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Exploring Bio Control Strategies to Control Insect Pests by Comparative In Silico Analysis of *Helicoverpa armigera* Juvenile Hormone Epoxide Hydrolase Enzyme with Selected Homologues Lepidopteran Insects

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Abstract: In silico analysis facilitates in understand the mechanism and behavior of a protein in the living system using the computational simulation. Reports reveal that more than 300 insect pests contribute for a considerable damage to the agriculture industry result in great loss to the country's economy. Among the damage caused by insect pests more than 25% of them are from the order lepidopterans. Extensive research on lepidopteran insects revealed that Juvenile Hormone Epoxide Hydrolase (JHEH) plays a key role in degenerative mechanism of Juvenile Hormone (JH), which is essential for Insect growth and reproduction. Because of the JHEH role in JH degenerative mechanism, in the present study the JHEH gene sequence is retrieved from sequence database and homologues lepidopterans were searched using Blastx for comparative analysis. Primary sequence analysis, secondary sequence analysis, functional domains and evolutionary relationship among the homologues sequence of Ha JHEH are studies using various bioinformatics tools to establish a base for insect pest biocontrol strategy using its own enzyme.

Key words: Comparative in silico sequence analysis, *Helicoverpa armigera* (*Ha*), Juvenile Hormone (JH), Juvenile Hormone Epoxide Hydrolase (JHEH), Lepidopteran insect pests biocontrol strategy.

I. INTRODUCTION:

Agriculture and its linked disciplines, such as poultry and dairy industries, play an important part in the economies of most emerging and agriculture-dependent countries, such as India. It is critical to keep up with new technology accessible globally in order to meet food needs in proportion to population growth. Despite advances in agricultural research and development, new challenges always appear in the form of unseasonable rains, natural disasters, new viruses, disease resistance developed by insects or pests, and so on. However, pest management is always regarded as a difficult task, as these pests develop resistance to chemical pesticides. Among these pests, the Lepidopteran species is regarded as the most destructive insect pest, attacking nearly all significant food crops as well as commercially important crops such as tomato, cabbage, lettuce, pea, pepper, peanut, sunflower, tobacco, cotton mulberry, and so on. According to reports, high levels of chemical pesticide use were having major detrimental consequences on the environment and human health by infiltrating the food chain. Biological pest control measures, on the other hand, were not producing substantial success in managing lepidopteran insect pests due to their strong reproducing capabilities.

New methods are now being investigated in order to control lepidopteran pests in an environmentally responsible manner. According to many research findings, Juvenile Hormone (JH) plays a vital function in controlling insect physiological activities such as behavior, diapauses, and so on [1]. JH affects the growth physiology of lepidopteran insect pests in the larval stage and the reproductive physiology at the adult stage. JH levels are regulated by the Juvenile Hormone Epoxide Hydrolase (JHEH) and the Juvenile Hormone Esterase (JHE) [2] & [3]. JHE performs ester hydrolysis, while JHEH performs epoxide hydrolysis, resulting in JH acid and JH diol [4].

Helicoverpa armigera, *Manduca sexta*, *Heiothis virescens*, *Spodoptera exigua*, *Trichoplusia ni* and *Bombyx mori* were chosen as prominent lepidopteran insect pests in this work to investigate their JH mechanism, as these pests cause significant

damage to both commercial and food crops. Understanding the JH mechanism, which plays a critical role in the growth and reproductive physiology of a lepidopteran insect, could be a possible target for pest management [5]. JHEH genes from these lepidopteran pests were used to study their structure, function, gene ontology, and pathway mechanism, using in-silico methods which are now available to examine the sequencing and functional features of an organism [6].

II. RESEARCH METHODOLOGY:

The JHEH sequences of the selected lepidopteran pests were obtained from the UNIPROT protein database and submitted to HMM scan and batch CDD search to determine the PFAM domain information. PROTPARAM was used to analyze physical-chemical properties, and SOPMA was used to analyze secondary structure. The protein sequence is predicted using the BLAST2GO functional gene ontology. Using the KEGG automatic annotation server is used to identify JHEH pathway mechanism.

