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Research Article

Biochemical Effects of *Euphorbia tirucalli* Latex Powder on *Oreochromis mossambicus* using Fourier Transform Infrared Spectroscopy

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ABSTRACT

Introduction: Aquatic ecosystems face increasing threats from natural and anthropogenic contaminants, including plant-based toxins such as *Euphorbia tirucalli* (*E. tirucalli*) latex, which pose specific risks to marine organisms. The present study aimed to evaluate the biochemical effects of *E. tirucalli* latex on the gills, liver, and kidneys of *Oreochromis mossambicus* (*O. mossambicus*) using Fourier transform infrared (FTIR) spectroscopy over a 28-day exposure period.

Materials and methods: A total of 54 *O. mossambicus* fish were collected from the Krishnagiri Reservoir, Tamil Nadu, India. The fish were divided into two groups, including the control group (Group A), maintained in clean water without latex exposure, and the second group exposed to lyophilized *E. tirucalli* latex at a concentration of 0.315 g/L for 28 days under continuous aeration (Group B). The latex of *E. tirucalli* was lyophilized and administered through water exposure following a 10-day acclimation period. The latex-induced biochemical alterations in gill, liver, and kidney tissues were assessed by FTIR spectral shifts in protein, lipid, and carbohydrate bands.

Results: The FTIR analysis revealed distinct, organ-specific biochemical alterations in response to latex exposure. The liver analysis in Group B exhibited a pronounced C=O ester stretch at 1745 cm^{-1}, indicating lipid peroxidation and oxidative stress, whereas the kidney indicated notable sugar and phosphate absorption bands (1084-1030 cm^{-1}) and a unique peak at 875 cm^{-1}, suggesting metabolic disturbance. Gill tissues in Group B displayed relatively moderate biochemical responses. Protein content analysis across different tissues in both experimental groups revealed significant variations, confirming that *E. tirucalli* latex disrupted protein metabolism.

Conclusion: The present study demonstrated the effectiveness of FTIR spectroscopy in detecting organ-specific biochemical changes, highlighting the toxic potential of *E. tirucalli* latex as an environmental hazard in aquatic ecosystems.

1. Introduction

Environmental contamination from plant-derived toxins has become a growing concern in aquatic ecosystems, particularly in areas such as Nedusalai Village, Tamil Nadu, India, where traditional medicinal plants, such as *Euphorbia tirucalli* (*E. tirucalli*), are extensively used and often discarded[1]. *Euphorbia tirucalli*, commonly known as the pencil tree, is a juicy, fleshy plant in the Euphorbiaceae family with well-documented medicinal and toxic properties[2]. *Euphorbia tirucalli* is widely recognized for its therapeutic applications, with its latex traditionally used as a carminative and laxative, and in the treatment of conditions such as leprosy, asthma, jaundice, and tumors[3]. The latex contains diterpenes and other bioactive compounds, such as phorbol esters, ingenol derivatives, and tigliane-type compounds, along with triterpenoids, including euphol and tirucallo, that exhibit cytotoxic, irritant, and genotoxic effect[4]. Yet, the potential consequences of *E. tirucalli* latex exposure on aquatic

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organisms, particularly fish, remain insufficiently explored.

Aquatic ecosystems may be contaminated by synthetic pesticides, which are often used in aquaculture operations to maintain water quality and control pests. The persistent application of synthetic and natural pesticides in aquatic environments may adversely impact organisms that are not the intended targets[5]. Aquatic ecosystems are particularly susceptible to contamination, as pollutants such as heavy metals, pesticides, hydrocarbons, and nutrient-rich fertilizers from industrial, urban, and agricultural activities are frequently released into freshwater and marine systems[5,6].

Fourier transform infrared (FTIR) spectroscopy is an advanced analytical tool that provides insight into the molecular makeup of biological tissues. The FTIR detects the unique vibrational patterns of chemical bonds, allowing scientists to identify and distinguish among different biomolecules with high precision[7]. The toxic effects of synthetic pesticides on the gill tissue of *Oreochromis mossambicus* (*O. mossambicus*), as detected by FTIR, reveal changes in lipids, proteins, carbohydrates, and biomolecules after exposure[8]. The FTIR spectroscopy can identify protein conformational shifts in gills under heavy metal stress, lipid peroxidation in membranes exposed to pesticides, DNA damage from pollutant exposure, and carbohydrate composition changes in toxin-affected tissues[9].

