Comparative study on the effect of the insecticide, Phorate on the COI (Cytochrome oxidase subunit 1) of Eudrilus eugeniae and Aedes aegypti using in silico techniques

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DOI: https://doi.org/10.22271/23487941.2023.v10.i6a.715

Abstract
The most crucial element of the global plan for managing diseases linked to mosquitoes is vector control, of which insecticide treatment is a crucial component. Insecticides affect an insect's nervous system, reproduction, or immune system. On the other hand, insecticides have a detrimental effect on soil microbial activity and counts, which is concerning for the environment. It also affects various other organisms such as earthworms present in the soil it. In this in silico research study, we carry out a comparative study on the effect of the insecticide, Phorate on the earthworm sps, Eudrilus eugeniae and on the mosquito, Aedes aegypti. Eudrilus eugeniae is native to tropical West Africa and is currently common in warm climates where it is grown in vermicompost. Many diseases like dengue, dengue hemorrhagic fever, dengue shock syndrome, yellow fever, chikungunya, and Zika virus infection are mostly spread by the mosquito Aedes aegypti. Primarily, we investigate the mechanism by which Phorate affects the earthworms in the soil and the mosquito. The protein sequence of Cytochrome oxidase, present in the extracellular region of Eudrilus eugeniae and Aedes aegypti, is introduced to Phorate using advanced automated molecular drug docking techniques. The results are validated in 3D view using advanced molecular visualization tools. The results obtained from our study shows that the 3D structure of Cytochrome oxidase subunit 1 binds with Phorate in the hydrophobic regions. Based on the binding affinity scores and 3D H-bond interactions between Cytochrome oxidase subunit 1 and Phorate, the level of inhibition between the protein and the receptor is analysed. The overall results conclude that the accumulation of Phorate in the soil leads to its binding with the extracellular region of the earthworm, Eudrilus eugeniae bringing about the degeneration of the species which is detrimental to soil fertility, whereas the effect of Phorate on Aedes aegypti is beneficial in controlling the species for the benefit of mankind.

Keywords: Eudrilus eugeniae, Phorate and Molecular drug docking

1. Introduction
Involved in important soil functions and associated ecosystem services, soil fauna may be harmed by insecticide use (McLaughlin and Mineau 1995; Blouin et al. 2013; Bertrand et al. 2015). Earthworms are ecosystem engineers and account for the majority of living biomass in terrestrial ecosystems [1-4]. In hardwood forests and pastures, their density can reach up to one tonne per hectare [3]. In addition to improving nutrient cycling, water regulation, and primary production, they alter the structure of the soil [6-9]. Additionally, they have been employed as model organisms in soil ecotoxicology for more than 30 years [10] and are acknowledged as indicators of soil biological activity [11].

The earthworm, Eudrilus eugeniae belongs to the phylum: Annelida, class: Clitellata, and family: Eudrilidae. An adaptable example of an anatomically complex earthworm with direct fertilisation is Eudrilus eugeniae. Originating in savannah soils, this tropical species from West Africa grows best on substrates that are rich in organic matter. Its life cycle can be completed in as little as 47 days, from cocoon to maturity. This worm's presence increased experimental pasture yields to 83.9%. Roots search for numerous pellet-like casts that are deposited on the soil's surface.
As organic material passes through its digestive system, microbial pathogens are reduced, and the resulting vermicompost product has improved microbial, enzymatic, and nutritional qualities. As a zoophilic tree-hole breeder (Ae. aegypti formosus), Aedes aegypti originated in Africa [12]. Domesticated or living in close contact to people, it is found in tropical and subtropical countries outside of Africa. It is thought that greater international trade is what allowed this human-adapted species to expand to Asia and the New World. Southeast Asian native Aedes albopictus originates originally from Bengal, India [13]. Africa, the Middle East, Europe, North and South America, and the Pacific Islands have all seen its expansion. Both Ae. Albopictus and Ae. aegypti are currently found in large parts of the world, including Southeast Asia [14, 15]. Throughout the year, dengue epidemics occur often and cyclically in the majority of Southeast Asian countries. [16, 17, 18, 19]

Phorate is an organic thiophosphate and an organothiophosphate insecticide. It has a role as an EC 3.1.1.7 (Acetylcholinesterase) inhibitor, an EC 3.1.1.8 (cholinesterase) inhibitor, an acaricide and an agrochemical. It is functionally related to an (Ethylsulfanyl) methanethiol. This research project's main goal is to compare the effects of the insecticide, Phorate on the earthworm species, Eudrilus eugeniae and the mosquito species, Aedes aegypti.

