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ARAŞTIRMA MAKALESİ **RESEARCH ARTICLE**

Phytochemical Effect of the Bitter Melon *Citrullus colocynthis* Extract on Various life Stages of the Lesser Grain Borer, *Rhyzopertha dominica*

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Abstract

Rhyzopertha dominica F. (Coleoptera: Bostrichidae), often known as the lesser grain borer, is a significant pest that infests stored grains in various parts of the world. Plant extracts show potential as active components for producing environmentally benign biopesticides as an alternative. However, their physicochemical qualities provide a significant challenge when creating commercial formulations. Various challenges associated with using chemical pesticides demand the implementation of alternative, environmentally friendly control methods. This study sought to assess the impact of extracts derived from the leaves, fruit pulp, and crude oil of the bitter melon, *Citrullus colocynthis* L., on different life stages of *R. dominica*. Different concentrations (0.0, 2.5, 5, and 10%) of these substances were tested at different stages of the insect's life cycle, including eggs, second- and fourth-stage larvae, and adult females and males. The spraying method was used to apply the extracts and oil as insect food, and the percentage of mortality was determined after 24, 48, 72, and 96 hours of exposure. The results revealed notable variations in the impact of the extracts evaluated. All examined extracts (fruit pulp, leaves, and seed oil) demonstrated a phytochemical efficacy of over 10% for all stages of the tested insect after 96 hours of exposure. The egg mortality rates were 72.9%, 68.8%, and 58.0%, respectively. The second larval stage had mortality rates of 87.0%, 83.8%, and 80.1%, respectively. The fourth larval stage had mortality rates of 96.6%, 65.8%, and 55.4%, respectively. The death rates for adult females were 41.8% and 38.2%, while for adult males, the rates were 33.1%, 45.0%, 44.1%, and 53.3%, respectively. The study demonstrated the potential of utilizing natural materials, specifically plant-derived substances, as alternatives to conventional pesticides for effectively managing stored grain pests, particularly lesser grain pests.

Keywords: Phenolic compounds, *Citrullus colocynthis*, *Rhyzopertha dominica*. Mortality rate, Grain borer

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1. Introduction

Stored grains serve as the primary reservoir of proteins and other important nutrients, rendering them a crucial sustenance resource (Poutanen et al., 2022). Wheat is a significant essential crop for human populations due to its global consumption (Erenstein et al., 2022). There are almost one thousand species of grain pests that infest various storage products worldwide. The predominant stored grain pests are classified into two orders, namely Coleoptera and Lepidoptera (Khare, 1994). Insect pests cause significant harm to stored grains, resulting in decreased quality and quantity and unpleasant odors. The primary determinant of the abundance of pests that infest stored grain is the presence of favorable temperatures that promote their rapid growth and ability to survive (Ahmad et al., 2021).

Rhyzopertha dominica, commonly known as the lesser grain borer, inflicts significant harm to different types of grains and poses a substantial threat during storage. Throughout multiple regions worldwide, this insect is one of the most critical storage insects, causing significant losses in grains of wheat, barley, rice, corn, and other crops (Javanmard et al., 2023).

Using chemical methods in pest control poses various issues, such as pollution and the development of insect resistance to these pesticides. To circumvent these problems, applying oils and extracts derived from various plants is a viable alternative to chemical pesticides. These alternatives are both safe and environmentally friendly and are employed in integrated management programs (Souto et al., 2021). There are more than 20,000 plant species that contain chemicals and act as insect growth inhibitors, repellents, and anti-reproduction agents (Sidauruk et al., 2022). Botanical pesticides offer numerous benefits in comparison to synthetic pesticides. These substances possess a distinctive mechanism of action, posing no harm to humans and pets, readily degrading in the natural environment, and causing no pollution. Their residues are easily eliminated and can be readily obtained from natural sources. These organic products may serve as a viable substitute for managing harmful pests (Ali et al., 2023). The bitter melon plant *Colocynthis citrullus*, which belongs to the Cucurbitaceae family, was chosen in the present study because of its multiple medicinal, veterinary, and agricultural uses, as it contains active compounds including glycosides, saponins, alkaloids, flavonoids, anthraquinone, and others (Ahmed et al., 2019).

