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Protective Efficacy of Dietary Yeast (*Saccharomyces cerevisiae*) Against Microplastic Toxicity in the Nile Tilapia (*Oreochromis niloticus*): Studies on Growth Performance, Gene Expression, Biochemistry, and Immune Response

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ABSTRACT

Microplastics (MPs) have received significant attention due to their harmful impact on fish production. This research investigated the protective effects of the yeast, *Saccharomyces cerevisia* (Sc) against MPs-induced toxicity in the Nile tilapia, focusing on growth performance, fed utilization, gene expression (IGF-1, IGF-2, and GH), biochemical markers, and immune responses. A total of 270 fingerlings were divided into nine groups; the control group received a basal diet, while the other groups were exposed to varying concentrations of Sc (2 and 4g/ kg) and MPs (10 and 50mg/ L). MPs exposure negatively impacted growth performance, feed efficiency, survival rates, and body composition. Nevertheless, dietary inclusion of Sc notably improved these parameters, particularly in groups receiving 4g/ kg Sc. Exposure to MPs led to down-regulation of gene expression in the liver and gills, whereas Sc supplementation increased gene expression, especially in the liver. Biochemical analysis showed increased levels of AST and ALP in most groups, with kidney function tests revealing elevated blood urea and serum creatinine. Additionally, cholesterol levels were significantly higher in group receiving 4g/ kg Sc. Immune response assessment indicated elevated lysozyme and antiprotease activities, with the highest levels observed in groups treated with 10mg/ L MPs plus 4g/ kg Sc and 50mg/ L MPs plus 4g/ kg Sc. The results suggest that *Saccharomyces cerevisia*, particularly at a concentration of 4g/ kg, acts as a beneficial probiotic that alleviates MPs toxicity, enhancing growth, gene expression, and immune responses in the Nile tilapia.

INTRODUCTION

Microplastics (MPs), defined as plastic particles or nanoparticles smaller than 5mm, have emerged as a significant environmental issue. These tiny particles are produced from the gradual breakdown of larger plastic items **(Walkinshaw *et al*., 2020; Hamed *et***

***al.*, 2021)**. Globally, about 300 million tons of plastic are used annually in a variety of industries, including food packaging **(Hamed *et al.*, 2021; Plastics Europe, 2023)**. Extensive use of plastics had resulted in widespread contamination of natural environments, with a significant portion of plastic waste entering aquatic ecosystems. This contamination occurs through multiple pathways, including direct discharge from ports and ships, riverine transport, wastewater, sewage, and atmospheric deposition **(Hamed *et al.*, 2020; Walkinshaw *et al.*, 2020; Thiele *et al.*, 2021)**. As a result, MPs are now common in marine environments, impacting nearly all marine species including fish **(Oliva-Hernandez *et al.*, 2021)**. In freshwater systems close to large urban areas, MS concentrations have reached 466,000 particles per square kilometer, with lake samples showing an average concentration of 5.51 ± 9.09mg/ L **(Lasee *et al.*, 2017; Hamed *et al.*, 2021)**. The resemblance of MPs in size and color to natural prey makes them susceptible to ingestion by fish, leading to toxic effects **(Yuan *et al.*, 2019)**. Microplastics have been found in fish gastrointestinal tract's and within the tissues of various invertebrates **(Van Cauwenberghe & Janssen, 2014; Hastuti *et al.*, 2019)**. The toxicity of MPs stems from excessive production of reactive oxygen species, which diminishes antioxidant defenses, resulting in oxidative stress, DNA damage, and other harmful outcomes in fish **(Hamed *et al.*, 2020)**. Several adverse effects associated with microplastic toxicity have been documented in fish, including liver stress, endocrine disruption, intestinal changes, and impaired predatory behavior **(Hamed *et al.*, 2020)**, as well as alterations in gene expressions and immune function **(Ma *et al.*, 2020)**. Furthermore, the accumulation of MPs in the food chain raises concerns about potential human health risks through ingestion **(Oliva-Hernandez *et al.*, 2021; Alberghini *et al.*, 2022)**. Given the multitude of harmful effects associated with MPs pollution on both fish and human health, it is crucial to develop strategies to control and mitigate these toxic effects. Research efforts are needed to identify natural products or medicinal plants that can neutralize or reduce the bioavailability of MS. Probiotics, particularly *Saccharomyces cerevisia* (Sc), have shown potential in promoting detoxification processes by removing or neutralizing toxins without leaving harmful residues **(Islam *et al.*, 2021; El-Bab *et al.*, 2022)**. Sc was recognized as an eco-friendly feed supplement that enhances fish performance and prevents various diseases in aquaculture species **(Yang *et al.*, 2021; El-Bab *et al.*, 2022; Abozaid *et al.*, 2024)**. The biological composition of Sc includes vital components such as zinc, selenium, glutathione, cysteine, β-glucan, and mannan oligosaccharides, all of which possess strong antioxidant, antimutagenic, and anticarcinogenic properties in animals exposed to various toxins **(Abd-El Moneim *et al.*, 2017)**. Glucan and mannan oligosaccharides have demonstrated the capacity to adsorb and bind toxicants, scavenge reactive free radicals, and exert protective effects against mutagenesis **(Oliveira *et al.*, 2013)**, thereby safeguarding animal cells and biomolecules, including genetic material, from damage **(Abd-El Moneim *et al.*, 2017; El-Bab *et al.*, 2022)**. Furthermore, Sc supplementation in diets contaminated with aflatoxin (AF) improved the expression

levels of neural and gonadal genes in quail birds compared to those fed AF-contaminated diets alone **(Eshak *et al.*, 2010)**. The Nile tilapia is a key species in freshwater aquaculture in Egypt and globally **(Hamed *et al.*, 2021)**. Previous research has shown that MPs negatively impact growth, antioxidant defenses, hematology, biochemistry, and DNA in the Nile tilapia **(Hamed *et al.*, 2020; Hamed *et al.*, 2021; Oliva-Hernandez *et al.*, 2021)**. However, the potential of Sc to counteract microplastic toxicity in this species has not been extensively studied. Thus, our investigation aimed to evaluate the protective role of Sc against MPs toxicity by assessing growth performance, biochemical parameters, immune response, and the expressions genes (IGF-1, IGF-2, and GH) in the Nile tilapia (*O. niloticus*).

