*Plant Archives* Vol. 21, Supplement 1, 2021 pp. 148-154 e-ISSN:2581-6063 (online), ISSN:0972-5210

Plant Archives

Journal homepage: http://www.plantarchives.org
doi link : https://doi.org/10.51470/PLANTARCHIVES.2021.v21.S1.027

# ANTIFUNGAL ACTIVITY OF CURCUMIN- SILVER NANOPARTICLES AGAINST *SAPROLEGNIA SPP.* IN COMMON CARP

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**ABSTRACT**

Over the years, nanotechnology has gained a rapidly growing in various aspects such as agricultural, environmental and health management. Since, little information is available relating using nanoparticles as antimicrobial agent. Thus, this work was initiated to assess the antifungal activity of curcumin-Silver nanoparticles (Cr-AgNPs) against pathogenic *Saprolegnia spp.* in common carp. Around of 120 *Cyprinus carpio*(20 fish treatment$^{-1}$) were infected with *Saprolegnia* ($2x10^4$ zoospore $l^{-1}$) and treated with different series (4, 8 and 12 mg $l^{-1}$) of Cr-AgNPs (size 30 nm) and a treatment as reference ("formalin; 0.15 ml $l^{-1}$, 30 min") and two treatments one acted as positive control "infected with *Saprolegnia spp.* without disinfectants" and one was served negative control without any disinfectants. After 14 days of treatment, results of blood sets, RBCs, WBCs count and hemoglobin content were recorded significantly ($P<0.05$) increased in T3 (12mg $l^{-1}$ of Cr-AgNPs) relative to C+ - group. Considerable changes in TP, albumin and globulin were observed in all Cr-AgNPs and formalin group in comparison to positive groups (C+). Albumin illustrated a significant decrease ($p<0.01$)in all Cr-AgNPs and formalin treatments relative to C+group.As a result, all the Cr-AgNPs treated groups showed highest survival rate than C+ group, the maximal survival was noticed in T3 (85%) indicating the antifungal efficacy of Cr-AgNPs against pathogenic *Saprolegnia.* This offers new evidence about the effectiveness of nanoparticles on aquatic species. Further studies are required of the antifungal and antibacterial activities of Cr-AgNPs against other pathogens in fish.

***Keywords*:** *Cyprinus carpio*, antimicrobial, curcumin, nanoparticles, hematology

## Introduction

Recently, fungal infections have considerably responsible for the highest mortality and morbidity as well as severe of commercial loss in aquaculture productions (Hassan *et al*., 2007; El-Ahl, 2010). Water mould (Saprolegniasis) is considered as an endemic disease affecting both cultured and wild fish industries across the world (Torto-Alalibo, 2005; Jalilpoor, 2006). This disease is usually causing focal lesion (patches) over the body and gills resulting destructive changes, loss of osmotic regulation and failure of respiration with a linked mortality rate of up to 50% (Pickering and Willoughby, 1982; Osman *et al*., 2008; Roberts, 2012; Van den Berg *et al*., 2013; Ashor, 2017; Fadhal, 2020). Frequently, Saprolegniasis is controlled using Malachitee green, but this compound was excluded in many countries for its recognized mutagenic and carcinogenic impacts (Alderman, 1985). Other products such hydrogen peroxide, formalin, and copper sulphate were also suggested for treating this pathogen. But, adverse effects, environmental impacts and bio-safety of these agents are unconvinced (Forneris *et al*., 2003; Zaki *et al*., 2003). Due to that, recently researchers have redrawn their efforts on some of the natural and alternative substances and traditional drugs such as curcumin. Noble metal nanoparticles have many applications in optical, electronic, chemical, biological, and medical fields due to their characteristic properties (Panigrahi, 2004; Ghosh, 2007). Curcumin, "*Curcuma longa* L.", is an ancient coloring spice of Asia" generally recognized as "turmeric," belongs to "*Zingiberaceae* family". Curcumin, is one such wonder that has paid the attention of researchers all over the world for different reasons involving antimicrobial and anti-inflammatory effects, wound remedial and lighting of the skin (Yang *et al*., 2005). Though, poorly aqueous soluble in natural pH and subsequently, rapid rate of metabolism, poorly bio-availability and absorption and fast systemic excretion delay the direct apply of curcumin as a medicine (Garcea *et al*., 2004). To exploit the potential therapeutic effect of this wonder agent, researches established on the application of chelating curcumin with metal (adjuvants) or mixing with other dietary agents, have been investigated. The antifungal activity of curcumin is owing to the "down regulation of 5,6 desaturase (ERG3) resulting to considerable reduction in ergosterol" of fungal cell. Therefore, biosynthetic precursors of ergosterol accumulate, leading to cell death through induction of reactive oxygen species (ROS)" (Sharma *et al*., 2010). Silver ions have been identified to carry awide spectrum of antimicrobial efficiency (Nasrollahi *et al*. 2011). Silver is low toxic to mammalian cells and very toxic to microbial agents via generation of ROS ($O_2^-$, OH, $O^-$) with consequent oxidative stress (Kim, 2007). Also, it has been stated that there is a damage of membrane integrity after treatment with Ag-NPs (Nasrollahi,*et al* 2011). The ultimate goal of this investigation was to assess the antifungal efficacy of Cr-AgNPs against pathogenic *Saprolegina spp.* in carp fish.

## Materials and Methods

### Preparation of the Curcumin-SilverNanoparticles (Cr-AgNPs)

Curcumin powder was bought from India. Solution was prepared following the procedure of Jagannathan *et al*. (2012). Firstly, 500 mg of curcumin powder was added to 100 ml of sterile deionized water. Then, the mixture was boiled for 30 min at 80°C. The heterogeneous mixture then filtered using "Whatman filter paper no". 1 to obtain a homogeneous solution.