The use of chemical pesticides in aquatic environments alters the habitat, causing fish to become confused and disturbed. The *O. mossambicus*, commonly known as Mozambique tilapia, is a widely studied freshwater species due to its ecological and economic relevance to Africa[10]. As a filter-feeding fish, it is especially susceptible to waterborne toxins, making it a suitable model for aquatic toxicology studies[11]. Freshwater species such as *Channa punctatus* are susceptible to the piscicidal effects of *E. tirucalli* latex and stem-bark extracts[12,13]. However, there is limited information on the precise mode of action of *E. tirucalli*, particularly its effects on fish metabolism. Fish are important bioindicators in aquatic environments because of their sensitivity to contaminants and environmental changes[14].

Evaluating the toxicological effects of *tirucalli* latex on aquatic organisms has gained increasing attention due to its bioactive properties and expanding applications[14]. Protein and lipid structural changes have been extensively analyzed using FTIR spectroscopy[15,16]. Therefore, the present study aimed to assess the biochemical alterations induced by *E. tirucalli* latex powder in the gills, liver, and kidneys of *O. mossambicus* after a 28-day exposure period, using FTIR spectroscopy as a non-destructive method to detect molecular-level changes in these tissues.

2. Materials and Methods

2.1. Ethical approval

Tissue sample collection from the fish in the present study was conducted in accordance with the National Animal Health Monitoring System (NAHMS) guidelines[17].

2.2. Study location

A total of 54 *O. mossambicus* were randomly collected using cast nets with minimal handling stress from a freshwater source at the Krishnagiri Reservoir Project (KRP) Dam, Krishnagiri, Tamil Nadu, India. The *O. mossambicus* measured 13-15 cm in length, with an average body weight of 22 ± 3.0 g. Fish were acclimated for 10 days in 20 L aerated containers filled with clean tap water at 23.0-26.5°C, pH 7.67, and a dissolved oxygen level of 7.41 mg/L. The experimental fish were fed a formulated diet daily at 5% of their body weight, consisting of commercial Raisa-brand feed (Black Market, India) and groundnut oil cake in a 3:1 ratio[18]. The proximate chemical composition of the diet was approximately crude protein (28-32%), crude lipid (6-8%), carbohydrate (nitrogen-free extract; 35-40%), crude fiber (6-8%), ash (8-10%), and moisture (8-10%). Water was renewed every 48 hours[19]. The latex of *E. tirucalli* was collected from Nedusalai Village, Krishnagiri Taluk, Tamil Nadu, India. Fresh stems and branches were aseptically incised using a sterile, sharp knife, and the exuded white, milky latex was carefully collected into clean glass tubes. The latex was lyophilized at -40°C, yielding a dry weight of 0.315 g and a wet weight of 1.37 g/mL, which was stored under refrigerated conditions for further experimental use[16]. For *E. tirucalli* latex, 96-hour LC_{50} values for freshwater fish vary from 1.2 to 1.8 g/L, depending on species, whether the latex is fresh or lyophilized, and the exposure conditions. In tilapia and other teleost models, LC_{50} values around 1.5 g/L have been reported for aqueous latex extracts[20].

After acclimation, two groups were established, each with 27 fish. The first group was the control group maintained in clean water (Group A), and the second group was exposed to lyophilized *E. tirucalli* latex (0.315 g/L)[21] for 28 days under continuous aeration (Group B). This grouping enabled detection of dose-dependent biochemical changes in the gill, liver, and kidney over 28 days, as reflected in alterations in FTIR spectral bands, including peak intensities and positions corresponding to proteins, lipids, and carbohydrates.

2.3. Sample preparation

The gills were exposed by lifting the operculum and then carefully removed using sterile scissors. The gills, liver, and kidney from each fish (six per group) were dissected under aseptic conditions. All tissues were rinsed with physiological saline (0.9% NaCl) to remove blood residues, blotted dry, and transferred to labeled Petri dishes for further processing. In addition, the samples were lyophilized for 12 hours at 23-26°C to ensure complete moisture[22]. The dried tissues were then ground into a fine, uniform powder using an agate mortar and pestle. Pre-dried potassium bromide (KBr; Techno Search Instruments, India) was thoroughly mixed with powdered tissue samples (as pellets) at a 1:100 ratio. Transparent pellets were subsequently prepared by applying 3000 psi of pressure for five minutes using a vacuum die (Techno Search Instruments Company, India). Each pellet measured

approximately 1 mm in thickness and 13 mm in diameter, making it suitable for infrared spectroscopic analysis[21].

