Punjab University Journal of Zoology

39(1): 61-67 (2024)
https://dx.doi.org/10.17582/journal.pujz/2024/39.1.61.67

Research Article

Investigating the Efficacy of Various Plant Essential Oils in Controlling Red Flour Beetle, *Tribolium castaneum* (Coleoptera: Tenebrionidae)

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Article History
Received: May 23, 2023
Revised: March 05, 2024
Accepted: March 27, 2024
Published: April 26, 2024

Authors' Contributions
MIU and ABMR conceived the tudy. MIU planned methodolog. KA and HL wrote manuscript. HL, KA, ES and ABMR collected data. SK, MA, KA, NP, ES and ABMR reviewed manuscript. MA did formal analysis. AA helped in experimentation.

Keywords
Essential oils, Insecticidal activity, Pest control, Stored product, Red flour beetle

Abstract | The red flour beetle, *Tribolium castaneum* (Herbst) (Coleoptera: Tenebrionidae), is an important insect pest that causes significant economic losses and food insecurity in stored grain products. This research aimed to assess the efficacy of plant essential oils against *T. castaneum*. The study found that cinnamon oil exhibited the highest insecticidal activity, with a 100% mortality rate for all tested concentrations and time intervals for adult beetles. Clove oil also displayed significant insecticidal activity, resulting in mortality rates between 68.0% to 86.6% for the highest concentration (6.0%). However, lemon and orange oils exhibited the lowest insecticidal activity against adult *T. castaneum*. Additionally, cinnamon oil demonstrated 100% mortality in the immature stage within 72 hours of the application of higher concentrations (6.0%), while a higher concentration of clove oil resulted in 88.0% larval mortality within 96 hours. Probit analysis demonstrated that cinnamon oil had the lowest LC_{50} values across all time intervals, indicating the highest potency in killing *T. castaneum*. Clove oil also had a relatively low LC_{50} value compared to orange and lemon oil. Furthermore, cinnamon and clove oils displayed negative effects on pupal formation and adult emergence of *T. castaneum*. Based on these findings, cinnamon and clove oils can be recommended as natural alternatives to chemical pesticides to control *T. castaneum* in grain storage facilities due to their high efficacy.

Novelty Statement | Our study reveals the remarkable insecticidal activity of cinnamon and clove oils, showing their potential as potent natural alternatives to conventional chemical pesticides. The findings underscore a promising avenue for sustainable pest management strategies, emphasizing the pivotal role of plant essential oils in mitigating economic losses and ensuring food security in grain storage facilities.

To cite this article: Ullah, M.I., Akram, K., Raza, A.B.M., Arshad, M., Khalid, S., Latif, H., Amjad, A., Perveen, N. and Sajid, E., 2024. Investigating the efficacy of various plant essential oils in controlling red flour beetle, *Tribolium castaneum* (Coleoptera: Tenebrionidae). *Punjab Univ. J. Zool.*, **39**(1): 61-67. https://dx.doi.org/10.17582/journal.pujz/2024/39.1.61.67

Introduction

The globally distributed red flour beetle, *Tribolium castaneum* (Herbst) (Coleoptera: Tenebrionidae), is a harmful insect pest that causes significant damage to stored grains and milled cereal products, especially in already damaged wheat (Campbell *et al*., 2010; McKay *et al*., 2019). This beetle feeds on a wide range of stored food products, including rice, oats, corn, and various cereals, and its infestation can lead to substantial quantitative and qualitative losses, turning affected products yellowish and emitting a pungent smell due to the release of carcinogenic liquid (Ahmad *et al*., 2022). Infestation by this beetle can

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result in up to 80% loss of stored grains during storage, leading to significant economic losses for farmers and food processors (Kuyu *et al.*, 2022). Moreover, the beetle's excreta, dead bodies, and body parts can contaminate the products, rendering them unsuitable for consumption by humans or animals and causing regulatory issues and costly product recalls for businesses (Astuti *et al.*, 2020).

