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In Silico Analysis and Comparative Modeling of Carboxylesterase in *Spodoptera litura*

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ABSTRACT

Esterase-derived metabolic resistance is a major issue in vector populations. Although many esterases may be involved in pesticide metabolism, the carboxylesterase gene family appears to contain the principal esterases engaged in insecticide metabolism. Numerous endogenous and exogenous ester-containing substances are hydrolyzed by these carboxylesterases. Agrochemicals, including pyrethroids, organophosphates, and carbamates, are found to be detoxified by them. At the proteome level, the three-dimensional (3D) architecture of proteins is extremely functional. In the current study, as no 3D structural details are available for Carboxylesterase in *Spodoptera litura, in silico* analysis is performed. Various physicochemical parameters, secondary structural elements, and 3D structural details are studied using computational techniques. The model predicted is found to be of good quality as evidenced by various tools of validation. Structure to function analysis, a fundamental premise in protein biology, demonstrates that structural information can be used to predict not only the functions of proteins but also various mechanisms involved. The predicted molecular model of Carboxylesterase described in this study could be useful to know more about substrate selectivity and pesticide metabolism. It may provide new insights regarding carboxylesterase-mediated pesticide resistance in the pest of choice regarding gene expression studies.

***Key words*:** Carboxylesterase, Resistance, Comparative modelling, *Spodoptera litura*

Insecticide resistance is thought to be caused in part by the indiscriminate application of insecticides [1]. Esterase-derived metabolic resistance is a major issue in vector populations since it has been linked to resistance to the two most often used insecticide classes, OPs and pyrethroids [2]. Although other esterases with other domains may be involved in pesticide metabolism, the carboxylesterase gene family appears to contain the principal esterases engaged in insecticide metabolism. Carboxylesterase is an essential enzyme involved in the destruction and detoxification of pyrethroid insecticides, and it works through hydrolysis by the esterase group enzyme [3]. Because of their significance in the metabolism of numerous agrochemicals and medicines, as well as their role in endogenous metabolism, interest in this class of enzymes has grown.

Spodoptera litura Fab. (Noctuidae: Lepidoptera) is a polyphagous agricultural pest with a wide geographic range that includes Asia, Africa, Australia, and New Zealand [4]. It had evolved resistance to the majority of common insecticides used around the world [5]. And moreover, in *Spodoptera litura*, esterase-mediated detoxification is discovered to be the primary mechanism for pyrethroid resistance [6]. For a better understanding of insecticide metabolic resistance and its genetic foundation, studies on structural characterization become inevitable. The main disadvantages of experimental approaches such as X-ray crystallography and NMR are that they are time-consuming and expensive. Hence, computational methods could be the best alternative. Since there is no 3D structure available for carboxylesterase of *Spodoptera litura* in the databases, to understand its structure and functional features, *in silico* structural characterization of the target enzyme carboxylesterase was carried out in the present study.

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MATERIALS AND METHODS

To perform the structural characterization and 3D structure prediction of carboxylesterase of *Spodoptera litura*, the following tools and software are used:

Sequence retrieval

The selected sequence of carboxylesterase of *S. litura* was retrieved from Uniprot Knowledge Base (UniProtKB)

with the Accession Number. Q1W2E4. This has 131 amino acids. The FASTA format of this selected protein sequence has been downloaded and used for further structural analysis.

Primary structure prediction

Physicochemical characterization

The amino acids sequence of the enzyme carboxylesterase of *S. litura* has been submitted to Expasy's ProtParam tool [7]. Various important biochemical parameters such as a number of hydrogen bonds, helices, strands, Isoelectric point (pI), molecular weight, instability index, aliphatic index and score for Grand average hydropathicity (GRAVY) are computed.

Secondary structure analysis

The secondary structure analysis of Q1W2E4 has been performed by the SOPMA server.

