Study on the Molecular Mechanism of *Artemisia annua* L / Induced Toxicity against *Tribolium casteneum* (Herbst.)

Mamata Deb

Abstract of Ph.D. Thesis In the Field of Zoology
for the Degree of Doctor of Philosophy

The Maharaja Sayajirao University of Baroda

NOVEMBER, 2019

Table of Contents

CHAPTER 1

1.0 INTRODUCTION

Agriculture has been the mainstay of the Indian economy since history and it continues to be the same for long. A recent report has cited that 17% of the world's total population is supported by our nation (Pandey, 2009). Unfortunately, the current yield is unable to meet the demand of the burgeoning population (Ali & Gupta, 2012). Cultivation of cereal grains like wheat, rice, barley, etc., which constitutes the staple food of our country is of great importance due to their continuous demand throughout the year. To meet this demand, more than 70% of the food grains are stored. However, grains in the storage are prone to different types of infestation and the list is dominated by insects due to abundant food resources, high moisture content and suitable temperature (Ahmed, 1983). This is to worry that 10-40% grain deterioration is reported every year in storage (Sharon et al., 2014).

Tribolium casteneum (Herbst, 1797) is a major pest of wheat grains and flour (Figure 1). The infestation of the grains intensifies with time due to the high reproductive potential of the beetle. The work of Ajayi & Rahman (2006), have quoted that approximately 40% devastation of wheat flour by the pest. Apart from the economic damages, its contamination also proposes major health concerns due to toxic quinone's secreted by them (Ladisch et al., 1967).

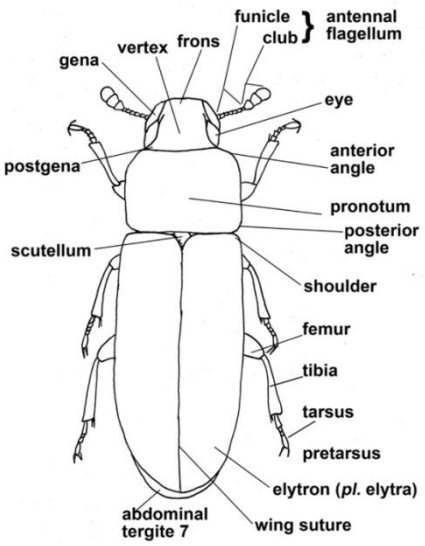


Figure 1: External morphology of Tribolium casteneum; Donitz et al., 2013

Control measures chiefly rely on fumigation through methyl bromide (CH_3Br) and phosphine (PH_3) (Bell, 2000). However, use of CH_3Br and PH_3 is discouraged worldwide (Anbar et al., 1996) due to its association in ozone layer depletion and resistance development in the pest respectively (Benhalima et al., 2004) (Okonkwo & Okoye, 2015). Replacement of these fumigants with an eco-friendly alternative was most important to control the pest which has inclined research towards the pesticidal plants. Plants are well known for possessing wide range of essential oils (Sasidharan et al., 2011). EOs contain a plethora of organic compounds that are relatively non-toxic for the environment and can be a potent alternative for the synthetic pesticides (Isman B., 2000).

Artemisia belonging to the Asteraceae family is used for its medicinal properties in Asian countries. *Artemisia* has been the subject of research interest for decades which is reflected in the wide range of studies conducted across the globe (Bora & Sharma, 2011). The review throws light in the fact that species of *Artemisia* possess either pharmacological or insecticidal properties. This demands further research in the same field to explore other species for their holistic benefit as a pesticide. Moreover, certainty of compound mediated toxicity inflicted in the mode of action is rarely evaluated. EOs or their purified compounds occasioned in structural symptoms point towards a broad range of neurotoxic mode of action. The substances attack multiple molecular targets including proteins like ion channels, receptors, enzymes, etc. and biomembranes to nucleic acids (Rattan, 2010). Taking these works into account, unveiling the biomolecular targets of *T. casteneum* affected by EOs of *A. annua* would add new dimension in pest management science.

Triticum aestivum is the most preferred staple food in the world. According to recent surveys, India stood as the second-largest producer of wheat. The grain is high in nutritional properties which include proteins, carbohydrates, minerals, fibres, and essential vitamins. Vitamins and minerals are present in high quantities. However, the list is dominated by selenium, manganese, phosphorus, copper and folate and many other vital to good health. The wheat kernel contains vital nutrients that lay profound metabolic effects in the human system. Insect infestation severely affects the nutritious contents of the grain. Padín et al., (2002) in his work has testified the damage of wheat grains by stored grains pests like *Tribolium castaneum, Sitophilus*

oryzae and *Acanthoscelides obtectus*. It severely damages the wheat grains chiefly the inner mass leaving a nutrition deficient grain coat.

1.1 Aim of the work

The aim of the work was "to evaluate the efficacy of Essential oils of *Artemisia annua* against *Tribolium casteneum* (Herbst, 1797)".

Objectives of the study

To fulfil the aim following objectives were undertaken.

1. Study of the biology of model organism, *Tribolium casteneum* (Herbst, 1797) in laboratory conditions.
2. Extraction, fractionation, and identification of chemical compositions of essential oils of *Artemisia annua.*
3. Identification of lethal doses (LD_{50} & LD_{90}) and repellency of the crude extracts against *Tribolium casteneum* employing different bioassays.
4. Understanding the differential tolerance in control and major lethal groups using different enzyme biomarkers.
5. Assessment of nutritional properties of insect free & insect infested wheat grain and wheat flour.

"Agriculture is locomotive of our economy and a prosperous rural economy based on agriculture will ultimately make the nation prosperous."

– **Sardar Vallabhbhai Patel**

CHAPTER 2

2.0 REVIEW OF LITERATURE

2.1 Literature documented from the state: Gujarat

While reviewing the literature in the state, unfortunate yet surprising fact of "no previous studies" came into light. Only a few preliminary works on the antifeedant properties of pulverised leaves has been published almost a decade before (Anita et al., 2012). However, extension of these works was needed for characterisation of novel plant compounds with insecticidal and repellent properties. The state lacks any report on the EOs of plant origin against any stored grain pest.

2.2 Literature documented from the nation: India

Scientist from different corners of India has documented a number of plant species whose EOs were efficient against *T. casteneum.* In a recent report by Brari & Thakur (2018) from Department of Biosciences, Himachal Pradesh University, Shimla, HP, the larvicidal potency of eight different plants EOs against larval *Tribolium casteneum* has been tested. Among all, *R. rugosa* EOs was most efficient against the *T. casteneum* larvae. However, treated larvae showed tolerance with maturity. Another work published recently Haider et al. (2017) from Department of Botany, Gorakhpur University, Gorakhpur, UP, has discussed about the insecticidal and repellent efficacy of *Tanacetum tomentosum* and *T. dolichophyllum* EOs against *T. casteneum*. The work emphasised *T. tomentosum* as the better plant variant than *T. dolichophyllum.* Mishra et al. (2016) from Department of Zoology, Gorakhpur University, Gorakhpur has taken the work one step ahead by evaluating the enzymatic activity of the treated groups. In the study, EOs of *Syzygium aromaticum* was found efficient in controlling the beetle. Also, the AChE activity of sub-lethal concentrations was reduced significantly compared to the control. In a relevant work, Haider et al. (2015) from Centre for Aromatic Plants, Dehradun, Uttarakhand, insecticidal activity of *Tanacetum nubigenum* collected from three different locations viz. TNG, TNB and TNM was evaluated for controlling *T. casteneum*. TNG was most effective and grain protectant among all the three varieties. This study presents an insight into the influence of climatic variations on the efficacy of different plant species.