2. Materials and Methods

2.1. Rhyzopertha dominica rearing

Infected wheat seed samples were obtained from the Department of Seed Testing and Certification laboratories in Al-Qadisiyah province. Samples with individuals of *R. dominica* were sent to the Natural History Museum-University of Baghdad for identification. Subsequently, *R. dominica* colonies were established in plastic containers (20 x 10 cm). Each container contained 400 grams of wheat grains of the Abaa 95 class, together with 10 g of dehydrated baker's yeast (Ali et al., 2009). Prior to infesting grains with insects, the grains underwent a two-week freezing process at -18 °C to eliminate any potential of various insect infestations. To collect insect eggs, 200 adult (100 males and 100 females) were introduced into each container. After two weeks, the containers were covered with tulle cloth and secured with a rubber band. They were then transferred to an incubator at 30 ± 2 °C and a relative humidity of 70 ± 5%. The insect colony was replenished after each generation and as required (Aziz et al., 2022). To obtain various developmental stages of the insect, a permanent colony was established using a nutrient medium composed of 2 kg of rice seeds, 2 kg of wheat seeds, and 10 g of dry baker's yeast. The seeds were ground using an electric grinder, and a portion of this nutrient medium was placed in glass containers (7 x 10 cm). Each container was then inoculated with 10 pairs of recently emerged adults. The openings of the containers were covered with tulle cloth and secured tightly with rubber bands to prevent adults from escaping. The insect colony was renewed after each generation. The specimen was placed in the incubator at the same temperature and relative humidity. The sexual organs determine the distinction between males and females. In female virgins, there are three prominent and spaced genital pieces at the abdominal end, while in males, there are two closely positioned pieces. A 40x power anatomical microscope was used to examine these anatomical differences. The pupae were placed in 10 cm Petri dishes, and filter papers moistened with water were added. The virgins and adults were then transferred to an incubator under specific conditions. A potassium hydroxide (KOH) solution saturated with distilled water was used to maintain the appropriate humidity in the incubator. This solution was placed in a sterile glass dryer at a 0.27 g/ml distilled water (Mebarkia et al., 2010).

2.2. Collection of plant samples

The bitter melon plant samples were collected during the flowering stage in March 2023 from different areas of the southwestern desert in Al-Muthanna Governorate, southwest of Iraq. The samples were identified as the *Citrullus colocynthis* plant belonging to the Cucurbitaceae family at the Iraqi National Herbarium/Seed Inspection and Certification/Ministry of Agriculture. Leaves were collected early in the morning and before sunrise, washed and cleaned well of dust, and dried naturally in the shade under room conditions. Fruits were collected from the same areas in the second half of April 2023 after the fruits had ripened well and dried under the same conditions in which the leaves were dried. Seeds were separated from the dry fruits. Leaves and fruit pulp were ground separately using an electric grinder and kept in plastic containers with a dark color until use.

2.3. Preparation C. colocynthis Extracts

The leaves of the examined plant were extracted using Harborne's technique to produce aqueous extracts (1973) by placing 20 g of powder separately in 150 ml of distilled water and mixing the mixture for one hour with a magnetic stirrer and leaving for 24 h and then filtering the extract using rarefied pressure and filter paper of 0.45 micrometer porosity. Then, the extract was concentrated with a rotary evaporator, and the concentrated extract was kept in opaque containers and stored in the refrigerator until use. Concerning oil extraction from the seeds, the seeds were initially dried at 50 °C for 24 h and then ground with an electric grinder. The oil extracted from the seed powder utilized petroleum ether as the organic solvent, with a boiling point of 40-60°C. The extraction process involved a ratio of 5 grams of seed powder to 100 milliliters of solvent. A Soxhlet apparatus operated at 50°C for 16 hours facilitated the extraction. Subsequently, the solvent was removed using a rotary evaporator at 50°C (Yaniv et al., 1991). To estimate the effectiveness of fruit pulp and dry leaf extracts, 10 g of raw extract was separately dissolved in 5 mL of 96% ethyl alcohol. The volume was then increased to 100 mL with distilled water, resulting in a 10% concentration of the basic solution (stock solution). These prepared solutions have 2.5%, 5%, and 10% concentration. In addition, 2.5 g of seed oil was mixed with dimethyl sulfoxide (Dimethyl sulfoxide) (DMSO) as a solvent and then homogenized with water at a 25 ml/L ratio. Citwett was added as a diffuser at a recommended 0.25 ml/L ratio. The volume was completed to 250 mL with distilled water, resulting in a standard solution with an oil concentration of 10,000 ppm. Solutions with 2.5%, 5%, and 10% concentration were also prepared.

