CHAPTER 5

5.0 DISCUSSION

The biology of *Tribolium casteneum* was studied thoroughly under the stereomicroscope for intricate characteristics. Seven larval instars were reported from the strain in the present study. Adults and larvae are the two damaging stage of the beetle whereas eggs and pupae are inert stages. However, the number of larval instars of the genus is variable i.e. from six to eleven, depending solely on the external conditions like temperature, humidity and food availability (Good, 1933). However, 7-8 larval instars are mostly reported from the species (Devi & Devi, 2015). The more the larval instars, the more damage it causes to the stored grains as they are voracious feeder. The enormous feeding is related with the growth and energy deposition required during metamorphosis. The beetle, however, shows two-fold damages; one by continuous feeding and simultaneous secretion of quinone's and second by the accumulation of the exuvia. This all reduces the nutritional efficiency of the grain and makes it unfit for the consumption (Hodges et al., 1995).

The oil yield of the *Artemisia annua* was found to be higher with the polar solvents over non-polar ones. The percent oil yield was found in the order of methanol> chloroform> n-hexane> petroleum ether. Lower oil yield of EOs with the nonpolar solvents were described previously (Mezzomo et al., 2010). The major thought behind selecting both the polar and non-polar solvents is to record better efficiency in drawing potential chemical constituents, which is unique to each solvent. The work of Haridasan et al. (2017) have reported the efficacy of methanol over petroleum ether derived EOs from *Vitex negundo* against *T. casteneum.* Another study has documented the efficacy of n-hexane derived EOs of camphor against four different stored grains pest (Obeng-Ofori, et al., 1998). Lee et al. (2004) has also used hexane as the solvent for eluting EOs from 42 species of Myrtaceae genus with highest oil yield (7.6%) recorded from the *M. thymifolia*. However, oil yield in other species was recorded in the range of <1. Majorly studies were focused in drawing EOs using a solvent, primarily the non-polar one, to check the efficacy against a

number of stored grains pests or a number of plant species is used against a single pest. However, very few attempts to use different solvents for eluting major insecticidal constituents are known to the best of our knowledge.

The major focus of the research was to decipher the influence of solvents in drawing potent chemical groups from the plant species. While discussing about the solvents, water would be the most preferable choice to the layman for its lower cost and easy availability. We also tried eluting the EOs with water through hydrodistillation. Extraction of EOs using water was successful. The difficulty with the solvent was the insolubility of EOs in acetone. Hence, I confined to the four solvents viz. methanol, chloroform, petroleum ether and n-hexane. In this study, all the four EOs derived of *A. annua* has demonstrated acute toxicity against *T. casteneum* adults as well as larvae. Results depicted the efficacy of non-polar solvents over polar ones. However, the degree of lethality varied with the time, concentration and solvent used to elute EOs.

Results of the present study have verified the efficacy of all the four solvent eluted EOs against the adult beetle in filter paper arena test. The methanolic EOs was found to be dose and time dependant. On the other hand, chloroform, petroleum ether and n-hexane EOs was found to be dose and time dependent till 12 hours. The repellency has decreased in these EOs by 2-3% after 24 hours. However, the degree of repellency recorded was extremely high i.e. >90%, hence this reduction does not questions the efficacy of the EOs. Liang et al. (2018) has reported the strong repellency of *Artemisisa ordosica* EOs against red flour beetle with 100% repellency at the concentration of 62.91nL/cm^2 in the filter paper arena test. Another piece of work has established the repellency of *Tanacetum nubigenum* EOs against the flour beetle (Haider et al., 2015). The filter paper arena test is choice based and depicts the preference made by the pest. The results of the other studies are continuous with ours and flour beetle is majorly seen to aggregate in the control half. This proves the efficacy of the plant-based EOs.

Repellency recorded through multi-arm olfactometer has shown the effectiveness of non-polar solvents over polar solvents at the same concentrations. Both the petroleum ether and n-hexane EOs have killed all the

test insects within one hour of exposure to that concentration. Hence, the doses of non-polar EOs were then reduced and repellency was detected. Methanolic EOs was weakest among all after 24 hours. The repellency was time and dose dependent in all the treatment sets except petroleum ether EOs. Jayakumar et al. (2017), in his recent publication has mentioned the use of multi-arm olfactometer. The highest EPI recorded in the study was -0.90 which is lower than our result i.e. -1 with the EOs derived from chloroform, petroleum ether and n-hexane. Sarada et al. (2018) has recently documented the repellency of pulse beetle assayed through multi arm olfactometer with strong activity by sweet flag rhizome bits (EPI -0.84). Another work has demonstrated the repellent potential of *Argemone mexicana, Prosopis juliflora* and *Tephrosia purpurea* on the flour beetle. The highest EPI recorded was -0.80 after 6 hours in the *T. purpurea* treatment sets (Pugazhvendan et al., 2009). The negative value which indicates the repellency showed that the repellency drawn with the *A. annua* was highest.

While evaluating the repellency of adult beetles, behavioural alteration like rapid abnormal movement in response to stress and their aggregation in the control half were penned down. Their aggregation below the filter paper was well depicted in the **Error! Reference source not found.**. This is the first report depicting the repellency of each EOs of *Artemisia annua* separately eluted with four different solvents. The results have established the strong repellency of the oil against the major pest, *Tribolium casteneum*.

The contact toxicity studies evaluated by the application of EOs topically have shown that the mortality is dose and time-dependent in all the EOs. In the methanolic EOs treatment sets, the larvae were more vulnerable than the adults with LD_{50} 1.24 mg adult^{-1} and 1.87 mg adult^{-1} respectively (Figure 1). However, adults were more susceptible to the chloroform EOs than the 14-days old larvae with LD_{50} 0.97 mg adult^{-1} and 1.57 mg adult^{-1} respectively (Figure 2). Among the non-polar solvents, petroleum ether has shown similar results like chloroform as depicted in the Figure 3 with adults (LD_{50}= 0.43 mg adult^{-1}) being more vulnerable than the larvae (LD_{50}= 0.60 mg adult^{-1}). Whereas the susceptibility of larvae (LD_{50}= 0.47 mg insect^{-1}) was more than the adults (LD_{50}= 0.71 mg adult^{-1}) towards the n-hexane EOs (Figure 4). It is

clear from the result of contact toxicity that all the EOs was effective in controlling the adults and larvae. However, it was effective in the following order of solvents i.e. petroleum ether> n-hexane >chloroform> methanol. While comparing the EOs, the non-polar solvents were found superior in controlling adults as well as larvae in the toxicity assays. However, petroleum ether demonstrated the best result.

Tapondjou et al. (2002) has evaluated the contact toxicity of the *Chenopodium ambrosioides* leaves against six different stored grains pest and found that the mortality was time and dose-dependent. Another study has demonstrated the efficacy of *Hyptis suaveolens* and *H. spicigera* against *Sitophilus granaries*. Result of contact toxicity studies have revealed the strong potential of both the oils which have resulted in LC_{100} at concentration as low as 0.4-0.6 µl/insects (Conti et al., 2011). In his work, Obeng-Ofori & Reichmuth (1997) has established the efficacy of eugenol, a common isolate of the EOs, in the contact toxicity assay drawing 100% mortality at 7 µ l/insect. In a similar study, the authors evaluated the toxicity of n-hexane derived EOs of two species of *Aloysia* viz. *A. citriodora* and *A. polystachya* against *Tribolium casteneum* and *Tribolium confusum* (Benzi et al., 2014). The study has shown the efficacy of non-polar solvent in eluting potent insecticidal components, which could effectively, controlled the pests. However, *T. casteneum* was found more tolerant to the EOs than the other species. The outcome can be justified by the fact of resistant development in the pest due to its continuous exposure to the synthetic fumigants (Champ & Campbell-Brown, 1970) (Opit et al., 2012). While comparing the LD_{50} values of the recent papers with our study, it was found that a higher concentration of non-polar solvent derived EOs was required to pose a lethal effect. The study is supported by the outcome of (Cao et al., 2018) where high LD_{50} values of β-Caryophyllene (41.7 µg cm-2), a major component of EOs, point towards resistance in the tested strain.

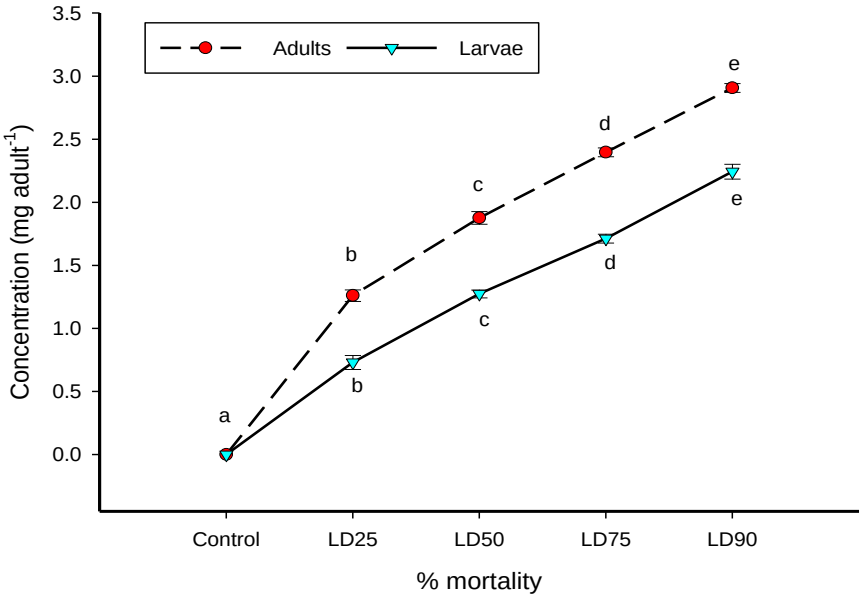


Figure 1: Comparison of percent mortlity between the adults and larvae of *T. casteneum* exposed to methanol derived EOs of *A. annua* in contact toxicity assays. Columns marked with different letters are significantly different (P<0.01; ANOVA and Tukey's honest post-hoc test).

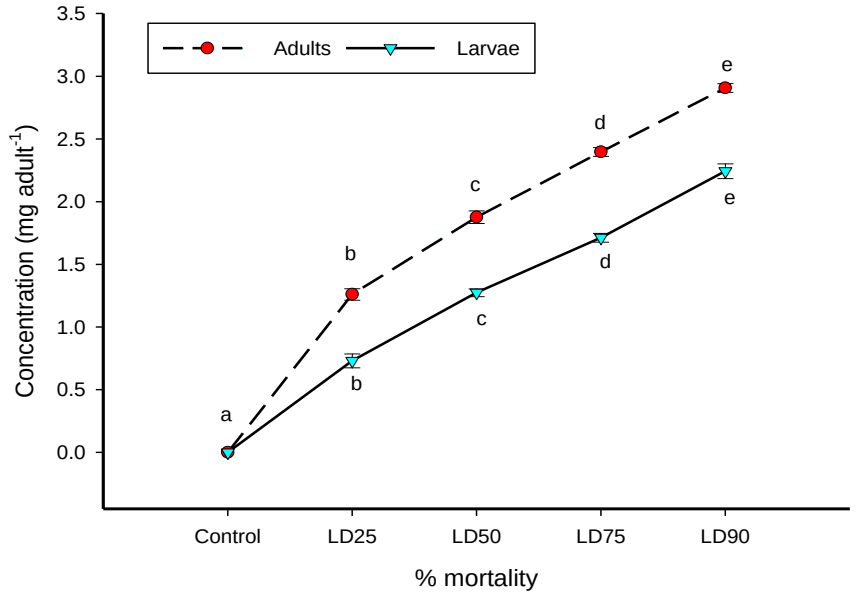


Figure 2: Comparison of percent mortlity between the adults and larvae of *T. casteneum* exposed to chloroform derived EOs of *A. annua* in contact toxicity assays. Columns marked with different letters are significantly different (P<0.01; ANOVA and Tukey's honest post-hoc test).

