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# Effect of Plant Extracts on Laboratory Organisms and Biodegrading Agents Found in the Industrial Effluent

Shalim, Rukhshana

University of Rajshahi

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# EFFECT OF PLANT EXTRACTS ON LABORATORY ORGANISMS AND BIODEGRADING AGENTS FOUND IN THE INDUSTRIAL EFFLUENT



A

THESIS

SUBMITTED TO THE INSTITUTE OF ENVIRONMENTAL SCIENCE  
UNIVERSITY OF RAJSHAHI, BANGLADESH  
IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR  
THE DEGREE OF  
DOCTOR OF PHILOSOPHY

Submitted By

**Rukhshana shalim**

B.Sc. (Hons.), M.Sc.

Registration No: 6913

Session: 2008-2009

Fourth Science Building  
Motihaar Green  
June, 2014

Institute of Environmental Science  
University of Rajshahi  
Rajshahi-6205  
BANGLADESH



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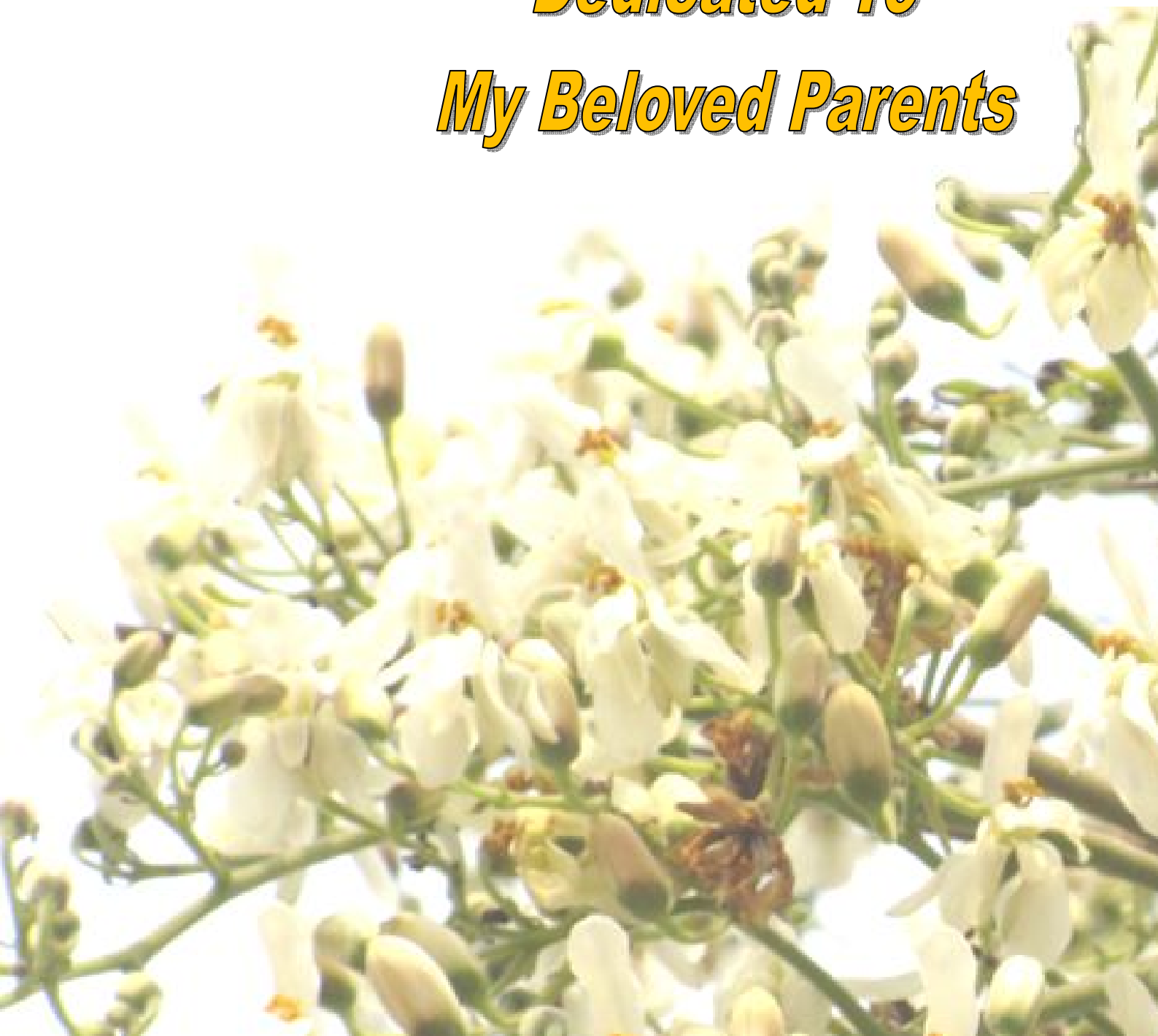
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Institute of Environmental Science  
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***Dedicated To  
My Beloved Parents***



## Declaration

*I hereby declare that the whole work submitted as a thesis entitled "Effect of plant extracts on laboratory organisms and biodegrading agents found in the industrial effluent" to the Institute of Environmental Science, University of Rajshahi for the Degree Doctor of Philosophy is the result of my own investigation. The thesis contains no materials which have been accepted for the award of any other degree or diploma elsewhere except, where due reference is made in the text.*

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## Certificate

*It is my pleasure to certify that the thesis entitled, “**Effect of plant extracts on laboratory organisms and biodegrading agents found in the industrial effluent**” submitted to the Institute of Environmental Science, University of Rajshahi by **Rukhshana Shalim** in partial fulfillment of the requirements for the degree of Doctor of Philosophy is a dissertation of the perfect study which she carried out in my laboratory with much success. It contains no materials previously published or written by any other person except, wherever, due references are made in the text of the thesis.*

*I hereby clarify that the authoress completed her work mostly under my direct supervision and contributed some new ideas and openings in our research field by adding most recent information in this line of the contemporary world.*

June, 2014  
Rajshahi University  
Rajshahi-1205

(Dr. Md. Nurul Islam)



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June, 2014

*Rukhshana Shalim*

## ABSTRACT

To determine whether or not the plant extracts affecting biodegrading agents that normalize industrial effluent sample plants known as bionormalizers *i.e.* *Carica papaya* Linn., *Moringa oliefera* Lam. and *Musa sapientum* L. were taken into consideration through dose mortality against the stored grain pest *Tribolium castaneum* (Hbst.), cytotoxicity against the brine shrimp *Artemia salina* nauplii and antibacterial activity against the 9 bacterial isolates from the tannery effluent and 7 certain other bacteria as laboratory test agents were done. Leaf, stem and roots of *C. papaya*; fruit, leaf, stem bark, stem wood, root bark and root wood of *M. oliefera*, and leaf, stem and roots of *Mu. sapientum* were extracted in petroleum ether, chloroform and methanol.

For *C. papaya* extracts against *T. castaneum* beetles the dose mortality was done through residual film assay to yield the highest and the lowest mortality for the CH<sub>3</sub>OH extract of roots (LD<sub>50</sub> 0.114mg cm<sup>-2</sup>) and CHCl<sub>3</sub> extracts of stem (LD<sub>50</sub> 2.053mg cm<sup>-2</sup>) after 48h of exposure, while the CHCl<sub>3</sub> extracts of leaf and root didn't offer any mortality to the beetles. According to the intensity of activity observed through dose mortality test against the adult beetles the potentiality of the Pet.E., CHCl<sub>3</sub> and CH<sub>3</sub>OH extracts could be arranged in a descending order: root (CH<sub>3</sub>OH) > stem (CH<sub>3</sub>OH) > leaf (CH<sub>3</sub>OH) > root (Pet.E.) > leaf (Pet.E.) > stem (Pet.E.) > stem (CHCl<sub>3</sub>) extract. For *M. oliefera* the highest and the lowest mortality have been observed for the CH<sub>3</sub>OH extract of root bark (LD<sub>50</sub> 0.276mg cm<sup>-2</sup>) and Pet.E. extract of root wood (LD<sub>50</sub> 0.629mg cm<sup>-2</sup>) after 48h of exposure. According to the intensity of activity observed through mortality of the adult beetles the potentiality of the Pet.E. and methanol extracts could be arranged in a descending order: root bark (CH<sub>3</sub>OH) > stem bark (CH<sub>3</sub>OH) > root bark (Pet.E.) > fruit (CH<sub>3</sub>OH) > root wood (CH<sub>3</sub>OH) > stem bark (Pet.E.) > stem wood (CH<sub>3</sub>OH) > root wood (Pet.E.) extract. For *Mu. sapientum* the highest and the lowest mortality have been observed for the CH<sub>3</sub>OH extract of stem (LD<sub>50</sub> 0.163mg cm<sup>-2</sup>) and Pet.E. extract of leaf (LD<sub>50</sub> 1.195mg cm<sup>-2</sup>) after 48h of exposure. According to the intensity of activity observed through

mortality of the adult beetles the potentiality of the extracts could be arranged in a descending order: stem (CH<sub>3</sub>OH) > root (CH<sub>3</sub>OH) > leaf (CH<sub>3</sub>OH) > stem (Pet.E.) > root (Pet.E.) > leaf (Pet.E.) extract.

For *C. papaya* against the *A. salina* nauplii the highest and the lowest mortality have been observed for the CHCl<sub>3</sub> extract of leaf (LC<sub>50</sub> 1.326ppm) and CH<sub>3</sub>OH extracts of leaf (LC<sub>50</sub> 183.443ppm) after 24h of exposure. According to the intensity of activity observed through dose mortality test against the adult beetles the potentiality of the Pet.E., CHCl<sub>3</sub> and CH<sub>3</sub>OH extracts could be arranged in a descending order: leaf (CHCl<sub>3</sub>) > root (CH<sub>3</sub>OH) > stem (CHCl<sub>3</sub>) > stem (Pet.E.) > stem (CH<sub>3</sub>OH) > root (CHCl<sub>3</sub>) > root (Pet.E.) > leaf (Pet.E.) > leaf (CH<sub>3</sub>OH) extract. For *M. oliefera* the highest and the lowest mortality have been observed from the CHCl<sub>3</sub> extract of root bark (LC<sub>50</sub> 4.197ppm) and CH<sub>3</sub>OH extracts of fruit (LC<sub>50</sub> 234.246ppm) after 24h of exposure respectively. According to the intensity of activity observed through dose mortality test against the adult beetles the potentiality of the Pet.E., CHCl<sub>3</sub> and CH<sub>3</sub>OH extracts could be arranged in a descending order: root bark (CHCl<sub>3</sub>) > root bark (CH<sub>3</sub>OH) > stem wood (CHCl<sub>3</sub>) > stem wood (CH<sub>3</sub>OH) > root wood (CHCl<sub>3</sub>) > stem wood (Pet.E.) > stem bark (CHCl<sub>3</sub>) > fruit (Pet.E.) > root bark (Pet.E.) > fruit (CHCl<sub>3</sub>) > root wood (Pet.E.) > fruit (CH<sub>3</sub>OH) > stem bark (CH<sub>3</sub>OH) > leaf (Pet.E.) > root wood (CH<sub>3</sub>OH) > leaf (CH<sub>3</sub>OH) extract. For *Mu. sapientum* the highest and the lowest mortality have been observed from the CH<sub>3</sub>OH extract of leaf bark (LC<sub>50</sub> 22.991ppm) and Pet.E. extract of leaf (LC<sub>50</sub> 127.604ppm) after 24h of exposure. According to the intensity of activity observed through dose mortality test against the adult beetles the potentiality of the Pet.E., CHCl<sub>3</sub> and CH<sub>3</sub>OH extracts could be arranged in a descending order: leaf (CH<sub>3</sub>OH) > leaf (CHCl<sub>3</sub>) > stem (CHCl<sub>3</sub>) > root (Pet.E.) > stem (Pet.E.) > root (CHCl<sub>3</sub>) > root (CH<sub>3</sub>OH) > stem (CH<sub>3</sub>OH) > leaf (Pet.E.) extract.

Bacterial strains were isolated from tannery effluent. Fifteen colonies were screened from initial level of effluent on nutrient agar media. Out of fifteen colonies, nine isolates were selected for biochemical test and other studies. Selected isolates were: two isolates from sample 1 (isolate 1 and 2), four from sample 2 (isolate 3, 4, 5 and 6) and three from sample 3 (isolate 7, 8 and 9). The isolates were determined as

1. *Bacillus cereus*, 2. *Klebsiella oxytoca*, 3. *Staphylococcus aureus*, 4. *Escherichia coli* (I), 5. *Escherichia coli* (II) 6. *Citrobacter freundii*, 7. *Proteus vulgaris*, 8. *Bacillus subtilis*, 9. *Salmonella typhimurium*. Physico-chemical characteristics of the tannery effluent were also determined along with the characterization of the found bacterial isolates.

The Pet.E., CHCl<sub>3</sub> and CH<sub>3</sub>OH extract of *C. papaya* (leaf, stem and root), *M. oleifera* (fruit, leaf, stem bark, stem wood, root bark and root wood) and *Mu. sapientum* (leaf, stem and root) were tested against 7 selected bacteria (2 Gram positive bacteria *Bacillus subtilis*, *Staphylococcus aureus* and 5 Gram negative bacteria *Escherichia coli*, *Klebsiella pneumoniae*, *Salmonella enteritidis*, *Shigella flexneri* and *Shigella sonnei*) to evaluate their antibacterial potential at a concentration of 200 and 400µg disc<sup>-1</sup> along with a standard antibiotic, Ampicillin 10µg disc<sup>-1</sup> and 9 isolates (from industrial effluent) to evaluate their antibacterial potential at a concentration of 400µg disc<sup>-1</sup> along with a standard antibiotic, Kanamycin 30µg disc<sup>-1</sup>.

Among the *C. papaya* extracts the root extracts showed the highest antibacterial activity. Only the *B. subtilis* (roots/CHCl<sub>3</sub> gave 20mm diam. for 400µg disc<sup>-1</sup>), *K. pneumoniae* (stem/CHCl<sub>3</sub> gave 09mm in diam. for 400µg disc<sup>-1</sup>) and *St. aureus* (leaf/Pet.E. gave 09mm in diam, stem/CHCl<sub>3</sub> gave 10mm in diam., roots/Pet.E. and CH<sub>3</sub>OH gave 15mm and 10mm in diam. respectively, while all the tests were for 400µg disc<sup>-1</sup>) were responsive among the selected test bacteria. Among the *M. oleifera* extracts the stem wood extracts showed the highest antibacterial activity. Only the (fruit/Pet.E. and stem bark/CHCl<sub>3</sub> both gave 09mm in diam. for 400µg disc<sup>-1</sup>), *K. pneumoniae* (fruit and stem wood/CHCl<sub>3</sub> gave 11 and 10mm in diam. both for 400µg disc<sup>-1</sup>) and *St. aureus* (fruit/Pet.E. and CHCl<sub>3</sub> gave 10mm and 09mm in diam., leaf/ Pet.E. gave 09mm in diam., and stem bark/Pet.E. and CH<sub>3</sub>OH gave 09 and 10mm in diam. respectively, stem wood/Pet.E. and CH<sub>3</sub>OH gave 10 and 12mm in diam. respectively, root bark/Pet.E. and CHCl<sub>3</sub> gave 11mm and 09mm in diam., and root wood/CHCl<sub>3</sub> gave 10mm in diam., while all the tests were for 400µg disc<sup>-1</sup>) were responsive among the selected test bacteria. Among the *Mu. sapientum* extracts the root extracts showed the highest antibacterial activity. Only the *St. aureus* (stem and roots/Pet.E. gave 08mm and 10mm in diam., for 400µg disc<sup>-1</sup>) were responsive among

the selected test bacteria. According to the susceptibility test bacteria could be arranged in a descending order of *St. aureus* > *B. subtilis* > *K. pneumoniae*.

The Pet.E., CHCl<sub>3</sub> and CH<sub>3</sub>OH extract of *C. papaya* (leaf, stem and root), *M. oliefera* (fruit, leaf, stem bark, stem wood, root bark and root wood) and *Mu. sapientum* (leaf, stem and root) were tested against 9 isolates viz. 1. *Bacillus cereus*, 2. *Klebsiella oxytoca*, 3. *Staphylococcus aureus*, 4. *Escherichia coli* (I), 5. *Escherichia coli* (II), (for the isolates 4 and 5 the strains were different but determination was not done) 6. *Citrobacter freundii*, 7. *Proteus vulgaris*, 8. *Bacillus subtilis*, 9. *Salmonella typhimurium*. Among the 9 Isolates Isolate 8 *B. subtilis* was highly responsive to the Pet.E. and CHCl<sub>3</sub> extracts of *C. papaya* stem (15 and 08mm), *M. oliefera* fruit (14 and 09mm) and root bark (10 and 16mm), and *Mu. sapientum* root (10 and 10mm) respectively; to the Pet.E. extracts of *C. papaya* root (11mm), *M. oliefera* root wood (11mm), *Mu. sapientum* stem (10mm), and to the CHCl<sub>3</sub> extract of *M. oliefera* stem bark (08mm). Next to the Isolate 8 it was Isolate 2 *K. oxytoca* responsive to the Pet.E. extract of *C. papaya* leaf (08mm), *M. oliefera* fruit (08mm), *Mu. sapientum* leaf (15mm) and root (10mm); and to the CHCl<sub>3</sub> extract of *C. papaya* leaf (08mm), *M. oliefera* stem bark (08mm), stem wood (08mm) and root bark (08mm); followed by the Isolate 1 *B. cereus* which was responsive to the Pet.E. extracts of *C. papaya* stem (16mm), *M. oliefera* fruit (10mm) and *Mu. sapientum* stem (8mm) and root (10mm); this was followed by Isolate 3 *St. aureus* which was responsive to the Pet.E. extracts of *C. papaya* stem (10mm), *M. oliefera* fruit (13mm) and *Mu. sapientum* root (08mm) and again the CHCl<sub>3</sub> extract of *M. oliefera* stem bark (08mm). This was followed by the Isolate 6 *Citrobacter freundii* where CHCl<sub>3</sub> extract of *C. papaya* stem (08mm) and Pet.E. extract of *Mu. sapientum* leaf (10mm) were found responsive. Isolate 4 *E. coli* (I) and 5 *E. coli* (II) (but different strains) and 9 *Sa. typhimurium* show response against Pet.E. extract of *C. papaya* stem (08mm), *M. oliefera* fruit (08mm) and CHCl<sub>3</sub> extract of *M. oliefera* stem wood (09mm) respectively. According to the susceptibility test bacteria could be arranged in a descending order of *B. subtilis* > *K. oxytoca* > *St. aureus* = *B. cereus* > *Citrobacter freundii* > *E. coli* (I) = *E. coli* (II) = *Sa. typhimurium*. Isolate 7 *P. vulgaris* was not responsive to any of the 12 extracts of the

3 test plants. For Kanamycin 30 $\mu$ g disc<sup>-1</sup> the inhibition zones for the Isolate 1, 2,3, 4, 5, 6, 7, 8 and 9 were 50, 35, 35, 40, 36, 42, 42, 55 and 40mm respectively. The differences of the clear zones given by Ampicillin 10 $\mu$ g disc<sup>-1</sup>(tested against the 7 bacteria) and Kanamycin 30 $\mu$ g disc<sup>-1</sup> (tested against the 9 isolates) were nearly the same in majority of the test cases.

The bacterial isolates found in the industrial effluent, *i.e.* *B. subtilis*, *St. aureus*, one of the *E coli* strains, another species of *Klebsiella*, *K. oxytoca*, another species of *Salmonella*, *Sa. typhimurium* were among the list of available 7 test bacteria of the Institute of Environmental Science (IES), R.U. and the response in that separate experiments nearly resembles the results got against the 9 isolates carried out afterwards. However, *E coli* among the 7 didn't show any response at all. The activity of the Pet.E., CHCl<sub>3</sub> and CH<sub>3</sub>OH extract of *C. papaya* (leaf, stem and root), *M. oliefera* (fruit, leaf, stem bark, stem wood, root bark and root wood) and *Mu. sapientum* (leaf, stem and root) were mild in activity in general, and *P. vulgaris*, *Sa. typhimurium*, both *E. coli* and *Citrobacter freundii* didn't give any considerable clear zone. Thus, it could be mentioned that *C. papaya*, *M. oliefera* and *Mu. sapientum* plant materials are not so much effective against the biodegrading bacteria and obviously they are helpful in biodegradation of industrial effluents causing very insignificant harm to biodegrading bacteria.



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## Abbreviations OF THE SPECIAL WORDS USED IN THE TEXT

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# U	=	Number of insects used	i.e.	=	That is
h	=	Hour(s)	% kill	=	Insects killed per cent
Fig.	=	Figure	# Kill	=	Number of insects killed
CHCl <sub>3</sub>	=	Chloroform	$\chi^2$	=	Chi-square
CH <sub>3</sub> OH	=	Methanol	LC <sub>50</sub>	=	Concentration required to kill 50% of test organisms
Pet.E.	=	Petroleum ether	LD <sub>50</sub>	=	Dose required to kill 50% of test organisms
diam	=	Diameter	$\mu$ g	=	Microgram
DMSO	=	Deuteromethyl sulphoxide	$\mu$ l	=	Microliter
df	=	Degree of freedom	mg	=	Milligram(s)
Cr %	=	Corrected mortality percentage	ml	=	Milliliter
Emp Probit	=	Empirical Probit	mm	=	Milimeter
<i>et al.</i> ,		And others (author)	cm <sup>2</sup>	=	Centimeter square
Expt Probit	=	Expected Probit	Weight	=	Weighting coefficient
TSI	=	Triple Sugar Iron	ppm	=	Parts Per Million

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## Chapter 1: Introduction

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### 1.3. Aims and Objectives of the research work

# INTRODUCTION

## 1. General Introduction

A plant is a bioreactor, since functionally it's a synthetic laboratory that produces different biomolecules as metabolites. Common among these metabolites are compounds with protective action against insects, such as alkaloids, non-proteic amino acids, steroids, phenols, flavonoids, glycosids, glucosinolates, quinones, tannins and terpenoids. Since plants may contain hundreds or even thousands of metabolites, there is currently a resurgence of interest in the vegetable kingdom as a possible source of new lead compounds for introduction into the therapeutical screening programs (Hostettmann *et al.*, 1995).

Amongst the most promising of the natural products investigated to date are the metabolites. Although only about 10,000 secondary plant metabolites have been chemically identified, the total number of plant chemicals may exceed 400,000. There are a vast commucopia of defense chemicals, comprising repellents, feeding and oviposition deterrents, growth inhibitors, sterilants, toxicants, etc. (Champagne *et al.*, 1989). Plant derived materials or phytochemicals, which once formed the basis of pest control technology, are again being scrutinized for potentially useful products or as models for new classes of insecticides (Kubo and Nakanishi, 1978; Champagne *et al.*, 1989; Klocke, 1989; Nawrot and Harmatha, 1994).

Massive use of these insecticides has had a long and difficult road because the earliest data gathering done by researchers among farmers and natives revealed a lot of practices based on superstition, which, when tested by scientific methods were not shown to be effective. After the World War II few plant and plant extracts replaced synthetic insecticides that had shown promising effects, and were of widespread use. When synthetic insecticides appeared in the 1940's some people thought that botanical insecticides would disappear forever but problems like environmental contamination, residues in food and feed and pest resistance brought them back to the fore. There is no

doubt botanical insecticides are an interesting alternative to insect pest control, and on the other hand only a few of the more than 250,000 plant species on our planet have been properly evaluated for this purpose. Moreover, botanicals, because of their low-mammalian toxicity, have received much attention as control agents against pests. For example, in 1950, Heal *et al.* reported approximately 2,500 plants in 247 families with some sort of toxic property against insects. But to use them it is not enough that the plant be considered as promising or even with proven insecticidal properties. According to Feinsein (1952), over 2000 species of plants representing 170 odd families are said to have some insecticidal values. There are many species of plants of these families in Bangladesh that are used as traditional medicine by the native people from the remotest antiquity. There are a lot of publications with lists of plants with insecticidal properties.

Biodegradation is a natural phenomenon where certain microorganisms play a vital role in the degradation procedure, however not being acquainted with synthetic chemicals that are dumped in the regular wastes the degradation process is being hampered and thereby environmental pollution takes place. Within the past few decades the world advanced rapidly with remarkable development in all sectors of science and technology and still going through heavy industrialization and industry-based commerce. It is remarkable now the extent of pollution that took place in different parts of the biosphere due to presence of non-degradable residual agents in the synthetic raw materials that are used in different industries. So, a worldwide awareness has been created for the investigation of nature-friendly substances or such other essential agents to run the developmental works, as well as to investigate biodegrading agents to disintegrate the pollutants already being deposited in the environment.

Industrialization in Bangladesh is an increasing phenomenon since 1960s that opened ever-growing volumes of industrial effluents that result in the widespread pollution of water and soil areas of the country. Despite taking safety precautions these industrial wastes are released to the nature. It is very common that nature itself possesses an efficient system for the recovery of polluted areas by means of microorganisms capable of assimilating the hydrocarbons of oils and grease materials. However, the micro-flora of nature is no longer capable of effectively recovering thousand tons of industrial effluents finding every year their way into the environment. As a result, like other



countries the people of Bangladesh also have found themselves under the real threat of ecological disaster. Among the industrial effluents oils, oil derivatives, detergents and dye are the major part to play the key role in causing pollution. It is now necessary to consider non-hazardous components for the industries that might not hamper the biodegrading micro-organisms. Analysis of the development of biotechnologies relating to the recovery of polluted water and soil ecosystems and equipment enables one to come to the conclusion that the processes based on the byproduct biodegradation under the action of microorganisms are ecologically efficient.

The pollution of the environment with toxic heavy metals is spreading throughout the world along with industrial progress. Microorganisms and microbial products can be highly efficient bioaccumulations of soluble and particulate forms of metals especially dilute external solutions. Microbes related technologies may provide an alternative or addition to conventional method of metal removal or metal recovery (Selvi *et al.*, 2012). In the past few decades, uncontrolled urbanization has caused a serious pollution problem due to the disposal of sewage and industrial effluents to water bodies. Unlike many other pollutants, heavy metals are difficult to remove from the environment (Ren *et al.*, 2009). Microbes play massive role in the bio-geochemical cycling of toxic heavy metals and also in cleaning up or remediating metal-contaminated environments. Microorganisms have acquired a variety of mechanisms for adaptation to the presence of toxic heavy metals (Rajbanshi, 2008).

Tanning is one of the oldest industries in the world. During ancient times, tanning activities were organized to meet the local demands of leather footwear, drums and musical instruments. With the growth of population, the increasing requirement of leather and its products led to the establishment of large commercial tanneries. Tanneries are typically characterized as pollution intensive industrial complexes which generate widely varying, high-strength wastewaters. Tannery wastewaters are highly complex and characterized by high contents of organic, inorganic and nitrogenous compounds, chromium, sulfides, suspended solids and dissolved solids (Durai and Rajasimman, 2011). Biological treatment of wastewater is evaluated as a good treatment method for industrial effluents. Treatment of wastes with bacteria involves the stabilization of waste by decomposing them into harmless inorganic solids either by

aerobic or anaerobic process. Biological processes play a major role in the removal of contaminants and they take advantage of the astonishing catabolic versatility of microorganisms to either degrade or to convert such compounds.

Bacterial biomass can be used as an economical option for removing chromium from the effluent by reduction and bioaccumulation. Microorganisms are advantageous for metal detoxification as they are easy to grow, resulting in a rapid production of biomass, and are part of natural environment (Faryal *et al.*, 2007). It is therefore advantageous to develop a bioprocess utilizing selected indigenous microbes that are both Cr<sup>6+</sup> resistant and Cr<sup>6+</sup> reducing.

In the wake of industrialization, consequent urbanization and ever increasing population, the basic amenities of life *viz.* air, water and land are being polluted continuously (Chhikara and Dhankhar, 2008). Leather tanning is one of the main sectors in Bangladesh's leather industry. Large amounts of chrome powder and chrome liquor are used during tanning process. More than 1,70,000 tons of chromium wastes are discharged to the environment annually as a consequence of industrial manufacturing activities (Kamaludeen *et al.*, 2003). Due to its high oxidation potential, it can easily penetrate biological membranes and cause health hazards (Chaudhary *et al.*, 2003). Feeds and fertilizer production from tanned skin-cut wastes (SCW) is the most direct phenomenon of chromium eco-toxicity leading to food chain contamination in Bangladesh (Rafiqullah *et al.*, 2008). The SCW is protein-rich and indiscriminately used to produce poultry and fish feeds, and organic fertilizer. It is reported that feed ingredients produced from SCW contained chromium at levels as high as 2.49% (Hossain *et al.*, 2007).

In the early reviews many kinds of bacteria were found that they live in the industrial effluent, and many kinds of dyes and heavy metals are released there. Bacteria live there and dissolve them. Bacterial strains ZA-6, W-61, KS-2 and KS-14 were isolated from agricultural soil irrigated with tannery effluents and subsequently identified by 16S rDNA sequencing as *Stenotrophomonas maltophilia*, *Staphylococcus gallinarum*, *Pantoea* sp. and *Aeromonas* sp., respectively. All isolates were examined for their resistance to hexavalent chromium and other heavy metal ions. These results suggest that chromate resistance and reduction in these bacteria are related (Alam and Ahmad, 2012).

A chromium resistant bacterial strain KUCr1 (*Bacillus firmus*) exhibiting potential Cr(VI) reducing ability under in vitro aerobic condition is reported. The bacterial strain showed varied degree of resistance to different heavy metal (Sau *et al.*, 2008). The bacterial isolates (*Bacillus* sp. JDM-2-1 and *Staphylococcus capitis*) can be exploited for the bioremediation of hexavalent chromium containing wastes, since they seem to have a potential to reduce the toxic hexavalent form to its nontoxic trivalent form (Zahoor and Rehman, 2009). *Bacillus* sp. ev3 could reduce 91% of chromium from the medium after 96 h and was also capable to reduce 84% chromium from the industrial effluents after 144h (Rehman *et al.*, 2008). Hexavalent chromium reduction and accumulation by *Acinetobacter* AB1 isolated from Fez tanneries effluents were tested. This strain was able to resist to concentrations as high as 400mg L<sup>-1</sup> of Cr(VI) (Essahale *et al.*, 2012). Three bacterial strains were isolated from effluents of leather (CMBL Cr13, CMBL Cr14) and steel (CMBL Cr15) industries for their possible use in Cr(VI) detoxification of industrial waste. Cr(VI) reducing ability of the three strains ranged from 70 to 80% after 3 days of incubation. The possible use of these bacteria in environmental cleanup is discussed by Shakoori *et al.* (1999). The tested tannery effluents had extremely high levels of all the tested parameters indicating high pollution potential, dangerous effects on the receiving environments and creating many treatment difficulties. Treatment of the tannery effluent was a function of time and bacterial species *Pseudomonas stutzeri* considered the most, while *Bacillus* sp. considered the least efficient in the treatment of tannery wastewater (El-Bestawy, 2013).

The pollution of the environment with toxic heavy metals is spreading throughout the world along with industrial progress. Microorganisms and microbial products can be highly efficient bioaccumulations of soluble and particulate forms of metals especially dilute external solutions. The five isolates were selected based on high level of heavy metal and antibiotic resistances. On the basis of morphological, biochemical analysis revealed that, the isolates were authentically identified as *Escherichia coli*, *Bacillus* spp., *Pseudomonas* spp., *Flavobacterium* spp. and *Alcaligenes* spp. The identified isolates were resistant to zinc (Zn), copper (Cu), chromium (Cr), mercury (Hg) and lead (Pb). These identified heavy metal resistant bacteria could useful for the bioremediation of heavy metal contaminated tannery effluents (Selvi *et al.*, 2012). Chromate tolerant heterotrophs and coliforms found in tannery effluent (Verma *et al.*, 2001). A strain

CSCr-3 with high Cr(VI) reducing ability under alkaline conditions was isolated from a chromium landfill and identified as *Ochrobactrum* sp. on the basis of 16S rRNA gene sequence analysis. The cells were rod shaped, Gram-negative and motile (He *et al.*, 2009). Waste water containing chromium ( $\text{Cr}^{6+}$ ) is by far the most important environmental challenge being faced. A total of 35 isolates have been selected as potential organism belonging to the species of *Moraxella* (14.3%), *Bacillus* (11.43%), *Streptococcus* (25.72%), *Staphylococcus* (5.7%), *Salmonella* (12.3%), *E. coli* (13.3%), *Enterobacter* (11.3%), *Hafnia alvei* (2.45%) and *Alcaligenes* (3.5%). The selected isolates were able to tolerate at least  $500\text{mg L}^{-1}$  of  $\text{Cr}^{6+}$  (Fakruddin *et al.*, 2009). Cr(VI) is a toxic, soluble environmental contaminant. Some bacteria are able to reduce hexavalent chromium to the insoluble and less toxic Cr(III), and thus chromate bioremediation is of considerable interest. An indigenous chromium-reducing bacterial strain, Rb-2, isolated from a tannery water sample, was identified as *Ochrobactrum intermedium*. on the basis of 16S rRNA gene sequencing. This bacterial strain can be useful for Cr(VI) detoxification under a wide range of environmental conditions (Batool *et al.*, 2012; Maruf *et al.*, 2012). High salinity (1–10% w/v) of tannery wastewater makes it difficult to be treated by conventional biological treatment. Salt tolerant microbes can adapt to these saline conditions and degrade the organics in saline wastewater. Four salt tolerant bacterial strains isolated from marine and tannery saline wastewater samples were identified as *Pseudomonas aeruginosa*, *Bacillus flexus*, *Exiguobacterium homiense* and *Staphylococcus aureus* (Sivaprakasam *et al.*, 2008).

Leather, a traditional export item of Bangladesh, enjoys a good reputation worldwide for its quality. This sector plays a significant role in the economy of Bangladesh in terms of its contribution to export and domestic market (Sharif and Mainuddin, 2003). In Bangladesh, tanning or the process of making leather is mostly carried out in the South-Western region of Dhaka city, occupying 25 hectares of land at Hazaribagh, where about 90% of tannery industries of Bangladesh are located. Due to lack of appropriate waste management practices, both solid wastes and liquid effluents from these industries are deposited at different low-lying locations of Hazaribag without proper treatments (Zahid *et al.*, 2006). Components of these wastes include rotten flesh, fat, blood and skin, toxic chemicals, dissolved lime, chromium sulfate, alkali, hydrogen sulfide, sulfuric acid, bleach, dyes, oil, formic acid, heavy metals, suspended solids,

organic matters and so on (Bhuiyan *et al.*, 2011; Zahid *et al.*, 2006). Consequently, soil sediment, groundwater and surface water of nearby the river Buriganga and the Turag are polluted heavily through percolation of the leachate from these dumping sites (Zahid *et al.*, 2006). Despite the toxic chemical load, a number of bacterial species are found to be abundant in the microflora of tannery effluents (Lefebvre *et al.*, 2006; Tripathi *et al.*, 2011). While the details of the mechanism of resistance of this microflora to toxic chemicals are yet to be deciphered, three possible mechanisms are likely to operate separately or in combination: a) efflux systems can reduce toxic chemical loads b) toxic chemicals can be degraded or converted to less toxic forms; c) toxic chemicals can be sequestered into complex compounds or compartments (Maruf *et al.*, 2012). In accordance with known bio-technologies, the polluted medium may be exposed to the action of biological preparation; including microorganisms either in the form of pure isolated cultures or pools of microorganisms, that is to say a combination or association of two or more organisms. To accelerate the degradation some plant materials may be used keeping these pools functional, however it is important to observe whether the bio-normalizing plant materials destroy them or not.

Therefore, the present study was planned on the isolation and characterization of bacterial strains from the tannery effluent and compares their response in the test plant extracts. In this investigation plant extracts were also tested to evaluate their efficacy against the laboratory organisms, *i.e.*, *Tribolium castaneum* (Hbst.) a stored product pest and *Artemia salina* (L.), the recognisable agent for cytotoxicity test and some selected pathogenic bacteria to potentiate the extracts by evaluating the range of their effectiveness. Three plant species, *viz.* *Carica papaya* (Linn.), *Moringa oleifera* (Lam.) and *Musa sapientum* (L.) were used to yield a conclusion regarding their effect on laboratory organisms and on the biodegrading organisms found in the industrial effluent.

## 1.1. Background information on the title plant species

### 1.1.1. *Carica papaya* Linn.

*C. papaya* (family: Caricaceae) is a tropical tree which is native to the tropics of the Americas but now widely cultivated in other tropical regions of the world, for its edible melon-like fruit, which is available throughout the year (Banerjee *et al.*, 2006).

#### 1.1.1.1. Origin and geographical distribution

Papaya is thought to have originated from Mexico and Central America. It was spread during the 16<sup>th</sup> century by the Spanish exploration from Central America to the Caribbean and the South East Asia. It is now widespread in the tropics in South America, Africa and Asia, and in the warm subtropics (Oceania) (Heuzé and Tran, 2012). It is grown worldwide in tropical and subtropical areas as a commercial crop, spreaded easily and become naturalized in many areas.

#### 1.1.1.2. Systematic position

Kingdom: Plantae

Division: Magnoliophyta

Class: Magnoliopsida

Subclass: Dilleniidae

Order: Violales

Family: Caricacea

Genus: *Carica*

Species: *C. papaya* Linn.



**Plate 1.1:** *C. papaya* fruits on tree

The species includes perplexingly variable forms, sometimes classified as different varieties of species. It has several synonyms, *i.e.*, *C. pinnatifada* Heilborn, *C. cubensis* Solms-Laub., *C. portorricensis* Urban, *C. peltata* Hooker et Arnott, *Papaya hermaphrodita* Blanco, *C. edulis* Bojer, *C. mamaya* Vellozo, *C. sativa* Tuss., *Carica*

*citriformis* Jacq., *Papaya communis* Noroña, *Papaya cucumerina* Noroña, *Papaya papaya* (L.) Karsten, *Papaya vulgaris* A.DC.

