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RESEARCH

Bioactive metabolites of *Lactobacillus plantarum* SB8 mitigate deltamethrin-induced oxidative stress and immune dysregulation in Nile Tilapia (*Oreochromis niloticus*)

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Abstract

Synthetic pesticides such as deltamethrin (DLM) pose severe risks to aquatic organisms, causing oxidative damage, immune dysregulation, and growth impairment in farmed fish. In this study, we investigated the protective role of *Lactobacillus plantarum* SB8, focusing on the bioactive metabolites present in its cell-free supernatant (CFS). In vitro, assays demonstrated that SB8 metabolites exerted strong antibacterial activity against *Aeromonas hydrophila* and *Pseudomonas fluorescens*. In vivo experiments with Nile tilapia (*Oreochromis niloticus*) revealed that dietary supplementation with SB8 markedly attenuated DLM-induced hematotoxicity, restoring leukocyte counts and protein profiles. Moreover, SB8 supplementation normalized antioxidant enzyme activities (SOD, CAT, GPx), reduced lipid peroxidation (MDA levels), and downregulated pro-inflammatory cytokines (IL-1β, IL-6) in liver tissue. GC–MS analysis of SB8 *L. plantarum* metabolites identified components such as lactic acid, acetic acid, phenolic derivatives, exopolysaccharides, peptides, and bacteriocins, which support antioxidant, antimicrobial, and immunomodulatory activities. These findings highlight that bioactive compounds secreted by *L. plantarum* SB8 can counteract pesticide-induced oxidative stress and inflammation, providing a promising molecular strategy for mitigating chemical toxicity in aquaculture species.

Keywords *Lactobacillus plantarum* · SB8 · Bioactive metabolites · Oxidative stress · Cytokine regulation · Deltamethrin toxicity

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Introduction

Aquaculture plays a pivotal role in global food security, particularly in countries such as Egypt, which is one of the leading producers of farmed fish in Africa (Abd El Tawab et al. 2020). However, the sustainability of this sector is increasingly threatened by environmental stressors, including drainage water contamination with pesticides, climate change, and disease outbreaks (Abdel-Hady et al. 2025). Among chemical pollutants, deltamethrin (DLM), a synthetic pyrethroid widely used in agriculture, is of particular concern due to its high toxicity to non-target aquatic organisms (Ahmadifar et al. 2020). Accumulation of DLM in fish tissues can impair growth, disrupt antioxidant defense systems, and compromise immune responses, thereby increasing susceptibility to infections (AWWA 2012; Aly et al. 2025). Moreover, consumption of contaminated fish poses significant risks to human health, including potential carcinogenic effects (Anyachor et al. 2025). Pesticide-induced oxidative stress and immune dysregulation are among the most critical mechanisms underlying DLM toxicity. Exposure to DLM generates excessive reactive oxygen species (ROS), which overwhelm antioxidant defenses such as superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx), leading to lipid peroxidation and cellular damage (Bendjeddou et al. 2012). At the immunological level, DLM exposure upregulates pro-inflammatory cytokines such as interleukin-1 β (IL-1β), contributing to chronic inflammation and immune suppression (Bradbury et al. 1988; Bernal-González et al. 2023). Probiotics, especially lactic acid bacteria such as *Lactobacillus plantarum*, are increasingly recognized as promising biotechnological tools for aquaculture (Bradford et al. 1976; Cao et al. 2021; Stabnikov et al. 2025). These microorganisms and their bioactive metabolites—including organic acids, bacteriocins, and antioxidant compounds—exert antimicrobial, antioxidant, and immunomodulatory effects (Cengiz et al. 2017; Chowdhury et al. 2020). In addition to improving growth and feed efficiency, probiotics can counteract xenobiotic-induced stress responses, enhancing fish resilience to environmental contaminants (Dawood et al. 2020a) Although the beneficial effects of probiotics on fish growth and immunity are well established, their role in mitigating pesticide-induced toxicity remains underexplored (Dawood et al. 2020b). This study aimed to investigate the bioeffect of metabolites of *Lactobacillus plantarum* SB8, which mitigate deltamethrin-induced oxidative stress and immune dysregulation in Nile tilapia (*Oreochromis niloticus*). Specifically, we assessed (i) the antibacterial activity of SB8 metabolites against aquatic pathogens, (ii) their ability to restore hematological and protein parameters, (iii) their modulation of antioxidant defenses and lipid peroxidation, and (iv) their effect on pro-inflammatory cytokine expression IL-1β and IL-6. By elucidating these mechanisms, we provide novel insights into the potential of probiotics as sustainable, bioactive interventions to improve aquaculture productivity and resilience in pesticide-contaminated environments.

