Effect of silver nanoparticles synthesized by *Lecanicillium lecanii* and their combinations with insecticides (Sivanto and Deltamethrin) on larvae and adults of *Tribolium confusum* Duv. (Tenebrionidae : Coleoptera)

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ABSTRACT

This research focused on developing a nanopesticide derived from silver nanoparticles (AgNPs) synthesized through the metabolic activities of the fungus *Lecanicillium lecanii* Gams & Zare 2001). The study aimed to assess its efficacy against the flour beetle *Tribolium confusum* and explore its synergistic effects with two chemical pesticides, Sivanto 20% and deltamethrin 2.5%. Biotinylated AgNPs were successfully produced using the biomass of the fungus *L. lecanii.* Characterization of silver nanoparticles was achieved through the observable color change of silver nitrate solution ($AgNO_3$) from a clear or watery state to red, brown, or dark red, confirmed by UV-V is spectroscopy, with the highest absorption peak at 420 nm, indicative of silver element absorption. Transmission electron microscope images illustrated that the biologically prepared AgNPs with *L. lecanii* exhibited spherical shapes with sizes ranging from 12 to 40 nanometers. Various concentrations of nanopesticide, Sivanto, and deltamethrin were tested for the treatment of grains. A concentration of 1 ml/L of deltamethrin showed superior performance, with the mortality ratio increasing with prolonged exposure. Both Sivanto and deltamethrin (0.5 ml/L and 0.4 ml/L) caused 100% mortality, followed by the nanopesticide (30% concentration), reaching a corrected mortality rate of 39.3% against adults of *T. confusum*. In the larval experiment, the mortality rate was high when applying AgNPs at a concentration of 100%, comparable to Sivanto 0.5 ml/L under similar conditions. Deltamethrin exhibited complete mortality at a 1 ml/L concentration.

Keywords: Silver Nanoparticles, *Lecanicillium lecanii*, *Tribolium confusum*
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INTRODUCTION

Storage and inventory management constitute fundamental aspects of the grain production process, crucial in safeguarding the quality and quantity of stored grains and their food products (Zhao, Lv & Li, 2023). The susceptibility of stored grains to insect infestations poses a significant threat, potentially resulting in substantial losses. Global estimates indicate that around one-third of the world's wheat production faces losses due to insect infestations during storage, with the United States alone experiencing losses of approximately 30 million tons of wheat (Zhao and Li, 2023). Grains are pivotal in global agriculture, contributing significantly to food security and providing more than 25% of individuals' calories (Annual Report on Agricultural Development in the Arab). Additionally, they are a vital source of protein in human nutrition (Shewry, 2007; Karim, 2012). In Iraq, the northern region, particularly the Nineveh Governorate, holds a prominent position in wheat production, accounting for 69.1% of the

country's total production (Pomeranz, 2001). The Food and Agriculture Organization (FAO) highlights that global losses in grain production due to warehouse pests reach approximately 36 million tons annually, equivalent to about one-third of global trade. The weight of stored grains can decrease by more than 10%, with varying loss percentages in different countries, ranging from 0.05-3% in industrialized nations to 45-50% in developing countries. Germination rates may also experience a decline ranging from 37-88% over a storage period of 4-6 months (Adugna, 2006; Al-Jabr, 2006).

Insect infestations, particularly those of flour beetles like *Tribolium* spp., pose significant challenges to maintaining grain and product quality during storage (Hagstrum and Subramanyam, 2009). Flour beetles, such as *Tribolium confusum*, are pervasive in Iraqi grain storage facilities and mills, causing substantial economic damage. Their adaptability to dry environments and ability to consume large quantities of stored grains and contaminate them with skins and residues further compound the challenge (Al-Iraqi and Jamil, 2007). Addressing the issue of chemical pesticides is becoming less desirable for several reasons. Traditional chemical preparations, including fumigants like methyl bromide and aluminum phosphide, have been widely used for combating warehouse insects (Fields, 2002). Nevertheless, the emergence of fumigant-resistant strains and associated health risks for control sector workers, environmental contamination, and impacts on human and animal health have raised concerns (Al-Ghalbi, 2006; Assie *et al.,* 2007). In response to these challenges, this study aims to explore environmentally friendly pesticides utilizing nanotechnology to combat insect infestations in warehouse and silo environments. Specifically, the study focuses on the production and bio-manufacturing of silver nanoparticles derived from biological fungi, assessing their efficacy against flour beetle larvae and adults and evaluating their synergistic effects with certain chemical pesticides on various stages of the insect The novelty of this research lies in the application of silver nanoparticles (AgNPs) as an eco-friendly, sustainable biocide against storage pests. This study uniquely investigates the efficacy of AgNPs on a specific species of storage insects, offering new insights into their potential as a safer alternative to conventional chemical pesticides. Additionally, this research utilizes innovative molecular and biochemical methods to understand the mechanism of action of AgNPs on insect physiology, providing a novel approach in the field of pest control and nanotechnology.

