Biospectra : ISSN: 0973-7057, Vol. 20(1), March, 2025, pp. 23-28

An International Biannual Refereed Journal of Life Sciences

Animal Science

ISSN : 0973-7057

Sub-lethal activity of *Citrus limon* plant essential oils against stored-grain insect pests *Sitotroga cerealella* (Olivier)

Pallavi Kumari & Arun Kumar*

University Department of Zoology, B.N. Mandal University, Madhepura, Bihar, India

Received : 14th November, 2024 ; Revised : 15th December, 2024

DOI:-https://doi.org/10.5281/zenodo.16065006

Abstract- Plant products in form of essential oils may serve as suitable alternatives to synthetic pesticides for repellent and antifeedant activity. In the present investigation *Citrus limon* leaf and peels oil were used for insecticidal activity. The maximum percent repellency (PR) was recorded 100% at 16µl of *C. limon* leaf and peel essential oil respectively against stored-grain insect pests *Sitotroga cerealella*. The consumption by third instar larva was significantly reduced to 68.12% and 59.64% of the control against *C. limon* leaf and peel oil respectively. These studies showed strong insecticidal activity of both essential oils and its potential role as a fumigant against *S. cerealella* for future as they are relatively safe, inexpensive, and are readily available in many parts of the world.

Keywords: *Citrus limon, Sitotroga cerealella*, instar, Antifeedant activity

INTRODUCTION

In India, agriculture is one of the most important sectors of the economy. About half of the country's population depends on agriculture as its principal source of income. However, various insect pests pose significant challenges to wheat production, particularly in storage conditions. These pests not only damage the quality of stored grains but also reduce their quantity with the waste materials they produce.[1,2] Wheat (*Triticum aestivum* L.) is a crucial cereal crop that provides a primary food source for billions of people around the world.[3] It has been estimated that India experiences annual storage losses of around 14 million tonnes of food grains, which translate to a financial loss of approximately $16 billion each year. Out of this staggering amount, losses attributed specifically to insect pests account for about $300 million.

*Corresponding author :
Phone : 9006991000
E-mail : prf.arukumar@gmail.com

One of the most significant pests affecting stored grains is *Sitotroga cerealella* (Olivier), commonly known as the Angoumois grain moth. This pest is particularly reported in Asian countries where traditional storage structures are commonly used.[4,5] Due to damage of stored-grain pest by stored insect pests in large scale last three decades, there is an urgent need to develop effective management strategies to minimize losses and ensure food security.

The use of synthetic insecticides for pest control in stored products has been a staple practice globally; however, this approach has led to significant environmental issues and the emergence of pest resistance,[6-8] conventional pesticides can inadvertently create long-term challenges that compromise ecological balance and agricultural sustainability.

As a response to these pressing challenges, there is a growing imperative to explore alternatives to chemical pesticides that are both effective and environmentally

friendly. Botanical insecticides, derived from plant extracts, are showing good alternative in this regard. Research has indicated that these natural products can serve as effective fumigants with notable insecticidal properties, offering sufficient control against pest infestations without the negative ramifications associated with synthetic chemicals.[9,10]

The advantages of botanical insecticides are manifold: they often exhibit lower toxicity to non-target organisms, biodegradable, and have the potential for rapid degradation in the environment, thereby minimizing ecological disruption. Furthermore, their accessibility and sustainability make them appealing options for pest management.

The lemon (*Citrus limon*), belonging to Rutaceae family, an evergreen tree is native of Asia found mainly in northerner area like Assam Myanmar and China. Citrus peels have several antioxidant components which have been known to have a variety of biological activities reported by different workers.[11,12]

In the present study, essential oil from *Citrus limon* (Family: Rutaceae) leaves and peel was extracted and evaluated to investigate their repellent and antifeedant activities on *Sitotroga cerealella*.

MATERIAL & METHODS

Plant Collection

Leaf and peels of *C. limon* were collected from the local area of Madhepura district, Bihar. The collected plant materials were air-dried at room temperature (20-25°C) for one week for extraction of essential oils.

Extraction of essential oils

The air-dried leaf and peels were grounded in mixer and subjected to hydrodistillation in 1L of distilled water in a Clevenger-type apparatus for 4 h. The essential oil was collected and dried over anhydrous sodium sulphate and stored in sealed Eppendorf tubes at 4-5°C for the further experimental works.

Insects rearing

The rice moth, *S. cerealella* was reared in the laboratory in glass jar (30 cm height, 20 cm diameter). Insects were fed crushed rice grain mixed with 5%(w/w) and kept in a rearing room at a temperature of 28±1ºC, a relative humidity of 65±5% and a photoperiod of 12 h light: 12 h dark. Newly emerged adults (2-3 days old) were used in the bioassays.