2.4. Fourier transform infrared spectroscopy

The FTIR spectroscopy was performed using a Thermo Scientific FTIR spectrophotometer in India. Sample pellets were scanned in the mid-infrared region (4000-400 cm^{-1}) at a resolution of 4 cm^{-1}, with air serving as the background for spectral calibration. All measurements were carried out at room temperature (22°C)[22].

2.5. Data analysis

Following the 28-day exposure period, FTIR spectra were obtained from the gill, liver, and kidney tissues of Group A and Group B. For each tissue type, characteristic absorption bands were identified based on standard functional group assignments. The frequency of each spectral band was calculated as the percentage of samples exhibiting that band in each experimental group (control versus treated). The FTIR spectra were analyzed by comparing peak positions, intensities, and functional group assignments between the control and treatment groups.

3. Results

The FTIR spectral profiles of *O. mossambicus* from Group A and Group B, treatment tissues exposed to *E. tirucalli* latex, revealed distinct, organ-specific biochemical alterations. Characteristic absorption bands were observed across the mid-infrared region (4000-400 cm^{-1}), corresponding to major biomolecular constituents including proteins, lipids, carbohydrates, and phosphate in all tissue samples. The gill spectra exhibited moderate protein and lipid-related absorption bands in Group B (Figure 1), with relatively fewer alterations compared to the liver (Figures 2) and kidney (Figure 3) tissues in Group A (Table 1). The amide A (3397 cm^{-1}) and amide B (3100 cm^{-1}) bands, attributed to N-H stretching vibrations of proteins, demonstrated slight intensity shifts in Group B, indicating mild stress responses in Group A (Table 1). Peaks in the region of 2920-2850 cm^{-1}, corresponding to C-H stretching vibrations of lipids, were present but not markedly altered in Group B gill tissues (Figure 1; Table 1).