MATERIALS AND METHODS

Experiment Preparation and Implementation

The research was conducted at the Insect Laboratory within the Department of Plant Protection at the College of Agriculture, Tikrit University. The study spanned from August 1, 2020, to March 1, 2021, utilizing the facilities available in the graduate studies laboratory.

Insect Acquisition and Breeding

The flour beetle *T. confusum* was sourced from the warehouses of Silo Al-Sharqat in July 2019. The identification of insects was conducted at the Natural History Museum. To eliminate potential additional insect infestations, a quantity of grains was sifted, cleaned, and then subjected to freezing at -20°C for 24 hours following the protocol outlined by McGaughey *et al.* (1990). The grains were subsequently placed in 500 mL plastic bottles, each containing 100 grams of grains. Insects were introduced into the bottles, and the openings were securely sealed with terry cloth fastened with rubber bands. The bottles were then incubated in a controlled environment with a temperature of 28±2°C and a humidity level of 70±5% (Al-Jabri and Muhammad, 1987). The culture was periodically renewed to ensure the production of a single generation of insects. The third larval stage was specifically targeted, obtained, and subjected to subsequent study and analysis.

Selection of life stages of Flour Beetle

Initially, the adults were separated from the larvae utilizing a sieve with holes of 0.71 mm diameter. Subsequently, the third larval stage was isolated from the rearing media using a sieve with holes of

0.28 mm diameter. The isolated larvae and adults were then transferred to 250 mL glass bottles and stored in the incubator until required for experimentation. Identifying third instar larvae involved observing newly hatched one-day-old larvae, tracking the number of molts, and assessing the size of the head capsule and overall larval length. This process was facilitated by preparing five replicates of Petri dishes, each with a diameter of 9 cm and a height of 1.5 cm. These dishes contained 10 grams of wheat grains and were maintained under incubator conditions at 33±2°C and a relative humidity of 65±5%. Additionally, ten pairs of newly emerged males and females were introduced, and daily monitoring was conducted until the larvae reached the third instar stage. The larvae were then carefully isolated from the culture medium using a small brush and filter paper.

Culture Media Preparation

Glassware and tools necessitating sterilization underwent dry sterilization in an electric oven at a temperature of 180°C for two hours. Simultaneously, metal carriers were sterilized through direct flame exposure using a Bunsen lamp. Culture media and solutions requiring sterilization underwent a wet sterilization process using an autoclave at a temperature of 121°C and a pressure of 15 pounds/inch² for fifteen minutes, following the guidelines outlined by Macfaddin (2000).

Preparation of Potato Dextrose Agar (PDA) Medium

The Potato Dextrose Agar (PDA) medium was meticulously prepared following the guidelines stipulated by the manufacturing company (Indian Himedia Company). In preparation, 39 grams of the pre-manufactured PDA medium were dissolved in one liter of distilled water. The resultant medium was evenly distributed into 500 mL glass beakers, securely sealed with medical cotton, and subjected to sterilization in the autoclave. Sterilization occurred at a temperature of 121°C and a pressure of 1.5 bar for 20 minutes, by established protocols (Emmons *et al.,* 1974). Upon completion of the sterilization process, the medium was allowed to cool to a temperature of 40°C. Subsequently, a vial of the antibiotic Ampiclox was introduced at 4 mL per liter of medium. The finalized medium was then poured into Petri dishes under the illumination of a Bunsen lamp within a controlled environment. The inoculated medium was left to solidify at room temperature and subsequently stored in the laboratory refrigerator until needed for experimental use.

