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RESEARCH ARTICLE

The efficacy of biogenic chitosan nanoparticles in controlling *Ichthyophthirius multifiliis* infection in Nile Tilapia (*Oreochromis niloticus*)

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ABSTRACT

Ichthyophthirius multifiliis (*I. multifiliis*) is a parasite that poses a serious hazard to global freshwater aquaculture. This study investigates the efficacy of biologically synthesized chitosan nanoparticles (ChNPs) as a sustainable antiparasitic alternative for controlling *I. multifiliis* in Nile tilapia. Thus, a total of 400 mono-sex Nile tilapia fingerlings were randomly distributed into 40 glass aquaria (eight groups, five replicates, 10 fish each). The groups included: control, T1–T3 (ChNPs at 80, 160, and 320 mg/kg), T4 (praziquantel-treated), T5 (*I. multifiliis*-infected), T6 (*I. multifiliis* + ChNPs, 320 µg/kg), and T7 (*I. multifiliis* + praziquantel, 320 µg/kg). ChNPs characterization showed a UV peak at 220 nm, a spherical shape in TEM, and an exact size of 80 nm in DLS. ChNPs (320 µg/mL) and showed strong antioxidant activity, scavenged 88% of DPPH, and showed 90% inhibition of *I. multifiliis* trophonts via direct lysis, as confirmed by TEM. In vivo, dietary ChNPs (320 µg/kg) enhanced growth performance, increasing final body weight by 29 % and improving FCR (4.0 vs. 4.9). Infected fish treated with ChNPs showed full survival (100 vs. 88.7% in untreated), reduced cortisol levels by 76%, and restored liver function, where reducing the ALT by 50 % compared to infected untreated fish. ChNPs modulated immune reaction, enhancing NBT activity (0.87 vs. 0.41 in controls) and upregulating immune genes [Interleukin-8 (CXCL8) and Type II interferons (IFNϒ)] in healthy fish, while suppressing hyperinflammation in infected fish. Histopathological examinations showed that ChNPs effectively reversed gill damage by significantly reducing hyperplasia and lowering trophont burden, revealing a therapeutic impact contrasted to that of praziquantel. It could be concluded that ChNPs has a dual-action therapeutic, directly targeting *I. multifiliis* and enhancing host health via immunomodulation and growth promotion. The efficacy, safety, and sustainability of ChNPs make them potent alternatives to chemical parasiticides in aquaculture.

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INTRODUCTION

Freshwater aquaculture continues to play a critical action in global food security and economic development, providing an important source of good cheap protein to millions all over the world (Boyd *et al.*, 2022; Alsulami *et al.,* 2025). Nile tilapia (*Oreochromis niloticus*) is one of the most widely cultured freshwater fish species due to its fast growth, adaptability to different ecological status, as well as consumer preference (Bonham, 2022). Nonetheless, the sustainability of tilapia aquaculture faces a major challenge from parasitic diseases, which adversely impact fish health, production efficiency, and economic viability (Abd El-Hack *et al.*, 2022; Metwaly *et al.*, 2025).

One of the most serious parasitic diseases in freshwater aquaculture is *I. multifiliis* that usually known as “Ich” or “white spot disease.” This ciliated protozoa causes severe disease in many freshwater fish species, including Nile tilapia (Uma, 2025). The parasite’s lifecycle involves three key phases: the infective free-swimming theront, the feeding trophont embedded in the fish’s epidermis and gills, and the reproductive tomont phase encysted in the environment (Kong *et al.*, 2024).

I. multifiliis infection produces severe clinical signs that include white cyst-like spots on the skin, fins, and gills, respiratory distress, behavioral changes such as flashing, lethargy and in most cases, the respiratory illness is life-threatening (von Gersdorff Jorgensen & Puspasari, 2022).

This disease has an economic cost to the aquaculture production in reducing the growth rate of fish, raising their mortality rates, and raising the costs of treatment (Mukaila *et al.*, 2023).

Treatment of *I. multifiliis* is not an easy task and the conventional approaches are mainly based on the use of chemical therapeutic agents such as formalin, copper sulfate, malachite green, and potassium permanganate that could be used to address the free-swimming amoeba in the water (Abou-Okada *et al.*, 2021). Nevertheless, such chemicals are usually problematic in their ecological toxicity, residue issues with different efficacies; besides, these treatments do not reach the trophont stage within the epidermis of fish (Elgendy *et al.*, 2024). Biological control approaches are receiving attention but can't provide any trustworthy applicalpe remedy to commercial aquaculture (Calcagnile *et al.*, 2024; Nolasco-Alzaga *et al.*, 2025). Moreover, the attempts at vaccinating dogs are still problematic because of the complex nature of the parasite life cycle and the variability of antigens (Rathor and Swain, 2024). Therefore, it is still imperative that novel, sustainable, and efficient methods of managing *I. multifiliis* infections are developed.

Nanotechnology has also become a new discipline that provides viable instruments to fight the aquatic pathogens. Nanoparticles can enhance the transfer of bioactive substances and their effectiveness due to their small size, intensive surface area, and the possibility to tune surface characteristics, which can also directly react to a pathogen to prevent infection (Ogunfowora *et al.*, 2021; Sharjeel *et al.*, 2024). Moreover, the use of nanoparticles will help boost the immunity of fish and minimize environmental pollution with traditional chemicals, which is in line with the trends of sustainable aquaculture (Saad *et al.*, 2022; Mahmud and Haque, 2025).

One of such nanomaterials is the chitosan nanoparticles (ChNPs), which have received significant interest due to their biocompatibility, biodegradability, and inherent antimicrobial qualities (Acharya *et al.*, 2025). Chitosan is a natural polysaccharide derived in chitin, which has antiparasitic effects of a general spectrum (Silva *et al.*, 2021). The bioactivity of CSNPs is also better than that of bulk chitosan as they are nanoscale, allowing them to better interact with the surfaces of parasites (Poznanski *et al.*, 2023). The effectiveness of ChNPs against various pathogens in aquaculture has been tested in different studies (Ahmed *et al.*, 2021; Eissa *et al.*, 2025); yet, there is a lack of research on the antiparasitic effectiveness of the compound, especially against *I. multifiliis*.

The past research on the *I. multifiliis* control has been biased towards the conventional use of chemicals as a means of control or immunology, and comparatively little research has been done on the possibility of nanotechnology-based products, such as ChNPs, to control parasites. As an illustration, although other studies have examined the application of silver and zinc oxide nanoparticles in combating fish parasites, the issue of toxicity and environmental safety restrictions its practical application (Nasr-Eldahan *et al.*, 2021). In the meantime, most studies on ChNPs have been conducted on antimicrobial activity in Nile tilapia and their immunostimulating properties (Aly *et al.*, 2023; Ponce *et al.*, 2024). Furthermore, ChNPs were proven to be effective against protozoan parasites through interference with cell membranes of parasites and increased synthesis of reactive oxygen species (ROS), causing the death of the parasites (Alkhalil, 2025). Furthermore, it is also proposed by the research on the mechanism of action that ChNPs are able to regulate host immune responses by stimulating macrophage activities and cytokine production, which improves the elimination of parasitic infection (Balasubramaniyan *et al.*, 2022). In aquaculture, the research has also shown that ChNPs enhance fish resistance to parasitic infections through enhancing mucosal immunity, decreasing parasite adhesion and invasion (Hossam-Elden *et al.*, 2024). Nevertheless, there is still a serious gap in the identification of the direct participation of ChNPs in the fight against *I. multifiliis* infections. This knowledge gap is also significant to the investigation of ChNPs as a safer and more effective antiparasitic drug that may help to increase the well-being of fishes and reduce the chemical residues in aquaculture.

Accordingly, this study directed to investigate the therapeutic effect of ChNPs in controlling *I. multifiliis* infection in Nile tilapia and study their effect on fish health parameters including survival rates under experimental infection conditions. Furthermore, the study explores the mechanisms by which ChNPs exert antiparasitic effects, contributing to a better understanding of their application in freshwater aquaculture parasites control.

MATERIALS AND METHODS

Production of *Jatropha dioica* extract: Fresh jatropha leaves of *Jatropha dioica* were harvested and dried for 7-10 days in a well-ventilated, shaded room. The leaves of the three tested species were crushed into fine powder and used in various investigations. Two hundred grams of powdered leaves were extracted with ethanol, HPLC grade (Advetchembio PVT. LTD, India) for 24h while the mixture was continuously shaken. Whitman's Filter paper (No. 1) was used to filter the extracts. The rotary evaporator (BUCHI Rotavapor® R-100, Switzerland) was used to concentrate the filtrate at 46°C. Finally, at 4 °C, the crude extracts were stored for further experiments (Omoregie and Folashade, 2013).

