Research Article

Efficacy of Essential Oils of Two Local Weed Plants in Controlling Major Stored Wheat Insect Pests *Rhyzopertha dominica* (Fabricius) and *Trogoderma granarium* (Everts) Under Laboratory Conditions

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Abstract | The research experiment was conducted in the laboratory to check the efficacy of essential oils of two indigenous plant species i.e., colocynth, *Citrulus colocynthis,* and Akk, *Calotropis gigantea* by using different 03 and 06ml/L concentrations of each plant seed oil against the lesser grain borer, *Rhyzopertha dominica*, and Khapra beetle, *Trogoderma granarium* infesting wheat grains. Observations were made on mortality, weight loss, and insect damaged grain. The results show the maximum mortality 62.0% of *R. dominica* and 34.3% of *T. granarium* at a 6ml/L concentrations of *C. colocynthis*. While lower mortality 1.8% and 5.2% were recorded at 3ml/L concentrations of *C.gigantea*. Whereas, weight loss and insect damage to grain were observed higher 28.3% 27.6% when wheat grains were treated with *C. colocynthis* on both pests. However, miminum weight loss and insect damaged grain 1.06%, 7.06%, and 2.0%, 12.1% were obtained when wheat grains were applied 3ml/L concentrations of extracted *C. gigantea* oil. Thus, it is concluded that colocynth extracted oil as most effective essential oil to protect wheat grains.

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Introduction

Wheat (*Triticum aestivum*) is the most dominant cereal crop in terms of cultivation and consumption all over the world (Ileke, 2011). Around 65-75% of Pakistan's entire wheat harvest is thought to be kept in storage or ware house (Zulfikar *et al.*, 2020). Due to high amount of proteins wheat is considered as the most vital crop around the world (Ileke, 2011). Significant losses caused to stored wheat due insect pests (Strbac, 2002; Kavita, 2004). These pest insect pests are responsible for both quantitative and qualitative adverse effects on cereal crops (Fornal *et al.*, 2007). The most frequently detected beetle pests in warehoused wheat grains with detrimental to the economy are, *Rhyzopertha dominica* (Bostrichidae: Coleoptera) which is a particularly harmful pest that infests all types of stored grains. The majority of this insect's whole life cycle passes inside grain kernels and where it feeds on the endosperm of grains (Rees, 2004; Edde, 2012) and another world's abundantly damaging insect pest of stored goods has been considered is *Trogoderma granarium* (Coleoptera: Dermestidae) (Lowe *et al*., 2000; Gharib, 2004).

Therefore, it is mandatory to minimize such losses by controlling pests on grains that are being stored. For this, in the past few decades, numerous scientists have designed various insect pest control methods to tackle these dangerous insect pests throughout the world. In Pakistan, synthetic pesticides are applied in huge quantity against insect pests of stored wheat grains (Khan and Marwat, 2004).

Local plants essential oils recently have attracted a lot of interest to protect stored grains against insect attack. They are natural substances made from various parts of plants, degrade quickly, and pose less of a threat to both human health and the environment (Ziaee and Moharramipour, 2013). Wong *et al*. (2005) for the insect repellency to the wheat grains, tested different oil extracts of pyrethrum, neem, pine, citronella, and garlic materials and obtained effective results in controlling wheat grain pests. Further, Yadav *et al*. (2008) assessed various concentrations of 0.1, 0.5, and 1.0 % (v/w) of plant essential oils to control *S. oryzae* on grains of wheat and observed at a concentration of 1.0 % of lemongrass, clove, karanj, and neem oils were most effective in lowering adult emergence, fecundity, longevity, weight loss, grain damage, and lengthening developmental period. The oil extracts of the plants *Luppia rugose, Vepris heterophylla*, and *Xylopia aethiopica* were utilized in Northern Cameroon for protecting the stored grains from insect pests (Ngamo *et al*., 2007). The biological activities of essential oils are concerned with their chemical nature, plant part, environmental factors, and the extraction technique (Angioni *et al*., 2006; Nerio *et al*., 2010; Zapata and Smagghe, 2010). In addition, it is necessary to find more efficient alternatives to ensure the security of the goods that are kept. In the light of above previously mentioned information. The present study was designed to ascertain the efficacy of essentialoils of indigenous plants against *R. dominica* and *T. granarium*.

Materials and Methods

The current research was carried out during the period from April, 2022 to December, 2022 at the Advanced Entomology Lab, Department of Zoology, University of Sindh, Jamshoro, Pakistan.