Preparation of Cr-AgNPs was done as labeled by Manonmani *et al*. (2015), 3 ml of curcumin solution and 10 ml of silver nitrate ($AgNO_3$; 0.1 M) were mixed in a 50ml conical flask and heated in a water bath at 60°C for 1 h. The formation of the Ag-NPs was checked and detected through a colour change appearance of the mixture that changed from "light-yellow to brownish". The mixture was extra optimized using "ultraviolet visible (UV-vis) spectroscopy (UV-2450, Shimadzu, Japan)". The synthetic solution was centrifuged for 15 min at 9000 rpm for 15 min. After, to get filtered and purified Cr-AgNPs, the compound was washed by ethanol and dried by vacuum oven (VO-27, Korea).Morphology of Cr-AgNPs were observed under "transmission electron microscopy "[(TEM)(JEM-2010 (Jeol)]" and size distribution of the prepared Cr-AgNPs were identified using the "UV–Vis absorption spectroscopy".

### Isolation and identification of "*Saprolegnia spp.*"from infected fish

Infected *C. carpio* that exhibited external fungal lesion "cotton -wool like lesion and ulcerations" over the surface of the body were collected. The infected parts were transferred to a sterilized "Sabouraud dextrose agar" containing sterile sesame seeds, *Sesamum indicum* and chloramphenicol. Culture dishes were incubated at 21ºC for 3-5 days with daily examination for any expected growth. Practicable fungal suspension of Saprolegnia was detected and validated at a level of $2\times10^4$ zoospores $l^{-1}$ using heamocytometer (Horwitz *et al*., 1975).

### Effect of Cr-AgNPs on *Saprolegnia spp.* in cultures media

For determination of Cr-AgNPs and formalin on the growth of *Saprolegnia spp*. in cultures media, 500 ml of SDA was prepared and divided into 5 beakers each one have 100 ml. and then autoclaved, the concentration of Cr-AgNPs (4, 8, 12, mg $l^{-1}$) were added for three beakers, the formalin (0.15 ml $l^{-1}$) for one beaker and one beaker left as a control. Then, 6 culture petri dishes were prepared for each concentration and were cultured by fungus. After that, incubated in 20 ºC for 15 day and observed the growth of fungus in all concentrations of Cr-AgNPs (Sykes *et al*., 1965).

### Fish rearing and experimental set up

Approximately, 120 juvenile *C. carpio* weighing 35 ± 5 g were bought from a local fish farm (Babylon, Iraq). Fish were acquainted for 15 day in laboratory conditions before initiation the experiment. Then, 120 fish (20 fish treatment-$^{1}$) were infected with *Saprolegnia* ($2x10^4$ zoospore $l^{-1}$) and treated with different series (4, 8 and 12 mg $l^{-1}$) of Cr-AgNPs for 30 min and a reference treatment ("formalin; 0.15 ml $l^{-1}$, 30 min for 3 d") and two treatments one acted as positive control "infected with *Saprolegnia spp.* without disinfectants" and one was served negative control without any disinfectants. Each of the six treated groups was reserved in 12 hr dark/light cycles and was fed daily the formulated diets at a percentage of 1% body mass during the experiment. Every day once feeding (about one hr) the tanks were cleaned and the remnants were removed. Water parameters (quality) were recorded during the experimental period as follows: ["Temperature (Cº) 20, Dissolved $O_2$ (6 mg /L) 6, pH 7.5"] as the optimize condition of carp fish.

### Blood indices and biochemical changes

Blood samples were collected from the caudal vein using a plastic syringe 3ml. Blood samples were transferred immediately to two sets of test tubes one have EDTA (as an anticoagulant) for investigating blood indices (Hb content, PCV value, RBC and WBC count). The second amount of blood samples were transferred to clean test tubes without adding EDTA and were permitted to clot for 3 hr. Serums were separated by centrifugation at 3000 rpm a for studying biochemical tests (Grant *et al*., 1987).

### Survival rate

Fish were monitored every day and the mortalities for the treated groups were recorded every day during the experimental period and the survival rate was calculated based on the following formula: "*Survival rate (%) =final number of fish survivor/initial number of fish rerared×100"*

### Statistical analysis

All analyses were carried out using Statistical Analysis System- SAS (2012) program. Significant level was detected using analysis of Variation-ANOVA) and Least significant difference –LSD) test. All results were presented as means±SE, P values less than 0.01 and 0.05 were represented significant.

## Results and Discussions

### Isolation and identification of "*Saprolegnia spp.*"

Following1-3 days incubation at 20 °C on SDA, the colonies of Saprolegnia appeared as rounded mass of fibers, brownish in the center and whitish in color and categorized by an extensive and abundant mycelium. Isolates were manifested by the presence of "separated non-septate hyphae", together with masses, different in width and length, translucent and has cell membrane. Such sporangia was filled with great amount of spores which disconnected from the "basal somatic hyphae" ( Fig.1).The present study indicated that the colonies of *Saprolegnia* after 48 hr. of incubation at 20 ºC appeared as circular and these observations are comparable to those recorded by Ashour *et al*. (2017) whom demonstrated that the Saprolegnia colony shape was rounded, whitish in color, (1-1.5 cm) with brownish color in center bevel to black. These finding are directly consistent with Bruno and Stamps (1987) and also are in agreement with Muhsin, (1989) who used sesame seeds for the growth of "*Saprolegnia spp"*. in as a substitute Of "*Cannabis sativa* L."

