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Subcellular localization of glutathione s transferase in *Papilio demoleus* larvae

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Abstract
GST activity was determined in different subcellular fraction of laboratory reared and field collected fifth instar larvae of *P. demoleus.* All the subcellular fractions and crude homogenate of field collected *P. demoleus* showed high levels of GST than laboratory reared *P. demoleus.* Nucleus fraction of laboratory reared *P. demoleus* showed 6.48 µM mg protein^{-1}min^{-1} and field collected *P. demoleus* showed 7.26 µM mg protein^{-1}min^{-1} GST activity, which was 1.12 fold higher. Mitochondrial fraction of laboratory reared and field collected *P. demoleus* showed 15.63 and 24.79 µM mg protein^{-1}min^{-1} enzyme activity. Mitochondrial fraction of field collected *P. demoleus* showed 1.58-fold increase in GST activity. Microsomal fraction of field collected *P. demoleus* showed 3.77-fold increase in GST activity. Microsomal fraction of laboratory reared *P. demoleus* showed 17.41 µM mg protein^{-1}min^{-1} GST activity and field collected *P. demoleus* showed 65.73 µM mg protein^{-1}min^{-1}. Cytosolic fraction of laboratory reared and field collected *P. demoleus* showed 58.72 µM mg protein^{-1}min^{-1} and 394.49 µM mg protein^{-1}min^{-1} GST activity, respectively. Thus, field collected *P. demoleus* showed 6.71-fold increase in GST activity in the cytosolic fraction.
Crude homogenate of laboratory reared and field collected *P. demoleus* showed 87.36 µM mg protein^{-1}min^{-1} and 579.16 µM mg protein^{-1}min^{-1} GST activity, respectively. The increase in GST activity in crude homogenate is almost 6.62 fold in field collected.
Thus, from above study it is clearly depicted that increased GST activity in crude homogenate was mostly attributed to increased GST activity in the cytosolic fraction. It can also be suggested that, primary location of GST is cytoplasm.

Keywords: *Papilio demoleus*, Glutathione S Transferase, subcellular fractions, xenobiotics, resistance

1. Introduction
The swallowtail lemon butterfly, *Papilio demoleus* is one of the major insect pests of citrus, and is found to attack the citrus plants in nurseries as well as in the orchards (Dohrrey and Butani, 1985; Singh and Singh, 1999; Narayanamma *et al.*, 2003) [4, 12, 8]. This pest was found in the orchards throughout the year and causes much more damage during three different seasons viz. summer, rainy and winter when the orchards put forth new shoot growth for blossoming (Arya Sunita, 2003) [1].
In the past, efforts were made to control this pest with chemical pesticides like dimethoate (Rogor), endosulfan, monocrotophos, etc. (Vyas, 1994; Shivankar and Singh, 1999) [13, 11]. But each and every life in the universe has been provided with a defence mechanism against toxicants, xenobiotics etc, without which it would not have been possible for them to survive. The mechanism by which insect pests develop resistance to insecticides involves the production of large amounts of detoxifying enzymes. Among them, Glutathione S Transferase was found to be involved in developing the insecticide resistance in many lepidopteran pests (Balabaskara *et al.*, 1989; Rajurkar and Khan, 2001) [2, 10]. Biotransformation of chemicals by the addition of glutathione, a reaction catalyzed by GST, is one of the most versatile protective mechanisms in eukaryotic cells. The enhanced water solubility of compounds, after the addition of glutathione, facilitates their excretion from cells and thereby prevents their accumulation in the body (Mohammed and Ahmed, 2007) [7]. The amount of detoxifying enzyme GST present in the insect body indicates the resistance power of insects to different groups of insecticides especially organophosphates. High enzyme activity is an important factor in development of tolerance to insecticide in the fields and thus, a serious problem in insect control (Zhang *et al.*, 2000) [14].

Mohammed and Ahmed (2007) [7] in their study showed that various organs of the Dhub possess variable amounts of GSH, GSSG and GST activity, which reflect one of the major pathways of detoxification of xenobiotics. They also confirmed the fact that the liver is the major organ responsible for the detoxification of various exogenous and endogenous toxicants in reptiles and had the highest antioxidant enzyme activity to counteract the oxidative damage.