MATERIALS AND METHODS

Fish and experimental protocols

The study was conducted at the Animal Production Department of the National Research Center (NRC), Egypt, and was financially supported by NRC under Grant No. 13050411. A total of 270 mono-sex Nile tilapia, with an initial average weight of 14.5 ± 1.5g, were obtained from the Abbassa Fish Hatchery in Egypt. They were acclimatized in aquaria within a controlled laboratory setting and fed a standard diet for two weeks before the start of the experimental feeding trial. Water quality was maintained according to the recommended standards **(APHA, 1998)** throughout the 60-day study with 10% of the water being replaced daily. The fish were divided into nine experimental groups, each receiving a specific diet incorporating two levels of Sc (2 and 4g/ kg) as mentioned in Table (1) and two levels of MPs levels at 10 and 50mg/ L. The diet groups were as follows: D1 (Control: 0mg/ L MPs + 0g/ kg Sc), D2 (0mg/ L MPs + 2g/ kg Sc), D3 (0mg/ L MPs + 4g/ kg Sc), D4 (10mg/ L MPs + 0g/ kg Sc), D5 (50mg/ L MPs + 0g/ kg Sc), D6 (10mg/ L MPs + 2g/ kg Sc), D7 (10mg/ L MPs + 4g/ kg Sc), D8 (50mg/ L MPs + 2g/ kg Sc), and D9 (50mg/ L MPs + 4g/ kg Sc). Each group was tested in triplicate, with 10 fish per replicate. The fish were fed twice daily at 3% of their weight. Fish carcass analysis, including dry matter, crude protein, crude lipid, and ash content, followed the protocols of the Association of Official Analytical Chemists **(AOAC, 2005)**.

Table 1. Feed formulation and proximate chemical analysis of the experimental diets

Ingredients	**Control (0.0 g)**	**Sc (2 g)**	**Sc (4 g)**
Sc*	0	0.2	0.4
Concentration	17	17	17
Soybean meal	40	40	40
Corn	28	28	28
Wheat bran	10	9.8	9.6
Oil	2	2	2
Salt	1	1	1
Vit. & Min.**	2	2	2
Chemical composition, % on dry matter basis			
DM	92	90.26	90.37
OM	93.66	93.23	93.44
CP	30.15	30.35	30.41
CF	6.55	6.66	6.38
EE	4.18	4.15	4.21
NFE***	52.78	52.07	52.44
Ash	6.34	6.77	6.56
GE(kcal/100g)****	445.66	444.10	445.36

*Sc: Saccharomyces cerevisiae (powder)

**Vit. & min. mixture/kg premix: Vitamin D3, 0.8 million IU; A, 4.8 million IU; E, 4 g; K, 0.8 g; B1, 0.4 g, riboflavin, 1.6 g; B6, 0.6 g, B12, 4 mg; pantothenic acid, 4 g; Nicotinic acid, 8 g; folic acid, 0.4 g biotin,20 mg , Mn, 22 g; Zn, 22 g; Fe, 12 g; Cu, 4g; I, 0.4 g, selenium, 0.4 g and Co, 4.8 mg.

***Nitrogen free extract (NFE) =100-(CP+EE+CF+ash).

****GE (Gross energy value) was calculated from their chemical composition, using the factors 5.6, 9.45, 4.00 and 4.00 (k cal/g) for protein, fat, fiber and NFE, respectively (Jobling, 1983)

Growth parameters

Body Weight Gain, BWG = Final weight - Initial weight

Survival Rate, SR% = (final fish number / start number) × 100

Specific Growth Rate, SGR = [(In final weight – In initial weight) / Experimental days] × 100

Feed Conversion Ratio, FCR = Dry matter intake / Body weight gain

Protein Efficiency Ratio, PER = TBWG / Crude protein intake

Protein Productive Value, PPV% = [(PR1 – PR0) / PI] × 100

Where, PR1 is the body protein at the end of the experiment, PR0 is the body protein at the start, and PI is the protein intake.

Gene expression analysis

Total RNA was extracted from liver and gill tissues using TRIzol Reagent (Invitrogen; Thermo Fisher Scientific). The RNA was quantified with a Nano Drop 2000 spectrophotometer (Thermo Fisher Scientific) and reverse transcribed into complementary DNA (cDNA) using a High Capacity cDNA Synthesis Kit (Applied Biosystems; Thermo Fisher Scientific), following the manufacturer's guidelines. Real-time quantitative PCR (qPCR) was performed using the Quant Studio™ 5 Real-Time PCR System, with β-Actin serving as the internal control for normalization. Each 20µl qPCR reaction contained 10µl SYBR Green I Master (Roche Diagnostics GmbH), 1µl each of forward and reverse primers, 2µl cDNA template, and 6µl nuclease-free water.

