INT. J. BIOL. BIOTECH., 21 (4): 483-491, 2024.

INSECTICIDAL ACTIVITY OF GREEN SYNTHESIZED ZINC OXIDE NANOPARTICLES FROM *CALOTROPIS PROCERA* AGAINST FALL ARMYWORM

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ABSTRACT

Spodoptera frugiperda J.E. Smith, commonly referred as fall armyworm, is a pest that infests crops such as maize, rice and sorghum. Nanotechnology is gradually being embraced in the agricultural sector because it is specific and safe. This research sought to determine the efficiency of green synthesized ZnO nanoparticles in the suppression of *S. frugiperda* under lab environment. The ZnO nanoparticles were synthesized from *Calotropis procera* (Aiton) Aiton leaves and their characterization was done by studying UV and FTIR. Five concentrations of ZnO NPs, ranging from 100 to 500 ppm, were prepared. The larvae of *S. frugiperda* were released on maize leaves dipped in different ZnO NPs concentrations. Mortality was the highest (85%) in the first instar larvae followed by 80%, 76%, 71%, 66% and 55% for the second, third, fourth, fifth and sixth instar larvae at the highest concentration of nanoparticles i.e. 500 ppm. Mortality correspondingly reduced as the concentrations of nanoparticles were decreased to their lowest levels. This study also revealed that the feeding preferences of *S. frugiperda,* the fall armyworm, are significantly affected when feeding on plants with different concentrations of ZnO nanoparticles. The study concludes that ZnO nanoparticles can regulate the population of *S. frugiperda* by causing larval mortality and structural deformations making them a promising tool in integrated pest management program. The first instar larvae had the highest mortality of 85% followed by 80%, 76% 71%, 66% and 55% for second, third, fourth, fifth and sixth instar larvae at 500ppm concentration. Frequency of mortality was reduced when the concentrations were reduced. The study concludes that ZnO nanoparticles can regulate the population of *S. frugiperda* by causing larval mortality and structural deformations making it a promising tool in integrated pest management program.

Key-words: Fall Armyworm, Characterization, Synthesis, Toxicity, Nanoparticles, Zinc Oxide

INTRODUCTION

Fall armyworm (FAW), or *Spodoptera frugiperda* J.E. Smith (Lepidoptera), belonging to the family Noctuidae, is a serious pest of maize crop, worldwide. This pest was first identified in the America, and from there spread to Asia-Pacific and Africa. It has become a significant global issue since 2018 (Deshmukh *et al.*, 2021). This pest is a polyphagous one affecting approximately 350 species of plants from 76 diverse families. Targets of fall armyworm are wheat, maize, barley, cotton, sorghum, soybean and tomato. It causes huge crop damages which in turn affects the economy in different parts of the world (Overton *et al.*, 2021). Its favourite plant is corn, and it can cause 15–73% yield losses. It targets the maize plant from seedling stage right until the onset of ear growth. There are two strains of FAW, the rice and the maize strains where the maize strain is more popular and affects different parts of the crop causing disadvantage to the farmer (Sun *et al.*, 2021).

Up to 1500 eggs can be laid in one place or in batches by a single female of the fall armyworms (Suby *et al.*, 2020). The eggs are usually laid on the underside of the leaves. The life cycle depends on temperature directly. In summer, it takes 30 days to complete the life cycle while in winter it prolongs up to 90 to 120 days. It goes through six instars, and the last three instars are the most dangerous ones. The most common symptoms of this disease include the formation of tiny holes at the surface of the leaves. Last three larval instars feed more enthusiastically on tissue and cause more damage as well. The sixth instar larvae migrate to the soil where they pupate for ten to twelve days and come out as adult. From the point of hatching, the FAW moths are capable of flying up to 500 km on the migratory winds (Ahmad and Ahmad, 2018). The later stage of larvae form windowpane on the leaves, while the younger forms feed on the chlorophyll of the leaves making the leaf surface to be white. Their presence is indicated by the fuzzy, sawdust-like frass on the surface of the leaf or near the base of the whorls of the leaf (Gebissa *et al.*, 2020).

