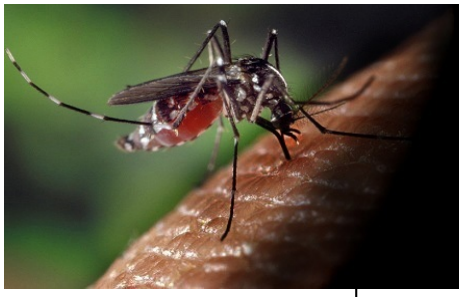

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Comparative study on the effect of the insecticide, Phorate on the COI (*Cytochrome oxidase subunit* 1) of *Eudrilus eugeniae* and *Aedes aegypti* using *in silico* techniques

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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 [5]. 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. *P*rotein 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*.

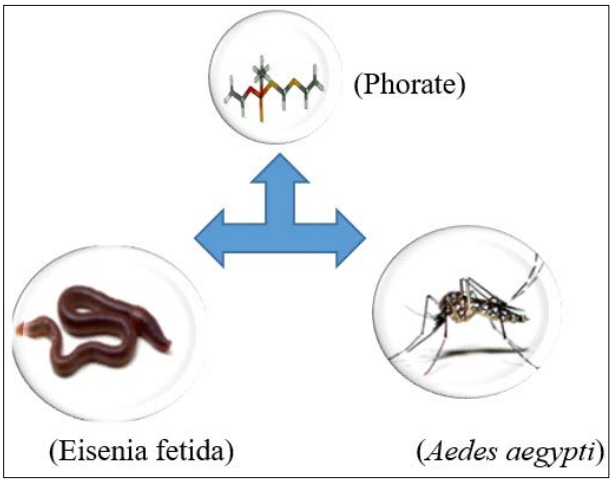

Fig 1: Flow chart of Methodology

Diagrammatic representation of molecular docking studies

3. Results

>QRZ20862.1 *Cytochrome oxidase subunit* 1, partial (mitochondrion) [*Eudrilus eugeniae*]
MIGAGMSLLIRIELSQPGAFLGSDQLYNTIVTAHAFLMI
FFLVMPVFIGGFGNWLLPLMLGAPDMAFPRL
NNLSFWLLPPSLILLVSSAAVEKGAGTGWTVYPPLASN
LAHAGPSVDLAIFSLHLAGASSILGAINFITT
VINMRWSGLRLERIPLFVWAVVITVVLLLLSLPVLAGAI
TMLLTDRNLNTSFFDPAGGGDPILYQHLFWF
FGH

Fig 2: FASTA sequence of *Cytochrome oxidase subunit* 1 of *Eudrilus eugeniae*

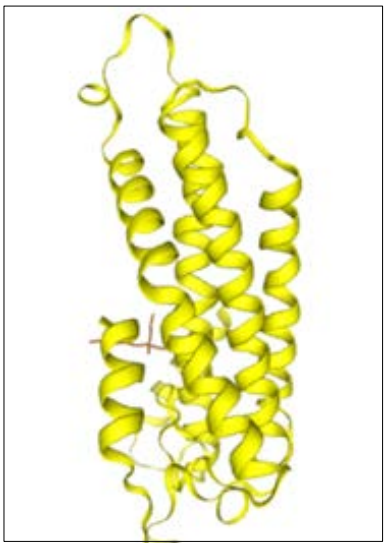

Fig 3: FASTA sequence of COI protein of *Eudrilus eugeniae* retrieved from NCBI database

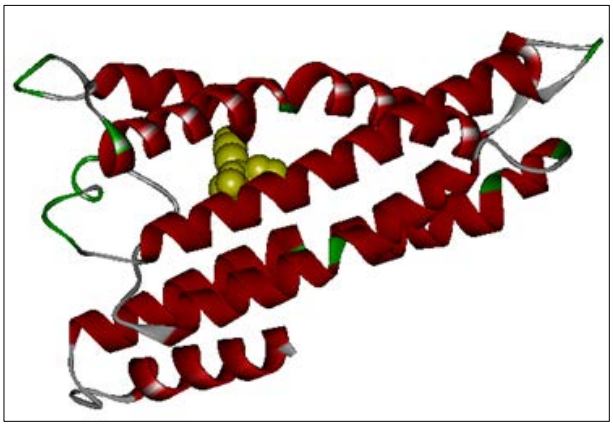

Fig 4: 3D complex form of *Cytochrome oxidase subunit 1* of *Eudrilus eugeniae* with Phorate using Discovery Studio software

