

Evaluation of insecticidal potential of *Citrus limon* leaf and peel essential oil against lesser grain borer, *Rhyzopertha dominica*

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Abstract

Lesser grain borer, *Rhyzopertha dominica* (Coleoptera: Bostrichidae) is a serious primary pest of stored grains and processed products feeding on cereals and processed products cryptically under storage and damage both quantitative and qualitative. For the management of this insect pest, several chemicals of synthetic nature have been used in different form. Since these synthetic chemicals affect environment, human health, non-target organism as well as quality of air, soil and water, researchers focused on green alternatives for this insect pest to reduce its population. In the present study, essential oil from *Citrus limon* (Family: Rutaceae) leaves and peel was extracted and evaluated to investigate their repellent, toxic, oviposition inhibitory, developmental inhibitory and antifeedant activities on *R. dominica*. These two essential oils repelled the adults in filter paper repellency assay and cause acute toxicity in both larvae and adults in fumigant and contact toxicity assays. These two essential oils reduce oviposition and interfere with the transformation of larva to adult besides inhibiting hatching of eggs when fumigated. Deterrence in feeding habit of *R. dominica* adults was also been recorded. In conclusion, these *C. limon* leaves and peel essential oils can used as the key components in eco-friendly insecticide formulation based on volatile organic chemicals.

Key words : *Rhyzopertha dominica*, Essential oil, Insecticides, Antifeedant activity.

Infestation of grain under storage by insects started with the beginning of storage practices leading to both quantitative and qualitative losses. In developing countries depending on traditional technologies for storing grains, such losses vary between 10-40%. These losses by insect pests at farm level approximate to 10% of total yield^{21,29}. These cause spoilage of food as well as raise concerns of security issues especially in developing countries. The lesser grain borer, *Rhyzopertha dominica* (Coleoptera: Bostrichidae) is a major

primary insect pest of stored grains with worldwide distribution. It attacks various grains, feed cryptically and considered as one of the most destructive pests. These can be seen in field and storage houses both¹. The adult insects are strong fliers and, therefore, can migrate from field to godowns and from one godown to another easily causing higher infestation. To manage these losses by controlling *R. dominica* population, several chemicals like organophosphates, organochlorines and phosphine were synthesized and used indiscriminately, but, besides getting success reducing its population, these insecticides have lead to resistance development in insects^{4,10,13,18}. These synthetic insecticides cause various chromosomal aberrations and formation of DNA adducts^{22,25,30}. These insecticides cause various environmental problems like thinning of ozone layer, contamination of soil, air and water, high persistence in the environment, toxic effects in non-target organisms like beneficial insects and a variety of health issues like neurotoxicity, carcinogenicity and teratogenicity^{3,23,27,34,35}.

These serious concerns lead to reduce the use of these synthetic products and exploration of new green and ecologically safe alternative methods. Essential oils are nowadays considered as alternatives of synthetic insecticides to protect stored products from insect infestation. The lemon (*Citrus limon*), an evergreen tree belonging to Rutaceae family, is native of Asia found mainly in northerner area like Assam, Myanmar and China. Citrus peels have a number of antioxidant components which been known to have a variety of biological properties including anti-cancer, anti-inflammatory, anti-obesity, immune-modulating, anti-osteoclastogenic,

anti-viral and neuroprotective effects^{2,12}. The essential oil extracted from peel contains forty-five volatile constituents representing 99.7% of total essential oil. The most abundant constituents are limonene (60.7%), β -pinene (12.6%), and γ -terpinene (10.3%). On the other hand, α -pinene (2.6%), sabinene (2.4%), and geranial (2.2%) are present in minor quantities²⁰. Citrus leaf oil contains twenty-seven compounds, and linalool (30.62%), geraniol (15.91%), α -terpineol (14.52%) and linalyl acetate (13.76%) are the main constituents¹⁶. Citrus peel components have been shown to have tumor growth inhibitory effects²⁴. Citrus essential oil is recognized for its bioactive compounds with potential antimicrobial properties that induce lysis of bacterial cell walls resulting in ingredient leakage and cell death^{12,32}. In the present study, essential oil from *Citrus limon* (Family: Rutaceae) leaves and peel was extracted and evaluated to investigate their repellent, toxic, oviposition inhibitory, developmental inhibitory and antifeedant activities on *R. dominica*.