Synthesis and characterization of ChNPs: The procedure for synthesizing chitosan nanoparticles (ChNPs) was modified according to El-Naggar *et al.* (2022a, b). Chitosan was first dissolved at 1% (w/v) in 1% (v/v) acetic acid. The pH was adjusted to 4.8±0.02 using 1 N NaOH at 25 °C. To confirm whole dissolution, the solution was stirred manually until a uniform the mixture, then shaken at 210 rpm overnight at 25 °C. After that, 10 mL of *Jatropha dioica* leaf extract was mixed with 10 mL of the chitosan solution (maintaining a 1:1 v/v ratio). This mix was then stirred at 110 rpm for 1 hour at 50 °C to ease the formation of ChNPs. Then, the suspension was centrifuged at 4000×g for 20 minutes, and the liquid stage was separated. The supernatant underwent a further centrifugation at 10,000×g for 10 minutes before being completely discarded. To purify the ChNPs via eliminating excess chitosan, the

resulting pellet was washed with 1% (v/v) acetic acid. It was centrifuged another time at 10,000×g for 10 minutes, and the washing step was repeated twice.

The color of the media begins to change simultaneously with Jatropha extract and eventually turns yellow within the 32-hour incubation period. Confirmatory characterization of chitosan nanoparticles was done using a UV-visible spectrophotometer (PG instrument, China T60U) at wavelengths ranging from 220 to 350 nm, Transmission electron microscopy (TEM, Leo 0430; Leica, Cambridge, UK) to analyze the dimension and the structural aspects morphology, shape, size, and distribution‖ of biogenic ChNPs. Green ChNPs were eventually centrifuged using a laboratory centrifuge (SIGMA 2-16KL) at 5000 rpm at 25 °C for 2–5 minutes. After separation, all cells were rinsed with sterile deionized distilled water and centrifuged again under the same previously established conditions until further analysis (Faramarzi *et al.*, 2020).

Biological activities of ChNPs: *Antioxidant activity*: The DPPH scavenging activity of ChNPs was estimated by Alsulami and El-Saadony (2024) with some modifications. After adding 100 µL of ethanolic DPPH to 100 µL of ChNPs (20, 40, 80, 160, and 320 µg/mL) and incubating for 15 minutes in the dark, the resulting color was assessed using a microplate reader (517 nm). The absorbance was incorporated into the subsequent equation.

$$\% \text{ antioxidant activity} = \frac{\text{OD control} - \text{OD sample}}{\text{OD control}} \text{x100}$$

***Antiparasitic activity*:** *I. multifiliis t*rophonts were directly harvested from infected fish for the assay. de Freitas Oliveira *et al.* (2021) evaluated the antiparasitic properties of ChNPs using the following methodology with some modifications: The culture of *I. multifiliis* at a concentration of 1×10^7 parasites/mL was distributed into 96-well plates. The wells were treated with ChNPs at levels of (20, 40, 80, 160, and 320 µg/mL) and kept for 24 hours at 28°C. After-incubation, resazurin reagent was added to every well. The viability of the parasites was estimated *via* colorimetric analysis, with the results expressed as a percentage of parasite mortality.

Experimental design: A 400 mono-sex of the Nile tilapia fingerlings (7.08±0.08) were used in the current study. The fish were transported to the experimental aquaria in tanks provided with oxygen. Immediately upon arrival, the fish were placed in glass aquaria filled with dechlorinated water and continuously aerated by a central air blower. The fish received the basal diet for three weeks to adapt to the new environment. At the beginning of the study, fish were randomly assigned to 40 glass aquaria (eight groups, five replicates, 10 fish per replicate). The first group was control, T1, T2, and T3 were Nile talipa fish treated with chitosan nanoparticles (80, 160, and 320 mg/kg), T4 was praziquantel-treated fish, T5 was *I. multifiliis* (IM) infected fish, T6 was IM-infected and treated with chitosan nanoparticles at 320 µg/kg, meanwhile, T7 was IM-infected fish and treated with praziquantel (320 µg/kg). Each glass aquarium (60 × 30 × 40 cm) contained 60 L of water. For 10 weeks, each group was fed its experimental diet two times daily (8:00 AM and 4:00 PM) until satiation, and daily feed intake was recorded. Using aerated and dechlorinated water, one-third of each tank was replaced twice daily. The remaining feed and feces were siphoned with the changed water. The experimental parameters included a water temperature of 26-30°C, ammonia levels below 0.2 mg/L, pH of 6.4, nitrite at 0 mg/L, total hardness of 144 mg/L, undetectable free chlorine, and total alkalinity of 120 mg/L. The lighting program was 12 hours of light and 12 hours of darkness. To challenge fish with *IM*, fish in T5, T6, and T7 were immersed in buckets containing 5 Liters of water with IM for 20 minutes. The infected fish were then placed in aquaria.

Fish and blood Sample collection: After 10 weeks of feeding experimental diets, the fish were anesthetized with 25 mg/L tricaine methane sulfonate (MS-222). Then, the fish were weighed individually to obtain the final weight. For assays requiring whole blood, five fish per group had blood drawn from the caudal vein into EDTA. Blood was also collected in anticoagulant-free tubes for serum preparation (5 fish per group). Then, samples were centrifuged at 3000 rpm for 15 minutes at 4°C to separate the serum, that was then frozen at −20°C until use. For gene expression analysis, gill samples were collected from three fish per group on Cellixi-RNA Guard reagent and frozen at -20°C until use. For histological analysis, gill specimens were dissected from the same site (40 fish, five per group) and immediately fixed in a 10% neutral buffered formalin until analysis.

Growth parameters: As demonstrated below, the fish growth performance parameters were computed in accordance with Abu-Elala *et al.* (2021). The estimation of survival rate and mean survival time was adopted following Khalil *et al.* (2023).

Body weight gain (BWG) = Final body weight (FBW)g - Initial body weight (IBW)g (1)

$$\text{Weight gain rate (WG \%)} = \frac{\text{FBW}-\text{IBW}}{\text{IBW}} \text{x100} \quad (2)$$

$$\text{Feed conversion ratio (FCR)} = \frac{\text{Feed intake (g)}}{\text{BWG (g)}} \quad (3)$$

$$\text{Speceific growth rate } \left(\frac{\text{SGR}}{\text{day}}\right)\% = \frac{100(\text{InFBW}-\text{InIBW})}{\text{No days}} \quad (4)$$

$$\text{Survival rate (SR \%)} = \frac{\text{No fish at the end of experiment}}{\text{No fish at the beganinng of experiment}} \text{x100} \quad (5)$$

Biochemical analysis protocol: Blood samples (3 mL) were gathered from the caudal vein via heparinized needles and then they were immediately transferred to Eppendorf tubes and kept at 4°C. Then samples were centrifugation (Sigma 3-30K, Germany) at 10,000 rpm for 5 minutes at 4°C to obtain serum. Biochemical parameters were estimated following Al-Deriny *et al.* (2020) and Dawood *et al.* (2020b), via using kits (Bio-Diagnostic Co) Absorbance measurements were performed calorimetrically using a BioTek Elx808 microplate reader (USA) at specified wavelengths. The following markers were measured: metabolic markers, including blood glucose (GLU, G3293, Sigma, USA), and hepatic enzymes, like Alanine transaminase (ALT), ALT100, Sigma, USA)

and Aspartate aminotransferase (AST), AST100, Sigma, USA). Immune function, i.e., respiratory burst activity via nitro blue tetrazolium (NBT) assay and lysozyme (LYZ, NBP3-21149, Novus Biologicals, USA) activity, and endocrine profiling, such as cortisol, reproductive hormones: Testosterone, Progesterone, Follicle-stimulating hormone (FSH, MBS035576, MyBiosource, USA), and growth hormone (GH, SL0052FI, Sunlong Biotech Co., LTD, China).

Gene expression

RNA isolation, cDNA synthesis, and real-time PCR: Gene expression in fish was analyzed using quantitative real-time PCR (qPCR) on a Step OnePlus Real-Time PCR System (Applied Biosystems) in a 96-well format. Each assay was performed in triplicate with DNA or cDNA templates, and amplification was adopted with Fast SYBR Green Master Mix (Applied Biosystems). For DNA-based assays, gill-derived DNA was tested with IM 18S primers to assess parasite infection levels, while tilapia β-actin served as the reference gene for normalization. Normalized values were calculated by determining the difference in cycle threshold (Ct) among β-actin and the parasite 18S gene, followed by expression analysis using the 2−ΔCT method.

