KAVAKA 60(2): 70-85 (2024) DOI: 10.36460/Kavaka/60/2/2024/70-85

Physical, Biological, and Immunological Changes of Tilapia, *Oreochromis Mossambicus* by Using Mushroom Silver Nanoparticles against *Aeromonas hydrophila*

V. Shyamala* and N. Uma Maheswari

**Department of Microbiology, Sengamala Thayaar Educational Trust Women's College (Autonomous), Sundarakkottai, Mannargudi - 614 001, Tamil Nadu, India. (Affiliated to Bharathidasan University, Tiruchirappalli, Tamil Nadu, India)*

**Corresponding author Email: shyamalamithun1985@gmail.com*

(Submitted on May 1, 2024; Accepted on June 6, 2024)

ABSTRACT

The current research aimed to study the study the green synthesis of silver nanoparticles (AgNO3) from *Pleurotus ostreatus* edible mushroom extract and its effects on haematological and immunological responses in tilapia, *Oreochromis mossambicus*, against *Aeromonas hydrophila*. The formation of *Pleurotus ostreatus* silver nanoparticles (PO-AgNPs) was evidenced by X-ray diffraction and scanning electron microscopy. The UV, GCMS, and FTIR analyseswere conducted on the synthesized PO-AgNPs. The experimental diets included: 0 (Control), T1 (0.1 µg/kg); T2 (0.5 µg/kg); T3 (1 µg/kg); T4 (5 µg/kg); T5 (10 µg/kg); T6 (20 µg/kg) diet of a mixture of PO-AgNPs respectively, for four weeks.During the trial,blood samples were taken fromeach group of each week up to four weeks. At the end of the trail, fish were challenged with 0.2 ml ($1x10^7$ cfu/ml) *A. hydrophila* pathogen by intra peritoneal injection. Results showed that feeding the tilapia with a PO-AgNPs supplemented diet significantly influenced immunological parameters, which were found to be higher in fish fed with an experimental diet than the control group ($P<0.05$).Statistically significant levels of serum total immunoglobulin were detected only in the fish group fed with 10µg/kg PO-AgNPs supplemented diet. The interaction between the DNA gyrase subunit B protein and two specific control antibiotics was examined through docking analysis. The survival rate was highest in the 10µg/kg *P. ostreatus* supplemented feeding group. The results suggested that fish fed with *P. ostreatus* mushroom extract supplemented diet had an enhanced immune response and decreased the mortality rate against *A. hydrophila*. Ligands commonly interacted with the protein, as indicated by the results obtained from molecular docking studies. These results indicated that 10µg/kg PO-AgNPs can be considered as a beneficial dietary supplement for improved heamatological, immunological response and diseases resistance in tilapia against *Aeromonas hydrophila*.

Keywords: *Oreochromis mossambicus*, *Aeromonas hydrophila*, *Pleurotus ostreatus*, Immune system, Protein, Silver nanoparticles.

INTRODUCTION

In recent years, many scientific reports, studies and researches have started for exploringthe possibility of synthesis of metallic nanoparticles (NPs) using different genera of edible and medicinal fungi and mushrooms owing to the innumerable bioactive compounds with diverse biological activities present within them. A wide variety of amino acids, proteins and polysaccharides present in the mushrooms have been utilized in the synthesis of both extracellular and intracellular silver, gold, selenium, lead and iron nanoparticles (Owaid and Ibraheem, 2017). The secreted compounds by the edible and medicinal mushrooms lead to form the nanoparticle so formed with high stability and good dispersion characteristics. Nano-tests with macro fungi (mushrooms) offer a promising and eco-friendly approach, to produce non-toxicandsilver nanoparticles (Moghaddam, 2015), thus our knowledge of mushroom synthesizing nanoparticles is increasing day by day.

The biosynthesis of metallic nanoparticles has been explored across different organisms, including bacteria (Prabhusaran *et al*., 2016), fungi (Al-Bahrani *et al*., 2017; Saxena *et al*., 2014), yeasts (Rahimi *et al*., 2016), algae and plants (Al Bahrani *et al*., 2018; Khan *et al*., 1981; Owaid, 2019). The first research used the synthesis of NPs from mushrooms started in 2004 when (Numata *et al*., 2004) produced nano fibers from the purified polysaccharides (β-1, 3-glucan) so-called Schizophyllan from the mushroom Schizophyllum commune.The medicinal mushroom *Pleurotus ostreatus* (Berk.) Pegl. (Oyster mushroom) was selected for this study due to its numerous medicinal properties. It has been linked to reducing cholesterol, lowering blood pressure, boosting the immune system, fighting tumors, and improving

liver function (Regula and Siwulski, 2007). These benefits are attributed to compounds like lentinan, eritadenine, L-ergothioneine (Smith *et al*., 2002; Bernas *et al*., 2006), and various antioxidants (Mau *et al*., 2002).

In order to control the proliferation of these bacteria, antibiotics are used widely inintensive aquaculture. But prolonged use of antibiotics could lead to many negative side effects such as antibiotic resistance in bacteria, antibiotic residues in the environment and fish products (Cabello, 2006). Therefore exploring new methods for preventing infectious diseases has become very urgent in rainbow trout culture. Immuno stimulants enhance resistance to infectious diseases by boosting both the acquired and innate immune responses (Galindo and Hosokawa, 2004 Various immune stimulants have shown efficacy in fish (Awad and Austin, 2010; Bilen *et al*., 2011; Binaii *et al*., 2014; Zahran *et al*., 2014; Tang *et al*., 2014; Wang *et al*., 2015).The aim of the study, was to determine the effects of dietary supplementation of *P. ostreatus* mushroom extract on the immune response and disease resistance of tilapia against *A. hydrophila* pathogen under natural environmental conditions of a commercial trout farm.The study included the development of three-dimensional homology models of selected white, brown, and soft fungal laccase protein sequences, examining their structural and functional properties using standard tools.

MATERIALANDMETHODS

Synthesis of Silver nanoparticles

Oyster mushroom *Pleurotus ostreatus* was collected from Tamil Nadu Rice Research Institute, Aduthurai, Kumbakonam, Thanjavur District, Tamil Nadu, India. Fresh mushrooms (5 g) were washed with distilled water, cut into small pieces, and placed in a 2L beaker with 500 mL double-distilled water. For the synthesis of silver nanoparticles, 10 ml of mushroom extract was added to 150 ml in a conical flask that containing 90 ml of a solution of 1 mM silver nitrate. The mixture was again incubated at 60˚C in the dark while being stirred at intervals of a different time interval. Over the period of 24 hours, the resulting reduction in silver ions (Ag^+) was periodically monitored. The reaction mixture's colour changed from light yellow to pale yellow and finally to dark brown after 4 hours of incubation indicating the formation of Ag-NPs (Gurunathan *et al*., 2013).

Characterization techniques

UV-Vis spectral analysis was done using an Elico UV-Vis spectrophotometer (Devika *et al*., 2012). Ag-NPs from fungus extracted samples were subjected to FTIR analysis using a Perk in Elemer Spectrum-1 to determine their chemical composition in the Mid Infrared (MIR) region of 400 – 4000 cm^{-1}. X-ray diffraction (XRD) measurements on the Ag-NPs were conducted using a Phillips PW 1830 instrument. The samples were cast into glass slides and biologically synthesized films. The instrument operated at a voltage of 40 kV and a current of 30 mA, utilizing Cu Kα radiation with a wavelength of 0.1541 nm. The measurements were taken with a step size of 0.02° 2θ over a range of 10-80°.Scanning Electron Microscopic (SEM) analysis was performed using an FEI QUANTA 200 FEGHR-SEM model, which was operated at 30 kV with an 8 mm working distance. A small amount of the sample was used to create thin films on a carbon-coated substrate. After placing the specimen on the sample holder and blotting away excess solution with a piece of paper, the film on the SEM was subjected to a mercury lamp for 5 min to dry.

GCMS analysis of Ag-NPs

By using the GC-MS technique, various volatile and semi-volatile compounds were analyzed in mushroom extract of *P. ostreatus* (control) and synthesized silver nano particles (Ag-NPs) from *P. ostreatus*. The analysis was carried out at the BGI Laboratory in Nigeria's Port Harcourt. The Agilent 6890N gas chromatography with an auto sampler linked to an Agilent mass spectrophotometric detector was used for GC analysis of the extracts. One microliter (1 µL) of the sample was injected in the pulsed spitless mode on to a fused silica column with 0.15 µm film thickness and dimensions of 30 m x 0.25 mm. In order to achieve a constant flow rate of 1 ml/min, helium gas was used as the carrier gas, and the column head pressure was maintained at 20 psi. The retention times of the volatile and semi-volatile components in the column were used to determine the identification time.The relative percentage of each extract constituent was expressed using peak area normalization. By comparing the components in the extract's retention in dices and mass spectra fragmentation patterns to those previously adopted by Jerome Jeyakumar and stored in the chemical library of the National Institute for Standard and Technology (NIST)

library version 2.4, the components were identified (Devika *et al*., 2012).

***In vivo* experiment**

Feed preparation and Experimental Design

Ingredients of the feed given during experiments are listed in **Table 1**. The fish were divided into seven experimental groups of 12 each in triplicate (7×12 x 3 = 252 fish) Control; T1 (0.1 µg/kg); T2 (0.5 µg/kg); T3 (1 µg/kg); T4 (5 µg/kg); T5 (10 µg/kg); T6 (20 µg/kg).The fish were fed *P. ostreatus* silver nanoparticles enriched diets at the specified concentrations twice a day at 10:00 a.m. and 2:00 p.m. Each group continued with their respective diets for the duration of the experiment. Water was exchanged daily (about 50%). The respective diets in each group were continued till the end of experiment. They were acclimated for 15 days in 100 L aerated fiber tanks with dechlorinated tap water, maintaining a temperature of 26–28 °C, pH 6.5–7.5, dissolved oxygen level 4.5–5.5 mg L^{-1}, and ammonia concentration 0.03–0.05 mg L^{-1}. At the end of weeks 1, 2, 3, and 4 post-treatment, six fish were randomly collected in each experimental group for evaluation and collection of blood samples for haematology and immunological assays.