Materials and methods

Diet components and chemical composition

The basal diet was formulated as follows (g/100 g): fish meal 10; soybean meal 44.4; wheat bran 10; yellow corn 18.6; rice bran 10; fish oil 5; dicalcium phosphate 1; vitamin–mineral premix 1. Proximate composition (as fed): dry matter 92.8%, crude protein 30.9%, crude lipid 7.1%, ash 7.2%; gross energy calculated using 5.65, 9.45 and 4.11 kcal/g for protein, lipid and carbohydrate respectively was ~ 437 kcal/100 g (≈4370 kcal/kg). Diet was supplemented with *L. plantarum* SB8 to reach 1×10^6–1×10^7 CFU/g feed; total feed offered over the 30-day trial (3% body weight/day) for 120 fish (initial mean weight 40.2 g) was ~ 5.07 kg.

Probiotic strain and culture conditions

The probiotic strain *Lactobacillus plantarum* SB8 with accession number (PQ157876) was obtained from the Faculty of Agriculture, Ain-Shams University (Cairo, Egypt). The strain was stored at –80 °C in tryptic soy broth with 30% glycerol until use. For experimental assays, the SB8 was cultured in de Man, Rogosa and Sharpe (MRS) broth at 30 °C for 24 h under shaking conditions (140 rpm) (Chowdhury et al. 2020).

Preparation of cell-free supernatant (CFS)

After incubation, the cells were harvested by centrifugation (4000 g), washed twice with phosphate-buffered saline (pH 7.2), and resuspended in the same buffer. The supernatant was then filtered through a 0.22 µm MF-Millipore® membrane filter (Sigma-Aldrich, St. Louis, MO, USA) according to Maccelli et al. (2020) and Khalil et al. (2024). To standardize the number of bacteria (10^6 to 10^7 CFU mL − 1) the absorbance at 600 nm was adjusted to 0.25 ± 0.05. Dilution plating was conducted to verify the relationship between absorbance at 600 nm and CFU/milliliter. The count/bacterial concentration was determined using the standard plate count method on MRS agar, and the bacterial suspensions were sprayed onto the feed with continuous mixing.

Metabolite analysis of SB8 Lactobacillus plantarum by Gas Chromatography–Mass Spectrometry

SB8 *Lactobacillus plantarum* preparation for metabolite analysis was performed by Gas Chromatography–Mass Spectrometry (GC–MS). GC–MS analysis of the *L. plantarum* culture supernatant methanol extract was performed using the Thermo/Finnigan Surveyor System. Mass spectrometric data were evaluated using data analysis software (Xcalibur Qual Browser 3.1; Thermo Electron, San Jose, CA, USA). Sample preparation and chromatographic separation were carried out following the method reported by Dawwam et al. (2022).

Antimicrobial assay

Pathogenic strains of *Aeromonas hydrophila* (ATCC 13037) and *Pseudomonas fluorescens* (ATCC 13525) were cultured on nutrient agar and diluted to 1.5×10^8 CFU/mL. The agar well diffusion method was employed by inoculating Mueller–Hinton agar plates with each pathogen, punching 6 mm wells, and adding 50 µL of SB8 CFS. Plates were incubated at 37 °C for 24 h, and inhibition zones were measured in three biological replicates across independent trials (Sahoo et al. 2015).

Experimental fish and husbandry

A total of 120 Nile tilapia (*Oreochromis niloticus*, average weight 40.2 ± 5.5 g) was collected from the National Research Centre's El-Noubarya fish farm (Egypt) and acclimated for two weeks. Fish were randomly distributed into four groups (30 fish/group) and maintained in aquaria under controlled conditions: temperature 26.7 ± 2.1 °C, pH 7.2–8.2, dissolved oxygen 6.9 ± 0.5 mg/L, ammonia 0.040 ± 0.005 mg/L, nitrite 0.06 ± 0.013 mg/L. Water parameters were monitored according to AWWA guidelines 2012.

Experimental design

Fish were randomly assigned to four experimental groups. The control group (NC) received only the basal diet as follows: The basal diet was contained (Fish meal 10%, Soybean meal 44.4%, Wheat bran 10%, Yellow corn 18.6%, Rice bran 10%, Fish oil 5%, Dicalcium phosphate 1% and Vitamins and minerals mixture 1%) with proximate chemical composition: (Dry matter 92.8%, Crude protein 30.9%, Ether extract 7.1%, Total ash 7.2% and Gross energy (kcal/100 g)^2 446). The SB8 group was fed the basal diet supplemented with *Lactobacillus plantarum* SB8 at a concentration of 10^6–10^7 CFU per gram of feed. The DLM group was exposed to deltamethrin at a concentration of 1.49 µg/L selected based on previously reported toxicological studies in Nile tilapia (Cengiz et al. 2017; Dawood et al. 2020a, b, c), with the pesticide solution renewed daily, while receiving the basal diet. Finally, the DLM + SB8 group was exposed to deltamethrin under the same conditions but was fed the SB8-supplemented diet (10^6 to 10^7 CFU/g feed). All groups were fed at 3% of body weight twice daily for 30 days (Cengiz et al. 2017).