For RNA-based studies, glyceraldehyde-3-phosphate dehydrogenase (GAPDH) was utalized as control. Gene expression was quantified applying the comparative Ct method (ΔΔCt). The ΔCt values were measured by subtracting the GAPDH Ct from that of the target gene, while ΔΔCt values were obtained via the ΔCt values of uninfected control samples. Relative expression (fold change) was estimated applying the 2−ΔΔCt method (Schmittgen and Livak, 2008). Each PCR reaction was adopted in a 10 µL total volume with 5 µL of 2× Fast SYBR Green Master Mix, 0.5 µL of each forward and reverse primer (10 µM), 3 µL of nuclease-free ultrapure water, and 1 µL of DNA or cDNA (25 ng/µL). Nuclease-free water was utilized as the no-template control (NTC). For initial validation and baseline threshold determination, cDNA at 25 ng/µL was used.

The thermal cycling protocol involved an initial denaturation step at 95°C for 20 seconds, then after that 40 amplification cycles at 95°C for 3 seconds and 60°C for 30 seconds. Melt curve analysis was formed with a cycle of 95°C for 15 seconds, 60°C for 1 minute, and then heating back to 95°C. Quantitative results were analyzed applying the 2−ΔΔCt method (Livak & Schmittgen, 2001).

Histological examination of gills: The skeletal gill samples (3 per group) were processed and then examined as described by Husseiny et al. (2021). Specimens were gradually dehydrated, cleared, then embedded in paraffin, and then they were cut into sections 5-7 µm thick via a rotary microtome. Two sections from each sample were loaded on glass slides, then they were stained with Hematoxylin and Eosin (H&E) finally, they were tested using bright-field microscope with a camera. Six photos of fixed cross-section areas were taken from different fields of each slide, and the numbers of cells were counted using the "Cell Counter" option of the ImageJ software.

Statistical analysis: The results were inspected for ANOVA assumptions, involving the normality and homogeneity of variance. One-way ANOVA and LSD test were used for normally distributed data. For non-normally distributed data, the Kruskal-Wallis test with pairwise comparisons was applied. The statistical analyses were performed with the help of SPSS 25s (IBM, USA). Confidence interval of 95% was maintained ($P<0.05$) for statistical significance.

RESULTS

The trophont stage of *I. multifiliis* possesses a characteristic morphology and the trophonts are usually large, microscopic to almost 1 mm in diameter and were rounded, oval or even spherical. The whole of their surface is thickly interlaced with lines of short, beating cilia, and when they see it they move in an unusual, steady rolling or moving motion. One of the hallmark diagnostic features is that they have very large, frequently faintly visible, horseshoe-shaped or C-shaped macronucleus (Fig. 1A, B). Although a smaller, more inconspicuous micronucleus is also present, the most easily visible under a light microscope is the macronucleus. At the feeding stage, trophonts can possess various food vacuoles, which illustrate their intake of host cellular debris, mucus and host cells. This size, shape, surface cilia, and the shaped macronucleus are what give specific microscopic characterization of *I. multifiliis* trophonts in infected tissues.

The UV absorbance spectrum of the ChNPs is indicated in Fig. 2 A. It demonstrates a strong, sharp absorption peak in the region of 220 nm, typical of chitosan and confirms the presence of the nanoparticles. Figure B is a TEM image which shows the morphology and the distribution of the ChNPs. As can be observed in the image, the size of ChNPs is quite uniformly distributed, with a distinct majority being spherical and well-dispersed, which is also denoted by the scale bar that shows 50 nm. Figure C presents an analysis of a size distribution by number which was probably measured by dynamic light scattering (DLS). The graph is a sharp, narrow peak which suggests that the ChNPs are of uniform, comparatively small sizes, most of the ChNPs being clustered around a certain diameter (seems to be approximately 80 nm, judging by the peak). Lastly, Figure D demonstrates zeta potential distribution of the ChNPs. The graph indicates that the negative zeta potential range (-27.33 mV) has a single and high peak, which means that the ChNPs are positively charged on the surface.

Fig. 3 once again shows the great antioxidant capability of ChNPs produced using *Jatropha dioica* extract as evidenced by the capacity to liberate DPPH free radicals. One notable concentration dependent effect is apparent: with increase in concentration of ChNPs, its DPPH scavenging activities increases exponentially. In the lowest level of 20 mg/mL, the ChNPs have approximately 30% scavenging activity. The level of this activity increases gradually but steadily to about 50 percent at 40mg/mL, almost 58 percent at 80mg/mL and approximately seventy three percent at 160mg/mL. The maximum antioxidant activity is observed at 320 mg/mL where the ChNPs have virtually 88 percent DPPH scavenging activities.

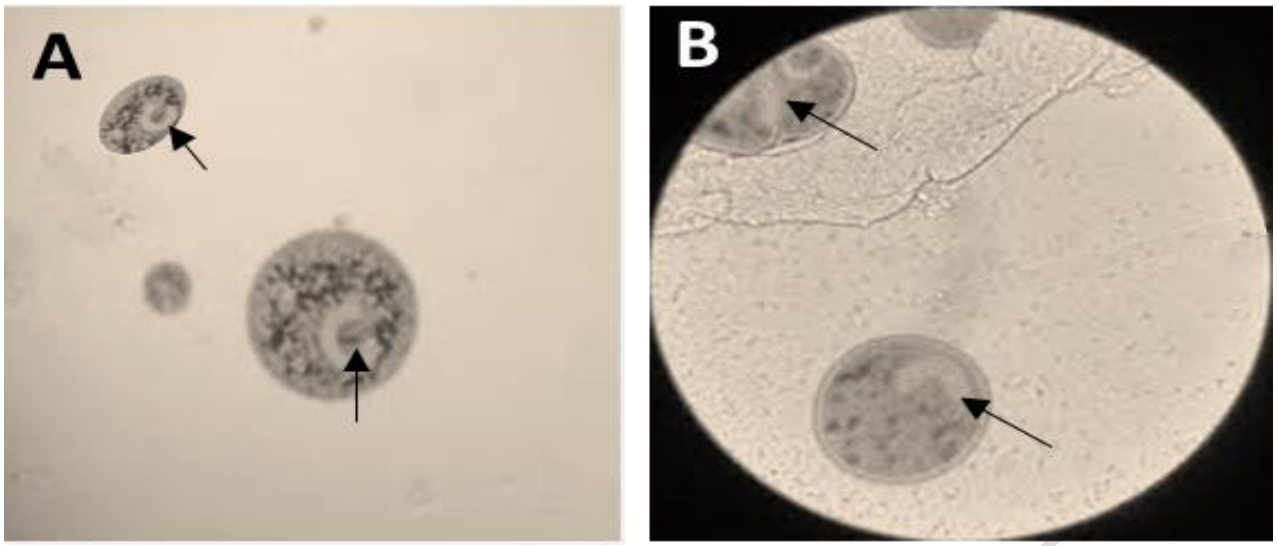

Fig. 1: Microscopic morphology of *Ichthyophthirius multifiliis* trophonts showing (A) individual parasites and (B) detailed cellular structure with visible nucleus.