2.4. Bioassay with R. dominica adults

The insects used in this study were bred in the laboratory for several generations. Newly emerged adults were collected from breeding vessels after isolating males from females in the pupae stage to perform the required treatments. Thirty adults, divided into three replicates, with ten adults for each replicate, males and females separately, were placed in 10 cm Petri dishes, and in each of them was placed a filter paper moistened with sterile distilled water. Adults were then transferred to Petri dishes measuring 10 cm, each containing wet filter paper, and 8 grams of food (sterilized wheat grains) were placed in each plate. A tulle cover covered each plate, and the plates were transferred to the incubator at a temperature of 32 ± 2°C and a relative humidity of 70 ± 5%. Finally, the replicate was numbered utilizing a Sharpie pen. Extracts of leaves, fruit pulp, and seed oil of *C. colocynthis* were applied to adults in various concentrations (2.5%, 5%, and 10%, respectively). The replicates were sprayed with 5 ml of the required concentrations separately with a hand sprayer, sized 15 ml. A control treatment was implemented using distilled water. *Rhyzopetha dominica* individuals displaying ataxia (disorganized, erratic movement) and individuals found in a supine position with legs raised or showing no movement were classified as deceased at 24, 48, 72, and 96 hours (Heinz-Castro et al., 2021). Generally, every experiment consisted of three replicates, with ten individuals in each replicate.

2.5. Bioassay with R. dominica larvae

The second and fourth larval stages of *R. dominica* were collected in 30 and evenly distributed into three replicates, with 10 larvae in each replicate.

2.6. Egg mortality rate (%)

A female of *R. dominica* was allowed to lay its eggs for 24 h. They were subsequently removed. Various concentrations of *C. colocynthis* extracts were applied to eggs, including 2.5%, 5%, and 10%. As a control treatment, distilled water was utilized in contrast to the various extracts derived from the *C. colocynthis* plant. The eggs were examined daily by using a dissecting microscope for ten days to ascertain the egg mortality rate (in percent). Eggs that successfully developed into larvae or erupted via a hatching hole were categorized as viable. Conversely, eggs that failed to develop into larvae or hatch due to the absence of a hatching opening were categorized as non-viable. Ten adults were utilized in each of the three replicates for each treatment.

2.7. Statistical analysis

ANOVA was done with GenStat 12th Edition. A two-way ANOVA was performed to assess different extraction activities of *C. colocynthis* on different *R. dominica* stages, and Tukey's HSD and least significant difference LSD separated means. The control treatment was excluded after Abbott's formula (Abbott, 1925) Corrected cumulative mortality for natural death. All analyses utilized a 0.05 significance level.

3. Results

Different extracts of C. *colocynthis* implemented in this research had diverse effects on the mortality rate (both adults and larval stages) and viability rate of *R. dominica* eggs, as shown below.

3.1. Effect of C. colocynthis extracts on R. dominica egg mortality

The study shows that extracts of *C. colocynthis* at different concentrations and exposure times significantly impact the mortality rates of *R. dominica* eggs, as shown in *Table 1*. The results also indicate an interaction between concentration and exposure time in reducing the viability of lesser grain borer eggs. It was observed that as the concentration of extracts increased, the mortality rates of eggs also increased. The highest percentage of mortality was found to be 56.85%, 46.91%, and 37.03% for fruit pulp extracts, leaves, and seed oil, respectively, at a concentration of 10%. On the other hand, the control treatment resulted in mortality percentages of only 4.35%, 4.20%, and 3.24% for fruit pulp extracts, leaves, and seed oil, respectively. The statistical analysis confirmed the

Table 1. Effect of Citrullus colocynthis extracts on the viability of egg of the lesser grain borer, Rhyzopertha dominica after 24, 48, 72, and 96 h of exposure

