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RESEARCH ARTICLE

ESTABLISHING CHIRONOMUS LARVAE AS AN ETHICALLY SOUND AND EFFICIENT SYSTEM FOR EARLY-STAGE ANTHELMINTIC SCREENING

Mayuresh Joshi

1. Department of Life Sciences, Kishinchand Chellaram College, HSNC University, Churchgate, Mumbai - 400020.

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Abstract

The escalating global challenge of helminthic infections, compounded by widespread anthelmintic drug resistance and the inherent limitations of traditional in vivo screening models (e.g., high cost, ethical concerns, low throughput), necessitates the urgent development of novel, efficient, and ethically sound drug discovery platforms. This paper proposes and elaborates on the strategic imperative for establishing Chironomus larvae (commonly known as bloodworms) as a robust in vitro model for early-stage anthelmintic efficacy screening. Chironomus species offer a unique confluence of biological and practical advantages. Their rapid life cycle, ease of laboratory cultivation, and remarkable robustness enable high-throughput screening, significantly reducing economic and logistical burdens. Crucially, their comparative physiological and anatomical commonalities with helminths, particularly concerning the chitinous cuticle, complete digestive system, complex nervous system, and conserved detoxification pathways, establish strong biological relevance, enhancing predictive value for identifying compounds active against parasitic worms. As invertebrates, their use aligns perfectly with the "3Rs" (Replacement, Reduction, Refinement) ethical framework. Leveraging the established precedent of Chironomus larvae in ecotoxicology and its amenability to advanced phenotypic profiling techniques, including high-content imaging and behavioral analysis, allows for detection of both overt and subtle "cryptic" anthelmintic effects. This multi-parametric approach provides comprehensive understanding of bioactivity. By embracing Chironomus larvae, this strategic shift in anthelmintic drug discovery not only addresses critical ethical concerns but also optimizes resource allocation, accelerating the identification of promising natural product candidates, such as those from potent traditionally reported plants that are known to be used for their anthelmintic properties, and contributing to the urgent global need for novel and effective treatments against parasitic helminths.

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Corresponding Author:-Dr. Mayuresh Joshi

Address:-Department of Life Sciences, Kishinchand Chellaram College, HSNC University, Churchgate, Mumbai - 400020.

Introduction:-

Need for Innovative Anthelmintic Drug

Helminthic infections continue to represent a significant and pervasive challenge in both human and veterinary medicine globally. These parasitic diseases contribute substantially to morbidity, mortality, and economic losses, particularly in agricultural settings where they threaten livestock productivity and welfare. The primary strategy for controlling helminthiasis has historically relied on a limited repertoire of anthelmintic drugs, which are administered for both prophylaxis and treatment(Zamanian & Chan, 2021)(Nixon, et al., 2020).

A critical and escalating concern is the widespread emergence and rapid dissemination of anthelmintic drug resistance. This resistance has been well-documented across all existing anthelmintic drug classes, severely compromising the efficacy of current treatments and threatening the sustainability of modern systems. For instance, recent data reveals an average prevalence of resistance to benzimidazoles at 86%, moxidectin at 52%, and levamisole at 48%. This alarming trend underscores a profound scarcity of new lead compounds and therapeutic options. Despite this urgent and unmet medical need for novel anthelmintics, the drug discovery pipeline remains remarkably sparse. Only three new drug classes have been introduced to the animal market since 2000(Galli, et al., 2025)(Zamanian & Chan, 2021)(Nixon, et al., 2020)(Geerts & Gryseels, 2000).

This critical disconnect between the high and growing demand for new anthelmintics due to widespread resistance and an almost stagnant drug discovery pipeline is not merely a scientific or biological problem; it carries profound economic and societal ramifications. The development of new drugs is notoriously time-consuming and expensive, with an estimated cost of \$1.2 billion over 15 years and a mere 5% success rate. The price of existing anthelmintic medications has also significantly increased, impacting healthcare costs, as evidenced by a 16-fold increase in annual costs for anthelmintic medications in the US, primarily driven by price increases in albendazole and mebendazole(Zamanian & Chan, 2021). This situation creates a substantial "anthelmintic treatment options gap," where the economic barriers to traditional drug discovery methods are too high to meet the urgent global need.