Source and extraction of indigenous plant oils

Fresh seeds of the two plant samples: *C. colocynthis* and *C. gigantea* were obtained from several sites in District Dadu. After washing with tape water, the samples were kept under the sun for drying and sundried 50g seeds of each test plant were individually grounded with the help of an electric blender (National, China) to gain fine powder and used for oil extraction. For the extraction of essential oils, subjecting plants seeds into Soxhlet apparatus (BOMEX, China). After extraction, a water bath (Julabo WS 100, Germany) was utilized to evaporate the extracting solvent (n-hexane) from the mixture to obtain the pure essential oil. The stock oils (Bitter apple or akk) were kept into the refrigerator at 4°C until for later use to prepare two different concentrations of Eos (03 and 06ml/L).

Collection and disinfestation of wheat grains

Wheat kernels (TD-1 variety) were taken from Johi City, District Dadu. The grains were cleaned of any damaged seeds and debris before being disinfested at 50°C in an oven for six hours to eliminate all life phases of beetle pests present in the grains. After that, grains were left to stabilize at room temperature for 24 hours and utilized for experimental purposes.

Establishment of stock culture and stored grain insect pests

The adults for *R. dominica* and *larvae* of *T. granarium* were acquired from wheat warehouses from the various localities of district Dadu and brought to lab and placed in glass jar having wheat grains for the establishment of stock culture.

Experimental procedure

The efficacy of the tested oil extracts in protecting wheat kernels or grains against attacks from the lesser grain borer and Khapra beetle was evaluated using the appropriate dosage for each type of oil. First, a glass jar's bottom was lined with Double Rings filter paper (11 cm) and glued horizontally inside for this purpose. After 20 minutes, varying solutions (3 and 6 ml/L) were applied individually using syringes to the filler paper in an attempt to distribute them evenly. Secondly to ensure a uniform coating of grains, 150g of wheat was added to a 250g plastic jar and shaken for 10 minutes with filter paper that had been treated with oil by the use of mixer/shaker machine (Heidolph Reax, 2000). Third, thirty unequal-sized larvae and adults of khapra beetle as well of lesser grain borer were introduced into every plastic jar along with control (without oil implement) as triplicate. In the end, jars were secured with muslin clothes through the use of double rubber bands in the lab at 26°C±2 and 69±5% of both temperature and relative humidity, respectively. The monthly readings were recorded over seven-month, and 50g of oil applied or without wheat grains from each jar were taken and examined for death rate, weight loss, and insect-damaged grains.

Data compilation

Corrected mortality: Monthly from each jar, the number of dead beetles calculated and recorded. The mortality rate in percent was corrected by the utilization of the Abbot formula (Abbot, 1925).

$$M_c = (M_o - M_e) \times 100\ (100\text{-}M_e)$$

M_o: observed mortality (%), M_e: controlled mortality (%), M_c: corrected mortality (%).

Weight loss

After removing the insects from every single jar, the number of damaged and still uninfected was calculated and weight loss concerning insects attacks was recorded by making use of the formula "Count and Weigh method" explained by (Adams and Schuton, 1978).

$$\text{Weight loss (\%)} = \frac{(W\mu \times Nd) - (Wd \times N\mu)}{Wu \times (Nd + Nu)} \times 100$$

Wµ: weight of undamaged grains, Nµ: number of undamaged grains, Wd: weight of damaged grains, Nd: number of damaged grains.

Insect damaged grain

A 50-gram sample of cleaned grain was gathered from everyone replication of the corresponding wheat variety. The following formula was used to calculate the percent damage caused by insects (Oparaeke and Daria, 2005).

$$\text{Insect damaged grain (\%)} = \frac{\text{Number of bored grains}}{\text{Total number of grains in sample}} \times 100$$

Statistical analysis

Using Statistix® version 8.1 software, analysis of variance (ANOVA) was applied to do statistical analysis on the collected data, and the least significant difference (LSD) was also used to identify significant treatment differences.

Results and Discussion

The comparative studies for the efficacy of different essential oils concentrations of *C. colocynthis* and *C. gigantea* were evaluated against the mortality rate of *R. dominica* and *T. granarium*. The data revealed that 6ml of oil extracted from *C. colocynthis* treated with TD-1 wheat variety was observed most effective exhibiting the highest mortality to the *R. dominica* followed by *T. granarium*. However, the non-significantly (P>0.05) highest mean percent mortality of *R. dominica* was observed followed by *T. granarium* in June-2022 at 06ml dosage of *C. colocynthis* in comparison to untreated wheat grains. Whereas, at a 3ml dosage of selected both oil extracts, lower mortality was recorded against the infestation of treated stored grain pests in the month of May, 2022 (Tables 1 to 4). The figures of mortality percent raised with the rise in concentration of essential oils.

