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PHYTOSYNTHESIS OF SILVER NANOPARTICLES USING *Aesculus hippocastanum* L. (KERNEL AND SHELL) AND EVALUATION OF THEIR LARVICIDAL ACTIVITY AGAINST *Plodia interpunctella* (HUBNER) (LEPIDOPTERA: PYRALIDAE)

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Abstract: The aim of this study was to synthesise silver nanoparticles (AgNPs) from horse chestnut organic wastes (kernel and shell) by phytosynthesis and to investigate the larvicidal activity of the obtained nanoproducts. In the characterisation tests (UV-Vis, FTIR, XRD, SEM, STEM, EDS), the physico-chemical structure of the synthesised AgNPs was clearly obtained. In order to determine the larvicidal activity of the synthesised AgNPs, topical application of four different doses of AgNPs (50, 100, 150 and 200 ppm) on second and fourth instar larvae of Indian meal moth (*P. interpunctella*) was carried out at two different temperatures (28-32 °C). The highest larvicidal activity was observed at the end of the fourth day, at the highest application dose (200 ppm), at 32 °C and in the nanoproduct obtained from the kernel (99% mortality rate for second instar larvae and 92% mortality rate for fourth instar larvae). It was observed that second instar larvae were more sensitive to AgNPs compared to fourth instar larvae, and as the applied temperature increased, the concentration values required for lethal effect and the exposure times required for killing decreased. According to the larvicidal activity data obtained, it was determined that the type of extract used in the synthesis, the temperature of the environment selected for application, the dose amounts applied and the exposure time are very important in this type of nano-insecticidal studies.

Keywords: Horse chestnut, Organic waste, AgNPs, Indian meal moth, Larvicidal activity

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1. Introduction

Many different moth species have the potential to damage stored products and one of the most dangerous of these, *Plodia interpunctella*, can cause irreversible and serious damage to stored nuts, dried fruits and foods, cereals and legumes if no action is taken (Kumar, 2017; Nasir et al., 2017). Although a certain success rate has been achieved in chemical control against this pest, the fact that the pest has developed resistance to most chemicals, the high rate of residue problems in the products after application and the resulting serious health risks for humans and animals have created new opportunities for alternative control methods against this pest (Attia et al., 1981; Pretty and Bharucha, 2015; Chellappandian et al., 2018).

As an alternative control, researchers have tried preparations obtained from plant-based drugs against this pest using different methods and have obtained very effective results (Elma et al., 2021; Prvulović et al., 2023). Biotechnological studies carried out to increase the effectiveness of herbal preparations even at low doses have led researchers to synthesise nanoparticles using herbal drugs and to test the nanoproducts formed in this way against pests (Adetunji and Oloke, 2024; Tirunagaru et al., 2024). The most well-known of the nanoparticles with different properties formed as a result of green synthesis processes are silver nanoparticles in metallic form and many storage pest species have been controlled using these synthesis products (Yasur and Rani , 2015; Abbood and Ali, 2020; Rehman et al., 2021; Anees et al., 2022).

The fact that the plants to be used for the green synthesis of silver nanoparticles can be easily found in nature and even the use of plant organic wastes in these synthesis processes have come to the fore as highly preferred studies in recent years (Kiani et al., 2023; Marcu Spinu et al., 2024). Horse chestnut (*Aesculus hippocastanum* L.) is abundant in natural forests and is even grown as an ornamental plant in many parts of the world as a landscape tree in many parks and gardens in cities (Lack, 2002). In studies on many different parts of this tree, it has been reported that they have a very high phytocompound content and even the fruits have a very rich variety of secondary metabolites (Kędzierski et al., 2016; Čukanović et al., 2020; Owczarek-Januszkiewicz et al., 2023). Due to these properties, horse chestnut has been the subject of many studies and even considered as

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organic waste and preferred in green synthesis processes of silver nanoparticles and some other metallic nanoparticles (Çolak et al., 2017; Demirezen et al., 2018; Küp et al., 2020).

The aim of this study is to synthesise silver nanoparticles by green synthesis from extracts of horse chestnut kernel and shell, which are considered as organic waste. The next step is to determine the larvicidal activity of the synthesised silver nanoparticles against *Plodia interpunctella*, an important storage pest. The data to be obtained as a result of the study will constitute a scientific resource for future nanotechnological studies of different nature using organic wastes with different properties against moths, which are warehouse pests and have similar properties.

2. Materials and Methods

2.1. Extraction of Plant Materials and Synthesis Process of Silver Nanoparticles

The kernels and shells of horse chestnut (*Aesculus hippocastanum* L.) were used in this study (September 2023, Latitude: 40°50′39′′ N, Longitude: 35°37′44′′ E, Altitude 522 m). Cleanly collected plant material was dried in a ventilation cabinet at room temperature for 60 days. Fifty gram of dried plant material was placed in a 500 mL Erlenmeyer flask, deionised water was added and placed in an ultrasonic water bath at 50 °C for 30 min. After cooling the solution, it was carefully filtered using sterile Whatman No.1 filter paper and prepared for silver nanoparticle synthesis (Hu et al., 2022). This solution was designed using silver nitrate (AgNO3, 99.9%, Sigma-Aldrich) similar to the method described by Küp et al. (2020) and silver nanoparticles were synthesised and AgNPs powder pellets were obtained from this synthesised solution.