The local names of the *C. papaya* are Fafay, Babaya in Arabic; Pappaiya, Papaya, Pepe (পেঁপেঁ) in Bengali; Thimbaw in Burmese; Pawpaw, Pawpaw tree, Melon tree, Papaya in English; Papaya, Lapaya, Kapaya in Filipino; Papailer, Papaye, Papayer in French; Papaya, Melonenbraum in German; Papaya, Papeeta in Hindi; Gedang, Papaya in Indonesian; Papaya, Betek, Ketalah, Kpaya in Malay; Pepol in Sinhala; Figueradel monte, Fruta bomba, Papaya, Papaita, Lechosa in Spanish; Pappali, pappayi in Tamil; Malakor, Loko in Thai; Du du in Vietnamese.



**Plate 1.2:** *C. papaya* trees

### 1.1.1.3. Morphological attributes

*C. papaya* tree is an erect, fast-growing tree measuring 7 to 8m tall, with copious latex and trunk of about 20cm in diameter (Duke, 1984). *C. papaya* plant has an erect branchless trunk and a palm like head of foliage at the top. The trunk remains somewhat succulent and soft wooded, and never develops true bark. The leaves are deeply incised and lobbed. Papaya is a very fast growing perennial herb. Male flowers are borne in clusters on stalks 90cm long; the flowers are funnel-shaped, about 2.5mm (0.1inch) long, and whitish, and the corolla is five-lobed, with 10 stamens in the throat. The female

flowers are considerably larger, on very short stalks, and often solitary in the leaf axils; they have five fleshy petals that are united toward the base and a large cylindrical or globose superior ovary that is crowned by five fan-shaped sessile stigmas. Most papaya trees bear male and female flowers on separate trees. There is no way of telling the sex until flowering. Some varieties are bisexual. Papaya is sensitive to salinity and frost, and does not tolerate water logging and flooding (Ecoport, 2009).

#### **1.1.1.4. Common and folkloric uses of *C. papaya***

The fruits, leaves, and latex are used medicinally. Papain, a major compound in the fruit and latex has been used in brewing and wine making and the textile and tanning industries. It also has been reported that papaya leaf extract is used as a prophylaxis against malaria; Papain also is used to treat arthritis. The level of the compounds varies in the fruit, latex, leaves, and roots. Papain is also applied topically (in countries where it grows) for the treatment of cuts, rashes, stings and burns. Women in India, Bangladesh, Pakistan, Sri Lanka, and other countries have long used green papaya as a folk remedy for contraception and abortion.

Papaya is a common man's fruit; available throughout the year in the tropics. It is referred to as the "medicine tree" or "melon of health". Papaya is filled with nutrients (Jackwheeler, 2003). Plants which are used for medicinal purposes are generally cheap and are best sources of pharmacologically active substances and are good resistance to bacterial activity (Basile *et al.*, 1999). In general medicinal purposes, the plant is used tremendously. Whole *C. papaya*, *i.e.* its fruits, seeds, bark and leaves are used for treatment and curing many diseases. The edible portion of the fruit of *C. papaya* (pawpaw) contains both macro and micro minerals like Na, K, Ca, Mg, Fe, Cu, Zn and Mn (OECD, 2010). The plant is a source of carotenoides, vitamin C, thiamine, riboflavin, niacin, vitamin B<sub>6</sub> and vitamin K (Bari *et al.*, 2006; Adetuyi *et al.*, 2008; USDA, 2009). The seed had recently been linked to cure sickle cell diseases (Imaga, *et al.*, 2009), poisoning related renal disorder (Olagunju, *et al.*, 2009) and as an anti-helminthes (Okeniyi *et al.*, 2007).



Leaves contain large amounts of alkaloids, carpaine and pseudocarpine which creates positive effects on heart as well as on respiration (Perry and Metzger, 1980). The leaves of papaya have been shown to contain many active components that can increase the total antioxidant power in blood and reduce lipid peroxidation level, such as papain, chymopapain, cystatin, tocopherol, ascorbic acid, flavonoids, cyanogenic glucosides and glucosinolates. Leaf extract of *C. papaya* is well known as an anti-tumor agent (Walter Last, 2008). The papaya fruit, as well as all other parts of the plant, contain a milky juice in which an active principle known as papain is present. Aside from its value as a remedy in dyspepsia and kindred ailments, it has been utilized for the clarification of beer (Ayoola and Adeyeye, 2010).

It contains medicinal properties and the major active ingredients recorded *viz.* carpaine, chymopapain and papain, a bactericidal aglycone of glucotropaeolin, benzyl isothiocyanate, aglycoside sinigrin, theenzyme myrosin, and carpasemine (Akah *et al.*, 1997; Jackwheeler, 2003; Eno *et al.*, 2000; Wilson *et al.*, 2002; Seigler *et al.*, 2002). Papaya plants are also produced for papain and chymopapain, two industrially important proteolytic enzymes found in the milky white latex exuded by fruits. In general, female fruits tend to exude more papain than hermaphrodite fruits. Papaya contains antifertility properties, particularly the seeds, (Lohiya *et al.*, 1999). A complete loss of fertility has been reported in male rabbits, rats and monkey's fed an extract of papaya seeds (Glazer and Smith, 1971; Lohiya *et al.*, 1999; Pathak *et al.*, 2000).

### **1.1.2. *Moringa oleifera* Lam.**

*Moringa oleifera* is the most widely cultivated species of monogeneric family, the Moringaceae. It commonly known as the Drumstick tree. *Moringa oleifera* is the best known of the thirteen species in the genus *Moringa*. These are *Moringa oleifera*, *M. arborea*, *M. borziana*, *M. concanensis*, *M. drouhardii*, *M. hildebrandtii*, *M. longituba*, *M. ovalifolia*, *M. peregrine*, *M. pygmaea*, *M. rivaie*, *M. ruspoliana* and *M. stenopetala* (Mahmood *et al.*, 2010).

### 1.1.2.1. Origin and geographical distribution

*M. oleifera* is indigenous to South Asia, where it grows in the Himalayan foothills from Northeastern Pakistan to Northern West Bengal, India (Sharma *et al.*, 2011). It has been introduced and become naturalized in other parts of India, Pakistan, Afghanistan, Bangladesh, Sri Lanka, Southeast Asia, West Asia, the Arabian Peninsula, East and West Africa, Southern Florida, throughout the West Indies, and from Mexico to Peru, Paraguay and Brazil. In Puerto Rico, it is grown chiefly as an ornamental and in fencerows and hedges and has become naturalized along roadsides on the coastal plains and lower foothills. The rapid growing tree was utilized by the ancient Romans, Greeks and Egyptians; it is now widely cultivated and has become naturalized in many locations in the tropics (Fahey, 2005; Sachan *et al.*, 2010).

### 1.1.2.2. Systematic position

Kingdom: Plantae

Division: Magnoliophyta

Class: Magnoliopsida

Subclass: Dilleniidae

Order: Capparales

Family: Moringaceae

Genus: *Moringa*

Species: *M. oleifera* Lam.



**Plate 1.3:** *M. oleifera* tree

The common name of *M. oleifera* are Drumstick tree, Horse radish tree, Mother's best friend, West Indian bean in English; Bean, Árbol del bean, Morango, Moringa in Spanish; Bèn ailé, Benzolive, Moringa in French; Sajina, (সজিনা, ছুড়ি) (Bangladesh). The local names of the *M. oleifera* are Rawag in Arabic; Dan-da-lun in Burmese; Malunggay in Filipino; Shajmah, Shajna, Segra in Hindi; Merunggai, Sajina in Malay;

Saijan, Sohanjna in Pakistan; Ma-rum in Thai; Shobhanjana in Sanskrit; Murangai, Murunga in Tamil.

### 1.1.2.3. Morphological attributes

*Moringa* is a slender softwood tree that branches freely and can be extremely fast growing. Although it can reach 3m heights in excess of 10m and a diameter of 20 to 40cm at chest height, it is generally considered a small to medium size tree (Radovich, 2009). The stem is normally straight but occasionally is poorly formed. The tree grows with a short, straight stem that reaches a height of 1.5 to 2m before it begins branching but can reach up to 3m (Foidl *et al.*, 2001). It has deep roots, and therefore it can survive in dry regions, and wide-open crown with a single stem. The extended branches grow in a disorganized manner and the canopy is umbrella shaped. Tripinnate compound leaves are feathery with green to dark green elliptical leaflets 1 to 2cm (0.4 to 0.8in) long. The tree is often mistaken for a legume because of its leaves. The alternate, twice or thrice pinnate leaves grow mostly at the branch tips. They are 20 to 70cm long, grayish-downy when young, long petiole with 8 to 10 pairs of pinnae each bearing two pairs of opposite, elliptic or obovate leaflets and one at the apex, 1 to 2cm long (Morton, 1991). Conspicuous, lightly fragrant flowers are borne on inflorescences 10 to 25cm (4 to 10in) long, and are generally white to cream colored, 2.5cm in diameter, borne in sprays, with 5 at the top of the flower, although they can be tinged with pink in some varieties. The flowers, which are pleasantly fragrant and 2.5cm wide are produced profusely in axillary, drooping panicles 10 to 25cm long (Sachan *et al.*, 2010). They are white or cream colored and yellow-dotted at the base. The five-reflexed sepals are linear-lanceolate. The five petals are slender-spatulate. They surround the five stamens and five staminodes and are reflexed except for the lowest. The fruits are trilobed capsules, and are frequently referred to as pods and fruits production in March and April.

### 1.1.2.4. Common and folkloric uses of *M. oleifera*

*M. oleifera*, commonly called the `drumstick tree`, is well known for its multi-purpose attributes, wide adaptability and ease of establishment. Its leaves, pods and flowers are

packed with nutrients, important both human and animals. *M. oleifera* possess highly therapeutic and pharmacological values, so its consumption in regular diet could possibly reduce the risk of degenerative diseases (Paliwal *et al.*, 2011c). *M. oleifera* is believed to possess numerous medical properties and is being used for the treatment of ascites, rheumatism (Anwar *et al.*, 2007), venomous bites (Mishra *et al.*, 2009), enhancing cardiac function (Limaye *et al.*, 1995), inflammation (Ezeamuzie *et al.*, 1996), liver disease (Rao and Misra, 1998), cancer, hematological, hepatic and renal function (Mazumder *et al.*, 1999). Almost all the parts of this plant: root, bark, gum, leaf, fruit (pods), flowers, seed and seed oil have been used for various ailments in the indigenous medicine of South Asia, including the treatment of inflammation and infectious diseases along with cardiovascular, gastrointestinal, hematological and hepatorenal disorders (Paliwal *et al.*, 2011b).

*Moringa* has been used in the traditional medicine passed down for centuries in many cultures around the world, for skin infections, anaemia, anxiety, asthma, blackheads, blood impurities, bronchitis, catarrh, chest congestion, cholera, conjunctivitis, cough, diarrhoea, eye and ear infections, fever, glandular, swelling, headaches, abnormal blood pressure, hysteria, pain in joints, pimples, psoriasis, respiratory disorders, scurvy, semen deficiency, sore throat, sprain, tuberculosis, for intestinal worms, lactation ,diabetes and pregnancy (Nikken *et al.*, 2003). The healing properties of *Moringa* oil have been documented by ancient cultures.



**Plate 1.4:** *M. oleifera* flowers and fruits \*\*

\*\* ([http://commons.wikimedia.org/wiki/File:Moringa\\_oleifera\\_sg.jpg](http://commons.wikimedia.org/wiki/File:Moringa_oleifera_sg.jpg))

It has tremendous cosmetic value and is used in body and hair care as a moisturizer and skin conditioner. It has been used also in skin preparations and ointments since Egyptian times (Ramachandran *et al.*, 1980; Sairam, 1999; Marcu, 2005).

It is vata and kapha suppressant. Due to its hot potency it is helpful in relieving from pain and also reduces inflammation. It is also helpful in curbing the infection in the body. It is very much effective in stimulating the nervous system. Due to pungent taste it is effective in treating the digestive disorders, worm infestation, and constipation. It stimulates heart and also increases the blood density because of its hot potency. It is also a good antitussive and helps in resolving from extra mucus in the respiratory tract because of its bitter nature. Due to hot potency it is helpful in maintaining the proper menstrual cycle. It is also helpful in relieving from skin related problems as it generates sweat in the body.

Its bark, roots, fruit, flowers, leaves, seeds, and gum are also used medicinally. Uses include as an antiseptic and in treating rheumatism, snake bites, and other conditions. Boiled leaves used to help increase lactation. Juice of the root with milk used for asthma, hiccups, gout, lumbago. In West Bengal, India, roots taken by women, especially prostitutes, for permanent contraception. The seed is often used to purify dirty or cloudy drinking water. In the Nile Valley, the name of the tree is `Shagara al Rauwaq` which means `tree for purifying` (Von Maydell, 1986).

*M. oleifera* is an anti-pyretic (Fahey, 2005; Fisher, 2011), anti-inflammatory (Hukkeri *et al.*, 2006; Holst, 2000; Sing and Kumar, 1999), and possesses a broad spectrum of anti-bacterial, anti-fungal, anti-viral and antibiotic abilities, which may certainly lighten the load on the immune system (Caceres *et al.*, 1992; Ezeamuzie *et al.*, 1996). A *Moringa* dietary protocol makes perfect sense to combat the ravages of Lyme disease (Kjaer *et al.*, 1979; Kurup and Narasimha, 1954; Viera *et al.*, 2010; Eilert *et al.*, 1981; Jahn *et al.*, 1986; Das *et al.*, 1957; Bennett *et al.*, 2003; Fahey *et al.*, 2001). According to Fuglie (1999) the many uses for *Moringa* include: alley cropping (biomass production), animal forage (leaves and treated seed-cake), biogas (from leaves), domestic cleaning agent (crushed leaves), blue dye (wood), fencing (living trees), fertilizer (seed-cake), foliar nutrient (juice expressed from the leaves), green manure (from leaves), gum (from tree

trunks), honey and sugar cane juice-clarifier (powdered seeds), honey (flower nectar), medicine (all plant parts), ornamental plantings, biopesticide (soil incorporation of leaves to prevent seedling damping off), pulp (wood), rope (bark), tannin for tanning hides (bark and gum), water purification (powdered seeds) (Adebayo *et al.*, 2011). Various parts of this plant such as the leaves, roots, seed, bark, fruit, flowers and immature pods act as cardiac and circulatory stimulants, possess antitumor, antipyretic, antiepileptic, anti-inflammatory (Kumar *et al.*, 2009), antiulcer, antispasmodic, diuretic, antihypertensive, cholesterol lowering, antioxidant, antidiabetic, hepatoprotective, antibacterial and antifungal activities and are being employed for the treatment of different ailments in the indigenous system of medicine, particularly in South Asia (Anwar and Rashid, 2007; Anwar and Bhangar, 2003; Fakurazi *et al.*, 2008; Paliwal *et al.*, 2011a). It is generally known in the developing world as a vegetable, a medicinal plant and a source of vegetable oil (Bennett *et al.*, 2003; Paliwal *et al.*, 2011b).

### **1.1.3. *Musa sapientum* L.**

*Musa sapientum* which is commonly called banana is a herbaceous plant of the family Musaceae. Akinyosoye (1991) reported that the plant is cultivated primarily for its fruits and to a lesser extent for the production of fibre. It is also believed to be an ornamental plant.

#### **1.1.3.1. Origin and geographical distribution**

In different countries about 300 varieties of bananas are grown, of which a vast majority have been growing in Asian, Indo- Malaysian and Australian tropics and are now widely found throughout the tropical and subtropical countries. India, Philippines, China, Ecuador, Brazil, Indonesia, Mexico, Costa Rica, Colombia, Thailand are the top banana producing countries. It is extensively grown and cultivated as a fruit plant all over Bangladesh.

The banana grows almost everywhere in the country throughout the year. The principal banana growing areas however, are Rangamati, Barisal, Rangpur, Dinajpur, Noakhali, Faridpur and Khulna (Rahman and Kabir, 2003).

### 1.1.3.2. Systematic position

Kingdom: Plantae

Division: Magnoliophyta

Class: Liliopsida

Subclass: Zingiberidae

Order: Zingiberales

Family: Musaceae

Genus: *Musa*

Species: *M. sapientum* L.

The local names of *Mu. sapientum* are Kala (কলা) in Bengali; Tawngyet byaw in Burmese; Dessert banana, Banana in English; Banane cultivée, Bananier des in French; Adamsfeige, Dessert banane in German; Banana in Japanese; Biu, Cau in Malay; Banana, Bananeira in Spanish; Vaazhai in Tamil.



**Plate 1.5:** *Mu. sapientum* tree

### 1.1.3.3. Morphological attributes

*Mu. sapientum* is the largest herbaceous flowering plant. Plants are normally tall and fairly sturdy and are often mistaken for trees, but their main or upright stem is actually a pseudostem that grows 6 to 7.6m tall, growing from a corm. Each pseudostem can produce a single bunch of bananas. After fruiting, the pseudostem dies, but offshoots may develop from the base of the plant. Leaves are spirally arranged and may grow 2.7m long and 60cm wide. They are easily torn by the wind, resulting in the familiar frond look. Each pseudostem normally produces a single inflorescence, also known as the *banana heart*. The inflorescence contains many bracts (sometimes incorrectly called petals) between rows of flowers.

#### 1.1.3.4. Common and folkloric uses of *Mu. sapientum*

Medicinal plants are frequently used in traditional medicine to treat different diseases in different areas of the world (Palombo, 2005). This indigenous knowledge, passed down from generation to generation, has significantly contributed to the development of different traditional systems of medicine (Jachak and Saklani, 2007), as well as helped in exploration of different medicinal plants to find the scientific basis of their traditional uses.

Young leaves of *Mu. sapientum* used for cool dressing of inflamed and blistered surfaces and as cool application for headaches. The trunk juice is applied to scalp for thinning hair. Cooked flower used for diabetes in India. Sap of the flower is used for ear-aches. In South-Western Nigeria, green fruits used for diabetes. The fruit of *Mu. paradisiaca* and *Mu. sapientum* is traditionally used in diarrhoea (unripe), dysentery, intestinal lesions in ulcerative colitis, diabetes (unripe), in sprue, uremia, nephritis, gout, hypertension, cardiac disease (Ghani, 2003; Khare, 2007). *M. sapientum* is also used in the treatment of excess menstruation with *Canna indica* L. var. *speciosa* (Partha and Hossain, 2007). Banana leaves (ashes) are used in eczema (Okoli, 2007), as cool dressings for blister and burns (Ghani, 2003). Stem juice of fruited plant is used for treating diarrhoea, dysentery, cholera, otalgia, haemoptysis and flower is used in dysentery, diabetes and menorrhagia (Ghani, 2003). The root is used as anthelmintic (Khare, 2007), blood disorders, venereal diseases (Ghani, 2003). The plant is also used in inflammation, pain and snakebite (Coe and Anderson, 1999).

Various researchers evaluated *Mu. sapientum* for its antidiarrhoeal activity (Block, 1941; Emery *et al.*, 1997; Rabbani *et al.*, 1999, 2001; Malik, *et al.*, 1991), antiulcerogenic (Goel *et al.*, 1985; Lewis *et al.*, 1999), hypoglycemic activity (Ojewole and Adewunmi, 2003; Rai *et al.*, 2009; Usha *et al.*, 1989; Singh *et al.*, 2007; Gomathy *et al.*, 1990), antidiabetic (Pari and Maheswari, 1999; Usha *et al.*, 1991), antitumoral and antimutagenic (Lohsoonthorn and Danvivat, 1995; Deneo-Pellegrini *et al.*, 1996; Murakami *et al.*, 1998; Botting *et al.*, 1999), antioxidant activity (Yin *et al.*, 2008; Mokbel and Hashinaga,



2005; Vijayakumar *et al.* 2008), diuretic activity (Jain *et al.*, 2007; Rizvi *et al.*, 2011; Sood *et al.*, 1985; Chodera *et al.*, 1991), wound healing activity (Agarwal *et al.* 2009), anti-allergic activity (Tewtrakul *et al.*, 2008) and have been also found to be effective in treatment of migraine (Guariso *et al.*, 1993) and hypertension (Osime *et al.* 1990; Perfumi *et al.* 1994; Orié, 1997; Sarkar *et al.*, 1999) antioxidant (Pari and Maheswari, 2000).

## 1.2. Background information on the test organisms

The whole project has been designed to carry on screening of the crude extracts of the test plant species on several test organisms for the detection of their biological activities by analyzing the data statistically that read on various parameters during the course of the work. The following test steps have been taken into consideration:

### 1.2.1. *Tribolium castaneum* (Hbst.)

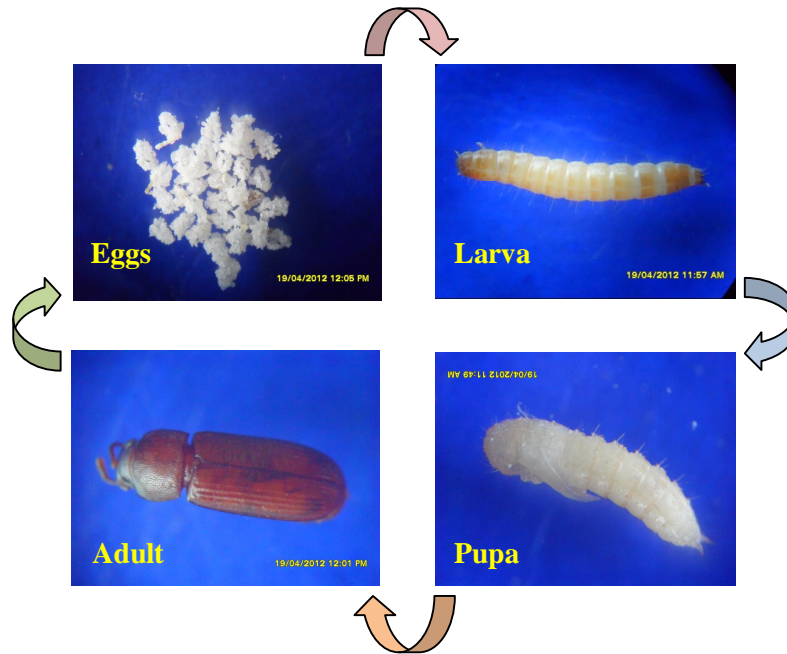
The red flour beetle is Indo-Australian origin and is found in temperate region, but will survive the winter in protected places, especially where there is central heat (Tripathi *et al.*, 2001). In the United States, it is found primarily in the southern states. *T. castaneum* is a worldwide and commonest stored pest of wheat-flour. It is commonly known as 'Rust-red flour beetle'. It is an insect of the family Tenebrionidae under the order Coleoptera. It is one of the serious pests of stored products. Mouth-parts of this pest insect are not adapted to feed on hard whole grains and they are thus found in almost any kind of flour, cracked grains etc. The specific food of *T. castaneum*, which includes whole-wheat flour, bran, rice flour, cornmeal, barley flour and oatmeal. It also feeds upon dried fruits, dried plant roots, nuts, chocolates, drugs, snuff, cayenne pepper, pulses and prepared cereal foods such as corn flakes (Metcalf and Flint, 1962). Not only pulses and millets, but cereals are also been attacked by this beetle (Purthi and Singh, 1950). *T. castaneum*, attack meal, crackers, beans, spices, pasta, cake mix, dried pet food, dried flowers and even dried museum specimens (Via, 1999; Weston and Rattlingroud, 2000). Although small beetles, about ¼ of an inch long, the adults are long-lived and may live for more than three years. The red flour beetle is reddish-brown in color and its antennae end in a three-segmented club (Bousquet, 1990).

**Table 1.1:** Developmental rates of *T. castaneum*

<b>Rearing Temperature</b>	<b>30°C</b>	<b>34°C</b>
Egg	3 days	2 days
Larva	20 days	15 days
Pupa	4 days	3 days
Reproductive Maturation	5 days	4 days
Total time egg to egg	32 days	24 days

The eggs are white, microscopic and often have bits of flour stuck to their surface. The slender larvae are creamy yellow to light brown in color. They have two dark pointed projections on the last body segment. The young larva is yellowish white and measures 1 mm in length. As it matures, it turns reddish yellow, becomes hairy and measures over 6 mm in length. Its head, appendages and the last abdominal segment are darker. The adult is a small reddish-brown beetle, measuring about 3.5mm in length and 1.2 mm in width. Its antennae are bent and bear a distinct club formed by the three enlarged terminal joints. The last antennal segment is transversely rounded. It was commonly found in the wild state in rotting wood and in loose bark of trees in India. This insect is now widely distributed all over the world mainly through commerce. The red flour beetle may be present in large numbers in infested grain, but are unable to attack sound or undamaged grain. Both the larva and adults cause damage. They are found in great numbers on infested materials and caused serious losses and considerable damage to flour and grains that have previously been attacked by other pests. Much of the damage done by *T. castaneum* is directly to kernels (germ and endoplasm). In case of severe infestation flour or other materials invaded may have a characteristics pungent odor as a result of the gaseous secretion exuded by the beetle. Such flour has an exceedingly low viscosity and its elasticity is markedly affected, which may cause gastric disturbance if used as food. In

severe infestation, the flour turn grayish and moldy and has a pungent, disagreeable odor making it unfit for human consumption (Good, 1936).



**Plate 1.6:** Life cycle of *T. castaneum*

Infested material will show many elongate reddish brown beetles, about 1/7 inch long crawling over the material when it is disturbed and brownish white (somewhat flattened) six-legged larval bedding on the inside of the grain kernels and crawling over the infested seeds. They are generally known among millers as “bran bugs”. *T. castaneum* contaminates more than they consume.

According to Khan (1981) this contamination results from;

- the presence of living or dead insects or insect parts;
- cast exuviae, egg shell and pupal cases;
- fecal and persistent odour; and
- webbing of food.

*Tribolium* species are major pests of stored grains and grain products in the tropics. Control of these insects relies heavily on the use of synthetic insecticides and fumigants, which has led to problems such as disturbances of the environment, increasing costs of application, pest resurgence, pest resistance to pesticides and lethal effects on non-target organisms in addition to direct toxicity to users (Jembere *et al.*, 1995). Thus, repellents, fumigants, feeding deterrents and insecticides of natural origin are rational alternatives to synthetic insecticides.

### 1.2.2. *Artemia salina* (Brine shrimp) nauplii

Brine shrimp lethality bioassay is a recent development in the bioassay for the bioactive compounds, which indicates cytotoxicity, as well as, a wide range of pharmacological activities (e.g. anticancer, antiviral, pesticidal, anti-AIDS, etc.) of the compounds. Bioactive compounds are almost always toxic in high doses. Pharmacology is simply toxicology at a lower dose or toxicology is simply pharmacology at a higher dose. Brine shrimp lethality bioassay is a bench top bioassay method for evaluating anticancer, anti-microbial and pharmacological activities of natural products. Natural product extracts, fractions or pure compounds can be tested for their bioactivity by this method.

Here *in vivo* lethality of a simple zoological organism (brine shrimp nauplii; Plate 1.6) is used as a convenient monitor for screening a fractionation in the discovery of new bioactive natural products. Generally, the median effective dose (ED<sub>50</sub>) values for cytotoxicity are one tenth (1/10) of median lethal dose (LC<sub>50</sub>) values in the brine shrimp test.



**Plate 1.7:** *A. salina* nauplius

The *A. salina* belongs to a genus of very primordial crustacean (crawfish - crayfish) the *Anostraca* (Fairy Shrimps). Crawfish of this genus just have a divided exoskeleton made of Chitin enhanced protein, no usual crust of chitin (escutcheon) as other crawfish have. There are many species within the genus of *Anostraca*, but the *A. salina* are very nice to grow, since the rate of successful hatches is very high. To carry on toxicity tests of certain materials these nauplii are very easy to grow from its marketed cysts and to set experiments thereby.

### 1.2.3. Agents for antimicrobial activity tests

The combination of the genetic versatility of microbes and the widespread overuse of antibiotics has led to increasing clinical resistance of previously sensitive microorganisms and the emergence of previously uncommon infections.

The principle of antimicrobial testing of plant extracts is based on the observation of the growth reduction of microorganisms after contact with plant tissues of extracts to be tested. So, it is very important to determine whether the crude extracts are active against various types of test organisms or not and thus a preliminary antibacterial screening of the crude extract was very much necessary. Therefore, screening was done against various pathogenic bacteria by disc diffusion assay method. The bacterial strains were cultured and used in the experiments carried out at the Microbiology Laboratory of the Institute of Environmental Sciences, University of Rajshahi, Rajshahi-6205, Bangladesh. Among the collected strains *Escherichia coli*, *Klebsiella pneumoniae*, *Salmonella enteritidis*, *Shigella flexneri*, *Shigella sonnei* were Gram negative and *Bacillus subtilis*, *Staphylococcus aureus* were Gram positive bacteria.

### 1.2.3.1. List of the test pathogenic bacteria

**Table 1.2:** List of the test pathogenic bacteria and their pathogenicity.

<b>Gram-negative Bacteria</b>		
<b>Sl. No.</b>	<b>Bacteria</b>	<b>Pathogenicity</b>
1.	<b><i>Escherichia coli</i></b>	Most <i>E. coli</i> strains are harmless, but some serotypes are pathogenic and can cause serious food poisoning in humans, and are occasionally responsible for product recalls gastroenteritis, urinary tract infections, and neonatal meningitis. In rare cases, virulent strains are also responsible for haemolytic-uremic syndrome, peritonitis, mastitis, septicaemia and Gram-negative pneumonia.
2.	<b><i>Klebsiella pneumoniae</i></b>	<i>K. pneumoniae</i> is a Gram-negative, non-motile, encapsulated, lactose-fermenting, facultative anaerobic, rod-shaped bacterium. Although found in the normal flora of the mouth, skin, and intestines (Ryan <i>et al.</i> , 2004). It can cause destructive changes to human lungs if aspirated.
3.	<b><i>Salmonella enteritidis</i></b>	Most cases of salmonellosis are caused by food infected with <i>S. enteric</i> . Secreted proteins are of major importance for the pathogenesis of infectious diseases caused by <i>Salmonella enteritidis</i> . A remarkable large number of fimbrial and non-fimbrial adhesins are present in <i>Salmonella</i> , and mediate biofilm formation and contact to host cells. Secreted proteins are also involved in host cell invasion and intracellular proliferation, two hallmarks of <i>Salmonella</i> pathogenesis (Gerlach <i>et al.</i> , 2009).

- 
- |                             |   |
|-----------------------------|---|
| 4. <i>Shigella flexneri</i> | In humans and other primates, <i>Shigella flexneri</i> is responsible for causing an acute bloody diarrhea known as shigellosis or bacillary dysentery (Jin <i>et al.</i> 2002). Aside from bloody diarrhea, other symptoms include fever and stomach cramps. The bleeding is due to destruction of the intestines. The bacteria destroy the intestinal epithelium, then continue to break down the intestinal mucosa in the cecum and rectum (Clark and Maurelli, 2007). |
| <hr/>                       |   |
| 5. <i>Shigella sonnei</i>   | <i>Shigella sonnei</i> is a species of <i>Shigella</i> . Together with <i>Shigella flexneri</i> , it is responsible for 90% of shigellosis (Mims <i>et al.</i> , 1993).   |
- 

### Gram-positive Bacteria

- 
- |                                 |  |
|---------------------------------|--|
| 6. <i>Bacillus subtilis</i>     | <i>B. subtilis</i> is only known to cause disease in severely immunocompromised patients, and can conversely be used as a probiotic in healthy individuals. It rarely causes food poisoning (Ryan <i>et al.</i> , 2004). |
| <hr/>                           |  |
| 7. <i>Staphylococcus aureus</i> | Wound infection, abscesses, endocarditis, septicaemia, osteomyelitis and food poisoning.   |
- 

## 1.3. Aims and objectives of the research work

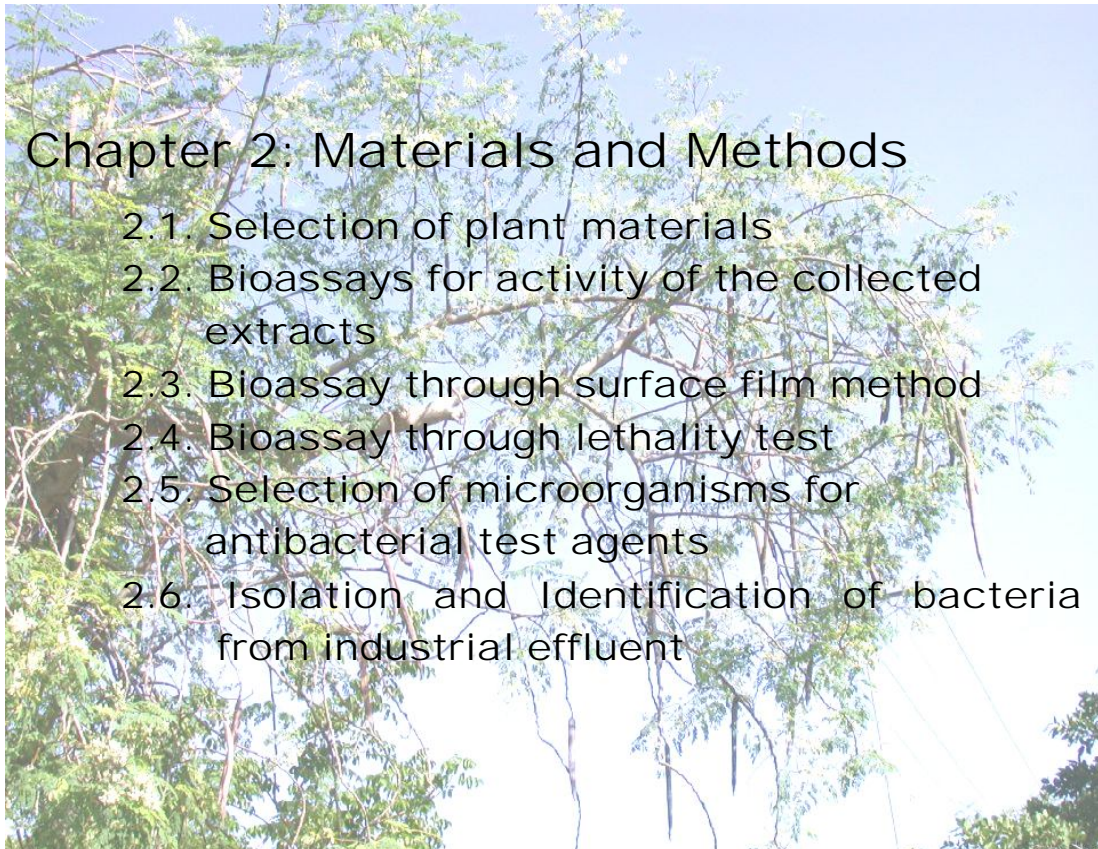
Quite a good number of plants have been identified and utilized for insecticidal and medicinal purpose till to date. But it is true that a large number of plants have still been untouched or less investigated from which significant results can be obtained to control the pest of crops and disease problems of human beings. *C. papaya*, *M. oleifera* and *Mu. sapientum* are such plants that have been studied phytochemically and studies have been done only on its medicinal properties, but in details works done till to date on its use

for the control of crop pests', as well as its function on biodegrading agents are scanty. Accordingly, a research topic entitled "Effect of plant extracts on laboratory organisms and biodegrading agents found in the industrial effluent" has been taken into consideration.

## Objectives of this work

1. To trace presence of bioactive potentials in *C. papaya*, *M. oleifera* and *Mu. sapientum* through primary screening:
  - by using the stored product pest *T. castaneum* to evaluate insecticidal effect of the extracts through dose-mortality assays by establishing LD<sub>50</sub> values;
  - by using *A. salina*, the recognized test agent for cytotoxic effect of the extracts by establishing LC<sub>50</sub> values;
  - by using human pathogenic bacteria to detect antibacterial activity;
2. Component analysis of the collected effluent.
3. Detection and identification of biodegrading agents (microorganisms) from industrial effluent.
4. Laboratory culture of the identified bacterial isolates and evaluation of plant extracts' impact on them.
5. To standardize the essences of the test materials through searching literature and web information in comparison with the results of the investigation to be carried out on their possible use in the contemporary pest control technology.
6. To comment on the future perspectives of the test plants depending on the achieved results.





## Chapter 2: Materials and Methods

- 2.1. Selection of plant materials
- 2.2. Bioassays for activity of the collected extracts
- 2.3. Bioassay through surface film method
- 2.4. Bioassay through lethality test
- 2.5. Selection of microorganisms for antibacterial test agents
- 2.6. Isolation and Identification of bacteria from industrial effluent

## Chapter 2

# Materials & Methods

After development of multimedia techniques natural resources have been used to be the potential source for safe, biodegradable and more beneficial drugs, remedies or pesticides for a sustainable environment on the planet. Insects, mites, algae, or even micro-organisms have also been subjected to yield active compound in this regard. But plants are the most suitable source for such an interesting propagation in the field of pesticide technology while some plants in different parts of the world are considered toxic and some are used in the traditional medicine. Literature search on the title plants offered some essential openings on these species bears repellent, and toxicological properties which were subjected by different researchers to go through screening with a view to develop natural non-hazardous biodegradable pesticides, bioformulators, etc.

### **2.1. Selection of plant materials**

In order to arrive at useful compounds in the shortest possible time, careful selection of plant material is obviously very important. Random collection is one method but it is more judicious to base the selection on certain criteria. By way of illustration, plants used in traditional medicine are more likely to provide pharmacologically active compounds (Huxtable, 1992). Similarly, folk use of toxic plants could be taken with desirable output. In case of very small plants, such as herbs, shrubs, grass, etc. normally the whole plant is subjected for extraction, because the distribution of constituents generally not vary too much. Being a large timber plant, the distribution of compounds in different parts of this plant is obviously different. The presence of constituents in the heart-wood may disappear in the leaves; similarly constituents in the roots may not be the same that present there in the fruits.

In this proposition different parts of the test plants, (i) *C. papaya* leaves, stems and roots; (ii) *M. oleifera* leaves, fruits, stem bark, stem wood, root bark, root wood, and (iii) *Mu. sapientum* leaves, stem and root have been collected for the detection of

toxic properties and bio-active constituents since the plants are well known as medicinal plants.

### 2.1.1. Preparation of plant materials for extraction

The fresh materials of *C. papaya*, *M. oleifera* and *Mu. sapientum* were collected from the campus of the University of Rajshahi and adjacent areas in the following way:

***C. papaya*:** After collection leaves and stem were cut into small pieces; roots were collected by digging up without damaging them and excess soil were removed without washing and cut into small pieces as thin as possible and spread out to dry without heaping the material together. It was done under the shade avoiding direct sunshine.