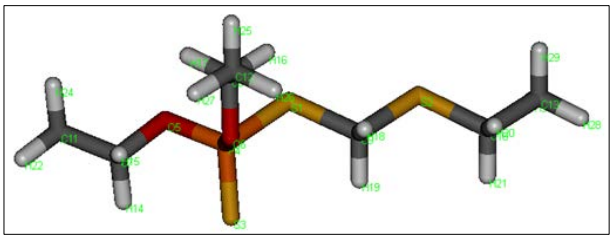

Fig 5: 3D *Phorate* Chemical compound in stick model with coloured atoms viewed using Discovery Studio software

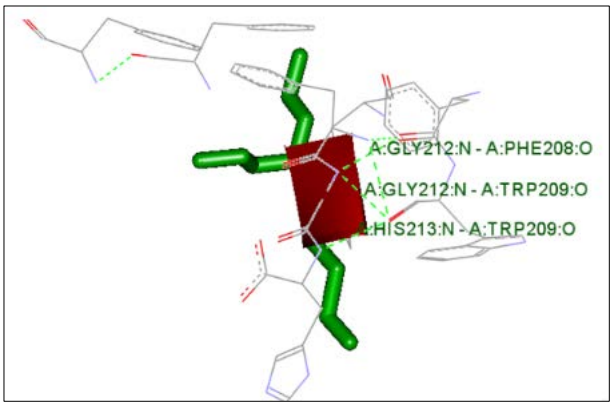

Fig 6: 3D H-bond interaction between *Cytochrome oxidase subunit 1* of *Eudrilus eugeniae* and *Phorate* with respective amino acid labels

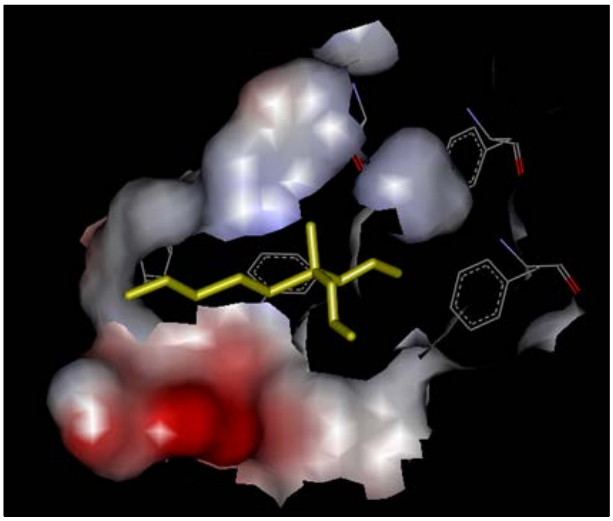

Fig 7: Van Der Waals interaction between *Cytochrome oxidase subunit 1* of *Eudrilus eugeniae* and *Phorate*

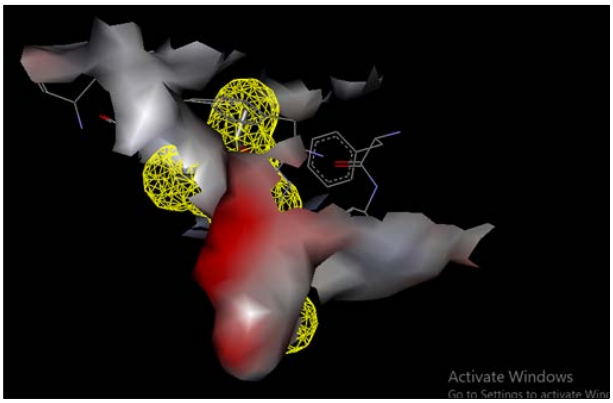

Fig 8: 3D complex view of *Cytochrome oxidase subunit 1* (COI) of *Eudrilus eugeniae* with *Phorate*. *Phorate* is represented in yellow colour in Electrostatic Model view

