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Insecticidal Activity of *Curcuma longa* Essential Oil and its Fractions against *Sitophilus oryzae* L. and *Rhyzopertha dominica* F. (Coleoptera)

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ABSTRACT

The essential oil of Curcuma longa L. distilled from its leaves was fractionated by column chromatography and tested in the laboratory for volatile toxicity against stored grain pests Sitophilus oryzae L.(Rice weevil) and Rhyzopertha dominica F. (lesser grain borer). The oil and its fractions were insecticidal in fumigant toxicity assay. The adults of R. dominica were most susceptible to fumigant action of all fractions of C. longa leaf oil at 0.1% and 0.05% concentration, while in case of S. oryzae, pure C. longa oil and its I-fraction at 0.1% concentration were found most effective. The C. longa oil and its fractions were analyzed by GC–MS, which contained terpinolene, α- phellandrene, limonene, myrcene and eucalyptol as the major compounds, while α-pinene, β-pinene, and linalool regarded as minor compounds. These compounds (purchased from Sigma & Aldrich) were also investigated for fumigant action against R. dominica and S. oryzae. Fractions with terpinolene, α-phellandrene, limonene and eucalyptol as major constituents were found highly effective against these storage insects and fractions which consist of all theses constituents together were also found effective. The fractions of essential oil which consists of more constituents, whether it was a major or a minor, was found often more toxic than any fraction with less constituents.

Key words: *Curcuma, Rhizopertha, Sitophilus, Monoterpenes, Fumigant, Leaf oil.*

INTRODUCTION

Stored grain Infested by various storage-product pests may occur at various stages from time of harvest to consumption by consumers. A range of methods are used by grain industries in developing countries to minimize insect infestation on stored grains, but usually rely on fumigants[15]. Fumigants should be biologically active, economical and eco-friendly, sufficiently volatile, less retention of residue in the grain, which is avoidable by other living organism. The use of phosphine (PH_3) is most common due to ready to use formulations, the relatively short-term hazard, and low retention of residues. However, PH_3 fumigation may become increasingly limited in use because resistance of stored grain insects to phosphine has now been discovered in more than 45 countries[2,3].

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Methyl bromide a broad spectrum fumigant has been declared as ozone- depleting substance and therefore, is being phased out completely. Essential oils (EO) and monoterpenes (MO) are potential sources of alternative compounds to currently used fumigants. EO and MO have low toxicity to warm-blooded animals, high volatility and strong toxicity to insect pests[10,11]. The essential oil of a plant may contain hundreds of different constituents but certain components will be present in larger quantities.

Turmerone and ar-turmerone, are major constituents of *C. longa* rhizome oil whereas leaf oil contains α- phellandrene (18.2%), 1, 8- cineole (14.2%) and p-cymene (13.3%) as major constituents[18]. The leaf and rhizome essential oil of *C. longa* contains 82.9% and 16.3% monoterpenes, respectively[12]. Monoterpenes have been well documented to be active as fumigants, repellents or insecticides toward stored grain insects[13,20]. In this paper, we report on population suppressant and toxic activities of *C. longa* leaf essential oil and its fractions against stored- product beetles *S. oryzae* and *R. dominica*.

MATERIAL AND METHODS

a) Test Insects culture. The stored grain insects are reared in the laboratory at 27 $\pm 1^0$C temperature and 70$\pm$5% relative humidity. Plastic jars of about 0.50 kg capacity were used for rearing purpose. At the center of the lid a hole of 1.8 cm. diameter was made and covered with 30 mesh copper wire net to facilitate aeration in the jar. The adult of *R. dominica* and *S. oryzae were* reared on the grain of wheat variety PBW-343. Before use, grain was disinfested in the oven at 60^0C for 12 hrs. After disinfestations the moisture content of the grain was measured and raised to 13.5 % by mixing water in the grain.

b) Plant Material, Extraction and Fractionation of essential oil: Fresh plant of *Curcuma longa* collected from different localities and air dried leaves were subjected to steam distillation to obtain the essential oil from leaves. The distillation process was carried out by using a Clevenger type of apparatus[6]. Anhydrous sodium sulphate was used to remove trace of moisture from essential oil and stored in air tight container in a refrigerator at 4 ^{0}C. Aliquots of essential oils were subjected to column chromatography through silica gel (60-12- mesh, Spectrochem) using hexane, hexane: Chloroform (1:1 v/v), Chloroform, Chloroform: ethyl acetate (1:1 v/v), Ethyl acetate and Ethyl acetate: acetone (1:1 v/v) sequentially as elute, to obtain fractions containing non-polar and polar compounds, respectively.