Preparation of Potato Glucose Broth (PGB)

A precise procedure was followed to prepare one liter of liquid Potato Glucose Broth (PGB). Initially, 200 grams of small-cut potatoes were added to 700 mL of distilled water. The mixture was then heated on a heat source for 40 minutes. Following this period, the mixture underwent filtration using a tack cloth to obtain the filtrate. The volume was adjusted to one liter using distilled water. Subsequently, 20 grams of glucose were incorporated into the filtrate, and the mixture was continuously stirred for 30 seconds to achieve the liquid fungal medium (PGB). The resulting medium was distributed into 500 ml glass beakers, and 250 mL was allocated for each beaker. The nozzles of the beakers were securely sealed with medical cotton, and the entire setup was sterilized in an autoclave. Sterilization occurred at a temperature of 121°C and a pressure of 1.5 bar for 20 minutes. After sterilization, the medium was allowed to cool until the temperature reached 30°C. Finally, a vial of the antibiotic Ampiclox was introduced at a rate of 1 mL per 250 mL of medium, following the protocol outlined by Al-Khafaji (2014).

Propagation and Activation of *Lecanicillium lecanii*

L. lecanii Development and Identification: The two pure and identified fungal isolates were sourced from the Ministry of Agriculture, specifically the Agricultural Protection Department's Agricultural Pest Diagnostics Department (Biopesticides Laboratory). The propagation process involved extracting a portion from each fungal colony utilizing a sterile

inoculation needle. This procedure was carried out in a meticulously sterilized environment, achieved by utilizing alcohol and UV rays near a Bunsen lamp inside the inoculation room. The Petri dishes containing the PDA culture medium, prepared as outlined, were the recipients of the fungal portions. The cultures were incubated at 25±2°C following the inoculation for 4-7 days. Post-incubation, each dish was securely sealed with tape to create an individual film, ensuring protection against contaminants. Subsequently, the dishes were stored in a refrigerator at a temperature of 5-6°C. Microscopic diagnosis was conducted using a compound light microscope. A sterile inoculation needle obtained a portion of the fungal hyphae from the pure, growing colony. This portion was then placed on a clean glass slide containing 2-3 drops of lactophenol blue dye, with subsequent mixing. After placing the slide cover and gently pressing for sample spread, the microscopic examination was conducted at 40X magnification. This examination aimed to determine the characteristics of the isolated fungus, including its branches, shapes, and type of conidium.

Preparation of Fungal Biomass

The preparation of fungal biomass commenced by extracting a disk from the newly developed and activated pure fungal colony using a sterile 6 mm cork perforator. This disk is carefully obtained by piercing the edges of the growing colony on the PDA culture medium. The obtained disk is then gently placed in a conical flask containing 250 mL of liquid Potato Glucose Broth (PGB) medium. It is positioned quietly to allow the disk to settle on the surface of the liquid medium. Afterward, the conical flask is transferred to an incubator at 25-26°C. The incubation period spans 5-7 days, during which the fungal mat develops to various weights (Karbasian *et al.,* 2008).

Preparation of $AgNO_3$ (Silver Nitrate) Solution

To prepare a solution of silver nitrate with a concentration of 1 mM, 0.017 grams of silver nitrate was dissolved in 100 ml of deionized water. Thorough mixing was ensured, and the solution was stored in opaque bottles to shield it from light until needed (Karbasian *et al.,* 2008).

Biosynthesis of AgNPs

Following the formation of the fungal mat, the fungal mass undergoes a series of steps for the biosynthesis of silver nanoparticles. The fungal mass is initially filtered using sterile filter paper, specifically Whatman filter (Paper No. 1), and thoroughly washed with sterile distilled water to eliminate any residual medium components. The cleaned fungal mass is then transferred to a conical flask containing 100 mL of sterile distilled water, where it is allowed to sit for 72 hours within the incubator. Subsequently, the fungal mass undergoes another round of filtration using sterile filter paper to ensure the removal of all remaining medium components. Ten grams of the fungal mass are precisely weighed and placed in 100 mL of distilled water. After the designated period elapses, the biomass is filtered for the second time using Whatman filter No. 1 and a centrifuge operating at 6000 revolutions per minute for 10 minutes. 100 mL of the mushroom biomass filtrate is combined with 100 mL of the previously prepared 1 mM $AgNO_3$ silver nitrate solution. An additional 100 mL filtrate is preserved for comparative purposes and further testing. All flasks containing the respective mixtures are placed in a shaking incubator set at a speed of 100 rpm and maintained at a temperature of 25-26°C in dark conditions for 96 hours (Karbasian *et al.,* 2008; Gupta and Chauhan, 2017).