Table 1: Composition of control and experimental diet

Experimental feed (kg/g $^{-1}$)							
Ingredients (g/kg^{-1})	**C**	**T_1**	**T_2**	**T_3**	**T_4**	**T_5**	**T_6**
Soya bean flour	400	400	400	400	400	400	400
Groundnut oil cake	250	250	250	250	250	250	250
Wheat bran	200	200	200	200	200	200	200
Wheat flour	40	40	40	40	40	40	40
Tapioca flour	100	100	100	100	100	100	100
Vitamin and mineral mix	10	10	10	10	10	10	10
P. ostreatus $AgNO_3$ Nanoparticles (µg/kg)	Absent	0.1	0.5	1	5	10	20

Collection of blood and serum separation

Five fish were randomly collected from each group every ten days. After being anesthetized with MS222 (Sigma, U.S.A.), blood was drawn from the caudal vein using a 2-mL syringe. The blood samples were transferred to plastic Eppendorf tubes containing an anticoagulant solution (heparin). These tubes were kept at 4 °C overnight before being centrifuged at 3000 g for 10 min to separate the serum. The collected serum was stored immediately at −86 °C until used for further assays.

Haematological parameters

The haemoatological parameters were measure dusing the diagnostic reagent kits according to the manufacturer's instructions (My BioSource Inc., San Diego, CA, USA). Hemoglobin (Hg) estimation was conducted using Sahli's method. Red Blood Cells (RBC) and White Blood Cells (WBC) were quantified using an improved

Neubaeur' schamper, following the methodologies described by McCord and Fridovich, (1969) and Wilchek and Bayer, 1990).

Immunological parameters

The neutrophil activity (mg ml^{-1}) was determined by nitro blue tetrazolium test (NBT, Sigma-Aldrich Chemical, St. Louis, MO, USA) according to Rook *et al*. (1985). Lysozyme activity was determined by turbidometric assay following Caruso *et al*. (2002) with lysozyme content calculated from a calibration curve.Total myeloperoxidase (MPO) content was measured. The serum protease enzyme levels were determined using a colorimetric assay, according to Montgomeryand Dymock, (1961). Serum bactericidal activity was measured as described by Kawahara *et al*. (1991).

Bacterial challenge

At the end of the experiment, 20 fish per treatment were divided into 2 replicates and subjected to a bacterial challenge test under the same conditions and AgNP levels. Pathogenic *A. hydrophila* was injected intra peritoneally in a dose of 0.1 ml (5

$\times 10^5$ cells ml^{-1}) previously isolated from moribund fish in the Rajiv Gandhi centre for Aquaculture, Tamilnadu, India, using VITEK®2-C15 automated system for bacterial identification (BioMerieux Inc., France) following manufacturer's instructions (Schaperclaus *et al*., 1992). The challenged fish were kept under observation for 14 days to record any abnormal clinical signs and the daily mortality rate. The relative percent of survival (RPS %) was calculated according to Amend (1981) as follows: RPS = 100[1−(% mortality in treated fish/ % mortality in the control fish).

Molecular docking

UNIPROT/SWISS-PROT is a created biological database of protein sequences. FASTA is software package for DNA and protein sequence alignment. SWISS-MODEL is a structural bioinformatics web-server dedicated to homology modeling of 3D protein structures. Pub Chem is a unique bioinformatics and cheminformatics resource that combines detailed drug data with comprehensive drug target information.

Blast

In bioinformatics, BLAST (basic local alignment search tool) is an algorithm and program for comparing primary biological sequence information, such as the amino-acid sequences of proteins or the nucleotides of DNA and/or RNA sequences.

Statistical analysis

All data were expressed as ameans ± standard error (SE). ANOVA was based on polynomial orthogonal contrasts. SPSS Version 17 for Windows (SPSS Inc., Chicago, Illinois, USA) was employed to calculate linear and quadratic regression equations to determine the effects of different concentrations of *P. ostreatus* AgNO3 nanoparticles in Nile tilapia. Duncan's multiple range test was used to identify the differences among mean data significance level of <0.05.

RESULTS

Synthesis of *P. ostreatus* AgNO3 nanoparticles

In the current study, the biosynthesis of silver nanoparticles was indicated by a color change in the solution from yellow to ruby-brown and finally to dark brown for the mushroom extract of *Pleurotus ostreatus*. The mushroom extract (1g) was used with varying concentrations of silver nitrate (1 mM, 5 mM, and 10 mM). After 48 hours of reaction, the solution's color change to dark brown confirmed the formation of silver nanoparticles.

UV-Visible analysis

The bio synthesized Ag-NPs from the mushroom extract shows the maximum absorbance of 0.916 in control, 1.865 in 1 mM, 2.916 in 5 mM and 3.822 in 10 mM at the peak of 380nm. The concentration 10 mM are highest peak in all wavelength at absorbance process were analyzed. The highest values are recorded at higher concentration (10 mM) issynthesized silver nanoparticles from *Pleurotus ostreatus* **(Figure 1)**. Further analysis will compare the control and highest concentration obtained previously.

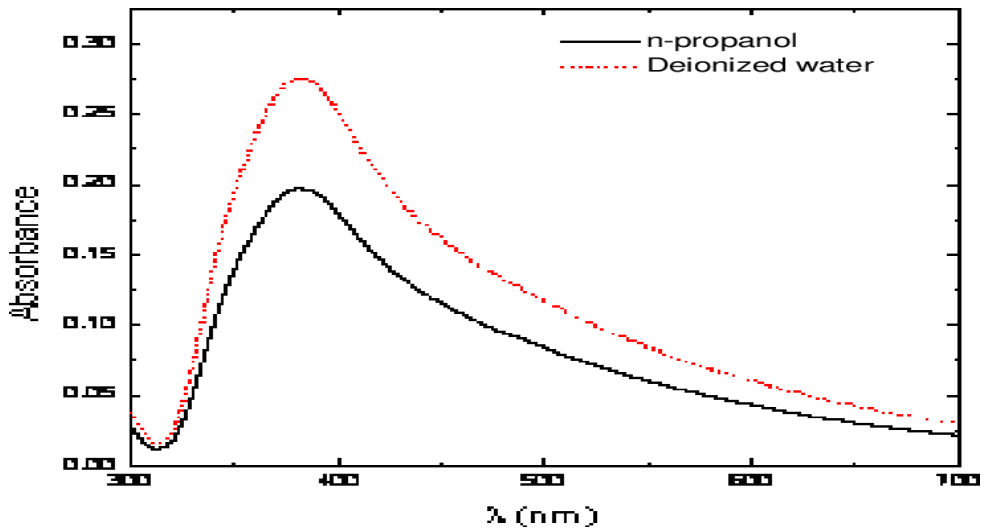


Figure 1: UV- visible spectrum

GCMS (Gas chromatography mass spectrometer) analysis

In the present study, the chromatograms of the samples from mushroom extract of *P. ostreatus* (control) and synthesized Ag-NPs of *P. ostreatus* (concentration 10mM),are represented by Figure, while the identified compounds and the irretention time, molecular formula, molecular weight, peak area (µg/kg), and activities related to medicinal uses are shownin **Figure 2**. From the results, each 10 different compounds were identified in *P. ostreatus* respectively. In the mushroom extract (control) the major compounds detected were Olean-12-en-3-ol, acetate, (3.beta.)(52.72 µg/kg), followed by Lup-20(29)-en-3-ol, acetate,(3.beta.) (18.28 µg/kg) and 9, 19- Cyclolanost-24-en-3-ol, (3.beta.) (15.67 µg/kg). The minimum compounds detected were 2H-1-Benzopyran-2-one,4-hydroxy-3-[1-(4-hydroxyphenyl)-3-oxobutyl]-$$2H-1-Benzopyran-2-one,4 hydrox) (0.01 µg/kg), 8-Carbethoxy-1-methyl-1,4,5,6,7,8-hexa hydro pyrrolo[2,3-b] azepin-4-one-3-carboxylic acid (0.1 µg/kg) and 2-[5-(4-Chloro phenyl)-2-methyl pyrimidin-4-yl]-5-methoxy phenol (0.2 µg/kg). The synthesized Ag-NPs from *P. ostreatus* are observed as a major compound by (Lup-20(29)-en-3-ol,acetate,(3.beta.)-) (28.96 µg/kg) and Tricyclo [4.2.1.1(2,5)] decan-9-one,10-cyano-10-trimethylsilyloxy-(15.78 µg/kg). The minimum compounds detected were (Cyclonona siloxane, octadeca methyl-) (4.53 µg/kg) and Methyl 5-amino-2-[(3-chlorophenyl)amino]-1,3-oxazole-4-carboxylate (5.55 µg/kg). Further compared with the control and concentration (10 mM), the synthesized Ag-NPs from *P. ostreatus* are presented at maximum area peak for 10 compounds (**Figure 2**).

