Study on the molecular mechanism of *Artemisia annua* L. induced toxicity against *Tribolium casteneum* (Herbst, 1797)

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1. Introduction

Agriculture has been the mainstay of the Indian economy since history and it continues to be the same for long. It supports about 17% of the world's population (Pandey, 2009). Indian agriculture is characterized by agro-ecological diversities in soil, rainfall, temperature, and cropping system which harbour different varieties of crops across the nation. Hence, the climatic conditions restrict the growth of a crop variety in a stipulated geological area. This constraint increases the awareness for proper management of the variety throughout its journey from harvesting in the fields until it reaches the end-users. However, varieties like stored grains demand an additional level of attention in the warehouses. Often due to lack of caution, storehouses experience a heavy loss of stored grains which poses a negative impact on the country's economy.

Wheat (*Triticum aestivum*) is the world's most favoured staple food along with rice and a most important source of carbohydrate in a majority of countries. Moreover, they are a rich source of essential nutrients including protein, fats, dietary fibres along with several B vitamins and minerals. Though the cultivation of wheat is restricted to Rabi season their demand continues across the year. This causes about more than 70% of the grain to be stored in the warehouses. In the sored conditions, they are very prone to different types of infestation mainly the insect's infestation.

Tribolium casteneum (Herbst, 1797) is a major pest of stored commodities across the globe (Good, 1933) (Semple, 1986) (Madrid et al., 1990). The beetle is a primary pest of wheat flour & different milled products (Leonard LeCato, 1975) and a secondary pest of wheat grains as can attack previously damaged grains only (Fao, 2013). Moreover, it is described to exploit 233 different commodities including flour, cracked wheat, and maize bran (Hagstrum, 2017). Due to its high reproductive potential, infestation intensifies rapidly and deteriorates the quality and quantity of the grains (Howe, 1965). Additionally, the beetle is known to secrete toxic quinone's of carcinogenic nature which turns the flour greyish thus poses a serious risk to human consumption (El-Mofty et al., 1989) (Ladisch et al., 1967). Apart from health concern, its contamination causes serious economic damage due to the loss of market price and nutritional efficiency (Shafique et al., 2006). It is estimated that about 40% weight loss in wheat flour is caused by red flour beetles (Ajayi & Rahman, 2006). Hence, controlling the pest is of utmost importance to reduce the economic burden in developing countries like India.

The use of chemical fumigants to control the pest is a widely practiced measure. However, the indiscriminate use of Phosphine and methyl bromide has triggered much negative impact on the environment which includes resistance towards insecticides, secondary pest outbreak, environmental pollution and negative health concern to the non-target animal including humans (Benhalima et al., 2004). Henceforth, the use of methyl bromide is banned worldwide due to its direct association in ozone layer depletion (Anbar et al., 1996) (Carter, et al., 2005). Moreover, phosphine is found to be least effective in controlling the different stored product pests due to the fast-growing resistance in insects (Pimentel et al., 2007). As the most reliable control measures are forced to be delisted from the category, the pressure to replace them with the best alternatives has taken the research community by storm.

Numerous studies are designed and conducted across the globe to find a better alternative to these existing fumigants. Among all, plant based researches are drawing global attention for possessing a wide range of essential oils and secondary metabolites (Sasidharan et al., 2011). They are proven to be effective against different groups of insects including stored grain pests (Isman, 2000) (Caballero-Gallardo et al., 2011). Moreover, they do not leave any residue into the environment and least toxic to non-target organisms including humans. Work of Yang & Tang (1988) has shown that the botanicals used for controlling the insect pest have a strong connection with the medicinal plants. This reduces the uncertainty associated with human health for consuming plant based treated commodities.

Genus *Artemisia* belongs to the family Asteraceae and widely used for its medicinal properties in Asian countries (Das, 2012). *Artemisia* which is native to southeast countries is commonly called as Qinghaosu. The plant is known to produce a wide range of essential oils and secondary metabolites which has potent insecticidal efficiency (Adeyemi, 2010).

Artemisia sieberi is reported to be effective against the different stored grain pests including *Tribolium casteneum* (Negahban et al., 2007). Reports on the fumigant toxicity of essential oils of *Artemisia scoparia* against *Tribolium casteneum* have established the insecticidal properties of the genus (Negahban et al., 2004). In a recent study, conducted in 2019, EOs of *Artemisia brachyloba* was evaluated for chemical possessions and its biological efficacy against *T. casteneum* was also testified (Hu, et al., 2019) *Artemisia annua*, the sole producer of Artemisinin, is mainly studied for its efficacy against the malarial parasites, Plasmodium (Dhingra et al., 1999). Artemisinin-based combination therapy has emerged as the most efficient antimalarial drug available in the market against MDR strains (Klayman, 1985)

(Eastman & Fidock, 2009). However, Very few have attempted to explain the efficacy of essential oils of *Artemisia annua* extracted with polar solvents like methanol against *Tribolium casteneum*. With regard to the existing literature, no previous reports on the infestation of the pest against the sound wheat grains have been reported.

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

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}) 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.

2. Material and Methods

2.1. Insect

Tribolium casteneum was reared in the defined culture media of wheat flour, wheat grain and yeast (6:3:1) respectively at 27±2˚C, 70±5 RH. Newly emerged adults of 1-10 days old, were used for the toxicity assays. Final larval stages i.e. 14 days old larvae were used in the experiment. All the experiments were conducted in the dark under the same temperature and humidity ranges.

2.2. Plant material

The dried, finely grounded leaves of the *Artemisia annua* was procured from the Botany department, The Maharaja Sayajirao University of Baroda, Vadodara, Gujarat, India. Plant powder was then stored in plastic bags at 4˚C until it is used for the extraction of essential oils.

2.3. Extraction and analysis of the chemical composition of essential oils

2.3.1. Extraction and analysis of essential oils

Dried plant powder was subjected to hydro distillation using a modified Clevenger-type apparatus. The essential oil was eluted using acetone free methanol with 1:12 plant material / solvent volume ratio. The conditions for hydro distillation are as follows: 16 hours at 65˚C for methanol. Anhydrous sodium sulphate was used to remove extra water from the extract. Solvents were evaporated by rotary evaporator at their boiling ranges. Oil yield was calculated on a dry weight basis (27.16% w/w). Then the extract was stored in airtight containers in a refrigerator at 4 ˚C until it is used.