2.1 Sequence Retrieval:

The sequence retrieval is done using UNIPROT- KB database. The European Bioinformatics Institute (EMBL-EBI), the Swiss Institute of Bioinformatics (SIB) and the Protein Information Resource (PIR) partner to develop UniProt by the UniProt Consortium. The three groups together employ about 90 employees for a variety of responsibilities, including database maintenance, software development, and user assistance. A number of jobs can be performed with the support of UniProt, including learning more about a protein of interest and comparing its protein sequence with those of other proteins, as well as interpreting a list of identifiers from an external database to UniProtKB or vice versa. Huge efforts are being made in the scientific community to learn as much as possible about the proteins encoded by these genomes as the number of fully sequenced genomes continually increasing. This endeavour is producing an enormous quantity of data and is important to many branches of research, including biology, medicine, and biotechnology. A current and comprehensive database of protein knowledge is provided by UniProt. By gathering, analysing, and structuring this data, the resource helps scientific discovery and saves the time of the researchers. The JHEH amino acid sequences of *Helicoverpa armigera and its homologues lepidopteran JHEH sequences of Manduca sexta, Heiothis virescens, Spodoptera exigua, Trichoplusia ni, and Bombyx mori* were obtained for further study using the UNIPROT-KB (Release 2014_02).

2.2 Analyzing the Physio- Chemical properties of *Ha* JHEH protein along with its homologues insects:

The molecular weight, theoretical PI, amino acid composition, atomic composition, instability index, gravity, grand average of hydrophobicity, and other properties of the JHEH protein were analyzed using the PROTPARAM tool. With the use of a protein sequence, this programme calculates several physico-chemical characteristics. Regarding the protein evaluation, no further details are necessary. A raw sequencing of the protein or its Swiss-Prot/TrEMBL accession number or ID can be used to identify it. Numbers and white space are not considered. An intermediary page will be displayed when you enter the accession number of a Swiss-Prot/TrEMBL entry, allowing you to choose the section of the sequence on which you would want to conduct the analysis. A selection of mature chains, peptides, and domains from the Swiss-Prot feature table are available, and there is also the option to input the start and end positions in two boxes.

2.3 Secondary Structure Analysis of Ha JHEH with its homologues lepidopterans:

JHEH protein secondary structure analysis is performed using Self Optimized Prediction Method with Alignment (SOPMA) tool. The SOAPMA tool gives the spatial arrangement of amino acid to its corresponding secondary structure of a protein i.e., arrangement of amino acid to the corresponding helix, beta bridges, extended stand, beta turns, and random coils. The Self-Optimized Prediction approach with Alignment, or SOPMA, is an enhancement of the SOPM approach. These techniques are based on the homologue approach developed by Levin et al. The enhancement occurs because SOPMA incorporates data from an alignment of sequences from the same family. Short homologous amino acid sequences will typically produce comparable secondary structures, according to this technique. There are 126 chains of non-homologous protein in its entire database. When a user enters an unidentified protein, the database will look for proteins with that protein's characteristics and evolutionary history.

2.4 Domain Search:

JHEH protein sequences were submitted to the HMMscan web version [7] to obtain PFam domain information and annotation of protein domains [8]. HMMscan searches sequences against protein profile datasets. The HMMscan examines a database of Hidden Markov Model (HMM) protein signatures for those that match a protein sequence, i.e., a protein sequence vs profile-HMM in the database. The input sequence can be submitted manually or uploaded to the programme in FASTA or UniProtKB/Swiss-Prot format, and the tool does not accept partly prepared sequences. To locate the family, Pfam searches the profile HMM against a large sequence collection based on UniProt Knowledgebase (UniProtKB). To construct the entire alignment, sequence areas that score higher than the selected threshold for each family are aligned to the profile HMM. The HMMER software package is used to create and search for profile HMMs. When a single profile HMM cannot identify all homologues in superfamily, additional entries may be generated to represent different sequence families within the superfamily.

2.5 Multiple Sequence alignment:

Multiple sequence alignment is a technique to identify evolutionary links and common patterns within genes. Multiple sequence alignment is the aligning of three or more biological sequences, such as DNA, RNA, or protein. Computational algorithms are used to produce and examine alignments. Multiple Sequence Alignment seeks to find structural or functional similarities between proteins by comparing them to another protein sequence. Multiple Sequence Alignment is important in identifying novel protein members by comparing them to comparable sequences. Alignments that are biologically good indicate homology and connections that may be used to extract relevant information. Multiple sequence alignment removes misplacements, insertion, and deletions from alignments, and the best alignment is obtained using the Dynamic Programming algorithm. Clustal Omega is a multiple sequence alignment programme that generates alignments between three or more sequences using seeded guide trees and HMM profile-profile algorithms. It generates various sequence alignments of divergent sequences that are physiologically relevant. Viewing Cladograms or Phylograms can reveal evolutionary links. Clustal Omega aligns sequences in the order indicated by the guide tree by default. This guide tree can be highly balanced or very unbalanced depending on sequence similarity. It has recently been demonstrated that properly balanced guide trees may provide high-quality alignments. Clustal Omega [9] is used for multiple sequence alignment to locate conserved domains in the selected five lepidopteran pests, and ENDscript server [10] is used for graphical representation of the multiple sequence alignment.