2.3 Literature documented across the globe: International platform

While extending the search further, the essential oils and its work was found to hit the global platform every now and then. While reviewing the work of Hu et al. (2019), School of Food Science and Technology, South China University of Technology, China, efficacy of *Artemisia brachyloba* EOs and its major constituents against *T. casteneum* could be recognised. The biochemical estimation has proved the reduction in the enzymatic level in the treatment groups. Authors have concluded the possibility of synergy in the effect of EOs. In a recent survey conducted in the Department of Agriculture, Food and Environment, University of Pisa, Italy, EOs of *Pimpinella anisum* were tested against *Tribolium casteneum* (Hashem et al., 2018). The scientist have modified and made use of nanoemulsions to mediate the toxicity of EOs on the pest and it was highly effective. Salem et al. (2017) from Tunisia has documented insecticidal properties of two well-known species viz. *Ricinus communis* and *Mentha pulegium* EOs against the adult beetles. *R. communis* EOs, rich in oxygenated monoterpene, was found more effective than the *M. pulegium*. Another work conducted in the same laboratory of Beijing Normal University, Beijing, China, has worked on the efficacy assessment of the isolates of *Juniperus formosana* against *T. casteneum* Guo et al. (2016). Results demonstrated the efficacy of EOs. Down the line, reports on bioactivity of EOs of *Zingiber purpureum* Rhizomes against *T. casteneum* was found during the literature search (Wang et al., 2015). The work belongs to the same lab of laboratory of Beijing Normal University, Beijing, China, has demonstrated the efficacy of isolates over the EOs.

"Nobody is qualified to become a statesman who is entirely ignorant of the problem of wheat."

— Socrates

CHAPTER 3

3.0 MATERIALS AND METHODS

3.1 Procurement & Rearing

Tribolium casteneum was collected from the laboratory cultures maintained in the division of Entomology of the Department of Zoology, The Maharaja Sayajirao University of Baroda, for the last 3 years. Pests were reared in the defined culture media which consisted of wheat flour, wheat grains and Baker's yeast in the ratio of 6:3:1 and maintained in the humidity chamber at 27±2˚C, 70±5 RH. Biology of the beetle was studied using computer assisted 13.1 mega pixel Catcam stereomicroscope.

3.2 Plant material

The dried, finely grounded leaves of the *Artemisia annua* was procured from the lab of Professor Neeta Pandya of Botany Department. Plant powder was then stored in Sterile Polyethylene Sampling Bags in the refrigerator at 4˚C until it is used for the extraction of EOs. 25 grams of plant powder was subjected to hydro-distillation in a Clevenger-type apparatus (Clevenger, 1928) for the extraction of EOs using methanol, chloroform, petroleum ether (40-60˚C) and n- hexane. Oil yield was calculated on a dry weight basis employing the Yield (%) formula.

$$\text{Yield (\%)} = \frac{W_{EO}}{W_I} \times 100$$

Extract were then stored in airtight plastic containers in a refrigerator at 4 ˚C until it is used for further experimentation. EOs were then analysed through GC-MS to unveil the chemical composition of the oil. Most constituents were identified by gas chromatography by comparison of their retention indices with those of the literature or with those of authentic compounds available in our laboratories.

3.3 Bioassays and Identification of Lethal doses

Repellency in insects was evaluated according to (Cosimi et al., 2009) in the filter paper arena test and PR was assessed. Behavioural bioassay was also assessed

through multi arm olfactometer based on the idea of. Jayakumar et al. (2017) and EPI was calculated.

3.3.1 Acute toxicity assays

Contact toxicity was evaluated following the method of Huang & Ho (1998) and fumigant toxicity was assessed as per the method suggested by López et al., (2008). Probit analysis (Finney, 1971) using Medcalc software was employed in analysing the dosage- mortality response in both the acute toxicity assays. Percentage mortality would be calculated using Abbott's formula (Abbott, 1925) to correct natural mortality, if any, in control groups.

3.4 Understanding biomolecular pathways

Quantitative analyses of biochemical constituents in viable (LD_{50}, LD_{90}) and control sets were assessed. Protein profiling by Biuret method (Reckon Diagnostics Pvt. Ltd.) and enzymatic activities of AChE, GST, GSH and LPO were performed following the methods of Ellman et al. (1961), Habig et al. (1974), Jollow et al. (1973) and Buege & Aust (1978) respectively. ANOVA and Tukey's Pairwise Comparison Test was employed using PAST statistical software package to compare means.

3.5 Quality Parameters

3.5.1 Feeding deterrence and % weight loss

Feeding deterrence potential of *Artemisia annua* was assessed against *T. casteneum*. Feeding ratio (Fr) and % Weight loss was calculated.

3.5.2 Flour disc Bioassay

Flour discs were prepared according to the method of Xie et al. (1996) with some modifications by Huang et al. (1997). Data collected after 72 h and then processed for calculating RGR, RCR, AE and FDI

3.5.3 Scanning Electron microscopy analysis

SEM measurements of damaged and undamaged grains were performed with JEOL JSM-7600F series. Details of the instrument are as follows: SEI resolution of 1.0nm at 15 kv 1.5nm at 1 kv, in GB mode; Magnification Low: 25X to 10,000X; High: 100X

to 1,000,000X at 4x5 photo size; Accelerating voltage: 0.1 to 30 kv; Probe Current Range: 1 pA to ≥ 200 nA).

3.5.4 Measure of PH

pH activity of *T. casteneum* infested and infestation free flour was evaluated following AACC International Method, 02-52.01

3.5.5 Iodine method for insect eggs in flour Objective

The evaluation of the presence of insect eggs and also to detect possible "carryover" infestation in infested and infestation free flour following the method of (AACC International Method, 28-44.01).

3.5.6 Crude Fat

The crude fat content of infested and uninfested flour samples was evaluated following AACC International Method, 30-10.01.

3.5.7 % Moisture content

The moisture content of the infested and uninfested grain samples were analysed following AACC International Method, 44-01.01.

3.5.8 Total Protein

Protein content of the infested and non-infested wheat grains was detected using Biuret kit.

3.5.9 Carbohydrate by GOD-POD

The carbohydrate content of the damaged and undamaged wheat grains was analysed by GOD-POD method.

3.5.10 Acid hydrolysis

The presence of insect fragments in infested and non-infested flour samples was evaluation following the method of AACC International Method, 28-41.03.

"I believe that for every illness or ailment known to man, that God has a plant out here that will heal it. We just need to keep discovering the properties for natural healing."

- Vannoy Gentles Fite

CHAPTER 4

4.0 RESULTS

4.1 Biology of the model beetle

Presence of seven larval instars was reported from the current study. Moreover, sexual dimorphic characters which are microscopic and prominent in the Figure 2.

4.2 Plant material

The average oil yield was found to be 27.16%, 19.28%, 1.36% and 3.68% in the methanolic, chloroform, petroleum ether and n-hexane EOs respectively. Result of GC-MS shows the presence of 13 different compounds from methanolic EOs accounting for 99.98% of the total oil (Table 1). 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%). 24 major compounds were identified in the chloroform derived EOs accounted for 97.11% of the total oil (Table 2). 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%) were found. Petroleum ether derived EOs were recorded to possess 16 different compounds which accounts for 98.15% of the total oil (Table 3). Among the major chemical constituents, 3,4-Hexadienal, 2-butyl-2-ethyl-5-methyl- (22.06%), Deoxyqinghaosu (10.84%), were enlisted. N-hexane EOs was found to contain 18 different compounds accounting for 97.11% of the total oil (Table 4). The major constituents were identified as 3,4-Hexadienal,2- butyl-2-ethyl-5-methyl-2 (20.98%), Cedran-diol, 8S,13- (8.29%).

