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STUDY ON THE IMPACTS OF GREEN SYNTHESIZED *PLEUROTUS OSTREATUS* MUSHROOM DIETARY SILVER NANOPARTICLES IN *Oreochromis mossambicus*

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ABSTRACT

Aquaculture is the best growing food production division in the globe. Infection outbreaks are also a most important problem for the aquaculture productions. Different types of technological development are uses to improve healthy aquaculture production and enhance the aquatic environment. Nanotechnology is most significant tool for the development of aquatic productions. The experiments were conducted using feed prepared with supplementation of dietary *P. ostreatus* mushroom $AgNO_3$ NPs on growth performance and survival rate in Tilapia (*O. mossambicus*). Fingerlings were stocked at a density of 10 nos/ tank. Experimental diets were formulated with 35% protein level. In this experiment 7 diets were prepared. Basal diet was considered as control. *P. ostreatus* mushroom $AgNO_3$ NPs were added at the rate of T1 (0.1%); T2 (0.5%); T3 (1%); T4 (5%); T5 (10%); T6 (20%) respectively. The experimental feed was given at the rate of 4% of body weight of the fish twice daily. After 10 days, feeding was decreased to 3% of body weight. After 30 days, Results showed that mean weight gain (%), Specific Growth Rate, Average Daily growth and survival were significantly higher in T5. The significantly lowest Feed Conversion Ratio was recorded in T1 respectively. The survival rate was higher in T5, but it was not significantly different among treatments. The results of the present investigation revealed that the supplementation of *P. ostreatus* mushroom $AgNO_3$ NPs in the diet of *O. mossambicus* fingerlings significantly affected the mean weight gain (%), Specific growth rate (SGR), Average Daily growth (ADG), food conversion rate (FCR) and survival rate. The present study was suggested that the addition of *P. ostreatus* mushroom $AgNO_3$ NPs to a supplement diet has the potential to enhance the growth performance and survival rate in tilapia fish.

Keywords: Mushroom, silver nanoparticles, Growth, food utilization, survival.

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INTRODUCTION

Aquaculture and fisheries supply about 15% of the average animal protein consumption to 2.9 billion people worldwide in, and is still increasing. Approximately 43.5 million people are directly employed within these sectors, and 520 million people indirectly derive their livelihoods from aquaculture and fisheries industries (Asche *et al*. 2015). Similarly, nanotechnology is no more a niche for researchers, but a really fast growing and impacting key economical field providing new nanoenabled products with novel and unique functions. The new-engineered nanoenabled products, improved by nanoparticles (NPs), have been the key factor for the success of the nanotechnology industry. With a size between 1 and 100 nm on at least one dimension, NPs present unique physico-chemical properties that differ from their bulk materials, such as a greater surface area to

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volume ratio, resulting in a larger reactivity. Due to their remarkable properties, NPs have been widely used in different fields such as energy and electronics, wastewater treatment, personal care products, and medicine and agriculture (Vale et al. 2016). Recently, nanotechnology has found several applications in aquaculture, but their implications are still unknown.

The benefits and applications of probiotics and prebiotics have been excessively reviewed in several studies, and their efficacy has been proven for use in aquaculture (Abdel-Latif *et al*., 2023). Prebiotics are non-digestible food constituents that promote the growth of beneficially important microbiota in the gastrointestinal tract and confer healthiness to the treated fish and shrimp (Yilmaz *et al*., 2022). Medicinal mushrooms are a functional feed ingredient for finfish and crustacean species (Mohan *et al.,* 2023). Several research studies have thrown light on their beneficial applications to improve growth, immunity, digestive enzymes, and the overall health conditions of several fish species (Harikrishnan *et al.,* 2011). The richness of medicinal mushrooms with numerous beneficial products such as hemicellulose, mannans, chitin, xylans, α-glucans, galactans, and β-glucans presents them as a best choice for use as prebiotics. Several mushroom products can be effectively utilized as a functional supplement in diets for finfish and shrimp, such as extract, mycelia liquid biomass, stalk waste extract, dry powder, and exopolysaccharides (Mohan *et al.,* 2023).