Extraction types	Concentration mg/mL	Exposure time/h				Con. Average
		24	48	72	96	
Fruit pulp	2.5	18.8	31.16	35.47	41.07	31.62a
	5	25.4	48	54.1	61.12	47.37b
	10	28.91	57.08	68.5	72.91	56.85c
Exp. average		24.39a	45.42b	52.99c	58.37d	
LSD (0.05)	Con. = 0.19		Exp. Time = 0.221		Con. x Exp. time = 0.380	
Leave extraction	2.5	12.9	19.9	27.47	30.15	22.59a
	5	22.06	35.09	46	54.9	39.53b
	10	24.1	40.9	52.8	68.8	46.91c
Exp. average		19.97a	31.97b	42.11bc	51.32d	
LSD (0.05)	Con. = 0.2712		Exp. Time = 0.3132		Con. x Exp. time = 0.542	
Seed oil	100	8.82	14.16	23.163	24.73	17.72a
	200	12.75	18.16	41.86	48.93	30.43b
	300	16.087	27.038	47.029	57.98	37.03b
Exp. average		12.55a	19.79b	37.35c	43.88d	
LSD (0.05)	Con. = 0.223		Exp. Time = 0.258		Con. x Exp. time = 0.446	

Means have different letters shows significant difference at the 0.05 according to Tukey's HSD test

significance of these observed differences. The highest mortality rates (72.91%, 68.85%, and 57.98%) were observed at a concentration of 10% after 96 h of exposure for fruit pulp, leaves, and seed oil extracts, respectively, compared to the mortality rates in the control treatment. This conclusion is based on the statistical analysis results indicating significant differences between the extracts. Increasing the extract concentration and the duration of exposure resulted in higher egg mortality rates in the tested insect.

3.2. Effect of C. colocynthis extracts on the survival of 2nd larval stage of R. dominica insect

There was a difference in the mortality rate of the second stage of *R. dominica* when bitter melon *C. colocynthis* extracts were used at different concentrations and for different exposure intervals (*Table 2*). The results show that there is a correlation between the concentrations of bitter melon extract *C. colocynthis* and the mortality rates. This correlation is positive, meaning that as the concentrations of fruit pulp extracts, leaves, and melon seed oil increase, the rate of mortality also increases. The fruit pulp, leaves, and melon seed oil extract had the highest mortality rates at a concentration of 10% (68.19%, 64.76%, and 55.29%, respectively) compared to the control treatment, which had mortality rates of 0.25%, 0.38%, and 0.83%, respectively. The type of extract significantly influenced the mortality rates of the 2nd larval stage, with the fruit pulp extract outperforming the extract of leaves and seed oil by 56.67, 51.03, and 42.84%, respectively. Statistical analysis confirmed the significance of the existing differences. Furthermore, the mortality rates of the second larval stage of the lesser grain borer exhibited a noteworthy correlation with the treatment duration. Specifically, the mortality rate increased as the treatment duration extended, culminating in a peak of 71.4, 65.24, and 51.43%, respectively, after 96 h, and a minimum of 34.81, 29.71, and 22.06%, respectively, after 24 h for fruit pulp, leaves, and seed oil of *C. colocynthis* extracts (*Table 2*).

Table 2. Effect of Citrullus colocynthis extracts on the mortality of 2nd larval stage of the lesser grain borer Rhyzopertha dominica after 24, 48, 72, and 96 h of exposure

Extraction types	Concentration mg/mL	Exposure time/h				Con. Average
		24	48	72	96	
Fruit pulp	2.5	29.2	42.8	47.9	53.3	43.34a
	5	36.18	58.3	65.3	73.9	58.47b
	10	38.97	66.9	79.84	87.03	68.19c
Exp. average		34.81a	56.03b	64.39bc	71.45d	
LSD (0.05)	Con. = 0.2086		Exp. Time = 0.2409		Con. x Exp. time = 0.417	
Leave extraction	2.5	19.3	37.8	36.15	39.80	33.30a
	5	32.8	53.01	62.2	72.15	55.05b
	10	36.96	63.1	75.15	83.8	64.76c
Exp. average		29.71a	51.35b	57.84c	65.24d	
LSD (0.05)	Con. = 0.25		Exp. Time = 0.29		Con. x Exp. time = 0.49	
Seed oil	100	18.8	28.3	32.1	34.8	28.53a
	200	21.12	41.7	55.933	60.04	44.69b
	300	26.2	47.1	66.3	80.8	55.29b
Exp. average		22.06a	39.30b	47.43bc	51.43c	
LSD (0.05)	Con. = 0.26		Exp. Time = 0.31		Con. x Exp. time = 0.54	

