained was stored at -20°C and used as enzyme source. The protein was estimated by Bradford method (1976).

Glutathione s transferase quantification

GST quantification was carried out by method described by kao et al (1989). 50 µl of 50 mM1-chloro -2-4 dinitrobenzene (CDNB) and 150 µl of 5 mM reduced glutathione (GSH) were added in 2.77 ml of PB (100 mM, pH 6.5 with 0.1 mM PTU). After adding 30 µl of enzyme stock to the above mixture the content was shaked gently and incubated for 2-3 minutes, at 25°C. The content was transferred into a 4 ml cuvette and placed in the samplke cuvette slot of the spectrophotometer (Hitachi, U 2001). The absorbance for 5 min at 340 ηm was recorded. The increase in absorbance over 5 minutes for calculation was taken into consideration. The samples were taken in triplicate. The enzyme activity was calculated as

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

µMmg protein^{-1} min^{-1}

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

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Staining for GST activity on native PAGE

Native 10% PAGE was run at 4°C for 4-5 hrs until the running front reaches the bottom of the gel. No SDS was added to any of the component of electrophoresis.

Staining solution-I : 100 ml 0.1 M sodium phosphate buffer, pH 6.5, containing 5.0 mM reduced glutathione and 1 mM each of CDNB (1-chloro-2, 4-dinitrobenzene) and Nitro Blue Tetrazolium (NBT)

Staining solution-II : 100 ml Tris HCL buffer, pH 9.6 containing 4 mM Phenozine methosulfate (PMS).

RESULTS

Median lethal Concentration of Rynaxypyr Against P. xylostella at F5 Generation

For determining LC_{50} value the homogeneous F_5 population was subjected to log dose probit (ldp) assay by leaf dip method of rynaxypyr against third instar larvae of P. xylostella. The data in the Table 8 indicated that LC_{50} value of 0.057 ppm was observed for rynaxypyr against third instar P. xylostella larvae of F_5 generation.

Table 1. Toxicity of rynaxypyr to homogeneous population of Plutella xylostella (F5 generation)

Insecticide	Probit analysis parameters				
	LC_{50} (ppm)	LC_{90} (ppm)	Fiducial limits of LC_{50}	Slope	Regression equation
Rynaxypyr	0.0575	0.7244	0.0366-0.0904	1.1647	Y = 6.445 + 1.1647X

Development of Resistance to Rynaxypyr

Generationwise studies against rynaxypyr revealed that the resistance increased with increase in the number of selection regimes under insecticide pressure. The LC_{50} value of F_{11} selected population of P. xylostella against rynaxypyr was found to be 2.0059 ppm. It was 34.88 fold greater as compared to unselected F_5 population of P. xylostella, (Table 2).

Table: 2 Selection responses of P. xylostella to rynaxypyr over generations

Sr. No.	Selected generation	Probit analysis parameters						
		LC_{50} (ppm)	LC_{90} (ppm)	Fiducial limits of LC_{50}	Chi. Square	Slope	Regression equation	Resistance ratio
1)	F_5	0.0575	0.7244	0.0366 - 0.0904	6.4320	1.1647	Y = 6.445 + 1.1647X	-
2)	F_6	0.1553	1.1395	0.1035 - 0.2287	4.4306	1.4809	Y = 6.1979 + 1.4809X	2.700
3)	F_7	0.6772	1.3810	0.5789 - 0.7922	2.7839	4.1420	Y = 5.7011 + 4.1420X	11.77
4)	F_8	1.0270	1.6884	0.9346 - 1.1303	1.9754	5.9465	Y = 4.9292 + 5.9465X	17.86
5)	F_9	1.2310	2.4054	1.0809 - 1.4019	2.5066	4.4062	Y = 4.6023 + 4.4062X	21.40
6)	F_{10}	1.7382	2.973	1.5431 - 1.9580	2.7200	5.4965	Y = 3.6803 + 5.4965X	30.22
7)	F_{11}	2.0059	3.9492	1.7935 - 2.2434	0.6498	6.6639	Y = 2.9854 + 6.6639X	34.88

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Table.3 Comparison of rynaxypyr resistance and GST activity in P. xylostella

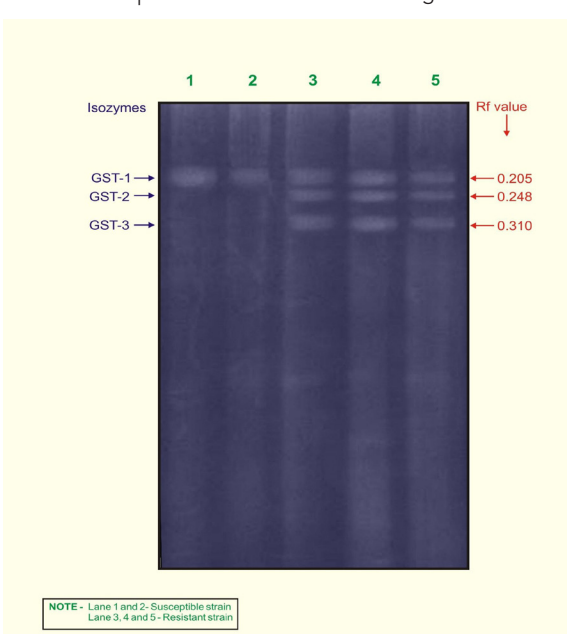
Sr. No.	Strain	LC_{50} (ppm)	Fold increase in LC_{50}	GST activity (µM mg protein^{-1} min^{-1}) (±SE)	Fold increase in GST activity
1)	Resistant	2.0059	34.88	4.202 ± 0.08	2.385
2)	Susceptible	0.0575	-	1.762 ± 0.06	-