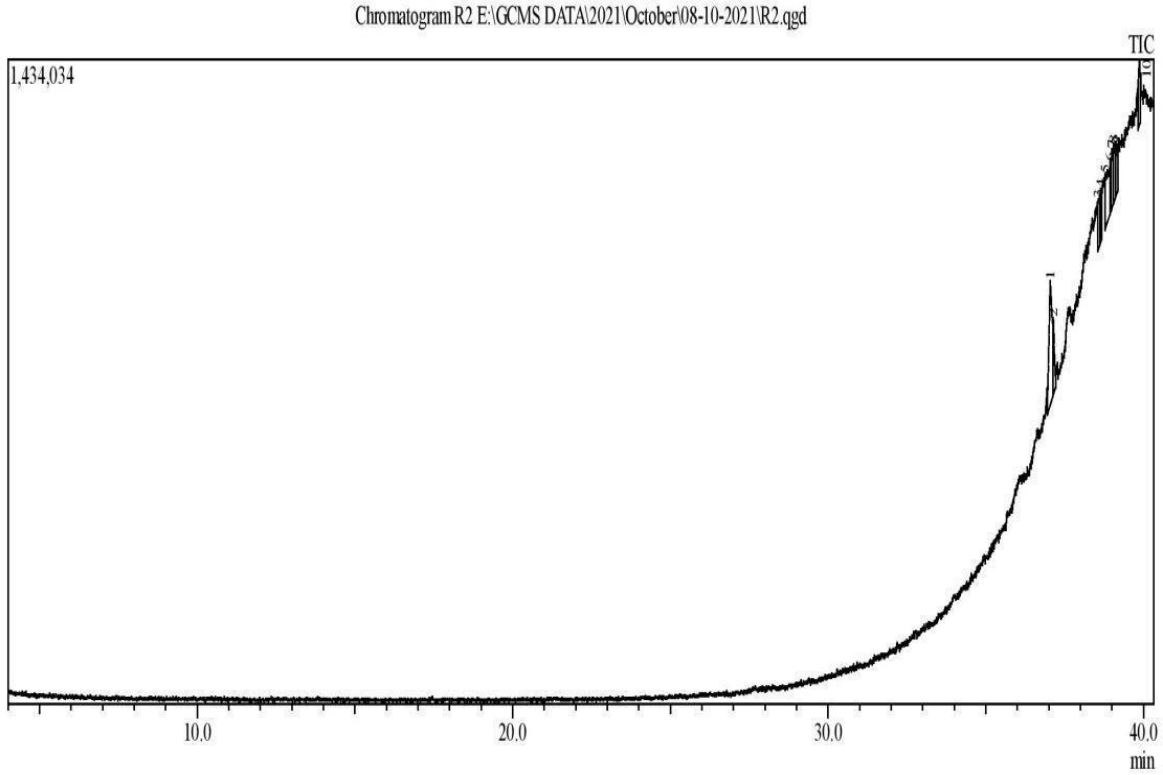


Figure 2: GC-MS analysis

Fourier Transform Infrared Spectrum (FTIR) analysis

FTIR spectral bands of the silver nanoparticles synthesized using *P. ostreatus* extract (concentration 10 mM) (**Figure 3**). The peaks in transmittance were observed at comparable spectral bands were observed at 3432.53, 2844.15, 2076.62, 1638.42, 1455.11, 1384.09, 1106.33, 1051.90, 1014.84 and 677.58 cm^{-1} in synthesized. Both the mushroom extract and the synthesized Ag-NPs exhibit similar transmittance bands. In particular, the strong peak at 1638.42 cm^{-1} indicates N-H bending vibrations, which suggests the presence of amines. The peak at 1051.90 cm^{-1} corresponds to S-O stretching vibrations in sulfoxides and sulfonic acids, indicating sulfur compounds, while the peak at 1014.84 cm^{-1} corresponds to C-X stretching vibrations in C-Cl, indicating halogen compounds

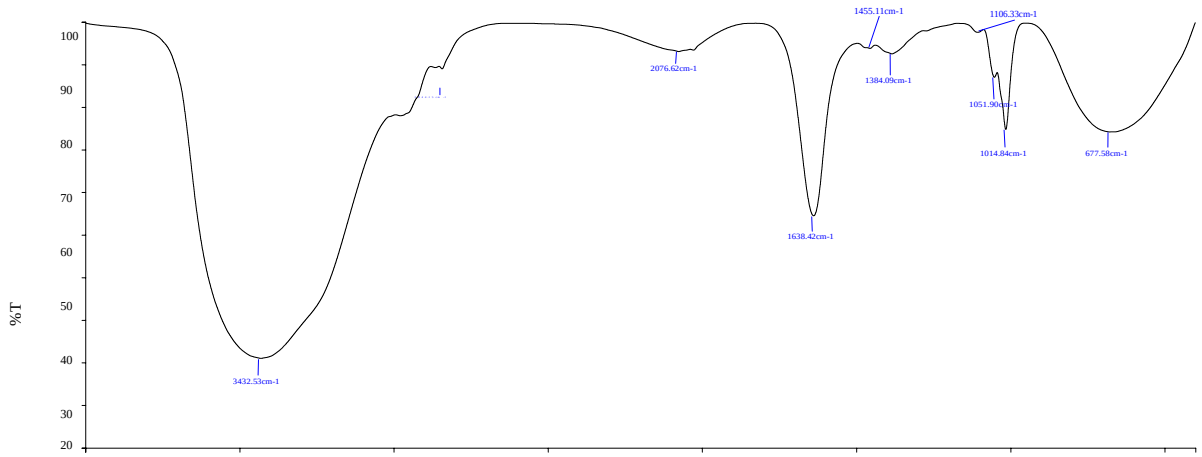


Figure 3: FT-IR analysis

Scanning Electron Microscopy (SEM) analysis

In the current study, for scanning electron microscopy (SEM), the surface morphology and topography of the Ag-NPs were investigated using different magnification forces **(Figure 4)**. SEM images revealed that nanoparticles with diameters of 100, 200, and 500 nm and formed relatively spherical shape agglomerates in mushroom extract (control). In contrast, the synthesized Ag-NPs from *P. ostreatus* extract (10 mM concentration) resulted in a sheet-like structure. The selected are a diffraction pattern recorded from one of the nanoparticles in the aggregates in shows that the silver particles are crystalline. Aggregates show that the silver particles are crystalline.

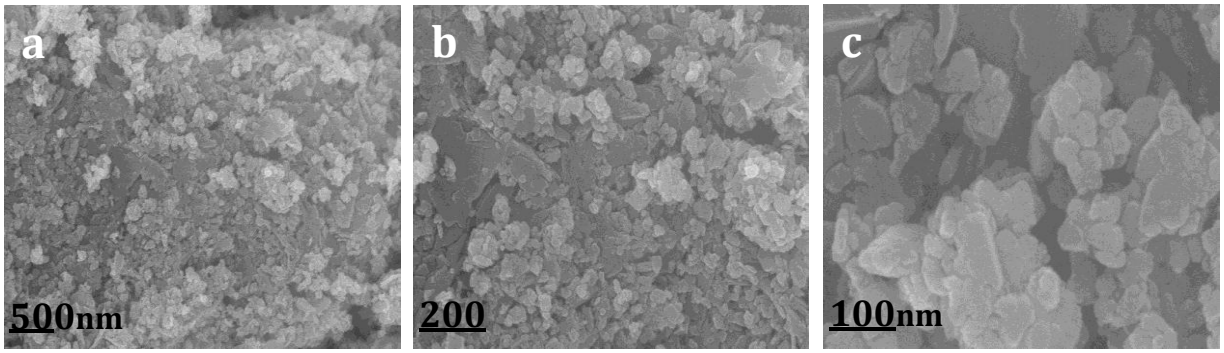


Figure 4: SEM micrographs

X-Ray Diffraction Analysis (XRD)

In the current study, the XRD plot (**Figure 5**) shows that at different diffraction angles, ranging from 20 to 80, different lattice planes of (111), (200), (220), (311), and (222) exhibit various crystalline lattices. Thus, it is completely obvious from the XRD pattern that the Ag-NPs were essentially crystalline. The diffraction's intensity was significantly higher than that of the other diffractions. In this case, the XRD diffraction measurements produced the four strong peaks seen in **Figure 4.**

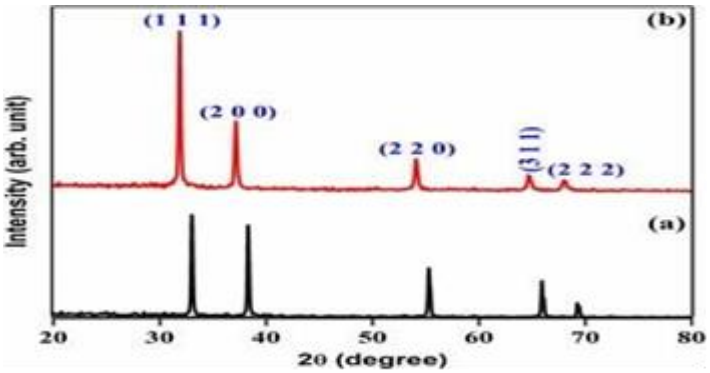


Figure 5: XRD analysis

Haematological parameters

The maximum Hb content was noted in T5 when compared to control. The erythrocyte count of the blood samples of T5 group was highest and differed significantly ($p<0.05$) with other treatment groups. Maximum of $7.02\pm0.38X10^{6}$ cells/cu mm^{-3} erythrocytes count was recorded in T5 and minimum of $5.03\pm0.15X\ 10^{6}$ cells/cu mm^{-3} erythrocyte count was recorded in control T3 **(Figures 6 to 13)**. The peak was elevated on the 21st day of post immunization.