### Cr-AgNPs size and characterization

The synthesized Cr-AgNPs observed under TEM had spherical morphology within average size 30 nm, in which few nanoparticles were conglomerated. The UV spectral analysis results in the current study indicated a prominent peak at 434.50 nm which is within the characteristic range of the silver nanoparticles (Fig. 2 A&B). Over the years, nanotechnology have performed unique success in different

areas, for instance, food, health management, agricultural and environmental applies (Bouwmeester *et al*. 2007; Kiruba Daniel *et al*. 2013). Silver particulates, and particularly Nano-silver in spherical shape, are recognized globally as a treatment or cure of fungal, viral and bacterial pathogens (Murr, 2009). Hence, silver is commonly applied to take benefit of its antimicrobial characteristics (Kawashita *et al*., 2000). The pathways involved in the antimicrobial activity of AgNPs have already been documented by Kim *et al*. (2007); Sanpui *et al*. (2008); Rai *et al*. (2009) which involve: destructive changes in the membrane structure of a microorganism, which increases its absorptivity and impairment the transportation function, causing in cell death, interaction with phosphorus and sulfur-containing complexes, such as DNA, damage of the reproduction capability of the DNA, inactivation of specific enzymes, affecting the respiratory chain causing induction of free radicals, and the release of the silver ions from the nanoparticles (Feng *et al*., 2000; Yamanaka *et al*., 2005; Yoshihiro, 2002; Song *et al*., 2006).

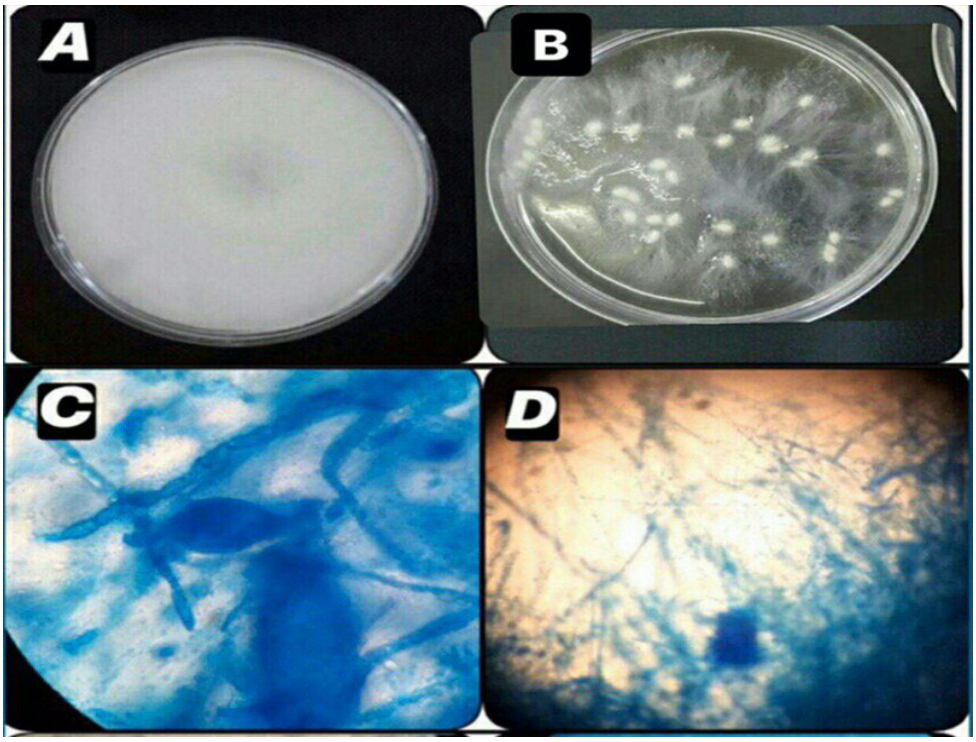


**Fig. 1:** A- *Saprolegnia spp.* colonies on SDA at 20ºC for 1-3 days started with cysts of long hairs with white- cottony color. B- Wet culture Saprolegnia growth on sesame seeds. C: part of sesame seed colony showed masses of zoosporangia filled with great numbers of zoospores blue; D-: the hyphae appeared profusely split and were non septate. "Lacto-phenol cotton blue" 40X.