4.3 Bioassays and Identification of Lethal doses

Methanolic EOs has demonstrated 91.63% of repellency at the highest concentration of 0.90 mg cm^{-2} (Table 5). The chloroform EOs have drawn 88.25% repellency at the same concentration (Table 6). On the other hand, the petroleum ether and n- hexane EOs has demonstrated 95% and 93.5% of repellency respectively at the highest concentration (Table 7) **(**Table 8**).**

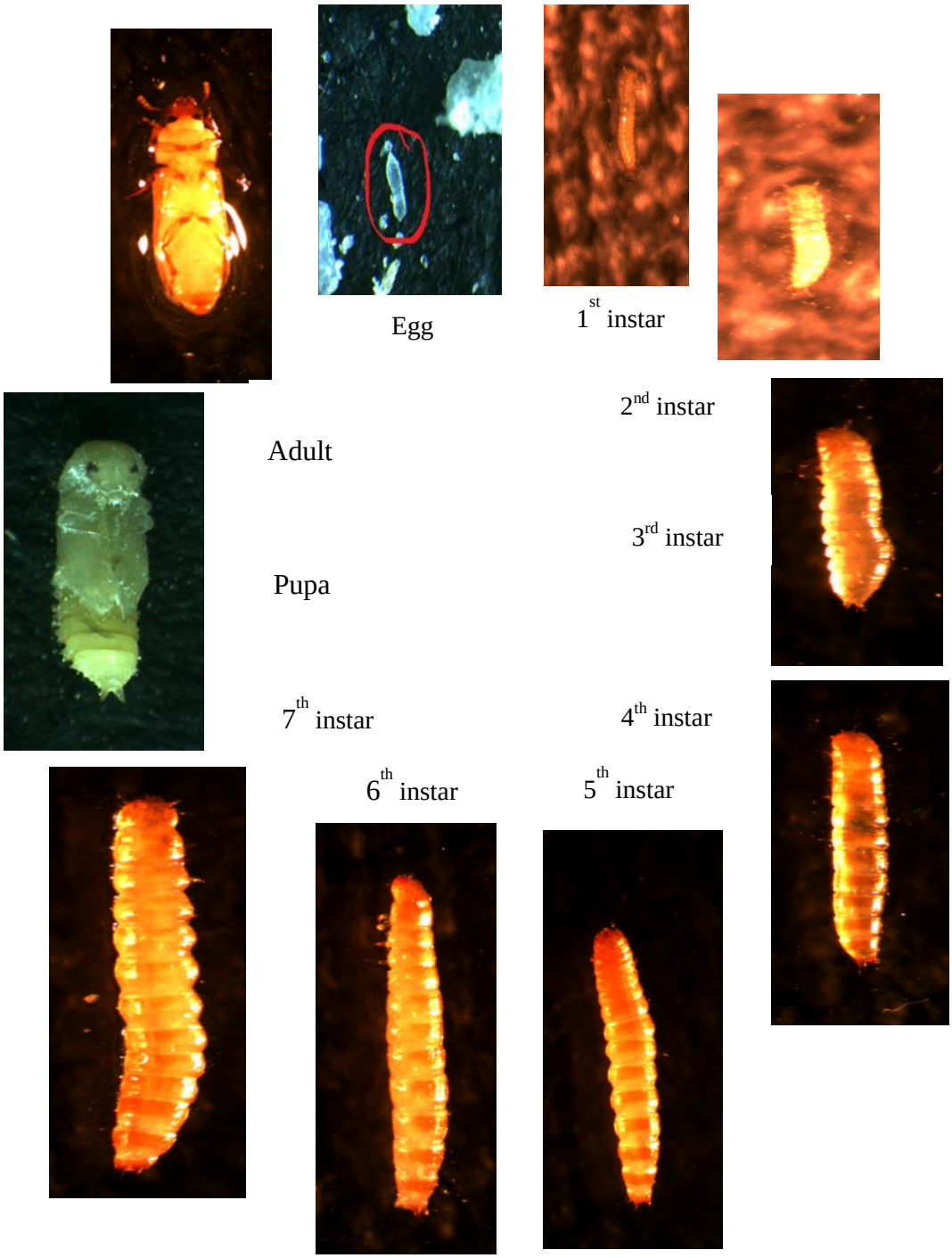


Figure 2: Different stages of *Tribolium casteneum* captured using the computer assisted 13.1 mega pixel catcam stereomicroscope with fine details.

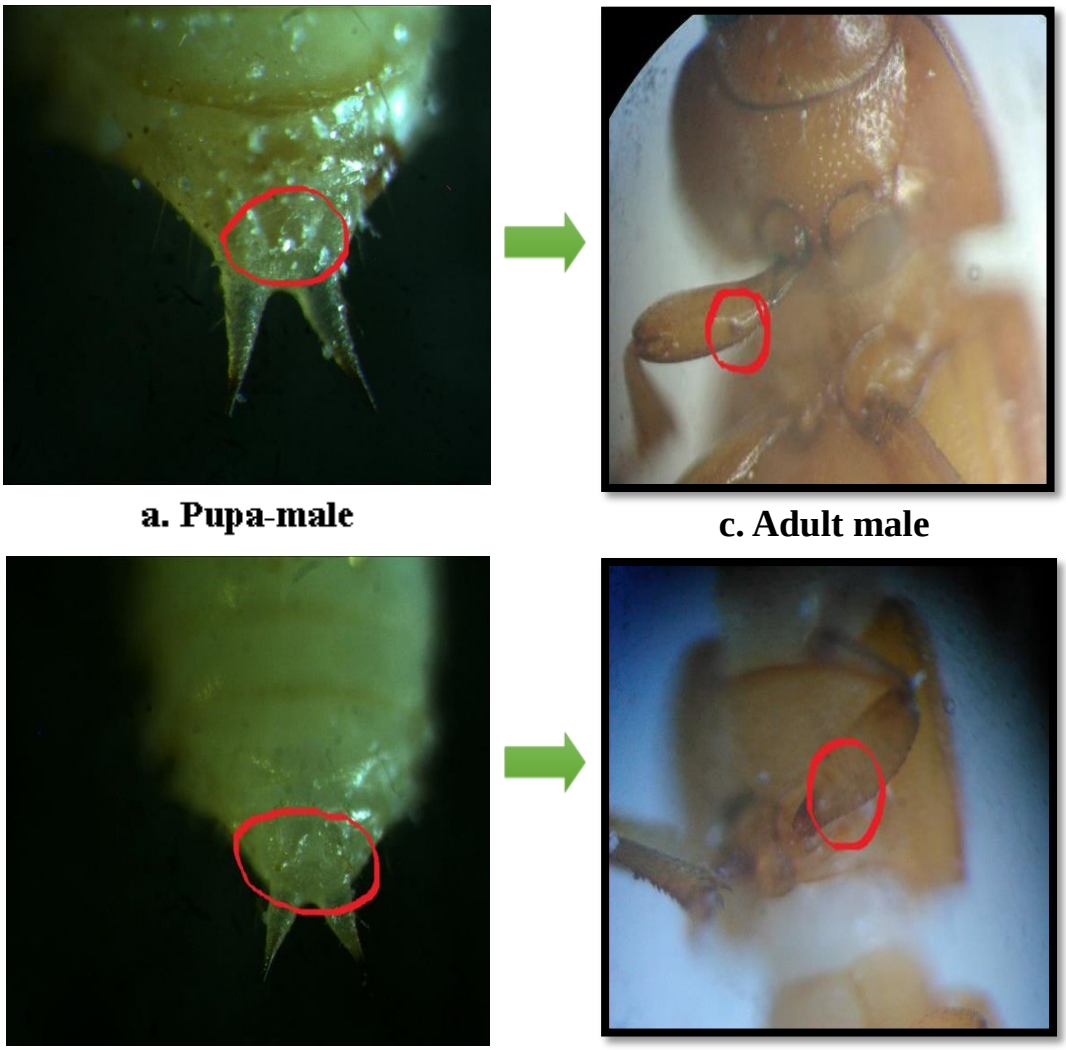


Figure 3: Sexually dimorphic characters of *Tribolium casteneum* **a***:* male pupa where genital papilla is stubby, **b**: female pupa where genital papilla is forked reaching the urogomphi, **c:** male adult marked by the presence of setiferous patch on the forefemur, **d:** setiferous patch absent in females

