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LONG-TERM EXPOSURE TOEMAMECTIN BENZOATE INDUCES ALTERATIONS IN TRANSAMINASE ACTIVITY IN ZEBRAFISH (*DANIO RERIO*)

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ABSTRACT

Emamectin benzoate (EMB), a macrocyclic lactone insecticide, is extensively used to control insect pests in agriculture and management of parasites in aquaculture farms. Owing to its widespread application, EMB has been detected in soil and water samples throughout the world and raised environmental pollution concerns. Due to itshigh efficiency, persistence, residue and bioaccumulation properties, EMB has caused adverse effects on aquatic organisms. Thus, in the current study, the toxicity of EMB on marker enzymes of zebrafish (*Danio rerio*) was assessed. Zebrafish were exposed to environmentally relevant concentrations of EMB (0.05, 0.1 and 1.0 mg/L) for a period of 35 days and the changes in the aspartate aminotransferase (AST) and alanine aminotransferase (ALT) activity were investigated in the liver and muscle of zebrafish. Results revealed a significant increase in AST and ALT activity in the liver and muscle of EMB exposed fish compared to control group. The alterations of enzymatic activity were found to be higher in liver compared to muscle. Throughout the study period, a dose-dependent elevation of enzyme (AST and ALT) activities was observed. The alterations of transaminases activity can be used as biomarkers for the monitoring of bio pesticides in the aquatic environment.

INTRODUCTION

Pesticides are commonly used in agriculture to protect the crops from insects, in aquaculture farm to control parasites and to control the vector diseases in human health(Veedu *et al.,* 2022; Rohani, 2023; Giang *et al.,* 2025).However, excessive use of different types of pesticides such as organochlorine, organophosphorus, pyrethroids, biopesticides etc.,disturb the function of nervous system, reproductive system, endocrine system etc., (Mostafalou and Abdollahi, 2012; Li *et al.,* 2025). Likewise, inappropriate usage of these pesticides may enter the aquatic environment and affect the non-target organisms such as fish (Ramesh *et al.,* 2023; Ramesh *et al.,* 2024; Khoshnood, 2024; Ramage *et al.,* 2025).Ullah *et al.*(2021) has reported thatpesticide residue in water bodies and their accumulation in aquatic organisms may pose a risk to biodiversity of aquatic ecosystem.

Avermectins (AVMs) are naturally synthetic compounds, produced from actinomycete *Streptomyces avermitilis*are extensively used as an insecticides to control insect pests particularly arthropods and nematodes (Zhang *et al.,* 2022; Pan *et al.,* 2023; Du *et al.,* 2023). In avermectin group, emamectin benzoate (EMB; 4″R)-4″-deoxy-4″-(methylamino)-avermectin B1 benzoate) is widely used as insecticides to control various pests in agriculture sector particularly lepidopteron pests (Mushtaq *et al.,* 1996; Abou-Zeid*et al.,* 2018; Giang *et al.,* 2025). EMB, a neurotoxic insecticide, act on the γ-aminobutyric acid (GABA) system of insects, activate chloride channel, disturb nerve conduction resulting paralysis and death of insects (Wolstenholme and Rogers, 2005; Gu *et al.,* 2023). Due to high efficiency and

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prolonged residual activity, EMB is extensively utilized for the control of many pests both in agriculture sector, aquaculture farms and forestry (Fırat and Tutus, 2020; Xu *et al.,* 2021; Gu *et al.,* 2023). The entry of EMB from agricultural runoff, waste feed from aquaculture farm and accumulation in the various environmental matrixes may pose a risk to human health and aquatic organisms (Bloodworth *et al.,*2019; Temiz, 2020; Gu *et al.,* 2023). Furthermore, the slow degradation of EMB may result in environmental persistence(Hamoutene *et al.,* 2023).EMB altered the normal physiological and biochemical systems of aquatic organisms such as fish (Julinta *et al.,* 2020; Lu *et al.,* 2022; Gu *et al.,* 2025; Guo *et al.,* 2025). However, studies on long-term effects of EMB on aquatic organisms are very limited.