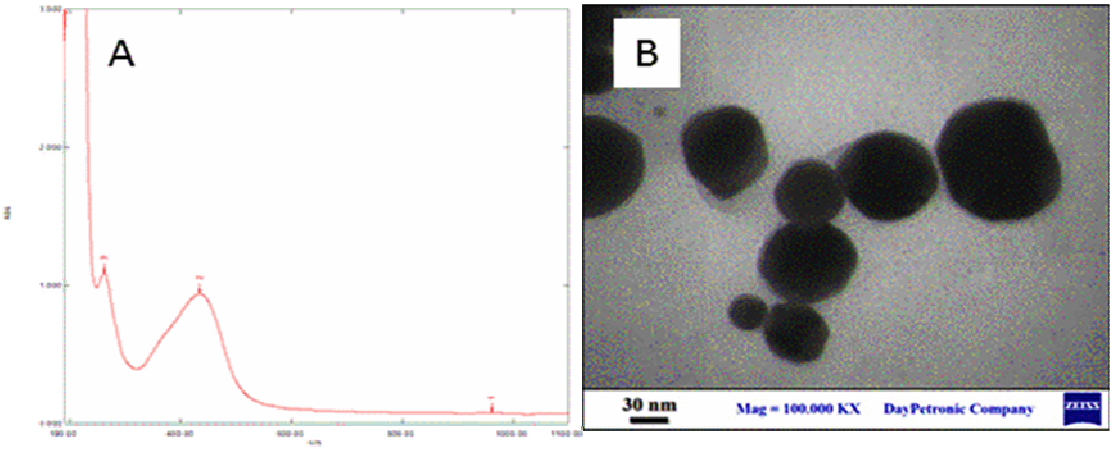


**Fig. 2:A:**Cr-AgNPs plasmon peak at 434.50 nm.; **B:** TEM image of Cr-AgNPs with the average size of 30 nm.

**Blood indices**

Results of blood indices are elucidated in Table 1. After 14 days from the infection with Saprolegnia and treatment with Cr-AgNPs, RBCs numbers of C+group registered significant decrease ($P< 0.01$) compared to C- group (normal). The highest mean was recorded in T3 which showed significant ($P<0.01$) increase relative to T1, T2,and to C+ group. PCV (%) registered considerable decrease ($P<0.05$) in C+ than C- group. But, PCV (%) exhibited significant increase ($P<0.05$) in T3 (treated with 12 mg $l^{-1}$ of (Cr-AgNPs)compared to T1, T2 and to C+ group respectively. Besides, there were significant differences ($P<0.05$) observed among all treated groups. For the Hb content after infection with *Saprolegnia spp.* showed significant decrease in C+relative to C- group. All experimental groups (T1, T2, T3 and T4) publicized

significantly increase relative C+ group. Interestingly, T1, T2, T3 and T4 were marginally increased from C- group but was not significant (P>0.05). On the other hand, WBCs numbers were found significantly (P<0.05) increased in T3 compared to control groups. Also, T3 documented significant increase relative to T2 and T4 respectively.

Results of RBCs count, PCV (%) and Hb content showed significant decreased in infected group (C+) relative to control group, this decreasing possibly due to anemia caused by the fungi mycelia that enter the blood vessels of infected fish and caused the hemorrhage, damage in hematopoietic tissues, haemodilution and osmotic-regulation imbalance, mucus release and lethargy. These results are in line with McLeay (1973); Tort (1987); Gill and Epple (1993) and Shah and Altindag (2004).

Shah *et al*. (2015) reported, that total RBCs, Hb concentration and hematocrit significantly decreased in the Saprolegnia- infected fish when compared with the normal ones. A possible elucidation for this could be that the mycelium of the Saprolegnia penetrates deeply causing injury resulting in the loss of blood (Juncey and Ross, 1982). These findings are match with findings observed by Zaki *et al*. (2008) who reported that "*Tilapia nilotica* infected with *Saprolegnia parasitica"* resulted in a considerable decrease in the RBCs numbers, Hb, and hematocrit. Similar results were also stated by Hatai *et al*. (1984) on naturally infected "Ayu, *Plecoglosus altivelis* by *Aphanomysis piscicida"*. Bruno and Munro (1986) documented that the experimental infection of rainbow trout and Atlantic salmon with "*Ranibacteriun salmoninarum"* resulted in the significantly decline of RBCs count and Hb level. Similar findings were also reported by Jamalzadah *et al*. (2009) who reported that fungal infected of "Salmo, *Trutta fario*" exhibited significant decline in the total erythrocyte count, Hb and PCV as compared to the values in normal ones. Wong *et al*. (2007) explored to gain further suggestion for the anti-inflammatory categories of Ag-NPs, in which they used both in vitro and in vivo models and found that AgNPs have the ability to down-regulate the quantities of inflammatory markers, indicating that Ag-NPs could suppress inflammatory events in the early stages of wound healing.

Quantity and quality of WBCs count is commonly used as pointer for diseases and immune response (Cagirgan, 1990). Changes in WBC have been reported to play a vital role in the evaluation of the fish health state. It is generally that leukocyte cells lower in healthy fishes and higher in infected fish, which could be used as a considerable indicator for infectious diseases (Gabriel *et al.,* 2004).

Results of current study observed significantly increase in WBCs count of *C. carpio* in C+ group compared to C- group. These findings reflect those of Jamalzadeh *et al*. (2009) who also reported significant increase in leucocyte count in fungal infected fish than healthy Caspian Salmon. Shah*et al*. (2015) also stated the changes in WBCs in rainbow trout in similar patterns as in the current study.

In the present study, the increases in WBC count in infected *C. carpio* (C+), are likely to be associated to a specific- immune response to fungal infection, this observation is are in line with Shah *et al.* (2015) who suggested that the increases in WBC count of "rainbow trout, *Oncorhynchus mykiss* affected by *Saprolegnia spp*." were believed as a response of cellular immune system to fungal infection In contrary, The present results of WBC count are in disagreement with Syed (2010) who observed that all blood parameters of "tench, *Tinca tinca* infected by *Saprolegnia spp*." decreased as response to infection include WBCs, the decreases in blood parameters of *Tinca tinca* were credited to stress-facilitated hormonal imbalance, damage in gill tissues due to direct action of the fungi, and skin lesions due to fungi could result rupturing of erythrocytes.

There was increase in WBCs count in all C-AgNPs S group (T3) and formalin group (T4) compared to control groups C+ and C- groups. This could owe that Cr-AgNPs and formalin like the other disinfectant which use for treatment the fish diseases, have a toxic effect on fish this toxicity lead to increase the immune response in fish and increase in WBCs count. The increase in WBCs count might be associated with stimulating the immune response and with an increase in antibody production which helps in survival and recovery of the fishes exposed to the toxicant (Seth and Saxena, 2003).