Without a fundamental shift towards more efficient, cost-effective, and ethically sustainable early-stage screening methodologies, the escalating problem of drug resistance will continue unchecked, leading to increased suffering, substantial economic losses, and severe public health crises, particularly in regions where helminthic infections are endemic like in India and especially in children. Therefore, the imperative for alternative models is not just scientific, but also economic and humanitarian(Geary, et al., 2015)(Lindrose, et al., 2022).

Inherent Limitations and Ethical Concerns Associated with Traditional In Vivo Screening Models

Historically, the discovery of most anthelmintic drugs has relied heavily on in vivo screening using complex animal models of infection. While these models have been productive in the past, capturing drug hits regardless of their mechanism of action, they are now recognized to possess significant inherent limitations. A major bottleneck is the requirement to harvest parasitic worms from vertebrate hosts or vectors, which is laborious, time-consuming, and severely restricts the throughput of screening assays. This limitation makes large-scale screening of compound libraries, including diverse natural product extracts, impractical and economically prohibitive(Zamanian & Chan, 2021).

The transition from in vitro to in vivo efficacy is complex and often challenging. Many anthelmintics require both host and parasite components for their full mechanism of action, or their effects may be mediated by host immune responses(Nixon, et al., 2020). Consequently, compounds with potent in vitro effects may not exhibit obvious phenotypes once worms are removed from the host environment, raising concerns about the predictive value of simplified in vitro assays. Conversely, some drugs effective in vivo, like ivermectin against filarial worms, may lack overt in vitro effects. Furthermore, the inherent complexity of in vitro environments designed to simulate host conditions typically hinders high-throughput capabilities(Zamanian & Chan, 2021)(Nixon, et al., 2020)(Galli, et al., 2025).

Further, the extensive use of animal models in parasitology research also raises substantial ethical concerns regarding the welfare of animal hosts. In response to these concerns, the scientific community widely adheres to the "3Rs" framework: Replacement (using alternative methods that do not involve animals), Reduction (minimizing the number of animals used), and Refinement (improving procedures to minimize suffering). The justification for animal use is increasingly scrutinized, requiring demonstration that scientific objectives cannot be achieved through alternative means(Lee, 2025)(Geary, et al., 2015).

Thus, the limitations of traditional in vivo screening are not just isolated issues but rather interconnected challenges that collectively exert immense pressure to find alternative models. Scientifically, the complexity of host-parasite interactions and drug pharmacokinetics in vivo means that even "hits" from simplified in vitro assays may not translate, but the in vivo models are definitely too cumbersome for initial broad screening.

This creates a powerful, synergistic drive, as ethical considerations demand alternatives, which, if successful, simultaneously address the economic unsustainability and throughput limitations of current methods. This can be relied upon to thereby accelerate the identification of promising compounds. Such an approach would further imply that any viable alternative model must offer tangible benefits across all three dimensions – ethical, economic, and scientific, to be widely adopted and impactful(Zamanian & Chan, 2021)(Geary, et al., 2015)(Lee, 2025)(Nixon, et al., 2020).

Thus, the current research paper proposes and elaborates on the strategic imperative for establishing *Chironomus* larvae as a robust in vitro model for early-stage anthelmintic efficacy screening.

The Strategic Imperative for Developing Novel and Ethically Sound Alternative Screening Platforms

Given the aforementioned challenges, there is a strategic and urgent imperative to develop and implement novel, high-throughput, and ethically sound screening platforms for anthelmintic drug discovery. Recent technological advancements offer promising avenues for this endeavor. These technologies allow for in-depth profiling of anthelmintic effects, moving beyond simple mortality to more nuanced biological responses(Galli, et al., 2025)(Geary, et al., 2015).

A recurring theme in the limitations of in vitro screening is the difficulty in replicating the complex in vivo environment, leading to a potential for missing "true positive" compounds or failing to capture the full spectrum of drug effects. This has been sought to be overcome through significant trends where technology can be leveraged to enhance the sophistication and predictive power of alternative models.

Thus, in line with the above requirements, the utility of *Chironomus* larvae as a model is not just about its inherent biological features, but also its amenability to these advanced techniques. By integrating *Chironomus* with high-content imaging and machine learning, researchers can move beyond basic survival assays to detect subtle behavioral, morphological, or physiological changes, thus capturing "cryptic phenotypes" that are crucial for understanding anthelmintic mechanisms and improving the correlation between in vitro and in vivo efficacy. Thus, the above proposition positions *Chironomus* larvae not just as a cheaper alternative, but as a scientifically advanced platform(Zamanian & Chan, 2021)(Geary, et al., 2015)(Nixon, et al., 2020).