Plant collection :

Leaves and peels of lemon, *Citrus limon* were collected locally from the market. The harvested material was air-dried at room temperature (20-25°C) for one week.

Essential oil extraction :

The essential oil was extracted by hydrodistillation of dried plant material in 1L of distilled water using a Clevenger-type apparatus for 4 h. The oil was dried over anhydrous sodium sulphate and stored in sealed eppendorff tubes at 4-5°C.

Insects :

The lesser grain borer, *Rhyzopertha dominica* beetle were reared in the laboratory in biochemical oxygen demand (BOD) climatic chambers in plastic containers with perforated plastic lids. Insects were fed wheat flour and kept in a rearing room at a temperature of $25\pm 1^{\circ}\text{C}$, a relative humidity of $65\pm 5\%$ and a photoperiod of 12 h light:12 h dark. Newly emerged adults (2-3 days old) were used in the bioassays.

Repellent Activity :

Repellent test was conducted in a glass Petri dish (9 cm diameter and 1.2 cm height) according to the method of Jilani and Saxena (1990) with some modifications¹⁹. Whatman No. 1 filter papers (diameter 9 cm) were cut in two halves. Test solutions of *C. limon* leaf and peel oils were prepared by dissolving 2, 4, 6 and 8 μl of essential oil in 1 ml of acetone (corresponding to 0.07, 0.14, 0.21 and 0.28 ml/ cm^2). Each essential solution was applied to half of filter-paper disc as uniformly as possible using a micropipette. The other half of the filter paper was treated with solvent only and was used as a control. The treated and control half discs were air dried to evaporate the solvent completely. Treated and untreated halves were then attached using adhesive tape and placed in Petri dishes. Twenty adults of mixed sex were released in the centre of each filter paper disc. The dishes were then covered and transferred to an incubator (25°C). Each concentration was replicated six times. Numbers of insects present on both the treated and untreated halves of the filter paper were recorded after 3h.

Fumigant toxicity :

Experimental solutions of *C. limon* leaf and peel oils were made in acetone. Ten *R. dominica* adults/larvae taken from laboratory culture were placed in glass petri dish (diameter 8.5 cm, height 1.2 cm) and added 2 gm of wheat flour. Experimental solution was applied in a filter paper strip (2 cm diameter) and left for few minutes to evaporate solvent. Now, treated filter paper strip was pasted on the inner surface of the petri dish cover, air tightened with parafilm and kept in rearing conditions in dark. After 24h and 48h of exposure, mortality in adults and larvae was recorded.

Contact toxicity :

Experimental solutions of *C. limon* leaf and peel oils were made in acetone. Experimental solution was applied on the inner surface of glass petri dish (diameter 8.5 cm, height 1.2 cm). Petri dish was left open for few minutes to evaporate solvent completely. Now, ten *R. dominica* adults/larvae were released in petri dish, covered and kept in rearing conditions in dark. After 24h and 48h of exposure periods, recorded the mortality in adults and larvae.

Oviposition inhibition :

Oviposition inhibitory activity *C. limon* leaf and peel oils was tested against *R. dominica* by fumigation. Twenty 1-2 week old adults of mixed sexes were placed in 2 gm of wheat flour in glass petri dish. Flour was spread uniformly along the whole surface of the petri dish. A paper strip (2 cm diameter) treated with different sub-lethal concentrations viz. 40

and 80% of 24h-LC₅₀ was pasted on the inner surface of the cover of each petri dish. All the closed petri dishes were kept in dark and six replicates were set for each concentration. After 24h of fumigation, the treated adults were transferred to fresh petri dish having fresh wheat flour. After six days of treatment, the adults were removed and discarded. The number of the larvae hatched in the flour was counted for the treated as well as for control groups. The counting was done for four days continuously. Percentage of oviposition deterrence (POD) was calculated as:

$$\text{POD} = [(E_C - E_T)/E_C] \times 100$$

E_C = number of adults emerged in control and
E_T = number of adults emerged in test.