Repellent Activity

Repellency assay was carried out in a Y-shaped glass tube (each arm with diameter of 2 cm and 10 cm length). Five grams of crushed rice mixed with different concentrations of *C. limon* leaf/peels oils was placed in one of the paired arms of the Y-tube (experimental arm) and the other arm contained only crushed rice (control arm). Twenty adult individuals of *S. cerealella* were released through the median arm of the Y-tube. The open ends of the tube were plugged with cotton wool and the experimental set up was left for three hours. After completion of the three hours, number of insects present in the experimental, control and median arms was counted. Each concentration was replicated six times.

Percent repellency (PR) was calculated using formula:

$$\mathbf{PR = [(N_{UT} - N_T / N_{UT} + N_T] \, 100}$$

Where, N_{UT} = number of insects in control arm and N_T = number of insects in experimental arm.

Antifeedant activity

Antifeedant activity of *C. limon* leaf and peels oils was tested in *S. cerealella* larvae. For this assay, food was prepared by crushing 10 gm of rice grains and mixing 5% (w/w) yeast powder in it. Now, food was mixed thoroughly with 0.3µl/gm, 0.6µl/gm and 1.2µl/gm *C. limon* leaf and peels essential oils. Now ten 3rd instar *S. cerealella* larvae were placed on Whatman filter paper No. 1 and then placed at the bottom of glass petridish (10 cm in diameter and 1.0 cm in height). Now, 10 gm of food prepared was spread around the larvae, cover the petri dish and kept in laboratory conditions applied for insect rearing. After the end of the larval life, antifeedant activity was calculated using:-

$$\mathbf{AFA = [C\text{-}T/C] \times 100}$$

where, C = consumption of food in control group, and T = consumption of food in treated group.

Data analysis

Correlation and linear regression analysis were conducted to define all concentration-response relationships.[13] Analysis of variance was performed to test the equality of regression coefficient.[13]

Repellency

Results of repellency assay indicated that essential oils of *C. limon* leaf and peel were repellent to *S. cerealella* adults. The percent repellency (PR) was recorded 36.33, 55.33, 98.25 and 100%, and 27.33, 51.66, 89.28 and 100% at 2, 4, 8 and 16µl of *C. limon* leaf and peel essential oil respectively (Table-1).

Antifeedant activity

C. limon leaf and peel oils reduced the consumption of food by *S. cerealella* as antifeedant activity was found to increase with increase in oil concentration. Consumption by third instar larva was significantly reduced to 68.12, 41.99 and 21.06%, and 59.64, 38.57 and 17.23% of the control at 0.3, 0.6 and 1.2 µl/gm of *C. limon* leaf and peel oil respectively (For *C. limon* leaf oil F = 111.30; and for *C. limon* peel oil F = 134.04; P<0.01; Table 2).

Table 1: Repellent activity of *Citrus limon* leaf and peels essential oils against stored grain insect pest *Sitotroga cerealella*

Concentration (vol:vol)	Leaf oil		Peel oil	
	Mean % of insect treated ±SE	(PI) Preference Index**	Mean % of insect treated ±SE	(PI) Preference Index**
2	36.33±1.12	-0.36	27.33±0.76	-0.27
4	52.33±2.54	-0.52	51.66±2.11	-0.51
8	98.25±2.71	0.98	89.28±2.96	-0.89
16	100±0.00	-0.10	100±0.00	-0.10

Percent repellency (PR) was calculated using formula: PR = $[(N_{UT} - N_T / N_{UT}+N_T] 100$, where N_{UT} = number of insects in control arm and N_T = number of insects in experimental arm.

Table 2: Effect of *C. limon* leaf and peel essential oils on food consumption by of *S. cerealella*

Oil	Concentration (µl/gm)	Food consumed (gm) (Mean±SE)	AFA	F-value** (df=3,20)
Control	-	7.31±0.23 (100)	-	-
***C. limon* leaf**	0.3	4.98±0.24 (68.12)	31.87	111.30
	0.6	3.07±0.13 (41.99)	58.00	
	1.2	1.54±0.12 (21.06)	78.93	
***C. limon* peel**	0.3	4.36±0.27 (59.64)	40.35	134.04
	0.6	2.82±0.16 (38.57)	61.42	
	1.2	1.26±0.11 (17.23)	82.76	

Six replicates were set for each concentration of essential oil and control

Values in parentheses indicate per cent change with respect to control taken as 100%

Antifeedant activity (AFA) = [C-T/C] × 100

Where, C = consumption of flour disc in control group, and T = consumption of flour disc in treated group