Chironomus Larvae Model: A Scientifically Robust and Ethically Advantageous Model System

To address the limitations of traditional anthelmintic drug discovery, the adoption of alternative model organisms is paramount. *Chironomus* larvae present a compelling candidate due to a unique combination of biological characteristics, practical advantages for laboratory cultivation, and established utility in related fields(Al-Shami, et al., 2011)(Halpern & Senderovich, 2014)(Montano Campaz, et al., 2022)(Maldonado, et al., 2021).

Biological Characteristics and Practical Advantages for Laboratory Cultivation

Chironomus species, commonly known as non-biting midges in their adult stage and "bloodworms" during their larval stage, are among the most abundant and diverse insect groups inhabiting freshwater ecosystems worldwide. They undergo a complete metamorphosis, encompassing four distinct life stages: egg, larva (with four instars), pupa (all of which are aquatic), and a terrestrial adult stage(Al-Shami, et al., 2011)(Montano Campaz, et al., 2022).

Chironomus larvae are remarkably easy to grow and maintain under controlled laboratory conditions, making them highly accessible for research(Montano Campaz, et al., 2022). A key advantage is their exceptionally short life cycle. This rapid turnover is invaluable for high-throughput screening and accelerating experimental cycles in drug discovery(Lee, et al., 2022)(Maldonado, et al., 2021). *Chironomus* larvae are ubiquitous, found across a vast array of aquatic environments, from pristine lakes to highly polluted waters(Al-Shami, et al., 2011). Further, many species are renowned for their high tolerance to adverse environmental conditions, including low oxygen levels and significant organic pollution(Montano Campaz, et al., 2022)(Khdre, et al., 2023)(Montano Campaz, et al., 2022).

This resilience allows them to survive and exhibit measurable responses even in the presence of potentially toxic compounds, providing a broad range for dose-response studies. Their widespread distribution, high species diversity, and responsiveness to environmental changes firmly established *Chironomus* larvae as key indicator organisms (Lee, et al., 2022)(Khdre, et al., 2023)(Liu, et al., 2025)(Hudson & Ciborowski, 1996)(Rossaro, et al., 2022).

The description of *Chironomus* as both highly tolerant to pollution and sensitive to environmental changes, reveals a strategic advantage for drug screening. Their tolerance means they can survive exposure to a wide range of concentrations of a test compound, including potentially crude plant extracts at higher doses without immediate mass mortality. This provides a broad experimental window to observe and quantify dose-dependent effects, including sublethal ones(Liu, et al., 2025).

Concurrently, their sensitivity to change ensures that even subtle anthelmintic effects, which may not be acutely lethal but impair fitness, can be reliably detected. This "tolerant sensitivity" allows for the generation of robust dose-response curves and the identification of nuanced phenotypic shifts, which is ideal for a pilot study on a complex natural product like the extract of traditionally reported medicinal plants.

Crucially, as an invertebrate, *Chironomus* larvae represent an ethically superior alternative to traditional vertebrate animal models in drug screening(Lee, 2025). Their use significantly mitigates the welfare concerns associated with animal experimentation, aligning perfectly with the globally accepted "3Rs" framework for humane research. The rapid life cycle, ease of laboratory culture, and high abundance of *Chironomus* larvae directly translate into substantial economic and logistical efficiencies for drug discovery. Compared to the astronomical costs (\$1.2 billion) and prolonged timelines (15 years) associated with traditional drug development and the limited throughput of in vivo screens (Zamanian & Chan, 2021)(Geary, et al., 2015), *Chironomus* offers a rapid, scalable, and significantly more cost-effective platform for initial screening.

In continuation to the above rationale, this model thus presents the opportunity for researchers to test a much larger number of extracts, fractions, or isolated compounds in a shorter period, thereby accelerating the early stages of the drug discovery pipeline. By efficiently triaging potential candidates at a lower cost, *Chironomus* helps de-risk the subsequent, more expensive, and ethically sensitive stages of drug development, making the overall process more sustainable and productive. Table 1 summarizes the key biological features that *Chironomus* larvae have that make it a possible alternative model organism for use.

Table 1:- Key Biological Features Relevant to Model Organism Use.