Immunological parameters

As presented in fig3, significant effect of mushroom AgNO3 nanoparticle s supplementation was observed on the serum levels of neutrophil activity at the end of the experiment. Lysozyme activity increased linearly ($P<0.05$) in the 10 µg/kg mushroom AgNO3 nanoparticles supplementation group compared to the control. Both lysozyme activity and myelo peroxidase activities increased linearly and quadratically ($P < 0.05$) in a concentration-de pendent manner in all the mushroom AgNO3 nanoparticles supplementation groups compared to the control group, with the10µg/kg concentration showing the highest values. Additionally, antiprotease and serum bactericidal activities were also significantly enhanced **(Figures 6 to 13)**.

Challenge with *A. hydrophila*

While the control group exhibited the highest mortality rate (75.5 µg/kg), the mortality rate decreased in a concentration-dependent manner in the mushroom AgNO3 nanoparticle groups (35.5 µg/kg for 10 µg/kg, 40.58 µg/kg for 20 µg/kg, and 15.84 µg/kg for 5 µg/kg supplementation). Among all A. hydrophila-challenged fish groups, the highest relative percentage survival (RPS) was observed in the group supplemented with 10 µg/kg mushroom AgNO3 nanoparticles (94.54 µg/kg), followed by the 20 µg/kg and 5 µg/kg supplementation groups, respectively **(Figures 6 to 13)**.

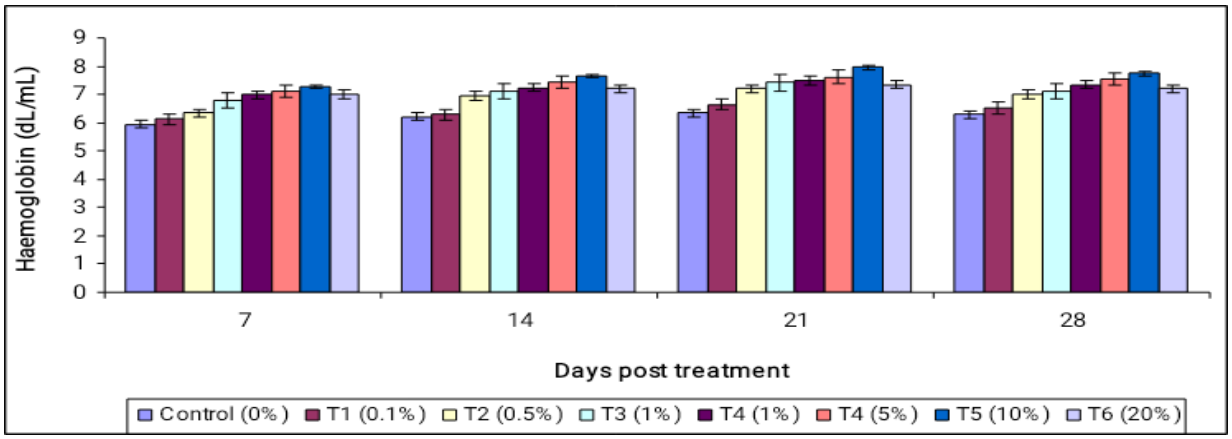


Figure 6: Effect of *P. ostreatus* mushroom AgNO3 nanoparticles on haemoglobin content in Tilapia (*O. mossambicus*

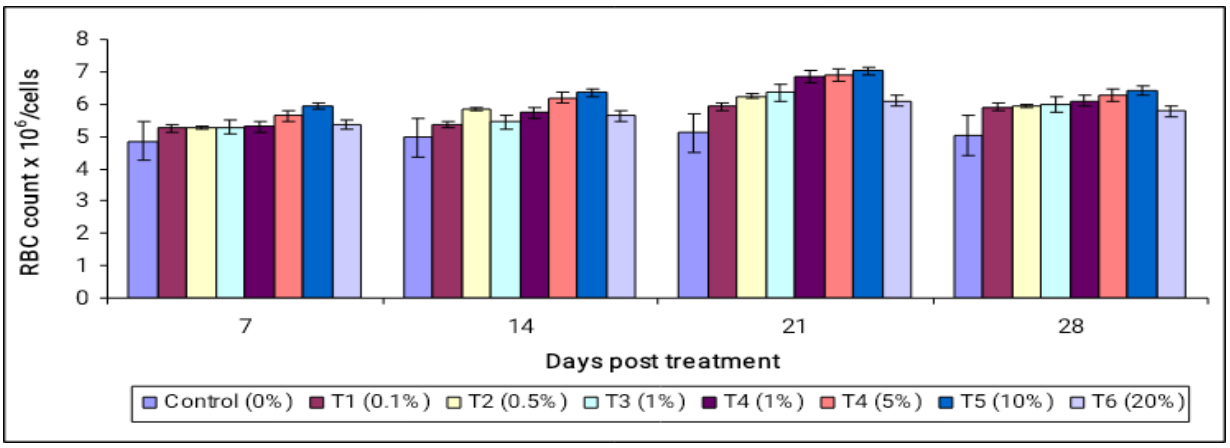


Figure 7: Effect of *P. ostreatus* mushroom AgNO3 nanoparticles on red blood cells content in Tilapia (*O. mossambicus*)

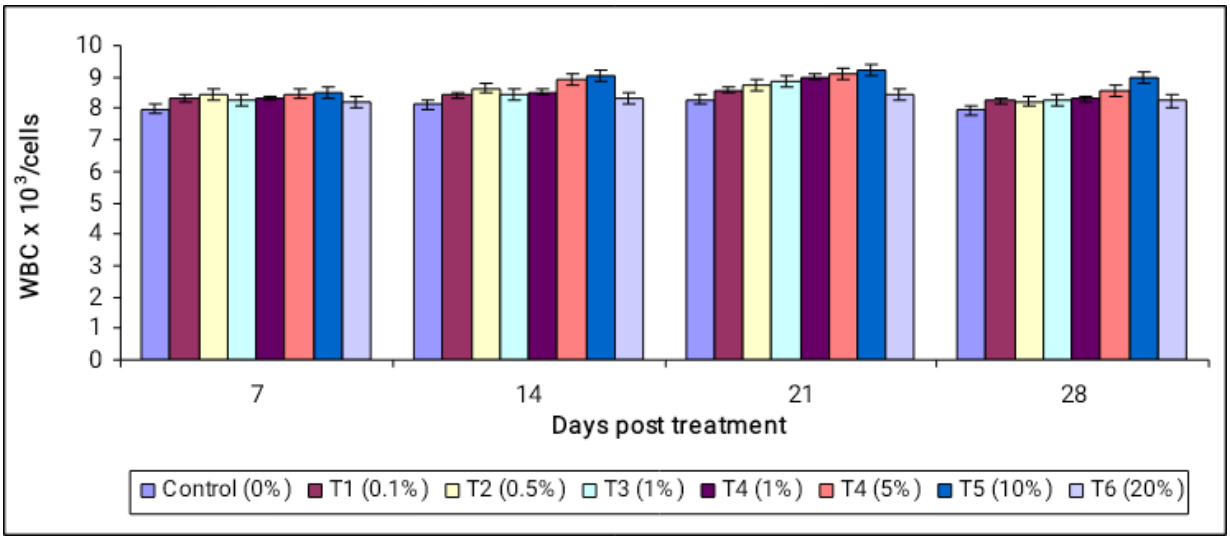


Figure 8: Effect of *P. ostreatus* mushroom AgNO3 nanoparticles on white blood cells content in Tilapia (*O. mossambicus*)

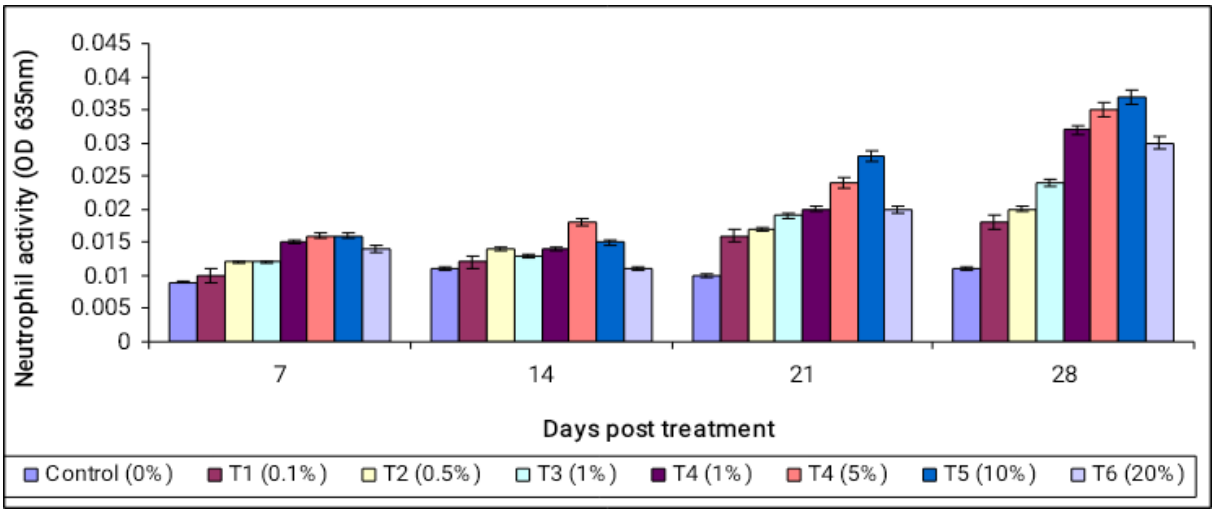


Figure 9: Effect of *P. ostreatus* mushroom AgNO3 nanoparticles on Neutrophil activity content in Tilapia (*O. mossambicus*)