The thermocycling conditions included an initial denaturation at 95°C for 10min, followed by 40 cycles at 95°C for 15 seconds, 58°C for 15 seconds (annealing), and 72°C for 30 seconds (extension). Gene expression relative to β-actin was calculated using the $2^{-\Delta\Delta Ct}$ method **(Livak & Schmittgen, 2001)**. The primer sequences used for gene expression analysis are provided in Table (2).

Table 2. Primer sequences used for gene expression analysis

Gene	Primer Sequences	Reference
GH	F: CTGTCTGTCTGTCTGTCAGTCGT R: AGAGGAGACGCCCAAACAC	**Rentier-Delrue *et al.*, 1989 [24]**
IGF-1	F: CCCGAACTTCCTCGACTTGA R: CCTCAGCCAGACAAGACAAAAA	**Wang *et al.*, 2019 [25]**
IGF-2	F: CCCCTGATCAGCCTTCCTA R:GACAAAGTTGTCCGTGGTGA	**Wang *et al.*, 2019 [25].**
β- actin	F: ACCCACACAGTGCCCATC R: CAGGTCCAGACGCAGGAT	**Monteiro, 2009 [26].**

F: forward R: reverse

Biochemical analysis

Blood samples were drawn from the caudal vein of four fish, which were euthanized using an overdose of 3-amino benzoic acid ethyl ester (Sigma-Aldrich, Basingstoke, U.K.). The collected blood was allowed to clot for two hours, followed by centrifugation at 1600 xg for 25min. The serum was then collected and stored at -20°C until analysis. Levels of alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), blood urea, creatinine, and total cholesterol (g/dL) were measured using commercially available kits (Spectrum Diagnostics, Egypt) following established protocols **(Wu, 2006)**. The completion of biochemical reactions was assessed according to kit instructions using a spectrophotometer (AGILENT CARY 100/300 Series UV-Vis, United States).

Immune parameters

Lysozyme activity

The turbidimetric assay for lysozyme activity followed the method outlined by **Parry *et al*. (1965)**. According to the manufacturer's protocol (Sigma-Aldrich), a suspension of *Micrococcus lysodeikticus* (0.2mg/ ml in 0.05 M sodium phosphate buffer, pH 6.2) was combined with 40 all of serum. The reaction took place at 25°C, with absorbance measured at 530nm after 0.5 and 4.5min. One unit of lysozyme activity was defined as the amount of sample that caused a decrease in absorbance of 0.001/min.

Antiprotease activity

Anti-trypsin activity was measured following the methods outlined by **Ellis (1987)** and **Lange *et al*. (2001)**. Briefly, a standard trypsin solution was mixed with serum and was allowed to incubate at 22°C for 10min. PBS and azocase in solution were then added. Following another incubation period, the reaction was stopped with trichloroacetic acid

(TCA), and the mixture was centrifuged. The absorbance of the resulting supernatant was measured at 410nm using a spectrophotometer.

Total protein

Total protein levels were determined using commercial biochemical kits (Bio-diagnostics, Egypt) and analyzed calorimetrically following the method outlined by **Cannon *et al*. (1974)**.

Statistical Analysis

Data were analyzed using one-way (ANOVA) with **SPSS (2020)**. Duncan's multiple range test was employed to compare mean differences (at a significance level of 5%).

RESULTS

Growth performances

The assessment of growth performance and feed utilization metrics presented in Table (3) shows that the diet labeled D3, which included 4g/ kg of *Saccharomyces cerevisia* (Sc), produced the highest growth performances, as reflected on WG and SGR. This was followed by the diet D2, containing 2g/ kg of Sc, in the absence of microplastics (MPs). These findings were statistically significant ($P< 0.05$) compared to the other treatments. The feed conversion ratio (FCR) was notably lower ($P< 0.05$) at 1.4± 0.01a in the 4g/ kg Sc group, with the control and D2 groups following closely, both showing an FCR of 1.5± 0.01b. In contrast, the group exposed to MPs exhibited asignificantly lower growth performance, with the highest FCR recorded at 1.9 in D5. The inclusion of Sc at 2g/ kg (D6) and 4g/ kg (D7) progressively improved growth parameters, indicating that higher Sc concentrations may counteract the detrimental effects of MPs. The feed utilization parameters aligned with the growth data, as a higher Sc concentration of 4g/ kg, led to improvements in PPR and PER. SR was adversely affected by the presence of MPs, dropping to 75% in D4 (10mg/ L MPs) and 70% in D5 (50mg/ L MPs). Yeast supplementation, at both 2 and 4g/ kg, significantly enhanced SR, achieving rates of 80% in D6, D7, and D8, and 85% in D9.

Table 3. Growth performances and feed utilization of Nile tilapia fed different diets supplemented with levels of Sc (*Saccharomyces cerevisiae*) in presence of two different levels of microplastics (MPs) for 60 days