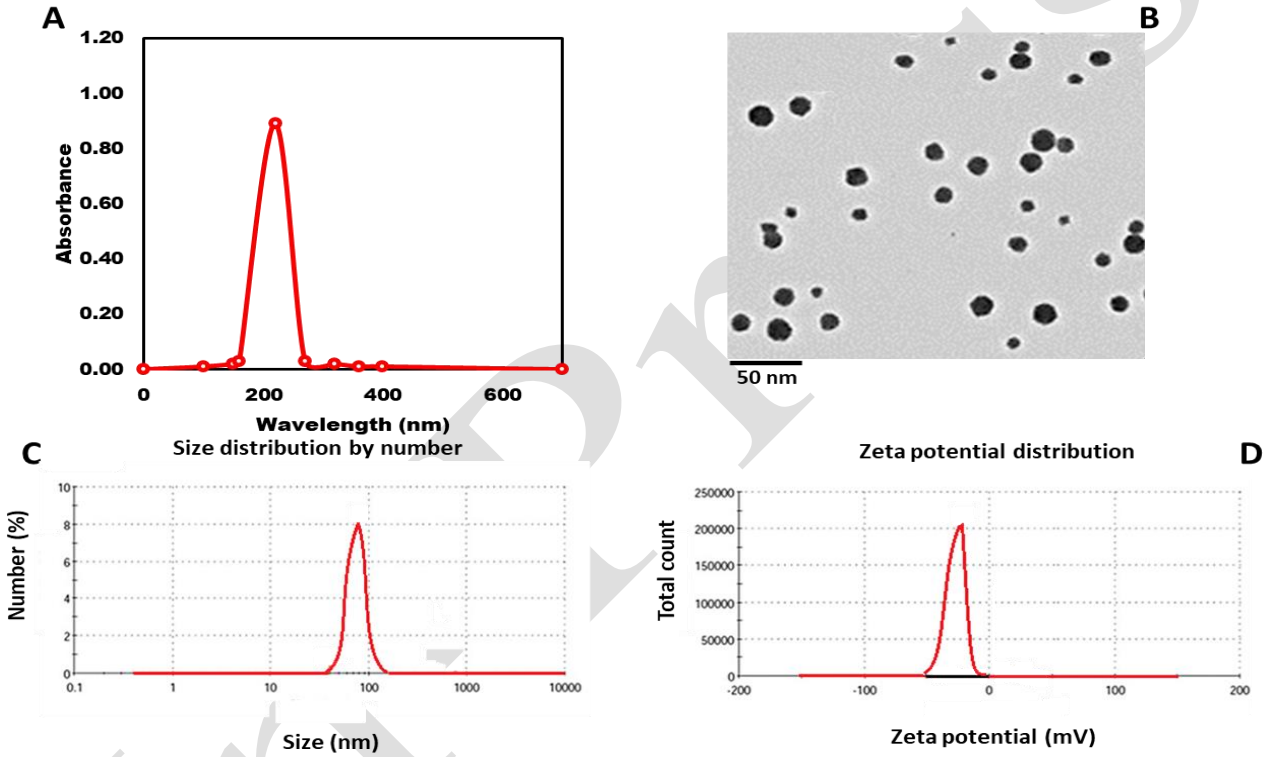

Fig. 2: Characterization of chitosan nanoparticles fabricated by *Jatropha dioica extract* (A) UV absorbance at 220 nm, (B) TEM to detect the shape of ChNPs, (C) zeta sizer of ChhNPs to detect the size, (D) zeta potential of ChNPs to detect the net charge.

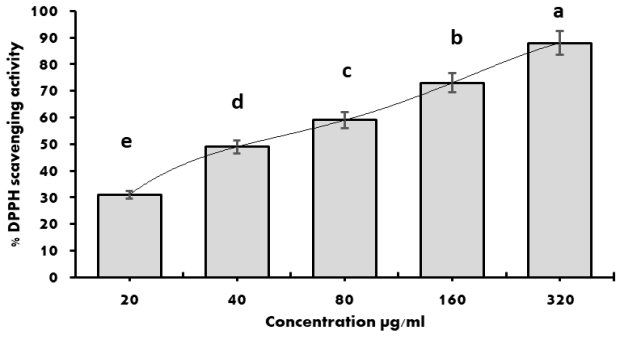

Fig. 3: Antioxidant activity of chitosan nanoparticles fabricated by *Jatropha dioica* extract against free radicals. Lowercase letters above columns indicate significant differences at P<0.05 using LSD.

Fig. 4 demonstrates the in-vitro effect of ChNPs on *I. multifiliis* trophonts, including morphological effects as well as dose-dependent responses. Fig. 4A and 4B revealed the morphological structures that were developed in the trophonts of *I. multifiliis* after being exposed to the ChNPs. The control or early exposure, as depicted in Fig. 4A, represents intact trophonts of characteristic shape of rounded or oval shapes, as indicated in the arrows. In contrast, Fig. 4B, which presumably was taken after the exposure to ChNPs, demonstrates serious damage and lysis of the trophonts, which appear as fragmented cellular debris or ghost cells, also shown by arrows. Such visual data has a high likelihood that ChNPs exhibit a direct destructive impact on the physical integrity of the parasite.

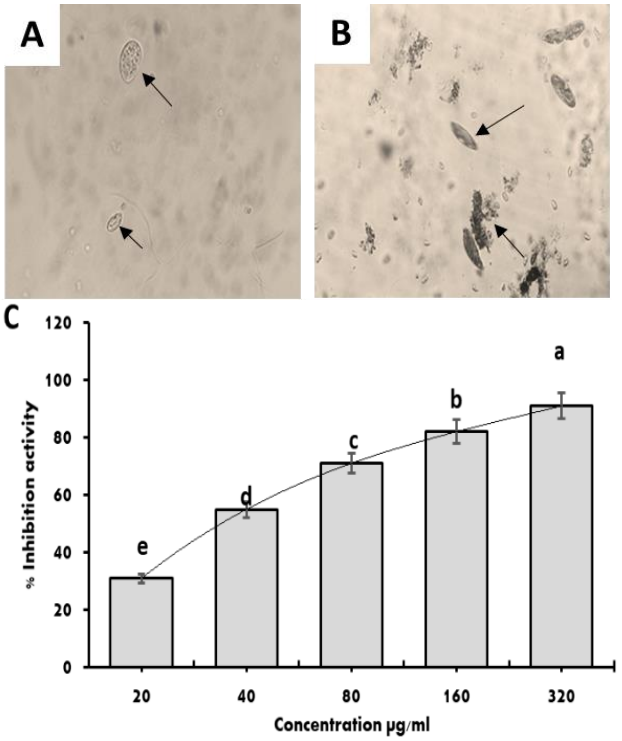

Fig. 4: In Vitro Morphological Changes (A, B) and Dose-Dependent Inhibition of *Ichthyophthirius multifiliis* by Chitosan Nanoparticles (C). Lowercase letters above columns indicate significant differences at P<0.05 using LSD.

Fig. 4C shows measurement of inhibition of *I. multifiliis* by ChNPs in a dose-dependent fashion. The x-axis depicts the progression of different levels of ChNPs (20 to 320mg/mL) whereas the Y-axis represents the percentage of inhibition activities. At the lowest level of concentration 20mg/mL, some 32 percent inhibition is observed. This inhibitory effect increases steadily to approximately 55, 70, and above 80 percent, respectively at a concentration of 40 and 80 and 160 mg/mL. The maximum level of inhibition of 90 in rate is attained at the maximum level of test of 320 mg/mL.

In terms of growth performance as presented in Table 2, Nile Tilapia fed on ChNPs diets was significantly improved compared to controls and infected groups. Final weight (FW) also significantly increased with increased ChNPs doses; FW increased as follows, 44.5±0.6 g in the control and 57.5±0.6 g in the T3 (320 mg/kg ChNPs), which is an increase of about 29%. This was followed by weight gain (WG) and specific growth rate (SGR) with the highest WG value of 37.6±0.5 g in T3 as compared to 24.7±0.5 g in controls representing 52 percent increase. FCR was increased and reduced by 4.9±0.1 in controls to 4.0±0.1 in T3, meaning improved feed efficiency.

All the ChNPs groups had a survival rate (SR) of 100% and untreated fish (T5) infected showed a lower survival rate of 88.7±0.5% illustrating the protective effect of ChNPs. ChNPs treatment (T6) in infected groups led to recovery in weight (FW 53.5±0.7 g) and WG (34.1±0.6 g) which is partially recovered as compared to praziquantel treated infected fish (T7) and this indicates superior therapeutic effect.

The biochemical and immunological parameters also indicate a positive effect of ChNPs in treated fish as shown in Table 3. Liver functioning markers such as glucose, ALT and AST were dramatically elevated in T5, glucose level was 126.3±2.5 mg/dL, ALT level was 82.5±1.8 U/L, and AST level was 152.4±3.5 U/L, which indicates liver damage. These values decreased significantly (P<0.05) with ChNPs treatment (T6) glucose down to 102.6±2.0 mg/dL, ALT down to 45.7±1.0 U/L, and AST down to 121.5±2.6 U/L showing an improvement in liver functions. Praziquantel treatment (T7) had moderate recovery (p=0.0356) which was not as effective as ChNPs.

Table 1: qPCR primers list, including gene symbols, ID and accession numbers. Orientation (F – forward, R – reverse) is followed by primer position.

