International Journal of Zoology Studies
www.zoologyjournals.com
E-ISSN: 2455-7269
Received: 19-09-2026, Accepted: 19-10-2026, Published: 03-11-2026
Volume 11, Issue 4, 2026, Page No. 28-35

Comparative toxicity of Neem, Pungam, and Coconut seed oils in Zebrafish (*Danio rerio*)

Azhagu Raj R*, Mowniga G S, Merson T
Department of Zoology, Crop Protection Research Centre (CPRC), St. Xavier's College (Autonomous), Palayamkottai, Tamil Nadu, India
Corresponding Author: Azhagu Raj R
DOI: https://doi.org/10.66856/ijzs.2026.11.4.11086

Abstract
The expanding use of plant-derived biopesticides in sustainable agriculture has increased the need to evaluate their unintended effects on aquatic vertebrates. This study examined the toxicological properties of three commercially available botanical plant seed oils from neem (*Azadirachta indica*), pungam (*Pongamia pinnata*), and coconut (*Cocos nucifera*) using on zebrafish (*Danio rerio*) as a model organism. The fishes and oils are punches from local aquarium and Palayamkottai market (latitude 8°4258 N and longitude 77°4420 E), Tirunelveli, Tamil Nadu, India. The acute toxicity was calculated over 96 hours through mortality trailing and opercular movement monitoring, while subacute effects were evaluated during 21-day exposure. Acute toxicity followed a concentration and time-dependent pattern, with neem oil showing the highest potency in 96-h on LC_{50} - 4.8 ppm and LC_{90} - 9.2 ppm, followed by pungam oil LC_{50} - 6.2 ppm and LC_{90} -10.8 ppm and coconut oil demonstrating the lowest hazard LC_{50} - 9.5 ppm and LC_{90} - 14.6 ppm. The opercular movement declined progressively with increasing dose and exposure duration, with neem and pungam oils causing the most marked respiratory depression. Sub-acute exposure showed high survival across all treatments, with only neem oil at the highest concentration producing mortality (20%). These findings indicate that while botanical plant seed oils are generally less hazardous than synthetic pesticides, neem oil despite its eco-friendly reputation can produce significant physiological and structural damage at elevated concentrations.

Keywords: *Danio rerio*, botanical biopesticides, Neem oil, Pungam oil, acute toxicity, sub- acute toxicity histopathology

Introduction
The global agricultural sector is increasingly turning to plant-based pest control products as alternatives to synthetic chemicals, driven by concerns about environmental persistence, non-target organism impacts, contaminated water or indirect exposure via agricultural runoff to rivers, and the development of pesticide resistance (Chaudhary *et al*., 2017; Roy Choudhury, 2016; Islam *et al*., 2026; Soliman and Abdelmawgood, 2026; Overmyer *et al*., 2007; and Pant *et al*., 2016) [5,7,15,16,19]. The synthetic chemicals are causing risks and serious impacts on other freshwater species (Chakraborty *et al*., 2025; Azhagu Raj *et al*.,2022, Azhagu Raj *et al*., 2023) [4.26,27]. Among the most widely used products, neem oil extracted from *Azadirachta indica* contains azadirachtin and other limonoids that interfere with insect growth and feeding behavior and are safe and sustainable for the environment (Nagini *et al*., 2024; and Sacco *et al*., 2025) [15,21]. The Pungam oil (*Pongamia pinnata*) provides karanjin and related compounds with comparable insecticidal properties, while the coconut oil (*Cocos nucifera*) serves primarily as a physical agent with minimal intrinsic toxicity to non-target species (Raghav *et al*., 2019; Wang *et al*., 2024) [18,24]. The *Danio rerio* (zebrafish) has become a standard model in aquatic toxicology due to its well-characterized genetics, rapid development, and sensitivity to environmental contaminants (Tanguay, 2018; Amaral *et al*., 2024; Azhagu Raj *et al*.2022,) [1,23,26]. This study aims to analyze the multiple botanical plant seed oils at different concentrations for experiments of mortality, respiratory physiology, and tissue-level pathology on zebrafish.

Materials and Methods
Test Animals and Maintenance
The zebrafish (*Danio rerio*) were obtained from a local aquarium supplier in Tirunelveli (latitude 8°4258 N and longitude 77°4420 E), Tamil Nadu, India (Figure 1), and transported to the laboratory in oxygenated containers. The fishes were acclimatized for four weeks in 70-L glass aquaria under controlled conditions: temperature 25 ± 1°C, 12:12 h light: dark cycle, dissolved oxygen >5 mg/L, and pH 7.2 ± 0.2. The fishes were fed commercial flake food twice daily. The water was renewed daily, and waste material was removed; feeding was stopped 24 hours before experiments began.

