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Research article

INHIBITION OF CARBOXYL ESTERASE BY BIO-PESTICIDES IN THE CONTROL OF COTTON BOLL WORM *HELICOVERPA ARMIGERA* (HUBNER)

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ABSTRACT: The noctuid moth species *Helicoverpa armigera* Hubner (Lepidoptera) is a major pest of cotton and other crops causing extensive damages to other crops such as okra, bhindi. These pests have developed resistance to synthetic pesticides which is mainly attributed to detoxification enzymes such as carboxyl esterase. Molecular modeling approaches were used to find the binding affinity of bio-pesticides azadirachtin, plumbagin, pyridalyl and tagitinin C. We report here that the compounds plumbagin and tagitinin C to be potential bio-pesticides to control the menace of this pest.

Key words: Helicoverpa armigera. Carboxylesterase, bio-pesticides, Molecular Docking

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INTRODUCTION

Indian economy is agro-based economy and agriculture is the mainstay as it constitutes the backbone of the rural livelihood security system [1]. Cotton (*Gossypium hirsutum*) is one of the major contributors to the agricultural economy of India. India is second largest producer of cotton after China, though it has largest area under cultivation [2]. There are several reasons attributed to this problem. The most important being the pest attack. Cotton crop is severely attacked by number of pests [3]. There are near about 1326 insects and mites all over the world and about 200 in India have been recorded as pests of cotton [4]. But due various reasons the production of cotton is declining per year. Among them one of the most important factors is the pest invasion, there is huge down fall in the production scale. *H. armigera* is the most prevalent pest of cotton crop which yields a huge loss every year [5].

The cotton bollworm, corn earworm or Old World (African) bollworm, *Helicoverpa armigera* (Hubner), is a moth, the larvae of which feed on a wide range of plants, including many important cultivated crops. It is a major pest in cotton and one of the most polyphagous and cosmopolitan pest species [6]. When the bolls are damaged, some will fall off and others will fail to produce lint or produce lint of an inferior quality. During the last 50 years, worldwide use of synthetic insecticides to control insect pests has led to both insecticide resistance and environmental problems [7]. Members of the carboxylesterase and cytochrome P450 monooxygenase super families are prominent candidates to confer metabolic resistance to pyrethroid insecticides. Both carboxylesterases and P450 enzymes have been shown to be involved in pyrethroid resistance in Australian *Helicoverpa armigera*, the noctuid species possessing by far the most reported resistance cases worldwide [8]. Carboxyl esterases are an important class of detoxification enzymes involved in insecticide resistance in insects [9].

Carboxyl esterase is mainly involved in the mediation of metabolic resistance of many insects to organophosphate (OP) insecticides [10]. Integrated Pest Management (IPM) is an environmentally friendly, common sense approach to controlling pests. Emphasis here is given to apt pesticide usage and thereby ensuring safety of non-target flora and fauna. Regulation of these pests using green methods such as bio-pesticides is widely practiced approach, as they are within the norms of IPM [11]. Bio-pesticides like Pyrydalyl [12] Tagitinin C [13], Plumbagin [14], Azadirachtin [15] are well known for their various properties like anti-microbial, anti-cancerous activity. So we performed molecular docking studies and found that all the bio-pesticides have affinity towards carboxyl esterase and hence inhibit their activity to control the pest. To find alternative pesticides to control the activity of carboxyl esterase so the detoxification could be arrested in *H. armigera*. To model the structure carboxyl esterase and to perform molecular docking of the modeled structure with the selected pesticidal compounds.

MATERIALS AND METHODS

Structures of the biopesticides were downloaded from the NCBI Pubchem database [16]. These compounds were Pyrydalyl, Tagitinin C, Plumbagin and Azadirachtin. These compounds were downloaded in sdf format and were further converted to the required PDB format using Open Babel software.

The sequence of the enzyme carboxylesterase was retrieved in a fasta format from the NCBI genbank [17] portal holding the accession number ABE01157.2 which was 537 amino acids long. This sequence was submitted to the Blastp database to search for the template against PDB which could form the basis for modeling the three dimensional structure by homology modeling.

Easy Modeller [18] application was used to model the structure. The modeled structure was validated by Ramachandran plot at the Rampage server. Once the modeled structure was found to be acceptable, it was submitted to the ligsite module to predict the binding site in this enzyme. Molecular docking was performed using Autodock version 4.2 [19] at default parameters. Enzyme model was prepared by adding hydrogens and then calculating the overall charges. Ligand conformation was set to default and the grid was generated in such a way that it covers the binding site reported by Ligsite [20]. Docking was performed by employing the genetic algorithm and every cycle of docking would generate 10 different poses and the best one was assessed based on the energy.