	Experimental diets								
Parameters	D1 (Control) 0 mg/L **MPs + 0 g/kg *Sc	D2 0 mg/L ** MPs + 2 g/kg *Sc	D3 0 mg/L** MPs + 4 g/kg *Sc	D4 10 mg/L ** MPs + 0 g/kg *Sc	D5 50 mg/L ** MPs + 0 g/kg *Sc	D6 10 mg/L ** MPs + 2 g/kg *Sc	D7 10 mg/L ** MPs + 4 g/kg *Sc	D8 50 mg/L ** MPs + 2 g/kg *Sc	D9 50 mg/L ** MPs + 4 g/kg *Sç
TIW	146.0 ±1.0^{b}	147.0 ± 1.0^{b}	145.0 ± 0.01^{ab}	147.5 ± 0.5^{b}	147.0 ± 2.0^{b}	147.5 ± 0.5^{b}	144.0 ± 2.0^{ab}	141.0 ± 1.0^{a}	145.5 ± 2.5^{ab}
TFW	515.0 ±0.01^{bc}	555.0 ± 0.01^{c}	689.0 ± 0.01^{d}	482.0 ± 0.01^{b}	408.0 ± 0.01^{a}	492.0 ± 0.01^{b}	434.0 ± 0.01^{a}	535.0 ± 35.0^{c}	517.5 ± 7.5^{bc}
TWG%	345.0 ± 0.01^{e}	408 ±1.0^{g}	500.0 ± 0.01^{h}	310.0 ± 0.01^{bc}	240.0 ± 0.01^{a}	310.0 ± 0.01^{c}	20.0 ± 20.0^{b}	325.0 ± 0.01^{d}	372.0 ± 5.0^{f}
FC	1.5 ± 0.01^{b}	1.5 ± 0.0 1^{b}	1.4 ± 0.01^{a}	1.7 ± 0.01^{bc}	1.9 ± 0.01^{d}	1.7 ± 0.01^{c}	1.8 ± 0.01^{c}	1.7 ± 0.09^{c}	1.5 ± 0.1^{a}
RGR%	0.67 ± 0.01^{bc}	0.74 ± 0.01^{c}	0.73 ± 0.01^{c}	0.64 ± 0.01^{ab}	0.59 ± 0.01^{a}	0.63 ±0.01^{ab}	0.62 ± 0.01^{ab}	0.61 ± 0.04^{abc}	0.72 ± 0.01^{c}
SGR	6.7 ± 0.05^{bc}	7.9 ± 0.07^{c}	9.6 ± 0.01^{c}	5.8 ± 0.03^{c}	3.8 ± 0.1^{a}	5.8 ± 0.03^{c}	4.9 ± 0.1^{b}	6.5 ± 0.1^{ab}	7.3 ± 0.03^{c}
PER	2.3 ± 0.01^{d}	1.0 ± 0.01^{a}	2.6 ± 0.01^{e}	2.1 ± 0.01^{c}	2.1 ± 0.01^{b}	2.1 ± 0.01^{c}	2.1 ± 0.01^{c}	1.9 ± 0.01^{c}	1.0 ± 0.01^{a}
PPV	1.5 ± 0.01^{b}	0.7 ± 0.02^{a}	1.4 ± 0.4^{b}	1.3 ± 0.02^{b}	1.3 ± 0.02^{b}	1.4 ± 0.01^{b}	1.3 ± 0.01^{b}	1.3 ± 0.1^{b}	0.6 ± 0.01^{a}
SR	100.0 ± 0.01^{b}	100.0 ± 0.01^{c}	100.0 ± 0.01^{c}	75.0 ± 5.0^{a}	70.0 ± 5.0^{a}	80.0 ± 10.0^{b}	80.0 ± 10.0^{b}	80.0 ± 5.0^{b}	85.0 ± 5.0^{b}

*Sc; *Saccharomyces cerevisiae*,**MPs; microplastics,
BW; body weight, SGR; specific growth rate, FCR; feed conversion ratio, RGR%; relative growth rate, PER; protein efficiency ratio, PPV; protein productivity value, SR; survival rate. Values with different alphabetical superscripts in a row differ significantly (P<0.05) among different diets. All values expressed as mean ± SD.

Impact of Sc and MPs on body composition

At the end of the experiment, the proximate composition of the Nile tilapia carcasses was evaluated. Yeast supplementation led to significant changes in the proximate composition of the fish. An increase in dry matter (DM) was observed in the D2 and D3 groups supplemented with 2 and 4g/ kg of Sc, respectively, compared to the control (D1). Conversely, MPs at 10 and 50mg/ L negatively affected DM in D3 and D4, where no yeast was added. However, the presence of the yeast in the MPs-exposed groups significantly improved DM, especially in D8 and D9 (50mg/ L MPs with 2g/ kg and 4g/ kg Sc). Crude protein (CP) levels peaked in D2 and D3 compared to the control and other groups, while crude fat (EE) followed a similar pattern, with D4 and D5 recording the lowest values. Ash content increased in parallel with MPs concentration, reaching its maximum in D5 (Table 4).

Table 4. Body composition of Nile tilapia fed different diets supplemented with two levels of Sc (*Saccharomyces cerevisiae*) in presence of two levels of microplastics (MPs)

Treatment	Proximate composition (%)			
	DM	**CP**	**EE**	**ASH**
D1 (0 mg/L MPs + 0 g/kg Sc)	28.2 ± 0.1^{d}	66.5 ± 0.4^{c}	18.8 ± 0.4^{c}	14.7 ± 0.3^{b}
D2 (0 mg/L MPs + 2 g/kg Sc)	28.7 ± 0.03^{d}	70.2 ± 1.2^{d}	19.3 ± 1.2^{d}	10.5 ± 0.5^{a}
D3 (0 mg/L MPs + 4 g/kg Sc)	29.1 ± 0.04^{e}	71.4 ± 0.7^{d}	19.6 ± 0.7^{d}	9.0 ± 0.3^{a}
D4 (10 mg/L MPs + 0 g/kg Sc)	27.8 ± 0.1^{bc}	63.3 ± 0.03^{b}	15.8 ± 0.2^{a}	20.9 ± 0.7^{d}
D5 (50 mg/L MPs + 0 g/kg Sc)	27.1 ± 0.02^{b}	62.1 ± 1.2^{b}	16.2 ± 1.6^{a}	21.7 ± 0.6^{d}
D6 (10 mg/L MPs + 2 g/kg Sc)	26.6 ± 0.1^{a}	65.6 ± 0.7^{dc}	16.6 ± 1.4^{b}	17.8 ± 0.3^{c}
D7 (10 mg/L MPs + 4 g/kg Sc)	26.5 ± 0.04^{a}	65.6 ± 1.9^{dc}	20.6 ± 0.6^{d}	13.8 ± 0.3^{b}
D8 (50 mg/L MPs + 2 g/kg Sc)	29.8 ± 0.2^{e}	61.6 ± 1.2^{b}	18.6 ± 0.3^{c}	19.8 ± 0.9^{d}
D9 (50 mg/L MPs + 4 g/kg Sc)	29.7 ± 0.3^{e}	58.1 ± 0.3^{a}	21.9 ± 0.3^{d}	20 ± 1.3^{d}