>BBO66047.1 *Cytochrome oxidase subunit* 1, partial (mitochondrion) [*Aedes aegypti*]
IRAELSHPGMFIGNDQIYNVIVTAHAFIMIFFMVMPIMI
GGFGNWLVPLMLGAPDMAFPRMNNMSFWMLP
PSLTLLLSSSMVENGAGTGWTVYPPLSSGTAHAGASV
DLAIFSLHLAGISSILGAVNFITTVINMRSSGI
TLDRLPLFVWSVVITAILLLLSLPVLAGAITMLLTDRNL
NTSFFDPIGGGDPILYQHLF

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

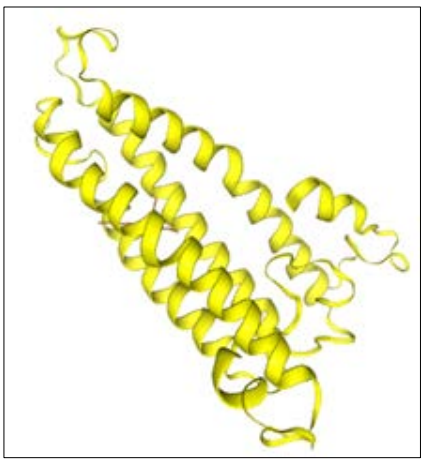

Fig 10: 3D structure of *Cytochrome oxidase subunit 1* of *Aedes aegypti*

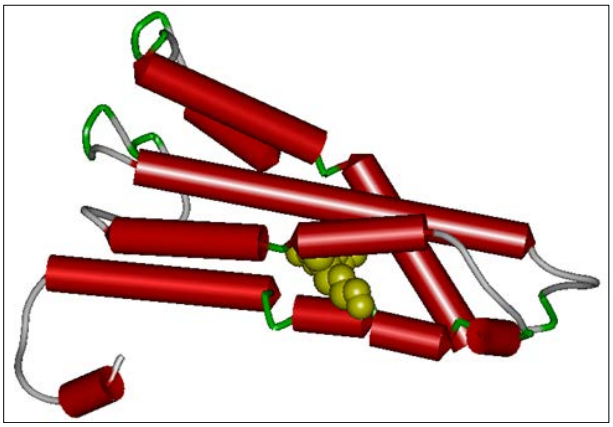

Fig 11: 3D complex form of *Cytochrome oxidase subunit* 1 of *Aedes aegypti* with Phorate using Discovery Studio software

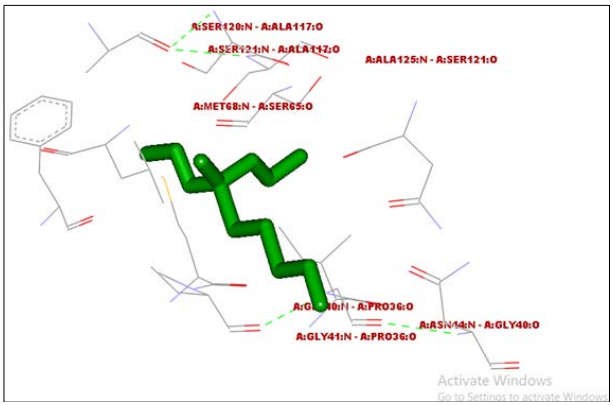

Fig 12: H-bond interaction between *Cytochrome oxidase subunit 1* of *Aedes aegypti* and *Phorate*