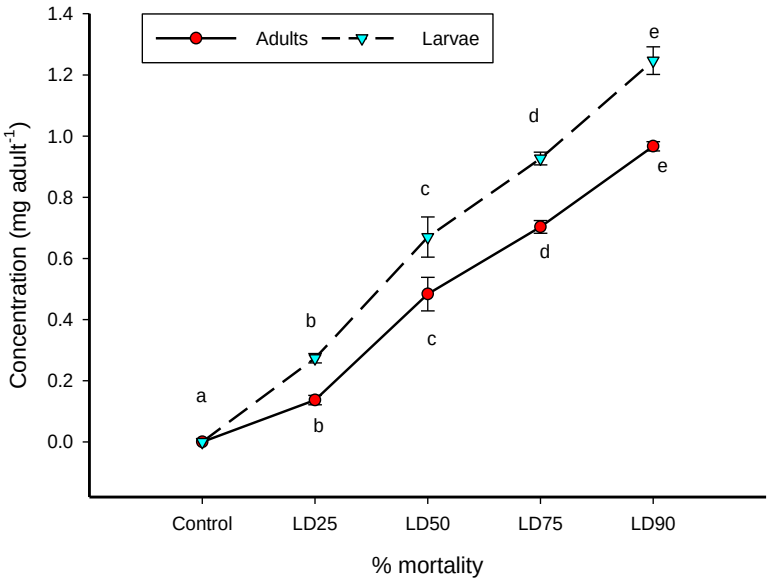


Figure 3: Comparison of percent mortlity between the adults and larvae of *T. casteneum* exposed to petroleum ether derived EOs of *A. annua* in contact toxicity assays. Columns marked with different letters are significantly different (P<0.01; ANOVA and Tukey's honest post-hoc test).

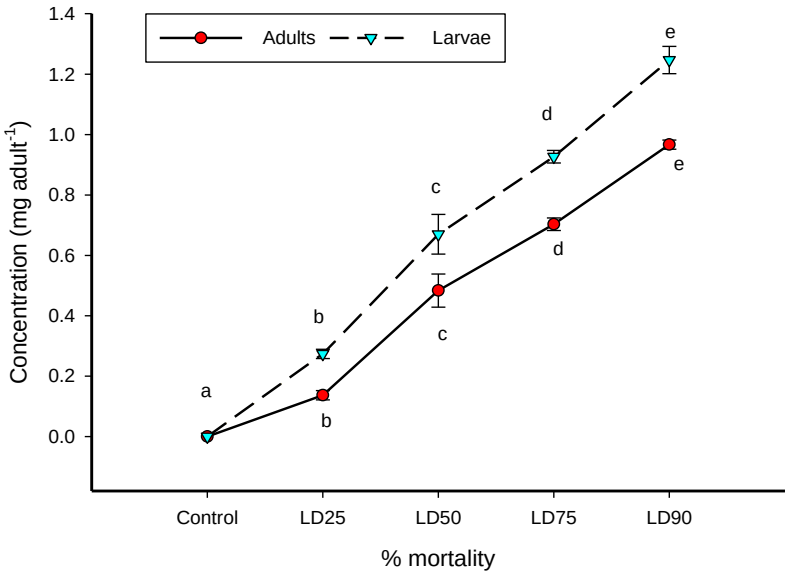


Figure 4: Comparison of percent mortlity between the adults and larvae of *T. casteneum* exposed to n-hexane derived EOs of *A. annua* in contact toxicity assays. Columns marked with different letters are significantly different (P<0.01; ANOVA and Tukey's honest post-hoc test).

When the EOs was tested as an effective fumigant against the beetle, a positive result was obtained. Comparing the response of the adults and larvae has revealed that the EOs had a profound effect which is well depicted in the lethal values. The toxicity was dose and time- dependent. Methanolic EOs has shown that adults are more resistant than the larvae with LD_{50} values of 1.64 mg L^{-1} air 1.35 mg L^{-1} air respectively (Figure 5). Results of chloroform derived EOs was continuous with the contact toxicity assays where the adults with LD_{50} value 0.97 mg L^{-1} air are more susceptible than the larvae which showed 50% mortality at 1.57 mg L^{-1} air (Figure 6). The non-polar solvents had killed complete population at the concentration range of 0.24-2.37 mg L^{-1} air, which is used for the polar solvents. Hence, the concentration was brought down to the range of 0.14- 1.42 mg L^{-1} air. In the petroleum ether EOs, the immatures (LD_{50}= 0.65 mg L^{-1} air) was more susceptible than the adults (LD_{50}= 0.81 mg L^{-1} air) as depicted in Figure 7. The lethal values of test insects exposed to n-hexane derived EOs have shown that adults were more tolerant than the larvae with LD_{50} values of 0.71 mg L^{-1} air and 0.47 mg L^{-1} air respectively (Figure 8). However, it was effective in the following order of solvents i.e. n-hexane > petroleum ether> chloroform> methanol. The comparison forms the basis and shows that the n-hexane as the best fumigant. However, petroleum ether EOs fumigant efficiency was superior to the methanol and chloroform EOs.

Results of previous studies have reported the benefits of EOs derived from 30 different plant species against *Lasioderma serricorne* (Kim et al., 2002). Horseradish oil, mustard oil and *Foeniculum* fruit extracts has drawn 100% mortality of the pest during fumigation in the completely sealed containers. The insecticidal potential of *Ocimum gratissimum* EOs against a wide range of stored grains pests was recorded with *T. casteneum* being the most tolerant one and seen to respond at very high concentrations of EOs (Ogendo et al., 2008). Our result differ from the work of (Negahban et al., 2007) where *Artemisia sieberi* has shown a lethal effect (LD_{90}) at a very low dose of 57.32 µl/L air. Cardiet et al. (2011) has found LD_{50} of clove oil at a lower concentration of 210 µl /l air against *Sitophilus oryzae* in the fumigant assay. Long before, the work of Shaaya et al. (1997) has validated the insecticidal

properties of different spices and herbs against stored grains pests including *T. casteneum*. The only thing which changed with time is the concentration of plant based EOs. Non-responsiveness of the pests in the pre-decided concentration has led to unveil more efficient means to pose toxicity on the pests. Moreover, results of fumigant toxicity assays have shown that *T. casteneum* was less susceptible to the EOs of *Pistacia lentiscus* which support our work (Bachrouch et al., 2010). 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 high dose required to display lethal effect.

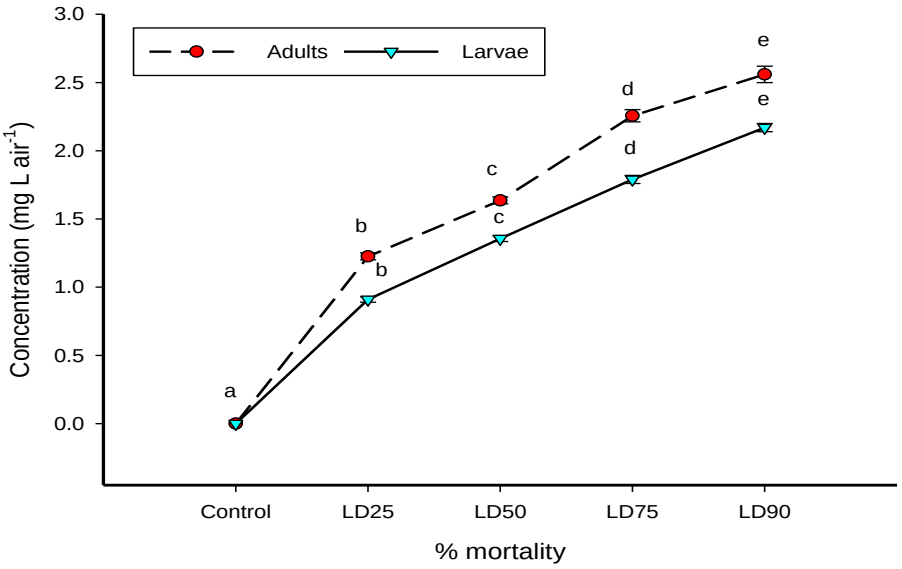


Figure 5: Comparison of percent mortlity between the adults and larvae of *T. casteneum* exposed to methanol derived EOs of *A. annua* in fumigant toxicity assays. Columns marked with different letters are significantly different (P<0.01; ANOVA and Tukey's honest post-hoc test).

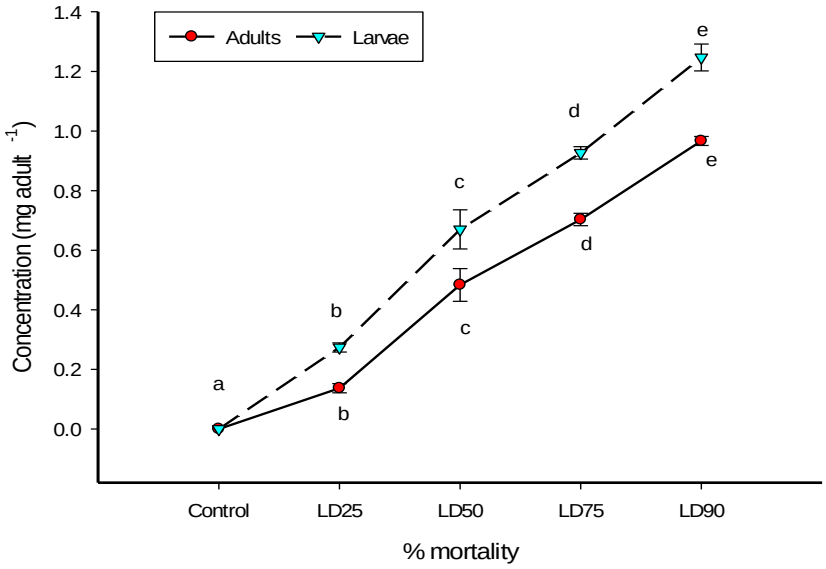


Figure 6: Comparison of percent mortlity between the adults and larvae of *T. casteneum* exposed to chloroform derived EOs of *A. annua* in fumigant toxicity assays. Columns marked with different letters are significantly different (P<0.01; ANOVA and Tukey's honest post-hoc test).