2.6 Phylogenetic Tree:

Phylogenetic trees are useful tools for organizing information about biological variety. A phylogenetic tree, also known as a phylogeny, is a diagram that displays the evolutionary paths of several species, creatures, or genes from a common ancestor. Phylogenies are important for organizing information about biological variety, structuring classifications, and offering insight into evolutionary events. To truly grasp the enormous evidence supporting the hypothesis of evolution, one must first understand phylogenies. Reading a phylogeny is similar to reading a family tree. The tree's root represents the ancestral lineage, while the branches' tips indicate the offspring of that ancestor. Phylogeny.fr is a free and easy-to-use web application for rebuilding and studying phylogenetic connections between molecular sequences. Phylogeny.fr executes and integrates multiple bioinformatics programmes to create an accurate phylogenetic tree from a set of sequences. Phylogenetic tree of the selected lepidopteran insect pests are generated using the web tool Phylogeny.fr [11].

2.7 Protein pathway analysis:

The JHEH protein pathway is explored with the help of KEGG automatic annotation server (KAAS) [12]. KEGG is a database resource for deriving high-level functions and utilities of biological systems, such as the cell, organism, and ecosystem, from molecular-level data, particularly large-scale molecular datasets generated by genome sequencing and other high-throughput experimental technologies. KAAS (KEGG Automatic Annotation Server) uses BLAST or GHOST comparisons against the manually curated KEGG GENES database to give functional annotation of genes. The end result includes KO (KEGG Orthology) designations and KEGG pathways that were constructed automatically. When the entire set of genes in a genome is known, KAAS works best. Prepare query amino acid sequences and assign orthologs using the BBH (bidirectional best hit) approach. A limited number of genes can also be targeted using KAAS. Prepare query amino acid sequences and use the SBH (single-directional homology) method. When the query contains a huge number of sequences and/or sequences from a variety of species, such as those from a metagenome sequencing study, the GHOSTX search and SBH (single-directional homology) technique are used.

III. RESULTS AND DISCUSSION

3.1 PROTPARAM Primary Structure Comparative analysis results:

PROTPARAM was used to perform the primary structure analysis of the five lepidopteran JHEH, and details such as amino acid composition, theoretical PI, number of negatively charged residues, positively charged residues, gravity, instability index, hydropathicity, etc and the results obtained are shown in Table No:1.

According to the PROTPARAM data, the *Helicoverpa armigera* JHEH protein has 462 amino acids, 7425 atoms, and a molecular weight of 52241.75 K. Da. The sequence has 48 negatively charged residues (Asp+Glu) and 48 positively charged residues (Arg+Lys), with a calculated PI of 7.17. The predicted half-life is 30 hours, with an instability index of 37.22, an aliphatic index of 95.39, and a gravity of -0.017.

Heliothis virescense JHEH protparam reported 463 amino acids with 7487 atoms and a molecular weight of 53129.42 K. Da. The sequence has 52 negatively charged residues (Asp+Glu) and 52 positively charged residues (Arg+Lys), with a predicted PI of 7.23. The predicted half-life is 30 hours, with an instability index of 33.71, an aliphatic index of 81.90, and a gravity of - 0.200.

Spodoptera exigua JHEH protein has 462 amino acids and 7414 atoms, with a molecular weight of 52458.75 K. Da. The sequence has 44 negatively charged residues (Asp+Glu) and 48 positively charged residues (Arg+Lys), with an isoelectric point of 8.64. The half-life is 30 hours, the instability index is 30.87, the aliphatic index is 88.66, and gravity is - 0.066.

Trichoplusia ni JHEH reported 463 amino acids with 7443 atoms and a molecular weight of 52365.64 K. Da. The sequence has 46 negatively charged residues (Asp+Glu) and 47 positively charged residues (Arg+Lys), with a calculated PI of 7.74. The projected half-life is 30 hours, the instability index is 27.24, the aliphatic index is 95.51, and gravity is - 0.048.

Bombyx mori JHEH has 461 amino acids and 7494 atoms, with a molecular weight of 52414.22 K. Da. The sequence has 50 negatively charged residues (Asp+Glu) and 52 positively charged residues (Arg+Lys), with an isoelectric point of 8.25. The projected half-life is 30 hours, the instability index is 41.54, the aliphatic index is 99.87, and gravity is - 0.044.