2. Methodology

2.1 Isolation of GST from subcellular fractions of fifth instar larvae: For study of the subcellular localization of GST, 10 fifth instar larvae were homogenized at 1500 rpm in the teflon tissue homogenizer in 2 ml of ice-cold sodium phosphate buffer (0.1 M pH 6.5) containing 0.1 M of EDTA, PTU, PMSF each and 10% glycerol. The crude homogenate was centrifuged at 470 x g for 10 min at 4°C to precipitate nuclei and cell debris, and the supernatant was decanted, and centrifuged at 10,000 x g for 20 min at 4°C to precipitate the mitochondrial fraction. The microsomal fraction was obtained by centrifuging the supernatant again at 105,000 x g for 90 min at 4°C. Supernatant at this step was used as cytosol fraction. After each step, supernatant was taken into fresh microcentrifuge tube to isolate next cell organelle fraction and the pellet from each centrifugation step was resuspended in 5 ml of 0.05 M phosphate buffer (pH 6.5) containing 0.5% (vol : vol) triton X-100 to aid in solubilizing the membrane bound enzymes.

2.2 Estimation of GST: Estimation of GST was carried out by the method described by Kao *et al.* (1989) [5]. Following protocol was followed:

1) 50 µl of 50 mM1 chloro-2, 4 dinitrobenzene (CDNB) and 150 µl of 50 mM reduced glutathione (GSH) were added in 2.77 ml (2770 µl) of phosphate buffer (100 mM pH 6.5, 0.1 mM PTU).
2) 30 µl of enzyme stock was added in the above mixture. Reaction was carried out in triplicate set.
3) The content were gently shaken and incubated for 2 minutes at 24ºC and then transferred to cuvette placed in sample cuvette slot of the UV Spectrophotometer.
4) 3 ml of the reaction mixture without enzyme was placed in the cuvette present in reference slot.
5) Absorbance was read for 5 min at 340 nm by employing time scan menu of the spectrophotometer.
6) The GST activity was calculated as follows:

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

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

3. Results and disscussion

GST activity was determined in different subcellular fractions of laboratory reared and field collected fifth instar larvae of *P. demoleus* using CDNB as substrate. Differential centrifugation of crude homogenate was carried out in high-speed centrifuge. Different subcellular fractions were isolated and reconstituted in 5 ml of ice-cold sodium phosphate buffer (0.05 M pH 6.5) containing 0.5% triton X - 100 (vol: vol) to aid in solubilizing the membrane bound enzymes.

From the data represented in the table 30, it can be clearly depicted that all the subcellular fractions and crude homogenate of field collected *P. demoleus* showed high levels of GST than laboratory reared *P. demoleus*. Nucleus fraction of laboratory reared *P. demoleus* showed6.48 µM mg protein^{-1}min^{-1} and field collected *P. demoleus* showed 7.26 µM mg protein^{-1}min^{-1} GST activity, which was 1.12 fold higher. Mitochondrial fraction of laboratory reared and field collected *P. demoleus* showed 15.63 and 24.79 µM mg protein^{-1} min^{-1} enzyme activity, respectively. This fraction of field collected *P. demoleus* showed 1.58 fold increase in GST activity. Microsomal fraction of field collected *P. demoleus* showed 3.77 fold increase in GST activity. This fraction of laboratory reared *P. demoleus* showed 17.41 µM mg protein^{-1}min^{-1} GST activity and field collected *P. demoleus* showed 65.73 µM mg protein^{-1}min^{-1}. Cytosolic fraction of laboratory reared and field collected *P. demoleus* showed 58.72 µM mg protein^{-1} min^{-1} and 394.49 µM mg protein^{-1}min^{-1} GST activity, respectively. Thus, field collected *P. demoleus* showed 6.71-fold increase in GST activity in the cytosolic fraction.

