

## Article

# Insecticidal Effect of Zinc Oxide Nanoparticles against *Spodoptera frugiperda* under laboratory conditions in Chiang Mai, Thailand

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**Simple Summary:** Fall armyworm has devastated several crops around the world especially maize that is widely grown and utilized globally. Also, it has been known to cause a lot of damage in rice fields. However, controlling this pest has been a challenge to farmers due to its ability to reproduce faster, development of resistance to synthetic chemicals among other factors. Moreover, synthetic chemicals are a threat to the environment and humanity. For these reasons, we are constantly looking for safer yet effective means of controlling this pest and nanotechnology comes in handy. Zinc Oxide nanoparticles have proved to be efficacious to several insect pests of which some are in the same genus as *S. frugiperda*. This study aimed to find out the insecticidal effects of ZnO nanoparticles on *S. frugiperda* under laboratory conditions. We observed deformations, reduced fecundity, reduced oviposition, and mortality on the insects were fed on food treated with several concentrations of ZnO nanoparticles yet the ones fed on control were normal in all the aspects. Therefore, we recommend ZnO nanoparticles for further studies with the aim of using them as an alternative control to fall armyworm.

**Abstract:** Fall armyworm *Spodoptera frugiperda* (J.E. Smith, 1797) is a major pest of corn, rice, and sorghum among other crops usually controlled using synthetic or biological insecticides. Currently, the new invention of nanotechnology is taking root in the agricultural industry as an alternative source of pest management that is target-specific, safe, and efficient. This study sought to determine the efficacy of commercial Zinc Oxide (ZnO) nanoparticles (NPs) towards *S. frugiperda* under laboratory conditions. ZnO NPs were diluted into different concentrations (100- 500ppm), where the baby corn used to feed the *S. frugiperda* larvae was dipped. The development of the insect feeding on food dipped in ZnO solution was significantly ( $p<0.05$ ) affected, and the number of days that the insect took to complete its life cycle had a significant difference compared to the control. There was a significant difference in the adults' emergence at all the concentrations of ZnO NPs compared to the control, with over 90% of the eggs successfully going through the cycle until adult emergence. Additionally, several malformations were observed throughout the lifecycle of the insect. Also, the fecundity of the females was greatly affected. The findings of this study suggest the possibility of exploitation of ZnO nanoparticles not only to eradicate *S. frugiperda* but to significantly reduce their population in the ecosystem through deformations, reduced fecundity, reduced oviposition, and hatchability of eggs. It will be a valuable tool in integrated pest management regimens.

**Keywords:** Malformations; nanotechnology; *Spodoptera frugiperda*; Zinc Oxide nanoparticles

## 1. Introduction

*Spodoptera frugiperda* Smith, 1797 otherwise, known as Fall armyworm (FAW) is a Lepidoptera in the family Noctuidae that was first mentioned by James Smith and Abbott in 1797 when it devastated sorghum crops in the Americas. Their statement read, "It is worthy the consideration of the husbandman whether, by studying the natural history of this formidable depredator, he could not get the better of it." In 1845 and 1854, this insect had gained fame by causing damage to corn, grass, sugarcane, and rice in western Florida and Georgia respectively. The spread became so robust that it invaded Loss Angeles, Missouri, Illinois, and Kansas. Sadly, one farmer alone underwent a loss of his crops that amounted to 19,000 dollars currently [1].