Manduca sexta JHEH protein has 462 amino acids and 7446 atoms, with a molecular weight of 52611.80 K. Da. The sequence has 50 negatively charged residues (Asp+Glu) and 47 positively charged residues (Arg+Lys), with a predicted PI of 6.47. The projected half-life is 30 hours, the instability index is 35.37, the aliphatic index is 95.97, and gravity is -0.110.

For a stable protein structure, the instability index should be less than 40. The PROTPARAM tool shows that the instability index of the *Ha* JHEH and its homologues is between 27.24 to 37.22 which acknowledge that the protein structures are with stable confirmation. The *Bombyx mori* JHEH instability index is 41.54 which is high normal and the protein shows stable confirmation.

The amino acid number, atoms number, gravity and the molecular weight are all much closer to *Ha* JHEH and to its corresponding homologues. The results are shown in the Table: 1.

3.2 SOPMA Comparative Analysis results:

SOPMA is used to analyze the secondary structure of five lepidopteran JHEHs. The percentages of Alpha helix, extended strand, Beta turn, and random coil are showed in Table No. 2.

The Helicoverpa armigera JHEH sequence revealed 36.8 percent alpha helix with 170 amino acids, 13.42% extended strands with 62 amino acids, 3.25% beta turns with 15 amino acids, and 46.54% random coils with 215 amino acids.

The JHEH sequence of Heliothis virescense revealed 36.44% alpha helix with 178 amino acids, 13.61% extended strands with 63 amino acids, 3.25% beta turns with 15 amino acids, and 44.71% random coils with 207 amino acids.

The JHEH sequence from Spodoptera exigua revealed 37.23% alpha helix with 172 amino acids, 14.29% extended strands with 66 amino acids, 3.25% beta turns with 15 amino acids, and 45.24% random coils with 209 amino acids.

The JHEH sequence of Bombyx mori revealed 35.36% alpha helix with 163 amino acids, 13.88% extended strands with 64 amino acids, 3.04% beta turns with 14 amino acids, and 47.72% random coils with 220 amino acids.

The JHEH sequence of Trichoplusia ni revealed 35.85% alpha helix with 166 amino acids, 14.90% extended strands with 69 amino acids, 3.02% beta turns with 14 amino acids, and 46.22% random coils with 214 amino acids.

The secondary structure of all five lepidopteran JHEHs is composed of 35.36 % to 38.44 % Alpha helix, 44.71 % to 47.72 % Random coils, 13.42 % to 14.90 % extended strands, and 2.38 % to 3.25 % beta strands.

TABLE 1: PROTPARAM results showing the primary structure analysis of *Ha* JHEH and its corresponding homologues lepidopteran insects

Organism	No. Amino Acids	No. Atoms	Mol.Wt (K.Dal)	Theoritical PI	(-) Charged Residues (Asp+ Glu)	(+) Charged Residues (Arg+ Lys)	Estimated Half-life	Instability Index	Aliphatic Index	Gravity
H.armigera	462	7425	52241.75	7.17	48	48	30	37.22	95.39	-0.017
H. virescens	463	7487	53129.42	7.23	52	52	30	33.71	81.90	-0.200
S.exigua	462	7414	52458.74	8.64	44	48	30	30.87	88.66	-0.066
Trichoplusia ni	463	7443	52365.64	7.74	46	47	30	27.24	95.51	-0.048
Bombyx mori	461	7494	52414.22	8.25	50	52	30	41.54	99.87	-0.044
M. Sexta	462	7446	52611.80	6.47	50	47	30	35.37	95.97	-0.110

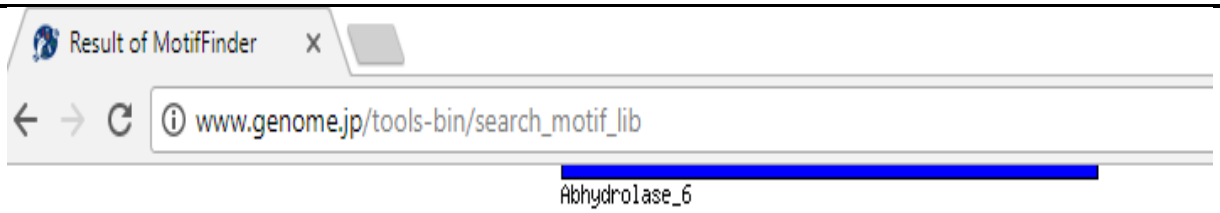
TABLE.2: SOPMA results showing the secondary structure analysis of *Ha* JHEH and its homologues lepidopteran insects.

