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Impact of *Cassia*-derived Zinc Oxide Nanoparticles on the Reproduction, Development, and Viability of *Bactrocera dorsalis*

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ABSTRACT

The fruit fly species *Bactrocera dorsalis* (Hendel, 1912) (Diptera: Tephritidae) is one of the most destructive pests impacting agricultural fruit production across tropical regions. The present study investigates the impact of *Cassia fistula* seed extract and its biosynthesized zinc oxide nanoparticles (ZnO NPs) on various developmental and reproductive parameters of *B. dorsalis*. The biosynthesis of ZnO NPs was confirmed through a shift in visual appearance and UV–Vis spectroscopy, with a prominent absorption peak at 372 nm, indicative of successful nanoparticle production. Fruit flies fed on *Cassia* seed-derived ZnO NP-supplemented food, ranging from 0.5 to 3.0 ml concentrations, significantly reduced egg-laying capacity and hatchability compared to the control group. Fecundity decreased from 37.12 ± 6.13 eggs in the control group to as low as 5.24 ± 0.67 eggs at the highest concentration, and hatchability declined from 99.6% to 3.4% at 3.0 ml. Viability estimation indicated a substantial decrease in adult emergence rates, from 99.9% in the control to 5.1% at 3.0 ml. Furthermore, the duration of development was prolonged with an increase in ZnO NP concentration, from 19 days in the control group to 21 days at 3.0 ml. Significant reductions were observed in pupal weight and length across treated groups, with weights decreasing from 15.10 ± 0.35 mg in control to 8.21 ± 0.05 mg at the highest concentration. This study demonstrates the potential of *Cassia* seed-derived ZnO nanoparticles as a bio-control agent in reducing reproductive success and development rates of *B. dorsalis*, highlighting their application in integrated pest management strategies.

Keywords: *Bactrocera dorsalis*; Fruit fly; *Cassia fistula*; Metal nanoparticles; Integrated pest management.

1. INTRODUCTION

Nanoparticles produced by green synthesis using different parts of plants have gained attention in recent research compared to the nanoparticles produced by physical or chemical methods (Hamed *et al*. 2023). The plant contains different phytoconstituents which act as reducing and stabilizing agents for the synthesis of nanoparticles and neutralize the use of chemicals and toxic solvents. These natural compounds not only facilitate the reduction of metal ions but also help in stabilizing the formed nanoparticles, enhancing their properties for diverse applications. Phenolic compounds, terpenoids, flavonoids, alkaloids, and saponins found in various plant extracts, are known for their reducing properties and ability to stabilize nanoparticles (Yadav *et al*. 2024; Dargah *et al*. 2024). Tannins present in *Arisaema dracontium* have been shown to effectively reduce metal ions and stabilize nanoparticles (Khattak *et al*. 2024). *Cassia fistula* L. (Family: Fabaceae), commonly known as the golden shower tree, is native to Asian countries and commonly found in South Africa, Mexico, and Mauritius. Various parts of the plant, including its leaves, flowers, bark, and fruits, possess significant pharmacological activities, making it a valuable resource in traditional medicine.

Bactrocera dorsalis Hendel (Diptera: Tephritidae), commonly known as the oriental fruit fly, is a significant pest affecting horticultural crops globally. Its polyphagous nature and ability to infest various fruits have led to substantial economic losses, particularly in the export market (Akheela *et al*. 2024). Effective management strategies are crucial to mitigate its impact, and recent studies have explored various control methods, including biological and molecular techniques (Damayanti *et al*. 2024; Yusof *et al*. 2024). While these control methods show promising results, the adaptability of *B. dorsalis* to predation risks, such as by *Hierodula patellifera* (mantis) suggests that further research is needed to understand its ecological interactions and improve recent control strategies (Liu *et al*. 2024). The eco-friendly control of *B. dorsalis* using green synthesized ZnO-*Cassia* nanoparticles presents a promising approach to pest management. Various studies have elucidated the potential of biogenic ZnO nanoparticles for various applications, including agriculture, but do not specifically address their use against *B. dorsalis* (Murali *et al.* 2023; Hamed *et al.* 2023). The study deals with the green synthesis of zinc nanoparticles using *C. fistula* seed and evaluates their anti-fecundity property on fruit fly species, *B. dorsalis*. The synthesis of ZnO NPs through plant extracts, particularly *C. fistula*, offers a biocompatible and environmentally safe alternative to conventional pesticides. This method not only minimizes environmental impact but also enhances the antibacterial properties of the nanoparticles, making them effective against various pathogens.