Fall armyworm is an insect pest of more than 100 plant species with a preference for graminaceous species mainly causing damage to economically important cultivated cereals such as maize, rice, sorghum, Bermuda grass, crabgrass, and also to vegetable crops and cotton [2]. The moth can fly up to 100 km per night [3]. In much of North and South America, FAW outbreaks have been sporadic but sometimes can be severe. This pest has caused millions of dollars of losses in Africa in the short period that it has invaded Africa and this is an indication of an impending tragedy if not controlled [4]. FAW is a threatening and major concern to researchers and agriculturalists all over the world. In 2018 it had been detected in 70% of maize crop of southern Karnataka state of India (2018) subsequently appearing in Bangladesh (2019), China (2019), Myanmar (2019), Sri Lanka (2019), and Thailand (2018) [5] and [6]. In Africa, it has been reported to have invaded over 90% of the countries by 2019 [7]. Rapid action is necessary as the pest can spread to other Asian countries due to suitable climatic conditions and the prominent cultivation of maize in this region. Its presence in China was confirmed when larvae found in corn in southwest Yunnan province (southwest China) were identified in January 2019 as FAW [5] and [8]. The fall armyworm, corn earworm, and true armyworm are identical within the Noctuidae family, except that FAW has four dark spots in the 8<sup>th</sup> abdominal segment and is marked with a white inverted 'Y' on its head. The adult male moths have grayish-brown mottled forewing light and dark splotches and the female adult having a noticeable spot near the end of each forewing, the hind wing is iridescent silver-white with a narrow dark border [9].

In the recent past, the world has been shifting rapidly to nanotechnology to provide solutions in the cosmetic, agricultural, pharmaceutical, food, and paint industries among others [10]. Nanoparticles are characterized by atomic or molecular size less than 100 nm [11]. Nanoparticles have gained popularity due to their unusual fascinating properties compared to their competitors in terms of applications [12]. Nanoparticles have proved to be efficacious against plant pathogens, weeds, and insect pests. Also, they have been added to the formulations of insecticides and insect repellents. Fortunately, the nanoparticles do not pose health hazards to the environment and public health compared to the traditional chemical pesticides [13];[14] and [15]. However, the detailed mechanisms of nanoparticles have not been understood to a convincing conclusion and therefore, needs in-depth examination or experimentations of their interaction with the biological systems before designing consumable products [11].

Zinc oxide nanoparticles (ZnO NPs) are among the significant metal oxides popular due to their chemical and physical peculiarity [16] and [17]. Notably, ZnO has proved to possess great potential in the biosynthesis of nanoparticles for clinical purposes compared to other oxides. Moreover, several studies have confirmed that ZnO nanoparticles can be synthesized using various plant extracts such as *Hibiscus rosasinensi* and *Cassia auriculata*

[18]. That notwithstanding, ZnO nanoparticles synthesized using plant extracts as reducing agents have proved to have sturdy antimicrobial efficacy against *Aspergillus* spp., *Pseudomonas aeruginosa*, *Staphylococcus aureus*, and *Klebsiella pneumonia* [19] and [20]. This study, therefore, sought to determine the efficacy of commercial ZnO nanoparticles towards *Spodoptera frugiperda* under laboratory conditions. Additionally, the study elucidated the developmental interference after the consumption of food dipped into ZnO nanoparticles solution of different concentrations.

## 2. Materials and Methods

### 2.1. Area of this study

The experiments were conducted at the Laboratory of Insect pathology, Department of Entomology and Plant Pathology, Faculty of Agriculture, Chiang Mai University, Muang District, Chiang Mai province, Thailand. The region is plain with an altitude of 311 m above sea level at a longitude of 98.97°E and a latitude of 18.77°N. Due to the invasive nature of the fall armyworm, all observations were recorded under quarantine facilities.

### 2.2. Fall armyworm rearing

Larvae, pupae, and adults of fall armyworm (FAW) use in each study are the laboratory strain maintained at the Insect Pathology Laboratory, Department of Entomology and Plant Pathology, Faculty of Agriculture, Chiang Mai University, Thailand. This colony originated from a wild strain kept larvae from the cornfield at Faculty of Agriculture, Chiang Mai University during the corn or maize growing and has been maintained in plastic containers (19 cm in width by 27 cm in length by 8 cm in height) on baby corn under laboratory room conditions at 26±1°C with a photoperiod of 12:12 h (dark: light) and 70±10% humidity.