2.3.2. Gas Chromatography-mass spectroscopy (GC- HRMS)

Gas chromatographic analysis was performed on an Agilent 7890N instrument equipped with a flame ionization detector and HP-5MS (30m × 0.25mm × 0.25μm) capillary column, while the essential oil components were identified on an Agilent Technologies Jeol mass spectrometer. The GC settings were as follows: the initial oven temperature was held at 60 °C for 1 min and ramped at 10 °C min^{-1} to 180 °C for 1 min, and then ramped at 20 °C min^{-1} to 280°C for 15 min. The injector temperature was maintained at 270°C. The samples (1μL) were injected neat, with a split ratio of 1:10. The carrier gas was helium at a flow rate of 1.0 mL min^{-1}. Spectra were scanned from 20 to 550 m/z at 2 scans s^{-1}. 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. The retention indices were determined with the homologous series of n-alkanes (C8–C24) under the same operating conditions. Further identification was made by comparison of their mass spectra on both columns with those stored in NIST 05 and Wiley 275 libraries or with mass spectra from literature. Component relative percentages were calculated based on GC peak areas without using correction factors.

2.4. Bioassays and Identification of Lethal doses

2.4.1. Repellency test

To evaluate the Repellency of the insect towards the essential oil different concentrations of the essential oils were dissolved in acetone to make the desired concentrations. Filter papers

measuring 7 cm in diameter were cut into two halves of equal measurement. Then one half was treated with the different concentrations of essential oils and the other half with the solvent only. Filter papers were allowed to air dry for 2 minutes. Then both the halves were attached underside with the cellotape and attached to the petriplate. 10 unsexed adults were released into the centre of the plate. Five replicates were maintained for each concentration. In controlled sets, one half is treated with the acetone and the other half with the water. Readings were taken at an interval of 1, 2, 3, 4, 5, 6, 12 and 24 hours. The insects were checked regularly for 3 days if any mortality is recorded. Percentage Repellency was calculated using the following formula:

$$PR = 2(C - 50)$$

Where, C is the percentage of insects recorded on the untreated half of the disc. Positive values expressed repellency and negative values attractancy. Results of PR were analysed using ANOVA and Tukey's honest significance test.

Repellency was also measured through the multi arm olfactometer. Details of the olfactometer are as follows: (middle glass chamber: 7 cm in diameter; arms: 1.5 cm in diameter & 14.5 cm in length). Filter papers were cut into two equal halves. Filter paper halves (3.5 cm) were then treated with the different concentrations of plant extract and fixed in the inner side of the cork of the arms. The half treated with the acetone was used as control. 20 unsexed adults were released in the centre and then the upper lid is covered with the muslin cloth. Movement of insects in different arms was recorded at 1, 2, 3, 4, 5, 6, 12 and 24 hours and Excess Proportion Index (EPI) was assessed using the following formula: (Sakuma & FUKUMI, 1985)

$$(EPI) = \frac{Nt - Nc}{Nt + Nc}$$

Where Nt is the number of insects present in the treated arms, Nc is the number of insects present in the control arm. Negative values expressed repellency and positive values attractancy.

2.4.2 Flour disc Bioassay

Flour discs were prepared according to the method of Xie et al. (1996) with some modifications by Huang et al. (1997). Aliquots of 200 µl of a stirred suspension of wheat flour in water (10 g in 50 ml) were decanted into a petri dish to form the disks. The discs were left in the fume hood overnight to dry and then they were transferred to incubators maintaining at 30±1˚C and 70-

80% RH to equilibrate for 24 h. The weight of the discs was ranged from 85 to 88 mg and the moisture content was recorded to be 11.3±0.1%.

Flour discs were treated with an aliquot of 5 µl of essential oils of *Artemisia annua* extracted with methanol. Five different concentrations of the extracts were prepared for treatment and acetone was used as control. The solvent was allowed to evaporate for one hour and then individual discs were transferred in plastic vials (diameter 4.2 cm height 6.8 cm). The weight of each plastic vials used was recorded. Ten unsexed previously starved adults were weighed and added to individual vials. Each treatment and control sets were maintained in triplicates. Plastic vials containing flour disks and insects were weighed again after 72 h. Nutritional indices were calculated according to Manuwoto & Mark Scriber (1982), Farrar et al., (1989) with slight modifications by Huang et al., (1997):

- Relative growth rate (RGR) = (A-B)/(B × day), where A is the weight of live insects on the third day (mg)/ number of live insects on the third day and B is the original weight of insects (mg)/original number of insects
- Relative consumption rate (RCR) = D/ (B × day), where D is the biomass ingested (mg)/number of live insects on the third day, and biomass ingested is the (original weight of flour disks 2 weight of flour disks on the third day
- Percentage efficiency of conversion of ingested food (ECI) = (RGR/RCR) × 100.
- For antifeedant effect (AE): the following formula was employed:
  AE (%) = [(C-T)/C] × 100, where C is the consumption rate of control discs and T is the consumption rate of treated discs.

2.4.3. Contact toxicity

To evaluate the contact toxicity 10 unsexed adults were taken in a plastic vial. Then they were kept in the freezer for 1 minute to unconscious them. Adults were then treated with different concentrations of essential oils. An aliquot of 5ul of essential oil was then topically applied in the thoracic region. After 2 minutes they were transferred in the plastic vials containing media. The mortality is recoded until 3 days.

2.4.4. Fumigant toxicity

Filter papers, whatman No. 1 (7 cm in diameter) were impregnated with the different concentrations of the Methanolic extract. Concentrations were ranged from 0.24 to 2.37 mg L

air^{-1}. Filter papers were allowed to air dry for 1-2 minutes to evaporate the solvent. The earlier report says that this much time is sufficient to evaporate the solvent. The impregnated paper was then sealed on the screw cap of the plastic vials (25mL). 10 unsexed adults were released for each concentration (1-14 days old). Each concentration was maintained in triplicates. Both control and vehicular control were also kept. Mortality was determined regularly for 3 days at an interval of 6 hours. Loss of antennal and leg movement was considered as an indication for mortality. Probit analysis (Finney, 1971) using Medcalc software was employed in analysing the dosage- mortality response in both the contact and fumigant toxicity tests.

2.5. Understanding biochemical pathways

Quantitative analyses of biochemical constituents in viable (LC_{50}, LC_{90}) and control sets were assessed. Protein profiling by Biuret method (Reckon Diagnostics P. LTD.) and enzymatic activities of Acetylcholine Esterase (AChE), Glutathione S Transferase (GST), Reduced Glutathione (GSH) and Lipid Peroxidases (LPO) were performed following the methods of Ellman et al. (1961), Habig et al. (1974), Jollow et al. (1973) and Buege & Aust (1978) respectively. Analysis of Variance (ANOVA) and Tukey's Pairwise Comparison Test were employed using PAST statistical software package to compare means.

2.6. Quality Parameters

2.6.1. Feeding deterrence and % weight loss

The feeding deterrence test performed was adopted from Owusu (2000) with some modifications by Iram et al. (2013). Wheat grains weighing 5 grams were transferred in plastic containers (25 ml). Three containers were supplemented with 0, 0.5 and 1 grams of powdered leaves of *Artemisia annua* respectively. 10 unsexed insects were then introduced in each vial and allowed to infest for three months. Triplicates were maintained for each set. After the completion of the assessment period, beetles were separated and the grains were reweighted and Feeding ratio (Fr) was calculated using the following formula:

$$Fr = 1 – FW/5,$$

Where, FW represents the final weight of grains after 90 days of continuous insect infestation.