c) GC-MS analysis of essential oil: GC-MS data was obtained on a Shimadzu GCMS-QP-2010 plus system using AB inno-wax column (60 m x 0.25 mm id, film thickness 0.25 m). Analysis was performed using the following temperature program: oven was kept isothermally at 80 ^{0}C for 2 min, increased from 80 ^{0}C to 200 ^{0}C at the rate of 4 ^{0}C/min with hold time of 2 min and from 180 ^{0}C to 230 ^{0}C at the rate of 25 ^{0}C/min with hold time of 13 minute. The injection temperature was 250 ^{0}C. Helium was used as a carrier gas with a flow rate of 0.3 ml/min and the split ratio was 100:0. Scan time and mass range were 0.05 sec and 40-600 m/z respectively. EI source and mass range were 70 eV and 40-650 amu. The volatile compounds in the essential oil were identified by calculating their retention time, relative to (C9-C18) n-alkenes, and from the data for the authentic compounds available in Willey, NIST and perfumery libraries and also by matching their mass spectrum fragmentation patterns with corresponding data stored in the mass spectra library of the GC-MS data system and their published mass spectra. The relative percentage amount of each identified compound was obtained from the electronic integration of its FID peak area.

d) Experimental Details

Effect of *C. longa* essential oil and its fractions on development of *S. oryzae and R. dominica:* The experiments were conducted twice on *S. oryzae and R. dominica* to confirm the efficacy of essential oils. Untreated grain was used as control. The experiments were conducted under controlled condition at 27$\pm 1^0$C temperature and 70$\pm$5 per cent relative humidity in the plastic vials (10×4 cm). Fifty

gram wheat grain (moisture content 13.5 per cent) was filled in plastic vials. Ten adults of *R. dominica and S. oryzae* (0-7 days old) were released in each vial. After 24 hrs of releasing the insects measured quantity of oil was poured on the absorbing mat, which was then placed inside the vial between the grains. Screw cap of vials were tightly closed and made completely airtight by sealing with parafilm wax strip. Each treatment was replicated thrice. Untreated grain was used as control.

Fumigant toxicity of different constituent of *C. longa* essential oil on *S. oryzae and R. dominica*: Major constituents of essential oils were tested at a of 100, 250, 350 and 500 µL L^{-1} according to the method of Lopez (2008). The fractions were applied by adding 2, 5, 7 and 10 µL on a 2 cm filter paper disc, which was placed on top of a 4 ml vial which was stood inside a tapered vial of 20 ml capacity. The insects were released in the bottom of the outer vial to avoid direct contact with product tested and to assess the toxicity of vapors. Experiment was replicated thrice. Two controls were used in experiment, one without application of product and another with organic solvent Hexane. Vials were placed in a chamber at $27\pm^{o}C$ in the dark and insect mortality was recorded after 6 h, 12 h and 18 h.

e) Statistical analysis: Data was analyzed in Completely Randomized Design after suitable transformation with log (X+1) or angular transformation. Data processing was conducted by STPR 3 program. Mortality data was analyzed for each exposure period according to the General Linier Model (GLM). Significant difference was identified by Duncan Multiple Range Test and entered in the tables. Data processing was conducted by SPSS 16.00 for Windows.

RESULT AND DISCUSSION

The efficacy of oils was classified in different categories on the basis of F_1 progeny production. In majority of the storage systems only a few individuals begin the infestation and the final loss greatly depends on the rate of their multiplication. Therefore, more emphasis was given to suppression of F_1 progeny. With this assumption products inhibiting more than 90 per cent F_1 progeny were classified as highly effective while inhibition of 80 to 89 and 70 to 79 per cent were ranked as moderately and less effective, respectively. Similarly, products showing less than 70 per cent F_1 progeny suppression were ranked as least effective for the control of the insect pests.

Effect of *C. longa* essential oil and their fractions on development of *S. oryzae* and *R. dominica*: The effect of *C. longa* oil and its fraction against *S. oryzae* is presented in Table 1.1 which indicated that all fractions of *C. longa* oil were found moderate to highly effective, however, complete mortality observed in pure *C. longa* oil by causing 100 % inhibition. At 0.05 and 0.025% concentration all treatments were found least effective, causing less than 70% inhibition. At 0.1% concentration I- fraction was found most effective after pure *C. longa* oil.