Tertiary structure prediction

For the enzyme carboxylesterase of *S. litura*, only the primary sequence is available. The data on 3D structural information is not available in any model of the structural databases such as PDB, Swiss model, etc. Hence comparative modeling or homology modeling has been performed to deduce the three-dimensional structure of the protein. SWISS-MODEL server is used to generate a 3D model of query protein Q1W2E4. This server performs modeling via sequence alignments for selection of the putative template protein to generate the 3D model of query sequence [8]. The template used in the present study is 4qwm.1A. with 55% sequence similarity. The generated model has been evaluated and validated by various evaluation tools and servers to elucidate the structural quality if the predicted protein. The 3D structure of query protein has been visualized using RASWIN visualization tool.

RESULTS AND DISCUSSION

Protein structure prediction is aided by *in silico* analysis in practically all research disciplines. Structure to function analysis, which is a fundamental premise in protein biology, demonstrates that structural information can be used to predict how proteins will perform. The creation of this protein's model and other functional analyses will place a strong emphasis on understanding the role of amino acids, which will aid in determining how these residues are involved in the protein-ligand interaction mechanism [9]. The physicochemical analysis of the predicted Carboxylesterase has been performed using ProtParam and results are shown in (Table 1). This protein has 131 amino acids with a molecular weight of 14552.56. The theoretical PI is recorded as 4.64. The total number of negatively charged residues (Asp + Glu, 16) is higher than the total number of positively charged residues (Arg + Lys, 10). The Instability index has been recorded as 49.20 which makes the protein is unstable. The aliphatic index is calculated as 75.11, revealed that this protein may be globular in nature. The grand average hydropathicity (GRAVY) is recorded as -0.278. The negative value indicates that the protein may be hydrophilic [10].

Table 1 Physicochemical parameters of carboxylesterase (Q1W2E4) of *S. litura*

Name of protein	Isoelectric point (pI)	Molecular weight (MW)	Negatively charged residues (-R)	Positively charged residues (+R)	Extinction coefficient (EC)	Instability index (II)	Aliphatic index (AI)	GRAVY
Carboxyl esterase	4.64	14552.56	16	10	14565	49.20	75.11	-0.278

The secondary structure of the protein is predicted using the SOPMA server (Fig 1). It is observed that random coil is predominant (70.99%), followed by extended strand (23.37%). Random coils have important functions in proteins for flexibility and conformational changes such as enzymatic turnover [11]. This may possibly be related to the enzymatic function of the protein. Protein 3D structure contributes to the understanding of protein function and active sites present if any. The SWISS-MODEL homology modeling program is used for the prediction of the three-dimensional structure of the Carboxylesterase enzyme of *S. litura* (Fig 2). PDB 4QWM1A has been selected as a template with 555.38% sequence identity to query sequence (Q1W2E4).

