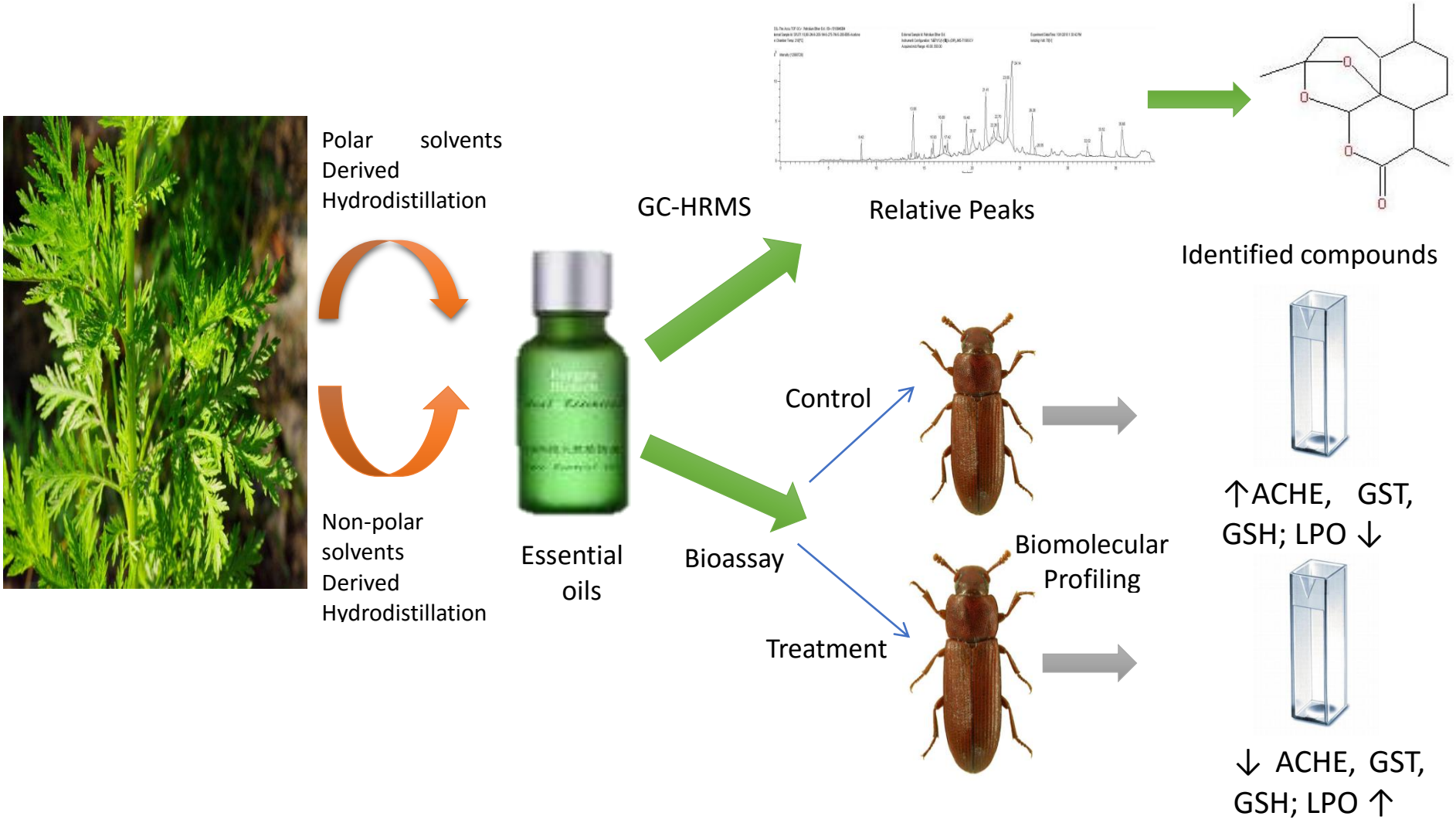
CHAPTER 4

1.0 RESULTS

Highlights

- Isolation and identification of chemical composition of *Artemisia annua* essential oils extracted with polar (methanol, chloroform) and non-polar (petroleum ether, n-hexane) solvents by GC-HRMS.
- *A. annua* solvent derived essential oils (EOs) treatment against *Tribolium casteneum* adults and larvae.
- Identification and comparison of lethal doses and repellency demonstrated by the EOs against *T. casteneum.*
- Quantitative assessment of biomolecules due to metabolic disturbances inflicted in the treatment sets.
- Quality assessment of the damaged and un-damaged grains by SEM imaging and AACC protocols
- Essential oils hold efficient insecticidal properties against the highly resistant strain.

GRAPHICAL ABSTRACT



1.1 Biology of the model beetle

Knowledge on the biology of *Tribolium casteneum*, the model beetle, was a major need of the work. The importance arises from the fact of varying degree of temperature and humidity that hit the different geographical locations has a profound effect on the insect's life cycle. Moreover, studying the key characteristics of the beetle helps us to evade any error in accurate identification.

1.1.1 Stage identification

T. casteneum is a holometabolous insect which have a complete life cycle. Four stages viz. Egg, larva, pupa and adult comprise their life cycle. The larval stage has varying number of larval instars depending on the abiotic factors. Each stage is morphologically and physiologically dissimilar. So morphometric and associated morphological characteristics were taken into account to establish a complete comparative exposition. As a few stage are microscopic and distinguishing characters of adults are fine enough to be observed by the naked eyes hence the study was done using the stereomicroscope (Computer assisted 13.1 mega pixel catcam stereomicroscope; Model no: LEICA MZ 16 A) at 200x magnification (Figure 1, Figure 2). Characteristics unique to each stage were recorded and are explained below.

Egg: Eggs, the first stage in the life cycle, marks the initiation of the generation of all the insects including red flour beetle. They are microscopic, oval in shape & pale white in colour. Flour particles often stick to their surface as they are sticky when laid which makes them more difficult to identify even under microscope. However, they were confirmed using standard references (Leelaja et al., 2007). Eggs are laid in the same culture media and measured about 0.088±0.004 mm in length and 0.056±0.005 mm in breadth (Table 1).

Larva: A total of seven larval instars of *T. casteneum* were recorded in the present study. They are campodeiform, slender in shape. Their dorsal surface is covered with fine bristles and the last abdominal segment is demarcated by the presence of anal cerci. Each larval stage is smaller in size from its

succeeding stage. Completion of incubation period leads to the emergence of minute 1st instar larva, though visible with naked eyes yet their inertness makes it difficult to visualize them. They are ivory white in colour and measures about 0.87±0.04 mm in length and 0.096±0.005 mm in breadth. 2nd instar is mobile, linen white in color and measured about 1.78±0.04 mm in length and 0.28±0.02 mm in breadth. The next instar i.e. 3rd is thread like, light yellowish in colour. They measure about 2.06±0.05 mm in length and 0.376±0.03 mm in breadth. 3rd instar develops into 4th instar which is light brownish in color. They measured about 2.852±0.06 mm in length and 0.45±0.05 mm in breadth. The next one is tortilla colored 5th instar immature which measured about 3.84±0.2 mm in length and 0.67±0.03 mm in breadth. 6th, the next moulted instar is larger and bulgy in appearance. Light brown in color and measured about 4.97±0.04 mm and 0.79±0.02 mm in breadth. The last instar i.e. 7th is highly mobile, heavy and tawny in color. They measured about 5.95±0.05 mm in length and 0.972±0.03 mm in breadth (Table 1).

Pupa: Based on the study, Pupal phase can be differentiated into three stages viz. Pre pupal, pupal & post pupal stage based on their progressive development. Pre pupal stage is light yellowish, smaller in size. The dorsal side possesses fine bristles. Their head region is broad and curved whereas the tail region always have shedding exuvia. Pupal stage recognised by the dark coloration and shorter size. Hind limbs developed on the ventral side and small eye spots are seen. The post pupal stages are demarcated by the presence of fully developed eyes, hind limbs and dark brownish coloration. Pupal stage show sexual dimorphism where females and males have forked and stubby genital papillae respectively. Moreover, the papillae in case of females are longer and reach the length of the urogomphi whereas in male it is small and restricted to the last abdominal segment (Figure 1). Pupa measured about 3.88±0.04 mm in length and 0.96±0.05 mm in breadth (Table 1).

Adult: Adults are dark brownish in colour. They have capitate type of antennae which are very prominent. They measure about 3.96±0.05 mm in length and 1.08±0.04 mm in breadth (Table 1). They shows sexual dimorphism where males possess setiferous patch in the forefemur which is absent in the females (Figure 2)

Table 1: Morphometric data of all the stages of *Tribolium casteneum* (Egg, Larva, pupa & Adult) of F1 generation

Sr. No.	F1 Gen.	Parameters	Morphometric data			
			Mean * (mm)	Range (mm)	±SD	SE
1	Egg	Length	0.088	0.08-0.09	0.004	0.001
		Width	0.056	0.05-0.06	0.005	0.001
2.	1st instar	Length	0.87	0.8-0.9	0.04	0.01
		Width	0.096	0.09-0.1	0.005	0.001
3.	2nd instar	Length	1.78	1.7-1.8	0.04	0.01
		Width	0.28	0.25-.3	0.02	0.008
4.	3rd instar	Length	2.06	2-2.1	0.05	0.01
		Width	0.376	.32-.4	0.03	0.01
5.	4th instar	Length	2.852	2.78-2.9	0.06	0.01
		Width	0.45	0.4-0.5	0.05	0.01
6.	5th instar	Length	3.84	3.6-4	0.2	0.06
		Width	0.67	0.64-.7	0.03	0.01
7.	6th instar	Length	4.97	4.9-5	0.04	0.01
		Width	0.79	0.75-.0.8	0.02	0.006
8.	7th instar	Length	5.95	5.9-6	0.05	0.01
		Width	0.972	0.93-1	0.03	0.01
9.	Pupa	Length	3.88	3.8-3.9	0.04	0.01
		Width	0.96	0.9-1	0.05	0.01
10	Adults (Both male & female)	Length	3.96	3.9-4	0.05	0.01
		Width	1.08	1-1.1	0.04	0.01

Mean of 10 individuals SD= sample standard deviation SE= Standard error of the mean

The biology was studied during post monsoon i.e. August to September. It was observed that the life cycle was completed rapidly within 22 days. The average temperature and humidity recorded was 34±6 ºC and 65±10 RH. The eggs metamorphose into first larval stage within 4-5 days. Each larval stage moulted to the next within one and half days and it metamorphose into pupal stage within 11 days. It took 4-5 days for pupal development and then adult emergence. However, the larval development reduces during winter when the temperature and humidity falls within the range of 25±4 ºC and 52±4 RH. It took the beetle 45 days to complete the life cycle.

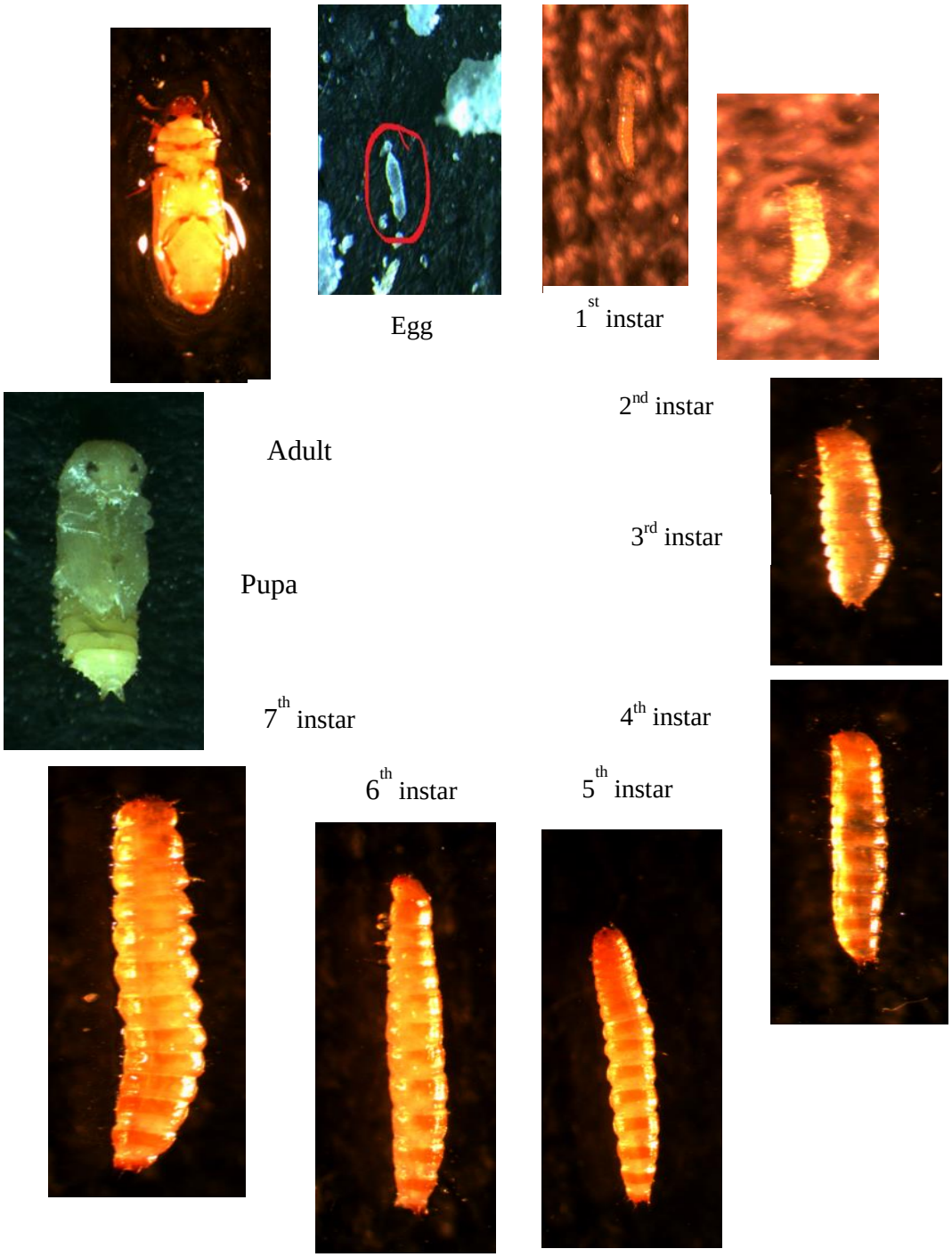


Figure 1: Different stages of *Tribolium casteneum* captured using the computer assisted 13.1 mega pixel catcam stereomicroscope with fine details

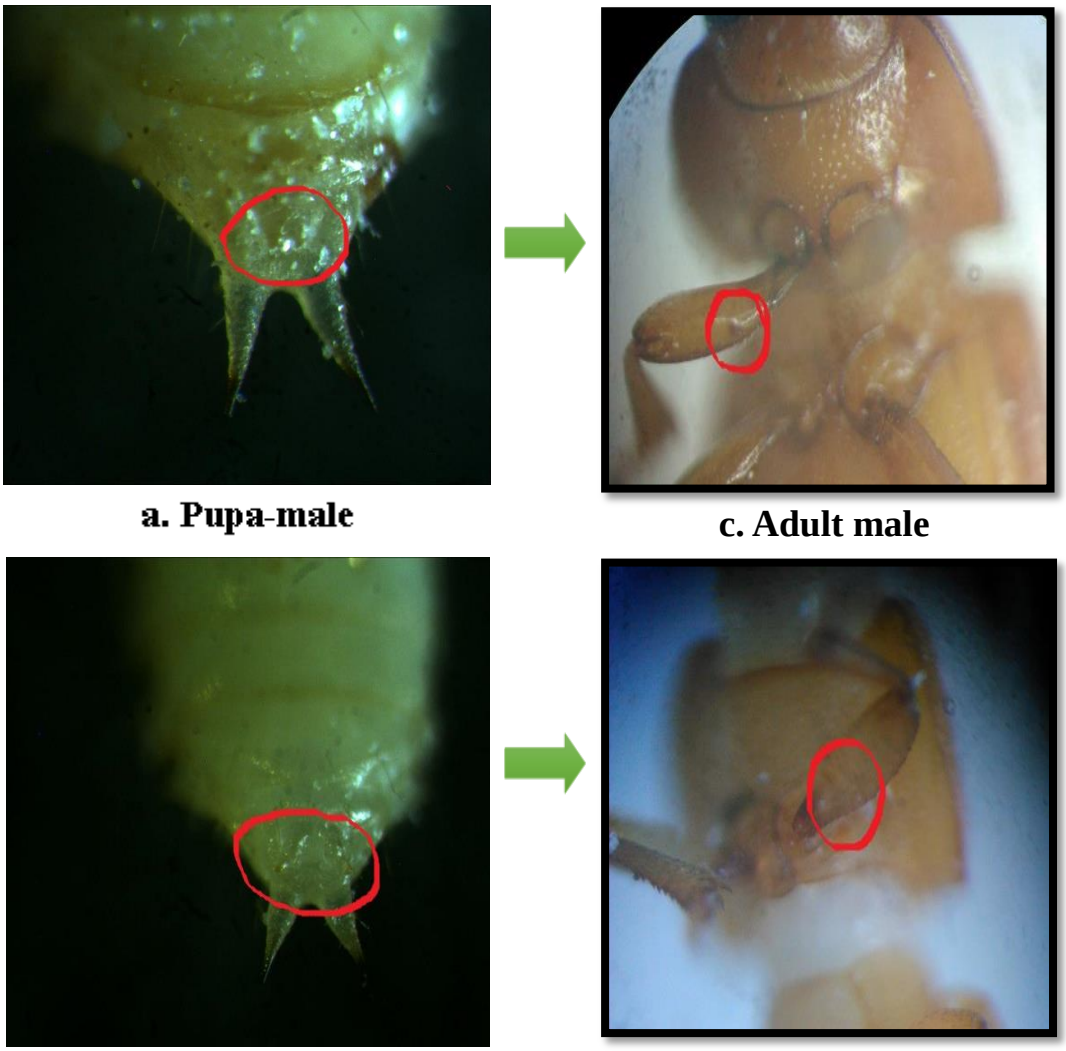


Figure 2: Sexually dimorphic characters of *Tribolium casteneum* **a:** male pupa where genital papilla is stubby, **b:** female pupa where genital papilla is forked reaching the urogomphi, **c:** male adult marked by the presence of setiferous patch on the forefemur, **d:** absence of setiferous patch in female adult.

1.2 Plant material

Artemisia anuua, the plant material was procured in powdered form. This was used to elute EOs using both polar (methanol, chloroform) and non-polar (petroleum ether, n-hexane) solvents through hydrodistillation.

1.2.1 Extraction and analysis of essential oils

The average oil yield from the solvent derived EOs of *A. annua* was different in all the four solvents and they are enlisted below:

Polar solvents

a. Methanolic extract = 27.16%
b. Chloroform extract =19.28%

Non-polar solvents:

a. Petroleum ether (40-60˚C) extract = 1.36%
b. n-Hexane extract = 3.68%

1.2.2 Gas Chromatography-mass spectroscopy

Major peaks determined in the solvent extracted EOs of *A. annua* were marked serially along with their retention indices and percent relative composition according to the order of their elution.

Methanolic crude extract

A total of 13 compounds were reported from methanolic EOs accounting for 99.98% of the total oil (Table 2). Major constituents identified in the oil were 1-Docosene (29.57%), I-Valine, N-Heptafluorobutyryl-, nonyl ester (22.99%), 3-Methylcyclopentadecylcarbamic acid, T- Butyl ester (12.12%) and 5α-Pregn-16-en-20-one,3β,12α-dihydroxy-,diacetate (5.33%). The relative content of the remaining nine minor components was ranged from 1.69 % to 4.67% (Figure 3). Moreover, when the chemical groups were compared, highest percentage of (39.72%) saturated hydrocarbons were reported. This was followed by Alcohols (7.63%), Oxygenated Sesquiterpene (5.07%) and Vitamins (3.49%). A major share of 44.07% was comprises of other

metabolites which could be identified using NIST 5 library and previous literature.

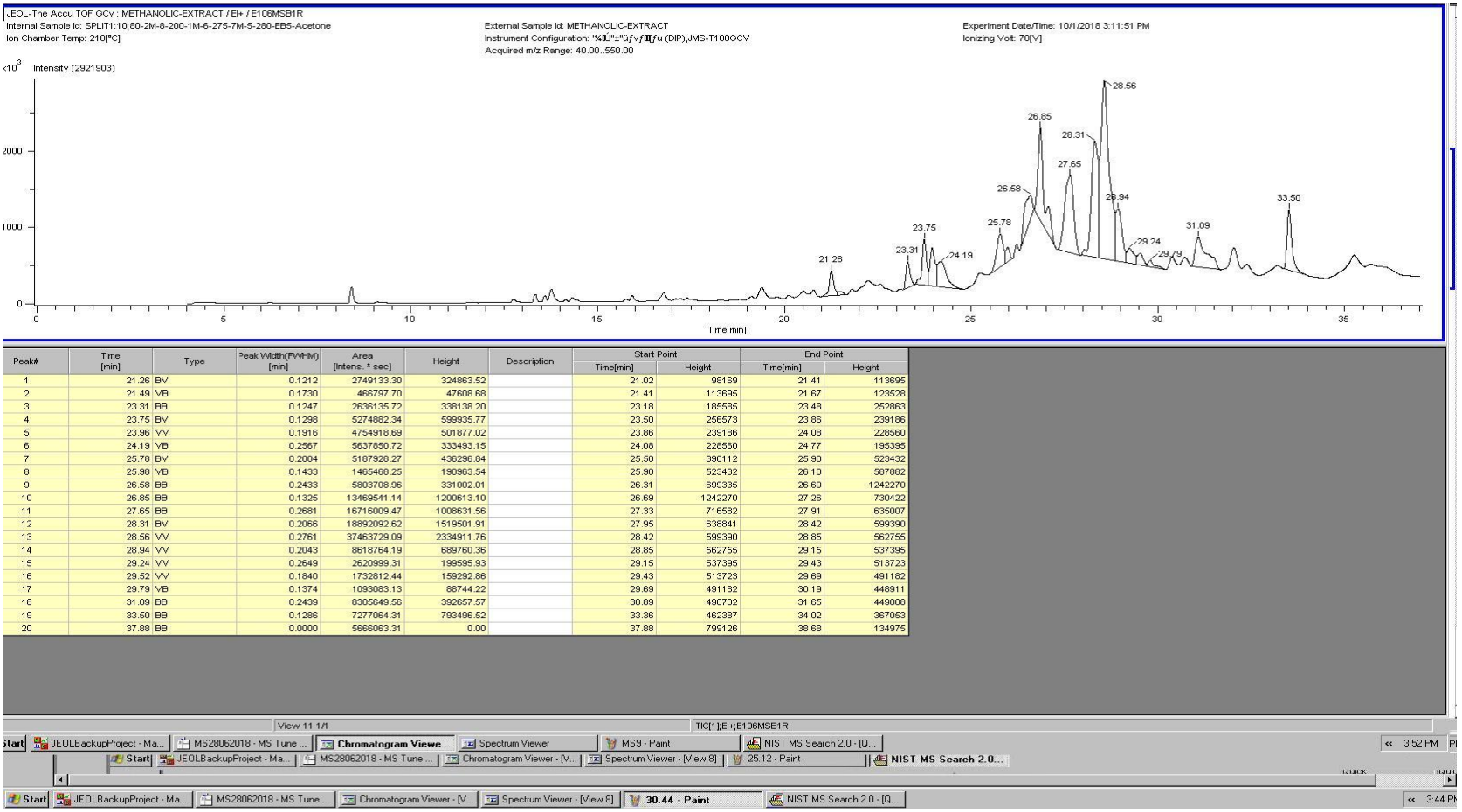
Chloroform crude extract

24 major compounds were identified in the chloroform derived EOs accounted for 97.11% of the total oil (Table 3). Among the major chemical constituents, Bicyclo (22.1) heptan-2-one,1,7,7, trimethyl- (15.35%) 3,4-Hexadienal, 2-butyl-2-ethyl-5-methyl- (10.26%), 2H-1-Benzopyran-2-one (8.20%), 2-(4a,8-Dimethyl-1,2,3,4,4a,5,6,7-octahydronaphthalen-2-yl)-prop-2-en-1-ol (6.01%) and 1-Ethyl-3-vinyl-adamantane (5.55%) were enlisted (Figure 4). Oxalic acid, allyl hexadecyl ester were existed in their stereoisomeric form hence reported with two different retention time in the analysis. The chemical groups found are arranged in the order of highest to lowest. The list includes oxygenated sesquiterpene (20.08%), ketones (17.86%), esters (14.13%), alcohols (12.97%), saturated hydrocarbons (9.91%), ethers (4.58%), and phenol (1.42%). Other metabolites which comprises of 16.14% were also reported in the EOs.

