Toxicological Effects of Silver and Zinc Oxide Nanoparticles on the Biological and Life Table Parameters of *Helicoverpa armigera* (Noctuidae: Lepidoptera)

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Abstract: American bollworm *Helicoverpa armigera* (Hubner) is a notorious pest of different agronomical and horticultural crops. Different synthetic insecticides are recommended to control *H. armigera* but widespread and repeated use has led to pesticide resistance in this pest. It is, therefore, necessary to develop a novel strategy to manage the population of *H. armigera*. Nanotechnology is the most effective and eco-friendly approach to mitigate this problem. In the present study, the bioefficacy of green synthesized nanoparticles and two different silver and zinc oxide nanoparticles with different concentrations, viz. 100, 125, 150, 175 and 200 ppm were used against the larvae. UV-vis spectrophotometer, SEM and EDX were used for nanoparticle characterization. Data were recorded daily. The result showed that in silver nanoparticles maximum larval mortality was 97%, while in zinc oxide nanoparticles, 82% was recorded against the 3rd, 4th and 5th instar of *H. armigera*. The effect of nanoparticles on demographic parameters was also evaluated, which increases the net reproductive rates, mean generation time and intrinsic rate in the control group compared to the treated population. After bioassay, larval and pupal duration was prolonged in the treated population compared to the control. The longevity of males, females and fecundity was also reduced. This technique will be a valuable tool in integrated pest management regimens.

Keywords: *Helicoverpa armigera*; silver; zinc oxide nanoparticles; life table

1. Introduction

*Helicoverpa armigera* is an important pest of main crops in many parts of the world as Asia, Europe, Oceania, Africa and recently in most countries of the Americas, including Brazil [1]. This pest is polyphagous and causes damage to many crops [2]. It attacks more than 200 plant species, including cotton [3,4], chickpea [5], tomato [6], maize [7] and cabbage [8], which are severely damaged by this pest. Some important species of the genus *Helicoverpa* viz. *Helicoverpa armigera* (Hubner), *H. assulta* (Guenee) and *H. peltigera* (Denis) also have great economic importance [9].

Due to its high fecundity, polyphagous nature and quick adaptation to synthetic insecticides, controlling this pest is difficult [10]. Different insecticides such as organophosphates, synthetic pyrethroids and biorational compounds have been used to control *H. armigera* [11].
However, due to the injudicious and frequent use of synthetic insecticides, the development of resistance against these pesticides has resulted [12,13]. In Pakistan, *H. armigera* has also attained resistance against cypermethrin and monocrotophos, as well as moderate resistance to endosulfan [13]. The second strategy, Bt crops, was also introduced to control this pest [14], but this pest has also gained resistance against Bt crops [15,16]. Implementation of more eco-friendly methods of control, including the use of nanotechnology, is urgently needed for this lepidopteran pest, which is economical and safer for the environment [17]. Nanoparticles (NPs) are specific in their mode of action and have a broad-spectrum activity [18].

Consequently, there is a rising need to develop ecologically acceptable nanoparticle production that does not rely on harmful ingredients. Several techniques have been used for the preparation of nanoparticles which include chemical, physical and biological methods. Among these methods, microorganisms, enzymes, and plant or plant extracts have been suggested as alternatives to chemicals [19]. Nanoparticles exhibit entirely new properties based on characteristics such as size, distribution and morphology compared to bulk material particles, and exhibit size up to 100 nm [20]. Nanoparticles such as silver, zinc oxide and gold and aqueous neem extract are more important and effective. The neem leaves have significant phytochemicals such as flavanones and terpenoids which help in stabilizing the nanoparticle and also act as a reducing and capping agent [21].

In addition, these nanoparticles have also been evaluated against insect control and its biological parameters such as survival rate, fertility and adult longevity [22,23]. The life table study involves the summering birth (age or stage-specific), reproductive and death rate data. This approach is better for predicting pest management programs [24–26]. Biological parameters mostly focused on the female population but without male inclusion may fail to examine the effects of control during the evaluation of demographic parameters such as average generation time, total reproductive rate and intrinsic rate of increase [26]. In population dynamics, both females and males are too important [26–30]. The objective of our study was to assess, for the first time, alterations in demographic parameters of progeny derived from *H. armigera* larvae treated with different concentrations of silver and zinc oxide accompanied by neem leaf nanoparticles.

