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Role of Glutathion-S-Transferase in Imparting Resistance in Plutella Xylostella (L.) Against Flubendiamide

KEYWORDS: Plutella xylostella, flubendiamide, Glutathion-S-Transferase, Insecticidal resistance.

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ABSTRACT *Present investigation was undertaken to biochemical study of Glutathion-S-Transferase between the resistance and susceptible strain of Plutella xylostella against flubendiamide. During development of resistance to flubendiamide, their was increase in resistance as generation wise. The resulting in development of resistance 16.62 fold at F10 generation. Similarly, In biochemical studies observed that increased level of Glutathion-S-Transferase (1.45 fold) in DBM resistant strain as compared to susceptible strain. From this experiment, confirmed that the involvement of GST enzyme play significant role in development of resistance to flubendiamide in Plutella xylostella.*

INTRODUCTION

Cruciferous are most important vegetable crop in human diet due to their economical as well as nutritive value (Talekar, 1992). But in recent year production of Cruciferous has been seriously affected by steady increase in pest such as *Plutella xylostella*. *Plutella xylostella* (L.) (Lepidoptera: plutellidae) shows resistivity to every class of insecticide used against it (Shelton *et al.*, 2000). Flubendiamide is new to crop protection developed by Nihon Nohyaku Co. Ltd., (Tokyo, Japan), represents as a novel class of insecticides, it disrupts proper muscle function in insects such as lepidopterious pest. Flubendiamide belongs to class of chemical pthalic acid diamides which act on rynodine receptors that massive release of Ca^{2+} from intracellular stores, which is an essential step in the muscle contraction process (Ebbinghaus *et al.*, 2007). Naturally Insect has system of detoxification of xenobiotics and chemical insecticides It carried out by detoxifying enzymes (Ugale, 2009). Glutathione-S-transferases (GST) are phase II detoxification isozymes. GST has been found to be involved in development of resistance in many insect against various insecticide groups.

MATERIALS AND METHODOLOGY

The objectives of this investigation was to developed resistance to insecticide and determine level of detoxifying enzyme associate with flubendiamide. Population of Plutella xylostella from different geographical locations from Akola District. (M. S. India). Rearing was done in Entomology Department, Dr. PDKV, Akola. Further studies were done at Biotechnology Centre, Dr. PDKV, Akola during the session 2010-11. The rearing procedure described by Lu and Sun, (1984) was followed. Continuous colonies of Plutella xylostella were reared in the laboratory under controlled conditions. Rearing done for upto F4 generations for establishing homogeneous laboratory population. The pupae were kept in oviposition chamber keeping mustard seedling so that adult emerged can utilize the seedling as oviposition substrate. The adults were provided with adult diet. After hatching, the neonate larvae feed on the mustard seedlings. Subsequently, the larvae were transferred to fresh seedling. Leaf dip method of bioassay as described by Tabashnik et al., (1987). In the present studies using cabbage leaves for assessing the resistance levels. Sterilized cabbage leaf disc about 5 cm diameter were dipped in a test solution for 10 seconds and dried it. Ten third instar larvae were released on each disc in an individual petriplate containing cabbage leaves where in blotting paper was placed at the bottom. Three replications were used for each concentration. Similarly For control ten larvae were released on cabbage leaf disc dipped in water only. The median lethal concentration LC_{50} value of insecticide flubendiamide determined by data subjecting to log probit analysis. For development of resistance to flubendiamide, the survival population after insecticide treatment had carried out on further high dose treatment upto maximum possible generations under continuous selection pressure of flubendiamide and the strain exhibiting high resistance was considered as resistant strain. Detection of insecticide resistance, as the resistance ratio (RR), calculated by the formula (Regupthy and Dhamu, 1990) LC_{50} of resistant strain (RS) divided by LC_{50} of susceptible strain (SS).

Enzyme Preparation

The third instar larvae (4.0 mg) were starved and chilled in refrigerator. Whole larvae were homogenized in sodium phosphate buffer (100 mM, pH 6.5). Buffer containing 0.1 mM of EDTA, PTU and PMSF each. The homogenate was centrifuged at 10,000 rpm for 15 minutes at 4°C. The resultant supernatant obtained was stored at -20°C and used as enzyme source, The protein was estimated by Bradford, (1976).

GST Quantification

Kao et al., (1989) described GST Quantification method. As 150 µl of reduced glutathione (GSH) were added in 2.79 ml PB (100 mM pH 6.0, 0.1 mM PTU), 50 mM 1-chloro-2, 4-dinitrobenzene (CDNB) added into it. 10 µl of enzyme stock of susceptible and resistant strains of the DBM was added in above mixture. The contents were gently shaken and incubated for 2 to 3 minutes at 20°C. Reaction was carried out in triplicate set. Control also took without enzyme source. It transferred to cuvette placed in sample cuvette slot in UV spectrophotometer. 3 ml of the reaction mixture used for taking absorbance. Absorbance was read at 340 nM in spectrophotometer. The GST activity was calculated as follows.

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

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

REASULTS AND DICUSSIONS

For determining LC_{50} value of homogeneous population, It was subjected to log dose probit analysis by leaf dip assay against third instar larvae of *P. xylostella*. This indicated that increased resistance with increase in the number of selection under insecticidal pressure. The LC_{50} value of F_5 population

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of *P. xylostella* against flubendiamide was found to be 0.38 ppm and in F_{10} selected population of *P. xylostella* against flubendiamide was found to be 6.35 ppm. It was 16.62 fold greater as compared to F_5 population of *P. xylostella*. It indicated in Table 1. Generation wise studies against flubendiamide revealed that the resistance increased with increase in the number of selection regimes under insecticide pressure. It correlated with Patil, (2009) reported the development of resistance in DBM to the extent of 44.54 fold to insecticide emamectin benzoate after seven selected generations.

Quantification of Glutathione-S-transferase

In Insecticidal treatment Glutathione-S-transferase plays an important role in imparting resistance to insects. In present study, the level of GST was increased in resistant as compare to susceptible population. It was found that, flubendiamide selection pressure increased in 1.45 fold GST activity of resistance DBM strain. In resistant strain GST activity was found to be 22.74 µM mg protein^{-1} min^{-1} while in susceptible strain 15.74 µM mg protein^{-1} min^{-1},. Glutathione-S-transferase plays an important role in imparting resistance to insects. (Results shown in table 2). This results correlated with Moharil, (2004) reported that 3.83 fold increase in GST activity was found in Cypermethrin resistant DBM as compared to susceptible and also Dukre, (2007) reported that 3.48 fold increase in GST activity in fenvalerate resistant population of DBM as compared to susceptible when selection pressure applied upto eight generation.

Table 1. Probit analysis result on LC_{50} and Resistance ratio values after to *P. xylostella* against flubendiamide

Sr. No.	Selected generation	Probit analysis parameters			
		LC_{50} (ppm)	Chi. Square	Slope	Resistance ratio
1)	F_5	0.38	1.37	0.61	-
2)	F_6	0.24	3.83	1.47	0.64
3)	F_7	0.79	1.79	1.58	2.07
4)	F_8	1.68	2.74	2.12	4.41
5)	F_9	5.12	1.41	1.87	13.38
6)	F_{10}	6.35	3.33	2.22	16.62

Table 2. Comparison of flubendiamide resistance and GST activity in *P. xylostella*

Strain	LC_{50} (ppm)	GST activity (µM mg protein^{-1} min^{-1}) (±SE)	Fold increase in GST activity
Resistant strain	6.35	22.74±0.54	1.45
Susceptible Strain	0.38	15.74±1.84	-

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