Synthetic insecticides, including malathion, deltamethrin, dichlorvos, and phospine tablets, have been primarily used to control *T. castaneum* (Paudyal *et al.*, 2016; Yao *et al.*, 2019). However, their repeated use has resulted in resistance, negative impacts on non-target organisms and humans, and the disruption of the biological control system (Zhu *et al.*, 2016; Nayak *et al.*, 2020).

To address these concerns, biopesticides have emerged as a promising alternative to synthetic insecticides due to their high content of bioactive chemicals. There is growing interest in developing plant-derived insecticides that are effective, safe, and sustainable alternatives to synthetic chemicals (Singh *et al.*, 2021; Nikolaou *et al.*, 2021). Plant-derived essential oils (EOs) demonstrate potential as effective insect control agents in stored grain, presenting a promising path for commercial application (Isman, 2020; Garrido-Miranda *et al.*, 2022; Azeem *et al.*, 2022). EOs contain volatile mixtures of monoterpene, sesquiterpene, and hydrocarbons, making them highly effective against target insect pests with low persistency in the environment and reduced likelihood of insect pest resistance. They have several advantages over synthetic insecticides, including less toxic to predators and parasitoids, less persistency level, and reduced risk of developing resistance among insect pests (Demeter *et al.*, 2021). EOs may also provide multiple entry points for pest control and may exert a variety of mechanisms of action at once, impairing the ability of insects to move or reproduce, or causing cellular toxicity (Oviedo *et al.*, 2021). Over 3000 EOs from plants have been identified that are being used to suppress the population of various insect species (Isman, 2020). These alternative biocontrol strategies could be instrumental in reducing the use of synthetic insecticides and promoting sustainable agricultural practices.

Using plant EOs against insect pests could contribute to the development of alternative pest control methods that are safe, effective, and environmentally friendly. Therefore, the primary objective of this study was to determine the comparative efficacy of plant EOs in controlling *T. castaneum*.

Materials and Methods

Insect culture

The mixed population of *T. castaneum* was collected from a local grain market of Sargodha. The population was brought to the Laboratory of Entomology, College of Agriculture, University of Sargodha and maintained under controlled conditions of 30±2°C temperature and 65±5% R.H. in the incubator. The culture of the tested insect was initiated on mild wheat flour. About 100 g of mild wheat flour was filled in glass jars (500g). A total of 50 pairs of adult beetles were released in each jar, covered with nylon cloth. The homogeneous population of the 3rd generation of *T. castaneum* was used for experimental purpose.

Plant essential oils

Four essential oils i.e., cinnamon, *Cinnamomum aromaticum* (Lauraceae), orange, *Citrus sinensis* (Rutaceae), lemon, *Citrus limon* (Rutaceae) and clove, *Syzygium aromaticum* (Myrtaceae) were tested against *T. castaneum*. Serial dilution (6.0% 3.0% and 1.50%) of each tested oil was prepared by following the method of Mondal *et al.* (2011).

Residual film bioassay against adults T. castaneum

The essential oils (1 mL volume of each concentration) were applied on the surface of a Petri dish (9 cm) and air dried for 1 h. Methanol was used as a control treatment. After application, Petri dishes were left to dry. Each concentration was replicated 5 times and 15 beetles were tested in each replication. The experiment was performed under controlled conditions of 30±2°C temperature and 65±5% R.H. Mortality data of the adult beetles were recorded after 24, 48, 72 and 96 h of application (Mondal *et al.*, 2011). Percent corrected mortality was calculated by Abbott's formula (Abbott, 1925).

$$Corrected\ mortality = \left[\frac{Mt - Mc}{100} - Mc\right] \times 100$$

Where, M_t = mortality data in treatment, M_c = mortality data in control

Fumigation bioassay against T. castaneum *larvae*

Most effective essential oils (cinnamon and clove oil) from residual film bioassay were selected to test efficacy against larvae of *T. castaneum*. Glass vials covered with stoppers were used for fumigation bioassay. Fifteen larvae were shifted to the vials and enclosed with nylon cloth. Methanol was used in the control treatment and the bioassay was carried out at room temperature with 14:10 h light: Dark condition. Mortality data were recorded after 24, 48, 72 and 96 h of application.