Generally, fungicides are used to control the population of fall armyworm (Ahmad et al., 2023; Ali *et al.*, 2023). Organophosphates, Carbamates, and pyrethroids are commonly used insecticides for control of FAW populations, and the two most critical factors affecting its control are the time of application and the stage of development. However, the applications of the chemicals are generally restricted because they are costly, persist in the environment, and have issues of pest resistance to the chemical control (Day *et al.*, 2017). Recently, scientists are focusing on alternative sustainable pest control strategies than using poisonous pesticides which are in use since many years (Hamza *et al.,* 2023; Maqsood *et al.,* 2023). One of these substitutes we use today is the use of green synthesized nanoparticles prepared by using natural materials (De *et al.,* 2014).

Application of nanotechnology is a relatively recent concept implemented in modern agriculture. Nanoparticles are defined herein as particles with sizes ranging between 10 and 100 nm with monodisperse, analogous, and morphologically similar particles. The applications of these nanoparticles in biology and agriculture are protection of crops, diagnosis of plant diseases and growth of plants (Jafer and Annon, 2018; Um-e-Aiman *et al.*, 2021; Waheed *et al.*, 2024). The synthesis of metal based nanoparticles using the reducing agents available in plant materials is called 'Green Nanoparticle Synthesis'. These ecofriendly, cost effective and structurally attractive nanoparticles are good pest repellent (Solorzano *et al.,* 2024). Plants contain tannins, flavonoids and phenolic chemicals, which make them a good source of nanoparticles (Rai *et al.*, 2008). Zinc oxide nanoparticles possess an extensive array of physical and chemical characteristics, making them one of the most significant metallic nanoparticles. Numerous plant extracts can be used to create zinc oxide nanoparticles (Pittarate *et al.,* 2021).

Calotropis procera (Asclepediaceae family), is a wild plant found in Asian deserts. It is acknowledged for its excellent insecticidal and pesticidal qualities. The extract can be prepared from any part of the plant. Previous research has indicated this extract is effective against sucking and lepidopterous pests (Prabhu *et al.,* 2012). The goal of this work was to prepare ZnO nanoparticles from leaf extract of *C. procera* as well as to determine their effectiveness and impact on the antifeedant activity of fall armyworm.

MATERIALS AND METHODS

Preparation of leaf extract of *C. procera*

Calotropis procera (Aiton) Aiton leaves were collected from the Lahore and left to dry under shade for three days. The dried leaves were grinded to produce a fine powder. Ten gram of this powder were soaked in 100 mL distilled water in 250-mL beaker to make a 10% (w/v) extract. The mixture was subjected to continuous heat and stirring for 20 min. Let the mixture to cool for some time, filtered and store it in refrigerator for further use.

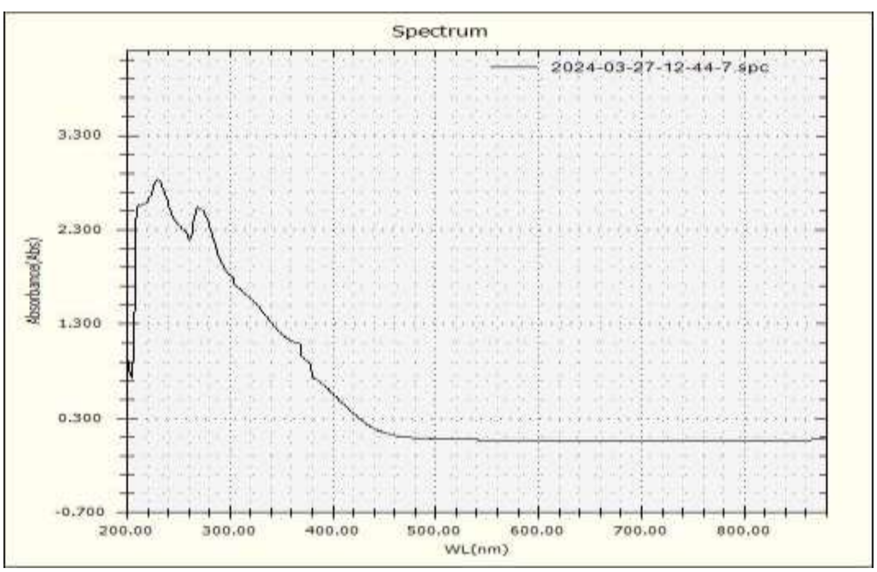

Fig. 1. UV Spectra of ZnO nanoparticles.