Fumigant toxicity of *C. longa* oil and its fraction against *R. dominica* is presented in Table 1.2. *R. dominica* was found most susceptible. All fractions at 0.1% concentration were found highly effective against *R. dominica*. At 0.05% concentration all treatments except fifth fraction of *C. longa* were highly effective causing up to 98.82 % inhibition. At lowest dose pure *C. longa* oil gave 79.96 % inhibition while all other treatments were found least effective. The above mentioned study was also supported by Triphati *et al*[19]., investigated that *C. longa* leaf oil was effective against stored grain beetles in both contact and fumigant in action and it is also found that the adult of *R. dominica* were highly susceptible for contact action of *C. longa* oil in comparison to other stored grain beetles, with LD_{50} Value of 36.71 g/mg weight of insects, while adult of *S. oryzae* were found more susceptible for fumigant action of *C. longa* oil with LC_{50} value of 11.36 mg/liter air. The volatile oil of *C. reticulata* and *C. longa* were tested against *S. oryzae* and resulted in 100% and 90% adult mortality after 24 h exposure period, respectively, Chayengia *et al*[4].

In support, Ashouri *et al*[1]., reported that turmeric and cinnamon powder showed a significant toxicity on *R. dominica* and *S. granarious* at 5% (W/W) concentration, but unable to cause 100% mortality. In conformity of our findings, Huang and Ho[7] evaluated that *C. longa* oil were two times more toxic than cinnamaldehyde. The effect of *C. longa* essential oil was also observed in growth rate, food consumption and food utilization by all three species and found significant reduction at higher concentration[5]. Similarly, the feeding-deterrent effect of *C. longa* oil was observed against *R. dominica* adults by Jilani and Saxena[8]. The ovicidal effect of *C. longa* oil observed by Jilani *et al*[9]., on *R. dominica*, *S. oryzae* and *T. castaneum* and found that eggs being killed by *C. longa* leaf oil.

Different constituent of *C. longa* essential oil and its fraction: Monoterpenoids, mainly terpinolene (41.12%) and α-phellandrene (21.46) with much smaller amount (0.47 to 8.49 %) linalool, β-pinene, Delta 3- carene, α-pinene, para-cymene, limonene, myrcene and limonene, are found in *C. longa* oil. The analysis of *C. longa* oil revealed that terpinolene, α- phellandrene, eucalyptol, myrcene and limonene were the main constituents (Table 1.3). Terpinolene, α-phellandrene, limonene and myrcene was also observed as major constituent in I- and II-fraction, however eucalyptol and linalool was absent in these fractions. Fraction three showed terpinolene and eucaptyol as a major compound, however, myrcene, α- phellandrene and limonene were present in trace amount. Fourth and fifth fraction consist eucalyptol and linalool as a major constituent while all other compounds were in trace amount (Figure 1-6).

Fumigant toxicity of major constituents of *C. longa* oil and its fractions against *S. oryzae* and *R. dominica*: The fumigant action of major constituents of essential oil against *S. oryzae* adults is presented in table 1.4 which indicates that among all constituents' α-pinene and β-pinene were highly effective against *S. oryzae* adults, causing 100% mortality within 6 h at a dose of 10 µl/15 ml. However, the insecticidal activity of these compounds was decreased with decreasing doses. Complete control was obtained after the 12 h exposure at a dose of 7.5 to 10 µl/15 ml with α-pinine and β- pinine while limonene and eucalyptol achieved 100% mortality at a dose of 5 µl/15ml and above. 100% mortality was obtained at highest dose with terpinolene and α-phellandrene after 12 h exposure. There was no mortality was found in untreated control for *S. oryzae* at the end of 18 h exposure period. The terpenoids α-pinine, β-pinene, sabinene, limonene, α-phellandrene and eucalyptol revealed good adulticidal activity against *S. oryzae* after 18 h exposure.

Fumigant toxicity of the constituents against *R. dominica* showed limonene, α-phellandrene and eucalyptol all gave complete control at highest dose of 10 µl/15 ml within 6 h, however, all other constituents were also found highly effective, causing 96.67% mortality. 100% mortality was obtained at a dose of 10 µl/15 ml with all Constituents except myrcene and after 12 h exposure. Limonene and eucalyptol caused 100% mortality at all dosages within 12 h while terpinolene and α-phellandrene achieved complete control at a dose of 5 µl/15 ml and above. Treatment with myrcene was less effective against *R. dominica.* Complete control was obtained after 18 h exposure at all dosages with α-pinene, β-pinene, limonene, α-phellandrene and eucalyptol while myrcene and terpinolene achieved 100% mortality at a dose of 5 µl/15 ml and above. No mortality was observed in untreated control treatment. (Table 1.5) This study was also supported by following scientist found that the major constituents of eucalyptus oil was 1-8 cineole (81.1%), limonene (7.6%) and α-pinene (4.0%), these oils were analyzed by GC-MS analysis. Lee *et al.* (2001) reported that these terpenes were most active against *S. oryzae* and same report given by Park *et al*[14]., for contact and fumigant activity of terpenes against adults of *C. chinensis* and *S. oryzae* (L.).

