Comparative Molecular Modeling of Insect Glutathione S-Transferases

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ABSTRACT
The evolution of resistance by an insect to an insecticide may involve several mechanisms. Many studies have shown that insecticide-resistant insects have elevated levels of glutathione S-transferases activity in crude homogenates, which suggests a role for GSTs in resistance. This prompted us to select the GSTs from *H.armigera, L.lineolaris* and *M.sexta* due to their economic importance. The 3D models for the GSTs from the insects were built using Modeller9V7, structure comparison between the GSTs was done using SwissPDBViewer and the models were docked with Piperonyl Butoxide (PB), TagitininC (TC), a phytochemical from *T.diversifolia*, Plumbagin (PL) and a comparative docking analysis was done. The results indicate that the compounds Piperonyl Butoxide was found to be more feasible in terms of docking energy closely followed by Tagitinin C and can be used in sync as potential regulator of insect GST activity.

Keywords: Gluatathione S-Transferase (GST), Homology Modeling, Structure Comparison, RMSD, Docking, Plumbgin, Tagitinin C & Piperonyl butoxide.

1. INTRODUCTION
Insecticides play a central role in controlling major vectors of diseases such as mosquitoes, sandflies, fleas, lice, tsetse flies, and triatomid bugs(1). Insecticide resistance is an increasing problem in many insect vectors of disease. Nearly 500 species of arthropod are now reported to resist insecticides or acaricides of atleast one chemical group, and in numerous cases such resistance renders control difficult or uneconomic over large areas. Resistance is a genetic phenomenon and therefore represents a parallel evolutionary phenomenon in several extremely diverse taxonomic groups, and aside from its economic significance, it provides an ideal model for investigating how organisms can respond to large scale exposure to xenobiotics(2).

Glutathione S-transferases (GSTs, EC 2.5.1.18) are a widely distributed family of detoxifying dimeric enzymes found in most forms of life. GST inactivates poisons by chemically bonding them to the tripeptide glutathione, making them soluble so that the body can easily excrete them. Pathogenic parasites also make their own GSTs to help them inactivate the drugs we use to combat them (4). Resistance to OPs is considered to be due to metabolism of these compounds by glutathione-S-transferases (5). Many studies have shown that insecticide-resistant insects have elevated levels of glutathione S-transferases activity in crude homogenates, which suggests a role for GSTs in resistance (6, 7). Multiple forms of these enzymes have been reported for mosquitoes, house fly, Drosophila, sheep blow fly, and grass grub (9, 10, 11). GSTs are also involved in intracellular transport, biosynthesis of hormones and protection against oxidative stress. In addition, they contribute to the removal of toxic oxygen free radical species produced through the action of pesticides. They have peroxidase (12, 13) and isomerase activity (14), they can inhibit the Jun N-terminal kinase (thus protecting cells against H_2O_2-induced cell death) (15), and they are able to non-catalytically bind a wide range of endogenous and exogenous ligands (16–18).

Three-dimensional structure of the target is essential for defining the active site and also for designing, improving, and docking of small ligands to the complex target protein. All cytosolic GSTs have the same basic protein folding, which comprises two domains. The N-terminal domain (domain I) adopts a α/β topology and provides the GSH-binding site (G-site) (19). It is currently believed that the residues which contribute to binding glutathione involve a network of specific polar interactions between GSH and G-site residues that are either conserved or conservatively replaced between classes. The C-terminal domain (domain II) is an all-helical structure and provides the structural element for recognition of the broad range of hydrophobic co-substrate [H-site (hydrophobic-substrate-binding site)], which lies adjacent to the G-site (19). It shows the greatest variability across the GST classes (20) and helps to define the substrate selectivity of the enzyme (19). The active site residue tends to be highly conserved within GST classes, but differs between classes. In most mammalian GSTs, the active site residue responsible for the GSH thiol residue activation in catalysis appears to be a tyrosine (27), but in the delta and epsilon insect GST classes, this role is performed by a serine residue (28 & 29).

