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Synthesis, Characterization, and Influence of Silver Nanoparticles against Phases of Musca Domestica

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ABSTRACT
The achievement of this research was to study the influence of AgNPs on the life stages of *Musca domestica*. The supernatant of *Trichoderma harzianum* successfully reduces $AgNO_3$ into AgNPs through the duration of incubation, as noticed by the color change from white to red. The absorbance of UV-visible light results in an absorbance peak at 418 nm for nanoparticle solutions of Ag particles. The SEM image confirmed the formation of AgNPs with an average particle size of 46.63 nm without aggregation or adhesion. The microscopy of energy-dispersive X-ray (EDX) approved the formation of AgNPs with a high amount of some atoms of carbon and oxygen derivatives from components of fungus extract used to reduce $AgNO_3$.

The concentration of AgNPs prepared with serial dilutions of 5, 10, 15, 20, and 25%, also with sterilized distilled water as a control, The influence of AgNPs on phases of *Musca domestica* has been studied. The eggs of the studied insects were affected by 20% of AgNPs, which showed an increase in incubation time of 11 hours without any hatched eggs at 25%. The larval stage of the insect was also influenced by AgNPs solution, which at 25% increased the duration of incubation to reach 11 hours with 27 larval insects dead, compared with 7 hours without any missing membrane in the larval stage of the insect. As well as the pupal phase of insects, we increased the period of incubation to 10 hours with 7 hours in control, and in addition, the number of pupal insects dead reached 23 in 25% concentration. Finally, the AgNPs also increased the number of adult insects to reach 27 at 25% concentration. The AgNPs approved the activity toward *Musca domestica* and therefore considered it a preferable option to biological control.

1. Introduction

One of the most prevalent flies that affects human life and habitat is the *Musca domestica*. The housefly of *Musca domestica*, which feeds on leftover food and animal waste, can be found in homes, living rooms, poultry farms, and horse stables (Cheng et al., 2021). The value of houseflies arises from their impact on human and some animal health, because of the ability of the fly to transport many microorganisms that cause many infections and diseases for many animals and humans, such as the Bird Flu virus that threads the human and animal populations all over the world (Barin et al., 2010), as well as the main cause of gastrointestinal diseases caused by houseflies that transport pathogen microorganisms such as bacteria of Campylobacter, Escherichia, and Enterococcus (Onwugamba et al, 2018). Additionally, the virus that caused newcastle disease in livestock was transported, which resulted in the extinction of domestic

animals (Watson et al.,2007). The fly that spreads the bacteria that causes trachoma in humans to blind them (Brewer, 2021) is to blame. The toxoplasma parasite can also be carried by houseflies, which are thought to be the primary source of congenital defects in fetuses that result in miscarriage in pregnant mothers (Kijlstra, 2004). Numerous research (Alam et al., 2004; Henning et al., 2005; Meerburg, 2007;) emphasized the role of houseflies in medical studies.

The housefly controlled which reduce the damage caused by the fly through removed it or prevented from reach the host by creating unsuitable condition for it reproduction or eliminating using a variety of methods. housefly control methods must reduce the losses they cause to humans and their property, including animals and plants (Malik et al., 2007). The numerous way used to control the pest one of them is chemical way that included used chemical and toxic materials. This materials have effect on life of human and animals in addition maybe killed all form of lives including the insects and most kind of housefly (Kabkaew et al., 2004). This prompted scientists to search for safer alternative ways to control pests, by resorting to safe control. The modern ways which using silver nanoparticles as safe control.

Silver nanoparticles are particles with a dimension of less than 100 nanometers and have special properties and these particles contains many atoms and ions that come together to form particles with a size ranging between 1 to 100 nanometers (Ahmed et al., 2018). The main reason to development product containing silver nanoparticles due to antimicrobial properties (Milić,et al. 2015). The numerous of biological methods have been discovered to synthesis nanoparticles (Suood et al., 2021; Suood et al., 2022). The common method to synthesis silver nanoparticle are methods those using fungi for many reason as mentioned in (Ahmed et al., 2018). The silver nanoparticles are used as alternative way instead of chemical way to control pest (Ghareeb et al., 2020). The studies mentioned the activity of silver nanoparticles with others pesticides to evaluation their activity toward some pests (Tyagi et al., 2020). On other hand some papers discussion the activity of silver nanoparticles on housefly (Gul et al., 2016), but no studies discuss the influence of AgNPs upon the phases of *Musca domestica*; therefore, the current study aims to include the ability of *Trichoderma harzianum* to synthesize silver nanoparticles and evaluate their effect on the control lifecycle of houseflies (*Musca domestica*).

