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Purification of Carboxylesterase Resistance to Flubendiamide in Plutella xylostella (L.)

KEYWORDS: Plutella xylostella, flubendiamide, Carboxylesterase, ammonium salt precipitation, size exclusion chromatography

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ABSTRACT *Present investigation was undertaken to study how Carboxylesterase play significant role in flubendiamide resistance in Plutella xylostella (Diamond back moth). The resistance strain was developed upto F10 generation resistance was increased to 16.62 fold. In ammonium salt precipitation method, CarE activity was found 18.5 and 9.31 fold pure in susceptible and resistance strain respectively. In size exclusion chromatography 30-60 percent saturation fraction of fraction 1 observed high specific activity than other fraction as 0-30 and 60-100 percent.*

Introduction

The diamondback moth, *Plutella xylostella* (L.) (Lepidoptera: plutellidae) is an oligophagous pest. The larva of this insect feeds on the foliage of cruciferous plant from the seedling stage to harvest and greatly reduce both yield 4 to 100 % and quality of the produce (Talekar and Sheltan, 1993). Dimondback moth has an extraordinary properties to develop resistance to every synthetic insecticide used to control in cruciferous. Flubendiamide belongs to class of chemical pthalic acid diamides shows extramly high activity against a broad spectrum of lepidopterious pest. It developed by Nihon Nohyaku Co. Ltd., (Tokyo, Japan) (Ebbinghaus, 2007). It disrupts proper muscle function in insects and therefore represents a unique and novel mode of action (Masaki et al. 2006). The study of detoxifying enzyme such as carboxylesterase (CarE), its behaviour with available synergist for enhancing the toxicity of insecticide and increases susceptibility in insect for control pest population (MaGhee, 1987).

MATERIALS AND METHODS

Under this study purification of CarE associated with flubendiamide resistance was carried out. The rearing procedure described by Lu and Sun (1984) was followed to maintained the culture of *P. xylostella*. Continuous colonies of *Plutella xylostella* from different geographical locations of Akola (MS) were reared in the laboratory under controlled conditions of temperature 25 ± 2°C and humidity 75 ± 5 percent. This studies were done at Biotechnology Centre, Dr. PDKV, Akola during the session 2010-11. Resistance ratio (RR) calculated by the formula (Reguppthy and Dhamu, 1990) as LC_{50} of resistant strain (RS) divided by LC_{50} of susceptible strain (SS). The third instar larvae were used for enzyme preparation (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. Centrifuged homogenate 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).

Purification of caroxylestarase

First purification step is Ammonium sulphate fractionation, The crude enzyme extract was brought to 30 percent saturation with gradual addition of solid ammonium sulphate. Constant stirring for 1h at 4°C Ammonium sulphate was slowly added to aliquot. The resulting precipitate was collected by centrifugation at 6000 rpm for 30 min (Roe, 2001). Pellet was resuspended in buffer containing sodium phosphate buffer pH 6.5, centrifuged at 10,000 rpm for 1h (Wang et al., 2007). The above step was repeated twice to obtain 60 percent and 100 percent ammonium sulphate saturation. Protein concentration was determined by the method of Bradford (1976). The supernatant obtained in the above step then loaded on to a Bio-Sil SEC-125 column (80×7.8cm) equilibrated with the buffer (pH 8.0) containing 10 mM Tris-HCl. The fractions eluted from this column containing protein peak were collected. Protein concentration was determined by the method of Bradford (1976).

RESULTS AND DISCUSSION

Present investigation was successful attempt, to study the and purification of CarE associated with flubendiamide. Generation wise studies against flubendiamide revealed that the resistance increased with increase in the number of selection regimes under insecticide pressure. The LC_{50} value of F_{10} selected population of *P. xylostella* against flubendiamide was found to be 6.35 ppm. The LC_{50} value was increases to 16.62 fold as compared to unselected F_5 population of *P. Xylostella* same as 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.