Once docking was completed, the interaction analysis was performed using the post dock analysis option of autodock; this gives detailed information on the interacting atoms and also the contributing energy by such an interaction to the total potential of the docked pose. These poses were finally visualized using the Pymol applications.

RESULTS

Blast P against PDB search resulted PDB ID: 4FNM|A which was Chain A of the Alpha-esterase-7 Carboxylesterase, E3, from the Blowfly *Lucilia Cuprina*. Statistics for this hit were 35% identity over 98% query coverage with an e-value of 7e-94. This modeled structure was found to consist of helices and sheets. Upon submission to the Rampage, the modeled structure was found to be having 98% of the residues to be in the allowed regions as was the requirement and the rest being in the additionally allowed regions meaning no residues in the disallowed regions.

Ligsite is a web server for the automatic identification of pockets on protein surface using the Connolly surface and the degree of conservation. Residues 128, 131, 135, 147, 152, 157, 161, 198, 252, 273, 281 and 290 were predicted to be actively involved in dictating the crucial activity for the modeled structure.

Table 1: Energies contributed by various interactions after the docking process in autodock.

Compound	Binding Score	Ligand Efficiency	Intermol Energy	Electrostatic Energy	Total Internal	Torsional Energy	Interacting Residue	H-Bond Distance
Plumbagin	-4.65	-0.33	-4.93	-0.02	-0.52	0.27	ASN161	2.195
Pyridalyl	0.75	0.03	-4.25	-0.03	1.87	2.74	GLU281	2.897
Tagitinin C	-4.67	-0.19	-6.11	0.02	-0.14	1.1	ASN161	2.15
Azadirachtin	520	10.2	494.06	0.18	23.51	3.29	TYR 131 & TYR147	1.98 & 2

Autodock is a molecular docking application with good accuracy in its predictions. Though there were interactions between all the four selected biopesiticides, it was only the compounds plumbagin and tagitinin C were found to be good enough to be concluded as the best. Because the binding energy was lower in this case in comparison to the other two azadirachtin and pyridalyl. Both Tagitinin C and Plumbagin reported the binding score to be approximately -4.65 (table 1) coupled with perfect ligand efficiency. Incidentally, both these compounds shared a similar mode of interaction where the residue ASN161 was found to be facilitating the hydrogen bond formation with approximately 2.1angstroms as the distance of it (Figure 1).