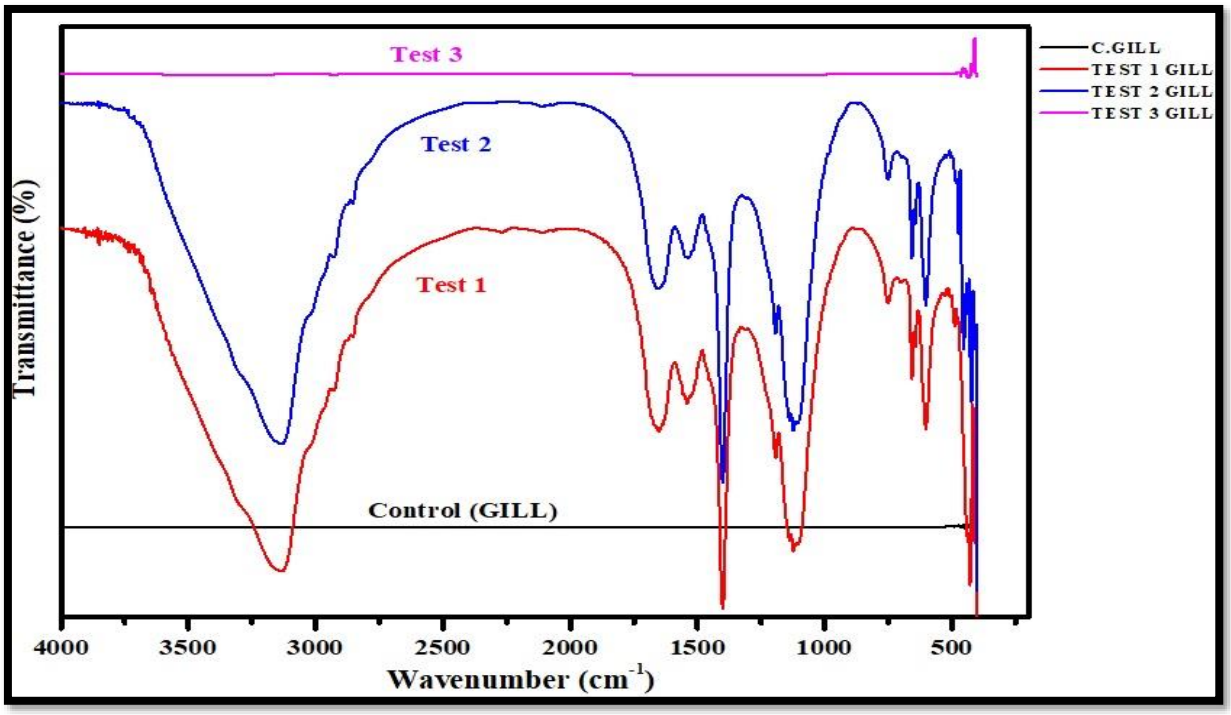

Figure 1. Fourier transform infrared spectrum of gill tissue in *Oreochromis mossambicus* exposed to *Euphorbia tirucalli* latex over a 28-day period. Spectral range: 4000-400 cm^{-1}. Control (GILL): Unexposed baseline spectrum (black line), Test 1: Gill tissue exposed to the lowest concentration or shortest exposure period (red line), Test 2: Gill tissue exposed to a moderate concentration or intermediate exposure period (blue line), Test 3: Gill tissue exposed to the highest concentration or longest exposure period (pink line).

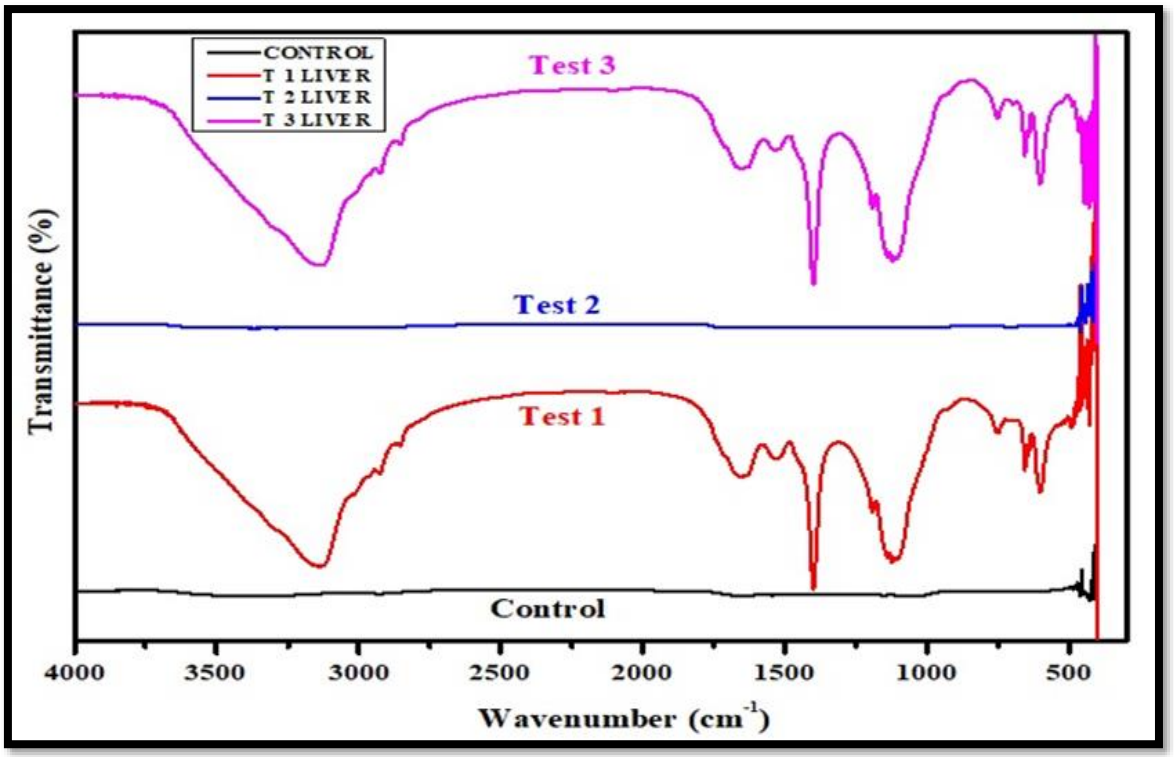

Figure 2. Fourier transform infrared spectrum of liver tissue in *Oreochromis mossambicus* exposed to *Euphorbia tirucalli* latex over a 28-day period. Spectral range: 4000-400 cm^{-1}. Control: Unexposed liver tissue (baseline spectrum; black line), Test 1: Liver tissue exposed to the lowest concentration or shortest exposure of *E. tirucalli* latex (red line), Test 2: Liver tissue exposed to a moderate concentration or intermediate exposure (blue line), Test 3: Liver tissue exposed to the highest concentration or longest exposure (pink line).