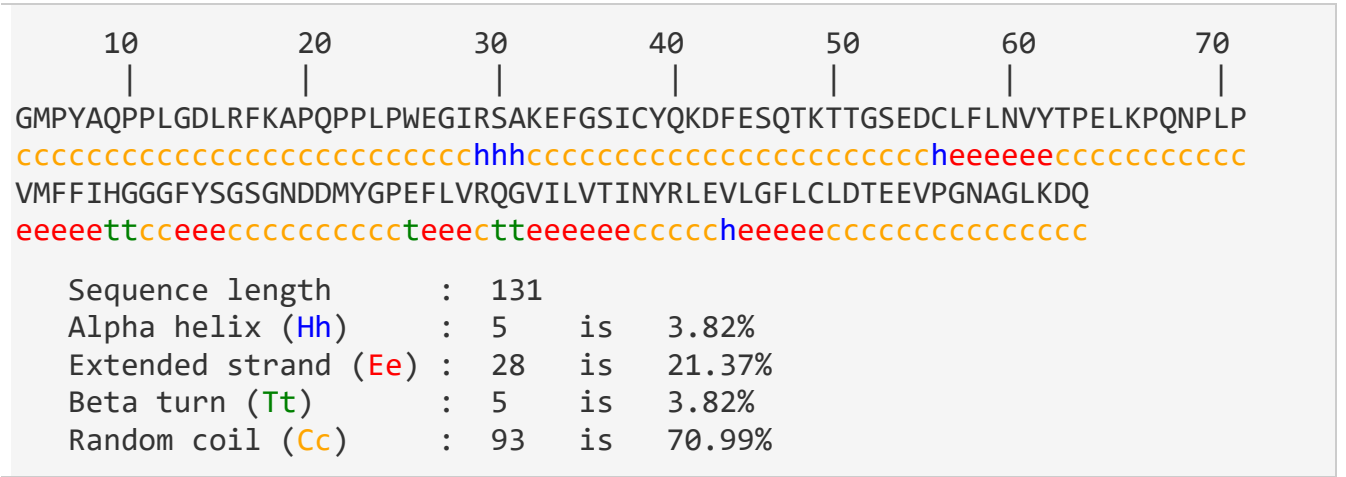

Fig 1 SOPMA result for Carboxylesterase Q1W2E4 showing secondary structural elements in the query protein

Using structurally solved proteins as references, the Z-score is utilized to estimate the model quality [12]. Z-Score was found similar to the non-redundant PDB structures (Fig 3). The stereochemical quality of the modeled protein has been analyzed by Ramachandran plot (Fig 4). This analysis shows that only 1.4% of residues in the generously allowed region,15.7% additionally allowed region, and 83.3% in the most favoured region, indicating that the model is found to be reliable and of good quality.

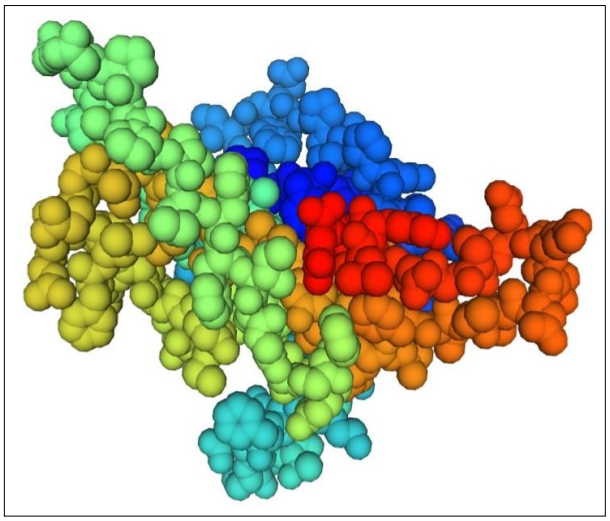

Fig 2 Predicted model of carboxylesterase of *Spodoptera litura* (Q1W2E4) by SWISS-MODEL

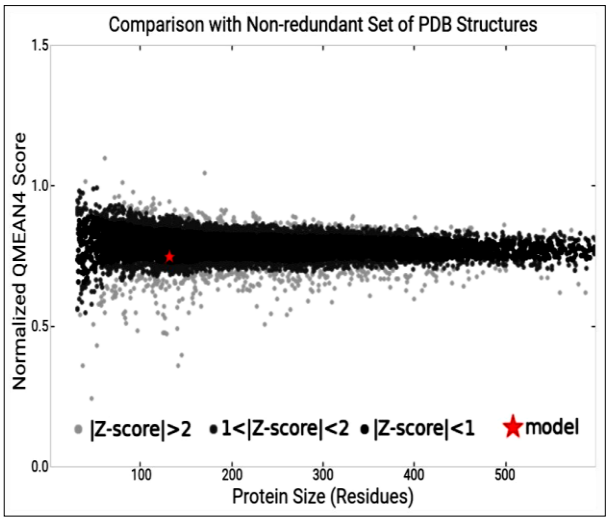

Fig 3 Showing the protein of the modeled query (in red colour) Q1W2E4 with its Z score. The modelled query lies well within the good scoring areas of other non-redundant PDB structures

