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Research Article

In silico docking studies on cytochrome P450 enzymes of *Helicoverpa armigera* (Hübner) and *Trichogramma cacoeciae* Marchal and implications for insecticide detoxification

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ABSTRACT: *In silico* docking of cytochrome P450 monooxygenase (CYP450) of an insect, *Helicoverpa armigera* (Hübner) and a parasitoid, *Trichogramma cacoeciae* Marchal was studied with two insecticides, monocrotophos and fenvalerate. The CYP450 sequences of *H. armigera* (CYP9A12), *T. cacoeciae* (CYP4G12) and a human microsomal sequence CYP3A4, as positive control were retrieved from NCBI's GenBank database. The structure, as predicted by SOPMA, of CYP450 in *H. armigera* contained 78.7% helix and 43.3% sheets, while that of *T. cacoeciae* contained 60.6% helix and 68.5% sheets. The three-dimensional molecular models of CYP450 of *H. armigera* and *T. cacoeciae* indicated that 96.5 and 97.2% residues, respectively, were in the most favored region. The docking studies revealed that the binding energy of *H. armigera* was -3.50 and -7.65 kcal/mole compared to the binding energy of *T. cacoeciae* -2.96 and -5.28 kcal/mole for monocrotophos and fenvalerate, respectively, inferring stronger interaction of *H. armigera* CYP450 with the insecticides and thereby higher potential for resistance in *H. armigera*.

KEY WORDS: Cytochrome P450, *Helicoverpa armigera*, *Trichogramma cacoeciae*, *in silico* molecular docking

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INTRODUCTION

The old world bollworm, *Helicoverpa armigera* (Hübner) is an important pest on many crops and has developed resistance to many groups of insecticides in many countries (Shen and Wu, 1995; Kranthi *et al*., 2002; Pedra *et al.,* 2004; Armes *et al*., 1996). *Trichogramma* spp. is among the most widely used natural enemies for the suppression of lepidopteran pests on many crops and is applied on 32 million ha of crops annually (Li, 1994). However, use of *Trichogramma* in integrated pest management programme is limited due to its susceptibility to various insecticides (Bull and House, 1983; Lopez and Morrison, 1985; Jalali *et al*., 2006).

Cytochrome (P450) monooxygenase is a diverse and important family of hydrophobic, heme containing, membrane associated enzymes involved in the metabolism of both endogenous and exogenous compounds such as hormones, fatty acids and steroids, and in the detoxification of xenobiotics such as drugs, pesticides and plant toxins. In insects, P450s metabolize and are inducible by a diverse array of lipophilic substrates. This generally results in the detoxification of the substrate to more soluble and less toxic forms. Thus, P450s play a key role in many aspects of insect biology and physiology (Feyereisen, 1999), and insecticide resistance (Scott, 1999). Insect genomes, including that of *H. armigera* (Pittendrigh *et al*., 1997), contain approximately 100 P450 genes classified in to 4, 6, 9, 12 and 28 CYP families (Feyereisen, 1999). In P450 genes, only a few like CYP6A1, CYP6D1 and CYP6G1 have been studied in detail as of insecticide resistance (Wheelock and Scott, 1992; Korytko and Scott, 1998; Sabourault *et al*., 2001; Daborn *et al*., 2002). Recently, CYP9A12 gene from *H. armigera* was found to exhibit considerable activity of O-demethylation against two model substrates (Yang *et al*., 2008) and CYP4G12 gene with similar activity was identified in *T. cacoeciae* (Tares *et al*., 2000).