### 2.3. Source of Zinc Oxide Nanoparticles

Extra pure zinc oxide (25-50 nm) nanoparticles were purchased from Green Nanotechnology (Chiangmai, Thailand). Complete characterization of the used particles is available at the manufacturer's website (<https://www.dataforthai.com/company>). Aqueous suspensions of nanoparticles are stable but usually, they suffer aggregation in water, hence the suspensions were vortexed for 10-20 min before use in the treatments.

### 2.4. Biological traits of fall armyworm reared on different concentration ZnO NPs

Due to the invasive status of this insect pest in Thailand, the egg batches used in this study were derived from the first generation of insect pathology laboratory (IPL) stocks from field-caught FAW females from the initial eggs provided by the cornfield at Mae-Hia Agricultural Training and Research Center, Faculty of Agriculture, Chiang Mai University as previously mentioned. Furthermore, different biological traits including the development, survival, reproduction, and ecological parameters of FAW fed on baby corn dip in ZnO NPs different concentrations (100, 200, 300, 400, 500 ppm.) Egg batches received from IPL were placed under controlled conditions and newly hatched larvae were individually placed and kept in plastic containers measuring (sauce cup; 6 cm × 3 cm; 2 oz.) and provided with baby corn weighing approximately 1-3 g dipped in ZnO NPs different concentrations daily and until they reached the pre-pupal stage. Each day, the development and survival of larvae were observed for each treatment. Larval stages were L1, L2, L3, L4, L5, and L6. Pupal weight, width, length, and deformed were recorded from the surviving insects, and sex determination was conducted at the pupal stage. Observations of insect biological parameters and development including duration of instars, sex, deformed, and longevity of individuals were recorded daily.

### 2.5. Oviposition

To determine which cumulative ZnO NPs difference concentrations in adults are preferred for oviposition. Oviposition substrate was used to build the resulting eggs corresponding to the treatment food they were offered as immatures. After adult emergence, a total of 30 FAW pairs were used for this experiment, with one virgin pair (one male and one female) released at the center of a plastic container covered with plastic cups (drinking cups; 11.5 cm in width × 14.5 cm in length; 300 oz.), for a total of five replications. Female adults were allowed to oviposit for 7 days and fed with a 10% honey solution provided in small plastic boxes. The source of food used to nourish adults were replaced at 2-3 days intervals. Folded pieces of paper (4 cm in width by 10 cm length) will be hung in the rearing cage for egg-laying. Egg masses, egg numbers, and percent egg hatching were recorded daily until the death of each individual.

### 2.6. Statistical analysis

Standard procedures were followed to record the data. The data collected was analyzed statistically using a one-way analysis of variance (ANOVA) with Duncan's posthoc test to assess the oviposition preference and pupal weight, length, and width of FAW among different concentrations of ZnO NPs. The treatment means were compared by the Least Significant Difference test (LSD) for their significance at the 0.05% probability level. The FAW egg masses and number of eggs recorded in the different concentrations of ZnO NPs were compared by paired T-test. Differences were considered significant at  $p < 0.05$ . The statistical analyses were conducted using the IBM SPSS Statistical Software package version 23.0 (Armonk, NY: IBM Corp; 2015).

## 3. Results

### 3.1. The development of FAW fed on baby corn dipped in different concentrations of ZnO nanoparticles

When the insect was fed on food dipped in ZnO nanoparticles, the mortality rate observed was not significant, however, there was a significant difference in the development of adults. The results obtained demonstrate the insecticidal activity of ZnO nanoparticles given the direct contact with the insect under a controlled environment in the assuming that no factors that interfere with the outcome. The insect feeding on food dipped in ZnO NPs solution was affected and the number of days that the insect took to complete its lifecycle had a significant difference compared to the control. A significant difference in the emergence of adults compared to the control that had over 90% of the eggs successfully going through the cycle till adult emergence (**Table 1, 2, and 3**). When the pupal weights were taken, we observed significant differences ( $p < 0.05$ ). The pupa fed on ZnO NPs treated baby corn weighed much less than the pupa from the control.