Feature	Description/Details
Life Cycle Type	Complete metamorphosis (aquatic immature stages)
Life Cycle Duration	Typically, 11-30 days (species-dependent)
Larval Instars	4 larval instars
Primary Habitat	Ubiquitous freshwater (sediment-dwelling)
Ease of Lab. Cultivation	Easy to culture and maintain; continuous supply
Environmental Tolerance	High tolerance to low O ₂ / pollution; sensitive to changes
Abundance	Widespread and highly abundant
Ethical Classification	Invertebrate (aligns with 3Rs: Replacement/Reduction)

Comparative Physiology and Anatomy - Relevance to Helminth Biology

The predictive power of any model organism stems from its biological relevance to the target pathogens. While *Chironomus* larvae are insects (Diptera), their fundamental biological processes and anatomical structures exhibit sufficient commonalities with helminths (specifically nematodes, given their shared Ecdysozoan lineage) to serve as a scientifically sound initial screening platform(Khdre, et al., 2023).

The Cuticle and Tegument: A Conserved Target for Anthelmintic Action

A primary and well-established target for many anthelmintic drugs is the external covering of helminths i.e., the cuticle in nematodes and the tegument in cestodes(Zamanian & Chan, 2021). Damage to these vital structures, such as surface erosion, degeneration of microtriches, or distortion of attachment organs, represents a hallmark effect of

effective antiparasitic compounds like albendazole and lupeol. Both insects, including Chironomus, and nematodes belong to the superphylum Ecdysozoa, a group characterized by the presence of a tough, flexible exoskeleton (cuticle) that must be periodically shed during growth (ecdysis)(Liu, et al., 2025).

The cuticle of Chironomus larvae is structurally composed of multiple overlapping layers of chitin fibers embedded within a protein matrix(Togawa, et al., 2004)(Verbruggen, et al., 2010). Similarly, the nematode cuticle is rich in both collagen and chitin, forming a protective outer armor that extends into parts of the digestive tract. This fundamental structural and compositional similarity in the chitinous cuticle between Chironomus and nematodes provides a plausible analogous target for potent compounds from plant-based extracts that may interfere with chitin synthesis, cuticle formation, or its structural integrity(Zamanian & Chan, 2021)(Liu, et al., 2025).

The shared fundamental composition of the cuticle in Chironomus larvae and nematodes means that biological target compounds present in extracts that disrupt chitin metabolism, cuticle integrity, or molting processes in Chironomus larvae could potentially exert similar detrimental effects on parasitic helminths. While the precise molecular targets or binding sites might differ, the presence of a structurally analogous and functionally critical external covering provides a relevant biological system for initial screening, allowing for the identification of a broad class of anthelmintics that operate via cuticle disruption. This avoids the need for a direct parasitic infection model while still probing a key anthelmintic target.

Digestive System and Nutrient Uptake

Parasitic helminths, particularly those residing in the host's gastrointestinal tract, acquire essential nutrients and drug molecules from their surrounding environment (e.g., host's digested food) primarily through passive diffusion across their tegument or cuticle(Zamanian & Chan, 2021). Chironomus larvae possess a complete digestive system, extending from an anterior mouth to a posterior anus. Their midgut is equipped with specialized cells that secrete a diverse array of digestive enzymes, including proteases, amylases, lipases, sucrases, and trehalases, which are essential for the degradation of ingested organic matter(Richardi, et al., 2015)(Chamani, et al., 2025).

A cylindrical peritrophic membrane, secreted by specialized cells in the cardia, lines the midgut lumen. This membrane serves to protect the midgut epithelium from abrasive food material and, crucially, facilitates the passage of water, salts, digestive enzymes, and digested food material in both directions through unique channels(Khdre, et al., 2023). This structure has been reported to influence the absorption and distribution of ingested substances(Khdre, et al., 2023)(Walshe, 2009)(Pery, et al., 2002).

This ingestion mechanism is highly relevant for assessing the anthelmintic potential of any potent plant extract. Helminths absorb drugs from their host environment(Zamanian & Chan, 2021). Chironomus larvae, as detritivores, actively ingest substances from their aquatic environment. Their complete digestive system, equipped with a range of digestive enzymes and a peritrophic membrane, means that ingested plant compounds will undergo a process of digestion and absorption too (Chamani, et al., 2025). This provides a more realistic early-stage screening environment than simple in vitro assays where compounds are directly exposed to the parasite without passing through a digestive system. Further, this can also be attributed to allow for the assessment of compounds that may require enzymatic activation or that exhibit differential stability or absorption within a biological gut environment, thereby enhancing the predictive value for potential in vivo efficacy against helminths.