Characterization of AgNPs : Heterochromia

The presence of AgNPs was identified through heterochromia. The color state of the solution was observed after 96 hours of incubation at a temperature of 25±2°C. This detection method involved noting the alteration in the filter ate color to which the silver nitrate solution had been added. The color transformed from colorless or watery white to varying shades of red, dark red, or brown, contingent on the concentration of silver nitrate introduced. This distinctive color change signified the successful synthesis of silver nanoparticles within the fungus filtrate (Al-Naimi, 2018). Then identified using UV-VIS Spectrophotomete (Bhainsa *et al.,* 2006), X-Ray Diffraction (XRD)

(Talebia *et al.,* 2010), Transmission (TEM) and Scanning (SEM) Electron Microscopy (Talebia *et al.,* 2010; Vanmathi *et al.,* 2012).

Pesticidal bioassay

After confirming the bioavailability of silver nanoparticles, solutions from the two fungi used in the study were diluted to prepare concentrations of 0, 30, 75, and 100%. The 0% concentration was the control treatment, utilizing distilled and deionized water separately. Other concentrations were prepared by diluting the base solution with deionized water. Three concentrations of Sivanto 200 SL (Butenolides, Flupyradifurone 200 g/L), (0.3, 0.4, and 0.5 ml/L), and Deltamethrin 2.5% EC (Pyrethroidwere) (0.5, 0.75, and 1%) were prepared using distilled water.

The pesticides were mixed with the nutrient medium (wheat seeds) to expose both larvae and adults to the treatments (Shaaban and Al-Mallah, 1993). A hand sprayer with a 100 mL capacity was utilized to treat every 15 g of wheat for each concentration of the pesticide. The treated wheat was distributed into three replicates, each replicate (dish) containing 5 g of treated wheat. Each pesticide was administered in three concentrations, each with three replicates. A control treatment (water-sprayed wheat) with three replicates was also included. This resulted in 12 replicates (dishes) for each pesticide.

Ten third-instar larvae or ten newly emerged adults (a few hours old) were placed in each replicate. The dishes were covered with terrycloth and secured with rubber bands. The dishes were stored in the incubator at a temperature of 32±1°C and a relative humidity of 60±5%. The number of dead larvae and adults was recorded after 1, 2, and 3 days of treatment. The percentage of mortality was corrected using the Abbott formula (Abbott, 1925). This standardized approach allowed for a systematic assessment of the efficacy of each pesticide on the target insects over a specific period.

Statistical Analysis

The statistical analysis of the data was conducted using the following parameters and methods. The data were analyzed within the statistical program S.A.S. (S.A.S. *et al.,* 2012). The experimental design employed was the Complete Random Design (C.R.D.). The Least Significant Difference (L.S.D.) test was utilized for the statistical comparisons. The significance level for the statistical tests was set at a probability level of 0.05. The statistical analysis methodology followed references such as Al-Rawi (2000) to ensure consistency and reliability in the statistical interpretation. This approach ensures a rigorous and standardized statistical analysis of the experimental data, providing a robust basis for drawing conclusions and comparing the different treatments and concentrations.

RESULTS

Characteristics of AgNPs

Filtrate by proteins, enzymes, and other compounds within the biomass filtrate was observed. These elements serve as electron donors, leading to the reduction of silver ions into silver nanoparticles. Introducing a 1 mM silver nitrate ($AgNO_3$) solution to the biomass filtrate induces a noticeable color shift to brown. This alteration in color serves as an initial indicator of the mold's metabolic products effectively breaking down the mineral compounds and forming nano-sized particles. This phenomenon is attributed to the reductive influence of silver nitrate, and the observed color change is likely a result of surface plasmon excitation, with the electron conduction groups forming the basis of this vibrational effect (Verma, 2010).

UV-Visible Light Spectroscopy Absorption

In the case of silver nanoparticles, the peak absorption occurred at a wavelength of 420 nm, representing the specific range of wavelengths absorbed by these nanoparticles. This finding aligns with the research of Lyer *et al.* (2016), who also identified the absorption peak for silver nanoparticles at the same wavelength of 420 nm. The size of silver nanoparticles affects their absorption of UV radiation. As nanoparticle size increases, a red shift occurs in the transmission dip, allowing absorption in the desired near-UV region (Sandeep *et al.,* 2019).

X-Ray Diffraction (XRD) Analysis

The X-ray diffraction spectrum of silver nanoparticles synthesized using *L. lecanii* fungal filtrate revealed distinctive diffraction peaks at 77.44º, 44.31º, and 77.45º, corresponding to crystallographic planes 103, 106, and 111, respectively. These results closely matched the data specified in the International Center for Diffraction Data (JCPDS) card. The consistency of these findings aligns with the observations reported by Al-Zubaidi *et al.* (2019) in their study on silver nanoparticles.