2. Materials and methods

2.1. Collection and identification of Musca domestica

The adult houseflies have been collected from some area in Tikrit city in Salad aldin governorate using web made from medical gauze, after that the collected flies transported into wood cages with 50 by 50 cm with wooden base, other sides of cage were covered by metal mesh. The food of flies were prepared from milk and sugar with equal volume then poured and dissolved in 20 ml of distilled water. The food were divided into several group in perti dish, medical cotton putted on surface of dishes to prevent the flies from stuck in food, all prepared putted inside the middle of wood cage (Hazfez et al., 1949). The wooden cage have been incubated in appropriate laboratory condition to get a new colony of flies for study, the flies were diagnosed in the University of Baghdad in Iraqi natural History Museum (1758).

2.2. Preparation of culture media for fungus

2.2.1. Potato Dextrose Agar

The Potato Dextrose Agar PDA was prepared according the instruction of company in liter, the volume were divided into several conical flask each containing 250 ml of media. The culture were sterilized depending on instruction that mentioned in (Emmons et al., 1974).one ml of ampiclox antibiotics was added in each conical flask after sterilization. The media was poured in petri dish at sterilization condition then waited cold to keep in refrigerator at 5 C until use.

2.2.2. Potato Glucose Broth

One liter of potato glucose broth PGB is made up of 200 g of sliced potatoes and 700 ml of distilled water. The fluid has been heated for 40 minutes before being filtered through medical mesh. After 20 seconds, 20 g of glucose was divided into conical flasks, which were subsequently sterilized (Emmons et al., 1974). Each flask received amplicox antibiotics that were introduced under sterilizing conditions.

2.3. Identification and subculture of Trichoderma harzianum

The strain of *Trichoderma harzianum* was obtained from the ministry of Science and Technology, Agricultural Research Department (mt648463.1,T7). The strain was implanted into a fresh PDA petri dish under laboratory conditions after being subcultured by taking a small amount of some mycelium with a sterilized needle. The inoculated PDA was incubated in an environment that was suitable to fungal growth, and the subculture PDA was kept in a temperature that was suitable to storage.

2.4. Regrowth of Trichoderma harzianum for the generation of biomass

Activation of the strain occurred on PDA days before biomass generation. To produce the fungal biomass required for the reduction of silver nitrate, a portion of a fresh, pure fungus colony was taken and drop into a few conical flasks of 250 ml of PGB medium, and incubated in a shaking incubator at 100 RPM/25 + 2 C for seven days (Sadowski et al., 2008).

2.5. Preparing the silver nitrate solution

The silver nitrate $AgNO_3$ was prepared at a concentration of 1 mM by dissolving 0.017g of $AgNO_3$ in 100 ml of sterilized distilled water. The solution was kept in a dark environment to avoid oxidation of the mixture by light (Karbasian et al., 2008).

2.6. Synthesis silver nanoparticles using Supernatant of Trichoderma harzianum

The biomass in section 2.4 was filtration using Whitman no.1. the biomass then washed three times by sterilized deionized water to remove residue of culture medium from mycelium and other waste of fungus. The biomass was grinded at sterilization region, then 10 g of wet biomass dropped in 100 ml of deionized water then incubated for 72 hours at 25 $\pm$2 C. After incubation the mixture was filtered again, 100 ml of supernatant was mix with 100 ml $AgNO_3$ at concentration 1 mM, and 200 ml of supernatant of fungus was prepared without any addition as control. All sample incubated at 100 RPM / 25 $\pm$ 2 C for 96 hours (Chauhan and Gupta, 2017).

2.7. Characterization of AgNPs

2.7.1. UV-VIS Spectrophotometer

After incubation, 2 ml of each sample were taken and tested using a UV-VIS spectrophotometer in the laboratory of the Department of Chemical Engineering, College of Engineering, University of Tikrit. The wavelength set up is 200–800 nm. (Bharathidasan and Panneerselvam, 2012).

2.7.2. Scanning Electron Microscopy SEM

The sample was prepared by taking 10 ml of supernatant, then centrifuged at 6000 RPM for 15 minutes. The pellet was collected and dried in an oven at 60 C for 20 minutes. The grinding powder was prepared to be tested using SEM to determine the shape and size of the nanoparticles of silver (Talebia *et al*., 2010).