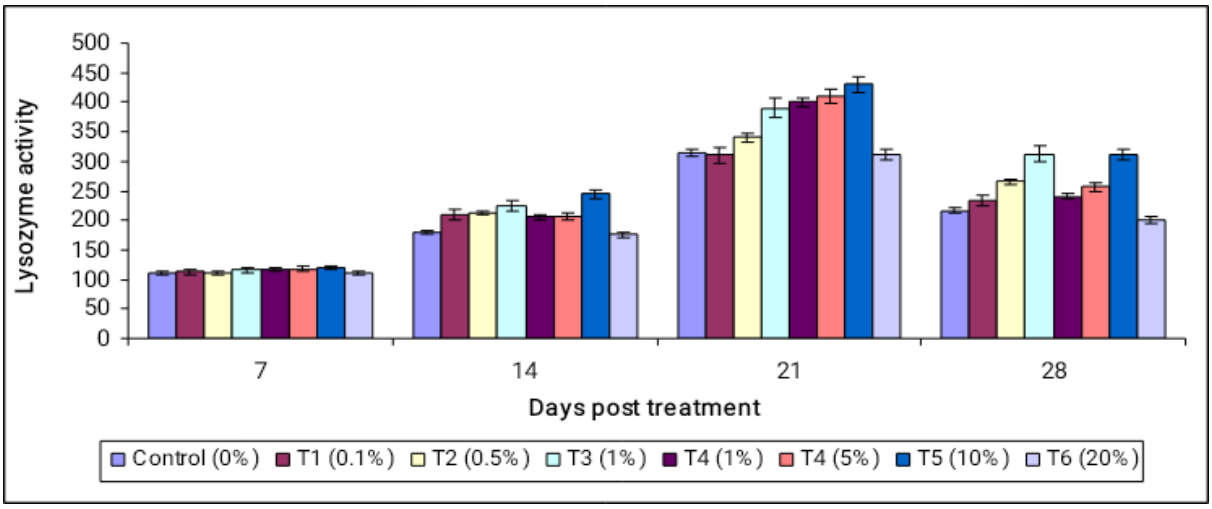


Figure 10: Effect of *P. ostreatus* mushroom AgNO3 nanoparticles on Lysozyme activity content in Tilapia (*O. mossambicus*)

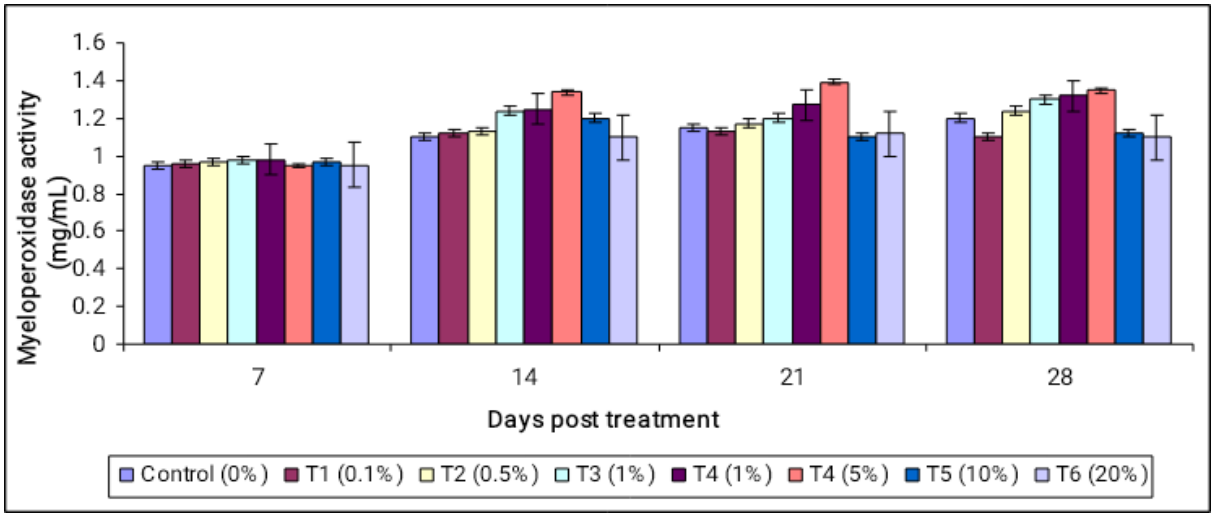


Figure 11: Effect of *P. ostreatus* mushroom AgNO3 nanoparticles on Myelo peroxidase activity content in Tilapia (*O. mossambicus*)

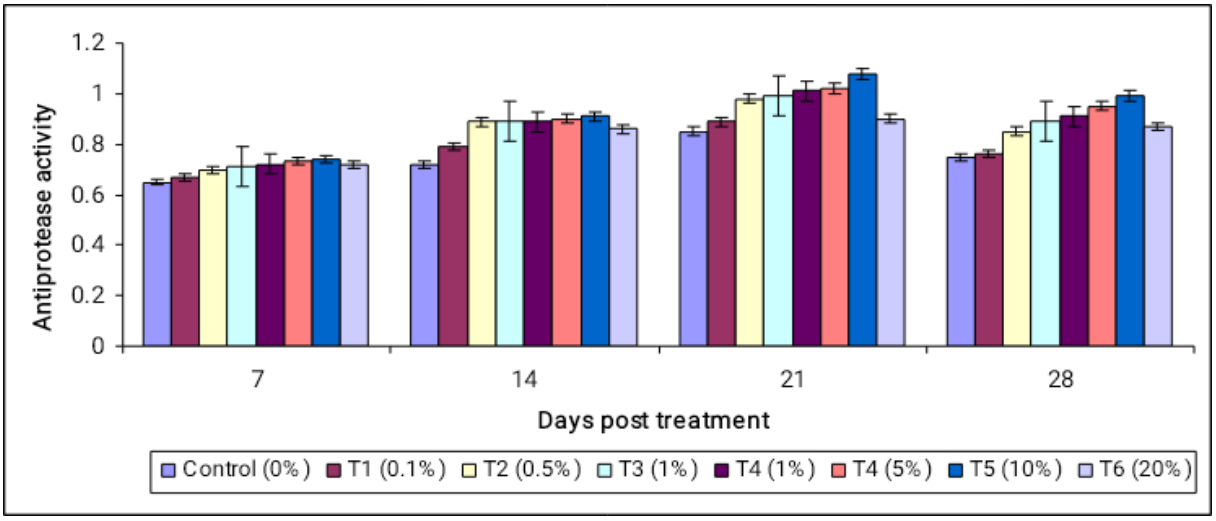


Figure 12: Effect of *P. ostreatus* mushroom AgNO3 nanoparticles on Antiprotease activity content in Tilapia (*O. mossambicus*)

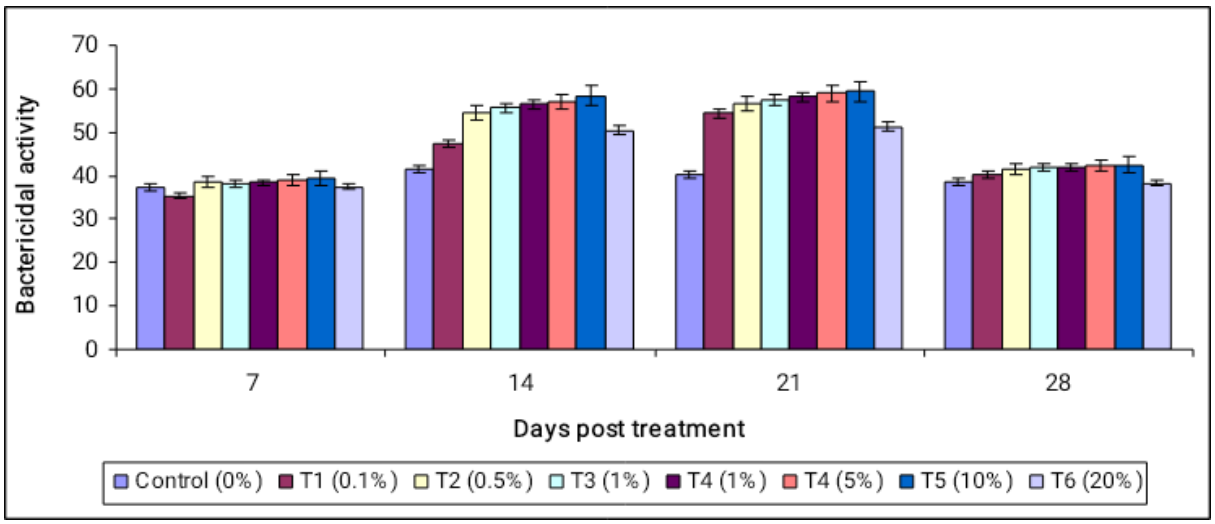


Figure 13: Effect of *P. ostreatus* mushroom AgNO3 nanoparticles on Bactericidal activity content in Tilapia (*O. mossambicus*)