Table 1: 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

Table 2: 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			

Table 3: 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			

Table 4: 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

Table 5: Repellency of methanol derived EOs of *A. annua* against *T. castaneum* adults using filter paper arena test

Solvent used	Conc (mg cm^{-2})	Duration of exposure (in) hour								% 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 6: Repellency of chloroform derived EOs of *A. annua* against *T. castaneum* adults using filter paper arena test

Solvent used	Conc (mg cm^{-2})	Duration of exposure (in) hour								% 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 7: Repellency of petroleum ether derived EOs of *A. annua* against *T. castaneum* adults using filter paper arena test

Solvent used	Conc (mg cm^{-2})	Duration of exposure (in) hour								% 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 8: Repellency of n-hexane derived EOs of *A. annua* against *T. castaneum* adults using filter paper arena test

Solvent used	Conc (mg cm^{-2})	Duration of exposure (in) hour								% 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.

EPI has ranged from -0.5 one hour post treatment to -0.9 after 24 hours in case of methanolic EOs (Table 9). On the other hand, chloroform derived EOs reported with an EPI ranged from -0.7 to -1 (Table 10). EPI was found to range from -0.6 to -1 in sets of petroleum ether eluted EOs treatment (Table 11). n- Hexane derived EOs treated sets showed steady increase in EPI ranged from -0.3 to -1 (Table 12).

Table 9: 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

Table 10: 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

Table 11: 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

Table 12: 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

4.4 Contact toxicity

When the lethal values of methanolic EOs were compared, significant ($p<0.01$) difference between the two stages was found as no overlap in 95% confidence interval

is prominent (Table 13). Significant ($p<0.01$) difference between the two stages at LD_{90} value was marked (overlap in 95% confidence interval) in the chloroform EOs (Table 14). When petroleum ether EOs treated sets were evaluated, significant difference between adult and larva was marked (Table 15). The relation between the two groups depicts a significant difference ($p<0.01$) between them. Moreover, the n-hexane EOs have shown significant relation between the adults and larvae at the lethal doses (Table 16).

Table 13: 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**	**$\chi 2$ (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	0.94-1.48	2.22	1.91-2.75	1.31±0.24	50.562 (1)*

Table 14: 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**	**$\chi 2$ (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)*

Table 15: 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	$\chi 2$ (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)*

Table 16: 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	$\chi 2$ (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; $\chi 2$= chi square; DF=degrees of freedom; *= Significant ($P < 0.01$); Lethal values are expressed as mean of five replicates.

4.5 Fumigant toxicity

The larvae were more susceptible to the methanolic EOs than the adults (Table 17). On the other hand, adults were found more susceptible to chloroform derived EOs (Table 18). The adults were recorded to be more resistant to the fumigants of petroleum ether EOs (Table 19). Same as methanolic EOs, n-hexane too was more effective against the larvae (Table 20).

Table 17: 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	$\chi 2$ (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)*

Table 18: 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 $adult^{-1}$)	95% Confidence interval	LD_{90} (mg $adult^{-1}$)	95% Confidence interval	Slope ± SE	$\chi 2$ (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)*

Table 19: Fumigant toxicity of petroleum ether 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	$\chi 2$ (DF)
Petroleum ether	Adult	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)*

Table 20: 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 $adult^{-1}$)	95% Confidence interval	LD_{90} (mg $adult^{-1}$)	95% Confidence interval	Slope ± SE	$\chi 2$ (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; $\chi 2$= chi square; DF=degrees of freedom; *= Significant ($P < 0.01$); Lethal values are expressed as mean of five replicates.

4.6 Biomolecular Profile

The protein, AChE, GST, GSH was found to decrease significantly in the lethal sets compared to control sets (Table 22, Table 23, Table 24, Table 25, Table 26, Table 27, Table 28, Table 29), On the other hand, 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}.

4.7 Quality Parameters

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. *Artemisia annua* was found to be effective against the *Tribolium casteneum* in minimizing the weight loss of the grains. (Table 21).

Table 21: 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
	1 g	0.36*	3.4

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

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

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

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

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.

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

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

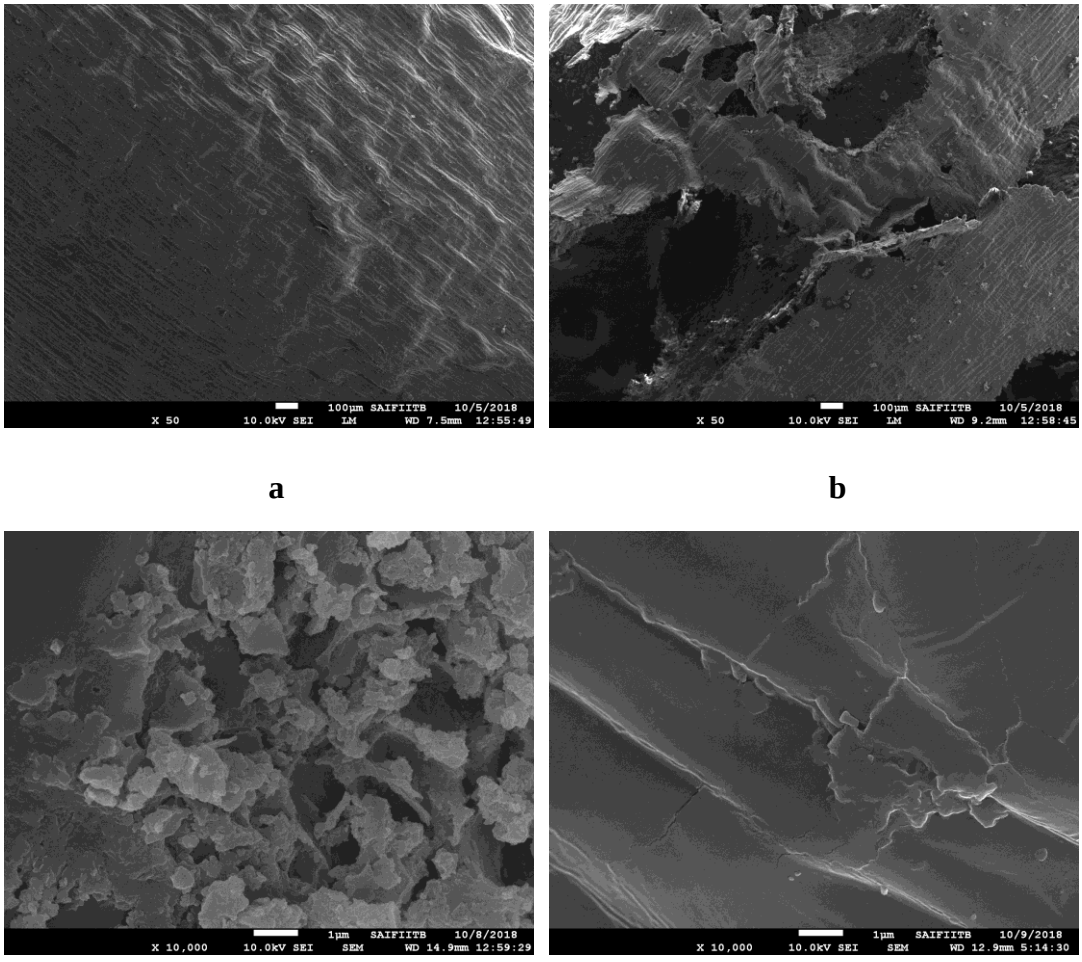
Table 28: Effect of petroleum ether derived EOs of *A. annua* on protein and enzymatic profile of *T. casteneum* subjected to fumigant toxicity bioassay.