Table 1. Fourier transform infrared spectral bands and functional group identification of gill, liver, and kidney tissues of *Oreochromis mossambicus* after a 28-day exposure to *Euphorbia tirucalli* latex powder

Wave number (cm^{-1})	Functional group / Band assignment	Tissue
3397	N-H stretching (Amide A and B); proteins	Liver
3143	N-H stretching (proteins), minor O-H from polysaccharides and hydrogen bonding	(-)
2924	CH_2 asymmetric; stretch is mainly lipids, with a little contribution from proteins, carbohydrates, and nucleic acids	Liver, Kidney
2851	CH_3 bending Lipids with slight protein contribution	Gill, Liver, Kidney
1656	C=O stretching (Amide I); α-helix proteins	Gill
1542	N-H bending and C-N stretching (Amide II); proteins	Gill, Liver
1400	CH_2 bending; primarily lipids	Gill
1234	PO_2^- asymmetric stretching; nucleic acids	Liver
1153	PO_2^- asymmetric stretching; phospholipids, nucleic acids	Liver
1078	C-O asymmetric stretching; glycogen and nucleic acids	Gill
1028	C-O-C asymmetric stretching; glycogen and nucleic acids	Gill
463	PO_2^- symmetric stretching; phospholipids and phosphodiesters	Liver
449	DNA base ring breathing vibrations	Liver
417	O-H bending vibration	Liver

(–): Indicates the absence of a detectable FTIR absorption band at the corresponding wavenumber in the analyzed tissue sample, -CH_2 asymmetric stretch: This is the asymmetric stretching vibration of methylene groups (-CH_2-), usually observed around 2920-2935 cm^{-1} in the FTIR spectrum, -CH_3 bending: Refers to the bending (deformation/scissoring) vibration of methyl groups (-CH_3), -PO_2^- asymmetric stretching: Refers to the asymmetric stretching vibration of the phosphate group (-PO_2^-) in the sugar-phosphate backbone of nucleic acids.

In contrast, the liver spectra in Group B displayed pronounced alterations in the ester and amide regions, reflecting substantial impacts on lipid and protein structures. Strong absorption bands were observed at 1740 cm^{-1} (C=O stretching of esters), 1650 cm^{-1} (amide I; C=O stretching of proteins), and 1540 cm^{-1} (amide II; N-H bending and C-N stretching), with higher intensities in Group B compared to Group A (Table 1; Figure 2 [Tests 2 and 3]). Additionally, bands in the 2920-2850 cm^{-1} region were more intense in Group B liver tissues than in Group A, indicating enhanced lipid degradation following latex exposure (Table 2). The kidney spectra of Group B exhibited distinct carbohydrate and phosphate-associated peaks, particularly in the 10801030 cm^{-1} region, corresponding to C-O and PO_4^- stretching vibrations (Table 1; Figure 3 [Tests 1 and 2]). These bands were more pronounced in kidney tissue than in gill and liver, suggesting kidney-specific biochemical responses to *E. tirucalli* latex exposure (Table 2).