In a recent study, potential of several P450s that confer insecticide resistance in *Drosophila melanogaster* (Meigen) were evolved by transgenic expression (Daborn *et al*., 2007). As expected, different profiles were observed where P450s were over expressed for different insecticides,

however, over expression did not increase survival of *D. melanogaster* on any of the insecticides. In the absence of crystal structures, understanding the complexity of insecticide resistance mediated by P450s relies on homology modeling (Jones *et al*., 2010). Such modeling as explained by Baudry *et al*. (2006) and de Graaf *et al*. (2005) has greatly improved in understanding complexity of insecticide resistance. Insecticide resistance related to CYP450 occurred due to over expression of the gene (Liu and Scott, 1998). Cis- and trans-regulating factors involved in CYP450 gene expression are also responsible for insecticide resistance in insects. In a study, it has been observed that expression of CYP6A1 in houseflies is influenced by a transacting factor (Carino *et al.*, 1994). In some resistant *Drosophila* strains, mutation in the transacting repressor gene increases expression of CYP6A8 (Maitra *et al.*, 1996). As described by Ranson *et al.* (2000a and 2000b), it is been observed that a mutational change from leucine to serine amino acid within a codon occurred frequently which is associated with pyrethroid resistance in many arthropods. In this study, the 3D protein structure for the genes CYP9A12 and CYP4G12 of *H. armigera* and *T. cacoeciae*, respectively, were constructed by using homology modeling and validated using Ramachandran plot (Ramachandran *et al*., 1963). Interaction levels between CYP9A12 and CYP4G12 and the ligand insecticides, – monocrotophos and fenvalerate, were studied using *in-silico* docking studies.

MATERIALS AND METHODS

Sequence alignment and Homology modeling

Molecular interactions of P450s were determined by constructing homology models using Swiss PDB viewer for *H. armigera* CYP9A12 [GenBank: AAQ73544.1] and *T. cacoeciae* CYP4G12 [GenBank: AAF29511.1] enzyme sequences. It is a method to predict the protein tertiary structure based on its primary amino acid sequences. Both P450 (CYP9A12 & CYP4G12) sequences were aligned against human microsomal P450 [PDB: 1TQN-A], whose 3D structure is well characterized (Baudry *et al*., 2003; Edgar, 2004; Yano *et al*., 2004; Jones *et al*., 2010). The comparative modeling follows a step wise procedure, *viz*., starting with a template structure search from PDB (http://www.pdb.org), aligning the query sequence to target sequence and constructing a model based on the alignment using the software. The modeling of the 3D structure of the enzymes was performed by homology modeling program, Swiss-PDB viewer (http://www.expasy.ch/spdbv). In order to determine homologous sequences to CYP450, the FASTA sequences of both the pest and parasitoid collected from NCBI's GenBank database, were submitted for BLASTp search against PDB. From the BLASTp analysis, human microsomal P450 CYP3A4, [GenBank: AAF13598] was selected and modeled structures were visualized using the Rasmol (http://www.openrasmol.org/). To know the structure composition (% of helix, sheets, loops and coils) of both the CYP450 enzyme sequences of *H. armigera* and *T. cacoeciae*, a secondary structure prediction tool SOPMA (Self-Optimized Prediction Method with Alignment) was used.

Validating the predicted models

The validation for modeled structures was performed using PROCHECK (http://www.ebi.ac.uk/thornton-srv/software/PROCHECK/) (Laskowski *et al*., 1993) to check for psi (Ø) and phi (Ö) torsion angles using the Ramchandran plot that provides a detailed check on the stereo chemistry of a protein structure. The Ramachandran plot is a way to visualize dihedral angles ö against ø of amino acid residues in protein structure and the white areas correspond to conformations where atoms in the polypeptide come closer than the sum of their Vander Waals radii (Ramachandran *et al*., 1963). These regions are sterically disallowed for all amino acids except glycine, which is unique in that it lacks a side chain. The red regions correspond to the allowed regions namely the alpha-helical and beta-sheet conformations where there are no steric clashes. The yellow areas show the partially allowed regions of left-handed helix wherein the atoms are allowed to come a little closer together.

The modeled structures for *H. armigera* and *T. cacoeciae* were compared using C-alpha match server, (http://bioinfo3d.cs.tau.ac.il/c_alpha_match/) (Bachar *et al*., 1993) where root mean square values were calculated by fitting the carbon backbone of the predicted models. The root mean square deviation (RMSD) is the measure of the average distance between the atoms (usually the backbone atoms) of superimposed proteins. In the study of globular protein conformations, one customarily measures the similarity in three-dimensional structure by the RMSD of the Cá atomic coordinates after optimal rigid body superposition.