2. Materials and Methods

2.1. Experimental Conditions and Insect Rearing

Larvae of *H. armigera* were collected from the cotton fields of Central Cotton Research Institute Multan and shifted to the rearing laboratory, Department of Entomology, Bahaud-din Zakariya University Multan, Punjab, Pakistan. To avoid cannibalism, these larvae were reared separately on an artificial diet in Petri dishes at 25 ± 2 °C, 75 ± 5% relative humidity (RH) and 10:14 h light: dark photoperiod [31]. The artificial diet was prepared with different ingredients such as chickpea flour (300 g), yeast (30 g), formaldehyde 10% (7.15 mL), choline chloride 20% (15 mL), vitamin mixture (5 g), agar (23 g) and distilled water (500 mL) [32]. After the emergence, adults were provided with 10% honey solution on a cotton swab. Three to four pieces of thin napkins were hung inside the cage as an oviposition substrate. The F1 generation was separated to obtain a uniform population.

2.2. Plant Sources and Preparation of Leaf Extract

Neem leaves were collected from the forest area of Bahaudddin Zakariya University Multan. After collection, leaves were completely washed with distilled water to remove contaminations from the leaves. After washing, leaves were sundried for 3–4 h. Twenty grams of these dried leaves were taken and cut into small pieces by scissors. These small, chopped leaves were put in a 100 mL beaker and 100 mL distilled water was added to the beaker. The beaker was covered with aluminum foil paper and then put into the water bath for boiling. The temperature of the water bath was set at 70 °C for 30 min. The beaker was removed from the water bath after 30 min and the extract was cooled at room temperature then filtered with the use of Whatman filter paper [33]. This extract was stored
at 4 °C temperature—this solution was used as a reducing agent or green synthesis Ag and ZnO NPs.

2.3. Synthesis of Silver and Zinc Oxide Nanoparticles

Neem extract was used for the synthesis of silver nitrate nanoparticles. To prepare a 1 mM solution, 169 mg silver nitrate and 100 mL distilled water were added to a 500 mL beaker. After stirring, 10 mL of the neem extract were added dropwise to the 90 mL of 1 mM AgNO$_3$ solution in the Erlenmeyer flask and heated for 30 min. The solution was changed to dark brown after 30 min, which confirms the reduction of Ag ions and indicates the formation of Ag NPs (silver nanoparticles); then, stirring was stopped and the solution was stored for further use [34]. This solution was added to the falcon tube, then these tubes were set in a centrifuge machine and the machine was run at 6000 rpm for 15 min. The solution was washed up to 5 times until dark brown pellets were obtained. These pellets were put into a Petri dish and dried at 70 °C for 24 h in the oven. Pellets were collected with the help of a spatula, crushed with mortar and pestle and stored in a small Eppendorf tube for further use. For the synthesis of ZnO NPs the same procedure was followed as silver NPs. In the case of zinc oxide, the prepared NPs were white in color instead of dark. Prepared NPs were preserved in air-tight vials for further use [35].

2.4. Characterization of Silver and Zinc Nanoparticles

2.4.1. Ultraviolet Spectroscopy

The ultraviolet-vis spectroscopy analysis confirmed Ag NPs and ZnO NPs synthesis based on their optical properties. Ultraviolet [36], visible spectroscopic analysis was done with the help of a spectrometer (Shimadzu 1800 Japan, Kyoto, Japan). The UV-visible spectrophotometer occurred ranging between 200 and 800 nm wavelengths. Spectra were obtained at 412 nm and 296 nm for AgNO$_3$ NPs and ZnO NPs, respectively.

2.4.2. SEM and EDX Analysis

SEM images were obtained by forming a smear of the aqueous solution of Ag NPs and ZnO NPs on aluminum foil paper. These smears were observed in non-contact mode using Nano-R2™ (Pacific Nanotechnology, Inc., Santa Clara, CA, USA) at a 0.5 Hz scanning rate and a voltage of 20 kV. The image indicated that nanoparticles were well distributed with the lowest agglomeration of nanoparticles. In EDX analysis the spectrum indicated the formation of Ag and ZnO NPs, respectively.

2.4.3. Larvicidal Activity

The larvicidal effect of biologically Ag and ZnO NPs was studied against all instars of $H. armigera$ using the immersion method. Different concentrations of each tested nanoparticle were incorporated into the larval diet. Almost 2 g diet was used for all instars. Five concentrations of 100 ppm, 125 ppm, 150 ppm, 175 ppm and 200 ppm of each NPs with four replications, including control of each treatment, were used in the bioassay. Ten larvae were exposed separately in each Petri dish of each replication and control. Control had simple diet or without any addition. Mortality data were recorded after 24 h of exposure to NPs. Larvae that showed no movement were considered as dead.

2.4.4. Life Table Parameters

To construct the life table of $H. armigera$, 80 newly ($\leq 24$ h) laid eggs were randomly collected from non-treated (control) and treated populations (100, 125, 150 and 175 ppm) and individually placed in Petri dishes supplied with an artificial larval diet at 25 ± 2 °C, 75 ± 5% relative humidity (RH) and 10:14 h light:dark photoperiod. The single egg was considered as replication in each treatment. Each population was reared separately and development periods were noted until adult formation. After adult emergence, insects were sexed (within 24 h) and one male and one female were placed into 20 × 25 cm per
plastic jar. Eggs were counted daily till the death of females. Adult longevity, oviposition period and duration of eggs hatching were also recorded [37].