For the assessment of % weight loss the following formula was employed:

$$\% \text{ Weight loss} = (U\ Nd) – (D\ Nu) / U\ (Nd + Nu) \times 100$$

Where, U = weight of undamaged grains, D = weight of damaged grains, Nu = number of undamaged grains, Nd = number of damaged grains.

2.6.2. Scanning Electron microscopy analysis

Scanning electron microscopic analysis was performed to evaluate the topographical characters of damaged and undamaged grain samples. Dried damaged and undamaged wheat grains were used as a specimen for the analysis. SEM measurements were performed with JEOL JSM-7600F series. Instrument details: 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. Results

3.1. Gas Chromatography-mass spectroscopy

Chemical composition of the methanolic extract of *Artemisia annua* has reported containing a wide array of groups (Figure 1). A total of 14 compounds were enlisted from the essential oils of methanolic extracts 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-, nonylester (22.99%), 3-Methylcyclopentadecylcarbamic acid, t- butyl ester (12.12%) and 5α-Pregn-16-en-20-one,3β,12α-dihydroxy-,diacetate (5.33%).

Figure 1: Gas chromatogram of Methanolic extract of *Artemisia annua* showing 1-Docosene, I-Valine, n-heptafluorobutyryl-, nonylester, 3-Methylcyclopentadecylcarbamic acid, t- butyl ester as the major constituents.

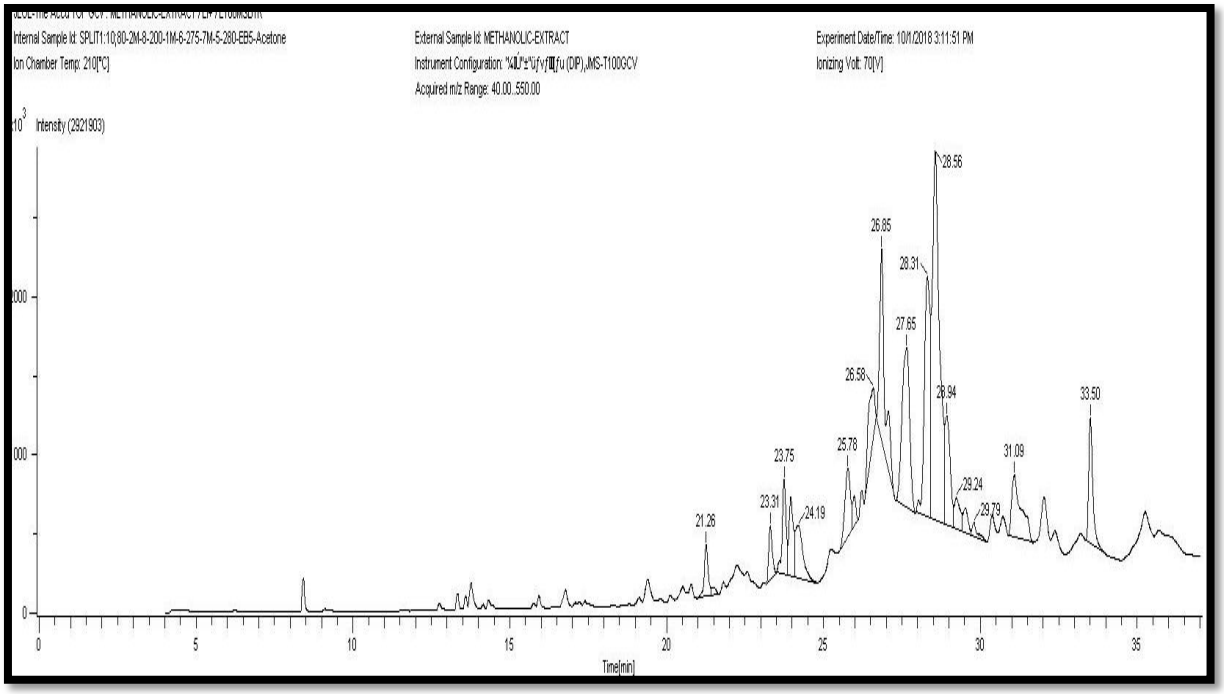

Table 1: Chemical composition of the essential oils of *Artemisia annua* extracted with methanol.

Sr. No.	Compounds	Formula	CAS no.	RI[a]	ID[b]	Relative content (%)[c]
1	Spiro(2,7)dec-4-ene,1,1,5,6,6,9,9-heptamethyl-10-methylene	$C_{18}H_{30}$	150397	1656	MS	1.76
2	Tricyclo(3.3.1.1.<3,7>)decane,tricyclo(3.3.1.1.<3,7>)decylidene	$C_{20}H_{28}$	30541-56-1	1825	MS	3.63
3	Deoxyqinghaosu	$C_{15}H_{22}O_4$	126036	1794	MS	1.69
4	3,4-Hexadienal, 2-butyl-2-ethyl-5-methyl	$C_{13}H_{22}O$	23739-80-2	-	MS	3.38
5	Phytol	$C_{20}H_{40}O$	150-86-7	2045	MS, RI	3.05
6	I-Valine, n-heptafluorobutyryl-, nonylester	$C_{18}H_{28}F_7NO_4$	320901	1807	MS, RI	22.99
7	5β,7βH,10α-Eudesm-11-en-1α-ol	$C_{15}H_{26}O$	25826-85-1	1651	MS	4.58
8	Nonadecane	$C_{19}H_{40}$	629-92-5	1910	MS, RI	3.72
9	3-Methylcyclopentadecylcarbamic acid, t- butyl ester	$C_{21}H_{41}NO_2$	191303	2600	MS	12.12
10	1-Docosene	$C_{22}H_{44}$	1599-67-3	2198	MS, RI	29.57
11	Vitamin E	$C_{29}H_{50}O_2$	59-02-9	-	MS	3.49
12	5α-Pregn-16-en-20-one,3β,12α-dihydroxy-,diacetate	$C_{25}H_{36}O_5$	5767-82-8	2732	MS	5.33
13	Squalene	$C_{30}H_{50}$	7683-64-9	2914	MS, RI	4.67
					Total	**99.98%**

[a]Retention indices were calculated using a homologous series of n-alkanes (C8–C24).
[b]Identification of volatile components was carried out by comparing MS spectrum and RIs of components with those of the authentic standards in NIST library and previous study.
[c]Results obtained by peak-area normalization.

3.2. Repellency test

Essential oils of *Artemisia annua* have strongly repelled the adult beetles. 91.63% of repellency was demonstrated by methanolic extract at the highest concentration of 0.90 mg cm^{-2} (Table 2). However, an insignificant increase in repellency with the increase in concentration was seen.

