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Research Article

Effects of Alcoholic Leaf and Seed Extracts of *Datura innoxia* on the Reproductive Performance and Behavioral Responses of Southern Cowpea Beetle, *Callosobruchus maculatus*

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ABSTRACT

Botanical insecticides are a sustainable option to synthetic compounds in controlling insect pests of stored grains and products. *Datura innoxia* contains bioactive alkaloids with established insecticidal and behavioral activity, however, its activity, against the southern cowpea beetle, *Callosobruchus maculatus*, is poorly studied. This research was carried out to explore the alkaloid profiles of alcoholic extracts of *D. innoxia* leaves and seeds on reproductive performance of *C. maculatus* as well as behavioral responses. The alkaloid richness of the plant was confirmed by the presence of two major tropane alkaloids in the leaf extract, hyoscyamine (5.1 ppm; retention time 3.68 min) and scopolamine (7.8 ppm; retention time 0.6 04 min) as revealed by high performance liquid chromatography analysis. The treatment of cowpea seeds using alcoholic leaf extracts caused a significant reduction in oviposition of *C. maculatus* females. Seed extracts performed better than leaf extracts. Treatments did not show any major variations among concentrations, which led to a high process of activity even in case of low doses. The assays conducted with repellency showed concentration-based effects, the highest repellency rates of 80 and 90 percent respectively were observed when leaf and seed extracts were used at a 3000 ppm concentration. The highly decreased reproduction and the effective repellencies can be linked to the toxicity of alkaloids, oviposition and disruption in insect-development. In general, the results demonstrate the potential of *D. innoxia*, especially seed extracts, as a green and efficient botanical protectant to preserve cowpea seeds.

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Introduction

Callosobruchus maculatus commonly known as the southern cowpea beetle is considered to be one of the most economically significant insect pests that cause considerable losses in stored grains. The attacks start in the field and get worse in the storage. Southern cowpea beetle is a leading cause of quantitative and qualitative losses, leading to the reduction of the seed weight and germination (Matos et al., 2020; Stathers et al., 2020).

As a result of the economic losses incurred by the insect pests, and the health hazards of overusing chemical pesticides to protect grains after harvest, emphasis on the use of natural pesticides in agriculture has been given (Saber et al., 2021). Use of botanical is one of the effective substitutes for insecticides considered to be simple and sustainable as compared to traditional

chemical pesticides which are based on one active ingredient. Comparatively, substances obtained in plants are a blend of compounds, which operate to influence behavioral and physiological functions. Literature has indicated that a lot of plants consist of lethal and repellent elements (Abdul-Rahman and Mohammed, 2019); Datura is among them.

The genus *Datura* (e.g., *Datura stramonium*, *D. innoxia* and *D. metel*) is very promising as a source of insecticides due to the high level of bioactive compounds, in particular, tropane alkaloids that include atropine, hyoscyamine, and scopolamine that disrupt the nervous system of insects and cause toxic effects (Sharma et al., 2021, 2023). *D. metel* has been reported to have adverse effects on the life cycle and survival of agricultural pests such as *Helicoverpa armigera* and mosquito species when used as methanolic seed extracts (Ali et al 2012; Adesina, 2022; Sharma et al., 2023). Extracts of *Datura* parts have been known to be effective against major pests in the stored grain protection scenario: acetone seed extracts of *D. stramonium* were found to have significant mortality against the rice weevil *Sitophilus oryzae* at higher concentrations and longer exposure times, which suggest dose-effective toxicity (Ali et al., 2012; Sharma et al., 2023). Contact toxicity against the khapra beetle (*Trogoderma granarium*) and rice weevil (*S. oryzae*) was also reported with laboratory experiments of *D. alba* leaf extracts with higher levels of the extract leading to significant mortality and demographic downfall across generations (Ali et al., 2012). Besides, *Datura* seed extracts have also been tested on repellency and inhibition of egg viability in cowpea weevil (*C. maculatus*) infestations, demonstrating fewer adult emergence and weight loss in stashed cowpea (Muhammad, 2017; Oyewole and Agwu, 2021; Akbar et al., 2024). Taken together, these results suggest the potential of *Datura* extracts as biologically friendly bio-insecticides to manage grain pests before and after storage both in the field and post-harvest grain storage, although their effect and mechanism of action differ depending upon species, plant part, solvent, and target pest.