Synthesis of zinc oxide nanoparticles from leaf extract of *C. procera*

The next procedures required the use of 50 mL of this extract. Subsequently, 1 M zinc nitrate and 5 M NaOH solution were prepared using distilled water. A solution was then prepared by mixing 50 mL of the zinc nitrate solution, 10 mL of the NaOH solution, and 50 mL of the *C. Procera* leaf extract. Then the mixture was stirred at 90 °C for 90 min of heating. Subsequently, the mixture was allowed to cool. Following this, the content was centrifuged at three thousand revolutions per minute. The mixture was then filtered after that. The filtrate was collected to determine UV spectrum with a spectrophotometer available at the Department of Chemistry, University of the Punjab.

The filter residues were then transferred into a China tray and dried in an oven at 90 °C for 40 min. The dehydrated leftovers were grounded into a fine powder using pastel and mortar. Finally, this powder was calcined at 550 °C for three hours. The required nanoparticles were present in the powder obtained after the calcination of the samples. Lastly, the features and the possible uses of these green synthesized nanoparticles were examined. The FTIR analysis was carried out in order to study the constituents of the nanoparticles. Following that, sonication was performed on the ZnO nanoparticles. To do this, 500 mL of water was mixed with 0.5 g of ZnO nanoparticles, which was then placed into a sonicator (Ray *et al*., 2019).

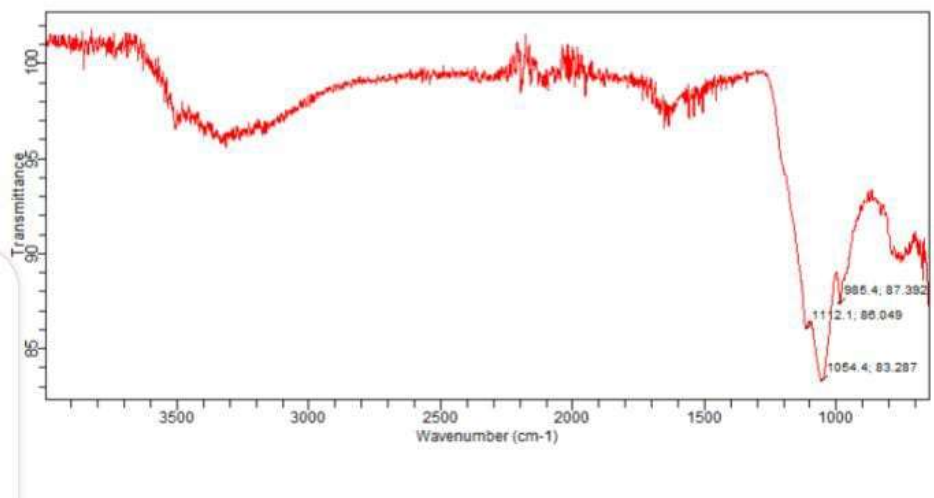

Fig. 2. FTIR spectrum of green synthesized ZnO nanoparticles.

Bioassay trials using ZnO nanoparticles

For the present study, we collected the larvae of FAW from the attacked field in the University of the Punjab Lahore. To prevent cannibalism, the larvae were transferred and reared in individual small boxes (sauce cups 6 cm × 3 cm) with lid having small holes. The laboratory was kept at 26 ± 1 °C, with a humidity of 70 ± 10 and a light/dark photoperiod of 14:10. Before they molted into pupae, the larvae were fed with newly chopped baby corn and corn leaves. Large rearing boxes were used for keeping the adults. The honey solution was used to feed the adults. For the oviposition, rearing cages of the adults were equipped with thin napkins. The eggs that were oviposited were incubated under optimal conditions, and upon hatching, they were carefully transferred to their designated containers.