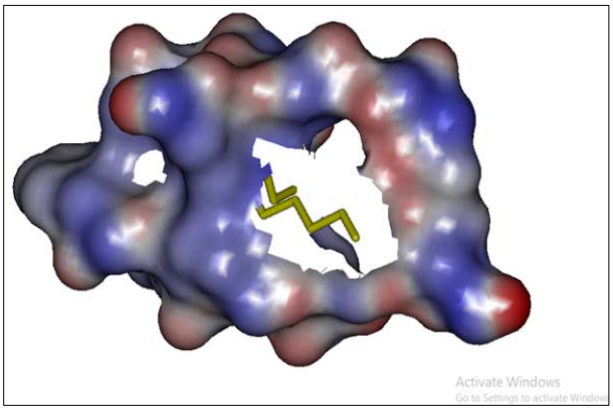

Fig 13: Van Der Waals interaction between *Cytochrome oxidase subunit 1* of *Aedes aegypti* and *Phorate*

Table 1: Drug docking summary

	Earthworm	**Mosquito**
Insecticide Compound 1	*Cytochrome oxidase subunit* 1 (COI) of *Eudrilus eugeniae* (QRZ20862.1)	*Cytochrome oxidase subunit* 1 (COI) of *Aedes aegypti* (BBO66047.1)
Phroate (CID: 4790)	-66.24 *kcal/mol.*	-65.21 *kcal/mol*

Table 1 Molecular binding affinity scores with units between *Phroate* 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 213 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 stricture 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, behavioural, and physiological changes in the reproductive, nervous, respiratory, and osmoregulatory organs of many soil organisms, including earthworms [28,29]. Earthworm organs go through a variety of chemical pathways, transport, adsorption, and desorption processes depending on the chemical makeup of the insecticides and the characteristics of the soil [30, 31].

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).

In 128 countries, about 3.9 billion people are susceptible to 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: PS50855). 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

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 is 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 [36-41].

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 [42-47].

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 Cytoplasmic 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, PS50855 (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: 117, 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 [48] 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 earth worm 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

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7. References

1. McLaughlin A, Mineau P. The impact of agricultural practices on biodiversity. Agric Ecosyst Environ. 1995;55:201-212.
2. Blouin M, Hodson ME, Delgado EA, Baker G, Brussaard L, Butt KR, *et al*. A review of earthworm impact on soil function and ecosystem services. Eur J Soil Sci. 2013;64:161-182.
3. Bertrand M, Barot S, Blouin M, Whalen J, de Oliveira T, Roger-Estrade J. Earthworm services for cropping systems. A review. Agron Sustain Dev. 2015;35:553-567.
4. Jones CG, Lawton JH, Shachak M. Organisms as ecosystem engineers. Oikos. 1994;69(3):373-386.
5. Lavelle P, Spain AV. Soil ecology. Amsterdam: Kluwer Scientific; c2001.
6. Lavelle P, Charpentier F, Gilot C, Rossi JP, Derouard L, Pashanasi B, *et al*. Effects of earthworms on soil organic matter and nutrient dynamics at a landscape scale over decades. In: Edwards CA, editor. Earthworm ecology. Boca Raton: CRC Press; c2004.
7. Seeber J, Seeber GUH, Langel R, Scheu S, Meyer E. The effect of macroinvertebrates and plant litter of different quality on the release of N from litter to plant on alpine pastureland. Biol Fertil Soils. 2008;44:783-790.
8. Bottinelli N, Henry-des-Tureaux T, Hallaire V, Mathieu J, Benard Y, Tran TD, *et al*. Earthworms accelerate soil porosity turnover under watering conditions. Geoderma. 2010;156:43-47.
9. Blouin M, Hodson ME, Delgado EA, Baker G, Brussaard L, Butt KR, *et al*. A review of earthworm impact on soil function and ecosystem services. Eur J Soil Sci. 2013;64:161-182.
10. Spurgeon DJ, Weeks JM, Van Gestel CAM. A summary of eleven years progress in earthworm ecotoxicology. Pedobiologia. 2003;47:588-606.
11. Paoletti MG. The role of earthworms for assessment of sustainability and as bioindicators. Agric Ecosyst Environ. 1999;74:133-155.
12. Brown JE, Evans BR, Zheng W. Human impacts have shaped historical and recent evolution in *Aedes aegypti*, the dengue and yellow fever mosquito. Evolution. 2014;68(2):514-525.
13. Delatte H, Gimonneau G, Triboire A, Fontenille D. Influence of temperature on immature development, survival, longevity, fecundity, and gonotrophic cycles of