Table 2: Repellency of essential oils of *Artemisia annua* extracted with methanol against *Tribolium castaneum* adults using the Filter paper arena test.

Conc.(mg cm^{-2})	**Duration of exposure (in) hour**								**Percent Repellency over 24 hours**
	1	**2**	**3**	**4**	**5**	**6**	**12**	**24**	
	Mean Repellency (% ± SD)								
0.54	53±12*	60±20*	57±29*	60*	60±35*	53±42*	80±20*	73±31*	**62.00**
0.63	53±12*	53±12*	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**

Means (±SEM) followed by * indicate non significance ($p < 0.05$) according to the ANOVA.

Repellency was found to be time and dose dependent in all the treated 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 to -0.9 in methanol derived essential oil treated sets (Table 3).

Table 3: Repellency of essential oils 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 the mean of five replicates ±standard deviation

3.3. Contact toxicity

Methanol derived essential oils of *Artemisia annua* were found to be toxic to *Tribolium castaneum* adults and 14 days old larvae when treated topically (Table 4). Neither of the chosen stages of flour beetle showed mortality in the control sets. Based on LD_{50} and LD_{90} values, *T. castaneum* adults were recorded to be more resistant than its larval counterparts to the essential oils as no overlap in 95% confidence interval is marked. Significant differences ($P < 0.01$) were recorded between the two stages treated with the given extract (Figure 2).

Table 4: Contact toxicity of essential oils of *Artemisia annua* applied topically to *Tribolium 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
Methanol	Adults	1.87	1.60- 2.13	2.94	2.59-3.54	1.18±0.2
	14 days old Larvae	1.24	0.94-1.48	2.22	1.91-2.75	1.31±0.24

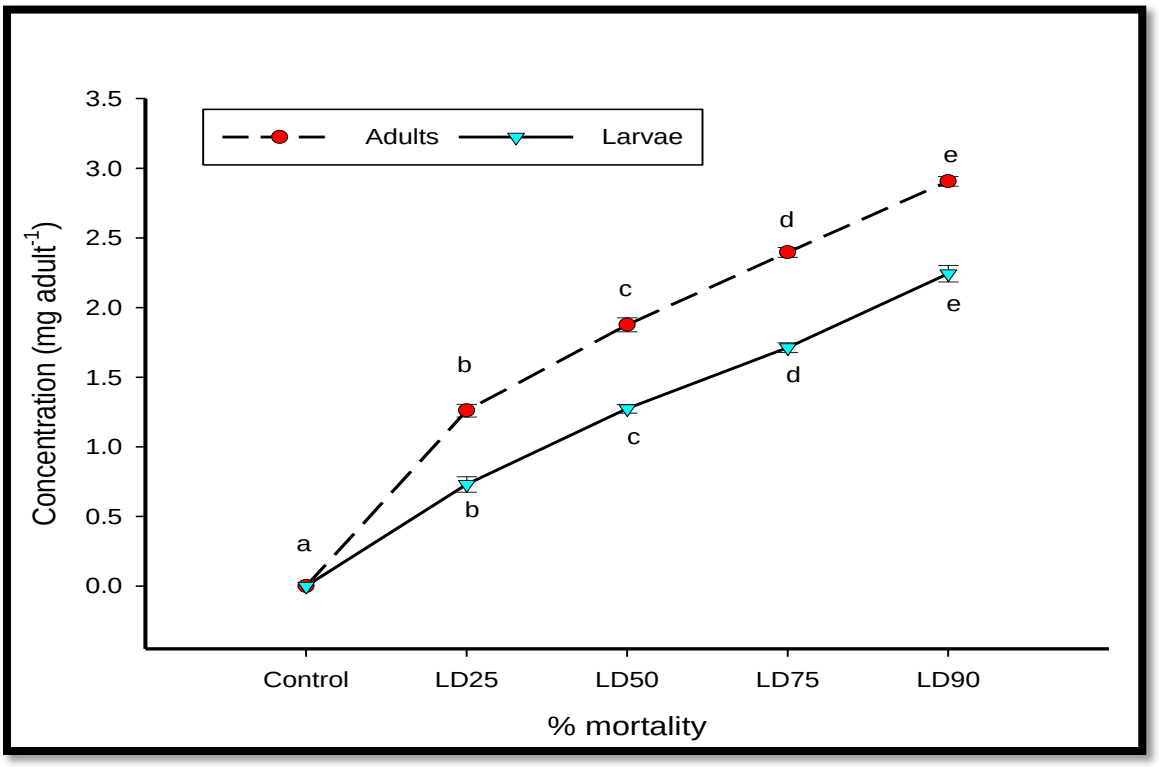

Figure 2: Mean (±SEM) percentage mortality of *T. casteneum* adults and 14- days old larvae undergone Contact toxicity bioassay with different concentrations of essential oils of *A. annua* after 72 h. Means (±SEM) followed by the same letters above bars indicate no significant difference ($p < 0.01$) according to the Chi-square test.

3.4. **Fumigant toxicity**

Solvent extracted essential oils of *Artemisia annua* also demonstrated fumigant toxicity against both the stages of the beetle. However, *T. castaneum* adults and larvae were found to be more susceptible to fumigant toxicity of the essential oil than to contact toxicity (Figure 3). Control mortality was zero. When LD_{90} values were compared then 14 days old larvae were found more vulnerable than the adults to fumigant toxicity of methanol derived extracts (Table 5).

Table 5: Fumigant toxicity of essential oils of *Artemisia annua* to *Tribolium castaneum* exposed for 24 h at 30˚C and 70±80% RH.