Petroleum ether crude extract:

Petroleum ether derived EOs were recorded to possess 16 different compounds which accounts for 98.15% of the total oil (Table 4). Among the major chemical constituents, 3,4-Hexadienal, 2-butyl-2-ethyl-5-methyl- (22.06%), Deoxyqinghaosu (10.84%), Acetic acid, (1,2,3,4,5,6,7,8-octahydro-3,8,8-trimethylnaphth-2-yl) methylester (9.68), 2-Isopropyl-4,4,7a-trimethyl-2,4,5,6,7,7a-hexahydro-benzofuran-6-ol (7.84%), 1-Eicosanol (7.68%) and 2H-1-Benzopyran-2-one (7.03%) were enlisted (Figure 5). It is important to mention that like chloroform extract, petroleum ether EOs also possessed stereoisomeric form of Isoaromadendrene epoxide and Spiro (4,5)decan-7-one,1,8-dimethyl-8,9-epoxy-4-isopropyl- and hence reported with two different retention time. In the EOs, oxygenated sesquiterpene (42.57%) holds highest percent among the chemical groups followed by esters (16.71%), saturated alcohols (5.84%), ethers (4.41%), hydrocarbons (4.26%), vitamins (3.36%), and other metabolites (19%).

On the other hand, chemical composition of the n-Hexane eluted EO was recorded to contain 18 different compounds accounting for 97.11% of the total oil (Table 5). The major constituents as depicted in the Figure 6 were identified as 3,4-Hexadienal,2- butyl-2-ethyl-5-methyl-2 (20.98%), Cedran-diol, 8S,13- (8.29%), 4,4-Dimethyladamantan-2-ol (7.37%), Bicyclo(2.2.1)heptan-2-one,1,7,7-trimethyl- (7.20), 2H -1- Benzopyran- 2-one (7.09), Deoxyqinghaosu (6.39%), 1,4-Methanoazulene-9-methanol,decahydro-4,8,8-trimethyl- (6.03). Oxygenated sesquiterpene (36.02%) was found as the major chemical group in the n-hexane derived EOs. Among other groups, alcohols (19.56%), esters (8.76%), saturated hydrocarbon (8.56%), ketones (7.2%), carboxylic acid (5.23%), aldehydes (3.67%), ethers (3.64%), and other metabolites (7.35%).



Peak#	Time [min]	Type	Peak Width(FWHM) [min]	Area [Intens. * sec]	Height	Description	Start Point		End Point	
							Time[min]	Height	Time[min]	Height
1	21.26	BV	0.1212	2749133.30	324863.52		21.02	98169	21.41	113695
2	21.49	VB	0.1730	466797.70	47608.68		21.41	113695	21.67	123528
3	23.31	BB	0.1247	2636135.72	338138.20		23.18	185585	23.48	252863
4	23.75	BV	0.1298	5274882.34	599935.77		23.50	256573	23.86	239186
5	23.96	VV	0.1916	4754918.69	501877.02		23.86	239186	24.08	228560
6	24.19	VB	0.2567	5637850.72	333493.15		24.08	228560	24.77	195395
7	25.78	BV	0.2004	5187928.27	436296.84		25.50	390112	25.90	523432
8	25.98	VB	0.1433	1465468.25	190963.54		25.90	523432	26.10	587882
9	26.58	BB	0.2433	5803708.96	331002.01		26.31	699335	26.69	1242270
10	26.85	BB	0.1325	13469541.14	1200613.10		26.69	1242270	27.26	730422
11	27.65	BB	0.2681	16716009.47	1008631.56		27.33	716582	27.91	635007
12	28.31	BV	0.2066	18892092.62	1519501.91		27.95	638841	28.42	599390
13	28.56	VV	0.2761	37463729.09	2334911.76		28.42	599390	28.85	562755
14	28.94	VV	0.2043	8618764.19	689760.36		28.85	562755	29.15	537395
15	29.24	VV	0.2649	2620999.31	199595.93		29.15	537395	29.43	513723
16	29.52	VV	0.1840	1732812.44	159292.86		29.43	513723	29.69	491182
17	29.79	VB	0.1374	1093083.13	88744.22		29.69	491182	30.19	448911
18	31.09	BB	0.2439	8305649.56	392657.57		30.89	490702	31.65	449008
19	33.50	BB	0.1286	7277064.31	793496.52		33.36	462387	34.02	367053
20	37.88	BB	0.0000	5666063.31	0.00		37.88	799126	38.68	134975

Figure 3: Gas chromatogram of the EOs of *Artemisia annua* extracted with methanol

Table 2: Chemical composition of the essential oils of *Artemisia annua* extracted with methanol

Peak No.	Compounds	RI[a]	RI[b]	ID[c]	Relative content (%)[d]
1	Spiro(2,7)dec-4-ene,1,1,5,6,6,9,9-heptamethyl-10-methylene	1656	-	MS	1.76
2	Tricyclo(3.3.1.1.<3,7>)decane,tricyclo(3.3.1.1.<3,7>)decylidene	1825	-	MS	3.63
3	Deoxyqinghaosu	1794	-	MS	1.69
4	3,4-Hexadienal, 2-butyl-2-ethyl-5-methyl	-	-	MS	3.38
5	Phytol	2045	2104	MS, RI	3.05
6	I-Valine, n-heptafluorobutyryl-, nonylester	1807	1786	MS, RI	22.99
7	5β,7βH,10α-Eudesm-11-en-1α-ol	1651	-	MS	4.58
8	Nonadecane	1910	1900	MS, RI	3.72
9	3-Methylcyclopentadecylcarbamic acid, t- butyl ester	2600	-	MS	12.12
10	1-Docosene	2198	2188	MS, RI	29.57
11	Vitamin E (α tocopherol)	-	-	MS	3.49
12	5α-Pregn-16-en-20-one,3β,12α-dihydroxy-,diacetate	2732	-	MS	5.33
13	Squalene	2914	2828	MS, RI	4.67
		Total			**99.98%**
	Grouped components (%)				
	Oxygenated Sesquiterpene			5.07	

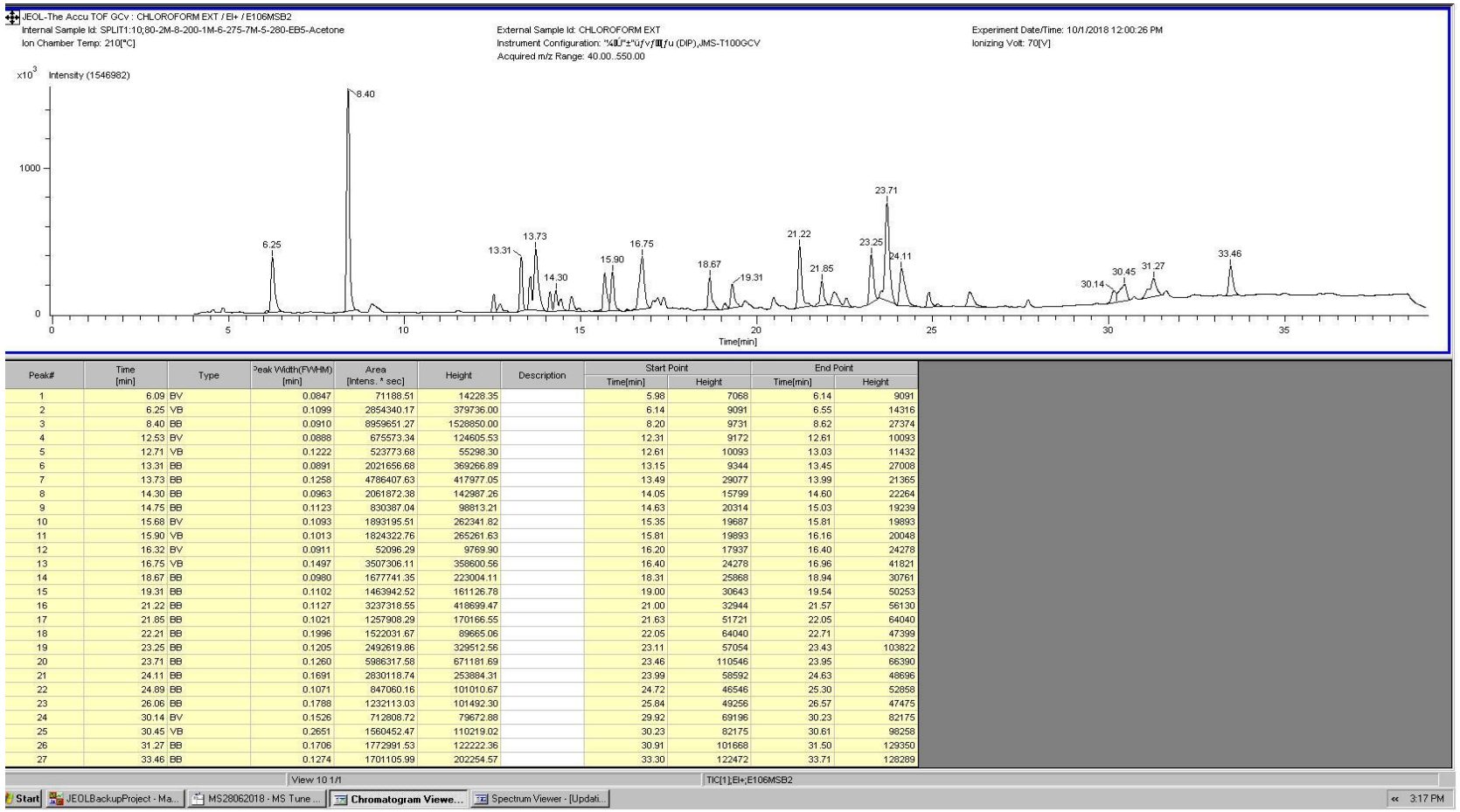
	Saturated Hydrocarbons	39.72
	Alcohols	7.63
	Vitamin	3.49
	Other Metabolites	44.07

[a]Retention indices were calculated using a homologous series of n-alkanes (C8–C24).

[b]Retention indices reported in previous studies

[c]Identification of volatile components was carried out by comparing Mass spectrum (MS) and Retention indices (RI) of components with those of the authentic standards in the NIST library and previous study.

[d]Results obtained by peak-area normalization



Peak#	Time [min]	Type	Peak Width(FWHM) [min]	Area [Intens. * sec]	Height	Description	Start Point		End Point	
							Time[min]	Height	Time[min]	Height
1	6.09	BV	0.0847	71188.51	14228.35		5.98	7068	6.14	9091
2	6.25	VB	0.1099	2854340.17	379736.00		6.14	9091	6.55	14316
3	8.40	BB	0.0910	8959651.27	1528850.00		8.20	9731	8.62	27374
4	12.53	BV	0.0888	675573.34	124605.53		12.31	9172	12.61	10093
5	12.71	VB	0.1222	523773.68	55298.30		12.61	10093	13.03	11432
6	13.31	BB	0.0891	2021656.68	369266.89		13.15	9344	13.45	27008
7	13.73	BB	0.1258	4786407.63	417977.05		13.49	29077	13.99	21365
8	14.30	BB	0.0963	2061872.38	142987.26		14.05	15799	14.60	22264
9	14.75	BB	0.1123	830387.04	98813.21		14.63	20314	15.03	19239
10	15.68	BV	0.1093	1893195.51	262341.82		15.35	19687	15.81	19893
11	15.90	VB	0.1013	1824322.76	265261.63		15.81	19893	16.16	20048
12	16.32	BV	0.0911	52096.29	9769.90		16.20	17937	16.40	24278
13	16.75	VB	0.1497	3507306.11	358600.56		16.40	24278	16.96	41821
14	18.67	BB	0.0980	1677741.35	223004.11		18.31	25868	18.94	30761
15	19.31	BB	0.1102	1463942.52	161126.78		19.00	30643	19.54	50253
16	21.22	BB	0.1127	3237318.55	418699.47		21.00	32944	21.57	56130
17	21.85	BB	0.1021	1257908.29	170166.55		21.63	51721	22.05	64040
18	22.21	BB	0.1996	1522031.67	89665.06		22.05	64040	22.71	47399
19	23.25	BB	0.1205	2492619.86	329512.56		23.11	57054	23.43	103822
20	23.71	BB	0.1260	5986317.58	671181.69		23.46	110546	23.95	66390
21	24.11	BB	0.1691	2830118.74	253884.31		23.99	58592	24.63	48696
22	24.89	BB	0.1071	847060.16	101010.67		24.72	46546	25.30	52858
23	26.06	BB	0.1788	1232113.03	101492.30		25.84	49256	26.57	47475
24	30.14	BV	0.1526	712808.72	79672.88		29.92	69196	30.23	82175
25	30.45	VB	0.2651	1560452.47	110219.02		30.23	82175	30.61	98258
26	31.27	BB	0.1706	1772991.53	122222.36		30.91	101668	31.50	129350
27	33.46	BB	0.1274	1701105.99	202254.57		33.30	122472	33.71	128289

Figure 4: Gas chromatogram of the essential oils of *Artemisia annua* extracted with chloroform

Table 3: Chemical composition of the essential oils of *Artemisia annua* extracted with chloroform

Sr. No.	Compounds	RI[a]	RI[b]	ID[c]	Relative content (%)[d]
1	2- Pyrrolidinone, 1-methyl-	920	1012	MS, RI	4.89
2	Bicyclo(2.2.1)heptan-2-one,1,7,7, trimethyl-	1121	1136	MS, RI	15.35
3	1- Chloroundecane	1340	1358	MS, RI	1.16
4	2H-1-Benzopyran-2-one, 3,4-dihydro	1392	1350	MS, RI	0.90
5	Bicyclo(7.2.0) undec-4- ene 4,11,11- trimethyl -8- methylene-	1494	1396	MS, RI	3.46
6	2H-1-Benzopyran-2-one	1374	1414	MS, RI	8.20
7	1,6-Cyclodecadiene, 1-methyl-5-methylene-8-(1-methyethyl)-	1515	1480	MS, RI	3.53
8	Phenol,2,4-bis(1,1-dimethyethyl)-	1555	1539	MS, RI	1.42
9	2-Undecanethiol,2-methyl-	1433	1410	MS, RI	3.24
10	Caryophyllene oxide	1507	1576	MS, RI	3.13
11	2-(4a,8-Dimethyl-1,2,3,4,4a,5,6,7-octahydronaphthalen-2-yl)-prop-2-en-1-ol	1745	1732	MS, RI	6.01
12	Oxalic acid, allyl hexadecyl ester	2433	-	MS	2.87
13	Pulegone	1212	-	MS	2.51
14	1-Ethyl-3-vinyl-adamantane	1216	-	MS, RI	5.55
15	Oxalic acid, allyl hexadecyl ester	2433	1514	MS	2.16
16	7-Hydroxy, 6- methoxy- 2H-1-benzopyran-2-one	1784	1924	MS, RI	2.61
17	Deoxyqinghaosu	1794	-	MS	4.27

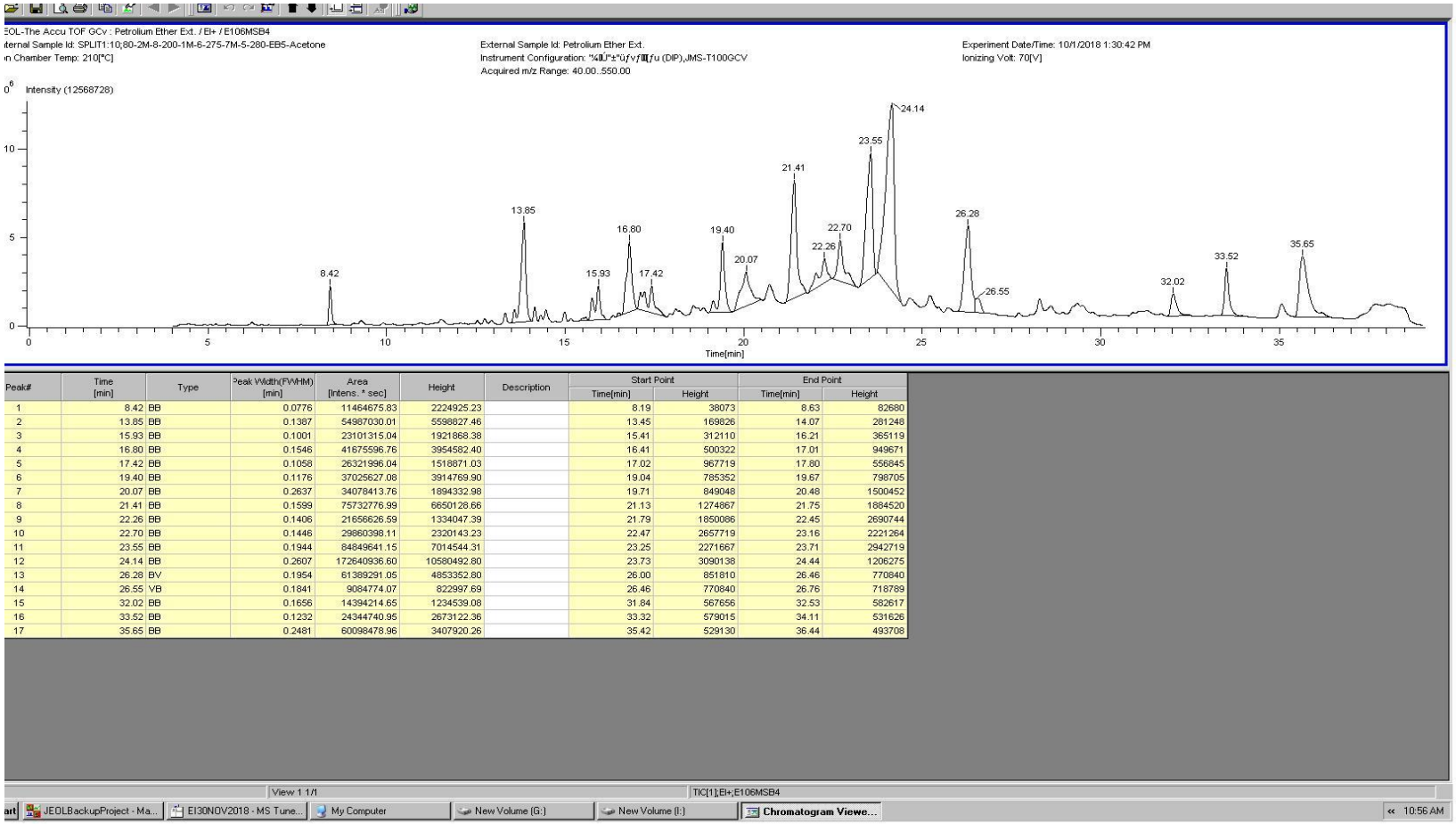
18	3,4-Hexadienal,2- butyl-2-ethyl-5-methyl	-	-	MS	10.26
19	Phytol	2045	2104	MS, RI	4.85
20	Oxirane(Tetradecyloxy)methyl-	1877	-	MS	1.45
21	1,4-Methanoazulene-9-methanol,decahydro-4,8,8-trimethyl-	1635	1712	MS, RI	2.11
22	Didodecyldimethylammonium	-	-	MS	1.22
23	1,3-Dimethyl-5-3(2-methoxycarbonyl-2 Acetamidoethyl)-1H-indol-2-yl 1-yl uracil	3566	-	MS	3.02
24	Squalene	2914	2818	MS, RI	2.92
		Total			**97.11**
	Grouped components (%)				
	Oxygenated Sesquiterpene	20.08			
	Ketones	17.86			
	Esters	14.13			
	Alcohols	12.97			
	Saturated Hydrocarbons	9.91			
	Ethers	4.58			
	Phenol	1.42			
	Other Metabolites	16.14			

[a]Retention indices were calculated using a homologous series of n-alkanes (C8–C24).

[b]Retention indices reported in previous studies

[c]Identification of volatile components was carried out by comparing Mass spectrum (MS) and Retention indices (RI) of components with those of the authentic standards in the NIST library and previous study.

[d]Results obtained by peak-area normalization.



Peak#	Time [min]	Type	Peak Width(FWHM) [min]	Area [Intens. * sec]	Height	Description	Start Point		End Point	
							Time[min]	Height	Time[min]	Height
1	8.42	BB	0.0776	11464675.83	2224925.23		8.19	38073	8.63	82680
2	13.85	BB	0.1387	54987030.01	5598827.46		13.45	169826	14.07	281248
3	15.93	BB	0.1001	23101315.04	1921868.38		15.41	312110	16.21	365119
4	16.80	BB	0.1546	41675596.76	3954582.40		16.41	500322	17.01	949671
5	17.42	BB	0.1058	26321996.04	1518871.03		17.02	967719	17.80	556845
6	19.40	BB	0.1176	37025627.08	3914769.90		19.04	785352	19.67	798705
7	20.07	BB	0.2637	34078413.76	1894332.98		19.71	849048	20.48	1500452
8	21.41	BB	0.1599	75732776.99	6650128.66		21.13	1274867	21.75	1884520
9	22.26	BB	0.1406	21656626.59	1334047.39		21.79	1850086	22.45	2690744
10	22.70	BB	0.1446	29860398.11	2320143.23		22.47	2657719	23.16	2221264
11	23.55	BB	0.1944	84849641.15	7014544.31		23.25	2271667	23.71	2942719
12	24.14	BB	0.2607	172640936.60	10580492.80		23.73	3090138	24.44	1206275
13	26.28	BV	0.1954	61389291.05	4853352.80		26.00	851810	26.46	770840
14	26.55	VB	0.1841	9084774.07	822997.69		26.46	770840	26.76	718789
15	32.02	BB	0.1656	14394214.65	1234539.08		31.84	567656	32.53	582617
16	33.52	BB	0.1232	24344740.95	2673122.36		33.32	579015	34.11	531626
17	35.65	BB	0.2481	60098478.96	3407920.26		35.42	529130	36.44	493708

Figure 5: Gas chromatogram of the essential oils of *Artemisia annua* extracted with petroleum ether

Table 4: Chemical composition of the essential oils of *Artemisia annua* extracted with petroleum ether

Peak No.	Compounds	RI[a]	RI[b]	ID[c]	Relative content (%)[d]
1	Oxirane, tetradecyl-	1702	-	MS	1.46
2	2H-1-Benzopyran-2-one	1374	1414	MS, RI	7.03
3	Caryophyllene oxide	1507	1576	MS, RI	2.95
4	Isoaromadendrene epoxide	1281	1590	MS, RI	5.32
5	Vitamin E (α tocopherol)	-	-	MS	3.36
6	Spiro(4,5)decan-7-one,1,8-dimethyl-8,9-epoxy-4-isopropyl-	1626	-	MS	4.73
7	Isoaromadendrene epoxide	1281	1590	MS,RI	4.35
8	Aceticacid,(1,2,3,4,5,6,7,8-octahydro-3,8,8-trimethylnaphth-2-yl)methylester	1763	-	MS	9.68
9	Spiro (4.5)decan-7-one,1,8-dimethyl-8,9-epoxy-4-isopropyl-	1626	-	MS	2.77
10	4-(1-Hydroperoxy-2,2-dimethyl-6-methylene-cyclohexyl)-pent-3-en-2-one	1835	1477	MS,RI	3.82
11	Deoxyqinghaosu	1794	-	MS	10.84
12	3,4-Hexadienal,2- butyl-2-ethyl-5-methyl-	-	-	MS	22.06
13	2-Isopropyl-4,4,7a-trimethyl-2,4,5,6,7,7a-hexahydro-benzofuran-6-ol	1604	-	MS	7.84
14	Heptacosane	2705	2700	MS, RI	1.15
15	Squalene	2914	2818	MS, RI	3.11
16	1-Eicosanol	2252	2276	MS, RI	7.68