Fish serve as valuable bioindicators for assessing the toxicological impacts and safety concerns of environmental pollutants.To evaluate the toxicity caused by xenobiotics in fish, biochemical parameter monitoring is most frequently used in aquatic eco-toxicological investigations (Kumar *et al.,* 2022; Nayak *et al.,* 2023; Mohanthi *et al.,* 2025). Biochemical stress indices are considered as potential biomarkers and are utilized as diagnostic tools to measure the effects of aquatic pollutants (Veedu *et al.,* 2022; Hossinian *et al.,* 2025). Furthermore, to evaluate the structural and functional changes of fish tissues under different stress scenarios, toxicity studies commonly use enzyme activities (Umamaheswari *et al.,* 2019; Ramesh *et al.,* 2023).

Enzymes such as aspartate aminotransferase (AST) and alanine aminotransferase (ALT)play a vital role in carbohydrate and protein metabolism during stress condition. In the field of aquatic toxicology, alterations of these enzyme activities are commonly used to assess the health condition and organ/tissue damage of aquatic organisms such as fish (Firat *et al.,* 2011; Umamaheswari *et al.,* 2019; Shahzadi *et al.,* 2024). BothALT and AST activities are commonly employed as liver specific enzymes and hepatotoxicity and histopathological changes can be easily measured within a short time (Bojarski *et al.,* 2025). Changes in the AST and ALT activity in various organ /tissues such as liver and muscle serve as vital biomarkers for assessing pesticide impacts on fish (Ramesh *et al.,* 2024).

Studies on emamectin benzoate toxicity on aquatic organisms are still scarce, particularly in fish. In addition, most of the previous studies have reported the EMB toxicity on aquatic organisms at acute or short-term exposure;studies on chronic or long-term exposure are scanty (Guo *et al.,* 2025). Hence, in the present investigation, the toxicity of emamectin benzoate on transaminase activity in liver and muscle of zebrafish was evaluated. Zebrafish is widely used to assess the impacts of pollutants on the environment and human due to its highly genetically homologous to humans, high reproductive capacity, high egg production, spawning in all seasons and sensitivity to environmental pollutants (Patton *et al.,* 2021; Mohanthi *et al.,* 2025). In this study, we assessedlong-term exposure (35 days) of EMB on AST and ALT activities on liver and muscle of a freshwater fish zebrafish *Danio rerio* at environmentally relevant concentrations (0.05 mg/L, 0.1 mg/L, and 1.0 mg/L). The findings of the present study may be used to monitor these pesticides in the aquatic environment.

MATERIALS AND METHODS

Test compound

Emamectin Benzoate, a formulation developed by Rilon, a subsidiary of Tata Company India Limited, was obtained from Shakti Agro Service Ltd., Coimbatore, India. AST-ALT – Enzyme kit was purchased from India Mart, manufactured by Proton Biological India Pvt Ltd, Bangalore, India.

Stock preparation

A stock solution was prepared by dissolving 1 g of emamectin benzoate in 1000 mL of distilled water, and a fresh solution was prepared for each experimental trial. Based on the desired exposure concentrations, the appropriate volumes of the stock solution were accurately added to the test tanks containing dechlorinated tap water.

Experimental animal collection and maintenance

Adult wild-type zebrafish (*Danio rerio*) were selected for the present study. Specimens were obtained from Siraco Fish Farm and acclimated for 15 days in glass aquaria containing dechlorinated tap water at the Toxicology Laboratory, Department of Zoology, Bharathiar University, Coimbatore, India. During the acclimation period, the fish were maintained under controlled laboratory conditions with a 12:12 h light-dark photoperiod. Fish were fed with commercial pellets at *ad libitum*. In the present study, physic-chemical parameters of the water such as temperature, 27.3 ± 1.2 °C; dissolved oxygen, 6.5 ± 0.4 mg L^{1}; pH, 7.1 ± 0.24; and total hardness, 18.4 ± 2.1 mg L^{1} was monitored daily and maintained at the constant level throughout the study period. Daily water renewal was carried out to ensure the removal of uneaten feed and metabolic waste. Following the acclimation period, zebrafish were maintained as stock for subsequent experimental procedures.