For the SB8-supplemented diet, *Lactobacillus plantarum* SB8 was cultured, adjusted to the target concentration, and added gradually to the ground basal diet. The mixture was homogenized thoroughly using a mechanical mixer to ensure uniform distribution of the bacterial suspension. The moistened mash was then pelletized and air-dried at room temperature before storage at 4 °C. The selected concentration of 10^6–10^7 CFU/g feed falls within the range of inclusion levels previously reported for *L. plantarum* in tilapia diets that improved antioxidant and immune responses (Van Nguyen et al. 2019; Dawood et al. 2020c; Gewaily et al. 2021).

Hematological and biochemical analyses

Blood was collected from the caudal vein of euthanized fish (MS-222, 100 mg/L) and analyzed for total white blood cells and differential counts (May–Grünwald–Giemsa staining). EDTA (ethylenediaminetetraacetic acid, 1.5 mg per ml of blood) was used as the anticoagulant for blood collection and subsequent hematological measurements. Serum was isolated for analysis of total protein, albumin, and globulin using commercial kits Pierce™ Bradford Protein Assay Kit (Cat. No. 23200), Thermo Scientific, Rockford, IL, USA. Albumin was quantified using the bromocresol green spectrophotometric method (Doumas et al. 1971). The experimental procedure involved incubating a known volume of sample (5 µL) with the BCG reagent and incubating at room temperature for a defined time (5 min). Subsequently, the absorbance was measured with a spectrophotometer at a wavelength of 630 nm using a blank for calibration. The final albumin concentration was determined by interpolation, comparing the absorbance of the unknown sample with a standard curve prepared with dilutions of albumin at known concentrations. The same procedure was performed for the total proteins content according to Bradford et al. (1976).

Antioxidant and oxidative stress markers

Liver samples were homogenized in PBS and assayed for superoxide dismutase (SOD) (Item No. 706002) Cayman Chemical (Ann Arbor, MI, USA), catalase (CAT) (Item No. 707002), Cayman Chemical (Ann Arbor, MI, USA), glutathione, peroxidase (GPx) (Item No. 707002) Cayman Chemical (Ann Arbor, MI, USA) and malondialdehyde (MDA) (Item No. 10009202) Cayman Chemical (Ann Arbor, MI, USA) using colorimetric kits Antioxidant Assay Kit (Peña-Llopis et al. 2003).

Superoxide dismutase (SOD) activity was estimated spectrophotometrically for 15 min at 340 nm as described in Guerriero et al. (2002). The basis of this technique is the oxidation of NADH mediated by superoxide radicals. Catalase activity was performed spectrophotometrically at 240 nm as described in Anyachor et al. (2023). The method is supported by the principle that catalase in the sample preparation splits hydrogen peroxide. Glutathione peroxidase (GPx) activity was determined spectrophotometrically at 340 nm as described in Guerriero et al. (2002) using the consecutive glutathione reductase reaction and oxidation of NADPH, with t-butyl hydroperoxide as substrate.

Malondialdehyde (MDA) level was assayed by using the procedure described in Anyachor et al. (2023). Under acidic medium, MDA reacts with the chromogenic reagent, 2-thiobarbituric acid (TBA), to form a pink colored complex at 532-nm absorbance.

Micronucleus test

Erythrocyte smears were stained with hematoxylin and eosin, and micronucleus (MN) frequency was assessed by counting 1000 cells per fish (10 fish per group). Morphological alterations were scored blindly, following established protocols counting the same number of cells in each fish ($n = 10$/group) (Schmidt 1975; Pascoe and Gatehouse 1986).

Cytokine gene expression

Total RNA was extracted from liver tissues, treated with DNase, and reverse-transcribed to cDNA. Real-time PCR was conducted using SYBR Green Master Mix and primers specific to IL-1β, IL-6, and β-actin (Table 1) (Cao et al. 2021). Gene expression levels were calculated by the $2 - \Delta\Delta CT$ method.

Statistical analysis

Data was analyzed using SAS software (SAS 1982). One-way ANOVA followed by Scheffé's test was applied to evaluate differences among groups. Values are expressed as mean ± SEM, and significance was set at $P < 0.05$.