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

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

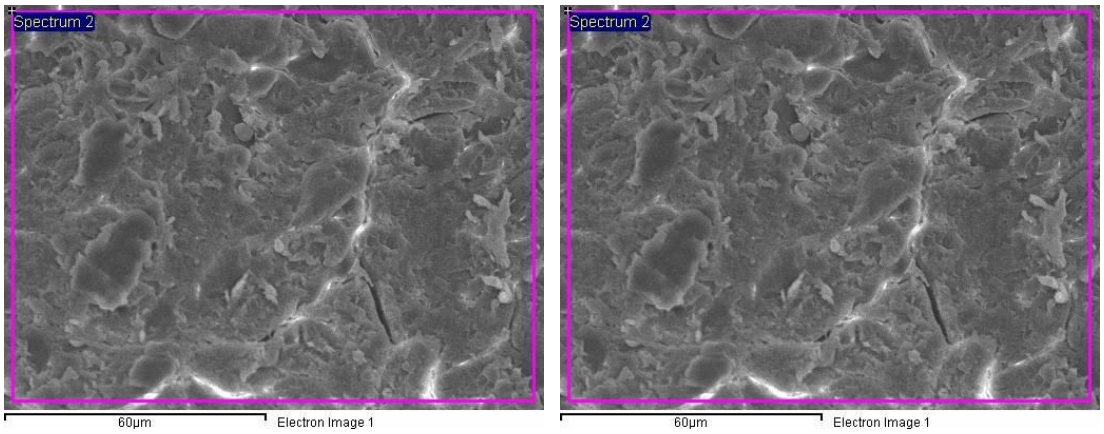
Mean (±SEM) followed by the same letters indicate no significant difference ($p< 0.01$) between different treated groups (Control, LD_{50} & LD_{90}) according to the Tukey's test.



a **b**

c **d**

Figure 4: a: Undamaged grain at 50x magnification; b: Damaged grain at 50x magnification **c:** Undamaged grain at 10,000x magnification; d: Damaged grain at 10,000x magnification



Undamaged Damaged

Figure 5: SEM/EDS data showing complex elemental mixture in the undamaged and damaged wheat grains

4.7.1 Scanning Electron Microscope (SEM) results:

The intact coat of the undamaged grain when compared with the infested grains, heavy loss of the bran as well as the endosperm at 50x magnification was evident (Figure 4). More detailed topography has been studied at 1000x magnification which evidenced the presence of starch globule throughout the surface of the undamaged grains (Figure 5). 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.

4.7.2 Flour disc Bioassay:

Table 30: 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 EOs 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

Table 31: 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 EOs 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

Table 32: 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 EOs 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

Table 33: 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 EOs 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).

The four different solvents were effective in the following order of increasing efficiency i.e. methanol < chloroform< petroleum ether < n-hexane (Table 30, Table 31, Table 32, Table 33). The growth and consumption rate is reduced in a dose dependant manner.

4.7.3 P^H result

P^H potentiometer meter and the value was recorded as

Undamaged sample = 7.1

Damaged sample = 4.98

4.7.4 Iodine method for insect eggs in flour

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

4.7.5 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%

4.7.6 % Moisture content

The results were as follows:

Moisture content of the undamaged flour = 12%

Moisture content of the damaged flour = 17%

4.7.7 Total Protein: Biuret Kit

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

4.7.8 Carbohydrate by GOD-POD

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

4.7.9 Acid hydrolysis

The number of insect fragments in the flour is as follows:

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

CHAPTER 5

5.0 DISCUSSION

The primary focus of the research was to decipher the influence of solvents in drawing potent chemical groups from the plant species. In this study, both the EOs of *A. annua* has demonstrated acute toxicity against *T. casteneum* adults and larvae. However, the degree of lethality varied with time, concentration, and solvent used to elute EOs. The contact toxicity studies have shown that the larvae were more susceptible than the adults to the methanolic EOs. However, adults were comparatively more susceptible to the petroleum ether EOs. While comparing the two EOs, adults, as well as larvae, were comparatively more susceptible to the petroleum ether EOs. In a similar study, the toxicity of n-hexane derived EOs of two species of *Aloysia* viz. *A. citriodora* and *A. polystachya* against *Tribolium casteneum* and *Tribolium confusum* was evaluated (Benzi et al., 2014). The study has shown the efficacy of non-polar solvent in eluting potent insecticidal components which could effectively control the pests. However, *T. casteneum* was found more tolerant of the EOs of *Aloysia* sp. While comparing the lethal values, it is found that a higher concentration of non-polar solvent derived EOs was required to pose a lethal effect in our study. The outcome can be justified by the fact of resistance development in the pest due to its continuous exposure to the synthetic fumigants. The study is supported by the outcome of Cao et al. (2018) where high LC50 values of β-Caryophyllene (41.7µg cm-2) point towards a resistant strain.

Testing of EOs as a fumigant against the beetle produced positive results. Comparing the response of the adult beetle revealed that the EOs derived with petroleum ether had a profound effect as depicted in LD_{50} values. The same was true for the larval stage as well. Our result differs from the work of Negahban et al., (2007) where *Artemisia sieberi* has shown a lethal effect (LD90) at a very low dose of 57.32 µl/l air. The work of Boyer et al. (2012) and Jagadeesan et al (2012) have demonstrated fast-growing resistance in the pest, which could be a probable reason for the high dose which was required to display lethal effects.

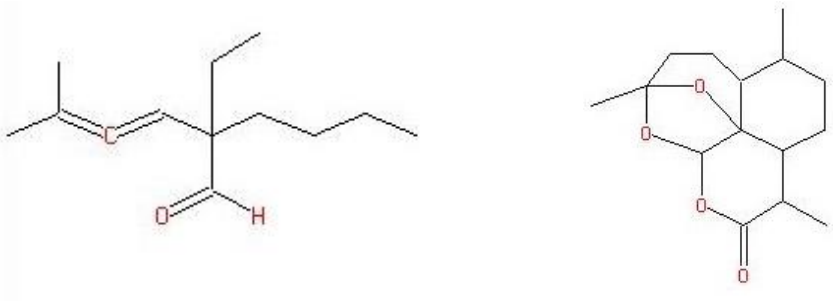
Results of the present study have verified petroleum ether derived EOs as a better insecticidal candidate for the control of *T. casteneum* and have added new dimensions to the previous findings (Tripathi et al., 2000). The success might be due to the

presence of novel compounds like 3,4-Hexadienal,2-butyl-2-ethyl-5-methyl- (22.06%), Deoxyqinghaosu (10.84%) in considerably higher percentage. Moreover, a lesser amount of compounds like Caryophyllene oxide and squalene, well known for their antimicrobial and anticancer properties, were also detected in both the extracts (Falowo et al., 2019) (Bui et al., 2014). However, further research is needed to claim a possible synergistic effect of different constituents present in petroleum ether EOs (Tak et al., 2015).