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

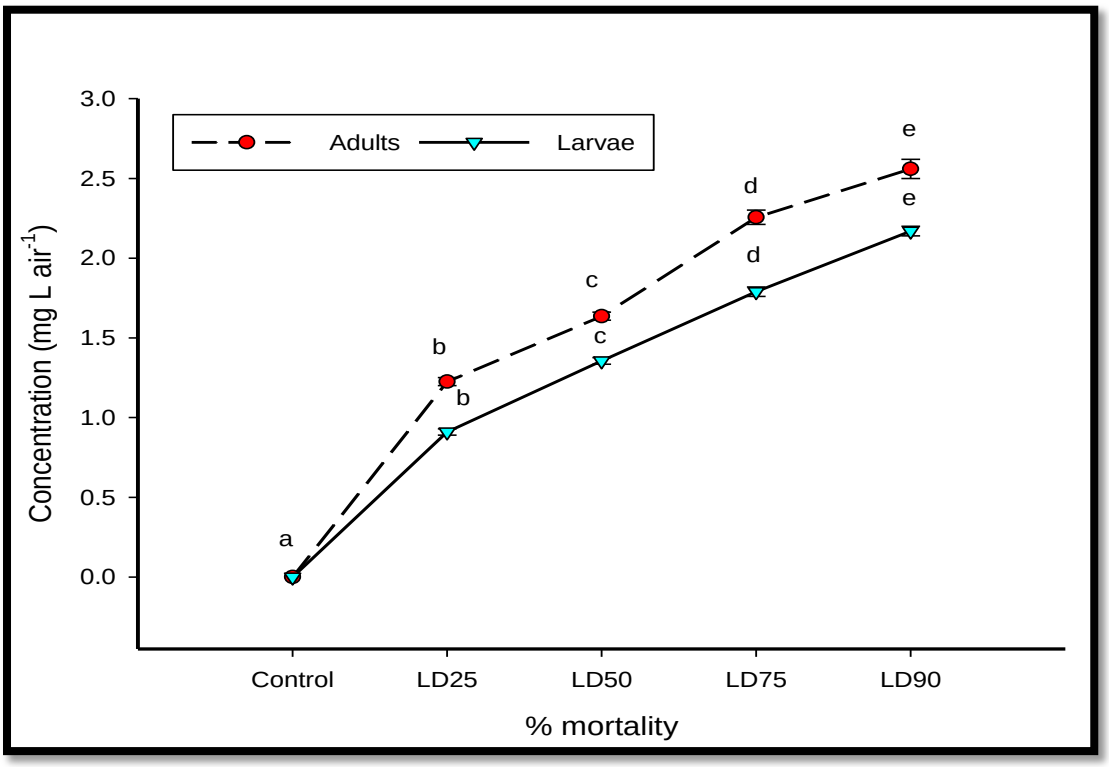

Figure 3: Mean (±SEM) percentage mortality of *T. casteneum* adults and 14- days old larvae undergone Fumigant toxicity bioassay with different concentrations of essential oils of *A. annua* after 72 h. Means (±SEM) followed by the same letters above bars indicate no significant difference ($p < 0.01$) according to Chi-square test.

3.5. Flour disc Bioassay

The relative growth rate (RGR) and Relative consumption rate (RCR) of the insect have 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 Methanolic 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 % have decreased significantly (*P<0.05*) when compared with the control value of 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 6).

Table 6: Nutritional & Feeding deterrence indices of adult *T. casteneum* exposed to essential oil derived from methanol treated flour discs.

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	0
	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

Means (±SEM) followed by * indicate no significant difference ($p < 0.05$) among the different groups according to the ANOVA.

3.6. Biochemical analysis

Quantitative assessment of protein and detoxifying enzymes were carried out in control and lethal sets of *T. casteneum* adults and larvae post treatment to record the changes in the bimolecular profile as an indicator of plant extract mode of action (Table 7 and 8). The activity of Acetylcholinesterase (AChE), Glutathione S-transferase (GST), Reduced glutathione (GSH)

and Protein were found to be downregulated in lethal doses than in control sets. Whereas, Lipid Peroxidase (LPO) level has upregulated in LD_{50} and LD_{90} than in control.

3.6.1. Biomolecular profiling of contact toxicity

The protein concentration of the whole body homogenate of the adults and 14 days old larvae treated topically with the methanol derived essential oils decreased significantly ($p<0.01$) from the control to LD_{50} and LD_{90} (Table 7). Similarly, AChE activity significantly ($p<0.01$) decreased from control sets to LD_{50} and LD_{90} as illustrated in Table 7. However, LD_{50} and LD_{90} in adults showed a slight and statistically insignificant ($p>0.05$) reduction in AChE level. On the other hand, larvae with the same treatment showed a significant relationship between the control and $LD_{90.}$

GST and GSH experienced significant ($p<0.01$) reduction within the control, LD_{50} and LD_{90} of both the life stages post treatment. However, an insignificant ($p>0.05$) reduction in the level of the enzymes was recorded between LD_{50} and LD_{90} sets (Table 7). However, the level of LPO was found to increase significantly ($p<0.01$) from control to LD_{50} and LD_{90} sets of *T. casteneum* adult and larvae in both the treatment.

Table 7: Effect of methanol derived essential oils of *A. annua* on protein and enzymatic profile of *T. casteneum* at the control and lethal doses of contact toxicity bioassays.

Extracts	Life stage	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

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

3.6.2. Biomolecular profiling of Fumigant toxicity

Protein level was reported to be significantly ($p<0.01$) decreased from control to LD_{50} and LD_{90} as illustrated in Table 8 in all the treatment sets. AChE activity was inhibited significantly in

the lethal groups when compared with the control, however, no insignificant reduction is seen between LD_{50} and LD_{90} sets. GST level in adults treated with both the essential oils has reported significant ($p<0.01$) reduction with the increased concentration. However, larvae have shown insignificant ($p>0.01$) reduction between LD_{50} and LD_{90}. On the contrary, LPO levels in the whole tissue homogenates were marked to be very high in treatment sets when compared with the control. Moreover, the LPO has significantly ($p<0.01$) upregulated with the increase in lethal doses.

Table 8: Effect of methanol derived essential oils of *A. annua* on protein and enzymatic profile of *Tribolium casteneum* at the control and lethal doses of fumigant toxicity bioassay.

Extracts	Life stage	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±3.51c	0.073±0.006b	0.07±0.007b	187.11±0.24c	35.83±0.151c

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

3.7. Feeding ratio and weight loss effects

Treatment with the *Artemisia annua* has shown the strong antifeedant action with the significantly lower feeding ratios to 0.36 at 1g and 0.39 at 0.5g when compared with the untreated set where the Fr was found to be 0.64. The feeding ratio decreased with the increase in the dose of the treatment. Statistical analysis of the data has shown the significant differences (*$P<0.05$*) between the control and treatment sets.

The efficiency of the botanical against the *Tribolium casteneum* was found to be effective in reducing the weight loss of the grains. Untreated grains experienced 30.08% of weight loss whereas significant reduction (*$P<0.05$*) in the weight loss of 6.46% and 3.4% were reported at the treatment doses of 0.5g and 1g respectively (Table 9).

Table 9: 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

3.8. SEM results

Images of the SEM analysis depict the clear structural differences between the undamaged and damaged sets. The undamaged grain shows structural integrity whereas the damaged grains are thoroughly punctured with the heavy loss of the bran as well as the endosperm at 25x magnification (Fig. 4& 5).