**Significant at P<0.01

DISCUSSION

The use of botanical products, particularly essential oils, as natural pesticides is gaining attention due to their significant activity against management of insect pests, especially in the realm of stored product protection and integrated pest management. Numerous studies have highlighted the insecticidal capabilities of various plants products as natural pesticides in form of essential oils.[14-17]

In the present study, essential oil extracted from the leaves and peels of *C. limon* showed significant insecticidal properties along with repellent and antifeedant effects. This translates to reduced damage inflicted by pests, specifically noted from observations of the consumption of flour disks by larvae of *S. cerealella*. Similar findings have been reported for essential oils from other plant species such as *Schinus molle*, *Alpinia conchigera*, *Zingiber zerumbet*, and *Curcuma zedoaria*, in relation to pests like *Tribolium castaneum* and *Sitophilus oryzae.*[18-21]

The repellent and anti-feeding actions of these essential oils can largely be attributed to their volatile nature, which results in relatively short persistence in the environment. Moreover, many essential oils exhibit neurotoxic effects on insect pests. Studies illustrate that these oils disrupt normal neurological functions by interfering with critical neuromodulators like octopamine and GABA (gamma-aminobutyric acid), ultimately impairing the insect's nervous system.[22,23] This neurotoxic mechanism reinforces the viability of using essential oils as a sustainable and eco-friendly means of pest control while minimizing the reliance on synthetic chemicals.

The findings by Ebadollahi *et al.* (2022)[24] suggest that essential oils derived from the leaves and bark of *Cinnamomum tamala* effectively reduce the activities of amylase and protease enzymes, which are crucial for the digestion and metabolism of nutrients in organisms such as the larvae of insects. Furthermore, these oils seem to

lower key indicators of larval feeding behavior, including the consumption index, relative consumption rate, and relative growth rate. This reduction in feeding activity highlights the potential of *C. limon* essential oils to minimize the damage caused by these pests on stored grains, ultimately leading to decreased quantitative and qualitative losses.

The damaged grains often suffer from microbial contamination, leading to deterioration, as well as a decline in essential nutrients like protein and carbohydrates. Therefore, utilizing *C. limon* essential oils not only assists in pest management but also helps maintain the quality of stored grains.

In summary, the essential oils obtained from the leaves and bark of *C. limon* exhibit repellent and antifeedant properties against *S. cerealella*. The oil is botanical in origin and are components of the human diet, their application in insecticide formulations is considered safe for human exposure. Moreover, their high fumigant activity indicates a lower risk of environmental persistence, reducing potential residual effects. Thus, the essential oils from *C. limon* can be developed into eco-friendly insecticide formulations based on volatile organic compounds, providing an effective and sustainable alternative for pest control.

REFERENCES

1. **Stathas I. G., Sakellaridis A. C., Papadelli M., Kapolos J., Papadimitriou K. & Stathas G. J. 2023.** The Effects of Insect Infestation on Stored Agricultural Products and the Quality of Food. *Foods.* **12(10):** 2046
2. **Kalpna., Hajam, Y. A., & Kumar, R. 2022**. Management of stored grain pest with special reference to *Callosobruchus maculatus*, a major pest of cowpea: A review, *Heliyon*, **8 (1):** e08703.
3. **Radhakrishna B., Harikala P., Shivalal N., Mukti R. P. 2024.** Climate resilient breeding for high yields and stable wheat (*Triticum aestivum* L.) lines under irrigated and abiotic stress environments. *Plant Stress*. **11:**100352.
4. **Basavanjali Nadagouda, S. Prabhuraj, A. Basavegowda & Shivaleela. 2020.** Biology of angoumois grain moth, *Sitotroga cerealella* (Olivier) (Gelechiidae: Lepidoptera) on paddy. *Journal of Entomology and Zoology Studies*. **8(5):**726-729.
5. **De M. & Dey S. R. 2022.** Variation in the stored grain pest *Sitotroga cerealella* (Olivier) infestation at low and high moisture storage conditions among some indigenous rice genotypes of West Bengal. *International Journal of Experimental Research and Review*. **28:**47-54.
6. **Elzen G. W. & Hardee D. D. 2003.** United State Department of Agricultural-Agricultural Research on managing insect resistance to insecticides. *Pest Management Science*. **59:** 770-776.
7. **Benhalima H., Chaudhry M. Q., Mills K. A. & Price N. R. 2004.** Phosphine resistance in stored-products insects collected from various grain storage facilities in Morocco. *Journal of Stored Product Research*. **40:**241-249.
8. **Islam M. S. & Talukdar F. A. 2005.** Toxic and residual effects of *Azadirachta indica*, *Tagetes erecta* and *Cynodon dactylon* extracts against *Tribolium castaneum. Journal of Plant Disease and Protection*. **112:**594-601.
9. **Mishra B. B. 2022.** Synergistic effects of natural plants products as essential oils against stored grain insect pests *Tribolium castaneum* (Herbst) (Coleoptera: Tenebrionidae) *International Journal of Agricultural Research*. **17(3):**116-121.
10. **Mishra B. B. & Tripathi S. P. 2011.** Repellent activity of plant derived essential oils against *Sitophilous oryzae* (Linnaeus) and *Tribolium castenium* (Herbst). *Singapore Journal of Scientific Research*. **1(2):** 173-178.
11. **Akarca G., Sevik R. 2021.** Biological activities of *Citrus limon* L. and *Citrus sinensis* L. peel essential oil. *Journal of essential Bearing Plants*. **24(6):**1415-1427.
12. **Farida K., Loucif M. C., Louiza H., & Malika B. 2023.** Antimicrobial and antioxidant activities of *Citrus limon* peel essential oils and their application as a natural preservative in fresh cream: Effects on oxidative and sensory properties. *Acta Universitatis Cibiniensis Series E: Food Technology* **1 Vol. XXVII**, no. 1.
13. **Sokal R. R., & Rohlf F. J. 1973.** Introduction to biostatistics. Freeman WH, San Francisco, California, USA.