Table 1.1 Effect of *C. longa* oil and its fractions on development of *S. oryzae*

Treat. NO.	*Curcuma longa*	Conc. % (v/w)	Amount in µl	Total no of adult emerged			% inhibition		
				I screening	II screening	Average	I screening	II screening	Average
1	FractionI	0.1	50 µl	2.67 (1.07)	2.33 (0.69)	2.50	98.57	98.68	98.63
2	Fraction II	0.1	50 µl	1.33 (0.54)	11.00 (1.89)	6.17	99.28	93.76	96.52
3	Fraction III	0.1	50 µl	19.00 (2.31)	31.67 (2.15)	25.34	89.78	82.04	85.91
4	Fraction IV	0.1	50 µl	15.00 (2.74)	0.33 (0.23)	7.67	91.94	99.81	95.88
5	Fraction V	0.1	50 µl	6.00 (1.76)	10.33 (1.79)	8.17	96.77	94.14	95.46
6	Oil	0.1	50 µl	0.00 (0.00)	0.00 (0.00)	0.00	100.00	100.00	100.00
7	FractionI	0.05	25 µl	43.33 (3.52)	70.67 (4.02)	57.00	76.70	59.92	68.31
8	Fraction II	0.05	25 µl	92.00 (4.34)	71.33 (3.97)	81.67	50.54	59.55	55.05
9	Fraction III	0.05	25 µl	90.33 (4.44)	119.67 (4.61)	105.00	51.43	32.13	41.78
10	Fraction IV	0.05	25 µl	108.00 (4.60)	131.33 (4.85)	119.67	41.94	25.52	33.73
11	Fraction V	0.05	25 µl	94.33 (4.35)	136.00 (4.72)	115.17	49.28	22.87	36.08
12	Oil	0.05	25 µl	63.33 (3.94)	139.33 (4.87)	101.33	65.95	20.98	43.47
13	FractionI	0.025	12.5 µl	114.00 (4.44)	61.00 (3.94)	87.50	38.71	65.41	52.06
14	Fraction II	0.025	12.5 µl	216.33 (5.37)	110.33 (4.67)	163.33	-16.31	37.43	10.56
15	Fraction III	0.025	12.5 µl	135.33 (4.67)	126.67 (4.40)	131.00	27.24	28.16	27.70
16	Fraction IV	0.025	12.5 µl	213.67 (5.36)	136.00 (4.72)	174.84	-14.87	22.87	4.00
17	Fraction V	0.025	12.5 µl	159.67 (5.06)	150.67 (5.02)	155.17	14.16	14.55	14.36
18	Oil	0.025	12.5 µl	136.33 (4.90)	143.00 (4.83)	139.67	26.70	18.90	22.80
19	Control			186.00 (5.17)	176.33 (5.18)	181.17			
	S.Em ±			(0.42)	(0.59)				
	CD at 5%			(1.21)	(1.68)				

**Data in parentheses indicate log (x+1) transformed values

Table 1.2 Effect of *C. longa* oil and its fractions on development of *R. dominica*

Treat. NO.	*Curcuma longa*	Conc. % (v/w)	Amount in µl	Total no of adult emerged			% inhibition		
				I screening	II screening	Average	I screening	II screening	Average
1	FractionI	0.1	50 µl	0.00 (0.00)	0.00 (0.00)	0.00	100.00	100.00	100
2	Fraction II	0.1	50 µl	0.00 (0.00)	0.00 (0.00)	0.00	100.00	100.00	100
3	Fraction III	0.1	50 µl	0.00 (0.00)	0.00 (0.00)	0.00	100.00	100.00	100
4	Fraction IV	0.1	50 µl	0.00 (0.00)	0.00 (0.00)	0.00	100.00	100.00	100
5	Fraction V	0.1	50 µl	16.67 (2.81)	0.00 (0.00)	16.67	90.18	100.00	95.09
6	Oil	0.1	50 µl	0.00 (0.00)	0.00 (0.00)	0.00	100.00	100.00	100
7	FractionI	0.05	25 µl	9.67 (1.52)	4.00 (1.60)	13.67	94.30	98.17	96.235
8	Fraction II	0.05	25 µl	9.33 (1.99)	22.00 (1.89)	31.33	94.50	89.92	92.21
9	Fraction III	0.05	25 µl	4.33 (1.66)	4.33 (1.67)	8.66	97.45	98.02	97.735
10	Fraction IV	0.05	25 µl	11.33 (2.50)	6.00 (1.76)	17.33	93.32	97.25	95.285
11	Fraction V	0.05	25 µl	138.67 (4.94)	118.67 (4.76)	257.34	18.27	45.65	31.96
12	Oil	0.05	25 µl	1.67 (0.77)	3.00 (1.30)	4.67	99.02	98.63	98.825
13	FractionI	0.025	12.5 µl	89.33 (4.42)	201.67 (5.36)	291.00	47.35	7.63	27.49
14	Fraction II	0.025	12.5 µl	122.00 (4.76)	208.33 (5.39)	330.33	28.10	4.58	16.34
15	Fraction III	0.025	12.5 µl	79.33 (4.32)	214.33 (5.40)	293.66	53.24	1.83	27.535
16	Fraction IV	0.025	12.5 µl	48.67 (3.79)	192.00 (5.30)	240.67	71.32	12.06	41.69
17	Fraction V	0.025	12.5 µl	123.33 (4.71)	208.00 (5.33)	331.33	27.31	4.73	16.02
18	Oil	0.025	12.5 µl	18.00 (2.35)	64.33 (4.03)	82.33	89.39	70.53	79.96
19	Control			169.67 (5.08)	218.33 (5.43)				
	S.Em ±			(0.40)	(0.23)				
	CD at 5%			(1.14)	(0.67)				