For each strain, the life table parameters were determined [38,39]. Briefly, the net reproductive rate ($R_0$), which means each female gives an average number of eggs in their whole life span, is calculated as the following equation [40]:

$$R_0 = \sum_{x=0}^{\infty} l_x m_x$$

The following procedure estimated the survival rate ($l_x$):

$$l_x = \sum_{j=1}^{k} s_{xj}$$

Age-specific fecundity $m_x$, determined as the following equation:

$$m_x = \frac{\sum_{j=1}^{k} s_{xj} f_{xj}}{\sum_{j=1}^{k} s_{xj}}$$

Intrinsic rate of increase ($r$), which was calculated by using iterative bisection methods and determined via the Euler–Lotka formula with zero-age indexing [41].

$$r = \sum_{x=0}^{\infty} e^{-r(x+1)} l_x m_x = 1$$

The finite rate of increase ($\lambda$) was determined as follows:

$$\lambda = e^r$$

Mean generation time ($T$) was estimated by the following method:

$$T = (\ln R_0) | r$$

The life expectancy $e_{xj}$ of age stage was estimated by the following procedure [38].

$$e_{xj} = \sum_{i=x}^{\infty} \sum_{y=j}^{\beta} S'_{iy}$$

The reproductive value $V_{xj}$ of age-stage was calculated by the following method [39].

$$V_{xj} = \frac{e^{r(x+1)}}{s_{xj}} \sum_{i=x}^{\infty} e^{-r(i+1)} \sum_{y=j}^{k} S'_{iy} f_{iy}$$

2.5. Data Analysis

The toxicological effect of different concentrations of synthesized NPs was determined by SPSS software [42]. The mortality was calculated using the formula

$$\text{Mortality} (\%) = \frac{\text{No. of dead specimens}}{\text{No. of exposed specimens}} \times 100$$

Developmental effects caused by tested NPs were analyzed through ANOVA and means were compared using Least Significant Difference at $p = 0.05$. All the statistical analyses were performed using SPSS Software. Age-stage, two-sex life table software analyzed adult longevity and fecundity [24]. The paired bootstrap procedure [43] (n = 100,000) was used to estimate the standard error and mean of life table principle at $p \leq 0.05$. TWO-SEX
MS Chart program was used for the analysis of the age stage, a two-sex life table [29]. The life table parameters $V_x$, $V_{xj}$, $s_{xj}$, $m_x$, $s_{xj}$, $l_x$, $m_x$ and $e_x$ were graphed with GraphPad Prism, version 8.

3. Results

3.1. Effect of Ag NPs and Zinc Oxide NPs on Mortality of H. armigera

In all treatments of Ag NPs for third instar larvae, mortality percentages were varied: 43%, 53%, 70%, 77% and 97% mortality were observed at 100, 125, 150, 175 and 200 ppm, respectively. Mortality for the fourth larval instar on various concentrations of silver NPs was recorded as 47%, 57%, 63%, 73%, and 93% at 100, 125, 150, 175 and 200 ppm, respectively. Mortality percentage varied within the treatment for the fourth larval instar. Similarly, in the fifth larval instar, 30%, 47%, 60%, 67% and 87% mortality was recorded at 100, 125, 150, 175 and 200 ppm, respectively. Mortality percentage varied within the treatment for the fifth larval instar. Results showed that with the increase in NPs concentrations mortality percentage was also increased in all tested instar larvae of H. armigera (Figure 1).

In the case of zinc oxide NPs, maximum mortality was observed in third instar larvae at 200 ppm. Zinc oxide NPs caused different percent mortalities at different concentrations in third instar larvae, e.g., 33%, 47%, 60%, 73% and 83% mortality at 100, 125, 150, 175 and 200 ppm, respectively. Similarly, on the fourth larval instar 43%, 57%, 63%, 73% and 87% mortality was recorded at 100, 125, 150, 175 and 200 ppm, respectively. Likewise, in the fifth larval instar, 13%, 37%, 47%, 60% and 70% mortality was recorded at 100, 125, 150, 175 and 200 ppm, respectively. Maximum mortality was observed at the highest concentration of 200 ppm in the third and fifth instar larvae. Ag NPs were more toxic for H. armigera as compared to zinc oxide nanoparticles (Figure 2).
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3.2. Toxicological Evaluation of Tested NPs on Larvae of H. armigera

The lower LC\textsubscript{50} value showed the high toxicity of a tested compound. LC\textsubscript{50} values of third, fourth, and fifth instar larvae against silver NPs were observed, i.e., 114.67, 112.07 and 130.89 ppm, respectively, while LC\textsubscript{90} values of 202.71, 228.08 and 240.90 ppm were observed, respectively. In the same way, LC\textsubscript{50} values of third, fourth and fifth instar larvae against zinc oxide NPs were observed at 127.79, 114.069 and 155.38 ppm, respectively, while LC\textsubscript{90} values were observed at 242.97, 271.48 and 276.87 ppm, respectively (Table 1).