Natural botanical substances, secondary metabolites are considered to be effective against the insects due to their friendly effect on the users. Plumbagin is a natural compound extracted from Plumbago rosea exhibiting filarial GST inhibition and antifilarial activity (30).

Tagitinin C, is a secondary metabolite extracted from the ethanolic extracts of *Tithonia diversifolia* are found to insecticidal activity against *Callosobrochus maculatus Frabiricius*. The ethanol extracts of the plants known to posses Antioxidant and reducing properties and glutathione S-transferases was found to be the potential target (31).

Piperonyl butoxide (PB) a pesticide synergist, a compound known for years to work well against pests, exhibited low toxicities to the herbivorous pest insects and ladybirds, but high toxicities to the parasitoids. The tolerance to the insecticides in 11 pest insects and natural enemies was found to be mainly associated with the tolerance to PB. PB showed the highest synergism with various substances in nine species of pest insects and natural enemies. It disrupts the metabolism of chemicals in insects and generally has a low toxicity in humans through any route of exposure (32).

Hence this study focuses on the comparison of the structures and comparative docking of GSTs from economically important insects *H.armigera, M.sexta, and L.lineolaris* with the three compounds mentioned above and its substrate glutathione, using bioinformatics tools and softwares.

2. MATERIALS AND METHODS

2.1 Data Collection:

The GST sequences of the insects were retrieved from the biological database NCBI which can be accessed using the URL: http://www.ncbi.nlm.nih.gov/. Using the protein name as query we have collected the sequences of Glutathione S- Transferases from *Lygus Lineolaris, Helicoverpa Armigera, and Manduca Sexta* which have the accession number as ABC46450, AAL23839, and AAF16718 respectively. The chemical structures of the compounds to be docked are obtained from pubchem database.

2.2 Template Selection:

The templates selection for the targets has been done using the BlastP (21). The downloaded sequences of the GSTs from various insects were uploaded to BlastP program individually and the programs were run by setting the parameters as default except dataset option as PDB to get the templates. The BlastP program was accessed at http://www.blast.ncbi.nlm.nih.gov/Blast.cgi.

2.3 Structural Modeling:

The 3D structures of target GSTs were modeled individually using the windows version of MODELLER9v7 program (22). MODELLER is used for comparative modeling of protein three-dimensional structures. ALIGN.PY, ALIGN2D.PY, MODEL-OUTPUTS.PY and MODEL EVALUATION.PY scripts were used to build models. Five structures per GST sequence were modeled and the one with the best DOPE score was selected for further analysis. The modeled structures were subjected to analysis by Ramachandran plot and further validation was employed using PROSA (23). The models after validation were docked with the various compounds using igemdock and the best dock was identified from the binding energy.

2.4 Structural Alignment:

Structural alignments were done using Swiss PDB-viewer and MAMMOTH and the mutilple structure alignment was performed using the tool MAMMOTH-mult (24). The outputs of a structural alignment are a superposition of the atomic coordinate sets and a minimal root mean square distance (RMSD) between the structures. The RMSD of two aligned structures indicates their divergence from one another.

3. RESULTS AND DISCUSSION

The templates for targets were listed in the Table: 1. The BlastP exercise showed required percent of identity with “Delta-Class GST from A DDT-Resistant Strain of the Malaria Vector *A.gambiae* (1PN9-B)”, “Epsilon-Class GST from the Malaria Vector *A.gambiae*(2IL3-B)” and “GST from *A.cracens* (1R5A-A)”. Table: 1 also shows the DOPE value, Z-score to assess the quality of the model and the RMSD values of the models with their respective templates. The modeled 3D structures were also found to be falling well in the allowed regions of the Ramachandran Plot, emphasizing the quality of the models. Z-score which indicates overall to an energy distribution derived from random conformations was also found to be perfect.

