Marine Science Center-University of Basrah

Mesopotamian Journal of Marine Sciences
Print ISSN: 2073-6428
E- ISSN: 2708-6097
www.mjms.uobasrah.edu.iq/index.php/mms

Effect of nanoparticles for silver and chitosan on growth, survival and molting of juvenile jinga shrimp *Metapenaeus affinis* (H. Milne Edwards, 1837) under laboratory conditions

Ashwaq T. Abbas ; iDAbdulhussein H. Ghazi* and Anteesar N. Sultan
Marine Science College, University of Basrah, Basra - Iraq
*Corresponding Author: *e-mail:* gabdulhussein@gmail.com

Article info.
✓ *Received: 21 November 2024*
✓ *Accepted: 4 February 2025*
✓ *Published: 29 June2025*

Key Words:
Growth indicators
Metapenaus affinis
Silver and chitosan nanoparticles

Abstract - In the study, the effect of silver and chitosan nanoparticles on growth parameters of *Metapenaeus affinis* were investigated. The highest weight was 1.88 ± 0.65 g in Ag NPs and 2.92 ± 0.90 g in Cs NPs, while in the control was 1.81 ± 0.74 g. The highest length in the Ag NPs was 6.22 ± 1.51 cm, chitosan reached 8.32 ± 1.61 cm, while it was 6.11 ± 1.35 cm for the control treatment. The weight gain rate in the silver NPs treatment was 0.58 ± 0.41 g, while in the Cs NPs reached 1.13 ± 0.86 g, and in the control reached to 1.81 ± 0.74 g. The highest relative growth rate (%) was achieved in the Cs NPs treatment 49.2 % compared to 44.6 % in Ag NPs and 2.39 % in the control. While the highest specific growth rate (%) was achieved in the Cs NPs, it was 1.24 and the lowest rate was 1.08 in the control treatment. The highest rate of daily growth rates (g/day) was recorded in the Cs NPs treatment, reaching 0.045 g/day. The highest survival and molting rate were achieved in the Cs NPs treatment, 76% and 28 % respectively, in the Ag NPs were 52 % and 26% respectively and in the control were 64 % and 18 % respectively. The green tea *Camellia sinensis* extract showed good results in manufacturing silver nanoparticles (Ag NPs) and chitosan nanoparticles. The results of the UV-visible spectrum examination showed that the absorbance of Ag NPs was 456 nm and Cs NPs were 271 nm. The atomic force microscope (AFM) examination revealed that the size of Ag NPs was 88.4 nm and Cs NPs was 43.33 nm.

تأثير الجسيمات النانوية للفضة والكيتوسان على النمو والبقاء والانسلاخ في يافعات الروبيان *Metapenaeus affinis* (H. Milne Edwards, 1837) تحت الظروف المختبرية

اشواق طالب عباس، عبد الحسين حاتم غازي وانتصار نعيم سلطان
كلية علوم البحار، جامعة البصرة، البصرة -العراق

المستخلص - في الدراسة الحالية، جرى اختبار تأثير جسيمات الفضة والكيتوسان النانوية على معايير النمو للروبيان *Metapenaeus affinis* ، تحقق أعلى وزن 1.88 ± 0.65 غرام في الفضة النانوية و2.92 ± 0.90 غرام في الكيتوسان بينما في السيطرة 1.81 ± 0.74 غرام. بلغ اعلى معدل طول في معاملة الفضة النانوية 1.51 ± 6.22سم، ووصل الكيتوسان إلى 8.32 ± 1.61 سم، بينما كان 6.11 ± 1.35 سم في السيطرة .كان معدل الزيادة الوزنية في اليافعات في معاملة الفضة النانوية 0.58 ± 0.41 غرام، بينما في الكيتوسان وصل إلى 1.13 ± 0.86 غرام، وفي السيطرة 1.81 ± 0.74 غرام. تم تحقيق أعلى معدل نمو نسبي (%) في معاملة الكيتوسان وبنسبة 49.2 % مقارنة بـ 44.6% في جسيمات الفضة النانوية و2.39% في االسيطرة. وتحقيق أعلى معدل نمو نوعي (%) في الكيتوسان كان 1.24 وأدنى معدل كان 1.08 في السيطرة .سجل أعلى معدل للنمو اليومي (غرام/يوم) في معاملة الكيتوسان ووصل إلى 0.045 غرام/يوم. وتحقق أعلى معدل بقاء ومعدل انسلاخ في معاملة الكيتوسان 76% و 28% على التوالي وفي الفضة النانوية 52% و26% على التوالي وفي السيطرة 64 % و 18% على التوالي. أظهر مستخلص الشاي الأخضر *Camellia sinensis* نتائج مقبولة في تصنيع جسيمات الفضة والكيتوسان النانوية، وأظهرت نتائج فحص طيف الأشعة فوق البنفسجية المرئي أن امتصاص الفضة النانوية كان 456 نانومتر والكيتوسان النانوي 271 نانومتر. أوضح فحص مجهر القوة الذرية (AFM) أن حجم جسيمات الفضة النانوية كان 88.4 نانومتر والكيتوسان 43.33 نانومتر.

DOI:https://doi.org/10.58629/mjms.v40i1.388,

الكلمات المفتاحية: جسيمات الفضة والكيتوسان النانوي، الروبيان Metapenaeus affinis ، مؤشرات النمو.

Introduction

Shrimp farming began in the mid-20 th century as a response to the increasing demand for shrimp in global markets and the shortage of supplies from natural sources (Yap, 1999). The early stages of shrimp farming included experimenting with different species of shrimp and developing techniques to improve growth and survival rates (Kumlu and Jones, 1995). Research and development played a major role in improving feeding methods, water management, and disease control, which contributed to increasing the productivity and quality of farmed shrimp (Chethurajupalli and Tambireddy, 2022; Nahavandi *et al*., 2022). Shrimp farming trends have increased significantly in recent year's worldwide, due to the increasing demand for shrimp as a source of protein and profitable economy (FAO, 2022). Studies related to shrimp in Iraq are still relatively few, Salman *et al*., (1990) studied the population structure, growth and some of its biological aspects in the Hammar Marsh area. Ghazi (2020) studied the food competition between the endemic *M. affinis* and the invasive prawn *Macrobrachium nipponense*. Abbas and Ghazi (2021) focus on the commercial shrimp landings of two penaeid shrimps in the main markets of Basrah Province, Iraq and others. New record of *Macrobrachium equidens* by (Hassan *et al*., 2023).