Protein-ligand docking

The modeled CYP450 enzymes of both the insects were docked with the insecticides, *viz.,* monocrotophos (Dimethyl *(E)*-1-methyl-2-(methylcarbamoyl) vinyl phosphate) and fenvalerate (RS) –alpha–Cyano–3-phenoxybenzyl (RS)-2-(4-chlorophenyl)-3-methylbutyrate as ligands using the software DockingServer (http://

www.dockingserver.com) (Bikadi and Hazai, 2009). Docking is a method which predicts the preferred orientation of one molecule to a second when bound to each other to form a stable complex. The process of classifying which ligands are most likely to interact favorably to a particular receptor based on the number of favorable intermolecular interactions such as hydrogen bonds and hydrophobic contacts and predicted free energy of binding. Docking Server, a web-based interface that handles molecular docking, from ligand and protein set-up through results representation integrating a number of software frequently used in computational chemistry. Docking simulations were performed using the Lamarckian Genetic Algorithm (LGA) (Morris *et al.*, 1998) and the Solis and Wets local search method (Solis and Wets, 1981).

RESULTS AND DISCUSSION

Protein structure prediction and homology modeling

Homology models for *H. armigera* CYP9A12 and *T. cacoeciae* CYP4G12 sequences were constructed (Fig. 1 and 2). Both P450 sequences were individually aligned with known three-dimensional structure of human microsomal P450 (CYP3A4, PDB id: 1TQN). Based on the sequence similarity analysis, CYP3A4 showed good similarity with both insect sequences. A medium level sequence conservation with 31% and 32% identity and 51% and 57% similarity for CYP9A12 and CYP4G12, respectively, were observed with template sequence, *i.e.*, CYP3A4, which is mammalian P450 clade 3, like CYP9A12, is known for its broad substrate specificity. The structure of P450s for two insects were determined and it contained 78.7% helix, 43.3% sheets and 12.4% turns for *H. armigera* and 60.6% helix, 68.5% sheets and 12.6% turns for *T. cacoeciae*. The quality of predicted structure for their internal consistency and reliability was assessed using PROCHECK (validated by Ramachandran Plot). The overall stereo chemical quality of the enzymes assessed for two genes showed that ≈ 96.5% and 97.2% of all residues were in core and allowed regions for CYP9A12 and CYP4G12, respectively, and only 3.5% and 2.8% of the residues were in disallowed regions (Table 1). These values are close to those models reported earlier, such as the model for the insects CYP12D1 and CYP6B1 (Baudry *et al*., 2003; Jones *et al*., 2010). The Ramachandran plot characteristics confirmed the quality of the modeled structure and retained the overall protein fold of the template CYP3A4, with large domain being á-helix and a smaller â-sheets rich N-terminal domain in CYP9A12, however, in CYP4G12; á-helix was not a predominant.

In order to determine whether the modeled structures are similar or not, C-alpha match server was used for comparison. It was noted that RMSD values for the structure alignment was <2Å between C-á atoms, suggested that ligand binding domain of both species are similar. Our observation corroborate with the findings for *Apis melifera* (Linnaeus) by Rocher and Marchand-Geneste (2008), who too reported minimal deviation, thereby suggesting the ligand binding domain of the species are similar.

Protein-ligand docking

The modeled structure of P450s were docked with two insecticides, *viz.*, monocrotophos and fenvalerate using Docking Server Force field parameters for energy

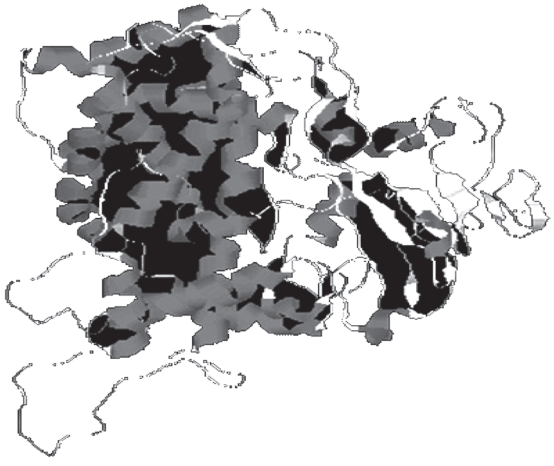

Fig. 1. 3D structure model of *Helicoverpa armigera* CYP9A12 enzyme. The structure is modeled by using SwissPDB viewer and the methodology used is Homology modeling