2.7.3. Scanning electron microscopy-energy-dispersive X-ray (SEM-EDX)

The powder was manufactured in accordance with the instructions in (Talebia et al., 2010) and

shipped to Iran for EDX testing, which is a helpful tool for determining the structure and morphology of silver nanoparticles.

2.8. The concentration of AgNPs used in the study

Several concentrations of AgNPs have been prepared (5, 10, 15, 20, and 25%) using deionized water. The control was prepared only from sterilized deionized water; the equation that has been used is mentioned, which is V mean volume and C concentration (George, 1968).

$V1 \times C1 = V2 \times C2$

2.9. Effect the AgNPs synthesized by supernatant of Trichoderma harzianum on life aspects Musca domestica

To study the influence of the AgNPs on some life aspects of *Musca domestica*, three duplicates of each concentration in Section 2.8 have been made. The pairs of both sexes of newly hatched *Musca domestica* were taken after the AgNPs of each concentration were sprayed on flies, then prepared in a petri dish that was closed by soft clothes with appropriate aeration and feed, and then incubated at 30 $\pm$ 2 C with good humidity. The average of laid eggs and inhibition of egg laying for each concentration was counted after the death of experimented flies using the equation mentioned in Abbott (1925), and the average of new membranes, fertility percentage, and decline in first-generation individuals was calculated using the equation in Tabu et al. (2012). Also, sprayed houseflies with sterilized distilled water were taken as control.

3. Results and discussion

3.1. Characterization of AgNPs

3.1.1. Visual observation

The results shown synthesized the AgNPs using *Trichoderma harzianum* as appeared in Figure 4-1, through transferred the color of solution from white to red color due to reduce the $AgNO_3$ to AgNPs (Verma et al., 2010).

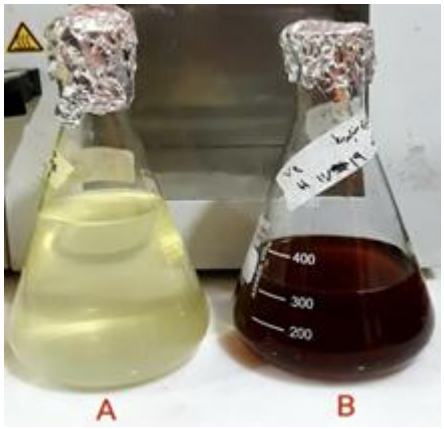


Figure 4-1. Visual observation of the AgNPs solution after incubation A. Fungus supernatant . B. Fungus supernatant after added the $AgNO_3$.

3.1.2. Absorption UV-Visible light spectroscopy

The results of current study shown ability of *Trichoderma harzianum* to produce the AgNPs through the sign of the ultraviolet and visible absorbance spectra within the range (800–200) nm of the $AgNO_3$ solution used to prepare AgNPs, which is one of the important techniques for detecting nanostructures as a result of irritation and vibrations in the plasmon (electron or hole) at the surface of the metal, Figure 4-2 shows the absorbance spectrum as a function of the wavelength of the $AgNO_3$ solution at a concentration of 1 mM after it was carried out with an

amount of 5 ml of the fungal supernatant and incubated for 96 hours. The absorption of silver and the concentration of silver ions in the solution, as well as the enzyme associated with the fungus represented by nitrate reductase, are responsible for the formation of AgNPs.

The results showed that the fungus of *Trichderma harzianum* has the ability to produce nanoparticles NPs, within the range 800-200nm, the (A) which represents the absorption spectrum of the wavelength of the $AgNO_3$ before adding the fungus supernatant to the solution and has an absorption peak at wavelength 300nm, (D) represents the ultraviolet and visible absorbance spectra of the $AgNO_3$ solution after mixing it with an amount of *Trichoderma harzianum* supernatant and incubating for 96 hours, the absorbance peak appeared at 418nm, and the curve (C) represents the absorbance spectrum after one hour of mixing the supernatant fungus with an $AgNO_3$ solution at 1mM. (B) represent the absorbance of supernatant of fungus only after incubation.

The absorption peak of 418 nm and peaks close to it were obtained by the Abod (2017), who confirmed that the absorption peaks of ultraviolet and visible rays of AgNPs prepared biologically using *Aspergillus niger* at the wavelength of 430nm.