Table 1: *Efficacy of essential oil of C. colocynthis plant against R. dominica in wheat.*

Month and year	3ml			6ml		
	Mortality rate (%)	Weight loss (%)	Damaged grains (%)	Mortality rate (%)	Weight loss (%)	Damaged grains (%)
May, 2022	$24.2^a \pm31.7$	$4.1^b \pm3.63$	$16.1^a \pm20.7$	$46.2^{ab} \pm22.6$	$3.13^b \pm3.35$	$11.3^b \pm4.61$
June, 2022	$45.0^b \pm22.6$	$1.06^a \pm0.11$	$7.06^e \pm3.49$	$62.0^a \pm28.8$	$0.96^d \pm0.15$	$5.0^a \pm3.46$
July, 2022	$40.0^c \pm35.1$	$1.31^d \pm1.26$	$9.3^e \pm2.06$	$60.0^b \pm21.7$	$1.0^c \pm0.98$	$6.23^b \pm2.05$
August, 2022	$37.0^d \pm15.3$	$1.83^c \pm1.44$	$10.6^d \pm4.0$	$55.0^c \pm33.0$	$1.2^c \pm0.91$	$7.2^c \pm2.81$
September, 2022	$35.0^a \pm30.3$	$2.0^b \pm1.0$	$11.6^a \pm9.45$	$52.2^d \pm34.9$	$1.36^a \pm0.28$	$8.13^d \pm5.58$
October, 2022	$32.0^b \pm16.7$	$2.58^c \pm15$	$13.1^c \pm8.15$	$50.1^a \pm24.0$	$1.63^b \pm0.6$	$9.1^c \pm3.85$
November, 2022	$28.3^d \pm32.4$	$3.1^c \pm1.15$	$14.3^b \pm2.85$	$49.1^b \pm25.6$	$2.43^d \pm0.51$	$9.95^b \pm5.4$
December, 2022	$25.2^c \pm15.5$	$3.5^b \pm3.96$	$15.0^c \pm6.06$	$47.1^d \pm7.35$	$2.9^b \pm3.37$	$10.2^b \pm2.96$
Control	$10.0^a \pm9.0$	$18.0^a \pm3.46$	$23.0^a \pm4.35$	$19.0^b \pm16.5$	$20.3^a \pm2.51$	$21.2^a \pm20.0$

Mean±SD within columns with the different letter(s) are significantly differ at (LSD test, P> 0.05).

Table 2: *Efficacy of essential oil of C. gigantea plant against R. dominica in wheat.*

Month and year	3ml			6ml		
	Mortality rate (%)	Weight loss (%)	Damaged grains (%)	Mortality rate (%)	Weight loss (%)	Damaged grains (%)
May, 2022	30.1^{ab} ±28.2	4.4^{b} ±3.84	17.2^{a} ±15.5	40.4^{ab} ±17.5	4.0^{b} ±3.46	13.0^{ab} ±9.16
June, 2022	24.1^{ab} ±15.5	5.23^{bc} ±1.64	18.2^{ab} ±6.17	37.3^{ab} ±15.2	4.2^{bc} ±5.2	13.9^{b} ±3.15
July, 2022	21.3^{a} ±27.4	5.63^{bc} ±3.19	19.3^{abc} ±1.41	34.1^{ab} ±15.3	5.12^{bc} ±3.62	14.2^{abc} ±3.57
August, 2022	18.7^{a} ±29.6	6.2^{b} ±1.73	20.2^{a} ±16.2	32.9^{ab} ±15.4	5.8^{b} ±5.46	15.2^{b} ±1.56
September, 2022	17.2^{ab} ±14.1	7.3^{b} ±3.31	21.8^{ab} ±3.27	30.4^{ab} ±14.1	6.1^{b} ±7.72	16.3^{ab} ±8.8
October, 2022	13.6^{ab} ±9.5	8.33^{b} ±3.51	23.2^{a} ±4.87	28.1^{ab} ±14.9	7.1^{b} ±7.71	18.0^{ab} ±3.0
November, 2022	10.1^{abc} ±7.85	11.3^{a} ±15.3	25.1^{a} ±6.87	23.1^{ab} ±12.0	8.1^{b} ±4.74	20.3^{a} ±11.4
December, 2022	5.2^{bc} ±5.89	16.2^{a} ±16.7	27.6^{a} ±2.68	20.5^{ab} ±9.34	11.2^{ab} ±9.88	25.3^{a} ±16.6
Control	8.0^{a} ±8.71	18.1^{a} ±3.63	30.0^{a} ±10.1	7.1^{b} ±5.00	17.1^{a} ±6.44	28.0^{a} ±22.5