Purification by ammonium salt fractionation

CarE purified from the crude enzyme obtained from third instar learvae of DBM. Crude enzyme obtained 0.023 mg of protein and CarE specific activity is 0.001 µM mg protein^{-1} min^{-1} of protein in susceptible strain and 0.0024 µM mg protein^{-1} min^{-1} activity in resistant strain. The enzyme source first partially purified and fractionated by ammonium salt precipitation (ASP). Due to gradual addition of ammonium sulphate responsible for protein precipitated. Partial purification was carried out in three fractions namely 0-30 percent, 30-60 percent, and 60-100 percent (ASP). In this step, detoxifying enzymes specific activity and fold purity were increased. In 0-30 percent saturation fractions, detoxifying enzyme CarE activity for susceptible strain was 0.005 µM mg protein^{-1} min^{-1} and for resistance strain was 0.002 µM mg protein^{-1} min^{-1} . In 30-60 percent saturation fractions showed high CarE activity for susceptible strain was 0.018 µM mg protein^{-1} min^{-1} and for resistance strain was 0.022 µM mg protein^{-1} min^{-1} . In 60-100 percent saturation fractions, detoxifying enzyme CarE activity for susceptible strain was 0.003 µM mg protein^{-1} min^{-1} and for resistance strain was 0.006 µM mg protein^{-1} min^{-1} (Table 1, Fig. 1). In CarE 30-60 per cent fraction contain high molecular weight proteins. This result correlated with Haubruge et al. (2002) reported that purification of carboxylestarase by 2.57 fold by ammonium salt saturation fraction from *Tribolium castaneum*.

Purification By Molecular exclusion chromatography

High moleculer weight protein of ASP fractions were loaded on to Bio-Sil SEC 125 column and the eluted fractions were

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collected. The fractions obtained were tested for their CarE activity. In 30-60 per cent saturation fraction of CarE gave high purity were loaded in column. The specific CarE activity of fraction 1 was 0.032 µM mg protein^{-1} min^{-1} in susceptible strain and 0.097 µM mg protein^{-1} min^{-1} in resistance strain. Resistance strain 97 fold purity and susceptible strain 32 fold purity. In CarE 30-60 per cent ammonium sulphate precipitated fraction 1 showed high specific activity as compared to fraction 2. From this concluded that fraction 1 showed high moleculer weight containing high specific activity. Result shown in table (Table 2, Fig 2) result same as Haubruge et al. (2002) reported that purification of purification of carboxylestarase by size exclusion chromatography is 2.42 fold in malathion resistance of *Tribolium castaneum*.

Table 1: Purification of Caroxylestarase from Plutella xylostella by ammonium salt precipitation method

Sr. No.	Ammonium sulphate precipitated fraction (%)		Protein (ug/ul)	Caroxylestarase specific activity (µM mg protein^{-1} min^{-1})	Fold purity
1	0-30	Susceptible	0.012	0.005	5.64
		Resistance	0.014	0.002	1.2
2	30-60	Susceptible	0.002	0.018	18.5
		Resistance	0.010	0.022	9.31
3	60-100	Susceptible	0.007	0.003	3.12
		Resistance	0.010	0.006	2.8

Table 2. Purification of CarE Activity by Size exclusion Chromatography

Strain	Fraction	Protein (ug/ul)	Enzyme activity(µM mg protein-1 min-1)	Fold purity
Susceptible	Fraction 1	0.014	0.032	32
	Fraction 2	0.07	0.006	6
Resistance	Fraction 1	0.003	0.097	97
	Fraction 2	0.09	0.003	3

Fig. 1: Partial Purification of Caroxylestarase from Plutella xylostella by ammonium salt precipitation method

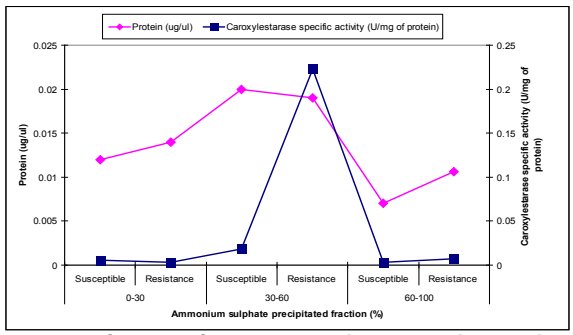
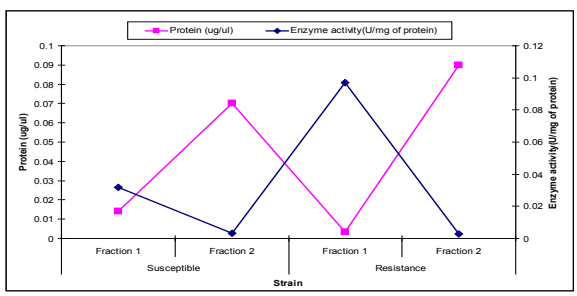


Fig 2. Purification of CarE Activity by Size exclusion Chromatography



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