Gene	ID	Accession number	Sequence (5'→ 3')
CIL8	CIL8 F258	ENSONIT00000085000_1	TCCCATCATTGGCTACAGGG
	CIL8 R396		TGCTTGCTCTAGCTCCTTGA
GATA3	GATA3 F704	ENSONIT00000022612_2	GCCTGATAGGTGGGTCATCT
	GATA3 R792		GCAGTTCACACACTCTCTACCT
IFNγ	IFNγ F438	XM_003448130.1	GAAACTTCTGCAGGGATTGG
	IFNγ R569		CTCTGGATCTTGATTTCGGG
ITK	ITK F1478	XM_003445827.5	ACGAGGTGAAGGTATCGGAC
	ITK R1565		TTGACAGGGAACTTGGAGCA
IL-1β	IL1β F91	KF747686.1	AAGGATGACGACAAGCCAAC
	IL1β R264		CGCTGTGCTGATGTACCAGT
GAPDH	GAPDH F106	XM_005455438.3	CATCCGGAGTCCCAAGACA
	GAPDH R172		CATTTTTGCCTGGGTTGGTT

Table 2: Growth performance of Nile tilapia (*Oreochromis niloticus*) infected with white spot (*Ichthyophthirius multifiliis*) and treated with chitosan nanoparticles (ChNPs) or praziquantel

Treatment	ChNPs (µg/Kg diet)	Antiparasitic Agent	IW (g)	FW (g)	WG (g)	SGR (%/day)	TFI (g/fish)	FCR	SR (%)
Control	--	--	19.8±0.3ab	44.5 ±0.6c	24.7 ±0.5c	0.49 ±0.01e	34.8 ±0.7e	4.9 ±0.1ab	100.0±0.0a
T1	80	--	20.3 ±0.2a	54.2 ±0.7ab	33.9 ±0.6bc	0.68 ±0.02b	50.5 ±0.8bc	4.5 ±0.1b	100.0±0.0a
T2	160	--	19.7 ±0.3ab	56.8 ±0.5a	37.1 ±0.4a	0.72 ±0.01a	51.8 ±0.7b	4.1 ±0.1c	100.0±0.0a
T3	320	--	19.9 ±0.2ab	57.5 ±0.6a	37.6 ±0.5a	0.73 ±0.01a	53.5 ±0.9a	4.0 ±0.1c	100.0±0.0a
T4	--	320	19.8 ±0.3ab	54.5 ±0.4ab	34.7 ±0.3b	0.69 ±0.01ab	51.2 ±0.6b	4.5 ±0.1b	100.0±0.0a
T5	--	--	19.2 ±0.3ab	47.8 ±0.5c	28.6 ±0.4c	0.51 ±0.01d	46.5 ±0.5d	5.1 ±0.1a	88.7±0.5b
T6	320	--	19.4 ±0.2ab	53.5 ±0.7b	34.1 ±0.6b	0.65 ±0.02bc	49.8 ±0.8c	4.4 ±0.1b	100.0±0.0a
T7	--	320	18.9 ±0.3b	51.5 ±0.6bc	32.6 ±0.5bc	0.63 ±0.01c	47.9 ±0.7d	4.2 ±0.1c	100.0±0.0a

IW: Initial Weight; FW: Final Weight; WG: Weight Gain; SGR: Specific Growth Rate; TFI: Total Feed Intake; FCR: Feed Conversion Ratio; SR: Survival Rate. Values with different superscript letters within a column differ significantly (P<0.05). Treatments: Control: Uninfected, untreated. T1–T3: ChNPs at 80, 160, 320 µg/Kg diet (no infection). T4: Praziquantel (320 µg/Kg; no infection). T5: Infected, untreated. T6: Infected + ChNPs (320 µg/Kg). T7: Infected + Praziquantel (320 µg/Kg). Means in the same column with different lowercase letters are significantly different at P≤0.05. Values are presented as mean±standard error (SE).

Table 3: Serum biochemical parameters, immune activity, and hormone levels of Nile Tilapia (*Oreochromis niloticus*) Infected with white spots and treated with chitosan nanoparticles (ChNPs) or praziquantel

Treatments	ChNPs (µg/Kg) diet	Antiparasitic	Liver function			Innate immune response			Hormones				Cortisol (µg/mL)
			Glucose (mg/dL)	ALT (U/l)	AST (U/l)	NBT (Abs)	LYZ (µg/mL)	Ig (µg/mL)	TP (mg/dL)	FSH (nIU/L) M	T (ng/mL) F	P4 (ng/mL)	GH	
Control	--	--	103.5±2.1b	55.8±1.2b	128.3±3.0c	0.41 ±0.02cd	1.31 ±0.05e	22.9±0.8d	44.8±1.5e	0.31±0.01d	0.97±0.03a	0.91 ±0.02a	0.51±0.02d	3.65±0.15b
T1	80	--	97.2±1.8c	46.5±1.0c	120.5±2.5cd	0.63 ±0.03b	1.54 ±0.06c	26.8±0.9c	60.2±1.8b	0.47±0.02bc	0.46±0.02d	0.50 ±0.02b	0.68±0.03c	2.55±0.12c
T2	160	--	94.8±1.6d	43.7±0.9cd	113.6±2.2e	0.67 ±0.03b	1.70 ±0.07b	30.8±1.0ab	62.1±1.9ab	0.50±0.02b	0.43±0.02de	0.44 ±0.02c	0.74±0.03b	2.32±0.11d
T3	320	--	92.7±1.5d	41.9±0.8d	104.3±2.0f	0.87 ±0.04a	1.81 ±0.07a	31.7±1.1a	64.8±2.0a	0.56±0.02a	0.39±0.02e	0.40 ±0.02d	0.87±0.04a	1.88±0.09f
T4	--	200	97.5±1.9c	47.0±1.0c	119.4±2.4d	0.64 ±0.03b	1.54 ±0.06c	28.1±0.9b	55.1±1.7c	0.49±0.02b	0.50±0.02cd	0.47 ±0.02c	0.77±0.03b	2.28±0.11e
T5	--	--	126.3±2.5a	82.5±1.8a	152.4±3.5a	0.20 ±0.01d	0.99 ±0.04f	14.8±0.6e	33.7±1.2f	0.20±0.01e	0.8±0.03b	0.55 ±0.02b	0.47±0.02e	9.85±0.45a
T6	320	--	102.6±2.0b	45.7±1.0c	121.5±2.6cd	0.51 ±0.02c	1.35 ±0.05e	22.8±0.8d	49.7±1.6d	0.44±0.02c	0.50±0.02cd	0.47 ±0.02c	0.61±0.03c	2.40±0.12d
T7	--	200	106.5±2.2b	51.7±1.1b	133.6±2.9b	0.49 ±0.02c	1.44 ±0.06d	26.4±0.9c	53.8±1.7c	0.47±0.02bc	0.58±0.02c	0.42 ±0.02cd	0.58±0.03d	2.54±0.12c

Liver Function: ALT (Alanine transaminase), AST (Aspartate transaminase). Immune Response: NBT (Nitroblue tetrazolium), LYZ (Lysozyme), Ig (Immunoglobulin), TP (Total protein). Hormones: FSH (Follicle-stimulating hormone), T (Testosterone), P4 (Progesterone), GH (Growth hormone). Treatments: Control: Uninfected, untreated. T1–T3: ChNPs at 80, 160, 320 µg/Kg diet (no infection). T4: Praziquantel (320 µg/Kg; no infection). T5: Infected, untreated. T6: Infected + ChNPs (320 µg/Kg). T7: Infected + Praziquantel (320 µg/Kg). Means in the same column with different lowercase letters are significantly different at P≤0.05. Values are presented as mean±standard error (SE).

Innate immunity was significantly improved (P<0.05) using chNPs. The increase in Nitroblue tetrazolium (NBT) activity increased nearly twice (112% increase) in T3 fish compared to controls (0.41±0.02 count). The level of Lysozyme (LYZ) increased by approximately 38 per cent at 1.31±0.05 to 1.81±0.07 ug/mL. Immunoglobulin (Ig) levels also improved by approximately 38% in T3 compared to controls. Notably, untreated infected fish had significantly depressed immune parameters (NBT at 0.20±0.01), which were restored to near-control levels with ChNPs treatment.

Stress indicators, particularly cortisol, showed dramatic shifts. T5 had cortisol levels of 9.85±0.45 µg/mL, about 170% higher than in the control (3.65±0.15 µg/mL), indicating high physiological stress. T6 showed significantly reduced (P<0.05) cortisol to 2.40±0.12 µg/mL, a 76% decrease compared to infected untreated fish, and even below control levels, suggesting strong stress mitigation. Praziquantel also reduced cortisol, but to a lesser extent (2.54±0.12 µg/mL). Hormonal profiles, including follicle-stimulating hormone (FSH), testosterone (T), progesterone (P4), and growth hormone (GH), demonstrated dose-dependent modulation with ChNPs supplementation. GH increased by about 70% in T3 (0.87±0.04) compared to controls (0.51±0.02), correlating with enhanced growth.