According to Holthuis (1980) the Jinga shrimp *M. affinis* is the most important species of the genus Metapenaeus. This species is widely distributed from Hawaii and the South China Sea to the Arabian Sea and Persian Gulf (Saoud *et al*., 2014; Khodanazary, 2019). *M. affinis* was frecorded in Iraq (Miquel, 1983).

In recent years, the term nanotechnology has emerged and has represented a huge leap in all sciences (Hochella, 2002). Reducing materials to the nano size gives them different features and properties, the most important of which is increasing the surface area and surface activity of the body, which leads to affecting the behavior of the basic units that make up that body, i.e. atoms and molecules, and thus different properties appear such as melting point, insulation, mass properties, heat transfer, reaction speed, and others (Baker *et al*., 2014 and Acedo-Valdez *et al*., 2017).

Nanoparticles (NPs) are manufactured in different ways, including physical, chemical and biological, but the first and second methods take a long time and use harmful materials and hazardous solvents that may be difficult to dispose of and have effects on the environment (Cheng *et al.,* 2016). Biosynthesis or so-called green synthesis is characterized by using the metabolites of microorganisms or plant extracts and does not require a lot of energy, low cost and does not contain risks as in physical and chemical materials (Zhang *et al.,* 2011 and Ghazal *et al.,* 2023).

Silver nanoparticles (Ag NPS) are widely used in nanotechnology because they act as antimicrobial agents, and have high thermal and electrical conductivity, and chemical stability (Bahabadi *et al*., 2017). Silver is non-toxic at low concentrations (Akter *et al*., 2018). On the other hand, chitosan is a natural, non-toxic organic compound that can be used in nanoparticles (Dash *et al*., 2011). Chitosan nanoparticles (Cs NPs) have received the attention of many researchers, such as No and Meyers,2000; Alishahi, 2011; Cheba, 2011; Sathiyabama and Parthasarathy 2016; Zhao *et al*., 2018 ; Budi *et al*., 2020 and Abdel-Warith *et al*., 2020).

The current study aims to manufacture two types of nanoparticles, including Ag NPs and Cs NPs, by using green tea extract, and then studying the effect of these nanoparticles on the growth, survival and molting of juvenile shrimp of *M. affinis.*

Materials and Methods

Preparation of green tea extract:

The green tea *Camellia sinensis* extract was prepared by placeing 4 g in a 250 ml glass beaker and dissolving in 100 ml of deionized water and heating it at 60 °C for half an hour. The extract was filtered using 45 micrometer filter paper and left to cool at laboratory temperature and stored in the refrigerator at 4 °C until use (Acedo-Valdez *et al*., 2017).

Preparation of Ag NPs:

Ag NPs were prepared according to Raheman *et al.* (2011) with some modifications, where 80 ml of silver nitrate solution was placed in a 500 ml conical glass flask and mixed with 20 ml of green tea extract *C. sinensis*, and incubated for 2 hours at room temperature in the dark, after which UV-visible spectroscopy was measured to track the bio reduction of silver ions in the solution (Figure 1).

Figure 1. Preparation of silver NPs solution by extraction using green tea plant *Camellia sinensis*, (right) and powder of silver NPs (left).

Preparation of Cs NPs

Abouzeed *et al*. (2015) and Budia *et al.* (2020) prepared chitosan, a number of samples of uneconomic shrimp of the genus Macrobrachium was dried, 62 g of it was taken. Sodium hydroxide was added for two hours at a temperature of 60 °C and at a concentration of 2% and washed well with water and dried at laboratory temperature to remove protein and carbohydrates. To obtain chitin, hydrochloric acid was added for four hours at laboratory temperature, then washed well with water and dried at laboratory temperature. To obtain natural chitosan, sodium hydroxide was added at a concentration of 50% for 2 hours at a temperature of 80 °C, then washed and filtered with filter paper (Figure 2).

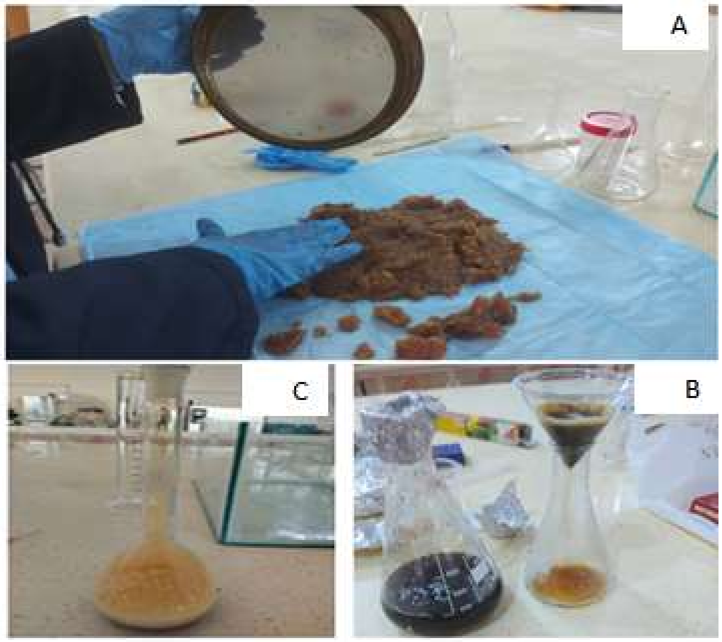

Figure 2. Steps for preparing nano chitosan in the laboratory: (A) drying shrimp, (B) chitin after adding HCl acid, (C) chitosan after adding NaO

Diagnosis of nanoparticles

UV-Visible spectrum diagnosis

This diagnosis aims is to confirm the formation of Ag NPs and Cs NPs based on the absorbance of the resulting solution. 3 ml of Ag NPs and Cs NPs solution were placed in the quart cell of the spectrophotometer and the nano range was recorded and then zeroed on the standard solution using distilled water for silver and dilute acetic acid for chitosan.

Atomic Force Microscope (AFM)

Atomic Force microscope is used to examine the size and distribution of nanoparticles and to know the shape of the surface roughness and thickness. Nanoparticles were prepared for AFM analysis by placing a drop of silicone solution and leaving it to dry at room temperature, then the dried solution was imaged using an AFM microscope (Rostamizadeh *et al*., 2018).