D. innoxia, also contains several biologically active compounds, particularly alkaloids. Alkaloids are toxic, biologically active molecules with insecticidal properties even at low concentrations. Studies have demonstrated that these compounds affect the neurotransmitter system, specifically acetylcholinesterase (ACh). Inactivation of the nervous system ultimately leads to the death of larvae (Raveen et al., 1917; Kabir, 2023).

The present study, therefore, aimed to know the effect of the alcoholic extract, both from leaves and seeds, of the *D. innoxia* on the egg production of female of southern cowpea beetles, as well as its effect on attracting and repelling the adults, to be used as available materials in preserving stored seeds until they are planted.

Materials and Methods

Insect culture

The laboratory population of the southern cowpea beetle was reared after obtaining infected cowpea seeds from the local markets in Baghdad. Southern cowpea beetle from healthy cowpea seeds were placed in cylindrical glass bottles, 10 cm high and 6 cm in diameter, and the nozzle was covered with an Orkanza cloth. The cover was fixed with a rubber band to prevent the exit of adults and placed in an incubator at a temperature of 28 ± 1°C and a relative humidity of 70 ± 5%. The colony was renewed after each generation (Zahraa and Kathier, 2025).

Collection of plant samples

The *D. innoxia* leaves were collected from the Botanical Garden of the College of Science, University of Baghdad in June 2023. The leaves and seeds were thoroughly cleaned from dust with water and spread on paper sheets in a shaded and dry place at room temperature, turning the leaves and seeds over occasionally to prevent rotting. These seeds were then finely pulverized after ensuring complete dryness using a clean, sterilized grinder. The ground material was then kept in a glass container till it was used in bioassays.

Preparation of alcoholic extract of *D. innoxia*

The alcoholic extract of *D. innoxia* leaves and seeds were prepared by macerating 70 g of ground leaves and seeds separately in 300 ml of hexane and left in a tightly sealed glass container covered with aluminum foil. It was constantly shaken for 1 h every day for one week to guarantee maximum extraction (Abubakar and Haque, 2020). The extract was then filtered through gauze and put in the Petri dishes to let the solvent to evaporate at a room temperature. The extract was prepared in the form of fine powder weighing 3.6 g and subsequently put in a glass container and stored at 20°C until their use in the bioassays.

High performance liquid chromatography analysis

The alcoholic extract of *D. innoxia* was analyzed by HPLC to determine the alkaloid content (Hyoscyamine and

Scopolamine) in a sample of 100-400 mg of *D. innoxia* leaf extract according to the following criteria:
HPLC model (SYKAM) Germany
Mobile phase = methanol: D.W = (80: 20)
Column = C18 – ODS (25cm × 4.6 mm)
Detector = UV – 254 nm
Flow rate = 1.0 ml / min

Effect of alcoholic extract of *D. innoxia* leaves and seeds on the fecundity of southern cowpea beetles

The insects were isolated at the age of 24 h and 10 adults were treated with the alcoholic extract at the concentration of (1000 ppm, 2000 ppm and 3000 ppm) by immersing them in 20 ml of the extract for 5 min. The samples were dried and placed on a filter paper with three replications for each concentration. Distilled water served as the control. The adults were then sprayed with 1000, 2000 and 3000 ppm of the extracts using a hand sprayer. During spraying, it was ensured that the insects were thoroughly covered with the extracts and incubated under normal incubation conditions of 28°C and 60 percent humidity. Data regarding mortality and fecundity of females were recorded after 7 days of treatment.

Effect of alcoholic extract of *D. innoxia* leaves and seeds on attraction and repellency of adults of southern cowpea beetles

Chemotropometer, a special device was used in this test to measure chemotaxis adapting from Folso's apparatus (Folsom, 1931). This device consists of a box, 80 cm long, 14 cm high, and 60 cm wide attached with a movable cover as shown in Figure 1. A transparent glass tube, 100 cm long and 11 cm in diameter, divided into centimeters passes through the two openings on opposite sides of the box. There is an opening in the middle of the tube used to introduce insects. This tube was placed inside the box, and a piece of cotton, immersed in 2.5 ml of the highest concentration (3000) of the alcoholic extract of *D. innoxia* leaves was placed at one end. On the other side of the tube, a piece of cotton was placed immersed in 2.5 ml of distilled water. The experiment was carried out with three replicates, 10 adults were introduced into each replicate. The numbers of moving insects in the tube were then recorded along a distance of 50 cm towards the two openings after 30 min. The attraction ratio and repulsion ratio were calculated according to the equation:

$$\text{Attraction ratio} = \frac{\text{Number of insects that moved towards the extract}}{\text{Total number of insects}} \times 100$$