Ovicidal assay :

In ovicidal assay, twenty five eggs were fumigated with test solutions prepared by diluting *C. limon* leaf and peel oils with acetone. A 100 µl aliquot of test solution was applied on filter paper strip (2.5 cm diameter) and the solvent was allowed to evaporate for 5 minutes. The filter paper was pasted to inner surface of screw cap of vial (3 cm in diameter and 10 cm in height). Cap of vial was screwed and incubated for 48h in conditions maintained for insect culture. After fumigation, eggs were allowed to hatch and number of eggs hatched was recorded after six days of treatment. Three different concentrations (20, 40 and 60 µl/L) of each essential oil were used and for each concentration of oil six replicates were set. Hatching inhibition rate (%HIR) was calculated as: %HIR = [(C-T)/C]X100

Where, C = Number of adults in control and T = Number of adults in test.

Developmental inhibition :

Developmental inhibitory activity of *C. limon* leaf and peel oil was tested against 4th instars larvae of *R. dominica*. Ten larvae were fumigated with different sub-lethal concentrations viz. 40 and 80% of 48h-LC₅₀ of volatile oil in petri dish for 48h as was done in larvicidal assay and then the treated larvae were transferred to fresh wheat flour in other Petri dish. Number of emerged adults from larvae was recorded. Six replicates were set for each concentration.

Antifeedant activity :

Antifeedant activity of *C. limon* leaf and peel oils was tested in of *R. dominica* adults. For this assay, 100 wheat grains were taken in a 100 ml plastic box and mixed thoroughly with three different concentration of *C. limon* leaf and peel oil and kept in laboratory conditions applied for insect rearing. After one month, percentage damage was calculated using a formula percentage of damaged cereals = number of damaged cereals/number of samples) x 100%. Frass results from damaged cereals was estimated by separating the damaged cereals and its frass. The frass obtained was weighed and percentage of frass production was calculated as (weight of frass/weight of grains initial) x 100%¹⁵.

Data analysis :

Chi-square test was applied to establish the repellent activity of the essential oils tested³¹. The LC₅₀ and EC₅₀ were calculated by POLO programme²⁸. Correlation and linear regression analysis were conducted

Table-1. Repellent effect of *C. limon* leaf and peel essential oils on *R. dominica*

Concentration (vol:vol)	Leaf oils			Peel oils		
	Untreated Mean±SE	Treated Mean±SE	χ^2 -value	Untreated Mean±SE	Treated Mean±SE	χ^2 -value
0.6	38.33±0.30	1.66±0.30	60.00*	39.33±0.27	0.66±0.27	62.60*
0.3	34.00±0.36	6.00±0.36	50.70*	36.33±0.31	3.33±0.31	51.40*
0.15	27.00±0.57	13.00±0.57	20.70*	29.66±0.49	10.33±0.49	28.40**
0.075	21.66±0.33	18.33±0.33	3.10 [#]	22.66±0.33	17.33±0.33	5.40 [#]

*Significant (P<0.01), **Significant (P<0.05), [#]Non-significant

Table-2. Fumigant toxicity of *C. limon* leaf and peel essential oils on *R. dominica*

Oil	Parameter	Exposure Period	LC ₅₀ (µl/L)	g-value	t-ratio	Heterogeneity	Regression Equation	Correlation Coefficient
<i>C. limon</i> leaf oil	Adult mortality	24h	89	0.29	3.67	0.32	Y = -3.52+1.38X	0.99
		48h	49	0.27	3.19	0.37	Y = -5.69+3.27X	0.98
	Larval mortality	24h	79	0.27	4.26	0.36	Y = -3.36+5.61X	0.98
		48h	42	0.24	3.36	0.34	Y = 2.36+4.93X	0.99
<i>C. limon</i> peel oils	Adult mortality	24h	96	0.25	3.56	0.38	Y = 3.22+1.97X	0.99
		48h	54	0.27	3.69	0.41	Y = -2.82+4.21X	0.99
	Larval mortality	24h	73	0.28	4.21	0.34	Y = -2.85+2.64X	0.98
		48h	39	0.25	3.95	0.37	Y = 3.95+2.17X	0.99