**Table 1:** Blood indices (mean± SE) of *C. carpio* which infected by Saprolegnia and treated with Cr-AgNPs and formalin.

| Treatments | RBC x $10^6$µl$^{-1}$ | PCV (%) | Hb (g/dl) | WBC x $10^3$ µl$^{-1}$ |
|---|---|---|---|---|
| **Control –ve non infected** | 2.006 ± 0.09$^{a}$ | 24.00 ±1.50$^{ab}$ | 7.05 ±0.30$^{a}$ | 19.36 ± 0.47$^{c}$ |
| **Control +ve infected** | 1.61 ± 0.06$^{b}$ | 17.66 ±1.76$^{c}$ | 5.48 ±0.39$^{b}$ | 21.68 ± 0.88$^{bc}$ |
| **T1 4 mg/l (Cr-AgNPs)** | 1.67 ± 0.08$^{b}$ | 20.00 ±2.31$^{bc}$ | 6.85 ±0.20$^{a}$ | 23.13 ± 0.37$^{ab}$ |
| **T2 8 mg/l (Cr-AgNPs)** | 1.72 ± 0.04$^{b}$ | 21.33 ±2.02$^{bc}$ | 7.07 ±0.26$^{a}$ | 22.75 ± 0.56$^{b}$ |
| **T3 12 mg/l (Cr-AgNPs)** | 2.08 ± 0.09$^{a}$ | 27.00 ±2.08$^{a}$ | 7.49 ±0.27$^{a}$ | 25.73 ± 0.52$^{a}$ |
| **T4 0.15 mg/l (Formalin)** | 1.98 ± 0.09$^{a}$ | 24.33 ±1.20$^{ab}$ | 7.27 ±0.39$^{a}$ | 22.88 ± 1.63$^{b}$ |
| **LSD value** | 0.251 ** | 5.578 * | 0.974 ** | 2.643 ** |

Means having with the different letters in same column differed significantly* (P≤0.05), ** (P≤0.01).

**Biochemical changes**

Effect of Cr-AgNPs treatment on biochemical changes (TP, albumin, and globulin) of *C. carpio* infected with Saprolegnia are summarized in Table 2. TP and albumin contents documented significant increase (P<0.01) in all treated groups (T1, T2, T3 and T4) relative to C+ group. The highest means of TP and albumin were recorded in T3 which was significantly different compared to T1 and T2 respectively. Besides, globulin level documented significant

increase (P<0.05) in treated groups (T1, T2, T3 and T4) relative to C+ group.

These results seem to be consistent with other research which noticed that total serum protein and albumin content in the *Saprolegnia spp.* infected rainbow trout decreased significantly as compared to the normal group. As discussed, this possibly owe to the alteration in the aerogram content in infected fish reflects the impact of "dermato/systemic mycosis" (Shah *et al.*, 2015). Hence, it can be established that stress caused by fungal infection leads to haemostatic imbalances in fish which is reflected in the blood and biochemical changes of infected fish. A similar pattern of findings was obtained by Mastan *et al.* (2009). Yang and Chen, (2003) have explained that this reduce in Tp and albumin in infected fish do to damage the blood vessel and leak in it lead to loss large amount of blood component which result to Hypoproteinaemia.

Results of total protein gives significant increasing (P<0.01) in all treatment group (T1, T2, T3 and T4) compared to C+ group, this increase due to the increase of albumin and globulin levels which could be related with a stronger "innate immune response" in fishes (Wiegertjes *et al.*, 1996). These findings are in line with the result of Johansson-Sjobeck and Larsson, (1978). Sung *et al.* (2003) elucidated that globulin increase due to immunological function and their numbers increase as a protective response in fish to stress when expose to the toxicity.

**Table 2:** Biochemical changes (Total protein, albumin and globulin levels) (M± SE) of *C. carpio* which infected with Saprolegnia and treated with Cr-AgNPs and formalin.

| Treatments | Albumin (g/dL) | TP (g/dL) | Globulin (g/dL) |
|---|---|---|---|
| **Control -ve non infected** | $1.703 \pm 0.04^{a}$ | $4.49 \pm 0.31^{b}$ | $2.787 \pm 0.34^{b}$ |
| **Control +ve infected** | $1.124 \pm 0.04^{d}$ | $3.24 \pm 0.24^{c}$ | $2.114 \pm 0.20^{c}$ |
| **T1 4 mg/l (Cr-AgNPs)** | $1.409 \pm 0.04^{c}$ | $4.256 \pm 0.23^{b}$ | $2.846 \pm 0.19^{b}$ |
| **T2 8 mg/l (Cr-AgNPs)** | $1.447 \pm 0.04^{bc}$ | $4.32 \pm 0.15^{b}$ | $2.875 \pm 0.17^{b}$ |
| **T3 12 mg/l (Cr-AgNPs)** | $1.643 \pm 0.04^{a}$ | $5.19 \pm 0.06^{a}$ | $3.548 \pm 0.02^{a}$ |
| **T4 0.15 mg/l (Formalin)** | $1.559 \pm 0.04^{ab}$ | $4.53 \pm 0.13^{b}$ | $2.972 \pm 0.16^{ab}$ |
| **LSD value** | 0.148 ** | 0.645 ** | 0.642 ** |

Means having with the different letters in same column differed significantly, ** (P≤0.01).