The results from mammoth-mult in table: 2 show that the RMSD score obtained after the structural comparisons to be 0.45 Å and the having the core percentage of 97.06, suggesting that 97.06% of the residues are in the core regions, giving us an idea about the similarity between the modeled structures. The clustering and final results had the RMSD ranging from 0.45 to 0.48 between the smooth core and the loose core respectively.

From the results of superposition of structures using SwissPDB-Viewer, we infer that the GSTs of *H.armigera*

Table: 1: Table showing the summary of the models built using the templates, % identity, DOPE value their RMSD value form their respective template and the Z-Score obtained from the ProSA.

Name	Template	Identity %	DOPE Value	RMSD	z-score
L.lineolaris **ABC46450**	1PN9-B	63%	-24960.72	0.20Å	-7.29
H.armigera **AAL23839**	1R5A-A	50%	-19101.43	0.36Å	-6.09
M.sexta **AAF16718**	1R5A-A	47%	-28293.72	0.30Å	-6.73

Table:2. Structure comparison results obtained from Mammoth mult.

MAMMOTH-mult v1.0

1 Name: pdb-001 oo Len: 170 ## Helicovarpa Armigera

2 Name: pdb-002 oo Len: 216 ## Lygus Lineolaris

3 Name: pdb-003 oo Len: 247 ## Manduca Sexta

//CLUSTERING:

DONE	g1	g2	#str	PSI	NALI	NORM	RMS	P-value	C-val	Z-scr	-ln(P)	secs
50%	2	3	2	95.81	206	216	1.58	0.4E-11	26.18	27.55	26.22	0.3
100%	1	2	3	100.00	169	170	1.36	0.1E-09	22.52	23.89	22.79	0.4

//RESULTS:

# OF	**NORM**		**AVG #**	**# CA**	**STRCT**	**LOOSE STRCT**	**LOOSE**	**INI**	**END**	**FINAL**	
STRUCT	**minCA**	**maxCA**	**OF CA**	**CORE**	**%CORE**	**%CORE**	**RMS**	**RMS**	**<lnP>**	**<lnP>**	**Z-scr**	**-lnP**
3	**170**	**247**	**211**	**165**	**97.06**	**124.26**	**0.45**	**0.48**	**23.74**	**23.83**	**23.20**	**22.14**

Table: 3. Pairwise structure comparison of Cα atoms, sequence similarity & identity score.

Pairwise Comarison		RMSD	Similarity	Identity
H.armigera	*L.lineolaris*	0.88Å	53.40%	36.80%
H.armigera	***M.sexta***	**0.42Å**	**61.90%**	**52.60%**
L.lineolaris	*M.sexta*	1.02Å	60.10%	36.80%

and *M.sexta* with RMSD score of 0.42Å to be structurally similar. This could be due to the fact that they share a good deal of sequence identity (table.3) and they were modeled with the same template.

The four best docked poses are shown in figure.1. The results of the docking studies in table.4 indicate that the binding site of different insects where the compounds TC, PB, PL & GSH, bind varies in all the three GSTs. This is synonymous with (27, 28 & 29). All the three inhibitors were found to bind at the same site involving the same residues of the GSTs of *H.Armigera.* And none of the GSH binding residues were involved in the binding of the inhibitors, suggesting a mechanism for a non competitive mode of inhibition in the GST model of *H.armigera.* Whereas the site of binding of the inhibitors was different in both the GST models of *M.sexta* & *L.lineolaris*. Further, we also noticed with this in vivo study the TagitininC shared almost similar binding site residues as that of the substrate GSH of the *L.lineolaris* model. The docking study reveals that the inhibitor PB was found to be most effective in terms of their binding energy amongst the GST models *H.armigera* & *L.lineolaris.* Whereas TagitininC had a good

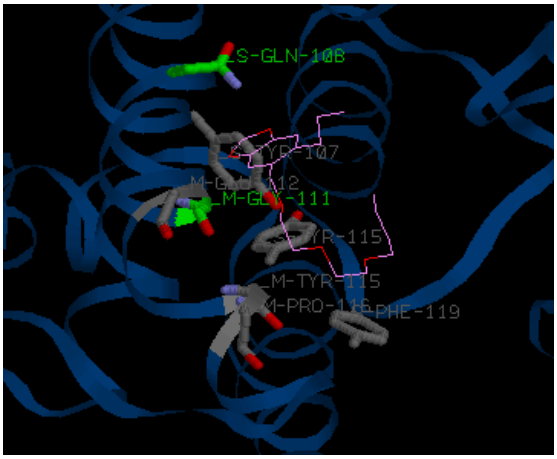
Table: 4. Residues involved in the binding site.