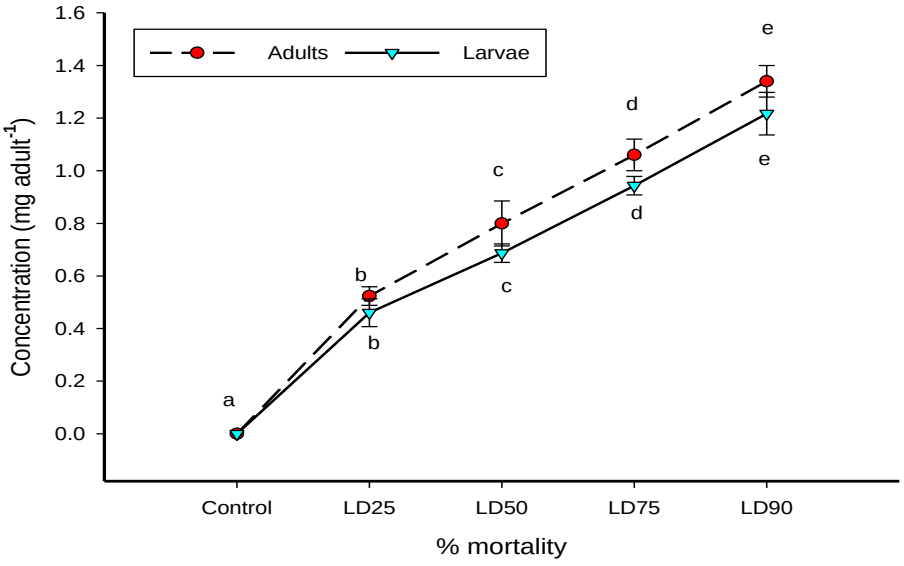


Figure 7: Comparison of percent mortlity between the adults and larvae of *T. casteneum* exposed to petroleum ether derived EOs of *A. annua* in fumigant toxicity assays. Columns marked with different letters are significantly different (P<0.01; ANOVA and Tukey's honest post-hoc test).

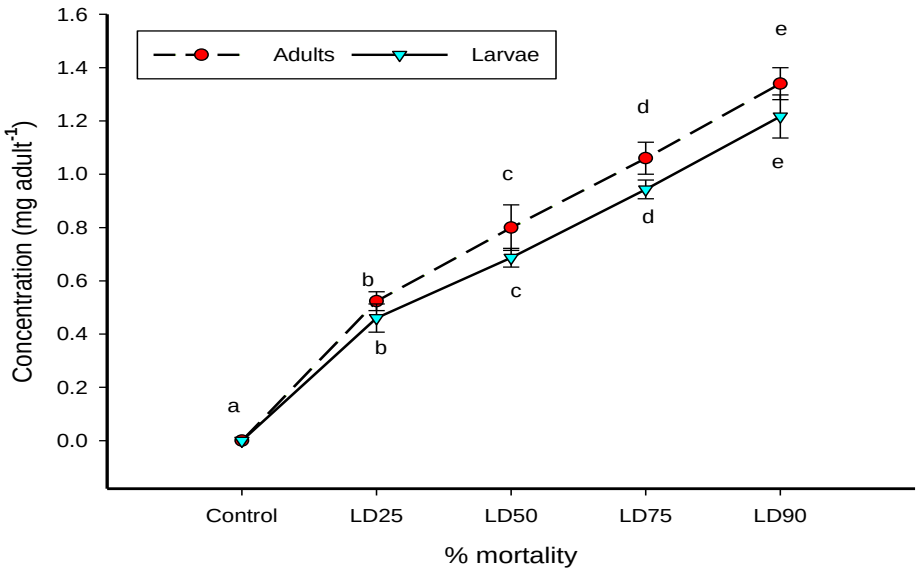


Figure 8: Comparison of percent mortlity between the adults and larvae of *T. casteneum* exposed to n-hexane derived EOs of *A. annua* in fumigant toxicity assays. Columns marked with different letters are significantly different (P<0.01; ANOVA and Tukey's honest post-hoc test).

Based on the results of our study and earlier reports (Cardiet et al., 2011) (Shaaya et al., 1997) EOs derived from plants is highly advantageous for the management of stored grains pest including the flour beetles. The outcome has specified the pesticidal action of EOs hence can be attributable to fumigant toxicity against the pest. Moreover, the fumigant action of *Artemisia annua* derived EOs, highly preferable mode in the storehouses, justifies the continuous release of the active insecticidal component is needed. Hence, the very use of this EOs is greatly recommended.

Further, the effect of the EOs on the biomolecular profile of *T. casteneum* adults and larvae was examined to gain an insight into the extent of metabolic disturbances inflicted in the treatment sets. Our experimental evidence indicates the action of EOs on the nervous system of *T. casteneum*. They work by disturbing the normal course of action of different detoxifying enzymes like AChE, GST, and LPO in the treated sets. While biomolecular profiling of LD_{50}, LD_{90} strains was conducted, decline in protein level with the increase in concentration was recorded in case of both the contact and fumigant toxicity bioassays (Figure 9, Figure 10, Figure 11, Figure 12). The results were continuous with a number of earlier investigations where scientists recorded

significant downfall in protein level in the treatment sets (Smirle et al., 1996) (Huang et al., 2004) (Macedo & Freire, 2011). Acetylcholine esterase, that works by clearing the neurotransmitter acetylcholine from the synaptic cleft. Reduction in the enzyme level, a major sign of toxicity, was reported in the lethal sets. The fall in the level was increased significantly with the increase in lethal concentration in the acute toxicity assays (Figure 13, Figure 14, Figure 15, Figure 16). The major detoxifying enzymes i.e. GST and GSH, has profound effect on establishing the normal homeostasis of the body. GST level in the methanolic EOs treatment sets showed significant reduction between the control and lethal sets (Figure 17). Whereas, chloroform EOs treatment sets has revealed significant reduction with the increase in concentration (Figure 18). The non-polar solvents derived EOs has demonstrated significant reduction in the lethal sets when compared with the control in both the contact and fumigant toxicity assays (Figure 19, Figure 20). GSH, in all the sets of the acute toxicity assays have demonstrated significant reduction with the increase in concentration. It was seen that the level of GSH has reduced in LD_{50} from control and reduced further in the LD_{90} sets (Figure 21, Figure 22, Figure 23, Figure 24). This signs point towards the oxidative stress that is inflicted in the sets due to the EOs treatment. Lipid peroxidase, LPO level is expected to rise with the accumulation of toxic molecules. In the study, results showed an increase in the level of LPO with the increase in concentration. The level increased significantly between all the three sets (Figure 25, Figure 26, Figure 27, Figure 28). The decrease in the level of proteins, life supporting enzymes viz. AChE, GSH, GSH and increase in the level of LPO are the major signs of oxidative stress. This could be reasoned due to the accumulation of molecules of EOs in the body thus disturbing the normal pathways. This claims the susceptibility of the test insects towards the *A. annua* EOs. Moreover, the most efficient solvent was n-hexane followed by petroleum ether to derive EOs. However, the polar solvents too had a satisfying result by increasing the oxidative stress.

Scientist working in the same trail showed similar results where Coumaran have inhibited the activity of AChE (Rajashekar et al., 2014) . Another set of experiments depicted contradictory results showing 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 were supported by the work of Koodalingam et al. (2011). LPO level in the study increased significantly in the treated sets thereby increasing the oxidative stress in the pest. Our findings are consistent with the result of Hasspieler et al. (1990).

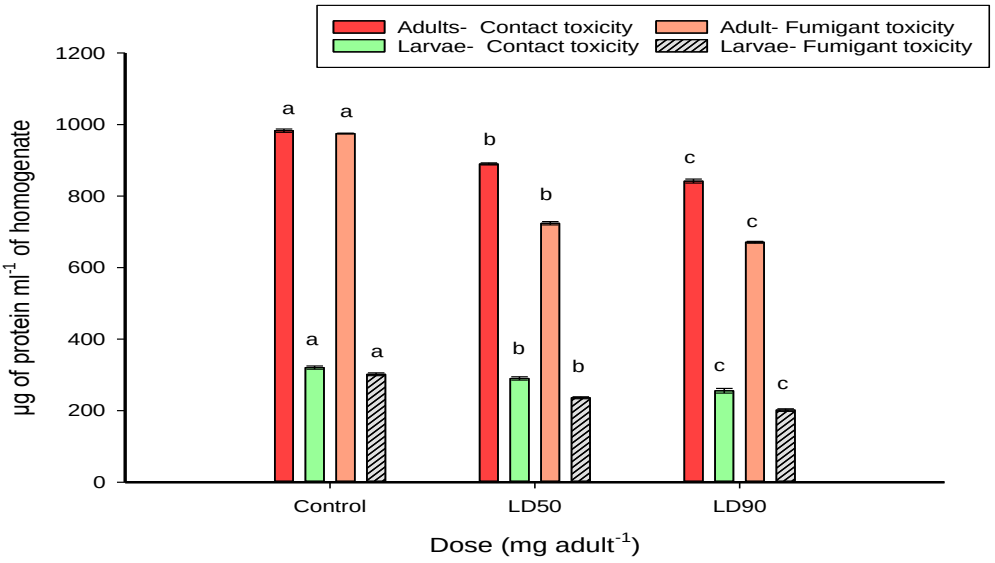


Figure 9: Protein activity (Mean±SE) in *T. casteneum* exposed to methanol derived EOs of *A. annua* at lethal doses. Columns marked with different letters are significantly different (P<0.01; ANOVA and Tukey's honest post-hoc test)

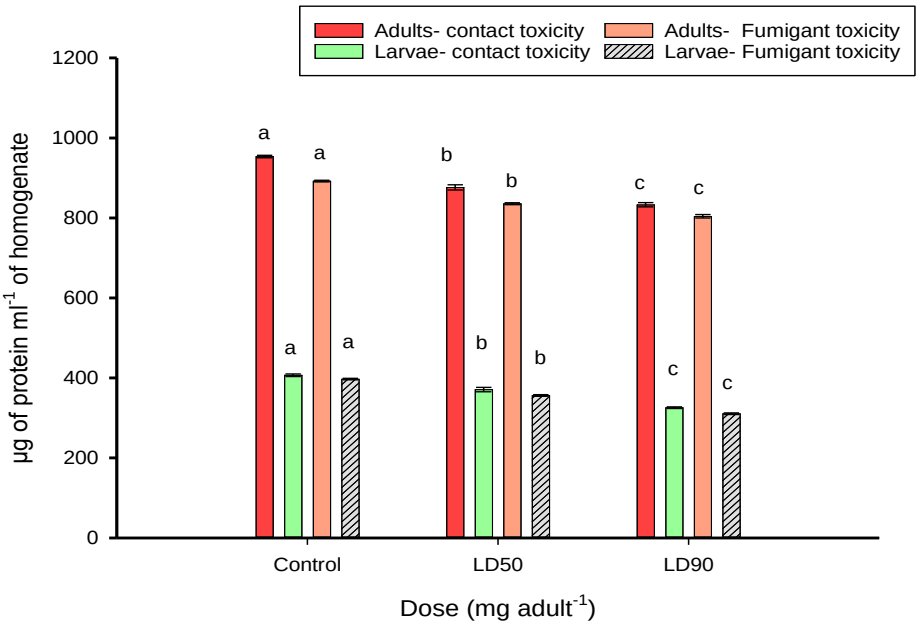


Figure 10: Protein activity (Mean±SE) in *T. casteneum* exposed to chloroform derived EOs of *A. annua* at lethal doses. Columns marked with different letters are significantly different (P<0.01; ANOVA and Tukey's honest post-hoc test)