Secondary Structural Analysis	Alpha Helix		Extended Strand		Beta Turn		Random coils	
	Amino Acid No.	%	Amino Acid No.	%	Amino Acid No.	%	Amino Acid No.	%
H.armigera	170	36.80	62	13.42	15	3.25	215	46.54
H. virescens	178	38.44	63	13.61	15	3.24	207	44.71
S.exigua	172	37.23	66	14.29	15	3.25	209	45.24
Bombyx mori	163	35.36	64	13.88	14	3.04	220	47.72
Trichoplusia ni	166	35.85	69	14.90	14	3.02	214	46.22
M. Sexta	170	36.80	64	13.85	11	2.38	217	46.97

3.3 HMM Scan comparative analysis results:

The Ha JHEH and its homologues, three motifs were identified: EHN (Epoxide Hydrolase N terminal), Abhydrolase_1 Alpha/beta hydrolase fold), and Abhydrolase_6 (Alpha/beta hydrolase family). All three motifs were identified in all lepidopterans, with slight difference in their sequential position. In Helicoverpa armigera, EHN motiff is located between 51 and 160, Abhydrolase_1 between 146 and 258, and Abhydrolase _6 between 147 and 441. In Heliothis Virescens, EHN motiff is located between 52 and 161, Abhydrolase_1 is located between 147 and 245, and Abhydrolase _6 is located between 148 and 440. In Spodoptara exigua, EHN motiff is located between 51 and 160, Abhydrolase_1 between 146 and 244, and Abhydrolase _6 between 147 and 437. EHN motiff is found in Trichoplusia ni between 51 and 160, Abhydrolase_1 between 146 and 247, and Abhydrolase _6 between 148 and 440. EHN motiff is found in Bombyx mori between 52 and 161, Abhydrolase_1 between 147 and 263 and Abhydrolase _6 between 148 and 441. EHN motiff is found in Manduca sexta between 52 and 161, Abhydrolase_1 between 147 and 270, and Abhydrolase _6 between 148 and 237.Helicoverpa armigera, Spodoptera, and Trichoplusia ni all have EHN that ranges from 51 to 160, with a slight variance in Heliothis virescens, Bombyx mori, and Manduca sexta having EHN that ranges from 52 to 161.Slight differences in the location of the Abhydrolase_1 and Abhydrolase_6 motifs were observed with the motiff mposition in all the lepidopterans.

The results from HMMscan showed that the Helicoverpa armigera and its homologues lepidopteran insect pests belong to members of the family EHN, superfamily EHN, and contain special multidomain site of abhydrolase_1 and Abhydrolase_6. The results are shown in Figure 1.



Pfam (3 motifs)

Query	Pfam	Position(Independent E-value)		Description
Helicoverpa	EHN	51..160(3.3e-28)	Detail	PF06441, Epoxide hydrolase N terminus
Helicoverpa	Abhydrolase_1	146..258(2.7e-14)	Detail	PF00561, alpha/beta hydrolase fold
Helicoverpa	Abhydrolase_6	147..441(2.7e-08)	Detail	PF12697, Alpha/beta hydrolase family
M.sexta_JHEH_Q25489	EHN	52..161(8.4e-28)	Detail	PF06441, Epoxide hydrolase N terminus
M.sexta_JHEH_Q25489	Abhydrolase_1	147..270(9e-14)	Detail	PF00561, alpha/beta hydrolase fold
M.sexta_JHEH_Q25489	Abhydrolase_6	148..237(0.022)	Detail	PF12697, Alpha/beta hydrolase family
H.virescens_JHEH_L7R9X8	EHN	52..161(6.5e-30)	Detail	PF06441, Epoxide hydrolase N terminus
H.virescens_JHEH_L7R9X8	Abhydrolase_1	147..245(7.8e-16)	Detail	PF00561, alpha/beta hydrolase fold
H.virescens_JHEH_L7R9X8	Abhydrolase_6	148..440(0.00097)	Detail	PF12697, Alpha/beta hydrolase family
Spodoptera	EHN	51..160(1.5e-29)	Detail	PF06441, Epoxide hydrolase N terminus
Spodoptera	Abhydrolase_1	146..244(3.6e-15)	Detail	PF00561, alpha/beta hydrolase fold
Spodoptera	Abhydrolase_6	147..437(0.01)	Detail	PF12697, Alpha/beta hydrolase family
Bombyx	EHN	52..161(2.9e-27)	Detail	PF06441, Epoxide hydrolase N terminus
Bombyx	Abhydrolase_1	147..263(3.1e-15)	Detail	PF00561, alpha/beta hydrolase fold
Bombyx	Abhydrolase_6	148..441(7e-08)	Detail	PF12697, Alpha/beta hydrolase family
Trichoplusia	EHN	51..160(2.2e-28)	Detail	PF06441, Epoxide hydrolase N terminus
Trichoplusia	Abhydrolase_1	146..247(5.9e-11)	Detail	PF00561, alpha/beta hydrolase fold
Trichoplusia	Abhydrolase_6	148..440(0.0005)	Detail	PF12697, Alpha/beta hydrolase family