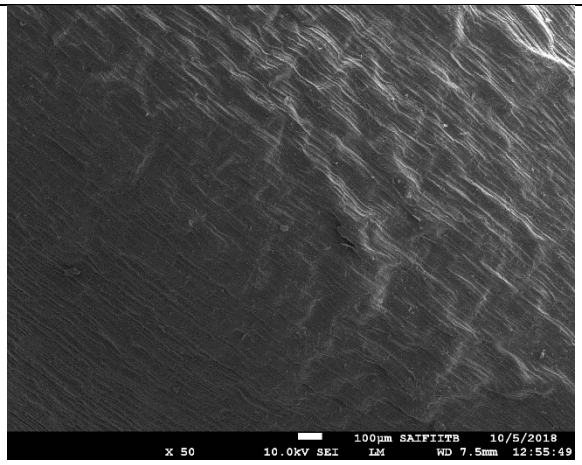

Fig 4: Undamaged grain 50x magnification

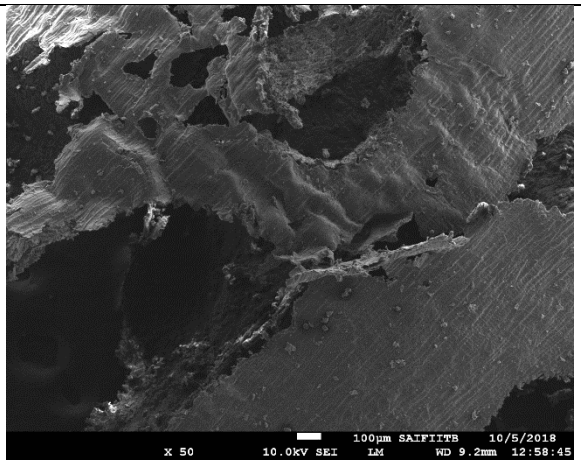

Fig 5: Damaged grain 50x magnification

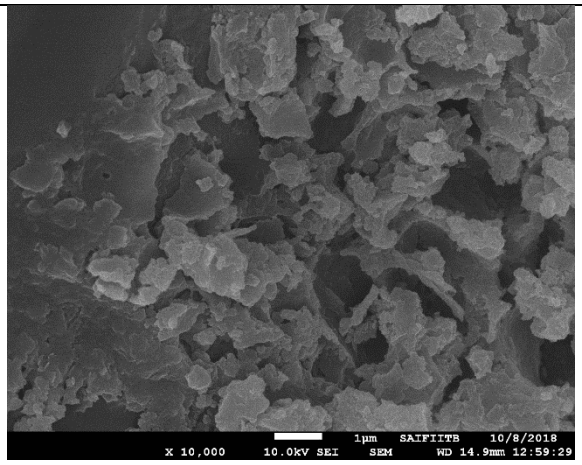

Fig 6: Undamaged grain at 10,000x magnification

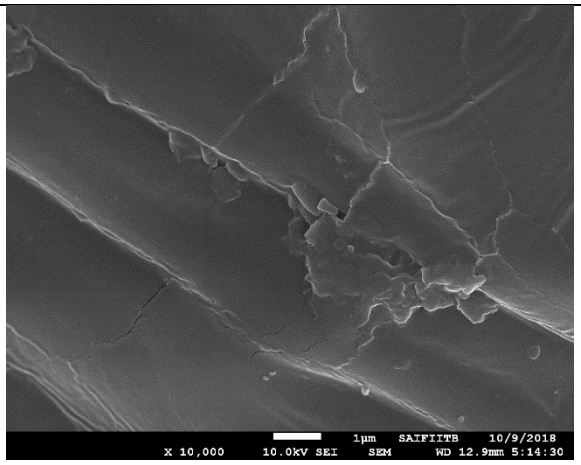

Fig 7: Damaged grain at 10,000x magnification

More detailed topography has been studied at 10,000x magnification which evidenced the presence of starch globule throughout the surface of the undamaged grains (Fig. 6). In damaged grains, starch molecules have been attacked by the pest which is confirmed by the smooth peripheral endosperm (Fig. 7).

4. Discussion

The essential oils of *Artemisia annua* were found to possess contact and fumigant toxicity as well as repellent activity against *Tribolium casteneum*. The insecticidal properties of the extract varied with the solvent used, the concentration of the oil and time of exposure.

The chemical composition of the plant extracts contains a wide range of natural components. However, lesser traces of characteristic compounds viz. Deoxyqinghaosu, Phytol and Squalene were recorded in the solvent extracted essential oils. However, the results were different than the previous studies (Tripathi et al, 2001) which might be due to climatic variations and influence of growth factors (Dangash et al, 2014).

Adults of *T. casteneum* were vulnerable to methanolic extract in the behavioural bioassay conducted with a filter paper arena test and multi arm olfactometer. Results were supported by the work of Engsontia et al. (2008) who have shown the presence of an expanded odorant receptor gene family in *Tribolium castaneum*. Moreover, EPI in both the experiment shows negative values which indicate that *Artemisia annua* is a repellent plant.

In the flour disc bioassay, the methanol derived essential oils of *A. annua* have shown significant antifeedant activity (85.15%). The results are consistent with the several reports of the earlier researcher. Stefanazzi et al., (2011) have proposed that the essential oils of *T. terniflora, C. citratus* showed antifeedant activity against *S. oryzae* and *Tribolium casteneum*. In a similar report by Liu & Ho (1999) has shown that the essential oils from *Evodia rutaecarpa* have proven to be a mild feeding deterrent against the *T. casteneum* when compared with their larval stage. However, no mortality has been recorded in the concentration ranges of 1.00-1.67mg disc^{-1} at the end of the third day. Similar results were documented in a previous study where no mortality was reported at the range of 6.8- 13.6 mg of cinnamaldehyde g $^{-1}$ of food (Huang & Ho, 1998). Moreover, essential oils of *A. annua* have significantly ($P<0.05$) reduced the relative growth rate, relative consumption rate and food utilization by the *Tribolium casteneum*. The curve of RGR and RCR decreases in a dose-dependent manner. The results are supported by the work of Huang et al., (2002) Eugenol, Isoeugenol, methyleugenol has shown a

significant reduction in RGR, RCR. Nevertheless, the efficiency of conversion of ingested food i.e. ECI% has decreased with the increase in doses when compared with the control set due to the negative growth rate and low rate of food consumption. The results are supported by the work of Xie et al., (1996) where Margosan-O has shown a significant reduction in ECI.

T. casteneum adults were more resistant than their larval counterparts to the contact toxicity when treated with the methanol derived essential oil. In a similar study where essential oil of *Elletaria cardamomum* extracted with n-hexane reported a significant difference between the larvae and adult beetle in acute toxicity assays (Huang et al., 2000). Moreover, larvae of *T. castaneum* did not follow the same trend as that reported in earlier work the essential oils of garlic (Ho et al., 1996) and nutmeg (Huang et al., 1997) where they gradually developed resistance. Lipid peroxidase level in the study has increased significantly in the treated sets which indicate the increase in oxidative stress in the pests. Our findings are consistent with the result of Hasspieler et al. (1990).