Gene expression analysis

The expression levels of IGF-1, IGF-2, and GH genes in the liver and gill tissues are shown in Figs. (1, 2).

Gene expression in liver tissues

1. **Effect of Dietary Sc:** Supplementation with Sc (2 or 4g/ kg) slightly elevated the expression of IGF-1, IGF-2, and GH genes compared to the control group. Significant increase was observed only in the GH gene at 2g/ kg of Sc.
2. **Effect of MPs (10 or 50mg/ L):** Exposure to 10 or 50mg/ L MPs resulted in a slight down-regulation of IGF-1, IGF-2, and GH gene expressions. These changes were generally not significant, except for a notable decrease in IGF-2 expression at 50mg/ L of MPs.
3. **Effect of MPs plus Sc:** The combination of 10mg/ L of MPs with Sc (2 or 4g/ kg) significantly improved the expression of IGF-1, IGF-2, and GH genes compared to 10mg/ L MPs alone. However, the improvement in IGF-2 expression was minor and not statistically significant in the 10mg/ L MPs plus 4g/ kg Sc group. Similarly, supplementation with Sc (2 or 4g/ kg) in a 50mg/ L MPs diet led to the significant up-regulation of all studied genes compared to 50mg/ L MPs alone.

Only IGF-1 showed a slight, non-significant improvement in the 50mg/ L MPs plus 4g/ kg Sc group.

Gene expression in gill tissues

1. **Effect of Dietary Sc:** Dietary Sc (2 or 4g/ kg) resulted in the up-regulation of IGF-1, IGF-2, and GH genes compared to the control group. There were significant increases in IGF-1 and IGF-2 at the dose 2g/ kg of Sc and in GH at the 4g/ kg dose of Sc.
2. **Effect of MPs (10 or 50mg/ L):** Treatment with 10mg/ L of MPs caused an insignificant down-regulation of all gene expressions, whereas the treatment with 50mg/ L MPs resulted in an insignificant up-regulation.
3. **Effect of MPs plus Sc:** Supplementing 10mg/ L of MPs with Sc (2 or 4g/ kg) significantly enhanced gene expression, except for IGF-1, which only showed a minor–improvement in the 10mg/ L MPs plus 4g/ kg Sc group. In contrast, no significant improvements were observed in gene expression when fish were treated with 50mg/ L MPs plus Sc (2 or 4g/ kg) compared to 50mg/ L MPs alone.

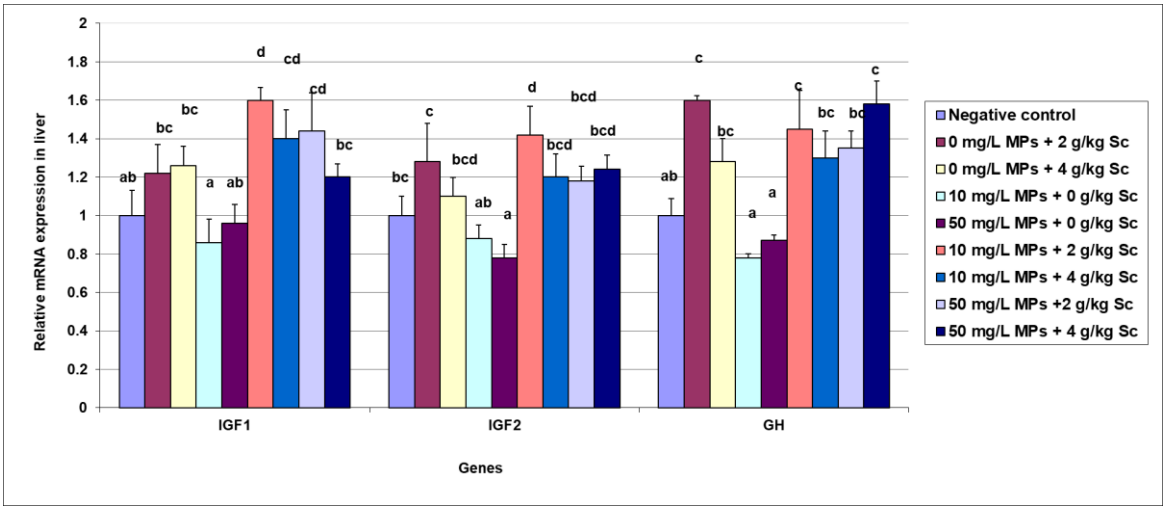

Fig. 1. Relative gene expression levels of IGF-I, IGF-2 and GH genes were determined by real-time PCR in Liver tissues of fish fed diets exposed to MPs (10 and 50mg/ L) and supplemented with Sc (2 and 4g/ kg). MPs= Microplastics, Sc= *Saccharomyces cerevisia*