Fig. 2. 3D structure model of *Trichogramma cacoeciae* CYP4G12. The structure is modeled by using SwissPDB viewer and the methodology used is Homology modeling

Table 1. Sequence identity scores with template CYP3A4 and Ramachandran plot statistics for the P450 models presented

Gene/ Organism	Sequence Identity (%)	Sequence similarity (%)	Ramachandran plot statistics: Core (%)	Allowed (%)	Generously allowed (%)	Disallowed (%)
	With CYP3A4 (Human)					
CYP9A12 (*H. armigera*)	35	51	67.7	24.4	4.4	3.5
CYP4G12 (*T. cacoeciae*)	31	57	78.7	17.6	0.9	2.8

minimization of ligands. The docked complex of the protein of both insects CYP9A12 (*H. armigera*) and CYP4G12 (*T. cacoeciae*) and ligands indicated that the former had more energetically favoured interaction. The presence of three hydrogen bonds and seven hydrophobic contacts were observed in *H. armigera* with binding free energy. It is noteworthy that in monocrotophos, hydrogen bonds are bound with alanine (at ALA304), threonine (at THR308) and cysteine (at CYS450) in *H. armigera*, thereby tightly holding the ligand. Further, more amino acid residues such as isoleucine (ILE162), methionine (MET246), phenylalanine (PHE455) and alanine (ALA456) (Table 2) (Fig. 3), were found holding the hydrophobic interactions with the insecticide apart from other clutching connections. However, in *T. cacoeciae,* the docked complex with the ligand showed deficiency of hydrogen bonds and less hydrophobic interactions at the active site, thus making the association between the parasitoid and the insecticide much weak (Fig. 4). Moreover the binding energy was much lower in *H. armigera*, *i.e.*, –3.50 kcal/mole compared to –2.96 kcal/mole in *T. cacoeciae*. The importance of

Table 2. Interaction table of docking of CYP9A12 (*Helicoverpa armigera*) and CYP4G12 (*Trichogramma cacoeciae*) with monocrotophos

H. armigera				***T. cacoeciae***			
H–bonds	Polar bonds	Hydrophobic interactions	Others	H–Bonds	Polar bonds	Hydrophobic interactions	Others
ALA (304) – N (1)	THR (308) – H (1)	ILE (162) – C (5)	ILE (162) – O (4)	NIL	ARG (121) – O (4)	MET (53) – C (2)	MET (53) – O (5)
THR (308) – N (1)		MET (246) – C (5)	MET (246) – O (2)		ARG (121) – O (1)	LEU (56) – C (7)	LEU (56) – O (5)
CYS (450) – O (5)		CYS (450) – C (4)	THR (308) – C (7)		ARG (121) – O (3)	TYR (123) – C (5)	THR (118) – C (6)
		PHE (455) – C (5)	PHE (443) – O (5)			PHE (126) – C (3)	ARG (121) – C (6)
		ALA (456) – C (1)	PHE (455) – O (1)			PHE (126) – C (5)	ARG (121) – P (1)
		ALA (456) –C (3)	PHE (455) – O (2)				ARG (121) – C (1)
		ALA (456) – C (4)	ALA (456) – O (5)				ARG (121) – C (3)
							TYR (123) – O (2)
							TYR (123) – O (3)

H–bonds = hydrogen bonds; ALA = alanine; THR = thrionine; CYS = cysteine; ILE = isoleucine; MET = methonine; PHE = phenylialanine; ARG = arginine; LEU = leucine; TYR = tyrosine