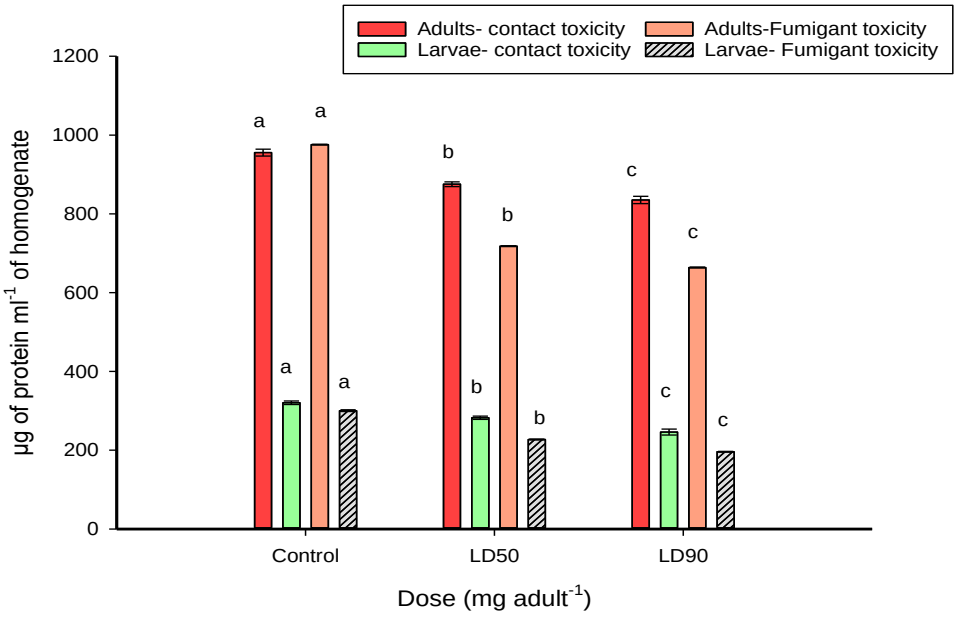


Figure 11: Protein activity (Mean±SE) in *T. casteneum* exposed to petroleum ether derived EOs of *A. annua* at lethal doses. Columns marked with different letters are significantly different (P<0.01; ANOVA and Tukey's honest post-hoc test)

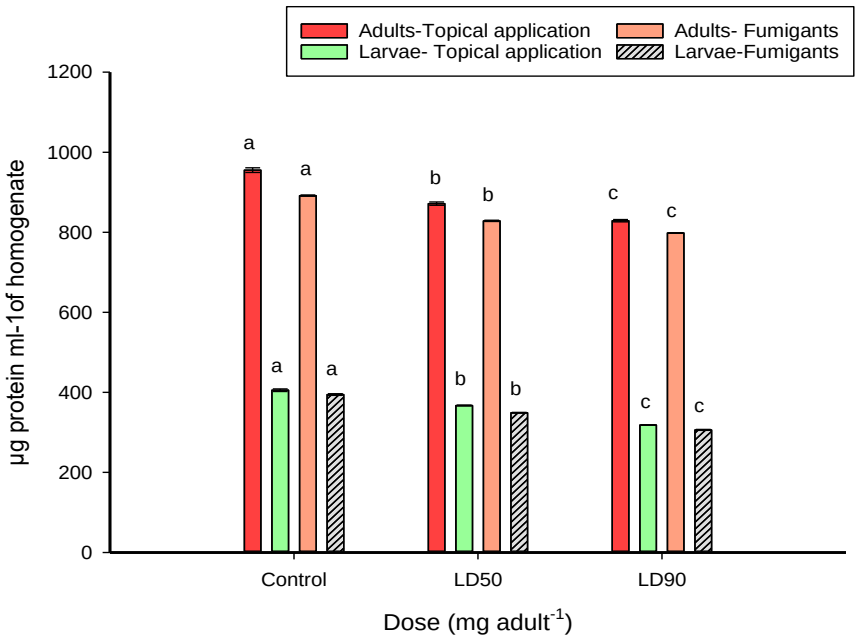


Figure 12: Protein activity (Mean±SE) in *T. casteneum* exposed to n-hexane derived EOs of *A. annua* at lethal doses. Columns marked with different letters are significantly different (P<0.01; ANOVA and Tukey's honest post-hoc test)

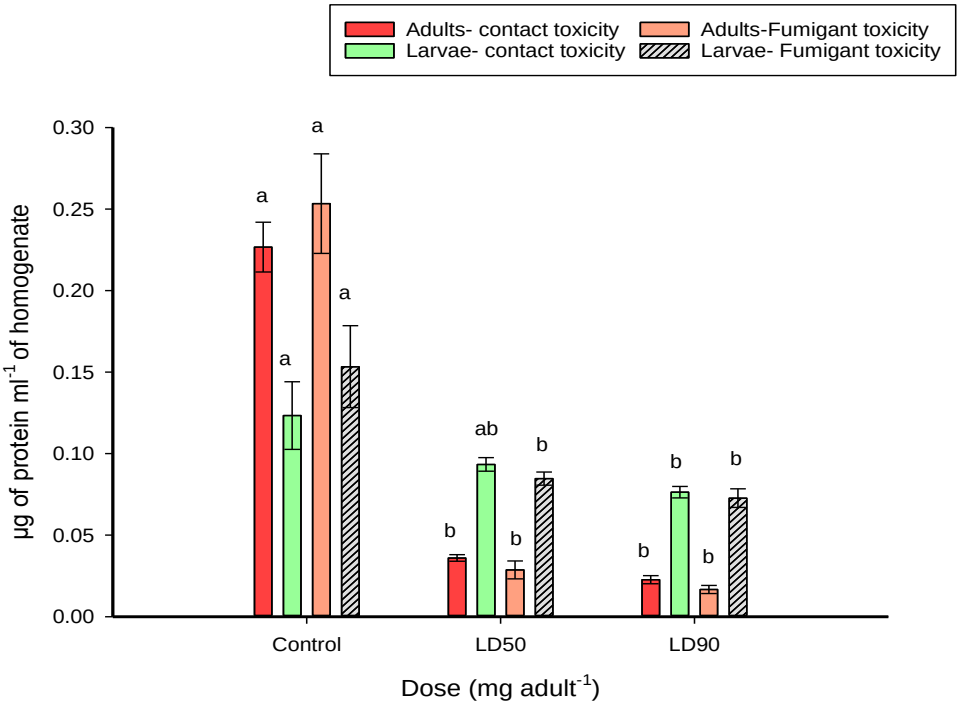


Figure 13: AChE activity (Mean±SE) in *T. casteneum* exposed to methanol derived EOs of *A. annua* at lethal doses. Columns marked with different letters are significantly different (P<0.01; ANOVA and Tukey's honest post-hoc test)

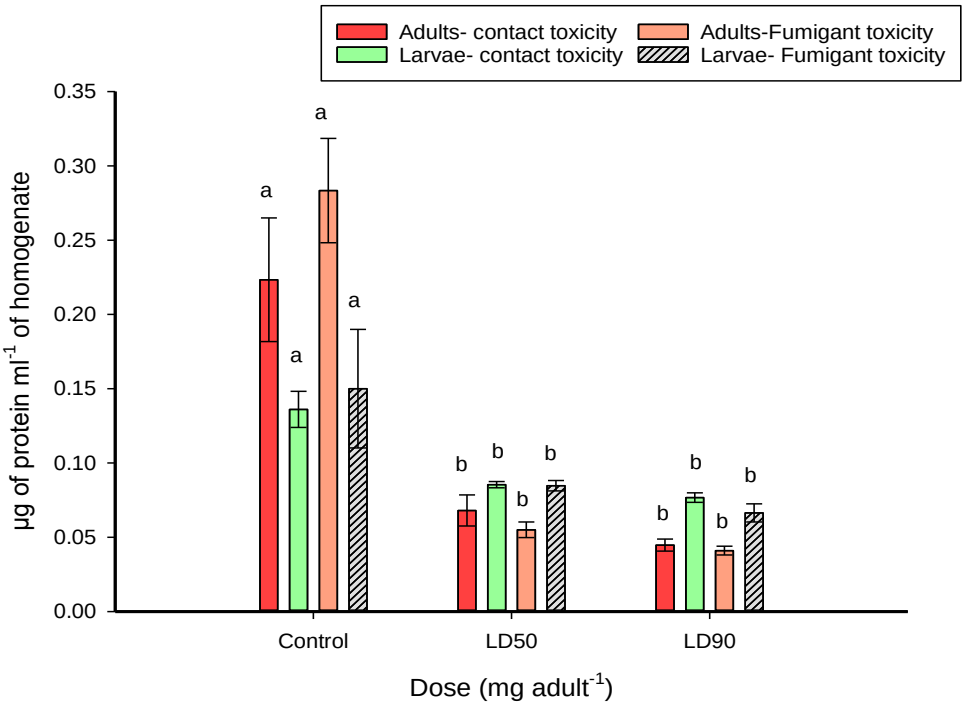


Figure 14: AChE activity (Mean±SE) in *T. casteneum* exposed to chloroform derived EOs of *A. annua* at lethal doses. Columns marked with different letters are significantly different (P<0.01; ANOVA and Tukey's honest post-hoc test)

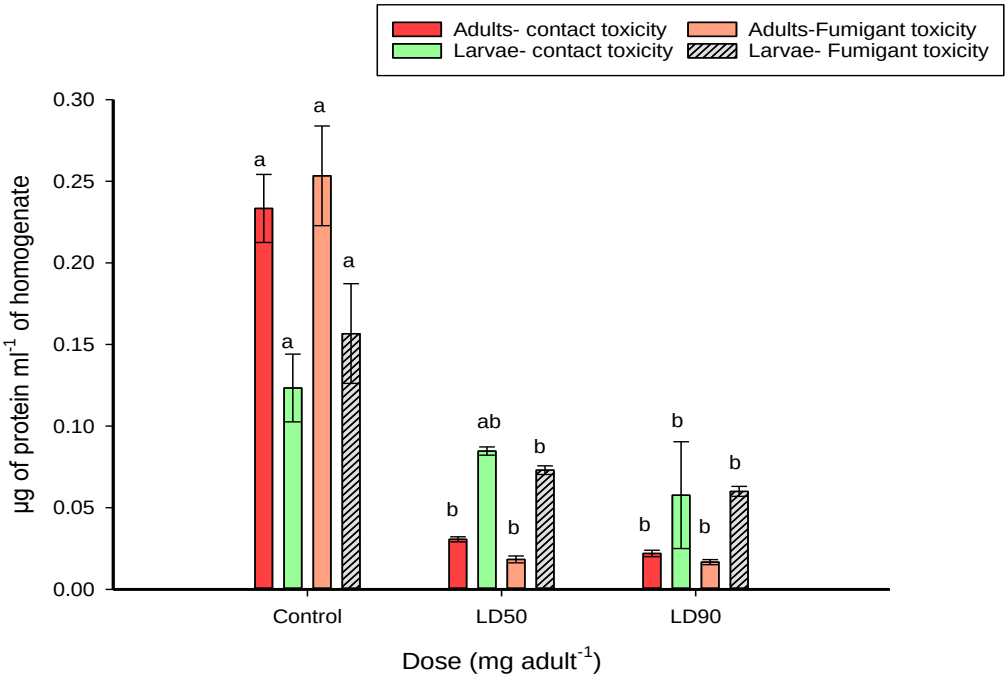


Figure 15: AChE activity (Mean±SE) in *T. casteneum* exposed to petroleum ether derived EOs of *A. annua* at lethal doses. Columns marked with different letters are significantly different (P<0.01; ANOVA and Tukey's honest post-hoc test)