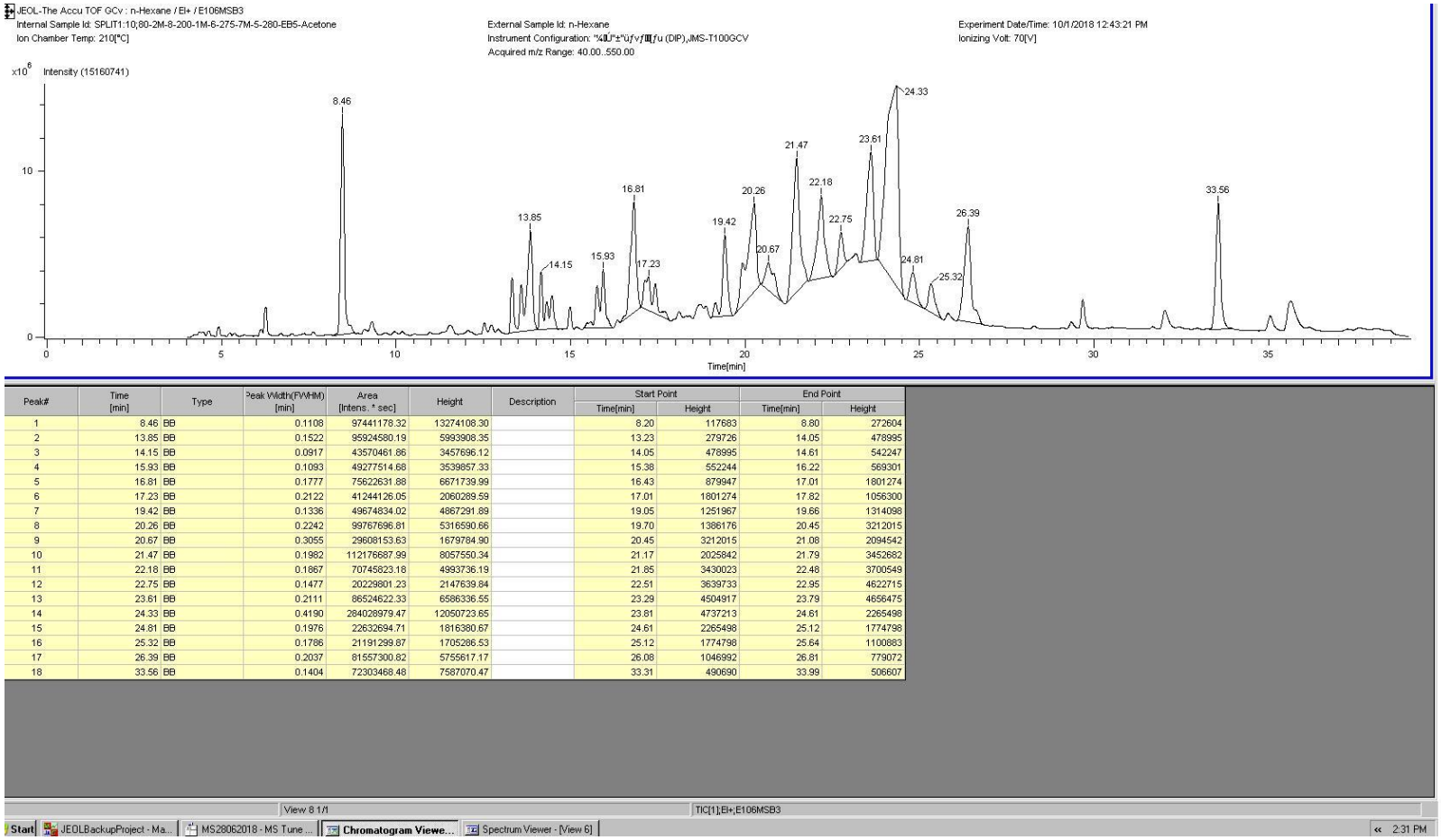
Total		**98.15%**
Grouped components (%)		
Oxygenated Sesquiterpene	42.57	
Saturated Hydrocarbons	4.26	
Alcohols	5.84	
Ethers	4.41	
Esters	16.71	
Vitamin	3.36	
Other Metabolites	19	

[a]Retention indices were calculated using a homologous series of n-alkanes (C8–C24).

[b]Retention indices reported in previous studies

[c]Identification of volatile components was carried out by comparing Mass spectrum (MS) and Retention indices (RI) of components with those of the authentic standards in the NIST library and previous study.

[d]Results obtained by peak-area normalization



Peak#	Time [min]	Type	Peak Width(FWHM) [min]	Area [Intens. * sec]	Height	Description	Start Point		End Point	
							Time[min]	Height	Time[min]	Height
1	8.46	BB	0.1108	97441178.32	13274108.30		8.20	117683	8.80	272604
2	13.85	BB	0.1522	95924580.19	5993908.35		13.23	279726	14.05	478995
3	14.15	BB	0.0917	43570461.86	3457696.12		14.05	478995	14.61	542247
4	15.93	BB	0.1093	49277514.68	3539857.33		15.38	552244	16.22	569301
5	16.81	BB	0.1777	75622631.88	6671739.99		16.43	879947	17.01	1801274
6	17.23	BB	0.2122	41244126.05	2060289.59		17.01	1801274	17.82	1056300
7	19.42	BB	0.1336	49674834.02	4867291.89		19.05	1251967	19.66	1314098
8	20.26	BB	0.2242	99767696.81	5316590.66		19.70	1386176	20.45	3212015
9	20.67	BB	0.3055	29608153.63	1679784.90		20.45	3212015	21.08	2094542
10	21.47	BB	0.1982	112176687.99	8057550.34		21.17	2025842	21.79	3452682
11	22.18	BB	0.1867	70745823.18	4993736.19		21.85	3430023	22.48	3700549
12	22.75	BB	0.1477	20229801.23	2147639.84		22.51	3639733	22.95	4622715
13	23.61	BB	0.2111	86524622.33	6586336.55		23.29	4504917	23.79	4656475
14	24.33	BB	0.4190	284028979.47	12050723.65		23.81	4737213	24.61	2265498
15	24.81	BB	0.1976	22632694.71	1816380.67		24.61	2265498	25.12	1774798
16	25.32	BB	0.1786	21191299.87	1705286.53		25.12	1774798	25.64	1100883
17	26.39	BB	0.2037	81557300.82	5755617.17		26.08	1046992	26.81	779072
18	33.56	BB	0.1404	72303468.48	7587070.47		33.31	490690	33.99	506607

Figure 6: Gas chromatogram of the essential oils of *Artemisia annua* extracted with n-hexane

Table 5: Chemical composition of the essential oils of *Artemisia annua* extracted with n-hexane

Sr. No.	Compounds	RI[a]	RI[b]	ID[c]	Relative content (%)[d]
1	Bicyclo(2.2.1)heptan-2-one,7,7-trimethyl-	1121	1146	MS, RI	7.20
2	2H -1- Benzopyran- 2- one	1374	1414	MS, RI	7.09
3	2-Isopropenyl-4α,8-dimethyl-1,2,3,4,4a,5,6,7-octahydronaphthalene	1502	1473	MS, RI	3.22
4	Caryophyllene oxide	1507	1576	MS, RI	3.64
5	Isoaromadendrene epoxide	1281	1590	MS, RI	5.59
6	Globulol	1530	1578	MS, RI	3.05
7	3-Cyclohexane-1-carboxaldehyde,1,3,4-trimethyl	1204	1171	MS	3.67
8	4,4-Dimethyladamantan-2-ol	1203	-	MS	7.37
9	2-Propen-1-ol,3-(2,6,6-trimethyl-1-cyclohexane-1-yl)-	1465	-	MS	2.19
10	Cedran-diol, 8S,13-	1786	-	MS	8.29
11	n-Hexadecanoic acid	1968	1942	MS, RI	5.23
12	4,8a-Dimethyl-6-(2-methyl-oxiran-2-yl)-4a,5,6,7,8,8a-hexahydro-1H-naphthalene-2-one	1742	-	MS	1.49
13	Deoxyqinghaosu	1794	-	MS	6.39
14	3,4- Hexadienal,2- butyl-2-ethyl-5-methyl-2	-	-	MS	20.98
15	Z,Z-5,16-Octadecadien-1-ol acetate	2193	-	MS	1.67
16	11,14,15,16- Tetraoxatetracyclo(10.3.1.0 (4,13).0(8,13))hexadecane-10-one,1,5,9-trimethyl	1903	-	MS	1.57
17	1,4-Methanoazulene-9-methanol,decahydro-4,8,8-trimethyl-	1635	1712	MS, RI	6.03

18	Squalene	2914	2818	MS, RI	5.34
		Total			**97.11**

Grouped components (%)

Oxygenated Sesquiterpene	36.02
Alcohols	19.56
Esters	8.76
Saturated Hydrocarbon	8.56
Ketones	7.2
Carboxylic acid	5.23
Aldehydes	3.67
Ethers	3.64
Other metabolites	7.35

[a]Retention indices were calculated using a homologous series of n-alkanes (C8–C24).

[b]Retention indices reported in previous studies

[c]Identification of volatile components was carried out by comparing Mass spectrum (MS) and Retention indices (RI) of components with those of the authentic standards in the NIST library and previous study.

[d]Results obtained by peak-area normalization.

1.3 Bioassays and Identification of Lethal doses

The major basis of the study was to evaluate the efficacy of EOs of *Artemisia annua* against *Tribolium casteneum*. Assessment of efficiency is measured in terms of repellency and acute toxicity assays. Generally, acute toxicity assays measured the contact and fumigant toxicity of the solvent derived EOs against both the damaging stage of red flour beetle i.e. larvae and adults.

1.3.1 Repellency test

The behavioural bioassay was evaluated through filter paper arena test according to Cosimi et al. (2009) .Repellency of the beetle towards the EOs was also assessed through multi-arm olfactometer (Jayakumar et al., 2017).

1.3.1.1. Filter paper arena test

In the behavioural bioassay, evaluated through filter paper arena test, one half of the filter paper was treated with EOs and other half with acetone. It has resulted in the aggregation of *T. casteneum* in the control half. When released initially they tend to move in all direction irrespective of treatment. However, with time they tend to aggregate in the control half. It is noticed that they accumulate in the underside of the filter paper. Once aggregated in response to the treatment, pests didn't moved until tapped with the paint brush. Moreover, they become sluggish and less mobile.

When evaluated with methanolic EOs, it has demonstrated 91.63% of repellency at the highest concentration of 0.90 mg cm^{-2} (Table 6). Among other concentrations, 0.54 mg cm^{-2}, 0.63 mg cm^{-2}, 0.72 mg cm^{-2}, and 0.81 mg cm^{-2} could draw 62, 80, 86.13, and 90.75% of repellency respectively. Repellency was time and dose dependent in the treatment sets. However, an insignificant increase ($p<0.01$) in repellency with the increase in concentration was seen.

In the chloroform derived EOs treatment sets, the repellency was found time and dose dependent upto the second highest concentration 0.81 mg $cm^{-}2$. The repellency was found to be 59%, 65.63%, 82.5%, and 90.75% at the concentrations of 0.54 mg $cm^{-}2$, 0.63 mg $cm^{-}2$, 0.72 mg $cm^{-}2$, and 0.81 mg

cm^{-}2 respectively **(**Table 7**).** The highest concentration of 0.90 mg cm^{-2} could repel 88.25% of the pests. Moreover, the percent repellency was insignificant ($p < 0.01$) with the increase in EOs concentration.

The EOs derived from petroleum ether has demonstrated 95% of repellency at the highest concentration of 0.90 mg cm^{-2} (Table 8). Repellency increased with time and dose in the treatment sets. The percent repellency of 0.54 mg cm^{-2} was 88.25%. 0.63 mg cm^{-2} could draw 82.13% repellency. EOs at 0.72 mg cm^{-2} showed 90.75% repellency. The highest repellency at the end of 24 hours was detected at 0.81 mg cm^{-2} (97.375%). Moreover, an insignificant increase ($p < 0.01$) in repellency with the increase in concentration was seen.

In the present study, repellency was more evident in the treatment sets of n-hexane derived EOs. Highest concentration of the EO (0.90 mg cm^{-2}) had demonstrated 93.5% of repellency by n-hexane derived EOs (Table 9). However, repellency was recorded to increase insignificantly ($p>0.01$) with the increase in concentration. The repellency at the concentrations of 0.54 mg cm^{-2}, 0.63 mg cm^{-2}, 0.72 mg cm^{-2}, and 0.81 mg cm^{-2} was recorded to be 72.625%, 85.875%, 87.5%, and 96.625% respectively.

Table 6: Repellency of methanol derived EOs of *A. annua* against *T. castaneum* adults using filter paper arena test

Solvent used	Concentration (mg cm^{-2})	Duration of exposure (in) hour								Percent Repellency over 24 hours
		1	2	3	4	5	6	12	24	
		Mean Repellency (% ± SD)								
Methanol	0.54	53±12*	60±20*	57±29*	60*	60±35*	53±42*	80±20*	73±31*	**62.00**
	0.63	53±12*	53±31*	73±23*	87±12*	87±23*	100*	100*	87±12*	**80.00**
	0.72	53±24*	73±23*	80±20*	83±21*	100*	100*	100*	100*	**86.13**
	0.81	80±20*	73±31*	87±12*	93±12*	93±11*	100*	100*	100*	**90.75**
	0.90	80±20*	80±20*	93±12*	87±12*	93±11*	100*	100*	100*	**91.63**

Mean (±SEM) followed by * indicate no significant difference ($p < 0.01$) according to the ANOVA.

Table 7: Repellency of chloroform derived EOs of *A. annua* against *T. castaneum* adults using filter paper arena test

Solvent used	**Concentration (mg cm^{-2})**	**Duration of exposure (in) hour**								**Percent Repellency over 24 hours**
		1	**2**	**3**	**4**	**5**	**6**	**12**	**24**	
		Mean Repellency (% ± SD)								
Chloroform	0.54	67±31*	26±31*	33±12*	40±35*	53±23*	80±20*	80±20*	93±12*	**59**
	0.63	40±35*	80±20*	73±31*	13±46*	53±12*	80±20*	93±11*	93±12*	**65.63**
	0.72	100*	60±35*	87±23*	67±23*	80±20*	80±20*	93±11*	93±12*	**82.5**
	0.81	80±20*	100*	93±12*	73±23*	100*	80±20*	100*	100*	**90.75**
	0.90	93±11*	67±24*	87±23*	73±31*	93±12*	93±12*	100*	100*	**88.25**

Mean (±SEM) followed by * indicate no significant difference ($p < 0.01$) according to the ANOVA.

Table 8: Repellency of petroleum ether derived EOs of *A. annua* against *T. castaneum* adults using filter paper arena test

Solvent used	**Concentration (mg cm^{-2})**	**Duration of exposure (in) hour**								**Percent Repellency over 24 hours**
		1	**2**	**3**	**4**	**5**	**6**	**12**	**24**	
		Mean Repellency (% ± SD)								
Petroleum ether	0.54	80±20*	80±20*	93±12*	93±12*	87±23*	87±23*	93±12*	93±12*	**88.25**
	0.63	60±20*	80*	67±12*	80*	80*	80*	80*	80*	**82.125**
	0.72	87±12*	87±12*	87±12*	93±12*	93±12*	93±12*	93±12*	93±12*	**90.75**
	0.81	93±12*	100*	100	93±12*	93±12*	100*	100*	100*	**97.375**
	0.90	100*	93±12*	93±12*	87±23*	87±23*	100*	100*	100*	**95**

Mean (±SEM) followed by * indicate no significant difference ($p < 0.01$) according to the ANOVA.

Table 9: Repellency of n-hexane derived EOs of *A. annua* against *T. castaneum* adults using filter paper arena test

Solvent used	**Concentration (mg cm^{-2})**	**Duration of exposure (in) hour**								**Percent Repellency over 24 hours**
		1	**2**	**3**	**4**	**5**	**6**	**12**	**24**	
		Mean Repellency (% ± SD)								
n-hexane	0.54	60*	67±12*	60±20*	87±12*	80±20*	60±20*	80±20*	87±11*	**72.625**
	0.63	80±20*	87±23*	80±20*	87±12*	87±12*	93±12*	93±12*	80±20*	**85.875**
	0.72	60±20*	93±12*	87±23*	87±23*	93±12*	87±12*	100*	93±11*	**87.5**
	0.81	87±12*	93±12*	100*	100*	93±12*	100*	100*	100*	**96.625**
	0.90	87±23*	87±23*	100*	87±23*	87±23*	100*	100*	100*	**93.5**

Mean (±SEM) followed by * indicate no significant difference ($p < 0.01$) according to the ANOVA.

1.3.2 Repellency test using multi-arm olfactometer

Repellency tested in the multi arm olfactometer has recorded the response of the pests towards the different EOs. The olfactometer where one arm was packed with the acetone treated filter paper considered as control. On the other hand, remaining four arms were introduced with different concentration of EOs. Number of insects in each arm was processed to calculate EPI.

Repellency was found to be time and dose dependant in the methanolic EOs treatment sets. As a sign of repellence adult beetles were seen to avoid the treated arms while aggregating in the control arm. EPI has ranged from -0.5 one hour post treatment to -0.9 after 24 hours. The EPI was -0.6 during 2nd, 3rd and 4th hours of treatment. EPI then increased to -0.7 after 5 hours followed by -0.8 in the successive hours (Table 10).

Table 10: Repellency of EOs of *Artemisia annua* extracted with methanol against *Tribolium castaneum* adults using Multi arm olfactometer bioassay

Conc. (mg cm^{-2})	**Duration of exposure (in hours)**							
	1	2	3	4	5	6	12	24
	Mean repellency (% ± SD)							
0	15±2.52	16±1.53	16±1	16	17±1	18±1.15	18±2.08	19±1
0.54	1±1	1	2±0.58	1	1	1±0.58	0±0.58	0±0.58
0.63	2±2.89	1±1.53	1±0.58	1±0.58	1	1	2±2.08	1±1.15
0.72	1±0.58	1±1.53	1±1	2±0.58	1±1	0±0.58	0	0
0.81	1±1.16	1±1.16	0±0.58	0±0.58	0	0	0	0
EPI	**-0.5**	**-0.6**	**-0.6**	**-0.6**	**-0.7**	**-0.8**	**-0.8**	**-0.9**

*Values are expressed as mean of five replicates ±standard deviation

On the other hand, chloroform derived EOs reported with an EPI ranged from -0.7 to -1 (Table 2). EPI continues to be -0.7 for the initial three hours which increased to -0.8 after 4 hours of treatment. It then falls to -0.7 again for the successive hours which then increased to -0.9 post 12 hours of treatment (Table 11). After 24 hours highest EPI (-1) was recorded.

Table 11: Repellency of EOs of *Artemisia annua* extracted with chloroform against *Tribolium castaneum* adults using Multi arm olfactometer bioassay

Conc. (mg cm^{-2})	**Duration of exposure (in hours)**							
	1	2	3	4	5	6	12	24
	Mean repellency (% ± SD)							
0	17±1.73	17±0.58	17±2.65	18±1	17±0.58	17±1	19±0.58	20±0.58
0.54	1±0.58	0	1±0.58	0	0	0±0.58	0±0.58	0
0.63	0±0.58	0	1±1	0	0	0	0	0
0.72	1±1.15	2±0.58	1±1.15	1±1	2±0.58	2±0.58	0±0.58	0±0.58
0.81	1±0.58	1±0.58	0±0.58	1±1	1±1	1±1	0	0
EPI	**-0.7**	**-0.7**	**-0.7**	**-0.8**	**-0.7**	**-0.7**	**-0.9**	**-1**

*Values are expressed as mean of five replicates ±standard deviation

EPI was found to range from -0.6 to -1 in sets of petroleum ether eluted EOs treatment (Table 12).

Table 12: Repellency of EOs of *Artemisia annua* extracted with petroleum ether against *Tribolium castaneum* adults using Multi arm olfactometer bioassay

Conc. (mg cm^{-2})	**Duration of exposure (in hours)**							
	1	2	3	4	5	6	12	24
	Mean repellency (% ± SD)							
0	16±3.1	15±7	16±3.5	17±4.0	15±4.6	17±4.9	18±1	20±0.6
0.18	2±2.89	4±6.66	2±2	2±3.46	2±2.65	2±2.52	1±1.15	0
0.27	1±1.15	0	1±0.58	1±0.58	1±1	0±0.58	1±0.58	0
0.36	0±0.58	0	0±0.58	0±0.58	0±0.58	0±0.58	0±0.58	0±0.58
0.45	1±1	1±0.58	1±0.58	0±0.58	2±2.08	1±0.58	0±0.58	0
EPI	**-0.6**	**-0.5**	**-0.6**	**-0.7**	**-0.5**	**-0.7**	**-0.8**	**-1**

*Values are expressed as mean of five replicates ±standard deviation

After one hour it dropped down to -0.5 which again increased to -0.6 in the next hour. EPI reaches -0.7 after four hour followed by -0.5 during five hour post treatment. After 12 hours it was recorded as -0.8 which after 24 hours increased to -1. Repellency was dose dependant in this treatment sets.

n-hexane derived EOs treated sets showed steady increase in EPI ranged from -0.3 to -1 (Table 13). EPI in this treatment was recorded to be -0.3, -0.6, -0.7, -0.8, -0.9 and -1 at 1st, 2nd, 3rd, 4th, 5th, 6th, 12th, and 24th hours respectively post treatment. However, repellency was recorded to be dose & time dependant in the treatment sets.

Table 13: Repellency of EOs of *Artemisia annua* extracted with n-hexane against *Tribolium castaneum* adults using Multi arm olfactometer bioassay.

Conc. (mg cm^{-2})	**Duration of exposure (in hours)**							
	1	2	3	4	5	6	12	24
	Mean repellency (% ± SD)							
0	13±2.7	16±1.2	17±1.2	17±1.5	18±1.2	18±1.2	19±2.1	20
0.18	2±0.58	2±1	2	2±0.58	1	1±0.58	1±1.15	0±0.58
0.27	3±2.65	1±1	1±0.58	1±0.58	1±1.15	0±0.58	0±1.15	0
0.36	1±1	1±1.73	0±1.73	0±1.73	0±1.73	1±1.73	0±2.30	0±2.30
0.45	1±0.58	0±0.58	0±0.58	0	0	0	0±0.58	0
EPI	**-0.3**	**-0.6**	**-0.7**	**-0.7**	**-0.8**	**-0.8**	**-0.9**	**-1**

*Values are expressed as mean of five replicates ±standard deviation

1.4 Contact toxicity

Topical application was employed to evaluate whether the insecticidal activity of the EOs of *A. annua* against *T. casteneum* adults and 14 days old larvae was attributable to contact toxicity. Ten different concentrations in the range of 0.33- 3.33 mg adult^{-1} of polar solvent derived EOs were tested. For non-polar solvents, the concentrations tested were in the range of 0.17- 1.67 mg adult^{-1}. The concentrations were decided after standardisation of each EOs. No

mortality has been recorded in the control sets treated with acetone. Hence, mortality was not corrected using Abbott's formula.

Table 14: Contact toxicity of methanol EOs of *A. annua* applied topically to *T. castaneum* at 30˚C and 70±80% RH.

Plant Extract	Life stage	LD_{50} (mg adult^{-1}	95% Confidence interval	LD_{90} (mg adult^{-1})	95% Confidence interval	Slope ± SE	χ2 (DF)
Methanol	Adults	1.87	1.60-2.13	2.94	2.59-3.54	1.18±0.2	51.735 (1)*
	14 days old Larvae	1.24	0.94-1.84	2.22	1.91-2.75	1.31±0.24	50.562 (1)*

LD_{50}= Lethal dose that kills 50% of the test organisms; LD_{90}= Lethal dose that kills 90% of the test organisms; χ2= chi square.

DF=degrees of freedom; *= Significant (P <0.01); Lethal values are expressed as mean of five replicates.

Table 15: Contact toxicity of chloroform derived EOs of *A. annua* applied topically to *T. castaneum* at 30˚C and 70±80% RH.

Plant Extract	Life stage	LD_{50} (mg adult^{-1})	95% Confidence interval	LD_{90} (mg adult^{-1})	95% Confidence interval	Slope ± SE	χ2 (DF)
Chloroform	Adults	0.97	0.48-1.29	2.30	1.93-3.02	0.96±0.2	32.174 (1)*
	14 days old Larvae	1.57	1.26-1.86	2.81	2.43-3.48	1.04±0.18	42.870 (1)*

LD_{50}= Lethal dose that kills 50% of the test organisms; LD_{90}= Lethal dose that kills 90% of the test organisms; χ2= chi square.

DF=degrees of freedom; *= Significant (P <0.01); Lethal values are expressed as mean of five replicates.

Table 16: Contact toxicity of petroleum ether derived EOs of *A. annua* applied topically to *T. castaneum* at 30˚C and 70±80% RH.