Studies on *A. Annua* EOs have deciphered the presence of volatile groups like sesquiterpene, coumarins, phenolic compounds, and flavones (Bora & Sharma, 2011). Among all, 1, 8- Cineole has gained considerable attention for being the core component of EOs which was responsible for insecticidal properties (Durden et al., 2011). The presence of oxygenated sesquiterpene, hydrocarbons, alcohols, vitamins, and diverse chemical groups in both the EOs was deciphered. Deoxyqinghaosu, 3, 4-Hexadienal, 2-butyl-2-ethyl-5-methyl, and Squalene were reported in both the EOs (Figure 6) with different percentage composition, and hence, we suggest their possible action in insecticidal activities (Chauhan et al., 2015). Moreover, additional components like esters (16.71%) and ethers (4.41%) in the petroleum ether EOs was a significant finding of our work. Their presence indicated the possibility of a synergistic effect for the higher insecticidal potential of the EOs. The results are supported by various scientists who portrayed EOs as the best management tools against stored grains pests (Sarwar & Salman, 2015) (Bett et al., 2016).

Further, we examined the effect of both the EOs on the bio-molecular profile of *T. casteneum* adults and larvae to gain an insight into the extent of metabolic disturbances inflicted in the treatment sets. Our experimental evidence has indicated the action of EOs on the nervous system of *T. casteneum*. They worked by disturbing the normal course of action of different detoxifying enzymes like AChE, GST, and LPO in the treated sets. While biomolecular profiling of LD50 and LD90 strains was conducted, the level of AChE, GST, and GSH were found to decrease, whereas the LPO level has increased. Scientists working in the same trail showed similar results where Coumaran has inhibited the activity of AChE (Rajashekar et al., 2014). Another set of experiments has depicted contradictory results with increased activity of GST in the multi-resistant strains of *T. casteneum* (Cohen, 1986). Moreover, the protein level has demonstrated a significant decrease in the lethal sets as compared to

the control group. The results are supported by the work of (Koodalingam et al., 2011). LPO level in the study increased significantly in the treatment sets thereby increasing the oxidative stress in the pest. Our findings are consistent with the result of Hasspieler et al. (1990) who emphasised on choosing a non-resistant strain for the study. The result dictates that the *Tribolium* strain used for toxicity assays was not resistant towards the *A. annua* EOs.



3, 4-Hexadienal,2-butyl-2-ethyl-5-methyl- Deoxyqinghaosu

Figure 6: Chemical structures of the major components of Essential oils of *Artemisia annua* grown in Indian climatic conditions

After establishing the efficacy of EOs of *A. annua* against the pest and their possible effect due to the major chemical groups, nutritional properties of the wheat was analysed. SEM images at 50x magnification clearly depict the structural loss to the damaged grains when compared with the undamaged ones. These findings conform by the work of Singh et al. (2013) where the wheat grains are significantly damaged by the stored pest *Rhyzopertha dominica*. The increase in microelement is also recorded which can be correlated with the work of Micu & Petanec (2011). However, decrease in potassium content can be explained by the fact that the aleurone layer which was heavily damaged.

In the flour disc bioassay, the essential oils extracted with the different solvents have shown significant antifeedant activity. Among the four different solvents used, n-hexane showed the best result followed by the petroleum ether> chloroform > methanol. The results are consistent with the several reports of earlier researcher. Stefanazzi et al. (2010) have proposed that the essential oils of *T. terniflora, C. citratus* showed antifeedant activity at the concentration of 4 mg disc^{-1} and *E. muticus* at both the concentration of 2 & 4 mg disc^{-1}.

"***Be stubborn on vision, but flexible on details***." **– Jeff Bezos**

REFERENCES

AACC International Method 02-52.01. *Hydrogen-Ion Activity (pH)—Electrometric Method*. doi.org/10.1094/AACCIntMethod-02-52.01.

AACC International Method 28-41.03. *Acid Hydrolysis Method for Extracting Insect Fragments and Rodent Hairs—Light Filth in White Flour*. doi.org/10.1094/AACCIntMethod-28-41.03

AACC International Method 28-44.01. *Iodine Method, for Insect Eggs in Flour*. doi.org/10.1094/AACCIntMethod-28-44.01.

AACC International Method, 30-10.01. *Crude Fat in Flour, Bread, and Baked Cereal Products Not Containing Fruit*. https://doi.org/10.1094/aaccintmethod-30-10.01. doi.org/10.1094/AACCIntMethod-30-10.01.

AACC International Method 44-01.01. *Percent Moisture*. doi.org/10.1094/AACCIntMethod-44-01.01.

Ahmed, H. (1983). Losses incurred in stored foos grains by insect pets- A review. *PPakistan Journal of Agriculture Research, 4*(3), 198–207.

Ajayi, F. A., & Rahman, S. A. (2006). Susceptibility of some staple processed meals to red flour beetle, *Tribolium castaneum* (Herbst)(Coleoptera: Tenebrionidae). *Pakistan Journal of Biological Sciences, 9*(9), 1744–1748.

Ali, M., & Gupta, S. (2012). Carrying capacity of Indian agriculture: Pulse crops. *Current Science, 102*(6), 874–881.

Anbar, A. D.; Yung, Y. L.; Chavez, F. P. (1996). Methyl bromide: Ocean sources, ocean sinks, and climate sensitivity. *Global Biogeochemical Cycles*, *10*(1), 175–190.

Anita, S., Sujatha, P., & Prabhudas, P. (2012). Efficacy of pulverised leaves of *Annona squamosa* (L .), *Moringa oleifera* (Lam .) and *Eucalyptus globulus* (Labill .) against the stored grain pest , *Tribolium castaneum* (Herbst .). *Recent Research in Science and Technology, 4*(2), 19–23.

Bell, C. H. (2000). Fumigation in the 21st century. *Crop Protection*, *19*(8–10), 563–

569. https://doi.org/10.1016/S0261-2194(00)00073-9

Benhalima, H., Chaudhry, M. Q., Mills, K. A., & Price, N. R. (2004). Phosphine resistance in stored-product insects collected from various grain storage facilities in Morocco. *Journal of Stored Products Research*, *40*(3), 241–249. https://doi.org/10.1016/S0022-474X(03)00012-2

Benzi, V., Stefanazzi, N., Murray, A. P., Werdin González, J. O., & Ferrero, A. (2014). Composition, Repellent, and Insecticidal Activities of Two South American Plants against the Stored Grain Pests *Tribolium castaneum* and *Tribolium confusum* (Coleoptera: Tenebrionidae) . *ISRN Entomology*, *2014*, 1–5. https://doi.org/10.1155/2014/175827

Bett, P. K., Deng, A. L., Ogendo, J. O., Kariuki, S. T., Kamatenesi-Mugisha, M., Mihale, J. M., & Torto, B. (2016). Chemical composition of *Cupressus lusitanica* and *Eucalyptus saligna* leaf essential oils and bioactivity against major insect pests of stored food grains. *Industrial Crops and Products*, *82*, 51–62. https://doi.org/10.1016/j.indcrop.2015.12.009

Bora, K. S., & Sharma, A. (2011). The genus Artemisia: A comprehensive review. *Pharmaceutical Biology*, *49*(1), 101–109. https://doi.org/10.3109/13880209.2010.497815

Boyer, S., Zhang, H., & Lempérière, G. (2012). A review of control methods and resistance mechanisms in stored-product insects. *Bulletin of Entomological Research*, *102*(2), 213–229. https://doi.org/10.1017/S0007485311000654

Brari, J., & Thakur, D. R. (2018). Larvicidal effects of eight essential oils against *Plodia interpunctella* and *Tribolium castaneum*, serious pests of stored products worldwide. *Journal of Entomological and Acarological Research*, *50*(1), 738–742. https://doi.org/10.4081/jear.2018.7469

Buege, J. A., & Aust, S. D. (1978). [30] Microsomal lipid peroxidation. In *Methods in Enzymology* (pp. 302–310). Cambridge, Massachusetts, United States: Academic Press, Elsevier.