Pupal formation and adult emergence

In this experiment, the most effective essential oils were tested on mature larvae to determine pupal formation and adult emergence by using diet incorporation bioassay. The experiment was replicated 5 times and 10 larvae were tested in each replication. About 50g wheat grains were treated with 1 mL of each solution in Petri dishes and air dried for 1 h. For control, wheat grains were treated with methanol. The Petri dishes were placed in an incubator at 30±2°C temperature and 65±5% R.H. Data on percent pupal formation and adult emergence were recorded on daily basis.

Statistical analysis

Data were analyzed by using two-way ANOVA by keeping essential oils and concentration as main treatments to check the significance. For corrected mortality of adults and larvae of *T. casaneum,* means were compared by Tukey HSD all-pairwise comparison test by using Minitab 17.0 software. Probit analyses were performed to assess the toxicity of essential oils by using POLO software.

Results and Discussion

The plant essential oils showed significant mortality of adult *T. casaneum* after 24 h (F = 4.43, $P < 0.05$), 48 h (F = 4.29, $P < 0.05$), 72 h (F = 4.30, $P < 0.05$), and 96 h (F = 10.84, $P < 0.001$). Cinnamon oil was highly effective causing 100% mortality of adult beetles after 48 h by using a higher concentration (6.0%). To a greater extent, the mortality rate was 95.9% at 24 h intervals by using a 6.0% concentration of cinnamon oil. By using 3.0% concentrations of cinnamon oil, the mortality rate was 74.6-85.3% and 64.2-71.9% mortality was observed with 1.50% concentration. Clove oil showed 81.3% mortality of adult beetles at 72 h and 86.6% at 96 h by using a higher concentration (6.0%). Lemon oil and orange oils were least effective with a maximum mortality rate of 46.5% and 25.3% respectively at 96 h intervals when a higher concentration of both oils was used (Table 1).

Table 1: Corrected mortality (means±SE) of adults *Tribolium castaneum* exposed to different concentrations of plant essential oils at different time intervals.

Treatments	Conc. (%)	24 h	48 h	72 h	96 h
Cinnamon oil	6.00	95.92±0.03a	100.0±0.00a	100.0±0.00a	100.0±0.00a
	3.00	74.64±0.32b	77.33±0.45b	82.65±0.41b	85.32±0.32b
	1.50	64.24±0.26c	64.65±0.32c	67.99±0.37c	71.98±0.26c
Clove oil	6.00	68.00±0.63c	78.65±0.95b	81.33±0.76b	86.64±0.83b
	3.00	52.26±0.26d	53.60±0.32d	60.40±0.44d	63.00±0.52d
	1.50	38.46±0.41e	43.45±0.52e	44.43±0.41e	25.28±0.48e
Orange oil	6.00	20.88±0.26g	24.00±0.33f	24.43±0.32f	25.28±0.36g
	3.00	7.973±0.02h	9.31±0.09g	11.99±0.03g	11.99±0.09h
	1.50	1.999±0.02i	3.33±0.06g	4.00±0.02h	7.31±0.02i
Lemon oil	6.00	40.00±0.12e	40.00±0.12e	44.77±0.15e	46.49±0.18e
	3.00	26.12±0.11f	28.27±0.12f	29.61±0.11f	34.62±0.13f
	1.50	20.88±0.12g	23.09±0.14f	25.69±0.17f	29.67±0.18g
Sign.		F= 4.43**	4.29**	4.30**	10.84***

** shows the significance at P < 0.05 and *** shows the significance at P < 0.001, means with similar letters within a column are not significantly different at P > 0.05,

Table 2: Median lethal concentration (LC_{50}) values for selected plant essential oils evaluated against adult *Tribolium castaneum* at different time interval.