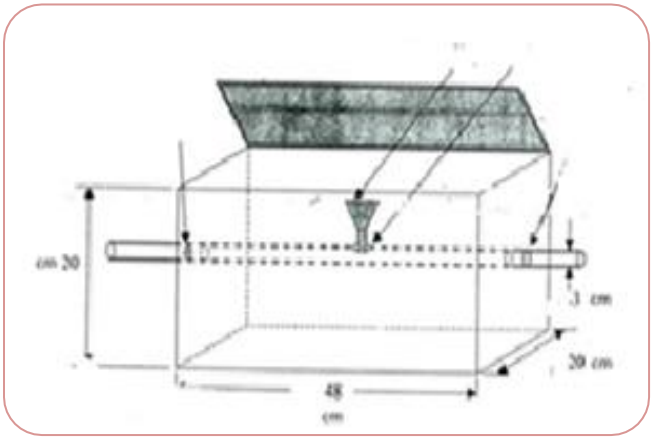

Figure 1. A schematic diagram of Chemotropometer.

Statistical analysis

To determine the effect of alcoholic extract of *D. innoxia* leaves and seeds on the fecundity of females of southern cowpea beetles, an ANOVA test was used. Tukey's test was used to compare egg count rates between different treatments. In addition, Two-Way Analysis of Variance was used to test the effect of alcoholic extract of *D. innoxia* leaves and seeds on the attraction and repellency of adults of southern cowpea beetles.

Results and Discussion

Detection of alkaloids in the alcoholic extract of *D. innoxia* leaves using HPLC

The alcoholic extract of *D. innoxia* leaves was found to contain two main alkaloids: Hyosyamine at 5.1 ppm at a retention time of 3.68 min and scopolamine at 7.8 ppm at a retention time of 0.604 min as shown in the Figure 2. Hyoscyamine and scopolamine are the predominant tropane alkaloids in the genus *D. innoxia*, which are found in all parts of the plant. When the components of the plant extracts of four *Datura* species (*D. innoxia, D. metel, D. stramonium,* and *D. stramonium* var. *tatula*) were analyzed using LC-MS (Liquid chromatography mass spectrometry), the results showed that *D. innoxia* had the highest concentration of scopolamine in all parts of the plant, while the concentration of hyoscyamine was highest in *D. stramonium* and lowest in *D. innoxia* (Jakabová et al., 2012).

Figure 3 shows that treating cowpea seeds with an alcoholic extract of *D. innoxia* had a significant effect on reducing egg numbers of *C. maculatus* females compared to the control. No significant differences were observed in the concentrations (1000, 2000, 3000 ppm) of the

alcoholic extract of leaf of D. innoxia. The lowest number of eggs laid by female of southern cowpea beetles was decreased to 15 eggs compared to the control (59 eggs) at 3000 ppm concentration. This indicates that treating cowpea seeds with an alcoholic extract of *D. innoxia* leaves leads to a reduction in the productivity of southern cowpea beetles, even at the lowest concentrations (1000 ppm) where the eggs number decreased to 29.3.

Figure 4 shows that treating cowpea seeds with an alcoholic extract of *D. innoxia* seeds had a significant effect in reducing the number of eggs to 6.6 eggs at 3000 ppm concentration, with no significant differences between the concentrations used in the treatments.