Table 1. Effect of various concentrations of synthesized silver and zinc oxide NPs coated with neem extract on the mortality of different larval instars of H. armigera.

| Nanoparticles | Larval Instars | N  | LC\textsubscript{50} | Lower Limit | Upper Limit | LC\textsubscript{90} | Lower Limit | Upper Limit | X\textsuperscript{2} | DF | Slope ± S. E | p  |
|---------------|---------------|----|---------------------|-------------|-------------|---------------------|-------------|-------------|---------------|----|---------------|----|
| Ag NPs        | 3rd instar    | 30 | 114.674            | 95.257      | 127.342     | 202.719            | 176.213     | 273.744     | 3.20          | 3  | 4.90 ± 0.04   | 0.362|
|               | 4th instar    | 30 | 112.073            | 84.693      | 127.509     | 228.086            | 188.701     | 361.791     | 0.608         | 3  | 5.41 ± 0.04   | 0.379|
|               | 5th instar    | 30 | 130.869            | 113.598     | 144.780     | 240.904            | 201.817     | 435.934     | 1.205         | 3  | 4.82 ± 0.04   | 0.752|
| ZnO NPs       | 3rd instar    | 30 | 127.792            | 108.708     | 142.106     | 242.977            | 201.746     | 379.737     | 0.247         | 3  | 4.49 ± 0.04   | 0.970|
|               | 4th instar    | 30 | 114.069            | 77.814      | 132.370     | 271.481            | 208.579     | 690.488     | 0.080         | 3  | 3.36 ± 0.06   | 0.994|
|               | 5th instar    | 30 | 155.387            | 140.875     | 174.502     | 276.874            | 226.954     | 435.934     | 0.608         | 3  | 5.41 ± 0.04   | 0.895|

Total number of larvae in each treatment = 40.

3.3. Life Table of H. armigera against Tested NPs

The egg duration of the control population was significantly longer than 100, 125, 150 and 175 ppm ($p < 0.05$). The larval duration of the control population was significantly shorter than that of the 100, 125, 150 and 175 ppm ($p < 0.05$). The pupal duration of all the tested populations was prolonged compared to the control ($p < 0.05$). The total egg to adult duration for male and female moths from the control population was significantly longer than for moths from all other treatments ($p < 0.05$). The male and female total longevity in 100, 125, 150 and 175 ppm was significantly shorter than those of the control populations. The fecundity per female was significantly lower in the 100, 125, 150 and 175 ppm populations ($p < 0.05$) but higher in control populations in the case of both tested nanoparticles (Tables 2 and 3).
Table 2. Effects of Ag NPs on biology of H. armigera.

| Nanoparticles | Parameters       | Control | 100 ppm | 125 ppm | 150 ppm | 175 ppm |
|---------------|-----------------|---------|---------|---------|---------|---------|
| Ag NPs        | Eggs duration   | 1.4 ± 0.09 a | 1.33 ± 0.09 a | 1.3 ± 0.09 a | 1.3 ± 0.09 a | 1.3 ± 0.09 a |
|               | 1st larval duration (d) | 1.9 ± 0.17 b | 2 ± 0 a | 2 ± 0 a | 2 ± 0 a | 2 ± 0 a |
|               | 2nd larval duration (d) | 2.3 ± 0.09 a | 2.38 ± 0.1 a | 2.38 ± 0.1 a | 2.32 ± 0.1 a | 2.37 ± 0.11 a |
|               | 3rd larval duration (d) | 3 ± 0 a | 3 ± 0 a | 3 ± 0 a | 3 ± 0 a | 3 ± 0 a |
|               | 4th larval duration (d) | 3.44 ± 0.1 a | 3.53 ± 0.12 ab | 3.47 ± 0.12 ab | 3.46 ± 0.14 bc | 3.8 ± 0.2 c |
|               | 5th larval duration (d) | 4.44 ± 0.1 a | 4.53 ± 0.51 ab | 4.47 ± 0.12 ab | 4.46 ± 0.14 bc | 4.8 ± 0.2 c |
|               | Total larval duration (d) | 15.33 ± 0.29 a | 15.58 ± 0.35 a | 15.42 ± 0.35 a | 15.38 ± 0.43 a | 16.4 ± 0.6 a |
|               | Pupal duration (d) | 5.94 ± 0.24 a | 6.12 ± 0.12 a | 6.46 ± 0.32 a | 6.54 ± 0.21 a | 6.68 ± 0.14 a |
|               | Pre-Adult duration (d) | 32.89 ± 0.39 a | 30.3 ± 0.33 a | 28.21 ± 0.46 a | 27.62 ± 0.49 a | 27.8 ± 0.66 a |
|               | Female longevity (d) | 31.33 ± 0.27 a | 27 ± 0.71 b | 26.9 ± 0.5 b | 26.29 ± 0.42 b | 26 ± 1.48 b |
|               | Male longevity (d) | 34.83 ± 0.27 a | 30.3 ± 0.33 b | 29.67 ± 0.44 b | 29.17 ± 0.31 b | 28.25 ± 0.63 b |
|               | Fecundity        | 785.27 ± 22.43 a | 291.56 ± 44.5 b | 269 ± 23.8 b | 170.43 ± 19.03 bc | 161 ± 0.43 c |