2. MATERIALS AND METHODS

2.1 Chemical and Reagents

The seeds and flowers of *Cassia fistula* were collected from the yard of Chandigarh University (altitude position as longitude 76°34'34.82" E latitude 30°46'18.35" N), Gharuan, Punjab, India. Analytical grade chemicals including Zinc nitrate ($Zn(NO_3)_2.6H_2O$) were used. Deionized water was utilized to perform the whole experimentation process.

2.2 Fly Stocks

Adult *B. dorsalis* were collected from the guava fruits procured from the guava orchard and taxonomically identified by the protocol of Taddei (Taddei *et al*. 2023). At room temperature, the *B. dorsalis* cultures were reared on an artificial diet inside a cage (90 cm × 45 cm × 45 cm). The adults were provided with an artificial rearing media containing a mixture of sugar (Dextrose-L) and protein hydrolysate (Protinex, Pfizer Ltd.) in a ratio of 3:1 (Ekesi *et al*. 2007) in a petri dish, and allowed to lay eggs on guava placed inside the cage at 25 ± 1.6 °C, 60.5 ± 3.8% relative humidity (RH) with 14 h light/10 h dark period.

2.4 Biosynthesis of ZnO Nanoparticles

Zn O NPs of *C. fistula* seed extract were prepared by the sol gel method for the zinc oxide nano particles synthesis (Hasnidawani *et al*. 2016) with slight modification. Briefly, C. fistula seeds were shade-dried, grounded, and preserved in an airtight container for further usage. The preserved seed powder was freshly extracted by the Soxhlet extraction method using 5 g of *C. fistula* seeds and 250 ml of ethanol as a solvent. The ethanolic solution was heated for 72 h at 80 °C to collect the ethanol-soluble phyto-constituents. The extracted liquid was collected, filtered and rotary-evaporated to obtain the concentrated extract.

To synthesize the ZnO NPs, 1 M solution of Zinc nitrate [$(Zn(NO_3)_2.6H_2O$] (aq.) was prepared. The solution was sonicated well so that $ZnNO_3$ was dissolved in the solvent. Around 20 ml of the zinc nitrate solution was taken in a beaker and stirred continuously (800-1000 rpm). Around 10% of the *C. fistula* seed extract in zinc nitrate solution was drop-wise added while stirring till the extract in the $ZnNO_3$ solution formed like a gel, which indicated the reduction of $ZnNO_3$ to ZnO. Approximately, 6 ml extract solution was used to reduce the zinc nitrate solution. The solution was stirred for 3 h and the sol-gel of zinc oxide was in a muffle furnace at 400 °C for 4 h in the crucible. A white-colored fine powder was observed which was further characterized for ZnO NPs formation.

2.5 Characterization of Biosynthesized Nanoparticles

2.5.1 UV Spectroscopic Characterization

Using a UV-245 Shimadzu model spectrophotometer, the UV-Vis spectra of the aqueous solution in the 200–800 nm range were measured to facilitate the bio-reduction of zinc ions to ZnO NPs.

2.3 Impact of *Cassia*-derived Zn NPs on Fruit Fly

Cassia seed-derived green Zn NPs had toxic effects on life history traits. To check the impact of *Cassia*–derived Zn NPs on *B. dorsalis* life history traits, Zn NPs derived from *Cassia* seeds were mixed in the fruit fly diet; the flies grown without Zn NPs diet were considered as control samples. Individuals of *B. dorsalis* were grown on different concentrations of *Cassia*-derived Zn NPs (0.5, 1, 1.5, 2, 2.5 ml concentrations). The eggs laid by 6-day-old flies within 24 hours are considered fecundity per day. To check the hatchability, 100 eggs from each normal diet-fed flies and *Cassia*-derived Zn NPs flies were placed on a black ethanol-soaked strip of paper on the surface of the fruit fly medium (10 × 10 eggs/vial). Furthermore, the viability (larval development into adult fly) of control and treated eggs was also tested (Singh *et al*. 2015). The impact of nanoparticles on developmental days from eggs to adults was investigated at 25 °C (Sharma *et al*. 2024). In addition, pupal weight, width, and length were measured using a stereomicroscope (Nikon, India) to check the effect of *Cassia* Zn NPs on the phenotypic characters of the fruit fly.

2.6 Statistical Analysis

The fecundity of fruit flies at different concentrations of ZnO NPs was compared by paired T-test. Differences were considered significant at $p < 0.05$. The recorded data were analyzed using (ANOVA) with Duncan's post hoc test to assess the fecundity and pupal weight, length, and width of *B. dorsalis* among different concentrations of Cassia ZnO NPs. Minitab 7.0 analytics software was used for data analysis.