2.2. Characterisation of the Materials Obtained

The spectra of the obtained materials (extract and AgNPs) were prepared using UV-vis spectrophotometer (Model UV 1601, Shimadzu). FTIR spectroscopy (Jasco 430 model FTIR spectrometer) was performed using KBr pellet method to determine the distribution of chemical functional groups of nanomaterials (AgNPs). XRD (X-ray diffraction) graphs of AgNPs were generated using XRD diffractometer (Rigaku SmartLab 9 kW diffractometer system). SEM (Scanning Electron Microscopy) and STEM (Scanning Transmission Electron Microscopy; carbon coated copper grating was used to visualise the nanoparticles in terms of shape, size and structure) were carried out using JEOL JSM-7001F device. EDS (Energy Dispersive X-ray Spectroscopy) imaging analyses were performed to determine the elemental distribution of the prepared AgNPs and EDS graph was obtained. In addition, in order to more clearly evaluate the dimensional distribution of silver nanoparticles synthesised from both extracts, size plots of nanoparticles were generated from TEM images using Image-J software.

2.3. Rearing and Larvicidal Bioassay

The 1-2-day old Indian meal moth larvae used in the experiments were obtained from a laboratory-reared pest colony (more than 5 generations). The colonies were reared in plastic containers (10 cm deep x 15 cm wide x 30 cm high) covered with a sturdy mesh with fine pores for ventilation in a rearing room at 26 ± 1 °C temperature, 60 ± 5% relative humidity and 16(L): 8(D) hours photoperiod. The diet consisted of a mixture of maize flour, milk powder, honey, glycerin and flour (2:1:1:1:1:1:1:1, w/w/w/w/w/w). Firstly, powdered silver nanoparticles were prepared with distilled water at 4 different doses of 50, 100, 150 and 200 ppm and bottled. Only 1 µL of the different doses of nanoparticles was applied topically to the anterior thorax of 1–2-day old second and fourth instar larvae (L2-L4 stages) using a micro-applicator. L2 and L4 stage larvae treated with nanoparticles were placed in sterile petri dishes (Ø 90 mm sterile petri dishes, Whatman No. 1 round sterile blotting paper to cover the petri dish and 5 grams of diet on it) in groups of 10 separately. This procedure was prepared according to a randomized block design with ten replications for each dose-temperature-nanoparticle type determined for the experiments. A control group was added to each of the groups prepared for each dose-temperature-nanoparticle type and larvae in the control group were treated with distilled water only. All plastic containers prepared for the experiments were placed in their own climatic environment (28 ± 1°C / 32 ± 1°C, 60 ± 5% relative humidity, 16 (L): 8 (D) hours) and monitored daily throughout the experiments and the larvae that died as a result of the observations made at the end of 24, 48, 72 and 96 hours following the treatment were recorded and these larvae were removed from the environment.

2.4. Statistical Analysis

Mortality rates in control groups were corrected using the Abbott formula (Abbott, 1925). In order to determine the lethal effects of synthesized silver nanoparticles on both life forms depending on different temperatures, the significance of the differences between the mortality rates depending on the dose-time pair was characterized by Duncan's multiple range tests (p<0.05) (One-Way ANOVA Test: SPSS-2017, Version-25.0). The mortality rates obtained (mean ± SE) were shown in percentage graphs (p<0.05). In addition, 50% and 90% lethal concentration (LC50 and LC90) values and 50% and 90% lethal time (LT50 and LT90) values and their 95% confidence intervals (95% CL) were calculated from the adjusted mortality rates using probit regressions and SPSS-17 (Version 25.0) software.

3. Results and Discussion

3.1. Characterisation Assessment

UV–VIS: The colour change in the form of darkening of the solution colour during the synthesis process is clear evidence that the silver ions undergo a reduction process and this is mainly due to the surface plasmon resonance

response (Küp et al., 2020; Hu et al., 2022). As a result of the surface plasmon resonance response during synthesis, a very high peak value is obtained in the graphs of nanoparticles using UV-vis spectrophotometry, while this is not the case for extracts (Küp et al., 2020; Hu et al., 2022). In this study, it was observed that silver nanoparticles synthesised using shell and kernel extracts formed an absorption spectrum band at 485 nm and 490 nm wavelength, respectively. However, the shell and kernel extracts used did not produce any peak value in the absorption spectrum band (Figure 1).

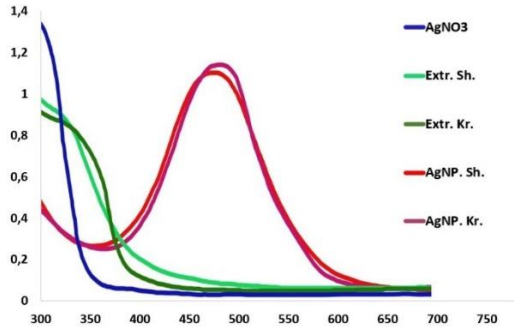

Figure 1. UV–Vis spectrum of Extracts (Sh.: Shell; Kr.: Kernel) and AgNPs (Sh.: Shell; Kr.: Kernel).

FTIR: While synthesising silver nanoparticles, a reduction process takes place due to the herbal components in the extract and this situation varies according to the content of the herbal components. This situation clearly shows itself in the FTIR test and when the spectroscopy of the synthesised nanoparticles and the extract is compared, it is seen that although the graphs are similar, there are differences due to reduction on the nanoparticle side (Chand et al., 2020; Salayová et al., 2021; Hu et al., 2022).When the FTIR test results of the nanoparticles synthesised using two different extracts in this study are compared, it is seen that the graphs are quite similar, but they differ from each other with some minor changes (Figure 2, a-b). This can be explained by some small differences in the functional groups of the plant compounds in the extracts used (Salayová et al., 2021). As a result of these small differences, two very similar nanoparticles were synthesised and as a result two different FTIR graphs were obtained (Figure 2, a-b). In similar studies where silver nanoparticles were synthesised, it was reported that the difference in the extract used caused changes in both the graphics and functional functions of the synthesised nanoparticles (Chand et al., 2020; Salayová et al., 2021; Hu et al., 2022).