**Table 1.** Development time and adult longevity (Mean±SE) of FAW fed on dip food (baby-corn) in ZnO NPs difference concentrations (100-500ppm).

Parameters	100		200		300		400		500		Control		df	F
	n	days	n	days	n	days	n	days	n	days	n	days		
Egg	80	2.50±0.000	80	2.50±0.000	80	2.50±0.00	80	2.50±0.000	80	2.50±0.000	80	2.50±0.000	-	-
L1	80	2.30±0.105	80	2.50±0.115	79	2.60±0.112	80	2.65±0.109	80	2.85±0.082	80	2.45±0.114	5	3.100
		a		ab		ab		bc		c		ab		
L2	80	2.15±0.167	80	2.25±0.160	79	2.30±0.147	80	2.35±0.109	80	2.55±0.114	80	2.00±0.126	5	1.796
		ab		ab		ab		ab		b		a		
L3	80	1.60±0.112	80	1.60±0.112	76	1.95±0.114	80	2.35±0.131	80	2.40±0.134	80	1.85±0.109	5	8.671
		a		a		a		b		b		a		
L4	73	1.50±0.115	77	1.55±0.135	73	1.55±0.114	76	1.75±0.123	80	1.85±0.820	80	2.05±0.135	5	3.292
		a		a		a		ab		ab		b		
L5	61	2.25±0.099	72	2.35±0.109	73	2.40±0.169	75	2.60±0.112	80	2.75±0.099	80	2.05±0.088	5	4.620
		ab		ab		ab		bc		c		a		
L6	39	2.00±0.126	34	1.90±0.176	34	2.50±0.154	37	2.80±0.172	40	3.30±0.164	76	2.20±0.156	5	11.241
		a		a		bc		c		d		ab		
Prepupa	39	1.50±0.115	34	1.55±0.114	34	1.60±0.134	37	1.65±0.131	40	2.10±0.143	76	1.45±0.114	5	3.505
		a		a		a		a		b		a		
Pupa	39	9.15±0.167	32	8.45±0.246	34	8.95±170	36	9.70±0.219	40	7.30±0.317	76	9.55±0.114	5	16.701
Adults	30	-	20	-	24	-	23	-	26	-	72	-	-	-
Female	22	8.7±0.524	11	8.45±0.515	16	7.85±0.554	14	5.2±0.374	17	5.05±0.394	39	13.15±0.284	5	42.962
		b		b		b		a		a		c		
Male	8	7.05±0.526	9	4.95±0.276	8	4.65±2.84	9	4.65±2.84	9	4.80±0.445	33	9.55±0.312	5	29.502
		b		a		a		a		a		b		

**Table 2.** Pupal weight, width, and Length (Mean±SE) of FAW fed on dip food (baby-corn) in ZnO NPs different concentrations (100-500ppm) and control