3 RESULTS

Since the shift in the visual state is the first indication of the production of nanoparticles, the biosynthesis process of ZnO NPs was verified by the gradual change of the gel from the liquid solution. The UV-Vis absorption spectra of the bio-produced nanoparticles showed a prominent peak at 372 nm (Fig. 1), which probably makes ZnO NPs unique. The basic range of zinc oxide nanoparticles ranges from 265-265 nm. The difference in absorption peaks is caused by particle size variation.

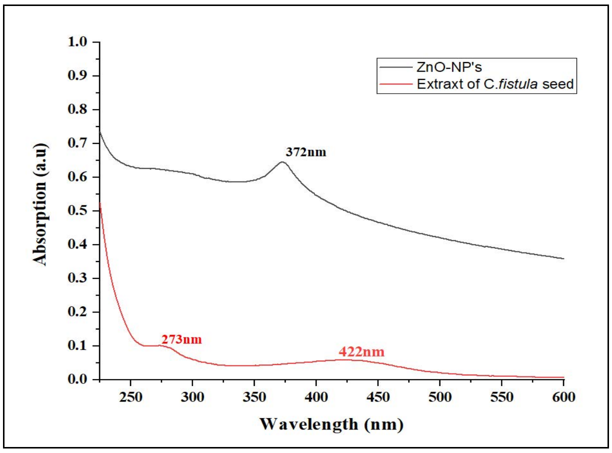

Fig. 1: UV Spectroscopic data for *Cassia fistula* seed extract and ZnO NPs

The biological methods for producing zinc oxide nanoparticles involve Soxhlet extraction with ethanol and *C. fistula* seed extract, heated to 80 °C for 72 minutes. A peak at 372 nm can be seen in the UV-Vis spectra data (Fig. 1) during the bioreduction of zinc nitrate to zinc oxide nanoparticles, stabilizing, and capping with seed extract. This is extremely comparable to the creation of zinc oxide nanoparticles by *Cassia fistula* seed.

3.1 Impact of *Cassia* seed-derived Zn NPs on *B. dorsalis*

There was a significant difference in fecundity of control *vs.* Zn NP-fed flies. Fecundity differs significantly among different concentrations of Zn NPs fecundity of control and crude extract treated flies is (37.12 ± 6.13), (30.06 ± 2.76) respectively and (26.45 ± 1.5, 18.00 ± 2.21, 13.34 ± 1.35, 6 .67 ± 3.12 and 5.24 ± 0.67 at 0.5, 1, 1.5, 2-, and 2.5-ml concentration of *Cassia*-derived Zn NPs, respectively) (Fig. 2a). The hatchability does not differ significantly in control (99.6%) *vs.* crude *Cassia* seed extract (97.9%) but differs significantly by 94.3 %, 55%, 30%, 25%, 19% and 3.4 % at 0.5, 1.0, 1.5, 2, 2.5, 3.0 ml concentrations of *Cassia*-derived Zn-NPs (Fig. 2b), respectively. The viability results showed that adult's emergence differs significantly in control and treated flies. Viability of control flies is 99.9% and 81.2% viability of flies treated with crude extarct, and whereas viability is 80.60%, 20.3%, 9.6%, and 5.1% at 0.5, 1.0, 1.5, 2, 2.5, 3.0 ml concentration of *Cassia* derived Zn NPs, respectively (Fig. 2d). Control flies developed in 460 hrs at 25°C and at 0.5 ml, 1.0 ml, 1.5 ml concentrations flies developed in 20 days whereas egg to adult takes 20 days at 2.0 ml, 2.5 ml concentrations and 21 days at 3.0 ml (Fig 2c). Table 2 summarizes the results of One-way Analysis of Variance (ANOVA) for the effects of *Cassia*-derived zinc oxide nanoparticles on the reproductive and developmental traits of *B. dorsalis*. Pupal weights and pupal length showed significant differences (P < 0.05) as larvae fed on *Cassia* ZnO NPs treated media had pupal weight much less and smaller than the pupa of the control food (Table 1).