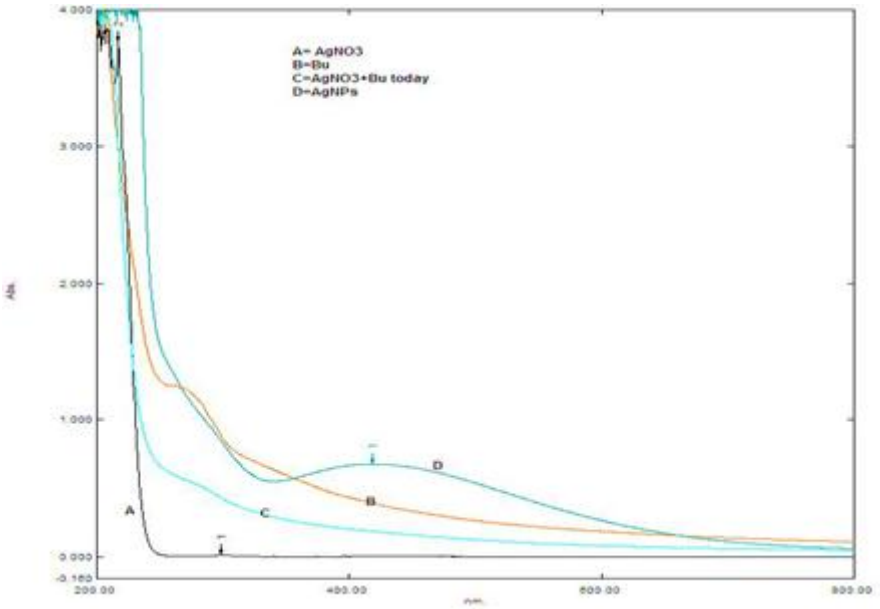


Figure 4-2. UV and visible absorbance spectra within the range 200–800nm of the $AgNO_3$ solution used to prepare AgNPs. A. absorbance spectra for the wavelength of $AgNO_3$ before adding the fungus supernatant to solution. D. absorbance spectra of ultraviolet and visible absorbance spectra for $AgNO_3$ after mixing with the supernatant of the fungus and incubating for 96 hours. B. represented the absorbance of the supernatant only. C. represents the absorbance of the solution after one hour of incubation.

3.1.3. Scanning Electron Microscopy

Through scanning electron microscopy (SEM), it is possible to know the shape, size, and distribution of nanoparticles with high accuracy at different magnifications. SEM images of AgNPs prepared using *Trichoderma harzianum* show the distribution of silver atoms that are in the form of spherical granules with an average size of 46.63nm for AgNPs. These granules are homogeneous and non-adhesive, which confirms the formation of nanoparticles, as shown in Figure 4_3. The results of SEM for the average particle size that we obtained in this study are carried out by the results obtained by some researchers, such as Hirpara et al. (2020), who showed the ability of *Trichoderma* sp. to produce AgNPs with a range size of 51–62 nm.

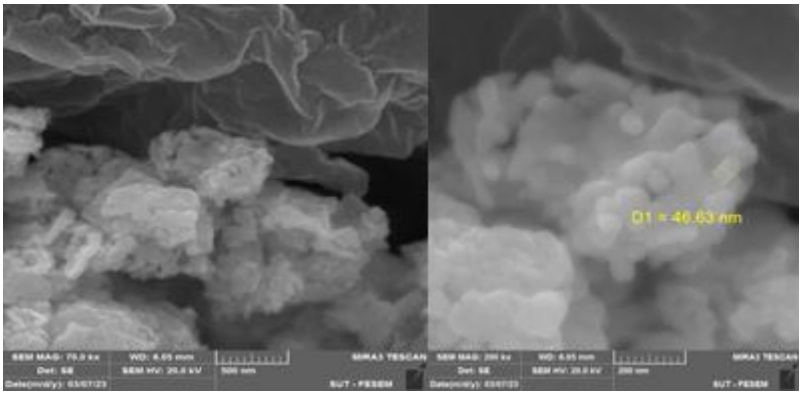


Figure 4-3. Shape, size, and distribution of AgNPs synthesized by fungus supernatant, SEM image with 25 0000 X magnification

3.1.4. Scanning electron microscopy-energy-dispersive X-ray (SEM-EDX)

In the EDX electron microscopy assay of the *Trichoderma harzianum* fungus solution to which $AgNO_3$ was added, the results of the current study showed the ability of *Trichoderma harzianum* to produce AgNPs. The chemical analysis of the sample gave carbon, oxygen, and silver as basic elements, in addition to sulfur, calcium, and phosphorus. The gold resulting from the process of plating the sample and the presence of carbon and oxygen are evidence that the sample is of organic origin, as shown in Figures 4–4.