### Survival rate

The highest percentage of survival was seen in T4 (90%) followed by T3 (12 mg $l^{-1}$ Cr-AgNPs) (85%) then T1 and T2 (65 and 60%) respectively and the survival percentage in C+ was 50%.The highest percentage of survival particularly T3 compared to infected group (C+) this indicated that Cr-AgNPs was very efficient disinfectant can depress and killing the Saprolegnia by rupturing the fungal-cell membrane (Amass *et al.,* 2001). This finding is in accordance with findings of Johari *et al.* (2014) who reported that the best antifungal effect of nanoparticles occurred in the highest levels. Commonly, death happens in fish, due to respiratory failure which consequences from general gill infection, organ dysfunction in some rarer cases and impaired osmoregulation is caused from injuries over a large surface (Bruno *et al.*, 2011).

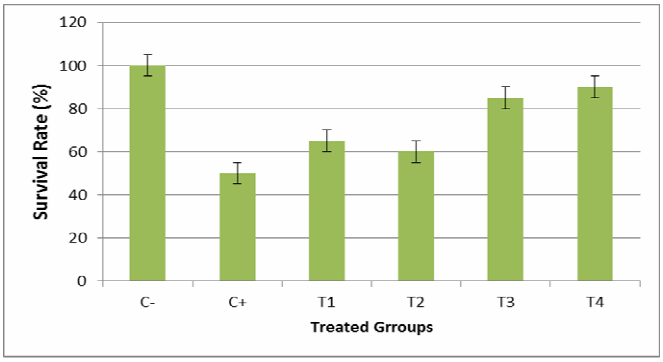


**Fig 3:** Survival rate (%) in *C. carpio* groups infected with Saprolegnia and treated with Cr-AgNPs after following 14 days.

## Conclusion

This is the first report on the antifungal properties of Cr-Ag-NPs against Saprolegniasis in carp fish make it a good substitute to formalin and malachite green, which are carcinogenic. To develop entire picture of Cr-AgNPs, further studies will be required can be explored to identify a potential antimicrobial candidate that can be used for the treatment of Saprolegniasis. Future investigations are required on the antifungal and antibacterial efficiencies of Cr-AgNPs against other pathogens in fish.

## References

Alderman, D. (1985). Malachite green: a review. *J. Fish Dis.*8:289–298.

Amass, S.F.; Glockling, S.L. and Luis, A. (2001). Evaluation of the efficacy of a peroxygen compound, Virkon™ S, as a boot-bath disinfectant. J Swine Health Prod. 9:121-123.

Ashour, A.A.; Mustafa, S.A. and Yassein, S. (2017). The efficacy of Virkon S in controlling the infection of Saprolegniasis in *Cyprinus carpio* L. Thesis of the College of Vet. Med. Baghdad University .p:47.

Bouwmeester, H.; Dekkers, S.; Noordam, M.Y.; Hagens, W.; Bulder, A.S.; de Heer, P.M. and Sips, A. (2007). Health impact of nanotechnologies in food production (No. 2007.014). RIKILT.

Bruno, D.W. and Munro, AL.S. (1986). Haematological assessment of Rainbow trout *Salmo giardnari Richordson* and Atlantic salmon, *Salmo salar* L.,

infected with *Ranibacterium salmonarium*. *J. Fish Dis.* 9:195– 204.

Bruno, D.W.; Van West, P. and Beakes, G.W. (2011). *Saprolegnia* and other oomycetes, In: Woo, P.T.K., Bruno, D.W. (Eds.) Fish Diseases and Disorders: Volume 3: Viral, Bacterial and Fungal Infections, 2nd Edition. CABI International, Wallingford, UK., 669-720.

Bruno, D.W. and Stamps, D.J. (1987). Saprolegniasis of Atlantic salmon (*Salmo salar* L.); fry. *J. Fish Dis.*10: 513-517.

Cagirgan, H. (1990). Immune system in teleost and its significance on diagnosis. J. Fisher. Aquat. Sci.7: 57-68.

El-Ahl, M.H.S. and Rasha (2010). Studies on fungi in fish and fish products and their control. Ph.D. Thesis. Cairo University, Egypt.

Fadel, A.A.; and Mustafa, S.A. (2020). Influence of Phytase Enzyme on Growth Performance, Immune Response and Histopathology in *Cyprinus carpio* L. Challenged with *Saprolegnia spp*. Master thesis.

Feng, Q.L.; Chen, G.Q.; Cui, F.Z.; Kim, T.N. and Kim, J.O. (2000). A mechanistic study of the antibacterial effect of silver ions on *E. coli* and *Staphylococcus aureus*. J.Biomed. Mater. Res.52: 662-668.

Forneris, G.; Bellardi, S.; Palmegiano, G.; Saroglia, M.; Sicuro, B.; Gasco, L. and Zoccarato, I. (2003). The use of ozone in trout hatchery to reduce saprolegniasis incidence. *Aquaculture.* 221: 157–166.

Gabriel, U.; Ezeri, G. and Opabunmi, O. (2004). Influence of sex, source, health status and acclimation on the haematology of *Clarias gariepinus* (Burch, 1822). *Afr. J. Biotechnol.* 3: 463-467.

Garcea, G.; Jones, D.J.; Singh, R.; Dennison, A.R.; Farmer, P.B.; Sharma, R.A.; Steward, W.P.; Gescher, A.J. and Berry, D.P. (2004). Detection of curcumin and its metabolites in hepatic tissue and portal blood of patients following oral administration. *Br. J. Cancer Mar.* 90: 1011-1015. DOI: 10.1038/sj.bjc.6601623.

Ghosh, S.K. and Pal, T. (2007). Interparticle coupling effect on the surface plasmon resonance of gold nanoparticles: from theory to applications. *Che. Rev.*107:4797-4862.