Glutathione-S-transferase plays an important role in imparting resistance to insects. In present study the level of GST is varied in susceptible and resistant (selected) population. It was found that rynaxypyr selection pressure resulted in 2.385 fold increase in level of GST activity. In susceptible strain it was 1.762 µM mg protein^{-1} min^{-1} while in resistant strain it was found to be 4.202 µM mg protein^{-1} min^{-1}.

Electrophoretic pattern of Glutathione-S-transferase from whole body homogenate of rynaxypyr resistant and susceptible strain of P. xylostella

The native PAGE of homogenates prepared from whole body homogenates of rynaxypyr resistant and susceptible strains was done, the electrophoretic pattern is presented in Plate1and in the Table

The study shows three GST isozymes having Rf values of 0.205, 0.248 and 0.310 denoted as GST-1, GST-2 and GST-3 respectively. According to mobility towards anode resistant and susceptible strains showed variation in banding pattern GST-1 with Rf value 0.205 was found in resistant as well as susceptible strain, while GST-2 and GST-3 were seen only in resistant strain. This might be happened due to induced expression of GST-2 and GST-3 genes.



GST isozyme pattern from whole body homogenate of Plutella xylostella

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Table4. Glutathione-S-transferase isozyme analysis of whole body homogenate of rynaxypyr resistant and susceptible strain of Plutella xylostella

Isozymes (Rf value)	Resistant strain	Susceptible strain
GST-1 (0.205)	+(D)	+(D)
GST-2 (0.248)	+(L)	-
GST-3 (0.310)	+(D)	-

Where,

L :	Light band	,	+ :	Present
D :	Dark band	,	- :	Absent

Discussion:

The diamondback moth has an extraordinary propensity to develop resistance to every insecticide used to control it in crucifers. Indiscriminate use of insecticide ,multiple generations of DBM per annum. Nirmal and Singh (2001) reported the development of resistance in DBM to the extent of 198-615 fold resistance to cypermethrin and 590 - 4576 fold resistance to fenvalerate. Cheng et al. (1981) reported 11 - 70 fold cypermethrin resistance to DBM in field condition.

Patil (2009) reported the development of resistance in DBM to the extent of 44.54 fold to insecticide emamectin benzoate after seven selected generations.

As rynaxypyr is a newly introduced insecticide in the Indian farming system. The reference regarding resistance to this molecule is not available. From past study of some insects on different molecules it is showed that when the insect get exposed to the molecule the resistance increased by many folds when the selection pressure is applied, same phenomenon get reported in this study.

Sannaveerappanvar (1995) reported 2.08 fold increase in GST activity in cypermithrin resistant DBM population from field as compared to susceptible strain. Similar results were reported by Moharil (2004) where 3.83 fold increase in GST activity was found in cyprmethrin resistant DBM.

Dukre (2007) reported 3.48 fold increase in GST activity in fenvalerate resistant population of DBM as compared to susceptible.

Patil (2009) reported 2.60 fold increase in GST activity in emamectin benzoate resistant strain of DBM as compared to susceptible when selection pressure given upto 10 generations.

Patil (2009) reported the presence of three GST isozymes out of which GST-2 and GST-3 (Rf values 0.30 and 0.31 respectively) were present in the emamectin benzoate resistant strain while susceptible strain showed only one i.e. GST-1 isozyme with Rf value 0.29.

Dukre (2007) observed two GST bands in SDS-PAGE of fipronil and cypermethrin resistant strains of P. xylostella. GST having molecular weight of 26 kDa and 32 kDa in DBM-FR strain, 25.2 kDa and 30 kDa in DBM-CR strain while susceptible strain showed 20.01 kDa and 34 kDa GST bands, it indicated heterodimer nature of GST.

In Drosophila melanogaster, GST enzyme showed two conspicuous protein bands in 12 percent SDS gel electrophoresis of purified enzyme from larvae, pupae and adult. Protein band with molecular weight of 23 kDa and 35 kDa were observed in the purified adult enzyme, (Hunaiti et al., 1995).

Thus, polymorphism obtained in GST isozymes pattern in present investigation can be exploited as a marker to differentiate rynaxypyr resistant and susceptible DBM population in its early stages of infestation and can be helpful to formulate the best IPM and IRM strategies.

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