Sample collection and study area

The study was conducted at the Marine Science College during 2023. Juveniles of M. affinis were obtained from the withdrawal area located near Al-Hammar Marsh, southern Iraq, this is an area that lies between 30º 39´ 34.27" N; 47º 39´ 13.81" E. by Kufa net using fishing boat for 15 minutes. Active and healthy juveniles were selected according to certain characteristics such as transparent body color, swimming behavior, and complete molting. Juveniles were placed in 25 liter plastic bags containing water from the collection area. One quarter filled with water and the rest with air and transferred to the Invertebrates Laboratory. Environmental factors such as dissolved oxygen, temperature, salinity, and pH were recorded in the field to prepare an aquatic environment similar to laboratory culture experiments (Figure 3).

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Figure 3. Shrimp collection net (A), (B) juveniles caught in the field, (C) transport bags.

Culture conditions

Aquaculture experiments were conducted for juveniles of *M. affinis* with an initial weight of 1.30 ± 0.33 g and an initial length of 5.27 ± 1.33 cm to know the effect of silver and chitosan nanoparticles on their growth, survival and molting rate. They were acclimatized for three days to the rearing tanks at a temperature 23±1 °C and salinity 4g / L (same salinity at natural environment). The juveniles were placed in tanks with a capacity of 80 liters and a density of 30 individuals per tank with three replicates. Figure 4.

The juveniles were fed daily at a rate of 10% of the body weight and twice daily, the first meal was provided in the early evening hours at a rate of 80% of the feeding rate, and in the morning the excess food remains were removed and 50% of the water quantity was replaced and then the second meal was fed at a rate of 20% of the feeding rate.

The feed was based on dry worms with a protein content of 60%, fat 6 %, fiber 5 %, ash 8% and moisture 5%. The concentration of silver NPs was 1.61 ppm (Lam, 2019), and 1.5 ppm of Cs NPs were added to the food and mixed well with water to make the food in the form of a paste. The Juveniles were weighed every week to determine growth indicators

Figure 4. Aquariums designed for laboratory culture of juvenile shrimp *Metapenaeus affinis*, treated with silver NPs and Cs NPs for two weeks

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Growth parameters

Length measurements were taken using a graduated ruler from the beginning of the rostrum to the end of the telson, and weighted. Weight gain, specific and relative growth rate indicators were calculated based on (Panigrahi *et al.,* 2017; Mondal *et al*., 2022 and Reyes-Avalos *et al*., 2023), as follows:

Weight gain (g) = Final weight (g) - Initial weight (g).

Relative growth rate (RGR) (%) = [Weight gain (g) / Initial weight (g)] x 100.

Specific growth rate (%) = [Natural logarithm of final weight (g) - Natural logarithm of initial weight (g) / period (day)] x 100.

Survival rate % = Number of live shrimp at the end of the experiment / Total number of shrimp at the beginning of the experiment x 100

Molting rate % = Number of molted shrimp / Total number of shrimp x 100

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Results

UV-Visible Spectrophotometer

The UV-visible spectrum of Ag NPs silver nanoparticles was measured after they started to form in the solution, where the solution turned brown. The absorption spectrum showed the appearance of an absorption peak at a wavelength of 456 nm, which was attributed to the presence of stable silver NPs that absorbs rays approximately within the range of 380-460 nm (Figure 1). The UV-visible spectrum of Cs NPs was measured after their formation in the solution, the absorption spectrum was recorded, and the analysis results showed the appearance of an absorption peak at a wavelength of 271 nm as a result of the formation of chitosan nanoparticles, which absorbs rays approximately within the range. Figures 5 and 6.

Atomic Force Microscope (AFM)

Figure 7 A shows that the size of the Ag NPs extracted by the green tea plant was 88.4 nanometers, and a set of surface elevations and depressions were seen, which are evidence of surface roughness, and the crystal appeared in an irregular and heterogeneous manner, while for the Cs NPs have a size of 43.33 nanometers Figure 7 B.

Transmission electron microscope (TEM)

Figure 8 showed chitosan nanoparticles. These particles appear becomes clear they have an irregular spherical shape, and the particles appear slightly clustered with the observation of the internal crystalline structures of the particles, which confirms that these particles are of a crystalline nature.

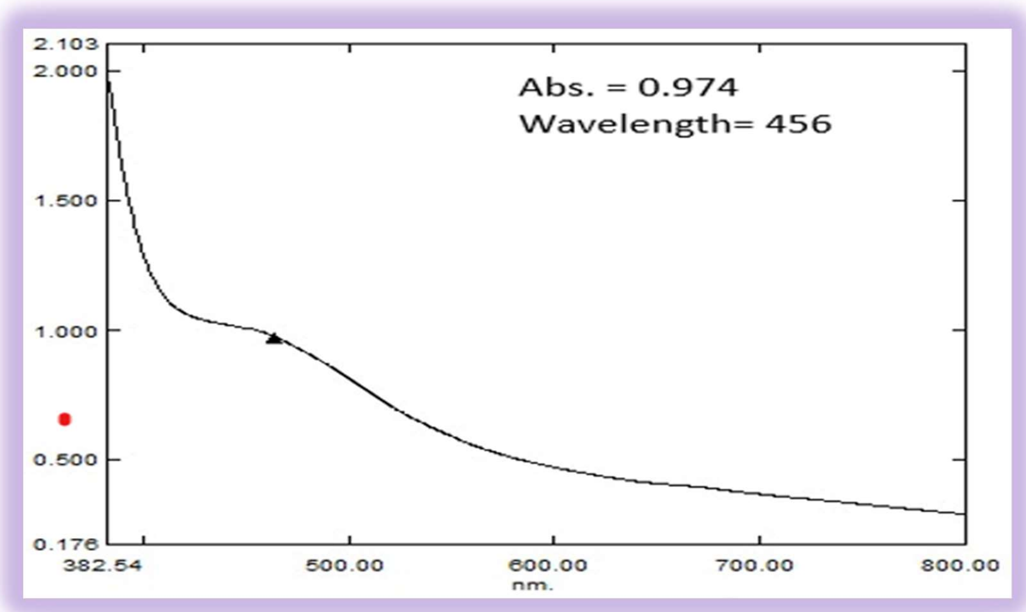

Figure 5. UV-visible Spectrophotometer spectrum examination of a sample of silver NPs prepared by using green tea *Camellia sinensis* extract