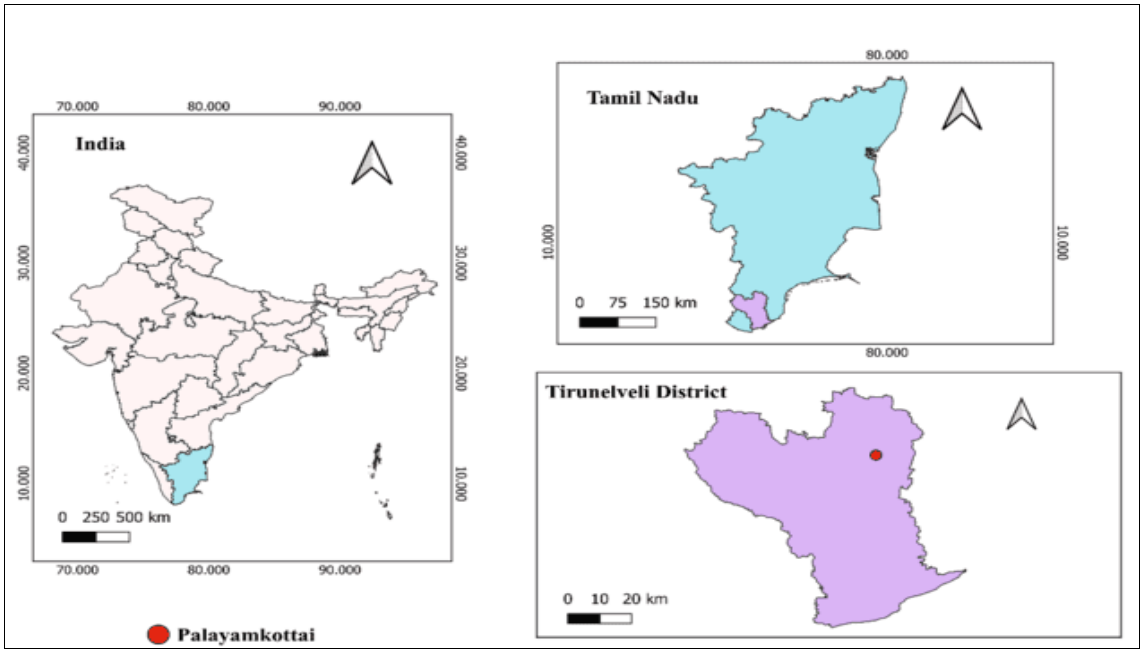


Fig 1: The study area Palayamkottai

Morphometric Measurements

The five fish were randomly selected for baseline measurements. The total length (tip of snout to end of caudal fin) was measured with digital calipers, and body weight was determined on an analytical balance after gently blotting surface water.

Operculum Movement Assessment

The Operculum beats per minute (BPM) were counted visually using a stopwatch for 60-second intervals. Baseline measurements were recorded prior to exposure and subsequently at 24-hour intervals (Day 1–4) for acute toxicity and on Days 1, 7, 14, and 21 for subacute toxicity. Each fish was assessed individually in triplicate, with mean values calculated per treatment group.

Test Materials

The commercial-grade neem oil (*Azadirachta indica*), pungam oil (*Pongamia pinnata*), and coconut oil (*Cocos nucifera*) were purchased from Palayamkottai market, Tirunelveli. Stock solutions (0.1 mL) were prepared by dissolving 10 mL emulsified oil with 2% Tween-20 emulsifier in 1 L distilled water. Working concentrations were prepared by serial dilution.

Acute Toxicity Test (96 h)

The ten trays (1.5 L capacity, 1 L working volume) were used for each treatment; it's based on preliminary range-finding tests. Three concentrations were selected for each oil: low (1 mL/L), medium (5 mL/L), and high (10 mL/L), and a control containing only emulsifier in dechlorinated water. Each aquarium held five fish (n = 5). Mortality was recorded at 24, 48, 72, and 96 hours, with dead fish removed immediately. Test solutions were completely renewed every 24 hours.

Sub-Acute Toxicity Test (21 days)

The subacute concentrations were prepared at a 1:10 dilution of acute concentrations: 0.1, 0.5, and 1.0 mL/L. And fishes were exposed to semi-static conditions with solution renewal every 48 hours. Survival rate was monitored daily, and fish were fed normally during the exposure period.

Histopathological Examination

The fish from each treatment and control group were randomly selected for histology analysis. The Gill and muscle tissues were dissected, rinsed in physiological saline, and fixed in 10% neutral buffered formalin for 48 hours. The tissues were processed through standard histological procedures: dehydration in graded ethanol series (70–100%), clearing in methyl benzoate, embedding in paraffin wax (56–58°C), sectioning at 5 µm, and staining with hematoxylin-eosin (Bancroft and Stevens, 1982; Bancroft *et al*., 1996) [2,3]. Sections were examined under an Olympus CH30 microscope, and photographs were taken.

Statistical Analysis

The mortality data were analysed by probit regression, and opercular movement data were analysed by two-way ANOVA followed by Tukey's test for multiple comparisons. Finally, all analyses were performed using SPSS v.16.0, with significance set at $p < 0.05$.

Results

Morphometric Characteristics

The experimental fish showed uni form morphometric parameters (Mean ± SD): total length 3.92 ± 0.26 cm; body weight 0.998 ± 0.14 g. The length-weight relationship indicated healthy, mature adults suitable for toxicity testing (Figure 2 A and B).



Fig 2: A – B zebrafish; C - Acute and D - Sub-Acute Toxicity of zebrafish against Neem, Pungam and Coconut Oil.

Opercular Movement

The control fish maintained stable opercular movement (78–80 beats/min) throughout the experiment, within the normal range for zebrafish. The treated groups showed progressive decline in opercular movement with increasing concentration and exposure time (Table 1; Fig. 3). The greatest reduction occurred in high-concentration neem oil (44 beats/min at 96 h; 45% reduction from baseline), followed by Pungam oil (52 beats/min; 35% reduction). Coconut oil produced minimal respiratory stress (67 beats/min at 96 h; a 16% reduction).