**Plate 2.1:** Chopped parts of *M. oleifera*

***M. oleifera*:** After collection the leaves were spread out to dry without heaping; fruits were picked up and cut into small pieces; after collection the stem bark and stem wood were separated from the stem and cut into small pieces as thin as possible. Roots were collected by digging up without damaging them and shake and brush away excess soil without washing. The root bark was collected by stripping out from

the root and after removal of the root bark the root wood was collected and cut into small pieces as thin as possible. Then the plant parts were dried thoroughly in a well-ventilated place.

***Mu. sapientum***: After collection leaves and stem were cut into small pieces; roots were collected by digging up without damaging and brush away excess soil without washing and cut into small pieces as thin as possible and spread out to dry.

### 2.1.2. Chemical extraction of the collected materials

There are basically two methods for extracting compounds from plant materials. Which one to choose, depends on whether the aim is to extract the more polar compounds (especially glycosides) which are present in the cell vacuole, or to obtain the less polar aglycones present on the surface of the plant, in aerial parts, heartwood or roots. In the present study three solvents petroleum ether (Pet.E.), chloroform ( $\text{CHCl}_3$ ) and methanol ( $\text{CH}_3\text{OH}$ ) were selected to extract for different parts of *C. papaya*, *M. oleifera* and *Mu. sapientum* separately.

All the plant materials were individually powdered in a grinder machine avoiding excess heat during grinding. The powdered materials, viz. leaves, stem and root of *C. Papaya*; leaves, fruits, stem bark, stem wood, root bark and root wood of *M. Oleifera*; leaves, stem and root of *Mu. sapientum* were weighed and placed in separate conical flasks to add sufficient amount of solvents maintaining a minimum ratio of 1:3 (1g dust: 3ml solvent). Then the conical flasks were put on an orbital shaker (Plate 2.2) for 48h and filtered (Plate 2.3) using 90mm Whatman filter paper. This was repeated for three times. Then the conical flasks were left open for evaporation for 24 to 48h before adding the next solvent.

The extraction was firstly done by the Pet.E. solvent followed by  $\text{CHCl}_3$  and  $\text{CH}_3\text{OH}$ . Extracts, thus obtained were concentrated on a rotary evaporator at  $40^\circ\text{C}$  and only as residues (extracts) left were collected in to glass vials and preserved in a refrigerator at  $4^\circ\text{C}$  with proper labeling (Plate 2.4). For each of the samples three solvents have been used separately and successively.



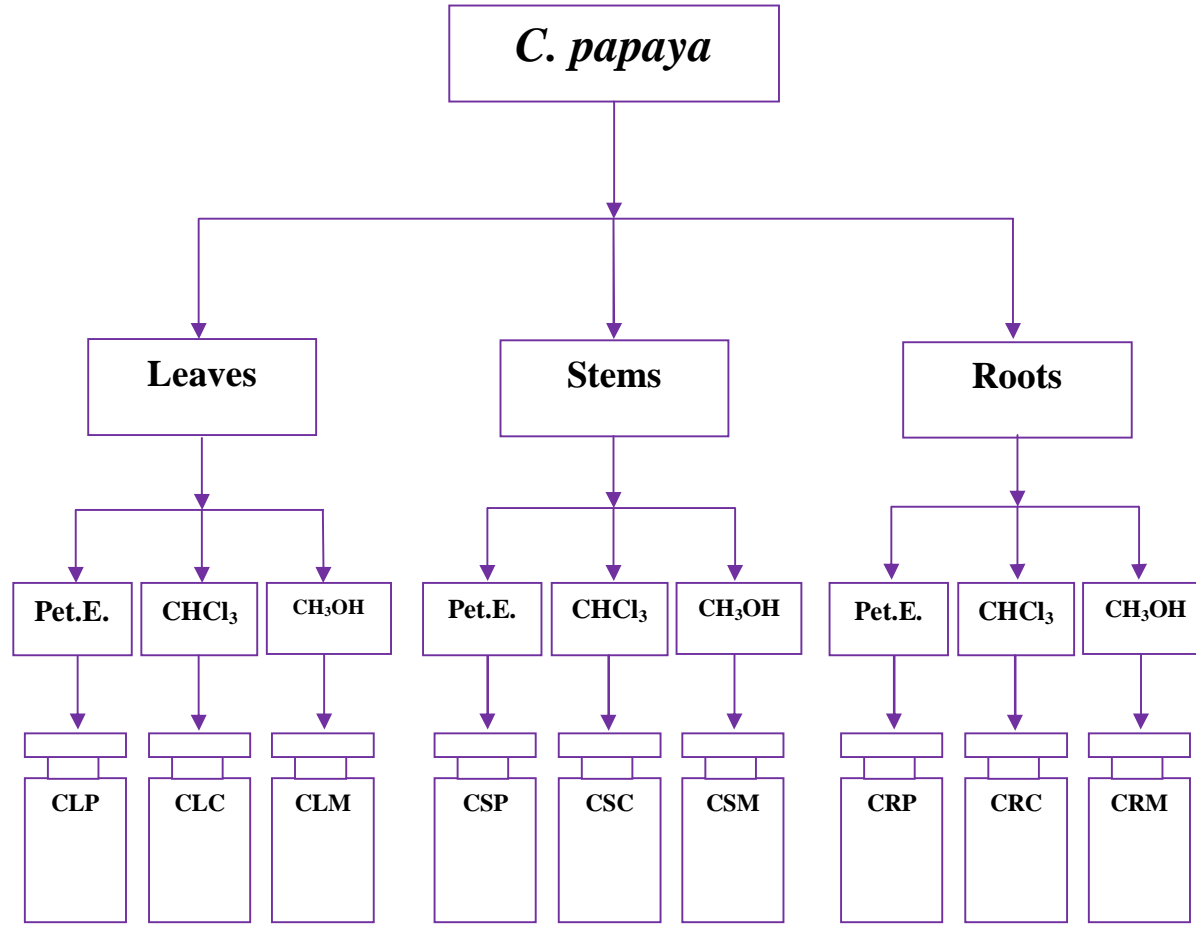
**Plate 2.2:** Shaking on the shaker



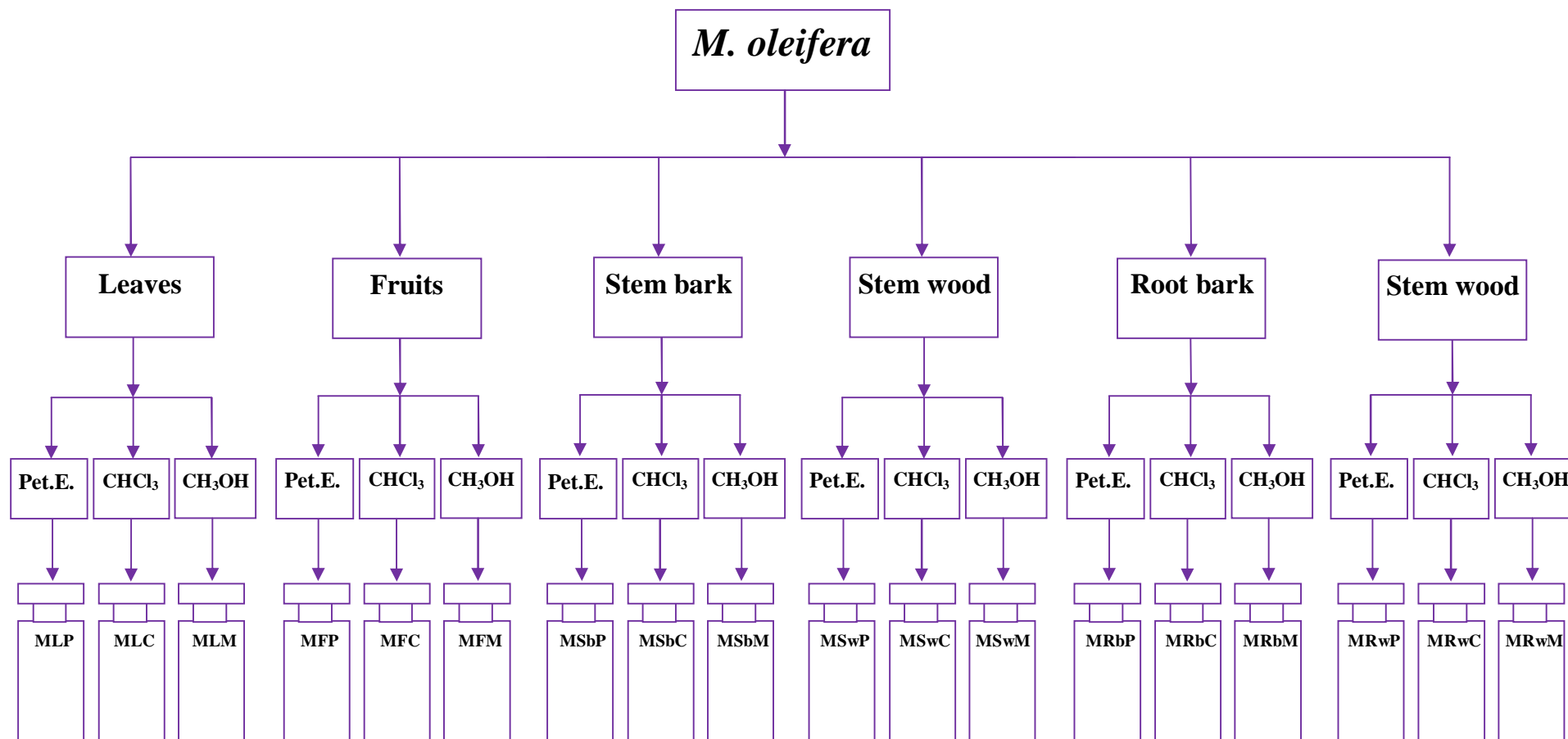
**Plate 2.3:** Filtration of extracts



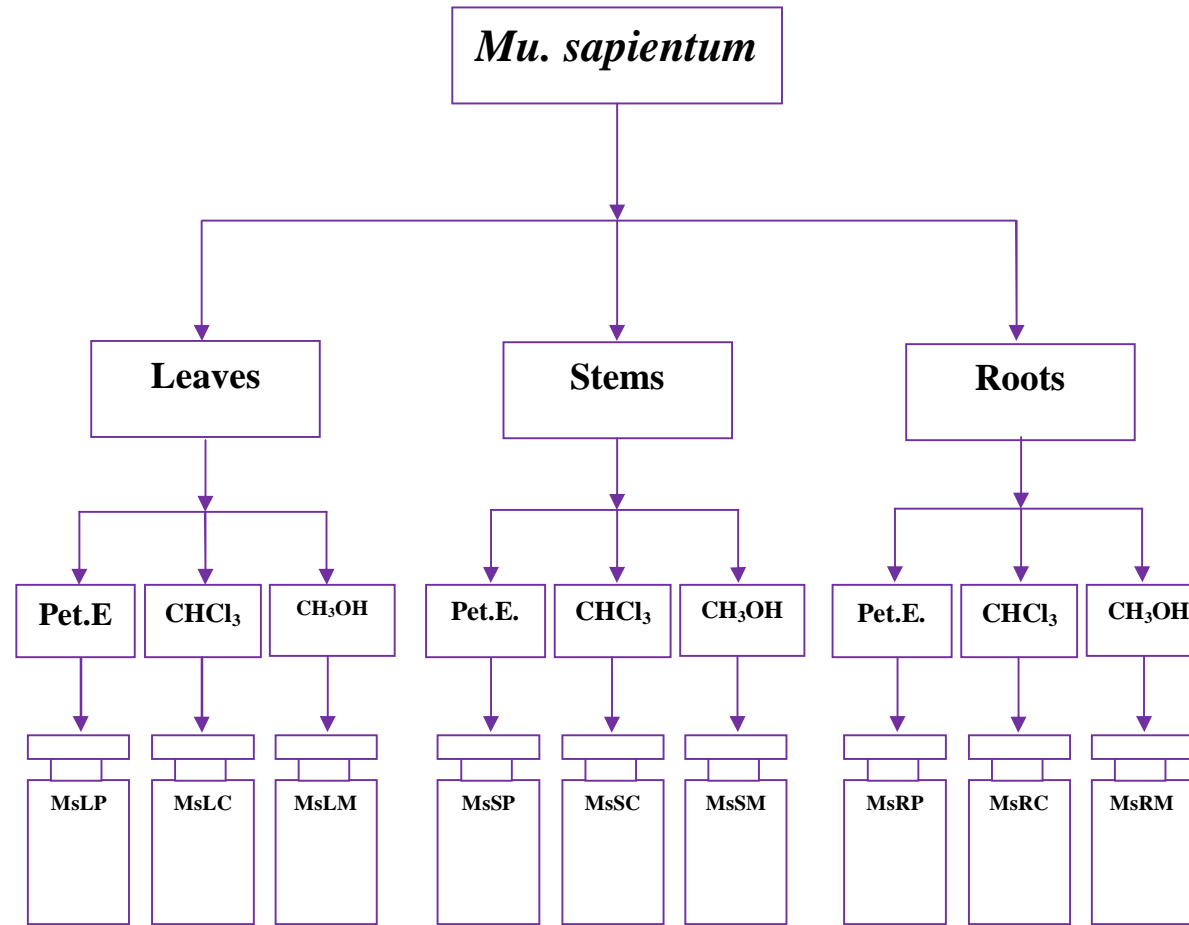
**Plate 2.4:** Extracts in vials with proper labeling



**Fig. 2.1:** Schematic diagram of extracts collection of *C. papaya* (leaves, stems and roots) by different solvents



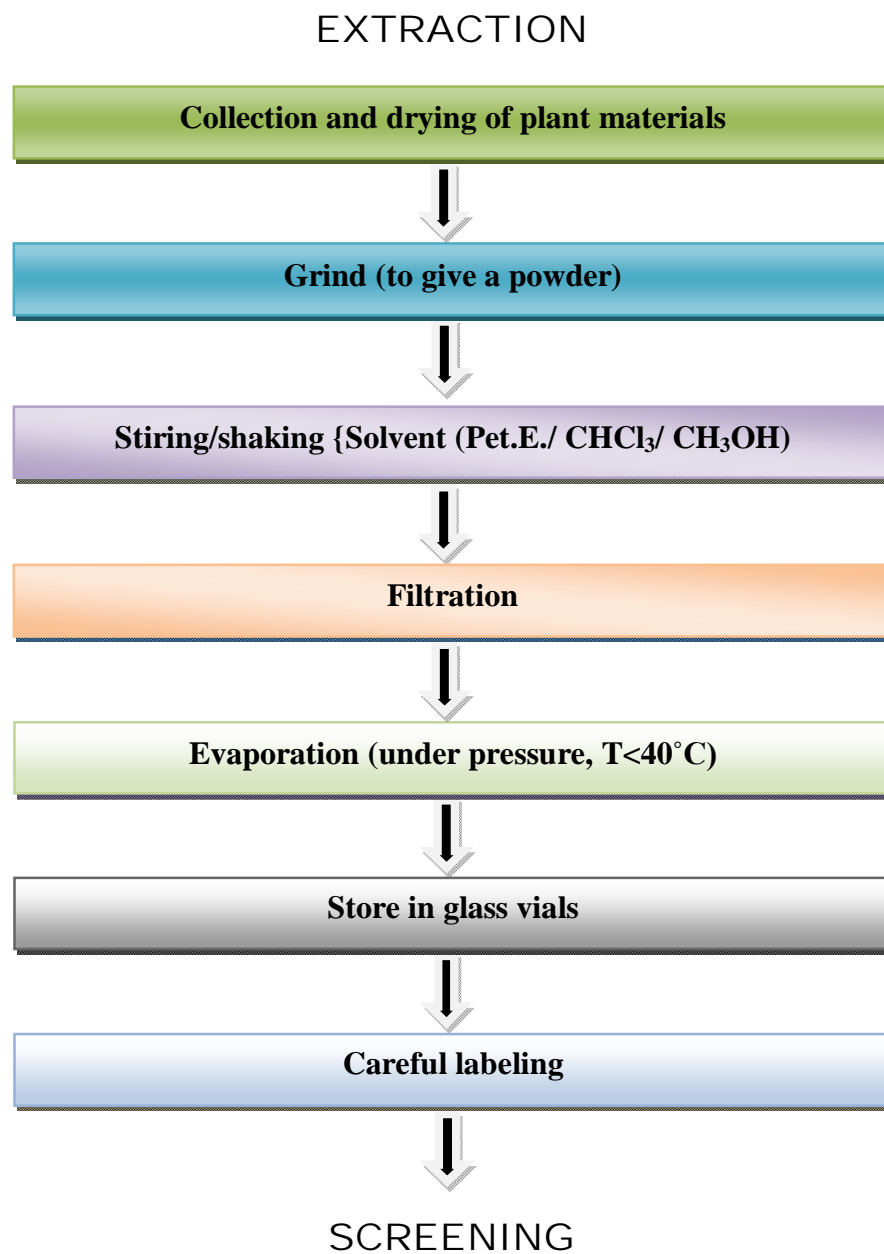
**Fig. 2.2:** Schematic diagram of extracts collection of *M. oleifera* (leaves, fruits, stem bark, stem wood, root bark and root wood) by different solvents



**Fig. 2.3:** Schematic diagram of extracts collection of *Mu. sapientum* (leaves, stems and roots) by different solvents



### 2.1.3. Extraction procedure for the Pet.E., CHCl<sub>3</sub> and CH<sub>3</sub>OH solvents



**Fig. 2.4:** Pathway of extraction

## 2.2. Bioassays for activity of the collected extracts

Bioassays are typically conducted to measure the effects of a substance on a living organism and are essential in the development of new drugs. It involves a procedure by which the potency or the nature of a substance is estimated by studying its effects on living matter. Bioassays could involve the use of *In Vivo* systems, *ex vivo* systems or *In Vitro* systems.

For the selection of bioassays to employ in research on plant constituents, the first step was to choose suitable target organisms. The complexity of the bioassay has to be designed as a function of the facilities and resources available. A list of bioassays taken in this investigation is shown in Table-2.1.

**Table 2.1:** A list of test agents used in different bioassays

Types of tests	Test agents
1. Insecticidal	1. <i>Tribolium castaneum</i> (Hbst.)
2. Cytotoxicity	2. <i>Artemia salina</i> (L.)
3. Antimicrobial (Antibacterial)	3. Seven pathogenic bacteria*
	4. Nine isolates from industrial effluent*

\*Names given in Table 2.2

### 2.2.1. Selection of test organisms

*T. castaneum* was selected to carry on bioassay for insecticidal potentials of the extractives of *C. papaya*, *M. oleifera* and *Mu. Sapientum*. It was chosen only because it is an easy cultivable and noble laboratory insect. The life histories made this insect as popular choice as test insects for biological studies. For brine shrimp lethality test *A. salina* nauplii was selected, since it is being used in such cases as a model test agent. A number of bacteria were selected to carry out further efficiency tests of the extractives.

#### 2.2.1.1. Collection of test organisms

Adult beetles of *T. castaneum*, used in the present investigation were taken from the stock cultures of the Crop Protection and Toxicology Laboratory, University of

Rajshahi, Rajshahi-6205, Bangladesh and reared as mass- cultures and subcultures to be used in the experimentations. The brine shrimp cysts were collected from any of the aquarium shops of Kataban, Dhaka, Bangladesh.

#### **2.2.1.2. Culture of the test insect *T. castaneum***

Mass cultures were maintained in plastic containers (1200ml) and sub-cultures in beakers (1000ml) with the food medium. The beakers were kept in an incubator at  $30^{\circ}\text{C} \pm 0.5^{\circ}\text{C}$  without light and humidity control. Each container and beaker contained 250g and 150g of food respectively. About 200 adults in each container and 100 adults in each beaker were introduced. The cultures were checked in regular intervals and eggs and larvae were separated to increase properly. A crumpled filter paper was placed inside each container and beaker for easy movement of the beetles. The containers and beakers were covered with pieces of muslin cloth tightly fixed with the help of rubber bands to avoid possible escape of the beetles.

#### **2.2.1.3. Preparation of food medium**

The whole-wheat flour was used as the food medium for the insect species. The flour was sterilized at  $60^{\circ}\text{C}$  for 24h in an oven. A standard mixture of whole wheat flour with powdered dry yeast in a ratio of 19:1 (Park and Frank, 1948; Park., 1962; Zyromska-Rudzka, 1966; Khalequzzaman *et. al.*, 1994) was used as food medium throughout the experimental period. Both flour and yeast were previously passed through a 250 micrometer sieve and sterilized. The prepared food was not used until at least 15 days after sterilization to allow its moisture content equilibrate with the environment (Khan, 1981).

#### **2.2.1.4. Collection of eggs**

About 500 beetles were placed in a 500ml beaker containing food medium. The beaker was covered with a piece of cloth and kept in an incubator at  $30 \pm 5^{\circ}\text{C}$ . In regular interval the eggs were collected by sieving the food medium by two sieves of 500 and 250 mesh separating the adults and eggs respectively following the methods of Khan and Selman (1981). Eggs were then transferred to Petri dishes (90mm in diameter) and incubated at the same temperature.

### **2.2.1.5. Collection of newly hatched larvae**

After 3-5 days, larvae hatched out in described conditions. Newly hatched larvae were then collected with a fine pointed camel hair brush and then shifted to the fresh food medium for culture. The larvae are yellowish white in color and long cylindrical shape. It appears 1mm in length after hatching and become 6-7mm at maturation.

### **2.2.1.6. Collection of mature larvae**

Most larvae had six instars as reported by Good (1936). According to Good (1936), the larval instars were determined by counting the number of exuviae (larval skin) deposited in the food medium. Two days old larvae was considered as 1<sup>st</sup> instar larva while 2<sup>nd</sup>, 3<sup>rd</sup>, 4<sup>th</sup> and 5<sup>th</sup> instars larvae were considered on 4<sup>th</sup>, 7<sup>th</sup>, 10<sup>th</sup> and 13<sup>th</sup> days from hatching respectively. Depending on these days according to larval instar 16 days old larvae have been considered as mature larvae. Larval cultures were maintained in an incubator in the same procedure at 30°C±5°C without light and humidity control. The food medium was replaced by three days interval to a fresh one to avoid conditioning by the larvae (Park, 1934).

### **2.2.1.7. Collection of adults**

A huge number of beetles were thus reared to get a regular supply of the newly formed adults. When sufficient adults produced in the sub-cultures, they were collected from the food medium. For this purpose some pieces of filter paper were kept inside the beaker on the food. Adults crawled upon the paper and then the paper was taken out with a forceps. Beetles were then collected in a small beaker (100ml) with the help of a fine camel-hair brush.

## **2.3. Bioassay through surface film method**

This is also one basic application method for doses of toxic substances to any insect population. The test material has been dissolved in an organic solvent with a certain concentration to apply to a Petri dish of known surface area. After application being volatile the solvent evaporates out immediately simply with the atmospheric temperature. Thus the ingredient goes to make a film on the surface of the Petri dish. Released insects within this captivity might have contact with the substance distributed evenly on the floor. However, being covered with the upper lid of the Petri dish there

could have a captive environment with the extract distributed even in the air inside and may cause mortality by suffocation. Mortality due to suffocation may cause promptly if there is any volatile bioactive principles in the test material. All extracts were diluted with the solvents in which they were extracted and the actual amount of extracted matter in a dose was recorded. The application of dose was carried out by residual film method (Busvine, 1971).

### 2.3.1. Preparation of doses with the crude extracts for the surface film test

In this investigation dose-mortality efficiency was evaluated through surface film experiment with series of doses applied on *T. castaneum* adults. All the three viz. Pet.E.,  $\text{CHCl}_3$  and  $\text{CH}_3\text{OH}$  extracts of the study plants were applied against *T. castaneum* adults. For each samples, an 'Ad Hoc' test was done before final experimentation. 50mg extract sample was weighed and taken in a small glass vial, and then 1ml of the same chemical (1ml Pet.E. for the Pet.E. extracts) was added to dissolve initially for preparing  $2.547\text{mg cm}^{-2}$  dose. This process was also maintained during final experiment. Separate vials were taken for each of the doses and the doses were maintained in three replications.

### 2.3.2. Application of doses in the surface film test

To conduct surface film activity test 50mm Petri dishes were taken for all doses and their replicates, 1ml of each of the doses were poured into the lower part of the Petri dishes and allowed them to dry out. Being volatile the solvent was evaporated out within a few minutes. Ten insects were released in each of the treated Petri dish. A control experiment by applying the only solvent to the lower part of the Petri dish was also set at the same time under the same condition (Plate 2.5).

**Doses selected for different extracts for the final experiment were as follows:**

- *C. papaya* leaf Pet.E.: 1.274, 1.019, 0.764, 0.510 and  $0.255\text{mg cm}^{-2}$ 
  - stem Pet.E.: 2.038, 1.783, 1.529, 1.274, 1.019 and  $0.764\text{mg cm}^{-2}$
  - root Pet.E.: 1.529, 1.274, 1.019, 0.764, 0.510 and  $0.255\text{mg cm}^{-2}$
  - stem  $\text{CHCl}_3$ : 4.076, 3.057, 2.038, 1.529 and  $1.019\text{mg cm}^{-2}$
  - leaf  $\text{CH}_3\text{OH}$ : 2.038, 1.529, 1.019, 0.764, 0.510, 0.255 and  $0.127\text{mg cm}^{-2}$
  - stem  $\text{CH}_3\text{OH}$ : 1.274, 1.019, 0.764, 0.510, 0.255 and  $0.127\text{mg cm}^{-2}$

- root CH<sub>3</sub>OH: 1.019, 0.764, 0.510, 0.255 and 0.127mg cm<sup>-2</sup>
- *M. oleifera* stem bark Pet.E.: 1.529, 1.274, 1.019, 0.764, 0.510 and 0.255mg cm<sup>-2</sup>  
 root bark Pet.E.: 1.529, 1.274, 1.019, 0.764, 0.510 and 0.255mg cm<sup>-2</sup>  
 root wood Pet.E.: 1.783, 1.529, 1.274, 1.019, 0.764, 0.510 and 0.255mg cm<sup>-2</sup>  
 fruit CH<sub>3</sub>OH: 2.038, 1.529, 1.019, 0.764 and 0.510mg cm<sup>-2</sup>  
 stem bark CH<sub>3</sub>OH: 1.274, 1.019, 0.764, 0.510, 0.255 and 0.127mg cm<sup>-2</sup>  
 stem wood CH<sub>3</sub>OH: 1.529, 1.274, 1.019, 0.764, 0.510 and 0.255mg cm<sup>-2</sup>  
 root bark CH<sub>3</sub>OH: 1.274, 1.019, 0.764, 0.510, 0.255 and 0.127mg cm<sup>-2</sup>  
 root wood CH<sub>3</sub>OH: 1.529, 1.274, 1.019, 0.764, 0.510 and 0.255mg cm<sup>-2</sup>
- *Mu. sapientum* leaf Pet.E.: 2.038, 1.783, 1.529, 1.274, 1.019 and 0.764mg cm<sup>-2</sup>  
 stem Pet.E.: 2.038, 1.529, 1.019, 0.510 and 0.255mg cm<sup>-2</sup>  
 root Pet.E.: 1.783, 1.529, 1.274, 1.019, 0.764, 0.510 and 0.255mg cm<sup>-2</sup>  
 leaf CH<sub>3</sub>OH: 1.529, 1.274, 1.019, 0.764, 0.510, 0.255 and 0.127mg cm<sup>-2</sup>  
 stem CH<sub>3</sub>OH: 0.510, 0.382, 0.255, 0.127 and 0.064mg cm<sup>-2</sup>  
 root CH<sub>3</sub>OH: 0.764, 0.637, 0.510, 0.382, 0.255 and 0.127mg cm<sup>-2</sup>

### 2.3.3. Observation of mortality in the surface film tests

After completing the all the arrangements treated Petri dishes were placed in a secured place at room temperature. The whole experiment was observed from time to time and mortality was observed by after ½, 6, 12, 24, 36 and 48h the data was recorded. A simple microscope was used to check each and every beetle by tracing natural movement of its organs. In some cases hot needle was taken closer to the bodies (without movement) to confirm death. Attention was also paid to recovery of the insects if occurred.

### 2.3.4. Statistical analysis

The mortality records of the residual film experiments done *T. castaneum* adults on adults were corrected by the Abbott's (1925) formula:

$$P_r = \frac{P_o - P_c}{100 - P_c} \times 100$$

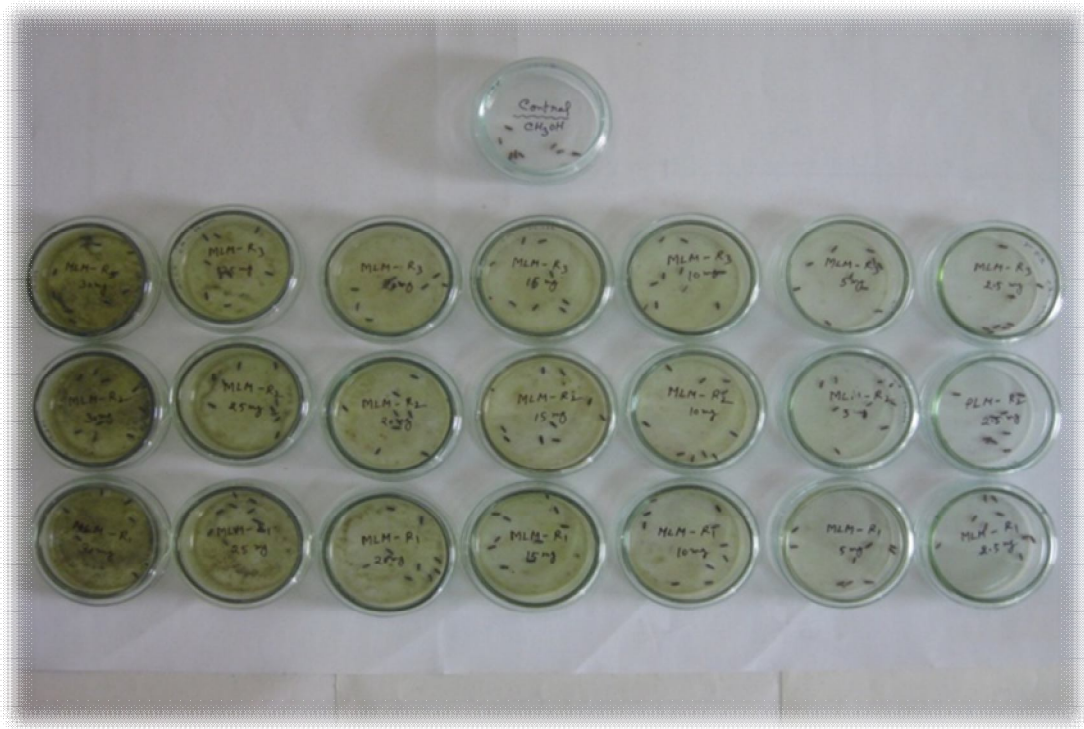
Where,

$P_r$  = Corrected mortality (%)

$P_o$  = Observed mortality (%)

$P_c$  = Control mortality (%), sometimes called natural mortality (%).

Then mortality percentages were subjected to statistical analysis according to Finney (1947) and Busvine (1971) by using 'computer software'. The dose-mortality relationship was expressed as a median lethal dose ( $LD_{50}$ ).



**Plate 2.5:** Bioassay with plant extracts on *T. castaneum* adults by surface film method

## 2.4. Bioassay through brine shrimp lethality test

### 2.4.1. Culture of *A. salina*

In the laboratory condition *A. salina* are very nice to grow, since the rate of successful hatches is very high. To conduct cytotoxicity test the brine shrimp nauplii were used because of its easy hatching and use in the experiment. The eggs (cysts) were collected from aquarium shops. For their easy hatching and use the requirements were as follows:

- Salt water: 38g salt per liter of water;
- Temperature: 26-28°C (80-82°F);
- Light: The beaker was placed near a window with sunlight before hatching;

- Aeration: Picking up some water carefully with a spoon and let it drop back into the beaker once a day [but a small aquarium pump with a little air-stone is better];
- Helpful Hint: Brine shrimp egg is sometimes very buoyant. Swirl the water to knock down eggs;

The cysts absorb water and if the sun is shining (a signal for growing algae and other plankton) they hatch after 24 to 48h, depending on their environment. Freshly hatched *A. salina* called nauplii and have a size of just 0.25mm. They molt like any other crawfish when they grow to adult they molt about 17 times. Freshly hatched nauplii were used in this experiment.

### **2.4.2. Experimental design for the lethality test**

Brine shrimp cysts were hatched in simulated seawater to get nauplii. Test samples are prepared by the addition of calculated amount of DMSO (dimethyl sulfoxide) for obtaining desired concentration of test sample. The nauplii were counted by visual inspection and were taken in Test-tubes containing 5ml of simulated seawater. Then samples of different concentrations were added to the pre marked test-tubes through pipettes. The test-tubes were left for 24h and then the nauplii were counted again to find out the cytotoxicity of the test agents and compared to the results with the control.

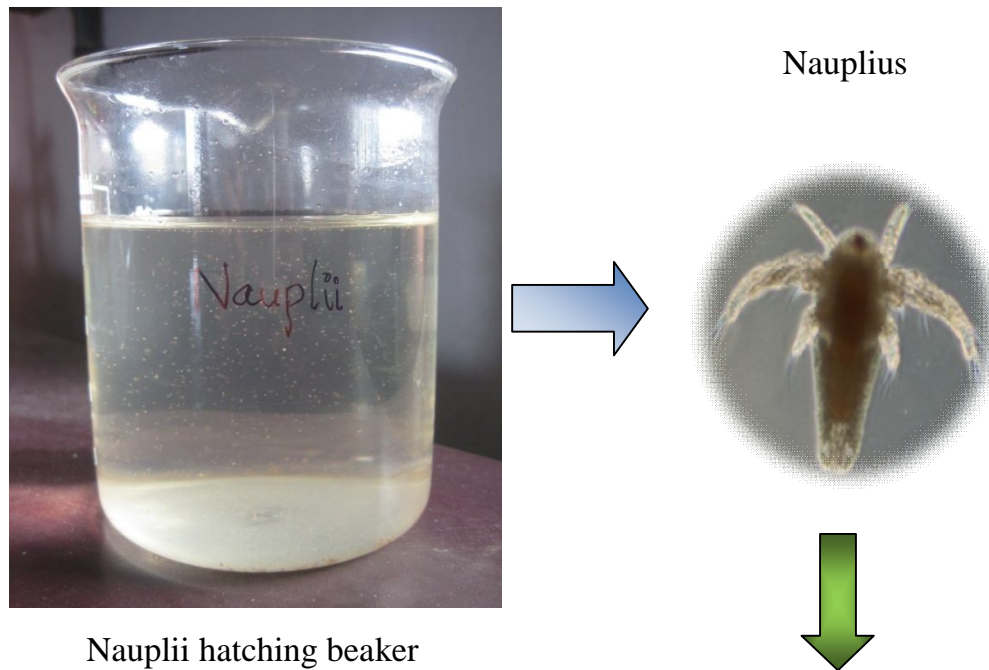
#### **Test materials:**

- Brine Shrimp (*A. salina*) cysts
- Iodine-free salt
- Small tank/ beaker to hatch the shrimp
- Pasteur pipette (1ml and 5ml)
- Test tubes (20ml)
- Magnifying glass

### **2.4.3. Preparation of simulated seawater (brine water)**

Since the lethality test involves the culture of brine shrimp nauplii that is, the nauplii should be grown in the seawater. Seawater contains 3.8% of NaCl. Accordingly 3.8% sodium chloride solution was made by dissolving sodium chloride (38g) in normal pond water (1000ml) and was filtered off.





**Plate 2.6:** Bioassay with plant extracts on *A. salina* nauplii by brine shrimp lethality test

#### 2.4.4. Hatching of the brine shrimp nauplii

Brine water was taken in a small tank and *A. salina* cysts ( $1.5\text{g L}^{-1}$ ) were added. Constant temperature ( $37^\circ\text{C}$ ) and sufficient light were maintained to give the sufficient aeration. After 24 hours, matured shrimp as nauplii was collected and used for the experiment.

#### 2.4.5. Experimentation of lethality test

The Pet.E.,  $\text{CHCl}_3$ , and  $\text{CH}_3\text{OH}$  extracts of different parts of *C. papaya*, *M. oleifera* and *Mu. sapientum* samples were applied against brine shrimp nauplii. For each samples, an 'Ad Hoc' test was done before final experimentation. Two milligram extract sample was weighed and taken in a small glass vial, then 1-2 drops of pure Dimethyl sulfoxide (DMSO) added to dissolve the extract initially and 1ml of pond water was taken into the vial to mix up the sample extract with water to prepare a 200ppm concentration. When it mixed up completely it was added to the test-tube (10ml marked) for conducting tests. This process was also maintained during final experiment. Separate vials were taken for each of the doses. The replicates were maintained for each of the concentrations.