Aedes albopictus, vector of chikungunya and dengue in the Indian Ocean. J Med Entomol. 2009;46:33-41.
14. Kraemer MU, Sinka ME, Duda KA. The global distribution of the arbovirus vectors *Aedes aegypti* and *Ae. Albopictus*. Elife. 2015;4:e08347. Published 2015 Jun 30.
15. Kraemer MU, Sinka ME, Duda KA. The global compendium of *Aedes aegypti* and *Ae. Albopictus* occurrence. Sci Data. 2015;2:150035. Published 2015 Jul 7.
16. Mia MS, Begum RA, Er AC, Abidin RD, Pereira JJ. Trends of dengue infections in Malaysia, 2000-2010. Asian Pac J Trop Med. 2013;6(6):462-466.
17. Struchiner CJ, Rocklov J, Wilder-Smith A, Massad E. Increasing dengue incidence in Singapore over the past 40 years: population growth, climate and mobility. PLoS ONE. 2015;10(8):e0136286.
18. Rajarethinam J, Ang LW, Ong J. Dengue in Singapore from 2004 to 2016: Cyclical Epidemic Patterns Dominated by Serotypes 1 and 2. Am J Trop Med Hyg. 2018;99(1):204-210.
19. Huy R, Buchy P, Conan A. National dengue surveillance in Cambodia 1980-2008: epidemiological and virological trends and the impact of vector control. Bull World Health Organ. 2010;88(9):650-657.
20. Schoch CL, Ciufo S, Domrachev M. NCBI Taxonomy: a comprehensive update on curation, resources and tools. Database (Oxford). 2020; 2020:baaa062.
21. Population density drive plant litter consumption by *Eudrilus eugeniae* (Oligochaeta) under tropical conditions. Integr Zool. 2021;16(2):255-269.
22. Singh S, Kumar V, Anil AG, Romero R, Ramamurthy PC, Singh J. Biodegradation of phorate by bacterial strains in the presence of humic acid and metal ions. J Basic Microbiol. 2022;62(3-4):498-507.
23. Yan Y, Tao H, He J, Huang S-Y.* The HDOCK server for integrated protein-protein docking. Nature Protocols; c2020.
24. Hole DG, Perkins AJ, Wilson JD, Alexander IH, Grice PV, Evans AD. Does organic farming benefit biodiversity? Biol. Conserv. 2005;122:113-130.
25. Mangala P, De Silva CS, Pathiratne A, Van Gestel CAM. Influence of Temperature and Soil Type on the Toxicity of Three Insecticides to *Eisenia Andrei*. Chemosphere. 2009;76:1410-1415.
26. PanGermany. Insecticide and health hazards. Facts and figures 116; c2012.
27. Rosell G, Quero C, Coll J, Guerrero A. Biorational insecticides in pest management. J of Insecticide Sci. 2008;33:103-121.
28. Mangala P, De Silva CS, Pathiratne A, Van Gestel CAM. Influence of Temperature and Soil Type on the Toxicity of Three Insecticides to *Eisenia Andrei*. Chemosphere. 2009;76:1410-1415.
29. De Silva PMCS, VanGestel CAM. Comparative sensitivity of *Eisenia andrei* and *Perionyx excavatus* in earthworm avoidance tests using two soil types in the tropics. Chemosphere. 2009;77:1609-1613
30. Thapar A, Zalawadia A, Pokharkar OV, Satam SS. Classification of insecticides and its damaging effects: a review. Biolife. 2015;4(1):13-24.
31. Baishya K. Impact of agrochemicals application on soil quality degradation-a review. ICSTM; c2015. p. 778-786.