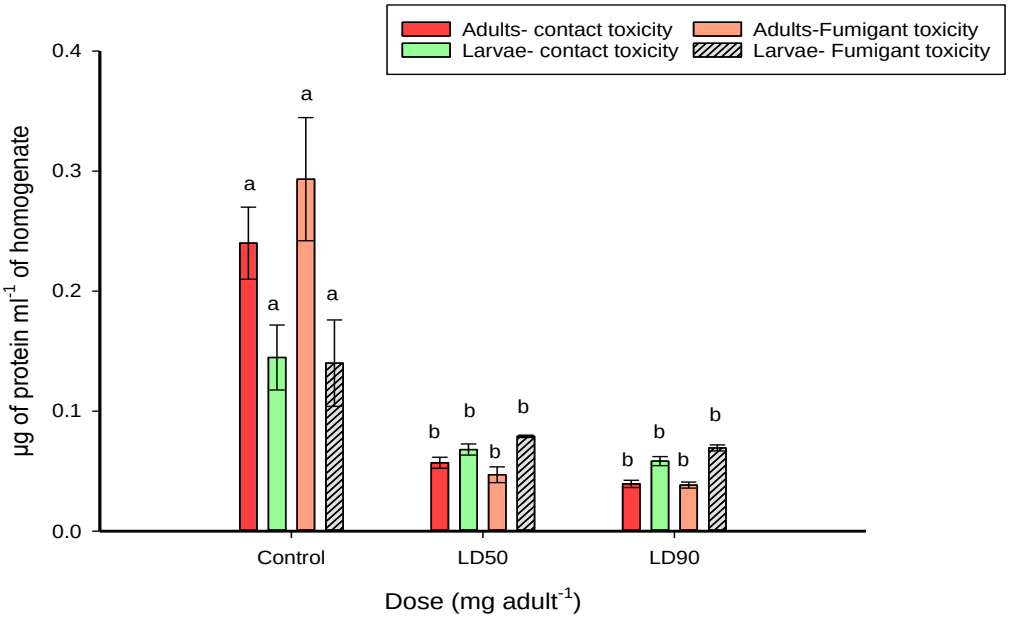


Figure 16: AChE activity (Mean±SE) in *T. casteneum* exposed to n-hexane derived EOs of *A. annua* at lethal doses. Columns marked with different letters are significantly different (P<0.01; ANOVA and Tukey's honest post-hoc test)

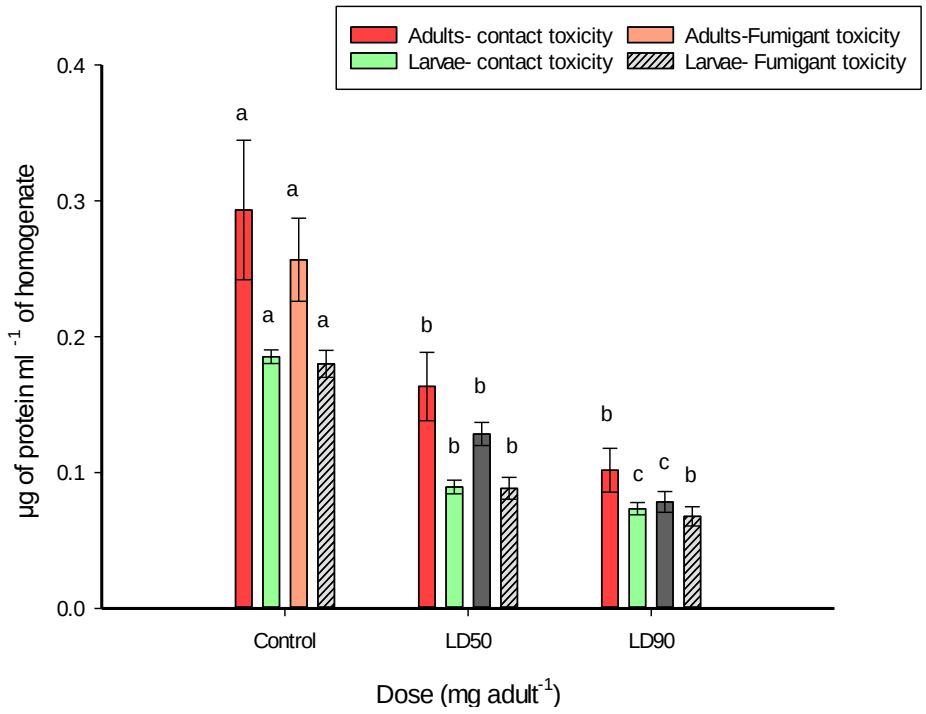


Figure 17: GST activity (Mean±SE) in *T. casteneum* exposed to methanol derived EOs of *A. annua* at lethal doses. Columns marked with different letters are significantly different (P<0.01; ANOVA and Tukey's honest post-hoc test)

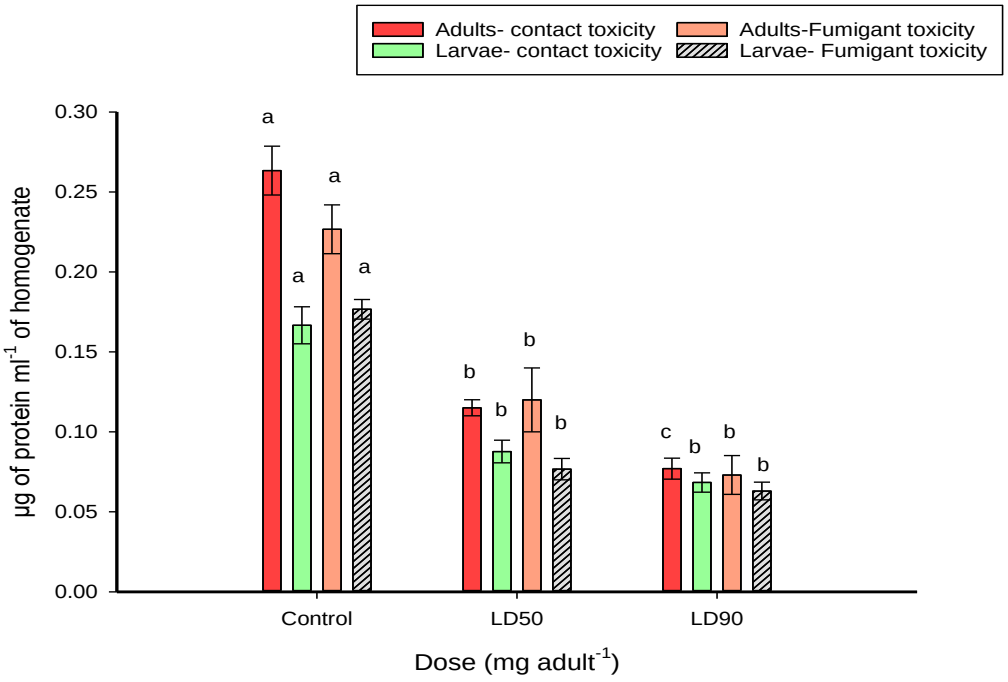


Figure 18: GST activity (Mean±SE) in *T. casteneum* exposed to chloroform derived EOs of *A. annua* at lethal doses. Columns marked with different letters are significantly different (P<0.01; ANOVA and Tukey's honest post-hoc test)

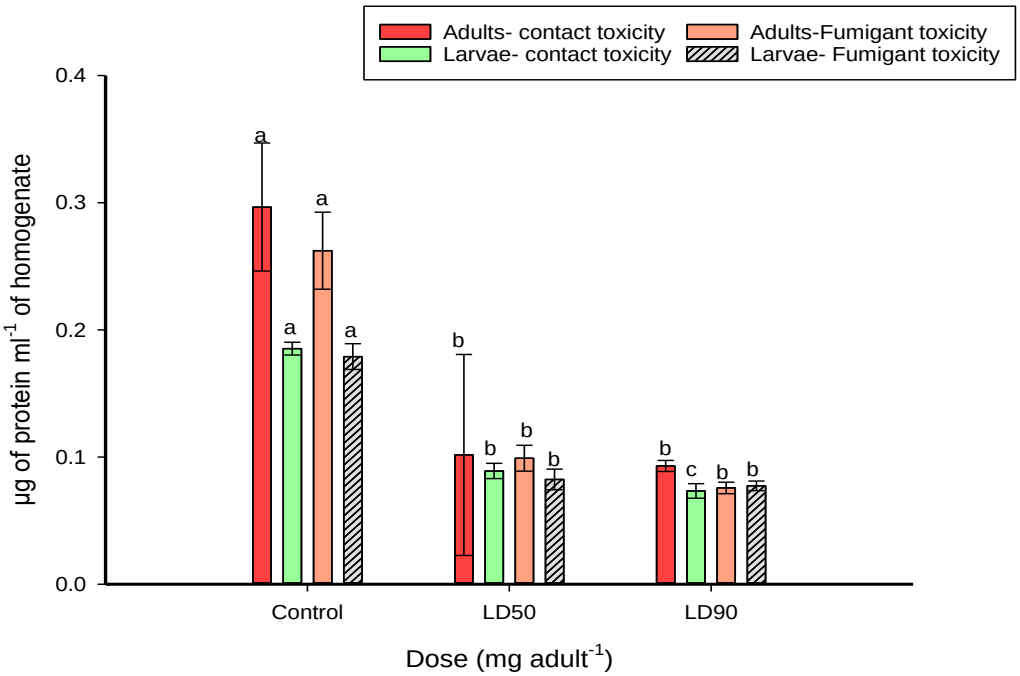


Figure 19: GST activity (Mean±SE) in *T. casteneum* exposed to petroleum ether derived EOs of *A. annua* at lethal doses. Columns marked with different letters are significantly different (P<0.01; ANOVA and Tukey's honest post-hoc test)

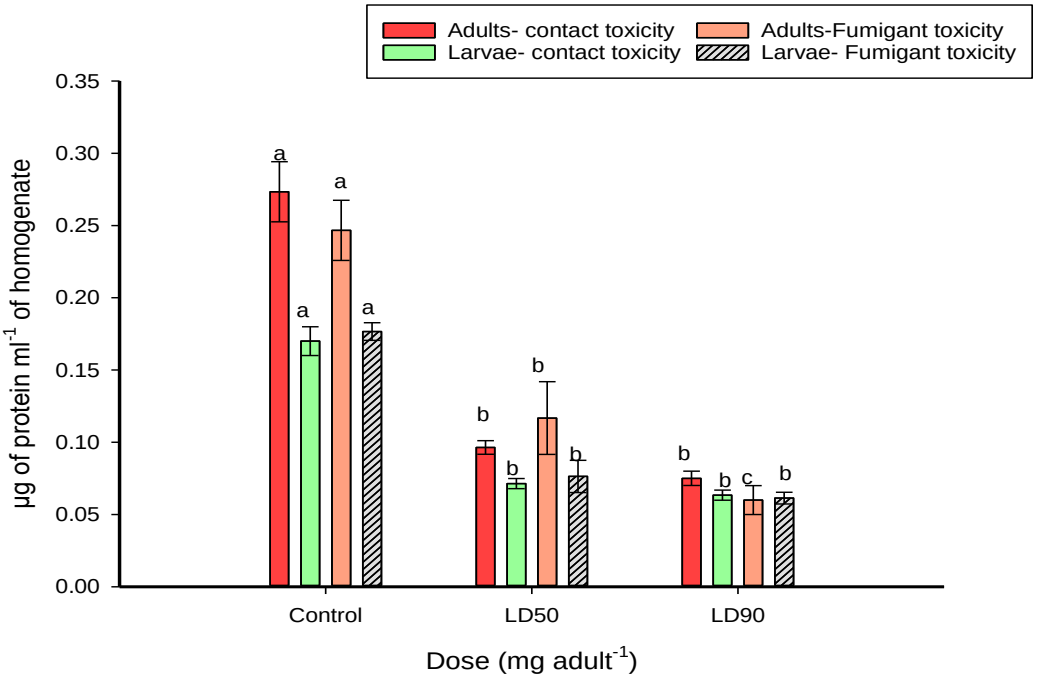


Figure 20: GST activity (Mean±SE) in *T. casteneum* exposed to n-hexane derived EOs of *A. annua* at lethal doses. Columns marked with different letters are significantly different (P<0.01; ANOVA and Tukey's honest post-hoc test)