XRD: As a result of XRD analysis to determine the refractive reflections of the synthesised AgNPs, 11 reflections were observed in nanoparticles produced from the shell and 19 reflections were observed in nanoparticles produced from the kernel (Figure 3, a-b). When the Miller index equivalents of the strongest of these Bragg reflections were calculated, it was determined that they corresponded to (110), (111), (200), (220), (311) and (222) planes, respectively (Ituen et al., 2020; Mahdi et al., 2024; Swathi et al., 2025). These strong reflections are very important in the characterisation of the synthesised silver nanoparticles and it is known that the Miller index expressed as '111' gives the strongest reflection (Ituen et al., 2020; Mahdi et al., 2024; Swathi et al., 2025).

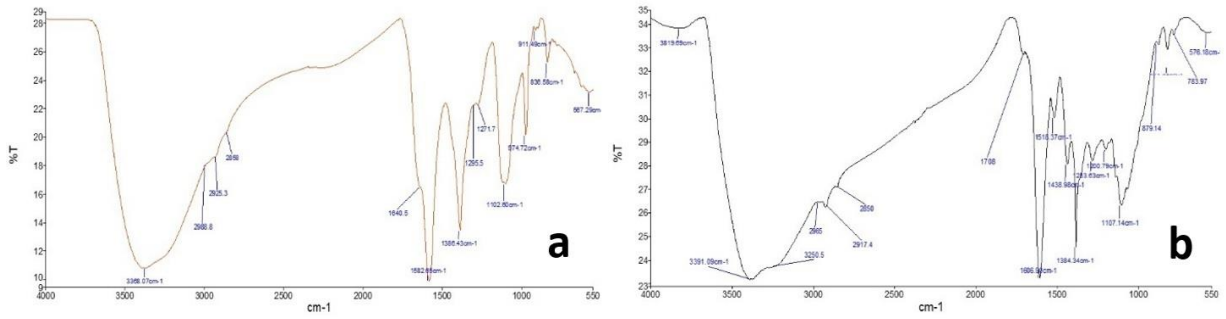

Figure 2. FTIR spectrum of Kernel AgNPs (a) and Shell AgNPs (b).

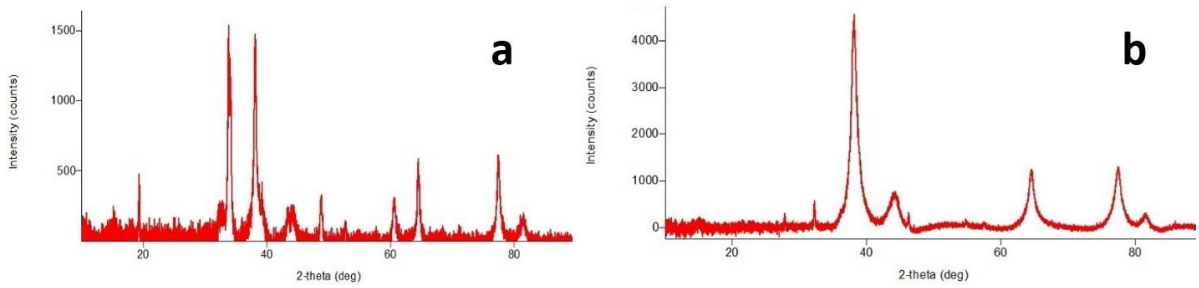

Figure 3. XRD pattern of Kernel AgNPs (a) and Shell AgNPs (b).

SEM, STEM and EDS: In this study, SEM scanning (X 50K magnification) and STEM scanning (X 100K magnification) images obtained from both nanoparticles revealed that they have a spherical structure and show clusters (Figure 4, a-b; Figure 5, a-b). In similar studies, it is stated that AgNPs generally exhibit the same appearance (Ali et al., 2023; Swathi et al., 2025). When the elemental distribution ratios and relationships of the synthesised silver nanoparticles were examined (EDS), it was determined that the silver (Ag) element had the highest density in both graphs (Shell: 65.07% and Kernel: 75.40%) and contained other elements at different ratios (Figure 6, a-b). In this study, the peak of Ag element at the highest intensity and above 3 keV in EDS graphs is a characteristic situation arising from the surface plasmon resonance of silver nanoparticles and the same situation is found in many similar studies (Chand et al., 2020; Ali et al., 2023). As in different AgNP synthesis and application studies, the sizes of nanoparticles were determined and their graphs were drawn on TEM images using Image J software. According to the results obtained, these size values for kernel-derived AgNPs ranged between 10-50 nm, while these size values for shell-derived AgNPs ranged between 5-40 nm (Figure 7, a-b). This dimensional difference is thought to be effective on larvicidal activity.