Table-3. Contact toxicity of *C. limon* leaf and peel essential oils on *R. dominica*

Oil	Parameter	Exposure Period	LC ₅₀ (µl/L)	g-value	t-ratio	Heterogeneity	Regression Equation	Correlation Coefficient
<i>C. limon</i> leaf oil	Adult Mortality	24h	0.09	0.28	3.59	0.33	Y = -3.02+1.10X	0.99
		48h	0.06	0.26	3.22	0.36	Y = 2.30+4.06X	0.99
	Larval mortality	24h	0.10	0.25	4.10	0.37	Y = -3.98+4.39X	0.99
		48h	0.07	0.27	3.46	0.38	Y = 2.94+3.20X	0.98
<i>C. limon</i> peel oils	Adult Mortality	24h	0.09	0.28	3.84	0.37	Y = -1.98+1.07X	0.98
		48h	0.05	0.24	3.31	0.40	Y = -2.01+3.24X	0.98
	Larval mortality	24h	0.08	0.28	4.08	0.36	Y = -2.11+2.32X	0.99
		48h	0.05	0.27	3.69	0.39	Y = 2.86+1.95X	0.98

Table-4. Effect of *C. limon* leaf and peel essential oils on oviposition potential of *R. dominica*

Oil	Concentration	Number of larvae emerged/adult (Mean±SE)	POD*	F-value**
Control	-	53.66±4.07 (100)	-	-
<i>C. limon</i> leaf oil	40% 48h-LC ₅₀	34.33±3.07(63.97)	36.02	29.54
	80% 48h-LC ₅₀	19.50±1.95 (36.33)	63.66	
<i>C. limon</i> peel oil	40% 48h-LC ₅₀	37.66±2.45 (70.18)	29.82	30.27
	80% 48h-LC ₅₀	22.16±1.40 (41.29)	58.70	

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

*Percentage of oviposition deterrence (POD) = $[(E_C - E_T)/E_C] \times 100$, E_C = number of adults emerged in control and E_T = number of adults emerged in test, **Significant at $P < 0.01$

Table-5. Effect of *C. limon* leaf and peel essential oils on viability of *R. dominica* eggs

Oil	Concentration (μl/L)	Number of larvae emerged (Mean±SE)	%HIR	F-value*
Control	-	23.50±0.33(100)	-	-
<i>C. limon</i> leaf oil	20	19.83±0.47(84.38)	15.62	93.85
	40	15.16±0.60(64.51)	35.49	
	60	9.83±0.87(41.83)	58.17	
<i>C. limon</i> peel oil	20	17.33±0.49(73.74)	26.25	204.76
	40	12.66±0.33(53.87)	46.13	
	60	8.66±0.55(36.85)	63.14	

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

Hatching inhibition rate (%HIR) was calculated as: $\%HIR = [(C - T)/C] \times 100$, Where,

C = Number of adults in control, T = Number of adults in test, *Significant at $P < 0.01$

Table-6. Effect of *C. limon* leaf and peel essential oils on development of *R. dominica*

Oil	Concentration	Number of adult emerged (Mean±SE)	F-value*
Control	-	8.50±0.22	-
<i>C. limon</i> leaf oil	40% 48h-LC ₅₀	6.16±0.30(72.47)	68.58
	80% 48h-LC ₅₀	3.66±0.29(43.05)	
<i>C. limon</i> peel oil	40% 48h-LC ₅₀	6.50±0.22(76.47)	84.57
	80% 48h-LC ₅₀	3.83±0.36(45.05)	