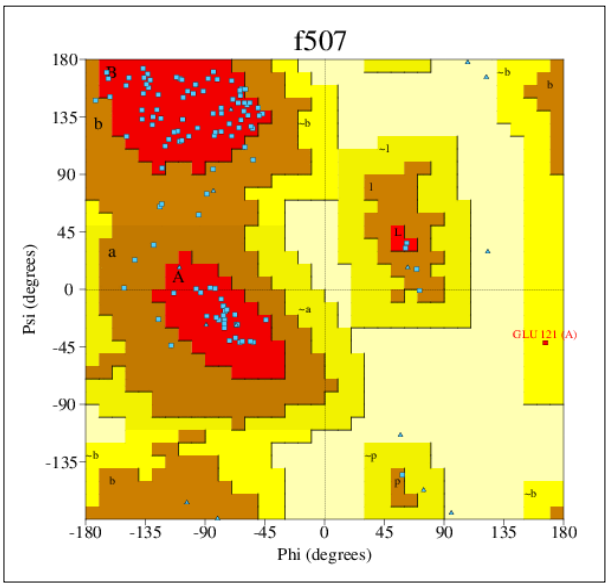

Fig 4 Ramachandran plot of query protein carboxylesterase of *Spodoptera litura*

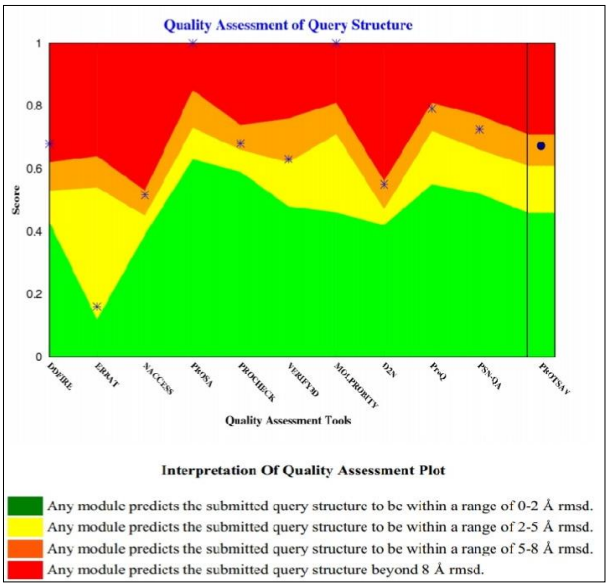

Fig 5 Estimated absolute quality of modeled protein carboxylesterase of *Spodoptera litura* with rmsd 5-8 A^0

	Residues	No. of percentage
Most favoured regions [A,B,L]	85	83.3*
Additional allowed regions [a,b,l,p]	16	15.7
Generously allowed regions [~a,~b,~l,~p]	1	1.0
Non-glycine and non-proline residues	102	100.0
End-residues (excl. Gly and Pro)	1	
Glycine residues	15	
Proline residues	13	
Total number of residues	131	

The protein structure analysis and validation tool (protSAV) revealed that the model lies within the allowed RMSD area 5-8A (Fig 5). The protein structure verification algorithm, ERRAT analyses the presence of various of non-bonded interactions between different atoms [13]. This shows that the predicted model has overall good quality of 87.619% (Fig 6).

Esterases play a key role in the metabolism of a variety of foreign and endogenous substances. Pheromones and other semiochemicals, which play important roles in

insect growth and behavior, are forms of esters that are hydrolyzed by esterases that primarily belong to the Carboxylesterase gene family in insects [14]. Metabolic resistance, the increased capacity to detoxify pesticides as a result of increased expression or activity of many enzyme families, gains importance as it is one of the important resistance mechanisms seen in insect pests [14]. Henceforth, esterases warrant special study as they can play a role in developing resistance to the principal chemicals used in vector and pest-control programs [15-16].