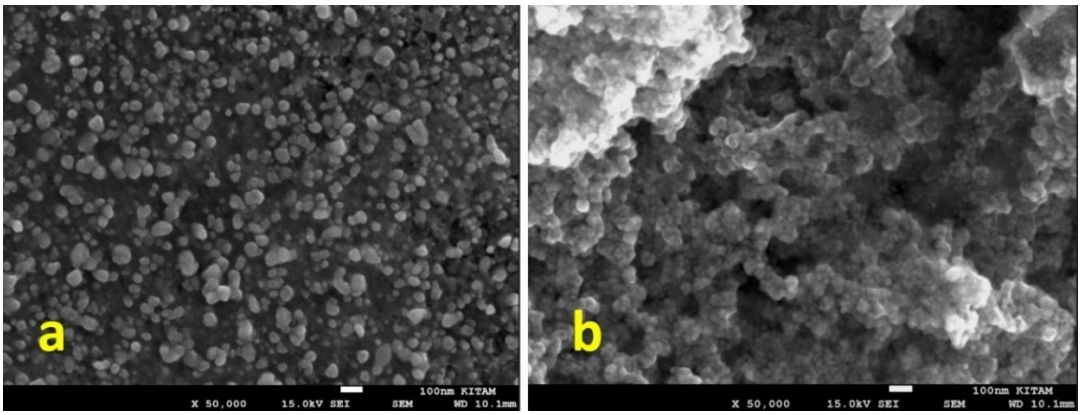

Figure 4. SEM image of Kernel AgNPs (a) and Shell AgNPs (b).

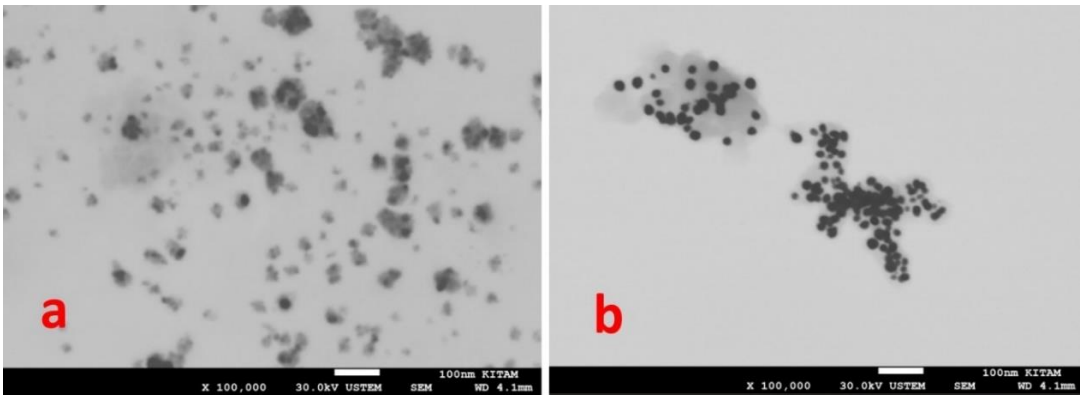

Figure 5. STEM image of Kernel AgNPs (a) and Shell AgNPs (b).

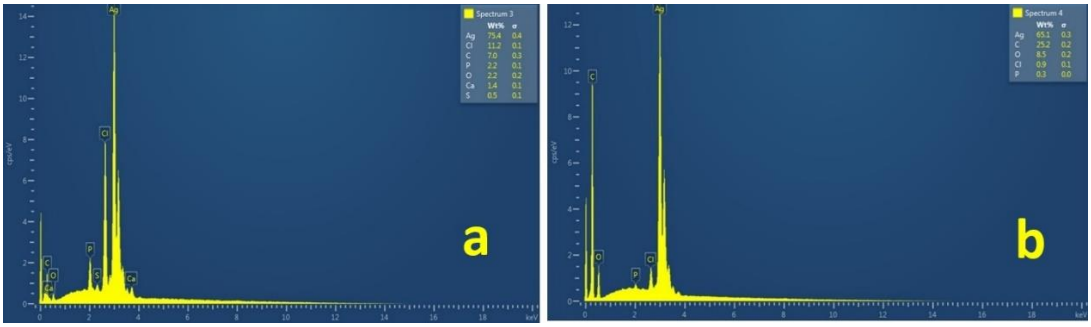

Figure 6. EDS spectrum of Kernel AgNPs (a) and Shell AgNPs (b).

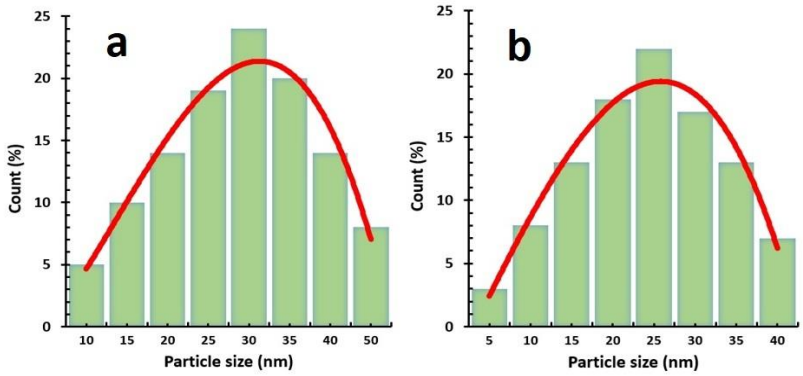

Figure 7. Particle diameter plot of Kernel AgNPs (a) and Shell AgNPs (b).

3.2. Larvicidal Activity Assessment

When the mortality rates resulting from the topical application of silver nanoparticles on the second and fourth larval stages of the pest were evaluated, it was determined that the larvicidal activity was more effective on the second stage larvae than on the fourth stage larvae based on the same conditions. At the end of the fourth day, when all treatments (in terms of dose-duration-temperature) were compared, it was observed that the second stage larvae lost more than the fourth stage larvae (Figure 8-15). In this study on larvicidal activity, it is understood that the strength of larvicidal activity increases with the increase in the amount of application dose and exposure time. The lowest level of larvicidal activity was observed in the lowest dose treatment at the end of the first day. On the other hand, the highest larvicidal activity was observed at the highest dose application and at the end of the fourth day (Figure 8-15). According to another result obtained from the experiments, the strength of larvicidal activity varies according to the ambient temperature of the application and the extract from which the nanoparticle was synthesised. The mortality rates observed at low temperatures are lower than those observed at high temperatures. In addition, the mortality rates obtained from the trials in which the nanoparticle prepared using the bark extract was applied were lower than the trials in which the nanoparticle prepared using the kernel extract was applied (Figure 8-15).