##### Concentrations selected for the different extracts for the final experiment:

- *C. papaya* leaf Pet.E.: 250, 200, 150 and 100ppm
  - stem Pet.E.: 75, 50, 25, 12.5 and 6.25ppm
  - root Pet.E.: 100, 80, 60, 40 and 20ppm
  - leaf  $\text{CHCl}_3$ : 200, 150, 100, 75, 50 and 25ppm
  - stem  $\text{CHCl}_3$ : 75, 50, 25, 12.5 and 6.25ppm
  - root  $\text{CHCl}_3$ : 100, 75, 50, 25, 12.5 and 6.25ppm
  - leaf  $\text{CH}_3\text{OH}$ : 100, 80, 60, 40 and 20ppm
  - stem  $\text{CH}_3\text{OH}$ : 200, 150, 100, 50 and 25ppm
  - root  $\text{CH}_3\text{OH}$ : 200, 150, 100, 50 and 25ppm
- *M. oleifera* fruit Pet.E.: 80, 70, 60, 50, 40 and 30ppm
  - leaf Pet.E.: 200, 150, 100, 75, 50 and 25ppm
  - stem wood Pet.E.: 80, 70, 60, 50, 40 and 30ppm
  - root bark Pet.E.: 100, 90, 80, 70, 60 and 50ppm
  - root wood Pet.E.: 100, 90, 80, 70, 60 and 50ppm

fruit  $\text{CHCl}_3$ : 200, 150, 100, 75, 50 and 25ppm  
stem bark  $\text{CHCl}_3$ : 100, 75, 50, 25, 12.5 and 6.25ppm  
stem wood  $\text{CHCl}_3$ : 75, 50, 25, 12.5 and 6.25ppm  
root bark  $\text{CHCl}_3$ : 50, 25, 12.5, 6.25, 3.13 and 1.56ppm  
root wood  $\text{CHCl}_3$ : 200, 150, 100, 50, 25, 12.5 and 6.25ppm  
fruit  $\text{CH}_3\text{OH}$ : 200, 150, 100, 50 and 25ppm  
leaf  $\text{CH}_3\text{OH}$ : 200, 150, 100, 50 and 25ppm  
stem bark  $\text{CH}_3\text{OH}$ : 90, 70, 50, 30 and 10ppm  
stem wood  $\text{CH}_3\text{OH}$ : 250, 200, 150, 100, 50, 25 and 12.5ppm  
root bark  $\text{CH}_3\text{OH}$ : 100, 50, 25, 12.5, 6.25 and 3.13ppm  
root wood  $\text{CH}_3\text{OH}$ : 300, 250, 200, 150, 100 and 50ppm

➤ *Mu. sapientum* leaf Pet.E.: 200, 150, 100, 75, 50 and 25ppm  
stem Pet.E.: 90, 70, 50, 30 and 10ppm  
root Pet.E.: 100, 80, 60, 40 and 20ppm  
leaf  $\text{CHCl}_3$ : 100, 75, 50, 12.5 and 6.25ppm  
stem  $\text{CHCl}_3$ : 50, 40, 30, 20 and 10ppm  
root  $\text{CHCl}_3$ : 80, 70, 60, 50 and 40ppm  
leaf  $\text{CH}_3\text{OH}$ : 200, 150, 100, 50 and 25ppm  
stem  $\text{CH}_3\text{OH}$ : 150, 100, 75, 50 and 25ppm  
root  $\text{CH}_3\text{OH}$ : 200, 150, 100, 50 and 25ppm

#### 2.4.6. Application of doses to the nauplii

In each of the five test tubes, 5ml brine water (3.8%) containing 10 brine shrimp nauplii with the help of a pasture pipette. Specific volume of each samples were transferred from the stock solution to the respective test tubes to get the target volume. The volume of DMSO should not exceed  $10\mu\text{l ml}^{-1}$  of the brine solution, because above this concentration toxicity due to DMSO may arise.

#### 2.4.7. Observation of lethality

The test tubes containing the nauplii along with the treated brine water were kept on a rack near the window in the laboratory. After 6, 12, 18 and 24h, the test tubes were

observed. The number of survived nauplii in each test tube was counted and the results were noted. From this, percentages of mortality of brine shrimp nauplii were calculated.

### 2.4.8. Analysis of data

Data were analyzed statistically by Probit analysis as done previously in surface film test and here the dose-mortality relationship was expressed as the median lethal concentration (LC<sub>50</sub>).

## 2.5. Selection of microorganisms for antibacterial test agents

Antimicrobial activity can be detected by observing the growth response of various microorganisms to the different plant extracts or isolated compounds from them, which are placed in contact with them. Seven pathogenic bacteria were selected for the antibacterial activity test, two of which were gram positive and the remaining were gram negative. The bacterial strains used for this investigation are listed in the following Table 2.2.

Sl. No.	Gram negative	Sl. No.	Gram positive
1	<i>Escherichia coli</i>	1	<i>Bacillus subtilis</i>
2	<i>Klebsiella pneumoniae</i>	2	<i>Staphylococcus aureus</i>
3	<i>Salmonella enteritidis</i>		
4	<i>Shigella flexneri</i>		
5	<i>Shigella sonnei</i>		
Nine isolates from the industrial effluents			
1	<i>Escherichia coli</i> I (Isolate 4)	1	<i>Bacillus cereus</i> (Isolate 1)
2	<i>Escherichia coli</i> II (Isolate 5)	2	<i>Bacillus subtilis</i> (Isolate 8)
3	<i>Klebsiella oxytoca</i> (Isolate 2)	3	<i>Staphylococcus aureus</i> (Isolate 3)
4	<i>Cytrobacter freundii</i> (Isolate 6)		
5	<i>Proteus vulgaris</i> (Isolate 7)		
6	<i>Salmonella typhimurium</i> (Isolate 9)		

### 2.5.1. Collection and culture of test bacteria

The pure cultures were collected from the microbiological research laboratory of the Department of Biochemistry, Rajshahi University.

#### 2.5.1.1. Culture media

A number of culture media are available to demonstrate the antibacterial activity. These are as follows:

- i) Nutrient agar medium
- ii) Nutrient broth medium
- iii) Mueller-Hinton medium
- iv) Tryptic Soy broth (TSB) medium
- v) Trypticase Soy agar medium
- vi) Staphylococcus defined medium
- vii) Adams and Roe medium
- viii) NTH agar or broth medium.

While the nutrient agar medium was adopted to conduct experiments in this investigation.

**Table 2.3:** The list of the composition of nutrient agar medium.

<b>Ingredient</b>	<b>Amount</b>
Bactopeptone	0.5g
Sodium chloride	0.5g
Bactoyeast extract	1.0g
Bactoagar	2.0g
Distilled water	100ml
<i>pH</i>	7.2±0.1 at 25°C

#### 2.5.1.2. Preparation of fresh culture of the pathogenic organisms

The nutrient broth medium was prepared and dispersed in a number of clean test tubes to prepare broth (5ml in each test tube). The test tubes were plugged with cotton and sterilized in an autoclave at 121°C and 15lbs sq<sup>-1</sup> inch pressure for 15min. After sterilization, the test tubes were kept in an inclined position for cool. The test organisms

were transferred to the nutrient broth from the pure cultures with the help of an inoculating loop in an aseptic condition. Burning the loop after each transfer of microorganism was done to avoid contamination very carefully. The inoculated broth was then incubated at 37.5°C for 24h to assure the growth of test organisms. These fresh cultures were used for the sensitivity tests.

## 2.5.2. Selection of test method

### (i) Primary assay

It is essentially a qualitative or semi-qualitative test that indicates the sensitivity or resistance of microorganisms to the compound. However, this technique cannot be used to distinguish between bacteriostatic and bactericidal agents (Reiner, 1980).

The primary assay can be done in three ways such as-

- A. Diffusion method
- B. Dilution method and
- C. Bio-autographic method.

Among these methods the disc diffusion method (Bauer *et al.*, 1966; Reiner, 1982) is widely acceptable for the preliminary evaluation of antimicrobial activity. It uses different concentrations of the agents absorbed on sterile filter paper discs. There is no standardized method for expressing the results of antimicrobial screening (Ayafar *et al.*, 1982). Some investigators use the diameter of the zone of inhibition or the minimum weight of extract that inhibits the growth of a microorganism. Disc diffusion is essentially a qualitative or semi-quantitative test indicating the sensitivity or resistance of microorganisms to the test material. No distinction between bacteriostatic and bactericidal activity can be made by this method (Reiner, 1982). However, the diffusion method was used in this investigation.

### Principles of the diffusion method

Diffusion assay (Barry, 1976) is based on the ability of antibiotics to diffuse from a confined source through the nutrient agar gel and create a concentration gradient. If the agar is seeded or streaked with a sensitive organism, a zone of inhibition will result where the concentration exceeds the minimum inhibitory concentration (MIC) for the particular organism.

In this method, measured amount of the test samples are dissolved in definite volumes of solvent to give solutions of known concentrations ( $\mu\text{g ml}^{-1}$ ). The sterile (BBL, Cocksville, USA) filter paper (5mm diameter) disc were impregnated with known amounts of the test substances and dried. These test material discs were placed on plates containing nutrient agar medium seeded with the test organisms. These plates were kept at low temperature ( $4^{\circ}\text{C}$ ) for 24h to allow maximum diffusion.

A number of events took place simultaneously which includes:

- i) The dried discs absorb water from the agar medium and the material under test was dissolved.
- ii) The test material diffuses from the discs to the surrounding medium according to the physical law that controls the diffusion of molecules through agar gel.
- iii) There was a gradual change of test material concentration in the agar surrounding each disc.

To determine the most optimal concentration of extracts to be used in this study, sterile 7.5mm filter paper disks were treated with 200 and 400 $\mu\text{l}$  Pet.E,  $\text{CHCl}_3$  and  $\text{CH}_3\text{OH}$  extracts (while the only solvent used as control). The bacteria were inoculated on full-strength Nutrient Agar (Qualigens Fine Chemicals, Prod # 58673) by suspending loops in sterile de-ionized water. The bacterial suspension was then smeared on agar plates with a sterile glass-rod to ensure the entire surface of the agar had an even coating of the bacterial suspension. The plates were divided into several areas and one filter paper disk was placed on each of the areas. The plates are then kept in an incubator ( $37^{\circ}\text{C}$ ) for 12 to 18h to allow the growth of the organisms. If any of the test material has antimicrobial activity, it will inhibit the growth of microorganisms just giving a clear distinct zone called 'Zone of Inhibition'. Biological activity of the *C. papaya*, *M. oleifera* and *Mu. sapientum* components on bacterial growth were quantified by measuring the diameter of the zones of inhibition in term of mm. The size of the inhibitory zones depends principally on the following factors:

- i) Intrinsic antimicrobial sensitivity of the test sample,
- ii) Growth rate of the test microorganisms,
- iii) Diffusion rate of the freshly seeded test organisms,

- iv) Concentration of the freshly seeded test organisms,
- v) Amount of test sample on disc,
- vi) Thickness of the test medium in the Petri dishes,
- vii) Composition of the culture medium,
- viii) Size of inocula,
- ix) Time of incubation,
- x) Temperature of incubation.

### **(ii) Secondary assay**

The simple assay quantifies the relative potency, such as minimum inhibitory concentration (MIC) of the lowest concentration of an antimicrobial agent required to inhibit the growth of the microorganisms *In Vitro*. It is done by serial dilution technique (Reiner, 1980).

### **Test materials used for the study**

- Pet.E., CHCl<sub>3</sub> and CH<sub>3</sub>OH extracts of different parts of *C. papaya*, *M. oleifera* and *Mu. Sapientum*.
- Ampicillin (10µg disc<sup>-1</sup>) as standard discs.

### **Apparatus and reagents to conduct antibacterial assay**

- i) Blank sterilized filter paper discs (diam. 5mm)
- ii) Petri dishes (diam. 120mm)
- iii) Test tubes
- iv) Inoculating loop
- v) Spirit burner and a match box
- vi) Sterile forceps
- vii) Sterile cotton
- viii) Laminar air flow unit (Biocraft & Scientific Industries, INDIA)
- ix) Micropipette (10 -100µl)
- x) Autoclave (ALP Co. Ltd. KT- 30L, JAPAN)
- xi) Incubator (Lab Tech, Model: LIB -030M, Korea)
- xii) Refrigerator
- xiii) Punch machine
- xiv) Beaker
- xv) Nutrient agar media (DIFCO)



- xvi) Solvent (Pet.E.,  $\text{CHCl}_3$  and  $\text{CH}_3\text{OH}$ )
- xvii) Vials
- xviii) Rectified spirit
- xix) Alcohol (95%)

### **2.5.3. Sterilization procedures**

The antibacterial screening was carried out in a laminar airflow unit and all types of precautions were highly maintained to avoid any type of contamination during the test. UV light was switched on for half an hour before working in the laminar hood to avoid any accidental contamination. Petri dishes and other glass-wares were sterilized in the autoclave at  $121^\circ\text{C}$  temperature and a pressure of 15lbs/sq inch for 15min. Micropipette tips, culture media, cotton, forceps, blank discs, etc were also sterilized.

### **2.5.4. Preparation of the test plates**

The test plates were prepared according to the following procedure:

- (i) The nutrient agar medium prepared in the previous section was poured in 15ml quantity in each in the clean test tubes and plugged with cotton.
- (ii) The test tubes and a number of Petri dishes were sterilized in an autoclave at  $121^\circ\text{C}$  and 15lbs/sq inch pressure for 15min and were transferred into laminar airflow unit and then allowed to cool to about  $45^\circ\text{C}$  to  $50^\circ\text{C}$ .
- (iii) The test organism was transferred from the fresh subculture to the test tube containing 15ml autoclaved medium with the help of an inoculating loop in an aseptic condition. Then the test tube was shaken by rotation to get a uniform suspension of the organism.
- (iv) The bacterial suspensions were immediately transferred to the sterile Petri dishes in an aseptic area. The Petri dishes were rotated several times, first clockwise and then anticlockwise to assure homogenous distribution of the test organisms. The media were poured into Petri dishes in such a way as to give a uniform depth of approximately 4mm.
- (v) Finally, after medium was cooled to room temperature in laminar airflow unit, it was stored in a refrigerator ( $4^\circ\text{C}$ ).

### **2.5.5. Preparation of discs containing samples**

For the preparation of discs containing samples, following procedure was utilized:

#### **(a) Sample discs**

Sterilized filter paper discs (5mm diam.) were taken by the forceps in to the plates. Sample solutions of desired concentrations were applied on the discs with the help of a micropipette in an aseptic condition. These discs were left for a few minutes in aseptic condition for complete removal of the solvent.

#### **(b) Standard discs**

These were used to compare the antibacterial activity of the test material. In the present study, Ciprofloxacin discs containing  $10\mu\text{g disc}^{-1}$  of antibiotic Ciprofloxacin were used as standard discs for comparison purpose.

### **2.5.6. Placement of the discs and incubation**

For the placement of the discs, the following procedure was utilized:

- (i) By means of a pair of sterile forceps, the sample impregnated discs were placed gently on the solidified agar plates seeded with the test organisms to ensure contact with the medium.
- (ii) The plates were then kept in a refrigerator at  $4^{\circ}\text{C}$  for 24h in order to provide sufficient time to diffuse the antibiotics into the medium.
- (iii) Finally, the plates were incubated at  $37.5^{\circ}\text{C}$  for 24h in an incubator.

### **2.5.7. Precaution**

The discs were placed in such a way that they were not closer than 15mm to the edge of the plate and far enough apart to prevent over lapping the zones of inhibition.

### **2.5.8. Measurement of the zones of inhibition**

After incubation, the antibacterial activities of the test samples were determined by measuring the diameter of inhibitory zones in term of mm with a transparent scale.

## **2.6. Isolation and Identification of bacteria from industrial effluent**

### **2.6.1. Collection of effluent**

The tannery effluent samples were collected from Rayerbazar sluice gate area under Dhaka City Corporation (South) adjacent to Hazaribagh tannery during March, 2014. The samples were collected in bags for bacteriological analysis and sterile plastic container for waste water analysis and transported to the laboratory. We collected three samples *viz.* sample 1: behind the sluice gate point of 500m far (waste water), sample 2: sludge of adjacent sluice gate point and sample 3: sludge with water of adjacent sluice gate point.

### **2.6.2. Physico-chemical analysis of effluent**

Physico-chemical analysis of water is the analysis of the physical and chemical properties of a sample of water, often to determine if the water is suitable for drinking or supporting aquatic life.

The remaining physico-chemical parameters (*pH*; Electrical Conductivity, EC; Total Dissolved Solids, TDS; Biological Oxygen Demand, BOD; Chemical Oxygen Demand, COD; heavy metal ions) were determined as soon as the sample was brought to the laboratory. Analysis of different metal ions in the effluent sample was determined by Atomic Absorption Spectrophotometer (AAS) as per the standard methods (APHA, 2005).

#### **2.6.2.1. *pH***

Measuring the *pH* for water analysis is an important physical parameter. The *pH* scale ranges from 0 to 14, with pure water at seven for neutral. If the water is under seven, that means there is acidic compound present. If it is above seven, there are alkalis present.

#### **2.6.2.2. Electrical Conductivity (EC)**

Electrical conductivity is used to measure water's ability to conduct an electrical current. Nutrients, minerals, metals and pollutants can affect this ability.

### **2.6.2.3. Total Dissolved Solids (TDS)**

Total dissolved solids (TDS) comprise inorganic salts and small amounts of organic matter that are dissolved in water. Many dissolved substances are undesirable in water. Dissolved minerals, gases and organic constituents may produce aesthetically displeasing colour, taste and odor. Some dissolved organic chemicals may deplete the dissolved oxygen in the receiving waters and some may be inert to biological oxidation, yet others have been identified as carcinogens.

### **2.6.2.4. Biological Oxygen Demand (BOD)**

The biological oxygen demand determination is a chemical procedure for determining the amount of dissolved oxygen needed by aerobic organisms in a water body to break the organic materials present in the given water sample at certain temperature over specific period of time.

### **2.6.2.5. Chemical Oxygen Demand (COD)**

The chemical oxygen demand (COD) test is commonly used to indirectly measure the amount of organic compounds in water. Most applications of COD determine the amount of organic pollutants found in surface water (e.g. lakes and rivers) or wastewater, making COD a useful measure of water quality. It is expressed in milligrams per liter ( $\text{mg L}^{-1}$ ) also referred to as ppm (parts per million), which indicates the mass of oxygen consumed per liter of solution.

### **2.6.2.6. Fluoride**

Fluoride is considered to be one of the major ions of seawater. Fluoride is used in certain industrial processes and consequently occurs in the resulting wastewaters.

### **2.6.2.7. Chloride**

Chloride anions are usually present in natural waters. A high concentration occurs in waters that have been in contact with chloride-containing geological formations. Otherwise, a high chloride content may indicate pollution by sewage or industrial wastes or by the intrusion of seawater or saline water into a freshwater body or aquifer.

### **2.6.2.8. Nitrite**

Nitrite is an unstable, intermediate stage in the nitrogen cycle and is formed in water either by the oxidation of ammonia or by the reduction of nitrate. Thus, biochemical

processes can cause a rapid change in the nitrite concentration in a water sample. In natural waters nitrite is normally present only in low concentrations (a few tenths of a milligram per litre). Higher concentrations may be present in sewage and industrial wastes, in treated sewage effluents and in polluted waters.

#### **2.6.2.9. Nitrate**

Nitrates generally occur in trace quantities in surface waters but may attain high levels in some ground waters. Nitrite in water is either due to oxidation of ammonium compounds or due to reduction of nitrate. It can be toxic to certain aquatic organisms even at concentration of  $1\text{ mg L}^{-1}$ . In excessive limits, it contributes to the illness known as methenoglobinemia in infants.

#### **2.6.2.10. Bromide**

Inorganic bromide is widely distributed in nature. Its natural physiological role in animal life is unknown. It was once used in sedatives and headache remedies like Bromo-Seltzer until it was withdrawn because of concerns about toxicity. When it shows up at elevated levels in freshwater, it is due to human activities.

#### **2.6.2.11. Phosphate**

Phosphate occurs in traces in many natural waters, and often in appreciable amounts during periods of low biologic productivity. Traces of phosphate increase the tendency of trouble some algae to grow in reservoirs. Waters receiving raw or treated sewage, agricultural drainage, and certain industrial waters normally contain significant concentrations of phosphate. Also phosphate is frequently added to domestic and industrial waters in various forms. Phosphate analyses are made primarily to control chemical dosage, or as a means of tracing flow of contamination.

#### **2.6.2.12. Sulphate**

Sulphate is an abundant ion in the earth's crust and its concentration in water can range from a few milligrams to several thousand milligrams per litre. Industrial wastes and mine drainage may contain high concentrations of sulphate. Sulphate also results from the breakdown of sulphur-containing organic compounds. Sulphate is one of the least toxic anions.

### 2.6.2.13. Concentration of metal ions in effluent

The presence of various heavy metals in industrial wastewaters is of serious concern because they are highly toxic, non-biodegradable, carcinogenic, and continuous deposition into receiving lakes, streams and other water sources within the vicinity causes bioaccumulation in the living organisms.

**Arsenic (As):** Arsenic is an element used for several human activities. It is a very poisonous element; prolonged exposure to it or ingestion of small amounts can cause long term effects, such as cancer. In nature, as is present in the form of various minerals, such as arsenopyrite ( $\text{FeAsS}$ ) and lollyngite ( $\text{FeAs}_2$ ). Leaks of these minerals into ground water can cause contamination.

**Chromium (Cr):** Chromium is the most abundant element in Earth's crust. Chromium compounds are found in the environment, due to erosion of chromium-containing rocks and can be distributed by volcanic eruptions. In larger amounts and in different forms, chromium can be toxic and carcinogenic. The most prominent example of toxic chromium is hexavalent chromium ( $\text{Cr(VI)}$ ). Abandoned chromium production sites often require environmental cleanup.

**Cadmium (Cd):** Cadmium can mainly be found in the earth's crust. It always occurs in combination with zinc. Cadmium is an extremely toxic metal commonly found in industrial workplaces. Due to its low permissible exposure limit, overexposures may occur even in situations where trace quantities of cadmium are found. Cadmium is used extensively in electroplating, although the nature of the operation does not generally lead to overexposures. Cadmium is also found in some industrial paints and may represent a hazard when sprayed. Operations involving removal of cadmium paints by scraping or blasting may pose a significant hazard.

**Cobalt (Co):** In nature, cobalt is frequently associated with nickel and both are characteristic components of meteoric iron. Free cobalt is not found in on Earth due to the amount of oxygen in the atmosphere and chlorine in the ocean. Oxygen and chlorine are abundant enough in the upper layers of the Earth's crust so as to make native metal cobalt formation extremely rare. It is one of the first transition metals. Small amounts of cobalt compounds are found in most rocks, soil, plants, and animals. Cobalt is the active center of coenzymes called cobalamins, the most common example

of which is vitamin B<sub>12</sub>. As such it is an essential trace dietary mineral for all animals. Cobalt in inorganic form is also an active nutrient for bacteria, algae and fungi.

**Copper (Cu):** Copper is essential to all living organisms as a trace dietary mineral. Copper compounds are used as bacteriostatic substances, fungicides, and wood preservatives. Hazards and risks associated with copper: copper metal powder is a fire hazard. Unless known otherwise, all copper compounds should be regarded as toxic. Pollution from industrial smoke is a problem.

**Iron (Fe):** Iron is an abundant element in the earth's crust, but exists generally in minor concentrations in natural water systems. The form and solubility of iron in natural waters are strongly dependent upon the pH and the oxidation- reduction potential of the water. Iron is found in the +2 and +3 oxidation states. In a reducing environment, ferrous (+2) iron is relatively soluble. An increase in the oxidation-reduction potential of the water readily converts ferrous ions to ferric (+3) and allows ferric iron to hydrolyse and precipitate as hydrated ferric oxide. The precipitate is highly insoluble.

**Lead (Pb):** Lead is also a hazardous element employed in many processes. Exposure or ingestion of lead can cause serious harm to the nervous system; young children seem particularly affected, as they may experience long-term effects, such as learning disabilities. In nature, lead is found in ores, such as galena (PbS), anglesite (PbSO<sub>4</sub>) and cerussite (PbCO<sub>3</sub>). Today, its main industrial application is in the production of batteries, especially for cars. Lead is also used in construction, due to its resistance to corrosion; some lead-based compounds are employed in the manufacturing of paints, and in many electronic devices.

**Manganese (Mn):** Manganese also functions in the oxygen-evolving complex of photosynthetic plants. The element is a required trace mineral for all known living organisms. In larger amounts, and apparently with far greater activity by inhalation, it can cause a poisoning syndrome in mammals, with neurological damage which is sometimes irreversible. Manganese compounds are less toxic than those of other widespread metals.

**Nickel (Ni):** Nickel is one of four elements that are ferromagnetic around room temperature. The metal is chiefly valuable in the modern world for the alloys it forms;

about 60% of world production is used in nickel-steels (particularly stainless steel). As a compound, nickel has a number of niche chemical manufacturing uses, such as a catalyst for hydrogenation. Enzymes of some microorganisms and plants contain nickel as an active site, which makes the metal an essential nutrient for them. Nickel can have an impact on human health through infectious diseases arising from nickel-dependent bacteria.

**Potassium (K):** Potassium is a relatively abundant element. Most industrial chemical application of potassium employ the relatively high solubility in water of potassium compounds. Potassium metal has only a few special applications, being replaced in most chemical reactions with sodium metal. Potassium is an extremely active metal, which reacts violently with oxygen and water in air. Potassium ions are necessary for the function of all living cells. Potassium ion diffusion is a key mechanism in nerve transmission, and potassium depletion in animals, including humans, results in various cardiac dysfunctions.

**Zinc (Zn):** Zinc is an essential and beneficial element in body growth. Concentrations above 5 mg/l may cause a bitter astringent taste and opalescence in alkaline water. Zinc most commonly enters the domestic supply from deterioration of galvanized iron and dezincification of brass. Zinc in water may also come from individual water pollution.

### **2.6.3. Isolation of bacteria from industrial effluent**

#### **2.6.3.1. Equipments and media for the isolation of bacteria**

- i) Petri dishes (90mm diam.)
- ii) Test tubes
- iii) Beaker
- iv) Inoculating loop
- v) Sterile cotton
- vi) Sterile forceps
- vii) Spirit burner and a match box
- viii) Eppendorf tube
- ix) Micropipette (10 $\mu$ l-100 $\mu$ l) and sterile tips
- x) ESCO, Class II Biological Safety Cabinet (Model: AC2- 4E1,Indonesia)



- xi) Autoclave (HIRAYAMA)
- xii) Incubator (JEIO TECH, Model: IB- 01E, Korea)
- xiii) Refrigerator (SHARP, Ag<sup>+</sup> Nano Deodoriger)
- xiv) Microwave oven
- xv) Mikrobiologie (Nutrient agar) Merck, Germany
- xvi) Different kinds of media and reagents
- xvii) Rectified spirit
- xviii) Ethanol (95%)
- xix) Vortex mixture
- xx) Nose mask and hand gloves
- xxi) Markers for labeling
- xxii) Racks and ice buckets
- xxiii) Waste containers



**Plate 2.7:** Biological safety cabinet in BCSIR microbiology laboratory, Rajshahi

### 2.6.3.2. Preparation of culture media

The instant nutrient agar (Mikrobiologie) medium was weighed and then reconstituted with distilled water in the McCartney bottle according to specification (28g L<sup>-1</sup>). It was then heated in a microwave oven to dissolve the agar until a transparent solution was obtained. The bottle was plugged with cap and sterilized in an autoclave at 121°C and 15lbs/sq inch pressure for 15min. After sterilization, the media transferred to Petri dishes kept in for solidification. These were then preserved in refrigerator at 4°C for further studies.

### 2.6.3.3. Isolation of single colony of bacteria from effluent

Effluent samples were serially diluted in sterile distilled water. Then 0.1ml of each effluent sample was taken in eppendorf tube then 0.9ml distilled water added and carefully shaken on a vortex mixture for few min. This solution was then serially diluted to the 10<sup>-7</sup> dilution, from which 0.1ml was separately plated onto nutrient agar medium and then incubated for 24 to 48h at 37°C. Discrete bacterial colonies that developed on agar plates were initially grouped on the basis of colony morphology. Selected bacterial isolates were further purified and sub-cultured. The pure cultures were identified based on gram staining and motility followed by their biochemical activities.

**Gram stain:** The stain makes use of the differing membrane structures between Gram positive and Gram negative organisms. The three stains (crystal violet, iodine solution and safranin) are used for this technique.

#### **Procedure:**

- The clean slide is taken and three drops of sterilized distilled water is dropped on three different places of the slide. A loop-full of isolate bacterial colonies are transferred with a sterilized loop one of the drop of distilled water on the slide and a very thin film is prepared by spreading uniformly. Then the loop is sterilized. A loop-full of bacterium is transferred from middle of the smear to the next water drop and thin film is prepared. In the same way bacterium is transferred from second to the third drop and thin film is prepared. This technique is better observation of each isolated bacterium. The film is fixed by passing it over the gentle flame for two or three times.

- Gently flood smears with crystal violet and let stand for 1min.
- Gently wash with tap water.
- Gently flood smears with the iodine solution and let stand for 1min.
- Gently wash with tap water.
- Decolorize with 95% ethyl alcohol. Do not over- decolorize. Add reagent drop by drop until alcohol runs almost clear, showing only a blue tinge.
- Gently wash with tap water.
- Counterstained with safranin for 45sec.
- Gently wash with tap water and blot dry with bibulous paper.
- At last the bacterial slide is examined under microscope.
- Gram positive cells will incorporate little or no counterstain and will remain blue-violet in appearance. Gram negative bacteria, however, take on a pink color and are easily distinguishable from the Gram positives.

**Motility test:** SIM (Sulfur Indole Motility Media) agar may also be used to detect motile organisms. Motility is recognized when culture growth (turbidity) of flagellated organisms is not restricted to the line of inoculation. Growth of non-motile organisms is confined to the line of inoculation. SIM tubes are inoculated with a single stab to the bottom of the tube and incubated at the 37°C temperature for 24 to 48h. If an organism is motile then the growth will radiate from the stab mark and make the entire tube appear turbid.

#### **2.6.3.4. Maintenance of stock culture**

The stock culture was maintained following the procedures of Carter (1979). Nutrient agar slants used for the maintenance of culture for each of the bacterial isolate. After growth of the organism in the slant, the sterile mineral oil was overlaid and culture was kept at room temperature for use as seed.

#### **2.6.4. Biochemical analyses**

Isolates were biochemically analyzed by; growth on MacConkey Agar and Mannitol salt agar; H<sub>2</sub>S production test, Gelatin liquefaction test, Citrate utilization test, Urease test, Indole production test, MR-VP test, Catalase test, Amylase test, Triple Sugar Iron Agar test and Carbohydrate fermentation test. The tests were used to identify the

isolates according to Bergey's Manual of Determinative bacteriology (Holt *et al.*, 1994).

#### **2.6.4.1. MacConkey agar test**

MacConkey agar is a widely used culture medium that is both selective and differential. A selective medium selects for the growth of some organisms, while inhibiting the growth of others. In the case of MacConkey agar, the presence of bile salts and crystal violet inhibits the growth of most Gram positive bacteria. A differential medium does not inhibit the growth of bacteria, but differentiates them based on some visible growth characteristic such as colony color. MacConkey agar contains lactose, a fermentable carbohydrate, and the pH indicator neutral red. When lactose is fermented, acid products lower the pH below 6.8 with the resulting colonial growth turning pinkish-red. If an organism is unable to ferment lactose, the colonies will be colorless or yellow. The medium thus differentiates between lactose-fermenting bacteria and lactose non-fermenters, which include potential pathogens.

#### **2.6.4.2. Hydrogen Sulfide Production test**

Sulfur indole media (SIM) is a differential medium. It tests the ability of an organism to do several things: reduce sulfur, produce indole and swim through the agar (be motile). Sulfur can be reduced to H<sub>2</sub>S (hydrogen sulfide) either by catabolism of the amino acid cysteine by the enzyme cysteine desulfurase or by reduction of thiosulfate in anaerobic respiration. If hydrogen sulfide is produced, a black color forms in the medium. The result is positive for H<sub>2</sub>S production. Absence of the colour is evidence of a negative reaction.

#### **2.6.4.3. Gelatin liquefaction test**

This test used to determine the ability of an organism to produce enzyme gelatinase, which liquefies gelatin. Gelatinase breaks down large proteins into smaller components, which can then enter the organism and be metabolized. Stab gelatin with organism using a straight wire and incubate at 37°C temperature for 24 to 48h. Place tubes in ice water bath for at least 30min. If gelatin is liquefied the results is positive. Gelatin is solid result is negative.

#### 2.6.4.4. Citrate test

The test used to determine if an organism is capable of using citrate as the sole source of carbon with production of the enzyme citratase. The media contains sodium citrate as the carbon source, and ammonium salts as the nitrogen source, with bromthymol blue as the pH indicator. An organism that uses citrate breaks down the ammonium salts to ammonia, which creates an alkaline pH. Stab and streak Simmons citrate agar slant with the organism and incubate at the 37°C temperature for 24 to 48h. Alkaline pH causes media to change from green to Prussian blue the result is positive. For no color change the result is negative.

#### 2.6.4.5. Urease test

This test used to determine the ability of an organism to split urea to form ammonia (an alkaline end product) by the action of the enzyme urease. Media also contains the pH indicator phenol red, which turns an intense pink at alkaline pH. Inoculate urea broth with the organism at 37°C temperature for 48h. After incubation phenol red turns to a deep pink color. This is a positive reaction for the presence of urease. Failure of the pink color is the result of negative reaction.

#### 2.6.4.6. Indole test

This test used to determine the ability of an organism to split indole from the amino acid tryptophan using the enzyme tryptophanase. Incubate broth at 37°C temperature with the organism for 24 to 48h. Add 10 to 12 drops of Kovacs Reagent. Red layer forms on surface of the media the result is positive. If yellow layer forms on the surface of the media the result is negative.

#### 2.6.4.7. MR-VP test

**Methyl Red test (MR):** Used the test to determine the ability of an organism to produce mixed acid end products from glucose fermentations. Some organisms produce large amounts of various acids (lactic, acetic, succinic, formic) plus H<sub>2</sub> and CO<sub>2</sub>. The large amounts of acids lower the pH to lower than 5. Inoculate MR broths with these organisms at 37°C temperature for 24 to 48hours. After incubation add 3 to 4 drops of Methyl Red reagent to each tube. Red color develops for positive and yellow color develops for negative reaction.

**Voges-Proskauer test (VP):** The test used to determine the ability of an organism to produce acetoin; 2,3 butanediol; and ethanol which causes less lowering of the *pH* than the methyl red positive organisms. VP test detects the presence of acetoin, which is a precursor to 2,3 butanediol. Inoculate VP broths with these organisms at 37°C temperature for 24-48 hours. After incubation add 18 drops of Barritt's Solution A (alphanaphthol) and 18 drops of Barritt's Solution B (KOH). Agitate vigorously for 1 to 2min. Let stand for 1 to 2h. Positive reaction for wine red (burgundy) color develops and negative for brown color develops

#### **2.6.4.8. Mannitol Salt Agar test (MSA)**

This type of medium is both selective and differential. The MSA will select for organisms which can live in areas of high salt concentration. The differential ingredient in MSA is the sugar mannitol. Organisms capable of using mannitol as a food source will produce acidic byproducts of fermentation that will lower the *pH* of the media. The acidity of the media will cause the *pH* indicator, phenol red, to turn yellow.

#### **2.6.4.9. Catalase test**

This test is used to identify organisms that produce the enzyme catalase. This enzyme detoxifies hydrogen peroxide by breaking it down into water and oxygen gas. Streak nutrient agar plate with the organism and incubate at 37°C temperature for 24 to 48h. Add a few drops of 3% H<sub>2</sub>O<sub>2</sub> on the colony, it is broken down and the oxygen produce bubbles. The bubbles resulting from production of oxygen gas clearly indicate a catalase positive and no bubbles indicate catalase negative.

#### **2.6.4.10. Amaylase test**

The test used to determine the ability of an organism to hydrolyze (break down) starch. The enzyme amylase breaks starch down into components more easily metabolized by the organism. Make a single streak of the organism on a starch agar plate and incubate at 37°C temperature for 24 to 48h. Drop a small amount of IKI (Gram's Iodine) onto the plate and rotate the plate gently. Iodine is an indicator of starch; in the presence of starch the iodine will turn blue/black. If positive, a zone of clearing appears adjacent to the streak line and negative no clearing; only a blue/black area surrounding the streak line.

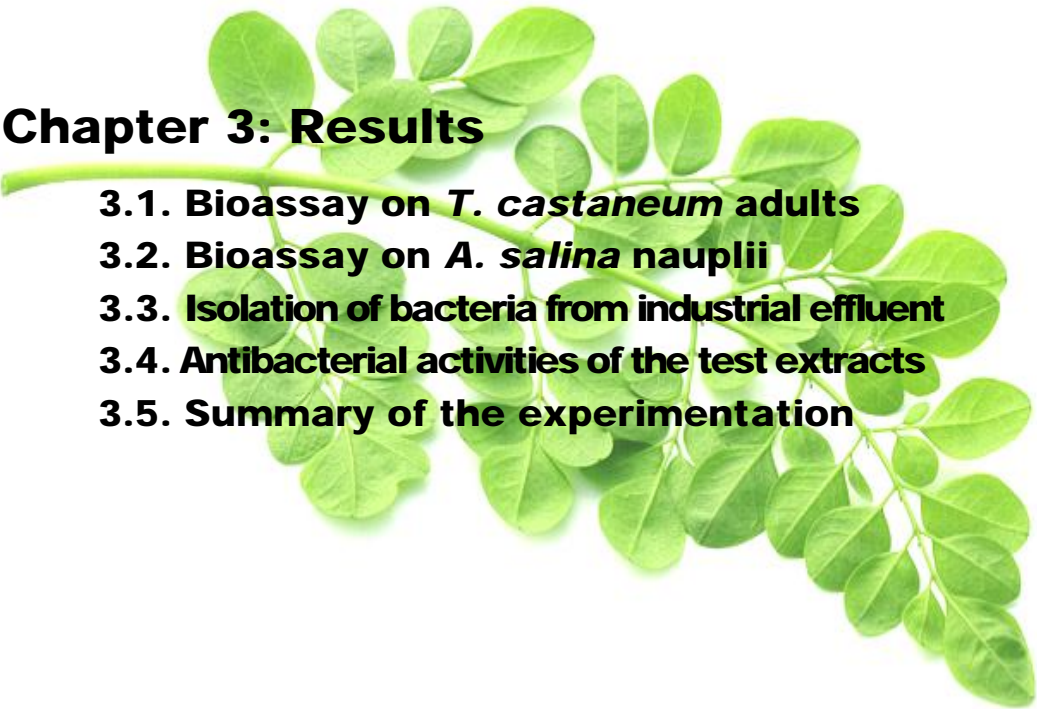
#### **2.6.4.11. Triple Sugar Iron (TSI) Agar test**

Triple Sugar Iron Agar is used for the differentiation of microorganisms on the basis of dextrose, lactose, and sucrose fermentation and hydrogen sulfide production. Inoculate the TSI slant by first stabbing the butt down to the bottom, withdraw the needle, and then streak the surface of the slant. Use a loosely fitting closure to permit access of air. Incubate at 37°C for 18 to 24h. After incubation observe the color and gas production.