Table 1 Primers sequences used for real-time PCR (qRT-PCR) of interleukin 1 (IL-1β), interleukin 6 (IL-6) and beta-action (β-Action)

Genes	Primer sequence	NCBI reference
IL-1β	F: 5′- caa gga tga cga caa gcc ag −3′ R: 5′- ggt agc gga cag aca tga ga −3′	JF957370.1*
IL-6	F: 5′- aat tcc ttc tgg ccc tga ca −3′ R: 5′- tcc tct gtc tcc tca cct ga −3′	XM_019350387.2*
β-Action	F: 5′- cca gcc ttc ctt cct tgg ta −3′ R: 5′- agg tgg ggc aat gat ctt ga −3′	KJ126772.1*

* Verified in the manuscript of Khalil et al. (2024)

Results

In vitro — antibacterial activity of Lactobacillus plantarum SB8 metabolites

The cell-free supernatant (CFS) derived from *L. plantarum* SB8 demonstrated antagonistic effects against two common fish pathogens, *Aeromonas hydrophila* (ATCC 13037) and *Pseudomonas fluorescens* (ATCC 13525). Inhibition zones ranged between 12.2–14.5 mm for *A. hydrophila* and 14.6–16.4 mm for *P. fluorescens*, confirming the antimicrobial potential of the bioactive metabolites produced by SB8.

In vivo — growth performance

Mean weight gain (MWG) for all fish groups revealed a significant difference among groups with respect to the initial weight (40.2 ± 5.5 g.). Fish in the DLM-exposed group had the lowest MWG (71.0 ± 0.9 g), representing a 17.4% reduction compared with the control group (86.0 ± 1.5 g, $P < 0.05$). In contrast, fish exposed to DLM but supplemented with SB8 showed a higher MWG (79.0 ± 2.1 g) than the DLM group. The SB8-only group exhibited the highest MWG (86.0 ± 1.8 g), comparable to the control.

Hematological parameters and micronucleus formation

Table 2 presents the hematological parameters of Nile tilapia. DLM exposure caused a reduction in white blood cell (WBC) number (–51.9%) and lymphocyte count (–38.1%) than controls ($P < 0.05$). Conversely, the percentages of heterophiles and monocytes were significantly higher in the DLM group. Supplementation with SB8 in DLM-exposed fish restored WBC and lymphocyte counts toward control values, while moderating neutrophil and monocyte percentages. In addition, DLM exposure markedly increased micronucleus (MN) formation, with an average of 29.5 MN per 10,000 erythrocytes compared to 8.6 in the control group (+ 243%, $P < 0.05$). SB8 supplementation statistically reduced MN frequency in DLM-exposed fish (16.7 MN per 10,000 erythrocytes), bringing values closer to those observed in controls.

Table 2 Hematological parameters of Nile Tilapia under the different treatments: white blood cell (WBC), lymphocyte count (Neutrophile, Lymphocytes, Monocytes and Eosinophils) measured in negative control, SB8 group, DLM group and S8 + DLM group

Treatments	WBC ($\times 10^9$/L)	Heterophiles (%)	Lymphocytes (%)	Monocytes (%)	Eosinophils (%)
NC	152.40 ± 8.21^a	55.94 ± 0.62^b	36.33 ± 0.74^a	5.10 ± 0.91^c	4.15 ± 0.35^{ab}
SB8	147.16 ± 9.32^a	54.25 ± 0.81^b	37.28 ± 1.04^a	4.86 ± 0.58^c	3.85 ± 0.60^b
DLM	112.81 ± 10.26^c	60.72 ± 1.12^a	22.50 ± 1.12^c	13.47 ± 1.13^a	5.17 ± 0.42^a
SB8 + DLM	134.52 ± 7.61^b	57.66 ± 1.24^{ab}	31.25 ± 0.92^b	8.29 ± 0.81^b	3.80 ± 0.38^b

NC (Negative Control): Fishes were fed only a basal diet; SB8 (Bacterial suspension): Fishes received the basal diet supplemented with the SB8 bacterial strain; *DLM* (Deltamethrin): Fishes were exposed to DLM and given the basal diet; *SB8 + DLM* (Bacterial suspension + Deltamethrin): Fishes were exposed to Bacterial suspension supplemented with Deltamethrin. Data are presented as the mean ± SEM for each experiment ($n = 10$). Values with different superscript letters (a, b, c) are considered markedly different ($P < 0.05$)

Table 3 Frequency of Micronucleus (MN) formation in Nile Tilapia Groups

Treatments	MnPCEs/PCEs
NC	8.6 ± 0.9^c
SB8	8.2 ± 0.4^c
DLM	29.5 ± 1.6^a
SB8 + DLM	16.7 ± 1.2^b

The experiment included four distinct groups: *NC* (Negative control): organisms were fed only a basal diet; *SB8* (Bacterial suspension): organisms received the basal diet supplemented with the SB8 bacterial strain; *DLM* (Deltamethrin): Organisms were exposed to DLM and fed the basal diet; *SB8 + DLM* (Bacterial suspension + Deltamethrin): organisms were exposed to DLM and also received the basal diet supplemented with the SB8 strain. The results are reported as the mean ± standard error of the mean (SEM) from 10 biological samples per group, across three independent experimental runs. Mean values that do not share common superscript letters (a,b,c) are considered statistically significant ($P < 0.05$)

Similarly, micronucleus frequency, a biomarker of genotoxicity, increased by + 243% in the DLM group, whereas SB8 co-supplementation reduced it by nearly half (Table 3), indicating protective effects at the genomic level.