A variety of medicinal mushrooms have been used for several finfish species. For instance, **Phellinus linteus** extract significantly enhanced growth, immune activity, and disease resistance in **Epinephelus bruneus** (Harikrishnan *et al.,* 2011). The white button mushroom (**Agaricus bisporus**) considerably boosted the immune responses (Khodadadian Zou *et al.,* 2016) and antioxidant capacity (Hoseinifar *et al*., 2019) of common carp. Another study showed that white button mushrooms augmented the growth, immune activity, antioxidative capacity, and stress resistance in Nile tilapia (Dawood *et al.,* 2020). The oyster mushroom (**Pleurotus ostreatus**) extract also stimulated the immune status and disease resistance of rainbow trout (Mithun *et al.,* 2019). Likewise, the eryngii mushroom (**Pleurotus eryngii**) recuperated the growth and immunity of koi carp fingerlings (Safari and Sarkheil, 2018). The present study was to determine the effects of dietary supplementation of *P.ostretus* mushroom silver nanoparticles on the growth response in tilapia against *A. hydrophila* pathogen.

Materials and methods

Fish

Tilapia fishes showing no signs of disease (under gross and microscopic examination of skin, gill, intestine and kidney tissues of representative samples), both sexes of average body weight of 150±0.23g and total length 15±0.31cm were obtained from the local fish farm, Swamimalai, Kumbakonam, Tamilnadu, India. Fishes were retained for acclimatization in glass tank 20 L capacity. Water was changed on alternate days. The fish were fed twice a day with a balanced diet prepared in our laboratory.

Preparation of mushroom extract

Mushroom extract was prepared according to the method described earlier (Kim *et al*., 2020). The 5g fresh mushrooms washed repeatedly with distilled water to remove any organic impurities present in it. The cleaned mushrooms were then crushed to small pieces with a sterilized knife. The small pieces of mushrooms were then taken into the 2L beaker containing 500mL double distilled water and thoroughly stirred for about half an hour and then the solution was filtered with filter paper. The resultant filtrate wash the extract of mushroom used as reducing and stabilizing agent for the reduction of Ag+ to Ag0.

Synthesis of $AgNO_3$ nanoparticles

Silver nanoparticle (AgNP) solution (99.5%purityasuncappedparticles-MKN-Ag-090) was purchased from M K Impex Corp. Different concentrations of AgNPs were maintained during water exchange by re-dosing with the respective concentrations according to the treatment (10, 20,

and 30 µg AgNPs L−1). The mixing of AgNP solutions with the tank water was conducted by continuous aeration, which also prevented the aggregation and precipitation of the tested material (Wang *et al*., 2011).

Feed Preparation

Ingredients of the feed given during experiments are presented in Table 1. The required ingredients were mixed with water to make dough followed by cooking in an autoclave. After cooling, vitamin and minerals were added. Finally, the dough was pressed through a hand pelletizer to get uniform size pellets (2 mm) and shade dried. The pellets were then kept at room temperature and shade dried for complete drying and then packed in clean plastic containers

Experimental Design

Healthy *O. mossambicus* (body weight 150±0.23g) were procured in local fish farm and the fish were examined for their health status upon arrival immediately than it was rinsed immediately with 0.1% KMnO4 solution to avoid infection. The fish were acclimated for 15 days in 100 L aerated fiber tanks with dechlorinated tap water and provided proper aeration. The water temperature 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 were maintained throughout the experimental period. The fish were divided into seven experimental groups of 12 each in triplicate (7×12x3=252fish) Control T1 (0.1%); T2 (0.5%); T3(1%); T4(5%); T5(10%); T6(20%) *P.ostreatus* Silver Nano particles enriched diets at the rate of above mentioned concentrations were exposure their body weight twice a day at 10.00 a.m. and 2.00 p.m. The water was exchange daily about 50% and uneaten feed were collected after 30 min of feeding for growth study. The respective diets in each groups were continued till the end of experiment.