An alkaline slant-acid butt (red/yellow) indicates fermentation of dextrose only. An acid slant-acid butt (yellow/yellow) indicates fermentation of dextrose, lactose and/or sucrose. An alkaline slant-alkaline butt (red/red) indicates dextrose or lactose were not fermented (non-fermenter). Cracks, splits, or bubbles in medium indicate gas production. A black precipitate in butt indicates hydrogen sulfide production.

#### **2.6.4.12. Carbohydrate fermentation test**

The test used to determine the ability of an organism to ferment a specific carbohydrate with or without the production of gas. The carbohydrates test was performed by inoculating a loop-full organism into the tubes containing different sugar media and incubated for 24h at 37°C. Phenol Red is used as an indicator in the media. At a neutral *pH*, the media is red; at a *pH* of less than 7, the media is yellow. Fermentation of the carbohydrate produces acid, causing the media to change from red to yellow.



## Chapter 3: Results

- 3.1. Bioassay on *T. castaneum* adults
- 3.2. Bioassay on *A. salina* nauplii
- 3.3. Isolation of bacteria from industrial effluent
- 3.4. Antibacterial activities of the test extracts
- 3.5. Summary of the experimentation



### 3.1. Bioassay on *T. castaneum* adults

#### 3.1.1. Effect of *C. papaya* extracts against *T. castaneum* adults by residual film assay

The Pet.E., CHCl<sub>3</sub> and CH<sub>3</sub>OH extracts of *C. papaya* leaf, stem and roots were tested against the adult beetles of *T. castaneum* through residual film assay. For the final application the doses were ranged between 4.076 to 0.127mg cm<sup>-2</sup> where the test insects were released to observe mortality or any sort of behavioral changes due to the action of the extracts compared to their controls. To trace acute toxicity an observation of mortality was made after ½h of application of doses, followed by 12h of intervals up to 48h. The data was subjected to probit analysis and the results have been presented in Tables 3.1 to 3.2 and Appendix Tables I-XXXIV.

The Pet.E. extract of *C. papaya* leaf, stem and roots showed mortality to the adult beetles of *T. castaneum* by giving the LD<sub>50</sub> values 1.202, 0.956, 0.891, 0.750 and 0.559mg cm<sup>-2</sup>; 1.636, 1.279, 0.980, 0.856 and 0.725mg cm<sup>-2</sup>; 2.336, 1.422, 0.853, 0.634 and 0.532mg cm<sup>-2</sup> for 6, 12, 24, 36 and 48h of exposure respectively. For the CHCl<sub>3</sub> extracts of stem the LD<sub>50</sub> values were 0.105, 4.745, 3.719, 2.759 and 2.053 mg cm<sup>-2</sup> for 6, 12, 24, 36 and 48h of exposure respectively while, the CHCl<sub>3</sub> extracts of leaf and root didn't offer any mortality to the beetles. For the CH<sub>3</sub>OH extracts of leaf the LD<sub>50</sub> values were 3.640, 1.562, 0.861, 0.607 and 0.419mg cm<sup>-2</sup> for 6, 12, 24, 36 and 48h of exposure respectively; followed by the stem and root extract with the LD<sub>50</sub> values 2.026, 0.945, 0.573, 0.343 and 0.274mg cm<sup>-2</sup>; 0.878, 0.414, 0.188, 0.129 and 0.114mg cm<sup>-2</sup> for the same exposure respectively.

The highest and the lowest mortality have been observed for the CH<sub>3</sub>OH extract of root (LD<sub>50</sub> 0.114mg cm<sup>-2</sup>) and CHCl<sub>3</sub> extracts of stem (LD<sub>50</sub> 2.053mg cm<sup>-2</sup>) after 48h of exposure respectively. Observation after ½h assured acute toxicity positively, however, the LD<sub>50</sub> values was simply larger.

According to the intensity of activity observed through dose mortality test against the adult beetles the potentiality of the Pet.E., CHCl<sub>3</sub> and CH<sub>3</sub>OH extracts could be arranged in a descending order: root (CH<sub>3</sub>OH) > stem (CH<sub>3</sub>OH) > leaf (CH<sub>3</sub>OH) > root (Pet.E.) > leaf (Pet.E.) > stem (Pet.E.) > stem (CHCl<sub>3</sub>) extract.

**Table 3.1:** LD<sub>50</sub> values, 95% confidence limits, regression equations and  $\chi^2$  values (along with their df) of the Pet.E. extracts of *C. papaya* leaf, stem and roots against *T. castaneum* adults

Solvent	Plant organs	Exposure (h)	LD <sub>50</sub> (mg cm <sup>-2</sup> )	95% confidence limits		Regression equations	$\chi^2$ values (df)
				Lower	Upper		
Pet.E.	Leaf	½	-	-	-	-	-
		6	1.202	0.973	1.485	Y = 0.900 + 3.797X	1.071 (1)
		12	0.956	0.819	1.116	Y = 1.406 + 3.665X	1.068 (3)
		24	0.891	0.777	1.021	Y = 1.178 + 4.024X	1.222 (3)
		36	0.751	0.666	0.845	Y = 0.869 + 4.719X	1.385 (3)
		48	0.559	0.430	0.729	Y = 2.326 + 3.576X	8.516 (3)*
	Stem	½	-	-	-	-	-
		6	1.636	1.492	1.793	Y = -1.809 + 5.610X	7.072 (4)
		12	1.279	1.167	1.402	Y = -0.676 + 5.128X	2.096 (4)
		24	0.980	0.859	1.119	Y = 0.553 + 4.486X	3.518 (4)
		36	0.856	0.738	0.993	Y = 0.433 + 4.896X	2.608 (4)
	Root	½	-	-	-	-	-
		6	2.336	1.229	4.440	Y = 0.998 + 2.924X	0.295 (2)
		12	1.422	1.044	1.936	Y = 2.473 + 2.192X	0.857 (3)
		24	0.853	0.719	1.012	Y = 2.516 + 2.668X	0.952 (4)
36		0.634	0.534	0.753	Y = 2.665 + 2.911X	3.230 (4)	
		48	0.532	0.439	0.646	Y = 2.914 + 2.872X	2.291 (3)

[\* Variance has been adjusted for heterogeneity, - No activity detected]

**Table 3.2:** LD<sub>50</sub> values, 95% confidence limits, regression equations and  $\chi^2$  values (along with their df) of the CHCl<sub>3</sub> and CH<sub>3</sub>OH extracts of *C. papaya* leaf, stem and roots against *T. castaneum* adults

Solvents	Plant organs	Exposure (h)	LD <sub>50</sub> (mg cm <sup>-2</sup> )	95% confidence limits		Regression equations	$\chi^2$ values (df)	
				Lower	Upper			
CHCl <sub>3</sub>	Leaf	-	-	-	-	-	-	
		½	-	-	-	-	-	
		6	0.105	2.6E-06	4248.959	Y = 3.920 + -1.102X	0.333(1)	
	Stem	12	4.745	2.348	9.591	Y = 4.114 + 1.311X	0.418 (3)	
		24	3.719	2.087	6.629	Y = 4.291 + 1.243X	0.237 (3)	
		36	2.759	1.980	3.846	Y = 4.288 + 1.615X	0.669 (3)	
		48	2.053	1.567	2.690	Y = 4.450 + 1.759X	0.823 (3)	
	Root	-	-	-	-	-	-	
	CH <sub>3</sub> OH		½	-	-	-	-	-
			6	3.640	1.783	7.431	Y = 0.273 + 3.028X	0.369 (2)
Leaf		12	1.562	1.062	2.298	Y = 2.015 + 2.501X	13.155(5)*	
		24	0.861	0.732	1.014	Y = 2.288 + 2.900X	7.521 (5)	
		36	0.607	0.516	0.712	Y = 2.561 + 3.116X	3.343 (5)	
		48	0.419	0.348	0.504	Y = 3.237 + 2.835X	6.106 (4)	
Stem		½	-	-	-	-	-	
		6	2.026	0.204	20.080	Y = 1.649 + 2.564X	6.327 (1) *	
		12	0.945	0.821	1.088	Y = 1.060 + 4.038X	3.127 (2)	
		24	0.573	0.494	0.664	Y = 2.069 + 3.868X	7.048 (3)	
	36	0.343	0.254	0.464	Y = 3.164 + 3.426X	12.787 (4)*		
	48	0.274	0.197	0.383	Y = 3.697 + 2.973X	10.914 (4)*		
Root	½	-	-	-	-	-		
	6	0.878	0.573	1.347	Y = 3.548 + 1.539X	4.060 (3)		
	12	0.414	0.331	0.518	Y = 3.567 + 2.323X	3.258 (3)		
	24	0.188	0.138	0.256	Y = 4.380 + 2.257X	1.721 (3)		
	36	0.129	0.082	0.204	Y = 4.783 + 1.937X	1.580 (3)		
	48	0.114	0.077	0.170	Y = 4.841 + 2.756X	0.214 (1)		

[\* Variance has been adjusted for heterogeneity, - No activity detected]

### 3.1.2. Effect of *M. oliefera* extracts against *T. castaneum* adults by residual film assay

The Pet.E., CHCl<sub>3</sub> and CH<sub>3</sub>OH extracts of *M. oliefera* fruit, leaf, stem bark, stem wood, root bark and root wood were tested against the adult beetles of *T. castaneum* through residual film assay. For the final application doses were ranged between 2.038 to 0.764mg cm<sup>-2</sup> where the test insects were released to observe mortality or any sort of behavioral changes due to the action of the extracts compared to their controls. To trace acute toxicity an observation of mortality was made after ½h of application of doses and followed by 12h intervals up to 48h. The data was subjected to probit analysis and the results have been presented in Tables 3.3 to 3.4 and Appendix Tables XXXV-LXXVII.

The Pet.E. extract of *M. oliefera* stem bark, root bark and root woods showed mortality to the adult beetles of *T. castaneum* by giving the LD<sub>50</sub> values 2.022, 1.055, 0.812, 0.628 and 0.466mg cm<sup>-2</sup>; 0.812, 0.610, 0.536, 0.427 and 0.365mg cm<sup>-2</sup>; 3.142, 2.041, 1.489, 0.983 and 0.629mg cm<sup>-2</sup> for 6, 12, 24, 36 and 48h of exposure respectively. Root bark Pet.E. extract showed acute toxicity by giving LD<sub>50</sub> 1.888mg cm<sup>-2</sup> after ½h of exposure. For the CH<sub>3</sub>OH extract of fruit, stem bark, stem wood, root bark and root wood the LD<sub>50</sub> values were 0.859, 0.606, 0.436, 0.420 and 0.382mg cm<sup>-2</sup>; 1.379, 0.786, 0.519, 0.392 and 0.308mg cm<sup>-2</sup>; 1.568, 1.350, 1.005, 0.691 and 0.535mg cm<sup>-2</sup>; 0.757, 0.552, 0.403, 0.312 and 0.276mg cm<sup>-2</sup>; 1.017, 0.847, 0.617, 0.474 and 0.393mg cm<sup>-2</sup> for 6, 12, 24, 36 and 48h of exposure respectively. Fruit and stem wood CH<sub>3</sub>OH extract showed acute toxicity by giving LD<sub>50</sub> 2.754 and 5.044mg cm<sup>-2</sup> after ½h of exposure respectively. The highest and the lowest mortality have been observed for the CH<sub>3</sub>OH extract of root bark (LD<sub>50</sub> 0.276mg cm<sup>-2</sup>) and Pet.E. extracts of root wood (LD<sub>50</sub> 0.629mg cm<sup>-2</sup>) after 48h of exposure respectively.

To consider acute toxicity of the extracts a reading of data is made after ½h of exposure, and in this case the result was positive, while the LD<sub>50</sub> values were comparatively larger. It is of course mentionable that the other test materials, viz. the Pet.E. extracts of fruit, leaf, stem wood; CHCl<sub>3</sub> extracts of fruit, leaf, stem bark, stem wood, root bark, root wood and CH<sub>3</sub>OH extracts of the leaf of *M. oliefera* didn't show any mortality against the adult beetles of *T. castaneum*.

According to the intensity of activity observed through mortality of the adult beetles the potentiality of the Pet.E. and methanol extracts could be arranged in a descending order: root bark (CH<sub>3</sub>OH) > stem bark (CH<sub>3</sub>OH) > root bark (Pet.E.) > fruit (CH<sub>3</sub>OH) > root wood (CH<sub>3</sub>OH) > stem bark (Pet.E.) > stem wood (CH<sub>3</sub>OH) > root wood (Pet.E.) extract.

**Table 3.3:** LD<sub>50</sub> values, 95% confidence limits, regression equations and  $\chi^2$  values (along with their df) of the Pet.E. extracts of *M. oliefera* stem bark, root bark and root woods against *T. castaneum* adults

Solvent	Plant organs	Exposure (h)	LD <sub>50</sub> (mg cm <sup>-2</sup> )	95% confidence limits		Regression equations	$\chi^2$ values (df)
				Lower	Upper		
Pet.E.	Stem bark	½	-	-	-	-	-
		6	2.022	1.113	3.675	Y = 2.622 + 1.821X	0.069 (3)
		12	1.055	0.864	1.289	Y = 2.507 + 2.437X	0.068 (3)
		24	0.812	0.663	0.994	Y = 2.985 + 2.215X	1.237 (4)
		36	0.628	0.517	0.763	Y = 2.972 + 2.540X	0.700 (4)
	Root bark	48	0.466	0.375	0.580	Y = 3.224 + 2.657X	0.869 (4)
		½	1.888	1.108	3.216	Y = 2.627 + 1.859X	0.303 (3)
		6	0.812	0.661	0.998	Y = 3.017 + 2.181X	1.203 (4)
		12	0.610	0.476	0.782	Y = 3.490 + 1.923X	0.937 (4)
		24	0.536	0.415	0.691	Y = 3.523 + 2.027X	1.204 (4)
	Root wood	36	0.427	0.319	0.572	Y = 3.693 + 2.073X	0.746 (4)
		48	0.365	0.270	0.493	Y = 3.716 + 2.286X	1.988 (4)
		½	-	-	-	-	-
		6	3.142	1.387	7.122	Y = 3.122 + 3.777X	0.055 (2)
		12	2.041	1.307	3.187	Y = 2.592 + 1.839X	0.201 (4)
Root wood	24	1.489	1.082	2.049	Y = 3.071 + 1.645X	0.098 (5)	
	36	0.983	0.772	1.251	Y = 3.320 + 1.693X	0.202 (5)	
	48	0.629	0.496	0.797	Y = 3.435 + 1.960X	2.029 (5)	

[- No activity detected]

**Table 3.4:** LD<sub>50</sub> values, 95% confidence limits, regression equations and  $\chi^2$  values (along with their df) of the CH<sub>3</sub>OH extracts of *M. oliefera* fruit, stem bark, stem wood, root bark and root woods against *T. castaneum* adults

Solvent	Plant organs	Exposure (h)	LD <sub>50</sub> (mg cm <sup>-2</sup> )	95% confidence limits		Regression equations	$\chi^2$ values (df)
				Lower	Upper		
CH <sub>3</sub> OH	Fruit	½	2.754	1.752	4.329	Y = 0.576 + 3.072X	1.239 (2)
		6	0.859	0.655	1.126	Y = 3.242 + 1.882X	0.825 (3)
		12	0.606	0.418	0.880	Y = 3.534 + 1.872X	1.610 (3)
		24	0.436	0.246	0.771	Y = 3.908 + 1.708X	0.496 (3)
		36	0.420	0.235	0.750	Y = 3.909 + 1.749X	1.020 (3)
		48	0.382	0.211	0.690	Y = 3.892 + 1.905X	0.334 (3)
	Stem bark	6	1.379	1.015	1.873	Y = 1.421 + 3.141X	0.428 (2)
		12	0.786	0.685	0.902	Y = 1.533 + 3.872X	1.321 (3)
		24	0.519	0.443	0.608	Y = 2.333 + 3.729X	2.155 (3)
		36	0.392	0.330	0.466	Y = 2.628 + 3.999X	2.905 (3)
		48	0.308	0.248	0.382	Y = 3.476 + 3.123X	0.212 (1)
	Stem wood	½	5.044	0.915	27.816	Y = 1.968 + 1.781X	1.101 (3)
		6	1.568	1.889	2.069	Y = 1.851 + 2.634X	1.717 (4)
		12	1.350	1.091	1.672	Y = 1.754 + 2.871X	4.086 (4)
		24	1.005	0.845	1.196	Y = 2.253 + 2.741X	3.473 (4)
		36	0.691	0.593	0.805	Y = 2.308 + 3.208X	2.150 (4)
		48	0.535	0.453	0.633	Y = 2.498 + 3.433X	2.760 (4)
	Root bark	6	0.757	0.580	0.989	Y = 3.414 + 1.804X	1.323 (4)
		12	0.552	0.433	0.704	Y = 3.604 + 1.881X	1.783 (4)
		24	0.403	0.316	0.515	Y = 3.809 + 1.967X	0.430 (4)
		36	0.312	0.239	0.408	Y = 4.010 + 2.004X	0.322 (4)
		48	0.276	0.218	0.350	Y = 3.918 + 2.452X	0.654 (4)
	Root wood	6	1.017	0.865	1.195	Y = 1.968 + 3.011X	1.662 (3)
		12	0.847	0.744	0.964	Y = 1.493 + 3.780X	2.922 (4)
24		0.617	0.524	0.726	Y = 2.472 + 3.199X	5.043 (4)	
36		0.474	0.399	0.562	Y = 2.600 + 3.554X	1.586 (4)	
48		0.393	0.317	0.487	Y = 3.117 + 3.166X	0.565 (2)	

### 3.1.3. Effect of *Mu. sapientum* extracts against *T. castaneum* adults by residual film assay

The Pet.E., CHCl<sub>3</sub> and CH<sub>3</sub>OH extracts of *Mu. sapientum* leaf, stem and roots were tested against the adult beetles of *T. castaneum* through residual film assay. For the final application doses were ranged between 2.038 to 0.127mg cm<sup>-2</sup> where the test insects were released to observe mortality or any sort of behavioral changes due to the action of the extracts compared to their controls. To trace acute toxicity an observation of mortality was made after ½h of application of doses and followed by 12h of intervals up to 48 hours. The data was subjected to probit analysis and the results have been presented in Tables 3.5 to 3.6 and Appendix Tables LXXVIII-CV.

The Pet.E. extract of *Mu. sapientum* leaf, stem and roots showed mortality to the adult beetles of *T. castaneum* by giving the LD<sub>50</sub> values 4.134, 2.865, 1.899, 1.489 and 1.195mg cm<sup>-2</sup>; 1.282, 1.009, 0.814, 0.751 and 0.582mg cm<sup>-2</sup>; 2.359, 1.568, 1.177, 0.849 and 0.718mg cm<sup>-2</sup> for 6, 12, 24, 36 and 48h of exposure respectively. Pet.E. stem extracts showed acute toxicity by giving LD<sub>50</sub> 9.289mg cm<sup>-2</sup> after ½h of exposure. For the CH<sub>3</sub>OH extract of leaf, stem and root the LD<sub>50</sub> values were 3.018, 1.804, 0.627 and 0.213 mg cm<sup>-2</sup>; 0.646, 0.318, 0.204 and 0.163mg cm<sup>-2</sup>; 1.935, 0.413, 0.073 and 0.205mg cm<sup>-2</sup> for 12, 24, 36 and 48h of exposure respectively. The highest and the lowest mortality have been observed for the CH<sub>3</sub>OH extract of stem (LD<sub>50</sub> 0.163mg cm<sup>-2</sup>) and Pet.E. extracts of leaf (LD<sub>50</sub> 1.195mg cm<sup>-2</sup>) after 48h of exposure respectively.

To consider acute toxicity of the extracts a reading of data was made after ½h of exposure, and in this case the result was positive, while the LD<sub>50</sub> values were comparatively larger. It is of course mentionable that the other test materials, viz. the CHCl<sub>3</sub> extracts of leaf, stem and roots of *Mu. sapientum* didn't show any mortality against the adult beetles of *T. castaneum*.

According to the intensity of activity observed through mortality of the adult beetles the potentiality of the extracts could be arranged in a descending order: stem (CH<sub>3</sub>OH) > root (CH<sub>3</sub>OH) > leaf (CH<sub>3</sub>OH) > stem (Pet.E.) > root (Pet.E.) > leaf (Pet.E.) extract.

**Table 3.5:** LD<sub>50</sub> values, 95% confidence limits, regression equations and  $\chi^2$  values (along with their df) of the Pet.E. extracts of *Mu. sapientum* leaf, stem and roots against *T. castaneum* adults

Solvent	Plant organs	Exposure (h)	LD <sub>50</sub> (mg cm <sup>-2</sup> )	95% confidence limits		Regression equations	$\chi^2$ values (df)
				Lower	Upper		
Pet.E.	Leaf	½	-	-	-	-	-
		6	4.134	1.852	9.229	Y = 0.732 + 2.641X	0.639 (4)
		12	2.865	1.799	4.561	Y = 1.107 + 2.672X	1.704 (4)
		24	1.899	1.532	2.354	Y = 1.252 + 2.932X	3.117 (4)
		36	1.489	1.299	1.707	Y = 1.079 + 3.343X	2.265 (4)
		48	1.195	1.063	1.343	Y = 0.606 + 4.079X	1.873 (4)
	Stem	½	9.289	0.295	292.334	Y = 3.232 + 1.826X	0.410 (1)
		6	1.282	1.030	1.597	Y = 2.211 + 2.518X	1.373 (3)
		12	1.009	0.827	1.230	Y = 2.342 + 2.648X	0.371 (3)
		24	0.814	0.671	0.986	Y = 2.442 + 2.809X	0.099 (3)
		36	0.751	0.624	0.903	Y = 2.316 + 3.066X	1.210 (3)
		48	0.582	0.469	0.723	Y = 2.961 + 2.665X	0.889 (3)
	Root	½	-	-	-	-	-
		6	2.360	1.630	3.416	Y = 3.138 + 4.994X	0.065 (2)
		12	1.568	1.258	1.953	Y = 1.808 + 2.671X	1.350 (4)
		24	1.177	1.007	1.375	Y = 1.906 + 2.889X	0.762 (5)
		36	0.849	0.730	0.987	Y = 2.310 + 2.897X	6.543 (5)
		48	0.718	0.614	0.840	Y = 2.373 + 3.068X	9.323 (5)

[- No activity detected]



**Table 3.6:** LD<sub>50</sub> values, 95% confidence limits, regression equations and  $\chi^2$  values (along with their df) of the CH<sub>3</sub>OH extracts of *Mu. sapientum* leaf, stem and roots against *T. castaneum* adults

Solvent	Plant organs	Exposure (h)	LD <sub>50</sub> (mg cm <sup>-2</sup> )	95% confidence limits		Regression equations	$\chi^2$ values (df)
				Lower	Upper		
CH <sub>3</sub> OH	Leaf	½	-	-	-	-	-
		6	-	-	-	-	-
		12	3.018	0.955	9.534	Y = 1.850 + 2.129X	0.073 (2)
		24	1.804	1.214	2.682	Y = 2.349 + 2.110X	0.083 (4)
		36	0.627	0.499	0.788	Y = 3.530 + 1.844X	3.366 (5)
		48	0.213	0.148	0.306	Y = 4.429 + 1.738X	3.287 (5)
	Stem	½	-	-	-	-	-
		6	-	-	-	-	-
		12	0.646	0.381	1.097	Y = 2.824 + 2.685X	2.625 (1)
		24	0.318	0.273	0.370	Y = -0.653 + 3.764X	7.230 (3)
		36	0.204	0.174	0.239	Y = 0.052 + 3.778 X	4.705 (3)
		48	0.163	0.139	0.191	Y = -0.083 + 4.191X	1.162 (3)
	Root	½	-	-	-	-	-
		6	-	-	-	-	-
		12	1.935	0.556	6.734	Y = 2.679 + 1.804X	2.559 (3)
		24	0.413	0.266	0.641	Y = 4.304 + 1.129X	1.012 (3)
		36	0.073	0.009	0.562	Y = 5.178 + 1.282X	0.408 (3)
		48	0.205	0.152	0.277	Y = 3.416 + 5.067X	8.706 (2)*

[\* Variance has been adjusted for heterogeneity, - No activity detected]

## 3.2. Bioassay on *A. salina* nauplii

### 3.2.1. Lethal effect of *C. papaya* extracts against *A. salina* nauplii

The Pet.E., CHCl<sub>3</sub> and CH<sub>3</sub>OH extracts of *C. papaya* leaf, stem and roots were tested against the one day aged *A. salina* nauplii through lethality assay. For the final application selected doses were ranged between 250 to 6.25ppm where the nauplii were released to observe lethality or any sort of behavioral changes due to the action of the extracts compared to their controls. Observation of mortality was made after 6h of application of the doses followed by 6h intervals up to 24h. The data was subjected to probit analysis and the results have been presented in Tables 3.7 to 3.8 and Appendix Tables CVI-CXXXIX.

The Pet.E. extract of *C. papaya* leaf, stem and roots showed lethality to the *A. salina* nauplii by giving the LC<sub>50</sub> values 4180.528, 1032.428, 363.954 and 102.701ppm; 3173.579, 115.180, 44.033 and 17.230ppm; 472.900, 187.898, 78.891 and 52.268ppm for 6, 12, 18 and 24h of exposure respectively. For the CHCl<sub>3</sub> extracts of leaf the LC<sub>50</sub> values were 372.025, 98.248 and 1.326ppm for 12, 18 and 24h of exposure respectively; followed by the stem extract 129.233, 76.026, 37.335 and 14.689ppm for 6, 12, 18 and 24h of exposure respectively; and also followed by the root extract 257.124, 156.739 and 40.072ppm for 12, 18 and 24h of exposure respectively. For the CH<sub>3</sub>OH extract of leaf, stem and roots the LC<sub>50</sub> values were 1370.555, 2665.86, 467.792 and 183.443ppm; 72.337, 45.542, 31.059 and 29.593ppm; 33.176, 22.699, 20.559 and 14.402ppm for 6, 12, 18 and 24h of exposures respectively. The highest and the lowest mortality have been observed for the CHCl<sub>3</sub> extract of leaf (LC<sub>50</sub> 1.326ppm) and CH<sub>3</sub>OH extracts of leaf (LC<sub>50</sub> 183.443ppm) after 24h of exposure respectively.

According to the intensity of activity observed through dose mortality test against the adult beetles the potentiality of the Pet.E., CHCl<sub>3</sub> and CH<sub>3</sub>OH extracts could be arranged in a descending order: leaf (CHCl<sub>3</sub>) > root (CH<sub>3</sub>OH) > stem (CHCl<sub>3</sub>) > stem (Pet.E.) > stem (CH<sub>3</sub>OH) > root (CHCl<sub>3</sub>) > root (Pet.E.) > leaf (Pet.E.) > leaf (CH<sub>3</sub>OH) extract.

**Table 3.7:** LC<sub>50</sub> values, 95% confidence limits, regression equations and  $\chi^2$  values (along with their df) of the Pet.E. extracts of *C. papaya* leaf, stem and root against *A. salina* nauplii

Solvent	Plant organs	Exposure (h)	LC <sub>50</sub> (ppm)	95% confidence limits		Regression equations	$\chi^2$ values (df)
				Lower	Upper		
Pet.E.	Leaf	6	4180.528	2.055	8503968	Y = 0.884 + 1.137X	1.183(2)
		12	1032.428	90.426	11787.66	Y = - 0.424 + 1.800X	2.728(2)
		18	363.954	178.161	743.498	Y = - 0.044 + 1.969 X	3.182(2)
		24	102.701	72.057	146.376	Y = - 0.094 + 2.532 X	0.423(2)
	Stem	6	3173.579	8.563	1176216	Y = 2.637 + 0.675X	1.190(3)
		12	115.180	52.497	252.707	Y = 2.308 + 1.306X	2.651(3)
		18	44.033	31.059	62.426	Y = 2.308 + 1.637X	1.255(3)
		24	17.230	13.384	22.181	Y = 2.324 + 2.165X	0.239(3)
	Root	6	472.900	28.533	7837.606	Y = 0.096 + 1.833 X	0.549(2)
		12	187.898	84.636	417.149	Y = 1.002 + 1.758X	5.227(3)
		18	78.891	50.355	123.598	Y = 0.524 + 2.359X	9.940(3)*
		24	52.268	38.031	71.833	Y = -1.410 + 3.731X	14.542(3)*

[\* Variance has been adjusted for heterogeneity]

**Table 3.8:** LC<sub>50</sub> values, 95% confidence limits, regression equations and  $\chi^2$  values (along with their df) of the CHCl<sub>3</sub> and CH<sub>3</sub>OH extracts of *C. papaya* leaf, stem and root against *A. salina* nauplii

Solvents	Plant organs	Exposure (h)	LC <sub>50</sub> (ppm)	95% confidence limits		Regression equations	$\chi^2$ values (df)
				Lower	Upper		
CHCl <sub>3</sub>	Leaf	6	-	-	-	-	-
		12	372.025	177.651	779.069	Y = -1.043 + 2.351X	0.530(3)
		18	98.248	71.704	134.620	Y = 2.168 + 1.422X	0.713(4)
		24	1.326	0.003	658.439	Y = 4.908 + 0.748X	0.145(2)
	Stem	6	129.233	62.656	266.551	Y = -0.908 + 2.798X	0.293(1)
		12	76.026	46.520	124.249	Y = 1.934 + 1.630X	2.224(3)
		18	37.335	27.146	51.348	Y = 2.350 + 1.686X	0.890(3)
		24	14.689	9.306	23.186	Y = 1.857 + 2.694X	8.407(2)*
	Root	6	-	-	-	-	-
		12	257.124	83.311	793.571	Y = 2.387 + 1.084X	0.500(4)
		18	156.739	73.267	335.307	Y = 2.321 + 1.221X	1.191(4)
		24	40.072	32.581	49.285	Y = 1.099 + 2.434X	3.798(4)
CH <sub>3</sub> OH	Leaf	6	1370.555	15.869	118373.2	Y = 1.905 + 0.987X	0.130(3)
		12	2665.860	3.857	1842682	Y = 2.753 + 0.656X	0.771(3)
		18	467.792	43.733	5003.815	Y = 2.436 + 0.960X	2.822(3)
		24	183.443	64.947	518.135	Y = 2.297 + 1.194X	2.109(3)
	Stem	6	72.337	47.977	109.066	Y = 2.814 + 1.176X	0.640(3)
		12	45.542	28.645	72.406	Y = 2.859 + 1.291X	4.548(3)
		18	31.059	21.390	45.098	Y = 1.909 + 2.072X	3.247(3)
		24	29.593	22.731	38.526	Y = 0.329 + 3.175X	0.389(1)
	Root	6	33.176	15.713	70.047	Y = 3.525 + 0.970X	1.446(3)
		12	22.699	9.084	56.720	Y = 3.632 + 1.009X	0.979(3)
		18	20.559	8.819	47.929	Y = 3.462 + 1.171X	0.695(3)
		24	14.402	3.945	52.573	Y = 3.833 + 1.008X	1.000(2)

[\* Variance has been adjusted for heterogeneity, - No activity detected]

### 3.2.2. Lethal effect of *M. oleifera* extracts against *A. salina* nauplii

The Pet.E., CHCl<sub>3</sub> and CH<sub>3</sub>OH extracts of *M. oleifera* fruit, leaf, stem bark, stem wood, root bark and root wood were tested against the one day aged *A. salina* nauplii through lethality assay. For the final application doses were ranged between 300 to 1.563ppm where the nauplii were released to observe lethality or any sort of behavioral changes due to action of the extracts compared to their controls. Observation of mortality was made after 6h of application of doses followed by 6h intervals up to 24h. The data was subjected to probit analysis and the results have been presented in Tables 3.9 to 3.11 and Appendix Tables CXL -CCI.

The Pet.E. extract of *M. oleifera* fruit, leaf and root woods showed lethality to the *A. salina* nauplii by giving the LC<sub>50</sub> values 81.213, 64.503, 61.055 and 58.228ppm; 562.916, 264.065, 228.101 and 123.482ppm and 103.841, 101.871, 90.784 and 78.393ppm for 6, 12, 18 and 24h of exposures respectively; followed by the stem wood and root bark extracts 72.589, 66.061 and 52.857ppm; 87.223, 76.028 and 68.485ppm for 12, 18 and 24h of exposure respectively. For the CHCl<sub>3</sub> extracts of fruit, stem bark, stem wood, root bark and root wood the LC<sub>50</sub> values were 237.446, 128.977, 87.433 and 70.318ppm; 83.813, 84.075, 65.847 and 54.301ppm; 55.125, 35.967, 31.297 and 24.774ppm; 131.175, 85.637, 13.549 and 4.197ppm; 1075.790, 5437.378, 131.251 and 42.595ppm for 6, 12, 18 and 24h of exposure respectively. For the CH<sub>3</sub>OH extract of fruit, leaf, stem bark, stem wood, root bark and root wood the LC<sub>50</sub> values were 1871.084, 442.596, 174.437 and 98.856ppm; 832.692, 491.176, 323.301 and 234.246ppm; 629.882, 351.887, 182.040 and 110.484ppm; 1368.165, 644.586, 51.143 and 35.876ppm; 139.280, 87.429, 44.824 and 24.119ppm; 3906.864, 941.192, 326.569 and 178.993ppm for 6, 12, 18 and 24h of exposure respectively. The highest and the lowest mortality have been observed for the CHCl<sub>3</sub> extract of root bark (LC<sub>50</sub> 4.197ppm) and CH<sub>3</sub>OH extracts of leaf (LC<sub>50</sub> 234.246ppm) after 24h of exposure respectively.

It is of course mentionable that the other test materials, viz. the Pet.E. extracts of stem bark didn't show proper activity and  $\text{CHCl}_3$  extracts of *M. oliefera* leaf didn't show any activity against the nauplii of *A. salina*. The Pet.E. extracts of *M. oliefera* stem bark was sticky and it could not dissolve in water properly.

According to the intensity of activity observed through dose mortality test against the adult beetles the potentiality of the Pet.E.,  $\text{CHCl}_3$  and  $\text{CH}_3\text{OH}$  extracts could be arranged in a descending order: root bark ( $\text{CHCl}_3$ ) > root bark ( $\text{CH}_3\text{OH}$ ) > stem wood ( $\text{CHCl}_3$ ) > stem wood ( $\text{CH}_3\text{OH}$ ) > root wood ( $\text{CHCl}_3$ ) > stem wood (Pet.E.) > stem bark ( $\text{CHCl}_3$ ) > fruit (Pet.E.) > root bark (Pet.E.) > fruit ( $\text{CHCl}_3$ ) > root wood (Pet.E.) > fruit ( $\text{CH}_3\text{OH}$ ) > stem bark ( $\text{CH}_3\text{OH}$ ) > leaf (Pet.E.) > root wood ( $\text{CH}_3\text{OH}$ ) > leaf ( $\text{CH}_3\text{OH}$ ) extract.