Nervous System and Behavioural Endpoints

The nervous system is a critical target for many established anthelmintics, with drug action often manifesting as disruptions in motility, coordination, or overall behavior (Zamanian & Chan, 2021). Chironomus larvae possess a complex central nervous system (CNS) with approximately 10,000 neurons, of which about 2,000 reside in the brain. Although numerically simpler than the adult insect CNS, it contains most neural cell types, offering a system of 'high complexity but with numerical simplicity'. Notably, the Chironomus larval CNS is one of the few fully reconstructed nervous systems, providing an unparalleled anatomical and connectomic basis for studying neurological effects(Janelia Research Campus, 2025).

Immunohistochemical studies have identified various putative neurotransmitters within the Chironomus nervous system, including 5-hydroxytryptamine (serotonin), tyrosine hydroxylase (involved in catecholamine synthesis), and neuropeptides such as methionine-enkephalin, proctolin, and bombesin(Johansson, et al., 1986). Calcium is also recognized as essential for neurotransmitter function(Wei, et al., 2024). Chironomus larvae also exhibit a range of

measurable behavioural endpoints, such as swimming abilities and exploration behavior, which have been demonstrated to be highly sensitive indicators of neurotoxicity, even revealing greater than predicted effects when combined with environmental stressors(Galli, et al., 2025). Many anthelmintics exert their effects by targeting the nervous system, leading to paralysis, uncoordinated movement, or other behavioral changes(Zamanian & Chan, 2021). Chironomus larvae possess a well-characterized central nervous system with identified neurotransmitters(Johansson, et al., 1986), providing a relevant biological substrate for neuro-active compounds. The ability to measure sensitive behavioral endpoints, coupled with the potential for high-content technological intervention promotes this animal model beyond simple mortality studies to detect subtle neurotoxic or paralytic effects, which are highly indicative of anthelmintic potential even if they do not immediately result in larval death. The detailed anatomical knowledge of the Chironomus CNS further supports the potential for mechanistic studies if promising neuro-active compounds are identified(Wei, et al., 2024)(Zamanian & Chan, 2021).

Detoxification Pathways

Finally, we know that, insects possess a robust array of detoxification enzymes, including cytochrome P450 monooxygenases (CYPs), glutathione S-transferases (GSTs), and carboxylesterases. These enzymes play crucial roles in neutralizing xenobiotics, such as pesticides and plant allelochemicals, through metabolic processes that modify, conjugate, and facilitate the excretion of these toxins(Chamani, et al., 2025). Nematodes also exhibit sophisticated detoxification mechanisms. Notably, some parasitic nematodes have acquired enzymes like cyanase through horizontal gene transfer from plants and bacteria, underscoring the critical importance of these pathways in their survival, adaptation, and the evolution of parasitism(Halpern & Senderovich, 2014). Understanding how Chironomus larvae metabolize the complex mixture of compounds within the selected medicinal plant extracts is crucial, as drug absorption, distribution, metabolism, and excretion (ADME) are key factors that profoundly influence a compound's effective concentration and ultimately its in vivo efficacy(Zamanian & Chan, 2021).

Plant extracts have been noted to contain a diverse array of secondary metabolites, some of which may be toxic or require metabolic activation. Both insects and nematodes possess analogous detoxification enzyme systems(Chamani, et al., 2025). While the specific enzyme isoforms and their activities will vary, the presence of these functional pathways in Chironomus means that the larvae will process the ingested plant compounds in a manner that shares functional similarity with how target helminths might handle them. This allows for a more realistic assessment of a compound's effective bioactivity after metabolic processing, rather than just its intrinsic activity against an isolated biological target.

This is particularly critical for natural product screening, as it helps identify compounds that are not rapidly detoxified by the test organism, thereby increasing the likelihood that they would also remain active against the target helminth or within a host environment. This contributes significantly to bridging the gap between initial in vitro findings and potential in vivo efficacy(Khdre, et al., 2023).