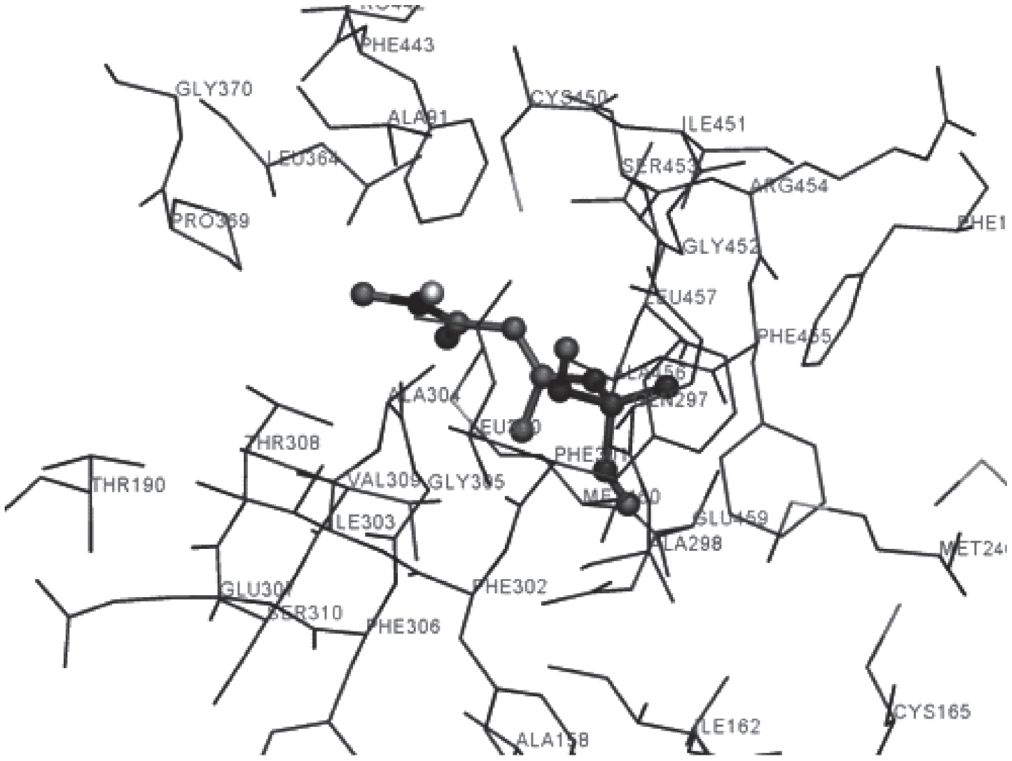

Fig. 3. Docking interaction of *Helicoverpa armigera* (CYP9A12) and monocrotophos. Protein docking ineraction of *H. armigera* (CYP9A12) and monocrotophos (ligand) showing the actively participated amino acids around protein active site. Amino acids participated in hydrogen bond inetractions were alanine, theorine, cystein. Other amino acids participated in docking interaction were isoleucine, methionine, phenylealanine and alanine