Figure. 1 HMM Scan results showing the Domain and protein family (Pfam) of *Ha* JHEH and its corresponding lepidopteran insects

3.4 KEGG analysis:

The insect JHEH sequences were then analysed using the KEGG automation server (KASS), and the server output revealed that the JHEH protein is involved in the Insect hormonal biosynthesis pathway number 00981. The hormonal pathway is showed in Figure: 2

3.5. Multiple Sequence Alignment Results using Clustal Omega tool:

The Clustal Omega results revealed conserved domains in all lepidopterans, and all conserved domains such as Putative Catalytic Triad (DH and E), Lid domain (Y and Y), and Oxyanion Hole (HGWP Motif) are visually depicted in the figure using the ENDscript server [10]. The results were showed in the Figure : 3.

3.6 Phylogenetic Tree analysis:

To analyze the Evolutionary relationship Phylogeny.fr is used. The evolutionary tree Figure: 4 showed that *Helicoverpa armigera* JHEH is showing much convergence with *Trichoplusia ni* JHEH and *Bombym mori* JHEH. *Heliothis virescens* JHEH and *Spodoptera exigua* JHEH are showing good convergence between them and a little divergence with *Helicoverpa armigera* JHEH. A significant divergence is seen between *Helicoverpa armigera* JHEH and *Manduca sexta* JHEH.