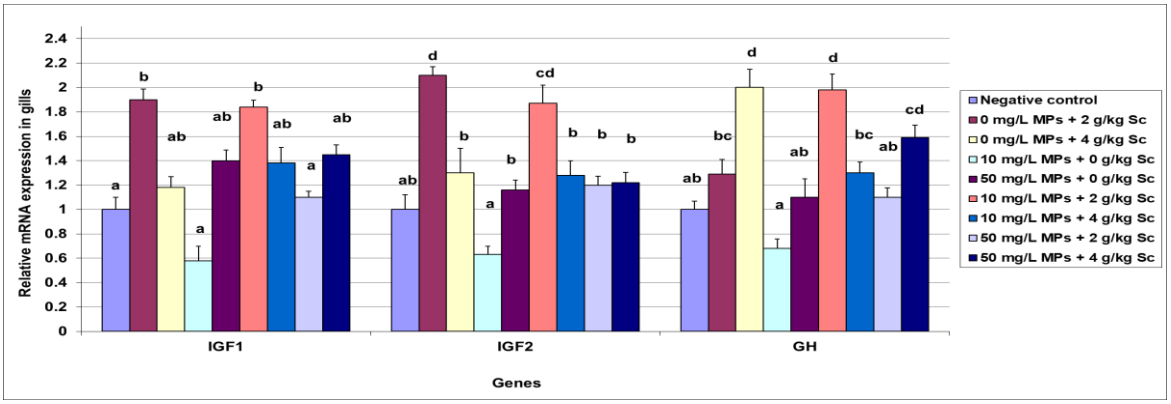

Fig. 2. Relative gene expression levels of IGF-I, IGF-2 and GH genes were determined by real-time PCR in gill tissues of fish fed diets exposed to MPs (10 and 50mg/ L) and supplemented with Sc (2 and 4g/ kg). MPs= Microplastics, Sc= *Saccharomyces cerevisia*

Biochemical analysis

Our investigation assessed the impact of different diets on biochemical markers in *O. niloticus*, as outlined in Table (5). The results revealed that AST levels increased across most experimental groups, except in the group fed 2g/ kg Sc, when compared to the control. ALT levels showed significant-rise ($P< 0.05$) only in groups fed diets containing 2g/ kg Sc and 50mg/ L MPs plus 4g/ kg Sc. Alkaline phosphatase (ALP) levels were notably higher in groups fed diets containing 10mg/ L MPs, 10mg/ L MPs plus 4g/ kg Sc, and 50mg/ L MPs plus 4g/ kg Sc compared to the control and other treatment groups. Kidney function markers, such as blood urea and serum creatinine, also displayed significant elevations ($P< 0.05$) in several treatment groups, particularly those receiving 2g/ kg Sc, 50mg/ L MPs, 50mg/ L MPs plus 2g/ kg Sc, and 50mg/ L MPs plus 4g/ kg Sc. Cholesterol levels were significantly higher ($P> 0.05$) in the group fed 4g/ kg Sc, although these increases did not reach statistical significance in other groups.

Immune parameters

The study also examined immune responses, revealing that fish exposed to microplastics, either alone or with yeast (excluding 50mg/ L MPs plus 2g/ kg Sc), showed increases in lysozyme (Fig. 3) and antiprotease activities (Fig. 4) compared to other groups. The group treated with 10mg/ L MPs plus 4g/ kg Sc had the highest antiprotease activity ($P< 0.05$), while the group fed 50mg/ L MPs plus 4g/ kg Sc had the highest lysozyme activity ($P< 0.05$). Similarly, the group fed 50mg/ L MPs plus 4g/ kg Sc exhibited the highest total protein levels (Fig. 5).

Table 5. Changes in biochemical parameters of Nile tilapia fed diets with different concentrations of MPs and Sc

Parameters	Experimental diets								
	D1	**D2**	**D3**	**D4**	**D5**	**D6**	**D7**	**D8**	**D9**
	0 mg/L **MPs + 0 g/kg *Sc	**0 mg/L **MPs + 2 g/kg *Sc**	**0 mg/L **MPs + 4 g/kg *Sc**	**10 mg/L **MPs + 0 g/kg *Sc**	**50 mg/L **MPs + 0 g/kg *Sc**	**10 mg/L **MPs + 2 g/kg *Sc**	**10 mg/L **MPs + 4 g/kg *Sc**	**50 mg/L **MPs + 2 g/kg *Sc**	**50 mg/L **MPs + 4 g/kg *Sç**
AST (IU/L)	129.5 ± 13.4^{ab}	226.5 ± 33.2^{cd}	107.5 ± 6.4^{a}	197.5 ± 4.9^{abc}	191.5 ± 4.9^{bc}	235 ± 35.4^{cd}	236 ± 17.0^{cd}	198.5 ± 13.4^{abc}	269.5 ± 13.4^{d}
ALT (IU/L)	12 ± 1.41^{a}	17 ± 1.4^{ab}	12 ± 1.4^{a}	13.5 ± 0.7^{a}	12.5 ± 0.7^{a}	12.5 ± 0.7^{a}	14.5 ± 0.7^{ab}	13.5 ± 0.7^{a}	20 ± 1.4^{b}
AlP(mg/dl)	72.5 ± 23.3^{a}	99 ± 2.8^{abc}	93 ± 8.5^{abc}	113 ± 7.1^{abc}	83 ± 11.3^{ab}	75.5 ± 3.5^{a}	121 ± 5.7^{bc}	98.5 ± 10.6^{abc}	129.5 ± 0.7^{c}
Creatinine (mg/dl)	0.2 ± 0.01^{a}	0.35 ± 0.07^{abc}	0.3 ± 0.01^{ab}	0.3 ± 0.01^{ab}	0.35 ± 0.1^{abc}	0.25 ± 0.1^{ab}	0.3 ± 0.01^{ab}	0.4 ± 0.01^{bc}	0.5 ± 0.01^{c}
Urea (mg/dl)	2.2 ± 0.28^{a}	3.3 ± 0.3^{ab}	2.6 ± 0.3^{ab}	2.75 ± 0.2^{ab}	3.1 ± 0.1^{ab}	2.05 ± 0.1^{a}	2.75 ± 0.4^{ab}	4.0 ± 0.3^{bc}	5.2 ± 1.0^{c}
Total Cholesterol (mg/dl)	130 ± 22.6^{a}	133 ± 8.5^{a}	251 ± 65^{b}	158.5 ± 4.9^{ab}	148.5 ± 4.9^{a}	175 ± 1.4^{ab}	175 ± 14.1^{ab}	161 ± 2.8^{ab}	177.5 ± 24.7^{ab}