Table 1: Operculum movement of Zebra fish per minute

S. No	Treatment	Concentration	Before Exposure	Day-1	Day-2	Day-3	Day-4
1	Control	–	79	80	78	79	78
2	Neem oil	Low	78	72	68	64	60
		Medium	79	68	62	56	50
		High	80	**64**	**58**	**50**	**44**
3	Pongam oil	Low	78	74	71	68	65
		Medium	79	71	67	62	58
		High	80	**67**	**62**	**56**	**52**
4	Coconut oil	Low	78	76	75	74	73
		Medium	79	75	73	71	69
		High	80	**73**	**71**	**69**	**67**

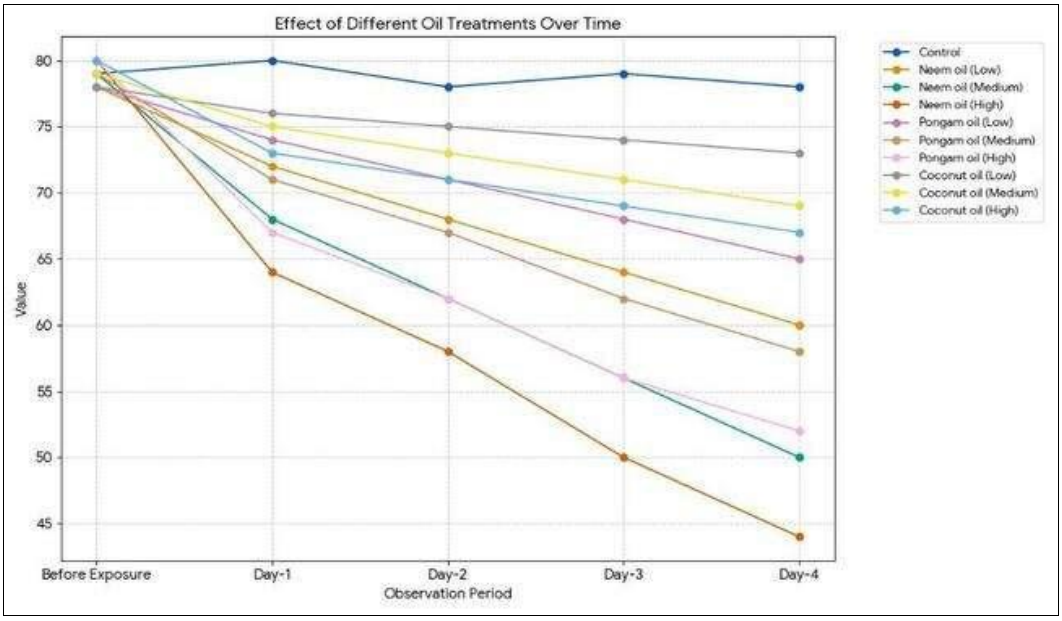


Fig 3: Operculum movement of Zebra fish per minute

Acute Toxicity

Mortality increased with both concentration and exposure time for all oils (Table 2; Figure 4). The neem oil showed the highest toxicity: at 10 mL/L, mortality began at 24 hours (2 of 5 fish) and reached 100% by 96 hours. The 5 mL/L treatment showed a delayed onset (48 hours) with complete mortality by 96 hours. The 1 mL/L treatment caused only minor mortality, with 2 out of 5 fish dying by 96 hours. And Pungam oil showed intermediate toxicity: the highest concentration caused 100% mortality by 96 hours, while the medium concentration caused 60% mortality. The lowest concentration was non-lethal. The coconut oil was the least toxic, and only the highest concentration caused mortality (40% at 96 hours), with lower concentrations producing no deaths (Figure 5).

Table 2 presents the LC_{50} and LC_{90} values from the probit analysis, along with the toxicity ranking, which indicates that neem oil has an LC_{50} of 4.8 ppm (95% CI: 3.9–5.7) and an LC_{90} of 9.2 ppm (slope: 2.45 ± 0.32), followed by Pungam oil with an LC_{50} of 6.2 ppm (95% CI: 5.1–7.4) and an LC_{90} of 9.2 ppm (slope: 2.45 ± 0.32). 10.8 ppm; slope: 2.10 ± 0.29), followed by coconut oil (LC_{50}: 9.5 ppm; 95% CI: 8.1–11.2; LC_{90}: 14.6 ppm; slope: 1.75 ± 0.25).

Table 2: LC50 and LC90 using probit analysis

Treatment	LC50	95% Confidence Limit		LC90	95% Confidence Limit		Slope ± SE	Intercept ± SE	χ^2
		LL	UL		LL	UL			
Neem Oil	4.8	3.9	5.7	9.2	7.8	10.6	2.45 ± 0.32	-3.12 ± 0.41	1.98
Pungam Oil	6.2	5.1	7.4	10.8	9.2	12.6	2.10 ± 0.29	-3.85 ± 0.38	2.15
Coconut Oil	9.5	8.1	11.2	14.6	12.8	16.9	1.75 ± 0.25	-4.90 ± 0.35	1.72