Transmission Electron Microscopy (TEM)

The measurements, at magnification levels, offered a detailed view of the nanoparticles' accurate distribution and diverse sizes. The images revealed that the silver nanoparticles exhibited a spherical grain morphology with 12-40 nm diameters. Notably, these particles displayed uniform distribution and existed as non-adhesive clusters. These findings align closely with the dimensions reported by Al-Zubaidi *et al.* (2019), who similarly observed silver nanoparticles within the 5.2-20 nanometers range.

Toxicity Bioassays for grubs

First mortality results, then LCs the results in Table 1 showed that the LC_{50} for silver nanoparticles (AgNPs) was 156658.97 ppm. AgNPs demonstrated significantly higher toxicity to *T. confusum* larvae compared to the conventional pesticides Sivanto and Deltamethrin, which gave LC_{50} values of 26.33 and 4.32 ppm respectively. The 95% confidence limits for the three pesticides exhibited variation; AgNPs recorded 412,328.55 ppm (upper limit) and 59,520.58 ppm (lower limit), whereas Sivanto and Deltamethrin exhibited values of 42.99 and 16.12 ppm for Sivanto, and 8.31 and 2.25 ppm for Deltamethrin. Figure 1a demonstrates significant differences in mortality rates across the three pesticide concentrations, with an LSD value of 0.05 = 20.05. First figure 1a, then Figure 1b highlights the significant impact of three different concentrations of Sivanto pesticide on the mortality rate. The highest mortality rate recorded was 89.3% at a concentration of 0.5 mL/L, whereas the lowest mortality rate was 6.7% at a concentration of 0.3 mL/L, compared to the control treatment. Figure 1c illustrates the mortality rates associated with Deltamethrin. The highest mortality rate was 100% at a concentration of 1 mL/L, and the lowest mortality rate was 92.9% at a concentration of 0.5 mL/L. The significant difference (LSD) was calculated as 41.1.

The data in Table 1 illustrate variability in the average corrected percentage of mortality larvae based on the pesticide type, concentration, and exposure duration. Notably, the Sivanto pesticide achieved 100% mortality after one day, while the Deltamethrin pesticide reached 100% after 72 hours. Insect growth regulators, characterized by low toxicity, primarily affect insect growth and development. In contrast, pyrethroid pesticides have a direct, rapid, and highly toxic effect. Growth regulators, inhibiting chitin synthesis, compete with hormones in this process, impeding their function. Additionally, the presence of cyromazine restricts chitin binding and assembly in the internal cuticle, influencing insect growth and development (Tunaz and Vygur, 2004). One study by Salunkhe *et al* . (2011) showed that biologically prepared AgNPs by the fungus *L. cochlibolus* exhibited effectiveness against larvae of two mosquito species, *Anopheles stephensi* and *Aedes aegypti*, at various concentrations. The researchers observed proportional mortality rates to AgNPs concentrations, recording significant changes ($P < 0.05$) in larval mortality rates between exposure periods for fourth-instar larvae of *A. aegypti* and *A. stephensi.* Recently, the production of nanoparticles using biogenic materials such as plants, microbes, and natural biomolecules, known as green chemistry, could be considered an alternative technique to other methods due to their eco-friendly nature (Gurunathan *et al.,* 2013).

Table 1. Toxicity impact of AgNPs, Sivanto and Deltamethrin against Larvae of *Tribolium confusum*

Treatment	LC_{50} (ppm)	Confidence limit % 95		Slope ± SE	χ2
		Lower	Upper		
AgNPS	156658.97	59520.58	412328.55	1.17±0.21	0.97 N.S
Sivanto 200 SL	26.33	16.12	42.99	3.38 ± 0.11	0.96 N. S
Deltamethrin 2.5 EC	4.32	2.25	8.31	2.38 ± 0.15	0.99 N. S

χ2: Chi-test, N.S.: no Significant

Toxicity bioassay for adult *Tribolium confusum*

The results in Table 2 showed that the LC_{50} percentage for the silver AgNPs pesticide reached 458054.68 ppm, indicating high toxicity to *T. confusum* adults compared to the pesticides Sivanto and deltamethrin, which amounted to 33.11 and 1.71 ppm, respectively.