Essential oil	Time (h)	LC_{50} (%)	Slope ± SE	Upper and lower 95%	X2 (df=13)*	P-value
Cinnamon oil	24	1.084	2.708±0.211	1.318-0.788	36.304	<0.05
	48	1.047	2.450±0.198	1.431-0.498	91.831	<0.05
	72	1.046	2.494±0.201	1.345-0.651	53.84	<0.05
	96	1.097	2.817± 0.227	1.347-0.790	31.317	<0.05
Clove oil	24	2.214	1.402±0.137	2.539 -1.868	16.409	<0.05
	48	2.08	1.740±0.141	2.512-1.593	46.905	<0.05
	72	2.225	1.529±0.138	2.533-1.900	17.315	<0.05
	96	2.374	1.459± 0.137	2.719-2.020	18.273	<0.05
Orange oil	24	19.447	1.547± 0.189	46.916-12.40	21.37	<0.05
	48	13.96	1.855±0.191	25.817-9.941	25.09	<0.05
	72	17.165	1.638 ± 0.19	37.456-11.40	22.67	<0.05
	96	23.772	1.348± 0.181	57.374-14.79	14.866	<0.05
Lemon oil	24	9.891	0.945 ± 0.14	16.39-7.236	9.024	<0.05
	48	10.816	0.880 ± 0.14	19.63-7.764	7.131	<0.05
	72	9.663	0.942± 0.139	15.901-7.219	8.167	<0.05
	96	10.124	0.95 ± 0.140	16.883-7.507	9.665	<0.05

Table 3: Percent larval mortality (means±SE) of *Tribolium castaneum* exposed to different concentrations of plant essential oils at different time intervals.

	Concentrations	24 h	48 h	72 h	96 h
Cinnamon oil	6.00	56.00 ±3.02a	76.00±3.01a	100.0±0.00a	100.0±0.00a
	3.00	38.00±3.02b	66.00±3.01b	84.00±3.00b	100.0±0.00a
	1.50	24.00±3.01cd	44.00±3.01cd	66.00±2.67c	76.00±2.88c
Clove oil	6.00	30.00±0.78bc	46.00±1.02c	64.00±1.11c	88.00±2.38b
	3.00	26.00±0.58cd	38.00±0.89d	45.80±1.53d	55.80±2.55d
	1.50	18.00±0.18d	28.00±0.32e	31.60±0.55e	37.60±1.01e
Sign.		6.87**	5.38**	5.11**	29.17***

** shows the significance at $P < 0.05$ and *** shows the significance at $P < 0.001$, means with similar letters within a column are not significantly different at $P > 0.05$.

Probit analysis also indicated the same trend in the effectiveness of essential oils against adult *T. casaneum*. The results showed cinnamon oil and clove oil as effective chemicals with lower LC_{50} values. In the case of cinnamon oil, the LC_{50} values were 1.08% (0.78-1.31%) at 24 h, 1.04% (0.49-1.43%) at 48 h, 1.04% (0.65-1.34%) at 72 h and 1.09% (0.79-1.34%) at 96 h. Similarly, LC_{50} values for clove oil are 2.21% (1.86-2.53%) at 24 h, 2.08% (1.59-2.51%) at 48 h, 2.22% (1.90-2.53%) at 72 h and 2.37% (2.02-2.71%) at 96 h (Table 2).

The results showed that essential oils had a significant effect on larvae of *T. casaneum* at 24 h (F = 6.87, $P < 0.05$), 48 h (F = 5.38, $P < 0.05$), 72 h (F = 5.11, $P < 0.05$) and 96 h (F = 29.1, $P < 0.001$). Cinnamon oils showed 100% mortality in the immature stage at 72 h with the application of higher concentration (6.0%). By using a 3.0% concentration of cinnamon oil, 84.0% mortality was recorded at 72 h which reached up to 100% at 96 h. A higher concentration of clove oil showed 88.0% larval mortality at 96 h (Table 3).