Bui, D. T., Nicolas, J., Maksimenko, A., Desmaële, D., & Couvreur, P. (2014). Multifunctional squalene-based prodrug nanoparticles for targeted cancer

therapy. *Chemical Communications*, *50*(40), 5336–5338. https://doi.org/10.1039/c3cc47427e

Butt, M. S., Nasir, M., Akhtar, S., & Sharif, K. (2004). Effect OF Moisture and Packaging on the Shelf Life of Wheat Flour. *Internet Journal of Food Safety*, *4*, 1–6.

Cao, J. Q., Guo, S. S., Wang, Y., Pang, X., Geng, Z. F., & Du, S. S. (2018). Contact toxicity and repellency of the essential oils of *Evodia lenticellata* huang and *Evodia rutaecarpa* (Juss.) Benth. Leaves against three stored product insects. *Journal of Oleo Science*, *67*(8), 1027–1034. https://doi.org/10.5650/jos.ess17251

Chauhan, N., Kumar, P., Mishra, S., Verma, S., Malik, A., & Sharma, S. (2015). Insecticidal activity of *Jatropha curcas* extracts against housefly, *Musca domestica. Environmental Science and Pollution Research*, *22*(19), 14793–14800. https://doi.org/10.1007/s11356-015-4686-1

Clevenger, J. F. (1928). Apparatus for the determination of volatile oil. *The Journal of the American Pharmaceutical Association 1912*, *17*(4), 345–349. https://doi.org/10.1002/jps.3080170407

Cohen, E. (1986). Glutathione-S-transferase activity and its induction in several strains of *Tribolium castaneum*. *Entomologia Experimentalis et Applicata*, *41*(1), 39–44. https://doi.org/10.1111/j.1570-7458.1986.tb02169.x

Cosimi, S., Rossi, E., Cioni, P. L., & Canale, A. (2009). Bioactivity and qualitative analysis of some essential oils from Mediterranean plants against stored-product pests: Evaluation of repellency against *Sitophilus zeamais* Motschulsky, *Cryptolestes ferrugineus* (Stephens) and *Tenebrio molitor* (L.). *Journal of Stored Products Research*, *45*(2), 125–132. https://doi.org/10.1016/j.jspr.2008.10.002

Durden, K., Sellars, S., Cowell, B., Brown, J. J., & Pszczolkowski, M. A. (2011). *Artemisia annua* extracts, artemisinin and 1,8-cineole, prevent fruit infestation by a major, cosmopolitan pest of apples. *Pharmaceutical Biology*, *49*(6), 563–568. https://doi.org/10.3109/13880209.2010.528433

Ellman, G. L.; Courtney, K. D.; Andres Jr, V.; Featherstone, R. M. (1961). A new and rapid colorimetric determination of acetylcholinesterase activity. *Biochemical*

Pharmacology, *7*(2), 88-95.

Falowo, A. B., Mukumbo, F. E., & Muchenje, V. (2019). Phytochemical Constituents and Antioxidant Activity of *Artemisia Afra* and *Bidens Pilosa* Essential Oil in Ground Pork. *Journal of Essential Oil-Bearing Plants*, *22*(1), 176–186. https://doi.org/10.1080/0972060X.2019.1574212

Guo, S., Zhang, W., Liang, J., You, C., Geng, Z., Wang, C., & Du, S. (2016). Contact and Repellent Activities of the Essential Oil from *Juniperus formosana* against Two Stored Product Insects. *Molecules*, *21*(4). https://doi.org/10.3390/molecules21040504

Habig, W. H.; Pabst, M. J.; Jakoby, W. B. (1974). Glutathione S-transferases the first enzymatic step in mercapturic acid formation. *Journal of Biological Chemistry*, *249*(22), 7130–7139.

Haider, S. Z., Mohan, M., Pandey, A. K., & Singh, P. (2017). Use of *Tanacetum tomentosum* and *Ta. dolichophyllum* essential oils as botanical repellents and insecticidal agents against storage pest *Tribolium castaneum* (Coleoptera: Tenebrionidae). *Entomological Research*, *47*(5), 318–327. https://doi.org/10.1111/1748-5967

Haider, S. Z., Mohan, M., Pandey, A. K., & Singh, P. (2015). Repellent and fumigant activities of *Tanacetum nubigenum* Wallich. ex DC essential oils against *Tribolium castaneum* (Herbst) (Coleoptera: Tenebrionidae). *Journal of Oleo Science*, *64*(8), 895–903. https://doi.org/10.5650/jos.ess15094

Hashem, A. S., Awadalla, S. S., Zayed, G. M., Maggi, F., & Benelli, G. (2018). *Pimpinella anisum* essential oil nanoemulsions against *Tribolium castaneum*—insecticidal activity and mode of action. *Environmental Science and Pollution Research*, *25*(19), 18802–18812. https://doi.org/10.1007/s11356-018-2068-1

Hasspieler, B. M., Arnason, J. T., & Downe, A. E. R. (1990). Modes of action of the plant-derived phototoxin α-terthienyl in mosquito larvae. *Pesticide Biochemistry and Physiology*, *38*(1), 41–47. https://doi.org/10.1016/0048-3575(90)90146-S

Hu, J., Wang, W., Dai, J., & Zhu, L. (2019). Chemical composition and biological activity against *Tribolium castaneum* (Coleoptera: Tenebrionidae) of *Artemisia*

brachyloba essential oil. *Industrial Crops and Products*, *128*, 29–37. https://doi.org/10.1016/j.indcrop.2018.10.076

Huang, Y., & Ho, S. H. (1998). Toxicity and antifeedant activities of cinnamaldehyde against the grain storage insects, *Tribolium castaneum* (Herbst) and *Sitophilus zeamais* Motsch. *Journal of Stored Products Research*, *34*(1), 11–17. https://doi.org/10.1016/S0022-474X(97)00038-6

Isman B., M. (2000). Plant essential Oils for Pest and disease management. *Crop Protection*, *19*, 603–608. Retrieved from http://citeseerx.ist.psu.edu/viewdoc/download?doi=10.1.1.456.3876&rep=rep1&type=pdf

Jagadeesan, R., Collins, P. J., Daglish, G. J., Ebert, P. R., & Schlipalius, D. I. (2012). Phosphine resistance in the rust red flour beetle, *Tribolium castaneum* (Coleoptera: Tenebrionidae): Inheritance, gene interactions and fitness costs. *PLoS ONE*, *7*(2). https://doi.org/10.1371/journal.pone.0031582

Jayakumar, M.; Arivoli, S.; Raveen, R.; Tennyson, S. (2017). Repellent activity and fumigant toxicity of a few plant oils against the adult rice weevil *Sitophilus oryzae* Linnaeus 1763 (Coleoptera: Curculionidae). *Journal of Entomology and Zoology Studies*, *5*(2), 324–335.

Jollow, D. J.; Mitchell, J. R.; Potter, W. Z.; Davis, D. C.; Gillette, J. R.; Brodie, B. B. (1973). Acetaminophen-induced hepatic necrosis. II. Role of covalent binding in vivo. *Journal of Pharmacology and Experimental Therapeutics*, *187*, 195–202.