Experimental design for long-term toxicity in adult zebrafish

For long-term exposure study, zebrafish were randomly assigned to four main groups, each comprising 125 individuals. Group 1 was maintained in emamectin benzoate (EMB)-free freshwater and served as the control. The water pH was maintained at 7.2. Groups 2, 3, and 4 were exposed to 0.05, 0.1, and 1 mg/L of EMB, respectively. The EMB was added following the removal of an equivalent volume of water. Feeding was discontinued 24 hours prior to the initiation of the experiment, which lasted for 35 days. Each group, including the control, was maintained in triplicate. Test media and treatment solutions were renewed every 24 hours throughout the experimental period. In the present study, the fish handling and experimental procedures were followed as per guidelines for the care and use of laboratory animals established by the organization for economic co-operation and development (OECD 36). Sampling was conducted at 7-day intervals (i.e., on days 7, 14, 21, 28, and 35) over the 35-day exposure period. No mortality was observed during the experimental duration. At each time point, tissue samples (liverand muscle) from both control and EMB-exposed fish were removed and processed for AST and ALT assay.

Sample preparation for AST and ALT assay

Liver, and muscle were excised from control and EMB treated groups and stored at ice cold condition. Then, 100 mg of liver, and muscle tissuesamples from control and EMB treated groupswere collected, subjected to homogenization with phosphate buffer saline (PBS). Then, the homogenates were centrifuged for 15 min at 5,000 rpm at 4 ^{0}C and the supernatant wascollected for enzymological (AST and ALT) assay.

Metabolising enzymes

AST and ALT activities were estimated in accordance with Reitman and Frankel (1957)method,using a commercial diagnostic kit,and in accordance with the manufacturer's instructions. Briefly, a reaction mixture consisting of 50 µL of supernatant, 400 µL of reagent R1, and 100 µL of reagent R2 was prepared, mixed thoroughly, and incubated for 10 seconds. The change in optical density (OD) was measured every 60 seconds over a 180-seconds period at 340 nm using a BioDrop spectrophotometer, with distilled water serving as the blank.For ALT activity, the same procedure was followed. The supernatant (50 µL) was combined with 400 µL of reagent R1 and 100 µL of reagent R2, mixed well, and incubated for 10 seconds. The change in OD was recorded every 60 seconds over a 240-seconds period at 340 nm, using distilled water as the blank.

Statistical analysis

The significance between control and emamectin benzoate (EMB) exposed groupswere calculated using one wayanalysis of variance (ANOVA) and Dunnett's post hoc tests. IBM SPSS software for Windows, version 16.0 was used for statistical analysis. Results are expressed as mean ± SE.The significance was kept at p < 0.001.

RESULTS

In the present study, zebrafish were exposed to different concentrations (0.05 mg/L, 0.1 mg/L, and 1.0 mg/L) of emamectin benzoate (EMB) for a period of 35 days and alterations of AST and ALT activities in the liver and muscle tissues were studied. During the study period the activity of both AST and ALT in the liver and muscle tissues was significantly elevated.

AST activity in liver

The AST activity in liver tissue of EMB treated fish was found to be increased significantly (*P*< 0.001) when compared to the control group (Fig.1).The AST activity was gradually increased as the exposure period extended, resulting in a maximum percent increase of 44. 21, 64. 36and 68.01at the end of the 35th day at 0.05 mg/L, 0.1 mg/L, and 1.0 mg/L concentrations, respectively. Throughout the study period, a concentration-dependent enzyme activity wasnoticed in both the tissues.