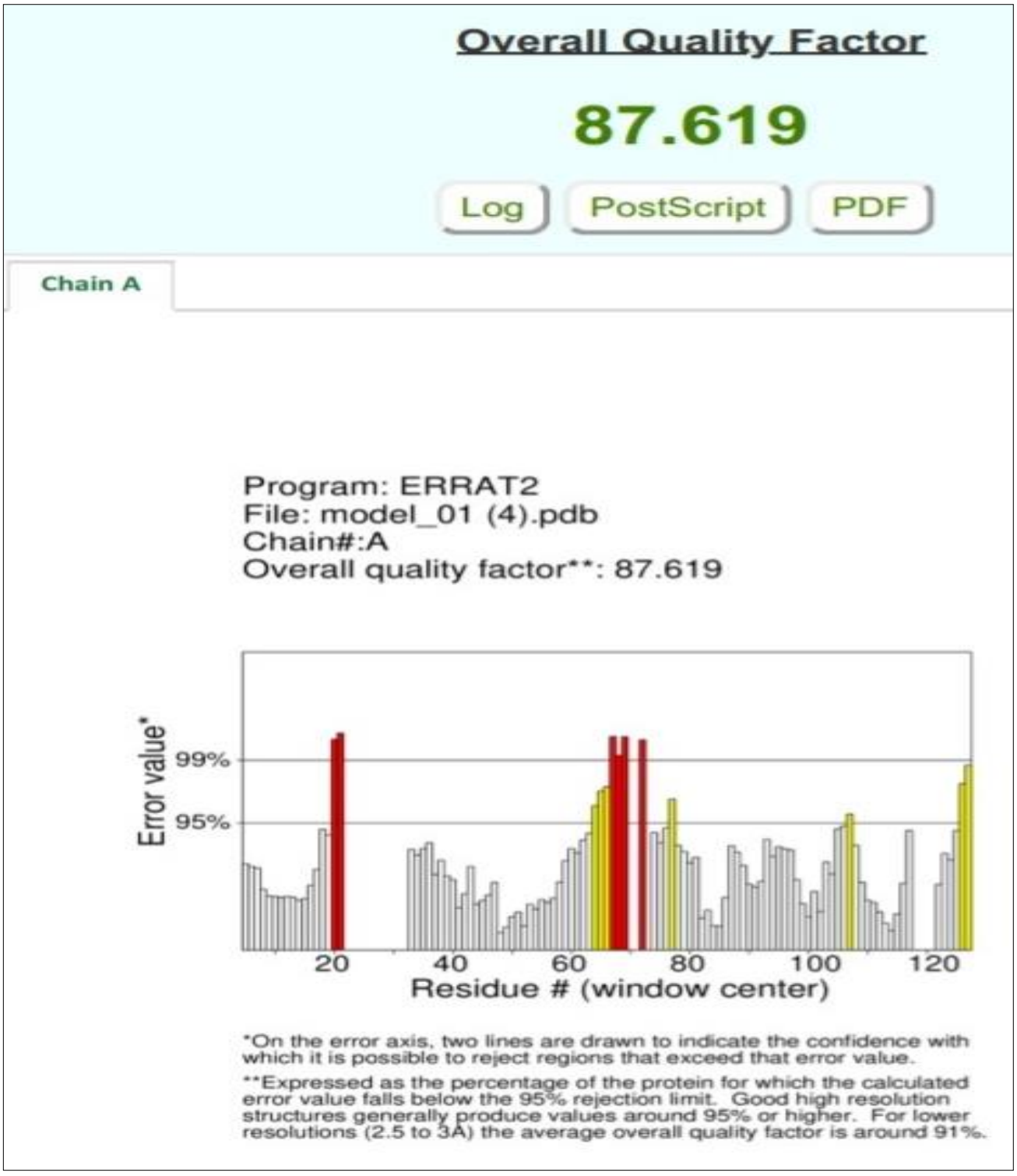

Fig 6 Overall quality of modelled protein of carboxylesterase of *Spodoptera litura* by ERRAT server

The carboxylesterase gene family appears to be evolving, and each insect species has its own set of detoxifying genes, with only a few orthologues seen in other insects [17]. In this study, the Carboxylesterase of *Spodoptera litura* (Q1W2E4) exhibits structural characterization and the comparative model generated is of good quality. The projected structural model may provide new insights into the biology of the protein and its function and insights into new aspects of detoxifying mechanism in the chosen insect pest.

The importance of carboxy-esterase in insect adaptation to new ecological niches, as well as the role of this esterase in specific metabolic pathways, has to be explored with the view of genomes, proteomics, and

metabolomics. The current study on the structural characterization of carboxylesterase in *Spodoptera litura* may be utilized for this investigation.