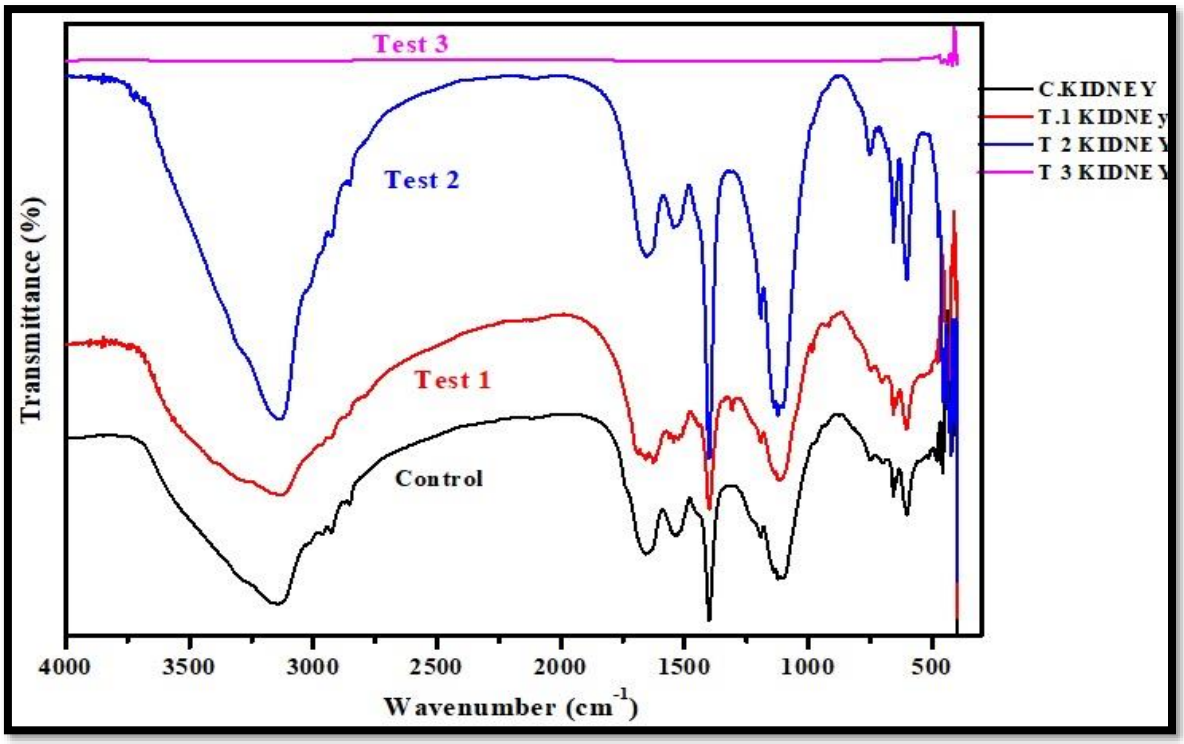

Figure 3. Fourier transform infrared spectrum of kidney tissue in *Oreochromis mossambicus* with *Euphorbia tirucalli* latex over a 28-day period. Spectral range: 4000-400 cm^{-1}. Control (black line): Represents the baseline or untreated kidney tissue of *O. mossambicus*, Test 1: Represents kidney tissue exposed to *E. tirucalli* latex under specific conditions for Test 1 (red line), Test 2: Represents kidney tissue exposed to *E. tirucalli* latex under different conditions for Test 2 (blue line), Test 3: Represents kidney tissue exposed to *E. tirucalli* latex under different conditions for Test 3 (pink line).

Table 2. Organ-specific observations and toxicological insights in *Oreochromis mossambicus* tissues exposed to *Euphorbia tirucalli* latex

Tissue	Major observations	Toxicological insight
Gill	Moderate protein/lipid bands, fewer distinct alterations	Possibly less affected; external tissue may buffer latex toxicity.
Liver	Strong ester and amide signals, significant lipid/protein change	Primary site of detoxification; highly vulnerable to latex-induced oxidative stress.
Kidney	High carbohydrate/phosphate signals, kidney-specific banding	Likely involved in the excretion of latex compounds; altered biochemical processing.

Compared with Group A, gill, liver, and kidney tissues of the treated fish showed consistent alterations in proteins, lipids, and carbohydrates, whereas changes in nucleic acids and oxidative stress markers were more pronounced in the liver and kidney of Group B, indicating metabolic and oxidative stress. Notably, the kidney tissue (Figure 3) exhibited strong absorption in the 1084-1030 cm^{-1} region corresponding to C-O and PO_4^- groups, along with a distinct peak at 875 cm^{-1}, suggesting organ-specific responses to latex exposure.