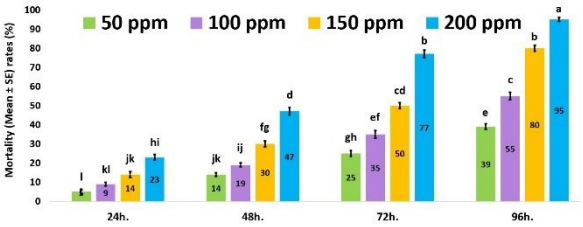

Figure 8. Daily mean (±SE) mortality rates caused by Kernel AgNP against second instar larvae of *Plodia interpunctella* at 28°C (Mortality rates were compared statistically using Duncan's multiple range test, $p < 0.05$. Each different letter indicates statistically significant difference between mortality rates).

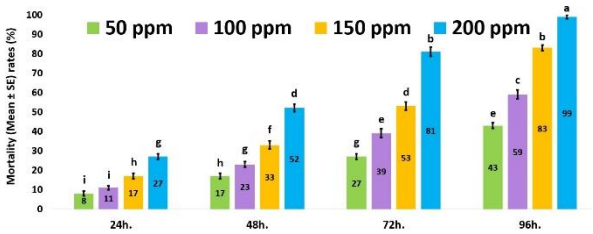

Figure 9. Daily mean (±SE) mortality rates caused by Kernel AgNP against second instar larvae of *Plodia interpunctella* at 32°C (Mortality rates were compared statistically using Duncan's multiple range test, $p < 0.05$. Each different letter indicates statistically significant difference between mortality rates).

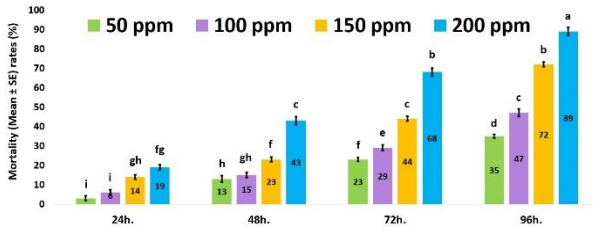

Figure 10. Daily mean (±SE) mortality rates caused by Kernel AgNP against fourth instar larvae of *Plodia interpunctella* at 28°C (Mortality rates were compared statistically using Duncan's multiple range test, $p < 0.05$. Each different letter indicates statistically significant difference between mortality rates).

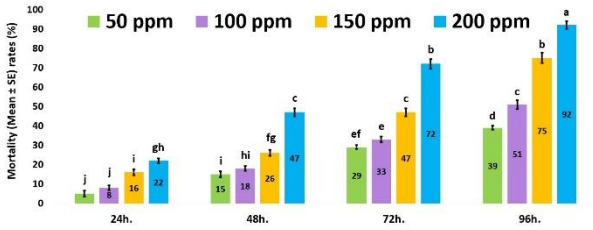

Figure 11. Daily mean (±SE) mortality rates caused by Kernel AgNP against fourth instar larvae of *Plodia interpunctella* at 32°C (Mortality rates were compared statistically using Duncan's multiple range test, $p < 0.05$. Each different letter indicates statistically significant difference between mortality rates).

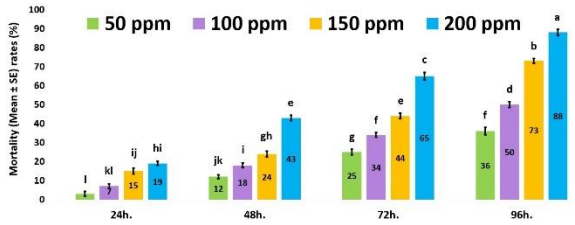

Figure 12. Daily mean (±SE) mortality rates caused by Shell AgNP against second instar larvae of *Plodia interpunctella* at 28°C (Mortality rates were compared statistically using Duncan's multiple range test, $p < 0.05$. Each different letter indicates statistically significant difference between mortality rates).

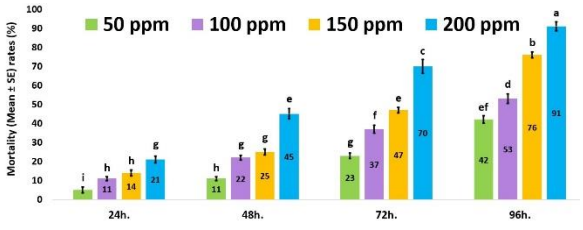

Figure 13. Daily mean (±SE) mortality rates caused by Shell AgNP against second instar larvae of *Plodia interpunctella* at 32°C (Mortality rates were compared statistically using Duncan's multiple range test, $p < 0.05$. Each different letter indicates statistically significant difference between mortality rates).

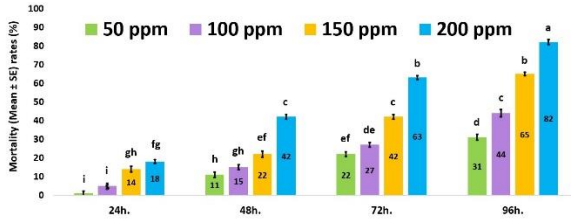

Figure 14. Daily mean (±SE) mortality rates caused by Shell AgNP against fourth instar larvae of *Plodia interpunctella* at 28°C (Mortality rates were compared statistically using Duncan's multiple range test, $p < 0.05$. Each different letter indicates statistically significant difference between mortality rates).