Plant Extract	Life stage	LD_{50} (mg adult^{-1}	95% Confidence interval	LD_{90} (mg adult^{-1}	95% Confidence interval	Slope ± SE	χ2 (DF)
Petroleum ether	Adults	0.43	0.22-0.58	0.98	0.82-1.29	2.33±0.49	37.148 (1)*
	14 days old Larvae	0.60	0.42-0.74	1.20	1.02-1.51	2.16±0.40	40.821 (1)*

LD_{50}= Lethal dose that kills 50% of the test organisms; LD_{90}= Lethal dose that kills 90% of the test organisms; χ2= chi square.

DF=degrees of freedom; *= Significant (P <0.01); Lethal values are expressed as mean of five replicates.

Table 17: Contact toxicity of n-hexane derived EOs of *A. annua* applied topically to *T. castaneum* at 30˚C and 70±80% RH.

Plant Extract	Life stage	LD_{50} (mg adult^{-1})	95% Confidence interval	LD_{90} (mg adult^{-1})	95% Confidence interval	Slope ± SE	χ2 (DF)
n-hexane	Adults	0.71	0.57-0.83	1.19	1.04-1.46	2.60±0.44	54.373 (1)*
	14 days old Larvae	0.47	0.25-0.61	1.04	0.87-1.35	2.24±0.46	37.06 (1)*

LD_{50}= Lethal dose that kills 50% of the test organisms; LD_{90}= Lethal dose that kills 90% of the test organisms; χ2= chi square.

DF=degrees of freedom; *= Significant (P <0.01); Lethal values are expressed as mean of five replicates.

The LD_{50} and LD_{90} values of methanolic EOs against *T. casteneum* adults calculated using probit analysis were 1.87 and 2.94 mg adult^{-1} respectively. Larval treatment demonstrated 1.24 mg larva^{-1} as LD_{50} and 2.22 mg larva^{-1} as the LD_{90} values (Table 14). When the lethal values were compared, significant ($p<0.01$) difference between the two stages was found as no overlap in 95% confidence interval is prominent. The lowest concentration did not show any mortality. Moreover, neither stage showed complete mortality even at the highest concentration. The mortality was dose dependant (Figure 7). However, in larval dose-response curve, plateau is obtained beyond the concentration of 2.25 mg larva^{-1} which confirms no further mortality is obtained within the tested concentration range (Figure 8).

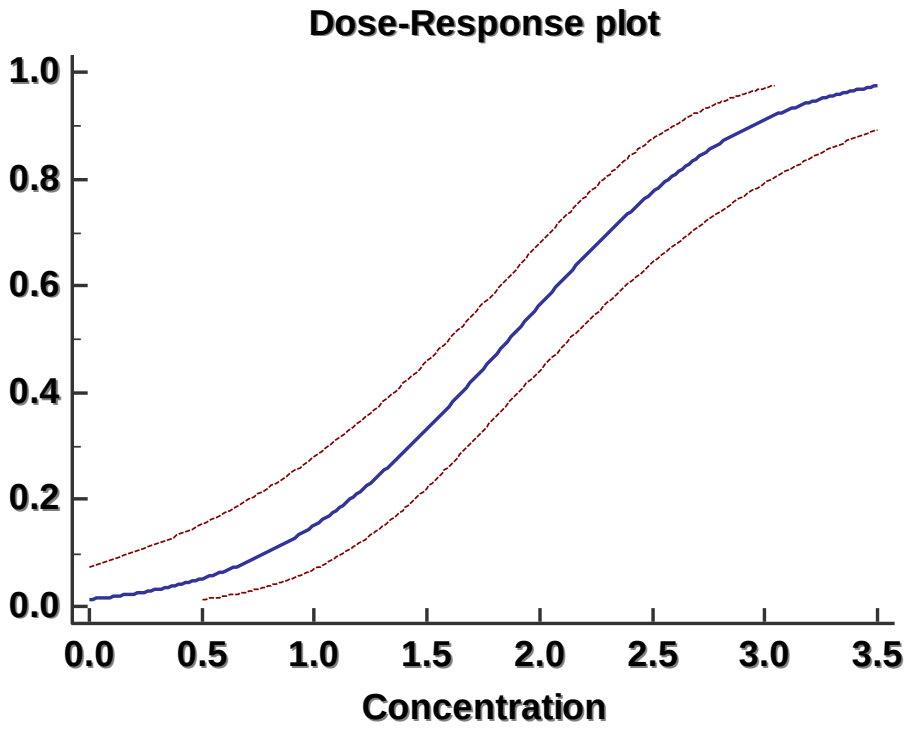


Figure 7: Dose- response relationship between the methanol derived EOs of *A. annua* against the *T. casteneum* (adult) in contact toxicity assays.The blue line marks the percent mortality and the red dotted lines depicts the 95% Confidence interval.

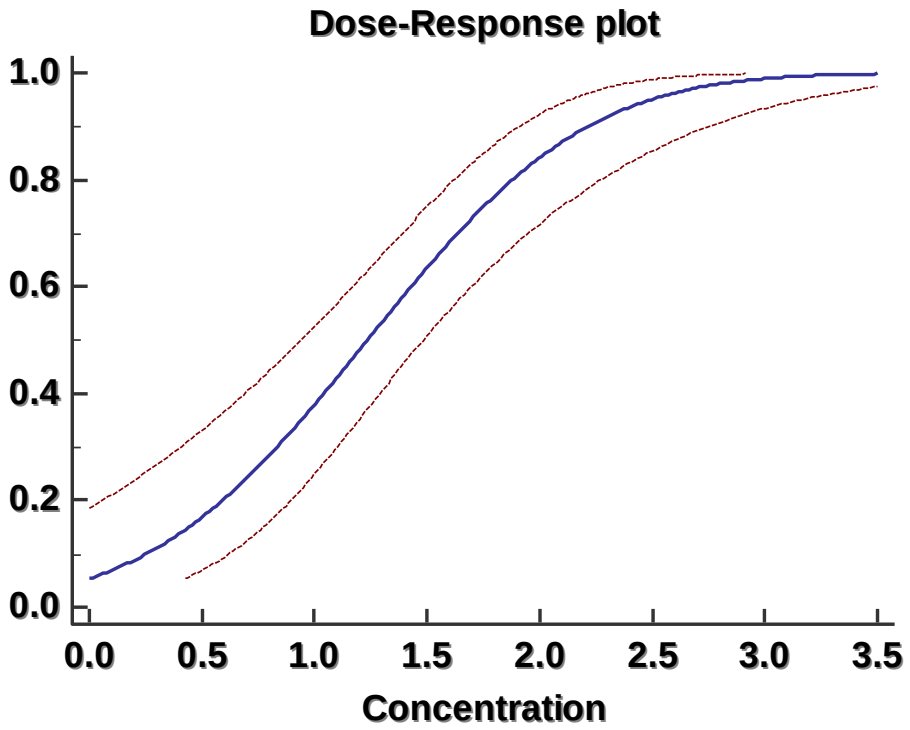


Figure 8: Dose- response relationship between the methanol derived EOs of *A. annua* against the *T. casteneum* (larvae) in contact toxicity assays. The blue line marks the percent mortality and the red dotted lines depicts the 95% Confidence interval.

In the chloroform treated EOs sets, LD_{50} value against adults was 0.97 mg adult^{-1} whereas LD_{90} obtained at 2.30 mg adult^{-1}. The immatures are more resistant and showed higher LD_{50} and LD_{90} values of 1.57 and 2.81 mg larva^{-1} respectively (Table 15). Significant ($p<0.01$) difference between the two stages at LD_{90} value was marked (overlap in 95% confidence interval). As depicted in the dose-response curve, mortality increases with the concentration. However, the adults were highly susceptible to the EOs and depicted LD_{50} at lowest concentration (Figure 9). On the other hand, gradual increase in mortality in larvae was recorded (Figure 10).

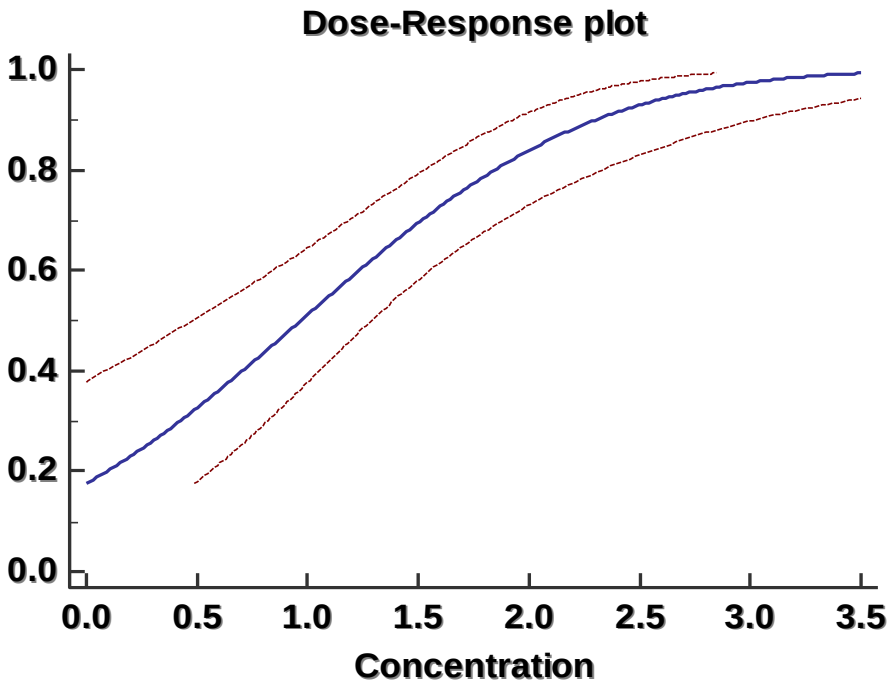


Figure 9: Dose- response relationship between the chloroform derived EOs of *A. annua* against the *T. casteneum* (adult) in contact toxicity assays. The blue line marks the percent mortality and the red dotted lines depicts the 95% Confidence interval.

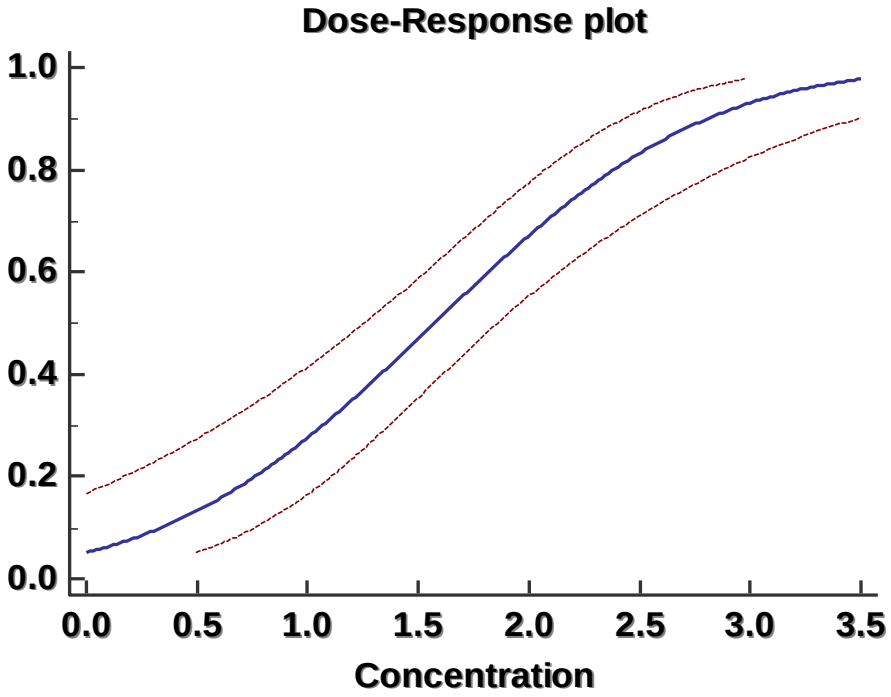


Figure 10: Dose- response relationship between the chloroform derived EOs of *A. annua* against the *T. casteneum* (larvae) in contact toxicity assays. The blue line marks the percent mortality and the red dotted lines depicts the 95% Confidence interval.

Petroleum ether EOs treated sets demonstrated LD_{50} value against adults at 0.43 mg adult^{-1} whereas LD_{90} obtained at 0.98 mg adult^{-1}. Their larval counterparts showed LD_{50} and LD_{90} values at 0.60 and 1.20 mg larva^{-1} respectively (Table 16). Significant ($p<0.01$) difference between adult and larva was marked (overlap in the 95% confidence interval). As depicted in the dose-response curve, mortality increases with the concentration. Adults showed 50% mortality at low concentration and 100% mortality was obtained at the highest concentration (Figure 11). In case of the larvae, lowest concentration demonstrated 20% mortality and highest concentration could draw 90% mortality (Figure 12).

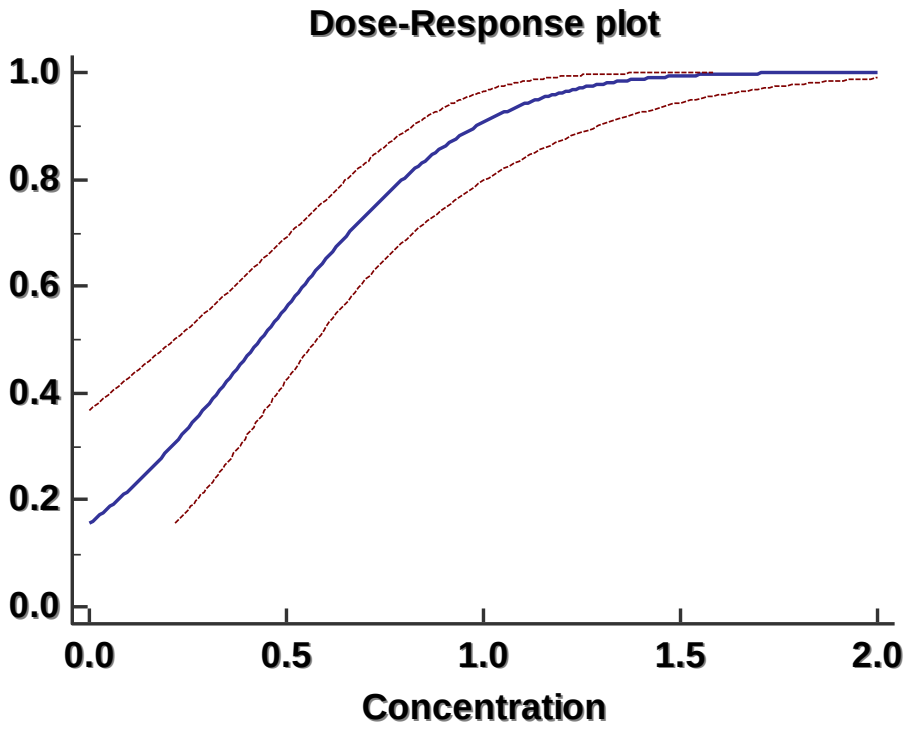


Figure 11: Dose- response relationship between the petroleum ether derived EOs of *A. annua* against the *T. casteneum* (adult) in contact toxicity assays. The blue line marks the percent mortality and the red dotted lines depicts the 95% Confidence interval.

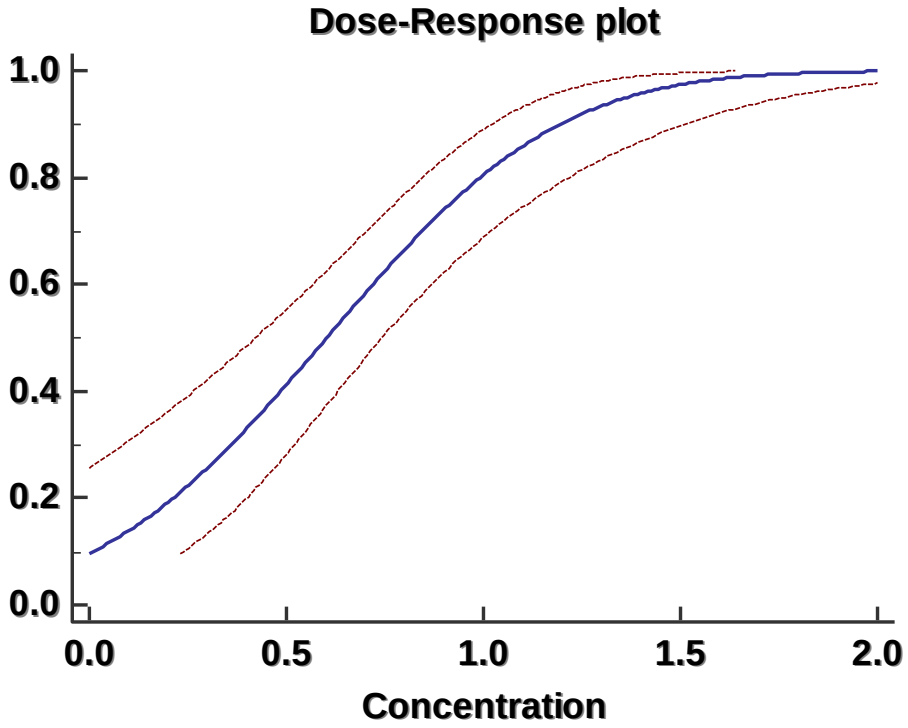


Figure 12: Dose- response relationship between the petroleum ether derived EOs of *A. annua* against the *T. casteneum* (larvae) in contact toxicity assays. The blue line marks the percent mortality and the red dotted lines depicts the 95% Confidence interval.

The LC_{50} and LC_{90} values of n-hexane derived EOs against *T. casteneum* was obtained at 0.71 mg adult^{-1} and 1.19 mg adult^{-1} respectively. The immatures were more susceptible to the EOs with LC_{50} recorded at 0.47 mg larva^{-1} and LC_{90} at 1.04 mg larva^{-1} (Table 17). The relation between the two groups depicts a significant difference ($p<0.01$) between them. Moreover, 95% confidence interval shows overlapping between the adults and larvae at the lethal doses. Mortality was dose- dependent. In adults, 10% mortality was recorded at lowest concentration which gradually increases and drawn 100% mortality at the highest concentration (Figure 13). On the other hand, the larval stage was vulnerable to the EOs and 50% mortality was found in the lower concentration (Figure 14).

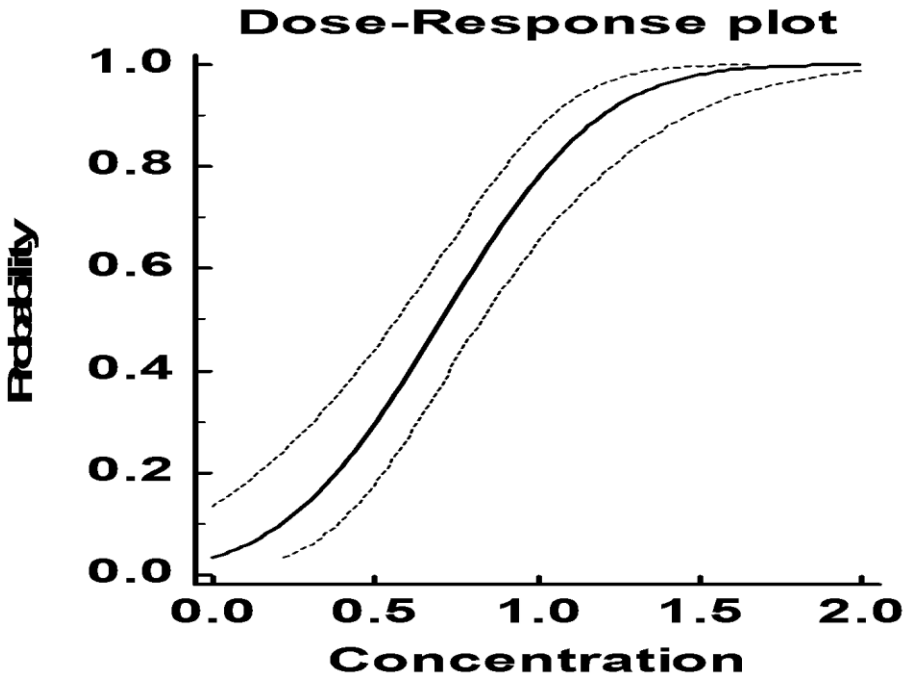


Figure 13: Dose- response relationship between the n-hexane derived EOs of *A. annua* against the *T. casteneum* (adult) in contact toxicity assays. The blue line marks the percent mortality and the red dotted lines depicts the 95% Confidence interval.

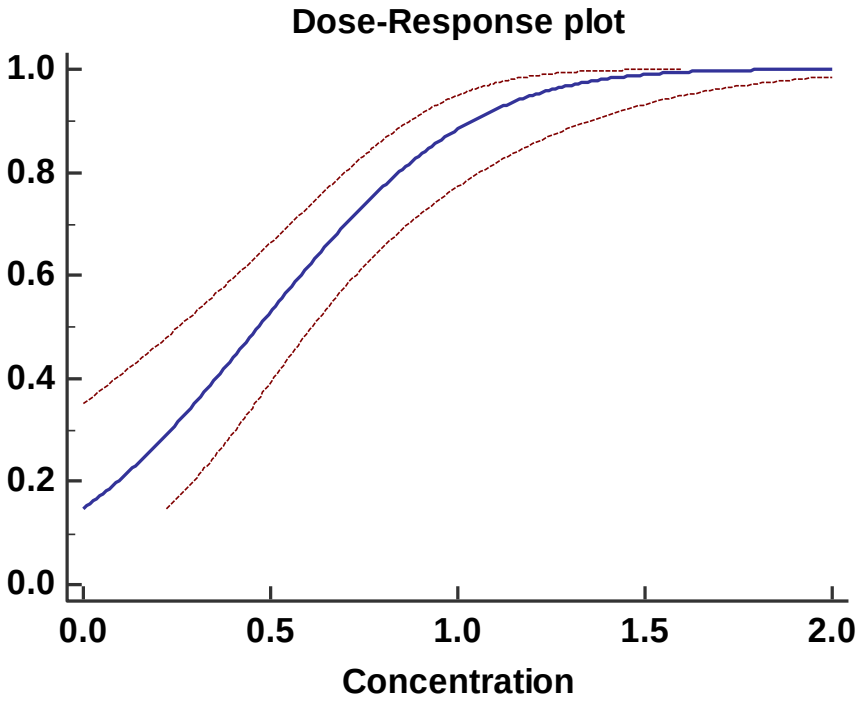


Figure 14: Dose- response relationship between the n-hexane derived EOs of *A. annua* against the *T. casteneum* (larvae) in contact toxicity assays. The blue line marks the percent mortality and the red dotted lines depicts the 95% Confidence interval.

1.5 Fumigant toxicity

In the acute toxicity assays, efficacy of EOs was evaluated via fumigation against the adults and larvae. The concentrations used for methanol and chloroform derived EOs was in the range of 0.24-2.37 mg L^{-1} air. On the other hand, ten concentrations in the range of 0.14- 1.42 mg L^{-1} air were employed for petroleum ether and n-hexane derived EOs. This concentration range was fixed after standardisation of the study. Control mortality was zero. Hence, mortality was not corrected using Abbott's formula. The mortality was then processed in the Medcalc software for probit analysis.

Methanolic EOs demonstrated LD_{50} in adult flour beetles at 1.64 mg L^{-1} air and LD_{90} at 2.51 mg L^{-1} air. However, in the immatures, 50% and 90% mortality was reported at the dose of 1.35 mg L^{-1} air and 2.14 mg L^{-1} air respectively (Table 18). Significant difference ($p<0.01$) between the treatment groups was observed (overlap in 95% confidence interval). As depicted in the dose-response curve, 20% adult mortality was reported at the lowest concentration (Figure 15). On the other hand, 40% larval mortality was recorded at the same concentration (Figure 16).