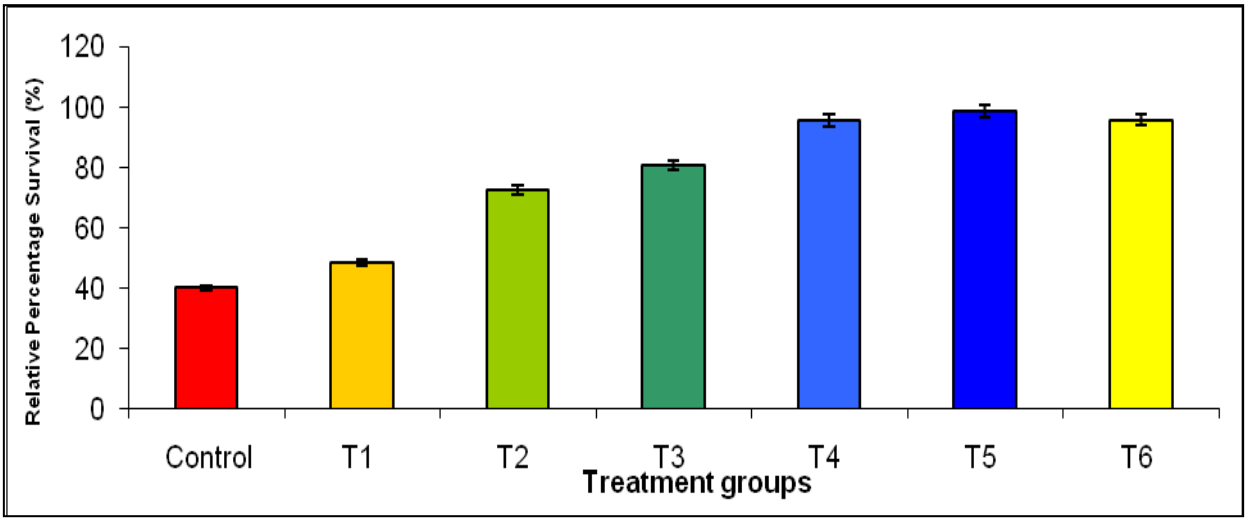


Figure 14: Effect of *P. ostreatus* mushroom AgNO3 nanoparticles on relative percent survival rate in Tilapia (*O. mossambicus*)

Molecular docking

Docking of (**Figure 14**) DNA gyrase subunit B protein with selected and two GC-MS compounds from synthesized silver nanoparticles of *P.ostreatus* antibiotic (Hexakis (trimethylsilyloxy) cyclotrisiloxane, Hexa decamethyl hepta siloxane) were performed by theuse of the tool Hex 5.1 (Fig 14). Red-Sore Disease antibiotics (Testosterone tridecanoate, Lupeolacetate) and (Hexakis (trimethylsilyloxy) cyclotrisiloxane, Hexadecamethyl heptasiloxane) have showed higher binding efficiency, ie-153.43,-135.78and-273.38,-158.37 respectively with DNA gyrase subunit B **(Figure 15 and Table 1**).

i) Hexakis (trimethylsilyloxy) cyclotrisiloxane

ii) Hexade camethylhepta siloxane

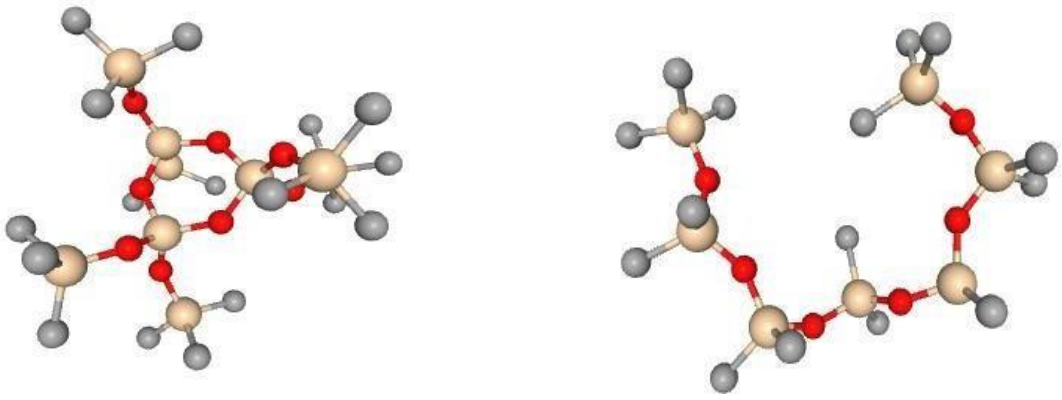
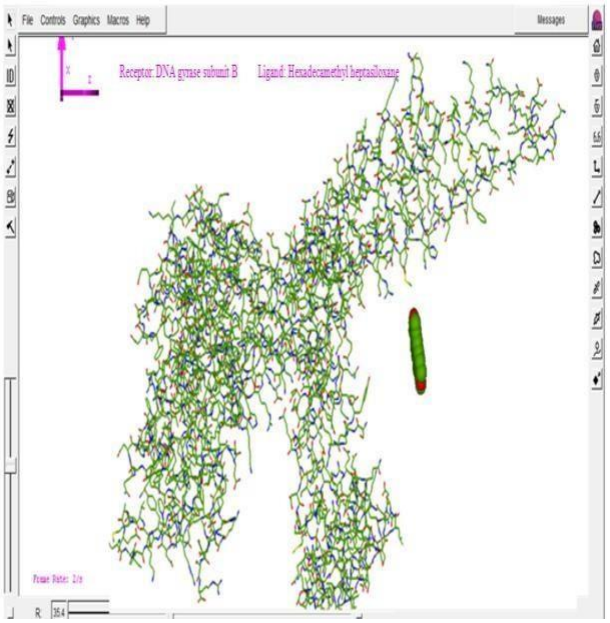


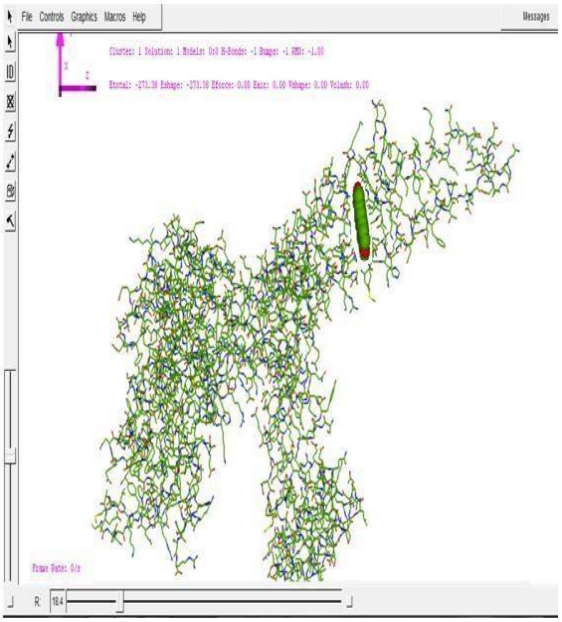
Figure 15: Structure of bioactive compounds identified from *P. ostreatus* and used for the analysis of molecular docking.

Table 2: Docking Results of DNA Gyrase Subunit B Protein (Synthesized silver nanoparticles from *P. ostreatus*)

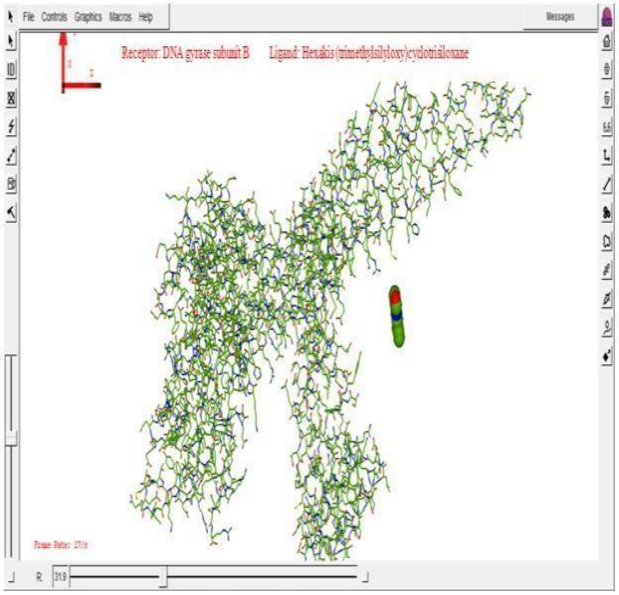
LIGAND	E.TOTAL	E.SHAP	E.FORCE	BUMPS	RMS
Hexakis (trimethylsilyloxy) cyclotrisiloxane	-273.38	-273.38	0.00	-1	- 1.00
Hexadecamethyl heptasiloxane	-158.37	-158.37	0.00	-1	- 1.00



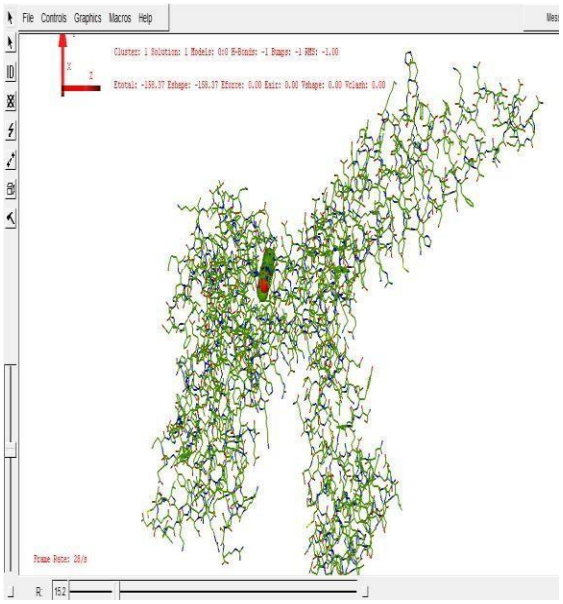
BEFORE DOCKING: DNA GYRASE SUBUNIT B AND HEXADECAMETHYL HEPTASILOXANE



AFTER DOCKING: DNA GYRASE SUBUNIT B AND HEXADECAMETHYL HEPTASILOXANE



BEFORE DOCKING: DNA GYRASE SUBUNIT B AND HEXAKIS (TRIMETHYLSILYLOXY) CYCLOTRISILOXANE



AFTER DOCKING: DNA GYRASE SUBUNITB AND HEXAKIS (TRIMETHYLSILYLOXY) CYCLOTRISILOXANE

Figure 16: Before and after docking

DISCUSSION

The observations suggested that the Ag^{+} ions were reduced extra cellularly. According to earlier reports, these Nobel metal particles exhibit silver's typical absorbance at about 430 nm (Nithya and Ragunathan, 2009).The silver nanoparticles were synthesized in small mono disperse form, as indicated by the peak attained at 440 nm (Huang, 2007; Wani *et al*., 2010).