**Data in parentheses indicate log (x+1) transformed values

Table 1.3: Major constituent of *C. longa* essential oil and its fractions

S. No.	R. Time	Area	Area %	Constituent name	S. No.	R. Time	Area	Area %	Constituent name
***C. longa* oil**					***C. longa* oil Fraction I**				
1	2.532	9230707	2.78	Alpha –Pinene	1	2.531	8478165	2.86	Alpha- pinene
2	3.262	2971653	0.89	Beta-Pinene	2	3.261	2945918	0.99	Beta-Pinene
3	3.744	21268508	6.40	Myrcene	3	3.742	19681909	6.63	Myrcene
4	3.909	71290799	21.46	Alpha –Fellandrene	4	3.906	66551390	22.43	Alpha- Phellandrene
5	4.095	18440281	5.55	Alpha-Terpinene	5	4.093	16889963	5.69	Alpha-Terpinene
6	4.382	7834527	2.36	Limonene	6	4.379	11057696	3.73	Limonene
7	4.527	28217737	8.49	Eucalyptol (1,8-cinole)	7	5.118	7545336	2.54	Delta 3- carene
8	5.121	8780181	2.64	Delta 3- careen	8	5.521	8183955	2.76	Para-Cymene
9	5.525	9973001	3.00	Para-Cymene	9	5.840	141194719	47.59	Terpinolene
10	5.841	136592202	41.12	Terpinolene	***C. longa* oil Fraction III**				
11	11.809	1576826	0.47	Linalool	1	3.260	200889	0.05	Beta-Pinene
***C. longa* oil Fraction II**					2	3.743	9611198	2.32	Myrcene
1	2.530	886078	0.37	Alpha-pinene	3	3.900	6529939	1.58	Alpha -phellandrene
2	3.261	669642	0.28	Beta-Pinene	4	4.091	9817021	2.37	Alpha-Terpinene
3	3.744	14349519	5.97	Myrcene	5	4.380	8130627	1.96	Limonene
4	3.901	21394056	8.90	Alpha –phellandrene	6	4.525	48808096	11.79	Eucalyptol
5	4.091	11512117	4.79	Alpha-Terpinene	7	5.522	14794987	3.57	Para-cymene
6	4.380	20630157	8.59	Limonene	8	5.854	272569072	65.83	Terpinolene
7	5.522	17295475	7.20	Para-Cymene	9	5.118	5686983	1.37	Beta-E-Ocimene
8	5.840	141196080	58.77	Terpinolene	10	11.233	569474	0.14	Camphor
***C. longa* oil Fraction IV**					***C. longa* oil Fraction V**				
1	4.387	298004	0.08	Limonene	1	4.375	297608	0.09	Limonene
2	4.540	267155858	75.75	Eucalyptol	2	4.527	127966930	38.87	Eucalyptol
3	11.248	1937879	0.55	Camphor	3	11.242	407565	0.12	Camphor
4	11.808	16201786	4.59	Linalool	4	11.809	16379134	4.98	Linalool
					5	19.805	110426230	33.54	P-cymen -8-ol
					6	24.477	19431355	5.90	Linalool Oxide

