

CHAPTER 7

7.0 SIGNIFICANT FINDINGS

- *Tribolium castaneum* is one of the major primary pest of wheat flour and secondary pest of wheat grains. It is known to damage a wide range of stored grains.
- Different control measures are implemented for controlling the pest. Majorly fumigation with methyl bromide and phosphine is widely practiced in the warehouses.
- Negative consequences of these chemicals have called for an alternative. Plant based compounds have emerged as a potent alternatives of these chemicals.
- Plant components are popularised due to its non-toxic effect towards the non-target animals including humans.
- *Artemisia annua* is popular in the field of medicine for its anti-malarial potential. The plant which is the only source of the Artemisinin is selectively cultivated in Gujarat, Kashmir valley and Madhya Pradesh.
- Different species of *Artemisia* has been established to hold an insecticidal property. A few preliminary studies have been conducted to evaluate the insecticidal properties of *A. annua*.
- In this piece of work, efficacy of different solvent derived essential oils against the *T. castaneum* has been evaluated. For elution of essential oils, both the polar and non-polar solvents were used to make the comparison between the essential oils stronger.
- Methanol and Chloroform were used among polar solvent and Petroleum ether and n-hexane were used among the non-polar solvent.
- In the study, biology of the flour beetle was studied and each stage was identified and photographed using stereomicroscope. Seven larval instars have been recorded. Moreover, sexual dimorphic characters were detected in the pupal and adult stages and were confirmed by the stereomicroscope.
- In the pupal stage, presence of genital papillae presents shows sexual dimorphism. In case of females the papillae is forked and reach the

length of the urogomphi. The males possess stubby and small genital papillae which is restricted to the last abdominal segment.

- Sexual dimorphism is prominent due to the presence of setiferous patch in the forefemur of males which is absent in case of the females.
- The average oil yield of the EOs of *Artemisia annua* was 27.16% with methanol, 19.28% with chloroform, 1.36% with petroleum ether and 3.68% with n- hexane.
- GC-MS results have shown the presence of 13 different compounds in the methanolic EOs which accounts for 99.98% of the total oil. Among the major constituents 1-Docosene (29.57%), I-Valine, N-Heptafluorobutyl-, nonyl ester (22.99%), 3-Methylcyclopentadecylcarbamic acid, T- Butyl ester (12.12%) etc. were reported.
- A total of 24 compounds were identified in the chloroform derived EOs accounted for 97.11% of the total oil. Bicyclo (22.1) heptan-2-one, 1,7,7, trimethyl- (15.35%) 3,4-Hexadienal, 2-butyl-2-ethyl-5-methyl- (10.26%) etc. were reported as the major components.
- Petroleum ether derived EOs were recorded to possess 16 different compounds which accounts for 98.15% of the total oil. Among the major chemical constituents, 3,4-Hexadienal, 2-butyl-2-ethyl-5-methyl- (22.06%), Deoxyqinghaosu (10.84%) etc. were in the list.
- 18 different compounds accounting for 97.11% of the total oil was recorded from n-hexane eluted EOs. The major constituents were identified as 3,4-Hexadienal, 2-butyl-2-ethyl-5-methyl-2 (20.98%), Cedran-diol, 8S,13- (8.29%) etc.
- Repellency as depicted in the filter paper arena test was maximum in the petroleum ether derived EOs with 95% at 0.90 mg cm^{-2} . This was followed by n-hexane EOs with 93.5% repellency, methanol with 91.63% repellency and finally chloroform with 88.25% repellency.
- EPI drawn with the methanolic EOs was -0.9 at the end of 24 hours. The EPI was recorded to be -1 after 24 hours with the other selected EOs.
- In contact toxicity assays, the toxicity effect was recorded in the order of methanol < chloroform < n-hexane < petroleum ether.