The sonicated ZnO nanoparticles were further diluted to get five different concentrations (100, 200, 300, 400 and 500 ppm) for bioassay. After this, the finely chopped corn leaves were rinsed in each concentration for five seconds. Larvae were later released on the diet. There was one control treatment, 5 treatments and three replicates for each of the treatments. Each replication used one larvae in one box and twenty larvae in total. Data were recorded at the interval of 1, 3, 5, 7 and 9 days. All the larval instars were used in order to study the larvicidal effect of the nanoparticles (Tahir *et al*., 2024).

Antifeedant activity

To determine the antifeedant activity, fresh maize leaves, 5 to 6 cm long were excised. For removal of the dust, leaves were first washed with tap water twice. Different concentrations of ZnO NPs were applied on the maize leaf. The samples were then left to dry for five to ten minutes. The control treatment comprised of the leaves that were not exposed to any treatment. In this study, 5 treatments, each with 3 replicates, were administered. Five larvae were released per replicate. The antifeedant action was observed 24 h after releasing the larvae. The following formula was used to measure the feeding deterrence index (FDI):

$$\mathbf{FDI} = \frac{\mathbf{C - T}}{\mathbf{C + T}} \ \mathbf{X\ 100}$$

C and T = Area of the leave consumed by larvae in control and nanoparticles treatment, respectively.

Statistical analysis

The experimental design employed was the completely randomized design (CRD). Analysis of variance was done using the Statistix 8. 1 software. The significance differences among the treatment means of the data results were checked using LSD test at $P \leq 0.05$. In the LC_{50} and LC_{90} experiments, Minitab 16 software was used.

RESULTS

Effect of ZnO NPs on mortality of FAW

The mortality of FAW varied with concentration of ZnO nanoparticles. On day 1, maximum mortality of 30.87%, 26.76%, 25.24%, 21.46%, 20.13%, and 20.23% were observed in the 1st through 6th instars at a concentration of 500 ppm as shown in the Fig. 3A. On day 3, the maximum mortality was increased to 53.34% for the 1^{st} instar, 45.76% for the 2^{nd}, 38.34% for the 3^{rd}, 36.45% for the 4^{th}, 30.11% for the 5^{th}, and 21.76% for the 6^{th} instar, all at 500 ppm (Fig. 3B). As depicted in Fig. 3C, by day 5, the highest mortality was recorded as 70.34% for the 1^{st}, 68.11% for the 2^{nd}, 55.23% for the 3^{rd}, 51.67% for the 4^{th}, 41.22% for the 5^{th}, and 36.24% for the 6^{th} instar, again at 500 ppm. On day 7, mortality remained high, with 76.34% in the 1^{st}, 76.23% in the 2^{nd}, 70.43% in the 3^{rd}, 66.23% in the 4^{th}, 60.15% in the 5^{th}, and 46.61% in the 6^{th} instar at 500 ppm shown in Fig. 3D. FAW mortality difference among the instars was the most pronounced on day 9, with 85.24% for the 1^{st}, 80.22% for the 2^{nd}, 76.45% for the 3^{rd}, 71.21% for the 4^{th}, 66.77% for the 5^{th}, and 55.43% for the 6^{th} instar, highlighting the impact of the 500 ppm concentration (Fig. 3E). In general, percentage mortality was directly proportional to the concentration of nanoparticles and minimum mortality of all the instars was represented by the control group.

Antifeedant activity of ZnO NPs

The ZnO nanoparticles showed notable antifeedant potential against 3^{rd}, 4^{th} and 5^{th} instar larvae of FAW after 24 h of nanoparticles application in maize leaves. The nanoparticles application 39–78% antifeedant effect against the 3^{rd} instar larvae. Likewise, in 4^{th} and 5th instar larvae, the antifeedant effect was in the range of 35-76% and 32–70%, respectively, as illustrated in Table 1. The lowest antifeedant activity was observed in the control group where leaves were treated with water.

Table 1. Antifeedant activity of *S. frugiperda 24* h after the application of ZnO nanoparticles.