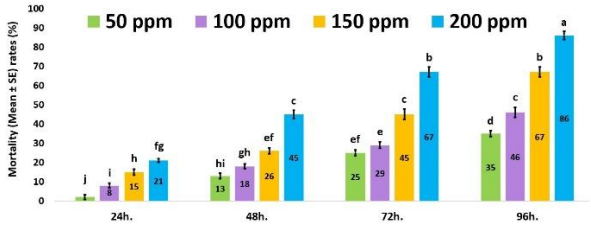

Figure 15. Daily mean (±SE) mortality rates caused by Shell AgNP against fourth instar larvae of *Plodia interpunctella* at 32°C (Mortality rates were compared statistically using Duncan's multiple range test, $p < 0.05$. Each different letter indicates statistically significant difference between mortality rates).

In this study, it was tried to determine the mortality rates of Ag nanoparticles synthesised from two different extracts against both life forms at different doses and different times by applying two different temperatures. When probit analyses were performed on the highest dose application (200 ppm) and the highest exposure duration (96 hours), lethal concentration and lethal duration values were determined (Table 1-2). According to these results, it was once again observed that second instar larvae were more sensitive to both extract derivatives Ag nanoparticles compared to fourth instar larvae. Furthermore, the concentration values required for lethality and the exposure times required for lethality decrease as the applied temperature increases. According to the information obtained from this study, it can be said that all factors such as larval stage status, dose amount applied, exposure time, structure of the extract from which the nanoparticle is synthesised and ambient temperature have a determining effect on larvicidal activity in such studies.

Synthesised AgNPs are highly effective in pest control studies by showing irreversible destructive effects on the physiology of the targeted pest (Suresh et al. 2018). Especially after AgNPs reach inside the cell, AgNP-related anomalies at the cellular level are the beginning of the end and trigger many cellular destruction events one after another (Roni et al., 2015). Vital cellular activities such as decreased levels of detoxifying enzymes, impaired function of many important neurotransmitter enzymes and irregular increases in the production of reactive oxygen species (ROS) are all adverse conditions caused by the entry of AgNPs into the cell and this process ultimately results in the death of the cell (Parthiban et al., 2019; El-Samad et al., 2022). AgNPs exhibit very strong larvicidal activity after adhering to the cuticle of insect larvae due to their nano-sized structures. After reaching the cells, AgNPs passing through the membrane can bind to sulfur and phosphorus groups in the cell, then cause abnormal cell activity and lead to the death of insect larvae (Morejón et al., 2018; Sutthanont et al., 2019; Kumar et al., 2022). Moreover, recent studies have shown that Ag+ exposure acts by disrupting the homeostasis of the ROS scavenging system, over-triggering ROS-mediated stress responses through excessive production of hydroxyl radicals, which has serious effects on cell destruction (Zhu et al., 2017; Li et al., 2020). It is also known that mitochondria manage ROS mainly through the mitochondrial antioxidant system, and it is reported that AgNPs play a role in cell destruction by causing excessive ROS production during this toxicity process, triggering DNA damage/deterioration, which is a critical and fatal situation, and ultimately leading to cell death through apoptosis (Gurunathan et al., 2022; Zhang et al., 2022). Wang et al. (2023) linked the toxicity of *Drosophila* larvae exposed to AgNPs to the increase in ROS and oxidative stress marker MDA (malon-dialdehyde) levels and stated that this led to DNA damage, decreased cell viability and an increase in MDA in larvae.

Table 1. LC50 and LC90 values obtained after topical application of biosynthesised AgNPs on *Plodia interpunctella* (Hubner) larvae.

Extract Type / Application Exposure Time	Life Period of Pest / Applied Temperature	LC50 (95% CL) (ppm)	LC90 (95% CL) (ppm)	Slope±SE	X^2 (df)
Kernel / 96 hour	*L_2 / 28 ℃*	79.918 (65.013 – 91.786)	182.847 (166.180 – 206.905)	0.012±0.001	8.762 (38)
	L_2 / 32 ℃	72.217 (57.655 – 83.630)	165.359 (150.739 – 186.057)	0.014±0.002	12.639 (38)
	L_4 / 28 ℃	94.748 (79.706 – 107.259)	213.367 (192.008 – 245.743)	0.011±0.001	8.209 (38)
	L_4 / 32 ℃	85.113 (68.968 – 97.917)	201.365 (181.421 – 231.399)	0.011±0.001	12.504 (38)
Shell / 96 hour	*L_2 / 28 ℃*	90.961 (74.553 – 104.165)	215.024 (192.667 – 249.529)	0.010±0.001	10.496 (38)
	L_2 / 32 ℃	79.265 (60.554 – 93.375)	204.467 (182.908 – 237.939)	0.010±0.001	12.615 (38)
	L_4 / 28 ℃	108.206 (92.760 – 121.846)	243.032 (215.590 – 287.091)	0.010±0.001	6.339 (38)
	L_4 / 32 ℃	99.292 (83.068 – 112.838)	231.268 (205.736 – 271.849)	0.010±0.001	11.620 (38)

*CL= Confidence limits, L_2= Second instar larvae, L_4= Fourth instar larvae, 28 / 32 ℃: Celsius of the temperature scale applied to the pest.

Table 2. LT50 and LT90 values obtained after topical application of biosynthesised AgNPs on *Plodia interpunctella* (Hubner) larvae.