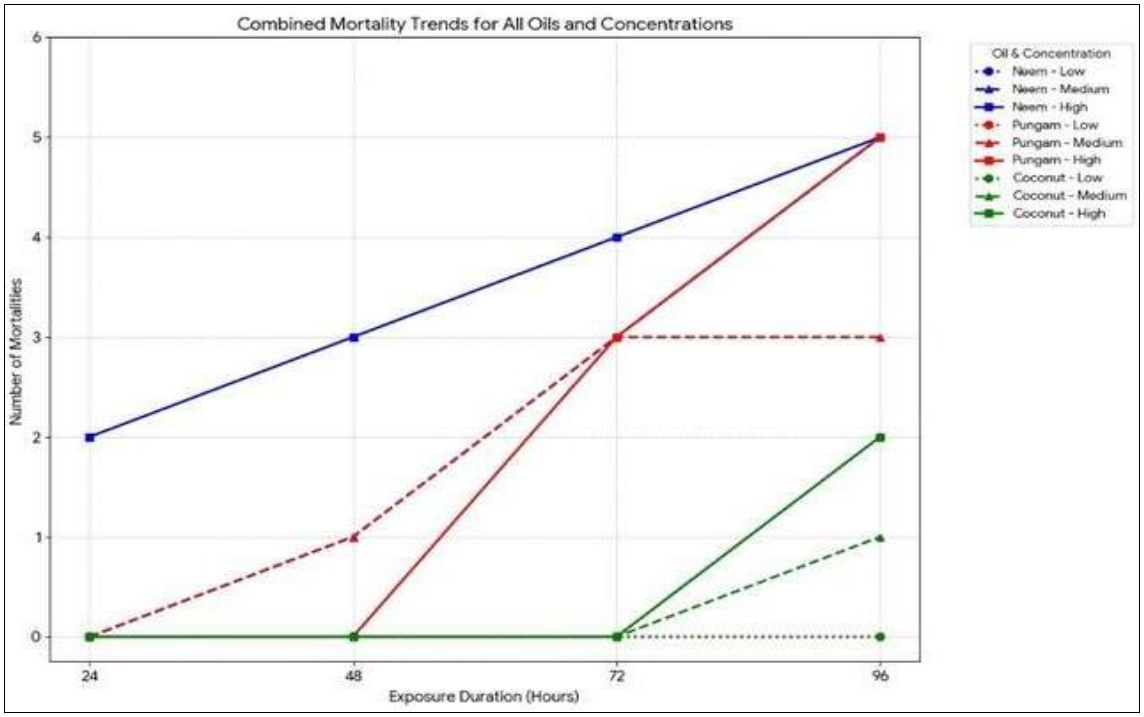


Fig 4: Acute toxicity of molly fish against biopesticides.

Subacute Toxicity

The survival remained high across most treatments during 21-day exposure (Table 3; Fig. 5). The Pungam and coconut oils caused no mortality at any concentration (100% survival), and Neem oil maintained 100% survival at 0.1 and 0.5 mL/L; however, the 1.0 mL/L concentration caused 20% mortality (1 of 5 fish), indicating a threshold for chronic neem oil effects.

Table 3: Subacute toxicity response of Zebra fish at different biopesticides.

S. No	Treatment Oil	Concentration	No. of Fish Exposed	No. of Fish Dead	Survival (%)
1	Neem Oil	Low	5	0	100
		Medium	5	0	100
		High	5	1	80
2	Pungam Oil	Low	5	0	100
		Medium	5	0	100
		High	5	0	100
3	Coconut Oil	Low	5	0	100
		Medium	5	0	100
		High	5	0	100

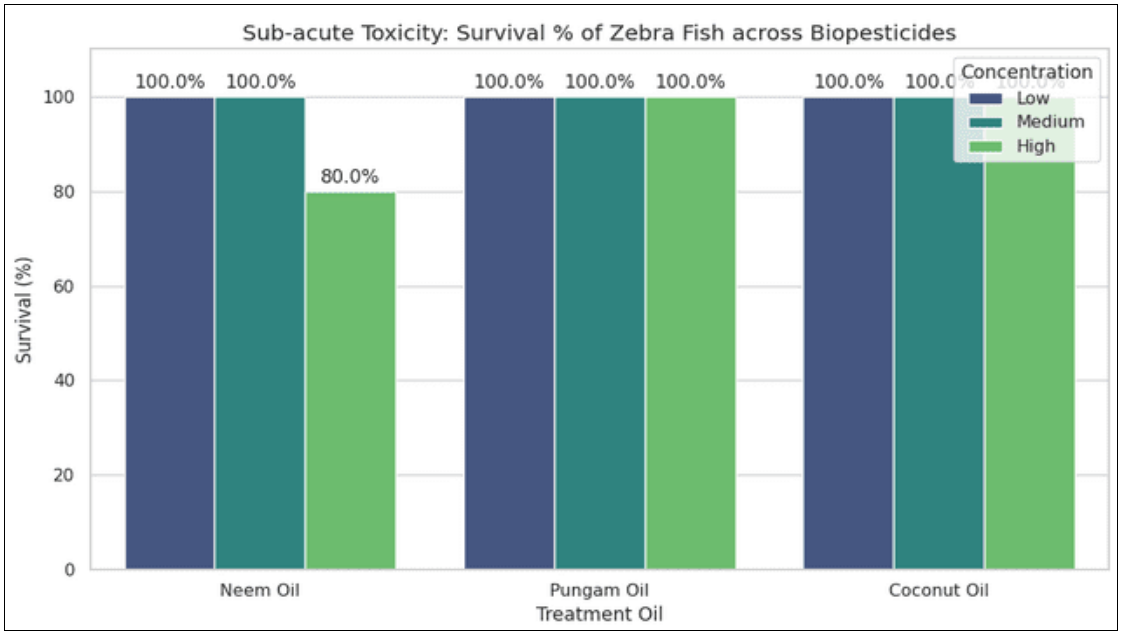


Fig 5: Subacute toxicity response of Zebra fish at different biopesticides

The Acute exposure on Gill and Muscle Tissue via Histopathological analysis:

The control had a normal gill structure with well-arranged primary and secondary lamellae, intact epithelial covering, and open interlamellar spaces (Fig. 6A). But acute exposure to 1 ml of neem oil early changes includes mild congestion, dilatation of filaments, lamellar aneurysm, slight epithelial hyperplasia, and initial lamellar fusion (Fig. 6B). Using 5 ml of neem oil was marked epithelial hyperplasia, degeneration of lamellae, slight edema, and congestion with blood vessel dilation (Fig. 6C). And severe necrosis, rupture of lamellae, extensive tissue damage, vascular congestion, and degeneration of gill filaments in 10 ml of neem oil (Fig. 6D). The control was normal striated muscle fibers with regular myofibrillar arrangement and central nuclei found (Fig. 7A). But after other concentrations of acute neem exposure are noted, 1 ml of neem oil has broken muscle fibers, loss of connective tissue, and myofibril clumping (Fig. 7B). Using 5 ml/L of neem oil thickened muscle fibers, wide vacuoles, and hemocytic infiltration (Fig. 7C). And extensive necrosis, internal hemorrhage, hyalinization and fragmentation, and massive hemocytic accumulation in 10/L ml (Fig. 7D).