- Adults (LD_{50} : 1.87 mg adult⁻¹) were found more resistant than the larvae (LD_{50} : 1.24 mg adult⁻¹) with the methanolic EOs.
- The chloroform derived EOs has shown that the adults (LD_{50} : 0.90 mg adult⁻¹) were more susceptible to the oil than the larvae (LD_{50} : 1.57 mg adult⁻¹).
- Results of petroleum ether EOs was consistent with the chloroform EOs where the adults were susceptible at low LD_{50} value of 0.43 mg adult⁻¹ than the larvae (LD_{50} : 0.60 mg adult⁻¹).
- The n-hexane derived EOs have demonstrated the larval instar (LD_{50} : 0.47 mg adult⁻¹) was susceptible than the adults (LD_{50} : 0.71 mg adult⁻¹).
- The toxicity effect in the case of fumigant toxicity was recorded in the order of methanol < chloroform < petroleum ether < n-hexane.
- The adults (LD_{50} : 0.97 mg L air⁻¹) were found more susceptible to the chloroform derived EOs than the larvae (LD_{50} : 1.57 mg L air⁻¹).
- In case of the other EOs viz. methanol, petroleum ether, n-hexane derived EOs the adults with the LD_{50} value of 1.64, 0.81, 0.71 mg L air⁻¹ respectively were more resistant than the larvae with the LD_{50} value of 1.35, 0.65, 0.47 mg L air⁻¹ respectively.
- The biomolecular profile of protein and various life-supporting enzymes has shown significant downfall in the treatment sets of both contact and fumigant assays compared to the control. Moreover, the reduction of enzymes like AChE, GSH and GST was more prominent in the treated sets with the increase in dose. LPO showed an increase in the level in LD_{90} followed by LD_{50} and control.
- The antifeedant action of *Artemisia annua* against *T. castaneum* was reported with a low feeding ratio in the treatment sets. The Fr of the untreated set was 0.64, whereas the treatment sets i.e. 0.5g and 1g of *A. annua* have drawn 0.36 and 0.39 Fr respectively.
- Heavy weight loss of 30.08% in untreated grains was observed whereas a significant reduction ($P < 0.05$) in the weight loss of 6.46% and 3.4% was reported in the treatment sets of 0.5g and 1g respectively.

- SEM images depict a clear structural difference between the undamaged and damaged wheat grains. The undamaged grain shows structural integrity whereas the damaged grains are thoroughly punctured by the beetles.
- More detailed topography was prominent at higher magnification like 1000x which evidenced the presence of starch globule on the surface of the undamaged grains. In damaged grains, starch molecules have been attacked by the pest.
- The EDX data has shown the presence of high carbon (39.21%) content in the undamaged grains. On the other hand, oxygen content was higher in the damaged grains with 59.27%.
- A few inorganic compounds like silicon, calcium and potassium in trace amount were also evidenced in the undamaged grain sample. Whereas, only potassium has been detected from the damaged grains.
- In the flour disc bioassay, relative growth rate (RGR) and relative consumption rate (RCR) of the insect has shown a significant reduction ($P < 0.05$) in the treated sets when compared with 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.
- FDI was dose dependant and the four solvents were effective in the following order of increasing efficiency i.e. methanol < chloroform < petroleum ether < n-hexane.
- The colour difference was very prominent with the white & clear undamaged flour and greyish damaged flour.
- The P^H , evaluated through the AACC International Method, 02-52.01, has a profound effect on the grain health. Higher P^H value point towards it's proneness to insect infestation. The value of the undamaged flour (7.1) was lower than the damaged flour (7.98).
- The damaged flour was evaluated for the presence of insect eggs via iodine method ((AACC International Method, 28-44.01) and it was

found to be loaded with 89 eggs. On the other hand, undamaged flour was free from such infestation.

- Percent crude fat, measured through AACC International Method, 30-10.01, is an integral component of the wheat flour and its accurate percent in the commodity is desired by the end-users. The damaged flour exhibited an exceptionally high percentage of crude fat (4.2%) than the undamaged flour (1.9%).
- Moisture content was analysed through the AACC International Method, 44-01.01 and it was found to be remarkably higher in case of the damaged flour i.e. 17%. This presents suitable condition for further deterioration of the flour.
- The protein content in the undamaged flour was 1.76 gm/dl and damaged flour was 0.961 gm/dl. This was significantly reduced in the damaged flour
- Carbohydrate content was extremely higher in damaged flour (19.44 mg/dl) than the undamaged flour (2.80 mg/dl).
- Presence of insect's fragments in the damaged and undamaged flour was evaluated through AACC International Method, 28-41.03 acid hydrolysis test. The result shows presence of 212 insect fragments per 50 grams of the damaged flour which is the DAL for the sample.