Similar results were reported in the fumigant toxicity bioassays. When LD_{90} values were compared 14 days old Larvae were more susceptible to the essential oil treatments compared to adults. However, our result was different from the work of Negahban et al., (2007) where *Artemisia sieberi* has LC_{90} at 57.32 µL L^{-1}air. The rationale behind this could be due to the fast growing resistance in pests (Zettler, 1991) (Dyte & Blackman, 1970).

In this study, we have examined the effect of methanol derived essential oils on the biomolecular 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 indicates the action of plant extracts on the nervous system of red flour beetle by inhibiting the action of different detoxifying enzymes including Acetylcholine esterase, Glutathione S Transferase and Reduced Glutathione in the treated sets. Rajashekar et al., (2014) has shown similar results where the Coumaran has inhibited the activity of AChE. Another set of experiments has shown the increased activity of GST in the multi-resistant strains (Cohen, 1986). This dictates that the pest is not resistant towards the *A. annua* extract. The result has shown that protein has experienced a significant downfall in treated when compared with the control sets. However, light and insignificant reduction in protein level was reported by (Koodalingam et al., 2011).

Antifeedant bioassay performed with the wheat grain has shown a significant reduction in feeding ratio with the increase in doses. The feeding ratio shows a steep decrease to almost half

at 0.5g dose when compared with the control. Moreover, % weight loss has been reduced to 80% when exposed to *Artemisia* leaves. Mortality is recorded in the treatment as well as control sets. Mortality in the control sets can be due to a gradual decrease in their food resources (Nathanson, 1975) (Lhaloui et al., 1988). However, this is not valid for the treated sets as the rate of oviposition was found to decrease and only a few adults were recorded to compete for the available food resources. A study by Iram et al., (2013) have shown that the leaves and peel of *C. reticulate* and leaves of *P. guajava* reported zero percent weight loss of the grain by antifeedant action against *T. casteneum*. In a similar study conducted by Padin et al, (2002) has recorded that *Tribolium casteneum* has reduced the weight of the grains by 1.9% after 16 weeks of bioassay where they are supplemented with the fungal formulation, milled rice, and *Beauveria bassiana*. Similar results were observed by Karunakaran et al, (2004) where they have studied the weight loss of the grains by *Tribolium casteneum* larvae. Thus, red flour beetle has emerged as a whole grain pest and efficiency of *Artemisia annua* open new dimensions in the field of grain management measures.

The undamaged and damaged grains from the previous experiment were processed further for topographical characterization using SEM-EDX. SEM images at 25x magnification depict the structural loss to the damaged grains when compared with the undamaged. Moreover, the images at 1000x magnification illustrate the intricacy of structural damage and the digestion of starch globules by the pest. These findings were confirmed by the work of Singh et al, (2013) where the wheat grains are significantly damaged by the stored pest *Rhyzopertha dominica*.

Conclusion

In conclusion, the present study has validated that the Methanol derived essential oils of *Artemisia annua* and the identified allelochemicals are potent in vivo suppressor of different biomolecules except for LPO in the contact and fumigant toxicity assays. Hence, there is potential for these compounds to be used in synergy by interfering with enzyme mediated detoxification in the target insects. Moreover, by probing the modes of action of fumigants through experiments, future research will evaluate if the enzyme inhibitors can act as synergists by increasing the toxicity of *Artemisia annua* against other stored grain pests. This study suggests that *Artemisia annua* which is used as a potent antimalarial drug can also be a potential grain protectant due to its antifeedant activity against the major grain pest, *Tribolium casteneum*. Moreover, it poses no threat to the environment and non-target animal including

human. Hence, further research to design a suitable formulation of the potent synthetic analogues of *Artemisia annua* would be a step in the right direction.

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References

Adeyemi, M. H. (2010). The potential of secondary metabolites in plant material as deterents against insect pests: A review. *African Journal of Pure and Applied Chemistry, 4*(11), 243-246.

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.

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.

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.

Buege, J. A. (1978). [30] Microsomal lipid peroxidation. In J. Abelson, *Methods in Enzymology* (Vol. 52, pp. 302-310). Cambridge, Massachusetts, United States: Academic Press, Elsevier.

Caballero-Gallardo, K., Olivero-Verbel, J., & Stashenko, E. E. (2011). Repellent activity of essential oils and some of their individual constituents against Tribolium castaneum Herbst. *Journal of Agricultural and Food chemistry., 59*(5), 1690-1696.

Carter, C. A., Chalfant, J. A., Goodhue, R. E., Han, F. M., & DeSantis, M. (2005). The methyl bromide ban: Economic impacts on the California strawberry industry. *Review of Agricultural Economics, 27*(2), 181-197.

Cohen, E. (1986). Glutathione-S-transferase activity and its induction in several strains of Tribolium castaneum. *Entomologia Experimentalis et Applicata,, 41*(1), 39-44.

Dangash, A. J., Pandya, N., Bharillya, A., Jhala, A., & Dharamchand, J. A. (2014). Impact of exogenous elicitors on artemisinin production and trichome density in Artemisia annua L. under subtropical conditions. *Notulae Scientia Biologicae, 6*(3), 349-353.

Das, S. (2012). Artemisia annua (Qinghao): a pharmacological review. *Int J Pharm Sci ReS, 3*(12), 4573-4577.

Dhingra, V., Rao, K. V., & Narasu, M. L. (1999). Current status of artemisinin and its derivatives as antimalarial drugs. *Life sciences, 66*(4), 279-300.

Dyte, C. E., & Blackman, D. G. (1970). The spread of insecticide resistance in Tribolium castaneum (Herbst)(Coleoptera, Tenebrionidae). *Journal of Stored Products Research, 6*(3), 255-261.

Eastman, R. T., & Fidock, D. A. (2009). Artemisinin-based combination therapies: a vital tool in efforts to eliminate malaria. *Nature Reviews Microbiology.*, *7*(12), 864-874.

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.

El-Mofty, M. M., Sakr, S. A., Osman, S. I., & Toulan, B. A. (1989). Carcinogenic effect of biscuits made of flour infested with Tribolium castaneum in Bufo regularis. *Oncology*, *46*(1), 63-65.

Engsontia, P., Sanderson, A. P., Cobb, M., Walden, K. K., Robertson, H. M., & Brown, S. (2008). The red flour beetle's large nose: an expanded odorant receptor gene family in Tribolium castaneum. *Insect biochemistry and molecular biology*, *38*(4), 387-397.

Fao, I. (2013). *WFP, The State of Food Insecurity in the World 2013—The Multiple Dimensions of Food Security.* Rome: FAO.

Farrar, R. R., Barbour, J. D., & Kennedy, G. G. (1989). Quantifying food consumption and growth in insects. *Annals of the Entomological Society of America*, *82*(5), 593-598.

Good, N. E. (1933). Biology of the flour beetles, Tribolium confusum Duv. and T. ferrugineum Fab. *Journal of Agricultural Research*, *46*(4), 327-334.