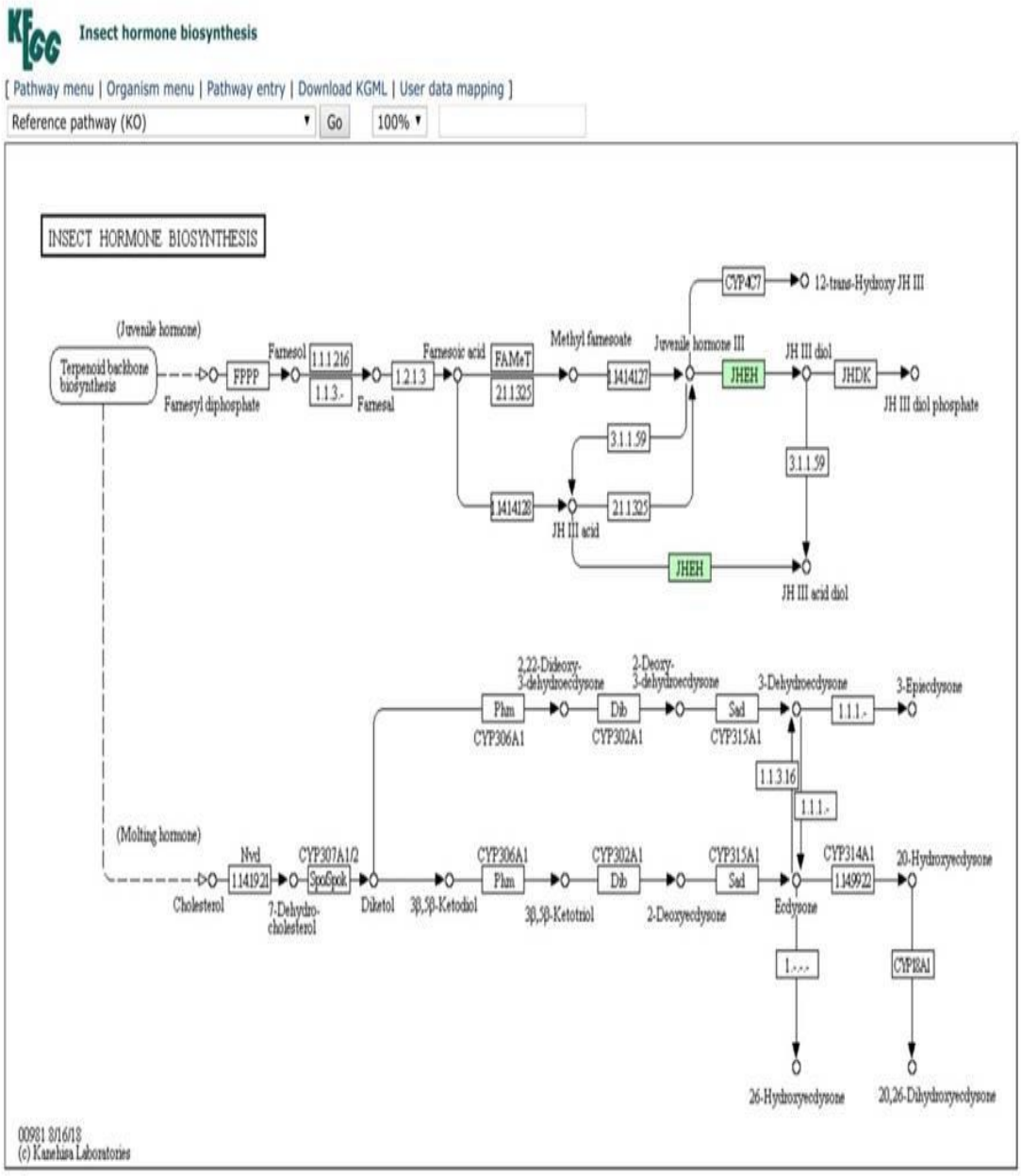


Figure. 2 KEGG results showing the role of *Ha* JHEH in the insect Hormone biosynthesis