	TAGITININ C	**PLUMBAGIN**	**PB**	**GLUTATHIONE**
	Energy (-79.6)	**Energy (-68)**	**Energy (-87.3)**	**Energy (-79.8)**
	H-S-ARG-23	H-S-ARG-23	H-S-ARG-23	H-S-ASN-124
	H-S-ASP-57	H-S-ASP-57	H-S-ASP-57	H-S-GLU-141
	V-M-SER-22	V-M-SER-22	H-M-SER-22	H-S-ASP-161
	V-S-ARG-23	V-S-ARG-23	H-S-ASP-156	V-M-ASN-124
	V-S-SER-115	V-S-SER-115	V-S-ASP-156	V-S-ASN-124
	V-M-VAL-118	V-M-VAL-118	V-M-VAL-118	V-S-PHE-140
	V-M-THR-119	H-M-THR-119	H-M-THR-119	V-S-LYS-144
	V-M-SER-115		V-M-THR-119	V-S-GLU-157
Helicoverpa Armigera	V-S-TYR-62	V-S-TYR-62	V-S-TYR-62	
	Energy (-76.7)	**Energy (-69.2)**	**Energy (-79.7)**	**Energy (-82.2)**
	H-S-ASP-34	H-M-THR-155	H-S-GLN-108	H-M-THR-8
	H-M-LEU-35	V-M-PRO-86	H-M-GLY-111	H-M-LEU-35
	V-S-THR-8	V-S-LYS-91	V-S-TYR-107	H-S-LYS-205
	V-M-ASN-153	V-M-ASN-153	V-M-GLU-112	V-M-MET-36
	V-S-ASP-34	V-S-ASN-153	V-M-TYR-115	V-S-LYS-205
	V-M-MET-36		V-S-TYR-115	V-M-ALA-212
	V-M-LYS-205		V-M-PRO-116	
	V-S-LYS-205		V-S-PHE-119	
	V-M-ALA-212			
Lygus Lineolaris	V-M-ASP-34			
	Energy (-80)	**Energy (-69.1)**	**Energy (-77.0)**	**Energy (-81.5)**
	H-M-PHE-17	H-S-ARG-26	H-S-ASN-65	H-S-LYS-35
	H-S-ASP-56	V-S-ARG-26	V-S-ARG-26	H-S-ARG-53
	V-M-VAL-14	V-M-ARG-28	H-S-ARG-28	H-S-GLU-58
	V-M-ASN-15	V-S-LEU-39	V-M-ASP-68	H-M-LEU-59
	V-S-ASN-15	V-M-PRO-41	V-M-ASN-65	V-M-ARG-53
	V-M-GLN-16	V-S-PHE-242	V-S-ASN-65	V-S-ARG-53
	V-S-GLN-16		V-M-MET-67	V-M-ASP-56
	V-M-ASP-56			V-S-ASP-56
	V-S-ASP-56			V-M-GLU-58
Manduca Sexta				V-S-GLU-58

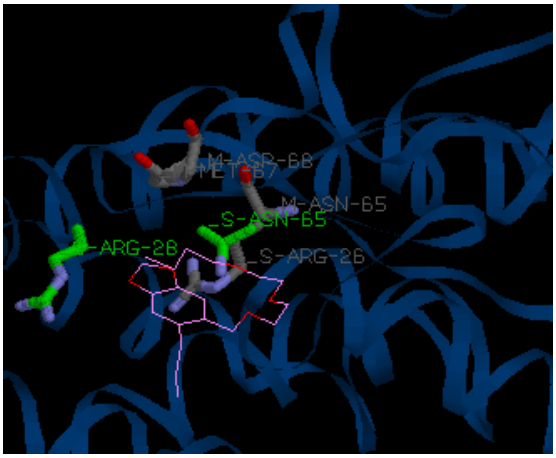
binding energy score with the model *M.sexta* than the PB, though the difference between the two in terms of binding energy was not huge.