Extract Type / Application Exposure Dose	Life Period of Pest / Applied Temperature	LT50 (95% CL) (hour)	LT90 (95% CL) (hour)	Slope±SE	X^2 (df)
Kernel / 200 ppm	*L_2 / 28 ℃*	48.470 (43.707 – 52.831)	87.784 (81.213 – 96.669)	0.033±0.003	12.272 (38)
	L_2 / 32 ℃	44.105 (39.448 – 48.281)	80.272 (74.314 – 88.271)	0.035±0.003	12.367 (38)
	L_4 / 28 ℃	54.552 (49.559 – 59.296)	98.977 (91.052 – 110.027)	0.029±0.003	8.623 (38)
	L_4 / 32 ℃	50.617 (45.542 – 55.281)	93.856 (86.431 – 104.123)	0.030±0.003	10.117 (38)
Shell / 200 ppm	*L_2 / 28 ℃*	55.597 (50.480 – 60.496)	101.775 (93.352 – 113.668)	0.028±0.003	15.967 (38)
	L_2 / 32 ℃	52.273 (47.234 – 56.969)	96.076 (88.429 – 106.691)	0.029±0.003	16.067 (38)
	L_4 / 28 ℃	58.516 (53.024 – 63.902)	109.730 (99.779 – 124.287)	0.025±0.003	10.180 (38)
	L_4 / 32 ℃	54.262 (48.733 – 59.445)	103.766 (94.658 – 116.894)	0.026±0.003	8.938 (38)

*CL= Confidence limits, L_2= Second instar larvae, L_4= Fourth instar larvae, 28 / 32 ℃: Celsius of the temperature scale applied to the pest.

In addition to the above-mentioned cellular destructive effects of the synthesised AgNPs on the targeted pest, this study also revealed that the destructive effects of the synthesised AgNPs on the targeted pest may vary depending on the nature of the extract used as raw material for the synthesis of AgNPs, the dose amount applied to the targeted insect and the duration of exposure to the applied dose, the biological period of the targeted insect and the ambient temperature at which the treatments were carried out.

As it is known, one of the safest synthesis routes of silver nanoparticles is the so-called green synthesis reactions in which plant extracts are generally preferred as raw materials (Anees et al., 2022; Ying et al., 2022). It has been stated by many researchers that the diversity and ratios of the secondary compounds of plant extracts play a role in the formation of AgNPs and that the potential of AgNP-induced effects on many biological activities varies

(Chand et al., 2020). In this study, two different organic wastes of the same plant were the source of extracts for green synthesis reactions and the small difference in the secondary compounds of these extracts was clearly seen in the graphs obtained because of FTIR tests (Figure 2, a-b). In addition, when the XRD graphs of the AgNPs formed because of the synthesis process are examined, it is noteworthy that there are some differences in the synthesized AgNPs (Figure 3, a-b). It is thought that the small differences between these two extracts are effective on larvicidal potential. Because according to the data obtained from this study, it was determined that the larvicidal power of AgNPs obtained from the kernel extract was higher than the AgNPs obtained using the shell extract (Figure 8-15).

Two other important factors affecting larvicidal activity are the amount of dose applied to the pest and the duration of exposure to the applied doses, and these two factors were found to be directly related to the mortality rates obtained in this study. Hazaa et al. (2021) tested AgNPs synthesised using *Borago officinalis* leaf extracts at eight different doses (100, 200, 400, 600, 800, 800, 1000, 2000 and 4000 mg/g) against *Spodoptera littoralis* (Lepidoptera: Noctuidae) 3rd instar larvae. At the end of 24 hours, 30% mortality rate was reached at the lowest dose application, mortality rates increased in direct proportion with increasing dose rates and mortality rate was recorded as 100% at the highest dose application. Anees et al. (2022) tested AgNPs synthesised from *Azadirachta indica* extract against *Helicoverpa armigera* (Hubner) (Lepidoptera: Noctuidae) second instar larvae at four different doses (500, 1000, 1500 and 2000 ppm). Post-treatment mortality rates were evaluated at increasing time intervals (24, 48, 72 and 96 hours) and accordingly, the mortality rates recorded at the lowest dose were 10%, 20%, 46.67% and 60%, respectively. The daily mortality rates increased as the applied dose amount increased and at the highest dose rate, 23.33%, 46.67%, 70% and 93.33% mortality rates were recorded, respectively.

In order to fully understand the efficacy of AgNPs against the targeted pest larvae, two different larval stages (second and fourth) were treated separately in this study and as a result, fourth stage larvae were found to be more resistant to AgNPs treatment than second stage larvae. Devi et al. (2014) applied AgNPs obtained from *Euphorbia hirta* (Euphorbiaceae) leaf extract at five different doses (2, 4, 6, 8 and 10 ppm) against I, II, III, IV, V and VI larvae of *Helicoverpa armigera* (Lepidoptera: Noctuidae), respectively. In the lowest dose application, mortality rates were recorded as 49%, 47%, 45%, 42%, 42%, 42%, 40% and 37% in order of larval stage. The mortality rates recorded in the highest dose application were 95%, 93%, 90%, 90%, 90%, 89%, 89%, 84% and 80% in order of larval stage. Bharani and Namasivayam (2017) applied AgNPs obtained from pomegranate (*Punica granatum*) fruits at five different doses (10, 25, 50, 75 and 100 µg) against I, II, III, IV, V and VI larvae of *Spodoptera litura* (Lepidoptera: Noctuidae), respectively. Dose-dependent mortality rates for first instar larvae were 26.7%, 53.4%, 66.4%, 86.2% and 100%, respectively. Dose-dependent mortality rates for third instar larvae were 16.4%, 34.5%, 54.2%, 72% and 86.4%, respectively. Dose-related mortality rates for fifth instar larvae were 4.2%, 10.2%, 27.2%, 51.2% and 61.3%, respectively. Jafir et al., (2021) applied AgNPs obtained from *Ocimum basilicum* plant extract at four different doses (100, 500, 1000 and 1500 mg/L) against 2nd, 3rd, 4th and 5th instar larvae of *Spodoptera litura* (Lepidoptera; Noctuidae). The mortality rates recorded as a result of the highest dose application were 96.67%, 91.67%, 81.67% and 78.33% for 2nd, 3rd, 4th and 5th instar larvae, respectively.