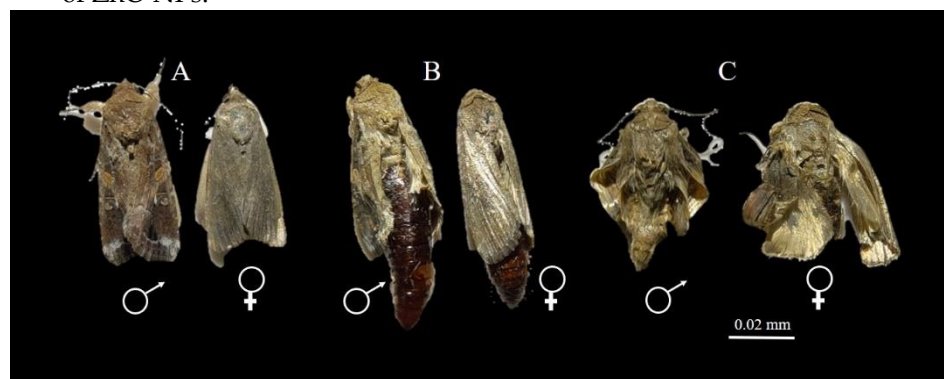
Treatments (ZnO NPs: ppm)	Parameters		
	Weight(g)	Width(cm)	Length(cm)
100	0.160±0.007 c	0.426±0.008 ab	1.397±0.031 a
200	0.130±0.005 b	0.432±0.006 c	1.416±0.025 a
300	0.125±0.005 ab	0.422±0.010 ab	1.376±0.024 a
400	0.120±0.006 ab	0.415±0.007 ab	1.383±0.022 a
500	0.113±0.006 a	0.407±0.007 a	1.407±0.029 a
Control	0.186±0.002 d	0.438±0.007 c	1.504±0.015 b
df	5	5	5
F	27.288	2.112	3.457

**Table 3.** Mean ( $\pm$ SE) percentages of pupa and adult normal/abnormal/dead of FAW fed on food (baby-corn) dipped in ZnO NPs different concentrations (100-500ppm)

Parameters	Treatments						d	F
	100	200	300	400	500	Control		
Pupa							f	
Normal	48.75±5.907 a	40.00±4.564 a	42.50±3.227 a	45.00±3.536 a	50.00±2.041 a	95.00±2.887 b	5	28.077
Abnormal	3.75±1.250 ab	5.00±2.041 ab	7.50±1.443 b	7.50±1.443 b	6.25±1.250 ab	2.50±1.443 a	5	1.846
Dead	7.50±1.443 abc	10.00±2.041 bc	5.00±0.000 ab	8.75±1.250 bc	11.25±2.394 c	2.50±1.443 a	5	4.080
Adults								
Normal	16.25±2.394 ab	15.00±2.041 a	17.50±1.443 ab	12.50±1.443 a	21.25±1.250 b	87.50±1.443 c	5	287.506
Abnormal	21.25±5.154 c	10.00±2.041 ab	12.50±2.500 abc	16.25±3.750 bc	11.25±3.146 abc	2.50±1.443 a	5	3.774

### 3.2. ZnO NPs induced morphological changes in the insect

Exposure to ZnO NPs caused morphological abnormalities in all stages of the life cycle from larvae to adults. **Figures 1 to 4** clearly show the malformations instigated after the ingestion of baby corn dipped in ZnO NPs. Each figure clearly shows the typical morphology and the malformations that are caused due to the ingestion of food dipped in ZnO NPs. Compared to the control, there were significant differences ( $p < 0.05$ ) in oviposition, larvae development, pupa development, and adult emergence. The pupal weight, length, and width were taken, and it was observed that there was a significant difference in the weight of all the other treatments compared to the control. Also, the pupal length had a significant difference when the control was compared to the different concentrations of ZnO NPs.