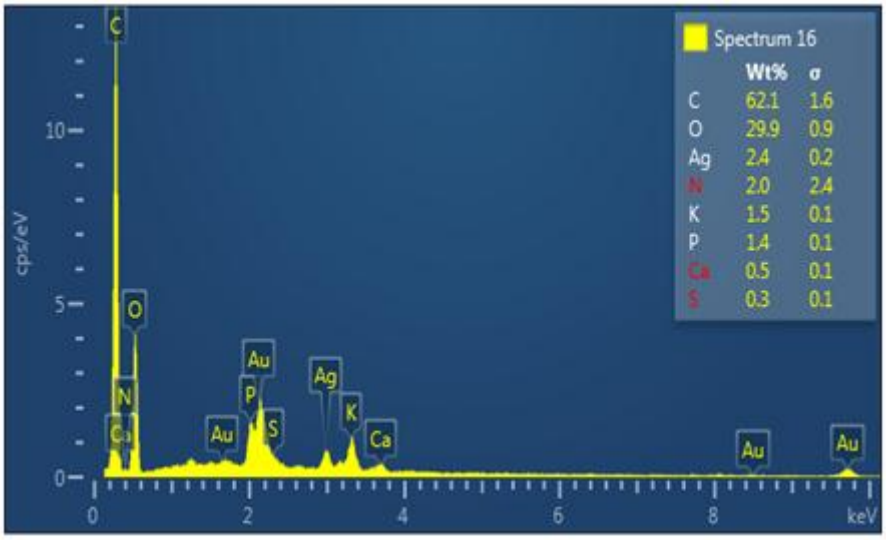


Figure 4-4. chemical analysis of AgNPs synthesized by fungus supernatant.

3.2. Effect of different concentrations of bio-prepared AgNPs upon the phases of the *Musca domestica*

3.2.1. Effect of AgNPs in eggs of *Musca domestica*

The results noted in Figures 4-5. indicate that AgNPs of fungus had the highest effect on the average incubation period of eggs for the test insect, as it was found that concentrations of 5%, 10%, 15%, and 20% prolonged the incubation period of eggs to reach 9, 12, 17 and 19 hours, respectively, at the same concentrations. Compared to the control coefficient, which recorded 7 hours, while at 25 none of them hatched.

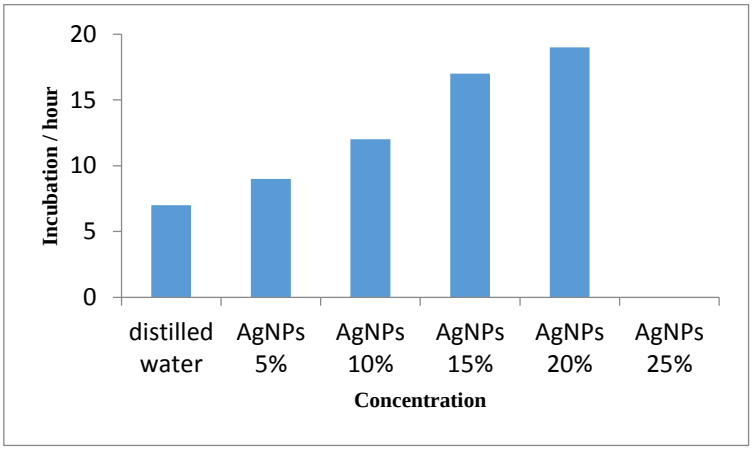
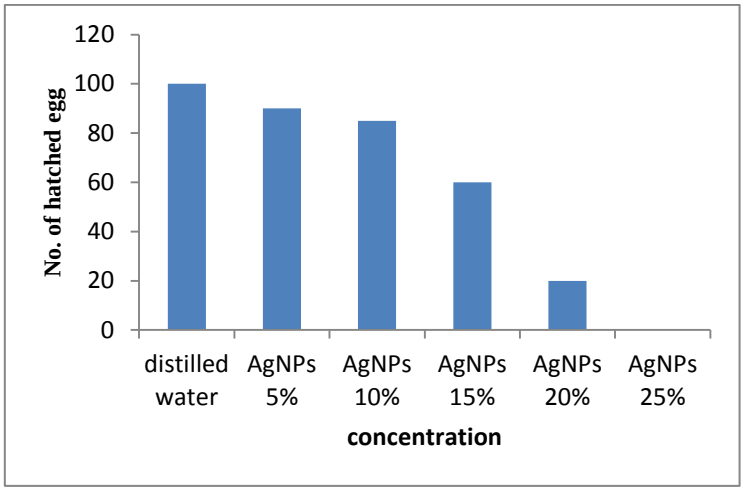


Figure 4-5. incubation of housefly eggs sprayed using AgNPs.