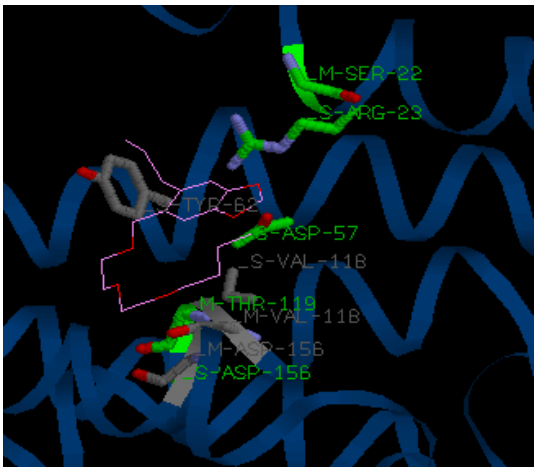
From this study we infer that the binding sites of individual GST models vary and the compounds TagitininC and Piperonyl Butoxide, an insecticide synergist, can be used together in synergism as an effective ingredient for GSTs.

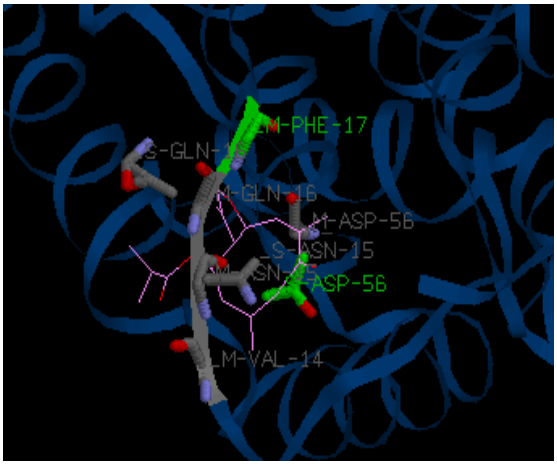
(A). PB docked with *L.Lineolaris* GST.



(B). PB docked with *M.Sexta* GST.







(C). PB docked with *H. Armigera* GST.

(D). Tagitinin C docked with *M.Sexta* GST.

Figure: 1. Docking of compound Tagitinin C with various insect GST models.

4. CONCLUSION

Insecticide resistance has become a major and serious issue of concern around the world affecting the farmers' community badly, ultimately affecting the overall production of food, crops and other economically important aspects. In this study, we have given importance to one of the enzymes responsible for detoxification Glutathione S-Transferase from *L.lineolaris*, *H.armigera*, and *M. sexta* insects. Homology modeling was done to construct the 3D models of the GSTs from these insects using MODELLER9v7. The general models were validated by ProSA, Ramachandran Plot and Superposition and multiple structure comparison techniques of SwissPDBViewer and Mammoth mult tools and the docking with secondary metabolites Tagitanin C, and Plumbagin , Piperonyl butoxide (PB) a pesticide synergist and the substrate glutathione was performed using igemdock. RMSD score obtained after the structural comparisons was found to be 0.45 Å and the having the core percentage of 97.06, suggesting that 97.06% of the residues are in the core regions, giving us an idea about the similarity between the modeled structures. Variation in the preference of binding modes is observed with the models *L.lineolaris* & *M.sexta* and the compound Piperonyl butoxide was found to bind well energetically than the other compounds. Tagitinin C had almost similar binding energetically and from this we can infer that these two compounds can be used in synergism. These findings may be relevant for the better understanding of the similarities between the enzymes involved in the insecticide resistance and for the further development of preventive strategies.