**Figure 1.** A Control male and female after emergence; B and C Malformed male and female after emergence.





**Figure 2.** A Control 5<sup>th</sup> instar larvae; B and C Malformed 5<sup>th</sup> instar larvae



**Figure 3.** A Control 6<sup>th</sup> instar larvae; B and C Malformed larvae 6<sup>th</sup> instar larvae.



**Figure 4.** A Control pupa; B and C Malformed pupa

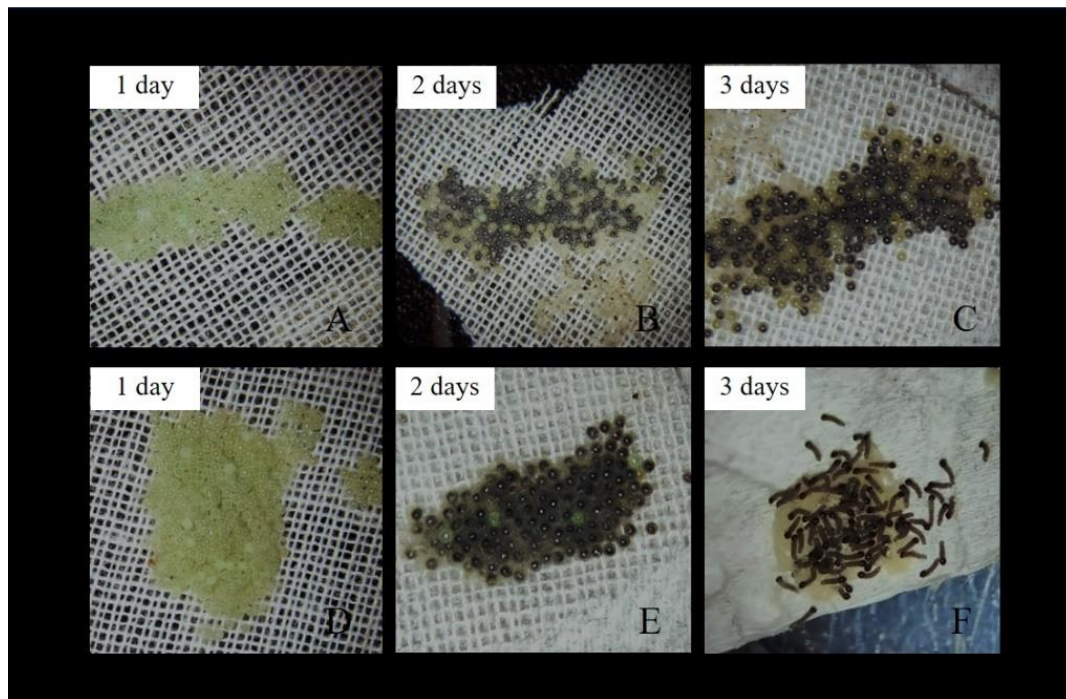
### 3.3. Mortality of *Spodoptera frugiperda* fed on baby corn dipped in ZnO NPs.

Our unpublished data showed mortality of about 40% of larvae after 10 days of feeding on baby corn dipped in ZnO NPs solution.

### 3.4. Effect ZnO NPs on Fecundity and Fertility of *Spodoptera frugiperda*

The effect of ZnO NPs on female fecundity and fertility is presented in **Tables 4 and 5 and Figure 5**. There were significant differences ( $p < 0.05$ ) in the number of females that were able to oviposit and the ones that did not oviposit due to ZnO NPs treatment. For example, in **Table 4**, out of 22 females fed on food dipped in ZnO NPs, only 7 (31.8%) proved to be reproductive, while over 74% of the females fed on control were oviposited. That notwithstanding, the number of eggs oviposited by the latter was much more than those oviposited by the former. Moreover, the hatchability of the eggs laid by the females who ingested ZnO NPs ranged between 1.92 to 7.64%, while the eggs oviposited by the

control hatched 96.4% **Table 5**. Additionally in **Figure 5**, it was observed that the eggs that were oviposited by females fed on baby corn dipped in ZnO NPs solution were not only a few by only a paltry hatched.