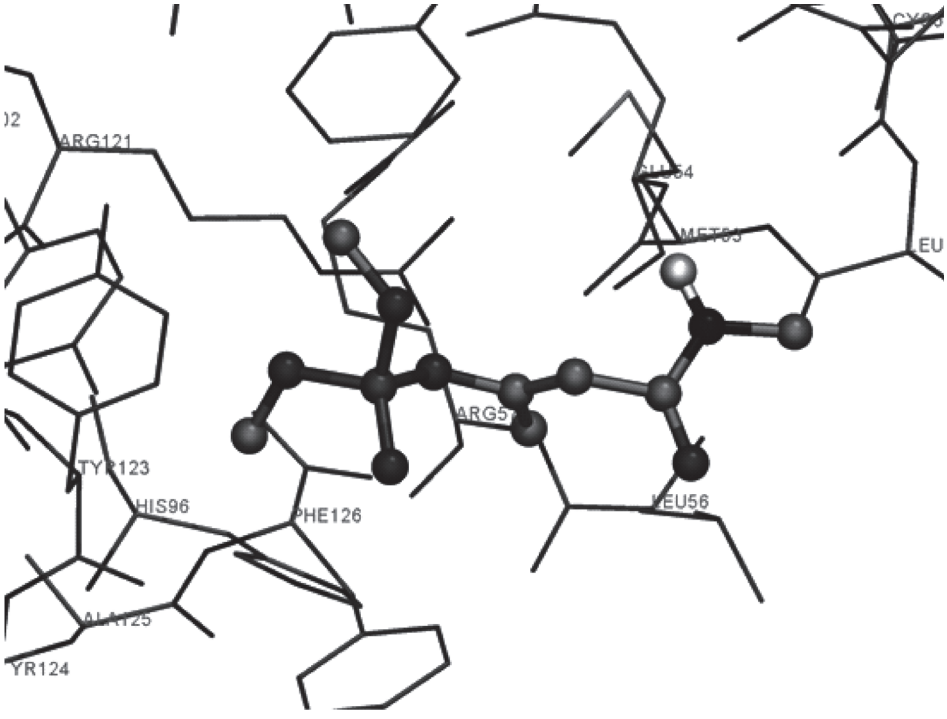

Fig. 4. Docking interaction of *Trichogramma cacoeciae* (CYP4G12) and monocrotophos. Protein docking ineraction of *T. cacoeciae* (CYP4G12) and monocrotophos (ligand) showing the actively participated amino acids around protein active site. Here hydrogen bond ineteractions were not obsercevd and amino acids participated in other interactions were arginine, methonine, leucine, phenylalanine, etc.

Table 3. Interaction table of docking of CYP9A12 (*Helicoverpa armigera*) and CYP4G12 (*Trichogramma cacoeciae*) with monocrotophos

H. armigera				*T. cacoeciae*				
H-bonds	Pi-Pi	Hydrophobic interactions	Others	Halogen bond	H-bonds	Pi-Pi	Hydrophobic interactions	Others
LEU (300) – N (1)	PHE (191) – C (18)	ALA (91) – C (9)	THR (190) – C (25)	ARG (448) – Cl (1)	NIL	PHE (126) – C (11)	LEU (56) – C (23)	PRO (61) – O (1)
	PHE (191) – C (15)	ALA (91) – C (17)	THR (190) – C (24)			PHE (126) – C (8)	LEU (56) – C (25)	THR (92) – C (11)
	PHE (193) – C (24)	ALA (92) – C (15)	ILE (303) – N (1)			PHE (126) – C (13)	LEU (56) – C (24)	THR (92) – C (4)
	PHE (193) – C (24)	ALA (304) – C (17)	ALA (304) – O (1)			PHE (126) – C (10)	PRO (60) – C (4)	THR (92) – C (5)
		CYS (450) – C (5)	ALA (304) – O (2)				PRO (61) – C (4)	THR (92) – C (13)
			ALA (304) – N (1)				PRO (61) – C (2)	THR (92) – C (8)
			THR (308) – C (4)				PRO (61) – C (16)	PHE (126) – O (2)
			CYS (450) – O (2)				PRO (61) – C (19)	PHE (126) – Cl (1)
							PRO (61) – C (18)	
							PRO (61) – C (20)	
							PRO (61) – C (21)	

H-bonds = hydrogen bonds; LEU = leucine; PHE = phenylalnine; ALA = alanine; CYS = cysteine; THR = thrionine; ILE = Isoleucine; ARG = arginine; PRO = proline

hydrogen bonds in the binding affinity of a target has been described (Panigrahi, 2008; Rocher and Marchand-Geneste, 2008) and these studies corroborates with the present study which indicates that hydrogen bonds responsible for higher resistance in *H. armigera* compared to *T. cacoeciae.*

In an analysis carried out with fenvalerate, *H. armigera* P450 interacted with one hydrogen bond and five hydrophobic interactions, with binding energy of –7.65 kcal/mole, where as no hydrogen bonds was observed and three types of interactions, *i.e.*, hydrophobic (Bachar *et al*., 1993), pi-pi (1) and some other, recorded between fenvalerate and CYP4G12 of *T. cacoeciae*. Moreover, the binding free energy between the CYP4G12 and fenvalerate in *T. cacoeciae* is much lower, *i.e.*, –5.28 kcal/mole as compared to the binding with monocrotophos. The lower the binding energy, the more stable the complex will be and more likely the possibility of binding will happen. Therefore, docking study indicated that P450 for *H. armigera* is having more stable docking confirmation with the fenvalerate ligand molecule than *T. cacoeciae* (Table 3) (Fig. 5 and 6).