The appearance of brown and yellowish solutions resulted in an absorbance intensity peak in gabove the solutions of a dark brown colour at the same ultra violet wavelength, indicating an increase in the concentration of nanoparticles (Ragunath *et al*., 2017). According to Shanmugam *et al*., (2014), a spectroscopic characterization study showed that

the UV absorption scale of nanoparticle silver manufactured by some types of mushrooms (*Pleurotus*) lies within the wavelength range of (430-420) nm. The highest UV absorption peak for silver nanoparticles produced by *P. florida* was found to beat 410nm.

According to Labrenz *et al*., (2000), the stretching of -OH in the extract's proteins, enzymes, or polysaccharides may be the source of these bonds. The -CH stretching of alkanes was the cause of the small band at 2931.60. At1442.66 and 1380.94, respectively, the analogous scissoring and bending vibration was seen. The stretching vibrations of the C=O functional groups of aldehydes, ketones, and carboxylic acids were implied by the medium band observed at 1720.39. The FTIR spectrum shown in Figure 3 identified amide bands, with bands at 1650 cm^{-1} and 1450 cm^{-1} due to the amide linkages of protein C=O and N-H stretch vibrations. These molecular vibrational positions align with previous literature on native proteins. The expansion of the bundles of peaks (111) and (200) reveals the nanoscale dimensions of the produced particles, with less intense unknown peaks potentially resulting from organic and biological filtrate components surrounding a massive surface From observations Remya *et al*., (2015) indicated that *P. djamor* oyster mushrooms produced nanoscale silver particles with a peak 111 that was stronger and higher than those of 200 and 220, while *P. ostreatus* oyster mushrooms produced particles that were more crystallised, with four peaks appearing at level 111. This is mostly because pH levels and the amount of filtrate used have an impact on the size and properties of the produced nano particles. The values of the diffraction peaks were used to calculate the mean value, or 45.26nm,for the average crystal size of the Ag-NPs. The crystalline structure of silver nanoparticles is explained by the four major peaks in Figure 5 with characteristic shifts of different angles (222), (111), (112), and (100) (Dauthal and Mukhopadhyay, 2016).

Blood is a fluid connective tissue circulating in the body that facilitates communication between the cells of different parts of the body. The study of the fish blood parameters are crucial for determining factors related to its physiological capacity (Well *et al.*, 2005). Blood's primary functions include oxygenation of tissues, nutrition, maintenance of acid-base balance, and removal of metabolic waste products. Dysfunctions in blood can severely impact physiological activities of the entire body. Physiological dysfunctions of in the body are reflected as alterations in blood constituents, which can be used as diagnostic indicators. Erythrocytes, leucocytes and thrombocytes being the essential cellular components of fish blood, their concentration is maintained within well-defined limits indifferent fish species unless the balance between production and elimination is disturbed by pathological process.

Generally, the erythrocytes not only pump out sodium and pump in potassium against electrochemical gradient but also reduce methaemoglobin to haemoglobin (Hb) to transport oxygen to the body tissues. Packed cell volume (PCV) or haematocrit (Hct) is a well-known index of anaemia is well known in clinical medicine. White blood corpuscles (WBC) are crucial for the defense mechanism, comprising granulocytes, monocytes, and lymphocytes. Granulocytes and monocytes function as phagocytes, while lymphocytes produce antibodies (Wedemeyer and McLeay, 1981).Thrombocytes are involved in coagulation of blood. Haematological variables of fish under stress are of great significance in assessing the impacts of pollutants in the biota of a particular ecosystem. Therefore, haematology has been widely used as potent bio indicator in aquatic toxicology (Sancho *et. al*, 2000).

The regular monitoring of the fish blood is a diagnostic tool in establishing the health status of the fish in farms. It helps in evaluating the response of different types of blood cells and its components in the conditions of physiological stress due to toxicity, as it quickly reflect the poor conditions of fish than other commonly measured parameters. The blood composition of a fish reflects to some extent to metabolic and other physiological processes. Accordingly, haematology can be used as clinical tool for the investigations of physiological and metabolic alterations in fish caused by pollution of the aquatic environment. In the present investigation, fish *O. mossambicus* on exposure to *P. ostreatus* mushroom AgNO3 nanoparticles at 10µg/kg concentration showed enhancement of different blood parameters indicating improved health and stress response.

Lysozyme activity is an another crucial component in the first line of defense in the immune system. Lysozyme has bactericidal effects by hydrolyzing linkages of bacterial cell wall peptidoglycans

resulting in bacteriolysis.in the present study fish fed diets supplemented with different levels of mushroom extract showed significantly higher lysozyme activities when compared to the control group. Myeloperoxidase (MPO), another group of enzyme which plays a role in the killing of microorganisms. In the study MPO activity of serum in the experimental groups showed an increase compared to the control especially after feeding with2µg/kg mushroom extract supplemented diets. The hematocrit values were increased with no statistical significance among the treated groups. The increased hematocrit, after 14th day post immunization, indicated the safety and efficacy of the immune stimulants used, as reduced hematocrit can indicate poor feed intake or infections (Blaxhall, 1972).

Computational advances played a significant influence in the drug development process. Virtual screening approaches are frequently and widely utilized to minimize the cost and time of drug development. Molecular docking, a technique to discover novel ligands for proteins structure is crucial in structure-based drug design, revealing the relationship between compounds and receptors. Natural products can help cure a wide variety of human ailments, including cancer, inflammatory, etc and various medicines are derived from natural products, e.g., an anticancer drug, paclitaxel (taxol) is derived from extracts of *Taxus brevifolia*. Plant-based medicines are the best potential options, given the rise of drug resistance in various diseases, and the negligible side effects of traditional treatments. This study investigated the binding capability of bioactive compounds from *P. timoriana* with key anticancer and anti-inflammatory targets. The higher negative docking score represented a high binding affinity between the receptor and ligand molecules, showing the higher efficiency of bioactive compounds. The docked ligand scores for anticancer ranged from -4.3 to -6.3 and from -5.3 to -6.0 foranti-inflammatory. In the present study, 3-n-Hexylthiolane, S, S-dioxide, and L-(+)-Ascorbic acid are the lead compounds showing the highest docking score among the subjected bioactive compounds, which also exhibited antibacterial, antioxidant potentials. The docking analysis also revealed that diverse energy sources were consistent and contributed to the overall strength of 3-n-Hexylthiolane, S, S-dioxide, and L-(+)-Ascorbic acid binding with each target protein. Methylbeta-L-arabinopyranoside had the lowest score while 3-n-Hexylthiolane, S,S-dioxide had the highest score as anticancer agents (Ralte *et al*., 2022).

CONCLUSION

It was concluded that, the supplementation of mushroom extract at certain levels in the diet of fish significantly decreased the mortality of *O. mossambicus* experimentally infected with *A. hydrophila* and enhance the non-specific immunity. Mineral nanoparticles are more effective than their bulk counterparts as feed supplements, in improving the growth and health of cultured fish. This higher level of effectiveness is interestingly observed with lower doses of nanomaterials which make them even more cost and material effective than the bulk materials. Higher doses of nanomaterials were found to be detrimental to fish growth and health. Different species and ages of fish were found to be differently impacted by nanomaterials of same nature, source and particle size. Conversely, same species and age of fish were affected in different ways by different ways by nanomaterials of different natures, sources and particle sizes. Hence for sustainable improvement in the growth and health of a particular fish by nanomaterial supplementation in feed, the particle size and dose of the nanomaterial is to be worked out. The effects of nanomaterials on catfish growth and health remain an unexplored research area in aquaculture nutrition.

ACKNOWLEDGEMENT

The authors would like to express their gratitude to Principal, STET College (Autonomous) of Arts and Science, Sundarkottai, mannargudi, Tamil Nadu, India, for the successful completion of Research work.

CONFLICTS OF INTEREST

The authors have no conflicts of interest to declare.

REFERENCES

Al-Bahrani, R., Raman, J., Lakshmanan, H., *et al*., 2017. Green synthesis of silver nanoparticles using tree oyster mushroom *Pleurotus ostreatus* and its inhibitory activity against pathogenic bacteria. *Materials Letters,* **186**:21-25.

Al-Bahrani, R.M., Muayad, S., Majeed, A. *et al.*, 2018. Phyto-fabrication,characteristics and

anti-candidal effects of silver nanoparticles from leaves of *Ziziphus mauritiana Lam*. *Acta Pharmaceutica Sciencia*, **56**:85-92.

Awad, E. and Austin, B. 2010. Use of lupin, Lupinus perennis, mango, *Mangifera indica* and stinging nettle, Urticadioica, as feed additives to prevent *Aeromonas hydrophil*a infection in rainbow trout, *Oncorhynchus mykiss* (Walbaum). *Journal of Fish Disease*, **33(5):**413-20.

Balashanmugam, P., Santhosh, S., Giyaullah, H., *et al*., 2013. Mycosynthesis, characterization and antibacterial activityof silver nanoparticles from *Microporusxanthopus*: a macro Mushroom. *International Journal of Innovative Research in Science, Engineering, and Technology*, **2(11)**:6262-6270.

Bernas, E., Jaworska, G., and Lisiewska, Z., 2006. Edible mushrooms as a source of valuable nutritive constituents. *Acta Scientiarum Polonorum Technologia Alimentaria*, **5(1)**:5-20.