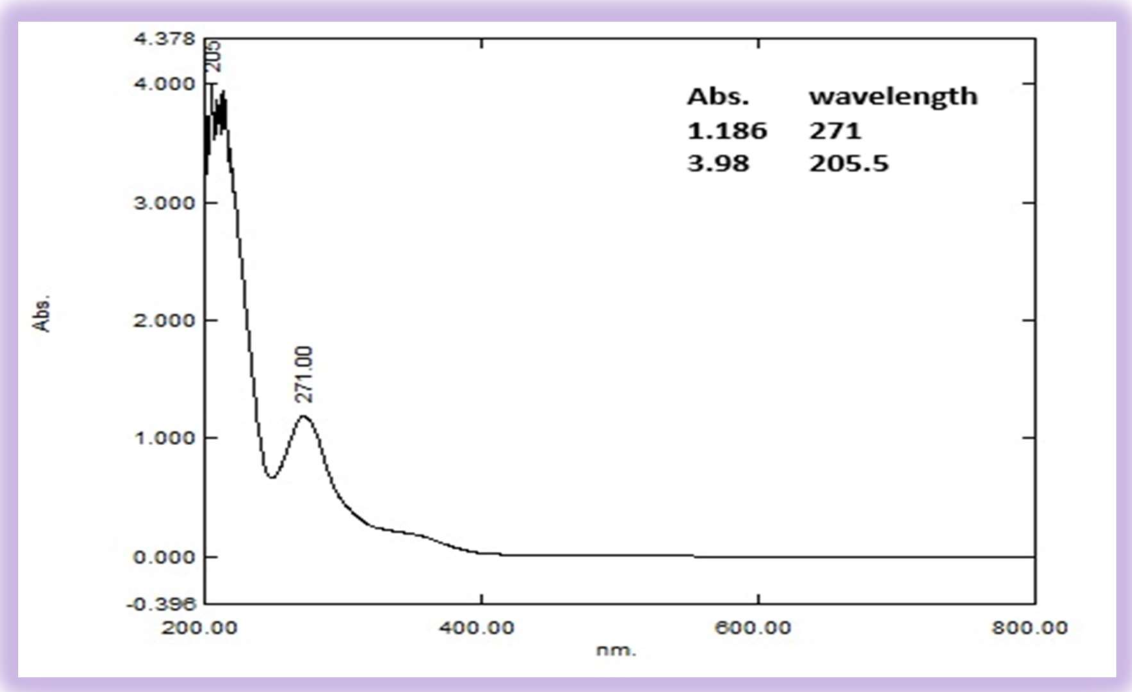

Figure 6. UV-visible Spectrophotometer spectrum examination of a sample of chitosan NPs prepared by using green tea *Camellia sinensis* extract.

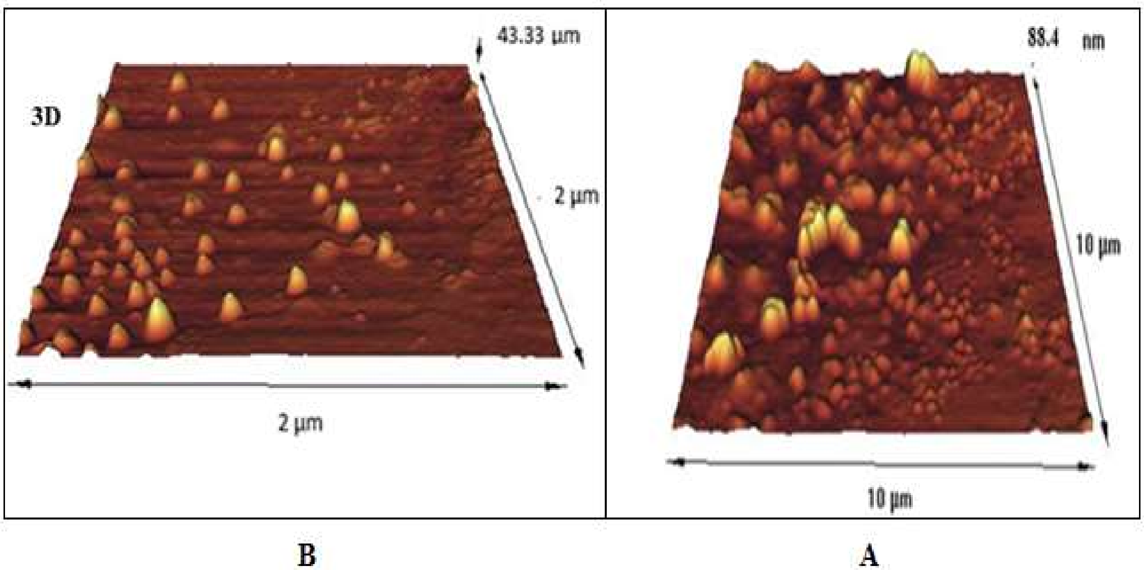

Figure 7. Characterization of atomic force microscope (AMF) prepared by using green tea *Camellia sinensis* extract using, (A) Ag NPs and (B) Cs NPs