AST activity in muscle

The AST activity in muscle tissue was zebrafish was decreased significantly (*P* < 0.001) after exposure to different concentrations of EMB compared to control group (Fig. 2). The significant increase in AST activity was found to be increased unto 28th day and then slightly recovered. The decrease in AST activity in muscle was found to be concentration dependent.

ALT activity in liver

The ALT activity in liver tissue was increased significantly (*P*< 0.001) in EMB treated groups in all the concentrations (Fig. 3).The elevation of ALT activity in liver tissue was directly proportional to the exposure period showing a per cent increase of 45.96, 78.50 and 86.98 at the end of 35th day in 0.05, 0.1 and 1 mg/L of EMB treated groups, respectively.

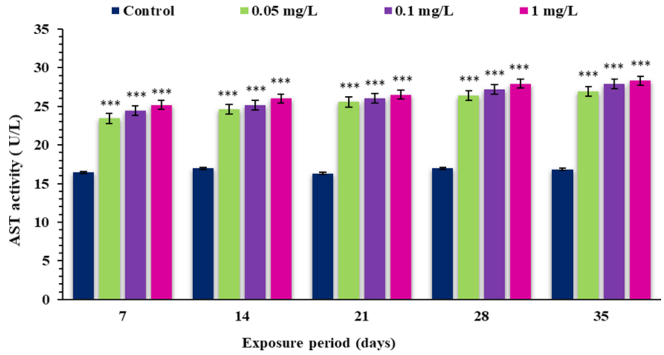

Fig. 1. AST activity in the liver of *Danio rerio* after exposure to different concentrations of EMB (0.05, 0.1 and 1 mg/L) for 35 days. All values are mean ± SE.***indicate statistical differences at the 0.001 significance level.

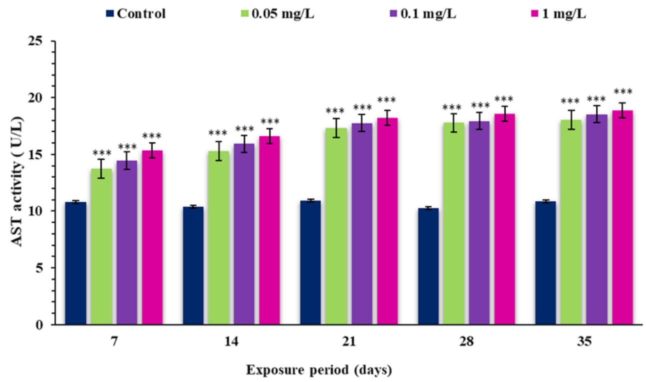

Fig. 2. AST activity in the muscle of adult zebrafish exposed to emamectin benzoate (0.05, 0.1 and 1 mg/L) for 35 days. Data were presented as mean ± SE. Statistically significant (*P*< 0.001) differences between control and EMB treated groups are indicated with an *** mark.

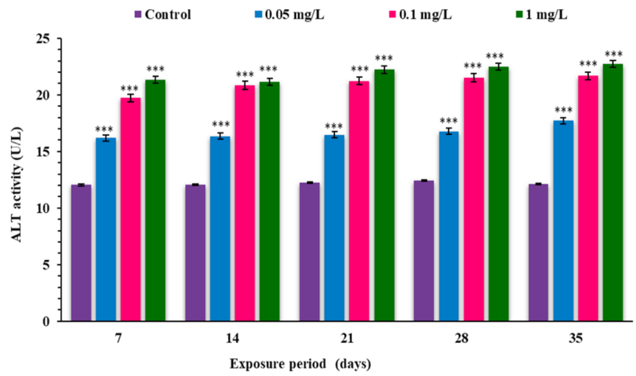

Fig. 3. ALT activity in the liver of adult zebrafish exposed for 35 days to different concentrations (0.05, 0.1 and 1 mg/L) of emamectin benzoate. Data are reported as mean ± SE. ***significant at *P* < 0.001.