Means have different letters shows significant difference at the 0.05 according to Tukey's HSD test

3.3. Effect of C. colocynthis extracts on the survival of 4th larval stage of R. dominica

The results showed that *C. colocynthis* fruit pulp, leaves, and seed oil extracts increased the mortality rate of the 4th larval stage of *R. dominica*, reaching their highest rates of 53.54, 43.80, and 34.22%, respectively, at 10%, compared to 0.50, 0.63, and 0.38% for the control. The type of extract significantly affected the mortality of the fourth-stage larvae. Specifically, the extract derived from fruit pulp performed better than those from leaves and seed oil (31.66, 25.00, and 18.98%, respectively). Larval mortality increased exponentially with exposure to bitter melon extract (*Table 3*).

Table 3. Effect of Citrullus colocynthis extracts on the mortality of the 4th larval stage of Rhyzopertha dominica after 24, 48, 72, and 96 h of exposure

Extraction types	**Concentration mg/mL**	**Exposure time/h**				**Con. Average**
		24	48	72	96	
Fruit pulp	2.5	15.90	28.15	32.03	37.90	28.50a
	5	22.09	44.9	51.09	58.24	44.09b
	10	25.8	53.8	64.9	69.57	53.54b
Exp. average		21.28a	42.29b	49.36bc	55.24c	
LSD (0.05)	Con. = 0.232		Exp. Time = 0.2684		Con. x Exp. time = 0.4658	
Leave extraction	2.5	9.5	17.28	24.59	27.2	19.67a
	5	18.1	31.9	42.2	51.2	35.90b
	10	22.10	37.5	49.8	65.8	43.80b
Exp. average		16.63a	28.92b	38.90ab	48.04c	
LSD (0.05)	Con. = 0.232		Exp. Time = 0.27		Con. x Exp. time = 0.464	
Seed oil	100	5.444	10.792	20.041	21.18	14.36a
	200	8.882	14.905	38.779	45.312	26.97b
	300	13.23	24.263	43.99	55.38	34.22c
Exp. average		9.19a	16.65b	34.27c	40.63d	
LSD (0.05)	Con. = 0.232		Exp. Time = 0.2684		Con. x Exp. time = 0.4658	

Means have different letters shows significant difference at the 0.05 according to Tukey's HSD test

3.4. Effect of C. colocynthis extracts on the mortality of adult females R. dominica insect

R. dominica adult female mortality was dramatically affected by the bitter melon plant *C. colocynthis* fruit pulp, leaves, and seed extract. As concentration and exposure times increased, correspondingly, mortality increased. Female mortality was highest (25.74, 23.18, and 18.73%) at 10% concentration compared to the control treatment, which had no mortality, and statistical analysis supported the findings. The extract type likewise influenced the mortality percentage rates of the 4th larval instar. Fruit pulp extract had the highest mortality rates, while seed oil treatment yielded the lowest mortality rates (11.96% and 14.96%, respectively). Leaf extract

Table 4. Effect of Citrullus colocynthis extracts on the mortality of adult females of Rhyzopertha dominica after 24, 48, 72, and 96 h of exposure

Extraction types	Concentration mg/mL	Exposure time/h				Con. Average
		24	48	72	96	
Fruit pulp	2.5	5.41	10.59	14.07	19.9	12.52a
	5	7.9	18.89	26.06	33.5	21.60b
	10	10.05	21.2	29.86	41.79	25.74c
Exp. average		7.80a	16.92b	23.33c	31.76d	
LSD (0.05)	Con. = 0.15		Exp. Time = 0.18		Con. x Exp. time = 0.3	
Leave extraction	2.5	3.911	6.92	11.20	14.41	9.11a
	5	6.555	14.11	22.943	30.019	18.40b
	10	8.755	17.85	27.903	38.213	23.18c
Exp. average		6.41a	12.96b	20.68c	27.55d	
LSD (0.05)	Con. = 0.232		Exp. Time = 0.27		Con. x Exp. time = 0.464	
Seed oil	100	3.09	4.9	8.9	11.9	7.26a
	200	5.03	6.9	20.01	26.8	14.71b
	300	5.9	10.8	24.9	33.1	18.73c
Exp. average		4.69a	7.60b	17.98c	23.98d	
LSD (0.05)	Con. = 0.09		Exp. Time = 0.10		Con. x Exp. time = 0.17	