Table 1.4 Fumigant toxicity of monoterpenes against *S. oryzae*

Exposure time	Dose µL L^{-1}	% Mortality of *Sitophilus oryzae*							
		α- Pinene	**βPinene**	**Myrcene**	**Limonene**	**Terpinolene**	**α-phyllendrene**	**Eucalyptol**	**Linalool**
6h	500	100.00^{e}	100.00^{c}	56.67^{c}	96.67^{d}	46.67^{b}	86.67^{cd}	96.67^{cd}	13.33^{b}
	350	96.67^{e}	100.00^{c}	46.67^{bc}	93.33^{d}	23.33^{a}	80.00^{c}	93.33^{c}	13.33^{b}
	250	56.67^{bc}	83.33^{b}	36.67^{b}	76.67^{c}	23.33^{a}	46.67^{b}	70.00^{b}	6.67^{a}
	100	10.00^{a}	56.67^{a}	3.33^{a}	23.33^{a}	20.00^{a}	16.67^{a}	20.00^{a}	3.33^{a}
12 h	500	100.00^{e}	100.00^{c}	83.33^{de}	100.00^{d}	100.00^{e}	100.00^{d}	100.00^{d}	30.00^{d}
	350	100.00^{e}	100.00^{c}	83.33^{de}	100.00^{d}	90.00^{de}	93.33^{cd}	100.00^{d}	30.00^{d}
	250	73.33^{cd}	86.67^{b}	46.67^{bc}	100.00^{d}	83.33^{d}	93.33^{cd}	100.00^{d}	20.00^{c}
	100	40.00^{b}	83.33^{b}	46.67^{bc}	53.33^{b}	60.00^{c}	60.00^{b}	73.33^{b}	20.00^{c}
18 h	500	100.00^{e}	100.00^{c}	96.67^{f}	100.00^{d}	100.00^{e}	100.00^{d}	100.00^{d}	90.00^{f}
	350	100.00^{e}	100.00^{c}	93.33^{ef}	100.00^{d}	96.67^{e}	100.00^{d}	100.00^{d}	86.67^{f}
	250	100.00^{e}	100.00^{c}	86.67^{def}	100.00^{d}	96.67^{e}	100.00^{d}	100.00^{d}	70.00^{e}
	100	86.67^{de}	86.67^{b}	80.00^{d}	100.00^{d}	96.67^{e}	93.33^{cd}	100.00^{d}	66.67^{e}
Control	Untreated	-	-	-	-	-	-	-	-

Table 1.5 Fumigant toxicity of monoterpenes against *R. dominica*

Exposure time	Dose µL L^{-1}	% Mortality of *Rhizopertha dominica*							
		α- Pinene	**βPinene**	**Myrcene**	**Limonene**	**Terpinolene**	**α-phyllendrene**	**Eucalyptol**	**Linalool**
6h	500	96.67^{c}	96.67^{c}	93.33^{def}	100.00^{c}	93.33^{e}	100.00^{b}	100.00^{c}	96.67^{c}
	350	93.33^{c}	96.67^{c}	90.00^{de}	96.67^{bc}	76.67^{c}	93.33^{b}	100.00^{c}	93.33^{c}
	250	83.33^{b}	93.33^{b}	66.67^{c}	93.33^{b}	63.33^{b}	86.67^{b}	93.33^{b}	73.33^{ab}
	100	56.67^{a}	83.33^{a}	36.67^{a}	76.67^{a}	46.67^{a}	46.67^{a}	70.00^{a}	56.67^{a}
12 h	500	100.00^{c}	100.00^{c}	96.67^{ef}	100.00^{c}	100.00^{e}	100.00^{b}	100.00^{c}	100.00^{c}
	350	96.67^{c}	96.67^{c}	86.67^{d}	100.00^{c}	100.00^{e}	100.00^{b}	100.00^{c}	96.67^{c}
	250	96.67^{c}	96.67^{c}	86.67^{d}	100.00^{c}	100.00^{e}	100.00^{b}	100.00^{c}	96.67^{c}
	100	73.33^{ab}	86.67^{ab}	46.67^{b}	100.00^{c}	83.33^{d}	93.33^{b}	100.00^{c}	83.33^{b}
18 h	500	100.00^{c}	100.00^{c}	100.00^{e}	100.00^{c}	100.00^{e}	100.00^{b}	100.00^{c}	100.00^{c}
	350	100.00^{c}	100.00^{c}	100.00^{e}	100.00^{c}	100.00^{e}	100.00^{b}	100.00^{c}	100.00^{c}
	250	100.00^{c}	100.00^{c}	100.00^{e}	100.00^{c}	100.00^{e}	100.00^{b}	100.00^{c}	100.00^{c}
	100	100.00^{c}	100.00^{c}	86.67^{d}	100.00^{c}	96.67^{e}	100.00^{b}	100.00^{c}	100.00^{c}
Control	Untreated	-	-	-					

*Mean in the same column followed by the same letters are not significantly different as determined by Duncan –test. - = 0.00 % mortality