Growth measurements

At end of the experiment, growth rate, specific growth rate, percentage increase in body weight, average daily growth was calculated using the following equations:

$$\text{Growth} = \text{Finalweight-Initialweight(mg)}$$

$$\text{Growth Rate} = \frac{\text{Weight gain}}{\text{No of days x initial weight}} \text{(mg.day}^{-1}\text{)}$$

$$\text{Specific Growth rate(\%)} = \frac{\text{Ln final weight-Ln initial weight}}{\text{Number of days}} \text{x100}$$

$$\text{Percentage of Increase in Bodyweight} = \frac{\text{Final weight-Initial weight}}{\text{Initial weight}} \text{x100}$$

$$\text{Average Daily Growth} = \frac{\text{Final body weight - Initial body weight}}{\text{No of feeding days}}$$

$$\text{Survival rate (\%)} = \frac{\text{Initial number of fish - mortality}}{\text{Initial number of fish}} \text{X100}$$

Statistical analysis

All data were expressed as a means ± standard error (SE). ANOVA was applied on the basis of polynomial orthogonal contrasts. Duncan's multiple range tests was used to identify the differences among the means at a significance level of 0.05.

Results

Synthesis of *P. ostratus* $AgNO_3$ nanoparticles

The silver nanoparticles was characterized by UV-visible (ELICO SL 159UV-VIS nano drop spectrophotometer) spectroscopy. The sample was found to showthepeakat371 nm which confirms the silver nanoparticles.

Different concentration of silver nitrate (mm)	Percentage of inhibition (%)
Control	0.958±0.11
1	1.667±0.68
5	1.947±0.55
10	2.304±0.09

Table 1: Biosynthesis of silver nanoparticles from Mushroom powder

The values are expressed in terms of (Mean ± Standard deviation)

Fourier Transform Infrared Spectrum (FTIR) analysis of *Pleurotus ostreatus*

Fourier Transform Infrared Spectrum showed vibration bands the absorption band at 732.95 cm -1 , 817.82cm -1 corresponded to C- H bending aromatic substitution. The band at 910.40 cm -1corresponded to C- H bending alkane mono substituted. The band at1026.13 cm -1 , 1203.58 cm -1 , 1249.87 cm-1and 1334.74cm-1corresponded to C-N stretching vibration of aromatic and aliphatic amines. The band at 1450.47cm -1 corresponded to C- H bending alkane CH2. The band at 1643.35cm-1 corresponded to N-H bending vibration of the amide I and II. The band at 1859.38 cm-1corresponded to an hydred stretching vibrations α,β - unsaturated, 5 membered rings. The band at 1913.39 cm -1, 2322.29 cm -1 corresponded to C-C multible bond steretching Alkene. The band at 2870.08 cm -1, 2947.23 cm -1 and 3078.39cm-1 corresponded to C-H stretching vibration. The band at 3170.97 cm -1, 3271.27cm -1 corresponded to N-H stretching Primary bonded, two bands. The band at 3410.15 cm-1, 3464.15 cm-1 and 3533.59 cm -1 corresponded to O-H stretching. The band at 3533.59cm-1, 3533.59 cm-1 corresponded to OH group of phenol. The band at 3849.92cm-1, 3965.65cm- 1 corresponded to N-H group of amines respectively

Growth study

The performance in growth of tilapia was influenced by *P. ostreatus* $AgNO_3$ as shown in Table 1. Upon completion of the trial, the AgNPs supplemented groups were exhibited a greater proportion of growth and weight gain in comparison with the control group ($P<0.05$). SGR exhibited a significant variance ($P < 0.05$) among the different treatments, with T5 having the greatest value, when compared to other treatments, was statistically higher ($P<0.05$) in the control treatment. The cumulative survival rate does not follow a specific order and the highest percentage recorded was in T4 and T5 and least survival rate in the control treatment.