**Table 3.9:** LC<sub>50</sub> values, 95% confidence limits, regression equations and  $\chi^2$  values (along with their df) of the Pet.E. extracts of *M. oleifera* fruit, leaf, stem wood, root bark and root woods against *A. salina* nauplii

Solvents	Plant organs	Exposure (h)	LC <sub>50</sub> (ppm)	95% confidence limits		Regression equations	$\chi^2$ values (df)
				Lower	Upper		
Pet.E.	Fruit	6	81.213	73.472	89.769	Y = -13.141 + 9.500X	2.324(2)
		12	64.503	59.359	70.093	Y = -6.344 + 6.269X	7.718(4)
		18	61.055	50.968	73.138	Y = -4.012 + 5.047X	14.216(4)*
		24	58.228	49.449	68.565	Y = -3.177 + 4.632X	10.610(4)*
	Leaf	6	562.916	173.615	1825.159	Y = 0.924 + 1.482X	2.313(4)
		12	264.065	157.754	442.018	Y = 0.390 + 1.904X	1.072(4)
		18	228.101	131.874	394.545	Y = 1.511 + 1.479X	3.902(4)
		24	123.482	97.195	156.878	Y = 0.584 + 2.111X	5.373(4)
	Stem wood	6	-	-	-	-	-
		12	72.589	65.01	81.052	Y = -5.838 + 5.824X	1.534(2)
		18	66.061	56.052	77.858	Y = -0.776 + 3.174X	0.936(4)
		24	52.857	42.282	66.077	Y = 1.697 + 1.917X	0.902(4)
	Root bark	6	-	-	-	-	-
		12	87.223	79.136	96.136	Y = -5.730 + 5.529X	1.344(4)
		18	76.028	70.6421	81.825	Y = -6.669 + 6.204X	1.158(4)
		24	68.485	63.149	74.270	Y = -5.501 + 5.721X	3.039(4)
Root wood	6	103.841	93.403	115.445	Y = -17.811+11.313X	1.462(1)	
	12	101.871	84.278	123.136	Y = -3.061 + 4.015X	0.543(4)	
	18	90.784	77.293	106.630	Y = -2.052 + 3.602X	0.304(4)	
	24	78.393	70.858	86.729	Y = -3.463 + 4.468X	0.114(4)	

[\* Variance has been adjusted for heterogeneity, - No activity detected]

**Table 3.10:** LC<sub>50</sub> values, 95% confidence limits, regression equations and  $\chi^2$  values (along with their df) of the CHCl<sub>3</sub> extracts of *M. oleifera* fruit, stem bark, stem wood, root bark and root woods against *A. salina* nauplii

Solvents	Plant organs	Exposure (h)	LC <sub>50</sub> (ppm)	95% confidence limits		Regression equations	$\chi^2$ values (df)
				Lower	Upper		
CHCl <sub>3</sub>	Fruit	6	237.446	161.576	348.943	Y = -0.858 + 2.466X	5.298(4)
		12	128.977	107.588	154.619	Y = -1.187 + 2.931X	5.079(4)
		18	87.433	74.593	102.482	Y = -0.823 + 2.999X	3.182(4)
		24	70.318	58.940	83.892	Y = -0.055 + 2.737X	3.087(4)
	Stem bark	6	83.813	68.777	102.137	Y = -1.573 + 3.418X	4.461(2)
		12	84.075	50.578	139.757	Y = 0.840 + 2.161X	8.485(3)
		18	65.847	46.821	92.605	Y = 2.080 + 1.606X	5.015(4)
		24	54.301	39.036	75.536	Y = 2.354 + 1.525X	3.471(4)
	Stem wood	6	55.125	41.454	73.304	Y = 0.992 + 2.302X	1.392(3)
		12	35.967	28.611	45.214	Y = 1.232 + 2.422X	2.568(3)
		18	31.297	25.256	38.783	Y = 1.164 + 2.565X	6.614(3)
		24	24.774	19.385	31.662	Y = 2.020 + 2.138X	5.710(3)
	Root bark	6	131.175	25.336	679.159	Y = 2.289 + 1.280X	0.034 (1)
		12	85.637	11.337	646.900	Y = 4.105 + 0.463X	0.719 (4)
		18	13.548	6.032	30.432	Y = 4.366 + 0.560X	3.692 (4)
		24	4.197	2.404	7.328	Y = 4.428 + 0.918X	3.160 (4)
	Root wood	6	1075.790	181.773	6366.888	Y = 1.530 + 1.145X	1.741 (4)
		12	5437.378	20.780	1422742	Y = 3.814 + 0.318X	1.079 (5)
		18	131.251	51.512	334.423	Y = 3.795 + 0.569X	1.173 (5)
		24	42.595	27.225	66.642	Y = 3.511 + 0.914X	2.003 (5)



**Table 3.11:** LC<sub>50</sub> values, 95% confidence limits, regression equations and  $\chi^2$  values (along with their df) of the CH<sub>3</sub>OH extracts of *M. oleifera* fruit, leaf, stem bark, stem wood, root bark and root woods against *A. salina* nauplii

Solvent	Plant organs	Exposure (h)	LC <sub>50</sub> (ppm)	95% confidence limits		Regression equations	$\chi^2$ values (df)
				Lower	Upper		
CH <sub>3</sub> OH	Fruit	6	1871.084	29.182	119968.4	Y = 2.011 + 0.914X	0.124 (2)
		12	442.596	147.538	1327.737	Y = 1.806 + 1.207X	0.215 (3)
		18	174.437	111.856	272.031	Y = 1.651 + 1.494X	0.490 (3)
		24	98.856	73.858	132.315	Y = 1.609 + 1.700X	0.301 (3)
	Leaf	6	832.692	145.449	4767.125	Y = 1.534 + 1.187X	3.188 (3)
		12	491.176	154.813	1558.362	Y = 1.610 + 1.260X	2.788 (3)
		18	323.301	147.414	709.045	Y = 1.611 + 1.350X	3.667 (3)
		24	234.246	128.558	426.820	Y = 1.724 + 1.382X	0.309 (3)
	Stem bark	6	351.887	66.673	1857.19	Y = 1.969 + 1.190X	0.098 (3)
		12	629.882	34.830	11390.93	Y = 2.886 + 0.755X	0.868 (3)
		18	182.040	62.790	527.766	Y = 2.484 + 1.113X	2.176 (3)
		24	110.484	21.730	561.736	Y = 3.374 + 0.796X	8.165 (3)*
	Stem wood	6	1368.165	88.930	21048.87	Y = 3.158 + 0.587X	0.705 (4)
		12	644.586	163.154	2546.632	Y = 2.974 + 0.721X	0.535 (5)
		18	51.143	37.301	70.121	Y = 2.539 + 1.440X	4.389 (5)
		24	35.876	26.237	49.057	Y = 2.470 + 1.627X	3.091 (5)
	Root bark	6	139.280	50.763	382.145	Y = 3.068 + 0.901X	3.223 (4)
		12	87.429	37.246	205.225	Y = 3.344 + 0.853X	1.244 (4)
		18	44.824	24.386	82.391	Y = 3.524 + 0.894X	0.278 (4)
		24	24.119	14.805	39.295	Y = 3.725 + 0.922X	0.019 (4)
Root wood	6	3906.864	9.375	1628051	Y = 2.256 + 0.764X	0.697 (3)	
	12	941.192	169.069	5239.537	Y = 2.330 + 0.898X	0.993 (4)	
	18	326.569	166.925	638.894	Y = 2.359 + 1.051X	0.761 (4)	
	24	178.993	126.707	252.856	Y = 2.108 + 1.284X	1.329 (4)	

[\* Variance has been adjusted for heterogeneity]

### 3.2.3. Lethal effect of *Mu. sapientum* of extracts against *A. salina* nauplii

The Pet.E., CHCl<sub>3</sub> and CH<sub>3</sub>OH extracts of *Mu. sapientum* leaf, stem and roots were tested against the one day aged *A. salina* nauplii through lethality assay. For the final application doses were ranged between 200 to 6.25ppm where the nauplii were released to observe lethality or any sort of behavioral change due to the action of the extracts compared to their controls. Observation of mortality was made after 6h of application of the doses and followed by 12h of intervals up to 24h. The data was subjected to probit analysis and the results have been presented in Table 3.12 to 3.13 and Appendix Table CCII-CCXXXVII.

The Pet.E. extract of *Mu. sapientum* leaf, stem and roots showed lethality to the *A. salina* nauplii by giving the LC<sub>50</sub> values 451.924, 228.629, 186.776 and 127.604ppm; 156.611, 82.246, 75.313 and 53.893ppm; 101.402, 72.270, 60.274 and 50.018ppm for 6, 12, 18 and 24h of exposure respectively. For the CHCl<sub>3</sub> extracts of leaf, stem and root the LC<sub>50</sub> values were 178.457, 69.203, 42.582 and 29.951ppm; 508.222, 177.202, 75.989 and 37.456ppm; 85.551, 76.793, 63.219 and 55.624ppm for 6, 12, 18 and 24h of exposure respectively. For the CH<sub>3</sub>OH extract of leaf, stem and roots the LC<sub>50</sub> values were 159.309, 71.492, 36.238 and 22.991ppm; 218.929, 496.210, 444.961 and 117.196ppm; 136.148, 121.240, 111.449 and 101.977ppm for 6, 12, 18 and 24h of exposure respectively.

The highest and the lowest mortality have been observed for the CH<sub>3</sub>OH extract of leaf bark (LC<sub>50</sub> 22.991ppm) and Pet.E. extracts of leaf (LC<sub>50</sub> 127.604ppm) after 24h of exposure respectively.

According to the intensity of activity observed through dose mortality test against the adult beetles the potentiality of the Pet.E., CHCl<sub>3</sub> and CH<sub>3</sub>OH extracts could be arranged in a descending order: leaf (CH<sub>3</sub>OH) > leaf (CHCl<sub>3</sub>) > stem (CHCl<sub>3</sub>) > root (Pet.E.) > stem (Pet.E.) > root (CHCl<sub>3</sub>) > root (CH<sub>3</sub>OH) > stem (CH<sub>3</sub>OH) > leaf (Pet.E.) extract.

**Table 3.12:** LC<sub>50</sub> values, 95% confidence limits, regression equations and  $\chi^2$  values (along with their df) of the Pet.E. extracts of *Mu. sapientum* leaf, stem and roots against *A. salina* nauplii

Solvent	Plant organs	Exposure (h)	LC <sub>50</sub> (ppm)	95% confidence limits		Regression equations	$\chi^2$ values (df)
				Lower	Upper		
Pet.E.	Leaf	6	451.924	175.893	1161.131	Y = 0.750 + 1.601X	1.585 (4)
		12	228.629	145.336	359.656	Y = 0.576 + 1.875X	4.046 (4)
		18	186.776	122.682	284.354	Y = 1.279 + 1.638X	3.885(4)
		24	127.604	96.646	168.478	Y = 1.114 + 1.845X	6.942 (4)
	Stem	6	156.611	40.830	600.719	Y = 0.715 + 1.952X	11.304(3)*
		12	82.246	45.604	148.327	Y = 1.090 + 2.041X	9.630 (3)*
		18	75.313	56.216	100.898	Y = 0.994 + 2.134X	6.186(3)
		24	53.893	44.060	65.919	Y = 0.508 + 2.594X	3.196(3)
	Root	6	101.402	82.580	124.513	Y = - 3.549 + 4.262X	3.934 (2)
		12	72.270	47.957	108.909	Y = -0.443 + 2.928X	14.482(3)*
		18	60.274	39.806	91.265	Y = -1.213 + 3.490X	23.227(3)*
		24	50.018	31.512	79.392	Y = 0.059 + 2.908X	20.423(3)*

[\* Variance has been adjusted for heterogeneity]

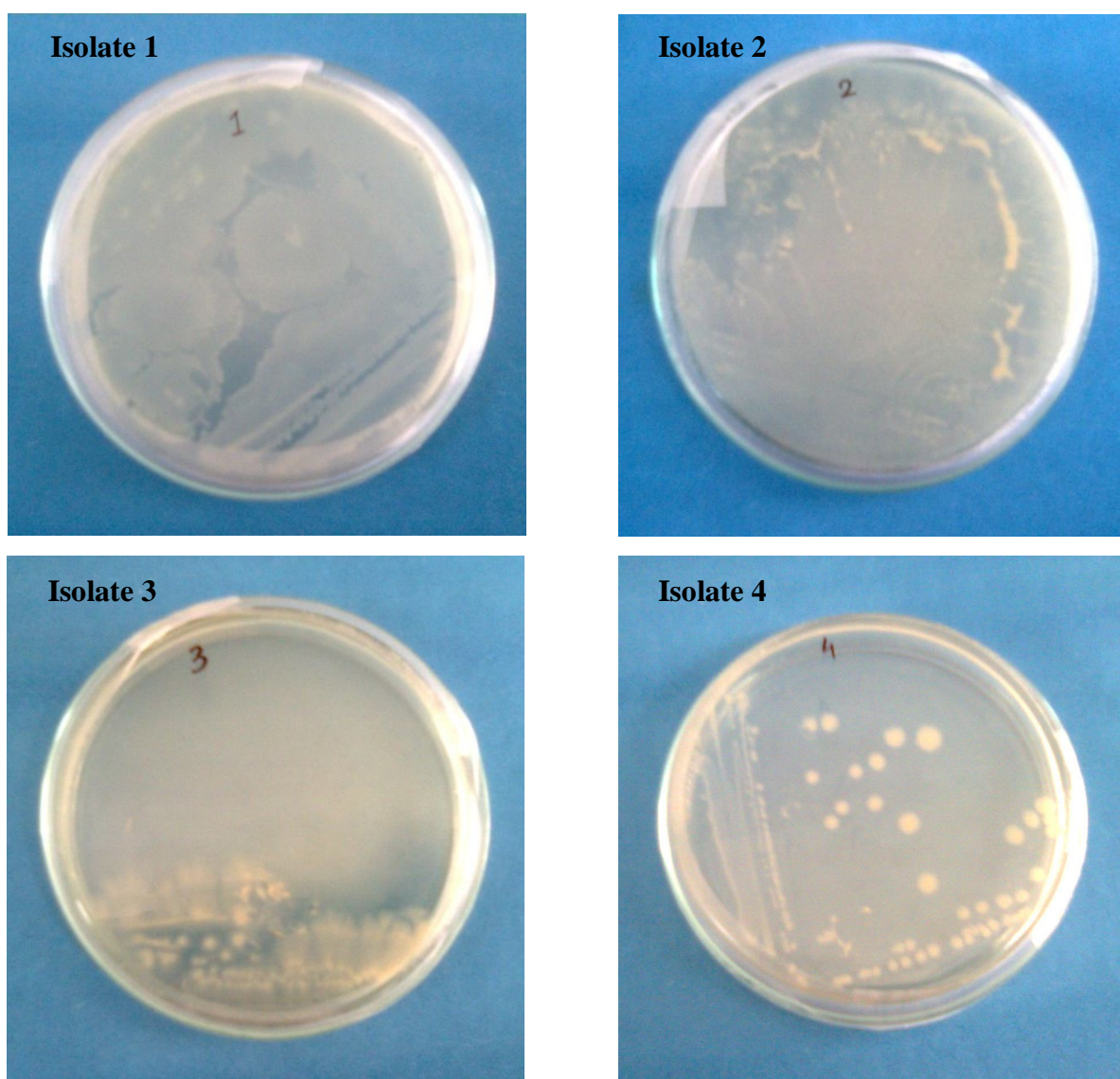
**Table 3.13:** LC<sub>50</sub> values, 95% confidence limits, regression equations and  $\chi^2$  values (along with their df) of the CHCl<sub>3</sub> and CH<sub>3</sub>OH extracts of *Mu. sapientum* leaf, stem and roots against *A. salina* nauplii

Solvents	Plant organs	Exposure (h)	LC <sub>50</sub> (ppm)	95% confidence limits		Regression equations	$\chi^2$ values (df)
				Lower	Upper		
CHCl <sub>3</sub>	Leaf	6	178.457	57.571	553.175	Y = 2.859 + 0.951X	1.307 (3)
		12	69.203	42.879	111.688	Y = 2.833 + 1.178X	2.161(3)
		18	42.582	31.807	57.007	Y = 2.364 + 1.618X	1.131 (4)
		24	29.951	23.238	38.605	Y = 2.250 + 1.863X	1.058(4)
	Stem	6	508.222	6.228	41472.81	Y = 3.195 + 0.667X	0.928 (3)
		12	177.202	12.424	2527.36	Y = 3.513 + 0.661X	0.032 (3)
		18	75.989	19.343	298.529	Y = 3.606 + 0.741X	0.938 (3)
		24	37.456	20.615	68.055	Y = 3.553 + 0.920X	0.756 (3)
	Root	6	85.551	64.249	113.915	Y = -0.994 + 3.102X	6.377 (3)
		12	76.793	57.357	102.816	Y = 0.474 + 2.401X	3.770 (3)
		18	63.219	53.453	74.768	Y = -0.269 + 2.926X	2.588 (3)
		24	55.624	47.824	64.695	Y = -0.562 + 3.187X	1.672 (3)
CH <sub>3</sub> OH	Leaf	6	159.309	72.878	348.243	Y = 3.264 + 0.788X	1.950 (3)
		12	71.492	36.131	141.460	Y = 3.707 + 0.697X	1.151 (3)
		18	36.238	10.980	119.600	Y = 4.109 + 0.571X	0.786 (3)
		24	22.991	7.828	67.523	Y = 3.844 + 0.849X	1.651 (3)
	Stem	6	218.929	115.126	416.325	Y = 0.654 + 1.857X	1.128 (3)
		12	496.210	57.228	4302.476	Y = 2.812 + 0.812X	4.145 (3)
		18	444.961	38.604	5128.763	Y = 3.281 + 0.649X	3.056 (3)
		24	117.196	48.361	284.009	Y = 2.572 + 1.174X	8.261(3)*
	Root	6	136.148	98.897	187.429	Y = 1.250 + 1.757X	0.948 (2)
		12	121.240	91.165	161.238	Y = 1.108 + 1.868X	3.090 (3)
		18	111.449	84.889	146.319	Y = 1.114 + 1.898X	3.262 (3)
		24	101.977	79.848	130.238	Y = 0.811 + 2.086X	2.296 (3)

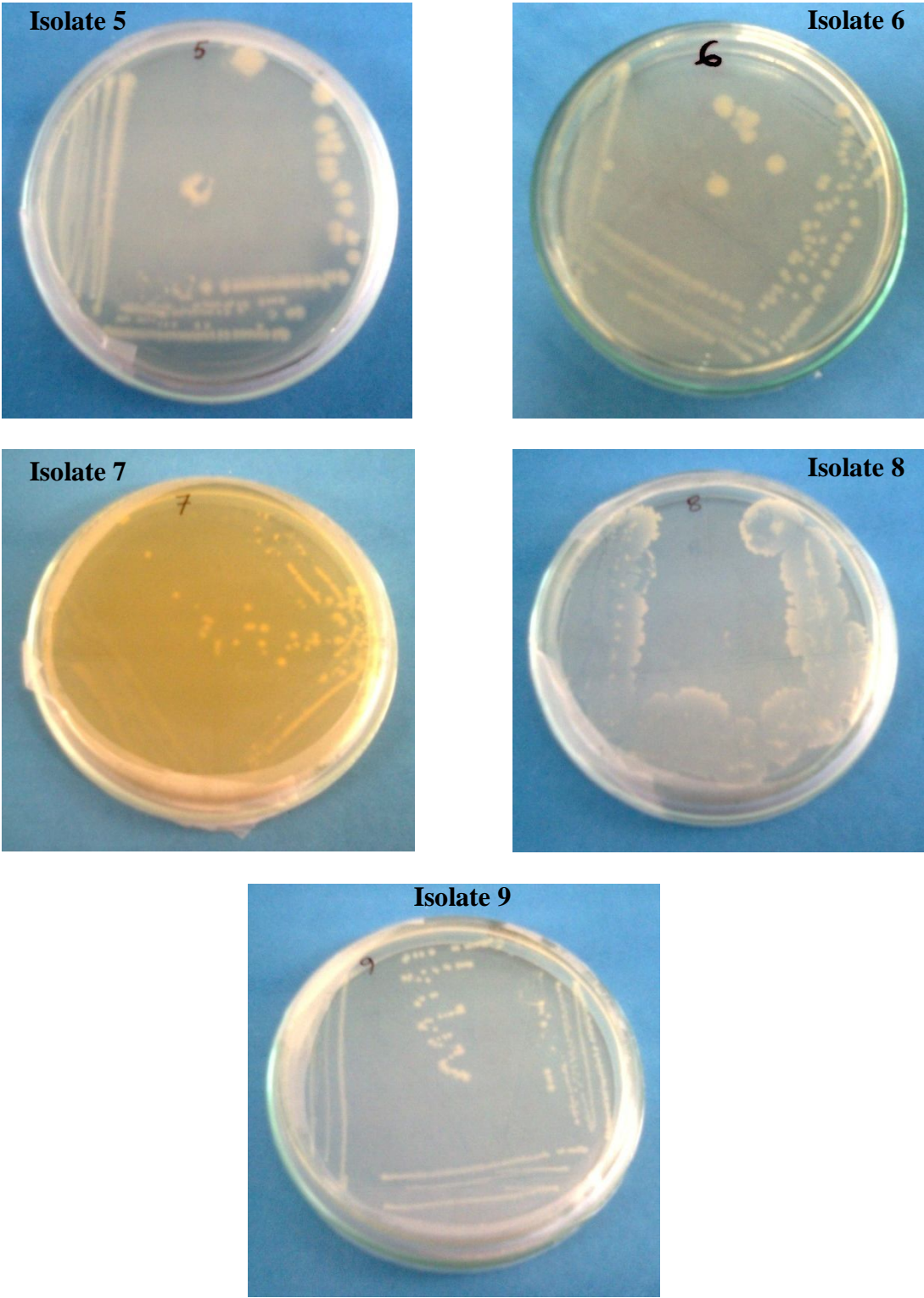
[\* Variance has been adjusted for heterogeneity]

### 3.3. Isolation of bacteria from industrial effluent

Bacterial strains were isolated from industrial (tannery) effluent. Fifteen colonies were screened from initial level of effluent on nutrient agar media. Out of fifteen colonies, nine isolates were selected for biochemical test and other studies (Table 3.14a to 3.14b). From the selected isolates two isolates were from sample 1 (Isolate 1 and 2), four from sample 2 (Isolate 3, 4, 5 and 6) and three from sample 3 (Isolate 7, 8 and 9). The rest of the isolates were rejected for their same colony characteristics.



**Plate 3.1a:** Bacterial isolates found in the industrial effluent



**Plate 3.1b:** Bacterial isolates found in the industrial effluent

**Table 3.14a:** Identification of bacterial isolates from industrial effluents

<b>Physiological and Biochemical Characteristics of bacterial isolates</b>									
Name of test	Sample 1		Sample 2			Sample 3			
	Isolate 1	Isolate 2	Isolate 3	Isolate 4	Isolate 5	Isolate 6	Isolate 7	Isolate 8	Isolate 9
<b>Physiological characters</b>									
Gram strain	+ve	-ve	+ve	-ve	-ve	-ve	-ve	+ve	-ve
Morphology	B	B	Co	B	B	B	B	B	B
Arrangement	C	S	I/C	S	S	S	S	C	S
Motility	+	-	-	+	+	+	+	+	+
<b>Biochemical characters</b>									
McConkey agar	-/-	+/+	-/-	+/+	+/+	+/+	+/-	-/-	+/-
H <sub>2</sub> S production	-	-	-	-	-	-	+	-	-
Gelatin liquefaction	+	-	+	-	-	-	-	+	-
Citrate test	+	+	-	-	-	+	-	+	-
Urease test	-	-	-	-	-	-	+	-	+
Indole test	-	+	-	+	+	-	+	-	-
Methyl Red test	-	+	+	+	+	+	+	-	+
Voges-Proskauer test	+	+	-	-	-	-	-	+	-
Mannitol agar	+/+	-/-	+/-	-/-	-/-	-/-	-/-	+/+	-/-
Catalase test	+	+	+	-	-	+	+	+	+
Amaylase test	+	-	-	-	-	-	-	+	-
TSI	Al/Al	A/AG	A/A	A/AG	A/AG	A/AG	A/A/ H <sub>2</sub> S	A/Al	Al/A/ H <sub>2</sub> S
Type strain	<i>Bacillus cereus</i>	<i>Klebsiella oxytoca</i>	<i>Staphylococcus aureus</i>	<i>E. coli</i> (I)	<i>E. coli</i> (II)	<i>Citrobacter freundii</i>	<i>Proteus vulgaris</i>	<i>Bacillus subtilis</i>	<i>Salmonella typhimurium</i>

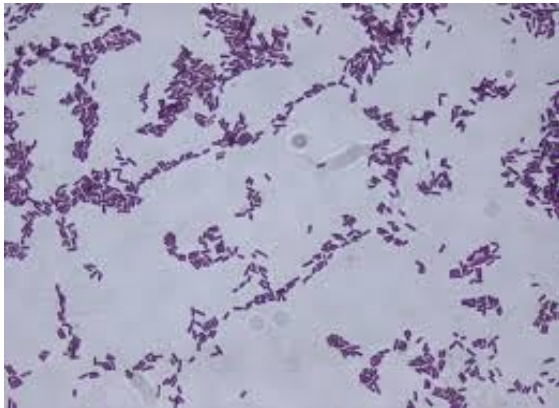
[**Reading chart:** +ve: Positive; -ve: Negative; A: Acid; AG: Acid/gas; Al: Alkaline; B: Bacilli; C: Chain; Co: Cocci; S: Single; **On plates:** ++ Growth and fermentation; +/- Growth and no fermentation; -/- No growth; **TSI:** (Slant/Butt of tube); H<sub>2</sub>S: Hydrogen sulfide]

**Table 3.14b:** Identification of bacterial isolates from industrial effluents

Acid production from carbohydrates by bacterial isolates									
Name of test	Sample 1		Sample 2			Sample 3			
	Isolate 1	Isolate 2	Isolate 3	Isolate 4	Isolate 5	Isolate 6	Isolate 7	Isolate 8	Isolate 9
Glucose	+	+	+	+	+	+	+	+	+
Lactose	-	+	+	+	+	+	+	-	-
Sucrose	+	+	+	+	+	-	+	+	-
Dextrose	+	+	+	+	+	+	+	+	+
Mannitol	-	+	+	+	+	+	+	-	+
Fructose	+	+	+	+	+	+	+	+	+
Arabinose	+	+	-	+	+	+	-	-	+
Cellobiose	+	+	+	+	+	+	+	+	+
Maltose	-	+	+	+	+	+	+	-	+
Xylose	-	+	+	+	+	+	+	-	+
Type strain	<i>Bacillus cereus</i>	<i>Klebsiella oxytoca</i>	<i>Staphylococcus aureus</i>	<i>E. coli</i> (I)	<i>E. coli</i> (II)	<i>Citrobacter freundii</i>	<i>Proteus vulgaris</i>	<i>Bacillus subtilis</i>	<i>Salmonella typhimurium</i>

[ + Acid produce; - No acid production ]

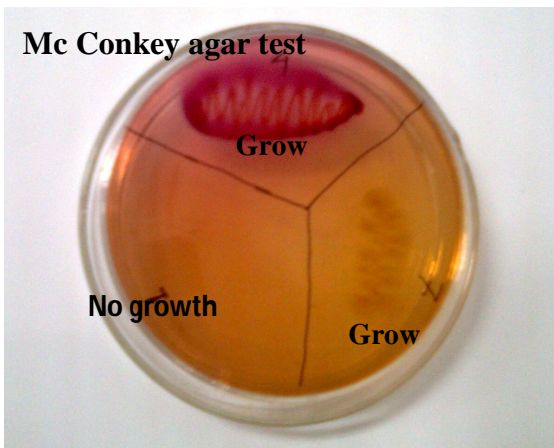




**Plate 3.2:** Gram staining (+ve)



**Plate 3.3:** Gram staining (-ve)



**Plate 3.4:** McConkey agar test



**Plate 3.5:** Manitol salt agar test



**Plate 3.6:** Catalase test (-ve)



**Plate 3.7:** Catalase test (+ve)

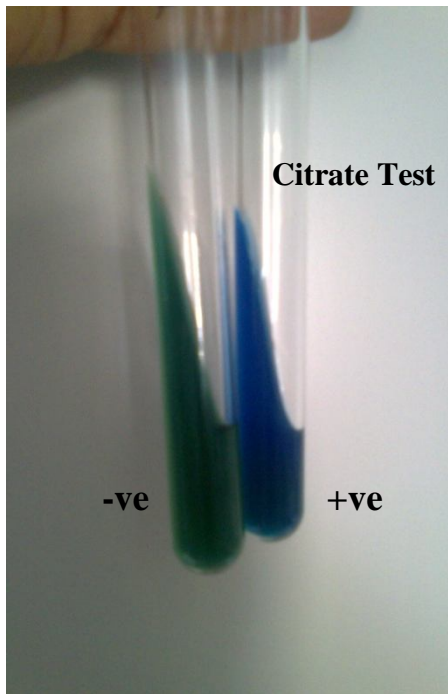


Plate 3.8: Citrate test

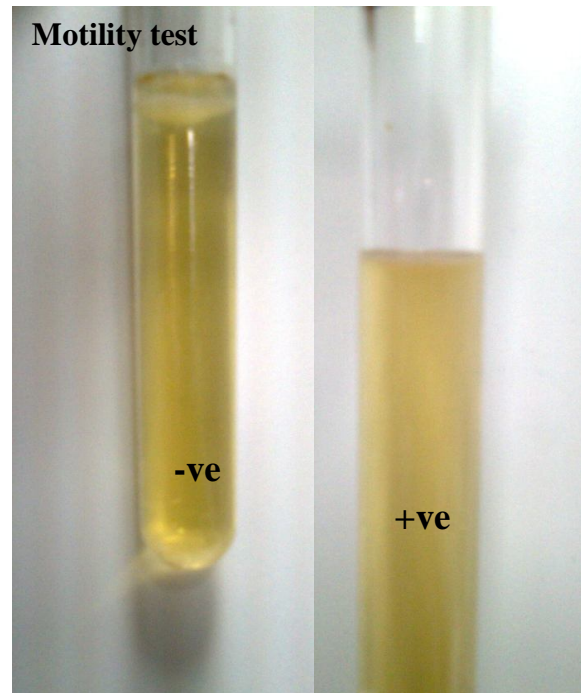


Plate 3.9: Motility test

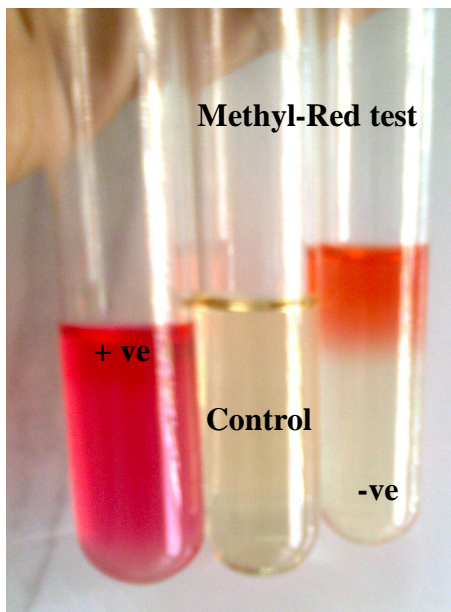


Plate 3.10: Methyl-Red test

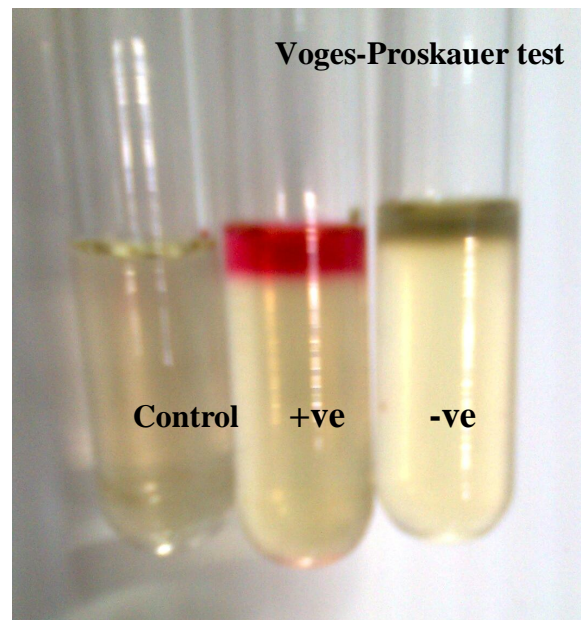
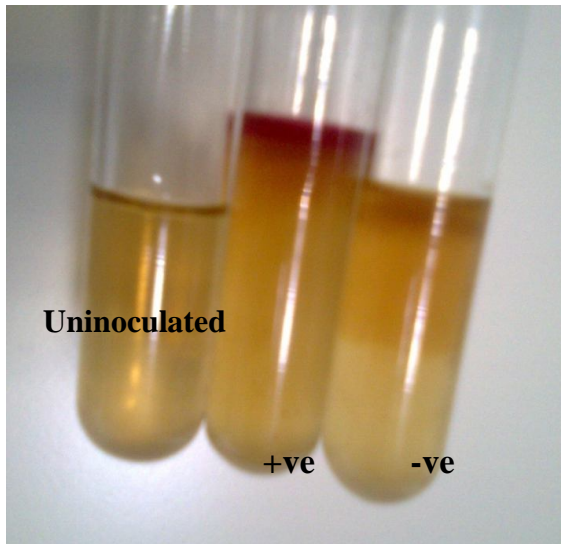


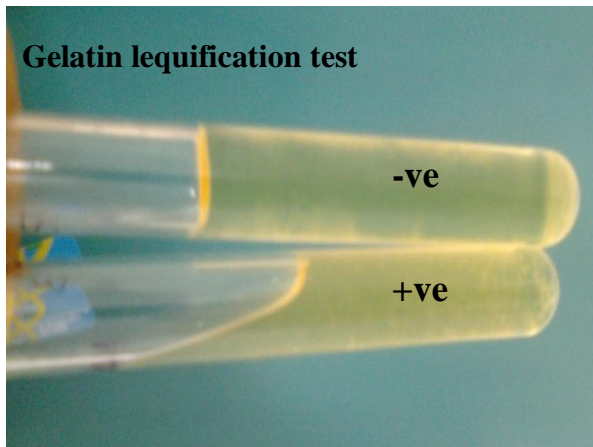
Plate 3.11: Voges-Proskauer test



**Plate 3.12:** Indole test



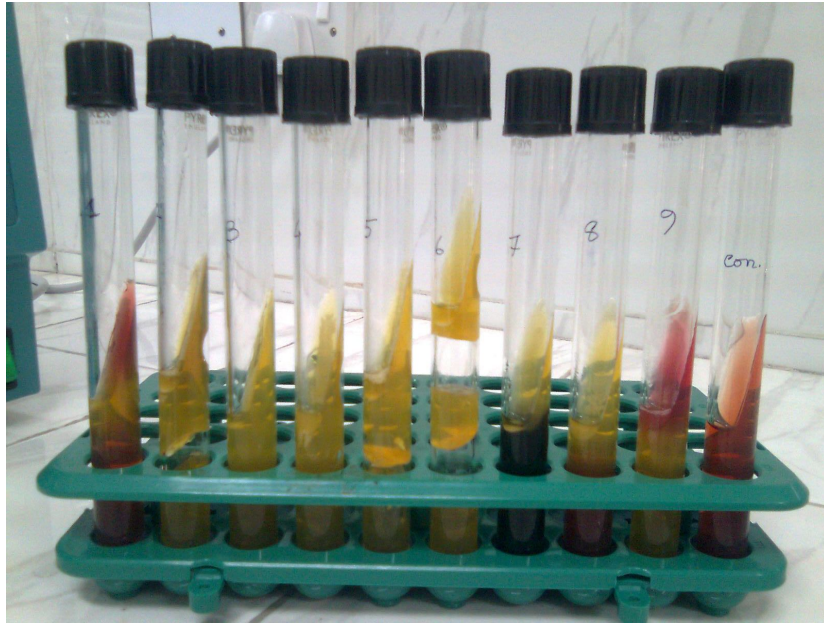
**Plate 3.13:** Urease test



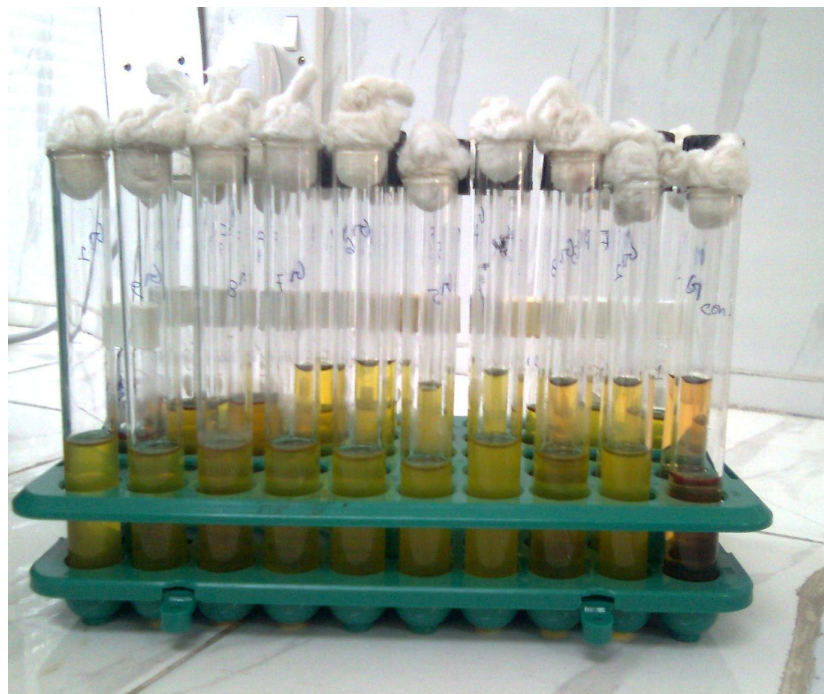
**Plate 3.14:** Gelatin liquefaction test



**Plate 3.15:** H<sub>2</sub>S production test



**Plate 3.16:** Triple sugar iron test



**Plate 3.17:** Carbohydrate fermentation test

### 3.3.1. Physico-chemical analysis of industrial effluent samples

The collected samples were analyzed in the laboratory of the Bangladesh Council of Scientific and Industrial Research (BCSIR), Dhaka and the Central Laboratory, University of Rajshahi, Bangladesh. The results are presented in the Table 3.15 and in Appendix (page, LXXXI – LXXXV).