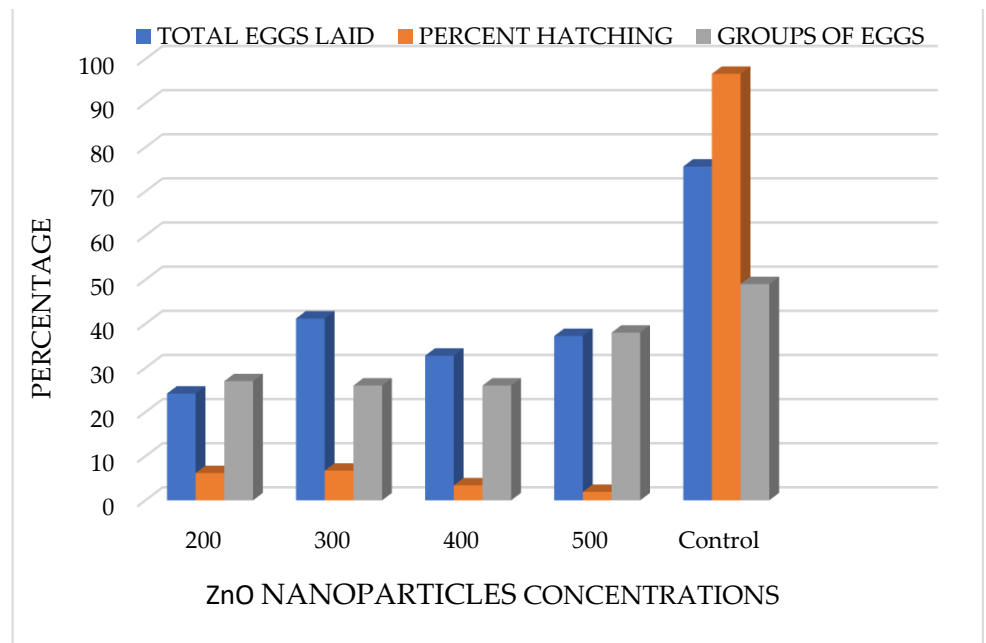
**Figure 5.** A, B, C=Eggs from adults fed on food dipped in ZnO NPs; D, E, F=Eggs from the adult fed on food dipped in control

**Table 4.** Adult preoviposition period (APOP), total preoviposition period (TPOP), oviposition period, fecundity, and percent hatching parameters.

Treatments ZnO NPs: ppm	Total fe- males	Reproductive females	APOP (days)	TPOP (days)	Oviposition pe- riod (days)
100	22	7	2.40±0.152 a	33.55±0.738 a	3.55±0.303 a
200	11	8	2.85±0.167 ab	33.45±0.709 a	3.50±0.366 a
300	16	9	2.85±0.167 ab	34.55±0.766 a	2.65±0.365 a
400	14	9	2.95±0.185 b	34.00±0.533 a	2.85±0.365 a
500	17	8	3.05±0.185 b	33.20±0.663 a	2.55±0.444 a
Control	39	29	2.60±0.152 ab	39.35±0.519 b	5.30±0.145 b
df	-	-	5	5	5
F	-	-	2.035	12.452	8.826

Moreover, the hatchability of the eggs laid by the females who ingested ZnO NPs ranged between 1.92 to 7.64% while the eggs oviposited by the control hatched 96.4% (**Figure 6**)





**Figure 6.** Graphical expression of eggs laid, percentage hatching, and groups of eggs by five pairs of *S. frugiperda* couples

### 3.5. Effects of ZnO NPs on the Longevity of *Spodoptera frugiperda*

When treated with different concentrations of ZnO NPs, there was a significant difference ( $p < 0.05$ ) in larval, pupal, male, and female adult longevity. The females feed on control lasted 13 days while the males lasted over nine and half days which was way longer than the rest who ingested ZnO NPs (Table 1)

## 4. Discussion

*Spodoptera frugiperda* has majorly been managed using synthetic pesticides and a few biopesticides for the longest time that it has been ravaging crops. However, the use of these synthetic pesticides has damaged the environment and caused resistance to insecticides [21]. It poses a significant challenge in controlling this insect and hence the need to come up with a more subtle but effective means of controlling *S. frugiperda* that will not be dangerous to the environment and at the same time does not cause resistance in the insect [22]. In this regard, ZnO NPs have proved to be a promising source of safe insecticides yet effective in controlling *S. frugiperda*. Also, it has been observed that it can manage several other insect pests at environmentally friendly and very low dosages [23]. Moreover, ZnO NPs were combined with Thiamethoxam and enhanced its efficacy in the management of *Spodoptera litura* [24]. The development of *S. frugiperda* was significantly jeopardized due to their exposure to ZnO NPs as displayed in **Table 1**. These observations corroborate other findings before with different insects such as rice weevil *Sitophilus oryzae* and *Tribolium confusum* where mortality was recorded due to exposure to ZnO NPs [25] and [26]. Also, when leaves infested by *Aphis nerii* were dipped into various concentrations of ZnO NPs, there was a significant effect on their development [23].