Crude homogenate of laboratory reared and field collected *P. demoleus* showed 87.36 µM mg protein^{-1} min^{-1} and 579.16 µM mg protein^{-1}min^{-1} GST activity, respectively. Increase in GST activity was almost 6.62 fold in field collected as compared to laboratory reared *P. demoleus*. Thus, from above study it can be clearly depicted that increased GST activity in crude homogenate was mostly attributed to increased GST activity in the cytosolic fraction. It can also be suggested that, primary location of GST is the cytoplasm (Fig. 1).

Lei and Peng (1990) [6] in a study on subcellular distribution of glutathione S-transferase (GST) in Chinese fetal liver at 4-8 months of age using 1-chloro-2,4-dinitrobenzene (CDNB) as substrate found GST activity to be 66 +/- 34 nmol/(min.mg protein) and that to mainly in the cytosol. Nishino and Ito (1990) [9] identified that the stimulated glutathione S-transferase activity was localized in mitochondrial and lysosomal fractions besides microsomes in rat liver. Among N-ethylmaleimide-treated sub-mitochondrial fractions, glutathione S-transferase activity was stimulated only in outer mitochondrial membrane fraction. Bhagwat, *et al.* (1998) [3] in their study on differential response of cytosolic, microsomal, and mitochondrial glutathione S-transferases to xenobiotic inducers also showed that cytosolic GST activity using 1-chloro-2, 4-dinitrobenzene (CDNB) as a substrate was 2-4 fold higher than that in the microsomal and mitochondrial fractions. Northern blot analysis also showed an increase in the GST-á mRNA level indicating a possible upregulation of the GST gene expression by the xenobiotic agent. The results suggest selectivity in the effects of different xenobiotics on the expression and catalytic activity of GST isoenzymes from different subcellular compartments of tissues.

Table 1: Distribution of GST activity in the subcellular fractions

Sub cellular fraction	GST-CDNB Conjugation (µM mg protein^{-1}min^{-1})		
	Laboratory reared	Field collected	Field collected / Lab. reared*
Nucleus	6.48 ± 0.12	7.26 ± 0.09	1.12
Mitochondria	15.63 ± 0.15	24.79 ± 0.05	1.58
Microsome	17.41 ± 0.03	65.73 ± 0.06	3.77
Cytosol	58.72 ± 0.08	394.49 ± 0.06	6.71
Crude	87.36 ± 0.12	579.16 ± 0.33	6.62

*Field collected/ Lab. reared: fold increase in enzyme activity

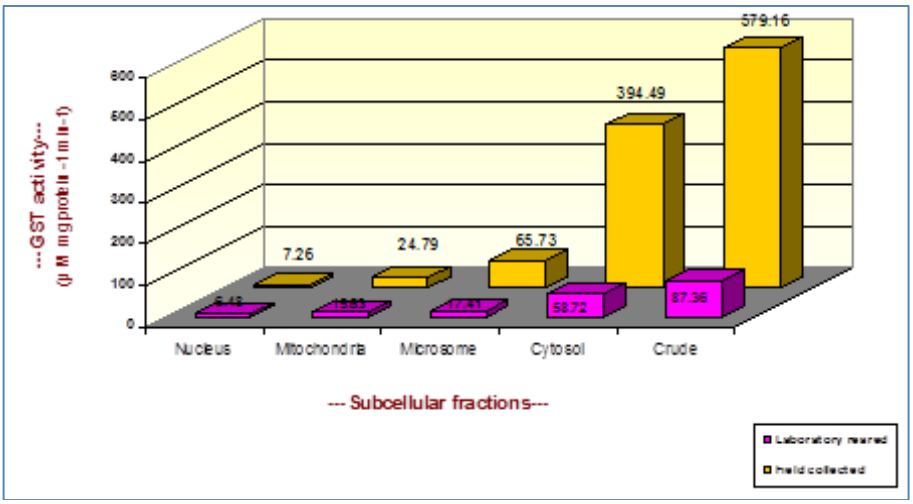

Fig 1: GST activity in different subcellular fractions of *P. demoleus*

4. Conclusion

Major GST activity was located in cytosolic fraction in both laboratory reared and field collected *P. demoleus* and also increase in GST activity was found to be highest (6.71 fold) in this fraction of field collected *P. demoleus*. Thus, it can be suggested that, primary location of GST is cytosol.

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