The use of vitamins as feed additives is recommended in the diets of farmed fish. *P.ostreatus* mushroom $AgNO_3$ nanoparticles is an important role as an immuno- stimulant and an antioxidant scavenger. In the present research, dietary supplementation of different concentrations of *P. ostreatus* mushroomAgNO3nanoparticles in Nile tilapia fingerlings significantly improved the growth performance of the fish in comparison to zero-level of P. ostreatus mushroomAgNO3 nanoparticles supplementation. This could be attributed to the role of *P. ostreatus* mushroom AgNO3nanoparticles in increasing the serum levels of growth hormone, enhancing the intestinal morphology, and improving the absorptive surface of the intestine in fish. Enhancement of growth rate and weight gain observed in Nile tilapia upon $AgNO_3$ nanoparticles supplementation could be ascribed to the silver nitrate nanoparticles induced stimulation of protein synthesis.

Discussion

In recent years, hematological parameters have been used to evaluate the response to applying additives and products of natural origin and determine tilapia's health status (Reda *et al.* 2016). Among the innovative products of natural origin is mushroom. These organisms improve the immune system and growth (Hleap-Zapata *et al.* 2021). The mushroom stalk waste of *Pleurotus pulmonarius* has been used in diets (23% protein) for tilapia tolerating inclusion levels of 5% maximum (Ahmed et al. 2017), a level lower than that found in our study with NPs. This study showed that the addition of 20% NPs meal in diets for tilapia improved the growth as well as health.

Table 2. Effects of mushroom nanoparticles supplement on the growth parameters of *Oreochromis niloticus* at 30 days of feeding the experimental diets. The inclusion of NPs in the diet of tilapia improved growth and survival parameters at the tested levels of 0.1 to 20% compared to the control. This difference with the control has been reported for other *Pleurotus spp.,*in the Nile tilapia feeding (Aderolu *et al.* 2015 (10-40g)). However, when tested with other species like *Pleurotus florida*, there were no differences in its growth with the control (Muin *et al.* 2014). The WG, SGR%, and survival obtained in this study have been higher than those found when including other *Pleurotus* species in diets for Nile tilapia (Aderolu *et al.* 2015, Khalafalla & EL-Sayed 2015, Srichanun *et al.* 2017), or similar to *P. florida* (Muin *et al.* 2014). However, when oyster mushroom stalk waste (*Pleurotus spp.)* has been used in Nile tilapia diets, higher yields have been obtained than those found in our study (Ahmed *et al.* 2017). The previous studies reported with *O. niloticus* were carried out in the ranges of weight studied (3-40 g) and time of the bioassay (60-70 days). The best response to the growth parameters in tilapia, compared to the control, could be because Pd has antioxidant, anti-inflam-matory, and antitumor substances (Serrano & Divina 2016).

It has been determined that mushrooms of the genus *Pleurotus spp.* produce β-glucan that at high levels can produce toxicity (Qinghui *et al.* 2007). Assays of mushroom stalk waste of *P. pulmonarius* in Nile tilapia have shown that levels up to 20% β-glucan do not induce mortality in feeding trials, and the content of β-glucan in our diets did not influence survival. This study demonstrated that *Pleurotus djamor* var. *roseus* mushroom binds to several other species (*P. sajor-caju, P. ostreatus, P. albidus* and *P. flabellatus*) when used in low concentration diets as a nutritional supplement for fish (Sartori *et al.* 2015). *P. djamor* increases the nonspecific immune response in Nile tilapia when using feed supplemented with oyster mushroom extract. Also, it was found that the supplementation of a mushroom meal in a proportion of 15 to 25% in the diet of the fish can improve the health status, growth, and survival of Nile tilapia, which is in accordance with what has been reported for the response of tilapia to the inclusion in the diet of other species of *Pleurotus spp.* (Srichanun *et al.* 2017, Safari & Sarkheil 2018). This positive response of Nile tilapia to the inclusion of NPs at low levels could be because the consumption of *Pleurotus* has shown immune-stimulating activity in fish (Abdullah *et al.* 2017).