Incase of protein-ligand docking, non-covalent bonds such as hydrogen bonds, hydrophobic interactions and ionic interactions play a vital role for the stability of the docked complex (Patil *et al*., 2010). According to a study for *D. melanogaster* (Liu and Scott, 1998), CYP6G1 active site cavity is surrounded by hydrophobic residues resembles structurally as well chemically to the molecular characteristics of DDT, therefore, forming a stable docked complex as compared to other insecticides like imidacloprid, nitenpyram, acetamiprid and malathion.

In the present study, both hydrogen bond and hydrophobic interactions were observed in the docking analysis for *H. armigera* and *T. cacoeciae* CYP450 with insecticides - monocrotophos and fenvalerate. In case of *T. cacoeciae*, though hydrophobic interactions were observed but hydrogen bonds were lacking, whereas, both hydrogen bonds and hydrophobic interactions

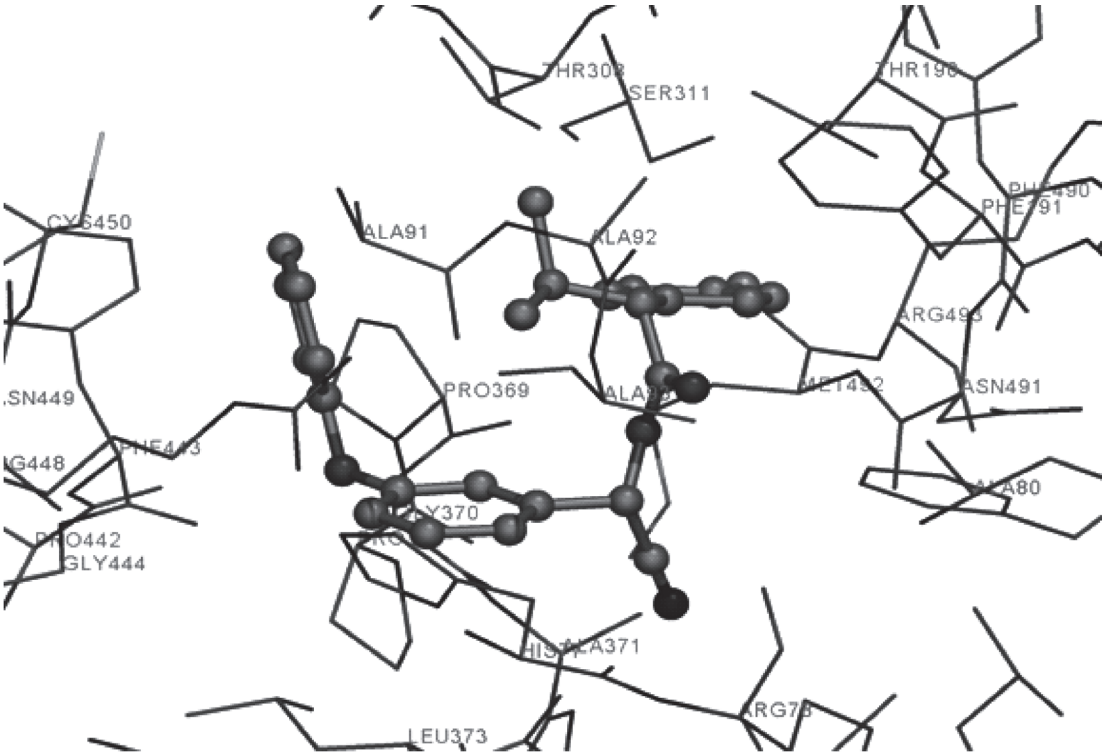

Fig. 5. Docking interaction of *Helicoverpa armigera* (CYP9A12) and fenvalerate. Protein docking ineraction of *H. armigera* (CYP9A12) and fenvalerate (ligand) showing the actively participated amino acids around protein active site. As compared to monocrotophos, here only one amino acid involved in hydrogen bond interaction with fenvalerate and other amino acids present were phenylalanine, alanine, thrionine, cystein, etc.