Antioxidant defense and lipid peroxidation

The activities of malondialdehyde (MDA) and the enzymatic antioxidants (superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx) in the liver are reported in Fig. 1. In DLM group, SOD, CAT, and GPx activities were statistically lower, while MDA levels were markedly increased compared with controls ($P < 0.05$). In DLM + SB8 fish, antioxidant enzyme activities were restored, and MDA levels reduced compared with the DLM-only group, approaching values recorded in controls.

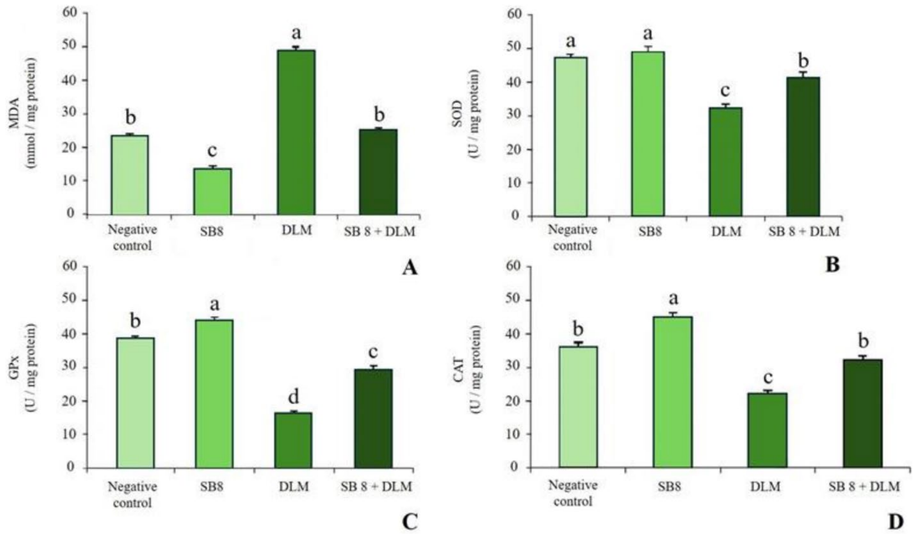

Fig. 1 The activity of MDA (**A**), SOD (**B**), GPx (**C**), CAT (**D**) oxidative stress enzymatic activities in the liver of Nile tilapia. Abbreviations: *Negative control*, fed only with basal diet; Bacterial suspension (*SB8*), fed with basal diet and the SB8; *DLM*, exposed to the DLM and fed with basal diet; *SB8 + DLM*, exposed to DLM and fed with basal diet, and supplemented with the SB8 strain. The data are presented as mean ± SEM following Scheffé-test of biological samples per group in each experiment ($n = 10$) of 3 independent runs. Mean values with not similar superscript letters (a,b,c,d) are significantly different ($P < 0.05$)

Table 4 Protein content levels among the Nile Tilapia groups

Treatments	Total protein (g/L)	Albumin (g/L)	Globulin (g/L)	A/G (ratio)
NC	39.41 ± 2.74^{a}	4.15 ± 0.50^{a}	39.60 ± 3.11^{a}	0.104 ± 0.01^{a}
SB8	41.49 ± 4.13^{a}	4.87 ± 0.48^{a}	44.19 ± 4.62^{a}	0.089 ± 0.01^{a}
DLM	23.54 ± 3.15^{c}	2.11 ± 0.87^{c}	21.71 ± 2.18^{c}	0.097 ± 0.01^{a}
SB8 + DLM	36.72 ± 5.17^{b}	3.81 ± 0.63^{b}	34.85 ± 1.91^{b}	0.109 ± 0.01^{a}

The analysis was conducted on four groups: a Negative control (NC) fed only a basal diet; Bacterial suspension (SB8) group fed the basal diet supplemented with the SB8 strain; DLM group exposed to DLM while on the basal diet; and an SB8 + DLM group that received the SB8 strain alongside the basal diet while also being exposed to DLM. Data are expressed as the mean ± SEM from 10 biological samples per group, across three independent trials. Mean values lacking similar superscript letters (a,b,c) show a statistically significant difference ($P < 0.05$)

Protein metabolism

The analyses of serum are summarized in Table 4. DLM-exposed fish exhibited significant decreases in total protein, albumin, and globulin contents compared to the negative control group.SB8 supplementation in DLM-exposed fish improved total protein, albumin, and globulin contents, restoring them close to control values. The albumin/globulin (A/G) ratio did not differ markedly among groups.