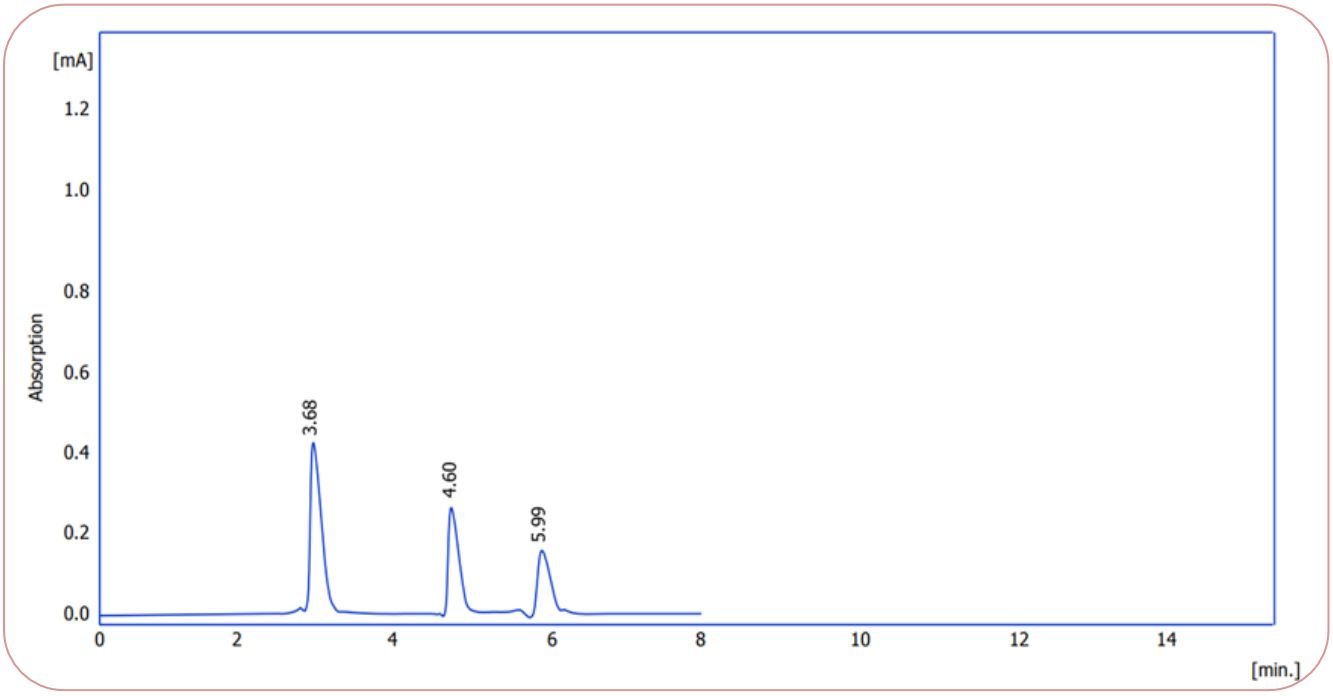

Figure 2. Major alkaloids identified in *D. innoxia* leaves by HPLC.

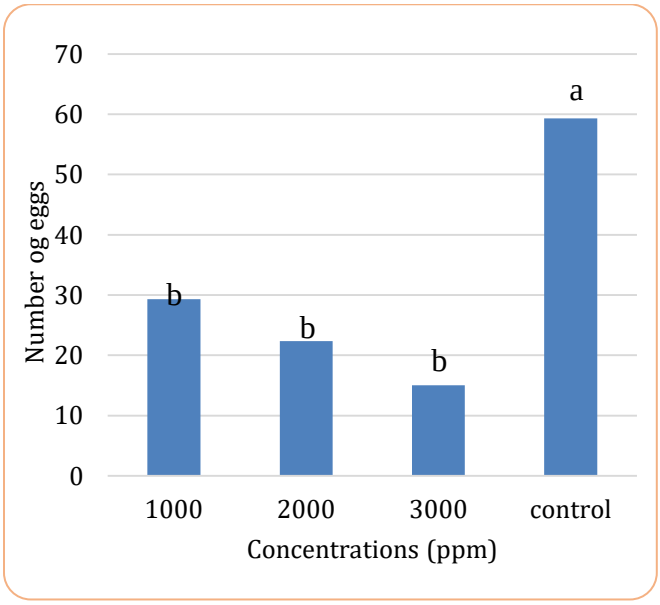

Figure 3. Effect of the alcoholic leaf extract of *D. innoxia* on the egg-laying capacity of the southern cowpea beetle.

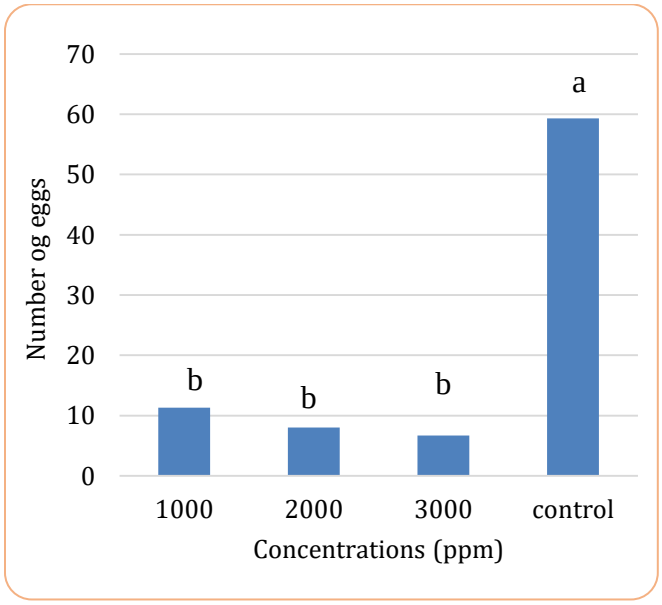

Figure 4. Effect of the alcoholic seed extract of *D. innoxia* on the egg-laying capacity of the southern cowpea beetle.