Means have different letters shows significant difference at the 0.05 according to Tukey's HSD test treatment reduced mortality to 12.97%. The results indicated a significant effect of the overlap between the type of extract and its concentration of the tested plant at different treatment periods, where the highest rate was (41.79, 38.21, and 33.14%) at the concentration of 10% after 96 h of exposure with the studied extracts, respectively, compared to the control treatment that did not record any percentage of mortality (*Table 4*).

3.5. Extracts of C. colocynthis affect R. dominica adult male mortality.

The results in *Table 5* illustrate the impact of varying treatment exposure times and concentrations of bitter melon *C. colocynthis* extracts on the mortality of adult males of *R. dominica*. The highest mortality rates (29.57, 26.1, and 20.40%) were observed for fruit pulp, leaves, and seed oil extracts, respectively, at a concentration of 10% after 96 h. In contrast, no deaths were recorded in the control treatment. There was a statistically significant difference in the percentage mortality when adult males were exposed to the various bitter melon extracts. At 17.34%, the fruit pulp extract had the highest mortality rate, followed by 15.77% for the leaf extract, and finally, it dropped to 11.45% when the seed oil extract was used. Adult male lesser grain borer mortality was found to vary significantly in response to the interaction between concentration and exposure duration to the bitter melon extract. An increase in the concentration and duration of the treatment was associated with a corresponding rise in the mortality rate of adult males.

Table 5. Impact of several concentrations of Citrullus colocynthis extracts on the percentage of adult male mortality of the lesser grain borer Rhyzopertha dominica following 24, 48, 72, and 96 h of exposure

Extraction types	Concentration mg/mL	Exposure time/h				Con. Average
		24	48	72	96	
Fruit pulp	2.5	7.26	12.043	21.31	23.95	16.14a
	5	8.90	20.55	28.13	36.95	23.64b
	10	11.26	25.23	36.72	45.05	29.57c
Exp. average		9.15a	19.28b	28.72c	35.32d	
LSD (0.05)	Con. = 0.12		Exp. Time = 0.28		Con. x Exp. time = 0.5	
Leave extraction	2.5	5.6	7.8	12.9	17.17	10.92a
	5	7.4	15.05	25.7	33.1	25.99b
	10	9.9	19.8	30.06	44.0	26.15c
Exp. average		7.66a	14.27b	30.72c	31.44d	
LSD (0.05)	Con. = 0.232		Exp. Time = 0.27		Con. x Exp. time = 0.464	
Seed oil	100	4.18	6.54	12.06	14.014	16.14a
	200	6.089	9.05	20.8	28.78	23.64ab
	300	7.34	12.8	26.02	35.33	20.37 b
Exp. average		9.15a	19.28b	28.72c	35.32d	
LSD (0.05)	Con. = 0.16		Exp. Time = 0.159		Con. x Exp. time = 0.34	

Means have different letters shows significant difference at the 0.05 according to Tukey's HSD test

4. Discussion

The current study aimed to assess the effectiveness of various *C. colonynthis* extracts in combating different stages of *R. dominica*, to minimize the quality and quantity loss of stored wheat grains. To achieve this objective, the effectiveness of fruit pulp, leaves, and seed oil from the *C. colocynthis* at various dosage levels was evaluated to determine the average percentage of mortality among the targeted pests. The study found that phenolic compounds from the fruit pulps, leaves, and seed oil of bitter melon *C. colocynthis* had different effects on the cumulative mortality of various stages of *R. dominica*.

The impact of bitter melon *C. colocynthis* on the mortality rate of adult stages (male and female) of the tested insect was found to be less significant. The plant extracts contain a variety of harmful chemicals which accumulate in the gut of the insect when examined for the effects on the second and fourth-instar larvae when they eat food contaminated with these extracts. This accumulation likely contributes to the observed effectiveness of the extracts.