5. REFERENCES

1. Janet Hemingway and Hilary Ranson, *Insecticide Resistance in Insect Vectors of Human Disease*, Annual Review of Entomology 2000, Vol. 45: 371-391.
2. I. Denholm, *Monitoring and Interpreting Changes in Insecticide Resistance*, Functional Ecology, Vol. 4, No. 5 (1990), pp. 601-608.

3. *Bull, D. L. "Factors that influence tobacco budworm, Heliothisvirescens, resistance to organophosphorous insecticides".* Bull.Entomol. Soc. Amer (1981) 27**:** 193-197.

4. Christopher M. Bruns, *Structural Molecular Biology & Protein Design*, the Scripps Research Institute, http://www.scripps.edu/~bruns/gst.html.

5. Whitten, C. J. and D. L. Bull. *Comparative toxicity, absorption and metabolism of chlorpyrifos and dimethyl homologue in methyl parathion-resistant and susceptible tobacco budworm*. Pestic. Biochem. Physiol.(1974) 4**:** 266-274.

6. Grant DF., *Evolution of glutathione S-transferase subunits in Culicidae and related Nematocera: Electrophoretic and immunological evidence for conserved enzyme structure and expression.* Insect Biochem. (1991) 21:435–45.

7. Grant DF, Dietze EC, Hammock BD, *Glutathione S-transferase isozymes in A. aegypti: purification,characterization, and isozyme specific regulation.* Insect. Biochem.(1991) 4:421–33.

8. Enayati AA, Ranson H, Hemingway J., *Insect glutathione transferases and insecticide resistance*. Insect Mol Biol. 2005 Jan; 14(1):3-8.

9. Clark AG, Dick GL, Martindale SM, Smith JN. *Glutathione S-transferases from the New Zealand grass grub,Costelytra zealandica*, Insect Biochem. 1985. 15:35–4.

10. Clark AG, Shamaan NA, Dauterman WC, Hayaoka T. *Characterization of multiple glutathione transferases from the housefly, Musca domestica (L).* Pestic.Biochem. Physiol. 1984, 22:51–59.

11. Toung YS, Hsieh T, Tu CD. *Drosophila glutathione S-transferase 1-1shares a region of sequence homology with maize glutathione S-transferase III.* Proc. Natl. Acad. Sci. USA 1990, 87:31–35.

12. Mannervik B., Danielson U. H. *Glutathione transferases – structure and catalytic activity.* CRC Crit. Rev. Biochem. 1988;23:283–337.

13. Zhao T., Singhal S. S., Piper J. T., Cheng J., Pandya U., Clark-Wronski J., Awasthi S., Awasthi Y. C. *The role of human glutathione S-transferases hGSTA1-1 and hGSTA2-2 in protection against oxidative stress*. Arch. Biochem. Biophys. 1999; 367:216–224.

14. Johansson A.-S., Mannervik B. *Human glutathione transferase A3-3, a highly efficient catalyst of double-bond isomerization in the biosynthetic pathway of steroid hormones*. J. Biol. Chem. 2001; 276:32061–32065.

15. Yin Z., Ivanov V. N., Habelhah H., Tew K., Ronai Z. *Glutathione S-transferase p elicits protection against H_2O_2-induced cell death via coordinated regulation of stress kinases.* Cancer Res. 2000; 60:4053–4057.

16. Bhargava M. M., Listowsky I., Arias I. M. Ligandin. *Bilirubin binding and glutathione S-transferase activity are independent processes*. J. Biol. Chem. 1978; 253:4112–4115.

17. Dulhunty A., Gage P., Curtis S., Chelvanayagam G., Board P. *The glutathione transferase structural family includes a nuclear chloride channel and a ryanodine receptor calcium release channel modulator*. J. Biol. Chem. 2001; 276:3319–3323.