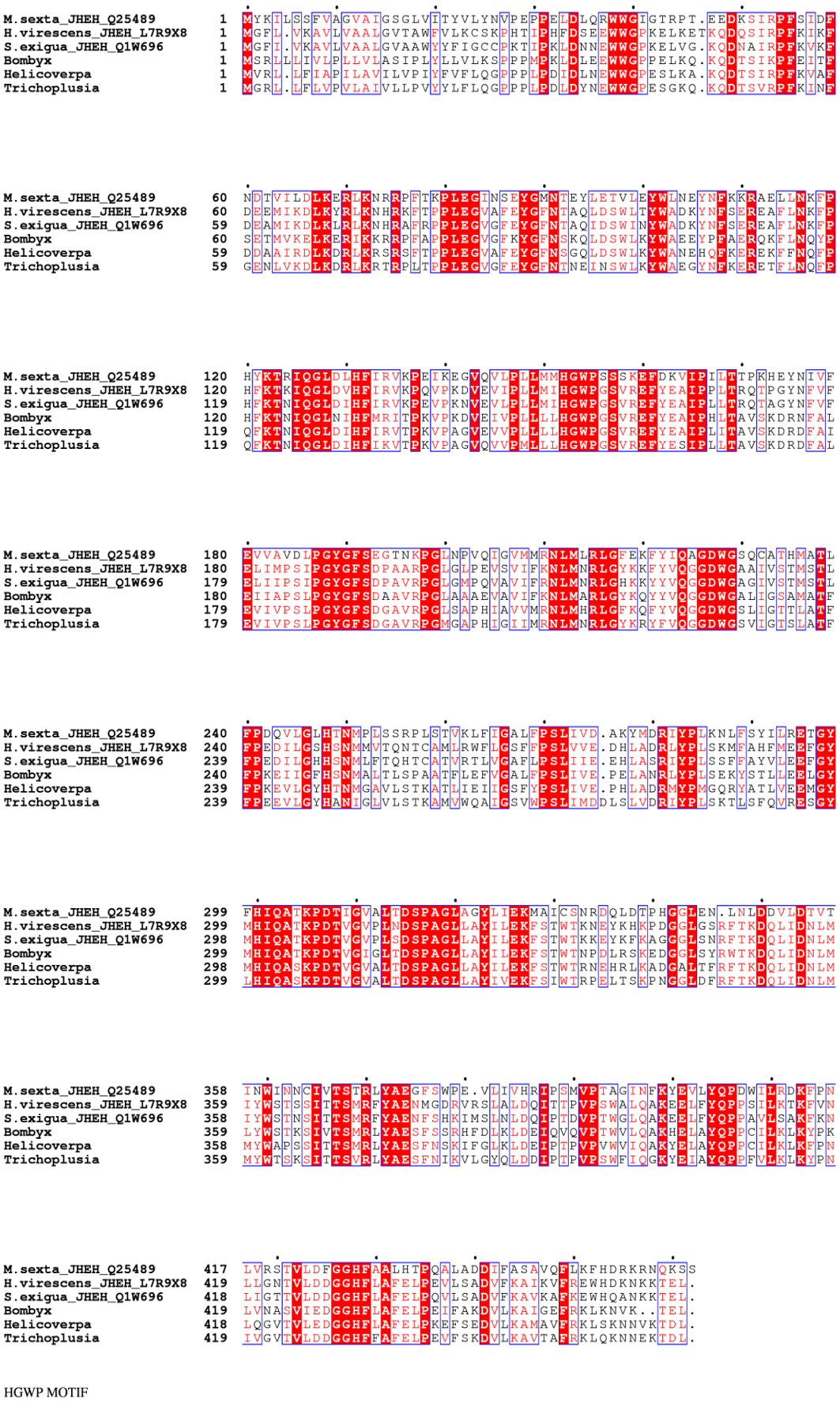


Figure. 3 Multiple sequence alignment of *Ha* JHEH with its selected lepidopteran insects showing conserved domains including Putative Catalytic triad (**D-226, H- 429 and E- 402**), Lid domain (**Y-297 and Y- 372**) and Oxyanion Hole (**HGWP Motif, 151 - 154**)

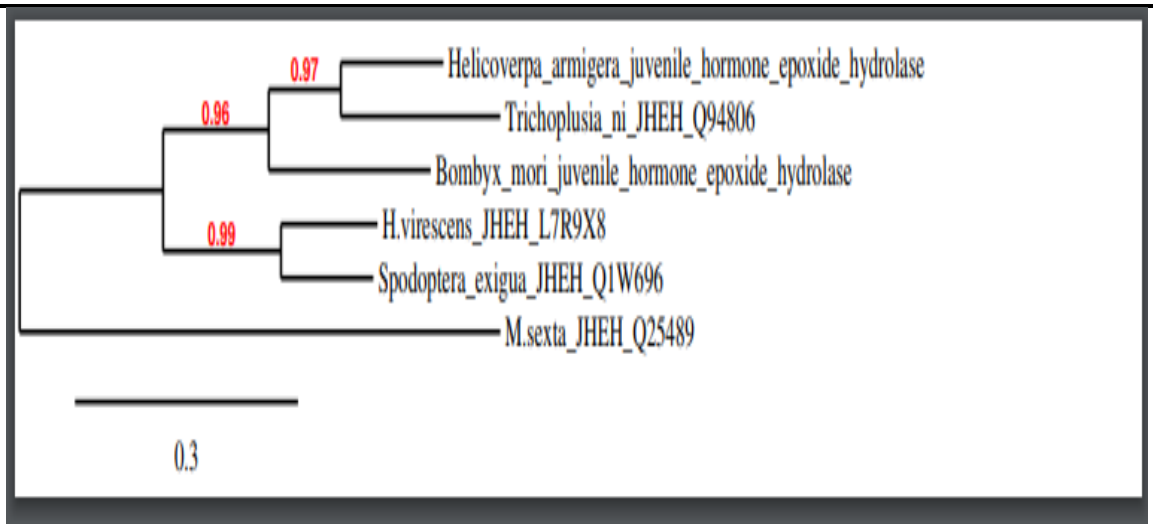


Figure. 4 Phylogenetic tree showing the evolutionary relationship between the *Ha* JHEH and its homologues insect JHEH

IV. CONCLUSION:

The primary structure analysis of five lepidopteran JHEHs was performed using PROTPARAM, revealing that the stability index of Ha JHEH and its homologues is between 27.24 to 37.22 indicating that the primary structure is stable. The secondary structure of all five JHEHs is composed of 35.36% to 38.44% Alpha helix, 44.71% to 47.72% Random coils, 13.42% to 14.90% extended strands, and 2.38% to 3.25% beta strands. The JHEH protein is involved in the Insect hormonal biosynthesis pathway number 00981, with conserved domains putative catalytic triad (D-H and E), Lid Domani (Y and Y) and Oxyanion Hole (HGWP) in all lepidopterans. The evolutionary tree Phylogeny.fr showed significant convergence between *Helicoverpa armigera* JHEH and *Trichoplusia ni* JHEH, while *Heliothis virescens* JHEH and *Spodoptera exigua* JHEH showed good convergence. Based on the findings on structural and functional analyses of the lepidopteran JHEH, the in silico analysis opens up new avenues for exploring prospective targets implicated in Xenobiotic metabolism. Furthermore, the research provides valuable insights into identifying and developing better JH analogues to control lepidopteran pests in an environmental friendly using biocontrol strategy.

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