Mean±SD within columns with the common letter(s) are not significantly differ at (LSD test, P< 0.05).

Table 3: *Efficacy of essential oil of C. colocynthis plant against T. granarium in wheat.*

Month and Year	3ml			6ml		
	Mortality rate (%)	Weight loss (%)	Damaged grains (%)	Mortality rate (%)	Weight loss (%)	Damaged grains (%)
May, 2022	10.2^{ab} ±9.38	7.0^{ab} ±2.0	19.1^{a} ±5.72	23.3^{ab}±17.4	4.16^{b} ±5.05	16.0^{abc} ±5.56
June, 2022	16.0^{a}±7.93	2.0^{c} ±0.87	12.1^{cd} ±2.81	34.3^{a} ±24.5	0.9^{b} ±0.98	9.1^{bc} ±2.0
July, 2022	15.2^{ab} ±9.71	2.8^{b} ±2.3	13.1^{ab} ±9.47	30.4^{ab} ±21.3	1.0^{b} ±0.0	10.1^{a} ±7.41
August, 2022	14.3^{ab} ±10.6	3.1^{b} ±3.63	14.2^{a} ±17.5	29.3^{ab} ±13.3	1.1^{c} ±1.0	11.0^{a} ±7.11
September, 2022	14.0^{a} ±12.0	3.8^{bc} ±0.91	15.2^{b} ±8.15	28.3^{ab} ±20.4	1.6^{bc} ±0.6	12.0^{b} ±8.88
October, 2022	13.2^{ab} ±8.58	4.2^{bc} ±2.02	16.2^{ab} ±13.8	27.1^{a} ±9.68	2.1^{b} ±1.96	13.2^{ab} ±3.36
November, 2022	12.3^{abc}±5.54	5.1^{b} ±2.59	17.1^{a} ±15.6	25.2^{ab} ±16.3	3.0^{b} ±2.64	14.2^{a} ±11.1
December, 2022	12.0^{a} ±9.16	6.1^{ab} ±8.83	18.2^{ab} ±11.4	24.3^{ab} ±10.0	3.1^{b} ±2.81	15.0^{abc} ±9.16
Control	2.0^{d} ±1.73	14.3^{a} ±4.04	22.1^{a} ±2.15	3.2^{c} ±2.07	25.3^{a} ±11.3	34.0^{a} ±21.2

Mean±SD within columns with the common letter(s) are not significantly differ at (LSD test, P < 0.05).

Table 4: *Efficacy of essential oil of C. gigantean plant against T. granarium in wheat.*

Month and year	3ml			6ml		
	Mortality rate (%)	Weight loss (%)	Damaged grains (%)	Mortality rate (%)	Weight loss (%)	Damaged grains (%)
May, 2022	1.8^{d} ±0.69	13.1^{ab} ±14.5	28.3^{a} ±4.01	10.3^{ab} ±11.3	9.96^{b} ±7.43	24.1^{a} ±17.4
June, 2022	5.1^{cd} ±3.77	6.9^{ab} ±5.19	20.1^{a} ±8.15	17.3^{ab} ±10.1	5.16^{b} ±5.1	17.6^{ab} ±13.6
July, 2022	4.3^{bcd} ±2.85	7.2^{bc} ±4.84	21.3^{a} ±9.71	17.1^{ab} ±7.9	5.33^{b} ±3.86	18.2^{ab} ±7.3
August, 2022	3.7^{cd} ±4.63	8.2^{ab} ±4.7	23.5^{a} ±11.2	15.2^{ab} ±11.0	5.9^{b} ±3.73	19.3^{a} ±14.4
September, 2022	3.0^{ab} ±2.0	9.3^{ab} ±8.02	24.6^{a} ±6.5	15.2^{ab} ±10.2	6.2^{b} ±3.93	20.1^{abc} ±10.3
October, 2022	2.1^{d} ±0.85	10.1^{b} ±7.7	25.3^{ab} ±1.13	13.1^{ab} ±6.89	6.8^{b} ±3.93	21.3^{a} ±17.9
November, 2022	2.0^{cd} ±1.0	11.3^{ab} ±12.0	26.3^{a} ±5.85	13.1^{ab} ±10.8	7.1^{b} ±3.15	22.1^{a} ±14.0
December, 2022	1.9^{b} ±0.85	12.3^{ab} ±10.1	27.9^{a} ±11.7	12.2^{ab} ±8.90	8.33^{b} ±6.5	23.9^{ab} ±13.8
Control	12.0^{a} ±10.5	18.0^{a} ±8.0	26.0^{a} ±5.29	2.0^{b}±1.0	14.0^{a} ±2.0	20.0^{a} ±8.66