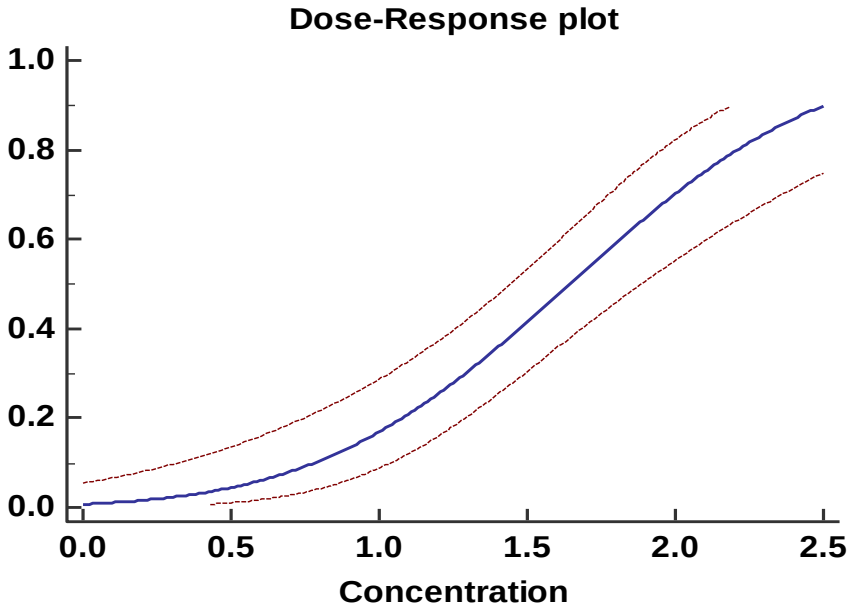


Figure 15: Dose- response relationship between the methanol derived EOs of *A. annua* against the *T. casteneum* (adult) in fumigant toxicity assays.The blue line marks the percent mortality and the red dotted lines depicts the 95% Confidence interval.

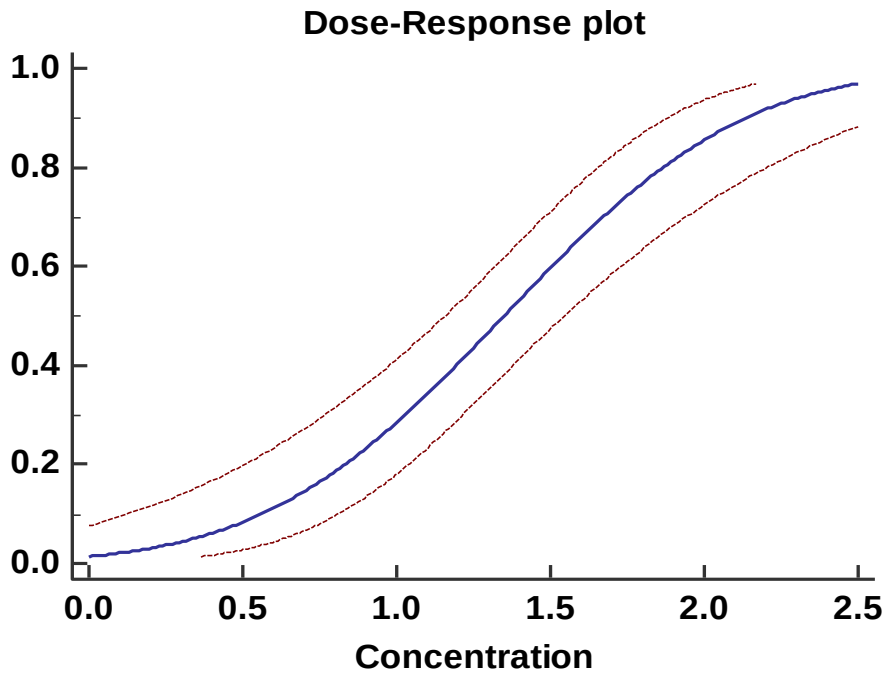


Figure 16: Dose- response relationship between the methanol derived EOs of *A. annua* against the *T. casteneum* (larvae) in fumigant toxicity assays. The blue line marks the percent mortality and the red dotted lines depicts the 95% Confidence interval.

T. casteneum adults were found more susceptible to chloroform derived EOs with a LD_{50} and LD_{90} value of 0.97 and 2.30 mg L^{-1} air respectively. A concentration of 1.57 mg L^{-1} air was required to draw 50% mortality in larvae. 90% mortality was obtained at 2.81 mg L^{-1} air (Table 19). The dose response relationship was highly significant ($p<0.01$) with an overlap in the 95% confidence interval. The tested concentration range recorded 20 to 90% mortality in the adults (Figure 17). On the other hand, 100% mortality in larval stage was reported with the highest concentration (Figure 18).

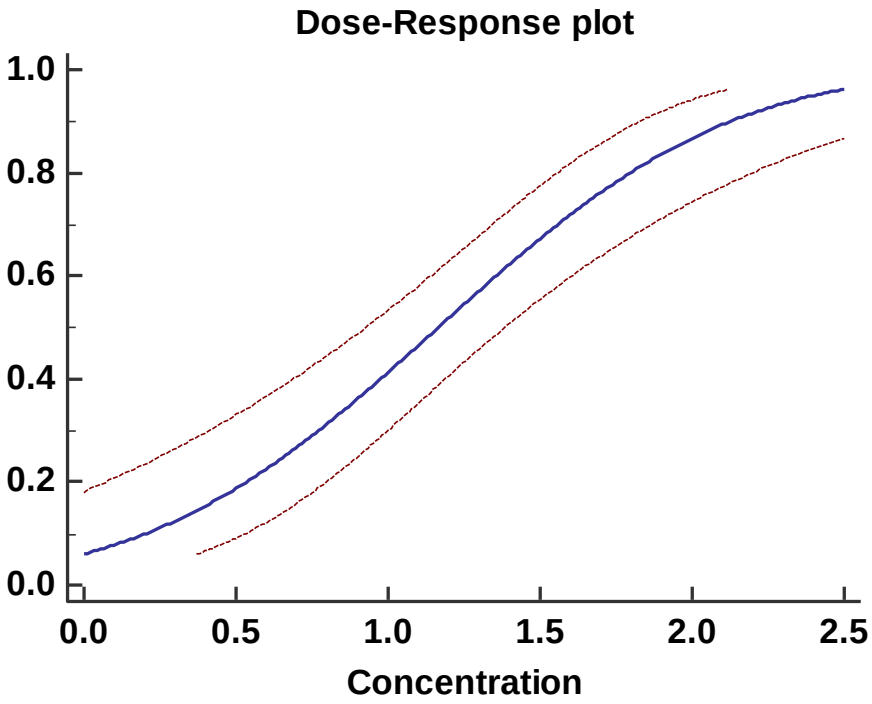


Figure 17: Dose- response relationship between the chloroform derived EOs of *A. annua* against the *T. casteneum* (adult) in fumigant toxicity assays.The blue line marks the percent mortality and the red dotted lines depicts the 95% Confidence interval.

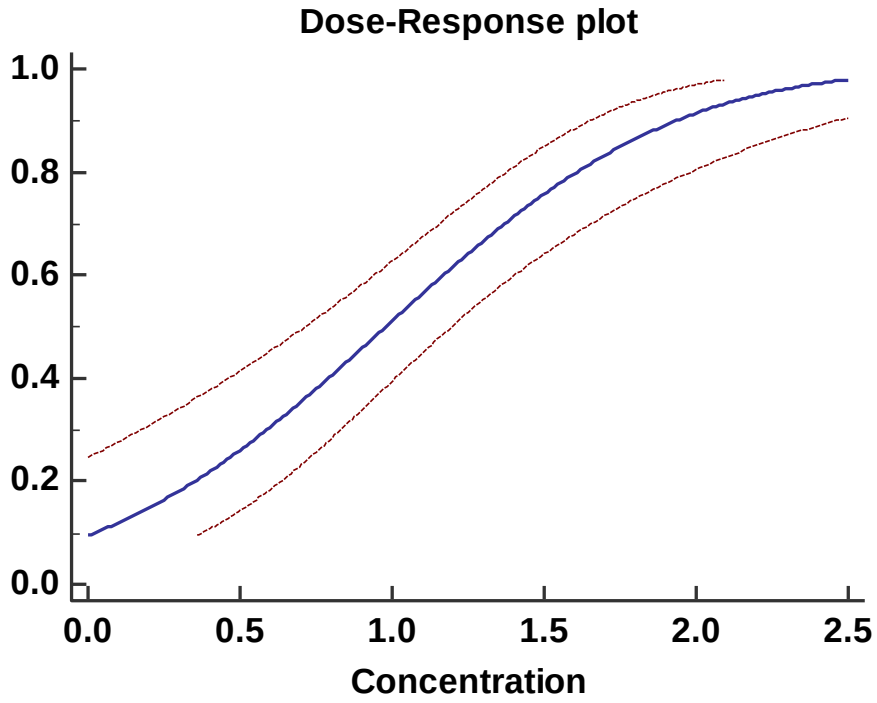


Figure 18: Dose- response relationship between the chloroform derived EOs of *A. annua* against the *T. casteneum* (larvae) in fumigant toxicity assays. The blue line marks the percent mortality and the red dotted lines depicts the 95% Confidence interval.

T. casteneum adults were recorded to be more resistant to the fumigants of petroleum ether EOs than their larval counterpart (Overlap in 95% Confidence interval). The dose-response relationship was highly significant ($p<0.01$) between the treatment sets. When LD_{50} values were compared, larvae were found more vulnerable at 0.65 whereas 50% adult mortality was found at 0.81 mg L^{-1} air. Similarly, LD_{90} in the adults and larvae reported at 1.28 and 1.13 mg L^{-1} air respectively (Table 20). As depicted in dose-response curve, the lower concentration demonstrated 10% mortality which gradually increases with the concentration. The highest concentration has drawn 90% mortality in both adults and larvae (Figure 19) (Figure 20).

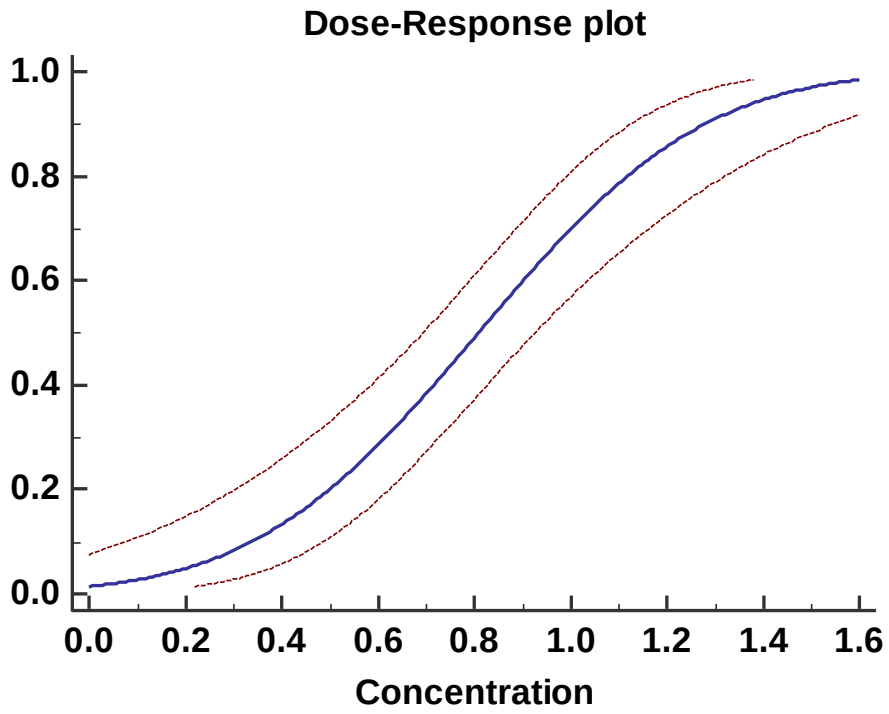


Figure 19: Dose- response relationship between the petroleum ether derived EOs of *A. annua* against the *T. casteneum* (adult) in fumigant toxicity assays. The blue line marks the percent mortality and the red dotted lines depicts the 95% Confidence interval.

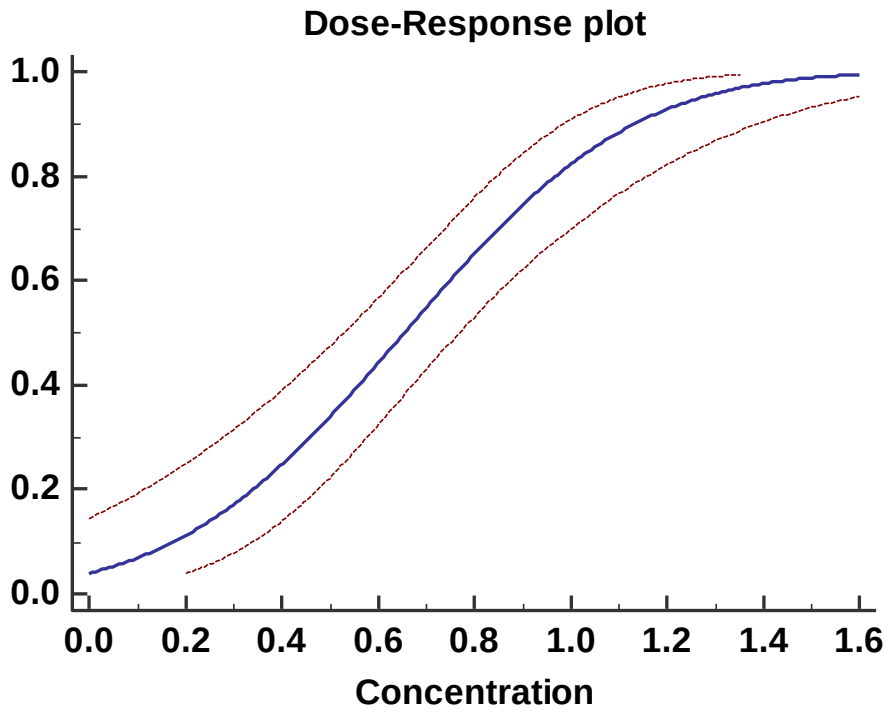


Figure 20: Dose- response relationship between the petroleum ether derived EOs of *A. annua* against the *T. casteneum* (larave) in fumigant toxicity assays. The blue line marks the percent mortality and the red dotted lines depicts the 95% Confidence interval.

Results of n-hexane derived EOs against the pest have demonstrated LD_{50} at 0.71 mg L^{-1} air in adults and at 0.47 mg L^{-1} air larvae. 90% mortality in adults and larvae was obtained at 1.19 mg L^{-1} air and 1.04 mg L^{-1} air respectively (Table 21). Highly significant ($p<0.01$) relationship between the treatment groups was observed. When lethal values were compared, the adults were found more resistant than the larvae as overlap in the 95% confidence interval was found. The adults showed, 10% mortality with the lowest concentration which gradually increases till 90% at the highest concentration (Figure 21). On the other hand, 100% mortality at the highest concentration was reported in the larvae (Figure 22).

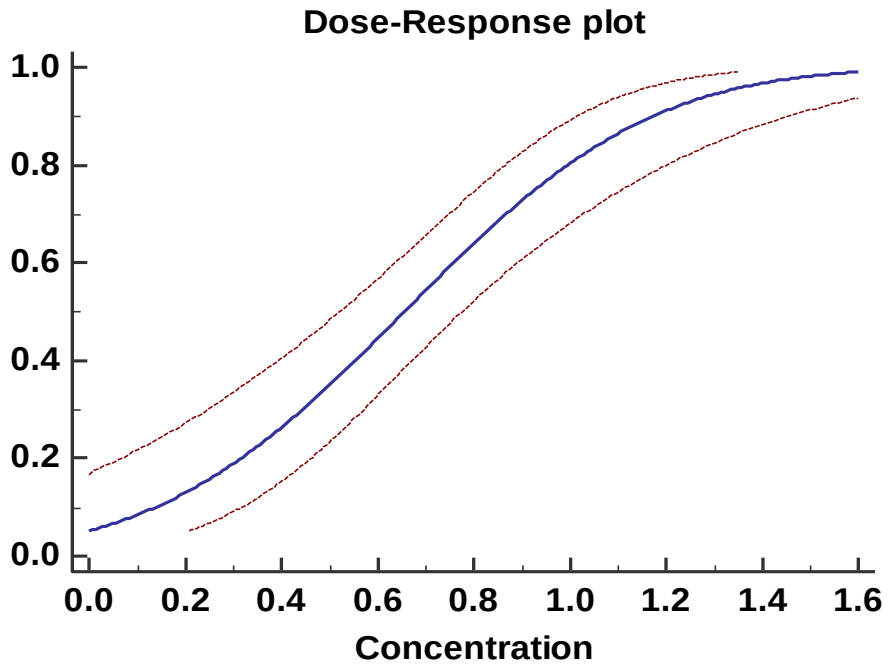


Figure 21: Dose- response relationship between the n-hexane derived EOs of *A. annua* against the *T. casteneum* (adult) in fumigant toxicity assays. The blue line marks the percent mortality and the red dotted lines depicts the 95% Confidence interval.

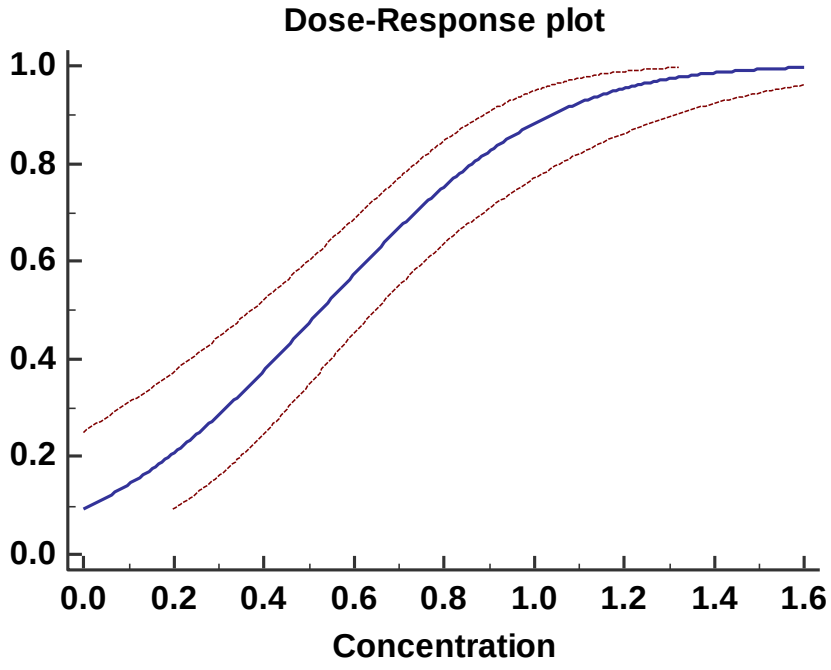


Figure 22: Dose- response relationship between the n-hexane derived EOs of *A. annua* against the *T. casteneum* (larvae) in fumigant toxicity assays. The blue line marks the percent mortality and the red dotted lines depicts the 95% Confidence interval.

Table 18: Fumigant toxicity of methanol derived EOs of *A. annua* to *T. castaneum* exposed for 24 h at 30˚C and 70±80% RH.

Extract	Life stage	LD_{50} (mg L^{-1} air)	95% Confidence interval	LD_{90} (mg L^{-1} air)	95% Confidence interval	Slope± SE	χ2 (DF)
Methanol	Adult	1.64	1.44- 1.89	2.51	2.19-3.10	1.49±0.27	41.486 (1)*
	14 days old Larvae	1.35	1.16-1.55	2.14	1.88-2.58	1.63±0.27	49.890 (1)*

LD_{50}= Lethal dose that kills 50% of the test organisms; LD_{90}= Lethal dose that kills 90% of the test organisms; χ2= chi square.

DF=degrees of freedom; *= Significant (P <0.01); Lethal values are expressed as mean of five replicates.

Table 19: Fumigant toxicity of chloroform derived EOs of *A. annua* to *T. castaneum* exposed for 24 h at 30˚C and 70±80% RH.

Plant Extract	Life stage	LD_{50} (mg L^{-1} air)	95% Confidence interval	LD_{90} (mg L^{-1} air)	95% Confidence interval	Slope ± SE	χ2 (DF)
Chloroform	Adults	1.17	0.93-1.38	2.13	1.83-2.68	1.33±0.24	37.944 (1)*
	14 days old Larvae	0.98	0.71-1.20	1.94	1.65-2.46	1.34±0.25	36.638 (1)*

LD_{50}= Lethal dose that kills 50% of the test organisms; LD_{90}= Lethal dose that kills 90% of the test organisms; χ2= chi square.

DF=degrees of freedom; *= Significant (P <0.01); Lethal values are expressed as mean of five replicates.

Table 20: Fumigant toxicity of petroleum ether derived EOs of *A. annua* to *T. castaneum* exposed for 24 h at 30˚C and 70±80% RH.

Plant Extract	Life stage	LD_{50} (mg L^{-1} air)	95% Confidence interval	LD_{90} (mg L^{-1} air)	95% Confidence interval	Slope ± SE	$\chi 2$ (DF)
Petroleum ether	Adults	0.81	0.69-0.93	1.28	1.13-1.55	2.72±0.46	49.968 (1)*
	14 days old Larvae	0.65	0.53-0.77	1.13	0.99-1.39	2.68±0.46	47.869 (1)*

LD_{50}= Lethal dose that kills 50% of the test organisms; LD_{90}= Lethal dose that kills 90% of the test organisms; $\chi 2$= chi square.

DF=degrees of freedom; *= Significant (P <0.01); Lethal values are expressed as mean of five replicates.

Table 21: Fumigant toxicity of n-hexane derived EOs of *A. annua* to *T. castaneum* exposed for 24 h at 30˚C and 70±80% RH.

Plant Extract	Life stage	LD_{50} (mg L^{-1} air)	95% Confidence interval	LD_{90} (mg L^{-1} air)	95% Confidence interval	Slope ± SE	$\chi 2$ (DF)
n-hexane	Adults	0.66	0.52-0.77	1.17	1.01-1.45	2.48±0.43	43.757 (1)*
	14 days old Larvae	0.53	0.37-0.65	1.04	0.89-1.31	2.50±0.47	40.603 (1)*

LD_{50}= Lethal dose that kills 50% of the test organisms; LD_{90}= Lethal dose that kills 90% of the test organisms; $\chi 2$= chi square.

DF=degrees of freedom; *= Significant (P <0.01); Lethal values are expressed as mean of five replicates.

1.6 Biomolecular Profile

Quantitative analysis of biomolecules was performed to assess the changes in their normal range on exposure to the different solvent derived EOs. Three groups were analysed and compared for biomolecular profiling viz. control, LC_{50} and LD_{90}. Alterations in the level of protein, AChE, GST, GSH, and LPO in the treatment sets was studied and their possible variation from the control was the main basis of this objective. The variation in the biomolecular level points towards the probable mode of action of the plants EOs.

1.6.1 Biomolecular profile of the control and treatment sets of Contact toxicity

Tissues obtained from the methanolic EOs treatment sets (Table 22), were evaluated for biomolecular profiling. The protein concentration of the whole body homogenate of adults and larvae decreased significantly ($p<0.01$) from control to lethal sets. A significant difference was observed between LD_{50} and LD_{90} sets as well. AChE activity significantly ($p<0.01$) decreased from control sets to lethal doses. However, the EOs depicted a slight and statistically insignificant ($p>0.01$) reduction in AChE level of adults between LD_{50} and LD_{90}. AChE level in the larval stage showed a significant ($p<0.01$) relationship between the control and LD_{90} group whereas LD_{50} was differing insignificantly from the control and LD_{90}. GST and GSH experienced a significant ($p<0.01$) reduction among the three groups i.e. control, LD_{50} and LD_{90} sets in both the adults and larvae. However, an insignificant ($p>0.01$) reduction in the GST level was recorded in the adults between LD_{50} and LD_{90} sets. The level of LPO was found to increase significantly ($p<0.01$) from control to lethal sets and also between the LD_{50} and LD_{90}.

In the Chloroform derived EOs treatment sets (Table 23), protein concentration of the whole body homogenate of adults and 14-days old larvae were found to be in the ranges of 326- 954 µg mL^{-1}. The protein concentration had decreased significantly ($p<0.01$) among the three sets i.e., control, LD_{50} and LD_{90}. Activity of AChE had significantly ($p<0.01$) decreased from control to lethal doses. However, the EOs depicted a statistically insignificant ($p>0.01$) reduction in the enzymatic level in the adults and larvae between

LD_{50} and LD_{90}. GST level has decreased significantly among the three sets whereas in the control it was 0.263 µmoles/min/mL of enzyme which was decreased to 0.115 µmoles/min/mL of enzyme in LD_{50} and 0.077 µmoles/min/mL of enzyme in LD_{90}. In the larvae, the enzymatic level was significantly reduced between the control and lethal sets whereas insignificant reduction is observed between the lethal groups. GSH experienced a significant ($p<0.01$) reduction among the three groups i.e. control, LD_{50} and LD_{90} sets in both the adults and larvae. The level of LPO was found to increase significantly ($p<0.01$) from control (17.64 nmole of MDA/gm of tissue) to lethal sets and also between the LD_{50} (35.68 nmole of MDA/gm of tissue) and LD_{90} (38.80 nmole of MDA/gm of tissue).