The results also indicated that the incubation period was directly affected by the concentrations as they increased, and therefore the concentration exceeded 20% in prolonging the incubation period if the incubation period for eggs treated with AgNPs was about 19 hours.

The results appeared in Figure 4-6, with the highest killing percentage at a concentration of 25% by 100% after sprayed with AgNPs. Perhaps the reason for the prolonged incubation period is due to the ability of nanomaterials to penetrate into the egg through the hilum (micopile), which may lead to toxicity of the embryo, in addition to the delay in the growth of embryonic cells inside the egg during the stages of cleavage and blastula, then the formation of germ layers, up to the stage of organization. (Rouhani, et al., 2012).



Figures 4-6 show numerous hatched eggs of houseflies after incubation.

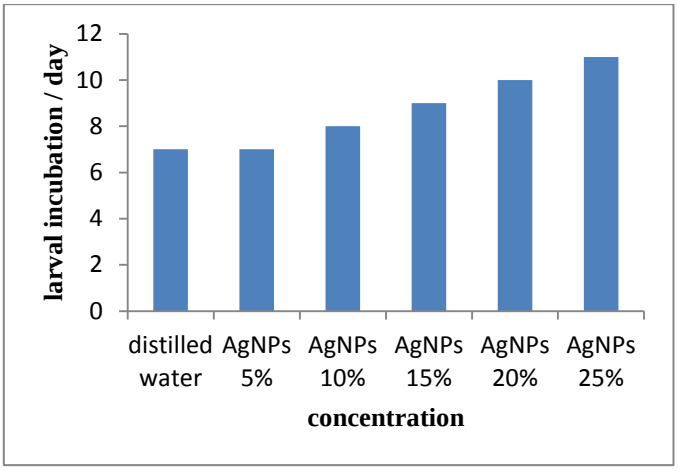
Another reason for the effect of NPs is that the ability of AgNPs to perish in eggs may be due to the physical and chemical properties and free ions emitted from AgNPs, their ability to penetrate the plasma membrane, and their high permeability and accumulation inside cells, which leads to a harmful effect on embryonic development. Addition to its ability to interfere with many of the insect's vital activities and destroy many vital molecules, such as enzymes, as well as the coagulation of proteins and the loss of its plasma membrane function and thus cell death (Rouhani, etal, 2013).

The results agreed with what was indicated by Rouhani et al. (2012), that nanomaterials affect the life of the southern cowpea beetle and thus act as insecticides. Mohammed et al. (2021) concluded that the incubation period for the eggs of the southern cowpea beetle was prolonged when treated with different concentrations of biologically prepared AgNPs, reaching 8.85 days at

100% concentration, compared to 6 days at 12% concentration.

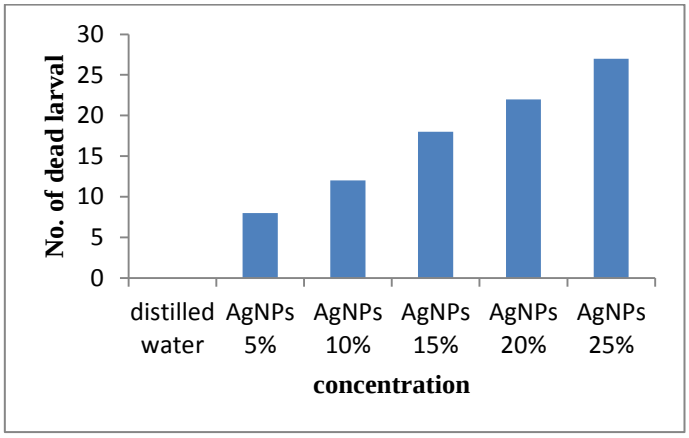
3.2.2. The effect of AgNPs on the duration of the larval stage / day and the number of dead larvae of *Musca domestica*

The results shown in Figure 4–7 showed the superiority of AgNPs after incubation upon larval stage. It is noted that AgNPs recorded a perfect effect on the average duration of the larval stage of the insect. Concentrations of 15%, 20%, and 25% of AgNPs were recorded after incubation. The effect of the treatment was greatest on the duration of the larval stage, which amounted to 9, 10, and 11 days, respectively.