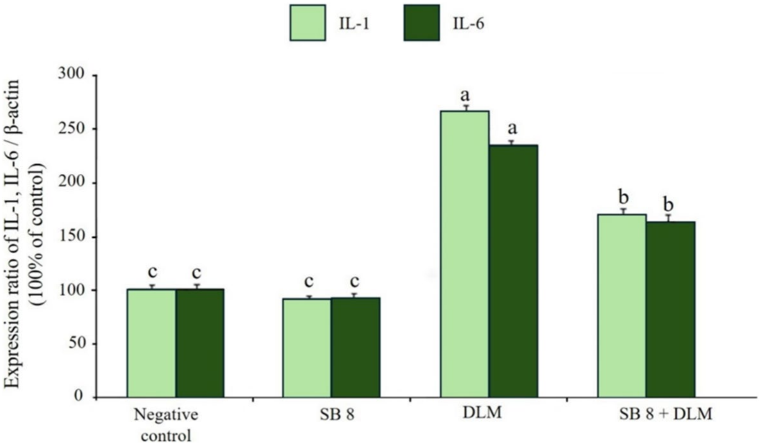

Fig. 2 Analysis of IL-1β and IL-6 Expression in Nile Tilapia Liver comparing four experimental groups: Negative Control: fed only a basal diet; Bacterial Suspension (SB8): fed the basal diet supplemented with the SB8 bacterial strain; DLM: exposed to DLM while on the basal diet; SB8 + DLM: Exposed to DLM and also supplemented with the SB8 strain in addition to the basal diet. The data presented represents the mean ± standard error of the mean (SEM) from 10 biological samples per group. Scheffé-test was used to determine statistical significance. Mean values that do not share the same superscript letters (**a**, **b**, **c**) are considered statistically different ($P < 0.05$)

Pro-inflammatory cytokine expression

Interleukin 1 and 6 expressions are presented in Fig. 2. The expression of both IL-1β and IL-6 was markedly upregulated in the DLM group compared to controls ($P < 0.05$). In contrast, fish in the DLM + SB8 group showed a significant downregulation of IL-1β and IL-6 expression, reaching levels similar to those of the control and SB8-only groups.

Discussion

The present study provides strong evidence that bioactive metabolites produced by *Lactobacillus plantarum* SB8 mitigate the deleterious impacts of deltamethrin (DLM) on Nile tilapia. By integrating microbiological, hematological, biochemical, and immunological endpoints, our findings highlight the potential of probiotic metabolites to function as molecular protectors against pesticide-induced toxicity.

Antimicrobial properties of the SB8 strain

The probiotic effects of lactic acid bacteria have been the focus of multiple studies concerning the guts of terrestrial animals (Vine et al. 2006). These results supported the antimicrobial activity of the SB8 strain against the two available bacterial pathogens used. Several studies have reported antagonistic impacts of *Lactobacillus* species on *Aeromonas hydrophila* and *Pseudomonas fluorescens* and the most active strains were *Lactobacillus acidophilus*, *Lactobacillus casei*, *Lactobacillius reuteri*, *Lactobacillus rhamnosus*, *Lactobacillus paracasei*, *Lactobacillus plantarum*, *Lactobacillus bulgaricus*, and *Lactobacillus fermentum*, *Propionibacterium acnes*, and *Lactococcus lactis* (Jabbar et al. 2008; Karska-Wysocki et al. 2010; Bendjeddou et al. 2012). The antagonistic effect of *Lactobacillus* species is attributed to both competitive exclusion and the secretion of acids or bacteriocin-like substances (Prince et al. 2012; Sikorska et al. 2013; Shu et al. 2013). Several studies have shown the protective benefits of supplementing Nile tilapia diets with *Lactobacillus*. This probiotic has been shown to effectively shield the fish from toxic compounds such as ammonium chloride, as well as from infections with bacteria like *Streptococcus agalactiae* (Van Nguyen et al. 2019).