Fig. 5 represents the immunomodulatory impacts of ChNPs on Nile tilapia infected with *I. multifiliis*. T5 showed significantly upregulated the expression of several crucial immune-related genes contrasted to healthy controls. Specifically, the expression of CLI8, GATA3, IFNϒ, ITK, and IL-1β were all notably elevated in infected fish. The treatment with ChNPs (T6, at 320 µg/kg) consistently led to a significant downregulation of these elevated gene expressions, bringing them closer to the levels observed in uninfected control fish. This indicates that the ChNPs are effective in mitigating the hyper-inflammatory or over-reactive immune response induced by the parasite. Conversely, in healthy fish (T1, T2, T3), ChNPs showed a dose-dependent impact, with a tendency to upregulate the expression of these immune genes at lower to moderate levels.

Fig. 6 shows histological examination of Nile Tilapia gills in the different experimental groups. Fig. 6A (Control): This image represents the healthy gill tissue. It shows normal gill lamellae with distinct, thin primary and secondary lamellae, well-organized epithelial cells, clear interlamellar spaces, and no apparent pathological changes or parasitic presence. Figures B, C, and D represent gills from healthy fish treated with elevating levels of ChNPs (80, 160, and 320 µg/kg, respectively). Compared to the control (6A), these gills largely retain their normal architecture. While there might be subtle, minor alterations (e.g., slight epithelial lifting or hypertrophy) as a response to the ChNPs exposure, they generally keep healthy lamellar structures, indicating that the ChNPs, at these levels, do not cause significant histopathological damage to healthy gill tissue. Fig. 6E represents gills from fish treated with praziquantel (T4). The gill architecture appears largely normal, similar to the control and ChNP-treated healthy groups. This suggests that praziquantel treatment alone does not induce significant morphological alterations in the gill tissue. Fig. 7F clearly shows severe histopathological changes in the gills due to *I. multifiliis* infection (T5). There is extensive hyperplasia of the gill lamellar epithelium, leading to severe lamellar fusion and effacement of the normal interlamellar spaces. The secondary lamellae appear thickened and blunted. Crucially, large, circular-to-oval trophonts of *I. multifiliis* are visible, embedded within the hyperplastic epithelium (though not explicitly labeled by an arrow, their characteristic morphology and location are consistent with the parasite's presence). There may also be evidence of inflammation, with an infiltration of inflammatory cells, and potentially mucous cell proliferation. This massive alteration severely compromises the gills' respiratory function. Fig.6G shows gills from infected fish treated with ChNPs (T6). Compared to the untreated infected group (6F), there is a noticeable improvement in gill architecture. While some epithelial hyperplasia and lamellar fusion may still be present, they are significantly less severe. The lamellae appear more organized, and the interlamellar spaces are less obliterated. Crucially, the presence of parasite trophonts is either absent or significantly reduced, indicating the efficacy of ChNPs in reducing parasitic burden and alleviating the associated pathological changes.

Fig. 6H shows gills from infected fish treated with antiparasitic drug (T7), like the ChNPs -treated group (G), there is a marked improvement in histopathological lesions in the untreated infected group (F). The gill lamellae show signs of recovery, with reduced epithelial hyperplasia and lamellar fusion, and restored interlamellar spaces.

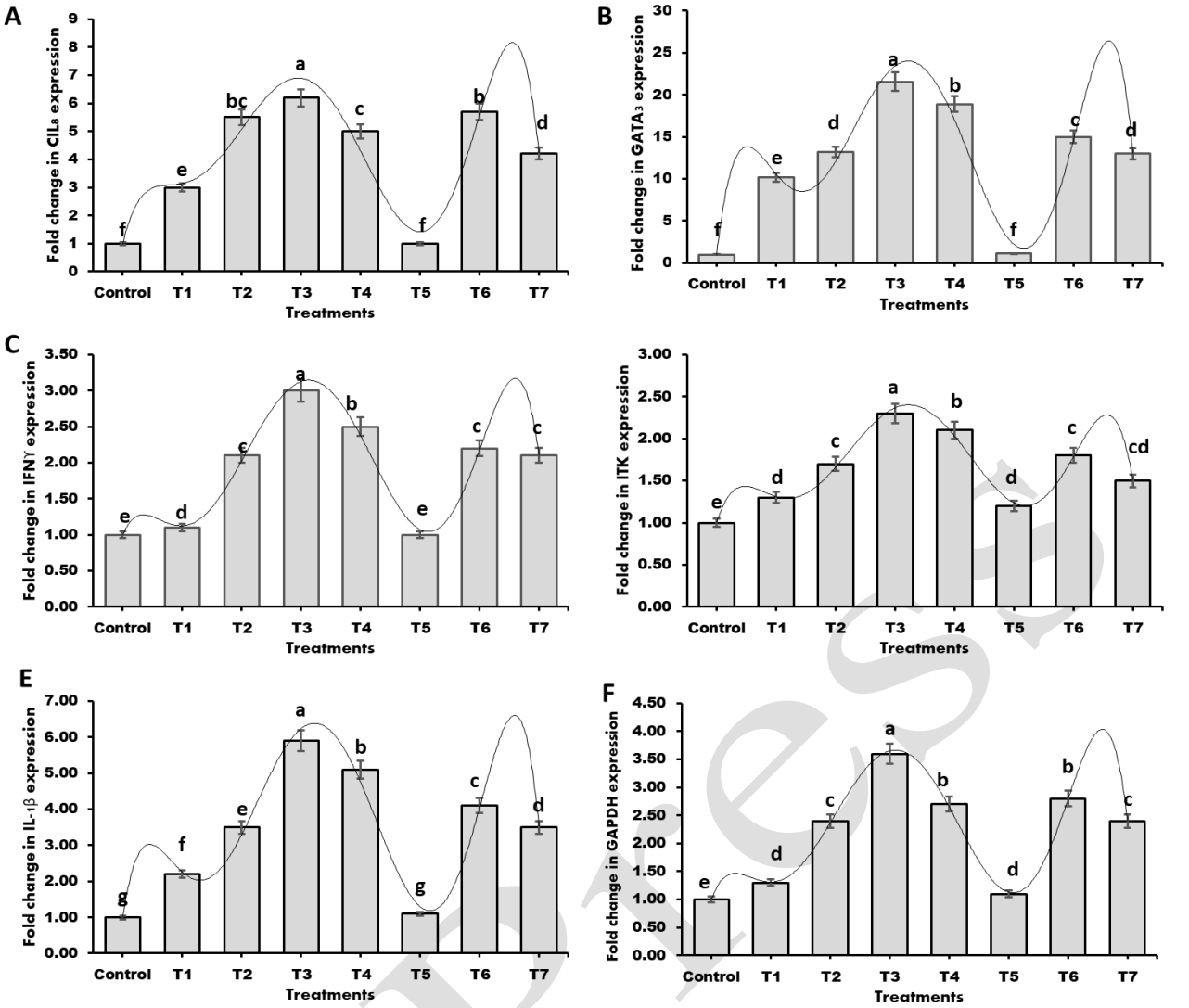

Fig. 5: The effect of ChNPs on the fold change in gene expression of (A) *CLI8*, (B) *GATA3*, (C) *IFNγ*, (D) *ITK,* (E) IL-1β, and (F) GAPDH in *Ichthyophthirius multifiliis* -infected Nile tilapia fish. the first group was control, T1, T2, and T3 were Nile talipa fish treated with chitosan nanoparticles (80, 160, and 320 mg/kg), T4 was praziquantel-treated fish, T5 was *Ichthyophthirius multifiliis* infected fish, T6 IM infected and treated with chitosan nanoparticles at 320 µg/kg, while T7 was white spot infected fish and treated with the antiparasitic drug (320 µg/kg). Lowercase letters above columns indicate significant differences at $p<0.05$ using LSD.