Bilen, S., Bulut, M., and Bilen, A.M., 2011.Immuno stimulant effects of Cotinu scoggyria on rainbow trout (*Oncorhynchus mykiss*). *Fish and Shell fish Immunology*, **30(2):**451-455.

Binaii, M., Ghiasi, M., Farabi, S.M.V., *et al.*, 2014. Biochemical and hemato-immunological parameters in *juvenile beluga* (Husohuso) following the diet supplemented with nettle (Urticadioica). *Fish and Shellfish Immunology*, **36(1)**:46-51.

Blaxhall, P.C., 1972.The haematological assessment of the health of fresh water fish. *Journal of Fish Biology*, **4(4)**:593-604.

Cabello, F.C., 2006. Heavy use of prophylactic antibiotics in aquaculture: A growing problem for human and animal health and for the environment .*Environmental Microbiology*, **8**:1137-1144.

Caruso, D.R., Mayer, J.D., and Salovey, P., 2002. Relation of an Ability Measure of Emotional Intelligence to Personality. *Journal of Personality Assessment*, **79(2)**:306-20.

Dauthal, P., and Mukhopadhyay, M., 2016. Noble metal nanoparticles: Plant-mediated synthesis, mechanistic aspects of synthesis and applications. *Industrial and Engineering Chemistry Research*, **12**:34-38.

Devika, R., Elumalai, S., Manikandan, E. *et al*., 2012. Biosynthesis of Silver Nanoparticles Using the Fungus *Pleurotus ostreatus* and their Antibacterial Activity. *Open Access Scientific Reports*, **1(12)**:557.

Galindo, V.J. and Hosokawa, H., 2004. Immuno stimulants: Towards Temporary Prevention of Diseases in Marine Fish. *Avancesen Nutrición Acuícola*, 273-319.

Gurunathan, S., Raman, J., AbdMalek, S.N. *et al.*, 2013. Green synthesis of silver nanoparticles using *Ganoderma neo-japonicum Imazeki*: A potential cytotoxic agent against breast cancer cells. *International Journal of Nanomedicine*, **8**:4399-4413.

Huang, J. 2007. Biosynthesis of silver and gold nanoparticles by novels undried *Cinnamomum camphora leaf. Nanotechnology*, **18**:105104.

Kawahara, A., Kohara, S., and Amano, M., 1989. Thyroid hormone directly induces hepatocyte competence forestrogen-dependent vitellogenin synthesis during the metamorphosis of *Xenopuslaevis. Developmental Biology*, **132(1)**:73-80.

Khan, S.M. and Ali, M.A., 1981. Cultivation of oyster mushroom (*Pleurotus*spp.) on cotton boll locules. *Mushroom Science*, **11**:691-692.

Mau, J., Lin, H., and Song, S., 2002. Antioxidant properties of several specialty mushrooms. *Food Research International*, **3**:519-526.

McCord, J.M. and Fridovich, I., 1969. Superoxide dismutase: An enzymic function for erythrocuprein (Homocuprein). *Journal of Biological Chemistry*, **244**:6049-6055.

Moghaddam, A.B., 2015. Nanoparticles Biosynthesized by Fungi and Yeast: A Review of Their Preparation, Properties, and Medical Applications. *Molecules*, **20(9)**:16540-16565.

Montgomery, H.A.C. and Dymock, J.F., 1961.The determination of nitrate in water. *Analyst*, **86**:414-416.

Nazeruddin G.M., Prasad,N.R., Prasad,S.R., Shaikh,Y.I., Waghmare,S.R.and Adhyapak

P., 2014. *Coriandrum sativum* seed extract assisted in situ green synthesis of silver nanoparticle and its anti-microbial activity. *Industrial Crops & Products* **60**:212-216.

Nithya, R., and Ragunathan, R., 2009. Synthesis of Silver Nanoparticle. Using *Pleurotus sajorcaju* and Its Antimicrobial Study. *Digest Journal of Nanomaterials and Biostructures*, **4(4)**:623-629.

Numata, K., Kemery, A., and Jordan, C., (2004).Thermal-Noise limit in the Frequency Stabilization of Lasers with Rigid Cavities. *Physical Review Letters*, **93**:250602.

Owaid, M.N., 2019. Green synthesis of silver nanoparticles by *Pleurotus* (oystermushroom) and their bioactivity: Review. *Environmental Nanotechnology, Monitoring and Management*, **12**:100256.

Owaid, M.N., Abed, I.A., and Al-saeedi, S.S.S., 2017. Nutraceutical Value of Four Oyster Mushroom Species, Higher Basidiomycetes. *Hacettepe Journal of Biology and Chemistry*, **45(1)**:117-123.

Prabhusaran, N., Manivannan, L., Pramila, M., *et al.*, 2018. Knowledge,Attitude And Practice of Personal Hygiene, Cleaning and Sanitation During Food Processing. *Ejpmr,* **5(7)**:455-461.

Ragunath, A., Parveen, N.A. and Kumutha kalavallir, A., 2017. Mycosynthesis of Silver Nanoparticles: characterization, Antioxidant and Anti-Inflammatory activity from *Pleurotus florida* (mont) singera macro fungi. *Asian Journal of Pharmaceutical and Clinical Research*, **10(9)**:186-191.

Rahimi, R., Mohammad pour, A.H., Dastani, M., *et al.,* 2016.The effect of nano-curcumin on HbA1c, fasting blood glucose, and lipid profile in diabetic subjects: a randomized clinical trial. *Avicenna Journal of Phytomedicine,* **6(5)**:567-577.

Ralte, L., Khiangte, L., Thangjam, N.M., *et al*., 2022. GC–MS and molecular docking analyses of phytochemicals from the under utilized plant, *Parkiati moriana* revealed candidate anti-cancerous and anti-inflammatory agents. *Scientific Reports*, **12**:3395.

Regula, J., and Siwulski, M., 2007. Dried Shitake (*Lentinulla edodes*) and Oyster (*Pleurotus ostreatus*) Mushrooms as a Good Source of Nutrient. *Acta Scientiarum Polonorum, Technologia Alimentaria*,**6:**135-142.

Remya, R.R., Rajasree, S.R., Aranganathan, L., *et al.,* 2015. An investigation on cytotoxic effect of bioactive AgNPs synthesized using *Cassia fistula* flower extract on breast cancer cellMCF-7. *Biotechnology Reports*, **8**:110-115.

Rook, G.A.W., Rook, B.R., Champion, J., *et al*., 1985. StanfordI-Are stricted activation by T-celllines of anti-tuberculosis activity inmurine macrophages. *Clinical and Experimental Immunology*, **59**:414-420.

Sancho, E., Ceron, J.J., and Ferrando, M.D., 2000. Cholin esterase activity and haematological parameters as biomarkers of sub lethalmolinate exposure in *Anguilla anguilla*. *Ecotoxicology and Environmental Safety*, **46**:81-86.

Saxena, R.K., von Wettberg, E., Upadhyaya, H.D., *et al.,* 2014. Genetic diversity and demo graphic history of *Cajanus* spp. illustrated from genome-wideSNPs. *PLoSOne*, **9**:e88568.

Schaperclaus, W., Kulow, H., and Schreckenbach, K., 1992. Fish diseases,Vol.I. A.A.Balkema/ Rotterdam, septicemia of fish. *Fish Disease Leaflet***, 68:**1-24.

Shanmugam, N., Rajkamal, P., Cholan, S., *et al.,* 2014. Biosynthesis of silver nanoparticles from the marine seaweed *Sargassum wightii* and their antibacterial activity against some human pathogens. *Applied Nanoscience*, **4**:881-888.

Smith, J., Rowan, N., and Sullivan, R., 2002. Medicinal mushrooms: Their therapeutic properties and current medical usage with special emphasis on cancer treatments. *University Strathclyde*, Glasgow, UK.

Tang, G., Gudsnuk, K., Kuo, S.H., *et al.,* 2014. Loss of mTOR-Dependent Macro autophagy Causes Autistic-like Synaptic Pruning Deficits. *Neuron,* **83**:1131-1143.

Vandrell, D., Balcazar, J.L., Imanol R.Z. *et al.,* 2006. Lactococcus garvieae in fish: A

review. *Comparative Immunology Microbiology and Infectious Diseases*, **29(4)**:177-98.

Wang, M., Moora, T.R., Talbot, J., *et al.*, 2014. The stoichiometry of carbon and nutrients in peat formation. *Global Biogeochemical Cycles*, **29**.

Wani, I.A., Khatoon, S., Ganguly, A., *et al.*, 2010. Silver nanoparticles: Large scale solvo thermal synthesis and optical properties. *Materials Research Bulletin*, **45(8)**:1033-1038.

Wedemeyer, G. and McLeay, D. 1981. Methods for determining the tolerance offishes to environmental stressors. 247-275 in A.D. Pickering, ed. Stress and fish. Academic Press, London, U.K.

Wells, R.M.G., Baldwin, J., Seymour, R., *et al.*, 2005. Red blood cell function and haematology in two tropical fresh water fishes from Australia. *Comparative Biochemistry and Physiology Part A. Molecular & Integrative Physiology*, **141(1)**:87-93

Wilchek, M. and Bayer, E.A., 1990. Avidin-biotintechnology. *Methods Enzymology*, **184**:5-45.

Zahran, E., Risha, E., Abdelhamid F., *et al.*, 2014. Effects of dietary Astragalus polysaccharides (APS) on growth performance, immunological parameters, digestive enzymes, and intestinal morphology of Niletilapia (*Oreochromis niloticus*). *Fish Shell fish Immunology*, **38(1)**:149-57.