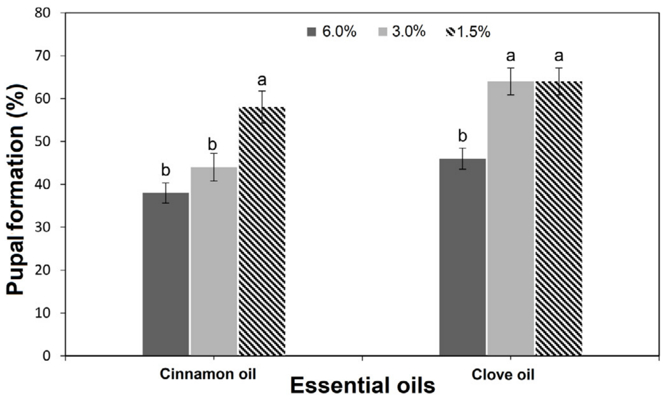

Figure 1: Percent pupal formation (means±SE) of *Tribolium castaneum* when exposed at the larval stage to different concentrations of cinnamon and clove oil

By using a higher concentration of cinnamon oil, only 38.0% of larvae were converted into pupae. Further 44.0% and 58.0% pupa formation were recorded at 3.0% and 6.0% concentrations of cinnamon oil, respectively. In the case of clove oil, 46.0% pupal formation was recorded by using 6.0% concentration, while 64.0% larvae were converted into a pupa by using 3.0% and 1.5% concentration (Figure 1). Only 22.0% adult emergence was recorded when 6.0% concentration of cinnamon oil was used. However, more number of adults emerged (34.0% and 40.0%) when lower concentration 3.0% and 1.5% concentration was used, respectively. In the case of clove oil, 30.0% of adults emerged at 6.0% concentration, while 44.0% and 50.0% adult emergence were recorded at 3.0% and 1.50% concentration respectively (Figure 2).

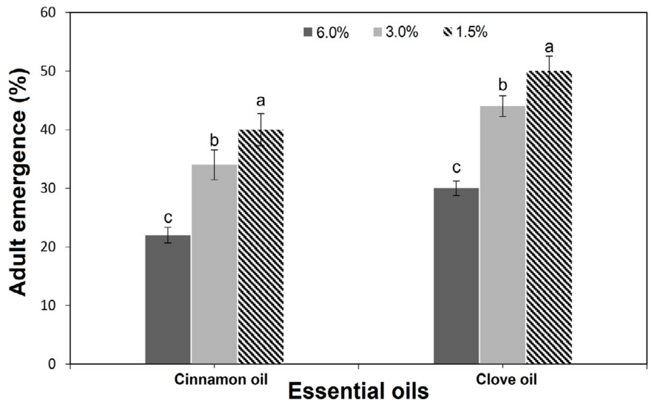

Figure 2: Percent adult emergence (means±SE) of *Tribolium castaneum* when exposed at the larval stage to different concentrations of cinnamon and clove oil.

The current study investigated the efficacy of different essential oils against *T. castaneum*, a common pest in stored products. Results showed that cinnamon and clove oils have insecticidal properties and significantly reduced the survival of both adults and larvae of *T. castaneum*. The effectiveness of cinnamon oil and clove oil against *T. castaneum* could be due to their ability to disrupt the insect's nervous system, leading to paralysis and eventual death (Tong and Bloomquist, 2013). This mechanism of action has been observed in other insecticidal compounds, including neonicotinoids and pyrethroids (Nauen *et al*., 2001).