Deltamethrin-induced toxicity and its physiological impact

Harsh environmental conditions like water temperature fluctuations, overstocking, nutritional deficiencies, and xenobiotics in aquaculture systems weaken fish immunity and increase their susceptibility to infection (Shourbela, et al. 2021). DLM is a well-known pyrethroid insecticide that has been regularly used in various agricultural activities (Bradbury et al. 1988; Wang et al. 2025). In the current work, DLM residuals induced remarkable toxic influences on *Oreochromis niloticus*. This is supported by a significant reduction in WBC and lymphocyte counts, and a significant increase in monocyte and eosinophil counts. The increase in white blood cell (WBC) count in tilapia count in SB8-supplemented tilapia counteracts the immunosuppression and is attributable to the potentiating (immunostimulatory) effect of the probiotics and their metabolites. In groups supplemented with *L. plantarum*, this increase reflects the potentiating (immunostimulatory) effect of probiotics and their metabolites, which act by mobilizing immune cells (leukocytes) to optimize defense function. Furthermore, the increase in the groups treated with *L. plantarum* reflects a well-documented immunostimulatory effect of probiotics and their metabolites, which activate and mobilize immune cells (such as leukocytes) to improve immune defense. Similarly, total protein levels show a significant increase. In fish exposed to stress (Deltamethrin) or treated with immunostimulants (*L. plantarum*), the increase in total protein is often due to an increase in immunoglobulins and acute phase proteins (such as albumin or globulins), which are critical components of the immune response and detoxification efforts. This increase could be a marker of immune activation and metabolic response to stress. The increase in protein levels, particularly in the DM group, probably reflects the increase in acute phase proteins (such as immunoglobulins) released during the systemic immune response and inflammation.

The observed decrease in SOD, CAT, and GPx enzymatic activities in the DLM-exposed group indicates tissue impairment due to overproduction of reactive oxygen species (ROS), leading to a weakened immune system and higher vulnerability to bacterial infections (Eder et al. 2006). Deltamethrin is highly toxic to fish, and several studies have reported LC_{50} values for *Oreochromis niloticus* in the low-µg/L range. Cengiz et al. (2017) reported an acute 96-h LC_{50} of 2.34 µg/L for Nile tilapia, indicating high sensitivity to this pyrethroid. Other available studies show comparable LC_{50} values, generally between 1–3 µg/L, depending on fish size, water quality, and formulation. SB8 remained viable and maintained the required concentration through plate count of SB8 (expressed as CFU/g) on MRS Agar, verified both immediately after preparation and at regular intervals (data not shown).

Based on these published LC_{50} ranges, our selected sublethal concentration of 1.49 µg/L corresponds to approximately 50–60% of the 96-h LC_{50}, making it an appropriate ecologically relevant stress dose that induces measurable physiological and immunological alterations without causing mortality. This concentration has also been successfully applied in previous research on DLM-induced toxicity in Nile tilapia (e.g., Dawood et al. 2020a, 2020c), ensuring comparability with the existing literature. The increased MDA activity within the DLM group might be explained by inhibited antioxidant enzyme production and enhanced free radical liberation (Rjeibi et al. 2016). Additionally, DLM exposure led to a significant decrease in total protein, albumin, and globulin levels, a higher incidence of micronucleus (MN) formation, and elevated IL-1β and IL-6 expression. These cytokines are known to suppress immune function by hindering T cell growth and activity (Mendivil et al. 2021) and inducing chronic inflammation that exhausts immune cells and disrupts immune communication (Eder et al. 2007; Bernal-González et al. 2023).

Molecular interpretation of antioxidant and cytokine modulation

The restoration of antioxidant enzyme activities (SOD, CAT and GPx) and the suppression of lipid peroxidation (MDA) observed in the SB8-treated groups are consistent with activation of redox-sensitive transcription factors such as Nrf2. In mammalian and fish models, probiotic metabolites have been shown to stimulate the Nrf2/ARE pathway, resulting in upregulation of antioxidant defenses and reduced oxidative stress (Dawood et al. 2016; Guerriero et al. 2018). Similarly, the downregulation of pro-inflammatory cytokines (IL-1β, IL-6) in SB8-supplemented fish indicates suppression of the NF-κB signaling pathway, which is a master regulator of inflammation and immune responses. Several studies have shown that *L. plantarum* metabolites, including short-chain fatty acids and bacteriocins, exert anti-inflammatory effects through inhibition of NF-κB activation (Cao et al. 2021; Bernal-González et al. 2023). This interpretation reinforces the molecular plausibility of our findings.

Bioactive metabolites of *L. plantarum*

The metabolites of SB8 *L. plantarum* extract include lactic acid, acetic acid, phenolic derivatives, exopolysaccharides, peptides, and bacteriocins (Dawwam et al. 2022). These molecules are known for their antioxidant, antimicrobial, and immunomodulatory activities, which align with the protective effects observed here. It is therefore reasonable to attribute the ameliorative effects of SB8 supplementation against DLM-induced toxicity to a synergistic action of these metabolites. Future work employing LC–MS or metabolomic profiling could provide direct evidence of their involvement.