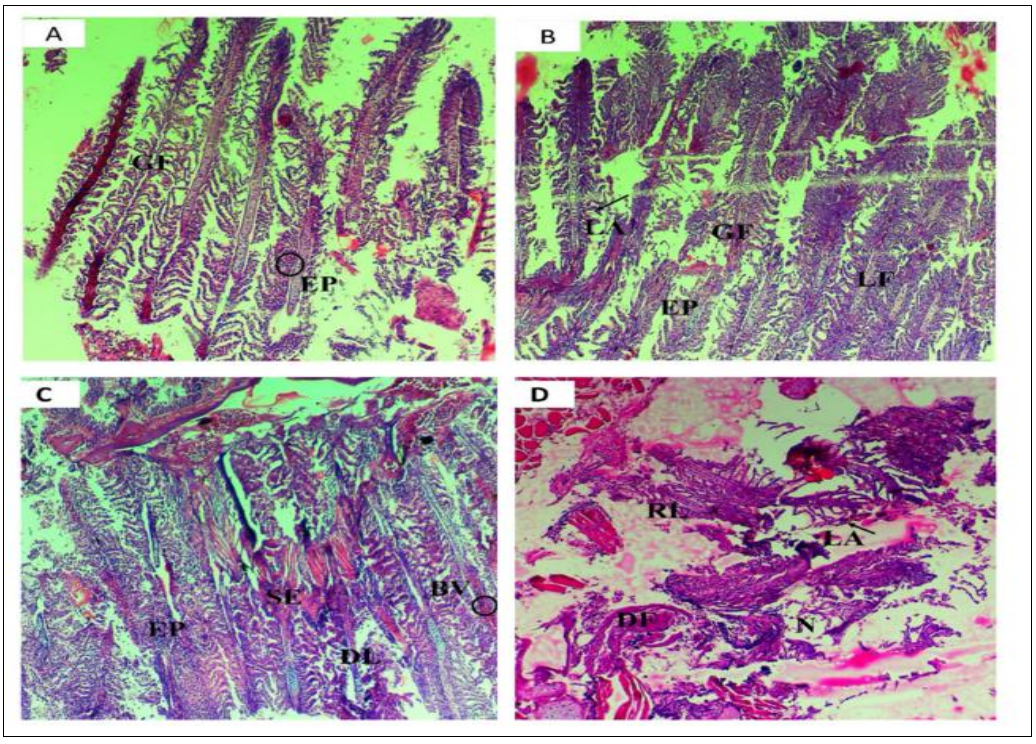


Fig 6: A – D; Microscopic view of L.S section of gills from *Danio rerio* (Zebra fish) at 10X magnification. (A) – Control group gill, B – D; Acute toxicity of neem seed oil experimental group from zebra fish gill (B) - 1ml/L, (C) – 5ml/L, and (D) -10 ml/L. (GF - Gill filament, EP - Epithelial proliferatio, LA - Lamellar aneurysm, LF - Lamellar fusion, RL -Ruptured lamellae, SE – Slight edema, DL - Degenerated lamellae, N – Necrosis).