The findings of the current study suggest that with greater concentration and exposure duration, the mortality rate of various life stages of *R. dominica* likewise increased. The findings of the current study agree with those of Alvi et al. (2018), who discovered that the extracts from the seeds and leaves of *Rhazya stricta* cause a considerable increase in the mortality rates for the khapra beetle, *Trogoderma granarium* and *R. dominica* when tested in a laboratory setting (Alvi et al., 2018). A study conducted by Ali and Baloch showed that the essential oils derived from the seeds of *C. colocynthis* possess insecticidal properties against *R. dominica*. This was achieved by enhancing insect mortality and reducing the percentage of seed damage and weight loss in wheat grains (Ali and Baloch, 2023).

A separate study conducted by Shabbir et al. found that the mortality effect was primarily observed in *C. colocynthis* at a concentration of 10% after 10 days, resulting in a mortality rate of 89.11%. The findings indicate that the plant extracts examined in this research could be valuable in developing repellent and toxicant compositions to combat the stored pest *Tribolium castaneum* (Shabbir et al., 2019). In another study by Ali et al., a plant-based extract of *C. colocynthis* exhibited a lethal impact on three stored pests *T. castaneum*, *T. granarium* and *Sitophilus granarius*. The extracts showed substantial inhibition of AChE, α-CE, β-CE, ACP, and ALP enzymes (Ali et al., 2021)This agrees with the current study's results, which showed that the extract of C. colocynthis impacted different stages of *R. dominica*.

Another study by Yaman and Şimşek (2022) evaluated the toxic effects of *Hypericum scabrum*, *Hypericum heterophyllum*, and Hypericum perforatum on adult *Rhyzopertha dominica* using ethanol extracts from their respective flowers, leaves, and stems, a major grain insect worldwide. The ethanol extracts at 10% concentration were tested for insecticidal action at 24, 48, and 72 hours. Results showed that after 72 hours, adult *R. dominca* were 44.8 to 88.9% dead, 26.0 to 78.8% alive, and 26.1 to 50.3% alive when exposed to extracts from H. perforatum, *H. heterophyllum*, and *H. scabrum*, respectively. A higher number of H. perforatum infections occur. (Yaman and Şimşek, 2022). The findings of (Yaman and Şimşek, 2022) I agreed with the findings of the current study, in which we found that the Adult of the lesser grain borer *R. dominica* was significantly affected by the ethanol extract of phytochemical compounds of the *C. colocynthis* plant.

In a study conducted by Yılmaz Erhan Koçak (2023), the phosphine resistance ratios of *R. dominica*, were determined, and analysis of dosage discrimination experiments revealed that out of the total of 15 populations examined, three populations were found to consist of specimens that were resistant to phosphine (Koçak and Yılmaz, 2023). Their finding highlighted the fact that it is advisable to utilize alternate methods for managing the lesser grain borer *R. dominica* to prevent the development of resistance to insecticides. For that, this study aimed to examine the phytochemical compounds of the bitter melon *C. colocynthis* against different stages of the lesser gain borer as a promising method to control the stubborn grain borer *R. dominica* without using chemical insecticides, which can harm the environment and increase insecticide resistance.

5. Conclusions

In conclusion, the fruit pulp, leaves, and seed oil of bitter melon *C. colocynthis* have shown promising environmentally friendly insecticidal properties against the smaller grain borer *R. dominica*. C. colocynthis extracts had a pronounced impact on the survival of both adult and larval stages of *R. dominica,* as well as the mortality rate of eggs deposited by females. Further investigation is necessary to ascertain the biological mechanisms by which these phytochemicals affect the lesser grain borer. This study can potentially contribute to the development of effective medicines using phytochemicals.

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Ethical Statement

There is no need to obtain permission from the ethics committee for this study.

Conflicts of Interest

We declare that there is no conflict of interest between us as the article authors.

Authorship Contribution Statement

Concept: Ali, A. N., Khudheyer, A., Mubasher, M.; Design: Ali, A. N., Khudheyer, A., Mubasher, M.; Data Collection or Processing: Ali, A. N., Khudheyer, A., Mubasher, M.; Statistical Analyses: Ali, A. N., Khudheyer, A., Mubasher, M.; Literature Search: Ali, A. N., Khudheyer, A., Mubasher, M.; Writing, Review and Editing: Ali, A. N., Khudheyer, A., Mubasher, M.

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