ALT activity in muscle

The alterations in the ALT activity in the muscle tissue of zebrafish exposed to emamectin benzoate (0.05, 0.1 and 1 mg/L) for 35 days is given in Fig. 4. The data obtained in the present study indicate that emamectin benzoate induced significantincrease in ALT activity in the muscle tissue compared to the control. The significant increase in the ALT activity was related to emamectin benzoate dose through the exposure period (35 days).

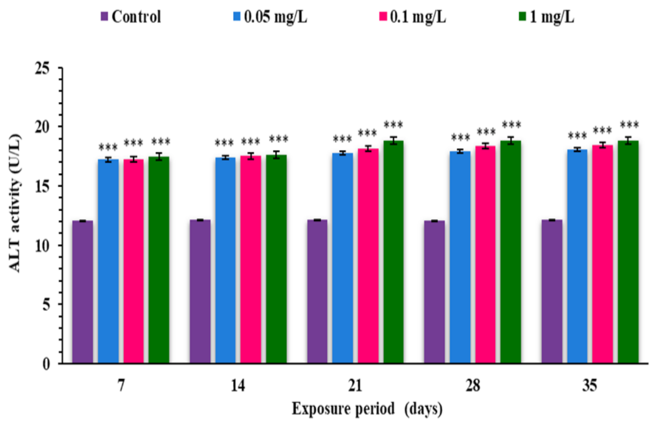

Fig. 4. ALT activity in the muscle of adult zebrafish exposed to emamectin benzoate (0.05, 0.1 and 1 mg/L) for 35 days. Data are reported as mean ± SE. ***significant at $P < 0.001$.

DISCUSSION

Around the world, pesticides are sprayed freely and carelessly, and eventually they wind up in aquatic bodies through direct application, surface run-off and leaching from agricultural fields, or industrial effluent discharge(Rohani, 2023). In the aquatic environment, pesticides are considered the most potent(Abd El Megid*et al.,* 2020) andbioaccumulate in fish and aquatic speciesthrough contaminated water and affect the aquatic organisms directly or indirectly (Veedu *et al.,* 2022; Nayak *et al.,* 2023; Li *et al.,* 2025).

Biopesticides typically exhibit efficacy comparable to that of synthetic pesticides. Emamectin benzoate (EMB), a biopesticide, isolated from soil microorganism isused in agriculture and forestry due to its broad-spectrum insecticidal properties and prolonged residual activity(Guo *et al.,* 2025). In addition, EMB is widely used in aquaculture to control parasitic infestations and crustacean ectoparasites (Xu *et al.,* 2021; Gu *et al.,* 2023; Rigos *et al.,* 2024). EMB is ranked among the top-selling pesticides worldwide because of its widespread usage (Tan *et al.,* 2020).The widespread usage of EMB can enter the aquatic environment which results in the detection of these pesticides in water bodies. For example, EMB has been detected insediments adjacent to salmon aquaculture farms (Cheng *et al.,* 2020) and in surface waters of in many countries (Tan *et al.,* 2021).The compound EMB exhibits slow degradation in the aquatic environments which result in persistence of EMB in the environment (Hamoutene *et al.,* 2023). Shi *et al.*(2024) has reported that EMB shows bio-accumulative properties, with an elimination half-life of 14 days in fish. As a result, increased attention has been given to the detection and its toxicity to aquatic organisms.

Fish serve as valuable bioindicators for assessing the toxicological impacts and safety concerns of environmental pollutants such as pesticides. Recently, zebrafish (*Danio rerio*) have been increasingly employed as bioindicators in toxicological research due to their small size, ease of laboratory maintenance (Qi *et al.,* 2017; Yang *et al.,* 2018), and its similarities with humans genomes (Zhao *et al.,* 2020). To evaluate the adverse effect of environmental contaminants in fish, biochemical biomarkers are commonly used in aquatic eco-toxicological investigations (Poopal *et al.,* 2020; Kumar *et al.,* 2022; Ramesh *et al.,* 2024). Changes in enzyme activities can provide valuable insights into fish populations' health status(EL-Gendy *et al.,* 2025). Generally,ASTand ALTare commonly used as enzymatic biomarkers to assess the structural and functional changes of fish under pesticide stress conditions (Kumar *et al.,* 2022; Banaee *et al.,* 2023).