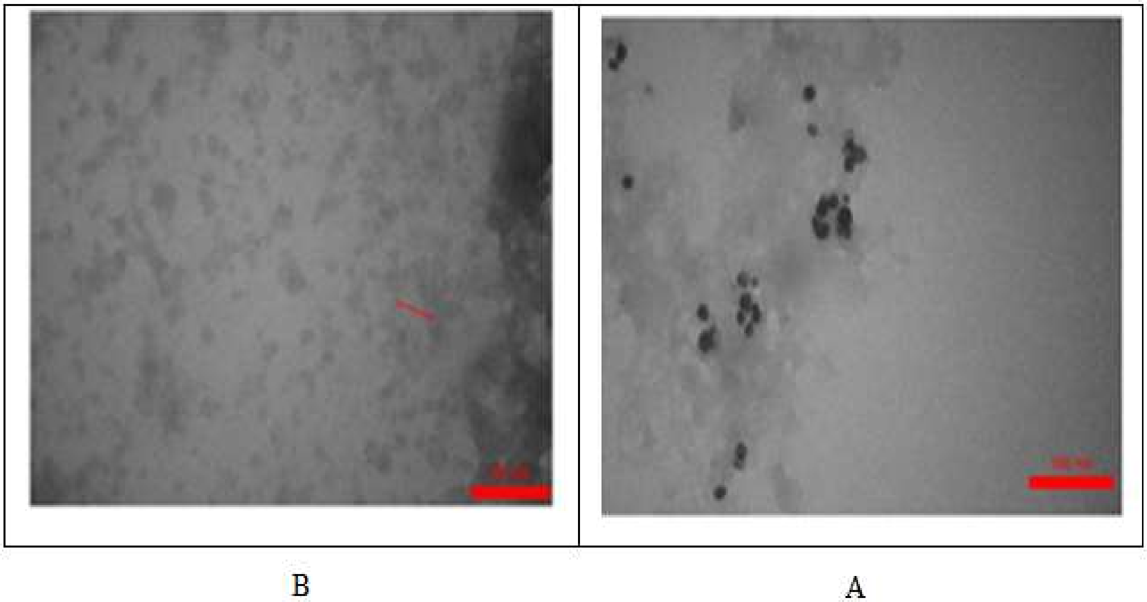

Figure 8. Characterization of transmission electron microscope (TEM) prepared by using green tea *Camellia sinensis* , (A) Ag NPs and (B) Cs NPs.

Length and weight

Figure 9 shows the average weight during two weeks of the experiment of culturing juvenile shrimp *M. affinis* treated with silver nanoparticles and chitosan with an initial weight average of 1.30 g. After the first week, the average weight was achieved in the silver treatment of 1.61 $\pm$ 0.43 g., and after the end of the second week; it reached 1.88 $\pm$ 0.65 g. As for the chitosan treatment, it reached 1.82 $\pm$ 0.67 g and 2.92 $\pm$ 0.90 g., respectively. In the juveniles fed with a diet not treated with nanoparticles (control), the weight in the first week reached 1.52 $\pm$ 0.53 g. and 1.81 $\pm$ 0.74 g. in the first and second weeks, respectively.

As for the average length, it reached 05.3 $\pm$ 1.22 cm in the first week of the Ag NPs treated juveniles, and then increased to 6.22 $\pm$ 1.51 cm in the second week. In Cs NPs, it reached 5.41 $\pm$ 1.00 cm and 8.32 $\pm$ 1.61 cm in the first and second weeks, respectively. As for the control treatment, it was 5.28 $\pm$ 1.51 cm and 6.11 $\pm$ 1.35 cm, respectively (Figure 10). Significant differences ($P>0.05$) were recorded in the second week in the length and weight of juveniles between the chitosan treatment and the silver and control treatments, while no significant differences ($P<0.05$) were observed between the silver and chitosan treatments.

Weight gain

The weight gain rate in the Ag NPs treatment ranged between 0.31 $\pm$ 0.11 g to 0.58 $\pm$ 0.41 g in the first and second weeks respectively, while in the Cs NPs treatment the increase reached 0.52 $\pm$ 0.11 g to 1.13 $\pm$ 0.86 g respectively, and in the control treatment it reached to 0.22 $\pm$ 0.06 g in the first week and 0.51 $\pm$ 0.36 g in the second week. Significant differences were recorded $P>0.05$ in the second week between the Cs NPs treatment with the Ag NPs and control treatments, and the silver NPs and control treatments did not differ significantly $P<0.05$ (Figure 11).

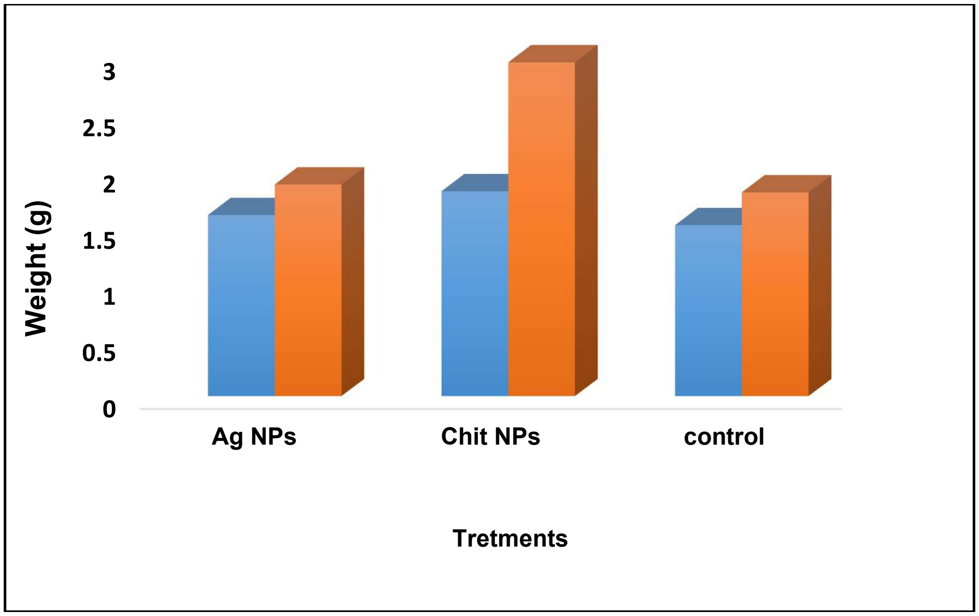

Figure 9. Average weight (g) of *Metapenaeus affinis* juveniles reared in the laboratory for two weeks using silver NPs and Cs NPs.