In the petroleum ether derived EOs treatment sets (Table 24), level of protein has decreased significantly ($p<0.01$) among the three groups from 320 µg mL^{-1} in control to 282 µg mL^{-1} in LD_{50} in larvae. This has further reduced to 246 µg mL^{-1} in LD_{90}. Protein concentration has similarly decreased in adults as well. AChE level has significantly ($p<0.01$) decreased from control to lethal sets. However, a statistically insignificant ($p>0.01$) reduction in the enzymatic level in both the sets between LD_{50} and LD_{90} was observed. GST level has decreased significantly among the three sets where in control it was 0.30 µmoles/min/mL of enzyme which was decreased to 0.102 µmoles/min/mL of enzyme in LD_{50} and 0.093 µmoles/min/mL of enzyme in LD_{90}. GST level was significantly reduced between the control and lethal sets of larvae but reduction was insignificant between the lethal groups. GSH has reduced significantly ($p<0.01$) among the three groups of both the adults and larvae. The level of LPO was found to increase significantly ($p<0.01$) from control to lethal sets and also between the LD_{50} and LD_{90}.

In the n-hexane derived EOs treatment sets (Table 25), level of protein has decreased significantly ($p<0.01$) among the three groups from 955 µg mL^{-1} in control to 872 µg mL^{-1} in LD_{50} in adults. This has further reduced to 829 µg mL^{-1} in LD_{90}. Protein concentration has decreased similarly in the larval sets as well. AChE level has significantly ($p<0.01$) decreased from control to lethal sets. However, a statistically insignificant ($p>0.01$) reduction in the AChE level between LD_{50} and LD_{90} was observed. GST level in the adults has

decreased significantly among the three sets where in control it was 0.273 µmoles/min/mL of enzyme which was decreased to 0.096 µmoles/min/mL of enzyme in LD_{50} and 0.075 µmoles/min/mL of enzyme in $LD_{90.}$ Enzymatic level in the larvae followed the same trend with 0.17 µmoles/min/mL of enzyme in control to 0.071 µmoles/min/mL of enzyme in LD_{50}; this further reduces in $LD_{90.}$with 0.063 µmoles/min/mL of enzyme. GSH has reduced significantly ($p<0.01$) among the three groups in both the adults and larvae. LPO was found to increase significantly ($p<0.01$) from control to lethal sets and also between the LD_{50} and $LD_{90.}$

Table 22: Effect of methanol derived EOs of *A. annua* on protein and enzymatic profile of *T. casteneum* subjected to contact toxicity bioassays.

Extracts	Life stages	Treatment	Protein (µg mL^{-1})	AChE (µmoles/min/mL of enzyme)	GST µmoles/min/mL of enzyme	GSH (µmoles/mg protein)	LPO (nmole of MDA/gm of tissue)
Methanol	Adult	Control	983±4.58a	0.227±0.015a	0.293±0.051a	238.03±0.252a	19.01±0.662a
		LD_{50}	890±3b	0.036±0.002b	0.163±0.025b	200.75±0.511b	35.24±0.655b
		LD_{90}	842±5.57c	0.023±0.003b	0.102±0.016b	186.56±0.225c	39.42±0.659c
	Larva	Control	320±4.51a	0.12±0.021a	0.185±0.005a	216.57±0.461a	15.42±0.445a
		LD_{50}	290±4.58b	0.093±0.004ab	0.089±0.005b	193.83±0.207b	29.01±0.355b
		LD_{90}	256±6.51c	0.076±0.004b	0.073±0.005c	180.43±0.184c	32.69±0.752c

Mean (±SEM) followed by the same letters indicate no significant difference ($p< 0.01$) between different treated groups (Control, LD_{50} & LD_{90}) according to the Tukey's test.

Table 23: Effect of chloroform derived EOs of *A. annua* on protein and enzymatic profile of *T. casteneum* subjected to contact toxicity bioassays.

Extracts	Life stages	Treatment	Protein (µg mL^{-1})	AChE (µmoles/min/mL of enzyme)	GST µmoles/min/mL of enzyme	GSH (µmoles/mg protein)	LPO (nmole of MDA/gm of tissue)
Chloroform	Adult	Control	954±3.06a	0.223±0.041a	0.263±0.015a	237.80±0.190a	17.64±0.252a
		LD_{50}	877±6.51b	0.068±0.010b	0.115±0.005b	197.97±0.887b	35.68±0.229b
		LD_{90}	833±5.29c	0.045±0.004b	0.077±0.007c	180.76±0.593c	38.80±0.116c
	Larva	Control	407±3.21a	0.136±0.012a	0.167±0.012a	209.88±0.781a	13.65±0.040a
		LD_{50}	371±5.51b	0.085±0.002b	0.088±0.007b	192.66±0.370b	29.35±0.03b
		LD_{90}	326±2.08c	0.077±0.003b	0.068±0.006b	179.35±0.221c	33.55±0.036c

Mean (±SEM) followed by the same letters indicate no significant difference ($p< 0.01$) between different treated groups (Control, LD_{50} & LD_{90}) according to the Tukey's test.

Table 24: Effect of petroleum ether derived EOs of *A. annua* on protein and enzymatic profile of *T. casteneum* subjected to contact toxicity bioassays.

Extracts	Life stages	Treatment	Protein (µg mL^{-1})	AChE (µmoles/min/mL of enzyme)	GST µmoles/min/mL of enzyme	GSH (µmoles/mg protein)	LPO (nmole of MDA/gm of tissue)
Petroleum ether	Adult	Control	956±8.737a	0.23±0.021a	0.30±0.050a	236.32±1.506a	19.01±0.662a
		LD_{50}	875±6.11b	0.031±0.002b	0.102±0.079b	198.19±0.422b	36.583±1.44b
		LD_{90}	835±9.292c	0.022±0.002b	0.093±0.004b	184.59±0.525c	41.273±1.217c
	Larva	Control	320±4.509a	0.123±0.021a	0.185±0.005a	216.28±0.815a	14.653±0.717a
		LD_{50}	282±4.163b	0.085±0.003ab	0.089±0.006b	190.55±0.478b	30.68±0.493b
		LD_{90}	246±7.55c	0.058±0.033b	0.073±0.006c	177.933±0.98c	32.833±0.588c

Mean (±SEM) followed by the same letters indicate no significant difference ($p< 0.01$) between different treated groups (Control, LD_{50} & LD_{90}) according to the Tukey's test.

Table 25: Effect of n-hexane derived EOs of *A. annua* on protein and enzymatic profile of *T. casteneum* subjected to contact toxicity bioassays.

Extracts	Life stages	Treatment	Protein (µg mL^{-1})	AChE (µmoles/min/mL of enzyme)	GST µmoles/min/mL of enzyme	GSH (µmoles/mg protein)	LPO (nmole of MDA/gm of tissue)
n-Hexane	Adult	Control	955±5.859a	0.24±0.03a	0.273±0.021a	238.137±0.745a	17.983±0.344a
		LD_{50}	872±4.042b	0.057±0.005b	0.096±0.005b	196.113±0.344b	37.077±0.785b
		LD_{90}	829±3c	0.039±0.003b	0.075±0.005b	179.92±0.934c	40.25±0.499c
	Larva	Control	405±3.215a	0.145±0.027a	0.17±0.01a	208.953±0.827a	13.313±0.61a
		LD_{50}	367±1b	0.068±0.005b	0.071±0.004b	190±1b	31.117±0.722b
		LD_{90}	318±0.577c	0.058±0.004b	0.063±0.004b	177.46±0.617c	34.61±0.535c

Mean (±SEM) followed by the same letters indicate no significant difference ($p< 0.01$) between different treated groups (Control, LD_{50} & LD_{90}) according to the Tukey's test.

1.6.2 Biomolecular profile of the control and treatment sets of Fumigant toxicity

Tissues obtained from the methanolic EOs fumigant toxicity assays (Table 26), were processed for biomolecular profiling. The protein concentration of the whole body homogenate of adults and 14 day old larvae has decreased significantly ($p<0.01$) from control to LD_{50} which further reduced to LD_{90} sets. Activity of AChE was found to decrease significantly ($p<0.01$) from control to lethal sets. However, the EOs depicted a statistically insignificant ($p>0.01$) reduction in AChE level between LD_{50} and LD_{90}. GST level in adults decreased significantly in all the groups from 0.257 µmoles/min/mL of enzyme in control to 0.128 µmoles/min/mL of enzyme in $LD_{50.}$ This in turn further reduces in LD_{90} to 0.078 µmoles/min/mL of enzyme. The enzymatic activity in larval sets was reduced significantly between the control and lethal sets but a statistically insignificant reduction between LD_{50} and LD_{90} was observed. GSH experienced a significant ($p<0.01$) reduction among the three groups i.e. control, LD_{50} and LD_{90} sets in both the adults and larvae. The level of LPO was found to increase significantly ($p<0.01$) in case of both adults and larvae from control to lethal sets and also between the LD_{50} and $LD_{90.}$

In the chloroform derived EOs fumigant toxicity sets (Table 27), protein concentration of the adults and larvae were found to decreased significantly ($p<0.01$) among the three sets i.e., control, LD_{50} and LD_{90}. The concentration in adults was 892, 836 and 804 µg mL^{-1} in control, LD_{50} and LD_{90} respectively. In larval sets, the concentration was as low as 397 µg mL^{-1} in control which reduced to 356 µg mL^{-1} in $LD_{50.}$ Further reduction to 310 µg mL^{-1} in LD_{90} was observed. Activity of AChE had significantly ($p<0.01$) decreased from control to lethal doses. However, the EOs represented a statistically insignificant ($p>0.01$) reduction in the AChE in both the adults and larvae between LD_{50} and LD_{90}. GST level has decreased significantly among the three sets where in control it was 0.227 µmoles/min/mL of enzyme which was decreased to 0.12 µmoles/min/mL of enzyme in LD_{50} and 0.073 µmoles/min/mL of enzyme in $LD_{90.}$ In the larvae, the enzymatic level was significantly reduced between the control and lethal sets whereas an insignificant reduction is observed between the lethal groups. GSH

experienced a significant ($p<0.01$) reduction among the three groups i.e. control, LD_{50} and LD_{90} sets in both the adults and larvae. The level of LPO in adults was found to increase significantly ($p<0.01$) from control (19.49 nmole of MDA/gm of tissue) to lethal sets and also between the LD_{50} (37.97 nmole of MDA/gm of tissue) and LD_{90} (42.38 nmole of MDA/gm of tissue). The larvae also follow the similar trend of LPO level.

In the petroleum ether EOs fumigant toxicity sets (Table 28), level of protein has decreased significantly ($p<0.01$) among the three groups from 976 µg mL^{-1} in control to 718 µg mL^{-1} in LD_{50} in larvae. This has further reduced to 664 µg mL^{-1} in LD_{90}. Protein concentration has similarly decreased in larvae as well. Activity of AChE has significantly ($p<0.01$) decreased from control to lethal sets. However, a statistically insignificant ($p>0.01$) reduction in the enzymatic level between LD_{50} and LD_{90} was observed in case of both the adults and larvae. GST level in adults has decreased significantly between the control and lethal sets where in control it was 0.262 µmoles/min/mL of enzyme which was decreased to 0.099 µmoles/min/mL of enzyme in LD_{50} and 0.076 µmoles/min/mL of enzyme in LD_{90}. GST level in larval sets followed the same trend. GSH was reduced significantly ($p<0.01$) among the three sets in case of both the adults and larvae. The level of LPO was found to increase significantly ($p<0.01$) from control to lethal sets and also between the two lethal sets i.e. LD_{50} and LD_{90}.

In the n-hexane derived EOs fumigant toxicity sets (Table 29), level of protein has decreased significantly ($p<0.01$) among the three groups from 892 µg mL^{-1} in control to 829 µg mL^{-1} in LD_{50} in adults. This has further reduced to 798 µg mL^{-1} in LD_{90}. Protein concentration has decreased similarly in the larval sets as well. AChE level has significantly ($p<0.01$) decreased from control to lethal sets. However, an insignificant ($p>0.01$) reduction in the AChE level between LD_{50} and LD_{90} was detected. GST level in the adults has decreased significantly among the three sets where in control it was 0.245 µmoles/min/mL of enzyme which was decreased to 0.117 µmoles/min/mL of enzyme in LD_{50} and 0.06 µmoles/min/mL of enzyme in LD_{90}. Enzymatic level in the larvae decreased significantly between control with 0.177 µmoles/min/mL of enzyme to lethal sets but decreased insignificantly between

LD_{50} (0.076 µmoles/min/mL of enzyme) and LD_{90} (0.061 µmoles/min/mL of enzyme). GSH has reduced significantly ($p<0.01$) among the three groups in both the adults and larval sets. The level of LPO was found to increase significantly ($p<0.01$) from control to lethal sets and also between the LD_{50} and LD_{90}.

Table 26: Effect of methanol derived EOs of *A. annua* on protein and enzymatic profile of *T. casteneum* subjected to fumigant toxicity bioassay.

Extracts	Life stages	Treatment	Protein (µg mL^{-1})	AChE (µmoles/min/mL of enzyme)	GST µmoles/min/mL of enzyme	GSH (µmoles/mg protein)	LPO (nmole of MDA/gm of tissue)
Methanol	Adult	Control	975±1a	0.253±0.031a	0.257±0.031a	235.73±0.161a	18.31±0.067a
		LD_{50}	724±4.58b	0.029±0.006b	0.128±0.009b	195.6±0.07b	36.09±0.035b
		LD_{90}	671±2.65c	0.017±0.003b	0.078±0.008c	178.81±0.165c	39.92±0.145c
	Larva	Control	302±4.04a	0.153±0.025a	0.18±0.01a	210.21±0.275a	14.65±0.04a
		LD_{50}	236±2.65b	0.085±0.004b	0.09±0.008b	201.5±0.475b	30.82±0.275b
		LD_{90}	202±3.512c	0.073±0.006b	0.07±0.007b	187.11±0.24c	35.83±0.151c

Mean (±SEM) followed by the same letters indicate no significant difference ($p< 0.01$) between different treated groups (Control, LD_{50} & LD_{90}) according to the Tukey's test.

Table 27: Effect of chloroform derived EOs of *A. annua* on protein and enzymatic profile of *T. casteneum* subjected to fumigant toxicity bioassay.

Extracts	Life stages	Treatment	Protein (µg mL^{-1})	AChE (µmoles/min/mL of enzyme)	GST µmoles/min/mL of enzyme	GSH (µmoles/mg protein)	LPO (nmole of MDA/gm of tissue)
Chloroform	Adult	Control	892±2.08a	0.283±0.035a	0.227±0.015a	230.36±0.542a	19.49±0.132a
		LD_{50}	836±2.52b	0.055±0.005b	0.12±0.02b	193.49±0.204b	37.97±0.076b
		LD_{90}	804±4.16c	0.041±0.003b	0.073±0.012c	175.81±0.201c	42.38±0.070c
	Larva	Control	397±2a	0.15±0.04a	0.177±0.006a	213.74±0.218a	15.88±0.087a
		LD_{50}	356±2b	0.085±0.004b	0.077±0.007b	203.18±0.251b	31.26±0.056b
		LD_{90}	310±2.08c	0.066±0.006b	0.063±0.006b	185.37±0.363c	38.9±0.053c

Mean (±SEM) followed by the same letters indicate no significant difference ($p< 0.01$) between different treated groups (Control, LD_{50} & LD_{90}) according to the Tukey's test.

Table 28: Effect of petroleum ether derived EOs of *A. annua* on protein and enzymatic profile of *T. casteneum* subjected to fumigant toxicity bioassay.

Extracts	Life stages	Treatment	Protein (µg mL^{-1})	AChE (µmoles/min/mL of enzyme)	GST µmoles/min/mL of enzyme	GSH (µmoles/mg protein)	LPO (nmole of MDA/gm of tissue)
Petroleum ether	Adult	Control	976±0.864a	0.253±0.031a	0.262±0.03a	235.81±0.227a	17.75±0.694a
		LD_{50}	718±1.035b	0.018±0.002b	0.099±0.01b	193±0.21b	38.167±0.666b
		LD_{90}	664±1.002c	0.017±0.002b	0.076±0.005b	175.33±0.534c	43.513±0.520c
	Larva	Control	300±2.081a	0.157±0.031a	0.179±0.01a	209.53±1.038a	13.543±1.06a
		LD_{50}	279±1.012b	0.073±0.003b	0.082±0.008b	197.993±0.99b	31.233±0.586b
		LD_{90}	196±0.946c	0.06±0.003b	0.077±0.004b	186.59±0.525c	35.667±0.586c

Mean (±SEM) followed by the same letters indicate no significant difference ($p< 0.01$) between different treated groups (Control, LD_{50} & LD_{90}) according to the Tukey's test.

Table 29: Effect of n-hexane derived EOs of *A. annua* on protein and enzymatic profile of *T. casteneum* subjected to fumigant toxicity bioassay.

Extracts	Life stages	Treatment	Protein (µg mL^{-1})	AChE (µmoles/min/mL of enzyme)	GST µmoles/min/mL of enzyme	GSH (µmoles/mg protein)	LPO (nmole of MDA/gm of tissue)
n-Hexane	Adult	Control	892±1.528a	0.293±0.051a	0.245±0.021a	229.62±1.45a	19.49±0.132a
		LD_{50}	829±1.528b	0.047±0.007b	0.117±0.447b	191.013±0.924b	39.23±0.584b
		LD_{90}	798±0.548c	0.038±0.003b	0.06±0.025c	173.15±0.678c	43.79±0.318c
	Larva	Control	394±2.082a	0.14±0.036a	0.177±0.006a	212.187±1.29a	15.073±0.753a
		LD_{50}	349±1.00b	0.079±0.001b	0.076±0.011b	200.52±0.501b	33.143±0.348b
		LD_{90}	306±1.172c	0.069±0.003b	0.061±0.004b	181.237±0.472c	39.477±0.408c

Mean (±SEM) followed by the same letters indicate no significant difference ($p< 0.01$) between different treated groups (Control, LD_{50} & LD_{90}) according to the Tukey's test.

1.7 Quality Parameters

Quality assessment of both the wheat grain and wheat flour was of prime importance to draw a complete picture of the present study. Prolonged infestation of the *T. casteneum* causes irreversible damage of the commodity. Visual alterations were evident. However, changes in the nutrition level needs detailed evaluation through standard protocols. Hence, evaluation through SEM/EDX photography and standard AACC methods is of great importance for quality assurance.

1.7.1 Feeding ratio and weight loss effects:

Treatment with the *Artemisia annua* has shown strong antifeedant action against red flour beetles. The feeding ratio has significantly lowered to 0.36 at 1g and 0.39 at 0.5g treatment sets when compared with the control set. The Fr of the untreated set was 0.64. The feeding ratio decreases with the increase in the dose of the treatment. Statistical analysis of the data has shown a significant relationship ($P<0.05$) between the control and treatment sets.

Artemisia annua was found to be effective against the *Tribolium casteneum* in minimizing the weight loss of the grains. Heavy weight loss of 30.08% in untreated grains was observed whereas a significant reduction ($P<0.05$) in the weight loss of 6.46% and 3.4% was reported in the treatment sets of 0.5g and 1g respectively (Table 30).

Table 30: Effect of *Artemisia annua* on the feeding ratio and % weight loss caused by *Tribolium casteneum*

Treatment	Dose	Feeding ratio	% Weight loss
***Artemisia annua* (Powdered leaves)**	0 g	0.64*	30.08
	0.5g	0.39*	6.46
	1g	0.36*	3.4

* indicate significant difference ($p < 0.01$) according to the ANOVA.

1.7.2 Weight by volume ratio:

Significant difference in weight is observed between the undamaged and damaged grains. The later was observed to retain only 8% of its total original weight.

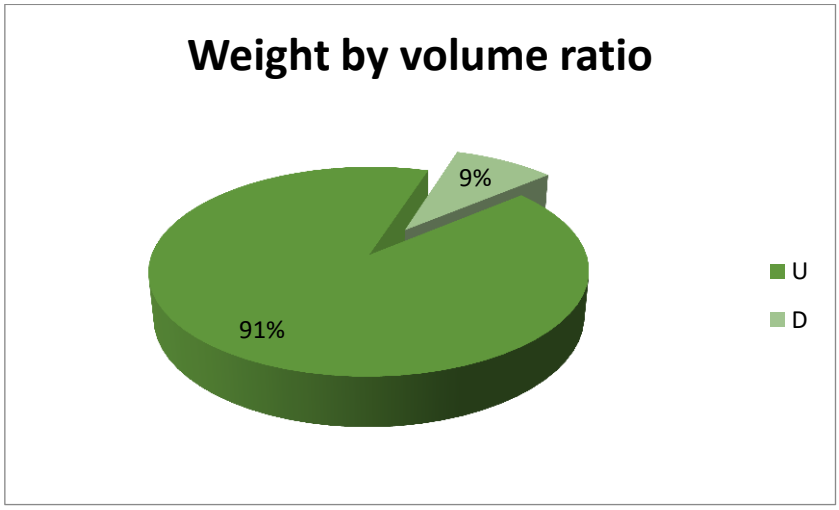


Figure 23: Difference in the weight by volume ratio of the undamaged and damaged grains infested with *Tribolium casteneum*

Weight by volume ratio showed extensive decrease of the damaged grains by 91% when compared with the undamaged grains. The reduction in weight was reported due to continuous feeding by the pest for a period of three months (Figure 23).

1.7.3 Scanning Electron Microscope (SEM) results:

Uninfested and *T. casteneum* infested grains were processed for SEM analysis to have an insight into more detailed topography. Images obtained from the SEM analysis depict a clear structural difference between the undamaged and damaged grains. The undamaged grain shows structural integrity whereas the damaged grains are thoroughly punctured by the beetle due to continuous nibbling. The infestation of the damaged grains was compared with the undamaged grains at different magnifications. The intact coat of the undamaged grain (Figure 24) when compared with the infested grains, heavy loss of the bran as well as the endosperm at 25x magnification was evident (Figure 25). The next magnification i.e. at 50x magnification, clearer picture of bran in undamaged grain was demonstrated (Figure 26). On the contrary, the bran is heavily damaged and deformed in the damaged grain (Figure 27).