d = stands for days. In columns same letters show statistically no difference among themselves (p < 0.05).

Table 3. Effects of ZnO NPs on biology of H. armigera.

| Nanoparticles | Parameters       | Control | 100 ppm | 125 ppm | 150 ppm | 175 ppm |
|---------------|-----------------|---------|---------|---------|---------|---------|
| ZnO NPs       | Eggs duration   | 1.01 ± 0.02 b | 1.4 ± 0.09 a | 1.33 ± 0.09 a | 1.33 ± 0.09 a | 1.2 ± 0.07 ab |
|               | 1st larval duration (d) | 2 ± 0 a | 2 ± 0 a | 2 ± 0 a | 2 ± 0 a | 2 ± 0 a |
|               | 2nd larval duration (d) | 2.4 ± 0.09 a | 2.44 ± 0.1 a | 2.38 ± 0.1 a | 2.4 ± 0.1 a | 2.43 ± 0.14 a |
|               | 3rd larval duration (d) | 3 ± 0 a | 3 ± 0 a | 3 ± 0 a | 3 ± 0 a | 3 ± 0 a |
|               | 4th larval duration (d) | 3.44 ± 0.1 a | 3.5 ± 0.1 a | 3.43 ± 0.11 a | 3.45 ± 0.11 b | 3.55 ± 0.16 a |
|               | 5th larval duration (d) | 4.4 ± 0.1 a | 4.5 ± 0.1 a | 4.43 ± 0.11 a | 4.45 ± 0.11 b | 4.55 ± 0.16 a |
|               | Total larval duration (d) | 15.33 ± 0.29 a | 15.5 ± 0.31 a | 15.3 ± 0.32 a | 15.36 ± 0.33 a | 15.64 ± 0.47 a |
|               | Pupal duration (d) | 5.24 ± 0.12 b | 6.24 ± 0.24 a | 6.34 ± 0.14 a | 6.46 ± 0.12 a | 6.64 ± 0.08 a |
|               | Pre-Adult duration (d) | 32.89 ± 0.39 a | 29.79 ± 0.31 a | 28.61 ± 0.24 a | 28.18 ± 0.35 a | 27.91 ± 0.67 a |
|               | Female longevity (d) | 31.33 ± 0.27 a | 29.17 ± 0.32 b | 27.85 ± 0.22 c | 27 ± 0.35 c | 26 ± 0.37 c |
|               | Male longevity (d) | 34.83 ± 0.27 a | 30.42 ± 0.47 b | 29.6 ± 0.22 b | 29.6 ± 0.22 b | 29.33 ± 0.8 b |
|               | Fecundity        | 869.73 ± 18.41 a | 415 ± 19.21 b | 279.54 ± 9.74 c | 156.75 ± 15.24 d | 75.8 ± 3.72 e |

d = stands for days. In columns same letters showed statistically no difference among themselves (p < 0.05).