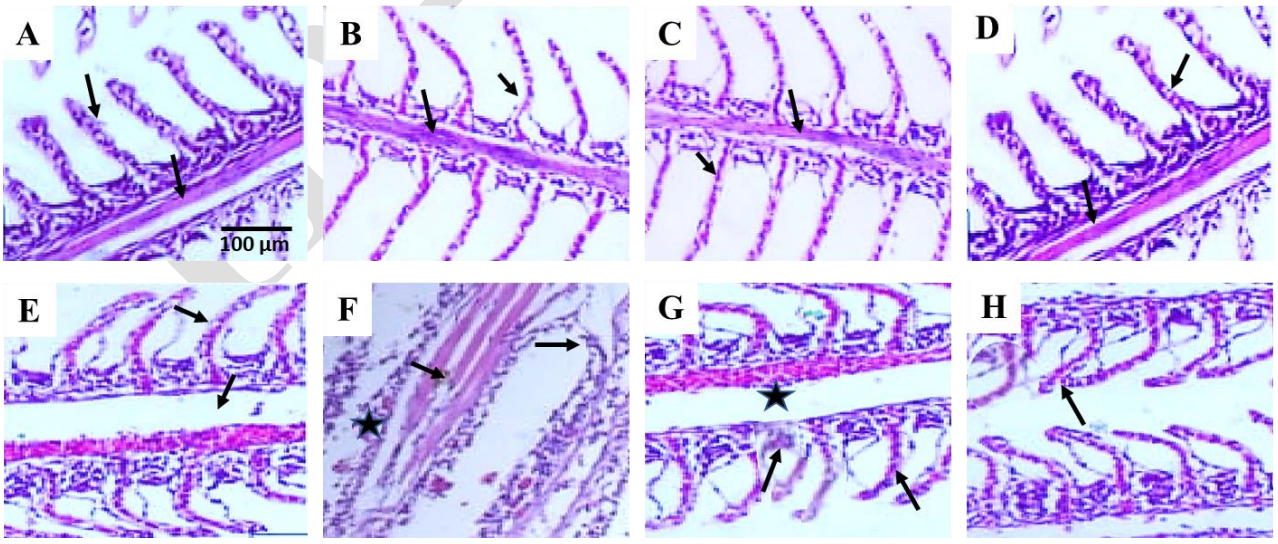

Fig. 6: The gills of *Ichthyophthirius multifiliis* -infected Nile Tilapia fish and treated with chitosan nanoparticles (ChNPs) and Praziquantel, where (A) the first group was control, (B-D) T1, T2, and T3 were Nile talipa fish treated with chitosan nanoparticles (80, 160, and 320 mg/kg), (E) T4 was praziquantel-treated fish, (F) T5 was *Ichthyophthirius multifiliis* infected fish, (G) T6 was *Ichthyophthirius multifiliis* infected and treated with chitosan nanoparticles at 320 µg/kg, while (H) T7 was *Ichthyophthirius multifiliis* infected fish and treated with the antiparasitic drug (320 µg/kg). Scale bar, 100 µm; H&E staining.

DISCUSSION

Parasitic diseases are one of the biggest problems in the world aquaculture as they incur serious losses. Recent estimations indicate that illness in the global aquaculture sector is estimated to cost the sector an estimated annual loss of US$ 6 billion with parasitic infections playing a significant role in this amount (Cain, 2022). The economic cost of diseases in the aquaculture sector was estimated to be US$ 2.48 billion, which forms 14.95% of the annual aquaculture production value (Patil *et al.*, 2025). The cost differs radically according to species and culture system as shrimp farms have the highest disease burden with US$ 1,224.82 per ton followed by marine fish with US$ 815.87 per ton (Patil *et al.*, 2025). I. The etiology of white spot disease is multifiliis, which is one of the most challenging parasites in freshwater aquaculture all over the world (Francis-Floyd *et al.*, 2023; Yang *et al.*, 2023). It is an enormous, solitary-celled ciliated protozoan which causes Ich or white spot disease and has an almost universal impact on freshwater fishes and poses a significant economic risk to commercial producers and ornamental fish industries (Bu *et al.*, 2025). Pathological effects on this type of parasitic disease in fish are dire, including tissue damage (lamellae of the gills are fused, or muscle is deteriorated), the incidence of immunomodulation, which undermines the natural defense mechanism, and chronic inflammation (involving several organ systems) (Abdel-Galil *et al.*, 2023; Abd Elraheem *et al.*, 2025).

Recent molecular research has disclosed that the genetic diversity of *I. multifiliis* strains in various geographical areas is high and studies conducted in China have indicated that there are considerable genetic diversities which can affect treatment efficacy (Yang *et al.*, 2023). Not only does the parasite have the capacity to develop white spots that are visible on the gills, fins, and skin of the fish and thus influence the physiological action of the organism, but also has an extremely negative impact on the aesthetic value of the affected fish (Bu *et al.*, 2025). The environmental conditions are significant in the outbreak of diseases, various researches revealed that fish in earthen ponds that have plentiful planktons are less prone to white spot disease as opposed to indoor culture systems with near clean water (Cao *et al.*, 2023).

The presence of natural predators has been attributed to this phenomenon, and studies reveal that cyclopoid copepods, such as *Mesocyclops* spp. and *Thermocyclops taihokuensis*, and *Paracyclopina* sp., can effectively predate on theronts, which are biologically controlled (Wang *et al.*, 2024). The use of chemical antiparasitics has been the major form of defense against fish parasites since time immemorial, but its application is becoming more problematic with the fears of toxicity, leftover environmental risks, and regulatory limitations. One of the most commonly used treatments, formalin, shows a high level of effectiveness compared to other ectoparasites but poses serious risks to fish, animals, and human health (Tancredo *et al.*, 2019; Mladineo *et al.*, 2021; Buchmann, 2022). Moreover, experiments on formalin toxicity in koi carp reported alarming safety factors, and LC50 was 191.34 mg/L in 6 hours and 135.44 mg/L in 24 hours. The FDA suggests the highest maximum levels of 250 mg/L when exposed to it in 1 hour but research indicates that 200 mg/L may cause death to the koi carp within less than 24 hours (Tancredo *et al.*, 2019). The use of formalin in the European Union has also been prohibited because of its risk of handling human beings since it is categorized as carcinogenic and allergenic.

Another possible chemical treatment method is chloramine T, which is rather limited by regulations due to the absence of approvals in various countries (Buchmann, 2022). Its compound demonstrates potency relative to other comparable ectoparasites, alongside toxicity of its own (Buchmann, 2022). Chemical agents using copper sulfate, H_2O_2, and potassium permanganate are often used, but each of them has a risk of ecological contamination and development of resistance (Dev *et al.*, 2024).

The reason behind this regulatory trend is the increased understanding of the health risks posed to humans by these chemicals, which leaves the industry with no choice but to find alternatives (Mladineo *et al.*, 2021).

ChNPs are also a new type of approach to parasitic control, which has direct anti-parasitic effects as well as health advantages due to antioxidant activity to the host (Dawood *et al.*, 2020a; Wu *et al.*, 2020; Hossam-Elden *et al.*, 2024a). In addition, the antioxidant property of ChNPs can be attributed to its ability to enhance the activities of antioxidant biomolecules and decrease oxidative stress indicators in fish tissues.

Several researches show that ChNPs in the diet cause a significant enhancement in the activity of important antioxidant enzymes, such as SOD, CAT, and GPx (Hossam-Elden *et al.*, 2024b). Optimal concentrations of ChNPs (1-3 g/kg diet) increased the total antioxidant capacity (TAC) and decreased concentrations of glutathione (GSH) and had a significant impact on reducing MDA, an important lipid peroxidation marker in Nile tilapia (Dawood *et al.*, 2020a; Wu *et al.*, 2020). Free amino groups of ChNPs are considered to be the source of antioxidant activity as they react with free radicals to form stable macromolecular radicals and ammonium groups (Munoz-Tebar *et al.*, 2023). This pathway safeguards the ROS, which are superoxide radicals, hydroxyl radicals and hydrogen peroxide, which are increased in parasitic diseases (Dawood *et al.*, 2020a; Munoz-Tebar *et al.*, 2023).

It may be observed that the antiparasitic functions of ChNPs may be activated through several ways. It is imputed to the negatively charged nanoparticles, which could be engineered by active compounds in Jatropha extract (Solis-Cruz *et al.*, 2023). Moreover, direct effects as disruption of cell membranes of parasites and disruption of pathogen metabolism (Wu *et al.*, 2020; Mahdy *et al.*, 2024). Furthermore, the negative surface charge may affect the impact with the biological membranes that are commonly negative. With negatively charged parasite membranes, this may decrease repulsion at the electrostatic scale or change the adhesion characteristics. In addition, the surface charge influences the colloidal stability, interactions with charged biomolecules, and, eventually, bioavailability (Ong *et al.*, 2019). Studies of fish-borne zoonotic trematodes indicate that ChNPs are more effective than silver and selenium nanoparticles, and LC50 values of 66mg/ ml of *Clinostomum* spp. metacercariae and 8 mg/ml of *Prohemistomum vivax* encysted metacercariae (Mahdy *et al.*, 2024).