Mean±SD within columns with the common letter(s) are not significantly differ at (LSD test, P < 0.05).

The result regarding weight loss and insect damaged grain mean percent induced by the activity of both insect grain pests on the oil treated and untreated grains of wheat as indicated in (Tables 1-4). The data recorded shows that maximum values of both treatments were found from June to August-2022 at applied lower to higher dosages of Eos. Nevertheless, weight loss and insect damaged grain treatments of mean percent decrease by the increase in concentrations of plants Eos. Non-significantly (P>0.05) oil treated values were observed to decline as compared to the untreated control. From June to July-2022 regarding peak mortality of both grain pests were recorded, but the weight loss and insect damaged grains from three months were noted in May, November, and December-2022. Also in similar months, lower mortality was observed, but the remaining two treatments declined noticed from June to August-2022. Moreover, at a moderate level, three treatments were found from September to October-2022 (Figures 1 and 2).

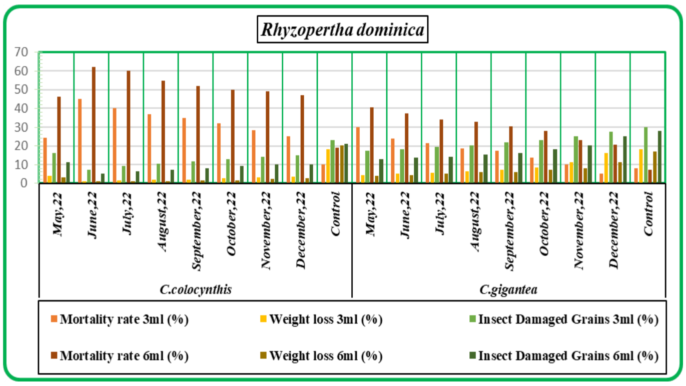

Figure 1: *Monthly (Mean) effect of varying amount (3 & 6ml) of essential oils against the R. dominica reared on treated wheat grain.*

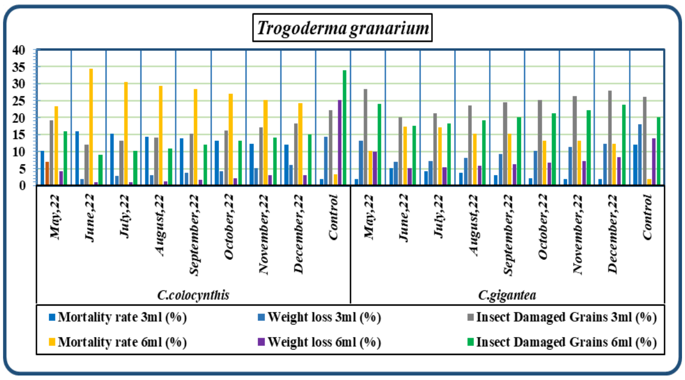

Figure 2: *Monthly (Mean) effect of varying amount (3 & 6ml) of essential oils against the T. granarium reared on treated wheat grain.*

The purpose of study was to find out the effectiveness of indigenous plant extracts or oils against two separate *R. dominica and T. granarium* pests to reduce both the quality and quantitative loss of stored wheat grains. For this objective, the efficacy of seed oil from the two test plants *C. colocynthis,* and *C. gigantea* at different dosage levels was assessed to determine the mortality means % of the selected pests. Our research indicates that oil extract from two chosen natural plants at 6ml generated the maximum percentage mortality of the test pests in June 2022. This outcome is consistent with that of Alvi *et al*. (2018), who observed that seed and leaf extracts of *Rhazya stricta* significantly raise mortality levels in the case of *T. granarium* and *R. dominica* species when used in a lab condition. Similar results were discovered by Al-Ameri *et al*. (2020), who noted that oil dosages of spearmint (*Mentha spicata*) when elevated than *T. granarium* mortality levels at various life stages were same observed to be higher in comparison to increasing rates of colocynth essential oil dosages. In accordance with Ogendo *et al*. (2004), *Tephrosia vogelii* extracts forced over fifty percent of induced mortality on *Sitophilus zeamais.*