**Table 3.15:** Physico-chemical characteristics of industrial effluent

SI. No.	Parameters	Results		
		Sample 1	Sample 2	Sample 3
01	pH	7.2400	7.2900	8.7400
02	EC (mS/cm)	5.3800	5.4600	14.8300
03	TDS (g L <sup>-1</sup> )	2.6900	2.7300	7.4100
04	Fluoride (ppm)	22.9330	20.2880	54.2170
05	Chloride (ppm)	1400.9380	1338.3800	1958.9190
06	Nitrite (ppm)	---	---	---
07	Nitrate (ppm)	1.9210	2.8350	3.4940
08	Bromide (ppm)	---	---	4.6130
09	Phosphate (ppm)	---	---	15.6000
10	Sulfate (ppm)	425.1200	16.5420	---
11	BOD (ppm)	286.00	1874.00	1768.00
12	COD (ppm)	320.00	1998.00	1854.00
13	Arsenic, As (ppm)	4.0134	1.7449	4.8583
14	Cadmium, Cd (ppm)	0.0163	0.0084	0.0084
15	Chromium, Cr (ppm)	0.4723	0.1607	0.5978
16	Cobalt, Co (ppm)	-0.0076	-0.0007	-0.0066
17	Copper, Cu (ppm)	0.0125	0.0137	0.0131
18	Iron, Fe (ppm)	0.0064	0.0095	0.1559
19	Lead, Pb (ppm)	1.1419	1.1938	1.0381
20	Manganese, Mn (ppm)	0.2604	0.2217	0.0620
21	Nickel, Ni (ppm)	0.1552	0.0887	0.0802
22	Potassium, K (ppm)	0.3225	0.3360	0.3557
23	Zinc, Zn (ppm)	0.0020	0.0056	0.0132

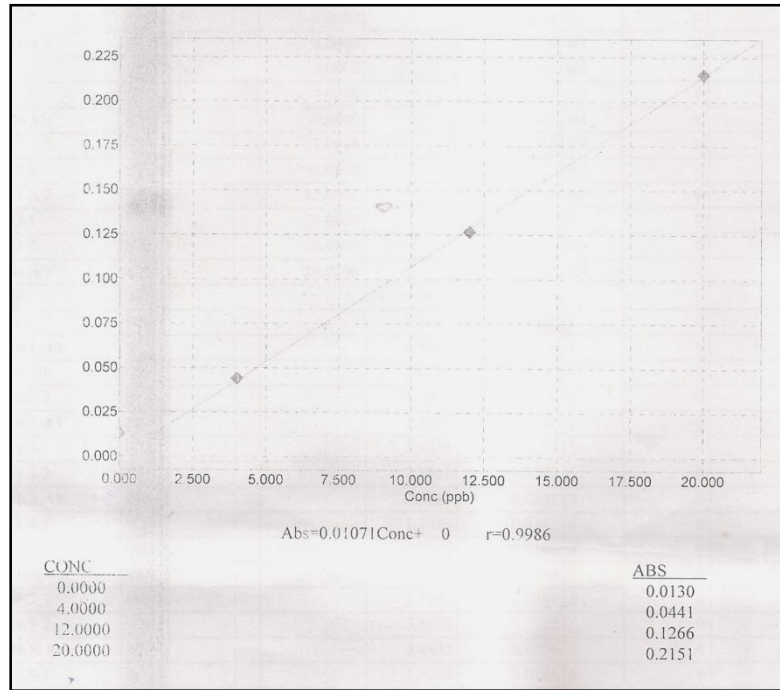


Fig 3.1: Calibration curve for the element arsenic (As)

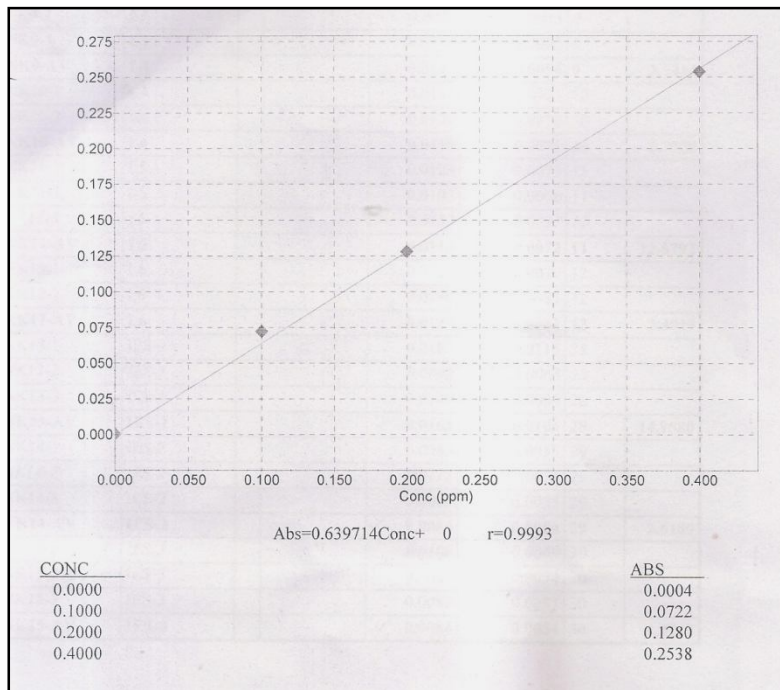


Fig 3.2: Calibration curve for the element cadmium (Cd)

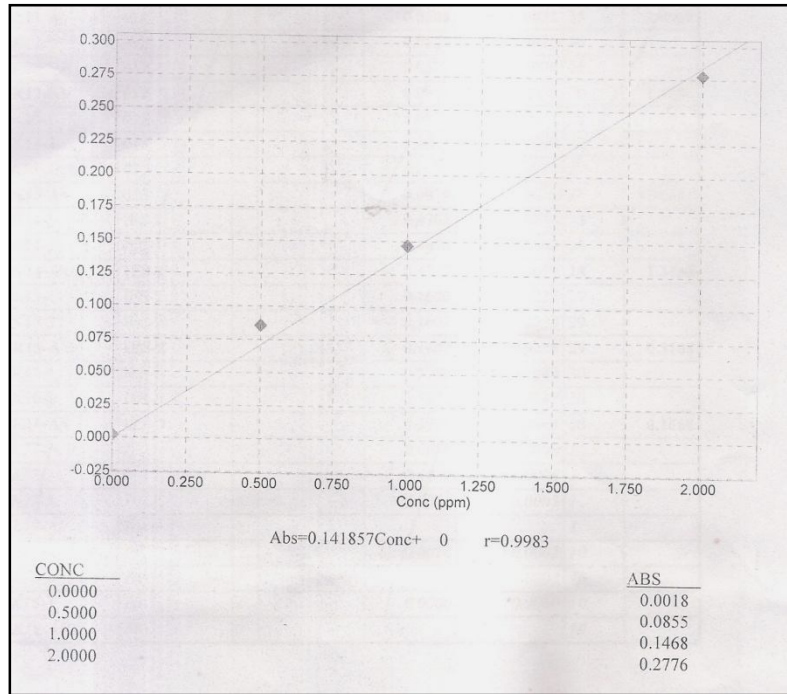


Fig 3.3: Calibration curve for the element chromium (Cr)

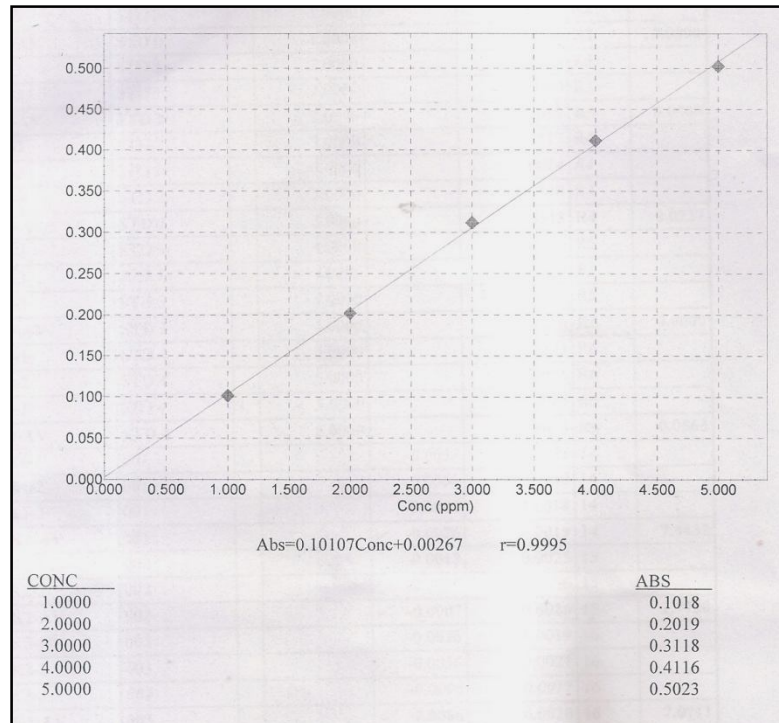


Fig 3.4: Calibration curve for the element cobalt (Co)

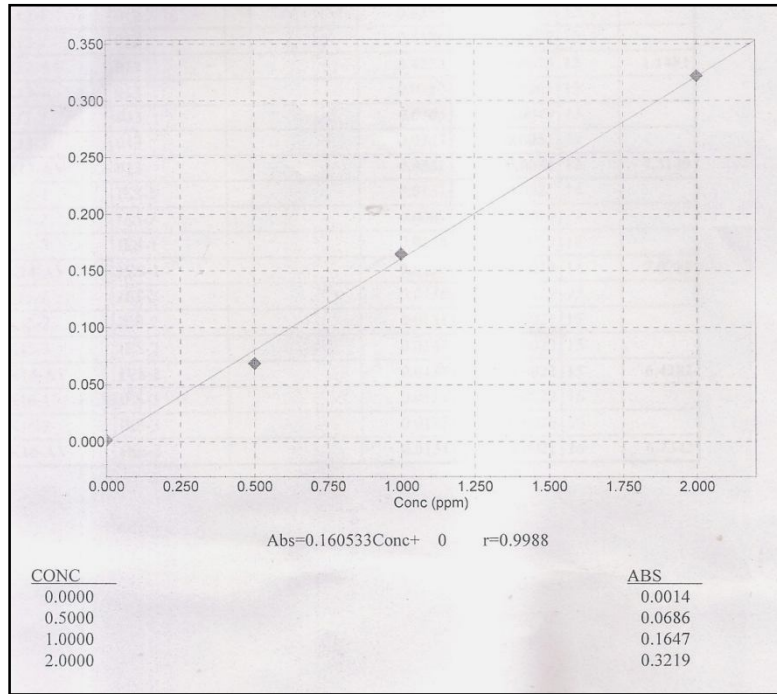


Fig 3.5: Calibration curve for the element copper (Cu)

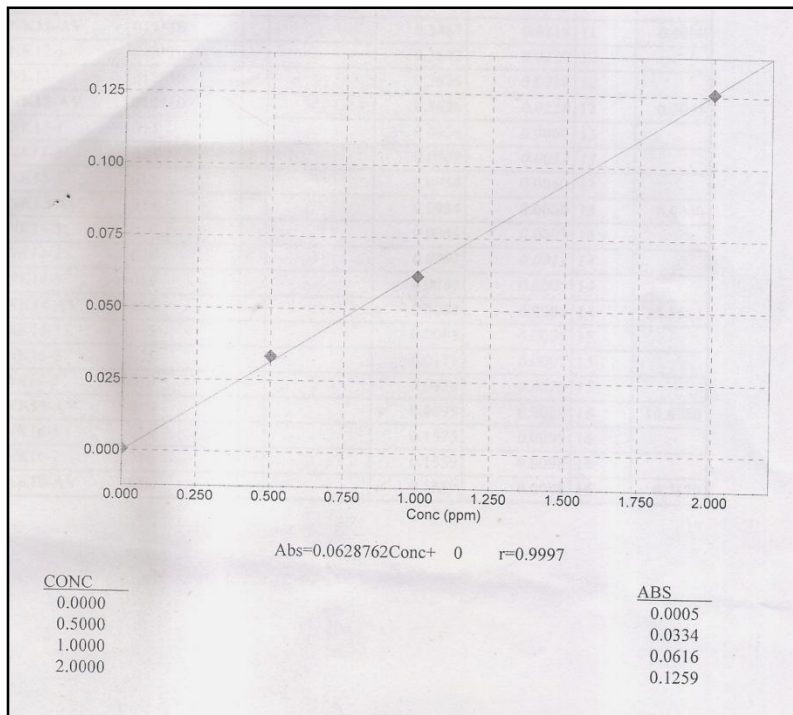


Fig 3.6: Calibration curve for the element iron (Fe)



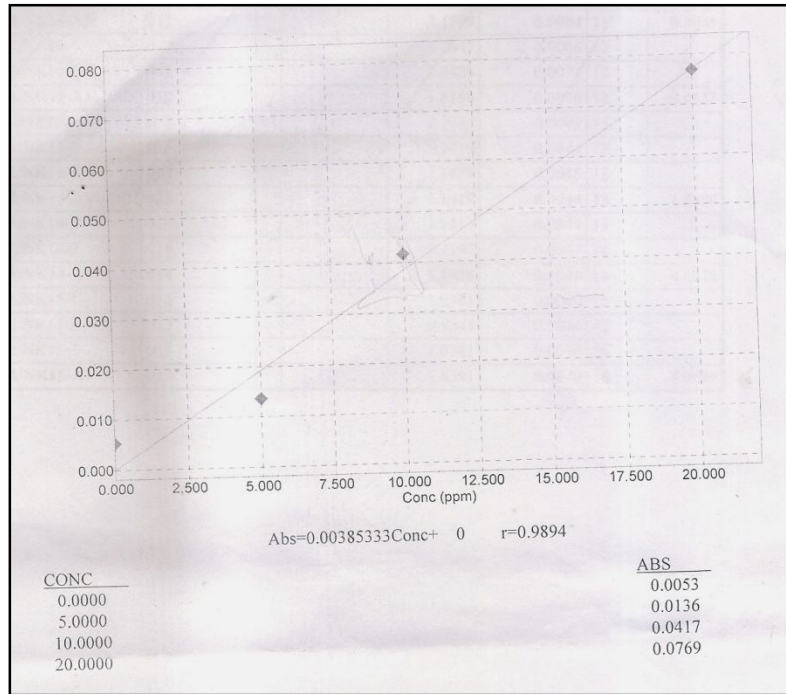


Fig 3.7: Calibration curve for the element lead (Pb)

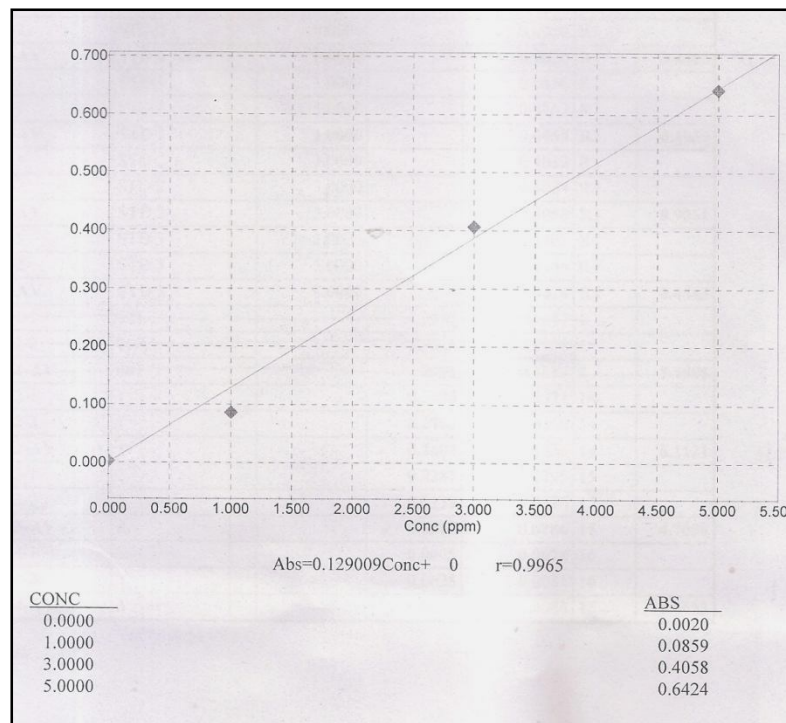


Fig 3.8: Calibration curve for the element manganese (Mn)

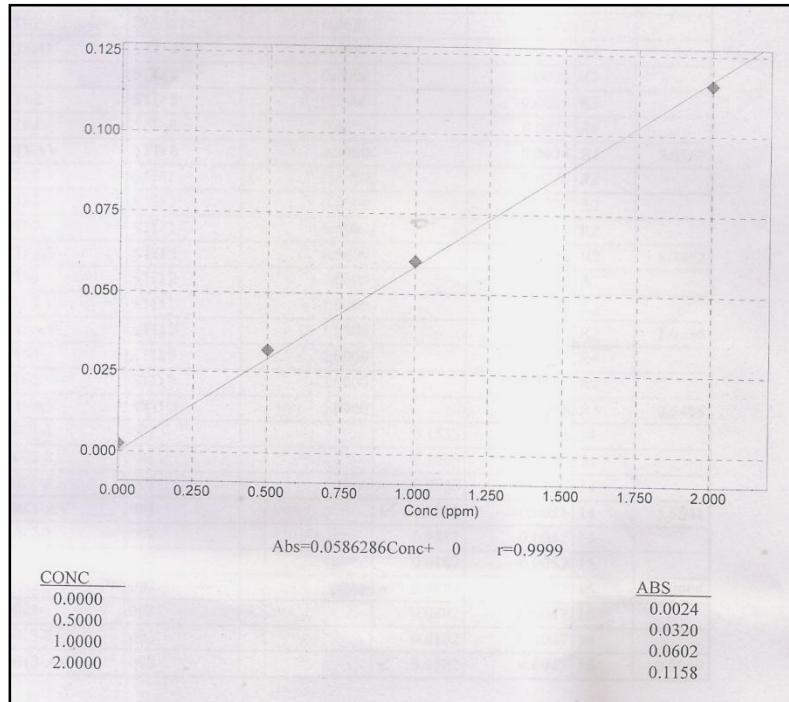


Fig 3.9: Calibration curve for the element nickel (Ni)

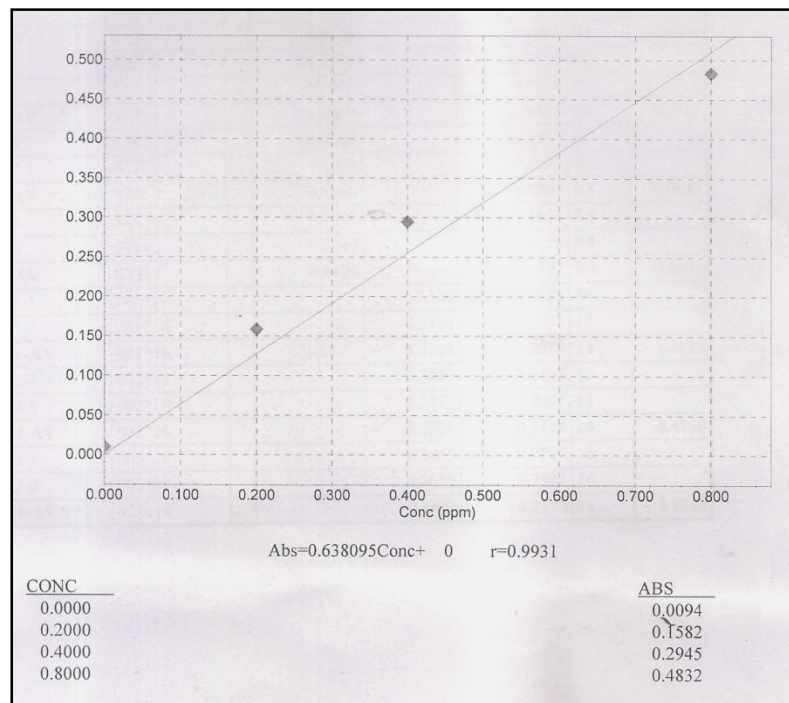


Fig 3.10: Calibration curve for the element potassium (K)

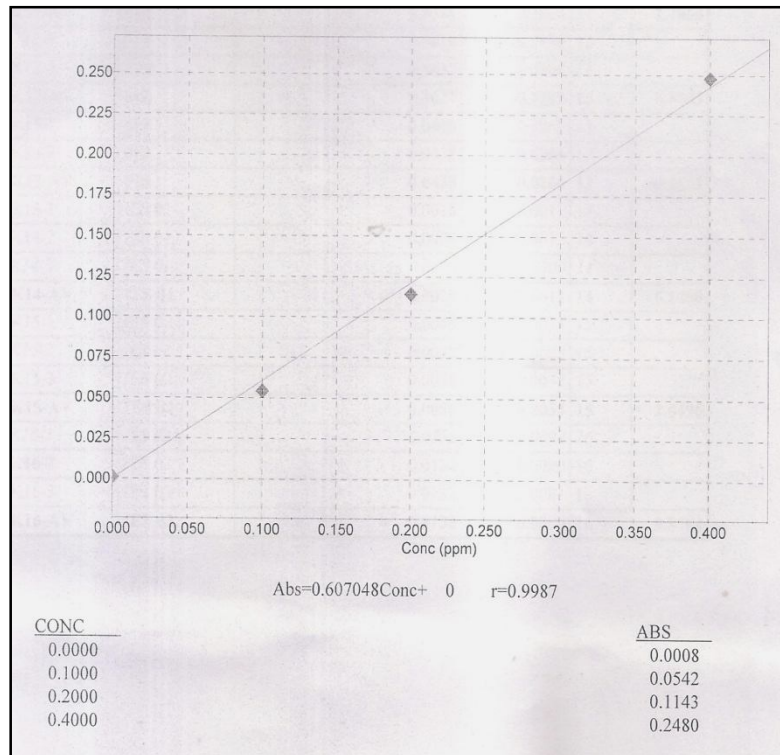


Fig 3.11: Calibration curve for the element zinc (Zn)

### 3.4. Antibacterial activities of the test extracts

The Pet.E., CHCl<sub>3</sub> and CH<sub>3</sub>OH extract of *C. papaya* (leaf, stem and root), *M. oliefera* (fruit, leaf, stem bark, stem wood, root bark and root wood) and *Mu. sapientum* (leaf, stem and root) were tested against 7 selected bacteria (2 Gram- positive bacteria *Bacillus subtilis*, *Staphylococcus aureus* and 5 Gram negative bacteria *Escherichia coli*, *Klebsiella pneumoniae*, *Salmonella enteritidis*, *Shigella flexneri* and *Shigella sonnei*) to evaluate their antibacterial potential at concentrations of 200 and 400µg disc<sup>-1</sup> along with a standard antibiotic, Ampicillin 10µg disc<sup>-1</sup>, 9 isolates (from industrial effluent) at a concentration 400µg disc<sup>-1</sup> along with a standard antibiotic, Kanamycin 30µg disc<sup>-1</sup>. The results obtained are shown in Tables 3.16 to 3.20.

#### 3.4.1. Antibacterial activity against the selected bacteria

##### 3.4.1.1. Antibacterial activity of the *C. papaya* extracts

Among the *C. papaya* extracts the root extracts showed the highest antibacterial activity. Only the *B. subtilis*, *K. pneumoniae* and *St. aureus* were responsive among the selected test bacteria.

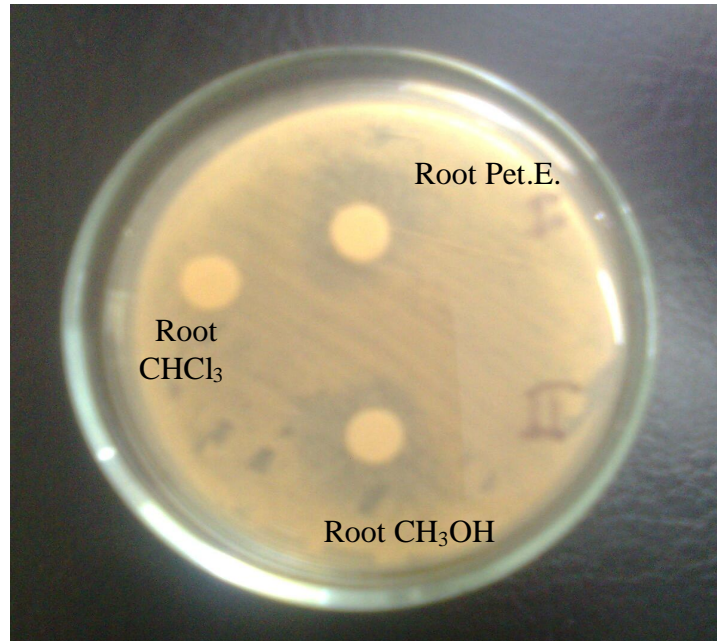
*St. aureus* was most susceptible against all three types of extracts by showing the inhibition zone of 9mm (leaf Pet.E.), 10mm (stem CHCl<sub>3</sub>), 15mm (root Pet.E.) and 10mm (root CH<sub>3</sub>OH) for 400µg disc<sup>-1</sup> application. While, *B. subtilis* only susceptible against the root CHCl<sub>3</sub> and *K. pneumoniae* against the stem CHCl<sub>3</sub> extracts by showing the inhibition zone of 20 and 9mm respectively for 400µg disc<sup>-1</sup> application.

*B. subtilis*, *K. pneumoniae* and *St. aureus* showed 40, 40 and 38mm inhibition zones for the standard Ampicillin 10µg disc<sup>-1</sup> respectively. While, *Sh. flexneri* and *Sh. sonnei* were not responsive against the extracts but they were responsive for the standard Ampicillin with the inhibition zone of 28 and 38mm respectively while, *E. coli* and *S. enteritidis* did not responsive for both the doses and standard Ampicillin. All the bacteria were not responsive against 200µg disc<sup>-1</sup> application.

**Table 3.16:** Antibacterial activity of the *C. papaya* extracts and the standard Ampicillin

Plant	Plant parts	Solvents	Doses ( $\mu\text{g disc}^{-1}$ )	Gram (+ve)		Gram (-ve)					
				<i>B. subtilis</i>	<i>St. aureus</i>	<i>E. coli</i>	<i>K. pneumoniae</i>	<i>Sa. enteritidis</i>	<i>Sh. flexneri</i>	<i>Sh. sonnei</i>	
<i>C. papaya</i>	Leaf	Pet.E.	200 $\mu\text{g disc}^{-1}$	-	-	-	-	-	-	-	-
			400 $\mu\text{g disc}^{-1}$	-	09	-	-	-	-	-	-
		CHCl <sub>3</sub>	200 $\mu\text{g disc}^{-1}$	-	-	-	-	-	-	-	-
			400 $\mu\text{g disc}^{-1}$	-	-	-	-	-	-	-	-
		CH <sub>3</sub> OH	200 $\mu\text{g disc}^{-1}$	-	-	-	-	-	-	-	-
			400 $\mu\text{g disc}^{-1}$	-	-	-	-	-	-	-	-
	Stem	Pet.E.	200 $\mu\text{g disc}^{-1}$	-	-	-	-	-	-	-	-
			400 $\mu\text{g disc}^{-1}$	-	--	-	-	-	-	-	-
		CHCl <sub>3</sub>	200 $\mu\text{g disc}^{-1}$	-	-	-	-	-	-	-	-
			400 $\mu\text{g disc}^{-1}$	-	10	-	09	-	-	-	-
		CH <sub>3</sub> OH	200 $\mu\text{g disc}^{-1}$	-	-	-	-	-	-	-	-
			400 $\mu\text{g disc}^{-1}$	-	-	-	-	-	-	-	-
	Root	Pet.E.	200 $\mu\text{g disc}^{-1}$	-	-	-	-	-	-	-	-
			400 $\mu\text{g disc}^{-1}$	-	15	-	-	-	-	-	-
		CHCl <sub>3</sub>	200 $\mu\text{g disc}^{-1}$	-	-	-	-	-	-	-	-
			400 $\mu\text{g disc}^{-1}$	20	-	-	-	-	-	-	-
		CH <sub>3</sub> OH	200 $\mu\text{g disc}^{-1}$	-	-	-	-	-	-	-	-
			400 $\mu\text{g disc}^{-1}$	-	10	-	-	-	-	-	-
	Ampicillin (10 $\mu\text{g disc}^{-1}$ )				40	38	-	40	-	28	38

[Zone of inhibition in mm]



**Plate 3.18:** Antibacterial activity test of *C. papaya* root extracts against *St. aureus* for 400 $\mu$ g disc<sup>-1</sup> application



**Plate 3.19:** Antibacterial activity test of *M. oleifera* extracts against *K. pneumoniae* for 400 $\mu$ g disc<sup>-1</sup> application along with standard Ampicillin 10 $\mu$ g disc<sup>-1</sup>

### 3.4.1.2. Antibacterial activity of the *M. oleifera* extracts

Among the *M. oleifera* extracts stem wood extracts showed the highest antibacterial activity. Only *B. subtilis*, *K. pneumoniae* and *St. aureus* were responsive among the selected test bacteria.

*St. aureus* was most susceptible against all six types of extracts by showing the inhibition zones of 10mm (fruit Pet.E.), 9mm (fruit CHCl<sub>3</sub>), 9mm (leaf Pet.E.), 9mm (stem bark Pet.E.), 10mm (stem bark CHCl<sub>3</sub>), 10mm (stem wood CHCl<sub>3</sub>), 12mm (stem wood CH<sub>3</sub>OH), 11mm (root bark Pet.E.), 9mm (root bark CHCl<sub>3</sub>) and 10mm (root wood CHCl<sub>3</sub>) for 400µg disc<sup>-1</sup> application. *B. subtilis* was susceptible against the fruit Pet.E. and stem bark CHCl<sub>3</sub> extracts by showing the inhibition zone of 9mm each for 400µg disc<sup>-1</sup> application and *K. pneumoniae* against the fruit CHCl<sub>3</sub> and stem wood CHCl<sub>3</sub> extracts by showing the inhibition zone of 11 and 10mm respectively for 400µg disc<sup>-1</sup> application.

*B. subtilis*, *K. pneumoniae* and *St. aureus* showed 40, 45 and 38mm inhibition zones for the standard Ampicillin 10µg disc<sup>-1</sup> respectively. While, *Sh. flexneri* and *Sh. sonnei* were not responsive against the extracts but they were responsive for the standard Ampicillin with the inhibition zone of 28 and 37mm respectively while, *E. coli* and *S. enteritidis* did not responsive for both the doses and standard Ampicillin. All the bacteria were not responsive against 200µg disc<sup>-1</sup> application.

**Table 3.17:** Antibacterial activity of the *M. oleifera* extracts and the standard Ampicillin

Plant	Plant parts	Solvents	Doses ( $\mu\text{g disc}^{-1}$ )	Gram (+ve)		Gram (-ve)					
				<i>B. subtilis</i>	<i>St. aureus</i>	<i>E. coli</i>	<i>K. pneumoniae</i>	<i>Sa. enteritidis</i>	<i>Sh. flexneri</i>	<i>Sh. sonnei</i>	
<i>M. oleifera</i>	Fruit	Pet.E.	200 $\mu\text{g disc}^{-1}$	-	-	-	-	-	-	-	
			400 $\mu\text{g disc}^{-1}$	09	10	-	-	-	-	-	
		CHCl <sub>3</sub>	200 $\mu\text{g disc}^{-1}$	-	-	-	-	-	-	-	
			400 $\mu\text{g disc}^{-1}$	-	09	-	11	-	-	-	
		CH <sub>3</sub> OH	200 $\mu\text{g disc}^{-1}$	-	-	-	-	-	-	-	
			400 $\mu\text{g disc}^{-1}$	-	-	-	-	-	-	-	
	Leaf	Pet.E.	200 $\mu\text{g disc}^{-1}$	-	-	-	-	-	-	-	
			400 $\mu\text{g disc}^{-1}$	-	09	-	-	-	-	-	
		CHCl <sub>3</sub>	200 $\mu\text{g disc}^{-1}$	-	-	-	-	-	-	-	
			400 $\mu\text{g disc}^{-1}$	-	-	-	-	-	-	-	
		CH <sub>3</sub> OH	200 $\mu\text{g disc}^{-1}$	-	-	-	-	-	-	-	
			400 $\mu\text{g disc}^{-1}$	-	-	-	-	-	-	-	
	Stem Bark	Pet.E.	200 $\mu\text{g disc}^{-1}$	-	-	-	-	-	-	-	
			400 $\mu\text{g disc}^{-1}$	-	09	-	-	-	-	-	
		CHCl <sub>3</sub>	200 $\mu\text{g disc}^{-1}$	-	-	-	-	-	-	-	
			400 $\mu\text{g disc}^{-1}$	09	10	-	-	-	-	-	
		CH <sub>3</sub> OH	200 $\mu\text{g disc}^{-1}$	-	-	-	-	-	-	-	
			400 $\mu\text{g disc}^{-1}$	-	-	-	-	-	-	-	
	Ampicillin (10 $\mu\text{g disc}^{-1}$ )				40	38	-	45	-	28	37

[Zone of inhibition in mm]



**Table 3.18:** Antibacterial activity of the *M. oleifera* extracts and the standard Ampicillin

Plant	Plant parts	Solvents	Doses ( $\mu\text{g disc}^{-1}$ )	Gram (+ve)		Gram (-ve)					
				<i>B. subtilis</i>	<i>St. aureus</i>	<i>E. coli</i>	<i>K. pneumoniae</i>	<i>Sa. enteritidis</i>	<i>Sh. flexneri</i>	<i>Sh. sonnei</i>	
<i>M. oleifera</i>	Stem Wood	Pet.E.	200 $\mu\text{g disc}^{-1}$	-	-	-	-	-	-	-	
			400 $\mu\text{g disc}^{-1}$	-	10	-	-	-	-	-	
		CHCl <sub>3</sub>	200 $\mu\text{g disc}^{-1}$	-	-	-	-	-	-	-	
			400 $\mu\text{g disc}^{-1}$	-	-	-	10	-	-	-	
		CH <sub>3</sub> OH	200 $\mu\text{g disc}^{-1}$	-	-	-	-	-	-	-	
			400 $\mu\text{g disc}^{-1}$	-	12	-	-	-	-	-	
	Root Bark	Pet.E.	200 $\mu\text{g disc}^{-1}$	-	-	-	-	-	-	-	
			400 $\mu\text{g disc}^{-1}$	-	11	-	-	-	-	-	
		CHCl <sub>3</sub>	200 $\mu\text{g disc}^{-1}$	-	-	-	-	-	-	-	
			400 $\mu\text{g disc}^{-1}$	-	09	-	-	-	-	-	
		CH <sub>3</sub> OH	200 $\mu\text{g disc}^{-1}$	-	-	-	-	-	-	-	
			400 $\mu\text{g disc}^{-1}$	-	-	-	-	-	-	-	
	Root Wood	Pet.E.	200 $\mu\text{g disc}^{-1}$	-	-	-	-	-	-	-	
			400 $\mu\text{g disc}^{-1}$	-	-	-	-	-	-	-	
		CHCl <sub>3</sub>	200 $\mu\text{g disc}^{-1}$	-	-	-	-	-	-	-	
			400 $\mu\text{g disc}^{-1}$	-	10	-	-	-	-	-	
		CH <sub>3</sub> OH	200 $\mu\text{g disc}^{-1}$	-	-	-	-	-	-	-	
			400 $\mu\text{g disc}^{-1}$	-	-	-	-	-	-	-	
	Ampicillin (10 $\mu\text{g disc}^{-1}$ )				40	38	-	45	-	28	37

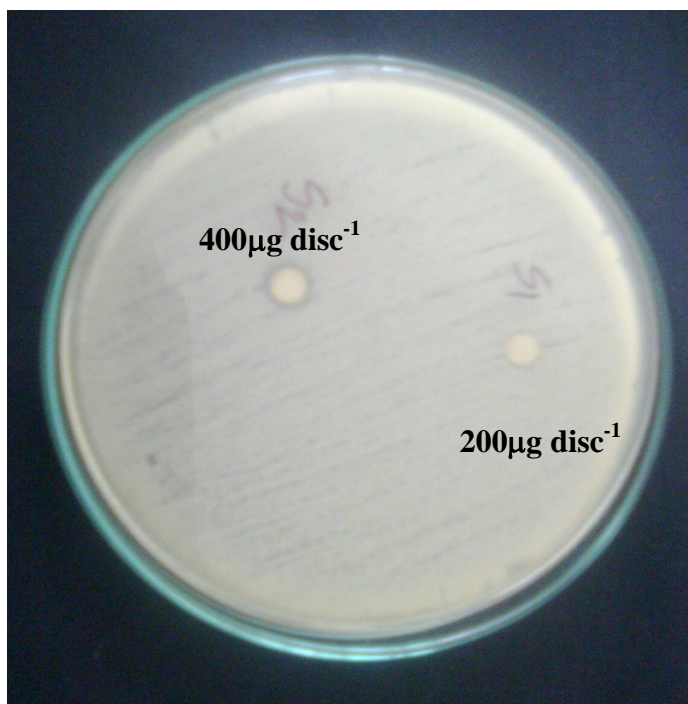
[Zone of inhibition in mm]

### 3.4.1.3. Antibacterial activity of the *Mu. sapientum* extracts

Among the *Mu. sapientum* extracts the root extracts showed the highest antibacterial activity. Only the *St. aureus* was responsive among the selected test bacteria.

*St. aureus* was susceptible against stem (Pet.E.) and root (Pet.E.) extracts by showing the inhibition zone of 8 and 10mm for 400 $\mu$ g disc<sup>-1</sup> application and also susceptible to the standard Ampicillin 10 $\mu$ g disc<sup>-1</sup> with an inhibition zone of 30mm.

*B. subtilis*, *K. pneumonia*, *Sh. flexneri* and *Sh. sonnei* were not responsive against the extracts but they were responsive for the standard Ampicillin with the inhibition zone of 40, 40, 28 and 37mm respectively. While, *E. coli* and *S. enteritidis* did not responsive for both the doses and standard Ampicillin. All the bacteria were not responsive to 200 $\mu$ g disc<sup>-1</sup> application.