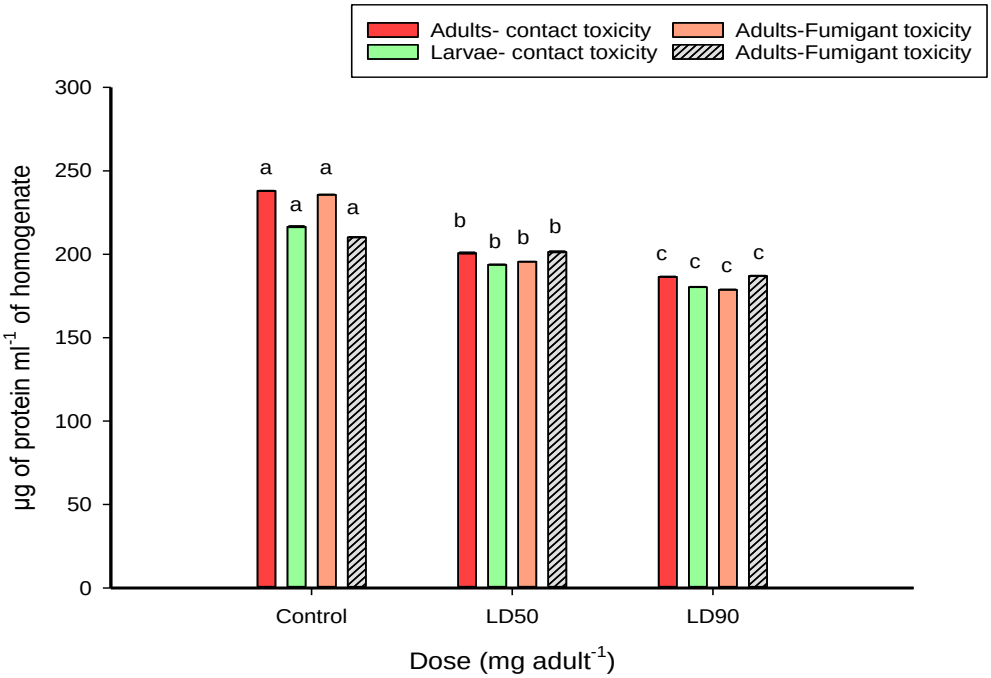


Figure 21: GSH activity (Mean±SE) in *T. casteneum* exposed to methanol derived EOs of *A. annua* at lethal doses. Columns marked with different letters are significantly different (P<0.01; ANOVA and Tukey's honest post-hoc test)

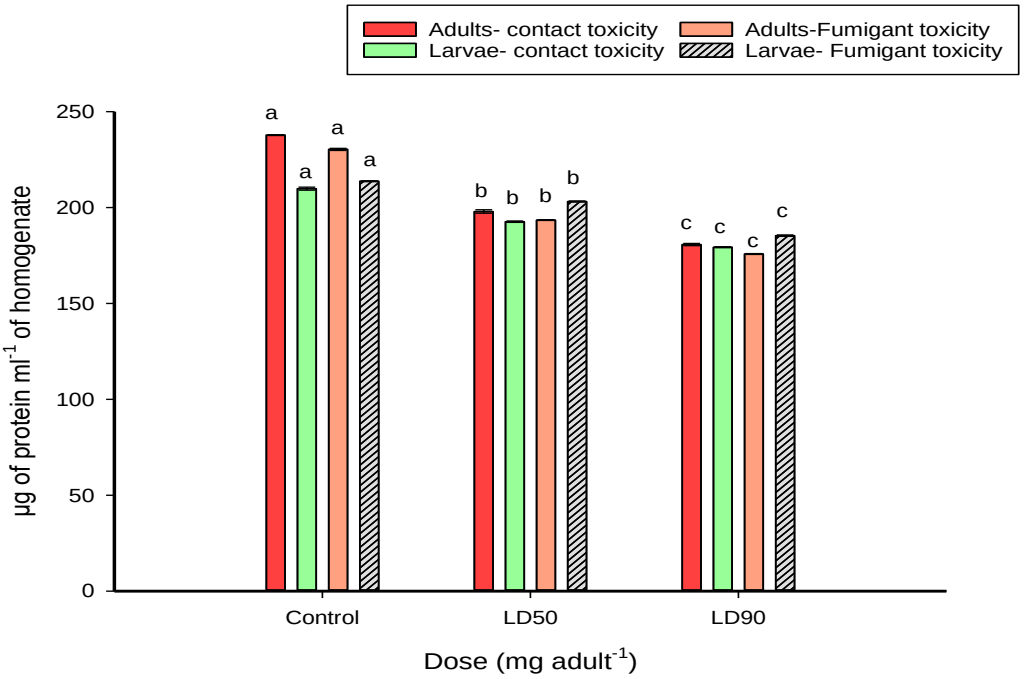


Figure 22: GSH activity (Mean±SE) in *T. casteneum* exposed to chloroform derived EOs of *A. annua* at lethal doses. Columns marked with different letters are significantly different (P<0.01; ANOVA and Tukey's honest post-hoc test)

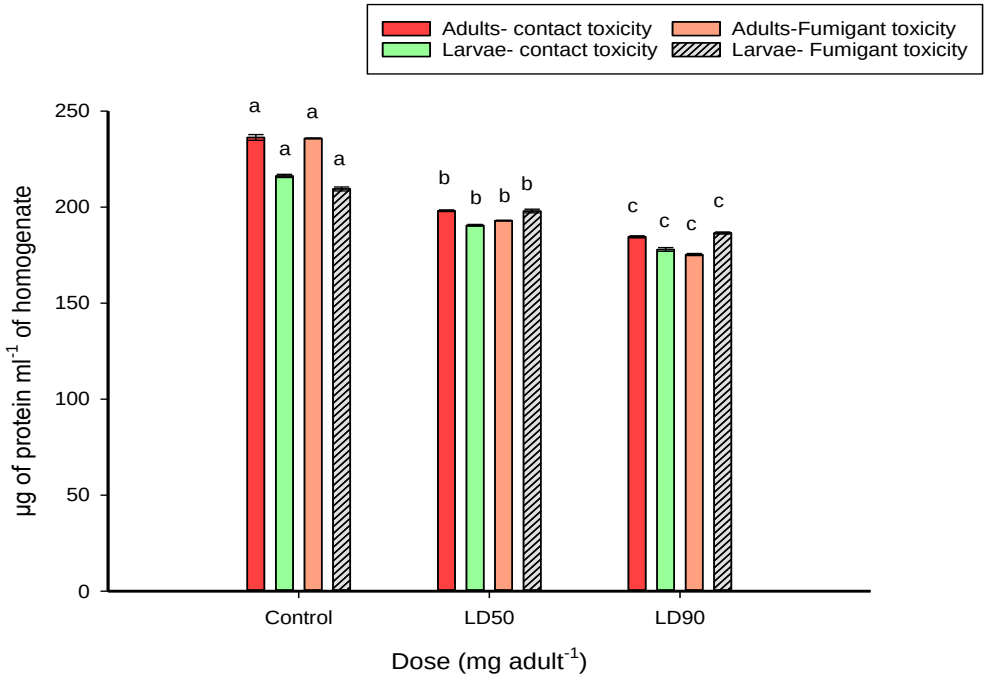


Figure 23: GSH activity (Mean±SE) in *T. casteneum* exposed to petroleum ether derived EOs of *A. annua* at lethal doses. Columns marked with different letters are significantly different (P<0.01; ANOVA and Tukey's honest post-hoc test)

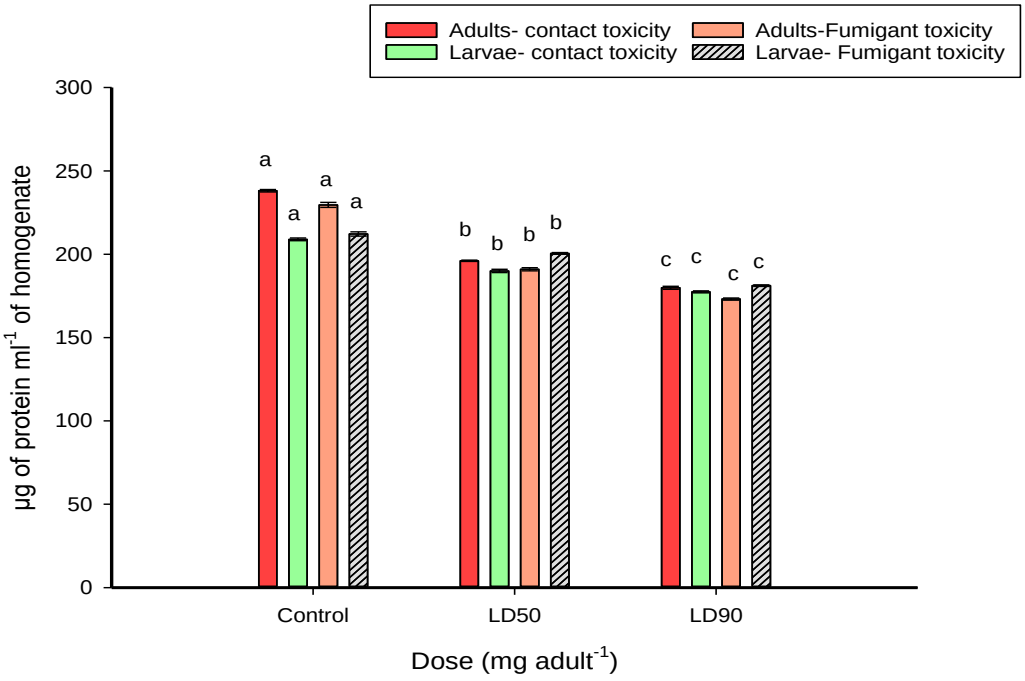


Figure 24: GSH activity (Mean±SE) in *T. casteneum* exposed to n-hexane derived EOs of *A. annua* at lethal doses. Columns marked with different letters are significantly different (P<0.01; ANOVA and Tukey's honest post-hoc test)