3.4. Effects of Ag and ZnO NPs on the Biological Parameter of Helicoverpa armigera

The intrinsic rate of increase (r) was inversely related to concentration, which varied from 0.21 to 0.17, 0.18, 0.14 and 6.60 in control, 100 ppm, 125 ppm, 150 ppm and 175 ppm, respectively. The mean finite rate of increase (λ) had a significant difference (per day) between control 1.23 and all treated concentrations of 100 ppm (1.18), 125 ppm (1.19), 150 ppm (1.15) and 175 ppm (1.06). The net reproduction rate (R₀) (offspring/individual) was high in control (392.6) and gradually decreased significantly at 100 ppm (87.46), 125 ppm (89.66), 150 ppm (39.76) and 175 ppm (5.36). Significant differences were also observed between mean generation times (T), 27.94 days for control and 25.94, 25.60, 25.28 and 25.43 days in 100 ppm, 125 ppm, 150 ppm and 175 ppm, respectively (p < 0.05). In the case of ZnONPs, the intrinsic rate of increase (r) was inversely related to concentration, which varied from 0.21, 0.18, 0.18, 0.15 and 0.10 in control, 100 ppm, 125 ppm, 150 ppm and 175 ppm, respectively. Mean finite rate of increase (λ) has a significant difference (per day) between control 1.24 and all treated concentrations of 100 ppm (1.20), 125 ppm (1.19), 150 ppm (1.17) and 175 ppm (1.10). The net reproduction rate (R₀) (offspring/individual) was high in control (434.86) and gradually decreased significantly at 100 ppm (166), 125 ppm (121.13), 150 ppm (62.7) and 175 ppm (12.6). Significant differences were also observed between
mean generation times ($T$) of (27.87) days for control and (27.00), (26.40), (25.88) and (25.24) days in 100 ppm, 125 ppm, 150 ppm and 175 ppm, respectively ($p < 0.05$) (Table 4).

### Table 4. Effects of Ag and ZnO NPs on demographic parameters of *H. armigera*.

| Nanoparticles | Parameters | Control | 100 ppm | 125 ppm | 150 ppm | 175 ppm |
|---------------|------------|---------|---------|---------|---------|---------|
| **Ag NPs**    | Intrinsic rate of increase ($r$) | 0.213730 | 0.1723 | 0.185601 | 0.14564 | 6.60483 |
|               | Net reproduction rate ($R_0$)    | 392.6333 | 87.4666 | 89.66666 | 39.7666 | 5.3666 |
|               | Mean length of a generation ($T$)| 27.94586 | 25.945590 | 25.60402 | 25.2876 | 25.4390 |
|               | Finite rate of increase ($\lambda$) | 1.23828 | 1.1880723 | 1.1919 | 1.15678 | 1.06827 |
|               | Birth rate (at SASD *)            | 0.24134 | 0.214441 | 0.2191 | 0.2031 | 0.15200 |
|               | Survival rate (at SASD *)         | 0.99694 | 0.973630 | 0.9727 | 0.95362 | 0.91627 |
|               | Death rate (at SASD *)            | 3.057097 | 2.63694 | 2.7215 | 4.6379 | 8.3728 |
| **ZnO NPs**   | Intrinsic rate of increase ($r$) | 0.21790 | 0.1892 | 0.181659 | 0.1598 | 0.1004 |
|               | Net reproduction rate ($R_0$)     | 434.8666 | 166 | 121.1333 | 62.7 | 12.6333 |
|               | Mean length of a generation ($T$) | 27.8788 | 27.0079 | 26.40596 | 25.88731 | 25.2416 |
|               | Finite rate of increase ($\lambda$) | 1.2434 | 1.2083 | 1.199205 | 1.1733 | 1.1057 |
|               | Birth rate (at SASD *)            | 0.24644 | 0.22366 | 0.21798 | 0.19609 | 0.1746 |
|               | Survival rate (at SASD *)         | 0.99702 | 0.98471 | 0.98122 | 0.9772 | 0.931025 |
|               | Death rate (at SASD *)            | 2.9758 | 1.52870 | 1.87748 | 2.2745 | 6.8974 |

SASD = Stage age stage distribution. * it shows good.

3.5. Age-Specific Survival Rate ($s_{xj}$), Life Expectancy ($e_{xj}$) and Age Stage Reproductive Value ($V_{xj}$) after Applying Tested NPs

The age stage survival rate ($s_{xj}$) indicates that in the first filial generation inclusive life span of *H. armigera* in the control group was longer but reduced after treatment of different concentrations (100, 125, 150, 175) ppm of nanoparticles (Figure 3A,B, respectively). A similar trend was also observed in age-stage life expectancy ($e_{xj}$), where treated group individuals had a lower life expectancy and overall life span (Figure 4A,B). Age stage reproductive rate ($V_{xj}$) showed the maximum reproductive value of a stage in life span; adult females from the control group showed the highest peak of reproductive value as compared to treated concentrations of nanoparticles (Figure 5A,B) in case of applying Ag NPs and ZnO NPs. Age stage survival rate ($l_{xj}$), fecundity ($m_{xj}$), life expectancy ($e_{xj}$) and reproductive value ($V_{xj}$) maximum in control as compared to the treated population is shown in Figure 6A,B.
Figure 3. Cont.
Figure 3. (A) Age-stage survival rate ($S_{xj}$) of 100, 125, 150, 175 ppm and control of NPs treated *H. armigera*. (B) Age-stage survival rate ($S_{xj}$) of 100, 125, 150, 175 ppm and control of NPs treated *H. armigera*. 
Figure 4. Cont.
Figure 4. (A) Age-stage life expectancy ($e_{xj}$) of 100, 125, 150, 175 ppm and control of NPs treated *H. armigera*. (B) Age-stage life expectancy ($e_{xj}$) of 100, 125, 150, 175 ppm and control of NPs treated *H. armigera*. 
Figure 5. Cont.
Figure 5. (A) Age-stage reproductive rate ($V_{xj}$) of 100, 125, 150, 175 ppm and control of NPs treated *H. armigera*. (B) Age-stage reproductive rate ($V_{xj}$) of 100, 125, 150, 175 ppm and control of NPs treated *H. armigera*.
Figure 6. (A) Age-specific survival rate ($l_x$), age-specific fecundity ($m_x$), age-specific reproductive value ($V_x$) and age-specific life expectancy ($e_x$). (B) Age-specific survival rate ($l_x$), age-specific fecundity ($m_x$), age-specific reproductive value ($V_x$) and age-specific life expectancy ($e_x$).
3.6. Analysis of Ag NPs
3.6.1. Visual and Ultraviolet Spectroscopy Observation