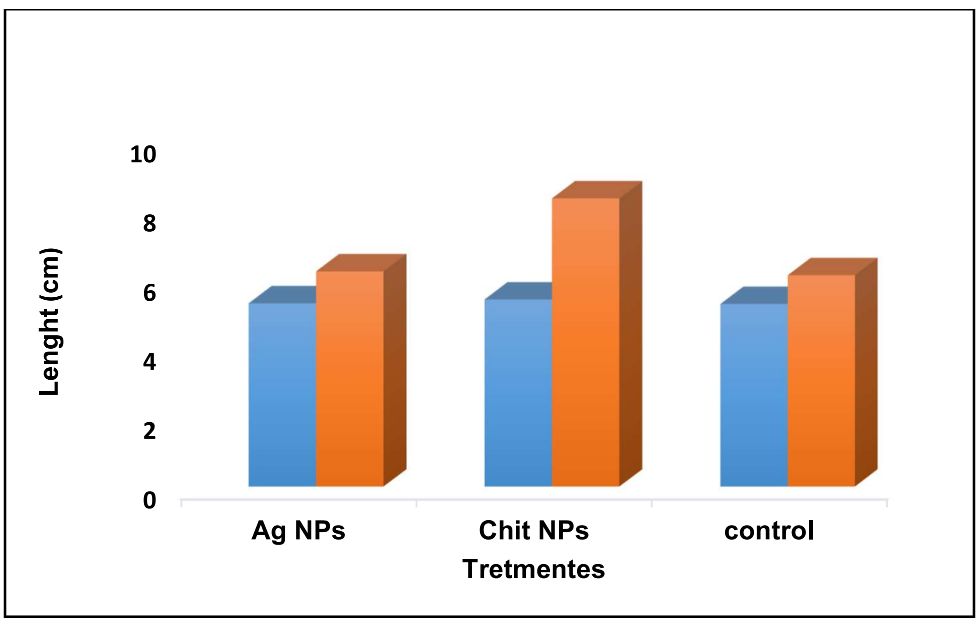

Figure 10. Average length (cm) of *Metapenaeus affinis* juveniles reared in the laboratory for two weeks using silver NPs and Cs NPs.

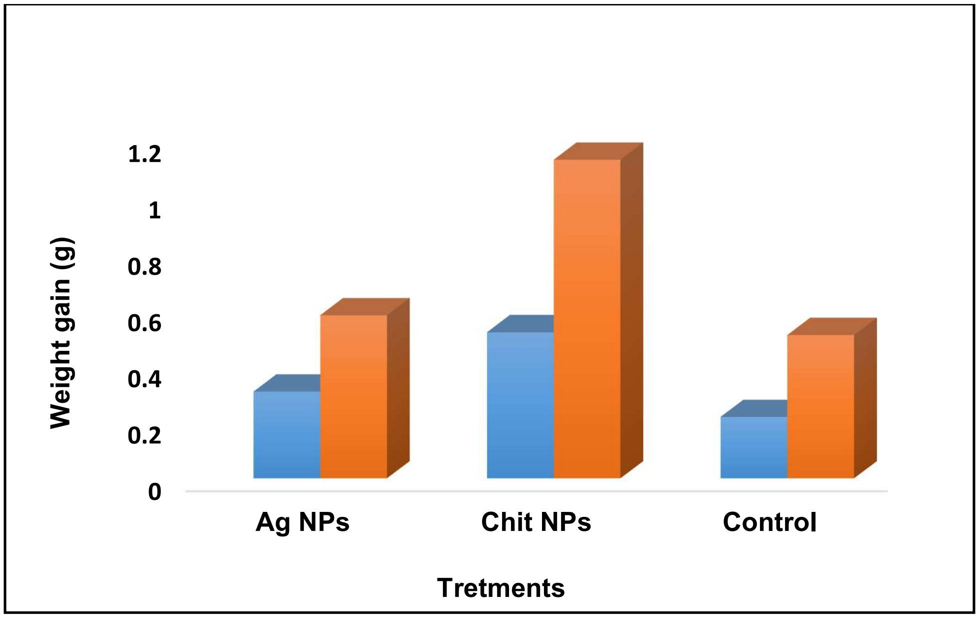

Figure 11. Weight gain rates (g) of *Metapenaeus affinis* juveniles reared in the laboratory for two weeks using silver NPs and Cs NPs.

Relative growth rate and specific growth rate

The highest percentage of relative growth rate (%) was achieved in the Cs NPs treatment and reached 49.2% compared to 44.6% in Ag NPs and 2.39% in juveniles fed on a diet free of nanoparticles (control). There were significant differences $P>0.05$ in the chitosan treatment compared to the rest of the treatments (Figure 12).

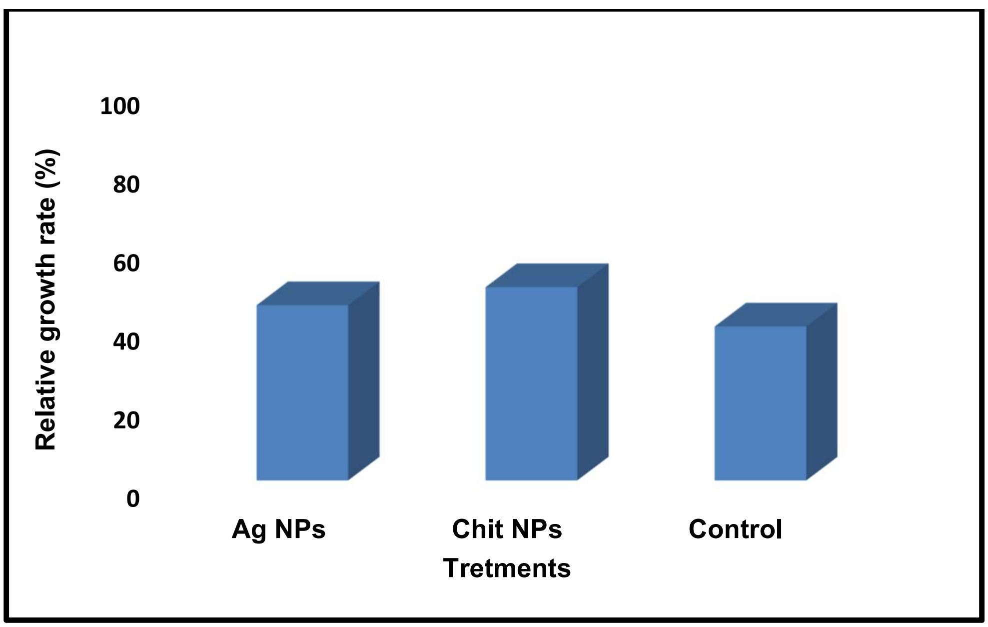

Figure 12. Relative growth rate (%) of *Metapenaeus affinis* juveniles reared in the laboratory for two weeks using silver NPs and Cs NPs.