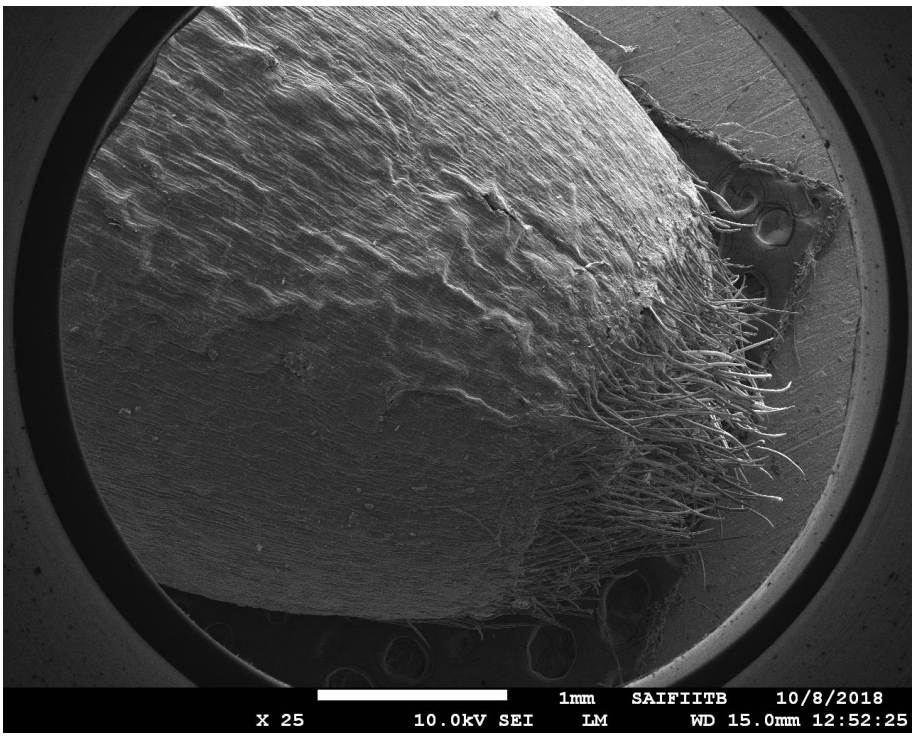


Figure 24: Undamaged grain 25x magnification

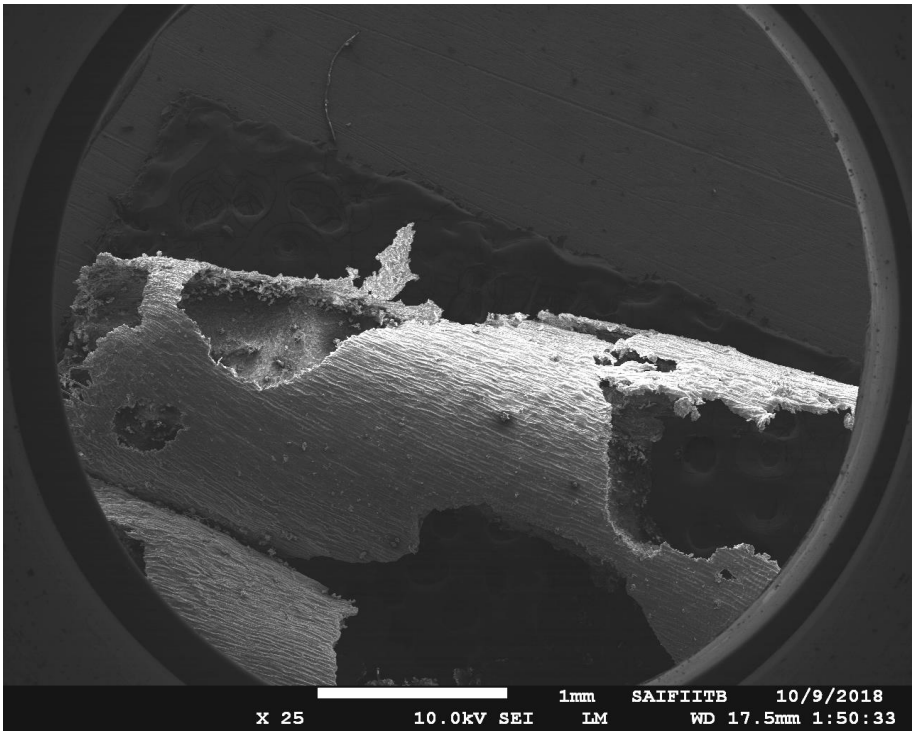


Figure 25: Damaged grain 25x magnification

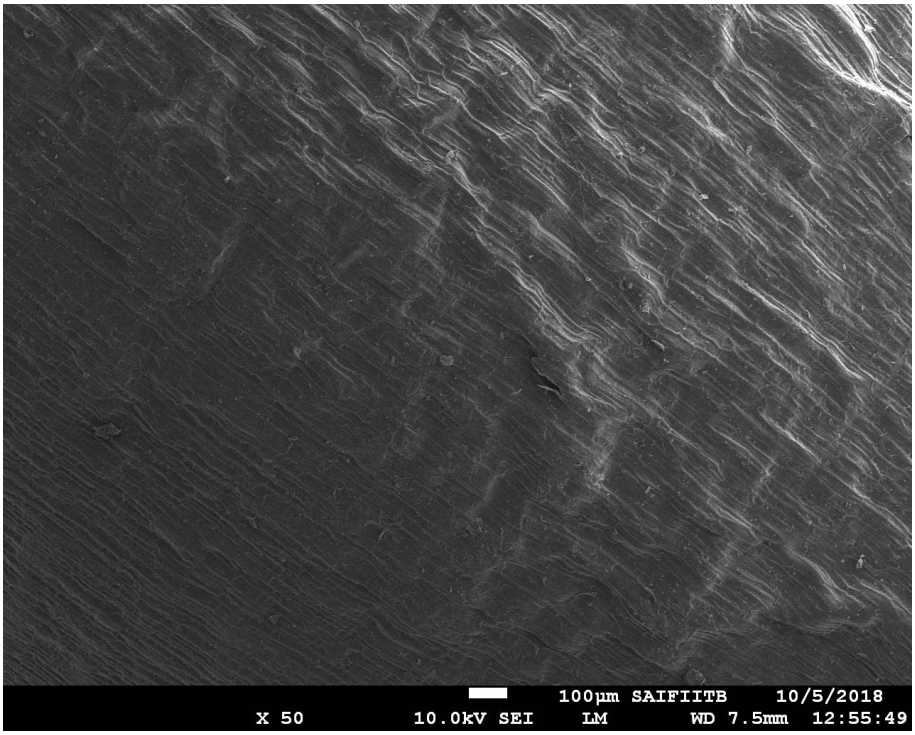


Figure 26: Undamaged grain 50x magnification

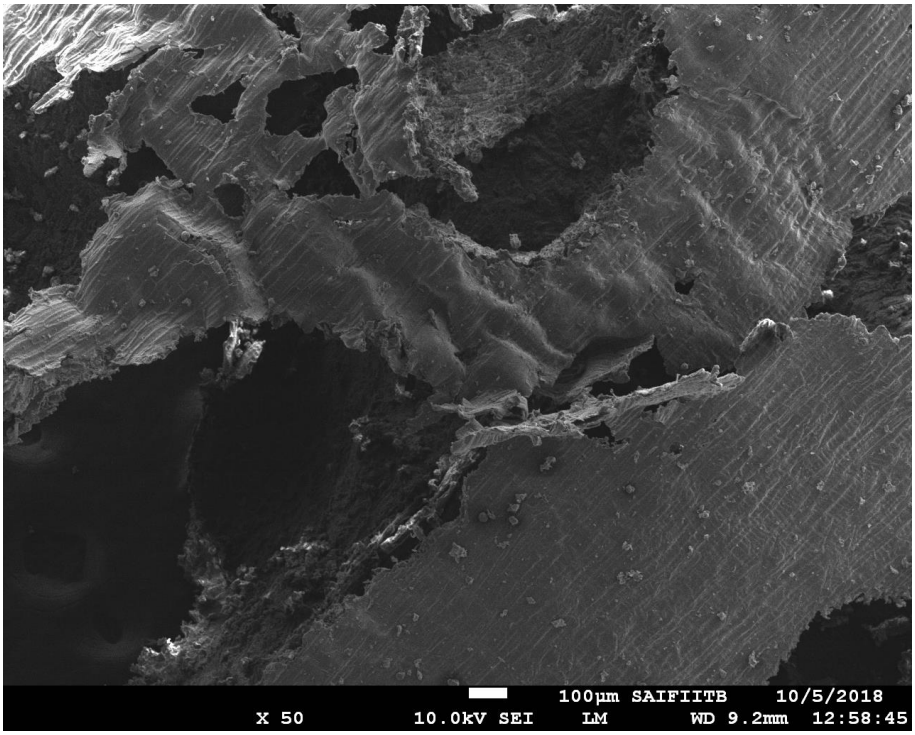


Figure 27: Damaged grain 50x magnification

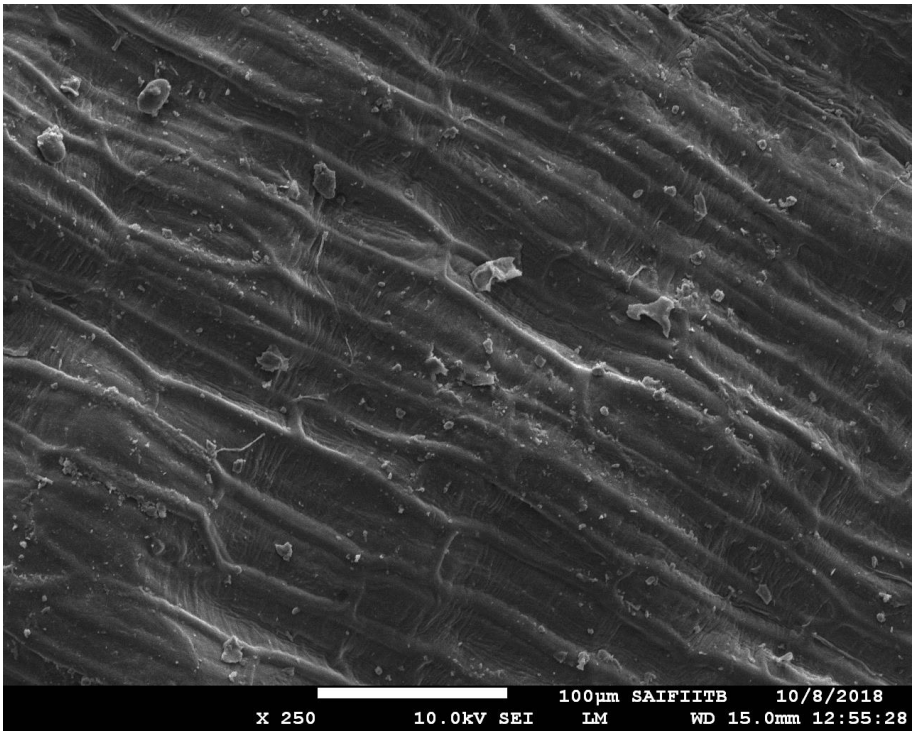


Figure 28: Undamaged grain 250x magnification

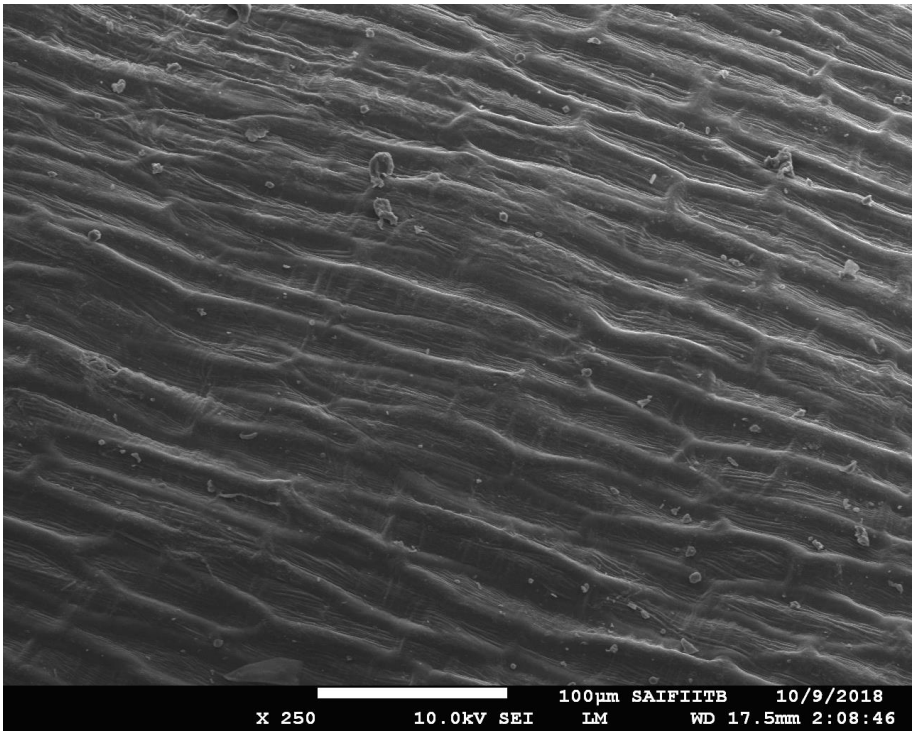


Figure 29: Damaged grain 250x magnification

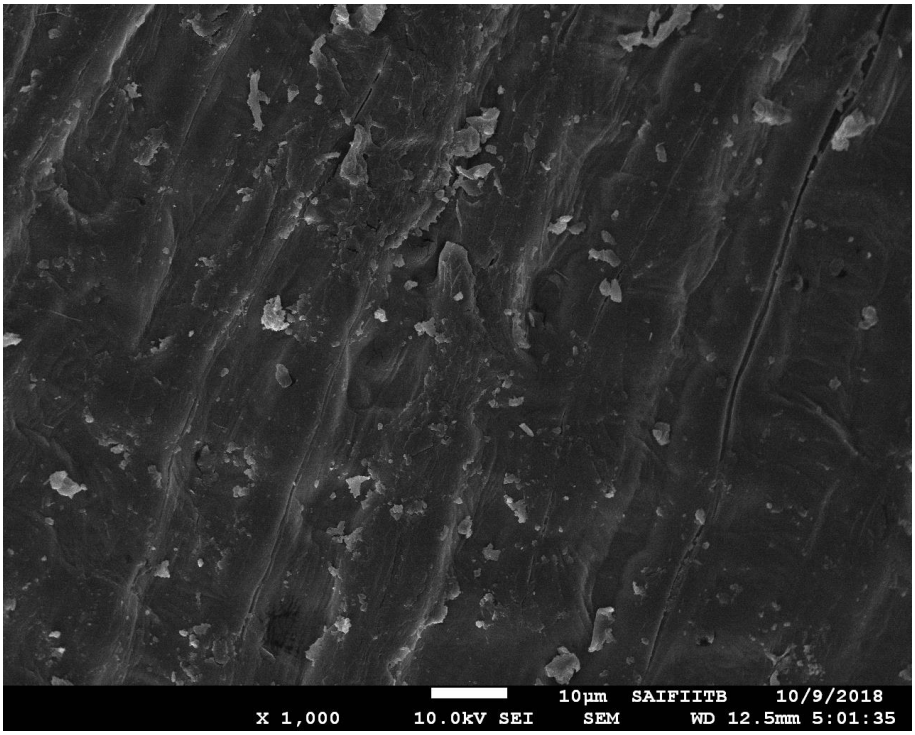


Figure 30: Undamaged grain 1,000x magnification

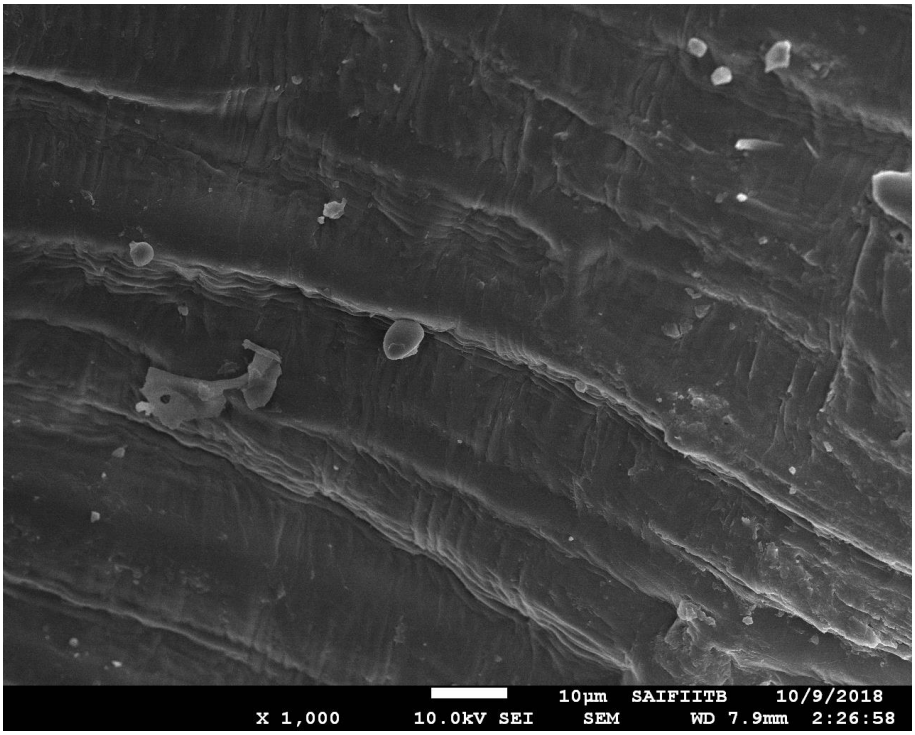


Figure 31: Damaged grain 1,000x magnification

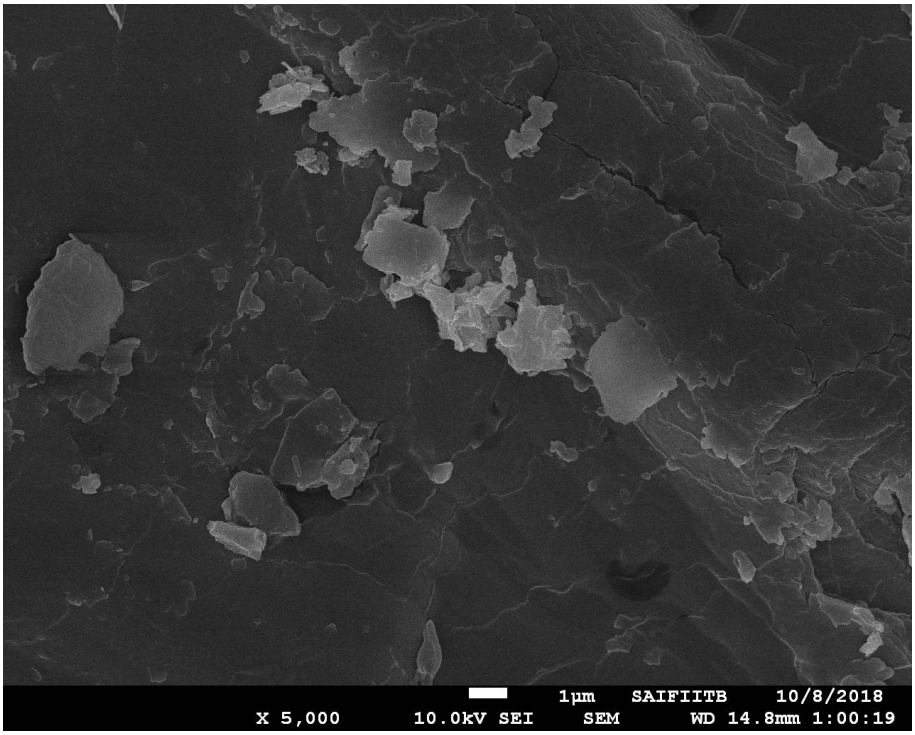


Figure 32: Undamaged grain 5,000x magnification

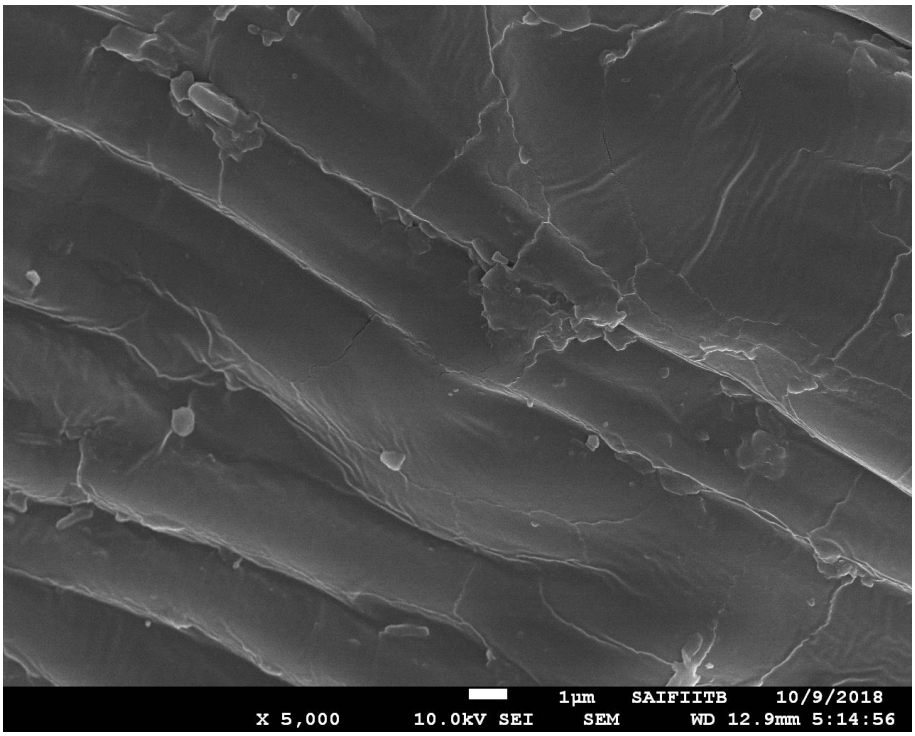


Figure 33: Damaged grain 5,000x magnification

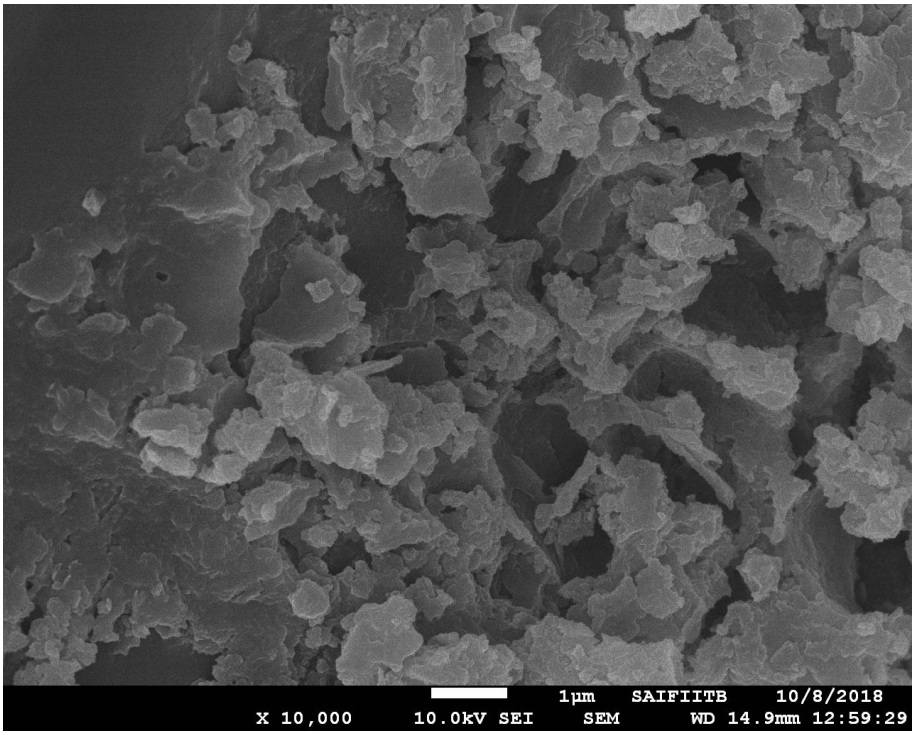


Figure 34: Undamaged grain 10,000x magnification

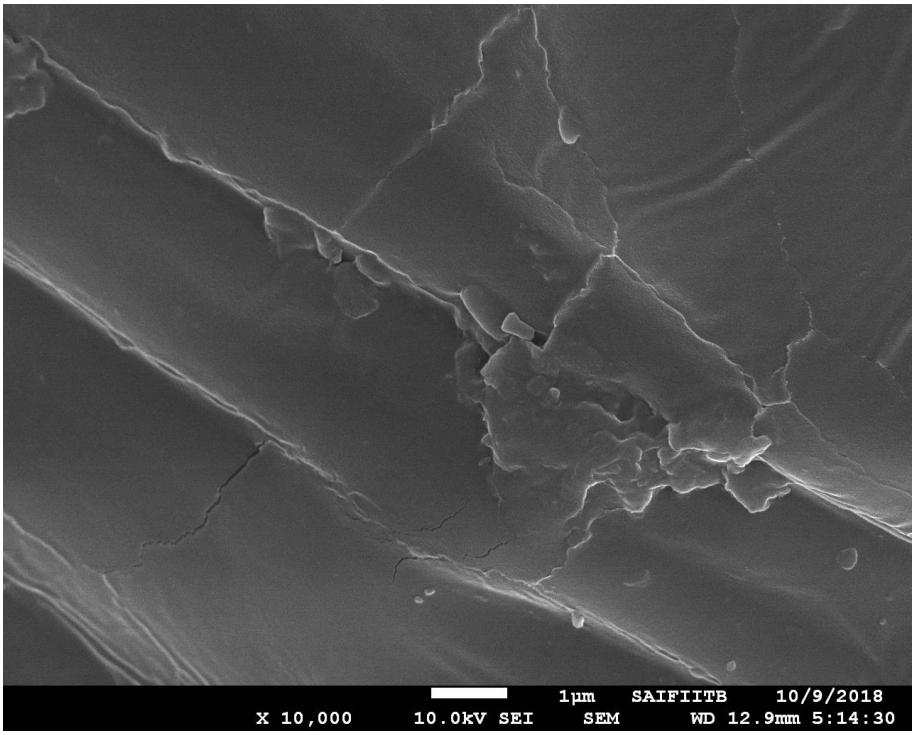


Figure 35: Damaged grain 10,000x magnification

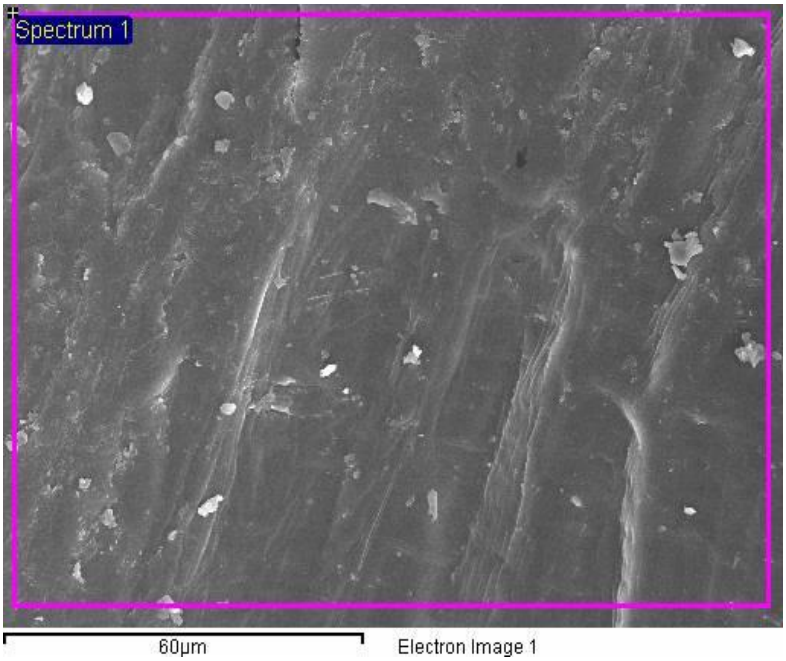
The next magnification in the series is 250x, which focused on the bran of the grain. The undamaged grain shows presence of starch globule in the form of spots (Figure 28). On the other hand, the presence of starch globule is reduced (Figure 29). More detailed topography has been studied at 1000x magnification which evidenced the presence of starch globule throughout the surface of the undamaged grains (Figure 30). In damaged grains, starch molecules have been attacked by the pest which is confirmed by the smooth peripheral endosperm (Figure 31). At 5000x magnification, patches of starch globules are highly prominent in undamaged grains (Figure 32) whereas a few globules are distributed in the endosperm of the damaged grains (Figure 33). Presence of starch globules in the form of crystals was more prominent in the undamaged grains at 10,000x magnification (Figure 34). The surface was plain and devoid of starch globules in the damaged grains at the same magnification (Figure 35).