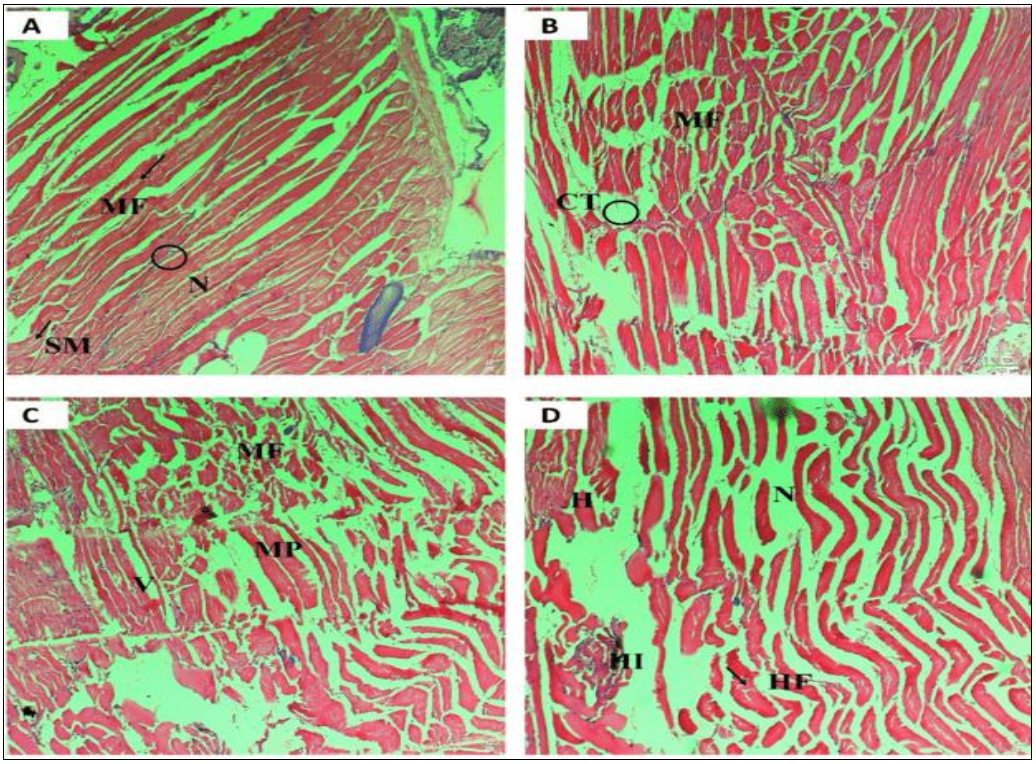


Fig 7: A – D Microscopic view of L.S section of muscle from Zebra fish (*Danio rerio*) at 10X magnification. (A) – Control group muscle, B – D; Acute toxicity of Neem seed oil experimental group from *D. rerio* muscle (B) - 1ml/L, (C) – 5ml/L, and (D) -10 ml/L (MF – Muscle fibre, N - Necrosis, SM - Skeletal muscle, CT - Connective tissue, MP - Muscle separation, V - Vacuolation, HI – Haemocyte infiltration, H – hyalinization, HF - Hypertrophied fibre, and Nu - Nucleus)

The Subacute exposure on Gill and Muscle Tissue via Histopathological analysis:

The subacute neem oil at 1.0 ml/L produced substantial but comparatively less severe gill damage, extensive lamellar fusion, and degeneration of lamellae (Figs. 8A, 8B). The muscle tissue showed necrosis with fragmented muscle fibers (Figs. 8 AA and BB), and these changes. While significant, they were less severe than those seen after acute exposure, suggesting some degree of adaptation or reduced bioavailability over longer periods.

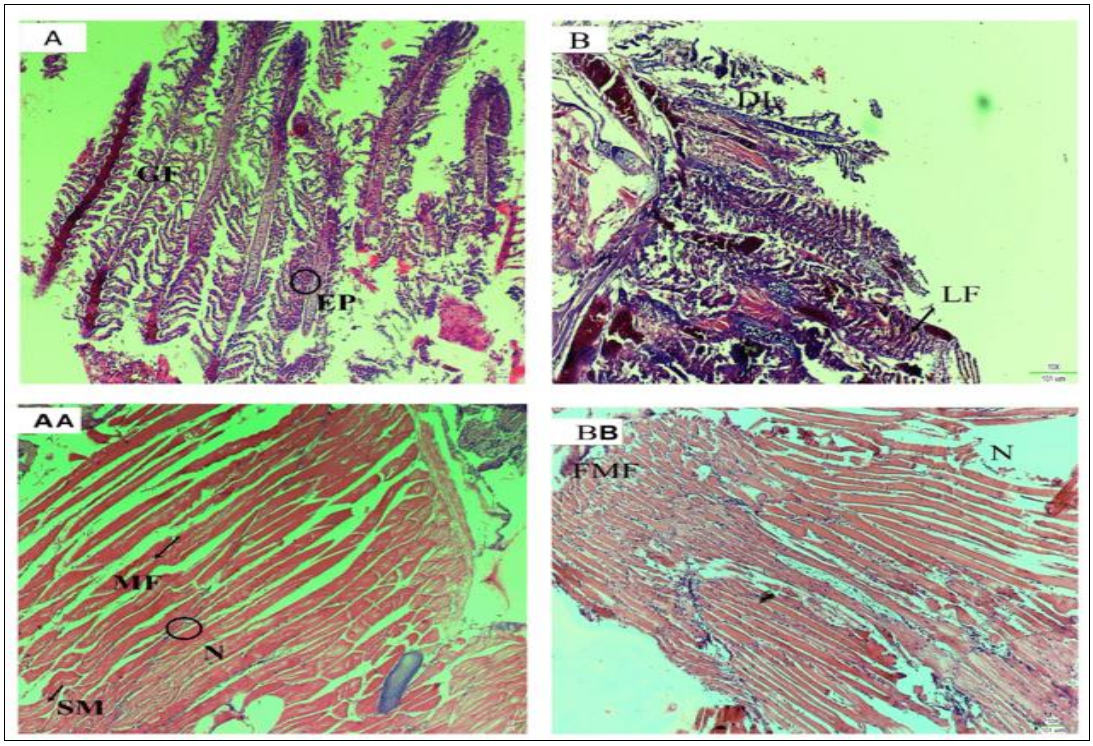


Fig 8: A – B: The microscopic view of the Zebra fish (*Danio rerio*) gills, **(A)** – Control group and **(B)** – Neem seed oil experimental group at 10 ml. **AA – BB**: The microscopic view of the Muscle tissue of Zebra fish, (**AA** – Control group and **BB -** Neem seed oil experimental group at 10 ml) (GF – Gill filament, EP – Epithelial proliferation, DL - Degenerated lamellae, LF – Lamellar fusion, MF – Muscle fibre, N – necrosis, SM – Skeletal muscle, and FMF – Fragmented muscle fibres).