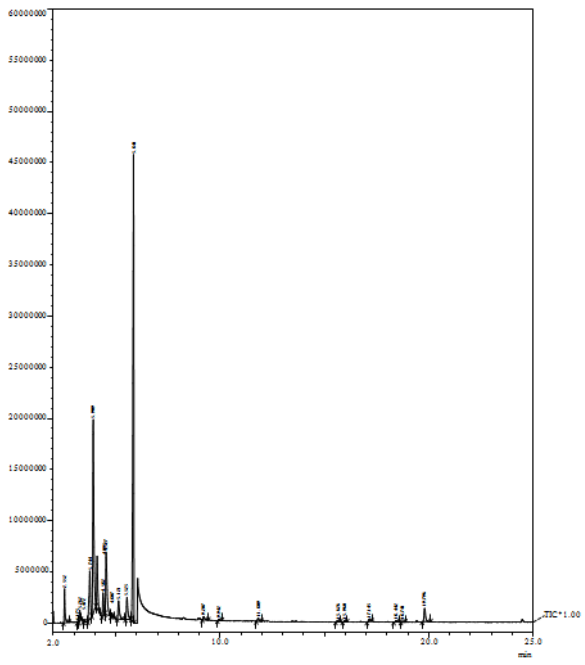

Fig. 1: Chromatogram of *C. longa* oil

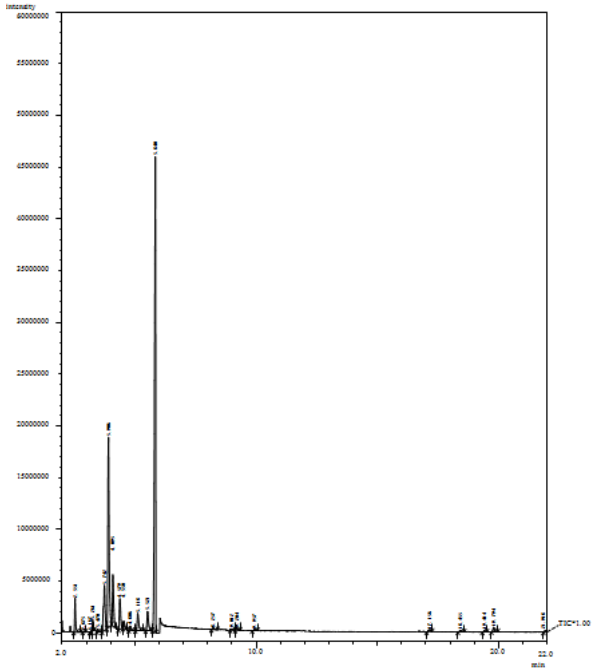

Fig. 2: Chromatogram of I- Fraction of *C. longa* oil

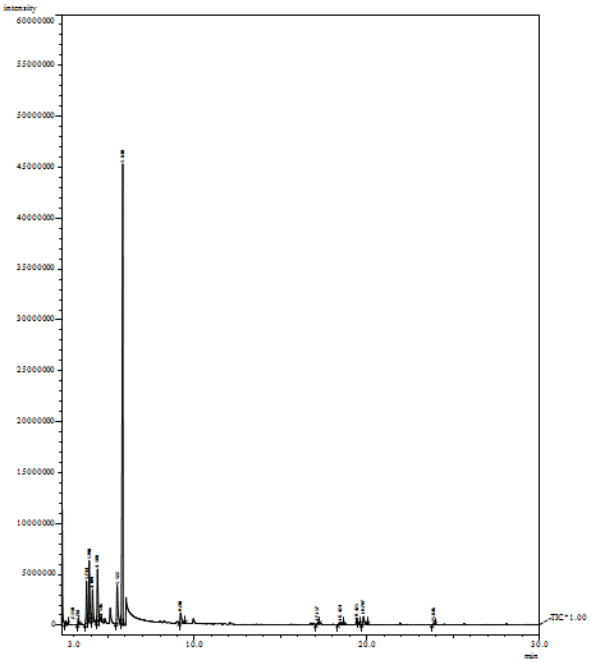

Fig. 3: Chromatogram of II- Fraction of *C. longa* oil

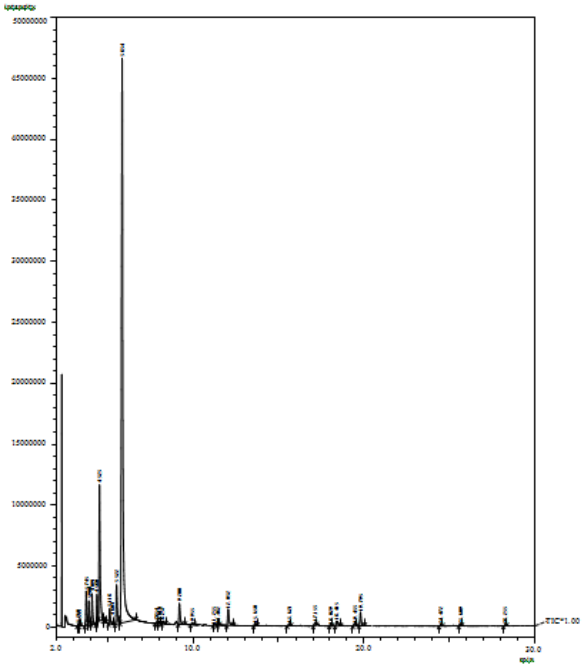

Fig. 4: Chromatogram of III- Fraction of *C. longa* oil

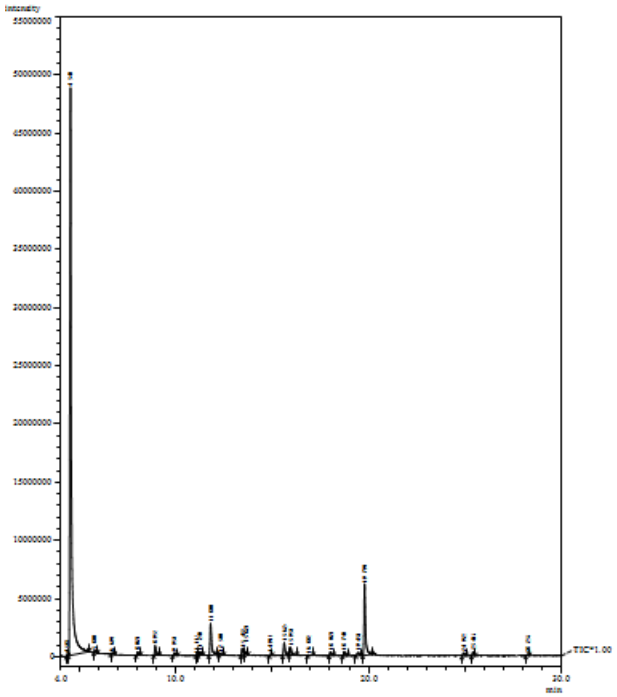

Figure: 5 Chromatogram of IV- Fraction of *C. longa* oil

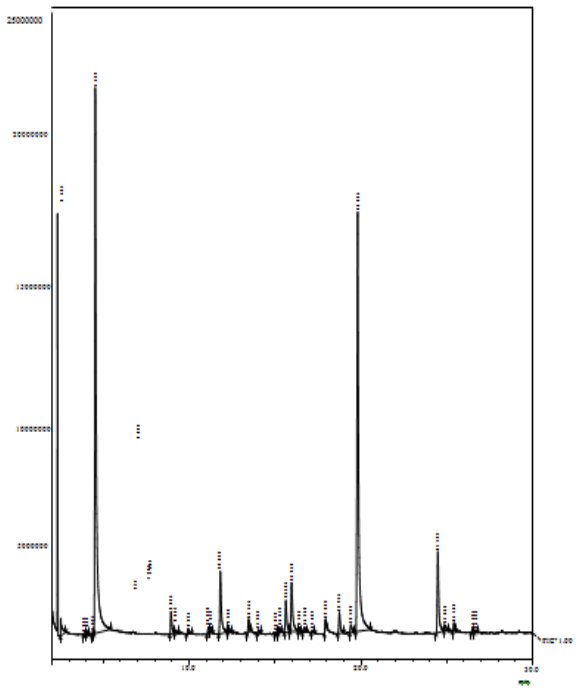

Figure: 6 Chromatogram of V- Fraction of *C. longa* oil

The impregnated-paper test of bornyl acetate, α -phellandrene and terpinolene caused 97, 97 and 87% mortality of stored grains beetles. Restello *et al*[16] reported that the major constituents of *Tagetes patula oil were* limonene (37.05%), terpinolene (32.61%), piperitone (14.40%), neophitadiene (5.91%), sabinene (2.88%), trans-ocimene (2.02%), β-cariphilene (1.98%), farnesol (1.84%), and α-pinene (1.30%) and this oil was efficient to control adult of *S. zeamais* adult. Similarly, 1, 8-cineole, camphor, eugenol, linalool, carvacrol, thymol, borneol, bornyl acetate and linalyl acetate were also evaluated for fumigant activity against adults of *S. oryzae*, *R. dominica* and *T. castaneum* and all these terpenes were found highly effective against stored grain beetles[17].

CONCLUSION

In conclusion, the current study shows that *C. longa* oil and its fractions possess toxic and fumigant activity against *S. oryzae* and *R. dominica*, but relatively higher doses more applications may be required *under* practical storage condition compared with doses of laboratory experiment. The major compounds of this oil were terpinolene α-phellandrene also found toxic against stored grain beetles.

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