4. Discussion

Environmental management of *E. tirucalli* latex exposure is critical for protecting aquatic organisms[15]. Preventing direct discharge into water bodies, ensuring proper disposal of plant residues, maintaining adequate water renewal and aeration, and establishing buffer zones around aquatic habitats are effective measures to minimize ecological risks and safeguard fish and marine life[12,13]. The differential expression of functional groups across the gill, liver, and kidney samples in the control and treatment groups underscored the organ-dependent toxicodynamic processes initiated by the latex. Protein content analysis across different tissues in both groups revealed notable variations, confirming that exposure to *E. tirucalli* latex disrupts protein metabolism. Comparable findings were reported by Palaniappan et al.[16], who examined arsenic-induced molecular alterations in kidney tissues of the edible fish *Labeo rohita* using FTIR spectroscopy. The similarity in biochemical alterations observed in the present study suggested comparable patterns of molecular disruption. The liver in the control group exhibited the most pronounced alterations, notably a strong C=O stretching peak (ester carbonyl), an established marker of lipid peroxidation. Similar observations were reported by Greco et al.[23], who indicated a notable consistency in the identification of peaks corresponding to similar regions including the lipid-associated carbonyl band, the CH_2/CH_3 stretching region, and the protein amide I and II bands, of the FTIR spectra, despite employing different analytical approaches. The enhanced amide bands further supported increased protein turnover or structural damage, consistent with the liver's role as the principal detoxification organ[23].

In the present study, the polysaccharide exhibited characteristic infrared absorption, including a prominent

peak near 1080 cm^{-1} attributed to C-O and P-O stretching vibrations and C-O-H bending. These findings align with those reported by Mondon et al al.[24], who similarly observed notable growth inhibition and liver necrosis in greenback flounder exposed to contaminated sediment and diet.

In comparison to the liver and kidney, the gills of experimental fish exposed to lyophilized *E. tirucalli* latex exhibited relatively mild spectral alterations. Nonetheless, variations in protein and carbohydrate bands indicated stress responses induced by exposure to *E. tirucalli* latex, similar to the findings of Fanta et al.[25]. As the main organ directly interacting with the environment, the gill tissue may initially act as a barrier to toxins but can still experience sublethal biochemical disruptions[25]. Exposure to *E. tirucalli* latex caused a marked decrease in the intensity of these amide bands, particularly under combined treatment conditions. This decline suggested that protein structures, especially those with α-helical structures, underwent remarkable conformational alterations or degradation in the exposed tissues (gills, liver, and kidneys) of experimental fish.

The Amide I region, between 1700 and 1600 cm^{-1} in liver spectra, was notably sensitive to changes in protein folding. A clear peak at 1659 cm^{-1} was typically attributed to α-helical structures, primarily arising from C=O stretching vibrations in peptide bonds, with minor contributions from C-N stretching and N-H bending, supporting the findings of Tiwari and Singh[13]. Additionally, measuring total protein content provided crucial insights into how stressed fish allocated energy and behaved physiologically. A decline in protein levels across organs may reflect depletion of readily available energy reserves resulting from prolonged exposure to pollutants, such as *E. tirucalli* latex, potentially impairing vital functions, including immune response, growth, and reproduction, as mentioned by Tiwari and Singh[13]. Notably, changes in protein absorption bands and overall protein content in vital organs, including muscle, liver, and gills, observed in the present study, provided strong evidence of physiological responses to environmental disruptions. These tissues served as reliable bioindicators of water quality and exposure to pollution because they are highly sensitive to external stimuli. Furthermore, FTIR spectroscopy was a useful, non-invasive method for biochemical monitoring, as demonstrated in the present study.

5. Conclusion

The FTIR spectral results indicated biochemical and structural alterations in the gill, liver, and kidney tissues of *O. mossambicus* following exposure to *E. tirucalli* latex powder. The present findings supported the potential toxicological effects of *E. tirucalli* latex on *O. mossambicus* and affirmed the importance of monitoring environmental releases. Further studies are recommended to investigate the molecular mechanisms of *E. tirucalli* latex and to offer more detailed insights into its toxic effects and side effects.

Declarations

Ethical considerations

The authors affirmed that it is an original study, written by all authors equally, and submitted to the journal for the first time. Ethical issues, including plagiarism, consent to publish, misconduct, data fabrication and/or falsification, and redundancy, have been checked by all the authors. The authors confirm that they have not used AI to write and prepare the present study.

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Availability of data and materials

The present study contains the original data used in this investigation, which may be obtained from the corresponding author upon reasonable request.

Authors' contributions

Data analysis was conducted by Manivelu Deivansigamani, Hassan Sulieman, and Vaiyapuri Kanmani, who also assisted with the study design and scheduling of experiments. Furthermore, samples were gathered from different areas by Moorthi Kanagaraj and Vaiyapuri Kanman. After reviewing the data analysis, all authors have read and approved the final edition of the manuscript.

Competing interests

There are no conflicts of interest in this study.

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