Protective role of *Lactobacillus plantarum* SB8

Importantly, supplementation with *Lactobacillus plantarum* SB8 mitigated these adverse effects. The expression of IL-1β and IL-6 was reduced in the SB8-supplemented/DLM group to values comparable with controls, consistent with Zahran et al. (2018). Similar studies have shown that pesticide exposure, such as to chlorpyrifos, markedly upregulated IL-1β and IL-6 in tilapia (Shourbela et al. 2021; Dawwam et al. 2022). Since pesticides like DLM are absorbed through gills, skin, and other tissues, they can accumulate and disrupt local immunity (Gipson et al. 2016; Ahmadifar et al. 2020). Probiotic supplementation has therefore been applied to improve the overall health of aquatic animals (Kim et al. 2010;

Vijayaram et al. 2022). *Lactobacillus* species, in particular, are valued for their safety, stability, and stress-mitigating properties, which enhance the overall health of aquatic fishes (Sikorska et al. 2013; Ismaeil et al. 2020). Previous work has shown that *Lactobacillus*-supplemented diets boost immune and antioxidant defenses in Nile tilapia exposed to DLM (Ismaeil et al. 2020), improving growth, biochemical and hematological parameters, and stress metabolism.

Enhancement of antioxidant and immune defenses

In the present study, supplementing the basal fish diet with SB8 improved growth, immune responses, hematological parameters, and oxidative stress resilience. SB8 enhanced antioxidant defenses, reduced oxidative damage, decreased MN frequency, and downregulated pro-inflammatory cytokines (IL-1β, IL-6). These findings are consistent with the established role of probiotics in enhancing antioxidant defenses against xenobiotics in aquacultured fish (Guerriero et al. 2003, 2004, 2018; Mendivil et al. 2021). The normalization of WBC, lymphocyte, neutrophil, monocyte, and eosinophil counts in the DLM + SB8 group confirmed SB8's capacity to counteract pesticide-induced toxicity. Restoration of SOD, CAT, and GPx activities, alongside reduced MDA levels, indicated that SB8 alleviated ROS-induced stress. This aligns with previous findings showing that probiotics enhance antioxidant enzyme expression and immune response under toxic stress (Dawood et al. 2016, 2020b, 2020c). By mitigating oxidative stress, SB8 improves antioxidant levels and thus supports reproduction and sustainability by limiting endocrine disruption (Guerriero et al. 2003; Gerriero et al. 2005).

Restoration of protein metabolism and immunostimulant activity

The observed increases in total protein, albumin, and globulin levels in the SB8 + DLM group suggest that SB8 contributes to restoring protein metabolism. These findings align with the immunostimulant properties of *Lactobacillus*, likely mediated by structural components such as peptidoglycan and lipopolysaccharides that promote T lymphocyte activation (Hasan et al. 2019; Gewaily et al. 2021). Hence, *Lactobacillus plantarum* SB8 not only counteracts DLM-induced physiological damage but also strengthens immune and metabolic function, promoting overall fish health and resilience under environmental stressors.

Sustainability and applied perspectives

From an applied perspective, the use of probiotics such as SB8 represents an eco-friendly and sustainable strategy for aquaculture. Unlike synthetic antioxidants or chemotherapeutics, probiotics act through natural bioactive molecules and are safe for both fish and consumers. By reducing oxidative damage, restoring immune balance, and enhancing growth even under chemical stress, SB8 supplementation improves fish resilience, which is a cornerstone for sustainable production in contaminated environments. This is particularly relevant in developing countries, where pesticide residues in aquatic systems remain a major challenge for food security (Abdel-Hady et al. 2025).

Limitations and future directions

While our results are robust, the study has some limitations. We did not directly characterize the SB8 metabolite profile or measure upstream signaling pathways (e.g., NF-κB and Nrf2). Nevertheless, our interpretation is strongly supported by the observed biochemical outcomes and by previous literature linking probiotic metabolites with these molecular mechanisms. Future studies should combine metabolomic analyses with transcriptomic or proteomic approaches to further clarify the mechanistic basis of probiotic-mediated protection. Additionally, expanding the experimental design to include multiple pesticide doses and longer exposure periods will enhance the ecological validity of the findings.

Conclusion

This study demonstrates that the bioactive metabolites of *Lactobacillus plantarum* SB8 confer substantial protection to Nile tilapia against Deltamethrin-induced toxicity. Dietary supplementation with SB8 restored hematological and protein parameters, enhanced antioxidant enzyme activity, reduced lipid peroxidation and downregulated pro-inflammatory cytokines. The incorporation of probiotics such as SB8 into aquaculture feed represents a sustainable and environmentally friendly strategy for mitigating the adverse effects of pesticides, thereby supporting fish welfare, and environmental and food safety. To consolidate the mechanistic understanding of this protection, future work should focus on characterizing the metabolic profile of SB8 and validating the involvement of the NF-kb and Nrf2 pathways at the transcriptomic or proteomic level.

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Data availability The authors declare that the data supporting this study are available within the paper. Source data are provided with this paper.

Declarations

Ethical approval The research described was performed on Nile tilapia (*Oreochromis niloticus*). This study was conducted in strict accordance with the guidelines of the Ethical Committee (Approval no. 06410923), National Research Centre, Egypt, on the care and use of animals for scientific purposes.

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