18. Lo Bello M., Nuccetelli M., Caccuri A. M., Stella L., Parker M. W., Rossjohn J., McKinstry W. J., Mozzi A. F., Federici G., Polizio F., et al. *Human glutathione transferase P1-1 and nitric oxide carriers: A new role for an old enzyme*. J. Biol. Chem. 2001; 276:42138–42145.

19. Armstrong R. N. *Structure, catalytic mechanism, and evolution of the glutathione transferases*. Chem. Res. Toxicol. 1997; 10:2–18.

20. Board P. G., Coggan M., Chelvanayagam G., Easteal S., Jermiin L. S., Schulte G. K., Danley D. E., Hoth L. R., Griffor M. C., Kamath A. V., et al. *Identification, characterization, and crystal structure of the Omega class glutathione transferases*. J. Biol. Chem. 2000; 275:24798–24806.

21. Altschul, Stephen F., Warren Gish, Webb Miller, Eugene W. Myers, and David J. Lipman (1990). *Basic local alignment search tool*. J. Mol. Biol. 215:403-10.

22. Eswar N, Webb B, Marti-Renom MA, Madhusudhan MS, Eramian D, et al. *Comparative protein structure modeling using MODELLER*. Curr Protoc Protein Sci. 2007 Chapter 2, Unit 2.9.

23. Wiederstein and Sippl, *ProSA-web: interactive web service for the recognition of errors in three-dimensional structures of proteins*. Nucleic Acids Research (2007) 35, W407-W410.

24. Lupyan D., Leo-Macias A., Ortiz AR. *A new progressive-iterative algorithm for multiple structure alignment*. *Bioinformatics*, (2005) 21, 3255-3263.

25. Dereeper A., Guignon V., Blanc G., Audic S., Buffet S., Chevenet F., Dufayard J.-F., Guindon S., Lefort V., Lescot M., Claverie J.-M., Gascuel O. *Phylogeny.fr: robust phylogenetic analysis for the non-specialist* Nucleic Acids Research. 2008 Jul 1; 36 (Web Server Issue):W465-9. Epub 2008 Apr 19.

26. Stephen V.Evans, Angharad M. R. Gatehouse and Linda E. Fellows. *Detrimental effects of 2,5-Dihydroxymethyl-3,4-dihydroxypyrrolidine in some tropical legume seeds on larvae of the bruchid Callosobruchus maculatus* , Entomol. Exp.appl. 37, 257-261 (1985).

27. Sheehan D, Meade G, Foley VM, Dowd CA (2001). *Review article,Structure, function and evolution of glutathione transferases: implications for classification of non-mammalian members of an ancient enzyme superfamily*. Biochem. J. 360: 1-16.

28. Ranson H, Hemingway J (2005). *Mosquito glutathione transferases. Review*. Methods Enzymol. 401: 226-241.

29. Udomsinprasert R, Pongjaroenkit S, Wongsantichon J, Oakley AJ, Prapanthadara L, Wilce MCJ, Ketterman AJ (2005). *Identification, characterization and structure of a new Delta class glutathione transferase isoenzyme.* Biochem. J. 388: 763-771.

30. Nisha M, Paily KP, Vanamail P, Abidha, Kalyanasundaram M, Balaraman K (2002) Macrofilaricidal activity of the plant Plumbago indica/rosea in vitro. Drug Dev Res 56(1):33–39.

31. Ayodele O. Kolawole, Ralphael E. Okonji and Joshua O. Ajele. *Inhibition of glutathione S-transferases (GSTs) activity from cowpea storage bruchid, Callosobrochus maculatus Frabiricius by some plant extracts*, African Journal of Biotechnology Vol. 9 (20), pp. 5516-5521.

32. Wu G, Miyata T, Kang CY, Xie LH. *Insecticide toxicity and synergism by enzyme inhibitors in 18 species of pest insect and natural enemies in crucifer vegetable crops.* Pest Manag Sci. 2007 May; 63(5):500-10.