2. Methodology

The methodology in this research study includes: 1. Protein sequence retrieval from the two species. 2. Chemical compound–protein docking. 3. 3D Molecular dynamics. (Fig: 1)

2.1 Protein Sequence Retrieval

The NCBI (National Centre for Biotechnology Information) [20] https://www.ncbi.nlm.nih.gov/ database was used to retrieve the potential protein sequence, Cytochrome oxidase subunit 1 (COI) (mitochondrion) in Eudrilus eugeniae (QRZ20862.1) [21] and Aedes aegypti (BBO66047.1) [22].

2.2 Chemical Compound-Protein Docking

Phorate is a systemic insecticide used to eradicate mites, insects, and nematodes. Extensive use of this organophosphate has engendered severe environmental concerns [23]. Hence, Phorate (CID: 4790) was selected using NCBI Pub Chem Compound Database (https://pubchem.ncbi.nlm.nih.gov/). The Cytochrome oxidase subunit 1 (COI) protein sequence of Eisenia fetida and Aedes aegypti was introduced to Phorate and the Molecular binding interactions was viewed using an advanced automated drug docking server called HDock server [23] http://hdock.phys.hust.edu.cn/.

2.3 3D Molecular Dynamics

The docking results were validated using the molecular visualization software called Discovery Studio. This software helps in viewing the intra molecular interaction between Phorate and the COI protein sequences of Eisenia fetida and Aedes aegypti.

Diagrammatic representation of molecular docking studies

3. Results
Fig 4: 3D complex form of Cytochrome oxidase subunit 1 of Eudrilus eugeniae with Phorate using Discovery Studio software

Fig 5: 3D Phorate Chemical compound in stick model with coloured atoms viewed using Discovery Studio software

Fig 6: 3D H-bond interaction between Cytochrome oxidase subunit 1 of Eudrilus eugeniae and Phorate with respective amino acid labels

Fig 7: Van Der Waals interaction between Cytochrome oxidase subunit 1 of Eudrilus eugeniae and Phorate

Fig 8: 3D complex view of Cytochrome oxidase subunit 1 (COI) of Eudrilus eugeniae with Phorate. Phorate is represented in yellow colour in Electrostatic Model view

Fig 9: FASTA sequence of Cytochrome oxidase subunit 1 of Aedes aegypti retrieved from NCBI database