	Antifeedant activity (%)		
Concentration (ppm)	**3^{rd} Instar**	**4^{th} Instar**	**5^{th} Instar**
100	$39.54±0.56^{D}$	$34.57±0.52^{D}$	$32.05±0.41^{D}$
200	$45.68±0.62^{C}$	$40.19±0.61^{C}$	$38.22±0.45^{CD}$
300	$52.56±0.65^{BC}$	$48.64±0.64^{B}$	$46.17±0.48^{B}$
400	$69.76±0.69^{AB}$	$64.08±0.68^{AB}$	$58.28±0.53^{B}$
500	$78.85±0.75^{A}$	$76.27±0.72^{A}$	$70.9±0.58^{A}$
Control	$12.17±0.23^{E}$	$10.01±0.52^{D}$	$9.95±0.38^{D}$

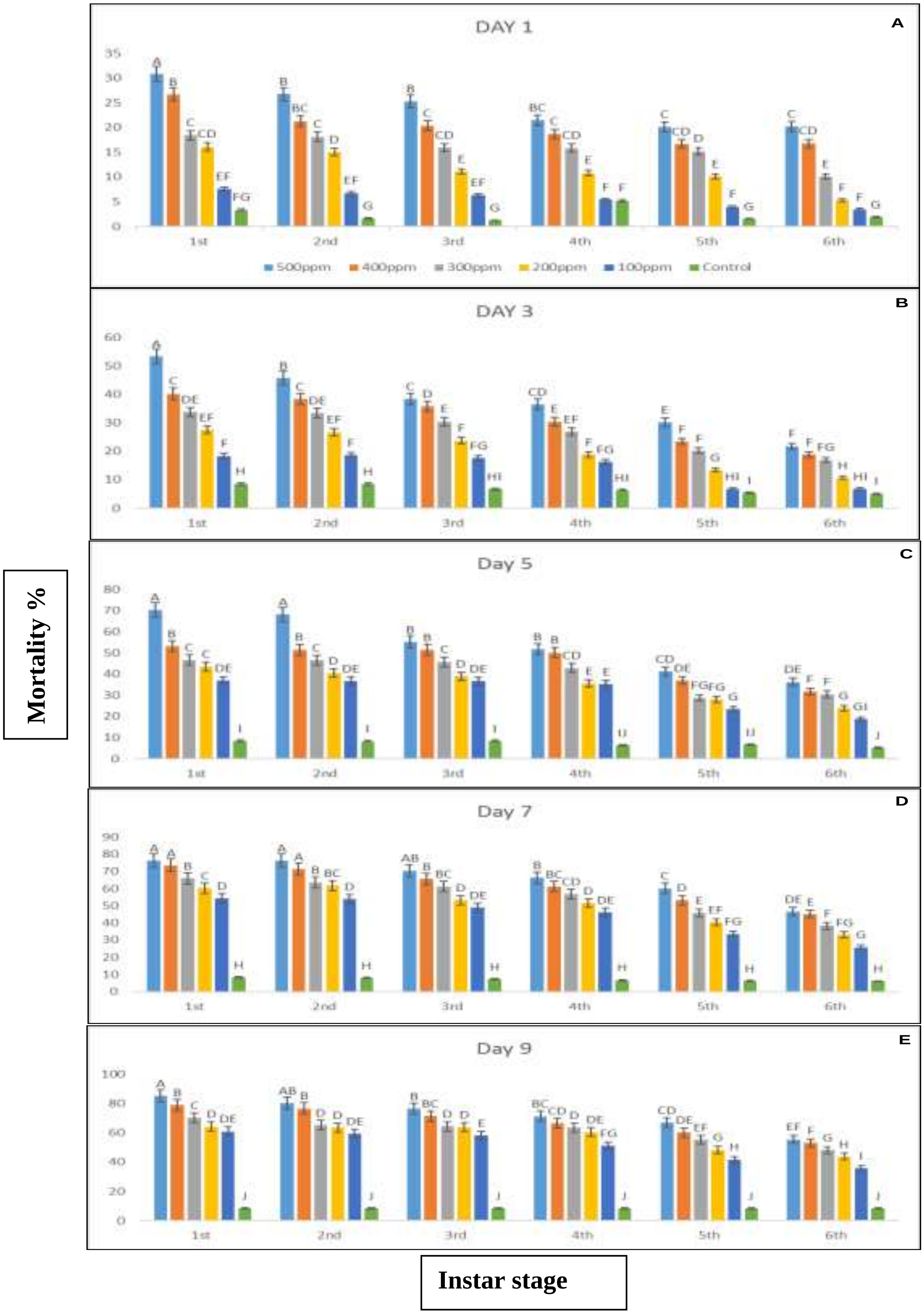

Fig. 3 (A-E). Percentage mortality of different instars of *S. frugiperda* at different time intervals after exposure to different concentrations of ZnO nanoparticles. Bars with different letters show significant difference as determined by LSD test at $P \leq 0.05$.