The synthesized Ag NPs were analyzed by using UV-vis spectroscopy. The color deviates to dark brown, signposting Ag-nanoparticles’ formation when a 1 M solution of AgNO$_3$ and aqueous leave extract of $A. indica$ was mixed with continuous stirring. The plasma resonance of Ag NPs showed a peak of 3.44 at 412.0 nm during UV–vis spectroscopy. This value indicated the absorption spectrum of Ag NPs (Figure 7).

![Figure 7. Ultraviolet–visible spectral analysis of synthesized silver-nanoparticles (Ag NPs) using $A. indica$ leaf extract.](image)

3.6.2. Scanning Electron Microscopy (SEM) and Energy-Dispersive X-ray (EDX) Analysis

Phase-contrast microscopy displayed the shape of Ag NPs. For further confirmation, SEM was utilized to measure the size of nanoparticles. Most Ag NPs’ sizes range between 10 nm and 70 nm and form a mass aggregate of 100 nm (Figure 8).

![Figure 8. Energy-dispersive X-ray (EDX) spectrum of green-synthesized silver nanoparticles using the leaf extract of $A. indica$.](image)
3.7. Analysis of ZnO NPs

3.7.1. Visual and Ultraviolet Spectroscopy Observation

The synthesized ZnO NPs were analyzed by using UV-vis spectroscopy. The color deviated from the lighting brown sign of the formation of ZnO NPs when a 1 M solution of zinc nitrate and aqueous leaf extract of *A. indica* was mixed with continuous stirring. The plasma resonance of ZnO NPs showed a peak of 2.68 at 296.0 nm during UV–vis spectroscopy. This value indicated the absorption spectrum of ZnO NPs (Figure 9).

![Figure 9. Ultraviolet-visible spectral analysis of synthesized ZnO NPs using *A. indica* leaf extract.](image)

3.7.2. Scanning Electron Microscopy (SEM) and Energy-Dispersive X-ray (EDX) Analysis

The surface morphology of synthesized ZnO NPs was explored by using SEM. Typical SEM micrographs display many agglomerated particles with irregular spherical morphology with an average size of 80–100 nm (Figure 10).

![Figure 10. Energy-dispersive X-ray (EDX) spectrum of green-synthesized ZnO NPs using the leaf extract of *A. indica*.](image)
4. Discussion

This study evaluated the insecticidal effect of ZnO and AgNO₃ on the development of American bollworms from larvae to adults. Many synthetic pesticides and a few biopesticides have been used to manage the *H. armigera* for a long time, as it has been destroying crops. However, the use of these artificial chemicals is a threat to the environment and humanity and the development of resistance to insecticides [44,45]. Managing this pest poses an important challenge, and therefore it is necessary to devise a more delicate but effective means of controlling *H. armigera* which will be safer for the environment and at the same time does not develop resistance to the American bollworm [46]. The current study is designed to appraise the most appropriate alternatives that can efficiently control this pest and are also ecofriendly. ZnO and Ag NPs have been ascertained to be a promising cradle of nontoxic pesticides that are effective in controlling *H. armigera*. Additionally, it has been shown that they can manage many other insect pests without poisoning the environment and low doses of about 200 ppm have the maximum insect mortality effect [44].

The process of extraction and use of nanoparticles (NPs) synthesized from plants are auspicious fields of research and pest management. Plant extracts are used efficiently as an important biological precursor for the biosynthesis of nanoparticles such as silver, zinc, gold, and iron [47]. These synthesized NPs are easy to apply to plants due to their size and shape [48]. Synthesized NPs have been applied in different aspects such as pest control due to being safer in use for humans and the environment. In recent research, the formation of zinc and silver NPs using neem extract was characterized by different techniques. These NPs of zinc and silver were applied to *H. armigera* to evaluate the effects of NPs on the growth and development of experimental species.