14. **Huang Y., Chen S. X. & Ho S. H. 2000.** Bioactivities of methyl allyl disulfide and diallyl trisulfide from essential oil of garlic to two species of stored-product pests, *Sitophilus zeamais* (Coleoptera: Curculionidae) and *Tribolium castaneum* (Coleoptera: Tenebrionidae). *Journal of Economic Entomology*. **93(2):** 537-543.

15. **Batish D. R., Singh H. P., Kohli R. K. & Kaur S. 2008.** *Eucalyptus* essential oil as a natural pesticide. *Forest ecology and management*. **256(12):**2166-2174.

16. **Cosimi S., Rossi E., Cioni P. L. & Canale A. 2009.** Bioactivity and qualitative analysis of some essential oils from Mediterranean plants against stored-product pests: Evaluation of repellency against *Sitophilus zeamais* Motschulsky, *Cryptolestes ferrugineus* (Stephens) and *Tenebrio molitor* (L.). *Journal of Stored Products Research*, **45(2):**125-132.

17. **Nerio L. S., Verbel J. O., & Elena S. 2010.** Repellent activity of essential oils: A review. *Bioresource Technology*. **101(1):**372-378.

18. **Chaubey M. K. 2012.** Biological effects of essential oils against Rice weevil *Sitophilus oryzae* L. (Coleoptera: Curculionidae). *Journal of Essential Oil-Bearing Plants.* **15(5):** 809-815.

19. **Cardiet, G., Fuzeau, B., Barreau, C., & Fleurat-Lessard, F. 2012**. Contact and fumigant toxicity of some essential oil constituents against a grain insect pest *Sitophilus oryzae* and two fungi, *Aspergillus westerdijkiae* and *Fusarium graminearum*. *Journal of Pest Science*. **85:**351–358.

20. **Mishra B. B., Tripathi S. P., & Tripathi C. P. M. 2012.** Repellent effect of leaves essential oils from *Eucalyptus globulus* (Mirtaceae) and *Ocimum basilicum* (Lamiaceae) against two major stored grain insect pests of Coleopterons. *Journal of Nature and Science*. **10(2):**50-54.

21. **Mishra B. B., Tripathi S. P., & Tripathi C. P. M. 2011.** Contact Toxicity of essential oil of *Citrus reticulata* fruits peels against stored grain pests *Sitophilous oryzae* (Linnaeus) and *Tribolium castenium* (Herbst). *World Journal of Zoology*. **6(3):**307-311.

22. **Enan E. E. 2005.** Molecular and pharmacological analysis of an octopamine receptor from American cockroach and fruit fly in response to plant essential oils. Arch. *Insect Biochemistry and Physiology*. **159:**161-171.

23. **Tong F. & Coats J. R. 2012.** Quantitative structure-activity relationship of monoterpenoid binding activities to the house flies GABA receptor. *Pest Management Science*, **68:**1122-1129.

24. **Ebadollahi A., Naseri B., Abedi Z., Setzer W. N., & Changbunjong T. 2022.** Promising Insecticidal Efficiency of Essential Oils Isolated from Four Cultivated *Eucalyptus* Species in Iran against the Lesser Grain Borer, *Rhyzopertha dominica* (F.). *Insects*, **13(6):**517.

***