One of the physical factors used in pest control is the temperature factor, which is an important abiotic factor, and studies have shown that it causes different adverse conditions in larvae depending on the ambient temperature (low and/or high) (Abarca et al., 2020; Sujatha et al., 2024). When the results of this study were evaluated in terms of mortality rates, it was determined that the larvicide activity values recorded because of the applications made at high temperatures were more effective than the applications made at low temperatures. It is thought that the most important factor that is effective in the emergence of this situation due to temperature increase is directly related to the cuticle, which is the outer layer of the insect (Ghaedi et al., 2024). The fat layer in the cuticle of insects is the primary layer responsible for preventing the evaporation of body water, therefore a higher percentage of fatty acids in the cuticle layer increases the insect's resistance to heat and with it minimises the loss of water from the body and thus prevents the insect from drying out (Woods et al., 2003; Gołębiowski et al., 2012; Ghaedi et al., 2024). When the percentage of fatty acids in the cuticle decreases for any reason, the natural resistance of the cuticle layer to heat decreases, the amount of water in the insect's body is rapidly lost, resulting in the death of the insect (Ghaedi et al., 2024). Particularly high temperatures (the higher the temperature, the more serious the situation) lead to thinning of the wax layer of the cuticle, then to deep damage to the cuticle, then to the destruction of the cuticle and finally to the death of the beetle (Lee, 2010; Gibbs, 2011). Therefore, this study reveals that the temperature of the environment where larvicide applications are made is an important criterion determining the direction and potential of control.

Nanoparticle synthesis is of great interest due to its wide range of applications such as medicine, pharmacy, agricultural activities. Green synthesis of AgNPs using plants has more advantages over other chemical methods in terms of its easy and effective procedure, non-pathogenic and non-toxic effect on non-target organisms (Benelli et al., 2017; Kumar et al., 2022). In addition to using plants or plant extracts, bacteria and fungi can be used in the biosynthesis of AgNPs and are considered an

excellent green synthesis option. However, using plant extracts in particular has many advantages, including availability of raw materials, non-toxic structure, and a wide range of ingredients. In addition, biosynthesized AgNPs also have antibacterial, antifungal, anticancer, and anti-inflammatory bioactivity properties (Nie et al., 2023). While it is important to consider how nanoparticles can harm the ecosystem, they can also have positive effects that deserve careful consideration. Synthesized Ag nanoparticles can positively impact the environment around them when used in a controlled manner, do not produce any toxic effects, and are safe (Esan et al., 2022; Yamini et al., 2023; Wang et al., 2024). Studies have reported that nanoparticles are used in different application forms in order to minimize environmental pollution and improve environmental health (Martínez et al., 2020). Although the use of silver nanoparticles is quite suitable for improving environmental remediation processes, it is very important that the synthesized silver nanoparticles have certain properties, especially the nano size is quite low (about 10 nm) in order to increase their performance (Silva-Holguín et al., 2024). As a result, nanoproducts consisting of AgNPs have been included in numerous environmental application studies due to their highly effective and safe nano-sized particle size and biocompatibility. Therefore, they have been effective against many contaminants including pathogens and heavy metal ions even at very low concentrations and have been determined to have no serious adverse effects on healthy organisms including soil microorganisms (Panda et al., 2023).

4. Conclusion

The results of this study, which was carried out to transform the kernel and shell of the horse chestnut tree, which finds its place in parks as an ornamental plant, from organic waste to a valuable nano-product and to determine the larvicide potential of this product, contain important information. In these studies, it was proved that the dose amount, exposure time, larval stages and ambient temperature, which determine the efficacy of synthesised AgNPs against *Plodia interpunctella* larvae, are effective on larvicidal activity. The development of more environmentally friendly and more effective products instead of existing synthetic chemical pesticides in the fight against pests and the use of nature's own organic wastes during these processes is very important in terms of showing the point reached by nanotechnology. It is thought that this nanoparticle-supported study carried out against an agricultural lepidopteran pest will set an example for similar studies to be carried out in the future and will constitute source information.

Author Contributions

The percentages of the author' contributions are presented below. The author reviewed and approved the final version of the manuscript.

	O.A.
C	100
D	100
S	100
DCP	100
DAI	100
L	100
W	100
CR	100
SR	100
PM	100
FA	100

C=Concept, D= design, S= supervision, DCP= data collection and/or processing, DAI= data analysis and/or interpretation, L= literature search, W= writing, CR= critical review, SR= submission and revision, PM= project management, FA= funding acquisition.

Conflict of Interest

The author declared that there is no conflict of interest.

Ethical Consideration

Ethics committee approval is not required for this study due to the use of research materials that are not defined as experimental animals (Scientific and Technological Research Council of Türkiye, Animal Experiments Local Ethics Committee Directive, 2018, Article 3-c).

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