Discussion

The present study on acute toxicity of neem oil 96-h LC_{50} - 4.8 and LC_{90} values are consistent with previous reports, although absolute values vary depending on product formulation and test species. Yadav *et al*. (2019) [25] reported that 96-h LC_{50} values declined from 3.155 to 1.717 mL/L using neem oil on zebrafish, and similarly, Papari Moghaddam *et al. (2023)* analysed 96-h LC_{50} 1.19 ml/L for NeemAzal in common carp. Also, the lower value of LC_{50} of 80 µL/L for purified azadirachtin from carp (Murussi *et al*., 2016) [14]. The Pungam oil's intermediate toxicity (LC_{50} - 6.2 ppm) during this study likely reflects the cytotoxic properties of karanjin, a furanoflavonoid known to disrupt mitochondrial function and generate oxidative stress in exposed organisms (Raghav *et al*., 2019) [17]. The coconut oil's low toxicity was observed from this study, finding an LC_{50} of 9.5 ml/L value, and it aligns with its established use as a dietary supplement in fish feeds without adverse effects on growth or survival (Wang *et al*., 2024) [24]. Mohamed *et al*. (2023) [13] reported that using the herbicide formulations on African catfish is attributed to gill damage that increases the diffusion barrier between water and blood. Konig *et al. (2023) and* Ramesh and Saravanan (2008) [9,19] reported that carvacrol (natural) pesticides were safer, and their replacement with fipronil (synthetic pesticides) had lower acute toxicity and pesticide hazards to fishes. Mariano *et al.* (2021) [12] observed that the biopesticide-based *Bacillus thuringiensis* water and diet affect *Arapaima gigas* fish health. Similarly, biopesticide-based Bt was toxic to the fish *Piaractus mesopotamicus* (Mariano *et al.,* 2019) [11]. Ismail and Mohamed (2012) [8] reported that the use of the biopesticide has shown no toxicity in rats compared to the control groups. Chukwuka *et al.* (2022) [6] observed that the toxin caused several effects on the *Mozambique tilapia*, including increased mucus secretion, increased opercular movement, and uncoordinated or irregular swimming. Leite *et al*., (2023) [10] reported that the short-term exposure to esfenvalerate affected the behaviour of the native species.

Conclusions

This study establishes a clear toxicity hierarchy among three botanical oil formulations in zebrafish, with neem oil posing the greatest hazard to non-target aquatic organisms. The integration of lethal concentration profiling, ventilatory monitoring, and diagnostic histopathology provides robust evidence that challenges the assumption of inherent safety for natural biopesticide products.

Declaration of AI Use

The author(s) acknowledge the use of Quillbot to assist with grammar checking.

Competing Interests

Authors have declared that no competing interests exist.

Acknowledgements

The authors acknowledge St. Xavier's College management providing the financial grant (SXCRG 25-2026/06) during this research work. The authors acknowledge DST-FIST (Ref. No. SR/FST/College-2017/95), Government of India, New Delhi, for providing an instrumentation facility to the Department of Zoology, St. Xavier's College, Palayamkottai.

Authors' Contributions

Conceptualization: RA. Data curation: RA. Formal analysis: GSM, RA. Investigation: RA, Methodology: GSM. Resources: RA. Supervision: RA. Visualization: RA, GSM. Project administration: RA. Software: GSM, TM. Validation: GSM. Writing – original draft: GSM. Writing – review and editing: GSM, RA.

References.

1. Amaral I, Antunes SC, Rebelo D, Carvalho AP, Rodrigues S. Biopesticide spinosad: Unraveling ecotoxicological effects on zebrafish, *Danio rerio.* Environmental Toxicology and Pharmacology, 2024:108:104458. https://doi.org/10.1016/j.etap.2024.104458
2. Bancroft JD, Stevens A. Theory and Practice of Histological Techniques. Churchill Livingstone, London. 1982: 2nd ed.
3. Bancroft JD, Stevens A, Turner DR. Theory and Practice of Histological Techniques. Churchill Livingstone, New York. 1996: (4th edition)
4. Chakraborty D, Ghosh S, Mandal AH, Roy T, Sadhu A, Paria DS, Faggio C. Sodium Dodecylbenzenesulfonate-induced oxidative stress and tissue damage in Tubifex tubifex: A comprehensive toxicological study. *Environmental Toxicology and Pharmacology,* 2025: 104778. https://doi.org/10.1016/j.etap.2025.104778
5. Chaudhary S, Kanwar RK, Sehgal A, Cahill DM, Barrow CJ, Sehgal R, Kanwar JR. Progress on *Azadirachta indica* based biopesticides in replacing synthetic toxic pesticides. *Frontiers in Plant Science*, 2017: 8:610. https://doi.org/10.3389/fpls.2017.00610
6. Chukwuka AV, Saha S, Mukherjee D, Banerjee P, Dhara K, Saha NC. Deltamethrin-induced respiratory and behavioral effects and adverse outcome pathways (AOP) in short-term exposed Mozambique tilapia, *Oreochromis mossambicus*. *Toxics*, 2022:10(11):701. https://doi.org/10.3390/toxics10110701
7. Islam S, Sohail M, Rohani MF, Hossain S, Islam SM. Pesticides-induced histopathological and biochemical alterations in fish: Impacts and restoration strategies. *Science of The Total Environment*, 2026:1045:181967. https://doi.org/10.1016/j.scitotenv.2026.181967
8. Ismail MF, Mohamed, H. M. (2012). Deltamethrin-induced genotoxicity and testicular injury in rats: comparison with biopesticide. *Food and Chemical Toxicology*, *50*(10), 3421-3425. http://dx.doi.org/10.1016/j.fct.2012.07.060
9. Konig, I., Iftikhar, N., Henry, E., English, C., Ivantsova, E., Souders II, C. L., and Martyniuk, C. J. (2023). Toxicity assessment of carvacrol and its acetylated derivative in early staged zebrafish (Danio rerio): Safer alternatives to fipronil-based pesticides?. *Comparative Biochemistry and Physiology Part C: Toxicology & Pharmacology*, *274*, 109762. https://doi.org/10.1016/j.cbpc.2023.109762
10. Leite, T., Mameri, D., Branco, P., Vieira, I., Oliveira, M., & Santos, J. M. (2023). Swimming under pressure: The sub-lethal effects of a pesticide on the behaviour of native and non-native Cypriniformes fish. *Fishes*, *8*(9), 462. https://doi.org/10.3390/fishes8090462
11. Mariano, W. S., Azevedo, S. B., Gomes, F. L., Lima, L. B., Moron, S. E., & Tavares-Dias, M. (2019). Physiological parameters of *Piaractus mesopotamicus*