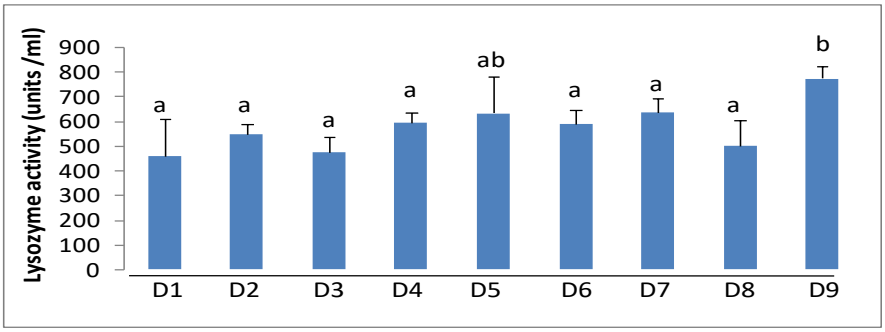

Fig. 3. Lysozyme activity of the Nile tilapia fed with different groups. The same letter is not significantly different (P> 0.05). The bars referred to the mean ± standard error.

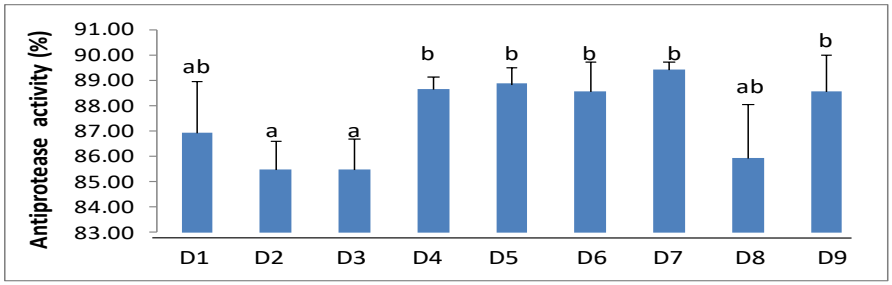

Fig. 4. Antiprotease activity of the Nile tilapia fed with different groups. The same letter is not significantly different (P> 0.05). The bars referred to the mean ± standard error

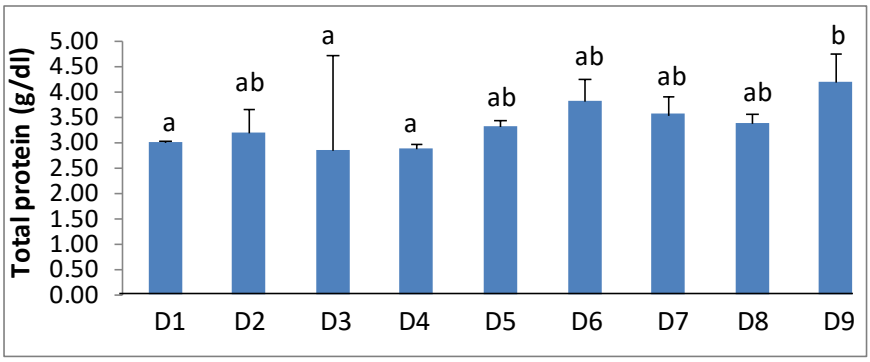

Fig. 5. Total protein of the Nile tilapia fed with different groups. The same letter is not significantly different ($P > 0.05$). The bars referred to the mean ± standard error.

DISCUSSION

Growth performance and feed utilization

Microplastics (MPs) have been shown to have toxic effects on various aquatic animals **(Banaee *et al.*, 2019)**. This study explored a novel approach to mitigating these effects in aquatic animals using probiotics, specifically yeast. SC, widely used in aquaculture feeds, offers an environmentally friendly alternative to antibiotics and has demonstrated potential for enhancing the growth performances of the Nile tilapia **(Abozaid *et al.*, 2024)**. Prior studies, such as **Abu-Elala *et al.* (2018)**, have reported the positive impact of dietary yeast on the Nile tilapia production **(Abozaid *et al.*, 2024)**. Furthermore, the adverse effects of MPs on these parameters can be mitigated by adding yeast to commercial diets, leading to increased survival rates. MPs present in aquatic environments pose significant risks to fish, disrupting their physiological processes **(Frank *et al.*, 2023)**. These particles can accumulate in the gastrointestinal tract, gills, and hepatopancreas of fish, resulting in oxidative stress, immune alterations, neurotoxicity, stunted growth, hormonal imbalances, behavioral changes, and damage to reproductive organs **(Dhar *et al.*, 2023; Subaramaniyam *et al.*, 2023)**.