Figures 4-7 show the incubation of housefly larval.

The results in Figure 4-8 revealed that the treatment with AgNPs exceeded the concentration by 25% after incubation, as it recorded a killing rate of 27 insects, while the lowest killing rate was at 5% with a concentration of 8 insects.



Figures 4-8 show the number of dead larval.

Maybe the reason for the effect of AgNPs on the lengthening of the larval stage is that the NPs act as inhibitors of the enzyme trypsin (necessary to complete the chemical reactions of protein digestion in the small intestine) and thus disrupt the processes of growth and reproduction. In addition, because the size of NPs is close to the size of cellular proteins, they have the ability to cross some barriers in vital systems (Paur, et al. 2011). It can enter the cell through phagocytosis or cell engulfment and thus influence the process of molting and many other physiological processes (Naresh, et al. 2012; Geiser et al. 2005).

The mechanism of the influence of NPs on the cell is represented by membrane rupture, protein oxidation, neurotoxicity, energy deficiency, and the formation of reactive oxygen species (ROS

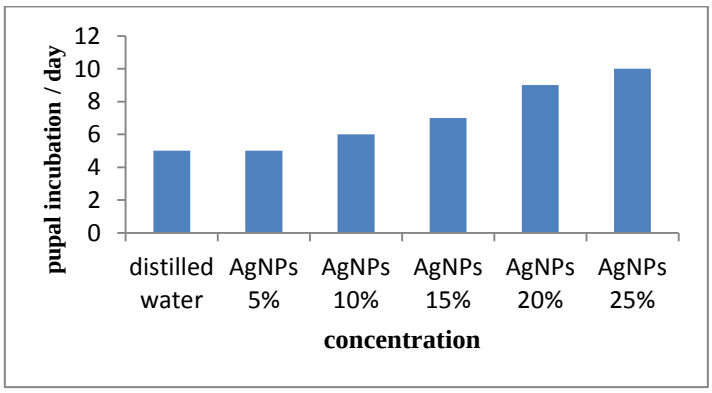
act as molecular indicators inside cells in response to external invaders and pathogens, and when their concentration is increased from the normal limit, it causes oxidative damage to proteins, which leads to cell membrane damage and cell death), the formation of free radicals that cause oxidative stress, and the release of toxic components. It affects the vital processes of the insect as it is linked to the enzyme esterase, which enters the metabolism of a variety of external and internal chemicals. Thus affecting the pathway of enzymes involved in metabolism (Madasamy et al., 2023).

These results are close to what Mao and his group concluded (2018): that lethal and sublethal doses of AgNPs have acute and chronic toxic effects on the growth and lifespan of the fruit fly D. melanogaster and that they may delay the growth of the insect stages as well as affect lethal doses of AgNPs. Also Raj and his group, (2017) stated that eating larvae on a diet containing AgNPs affects their development and causes a significant decrease in the percentage of larvae that reach adulthood, which affects the rate of emergence of adults.

Mahmood (2017) also conducted biological tests on the effect of AgNPs prepared biologically by the insect pathogenic fungus *Entomophthora muscae* on the housefly *Musca domestica*. The test results showed that these particles had high toxicity towards the larval stages of the housefly insect, and the larvae of the first age were the most They were affected by a killing rate that ranged between 95 and 100% in all tested concentrations, which indicates the possibility of using AgNPs in insect control.