(Osteichthyes: Characidae) exposed to a biopesticide based on *Bacillus thuringiensis*. *Anais da Academia Brasileira de Ciências*, *91*(02), e20180474. http://dx.doi.org/10.1590/0001-3765201920180474
12. Mariano, W. S., Oliveira-Lima, J., Santuci, M. A., Lima, L. B., Moron, S. E., & Tavares-Dias, M. (2021). Can a biopesticide based on *Bacillus thuringiensis* affect the physiology and histomorphology of Arapaima gigas?. *Anais da Academia Brasileira de Ciências*, *93*(suppl 4), e20201715. https://doi.org/10.1590/0001-376520212020171
13. Mohamed, I.A., Fathy, M., Farghal, A.I.A., Temerak, S.A.H., Abd El-Ghaffar, S.Kh., & Idriss, S.K.A. (2023). Behavioral and histopathological changes of *Clarias gariepinus* as a predatory fish against Culex pipiens larvae following exposure to sublethal concentration of quinclorac and bensulfuron-methyl based herbicide. *Hellenic Plant Protection Journal*, 16(2), 67–78.
14. Murussi, C. R., Menezes, C. C., Nunes, M. E., Araújo, M. D. C. S., Quadros, V. A., Rosemberg, D. B., & Loro, V. L. (2016). Azadirachtin, a neem-derived biopesticide, impairs behavioral and hematological parameters in carp (*Cyprinus carpio*). *Environmental toxicology*, *31*(11), 1381-1388. https://doi.org/10.1002/tox.22143
15. Nagini, S., Palrasu, M., & Bishayee, A. (2024). Limonoids from neem (*Azadirachta indica* A. Juss.) are potential anticancer drug candidates. *Medicinal Research Reviews*, 44(2), 683–729. https://doi.org/10.1002/med.21988
16. Overmyer, J. P., Rouse, D. R., Avants, J. K., Garrison, A. W., DeLorenzo, M. E., Chung, K. W., ... & Black, M. C. (2007). Toxicity of fipronil and its enantiomers to marine and freshwater non-targets. *Journal of Environmental Science and Health, Part B*, *42*(5), 471-480. https://doi.org/10.1080/03601230701391823
17. Pant, M., Dubey, S., & Patanjali, P.K. (2016). Recent advancements in bio-botanical pesticide formulation technology development. In: V. Veer & R. Gopalakrishnan (Eds.), Herbal Insecticides, Repellents and Biomedicine (pp. 117–126). Springer, New Delhi. https://doi.org/10.1007/978-81-322-2704-5_7
18. Raghav, D., Mahanty, S., & Rathinasamy, K. (2019). Biochemical and toxicological investigation of karanjin, a bio-pesticide isolated from Pongamia seed oil. *Pesticide Biochemistry and Physiology*, 157, 59–68. https://doi.org/10.1016/j.pestbp.2019.03.011
19. Ramesh, M., & Saravanan, M. (2008). Haematological and biochemical responses in a freshwater fish *Cyprinus carpio* exposed to chlorpyrifos. *International journal of integrative biology*, *3*(1), 80-83.
20. Roy Choudhury, R. (2016). Neem products. In: Omkar (Ed.), Ecofriendly Pest Management for Food Security (pp. 545–562). DOI: 10.1016/C2014-0-04228-1
21. Sacco, D., Velíšek, J., & Mikušková, N. (2025). Toxicological effects of azadirachtin on aquatic species: A review of its role in biopesticides. *Aquatic Toxicology*, 107547. https://doi.org/10.1016/j.aquatox.2025.107547
22. Soliman, A. M., & Abdelmawgood, I. A. (2026). Mechanisms of pesticide toxicity in freshwater fish: Insights into the emerging role of programmed cell death. *Pesticide Biochemistry and Physiology*, 107063. https://doi.org/10.1016/j.pestbp.2026.107063
23. Tanguay, R.L. (2018). The rise of zebrafish as a model for toxicology. *Toxicological Sciences*, 163(1), 3–4.
24. Wang, K., Song, T., Ke, L., Sun, Y., & Ye, J. (2024). Dietary high levels of coconut oil replacing fish oil did not affect growth, but promoted liver lipid deposition of orange-spotted groupers (*Epiphelus coioides*). *Animals*, 14(11), 1689. https://doi.org/10.3390/ani14111534
25. Yadav V, Ahmad S, Zahra K. Assessment of the protective effects of Moringa oleifera leaf extract against neem-oil induced toxicity in zebra fish, *Danio rerio*. Journal of Pharmacognosy and Phytochemistry, 2019:8(3):4263–4270.
26. Azhagu Raj R., Saravanakarthick. P., Palavesam A., Prakasam A. and Balachandar M.: Effect of solar radiation on freshwater fish *Puntius bimaculatus* (Bleeker) (Cypriniformes: Cyprinidae). *Intern. J. Zool. Invest.* 8(2): 218-222, 2022. https://doi.org/10.33745/ijzi.2022.v08i02.028.
27. Azhagu Raj R & Hemalatha K & Sudhakani B & Ari Karan M & Jeya Murugan E & Balachandar M, 2023. "Impact of Household Chemicals on the Ornamental Fish *Poecilia sphenops* (Valenciennes)," International Journal of Scientific Research in Science, Engineering and Technology. 2023, 10(2), 35-40.
28. Azhagu Raj, Krishna Kumar, Palavesam Arunachalam, Muthupandi Sankar, Prathap Selvaraj, and Sohan Jheeta. "Deleterious Effect of Ultraviolet Radiation on *Glossogobius giuris:* A Short Experimental Study" *Sci.* 2022 4, no. 1: 12. https://doi.org/10.3390/sci4010012.