Fig 10: 3D structure of Cytochrome oxidase subunit 1 of Aedes aegypti

Fig 11: 3D complex form of Cytochrome oxidase subunit 1 of Aedes aegypti with Phorate using Discovery Studio software
target, biodegradable, and somewhat environmentally benign
used in agriculture should only be poisonous to the species they
of the insecticides and the characteristics of the soil [30, 31].
and desorption processes depending on the chemical makeup
through a variety of chemical pathways, transport, adsorption,
organisms, including earthworms [28, 29]. Earthworm organs go
nervous, respiratory, and osmoregulatory organs of many soil
insecticides used in agriculture land cause morpholo
gical, contaminating the soil and harming a variety of invertebrates,
ecosystems, including earthworms. In addition to
destroy innocuous creatures that are vital to different
behavioural, and physiological changes in the reproductive,
in toxicity, significant population harm, and soil pollution [24,
Unfortunately, the majority of insecticides are non-specific and
in agriculture and the careless application of insecticides
have a detrimental effect on earthworms. Expansion of
various literature studies have proved that certain insecticides
have a detrimental effect on earthworms. Expansion of
agriculture and the careless application of insecticides
frequently have negative effects on the soil ecology, resulting
in toxicity, significant population harm, and soil pollution [24,
Insecticide spending is estimated to reach $38 billion
worldwide annually [26] (Pan-Germany, 2012). Insecticides
contracting dengue. [32] The illness is currently the fastest-
growing virus spread by mosquitoes and affects the majority of
tropical and subtropical regions on Earth [33]. 2010 saw an
estimated 390 million infections, of which 96 million had
observable symptoms. The data used to create these estimates
came from a variety of sources, including news articles,
surveillance footage, published books, and expert
consultations. The estimates have wide confidence intervals
since the data are not all the same quality and completeness.
The estimates do, however, reflect an international consensus
among specialists that indicates the number of infections is
rising over time and spreading geographically. The World
Health Organisation (WHO) wants to cut dengue's morbidity
and mortality rates by at least 50% and 25%, respectively,
between 2010 and 2020 [34].
In this research study, protein profiling studies were carried out
on the Cytochrome oxidase subunit 1 (mitochondrion) sequence of
Eudrilus eugeniae. The larger domain regions involved in the protein sequence are in the range 1-213 which
is specific to Cytochrome c oxidase (Interpro: IPR023616 and
Pfam: PS050855). Previous research studies have proved the
molecular function of Cytochrome oxidase subunit 1. The final
stage in the respiratory chain is catalysed by oligomeric integral membrane protein complexes called cytochrome c oxidase (EC
1.9.3.1) [35], which transfers electrons from cytochrome c or a
quinol to oxygen. Certain terminal oxidases produce a
transmembrane proton gradient that crosses the inner
membrane of the mitochondria in eukaryotes or the plasma
membrane in prokaryotes.
The Aedes aegypti mitochondrial sequence, or Cytochrome oxidase subunit 1, was subjected to protein profiling
investigations in this study. The range 1-199, which is unique
to cytochrome c oxidase, contains the bigger domain sections
that are involved in the protein sequence (Scan Prosite:
PS00008). Previous investigations have demonstrated the
molecular role of subunit 1 of cytochrome oxidase. An appreciable number of eukaryotic proteins are acylated by the
covalent addition of myristate (a C14-saturated fatty acid) to
their N-terminal residue via an amide linkage. The functional

<table>
<thead>
<tr>
<th>Earthworm</th>
<th>Mosquito</th>
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<tbody>
<tr>
<td>Insecticide Compound 1</td>
<td>Cytochrome oxidase subunit 1 (COI) of Eudrilus eugeniae (QRZ20862.1)</td>
</tr>
<tr>
<td>Phorate (CID: 4790)</td>
<td>-66.24 kcal/mol</td>
</tr>
</tbody>
</table>

Table 1 Molecular binding affinity scores with units between Phorate and Cytochrome oxidase subunit 1 of Earthworm and Mosquito

4. Discussion
Cytochrome oxidase subunit 1 (mitochondrion) sequence of Eudrilus eugeniae [QRZ20862.1] is retrieved using NCBI database
the nucleotide sequence contains 642 bp and its corresponding translated amino acid sequence has 199 aa (Fig: 2, 3). Cytochrome oxidase subunit 1 (mitochondrion) sequence of Aedes aegypti [BBO66047.1] is retrieved using NCBI database
the nucleotide sequence contains 599 bp and its corresponding translated amino acid sequence has 199 aa (Fig: 9, 10) The 3D structure of the Phorate was viewed in discovery studio software in order to perform drug docking studies. (Fig: 5).
Various literature studies have proved that certain insecticides have a detrimental effect on earthworms. Expansion of
agriculture and the careless application of insecticides frequently have negative effects on the soil ecology, resulting
in toxicity, significant population harm, and soil pollution [24, 25]. Insecticide spending is estimated to reach $38 billion
worldwide annually [26] (Pan-Germany, 2012). Insecticides used in agriculture should only be poisonous to the species they
target, biodegradable, and somewhat environmentally benign [27].
Unfortunately, the majority of insecticides are non-specific and
destroy innocuous creatures that are vital to different
ecosystems, including earthworms. In addition to
contaminating the soil and harming a variety of invertebrates,
insecticides used in agriculture land cause morphological,
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Cytochrome oxidase subunit 1 (mitochondrion) sequence of Aedes aegypti (BBO66047.1) is retrieved using NCBI database
the nucleotide sequence contains 599 bp and its corresponding translated amino acid sequence has 199 aa (Fig: 8, 9).
motifs present at the N-myristoylation site. To conduct docking studies, we used Discovery Studio software to model the 3D structure of Phorate. Fig.4.