Gill, T.S. and Epple, A. (1993). Stress-related changes in the haematological profile of the American eel (*Anguilla rostrata*). *Ecotoxicol. Environ. Safe.* 25: 227-235.

Grant, J.; Grant, R. and Grant, C. (1987). Grant & Hackh's chemical dictionary: American, international, European and British usage: containing the words generally used in chemistry, and many of the terms used in the related sciences of physics, medicine, engineering, biology, pharmacy, astrophysics, agriculture, mineralogy, etc., based on recent scientific literature. McGraw-Hill.

Hassan, A.A.; Hammad, A.M.; El-Barawy, A.M. and Manal, A.H. (2007). Incidence of aflatoxigenic fungi in frozen and canned fishes and trials to inhibit aflatoxin production by use of some minor elements and lupinustermis seeds. *Egypt. J. Appl. Sci.,* 22: 351-360.

Hatai, K.; Takahashi, S. and Egusa, S. (1984). Studies on the pathogenic fungus of mycotic granulomatosis-IV. *Fish Pathol.*19:17–23.

Horwitz, W.; Sezel, H. and Park, D.L. (1975). Official methods of analysis of the association of official analytical chemists. 12th ed. George Banta company, Inc. Menasha, Wisconsin.1857–1935.

Jagannathan, R.; Abraham, P.M. and Poddar, P. (2012). Temperature-dependent spectroscopic evidences of curcumin in aqueous medium:A mechanistic study of its solubility and stability. *J Phys Chem B.*116:14533-14540.

Jalilpoor, J.; Shenavar Masouleh, A. and Masoumzadeh, M. (2006). Fungal flora in *Acipenser persicus* eggs with particular emphasis on *Saprolegnia sp*.(Oomycetes) and mortality during mass incubation at the Shahid Beheshti hatchery. *J. Appl. Ichthyol.,* 22: 265-268.

Jamalzadeh, H.R.; Keyvan, A.; Ghomi, M.R. and Gherardi, F. (2009). Comparison of blood indices in healthy and fungal infected Caspian salmon (*Salmo trutta caspius*). *Afr. J. of Biotechnol.* 8: 319-322.

Johansson-Sjobeck, M.L. and Larsson, A. (1978). The effect of cadmium on the hematology and on the activity of delta-amino leverlinic acid dehydratase (ALA-D) in blood and hematopoietic tissues of the flounder, *Platichthys flesus* (L.). *Environ. Res.*17:191-204.

Johari, S.A.; Kalbassi, M.R. and Yu, I.J. (2014). Inhibitory effects of silver zeolite on in vitro growth of fish egg pathogen, *Saprolegnia sp. J. Coast. Life Med.2*: 357-361.

Juncey, K. and Ross, B. (1982). A guide to tilapia feed and feeding. Institute of aquaculture Univ. of Striling, Scotland. P:111.

Kawashita, M.; Tsuneyama, S.; Miyaji, F.; Kokubo, T.; Kozuka, H. and Yamamoto, K. (2000). Antibacterial silver-containing silica glass prepared by sol–gel method. *Biomaterials.* 21: 393-398.

Kim, J.S.; Kuk, E.; Yu, K.N.; Kim, J.H.; Park, S.J.; Lee, H.J. *et al*. (2007). Antimicrobial effects of silver nanoparticles. *Nanomedicine.* 3:95-101.

Kiruba Daniel, S.C.G.; Vinothini, G.; Subramanian, N.; Nehru, K. and Sivakumar, M. (2013). Biosynthesis of Cu, ZVI, and Ag nanoparticles using *Dodonaea viscosa* extract for antibacterial activity against human pathogens. *J. Nanopart Res.*15:1319–1328.

Manonmani, P.; Ramar, M.; Geetha, N.; Arasu, M.V.; Erusan, P.R.; Mariselvam, R. *et al*. (2015). Synthesis of silver nanoparticles using natural products from *Acalypha indica* (Kuppaimeni) and *Curcuma longa* (turmeric) on antimicrobial activities. *IJPRBS.*4:151-164

Mastan, S.; Priya, G.I. and Babu, E. (2009). Haematological profile of *Clarias batrachus* exposed to sublethal concentrations of lead nitrate. *Internat. J. Haematol.* 6: 322–328.

McLeay, D.J. (1973). Effects of a 12-hr and 25-day exposure to kraft pulp mill effluent on the blood and tissues of juvenile coho salmon, *Oncorhynchus kisutch*. *J. Fish. Res.* Board Canada.30: 395-400.

Muhsin, T.M. (1989). Polish achieve hydrobiology. College of Sciences. Univ. of Basrrah, Iraq.

Murr, L.E. (2009). Nanoparticul, ate materials in antiquity: The good, the bad and the ugly. *Materials Characterization.* 60:261-270.

Nasrollahi, A.; Pourshamsian, K. and Mansourkiaee, P. (2011). Antifungal activity of silver nanoparticles on some fungi. Int. J. Nano. Dim.1: 233-239.

Osman, H.A.; Solman, W.E.; Noor El-Deen, A.E. and Mohamed, L.A. (2008). Induction of Saprolegniosis in *Oreochromis niloticus* with special reference to its biological control. *Global Vet.* 2: 32–37.

Panigrahi, S.; Kundu, S.; Ghosh, S.; Nath, S. and Pal, T. (2004). General method of synthesis for metal nanoparticles. *J. Nanoparticle Res.,* 6: 411-414.