To put it briefly, the present study's findings revealed that supplementing diets with 10 % NPs supplement diet had significantly upgraded growth and feed utilization. In addition, there was a trend towards a high final weight, WG, SGR%, and survival in the diets with up to 10% inclusion of *P. ostreatus* meal. Even in the groups that presented alterations in the proportions of defense blood cells, there was no damage to growth rates, which can be considered positive. In a future trial, it would be interesting to test the effect of these treatments followed by an experimental infection.

Parameters	Treatment groups						
	Control	T1 (0.1%)	T2 (0.5%)	T3 (1%)	T4 (5%)	T5 (10%)	T6 (20%)
Initial Weight (g)	5.15±0.22	5.12±0.23	5.1±0.24	5.17±0.27	5.19±0.28	5.14±0.24	5.44±0.25
Final Weight (g)	5.85±1.21	6.16±1.25	6.22±1.22	6.37±1.22	6.61±1.24	6.67±1.28	6.44±1.27
Growth (g)	0.26±0.01	0.40±0.022	0.44±0.023	0.56±0.022	0.69±0.021	0.59±0.022	0.43±0.021
Growth Rate (g/day)	0.0017±0.001	0.0026±0.001	0.0029±0.0023	0.0036±0.001	0.0044±0.001	0.0038±0.001	0.0026±0.0012
Specific Growth Rate (%)	0.15±0.21	0.22±0.12	0.24±0.17	0.31±0.18	0.30±0.21	0.38±0.25	0.23±0.24
Percentage Increase Body Weight	4.91±1.22	6.57±1.24	7.18±1.26	8.88±2.44	9.438±2.41	10.84±3.24	6.74±2.54
Average Daily Growth (g/day)	0.92±0.21	1.33±0.12	1.49±0.13	1.88±0.17	1.99±0.18	2.23±0.17	1.44±0.15
Survival Rate (%)	60±2.45	70±2.54	80±3.24	90±3.44	100±5.74	100±5.98	90±6.45

Table 2: Growth characteristics of Tilapia fingerlings on the treated with *P. ostratus* mushroom $AgNO_3$ nanoparticles on 30th day post treatment

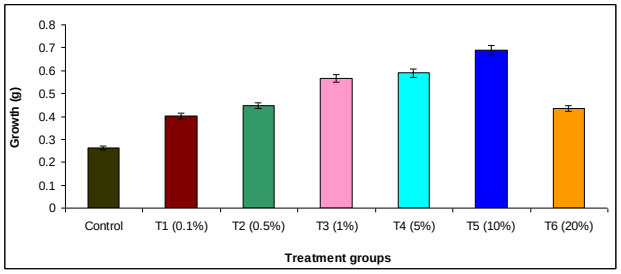

Fig 1: Effect of *P.ostreatus* mushroom $AgNO_3$ nanoparticles supplement on growth performance in tilapia

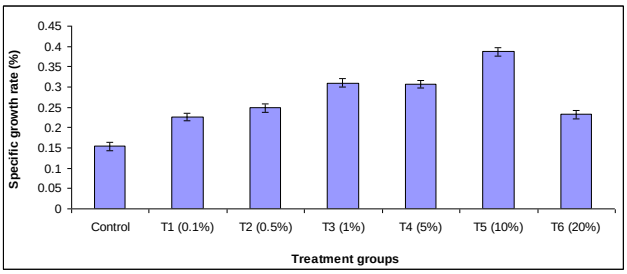

Fig 2: Effect of *P.ostratus* mushroom AgNO3 nanoparticles supplement on specific growth rate in tilapia

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