The Figures 3 and 4 show that the effects of the *D. innoxia* seed extract was superior in reducing the number of eggs of *C. maculatus*. Based on this result, we can recommend using the alcoholic extract of *D. innoxia* seeds in treating stored cowpea seeds until they are planted in the field preventing their damage by insect pests in storage. This effect can be attributed to plant secondary metabolites, particularly alkaloids, which are known to either retard or accelerate insect development and disrupt their life cycle through various mechanisms. Previous studies have demonstrated that alkaloids possess toxic and anti-nutritional properties against storage pests (Adesina and Mobolade-Adesina, 2020).

These findings align with reports by Xie et al. (2020) and Ali and Jazaa (2022), which indicated that higher alkaloid concentrations enhanced repellent activity and increased effectiveness against the immature stages of *C. maculatus*. Therefore, elevated levels of alkaloids may function as ovicidal agents (active against eggs) and larvicidal agents (affecting larvae). This effect is likely associated with the strong odor of alkaloids, which deterred the females from oviposition.

In a study conducted by Jassim (2023), where the alkaloids extract of *Moringa oleifera* leaves were studied on the development, fertility and demography of the *C. maculatus*, the life tables curve showed that the survival rate, the ratio of the females and the productivity was affected in a negative way causing a decline in the whole insect population.

Table 1 shows the attractant and repellent effect of the alcoholic leaves and seeds extract of *D. innoxia* on the adults of *C. maculatus*. The highest repellent rate was recorded at a concentration of 3000 ppm (80% and 90%) of alcoholic leaves and seeds extract of *D. innoxia* respectively. On the other hand, the lowest repellent rate found at a concentration of 1000 ppm (50% and 40%). The reason for the repellent effect is that the plant extracts have a pungent odor that repels the insect. This is consistent with a similar study in which a 1% concentration of *D. stramonium* leaf extract was effective when used as a repellent against several species of mosquitoes, *Aedes aegypti, Anopheles stephensi, and Culex quinquefasciatus*, demonstrating the effectiveness of *D. stramonium* leaf extract as a mosquito repellent (Swathi, 2010). In another study, when the alkaloid extract of *M. oleifera* leaves was tested against southern cowpea beetle, the repellency rate was 55% at a concentration of 3000 ppm (Jassim, 2023). The repellency of *C. maculatus* by different plant extracts (*Nicotiana tabacum*, *N. rustica*, *Azadirachta indica*, *Thuja orientalis*, and *Melia azedarach*) increased with the increasing dose and time (Akbar et al., 2024).

These results showed the toxicity of alcoholic extract of the leaves and seeds of plant to the southern beetle, the importance of this research lies in encouraging the use of plant extracts in preserving stored seeds. Therefore, they are a environmental friendly pest control methods instead of using conventional chemical pesticides which have negative effects on the environment.

Table 1. Attractant and repellent effects of alcoholic leaf and seed extracts of *D. innoxia* on adult *C. maculatus*

Treatments	Concentration	Repellent	Attractant
Alcoholic extract of *D. innoxia* leaves	1000	50	50
	2000	70	30
	3000	80	20
Alcoholic extract of *D. innoxia* seeds	1000	40	60
	2000	80	20
	3000	90	10
Critical Values for the Tukey HSD Test HSD[.05]		Rows = 1.54, Columns = 1.03	

Authors' Contributions

SAK and SAK conceptualized and designed the study, conducted data analysis, and prepared the original draft of the manuscript. RAAA provided critical guidance, constructive feedback, and substantive suggestions to enhance the content and clarity of the manuscript. All authors reviewed and approved the final version of the manuscript and take responsibility for all aspects of the work, ensuring its accuracy, integrity, and compliance with ethical standards.

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Conflict of Interest

The authors declare no conflict of interest.

Sustainable Development Goals Targeted

SDG 2: Zero Hunger
SDG 12: Responsible Consumption and Production
SDG 15: Life on Land

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