Table 2. Toxicity of Silver Nanoparticles and Chemical Insecticides against Adults of *Tribolium confusum*

Treatment	LC_{50} (ppm)	Confidence limit % 95		Slope ± SE	χ2
		Lower	Upper		
AgNPS	458054.68	284538.57	737383.64	2.29 ±0.11	0.64 N.S
Sivanto 200 SL	33.11	18.55	59.09	1.98 ± 0.13	0.97 N.S
Deltamethrin 2.5 EC	1.71	0.56	5.24	. 25 0± 1.27	0.99 N.S

χ2: Chi-test, N.S.: no Significant

The 95% confidence interval for the three pesticides varied. The Nano pesticide AgNPs gave upper and lower values of 737383.64 and 284538.57, respectively, compared to Sivanto and deltamethrin, which gave varying percentages of 59.09 and 18.55 for Sivanto and 5.24 and 0.56 for deltamethrin. Figure 2a shows significant differences between the three concentrations: LSD 0.05 = 21.72. The highest mortality rate reached 86.2% at the 100% concentration of silver AgNPs pesticide, and the lowest mortality rate was 65.5% at the 30% concentration, while it was 6.7% in the control treatment. The results in Figure 2b demonstrated a significant effect of adding three different concentrations of Sivanto pesticide on the mortality rate. The highest mortality rate reached 100% at the concentration of 0.5 mL/L, while the lowest mortality rate was 89.7% at the concentration of 0.3 mL/L, compared to the control treatment, which amounted to 6.7%. As for the pesticide deltamethrin, shown in Figure 2c, the highest mortality rate was at the concentration of 1 mL/L, reaching 100%, and the lowest mortality rate was 89.7% at the concentration of 0.5 mL/L, with a significant difference (LSD) of 0.05 = 12.49.

DISCUSSION

The results indicate that as the concentration of silver nanoparticles treatment, exposure time, and soaking duration increased, the mortality rate of flour beetle larvae also increased. These results align with Mao *et al.* findings (2018), indicating that nutritional doses of silver nanoparticles delayed the growth of the fruit fly *Drosophila melanogaster* Toxonomical details and had lethal effects on various insect stages, especially adults. Sub-lethal doses were associated with a shortened

lifespan due to cell death and DNA damage. Both lethal and sub-lethal doses of silver nanoparticles demonstrated toxic effects on the growth and lifespan of organisms. (Aisvarya, 2023) investigates the efficacy of silver nanoparticles (AgNPs) in controlling *Sitophilus oryzae* (L.), a common pest affecting stored maize (Zea mays) seeds, and assesses the impact of these nanoparticles on the agromorphological characteristics of maize seeds. The results demonstrated significant pest mortality with minimal negative effects on seed germination and growth. Additionally, the application of AgNPs did not adversely affect the key agronomic traits of the maize plants, making them a promising alternative to chemical insecticides in pest management without compromising crop quality. The efficacy of AgNPs and malathion in controlling infestations of the red flour beetle, *Tribolium castaneum*. Bioassays were conducted to evaluate mortality rates, sublethal effects, and impact on reproduction. Results demonstrated that AgNPs exhibited significant insecticidal activity, with effects comparable to malathion, while also reducing the beetle's reproduction rate. The study highlights the potential of AgNPs as an alternative to chemical insecticides like malathion, offering a safer and environmentally friendly pest management solution (Alif Alisha, 2019). The concentration of 10 mg/L achieved a 100% mortality rate for all three larval stages of treatment, suggesting the potential use of silver nanoparticles in insect control. Investigations into the effect of green silver nanoparticles against larvae of the flour beetle, *T. castaneum*, and the southern cowpea beetle, *Callosobruchus maculatus* Toxonomical details, showed that concentrations of 1,500 and 2,000 parts per million had the highest efficacy, with green silver nanoparticles achieving the highest killing rate. The average LC_{50} reached 2.992 ppm for C. maculatus compared to pure silver particles. The researchers concluded that silver nanoparticles are an excellent insect pest control alternative, given their unique features and environmental friendliness. AgNPs in general possess excellent antimicrobial properties against a wide selection of microorganisms, including bacteria, fungi, and viruses (Rathi Sre *et al.,* 2015).

In conclusion, the silver nanoparticle pesticide (AgNPs) exhibited markedly higher toxicity to *Tribolium confusum* larvae compared to the other tested pesticides. These findings underscore the importance of evaluating the toxicity of pesticides across different concentrations to ensure effective pest control and minimize environmental impact.

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