32. Kraemer MU, Sinka ME, Duda KA, *et al*. The global distribution of the arbovirus vectors *Aedes aegypti* and *Ae. Albopictus*. Elife. 2015;4:e08347. Published 2015 Jun 30.
33. Kraemer MU, Sinka ME, Duda KA. The global compendium of *Aedes aegypti* and *Ae. Albopictus* occurrence. Sci Data. 2015;2:150035. Published 2015 Jul 7.
34. Raquin V, Lambrechts L. Dengue virus replicates and accumulates in *Aedes aegypti* salivary glands. Virology. 2017;507:75-81.
35. National Center for Biotechnology Information. PubChem Enzyme Summary for Enzyme 1.9.3.1, Cytochrome-c oxidase (EC 1.9.3.1). Retrieved October 27, 2023 from; c2023.
36. Remmert M, Biegert A, Hauser A, Soing J. HHblits: lightning-fast iterative protein sequence searching by HMM-HMM alignment. Nat Methods. 2011;9:173-175.
37. Pearson WR, Lipman DJ. Improved tools for biological sequence comparison. Proc Natl Acad Sci USA. 1988;85:2444-2448.
38. Sievers F, Wilm A, Dineen D, Gibson TJ, Karplus K, Li W, *et al*. Fast, scalable generation of high-quality protein multiple sequence alignments using Clustal Omega. Mol Syst Biol. 2011;7:539.
39. Larkin MA, Blackshields G, Brown NP, Chenna R, McGettigan PA, McWilliam H, *et al*. Clustal W and Clustal X version 2.0. Bioinformatics. 2007; 23:2947-8.
40. Marti-Renom MA, Stuart A, Fiser A, Sanchez R, Melo F, Sali A. Comparative protein structure modeling of genes and genomes. Annu Rev Biophys Biomol Struct. 2000;29:291-325.
41. Berman HM, Westbrook J, Feng Z, Gilliland G, Bhat TN, Weissig H, *et al*. The Protein Data Bank. Nucleic Acids Res. 2000;28:235-242.
42. Astalakshmi P, Basheera John M, Kavinilavu G, Vimala D. Identification of the efficiency of Pentane on the bacterial and insecticide proteins of *Aedes aegypti* and *Aeromonas hydrophila* by *In silico* methods. Int J Mosq Res. 2023;10(3):47-53.
43. P Astalakshmi, M Basheera John, Kavinilavu G, Vimala D. In silico study on Hexadecanoic acid against the outer membrane protein transport protein of Culex quinquefasciatus and *Aeromonas hydrophila*. Int J Mosq Res. 2023;10(4):07-14.
44. Nijanthi P, Santhi S, Balaji Munivelan. Molecular dynamics studies on the arginine kinase protein of Aedes sollicitans: Against the natural chemical compound, Gedunin. Int J Mosq Res. 2023;10(2):10-14.
45. Maithreyee S1, Prabha V1. Molecular Interactions between Anti-Inflammatory Drug with Colorectal Cancer (MSH2) Protein Using In-silico Studies. Solovyov Studies ISPU. 2023, 71(10).
46. Nithya G, Prabha V. Identification of a plant derivative (*Hibiscus cannabinus*) for mosquito (*Anopheles darlingi*) control using in silico protein-protein docking techniques. Int J Mosq Res. 2023;10(4):25-29.
47. Balaji Munivelan. Identification of the mutated sites present in the transmembrane regions of SCN1A_HUMAN (Sodium Voltage-Gated Channel Alpha Subunit 1) using *In silico* techniques. Int. J Pharm. Int. Biosci. 2020;5(1):1-6.
48. Udenwobele DI, Su RC, Good SV, Ball TB, Varma Shrivastav S, Shrivastav A. Myristoylation: An Important Protein Modification in the Immune Response. Front Immunol. 2017;8:751. Published 2017 Jun 30.