Established Precedent: Chironomus as a Model in Ecotoxicology and Pharmacological Screening

Chironomus species have a long and well-established history as model organisms in ecotoxicology, particularly for evaluating the toxic effects of various environmental pollutants. Chironomus riparius, for example, is a suggested model organism by the Organization for Economic Cooperation and Development (OECD) for acute and chronic chemical toxicity tests(Montano Campaz, et al., 2022)(Lee, et al., 2022). Their utility spans from molecular-biochemical levels to population-level assessments, covering impacts from heavy metals, pesticides, and microplastics(Liu, et al., 2025). A wide array of standardized and quantifiable endpoints have been developed and validated in these studies, including mortality, growth inhibition, emergence rates, development time, and specific morphological deformities, as well as behavioral changes(Hudson & Ciborowski, 1996)(Veroli, et al., 2014).

The extensive and well-documented use of Chironomus is a tremendous asset for developing anthelmintic screening assays(Montano Campaz, et al., 2022)(Lee, et al., 2022). This means that standardized protocols for laboratory rearing, exposure setups, and the assessment of various endpoints are already well-established and validated(Khdre, et al., 2023)(Montano Campaz, et al., 2022). This significantly reduces the initial development time, effort, and cost required to set up a pilot anthelmintic study. This established precedent makes Chironomus a "low-hanging fruit" for rapid and reliable adoption in a new screening context, allowing researchers to focus directly on the anthelmintic potential of traditionally reported medicinally potent plants rather than on model development.

High-Throughput Screening Potential

The inherent biological characteristics of *Chironomus* larvae, including their short life cycle, ease of laboratory culture, and relatively small size, make them exceptionally amenable to miniaturization of screening platforms. This characteristic is crucial for enabling the testing of a large number of compounds or natural product fractions in a high-throughput manner. The potential for *Chironomus* to be integrated into high-throughput screening (HTS) workflows has been explicitly acknowledged (Halpern & Senderovich, 2014) (Lee, et al., 2022).

A significant limitation of traditional in vitro anthelmintic screens is their tendency to miss compounds that do not induce "overt phenotypes" but nonetheless disrupt crucial subtle processes for parasite survival (Zamanian & Chan, 2021). *Chironomus* larvae, however, offer a rich array of quantifiable sublethal and behavioral endpoints (Hudson & Ciborowski, 1996) (Veroli, et al., 2014). Further, the ability to assess a broad spectrum of endpoints, ranging from conventional measures like survival, growth, and emergence to more nuanced, non-conventional parameters such as adult size, swimming abilities, and exploration behavior, provides a comprehensive picture of compound effects. This multi-parametric approach increases the likelihood of identifying novel mechanisms of action (Rossaro, et al., 2022).

When combined with the power of high-content imaging and machine learning, this model can move beyond simple mortality or gross motility inhibition. It can detect and quantify subtle changes in behavior (e.g., altered swimming patterns, reduced exploration), morphology (e.g., cuticle damage, mouthpart deformities), or developmental progression (e.g., delayed emergence). This capability to identify "cryptic phenotypes" means that *Chironomus* can serve as a sophisticated phenotypic screening tool, potentially identifying novel anthelmintic mechanisms that might not cause immediate death but severely impair the organism's fitness or reproductive capacity, which are equally critical for parasitic control. This enhanced phenotypic profiling capability directly contributes to improving the predictive value of early-stage screening for subsequent in vivo efficacy (Pery, et al., 2002) (Chamani, et al., 2025).

Rationale for Utilizing *Chironomus* Larvae in Assessing Anthelmintic Potential of traditionally reported plant extracts

The strategic integration of *Chironomus* larvae into assessment of anthelmintic potential of traditionally reported medicinal plants is justified by a confluence of practical advantages, its biological relevance, and its capacity to address common challenges in natural product screening.

Strategic Advantages for Initial Screening of Plant Extracts

Chironomus larvae provide an exceptionally cost-effective and high-throughput platform for the initial, broad-spectrum screening of complex natural product such as those derived from plant extracts. Such plant extracts would act as potent reservoirs of natural phytochemicals, meaning its active principles are unknown, and initial screening involves complex mixtures. Utilizing *Chironomus* larvae for this early-stage screening would allow for rapid, inexpensive, and high-throughput triage of extracts and their fractions (Geary, et al., 2015). This would enable the current proposed model to efficiently identify promising leads or active fractions before committing significant financial and ethical resources to more complex, costly, and ethically sensitive vertebrate models or downstream drug development (Geary, et al., 2015).

Addressing Practical Challenges Associated with Plant-Derived Compounds in In Vitro Assays

One well-documented practical challenge in conducting in vitro tests with plant extracts is their solubilization. Often, higher amounts of solvent are required, which can itself induce toxicity in sensitive organisms, leading to false-positive results. Furthermore, high concentrations of plant extracts can result in dark coloration or the presence of particulate matter, severely hindering the visual assessment and counting of larvae or eggs in assays (Hudson & Ciborowski, 1996).