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

*Significant at $P < 0.01$

Table-7. Effect of *C. limon* leaf and peel essential oils on per cent cereal damage and frass production by of *R. dominica*

Oil	Concentration ($\mu\text{l}/100$ cereal)	Per cent Grain damage (Mean $\pm$ SE)	F-value** (df=3,20)	Per cent frass production (Mean $\pm$ SE)	F-value** (df=3,20)
Control	-	74.16 $\pm$ 3.44 (100)		6.63 $\pm$ 0.29 (100)	-
<i>C. limon</i> leaf oil	1	47.33 $\pm$ 2.13 (63.82)	75.18	4.60 $\pm$ 0.13 (69.38)	78.07
	2	33.83 $\pm$ 2.12 (45.61)		2.75 $\pm$ 0.12 (41.47)	
	4	14.66 $\pm$ 1.32 (19.76)		1.65 $\pm$ 0.02 (24.88)	
<i>C. limon</i> peel oil	1	44.66 $\pm$ 1.98 (60.22)	77.21	4.67 $\pm$ 0.16 (73.77)	110.45
	2	29.50 $\pm$ 2.17 (39.77)		3.06 $\pm$ 0.12 (48.34)	
	4	10.83 $\pm$ 1.28 (14.60)		1.70 $\pm$ 0.17 (26.85)	

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%, **Significant at $P < 0.01$

to define all concentration-response relationships³¹. Analysis of variance was performed to test the equality of regression coefficient³¹.

Repellency :

Chi-square analysis indicated that *C. limon* leaf and peel oils were repellent to *R. dominica* adults. These *C. limon* leaf and peel oils showed significant repellent activity even at low concentrations as the hypothesis of the ratio 1:1 was rejected (Table-1).

Fumigant toxicity :

In adults, median lethal concentrations were recorded 86 and 49 $\mu\text{l}/\text{L}$, and 96 and 54 $\mu\text{l}/\text{L}$ air against *R. dominica* adult for *C. limon* leaf and peel oils after 24h and 48h exposure period respectively (Table-2). On the other hand, median lethal concentrations were 79 and 42 $\mu\text{l}/\text{L}$, and 73 and 39 $\mu\text{l}/\text{L}$ air against *R.*

dominica larvae for *C. limon* leaf and peel oils after 24h and 48h exposure period respectively (Table-2). The index of significance of potency estimation, g-value indicates that the mean value is within the limits of all probability levels ($P < 0.1$, 0.5 and 0.01) as it is less than 0.5. Values of t-ratio greater than 1.6 indicate the significance of regression. Values of heterogeneity factor less than 1.0 denotes that model fits the data adequate. Regression analysis shows concentration-dependent mortality in *R. dominica* adults and larvae (Table-2).

Contact toxicity :

In adults, median lethal concentrations were 0.09 and 0.6 $\mu\text{l}/\text{cm}^2$, and 0.10 and 0.07 $\mu\text{l}/\text{cm}^2$ area against *R. dominica* adult for *C. limon* leaf and peel oils after 24h and 48h exposure period respectively (Table-3). On the other hand, LC_{50} values were 0.09 and 0.0.05

$\mu\text{l}/\text{cm}^2$, 0.08 and 0.05 $\mu\text{l}/\text{cm}^2$ against *R. dominica* larvae for *C. limon* leaf and peel oils after 24h and 48h exposure period respectively (Table-3). Regression analysis showed concentration-dependent mortality in *R. dominica* adults and larvae by *C. limon* leaf and peel oils (Table-3).

Oviposition inhibition :

The oviposition potential of the *R. dominica* was decreased significantly when fumigated with the *C. limon* leaf and peel oils. The oviposition was reduced to 63.97 and 36.33%; and 70.18 and 41.29% when *R. dominica* adults were fumigated with 40 and 80% of 48h-LC₅₀ of *C. limon* leaf and peel oils respectively (For *C. limon* leaf oil F = 29.54; and for *C. limon* peel oil F = 30.27; P<0.01; Table-4).