Gene expression analysis

In this study, we observed altered expressions of IGF-1, IGF-2, and GH genes in the liver and gill tissues of fish exposed to MPs (10 and 50mg/ L). RT-qPCR analysis revealed down-regulation of gene expressions in liver tissues at both 10 and 50mg/ L of MPs, and in gill tissues at 10mg/ L. Interestingly, exposure to 50mg/ L of MPs led to up-regulation of all genes in gill tissues. Our findings are consistent with those of **Feng *et al.* (2022)**, who reported significant down-regulation of the Fen1 gene in the zebrafish embryos treated with 100, 200, and 400mg/ L of polystyrene nanoparticles. Similarly, **Espinosa *et al.* (2017)** found decreased expression of peroxiredoxin 5 and heat shock

protein 90 genes in the gilthead seabream exposed to polyvinyl chloride MPs. On the other hand, the up-regulation observed in our study aligns with that of **Limonta *et al.* (2021)**, who reported significant up-regulation of the CYP2P8 gene in the zebrafish exposed to MPs. The genotoxic effects of MPs, evidenced by altered gene expressions, may be due to the toxic chemical structures within the MPs matrix, such as bisphenol A (BPA), polybrominated diphenyl ethers (PBDE), nonylphenol (NP), and octylphenol (OP) **(Alberghini *et al.*, 2022)**. These chemicals can induce oxidative stress and inflammatory responses by generating reactive oxygen species (ROS) and nitric oxide (NO), leading to cellular damage and impaired gene expressions **(Rahman *et al.*, 2021)**. **Allard and Colaiácovo (2010)** demonstrated that BPA treatment caused down-regulation of the DSBR gene in mammalian cells. Similarly, **Aboelhassan *et al.* (2022)** found that BPA exposure in rats decreased nucleic content and reduced gene expressions. In the African catfish, MPs exposure resulted in a reduced carbohydrate content and adverse effects on metabolism, impairing gene expressions **(Sayed *et al.*, 2023)**. In contrast, our results show that supplementing a MPs-containing diet with *Saccharomyces cerevisia* (Sc) can mitigate the adverse effects of MPs and improve the expression of IGF-1, IGF-2, and GH genes in liver and gill tissues. To our knowledge, this is the first study to report Sc's protective role against MPs-induced genotoxicity in fish. However, **Eshak *et al.* (2010)** found that quail birds fed a diet supplemented with Sc showed significant improvement in neural and gonadal gene expressions compared to birds on an aflatoxin B-treated diet. Additionally, **El-Bab *et al.* (2022)** reported significant up-regulation of IGF-1 and IL-1B genes in the seabream fish fed a diet supplemented with 4g/ kg Sc. **Islam *et al.* (2021)** also demonstrated improved growth performance and feed utilization in *O. niloticus* fed a diet with 4g/ kg Sc. Sc cells contain high levels of zinc, selenium, vitamins, carotenoids, minerals, essential amino acids, β-D-glucans, and mannan oligosaccharides, which are potent antioxidants and antimutagenic agents. These components help inhibit oxidative processes, scavenge free radicals, and prevent DNA oxidative damage, leading to genomic stability and maintenance of gene expressions **(Brown & Gordon, 2003; Van Breda *et al.*, 2005)**.

Biochemical analysis

Our study revealed significant changes in key biochemical markers. Specifically, levels of alanine aminotransferase (ALT) and aspartate aminotransferase (AST) fluctuated significantly among the diet groups containing MPs, indicating potential liver stress or damage. Additionally, variations in alkaline phosphatase activity, blood urea, creatinine, and total cholesterol suggest disruptions in metabolic and renal functions. These findings align with those of **Gholamhosseini *et al.* (2023)**, who observed increased AST, ALT, and ALP activities in the crayfish exposed to polyethylene MPs. **Choi and Kim (2023)** also reported significant changes in plasma components, including AST, ALT, and ALP, in crucian carp exposed to polyamide MPs. Elevated blood urea levels in fish-fed MPs

diets suggest possible renal stress or dysfunction **(Wang *et al.*, 2020; Lu *et al.*, 2021)**. Moreover, increased serum creatinine levels indicate potential kidney impairment **(Zhang *et al.*, 2019; Li *et al.*, 2020)**.

Immune responses

MPs are recognized as foreign particles by the immune system, which can either stimulate immune responses or suppress immune function by inducing immunotoxicity. For example, common carp exposed to MPs or cadmium showed altered immunological parameters, with more significant changes observed when exposed to both simultaneously **(Banaee *et al.*, 2019)**. Another study discovered immunosuppressant in juvenile carp exposed to polyamide MPs **(Choi *et al.*, 2023)**. Conversely, dietary intake of polyvinylchloride MPs enhanced the innate immune response of the gilthead seabream, increasing peroxidase and immunoglobulin levels and decreasing phagocytosis **(Espinosa *et al.*, 2017)**. Our results also indicated enhanced immune parameters (lysozyme and antiprotease activities and total protein) in fish administered MPs alone or in combination with yeast. Previous studies have shown that yeast supplementation in the Nile tilapia diets enhances antioxidants, immune function, growth performance, and resistance to Aspergillus flavus infection **(Abdel-Tawwab *et al.*, 2020; Banu *et al.*, 2020)**.

CONCLUSION

Our findings suggest that dietary supplementation with yeast, *Saccharomyces cerevisia* is an effective probiotic strategy for mitigating the toxic effects of MPs. Yeast supplementation significantly improved growth performance and growth-associated gene expressions, with partial improvements in biochemical and immune parameters. The 4g/ kg dose was generally more effective than the 2g/ kg dose, but both levels provided beneficial protection against MPs toxicity.

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