According to the investigation of Don-Pedro (1989), the death of insect pests resulting from the extraction of oils may be caused by either of anoxia or a disruption in their normal breathing that leads to suffocation. Moreover, in lab research done by Laznik *et al*. (2012) who found rosemary caused a 60% higher mortality rate in adult granary weevil pests among essential oils from four plant species than the control. The same ratio leading to the control group in comparable outcomes to the oil dosage rate of plants on treated wheat grains was recorded in current studies.

In this research, we assessed the cumulative weight loss resulting from *R. dominica* and *T. granarium* on wheat grain of treated and untreated. At the higher dosage of 6ml, mean percent figures for weight loss in wheat kernels against the damage of both insect pests were noted lowest in the month of June-2022. The same results have been found by Chougourou *et al*. (2015), who stated that the lowest percentage of weight loss was produced by 7.5g at higher dosages of *C. ambrosioides*. Furthermore verified (Jakhar and Jat, 2010) that, weight loss in wheat grain was elevated with lower neem oil dosages but minimized with higher concentrations (0.1 to 1.0 ml/100g seeds). In addition, compared to the untreated control treatment, mean percent figures of weight loss were found to be significant (P<0.05) lower in oil applied grain caused by examined insect species in our obtained record. Asawalam and Onu (2014) also reported a similar observation.

In the final test, insect damaged grain parameter caused by both tested beetle pests on the oil treated and untreated wheat grains was studies. The results of the measurement of that parameter were significantly different from the untreated control and were seen to be decreased on the damage of wheat grain caused by the test insects, *T. granarium* and *R. dominica* at increased 6ml extracted oil of two specified *C. colocynthis,* and *C. gigantea* plants in June, 2022.

The results are in line with Kumawat and Naga (2013), who determined that lower dosages of eucalyptus oil and karanj oil, each at 0.5 percent, resulted in less grain damage from *R. dominica* than higher dosages of both products, each at 0.1 percent, which caused the most insect-damaged grain on treated food kernels. As described by Singhamony *et al.* (1986) that, wheat was extremely effectively protected by the infestation of *R. dominica* whenever treated with Karanj oil at levels ranging from 25 to 100 ppm. Moreover, reports of Hagstrum and Subramanyam (2006) demonstrated that after one and two weeks lower damage in stored grains is expected since food pests due to the shorter period of time when exposed to the grain. Further, the findings of Huang *et al.* (2007) demonstrated that after one and two weeks lower damage in stored grains is expected since food pests due to the shorter period of time when exposed to the grain. Sunilkumar (2003) discovered more harmful values at 30 and 60 DAT, this greater harm may have been brought on by the diverse laboratory environment. Additionally, the outcomes of this research showed that the seeded oil extract of the two trial plants had insecticidal potential towards the damaging effect of *R. dominica* and *T. granarium* on the preservation grains of wheat for an extended period.

Conclusions and Recommendations

It was shown that essential oils extracted from the seeds of *C. colocynthis* and *C. gigantea* had insecticidal potential against *R. dominica,* and *T. granarium* by increasing insect mortality and lowering the percent values of seed damage and weight loss in wheat grains. Among these two extracted oils, *C. colocynthis* at 06 ml/L might be an excellent replacement for traditional chemical control management. To regard weight loss and damaged grain, it was found that wheat variety TD-1 was resistant to *T. granarium* while vulnerable to *R. dominica.* Hence, it is suggested that examination of tested plants concerning at any extracted dosage could helpful to protect against damage rate from the attack of insect pests in stored products.

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Novelty Statement

In the present study, pure seed oils of *C. colocynthis* and *C. gigantea* were tested as grain protectants versus stored grain pests would be helpful especially in the advancement of pest management strategies.

Author's Contribution

Shamsher Ali: Principal author conducted experiment; data analyzed and wrote the manuscript.
Naheed Baloch: Conceived, designed and supervised the work.

Conflict of interest
The authors have declared no conflict of interest.

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