The scanning electron microscopy shows the very tragic consequences of ChNPs on the ultrastructure of parasites,

which includes the destruction of transverse ridges, shrinkage of the integument, and blebs on the tegument surface (Kishik *et al.*, 2019). This might be explained by the fact that under 18.7/9.6nm nanoparticles will penetrate parasite cuticles, resulting in mortality due to increased permeability of the membrane (Mahdy *et al.*, 2024).

ChNPs demonstrate potent immunomodulatory impacts via upregulation of defense-related genes, representing a sophisticated approach to improve fish immune response (Wu *et al.*, 2020; Saleh *et al.*, 2022). Studies on rainbow trout show that therapeutic intervention with ChNPs results in significant upregulation of inflammatory-mediator genes, like IL-1β, TGF-β, lysozyme II, and immunoglobulin M (Saleh *et al.*, 2022).

ChNPs are capable of modulating the gene expression of more than just simple immune reactions, various studies report the upregulation of interferon induced genes, chemokine receptors, T-cell receptors, MHC class I and II molecules (Wu *et al.*, 2020). CNPs enhance the expression of immune-related genes (MX2, IFN, CXCR, TCR, MHC-Ia, and MHC-IIa) in the field of DNA vaccines, and some of them demonstrate 10-fold increased expression levels (Wu *et al.*, 2020).

Of particular interest is the long-term effects of ChNPs on genes. It was found that the enhancement of gene expression may be observed even weeks and months after the treatment, and this effect of long-term immunity is caused by the stability of the chitosan-encapsulated delivery systems and their capacity to supply the bioactive substances on a long-term basis (Li *et al.*, 2023) and this long-term effect is attributed to the stability of the chitosan-encapsulated delivery systems.

In mechanistic research, ChNPs were observed to stimulate several immunes signaling cascades at the same time (Ghattas *et al.*, 2025). ChNPs stimulate innate and adaptive immunity with the highest upregulation of the various classes of genes occurring at various period points following-administration (Dmour and Islam, 2022). As an illustration, innate immune genes such as IFN-U and MX are also at their peak at 3-7 days, whereas adaptive immune genes such as CD4, IgM, and IgT peak at 15-30 days (Wu *et al.*, 2020).

The hematological and biochemical effects of ChNPs give sufficient evidence to the positive effect they have on fish health and immune system (Dawood *et al.*, 2020a; Hossam-Elden *et al.*, 2024a; Hossam-Elden *et al.*, 2024b). Thorough investigations indicated that dietary ChNPs have significant benefits on the improvement of various blood parameters pointing to the increase in physiological status and immune capacity. The hematological changes are characterized by a substantial rise in the number of RBCs, WBCs, and hemoglobins (Hossam-Elden *et al.*, 2024a). The increase in WBC levels, especially that of lymphocytes and neutrophils, is an indicator of increased production and the availability of immune cells to combat pathogens (Wu *et al.*, 2020). The research of other fish species indicates that ChNPs increase lymphocyte counts by 20-40 percent as compared to the controls (Say *et al.*, 2023).

Biochemical studies of serum indicate that there are elevations of protein metabolic indicators. The supply of ChNPs increases the levels of total protein, albumin, and globulin significantly, which means that protein synthesis and the general work of the metabolism are improved (Dawood *et al.*, 2020a; Hossam-Elden *et al.*, 2024a). These alterations indicate improved nutritional conditions and increased liver performance because the synthesis of these proteins is mostly carried out in the hepatic tissues.

The treatment with ChNPs has dramatic changes on the immune-related biochemical parameters. Serum lysozyme activity, which is the major parameter of non-specific immunity, rises significantly in fish treated with ChNPs (Hossam-Elden *et al.*, 2024b). Cellular immunity measures are phagocytic activity and phagocytic index which are greatly increased. The amount of nitric oxide, which points to the activation of the macrophages, also improved with the provision of ChNPs (El-Hamid *et al.*, 2024).

The stress-reducing applications of ChNPs are also possible and it has been found that the activities of the stress-associated enzymes of glutamic oxaloacetic transaminase (GOT) and glutamic pyruvic transaminase (GPT) are lowered (Zhang *et al.*, 2024). Such decrease demonstrates the decreased tissue destruction and the increased capacity of stress, which is especially promising in intensive aquaculture systems. The benefits of ChNPs become apparent when evaluated against more conventional methods of treatment concerning such parameters as efficacy, safety, environmental effects, and regulatory approval (Mladineo *et al.*, 2021; Mahdy *et al.*, 2024). The Separation ChNPs have better therapeutic indices than chemical counterparts, and the effective dose range and safety range are much broader.

When compared to traditional chemicals on a direct antiparasitic effect, ChNPs tend to be superior to traditional chemicals against *Clinostomum larvae*, ChNPs were able to demonstrate LC50 of 66 mg/ml, and no withdrawal period or environmental cleanup is necessary (Mahdy *et al.*, 2024). Conversely, formalin needs concentrations of 150-250 mg/L and has high toxicity levels to the fish and the environment (Tancredo *et al.*, 2019).

ChNPs and chemical treatments vary in their mode of action. Whereas formalin, which is a chemical, induces non specific cellular damage to both parasites and the host tissue, ChNPs show specific targeting and low host toxicity. The nanoparticle system allows delivering controlled release and targeting, reducing systemic exposure, and preserving antiparasitic potency (Mathews *et al.*, 2021). One of the important areas of differentiation is environmental impact. Formal and other chemicals need to be diluted prior to environmental release to 1 mg/L and can remain in water bodies but, ChNPs, as a bio degradable particle, does not build up in the environment (Bhoopathy *et al.*, 2021). Such environmental friendliness complies with the best practices of sustainable aquaculture and regulatory trends that support greener treatment.

The economic comparison of various treatment methods demonstrates that there are big differences in the cost-effectiveness and sustainability in the long term (Patil *et al.*, 2025). Although the initial costs of ChNPs might be more expensive than the traditional chemicals, the overall effects of the product as far as increased mortality rates, enhanced growth performance, and completely removed withdrawal periods are more beneficial in terms of economics.

ChNPs have prophylactic benefits of long-term immune enhancement and decreased disease rates, which could lower treatment costs, although with an initial investment increase. The new regulatory environment prefers more ecologically favorable options of treatment,

such as ChNPs, where the ecological impact and the issue of residue can be eliminated (Mladineo *et al.*, 2021; Buchmann, 2022). With bans and restrictions on traditional chemicals, alternative treatment is no longer only highly environmentally preferable but necessary as well in terms of economics to enter markets.

Integrated approaches involving the application of several treatment modalities at once are the future of the control of aquaculture parasites (Gadallah *et al.*, 2024). The combination of probiotics, prebiotics, and plant extracts with ChNPs also demonstrates specific potential, generating synergistic effects that make the overall effect more effective without jeopardizing effectiveness and sustainability (El-Hamid *et al.*, 2024).

Another promising direction is the development of specific delivery systems based on the usage of the ChNPs as carriers of conventional medicine (Mathews *et al.*, 2021). This could be used to decrease the levels of required drugs and enhance their effects through controlled release and directing delivery to areas of infection. The use of copepod in the control of *I. multifiliis* is research on biocontrol methods with innovative approaches that supplement the nanotechnology solutions. These bio-control strategies have offered as alternatives efficient cost-effective solutions that are complementary to the other modes of therapy.

Conclusions: ChNPs (320 ug/mL) had a high antioxidant potential and inhibition of *I. multifiliis* trophonts directly by lysis. In vivo, the dietary ChNPs (320 ug/kg) enhanced growth, FCR, and survival rate, and decreased cortisol and ALT in the infected fish. ChNPs increased the NBT activity, increased the expression of CXCL8 and IFNg in healthy fish, and reduced the inflammation of infected fish. Histologically, ChNPs had less hyperplasia and trophont load on the gill compared to praziquantel. By summing up, it can be concluded that ChNPs are a dual-purpose direct antiparasitic agent and host immuno-growth promoting agent which is a safe, effective, and sustainable alternative to chemical parasiticides in aquaculture.

Author contribution statement: MNA: conceptualization, visualization, methodology, writing the original draft, writing-review, and editing.

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