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.

Hagstrum, D. (2017). Infestation records. In K. T. Hagstrum D, *Atlas of stored-product insects and mites* (pp. 474-483). Minnesota: Elsevier.

Hasspieler, B. M., Arnason, J. T., & Downe, A. E. (1990). Modes of action of the plant-derived phototoxin α-terthienyl in mosquito larvae. *Pesticide Biochemistry and Physiology*, *38*(1), 41-47.

Ho, S. H., Koh, L., Ma, Y., Huang, Y., & Sim, K. Y. (1996). The oil of garlic, Allium sativum L.(Amaryllidaceae), as a potential grain protectant against Tribolium castaneum (Herbst) and Sitophilus zeamais Motsch. *Postharvest Biology and Technology*, *9*(1), 41-48.

Howe, R. W. (1965). A summary of estimates of optimal and minimal conditions for population increase of some stored products insects. *Journal of Stored Products Research, 1*(2), 177-184.

Hu, J., Wang, W., Dai, J., & Zhu, L. (2019). Chemical composition and biological activity against Tribolium castaneum (Coleoptera: Tenebrionidae) of Artemisia brachyloba essential oi. *Industrial Crops and Products*, *128*, 29-37.

Huang, Y., Lam, S. L., & Ho, S. H. (2000). Bioactivities of essential oil from Elletaria cardamomum (L.) Maton. to Sitophilus zeamais Motschulsky and: Tribolium castaneum (Herbst). *Journal of Stored Products Research*, *36*(2), 107-117.

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.

Huang, Y., Ho, S. H., Lee, H. C., & Yap, Y. L. (2002). Insecticidal properties of eugenol, isoeugenol and methyleugenol and their effects on nutrition of Sitophilus zeamais Motsch.(Coleoptera: Curculionidae) and Tribolium castaneum (Herbst)(Coleoptera: Tenebrionidae). *Journal of stored product research*, *38*(5), 403-412.

Huang, Y., Tan, J. M., Kini, R. M., & Ho, S. H. (1997). Toxic and antifeedant action of nutmeg oil against Tribolium castaneum (Herbst) and Sitophilus zeamais Motsch. *Journal of Stored Products Research, 33*(4), 289-298.

Iram, N., Arshad, M., & Akhter, N. (2013). Evaluation of botanical and synthetic insecticide for the control of Tribolium castaneum (Herbst)(Coleoptera: Tenebrionidae. *Bio Assay, 8*(3), 1-10.

Isman, M. B. (2000). Plant essential oils for pest and disease management. *Crop protection, 19*(8-10), 603-608.

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*(1), 195-202.

Karunakaran, C., Jayas, D. S., & White, N. D. (2004). Identification of wheat kernels damaged by the red flour beetle using X-ray images. *Biosystems Engineering, 87*(3), 267-274.

Klayman, D. L. (1985). Qinghaosu (artemisinin): an antimalarial drug from China. *Science, 228*(4703), 1049-1055.

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.

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* (pp. 213-219). Pennsylvania Academy of Science.

Leonard LeCato, G. (1975). Red Flour Beetle: Population Growth on Diets of Corn, Wheat, Rice or Shelled Peanuts Supplemented with Eggs or Adults of the Indian Meal Moth. *Journal of Economic Entomology, 68*(6), 763-765.

Lhaloui, S., Hagstrum, D. W., Keith, D. L., Holtzer, T. O., & Ball, H. J. (1988). Combined influence of temperature and moisture on red flour beetle (Coleoptera: Tenebrionidae) reproduction on whole grain wheat. *Journal of economic entomology, 81*(2), 488-489.

Liu, Z. L., & Ho, S. H. (1999). Bioactivity of the essential oil extracted from Evodia rutaecarpa Hook f. et Thomas against the grain storage insects, Sitophilus zeamais Motsch. and Tribolium castaneum (Herbst). *Journal of Stored Products Research, 35*(4), 317-328.

Madrid, F. J., White, N. D., & Loschiavo, S. R. (1990). Insects in stored cereals, and their association with farming practices in southern Manitoba. *The Canadian Entomologist, 122*(3), 515-523.

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.

Nathanson, M. (1975). The effect of resource limitation on competing populations of flour beetles, Tribolium spp.(Coleoptera, Tenebrionidae). *Bulletin of Entomological Research, 65*(1), 1-12.

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.

Negahban, M., Moharramipour, S., & Yousefelahi, M. (2004). Efficacy of essential oil from Artemisia scoparia Waldst. & Kit. against Tribolium castaneum (Herbst) (Coleoptera:Tenebrionidae). *In*

Proceedings of The Fourth International Iran & Russia Conference (Agricultural and natural resource) (pp. 261-266). Viietnam: Sharekord.

Owusu, E. O. (2000). Effect of some Ghanaian plant components on control of two stored-product insect pests of cereals. *Journal of Stored Products Research, 37*(1), 85-91.

Padın, 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.

Pandey, M. M. (2009). *Indian agriculture–an Introduction. Submitted to Fourth Session of the Technical Committee of APCAEM.* Bhopal, India.: APCAEM, Thailand .

Pimentel, M. A., Faroni, L. R., Tótola, M. R., & Guedes, R. N. (2007). Phosphine resistance, respiration rate and fitness consequences in stored-product insects. *Pest Management Science: formerly Pesticide Science, 63*(9), 876-881.

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*, 1-6.

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.

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.

Semple, R. (1986). Problems relating to pest control and use of pesticides in grain storage: the current situation in ASEAN and future requirements. *In Pesticides and humid tropical grain storage systems* (pp. 45-75). Manila, Philippines: Proceedings International Symposium.

Shafique, M., Ahmad, M., & Chaudry, M. A. (2006). Feeding preference and development of Tribolium castaneum (Herbst.) in wheat products. *Pakistan Journal of Zoology, 38*(1), 27-31.

Singh, P., Satya, S., & Naik, S. N. (2013). Effect of insect infestation on quality parameters of wheat. *In International Conference on Food and Agricultural Sciences* (pp. 79-82). Singapore: IACSIT Press.

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.

Tripathi, A. K., Prajapati, V., Aggarwal, K. K., & Kumar, S. (2001). Toxicity, feeding deterrence, and effect of activity of 1, 8-cineole from Artemisia annua on progeny production of Tribolium castanaeum (Coleoptera: Tenebrionidae). *Journal of Economic Entomology,, 94*(4), 979-983.

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.

Yang, R. Z., & Tang, C. S. (1988). Plants used for pest control in China: a literature review. *Economic Botany., 42*(3), 376-406.

Zettler, L. J. (1991). Pesticide resistance in Tribolium castaneum and T. confusum (Coleoptera: Tenebrionidae) from flour mills in the United States. *Journal of Economic Entomology, 84*(3), 763-767.

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