While *Chironomus* assays would have to inherently encounter these challenges, their robust nature and documented tolerance to various environmental stressors (Khdre, et al., 2023) provides a wider therapeutic window for testing a broader range of extract concentrations compared to highly sensitive parasitic helminths in direct in-vitro culture. This diversified approach would increase the overall reliability and comprehensiveness of the screening study, ensuring that potential anthelmintic activity is not missed due to technical limitations inherent in working with complex natural products.

Conclusion:-

Chironomus - A Forward-Thinking Approach to Anthelmintic Drug Discovery

In conclusion to the introduction of this model, the proposed utilization of *Chironomus* larvae as a model organism for the pilot assessment of anthelmintic potential represents a scientifically sound, ethically responsible, and strategically advantageous approach to addressing the critical global challenge of helminthiasis and anthelmintic resistance.

The inherent biological and practical advantages of *Chironomus*, including their rapid life cycle, ease of cultivation, and robust nature, position them as an ideal, cost-effective, and high-throughput platform for early-stage screening. Furthermore, their comparative physiological and anatomical commonalities with helminths, particularly concerning the cuticle, digestive system, nervous system, and detoxification pathways, establish a strong biological relevance for identifying compounds with anthelmintic properties.

Leveraging the extensive precedent of *Chironomus* in ecotoxicology and its amenability to advanced phenotypic profiling techniques, including high-content imaging and behavioral analysis, enables the detection of both overt and subtle "cryptic" anthelmintic effects. This multi-parametric approach enhances the predictive value of the initial screen, allowing for a more comprehensive understanding of the bioactivity of selected plant extracts.

By embracing *Chironomus* larvae, this study model would not only contribute to the ethical imperative of reducing animal use in research but also strategically optimizes resource allocation in the arduous and costly process of drug discovery. This forward-thinking approach thus would become crucial for efficiently identifying promising natural product candidates and accelerating their progression through the anthelmintic development pipeline, ultimately contributing to the urgent need for novel and effective treatments against parasitic helminths.

References:-

1. Al-Shami, S., Salmah, R. C., Hassan, A. A. & Azizah, N., 2011. Evaluation of mentum deformities of *Chironomus* spp. (Chironomidae: Diptera) larvae using modified toxic score index (MTSI) to assess the environmental stress in Juru River Basin, Penang, Malaysia. *Environmental Monitoring and Assessment* , 177(4), pp. 233 - 44.
2. Chamani, M. et al., 2025. From Digestion to Detoxification: Exploring Plant Metabolite Impacts on Insect Enzyme Systems for Enhanced Pest Control. *Insects*, 16(4), p. 392.
3. Galli, G. et al., 2025. High-Throughput Screening of Five Compound Libraries for Anthelmintic Activity and Toxicity Leads to the Discovery of Two Flavonoid Compounds. *International Journal of Molecular Sciences*, 26(4), p. 1595.
4. Geary, T. G., Sakanari, J. A. & Caffrey, C. R., 2015. Anthelmintic drug discovery: into the future. *Journal of Parasitology*, 101(2), pp. 125 - 33.
5. Geerts, S. & Gryseels, B., 2000. Drug Resistance in Human Helminths: Current Situation and Lessons from Livestock. *Clinical Microbiology Reviews*, 13(2), pp. 207 - 222.
6. Halpern, M. & Senderovich, Y., 2014. Chironomid Microbiome. *Microbial Ecology*, 70(1), pp. 1 - 18.
7. Hudson, L. A. & Ciborowski, J. J., 1996. Teratogenic and genotoxic responses of larval *Chironomus salinarius* group (diptera: Chironomidae) to contaminated sediment. *Environmental Toxicology and Chemistry*, 15(8), pp. 1375 - 1381.
8. Janelia Research Campus, 2025. The Larval Nervous System. [Online]
9. Available at: <https://www.janelia.org/project-team/flylight/research/the-larval-nervous-system>
10. [Accessed 30 06 2025].
11. Johansson, I., Olsson, A. & Wieslander, L., 1986. The distribution of putative neurotransmitters in the nervous system of the dipteran *Chironomus tentans* insect larva: An immunohistochemical study using antisera to 5-hydroxytryptamine, tyrosine hydroxylase, methionine-enkephalin, proctolin and bombesin. *Neurochemistry International*, 8(3), pp. 311 - 26.
12. Khdre, A. M., Ramadan, S. A. & Ashry, A., 2023. *Chironomus* sp. as a Bioindicator for Assessing Microplastic Contamination and the Heavy Metals Associated with It in the Sediment of Wastewater in Sohag Governorate, Egypt. *Water Air and Soil Pollution*, 234(161).
13. Lee, C. Y. et al., 2022. Passability of Chironomid Larvae in Granular Activated Carbon. *International Journal of Environmental Research and Public Health*, 19(2), p. 1005.
14. Lee, S., 2025. Ethics in Parasitology Research. [Online]