Synthesized NPs help to reduce adverse effects of insecticides on the environment and the formation of ecologically safer formulations for insect pest control. In the present study, pest mortality was increased with the increase in NPs concentrations. Similar findings were proposed by Devi et al. [3], who observed a direct relation of concentration of NPs with mortality of *H. armigera*. The LC₉₀ values for silver and zinc oxide NPs were 114.6, 112.73, 130.869 ppm, and 108.7, 77.81, and 140.875 ppm, respectively, for third, fourth and fifth instar larvae and LC₉₀ values of silver and zinc oxide NPs were 202.72, 228.08 and 240.90 ppm, and 379.73, 690.48, 435.93, respectively, for third, fourth, and fifth instar larvae. Similar to our findings, Priyadarshini et al. [49] observed LC₅₀ values of 169.11 and 197.40 ppm and LC₀ₕ values of 331.42 and 371.34, respectively, against *Anopheles stephensi* Liston after applying different concentrations of NPs.

In the current study, silver and zinc oxide NPs prolonged the larval and pupal duration. Our results of larval and pupal duration were similar to the findings of Manimegalai et al. [50], which showed the pupal duration of *Spodoptera littura* (Fabricius) and *H. armigera* was prolonged after applying NPs. Male and female longevity was shorter than control after applying these NPs. Similarly, Ammar and Abd-ElAzeem [51] showed reduced longevity for males and females of *Earias insulana* (Frauenfeld) after applying gelatin copper bio NPs. Morphological and genetic characteristics of *H. armigera* were similar to the species studied by Queiroz-Santos et al. [52].

The current results showed the variations in the life table parameters (R₀, λ, T and r) among the *H. armigera* populations. The R₀, λ, T and r of the control populations were higher than other tested populations. The parameters R₀, r and λ indicated the growth potential for estimating pest population—a wider understanding than that provided by individual life history parameters [53], because the life table parameters such as λ and r depend upon the growth and fecundity of the individuals. Hence, the significant differences in such parameters affect the growth rates of the populations [54]. The lower increase rates of populations in this work could be attributable to decreased fecundity of females. In present outcomes, the highly significant variations in demographic parameters of many insect pests have previously been described in *M. femurrubrum* [54], *P. crisonalis* [55] and *P. xylostella* [56]. The life table parameters such as Vₓj, sₓj, eₓj, eₛ, mₓs, lₓ and Vₛ are important indicators for calculating the biological fitness of the insect pest populations.
The aqueous solution of AgNO$_3$ turned into yellowish-brown color after 1 h of A. indica leaf extract addition. In ultraviolet spectral analysis, synthesis of silver nanoparticles was monitored. UV-vis spectrophotometer showed the silver NPs’ color excitation and characteristic surface plasmon resonance band along with a wavelength of 412 nm. This is similar to the finding of Jafir et al. [57], who observed the silver NP’s wavelength ranging from 200 to 700 nm. The morphological analysis, such as the size and shape of Ag NPs, was analyzed using phase contrast microscopy and SEM. Images of synthesized Ag NPs showed uniformity in size and shape. The nanoparticles were found to be aggregated as a round mass of 100 nm. Moreover, when magnification was increased in SEM, small-sized nanoparticles ranging from 10 nm to 70 nm were observed clearly. A similar shape was observed in Ag NPs synthesized from the leaf extract of Phyllanthus niruri [58]. Energy-dispersive X-ray spectroscopy provides information on the composition of Ag NPs. Our result is similar to the findings of Fayaz et al. [59], who observed a peak in silver NPs synthesized from the fungus Trichoderma viridae. The plasma resonance of ZnO NPs showed a peak of 2.68 at 296.0 nm during UV–vis spectroscopy. Likewise, the study of Shukla et al. [60] observed peaks ranging between 240 and 380 nm of synthesized ZnO NPs. In our current study the size and shape of the ZnO NPs were explored by using SEM and EDX analysis ranging between 80 and 100 nm. This is similar to the findings of Umar et al. [61], who also explored the size ranging between 80 and 112 nm and the shape of ZnO NPs.

5. Conclusions

These research findings highlighted the control tactics and provided basic yet important time-specific and age-specific information to understand better H. armigera population dynamics under the influence of NPs. In the current research, the findings can be drawn that NPs have long-lasting effects on the biology of H. armigera and promise an ecologically safe control technique. For the most part, adult longevity and female fecundity was significantly affected after using NPs. Because of the influence of NPs on demographic parameters, they can be integrated with effective pest control strategies. After using Ag NPs and ZnO NPs, 96% and 82% mortality were recorded, respectively. It can also be concluded that the resources obtained from plants can be efficiently used to produce Ag NPs and ZnO NPs and could be utilized in various fields such as biomedical and nanotechnology.

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