It has been established that when there are malformations in the insect population, the population is greatly reduced beneath economic levels and that is exactly what we observed in this experiment. There were several deformations observed throughout the lifecycle of the insect as depicted in **Figures 1 to 4**. Causing deformity in the insect pest

population in the integrated pest management program is one of the important aspects in controlling pest population below the economic damage level. Apart from ZnO NPs, silica nanoparticles have been observed to reduce pests when sprayed in infested plants [15].

Longevity has been considered as one of the vital characteristics of an insect's life cycle due to the role it plays in maintaining population size. Observably, our results show that when the insects were fed on food dipped in different concentrations of ZnO NPs, the longevity of adults had a significant difference when compared to insects fed on food dipped in control **Table 1**. Previous studies recorded that when the insect pests are exposed to insecticides, their longevity is distorted hence the population plummets [24].

Oviposition was greatly reduced due to ingestion of ZnO NPs as compared to the specimen fed on control-treated baby corn. That notwithstanding, the number of eggs laid by the females who were fed on baby corn dipped in ZnO were fewer than those fed on food dipped in control. Moreover, the hatchability of eggs was significantly reduced in all the treatment groups compared to the control [27]. Considerably, as a substitute for killing adult insects, it would be beneficial to regulate or manage the insect pest population by plummeting the egg-laying capacity of females and the hatching of the same.

## 5. Conclusions

ZnO NPs demonstrate a promising potential to be used as an alternative to the more lethal insecticides. Although the environmental effects of using ZnO nanoparticles as an insecticide should be studied further, one obvious benefit of using them as insecticides is the low risk of developing resistance by the insects in long-term uses. Additionally, Zn falls within the micronutrients in the diet of human beings and animals and, therefore, when ingested by humans and animals, they tend to benefit rather than harm them.

The findings of this study suggest the possibility of exploitation of ZnO nanoparticles not only to eradicate *S. frugiperda* but to significantly reduce their population in the ecosystem through deformations, reduced fecundity, reduced oviposition, and hatchability of eggs. It is a valuable tool in integrated pest management regimens.

**Supplementary Materials:** The following are available online at [www.mdpi.com/xxx/s1](http://www.mdpi.com/xxx/s1), Figure S1: title, Table S1: title, Video S1: title.

**Author Contributions:** Conceptualization, S.P.; P.K.; and A.R.; methodology, S.P.; P.K. and M.T.; software, S.P.; validation, S. P.; P.V.; A.R. and S.M.; formal analysis, S.P.; J.R.; and S.M.; investigation, S.P.; P.K.; and M.T.; resources, P.K.; M.T.; and S.M.; data curation, S.P.; J.R.; P.K. and P.V.; writing—original draft preparation, S. P.; J.R.; writing—review and editing, J.R.; S.P.; P.K.; and P.V.; visualization, S.P.; A.R. and P.K.; supervision, M.T.; P.K.; S.M.; and P.V.; project administration, M.T.; P.K.; and S.M.; funding acquisition, P.K.; and M.T. All authors have read and agreed to the published version of the manuscript.”.

**Funding:** This research received no external funding.

**Institutional Review Board Statement:** Not applicable

**Data Availability Statement:** Not applicable

**Acknowledgments:** We would like to acknowledge the Teaching Assistant and Research Assistant (TA/RA) and Presidential scholarships from the Graduate School, Chiang Mai University. This research work was partially supported by Chiang Mai University.

**Conflicts of Interest:** The authors declare no conflict of interest. The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, or in the decision to publish the results.

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