4.1 Molecular Drug Docking
In this docking study, HDOCK server was used to dock the protein sequence of Eudrilus eugeniae – COI (Cytochrome oxidase subunit 1) with Phorate (Fig: 6, 7, 8). Next, HDOCK server was used to dock the protein sequence of Aedes aegypti – COI (Cytochrome oxidase subunit 1) with Phorate (Fig: 10). The HDOCK server is a state-of-the-art platform that includes biological data and is useful for template-based modelling, homology search, macromolecular docking, structure prediction, and task management for dependable and efficient protein-protein docking. Previous HDOCK server proved.

When information about receptor and ligand molecules is supplied, the server automatically predicts the interaction between them using a hybrid algorithm that combines template-free and template-based docking. Unlike other similar docking servers, the HDOCK server employs a hybrid docking strategy that enables experimental data about the protein-protein binding site and small-angle X-ray scattering to be included during the docking and post-docking processes. It can also accept amino acid sequences as input. The molecular interaction studies between Eudrilus eugeniae – COI and Phorate at different binding amino acid sites have also been discussed. The binding score between Eudrilus eugeniae – COI and Phorate is -66.24 kcal/mol (Table: 1) and that between Aedes aegypti– COI and Phorate is -65.21 kcal/mol. Our results are consistent with those of earlier research studies.

The amino acids of Eudrilus eugeniae – COI interacting with Phorate are GLY:212, PHE: 208, GLY: 212, TRP: 209, HIS: 213, TRP: 209. These findings are consistent with our most recent In silico research work, which also shown that Histidine (H) residues play a major role in the binding of Phorate. Figures 3, 4, 5, 6 and 7 clearly explain H bond interactions. According to our research, the structural domain ranges 184– 213 (Non Cytosplasmic Domain) are directly impacted by the binding amino acids.

The membrane-bound protein is present in the extracellular region of the earthworm. The drug binds to this structural domain. The structural domains of Eudrilus eugeniae – COI are IPR023616, PSS0855 (Cytochrome c oxidase (EC 1.9.3.1)). Hence, it can be understood that high concentration of Phorate in the soil may result in its accumulation in the soil. This will have an indirect effect on the earthworm.

The amino acids of Aedes aegypti – COI interacting with Phorate are GLY: 40, PRO: 36, GLY: 41, PRO: 36, ASN: 44, GLY: 40, MET: 68, SER: 65, SER: 120, ALA: 117, SER: 120, ALA: 117, SER: 121, ALA: 125, SER: 121. The structural domains of COI of Aedes aegypti are Prosite: PDOC00008, PS00008 (N-myristoylation).These findings are consistent with our most recent In silico research work, which also showed that GLY: 40 and PRO: 36 residues play a major role in the binding of Phorate. Figures 11, 12 and 13 clearly explain H bond interactions. According to our research, the structural motif ranges 40-36 are directly impacted by the binding amino acids. Certain amino acids like GLY: 40 and PRO: 36 are present in the motif region. This leads to the down-regulation of the mosquito protein.

5. Conclusion
During this entire In silico research work, we focus on how insecticide, such as, Phorate, indirectly affect mosquito and earthworm sps such as Aedes aegypti and Eudrilus eugeniae respectively. Phorate was chosen for our study as it is an insecticide which is widely used in India. The results obtained from molecular drug docking studies between the chemical compound, Phorate and Cytochrome oxidase subunit 1 (COI) present in Eudrilus eugeniae and Aedes aegypti show the inhibitory effect between the drug and the receptor. This inhibition takes place at the extracellular region of the species and affects the various biological functions of the earthworm and mosquito. Therefore, we draw the conclusion that whereas phorate is detrimental to earthworms, it helps regulate mosquito populations.

6. Acknowledgement
The authors acknowledge the help extended by Dr. Balaji Munivelan, PhD., CEO and Senior Bioinformatician (bioinfobalaji@gmail.com), ABS Geno-informatics, Chennai, for his contribution towards In silico drug docking studies.

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