15. Available at: <https://www.numberanalytics.com/blog/ethics-parasitology-research-practices>
16. [Accessed 30 06 2025].
17. Lindrose, A. R., Fraser, J. A., Hickey, P. W. & Mitre, E., 2022. Costs and Prescribing Patterns of Anthelmintics in the United States Military: A Retrospective Analysis. *Open Forum Infectious Diseases*, 9(3), p. ofac040.
18. Liu, W. B. et al., 2025. Trends and Emerging Hotspots in Toxicology of Chironomids: A Comprehensive Bibliometric Analysis. *Insects*, 16(6), p. 639.
19. Maldonado, R. R., Marie, B. & Ramirez, A., 2021. Rearing methods and life cycle characteristics of *Chironomus* sp. Florida (Chironomidae: Diptera): A rapid-developing species for laboratory studies. *PLoS One*, 16(2), p. e0247282.
20. Montano Campaz, M. K., G-Dias, L. & Restrepo, B. T., 2022. *Chironomus columbiensis* (Diptera: Chironomidae) as test organism for aquatic bioassays: Mass rearing and biological traits. *Anais da Academia Brasileira de Ciencias*, 94(3), p. e20210389.
21. Nixon, S. A. et al., 2020. Where are all the anthelmintics? Challenges and opportunities on the path to new anthelmintics. *International Journal for Parasitology: Drugs and Drug Resistance*, Volume 14, pp. 8-16.
22. Pery, A. et al., 2002. A modeling approach to link food availability, growth, emergence, and reproduction for the midge *Chironomus riparius*. *Environmental Toxicology and Chemistry*, 21(11), pp. 2507 - 2513.
23. Richardi, V., Vicentini, M., Rebecchi, D. & Favaro, L. F., 2015. Morpho-histological characterization of immature of the bioindicator midge *Chironomus sancticarloi* Strixino and Strixino (Diptera, Chironomidae). *Revista Brasileira de Entomologia*, 59(3), pp. 240 - 250.
24. Rossaro, B., Marziali, L. & Boggero, A., 2022. Response of Chironomids to Key Environmental Factors: Perspective for Biomonitoring. *Insects*, 13(10), p. 911.
25. Togawa, T., Nakato, H. & Izumi, S., 2004. Analysis of the chitin recognition mechanism of cuticle proteins from the soft cuticle of the silkworm, *Bombyx mori*. *Insect Biochemistry and Molecular Biology*, 34(10), pp. 1059 -67.
26. Verbruggen, F., Heiri, O., Reichart, G. J. & Leeuw, J. D., 2010. Effects of chemical pretreatments on ?? 18 O measurements, chemical composition, and morphology of chironomid head capsules. *Journal of Paleolimnology*, 43(4), pp. 857 - 872.
27. Veroli, A. D., Santoro, F., Pallottini, M. & Selvaggi, R., 2014. Deformities of chironomid larvae and heavy metal pollution: From laboratory to field studies. *Chemosphere*, Volume 112, pp. 9 - 17.
28. Walshe, B. M., 2009. The feeding habits of certain Chironomid larvae (subfamily Tendipedinae). *Proceedings of the Zoological Society of London. Journal of Zoology*, 121(1), pp. 63 - 79.
29. Wei, F., Gu, W., Zhang, D. & Wo, S., 2024. Paralysis caused by dinotefuran at environmental concentration via interfering the Ca²⁺–ROS–mitochondria pathway in *Chironomus kiiensis*. *Frontiers in Public Health*, Volume 12, pp. 1 - 9.
30. Zamanian, M. & Chan, J. D., 2021. High-content approaches to anthelmintic drug screening. *Trends in Parasitology*, 37(9), pp. 780 – 789.