3.2.3. Effect of AgNPs on pupal stage day and number of dead pupae

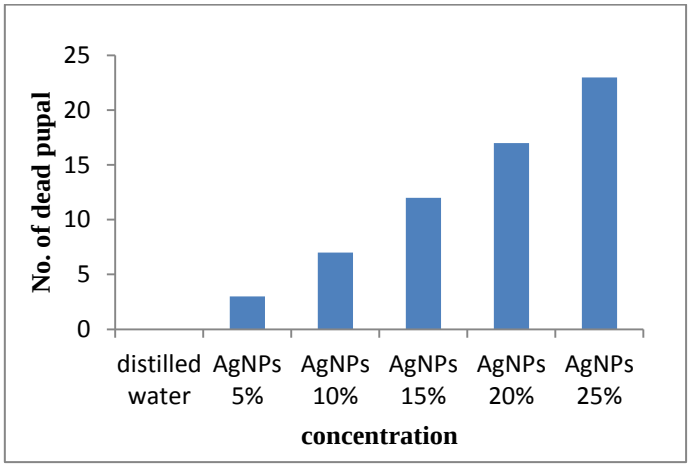
The results in Figure 4-9 revealed the effectiveness of the AgNPs after incubation, during which they reached the incubation period of the larval stage of 10 day in 25% of AgNPs. Concentrations of 15%, 20%, and 25% of AgNPs after of treatment reveald the highest effect on the duration of the pupal stage, which amounted to 7, 9, and 10 days, respectively. While it had less effect on the duration of the pupal phase at 5%, which reached 5 days.



Figures 4-9 show the number of pupal incubation day.

Results in Figure 4–10 showed the effect of treatment with AgNPs at a rate of killing 23 insects, while the lowest concentration recorded 3 insects. The effect of AgNPs on the pupal stage is due to the toxic effect of NPs on the inside of the insect's cuticle, which affected the vital processes of the insect, including a decrease in the liberation of phosphorus, which is important in energy metabolism, in addition to a decreased in metabolism level.

The results of the study were the same as those mentioned by Mall (2022), except for the species of insect that confirmed the effect of AgNPs on the southern cowpea beetle, as the period of the pupal stage increased by an average of 9 days at a concentration of 100%, compared to the control coefficient, which amounted to 3 days.



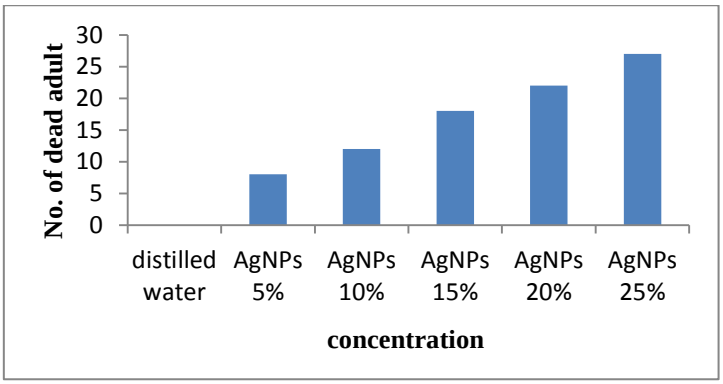
Figures 4-10 appeared the number of dead pupal of houseflies.

3.2.4. The effect of AgNPs on the death of adult Musca domestica

The results of death of adult showed the superiority of AgNPs, the number of killed insects reached 27 insects, respectively (Figure 4-11).

While the concentrations were recorded as 5%, 10%, 15%, and 20% when treated with AgNPs 8, 12, 18, and 22, adults.

It is clear from what was mentioned that the percentage of killing is directly proportional to the concentration used, and through the concentrations used for AgNPs, it is revealed that the concentration exceeds 25%. As shown by Chakravarthy, (2012), the reason for the death of insects is due to the fact that nanoparticles can cause distortions or anomalies in chromosomes and DNA damage, which leads to cell damage and death.



Figures 4–11 show the number of dead adult houseflies.

Mao et al. (2018) indicated that nutritional doses of AgNPs have an effect on the fruit fly insect Drosophila melanogaster, as they may lead to a delay in growth and the lethal effect of the stages of the insect, especially modern adults, and DNA damage, and added that lethal and sub-lethal doses of AgNPs have toxic and acute effects on the growth and lifespan of the organism. Singh et al. (2021) also confirmed that ingestion of AgNPs by D. melanogaster larvae can lead to failure of melanogenesis, decreased body size, impaired motility in adult flies, and decreased female fertility.

Conclusions

The *Trichoderma harzianum* successfully transferred the $AgNO_3$ to AgNPs when the period of incubation finished. The average size of AgNPs revealed an average of 46.63 nm without any aggregation or adhesion.

The period of incubation of eggs of *Musca domestica* increased with an increase in the concentration of AgNPs, while the number of hatched eggs decreased when the concentration increased. Addition the numerous of death of larval increased at AgNPs increased. The pupal is also influenced by AgNPs at high concentrations. The adults of houseflies as well as effects when using AgNPs. Finally, the AgNPs are considered the perfect agent that can be used as biological control for some insects.

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