Pickering, A.D. and Willoughby, L.G. (1982). Saprolegnia infections of salmonid fish. In Microbial Diseases of Fish, Edit Roberts, RJ Academic Press, London.271–298.

Rai, M.; Yadav, A. and Gade, A. (2009). Silver nanoparticles as a new generation of antimicrobials. *Biotechnol. Adv.* 27: 76-83.

Sanpui, P.; Murugadoss, A.; Prasad, P.V.D.; Ghosh, S.S. and Chattopadhyay, A. (2008). The antibacterial properties of a novel chitosan–Ag-nanoparticle composite. *Int. J. Food Microbiol.* 124: 142- 146.

Seth, N. and Saxen, K.K. (2003). Hematological responses in a freshwater fish *Channa punctatus* due to fenvalerate. *Bull Environ Contam Toxicol.* 71: 1192-1199.

Shah, A.F.; Bhat, A.S.; Bhat, F.A.; Balkhi, M.H.; Abubakr, A. and Ahmad, I. (2015). Alteration in haemato-biochemical profiles of rainbow trout *Oncorhynchus mykiss* affected by *Saprolegnia* spp- A potential constraint for culture of trout in Kashmir Himalaya. *Iranian J. Fisheries Sci.* 14: 970-984.

Shah, S.L. and Altindag, A. (2004). Haematological parameters of tench, (*Tinca tinca* L.) after acute and chronic exposure to lethal and sublethal mercury treatments. *Bull. Environ. Contam. Toxicol.*73: 911-918.

Sharma, M.; Manoharlal, R.; Puri, N. and Prasad, R. (2010). Antifungal curcumin induces reactive oxygen species and triggers an early apoptosis but prevents hyphae development by targeting the global repressor TUP1 in *Candida albicans*. *Biosci Rep.,* 30: 391-404.

Song, H.Y.; Ko, K.K.; Oh, L.H. and Lee, B.T. (2006). Fabrication of silver nanoparticles and their antimicrobial mechanisms. *European Cells and Materials,* 11: 58.

Sung, H.J.; Doo, S.S.; Mi-Seon Park, Q.; Tae, J.O. and Yoon, K. (2003). Effects of formalin on haematological and blood chemistry in olive flounder, *Paralichthys olivaceus*(Temminck et Schlegel). Blackwell Publishing Ltd, *Aqua. Res.,* 34: 1269-1275.

Syed, L.S. (2010). Impairment in the haematological parameters of tench (*Tinca tinca*) infected by *Saprolegnia* spp. *Turk. J. Vet. Anim. Sci.* 34: 313-318.

Sykes, S. (1965). Disinfectant and Sterilization. 2nd ed. Edited by Bunburg, printed in Great Britian for E. and F.N. Ltd. Spon, at the pitman press.

Tort, L.; Torres, P. and Flos, R. (1987). Effects on dogfish haematology and liver composition after acute copper exposure. Comp. Biochem. Physiol., 87: 349-353.

Torto-Alalibo, T.; Tian, M.; Gajendran, K.; Waugh, M.; van West, P. and Kamoun, S. (2005). Expressed sequence tags from the oomycete fish pathogen *Saprolegnia parasitica* reveals putative virulence factors. *BMC Microbiol.*5: 46.

Van Den Berg, A.H.; McLaggan, D.; Diéguez-Uribeondo, J. and Van West, P. (2013). The Impact of The Water Moulds *Saprolegnia diclina* and *Saprolegnia parasitica* on Natural Ecosystems and The Aquaculture Industry. *Fungal Biol*. Reviews.27:33-42. https://doi.org/10.1016/j.fbr.2013.05.001

Wiegertjes, G.F.; Stet, R.M.; Parmentier, H.K. and van Muiswinkel, W.B. (1996). Immunogenetics of disease resistance in fish: a comparative approach. *Develop. & Comp. Immunol.* 20: 365-381.

Wong, C.K.; Cheung, P.F.; Ip, W.K. and Lam, C.W. (2007). Intracellular signaling mechanisms regulating toll-like receptor-mediated activation of eosinophils. *Am. J. Resp. Cell Mol. Biol.* 37: 85–96.

Yamanaka, M.; Hara, K. and Kudo, J. (2005). Bactericidal action of a silver ion solution on *Escherichia coli*, studied by energy-filtering transmission electron microscopy and proteomic analysis. *Appl. Environ. Microb.*71:7589-7593.

Yang, F.; Lim, G.P.; Begum, A.N. *et al*.(2005). Curcumin inhibits formation of amyloid beta oligomers and fibrils, binds plaques, and reduces amyloid in vivo. *J. Biol. Chem.* 280: 5892-5901. doi:10.1074/jbc.M404751200

Yang, J.L. and Chen, H.C. (2003). Effects of gallium on Common carp (*Cyprinus carpio*) acute toxicity, serum biochemistry and erythrocyte morphology. *Chemosphere.*53:877–922.

Yoshihiro, I (2002). Bactericidal activity of Ag zeolite mediated by reactive species under aerated conditions. J. Inorg. Biochem. 92: 37-42.

Zaki, M.; Osfor, M.; Bayumi, F. and Gheit, F. (2003). Impact of low dietary carbohydrate diets on some nutritional and clinicopathological parameters of with *Saprolegnia parasitica* and exposed to copper sulphate. *Bull. Nat. Res. Cent.* 28: 245–257.

Zaki, M.S.; Fawzi, O.M. and Jackey, J. (2008). Pathological and biochemical studies in *Tilapia nilotica* infected with *Saprolegnia parasitica* and treated with potassium permanganate. *J. Agric. Environ. Sci.* 3: 677–680.