The specific growth rate of shrimp *M. affinis* ranged between 1.08-1.24, as the highest rate was achieved in the Cs NPs treatment and reached 1.24, and the lowest rate in the control treatment, reached 1.08, while in juveniles treated with Ag NPs, the specific growth rate was 1.15. No significant differences P<0.05 were recorded between the three treatments in the specific growth rate (Figure 13).

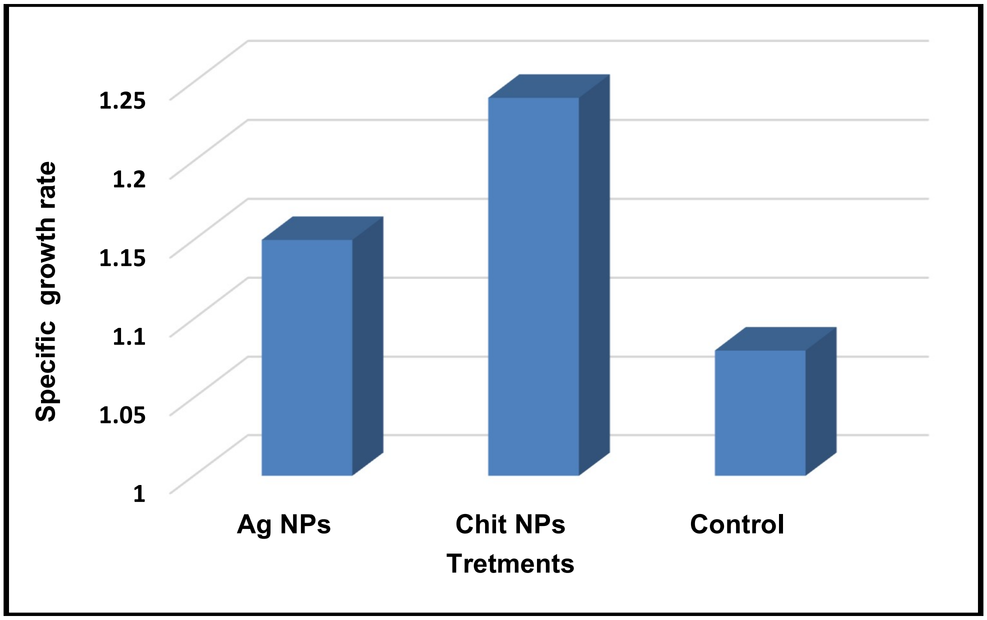

Figure 13. Specific growth rate (%) of *Metapenaeus affinis* juveniles reared in the laboratory for two weeks using silver NPs and Cs NPs.

Daily growth rate

Figure 14 shows the daily growth rates (g/day) were close in the three treatments, and the highest rate was recorded in the Cs NPs treatment (0.045 g/day), and the daily growth rate was equal in the control and Ag NPs treatments (0.041 g/day). No significant differences (P<0.05) were recorded between the three treatments in the daily growth rate.

Survival rate and molting rate

Figure 15 shows the survival rates in juveniles of the Jinga shrimp during the three-week experiment. The highest survival rate was achieved in the Cs NPs treatment, reaching to 76% compared to 64% in the control treatment, and Ag NPs achieved the lowest survival rate, reaching 52%.

Growth indicators through repeated molting process indicate that juveniles raised under the influence of nanoparticles treated with Cs NPs achieved the highest molting index at a rate of 28% compared to a molting rate of 26% in the treatment in which Ag NPs was used and a rate of 18% in the control tanks. No significant differences were recorded (P<0.05) in the survival rates and molting rates between the Cs NPs treatment and the control, while the silver NPs treatment differed significantly from them P > 0.05 (Figure 16).

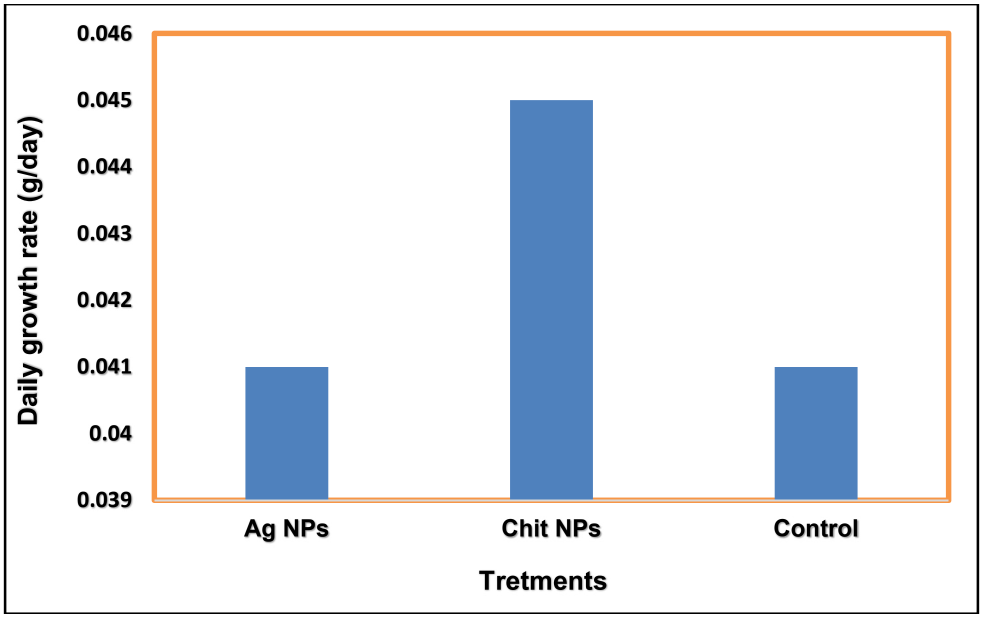

Figure 14. Daily growth rate (g/day) of *Metapenaeus affinis* juveniles reared in the laboratory for two weeks using silver NPs and Cs NPs.