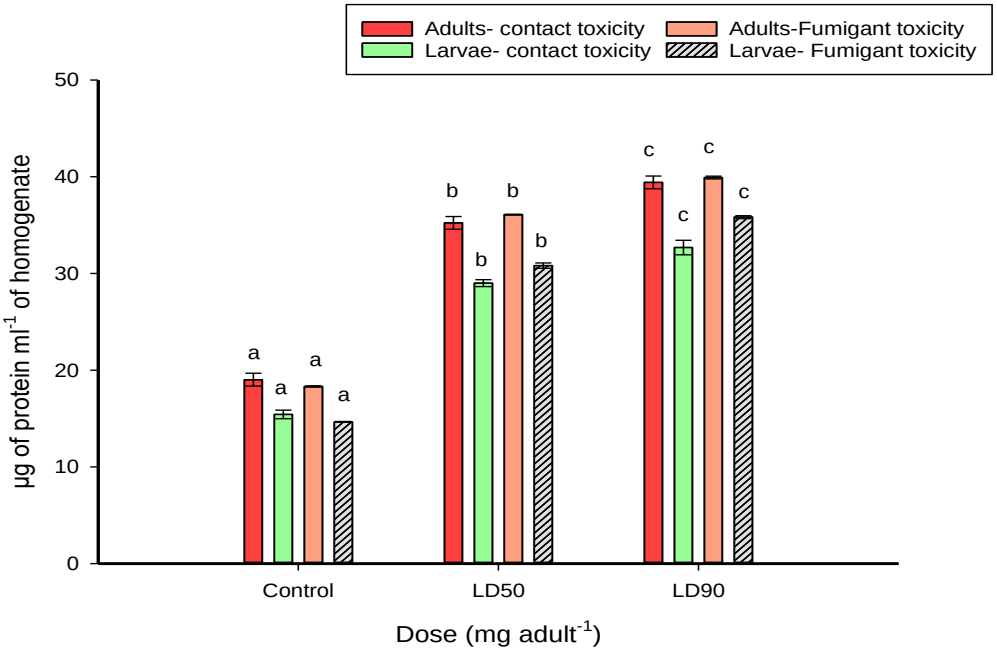


Figure 25: LPO activity (Mean±SE) in *T. casteneum* exposed to methanol derived EOs of *A. annua* at lethal doses. Columns marked with different letters are significantly different (P<0.01; ANOVA and Tukey's honest post-hoc test)

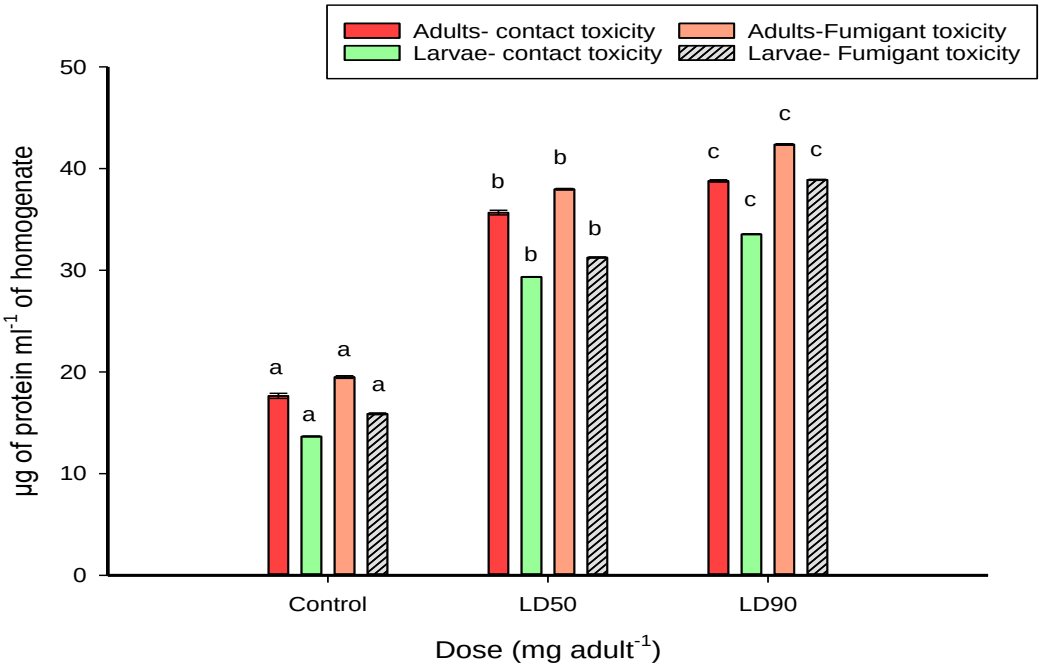


Figure 26: LPO activity (Mean±SE) in *T. casteneum* exposed to chloroform derived EOs of *A. annua* at lethal doses. Columns marked with different letters are significantly different (P<0.01; ANOVA and Tukey's honest post-hoc test)

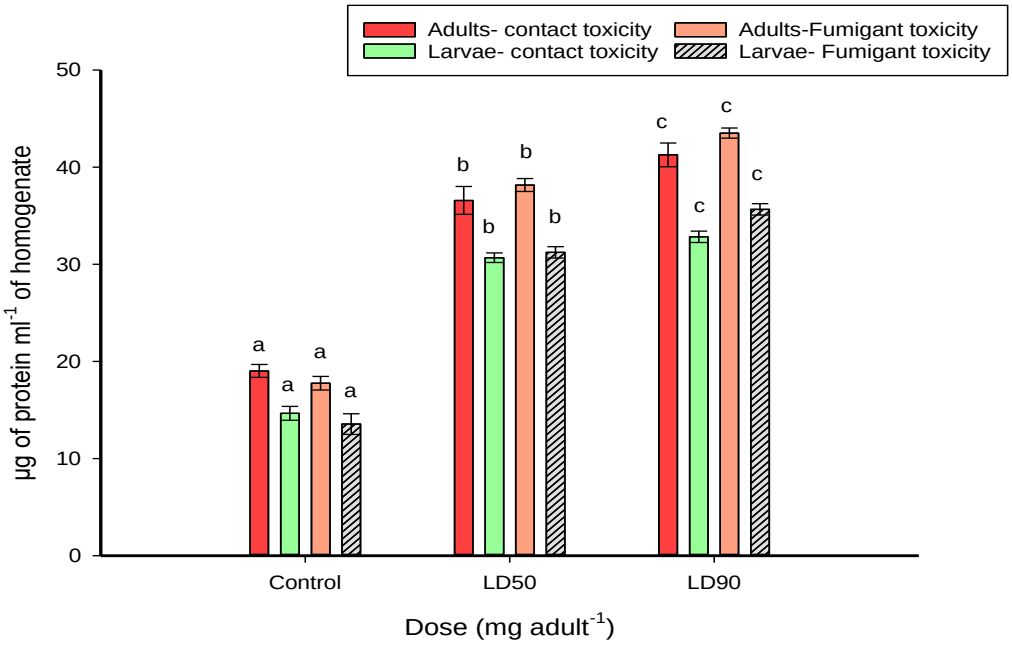


Figure 27: LPO activity (Mean±SE) in *T. casteneum* exposed to petroleum ether derived EOs of *A. annua* at lethal doses. Columns marked with different letters are significantly different (P<0.01; ANOVA and Tukey's honest post-hoc test)

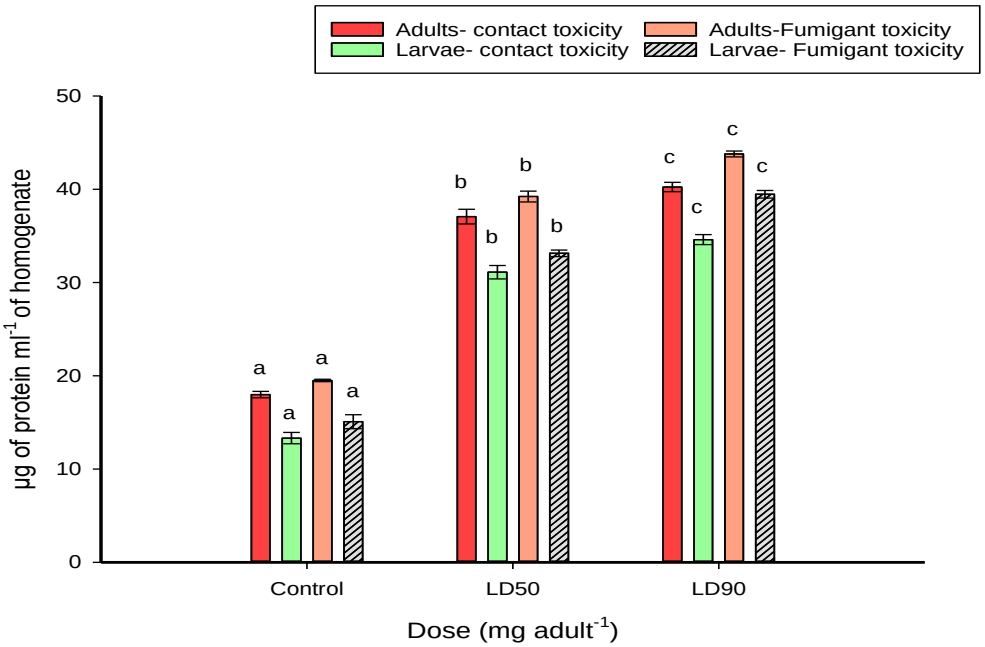


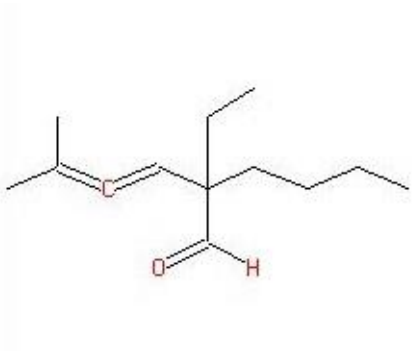
Figure 28: LPO activity (Mean±SE) in *T. casteneum* exposed to n-hexane derived EOs of *A. annua* at lethal doses. Columns marked with different letters are significantly different (P<0.01; ANOVA and Tukey's honest post-hoc test)

A. annua showed potent contact, fumigant and repellent activity against *T. casteneum* with the EOs. However, the insecticidal properties of the EOs varied with solvents and the life stages of the red flour beetle. Hence the curiosity to unveil the chemical groups present in EOs led to the GC-MS

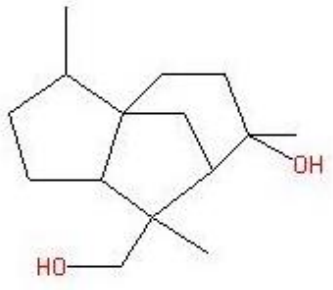
analysis. The previous reports have already dictated the importance of chemical groups in the insecticidal properties of EOs. Based on these studies, the EOs were processed for fractionation and identification of different components present in the oil.

Our results are quite different from the previous reports. The study has verified non-polar solvent 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). *A. annua* is well established for possessing Artemisinin, a potent antimalarial component. However, 1,8 -cineole has emerged as a major insecticidal candidate in various studies (Tripathi et al., 2001). Conversely, GC-MS analyses of the present study have clearly depicted the presence of some novel component in the EOs of *A. annua* in excessively high amount. Oxygenated sesquiterpene was the major chemical group in both the non-polar solvent derived EOs. The group was also reported in the methanol and chloroform EOs, but in very low amount.