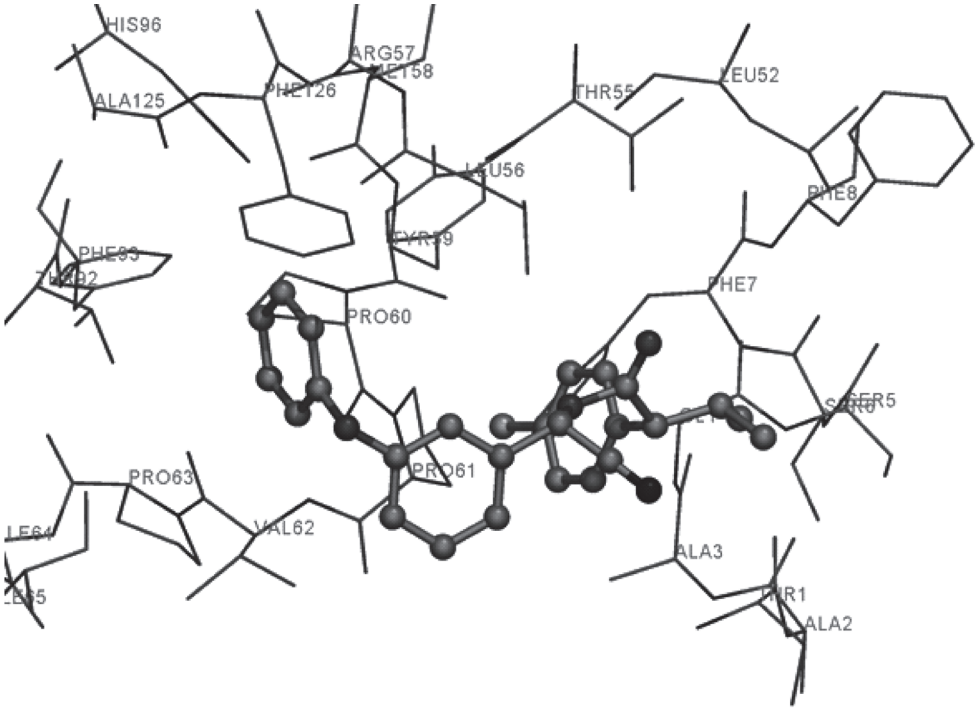

Fig. 6. Docking interaction of *Trichogramma cacoeciae* (CYP4G12) and fenvalerate. Protein docking ineraction of *T. cacoeciae* (CYP4G12) and fenvalerate (ligand) showing the actively participated amino acids around protein active site. Hydrogen bond interactions were mising amino acids participated in other interactions were proline, thrionine, phenylalanine, etc.

were present for *H. armigera* CYP450 docked complex. The role of hydrogen bonds in docking interactions may be considered as one of the factor for insecticide interactions on insects. Thus, it can be possible for *H. armigera* being more resistant towards insecticide as compared to *T. cacoeciae,* as the CYP450 of *T. cacoeciae* is less reactive due to lack of H-bonds. The homology modeling and 3D structure comparison of cytochrome P450 of both *H. armigera* and *T. cacoeciae* showed that they were similar as the Root Mean Square Deviation (RMSD) value for both the structures was less than 2Å, *i.e.*, 1.12Å (as per C-alpha match server), however, the docking studies revealed that *H. armigera* is more interactive which indicated the potential of the pest to easily detoxify the insecticides monocrotophos and fenvalerate compared to *T. cacoeciae*. Molecular docking studies could add value to insecticide discovery programs through predicting the potential for resistance development in economically important insect species.

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