Table 1. Pupal weight, width, and length (Mean ± SE) of *B. dorsalis* fed on *Cassia* Zn NP-supplemented food with different concentrations (100–500 ppm)

Treatments	Parameters		
	Weight (mg ± SD)	Width (mm ± SD)	Length (mm ± SD)
C-E	14.10^{c} ± 0.3	1.01^{a} ± 0.03	3.00^{b} ± 0.35
0.5	11.12^{a} ± 0.30	1.04^{a} ± 0.001	2.91^{a} ± 0.11
1	10.50^{b} ± 0.29	1.05^{a} ± 0.002	2.90^{a} ± 0.012
1.5	9.63^{b} ± 0.21	1.07^{a} ± 0.003	2.89^{a} ± 0.013
2	9.00^{b} ± 0.20	1.02^{a} ±0.007	2.90^{a} ± 0.015
2.5	8.11^{b} ± 0.19	1.03^{a} ±0.002	2.88^{a} ± 0.017
3	8.21^{b} ± 0.05	0.98^{a} ±0.001	2.76^{a} ± 0.029
Control	15.10^{c} ± 0.35	1.04^{a} ±0.03	3.03^{b} ± 0.35
df	7	7	7
F	29.3278	2.012	3.425
P	0.0001	0.6	0.001

Note: Means that do not have a letter are significantly different

Table 2. One-way Analysis of Variance (ANOVA) results for the effects of *Cassia*-derived zinc oxide nanoparticles on reproductive and developmental traits of *B. dorsalis*

Trait	*F* (df, error)	*P* value
Fecundity	881.73 (7, 192)	0.001
Hatchability	2952.48 (7, 192)	0.001
Viability	2395.49 (7, 192)	0.0001
Developmental Duration	32.94 (7, 192)	0.01

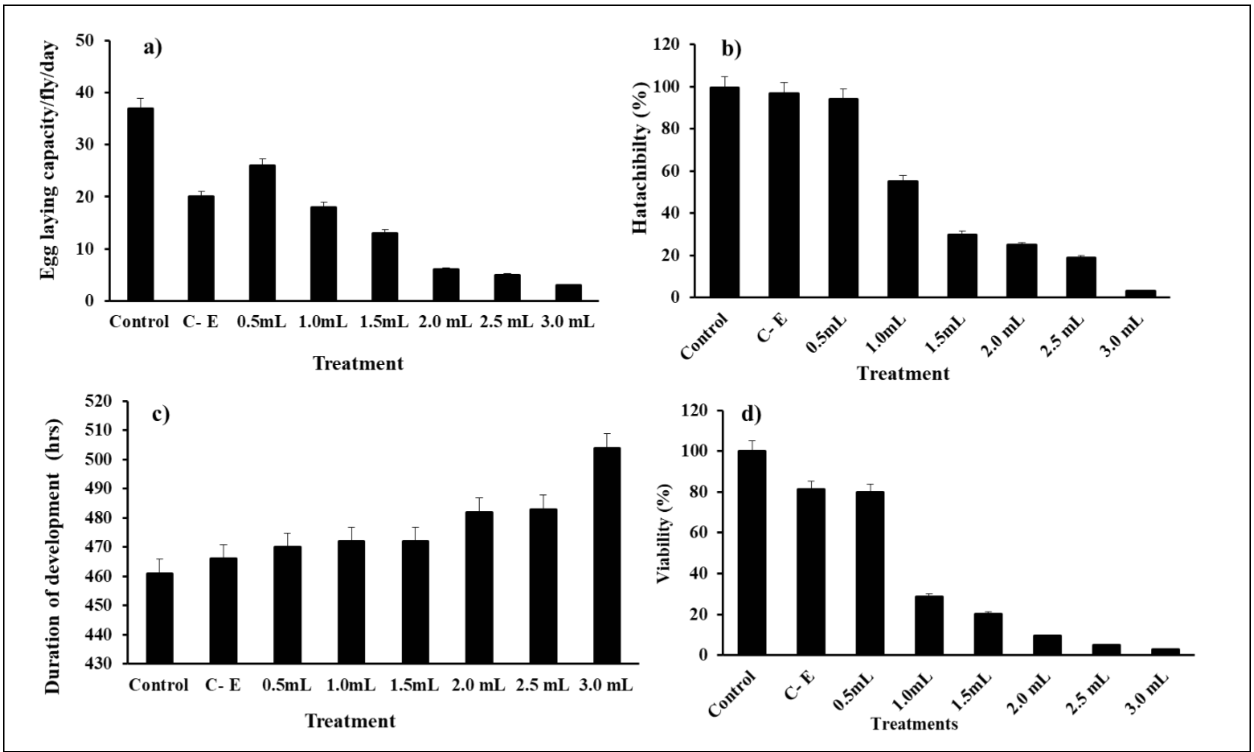

Fig. 2: Effect of *Cassia* seed extract (C-E) and *Cassia*-derived ZnO (ranging from 0.5 to 3 ml) treatment against the control group on: (a) the egg-laying capacity, (b) hatchability, (c) viability, and (d) developmental time of *Bactrocera dorsalis*

Error bars represent the standard deviation within the dataset (SD).