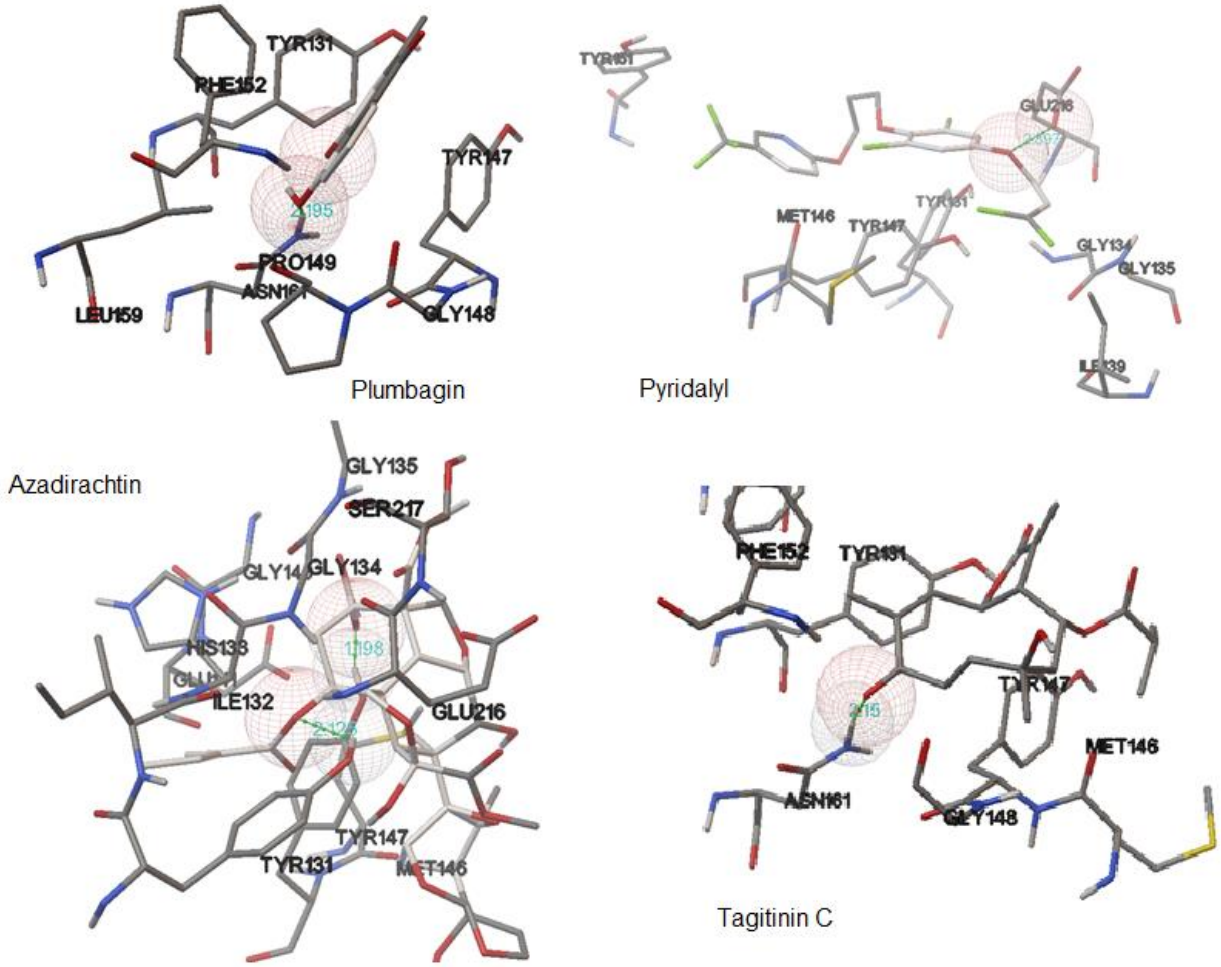

Figure 1: Showing the binding of the bio-pesticides with the modeled structure of carboxyl esterase using autodock.

DISCUSSION

Cotton is the important crop of commercial importance in the Indian subcontinent. Severe loses were reported due to the incidence of pest attacks. *Helicoverpa armigera* is a lethal pest of cotton and was found to be developing resistance to various classes of pesticides. Carboxyl esterase is vital enzyme which acts as a defense mechanism in insects where it just metabolizes the pesticdes and thereby detoxifying it. These class of enzymes such as Glutathione S transferase, Cytochrome P450 Mono oxygenase have undergone various changes genetically over the period of evolution, thereby, developed resistance to almost all class of synthetic pesticides.

Integrated Pest Management strategies involve usage of less of no pesticides and bio-control measures such as entomopathogens, microorganisms and bio-pesticides derived from the plant and microbes. In this study, we tried to find the binding affinity of few of the bio-pesticides in arresting activity of this enzyme. One of the main advantages of selecting bio-pesticides is they are not considered to be causing any side effects and they are target specific and also since they are biologically, derived; their over usage may not significantly affect the soil quality as is the case with synthetic pesticides.

Molecular modeling approaches have significantly reduced the time and money in the diverse fields of health and agriculture. These are the theoretical approaches based strongly on the principles of physics and chemistry, which try to emulate the possible interactions between the drug / pesticide like compounds before they could be tested for their efficacy in wet labs where vast money is spent. We found in this study, that Tagitinin C and Plumbagin were reported to be having binding affinity with energy and related parameters being in the acceptable limits. These are calculated based on the concepts of molecular mechanics force field which involves the energy contribution of all the interactions involved. Often, interaction between any compound and its target structure is mainly Van der Waals, electrostatic and/or hydrogen bond interaction.

Though there were two hydrogen bonds formed between the modeled structure and neem based derivative azadirachtin, it was not a favourable result as its overall binding energy is in positive meaning the complex to be in excited state. Plumbagin and Tagitinin C as could be seen witnessed fared better in terms or energy and its related terms. Here the interaction between these two compounds and the modeled structure was due to a hydrogen bond formed involving residue Asn161 in either cases.

CONCLUSION

Cotton farmers are facing heavy losses due to the menace pesticides, especially the noctuid species Helicoverpa armigera Hubner. Existing measures of control have all been failing and are not in accordance with IPM, as the pests have developed resistance to the synthetic pesticides. Carboxyl esterase is a detoxification enzyme known to be metabolizing pesticides due to its exposure to heavy pesticides usage. Therefore, bio-pesticides such as Azadirachtin, Plumbagin, Tagitinin C and Pyridalyl were used to find their binding affinity to carboxylesterase and therefore, inhibiting its activity. We used the molecular modeling and docking approaches and found that Tagitinin C and Plumbagin to be having better interaction with the enzyme and so, could be used as a control measure to Helicoverpa armigera.

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