Fig. 4. Control (left) and malformed (right) 5th instar larvae.

Fig. 5. Control (left) and malformed pupae (right)

Fig. 6. Control (left) and malformed adult (right).

Morphological changes induced in FAW due to ZnO nanoparticles application

ZnO NP treatment elicited extensive direct physical developmental abnormalities in the larvae, juveniles, and adults of both male and female suppressing their normal body development. In Fig. 4, 5 and 6 the superimposed correlation between the consumption of baby corns and leaves treated with zinc oxide NPs and the presence of some physical abnormalities is shown. The aura of each life stage that was nourished on control is described by each picture intervention with the distortions occasioned by ingesting food that was prepared through dipping in ZnO NPs.

DISCUSSION

The purpose of this study was to ascertain how ZnO NPs insecticidal properties affect the fall armyworm's control. So long as *S. frugiperda* has been destroying crops, synthetic pesticides and a few biopesticides have been the main methods of management. However, the usage of these synthetic pesticides has harmed the ecosystem and led to insecticide resistance (Rouhani *et al.*, 2012). Studies on navigation of the insecticidal activity of Ag Nps have established that the compounds can help in controlling agriculture pests such as *Spodoptera littoralis* (Santos *et al*., 2021). Also, thiamethoxam formulation amplified the efficacy of ZnO NPs when used in combination to suppress *Spodoptera littoralis* (Jameel *et al.*, 2021). These findings are in line with numerous prior studies against many insects including *Sitophilus oryzae*, rice weevil, *Trialeurodes vaporariorum*, where simple exposure to ZnO NPs killed the pests (Haroun *et al.*, 2020). One other work that used biosynthesized Ag NPs showed larvicidal toxic effect on *Culex quinquefasciatus*, *Aedes aegypti*, and *Anopheles stephensi* (Kalaimurugan *et al.*, 2019). Likewise, upon assessing green copper nanoparticles on *Culex quinquefasciatus*, *Anopheles stephensi* and *Aedes aegypti*, the LC_{50} and LC_{90} were fairly impressive (Vivekanandhan *et al.*, 2021).

In the course of the trial, the highest mortality of 85% was recorded at first larval instar of FAW in 500 ppm concentration. These findings were also in agreement with the study conducted by Asghar *et al.* (2022), which established that when ZnO nanoparticles were applied to lepidopteran insects, the highest mortality index was 82%. This research supports the application of biosynthesized nanoparticles in undertaking the pest management to reduce the yearly impact of these pests on crops. Different concentrations of green synthesized ZnO NPs affected the survival percentage of *S. frugiperda*. Therefore, it will be quite useful as a pest control tool in future.

It is a well-established fact that populations of insects with body deformities are greatly controlled below economical thresholds. Results of the present study showed that there were numerous deformations on the body of the insect at different stages of life cycle of FAW. One of the prevention activities, which can be practiced in an integrated pest management program in order to check the pest population below the level of economic importance is to induce deformity in the insect pest population. In fact, it has been ascertained that spraying the affected plant part with silica nanoparticles alongside ZnO nanoparticles significantly reduce pest population (Thabet *et al.*, 2021).

Conclusion

In the context of control of *S. frugiperda*, green synthesized ZnO NPs can be considered as having a very promising substitute for hazardous pesticides. The minimal likelihood of insects developing resistance to ZnO nanoparticle insecticides after prolonged use renders them an optimum choice, notwithstanding potential environmental ramifications. Consequently, this work indicates that ZnO nanoparticles have the possibility of controlling and significantly reducing the number of *S. frugiperda*. Therefore, it is very useful in integrated pest control program.

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(*Accepted for publication October 2024*)