4. DISCUSSION

Traditional methods for controlling *B. dorsalis* such as synthetic pesticides, methyl eugenol (a male attractant), and the male sterile technique, though quite promising, have shown significant drawbacks. Synthetic pesticides, in particular, have led to adverse health effects in humans, environmental pollution, and increased resistance among insect populations. Consequently, the need for safer and more sustainable pest control methods has intensified, especially as the global emphasis shifts towards eco-friendly agricultural practices (Rajula *et al.* 2020; Rouhani *et al*. 2012; Guan *et al*. 2008). The combination of *Cassia* with zinc nanoparticles (Zn NPs) offers a promising solution. *Cassia* Zn NPs leverage the natural insecticidal properties of *Cassia* such as its antimicrobial, antifungal, and insect-repellent qualities, enhanced by the physical and chemical attributes of Zn NPs. Zinc oxide might have increased the effectiveness of *Cassia* through its unique size and reactivity with the constituents of the extract as investigated by Mazhar *et al*. 2024, allowing for better penetration and interaction with insect physiology, thus improving mortality rates and reducing reproduction metrics. Studies on other insect pests, such as *Sitophilus oryzae* (rice weevil) and *Trialeurodes vaporariorum* (whitefly), also support the efficacy of Zn NPs in achieving higher mortality rates due to their nano-scale interaction with insect bodies (Malaikozhundan *et al*. 2017).

4.1 Mechanism of Action on *B. dorsalis*

When B. dorsalis feeds on *Cassia* Zn NPs at different concentrations, the nanoparticles induce stress responses that interfere with vital processes such as egg production and hatching. A significant reduction in egg-laying capacity was observed as the concentration of the *Cassia* ZnO NPs increased, with the lowest capacity recorded at the 3.0 ml concentration. A marked decline in hatchability and an increase in the duration of development was also observed as the concentration of the treatment increased, with near-complete inhibition of egg hatchability and longest developmental duration at the highest concentration of 3.0 ml, pointing to a reproductive inhibition effect which is crucial in integrated pest management (IPM). The results align with previous research on the chronic reproductive effects of nanoparticles such as nanoceria, also known as cerium oxide nanoparticles (CeO_2 NPs) in *Aedes aegypti* (Doshi *et al*. 2020), suggesting that Zn NPs could also impair egg viability and developmental stages, thus reducing population growth over time.

4.2 Advantages of Green-synthesized Nanoparticles

Green synthesis of nanoparticles, as demonstrated with *Cassia* Zn NPs, offers a sustainable alternative to chemically synthesized nanoparticles. By using plant extracts, the synthesis process avoids hazardous chemicals, reduces environmental impact, and yields nanoparticles with effective bioactivity against pests. This aligns with current agricultural trends toward minimizing environmental footprints while addressing pest-resistance issues (Prasad *et al*. 2017).

In summary, the use of *Cassia* Zn NPs provides a potent, eco-friendly alternative to conventional pesticides for *B. dorsalis* management, addressing both ecological and agricultural sustainability. Future research will explore the scalability of these findings, investigate potential non-target effects, and consider the broader applicability of *Cassia* Zn NPs in managing other economically significant pests.

5. CONCLUSION

Cassia Zn NPs can be used as an alternative to the more lethal insecticides for controlling *B. dorsalis*. *Cassia* Zn NPs decrease the population size of *B. dorsalis* by reducing fecundity, hatchability, and viability; hence, it is a promising tool for integrated pest management of fruit flies. Green synthesized ZnO *Cassia* nanoparticles demonstrate promising potential in controlling the fruit fly pest, *B. dorsalis*. The eco-friendly synthesis process and effective pest control properties make it a viable alternative to chemical pesticides, contributing to sustainable agricultural practices. Further studies are recommended to optimize the synthesis process, evaluate long-term effects, and assess the impact on non-target organisms and the environment.

AUTHOR CONTRIBUTION STATEMENT

Manuscript conceptualization and drafting were done by Seema and Saranagatt. Manuscript editing and interpretation of data was done by Seema and Aanchal.

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CONFLICT OF INTEREST

The authors have no relevant financial or non-financial interests to disclose.

DATA AVAILABILITY

All data generated or analyzed during this study are included in this published article.

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