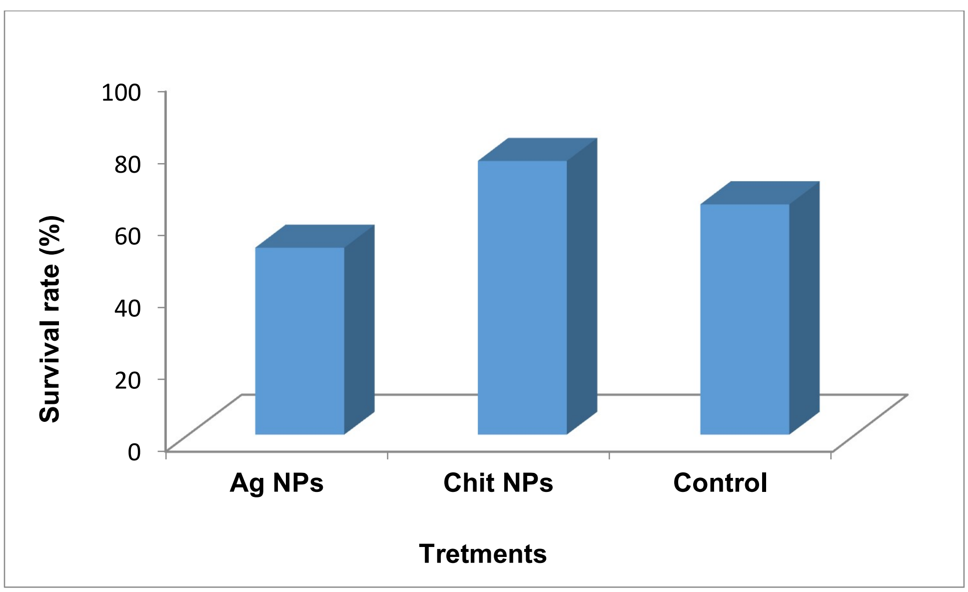

Figure 15. Survival rate (%) in juvenile *Metapenaeus affinis* shrimp treated with silver NPs and Cs NPs for two weeks.

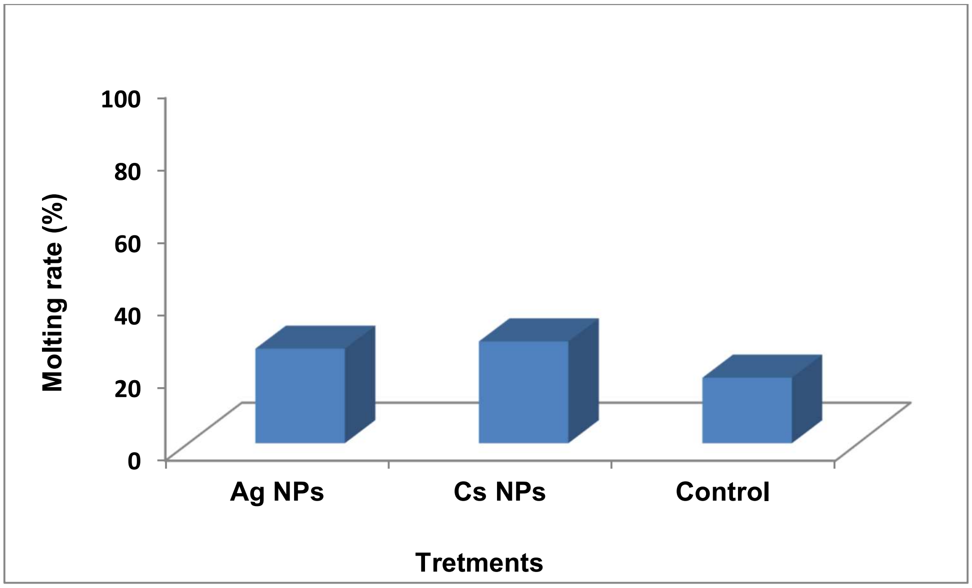

Figure 16. Molting rate (%) in juvenile *Metapenaeus affinis* shrimp treated with silver NPs and Cs NPs for two weeks.

Discussion

Chitosan nanoparticles, silver nanoparticles and other nanoparticles can be incorporated into the feed used to feed shrimp, or added directly to the water, which enhances growth opportunities and overall health (Xu and Du 2003 ; Borrege *et al*., 2016). The current study achieved the best growth in shrimp larvae using Cs NPs. To know the reason, it requires a good understanding of how Cs NPs biological environment of the larvae. Chitosan is a natural polymer derived from chitin, characterized by its antibacterial and growth-stimulating properties, especially when reduced to the nanoscale, its effectiveness increases due to the increased surface area, which leads to improving the efficiency of the digestive system of shrimp juveniles and enhances the absorption of nutrients necessary for growth (Abdou *et al*., 2008 and Reyes-Avalos *et al.,* 2023). Nano-chitosan is also characterized by its antibacterial properties that work to reduce bacterial diseases that affect juveniles, which increases survival rates and increases growth opportunities (Xu and Du 2003). In addition, Cs NPs enhance the immunity of juveniles against various diseases and other parasites, which enables them to grow better under laboratory conditions. However, understanding the actual effects of Cs NPs on shrimp growth requires continuous scientific experiments to measure the effect of Cs NPs on parameters such as growth rate, survival rate, food conversion efficiency, and molting (Weerakody *et al*., 2008). On the other hand, lower growth and survival rates were recorded when using silver NPs, and the reason may be that the effect of Ag NPs on the growth of shrimp juveniles can be complex, as silver NPs has effect on aquatic organisms, including shrimp larvae, which may be harmful in some cases and requires studies on safe concentrations (Wei *et al*., 2014; Borrego *et al.,* 2016). Studies have shown that Ag NPs can be toxic to aquatic organisms, including shrimp juveniles and larvae, which can lead to the death of the larvae or delay in their growth and development (Lam, 2019). Toxicity depends on several factors such as particle size, concentration, and the time during which the larvae are exposed to silver NPs (Vaseeharan *et al*., 2010; Franci *et al*., 2015). Ag NPs may cause damage to shrimp cells through the production of free radicals and oxidation, which leads to damage to proteins and nucleic acids. In addition, silver NPs may affect the immune system of juveniles, making them more susceptible to diseases (Kim *et al*., 2004). Sometimes, Ag NPs accumulations in the aquatic environment can have negative effects on the ecosystem as a whole, which may indirectly affect the growth and development of shrimp larvae and juveniles (Hsu and Wu 2010).

Conclusion

We conclude from the current research, that green tea extract has the ability to radiation silver and chitosan and manufacture nanoparticles for them, and chitosan particles showed the best growth rates, survival and molting rates in juveniles of the *M. affinis* shrimp.

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