Koodalingam, A., Mullainadhan, P., & Arumugam, M. (2011). Effects of extract of soapnut Sapindus emarginatus on esterases and phosphatases of the vector mosquito, *Aedes aegypti* (Diptera: Culicidae). *Acta Tropica*, *118*(1), 27–36. https://doi.org/10.1016/j.actatropica.2011.01.003

Ladisch, R. K.; Ladisch, S. K.; Howe, P. M. (1967). Quinoid secretions in grain and flour beetles. *In Proceedings of the Pennsylvania Academy of Science*, 213–219. Pennsylvania: Pennsylvania Academy of Science.

López, M. D., Jordán, M. J., & Pascual-Villalobos, M. J. (2008). Toxic compounds in essential oils of coriander, caraway and basil active against stored rice pests.

Journal of Stored Products Research, *44*(3), 273–278. https://doi.org/10.1016/j.jspr.2008.02.005

Manuwoto, S.; Mark Scriber, J. (1982). Consumption and Utilization of Three Maize Genotypes by the Southern Armyworm. *Journal of Economic Entomology*, *75*(2), 163–167. https://doi.org/10.1093/jee/75.2.163

Micu, L. M., & Petanec, D. I. (2011). Research regarding the changes in the content of micro-elements (Cu, Zn, Mn) in stored wheat after infestation by *Rhizopertha dominica* F. *Rom. Agri. Res*, *28*, 2067–5720.

Mishra, B. B., Tripathi, S. P., & Tripathi, C. P. M. (2016). *Impact of Syzygium aromaticum (L .) essential oil as fumigant against Tribolium castaneum (Coleoptera : Tenebrionidae)*. *Journal of Entomology and Zoological Studies*, *4*(6), 811–816.

Negahban, M., Moharramipour, S., & Sefidkon, F. (2007). Fumigant toxicity of essential oil from *Artemisia sieberi* Besser against three stored-product insects. *Journal of Stored Products Research*, *43*(2), 123–128. https://doi.org/10.1016/j.jspr.2006.02.002

Okonkwo, E. U., & Okoye, W. I. (1996). The efficacy of four seed powders and the essential oils as protectants of cowpea and maize grains against infestation by Callosobruchus maculatus (Fabricus)(Coleoptera: Bruchidae) and Sitophilus zeamais (Motschulsky)(Coleoptera: Curculionidae) in Nigeria. *International Journal of Pest Management*, *42*(3), 143–146. https://doi.org/10.1080/09670879609371985

Padín, S., Dal Bello, G., & Fabrizio, M. (2002). Grain loss caused by *Tribolium castaneum, Sitophilus oryzae* and *Acanthoscelides obtectus* in stored durum wheat and beans treated with *Beauveria bassiana*. *Journal of Stored Products Research*, *38*(1), 69–74. https://doi.org/10.1016/S0022-474X(00)00046-1

Pandey, M. (2009). Indian Agriculture – an Introduction. *Apcaem*, (February), 1–39. Retrieved from http://un-csam.org/Activities Files/A0902/in-p.pdf

Rajashekar, Y., Raghavendra, A., & Bakthavatsalam, N. (2014). Acetylcholinesterase inhibition by biofumigant (Coumaran) from leaves of *Lantana camara* in stored

grain and household insect pests. *BioMed Research International*, *2014*, 1–6. https://doi.org/10.1155/2014/187019

Sakuma, M.; Fukumi, H. (1985). The linear track olfactometer: an assay device for taxes of the German cockroach, *Blattella germanica* (L.)(Dictyoptera: Blattellidae) toward their aggregation pheromone. *Applied Entomology and Zoology*, *20*(4), 387–402.

Salem, N., Bachrouch, O., Sriti, J., Msaada, K., Khammassi, S., Hammami, M., … Mediouni Ben Jemaa, J. (2017). Fumigant and repellent potentials of *Ricinus communis* and *Mentha pulegium* essential oils against *Tribolium castaneum* and *Lasioderma serricorne*. *International Journal of Food Properties*, *20*, S2899–S2913. https://doi.org/10.1080/10942912.2017.1382508

Sarwar, M., & Salman, M. (2015). Toxicity of oils formulation as a new useful tool in crop protection for insect pests control. *International Journal of Chemical and Biomolecular Science*, *1*(4), 297–302. Retrieved from http://files.aiscience.org/journal/article/pdf/70420059.pdf

Sasidharan, S.; Chen, Y.; Saravanan, D.; Sundram, K. M.; Latha, L. Y. (2011). Extraction, isolation and characterization of bioactive compounds from plants' extracts. *African Journal of Traditional, Complementary and Alternative Medicines.*, *8*(1), 1–10. https://doi.org/10.1007/978-3-642-56936-4_2

Sharon, M., Abirami, C. V. K., & Alagusundaram, K. (2014). Grain storage management in India. *Journal of Postharvest Technology*, *02*(01), 012–014. Retrieved from www.jpht.info

Singh, P., & Santosh Satya, and S. N. N. (2013). Effect of Insect Infestation on Quality Parameters of Wheat. *In International Conference on Food Security and Nutrition IPCBEE*, *67*, 35–39. https://doi.org/10.7763/IPCBEE

Stefanazzi, N., Stadler, T., & Ferrero, A. (2011). Composition and toxic, repellent and feeding deterrent activity of essential oils against the stored-grain pests *Tribolium castaneum* (Coleoptera: Tenebrionidae) and *Sitophilus oryzae* (Coleoptera: Curculionidae). *Pest Management Science*, *67*(6), 639–646. https://doi.org/10.1002/ps.2102

Tak, J. H., Jovel, E., & Isman, M. B. (2015). Comparative and synergistic activity of R*osmarinus officinalis* L. essential oil constituents against the larvae and an ovarian cell line of the cabbage looper, *Trichoplusia ni* (Lepidoptera: Noctuidae). *Pest Management Science*, *72*(3), 474–480. https://doi.org/10.1002/ps.4010

Tripathi, A. K., Prajapati, V., Aggarwal, K. K., Khanuja, S. P. S., & Kumar, S. (2000). Repellency and Toxicity of Oil from *Artemisia annua* to Certain Stored-Product Beetles. *Journal of Economic Entomology*, *93*(1), 43–47. https://doi.org/10.1603/0022-0493-93.1.43

Wang, Y., You, C. X., Yang, K., Wu, Y., Chen, R., Zhang, W. J., … Han, J. (2015). Bioactivity of Essential Oil of *Zingiber purpureum* Rhizomes and Its Main Compounds against Two Stored Product Insects. *Journal of Economic Entomology*, *108*(3), 925–932. https://doi.org/10.1093/jee/tov030

Xie, Y. S.; Bodnaryk, R. P.; Fields, P. G. (1996). A rapid and simple flour-disk bioassay for testing substances active against stored-product insects. *The Canadian Entomologist*, *128*(5), 865–875.

Publications from the Thesis

Deb, M., & Kumar, D. (2019). Chemical Composition and Bioactivity of the Essential Oils Derived from *Artemisia Annua* Against the Red Flour Beetle. *Biosciences Biotechnology Research Asia*, *16*(2), 463-476.

Deb, M., & Kumar, D. (2019). Bioactivity and efficacy of essential oils extracted from *Artemisia annua* against *Tribolium casteneum* (Herbst. 1797) (Coleoptera: Tenebrionidae): An eco-friendly approach. *Ecotoxicology and Environmental Safety, In Press, Corrected Proof, Available online 22 November 2019.*