CONCLUSION

Model development by *in silico* methods will give great focus on understanding the role of amino acids present in the protein and helps in deciphering the involvement of these residues during the mechanism of protein-ligand interaction in various mechanisms involved. The structural characterization in the present study reveals that the comparative model of Carboxylesterase suggested for *Spodoptera litura* (Q1W2E4) is good. The predicted structural model may reveal new information about the protein's biology and function, as well as new hopes and insights into new facets of carboxylesterase mediated detoxifying mechanism in *Spodoptera litura* in the future.

LITERATURE CITED

1. Kranthi KR, Jadhav DR, Kranthi S, Wanjari RR, Ali SS, Russell DA. 2002. Insecticide resistance in five major insect pests of cotton in India. *Crop Protection* 21(6): 449-460.
2. Bass C, Field LM. 2007. Gene amplification and insecticide resistance. *Pest Management Science* 67(8): 886-890.
3. Mohan L, Sharma P, Srivastava CN. 2007. Comparative efficacy of *Solanum xanthocarpum* extracts alone and in combination with a synthetic pyrethroid, cypermethrin, against malaria vector *Anopheles stephensi*. *Southeast Asian Journal of Tropical Medicine and Public Health* 38(2): 256-260.
4. Weinberger K, Srinivasan R. 2009. Farmers' management of cabbage and cauliflower pests in India and their approaches to crop protection. *Journal of Asia-Pacific Entomology* 12(4): 253-259.
5. Saleem MA, Ahmad M, Ahmad M, Aslam M, Sayyed AH. 2008. Resistance to selected organochlorin, organophosphate, carbamate and pyrethroid, in *Spodoptera litura* (Lepidoptera: Noctuidae) from Pakistan. *Journal of Economic Entomology* 101(5): 1667-1675.
6. Shankarganesh K, Walia S, Dhingra S, Subrahmanyam B, Babu SR. 2012. Effect of dihydrodillapiole on pyrethroid resistance associated esterase inhibition in an Indian population of *Spodoptera litura* (Fabricius). *Pesticide Biochemistry and Physiology* 102(1): 86-90.
7. Gasteiger E, Hoogland C, Gattiker A, Wilkins MR, Appel RD, Bairoch A. 2005. Protein identification and analysis tools on the ExPASy server. *The Proteomics Protocols Handbook.* pp 571-607.
8. Arnold K, Bordoli L, Kopp J, Schwede T. 2006. The SWISS-MODEL workspace: a web-based environment for protein structure homology modelling. *Bioinformatics* 22(2): 195-201.
9. Mishra M, Pant AB, Srivastava P. 2013. 3D structure prediction with functional site identification of fatty acid retinal binding (FAR) protein: A target against filarial fight. *International Journal of Current Microbiology and Applied Sciences* 2(11): 123-131.
10. Filiz E, Koç I. 2014. In silico sequence analysis and homology modeling of predicted beta-amylase 7-like protein in *Brachypodium distachyon L. Journal of BioScience and Biotechnology* 3(1): 61-67.
11. Buxbaum E. 2007. *Fundamentals of Protein Structure and Function*. Vol. 31. New York: Springer. pp 23.
12. Benkert P, Künzli M, Schwede T. 2009. QMEAN server for protein model quality estimation. *Nucleic Acids Research* 37(2): 510-514.
13. Colovos C, Yeates TO. 1993 Verification of protein structures: Patterns of nonbonded atomic interaction. *Protein Science* 2(9): 151-1519.
14. Montella IR, Schama R, Valle D. 2012. The classification of esterases: An important gene family involved in insecticide resistance- A review. *Memorias do Instituto Oswaldo Cruz* 107: 437-449.
15. Andrew Y, LiFelix D, Pruett GH. 2007. Involvement of esterases in diazinon resistance and biphasic effects of piperonyl butoxide on diazinon toxicity to *Haematobia irritans irritans* (Diptera: Muscidae). *Pesticide Biochemistry and Physiology* 87(2): 147-155.
16. Nauen R. 2007. Insecticide resistance in disease vectors of public health importance. *Pest Management Science* 63(7): 628-633.
17.Oakeshott J, Claudianos C, Campbell PM, Newcomb RD, Russell R. 2010. Biochemical genetics and genomics of insect esterases. *Comprehensive molecular insect Science* 5: 229-234.