Both cinnamon oils and clove oils were effective in controlling both larvae ad adults of *T. castaneum*. In addition, the probit analysis also showed the higher effectiveness of these two oils as the LC_{50} value was lower as compared to others. Cinnamon oil, extracted from the bark of Cinnamomum trees, has been reported to have insecticidal properties against a variety of stored grain insect pests. Several studies have investigated the efficacy of cinnamon oil against these pests, and the results have shown promising effects. Abbasipour *et al.* (2011) investigated the effect of cinnamon oil on *Sitophilus granarius* (Curculionidae: Coleoptera) and found that it was highly effective in reducing adult emergence and weight loss in wheat grains. Similarly, Bakkali *et al.* (2008) found that cinnamon oil was effective in controlling the growth of *Rhizopertha dominica* (Bostrichidae: Coleoptera) in stored wheat grains. In addition, several studies have reported the insecticidal properties of cinnamon oil against other stored grain insect pests such as *T. castaneum*, *Callosobruchus chinensis* (Chrysomelidae: Coleoptera), and *R. dominica* (Mahmood *et al*., 2015). Cinnamaldehyde, the main component of cinnamon oil, is believed to be the active ingredient responsible for its insecticidal properties (Dhingra and Sharma, 2019). The mode of action of cinnamon oil on stored grain insect pests is not fully understood, but it is believed to involve the disruption of the insect's nervous system. This mechanism of action has been observed in other insecticidal compounds, including synthetic pyrethroids (Nauen *et al*., 2001).

Clove oil, extracted from the dried flower buds of *Syzygium aromaticum*, is known for its antimicrobial, antifungal, and insecticidal properties. Several studies have investigated the efficacy of clove oil against stored grain insect pests, and the results have shown promising results. Kumar *et al.* (2015) investigated the effect of clove oil on *S. oryzae* and found that it was highly effective in reducing adult emergence and weight loss in stored rice grains. Similarly, Tripathi *et al.* (2017) found that clove oil was effective in controlling the growth of *S. oryzae* and *R. dominica* in stored wheat grains. In addition, several studies have reported the insecticidal properties of clove oil against other stored grain insect pests such as *Tribolium castaneum, Callosobruchus chinensis*, and *Cryptolestes ferrugineus* (Laemophloeidae: Coleoptera) (Tacconelli *et al*., 20018; Ayvaz *et al*., 2010). Eugenol is the main component of clove oil, and is an active ingredient responsible for its insecticidal properties (Araujo *et al*., 2018). The clove oil disrupts the insect's nervous system and inhibits the digestive enzymes (Ayvaz *et al*., 2010).

The higher concentrations (6.0%) of both essential oils were effective as the mortality rate was higher, and the percent pupal formation and adult emergence was reduced. Saeed *et al.* (2018) investigated the effect of cinnamon oil on the pupal formation and adult emergence of *T. castaneum* and found that it was highly effective in reducing both. Similarly, another study by Kiran *et al.* (2016) found that cinnamon oil was effective in inhibiting the development of *T. castaneum* larvae, thereby reducing the number of pupae formed and adult emergence. Araujo *et al.* (2018) investigated the effect of clove oil on *T. castaneum* and found that it caused a significant reduction in the number of adult insects emerging from treated grains.

Essential oils are complex mixtures of volatile compounds, and the specific composition can vary widely depending on factors such as plant species, geographic origin, and extraction methods. Future research could investigate deeper into the specific mechanisms of action of individual components within essential oils to explain their contributions to insect control efficacy. The use of EOs as an alternative to insecticides is gaining popularity due to their low toxicity and eco-friendliness (Regnault-Roger *et al*., 2012). The findings of this study suggest that cinnamon oil and clove oil could be potential candidates in controlling the population of *T. castaneum* infestations in stored products.

Conclusions and Recommendations

In conclusion, it can be said that cinnamon and clove oil are effective natural alternatives to synthetic insecticides for the control of *T. castaneum*, a serious pest of stored grain products. Overall, the use of cinnamon and clove oil could offer a safe, effective, and environmentally friendly alternative to synthetic insecticides for the control of *T. castaneum*. Further, it would be useful to investigate the possible effects of essential oils on the quality of stored grains and their potential impact on human health and the environment.

Acknowledgments

The authors are thankful to Department of Horticulture for providing research facilities.

Conflict of interest

The authors have declared no conflict of interest.

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