Ovicidal activity :

The percentage of egg hatching was decreased significantly with an increase in concentration of *C. limon* leaf and peel oils. Egg hatching was reduced to 84.38, 64.51 and 41.83%; and 73.74, 53.87 and 36.85% of the control when eggs were fumigated with 20, 40 and 60 $\mu\text{l}/\text{L}$ of *C. limon* leaf and peel oils respectively (For *C. limon* leaf oil F = 93.85; and for *C. limon* peel oil F = 207.76; P<0.01; Table-5).

Developmental inhibition :

The percentage of larvae transformed into the adult stage was decreased significantly with an increase in concentration of *C. limon* leaf and peel oils. Adult emergence was reduced to 72.47 and 43.05%; and 76.47 and

45.05% of the control when larvae were fumigated with 40 and 80% of 48h-LC₅₀ of *C. limon* leaf and peel oils respectively (For *C. limon* leaf oil F = 68.58; and for *C. limon* peel oil F = 84.57; P<0.01; Table-6).

Damage of Cereal Grains :

C. limon leaf and peel oils reduced the damage caused by *R. dominica* as percent grain damage and percent frass production was reduced in experimental condition. At 6 $\mu\text{l}/100$ wheat grain of *C. limon* leaf and peel oil, percent of damaged cereals found was 19.76 and 14.60% of the control respectively (For *C. limon* leaf oil F = 75.18; and for *C. limon* peel oil F = 77.21; P<0.01; Table-7). The percent frass production was reduced to 24.88 and 26.85% of the control at 6 $\mu\text{l}/100$ wheat grain of *C. limon* leaf and peel oil respectively (For *C. limon* leaf oil F = 78.07; and for *C. limon* peel oil F = 110.45; P<0.01; Table-7).

In early attempts for the management of economic losses, several volatile products of botanical origin have been reported for their repellent, toxic, oviposition inhibitory, developmental inhibitory and antifeedant activities against a variety of coleopteran insect pests of stored grains and products. In the present study, *C. limon* leaf and peel essential oils repelled adults and caused acute toxicity in adults as well as larvae of *R. dominica*. The repellent and rapid toxicity of the essential oils is caused due to vapour action showing their low persistence in the environment and probable neurotoxicity. *Eucalyptus microtheca*, *E. procera*, *E. spatulata* and *E. torquata* essential oils have been reported for their insecticidal effects against the adults of

R. dominica by vapour action⁹. Several other essential oils and pure volatile compounds have been known for their neurotoxicity in coleopteran insect pests⁵⁻⁷. These oils interfere with neuromodulator octopamine or GABA-gated chloride channels and cause disruption and break down of nervous system in insects^{11,17,33}.

C. limon leaf and peel oils inhibit oviposition and egg hatching by vapour action in *R. dominica*. The mode of action of these volatile chemicals has not yet been established but it appears that oviposition inhibition and development inhibition may be due to the suffocation and inhibition of different biosynthetic processes of the insect metabolism⁸. In a study, essential oils have been reported to reduce total protein, glycogen, and lipid contents and digestive amylase and protease enzyme activities as well as consumption index, relative consumption rate, and relative growth rate in *R. dominica*⁹.

C. limon leaf and peel oils reduce grain damage and frass production produced by adult and larvae of *R. dominica*. The grains were damaged by feeding action of insects making holes and cracks resulting in frass production. This damage in stored grains is responsible for quantitative losses and consequently market value²⁶. The damage to grains causes deterioration and contamination in damaged grains due to microbial action as well as reduction in protein and carbohydrates level. In conclusion, *C. limon* leaves and peel essential oils show repellent, toxic, oviposition inhibitory, developmental inhibitory and antifeedant activities on *R. dominica*. Since these two volatile oils are of botanical origin and are parts of human diet, these are safe

for human when used in insecticide formulation. Their high fumigant action shows its low persistence in the environment, thus, elimination residual properties. Thus, these *C. limon* leaves and peel essential oils can be used in developing eco-friendly insecticide formulation based on volatile organic chemicals.

Conflict Of Interest :

The author has no conflict of interest.

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