1.7.4 SEM-EDX mapping of undamaged and damaged wheat grain

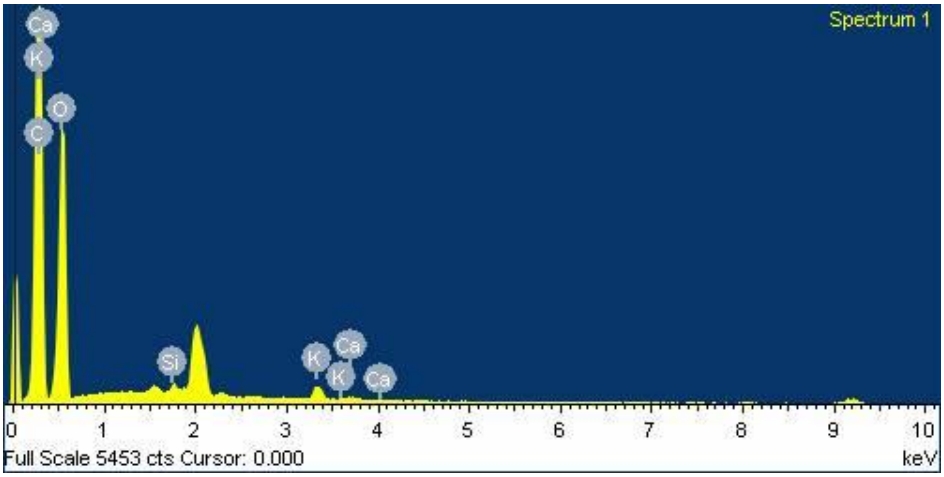
EDS qualitative and quantitative analyses have provided the basic compositional information about the samples. Moreover, it also establishes the relationship of various compounds with each other. In the study, SEM/EDS data have provided information of various inorganic compounds present in the undamaged (Figure 36) and damaged grains (Figure 37). The exceptionally high carbon and oxygen content in the samples confirms the presence of carbonaceous or organic compounds. Moreover, a few inorganic compounds in trace amount were also evidenced in the undamaged grain sample. Among inorganic compounds, silicon, calcium and potassium in undamaged grains was found whereas only potassium has been detected from the damaged grains.

The EDX (Energy Dispersive X-Ray Analyser) data has shown the presence of 39.21% of carbon compound in undamaged grain. Whereas, an increase in the carbon content by 2% in the damaged grains (40.50%) was reported. Trace elements like potassium detected in both the undamaged (1.76%) and damaged grains (0.23%). Data has shown a steep reduction in the potassium

content in the damaged grains by 1% when compared with the EDX result of the undamaged samples. No residue of calcium and silicone was detected in the damaged grain. However, undamaged grain has revealed the presence of 0.66% of silicone and 0.42% of calcium of the total weight in it. Oxygenated content was 59.27% in damaged grains and 57.95% in undamaged grains. It was calculated to be increased in the damaged grains by 3%.



a

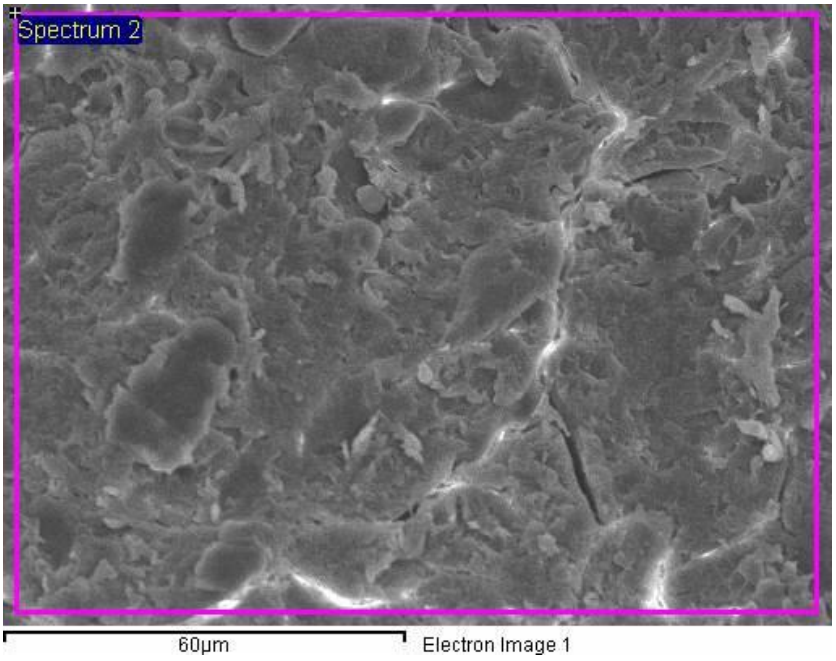


b

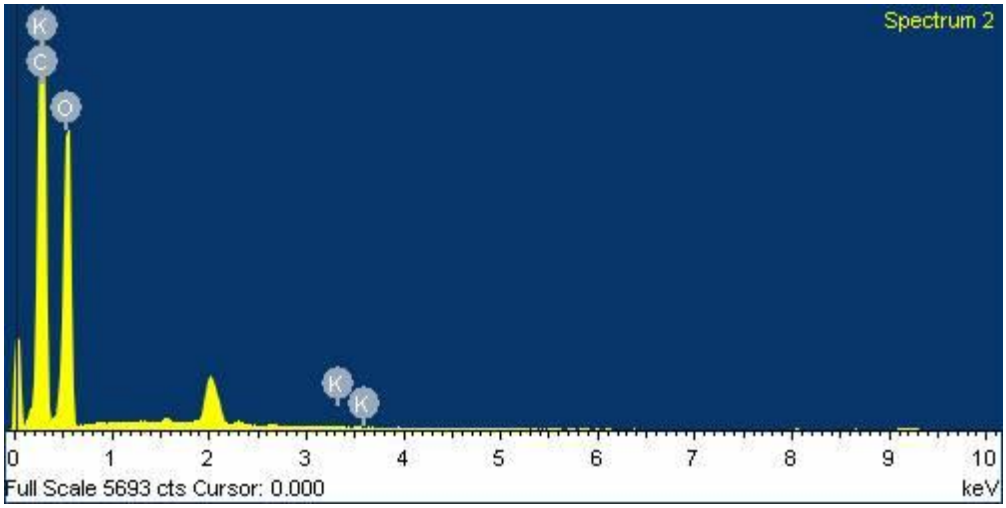
Element	Weight%	Atomic%
C K	39.21	46.87
O K	57.95	52.00
Si K	0.66	0.34
K K	1.76	0.65
Ca K	0.42	0.15
Totals	100.00	

c

Figure 36: SEM/EDS data showing complex elemental mixture in the undamaged wheat grains. a: surface view, b: relative content of different nutrient, c: % composition of elements.



a



b

Element	Weight%	Atomic%
C K	40.50	47.61
O K	59.27	52.30
K K	0.23	0.08
Totals	100.00	

c

Figure 37: SEM/EDS data showing complex elemental mixture in the damaged wheat grains. a: surface view, b: relative content of different nutrient, c: % composition of elements.

1.7.5 Flour disc Bioassay:

The relative growth rate (RGR) and relative consumption rate (RCR) of the insect has shown a significant reduction ($P<0.05$) in the treated sets when compared to the control at the end of the third day. On the contrary, the percentage of efficiency of conversion of ingested food (ECI %) has decreased significantly with the increase in doses when compared with the control due to the negative growth rate and low rate of food consumption. The four different solvents were effective in the following order of increasing efficiency i.e. methanol < chloroform< petroleum ether < n-hexane. The growth and consumption rate is reduced in a dose dependant manner.

The methanolic EOs extract at the highest concentration chosen i.e. 1.67 has significantly reduced the RGR to -0.723 mg/mg/day than the control where the value was calculated to be 0.474. Similarly, RCR was found to be reduced from 0.227 to 0.075 mg/mg/day at the extreme concentrations chosen. The rate of consumption at the control set was 0.505. The ECI% has decreased significantly ($P<0.05$) when compared with the control value 93.861. Significant feeding deterrence has been exhibited by the extract which gradually increased from 55.05% to 58.4%2, 66.93%, 75.84% and 85.15% at the concentration of 1, 1.17, 1.33, 1.5 and 1.67 respectively (Table 31).

Chloroform EOs has significantly reduced RGR to -0.765 at the highest concentration of the extract 1.67 mg/disc when compared with the control set (0.452 mg/mg/day). Similarly, RCR shows gradual decrease from 0.208 to 0.065 at the concentrations 1 and 1.67 mg/mg/day respectively. Rate of consumption was very high in the control set 0.512 mg/mg/day. ECI% has decreased significantly from the control 88.28. Like methanol chloroform also showed significant feeding deterrent activity against the pest. Moreover, feeding deterrence index (FDI %) increased in a dose-dependent manner. The FDI % was 59.38, 65.82, 71.68, 78.13 and 87.30 for 1, 1.17, 1.33, 1.5 and 1.67 mg/disc respectively (Table 32).

Similarly, results of RGR with the petroleum ether EOs have shown decrease when compared with the control (0.394 mg/mg/day). A concentration of 1, 1.17, 1.33, 1.5 and 1.67 mg/disc has resulted in -0.469, -0.582, -0.583, -0.626 and -0.685 mg/mg/day respectively. RCR values are also consistent as they

decreased with the increase in doses. They have decreased from 0.223 to 0.045 mg/mg/day at the highest and lowest concentration. ECI% has significantly decreased to -1522.22 from the control (80.40). Feeding deterrence index has significantly reduced the feeding by 90.82% at 1.67 mg/disc (Table 33).

RGR with the n-hexane EOs were found to be -0.635 mg/mg/day at 1 mg/disc and -0.876 mg/mg/day at 1.67 mg/disc which are significantly low compared to control i.e. 0.394 mg/mg/day. Similarly RCR has experienced a downfall from 0.571 mg/mg/day at the control set to 0.033 mg/mg/day at the highest dose. ECI% has also decreased with the increase in dose of essential oil. Significant feeding deterrent activity was exhibited by n-hexane at different concentrations in a dose-dependent manner. The feeding deterrence indices (FDI %) increased gradually from 76.36%, 81.09%, 83.71%, 88.97% & 94.22% at the 1, 1.17, 1.33, 1.5 and 1.67 mg/disc respectively (Table 34).

Table 31: Nutritional and feeding deterrence indices of adult of *Tribolium casteneum* exposed to flour discs treated with the methanolic EOs of *Artemisia annua.*

Solvent used for essential oil extraction	Conc. Of extract (mg/disc)	RGR (mg/mg/day)	RCR (mg/mg/day)	ECI (%)	FDI (%)
Methanol	0	0.474±0.001	0.505 ±0.002	93.861	
	1	-0.543±0.002**	0.227±0.00**	-239.21**	55.05
	1.17	-0.594±0.002**	0.212±0.002**	-280.19**	58.42
	1.33	-0.626±0.001**	0.167±0.002**	-374.85**	66.93
	1.5	-0.664±0.002**	0.122±0.002**	-544.26**	75.84
	1.67	-0.723±0.002**	0.075±0.001**	-964.00**	85.15

RGR (relative growth rate)

RCR (relative consumption rate)

FDI (feeding deterrence indices)

ECI (efficiency of conversion of ingested food)

**significantly different ($P<0.05$) when compared to control (ANOVA).

Table 32: Nutritional and feeding deterrence indices of adult of *Tribolium casteneum* exposed to flour discs treated with the chloroform EOs of *Artemisia annua.*

Solvent used for essential oil extraction	**Conc. Of extract (mg/disc)**	**RGR (mg/mg/day)**	**RCR (mg/mg/day)**	**ECI (%)**	**FDI (%)**
Chloroform	0	0.452±0.002	0.512 ±0.002	88.28	
	1	-0.551±0.002**	0.208±0.003**	-264.90**	59.38
	1.17	-0.636±0.003**	0.175±0.002**	-363.43**	65.82
	1.33	-0.665±0.002**	0.145±0.003**	-458.62**	71.68
	1.5	-0.732±0.002**	0.112±0.002**	-653.57**	78.13
	1.67	-0.765±0.002**	0.065±0.002**	-1176.92**	87.30

RGR (relative growth rate)

RCR (relative consumption rate)

FDI (feeding deterrence indices)

ECI (efficiency of conversion of ingested food)

**significantly different ($P<0.05$) when compared to control (ANOVA).

Table 33: Nutritional and feeding deterrence indices of adult of *Tribolium casteneum* exposed to flour discs treated with the petroleum ether EOs of *Artemisia annua*.

Solvent used for essential oil extraction	Conc. Of extract (mg/disc)	RGR (mg/mg/day)	RCR (mg/mg/day)	ECI (%)	FDI (%)
Petroleum ether	0	0.394±0.001	0.490±0.002	80.40	
	1	-0.469±0.001**	0.223±0.002**	-210.31**	54.49
	1.17	-0.582±0.007**	0.175±0.003**	-332.57**	64.29
	1.33	-0.583±0.007**	0.126±0.003**	-462.70**	74.29
	1.5	-0.626±0.005**	0.077±0.003**	-812.99**	84.29
	1.67	-0.685±0.005**	0.045±0.003**	-1522.22**	90.82

RGR (relative growth rate)

RCR (relative consumption rate)

FDI (feeding deterrence indices)

ECI (efficiency of conversion of ingested food)

**significantly different ($P<0.05$) when compared to control (ANOVA).

Table 34: Nutritional and feeding deterrence indices of adult of *Tribolium casteneum* exposed to flour discs treated with the n-hexane EOs of *Artemisia annua.*

Solvent used for essential oil extraction	Conc. Of extract (mg/disc)	RGR (mg/mg/day)	RCR (mg/mg/day)	ECI (%)	FDI (%)
n- hexane	0	0.394±0.001	0.571 ±0.002	81.61	
	1	-0.635±0.005**	0.135±0.001**	-470.37**	76.36
	1.17	-0.732±0.008**	0.108±0.003**	-677.77**	81.09
	1.33	-0.786±0.005**	0.093±0.002**	-841.94**	83.71
	1.5	-0.835±0.005**	0.063±0.002**	-1325.40**	88.97
	1.67	-0.876±0.006**	0.033±0.002**	-2654.55**	94.22

RGR (relative growth rate)

RCR (relative consumption rate)

FDI (feeding deterrence indices)

ECI (efficiency of conversion of ingested food)

**significantly different ($P<0.05$) when compared to control (ANOVA).

1.7.6 Flour colour

Analysing the flour colour is an important protocol while performing quality control evaluation of wheat flour. The colour is known to have a profound effect on the end-products as well. It is among one of the major specifications asked by the end-users.

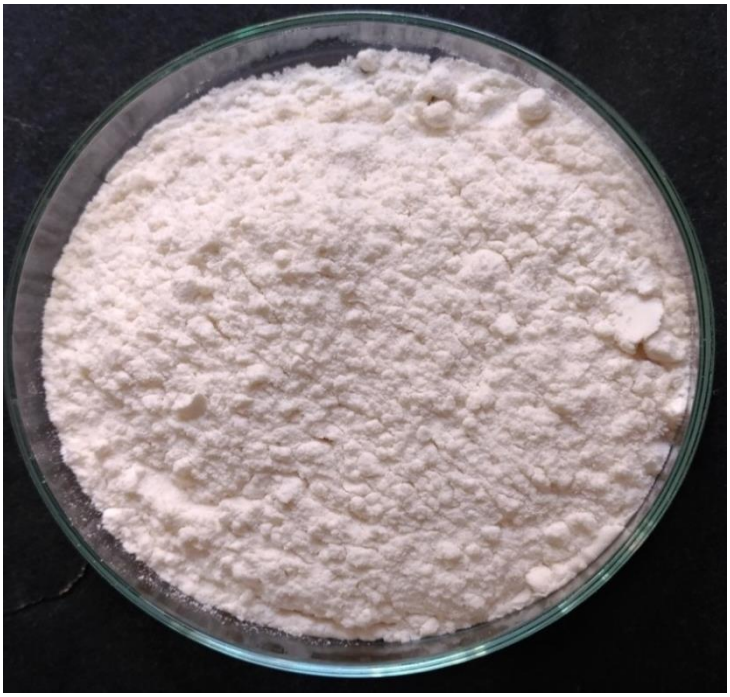


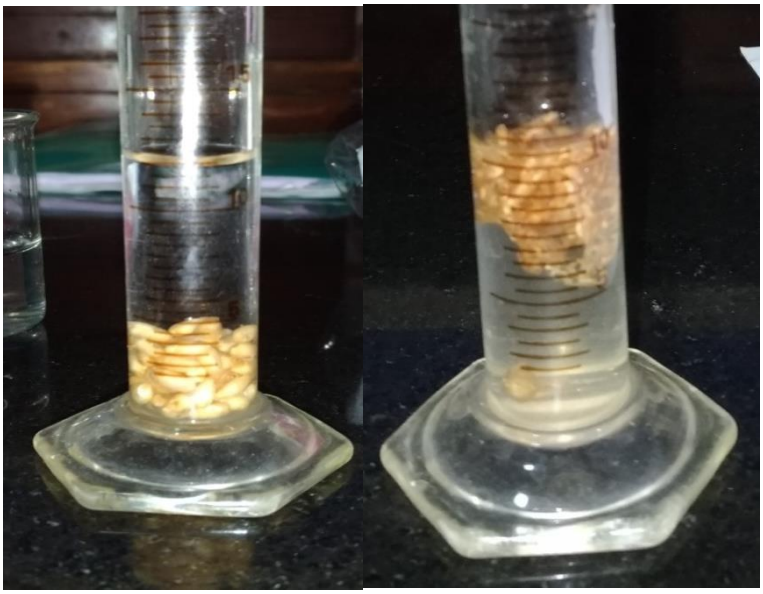
Figure 38: Undamaged wheat flour

Figure 39: *T. casteneum* infested wheat flour

By conventions, white coloured flour is more preferable by the consumers. The flour from the damaged set, where the insect was allowed to infest the flour continuously for three months, was found to lose its aesthetic value. The damaged grain turned greyish in colour which is due to the toxic quinone's generally secreted by the red flour beetle. On the other hand, the undamaged flour is white & clear.

1.7.7 Weight difference

Difference in weight was highly prominent. The undamaged grain was heavy and settled down below the water. In this case, the bran and endosperm region remains intact and hence contribute to the weight of the grains. On the other hand, the damaged grains were floating due to loss in weight.



A B

Figure 40: A- undamaged grains are heavy and immerged in water; B- damaged grains are thoroughly punctured and floated in water

1.7.8 P^H result

P^H determination is an important criterion for quality assurance of wheat flour. The flour is known to release no gases upon suspension in water that may deviate the P^H range from the normal. The P^H of the infested and uninfested flour was measured using P^H potentiometer meter and the values were recorded as:

Undamaged sample = 7.1

Damaged sample = 4.98

1.7.9 Iodine method for insect eggs in flour

Iodine methods for insect eggs determination is one of the standard methods for finding out any carryover in the samples. It is already mentioned in the result section that the eggs of *T. casteneum* are microscopic and cannot be noticed with the naked eyes. Hence, its determination in the samples can be improved by colouring them with the iodine for the ease of noticing them. The results of iodine method have demonstrated that the undamaged flour was free from any eggs and was healthy. But, a large number of eggs were detected in the damaged flour sample which were differentially coloured in wine red colour. The number of eggs found in each category is as follows:

Number of insect eggs in undamaged set = Nil

Number of insect eggs in damaged set = 89

1.7.10 Crude Fat

The crude fat content of the damaged and undamaged flour were evaluated and it was found to be as follows:

Crude fat in the flour free from infestation = 1.9%

Crude fat in the infested flour free = 4.2%

1.7.11 Percentage Moisture content

Moisture content determination is another simple yet very vital criterion for establishing quality of the wheat flour. Moreover, it is also an indicator of flour health in storage. When moisture content of both the *T. casteneum* infested and infestation free flour was analysed the undamaged grains showed higher percentage of moisture content. The results were as follows:

Moisture content of the undamaged flour = 12%

Moisture content of the damaged flour = 17%

1.7.12 Total Protein: Biuret Kit

Determination of protein content was a key factor for quality assurance and forms a basis of undamaged grains. Hence, comparing the result of damaged grains with that of the undamaged grains gives a clear picture of the infestation level. In the present work, protein content was found to reduce

drastically from the undamaged grains. The estimation results are depicted below:

Total protein content of undamaged grains= 1.76 gm/dl

Total protein content of damaged grains= 0.961 gm/dl

1.7.13 Carbohydrate by GOD-POD

Another vital biomolecule is carbohydrate, whose presence in correct proportion in the edibles like wheat flour is desirable. Any deviation from the normal range has direct effect on consumer's health. In our study, we could evidence abnormally high carbohydrate content in the damaged grain. The spectroscopic results were analysed and carbohydrate level was determined.

Starch content of undamaged grains= 5.80 mg/dl

Starch content of damaged grains= 1.44 mg/dl

1.7.14 Acid hydrolysis

Presences of insect fragments in both the damaged and undamaged wheat flour were evaluated through the acid hydrolysis test. Huge fluctuation was noted in the *T. casteneum* damaged flour which was obvious due to its continuous infestation for three months. Results have shown 3 fragment counts in the undamaged flour whereas 212 fragments were evidenced from the damaged flour. Moreover, the number of fragments detected per 50 grams of wheat flour was more than 75 in case of damaged samples. Hence, this sets the DAL (defect action level) level for wheat sample.

Insect fragments per 50 grams of undamaged flour= 3

Insect fragments per 50 grams of damaged flour= 212

"Pharmaceuticals are regulated. Pesticides are, as well as food, save the occasional salmonella outbreak. But chemicals and their witch's brew of ingredients continue to augment American industry without anyone quite knowing their makeup and possible toxicity. And that needs to change."

- Suzy Shuster