AST catalyzes the interconversion of aspartate and α-ketoglutarate to oxaloacetate and glutamate and is present in the organs/tissue such as liver, brain, heart, kidney muscle etc. Generally, the enzyme ALT catalyzes the transfer of an amino group from L-alanine to α –ketoglutarate and is found in serum and in various organ/tissues.AST and ALT can be used as critical markers of chemical induced toxicity in liver. Furthermore, the impact of pesticide toxicity on aquatic organisms can be assessed bymeasuring these enzyme activities in key tissues of fish (Kumar *et al.,* 2022).Fish liver, the most important organ of essential metabolic pathways and rich in transaminaseshas a highsensitive to environmental contaminants (Gül *et al.,*2004; Rohani, 2023)and plays an important role in purifying the toxic chemicals in blood by changing their chemical structure (Soufy *et al.,* 2007).EL-Gendy *et al.,* (2025) has reported that the cells of liver and membrane may be damaged during biotransformation of toxicants.

Pesticide toxicity has been shown to enhance AST and ALT activities in the vital tissues of fish species such as *C. mrigala* (Ghayyur *et al.,* 2021) and *L. rohita* (Nayak *et al.,* 2023). The significant increase of AST and ALT activity reflects increased energy production through the conversion of amino acids into intermediates of the tricarboxylic acid via oxidative deamination and active transamination processes, utilizing glucose during stress condition (Al-Ghanim *et al.,* 2012; Nayak *et al.,* 2023). In the present investigation, zebrafish exposed to EMB showed noticeably higher levels of AST and ALT activity in the liver and muscle tissue of zebrafish than the control group, indicating the hepatic damage due to EMB toxicity. Similar observation have been reported in rat liver exposed to abamectin(Hsu *et al.,* 2001; Wolstenholme and Rogers, 2005;Khaldoun Oularbi *et al.,* 2013).

The elevation of transaminase levels in the liver indicates potential hepatic impairment or alterations in carbohydrates and protein metabolism due to toxicity of chemical substances (Ogueji *et al.,* 2020; EL-Gendy et *al.,* 2025; Mansour *et al.,* 2025). Elevation of AST and ALT activities in the tissue or organ such as liver, kidney, muscle and gill indicate even minor cellular damage (Das *et al.,* 2004). Incypermethrin exposed fish *Catla catla,* ALT activity was found to be increased in the liver tissue indicating increased protein catabolism and hepatocellular damage (Vani *et al.,*2012). Alterations in the liver enzymes due to pesticide toxicity may be due to formation of reactive intermediates that can damage cellular components or oxidative stress or endocrine disruption (Sharafeldin and Nagy, 2015).

Furthermore, oxidative stress caused by pesticides may also cause an elevation of AST and ALT activity (Li *et al.*, 2021). In the present study, the elevation of AST and ALT activity in the EMB exposed fish might be due to accumulation of EMB in the liver and muscle tissue of fish resulting in damage to these organs/tissue or an attempt to meet the energy demand caused by EMB toxicity. We conclude that alterations in AST and ALT activity in liver and muscle of EMB treated fish may be due to damage to these organs which in turn affect the survival and growth of aquatic organisms such as fish.

CONCLUSION

In the present investigation EMB exposure alters the AST and ALT activities in the liver and muscle of zebrafish at environmentally relevant concentrations. The alteration in the AST and ALT activity was higher in liver tissue followed by muscle which may be due to its role in detoxification of the pollutants. The changes in the activity of transaminases can be employed as potential biomarkers for the monitoring of pesticides in the aquatic environment.

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