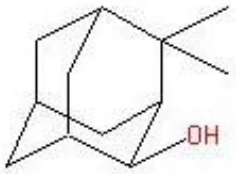
The success of non-polar solvent EOs might be due to the presence of some novel compounds in considerably higher percentage (Figure 29). 20.98% of 3,4- Hexadienal,2- butyl-2-ethyl-5-methyl-, 8.29% of Cedran-diol, 8S,13-, 7.37% of 4,4-Dimethyladamantan-2-ol, 7.20% of Bicyclo(2.2.1)heptan-2-one,7,7-trimethyl-, 7.09% of 2H -1- Benzopyran- 2- one and 6.39% of Deoxyqinghaosu was reported in the n-hexane EOs. The petroleum ether EOs was reported with high percentage of 3,4-Hexadienal, 2- butyl-2-ethyl-5-methyl- (22.06%), Deoxyqinghaosu (10.84%), Aceticacid,(1,2,3,4,5,6,7,8-octahydro-3,8,8-trimethylnaphth-2-yl)methylester (9.68%), 2-Isopropyl-4,4,7a-trimethyl-2,4,5,6,7,7a-hexahydro-benzofuran-6-ol (7.84%), 1-Eicosanol (7.68%) and 2H-1-Benzopyran-2-one (7.03%). On the other hand, chloroform EOs was found to contain Bicyclo(2.2.1)heptan-2-one,1,7,7, trimethyl- (15.35%), 2H-1-Benzopyran-2-one (8.20%), and 3,4-Hexadienal,2- butyl-2-ethyl-5-methyl (10.26%). Very low amount of Deoxyqinghaosu (4.27%) was recorded in the EOs. Methanolic EOs has documented 1-Docosene (29.57%), I-Valine, n-heptafluorobutyryl-, nonylester (22.99%), 3-Methylcyclopentadecylcarbamic acid, and t- butyl ester (12.12%). The lowest level of Deoxyqinghaosu i.e. 1.67% is recorded from the EOs.



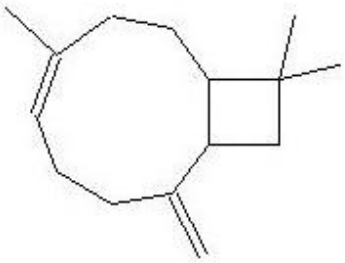
3,4-Hexadienal,2-butyl-2-ethyl-5-methyl-



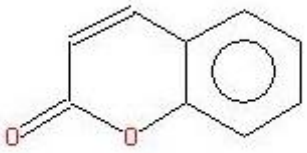
Cedran-diol,8S,13-



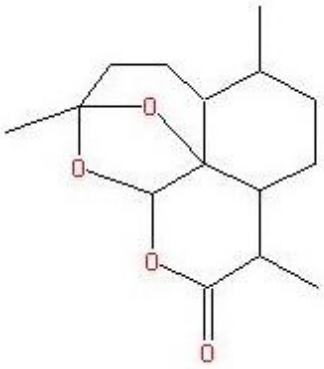
4, 4-Dimethyladamantan-2-ol



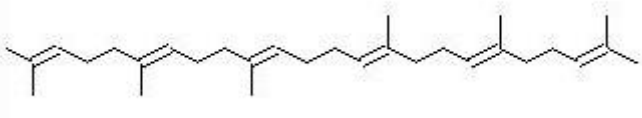
Bicyclo(2.2.1)heptan-2-one,7,7-trimethyl-



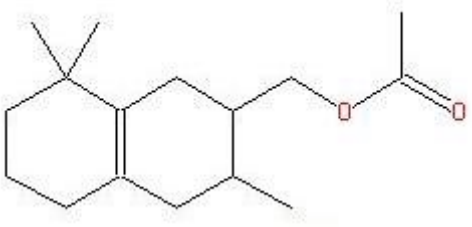
2H -1- Benzopyran- 2- one



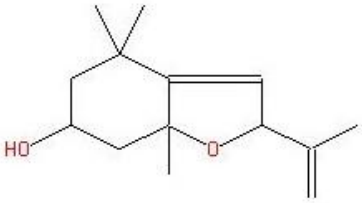
Deoxyqinghaosu



Squalene

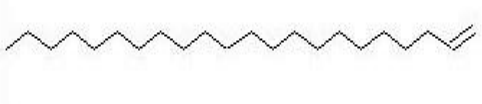


2-Isopropyl-4,4,7a-trimethyl-2,4,5,6,7, octahydro-7a-hexahydro-benzofuran-6-ol

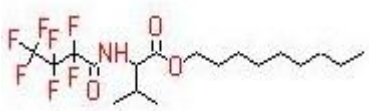


Aceticacid,(1,2,3,4,5,6,7,8-3,8,8-trimethylnaphth-2-yl)methylester

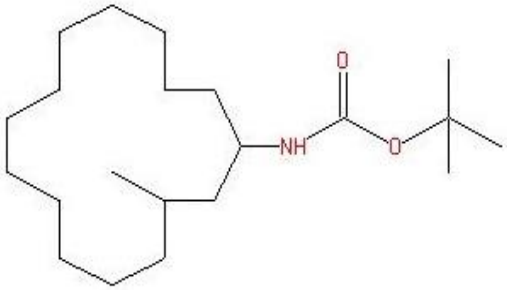
1-Eicosanol



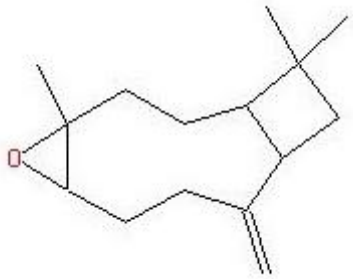
1- drocosene



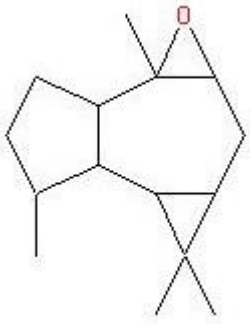
I-Valine, n-heptafluorobutyryl-, nonylester



Methylcyclopentadecylcarbamic acid, and t- butyl ester



Caryophyllene oxide



Isoaromadendrene epoxide

Figure 29: Chemical structures of the major components of EOs of *Artemisia annua* grown in Indian climatic conditions

Studies on *A. annua* EOs deciphered the presence of volatile groups like sesquiterpenes, coumarins, phenolic compounds and flavones (Bora & Sharma, 2011). Among all, 1, 8- Cineole has gained considerable attention for being the core component that is 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 non-polar EOs with different percentage composition and hence, we suggest their possible action in insecticidal activities (Chauhan et al., 2015). Excessive percentage of oxygenated sesquiterpene in the non-polar solvent derived EOs indicates the better pesticidal properties of the oil (Khani & Heydarian, 2014). Moreover, additional components like esters (16.71%) and ethers (4.41%) in the petroleum ether EOs was a major finding in our work. Their presence could be a possibility for higher insecticidal potential in the EOs. The results are supported by various scientists who portrayed EOs as the best management tool against stored grains pests (Sarwar & Salman, 2015) (Bett et al., 2016).

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. The antifeedant bioassay performed with the wheat grain has shown significant reduction in feeding ratio with the increase in doses. Feeding ratio shows steep decrease to almost half at 0.5g dose when compared with the control. Moreover, % weight loss of the wheat grain (whole wheat kernel) was found to be 30.08 in the set which was not supplemented with *Artemisia* leaves. However, % 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 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 showed 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 Padín 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 are processed further for topographical characterization using SEM-EDX. SEM images at 25x magnification clearly depict the structural loss to the damaged grains when compared with the undamaged ones. Moreover, the images at 1000x magnification illustrate the intricacy of structural damage and digestion of starch globules by the pest. These findings conform by the work of (Singh el., 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 EOs extracted with the four different solvents viz. methanol, chloroform, n-hexane and petroleum ether (40-60˚C) has shown significant antifeedant activity. Among the four different solvents used, n-hexane showed the best result with 94.22% antifeedant action at the concentration of 1.67mg disc^{-1} which is followed by the Petroleum ether (90.82%) > chloroform (87.30%) > methanol (85.15%).

The results are consistent with the several reports of earlier researcher. Stefanazzi et al. (2011) 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}. In a similar report by Liu & Ho (1999) has showed 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 with the four

solvents in the concentration ranges of 1-1.67mg disc^{-1} at the end of the third day.

The mortality results were supported by the work of Huang & Ho (1998) where no mortality was reported at the range of 6.8- 13.6 mg of cinnamaldehyde g^{-1} of food. 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* even at the lower concentration of 1mg disc^{-1} when compared with the control. Authors have also worked at the lower concentration range of 0.17-0.83 mg disc^{-1} which showed negative growth rate of the insect and lowered food consumption rate.

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 showed significant reduction in RGR, RCR. Nevertheless, 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 showed significant reduction in ECI. This study suggests that *Artemisia annua* which is used as a potent antimalarial drug can also be a potential grain protectant too due to its antifeedant activity against the major grain pest, *Tribolium casteneum*. Moreover, it poses no threat to environment and non-target animal including human. Hence, further research to design a suitable formulation of the potent synthetic analogues would be a step in the right direction.

Among other parameters, flour colour which has changed drastically within three months is a major concern and supported by the work of (Sánchez-MariñEz et al., 1997). The grey colour of the flour not only reduces its aesthetic value but also poses serious threat to consumption (Gorham, 1979) (Bakula et al., 2011). Hence, the wheat flour is discarded which is very tough to sustain by the developing and under developed countries (Weaver, 1994) (Müller & Krawinkel, 2005). P^H value also deviated from the normal neutral range which is due to the accumulation of uric acid content (Pingale et al., 1954). Moisture content of wheat flour, an important criterion for the quality

assessment protocol, was found to increase significantly in the damaged grains compared to the undamaged ones. It is an indicator of the storage capacity of the grains. High moisture content i.e. >14% attracts infestation causing agents like bacteria, mould, insects etc. Hence, lower moisture content of the grains is preferred. Our result showing a very high level of moisture content of the flour, attributable to the *T. casteneum* infestation, supported by the work of Butt et al. (2004). Moreover, our result depicts the reduction in the protein and carbohydrate contents of the damaged grains compared to the undamaged grains. The outcome is supported by the work of Jood & Kapoor (1993a) and Jood et al. (1993b). In a similar study conducted by Bamaiyi et al. (2006) has reported the heavy nutritional loss of wheat grains by *Callosobruchus maculatus* infestation. Another study has documented the nutritional loss of stored grains like chickpea, green gram and pigeon pea infested with *Callosobruchus chinensis* (Modgil & Mehta, 1996).

Results of the repellency and toxicity assays have proved the efficacy of EOs of *A. annua* against *T casteneum*. Though our research interest revolves around the selection of proper solvent for eluting oil still this is the first report of the *Artemisia annua* EOs against the flour beetles. Further, the biomolecular studies of the treatment sets have confirmed the involvement of EOs on the lethal effect in the form of oxidative stress. Scientists working in the same aspect, majorly emphasizes on the chemical constituents that made up the EOs. However, as seen in the review of literature, individual components are given importance.

The present study has verified the efficacy of petroleum ether and n-hexane derived EOs as a better insecticidal candidate for the control of *T. casteneum* and has added new dimensions to the previous findings (Tripathi et al., 2000). This 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. Presence of Deoxyqinghaosu in the extract was an obvious outcome (Li, 2012) (Ni et al., 2012). Correlated to the fact, relatively higher Percent composition of Deoxyqinghaosu in n-hexane and petroleum ether EO can be attributable to the better repellent activity and was reported for the first time in the present study from filter paper arena tests.

Moreover, 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).

In conclusion, the present study has validated the insecticidal potential of non-polar solvent derived EOs of *A. annua*. Moreover, the identified allelochemicals of EOs are potent in vivo suppressor of life supporting biomolecules in the acute toxicity assays. Hence, there is a potential for these compounds to be used in synergy to interfere with enzyme mediated detoxification process in the target insects. Additionally, by probing the modes of action of fumigants through experiments, future research will evaluate if the enzyme inhibitors can act as synergists by employing potent non-polar solvents to elute EOs from *A. annua* to increase toxicity against other stored grain pests.

"Be stubborn on vision, but flexible on details."

– Jeff Bezos