Strain of *St. aureus* on *Mu. sapientum* (root) extracted in Pet.E. for 200 and 400 $\mu$ g disc<sup>-1</sup>

**Plate 3.20:** Antibacterial activity test of *Mu. sapientum* extracts

**Table 3.19:** Antibacterial activity of the *Mu. sapientum* extracts and the standard Ampicillin

Plant	Plant parts	Solvents	Doses ( $\mu\text{g disc}^{-1}$ )	Gram (+ve)		Gram (-ve)					
				<i>B. subtilis</i>	<i>St. aureus</i>	<i>E. coli</i>	<i>K. pneumoniae</i>	<i>Sa. enteritidis</i>	<i>Sh. flexneri</i>	<i>Sh. sonnei</i>	
<i>Mu. sapientum</i>	Leaf	Pet.E.	200 $\mu\text{g disc}^{-1}$	-	-	-	-	-	-	-	-
			400 $\mu\text{g disc}^{-1}$	-	-	-	-	-	-	-	-
		CHCl <sub>3</sub>	200 $\mu\text{g disc}^{-1}$	-	-	-	-	-	-	-	-
			400 $\mu\text{g disc}^{-1}$	-	-	-	-	-	-	-	-
		CH <sub>3</sub> OH	200 $\mu\text{g disc}^{-1}$	-	-	-	-	-	-	-	-
			400 $\mu\text{g disc}^{-1}$	-	-	-	-	-	-	-	-
	Stem	Pet.E.	200 $\mu\text{g disc}^{-1}$	-	-	-	-	-	-	-	-
			400 $\mu\text{g disc}^{-1}$	-	08	-	-	-	-	-	-
		CHCl <sub>3</sub>	200 $\mu\text{g disc}^{-1}$	-	-	-	-	-	-	-	-
			400 $\mu\text{g disc}^{-1}$	-	-	-	-	-	-	-	-
		CH <sub>3</sub> OH	200 $\mu\text{g disc}^{-1}$	-	-	-	-	-	-	-	-
			400 $\mu\text{g disc}^{-1}$	-	-	-	-	-	-	-	-
	Root	Pet.E.	200 $\mu\text{g disc}^{-1}$	-	-	-	-	-	-	-	-
			400 $\mu\text{g disc}^{-1}$	-	10	-	-	-	-	-	-
		CHCl <sub>3</sub>	200 $\mu\text{g disc}^{-1}$	-	-	-	-	-	-	-	-
			400 $\mu\text{g disc}^{-1}$	-	-	-	-	-	-	-	-
		CH <sub>3</sub> OH	200 $\mu\text{g disc}^{-1}$	-	-	-	-	-	-	-	-
			400 $\mu\text{g disc}^{-1}$	-	-	-	-	-	-	-	-
	Ampicillin (10 $\mu\text{g disc}^{-1}$ )				40	30	-	40	-	28	37

[Zone of inhibition in mm]

### 3.4.2. Antibacterial activity against the isolates from the effluent

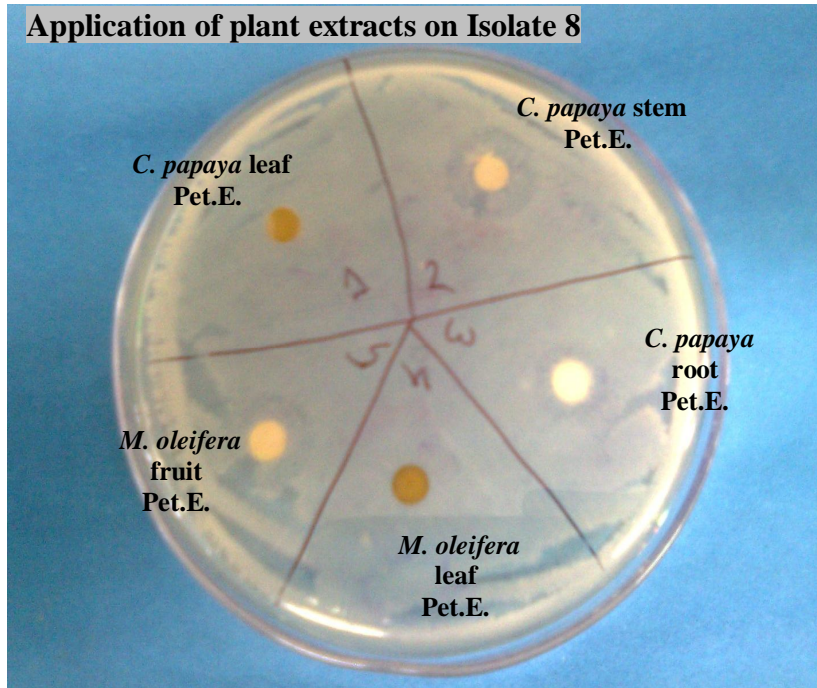
The Pet.E.,  $\text{CHCl}_3$  and  $\text{CH}_3\text{OH}$  extract of *C. papaya* (leaf, stem and root), *M. oliefera* (fruit, leaf, stem bark, stem wood, root bark and root wood) and *Mu. sapientum* (leaf, stem and root) were tested against 9 isolates viz. Isolate 1 (*Bacillus cereus*), Isolate 2 (*Klebsiella oxytoca*), Isolate 3 (*Staphylococcus aureus*), Isolate 4 and 5 (*Escherichia coli*), Isolate 6 (*Citrobacter freundii*), Isolate 7 (*Proteus vulgaris*), Isolate 8 (*Bacillus subtilis*) and Isolate 9 *Salmonella typhimurium* at a concentrations of  $400\mu\text{g disc}^{-1}$  doses. Kanamycin  $30\mu\text{g disc}^{-1}$  is used as the standard antibiotic. The results obtained are shown in Tables 3.28.

Among the 9 Isolates Isolate 8 *Bacillus subtilis* was highly responsive to the Pet.E. and  $\text{CHCl}_3$  extracts of *C. papaya* stem (15 & 08mm), *M. oliefera* fruit (14 & 09mm) and root bark (10 & 16mm), and *Mu. sapientum* root (10 & 10mm) respectively; to the Pet.E. extracts of *C. papaya* root (11mm), *M. oliefera* root wood (11mm), *Mu. sapientum* stem (10mm), and to the  $\text{CHCl}_3$  extract of *M. oliefera* stem bark (08mm). Next to the Isolate 8 it was Isolate 2 *Klebsiella oxytoca* responsive to the Pet.E. extracts of *C. papaya* leaf (08mm), *M. oliefera* fruit (08mm), *Mu. sapientum* leaf (15mm) and root (10mm); and to the  $\text{CHCl}_3$  extract of *C. papaya* leaf (08mm), *M. oliefera* stem bark (08mm), stem wood (08mm) and root bark (08mm); followed by the Isolate 1 *Bacillus cereus* which was responsive to the Pet.E. extracts of *C. papaya* stem (16mm), *M. oliefera* fruit (10mm) and *Mu. sapientum* stem (8mm) and root (10mm); this was followed by Isolate 3 *Staphylococcus aureus* which was responsive to the Pet.E. extracts of *C. papaya* stem (10mm), *M. oliefera* fruit (13mm) and *Mu. sapientum* root (08mm) and again the  $\text{CHCl}_3$  extract of *M. oliefera* stem bark (08mm). This was followed by the Isolate 6 *Citrobacter freundii* where  $\text{CHCl}_3$  extract of *C. papaya* stem (08mm) and Pet.E. extract of *Mu. sapientum* leaf (10mm) were found responsive. Isolate 4 and 5 *Escherichia coli* and 9 *Salmonella typhimurium* show response against Pet.E. extracts of *C. papaya* stem (08mm), *M. iloefera* fruit (08mm) and  $\text{CHCl}_3$  extract of *M. oliefera* stem wood (09mm) respectively. Isolate 7 *Proteus vulgaris* was not responsive to any of the 12 extracts of the 3 test plants. For Kanamycin  $30\mu\text{g disc}^{-1}$  the inhibition zones for the Isolate 1, 2, 3, 4, 5, 6, 7, 8 and 9 were 50, 35, 35, 40, 36, 42, 42, 55 and 40mm respectively.

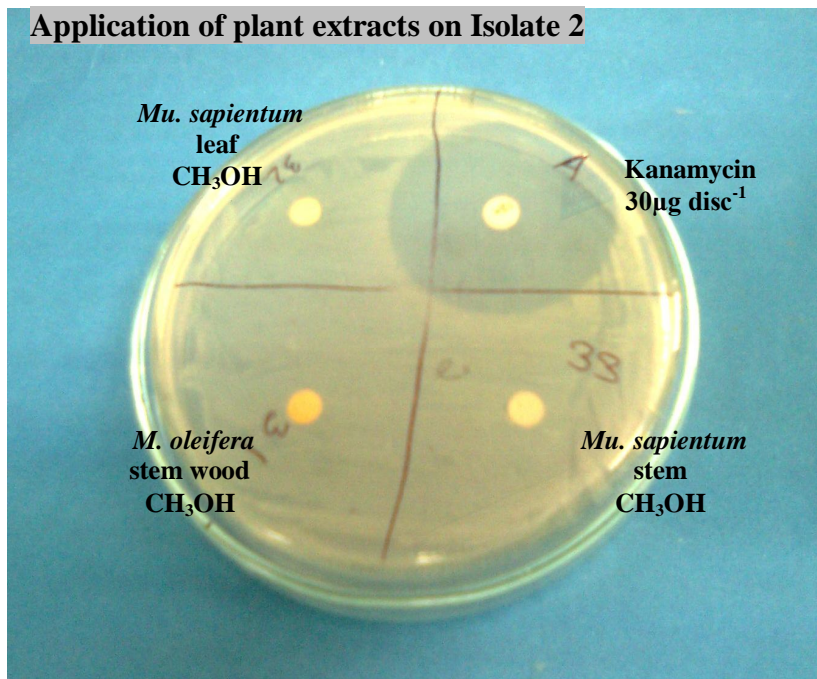
**Table 3.20:** Antibacterial activity of *C. papaya*, *M. oleifera* and *Mu. sapientum* extracts against nine isolates for 400 $\mu$ g disc<sup>-1</sup> application

Plants	Solvents	Plant parts	Isolate 1	Isolate 2	Isolate 3	Isolate 4	Isolate 5	Isolate 6	Isolate 7	Isolate 8	Isolate 9	
<i>C. papaya</i>	Leaf	Pet.E.	-	08	-	-	-	-	-	-	-	
		CHCl <sub>3</sub>	-	08	-	-	-	-	-	-	-	
		CH <sub>3</sub> OH	-	-	-	-	-	-	-	-	-	
	Stem	Pet.E.	16	-	10	08	-	-	-	-	15	-
		CHCl <sub>3</sub>	-	-	-	-	-	-	08	-	08	-
		CH <sub>3</sub> OH	-	-	-	-	-	-	-	-	-	-
	Root	Pet.E.	-	-	-	-	-	-	-	-	11	-
		CHCl <sub>3</sub>	-	-	-	-	-	-	-	-	-	-
		CH <sub>3</sub> OH	-	-	-	-	-	-	-	-	-	-
<i>M. oleifera</i>	Fruit	Pet.E.	10	08	13	-	08	-	-	-	14	-
		CHCl <sub>3</sub>	-	-	-	-	-	-	-	-	09	-
		CH <sub>3</sub> OH	-	-	-	-	-	-	-	-	-	-
	Leaf	Pet.E.	-	-	-	-	-	-	-	-	-	-
		CHCl <sub>3</sub>	-	-	-	-	-	-	-	-	-	-
		CH <sub>3</sub> OH	-	-	-	-	-	-	-	-	-	-
	Stem bark	Pet.E.	-	-	-	-	-	-	-	-	-	-
		CHCl <sub>3</sub>	-	08	08	-	-	-	-	-	08	-
		CH <sub>3</sub> OH	-	-	-	-	-	-	-	-	-	-
	Stem wood	Pet.E.	-	-	-	-	-	-	-	-	-	-
		CHCl <sub>3</sub>	-	08	-	-	-	-	-	-	-	09
		CH <sub>3</sub> OH	-	-	-	-	-	-	-	-	-	-
	Root bark	Pet.E.	-	-	-	-	-	-	-	-	10	-
		CHCl <sub>3</sub>	-	10	-	-	-	-	-	-	16	-
		CH <sub>3</sub> OH	-	-	-	-	-	-	-	-	-	-
Root wood	Pet.E.	-	-	-	-	-	-	-	-	11	-	
	CHCl <sub>3</sub>	-	-	-	-	-	-	-	-	-	-	
	CH <sub>3</sub> OH	-	-	-	-	-	-	-	-	-	-	
<i>Mu. sapientum</i>	Leaf	Pet.E.	-	15	-	-	-	10	-	-	-	
		CHCl <sub>3</sub>	-	-	-	-	-	-	-	-	-	
		CH <sub>3</sub> OH	-	-	-	-	-	-	-	-	-	
	Stem	Pet.E.	08	-	-	-	-	-	-	-	10	-
		CHCl <sub>3</sub>	-	-	-	-	-	-	-	-	-	-
		CH <sub>3</sub> OH	-	-	-	-	-	-	-	-	-	-
	Root	Pet.E.	10	10	08	-	-	-	-	-	10	-
		CHCl <sub>3</sub>	-	-	-	-	-	-	-	-	10	-
		CH <sub>3</sub> OH	-	-	-	-	-	-	-	-	-	-
Kanamycin 30 $\mu$ g disc <sup>-1</sup>			50	35	35	40	36	42	42	55	40	

[Zone of inhibition in mm]



**Plate 3.21:** Antibacterial activity of different extracts against Isolate 8 for  $400\mu\text{g disc}^{-1}$  application



**Plate 3.22:** Antibacterial activity of different extracts against Isolate 8 for  $400\mu\text{g disc}^{-1}$  application with standard antibiotic Kanamycin  $30\mu\text{g disc}^{-1}$

### 3.5. Summary of the experimentation

For the detection of bioactive potentials of *C. papaya* (leaf, stem and root), *M. oleifera* (fruit, leaf, stem bark, stem wood, root bark and root wood) and *Mu. sapientum* (leaf, stem and root) extracted in Pet.E., CHCl<sub>3</sub> and CH<sub>3</sub>OH solvents insecticidal, brine shrimp lethality and antibacterial activity tests have been carried out. A total outcome of the bioassays carried out is represented in the Table 3.21 to 3.23 given below:

**Table 3.21:** Result summary of *C. papaya* extracts

Plant	Solvents	Plant parts	Antibacterial activity																	
			Dose mortality ( <i>T. castaneum</i> )									Brine shrimp lethality ( <i>A. salina</i> )								
			Selected Bacteria						Nine Isolates											
			Gram (+ve)			Gram (-ve)			Gram (+ve)			Gram (-ve)								
			<i>B. subtilis</i>	<i>St. aureus</i>	<i>E. coli</i>	<i>K. pneumoniae</i>	<i>S. enteritidis</i>	<i>Sh. flexneri</i>	<i>Sh. sonnei</i>	<i>B. cereus</i> (Iso 1)	<i>S. aureus</i> (Iso 3)	<i>B. subtilis</i> (Iso 8)	<i>K. oxytoca</i> (Iso 2)	<i>E. coli</i> (Iso 4)	<i>E. coli</i> (Iso 5)	<i>C. freundii</i> (Iso 6)	<i>P. vulgaris</i> (Iso 7)	<i>S. typhimurium</i> (Iso 9)		
<i>C. papaya</i>	Pet.E.	Leaf	+	+	-	+	-	-	-	-	-	-	+	-	-	-	-	-		
		Stem	+	+	-	-	-	-	-	-	+	+	+	-	+	-	-	-	-	
		Root	+	+	-	+	-	-	-	-	-	-	+	-	-	-	-	-	-	
	CHCl <sub>3</sub>	Leaf	-	+	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	
		Stem	+	+	-	+	-	+	-	-	-	-	+	-	-	-	+	-	-	
		Root	-	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
	CH <sub>3</sub> OH	Leaf	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
		Stem	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
		Root	+	+	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	

[Iso = Isolate; (+) = active; (-) = Not active]

Table 3.22: Result summary of *M. oleifera* extracts

Plant	Solvents	Plant parts	Dose mortality ( <i>T. castaneum</i> )	Brine shrimp lethality ( <i>A. salina</i> )	Antibacterial activity																
					Selected Bacteria						Nine Isolates										
					Gram (+ve)		Gram (-ve)				Gram (+ve)			Gram (-ve)							
					<i>B. subtilis</i>	<i>St. aureus</i>	<i>E. coli</i>	<i>K. pneumoniae</i>	<i>S. enteritidis</i>	<i>Sh. flexneri</i>	<i>Sh. sonnei</i>	<i>B. cereus</i> (Iso 1)	<i>S. aureus</i> (Iso 3)	<i>B. subtilis</i> (Iso 8)	<i>K. oxytoca</i> (Iso 2)	<i>E. coli</i> I (Iso 4)	<i>E. coli</i> II (Iso 5)	<i>C. freundii</i> (Iso 6)	<i>P. vulgaris</i> (Iso 7)	<i>S. typhimurium</i> (Iso 9)	
<i>M. oleifera</i>	Pet.E.	Fruit	-	+	+	+	-	-	-	-	-	+	+	+	+	-	+	-	-	-	
		Leaf	-	+	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
		S. bark	+	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
		S. wood	-	+	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
		R. bark	+	+	-	+	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-
		R. wood	+	+	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-
	CHCl <sub>3</sub>	Fruit	-	+	-	+	-	+	-	-	-	-	-	-	+	-	-	-	-	-	-
		Leaf	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
		S. bark	-	+	+	+	-	-	-	-	-	-	+	+	+	-	-	-	-	-	-
		S. wood	-	+	-	-	-	+	-	-	-	-	-	-	+	-	-	-	-	-	+
		R. bark	-	+	-	+	-	-	-	-	-	-	-	-	+	+	-	-	-	-	-
		R. wood	-	+	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	CH <sub>3</sub> OH	Fruit	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
		Leaf	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
		S. bark	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
		S. wood	+	+	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
		R. bark	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
		R. wood	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

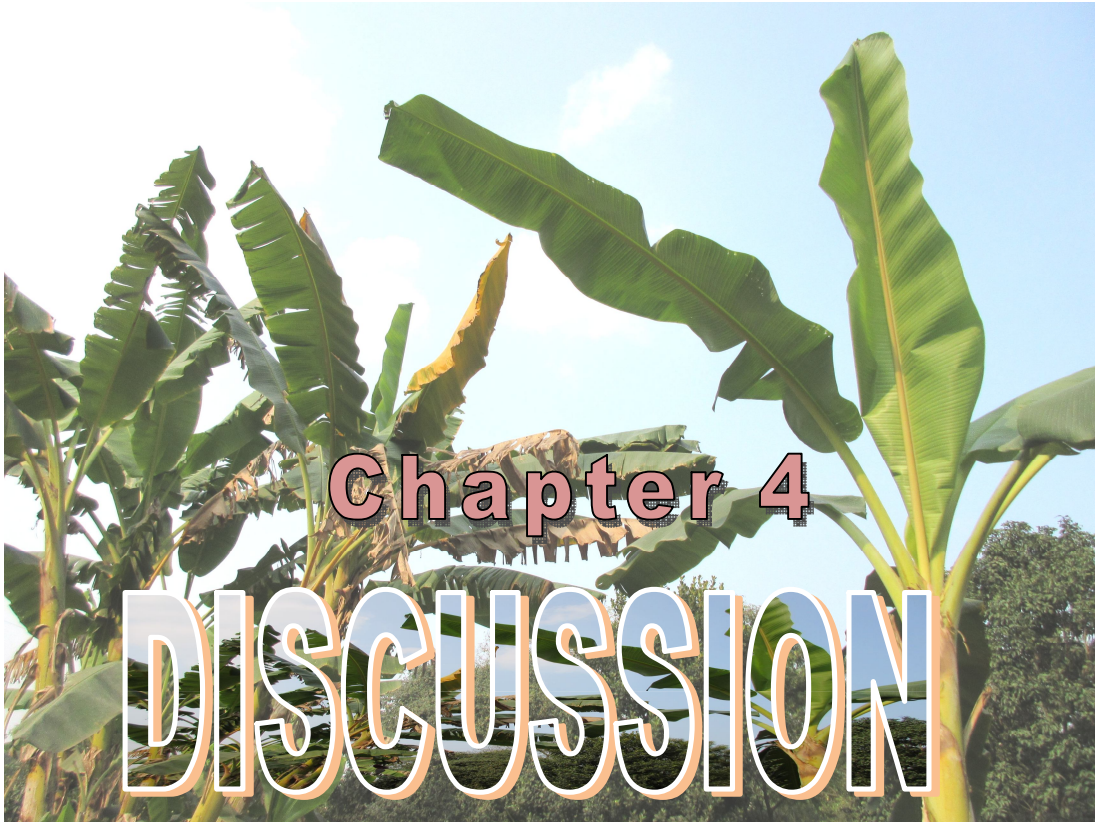
[Iso = Isolate; S. bark = Stem bark; S. wood = Stem wood; R. bark = Root bark; R. wood = Root wood; (+) = active; (-) = Not active]



Table 3.23: Result summary of *Mu. sapientum* extracts

Plant		Solvents	Plant parts	Dose mortality ( <i>T. castaneum</i> )	Brine shrimp lethality ( <i>A. salina</i> )	Antibacterial activity															
						Selected Bacteria						Nine Isolates									
						Gram (+ve)		Gram (-ve)				Gram (+ve)			Gram (-ve)						
						<i>B. subtilis</i>	<i>St. aureus</i>	<i>E. coli</i>	<i>K. pneumoniae</i>	<i>S. enteritidis</i>	<i>Sh. flexneri</i>	<i>Sh. sonnei</i>	<i>B. cereus</i> (Iso 1)	<i>S. aureus</i> (Iso 3)	<i>B. subtilis</i> (Iso 8)	<i>K. oxytoca</i> (Iso 2)	<i>E. coli</i> (Iso 4)	<i>E. coli</i> (Iso 5)	<i>C. freundii</i> (Iso 6)	<i>P. vulgaris</i> (Iso 7)	<i>S. typhimurium</i> (Iso 9)
<i>Mu. sapientum</i>	Pet.E.	Leaf	+	+	-	-	-	-	-	-	-	-	-	-	+	-	-	+	-	-	
		Stem	+	+	-	+	-	-	-	-	-	-	+	-	+	-	-	-	-	-	-
		Root	+	+	-	+	-	-	-	-	-	-	+	+	+	+	-	-	-	-	-
	CHCl <sub>3</sub>	Leaf	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
		Stem	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
		Root	-	+	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-
	CH <sub>3</sub> OH	Leaf	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
		Stem	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
		Root	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

[Iso = Isolate; (+) = active; (-) = Not active]



## Chapter 4

# Discussion

Plants known as bionormalizers viz. *Carica papaya* Linn., *Moringa oleifera* Lam. and *Musa sapientum* L. were taken into consideration to determine whether or not materials of these plants affecting biodegrading bacteria that take part in normalization of industrial effluent. The Pet.E., CHCl<sub>3</sub> and CH<sub>3</sub>OH extracts of *C. papaya* (leaf, stem and root), *M. oleifera* (fruit, leaf, stem bark, stem wood, root bark and root wood) and *Mu. sapientum* (leaf, stem and root) were the test materials. The title experiments were carried out against the bacterial isolates (9 isolates found in collected tannery effluent were determined as 1. *Bacillus cereus*, 2. *Klebsiella oxytoca*, 3. *Staphylococcus aureus*, 4. *Escherichia coli* (I), 5. *Escherichia coli* (II), 6. *Citrobacter freundii*, 7. *Proteus vulgaris*, 8. *Bacillus subtilis*, 9. *Salmonella typhimurium*). A parallel supporting experiment was also set against 7 bacteria (2 Gram- positive bacteria *B. subtilis*, *St. aureus* and 5 Gram negative bacteria *E. coli*, *K. pneumoniae*, *Sa. enteritidis*, *S. flexneri* and *S. sonnei* which were available in the laboratory of IES, RU. Physico-chemical characteristics of the tannery effluent were also determined along with the characterization of the found bacterial isolates to have a complete knowledge on the effluent and its functional relationship with the bacteria to be certain whether or not they play a role in biodegradation. Besides, potentiation of the test plants were done through insecticidal activity test against the red flour beetle, *Tribolium castaneum* (Hbst.) through dose-mortality assay, and against the brine shrimp, *Artemia salina* L. nauplii through cytotoxicity test. The results were found promising in terms of causing less harm to the biodegrading bacteria, while it is popularly known that the test plants themselves take part in bionormalization (Von Maydell, 1986).

The results receive supports from the results achieved by the previous researchers, however all the isolates found in the effluent may not be the same in their attributes to response against the extractives of the test plants, *i.e.* the bionormalizer plants.

For *C. papaya* Pet.E. extract of leaf found active against *St. aureus*, CHCl<sub>3</sub> extracts of stem found active against *St. aureus* and *K. pneumoniae*; and the Pet.E., CHCl<sub>3</sub> and CH<sub>3</sub>OH extracts of roots were found active against *B. subtilis* and *St. aureus* among the 7 test bacteria (of IES laboratory) *B. subtilis*, *St. aureus*, *E. coli*, *K. pneumoniae*, *Sa. enteritidis*, *S. flexneri* and *S. sonnei*. Again, among the 9 isolates from the tannery effluent Pet.E. and CHCl<sub>3</sub> extracts of leaf showed activity against *K. oxytoca*; Pet.E. and CHCl<sub>3</sub> extracts of stem showed activity against *B. cereus*, *St. aureus*, *E. coli* (I), *Citrobacter freundii* and *B. subtilis*; Pet.E. extract of roots showed activity against *B. subtilis* only, however no activity was traced against *E. coli* (II), *P. vulgaris* and *Sa. typhimurium* at all.

Alabi *et al.* (2012) found aqueous, ethanol and acetone extracts of dried and fresh leaves of *C. papaya* not to show active against majority of almost 11 test bacteria: *E. coli* (3 strains), *K. pneumoniae*, *E. faecalis*, *S. aureus* (3 strains), *S. pyogenes*, *P. aeruginosa*, *K. oxytoca*, *P. vulgaris*. Among them only *S. aureus* was responsive at 50 and 100mg/ml of the aqueous extract of the dried leaves of *C. papaya*. The ethanol extract of the same didn't show any activity at doses 25 and 50mg/ml, and even at 100mg/ml *K. oxytoca* and 2 strains of *S. aureus* didn't response; while for the acetone extract *K. pneumoniae*, *E. faecalis*, *E. coli* and *S. aureus* were responsive at 100mg/ml concentration. For the fresh leaf extract the scenario was more disappointing in terms of strong activity, where one strain of *S. aureus* found responsive to aqueous extract at 50 and 100mg/ml, 2 strains of *E. coli* and *K. oxytoca* were responsive for ethanol extract at only 100mg/ml concentration; while for the acetone extract of the same only *S. aureus* was responsive at 100mg/ml concentration. They also carried out another test experiment on the same extracts against 6 fungi *Epidermophyton floccosum*, *Aspergillus niger*, *Aspergillus flavus*, *Trichophyton metagrophytes*, *Candida albicans* and *Aspergillus carbonarius* for both dried and fresh leaf extracts for 25, 50 and 100mg/ml concentrations, and only three of them: *A. flavus*, *T. metagrophytes* and *C. albicans* were responsive at 100mg/ml of the aqueous extract with 2, 1 and 1 mm of inhibition zones respectively. The MIC was recorded with 100mg/ml concentration of most of the extracts against the bacteria and 3 fungi (Alabi *et al.*, 2012). Anibijuwon and Udeze (2009) showed the brown colored hot water extract of *C. papaya* leaf was

found active against *P. aeruginosa* while the ethanol and the methanol extracts were not active at all. Nirosha and Mangalanayaki (2013) tested leaf and root extracts of *C. papaya* against *E. coli*, *P. aeruginosa*, *S. typhi*, *S. aureus* and *B. subtilis* extracted in water, ethanol and ethyl acetate. They got no response for the water extracts, while at concentrations 100, 150, 200 and 250mg/ml the clear zones for ethanol and ethyl acetate extracts were not so much promising. Orhue and Momoh (2013) tested *C. papaya* seed, leaf and peel extracted in water, ethanol, 1% HCL, acetone and petroleum against the bacteria *S. aureus*, *E. coli* and *P. aeruginosa*, and got no response for the water extract. Peter *et al.* (2014) found a high dose (100mg/ml DMSO) of 70% methanolic and the aqueous extracts of *C. papaya* seeds to yield 3 and 2mm inhibition zones.

For *M. oleifera* Pet.E. extract of fruit was found active against *B. subtilis* and *St. aureus* and the  $\text{CHCl}_3$  extract of the same was active against *St. aureus* and *K. pneumoniae*; the Pet.E. extract of leaf was active against *St. aureus*. While the Pet.E. and  $\text{CHCl}_3$  extracts of stem bark were active against *B. subtilis* and *St. aureus*. Pet.E.,  $\text{CHCl}_3$  and  $\text{CH}_3\text{OH}$  extracts of the stem wood were active against *St. aureus* and *K. pneumoniae*; Pet.E. and  $\text{CHCl}_3$  extracts of the root bark and the  $\text{CHCl}_3$  extract of the root wood found active only against *St. aureus*, and no activity was traced against *E. coli*, *Sa. enteritidis*, *Sh. flexneri* and *Sh. sonnei*. Moreover, all the responses were achieved for  $400\mu\text{g disc}^{-1}$  which is obviously a higher dose

Rockwood *et al.* (2013) tested leaf and seed extracts of *M. oleifera* against *B. spaericus*, *B. subtilis*, *B. megaterium*, *B. cereus*, *M. smegmatis*, *S. aureus*, *A. faecalis*, *E. aerogenes*, *E. coli*, *K. pneumoniae*, *P. mirabilis*, *P. vulgaris* and *S. flexneri* and they got only *B. spaericus* to response against the leaf extract and *B. spaericus*, *M. smegmatis*, *S. aureus*, *A. faecalis* to response against the seed extract; and it shows that *M. oleifera* leaf and seed extracts have no effect on *B. subtilis*, *B. megaterium*, *B. cereus*, *M. luteus*, *E. aerogenes*, *E. coli*, *K. pneumoniae*, *P. mirabilis*, *P. vulgaris* and *S. flexneri*. Singh (2013) revealed that aqueous, hexane, ethanol and methanol extracts of *M. oleifera* leaf gave inhibition zones against *P. aeruginosa*, *S. aureus* and *E. coli*, however there were some 7, 8, and 9mm diam. clear zones which are negligible in terms of strength of activity where the disc used was 6mm in diam. Patil and Jane (2013) carried out a test

of methanol, petroleum ether acetone and chloroform extracts of the roots of *M. oleifera* on sensitivity of otitis media pathogens *K. pneumoniae*, *S. aureus*, *P. aeruginosa* and *S. pneumoniae* and the results showed that the petroleum ether and chloroform extracts had no effect on all the test bacteria, and the methanol extract didn't inhibit *K. pneumoniae* and *S. aureus*; and the acetone extract didn't inhibit *P. aeruginosa* and *S. pneumoniae*. For *S. pneumoniae* and *E. coli* the MIC were 16mg/ml, however, high MIC may be an indication of low efficacy or that the organisms have the potential for developing resistance to the bioactive compounds (Anibijuwon and Udeze, 2009). Onsare *et al.* (2013) found that aqueous extracts of seeds and seeds kernel of *M. oleifera* were active against a number of bacteria except *S. flexeneri*, *S. typhimurium* and *E. coli*.

For *Mu. sapientum* Pet.E. extracts of stem and root showed activity against *St. aureus* both for 400µg disc<sup>-1</sup> among the 7 test bacteria, and Pet.E. extracts of leaf, stem and roots found active against *K. oxytoca* and *Citrobacter freundii*, *B. cereus* and *St. aureus*, and *B. cereus*, *K. oxytoca*, *St. aureus* and *B. subtilis* respectively; while the CHCl<sub>3</sub> extracts of the root also found active against *B. subtilis* among the 9 isolates found in the tannery effluent. However, the *Mu. sapientum* extractives didn't show any activity against the 5 isolates, *i.e.* *E. coli* (I), *E. coli* (II), *Citrobacter freundii*, *P. vulgaris* and *Sa. typhimurium*.

Hashempour (2014) tested aqueous extract of *Mu. sapientum* on *Sa. typhimurium* and *C. albicans* and found that it inhibited the growth of the bacteria but couldn't stop growth of the fungus. Rashid and Sajid (2013) tested methanolic extract of *Mu. sapientum* to yield that the LD<sub>50</sub> must be bigger than 5000mg/kg body weight on mice. Venkatesh *et al.* (2013) found MIC 4mg/ml for *E. coli* which means this plant can hardly suppress the growth of *E. coli* with usual doses. The activity of *Mu. sapientum* extractives were more less similar to those of the results found in case of *C. papaya* and *M. oleifera*.

For further potentiation of the extractives insecticidal and brine shrimp lethality tests were also carried out as part of this investigation. Plants having medicinal value indicate occurrence of biopotential components in them, since curing disease means elimination of the causal agents of those diseases, *i.e.* the pathogenic bacteria, fungi,

virus, certain protozoans, helminthes, etc. However, all the 3 test plants are well known for their medicinal uses. Papaya the "medicine tree" or "melon of health" is filled with nutrients (Jackwheeler, 2003) and have antibacterial activity (Basile *et al.*, 1999), cure sickle cell diseases (Imaga, *et al.*, 2009) and poisoning related renal disorder (Olagunju, *et al.*, 2009), and also known as an anti-helminthes (Okeniyi *et al.*, 2007). It contains antifertility properties, particularly the seeds, (Lohiya *et al.*, 1999). A complete loss of fertility has been reported in male rabbits, rats and monkeys fed an extract of papaya seeds (Glazer and Smith, 1971; Lohiya *et al.*, 1999; Pathak *et al.*, 2000). It has hypoglycemic and hypolipidemic (Banerjee *et al.*, 2006; Adeneye and Olagunju, 2009), purgative (Akah *et al.*, 1997), antifertility (Bodharkar *et al.*, 1974; Das, 1980; Udor and Kehinde, 1999; Ekanem and Okoroinkwo, 2003; Ayotunde and Ofem, 2008), antibacterial (Eneruwa, 1982), Dengue virus suppressive (Nikkon *et al.*, 2003) activities.

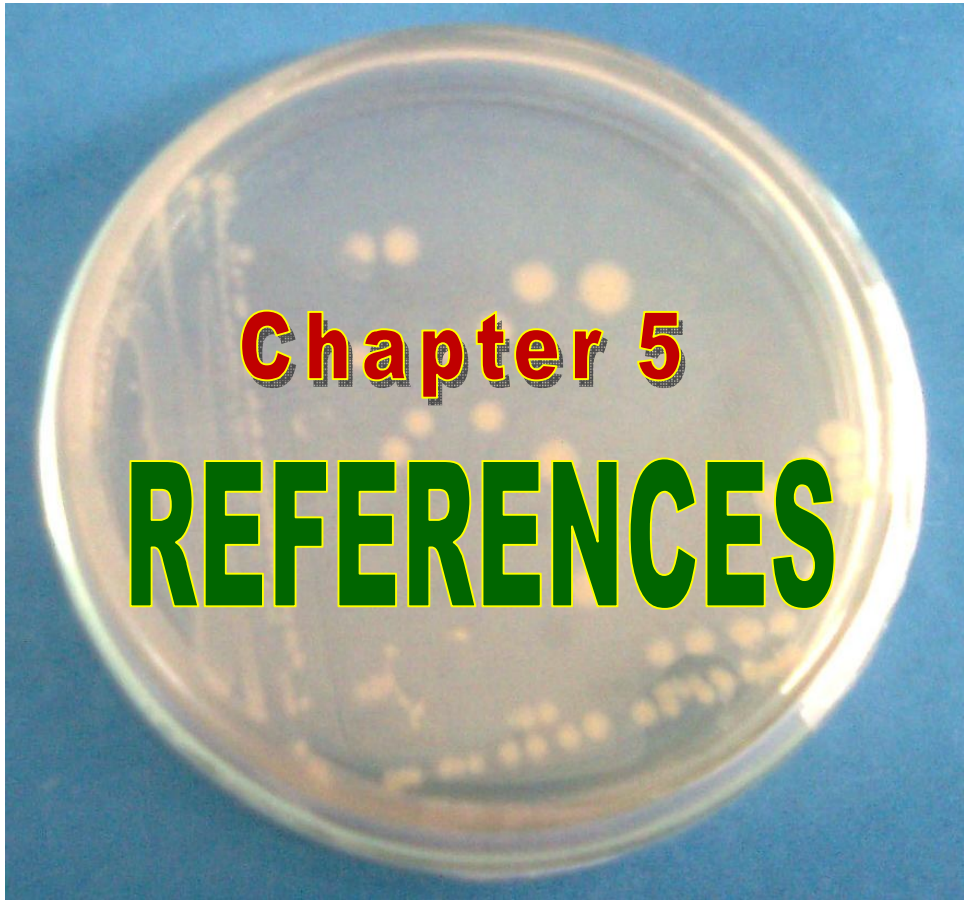
*M. oleifera* reduce the risk of degenerative diseases (Paliwal *et al.*, 2011a) and is being used for the treatment of ascites, rheumatism (Anwar *et al.*, 2007), venomous bites (Mishra *et al.*, 2009), enhancing cardiac function (Limaye *et al.*, 1995), inflammation (Ezeamuzie *et al.*, 1996), liver disease (Rao and Misra, 1998), cancer, hematological, hepatic and renal function (Mazumder *et al.*, 1999), and heals cardiovascular, gastrointestinal, hematological and hepatorenal disorders (Paliwal *et al.*, 2011a). *Moringa* has been used in the traditional medicine passed down for centuries in many cultures around the world, for skin infections, anaemia, anxiety, asthma, blackheads, blood impurities, bronchitis, catarrh, chest congestion, cholera, conjunctivitis, cough, diarrhoea, eye and ear infections, fever, glandular, swelling, headaches, abnormal blood pressure, hysteria, pain in joints, pimples, psoriasis, respiratory disorders, scurvy, semen deficiency, sore throat, sprain, tuberculosis, for intestinal worms, lactation, diabetes and pregnancy (Nikkon *et al.*, 2003). The seed is often used to purify dirty or cloudy drinking water (Von Maydell, 1986; Gassenschmidt *et al.*, 1995; Katayon *et al.*, 2005; Kebreab *et al.*, 2005). It is also reported as anti-inflammatory (Caceres *et al.*, 1992; Rao and Misra, 1998), antimicrobial (Caseres *et al.*, 1991; Dahot, 1998; Jabeen *et al.*, 2008), antibacterial (Viera *et al.*, 2010), antioxidant (Fakurazi *et al.*, 2008; Paliwal *et al.*, 2011c), anticancer, antibiotic (Kurup and Rao, 1954), cardiovascular,

antispasmodic and antipyretic (Hukkeri *et al.*, 2006), hepatoprotective (Mazumder *et al.*, 1999), anti-ulcer (Akhtar and Ahmad, 1995), diuretic, antiurolithiatic, and anthelmintic by Farooq *et al.*, 2012.

*M. sapientum* has wound healing (Agarwal *et al.*, 2009) antidiarrheal (Emery *et al.*, 1997), antidiuretic (Jain *et al.*, 2007), antibacterial and antioxidant (Mokbel and Hashinaga, 2005), Hypoglycemic (Pari and Maheswari, 1999; Ojewole and Adewunmi, 2003; Singh *et al.*, 2007; Rai *et al.*, 2009), antihyperglycemic (Pari and Maheshwari, 2000) activities.

Thus, the target output of this investigation matches the hypothesis how these plants play role as bionormalizer causing 'no' or 'less' harm to the biodegrading agents, *i.e.* the bacteria. The diam. of the inhibition zones resulted in the antibacterial activity tests against the 9 isolates found in the tannery effluent were all within the range of 8 to 16mm for the size of the disc inoculated 6mm (included). It is obvious from the antibacterial activity tests that some of the bacteria found in the isolates were not affected by the extractives of the test plants, while some were responsive depicting very poor effect on them. These mildly active small molecules or secondary metabolites may be the components of the defense mechanism of the plants for their survival. Thus, it could be assumed that the bacteria which were survived the application of extracts may play an important role in degradation of the effluent making it gradually easy for the invasion of the other bacteria, as well as welcoming in near future the ones who were inhibited. The result of this investigation along with the outcome of certain other previous investigators suggest that 'among the components of the bionormalizer plants there are bioactive compounds, and those are effective against some of the biodegrading bacteria, but of course not against certain other members of the same group; thereby ensures survival of the plants working simultaneously besides the bacteria taking part in bionormalization'.





**Chapter 5**

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## Chapter 5

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# APPENDICES

## Appendices

**Appendix Table I:** Dose-mortality effect of leaf extract (Pet.E.) of *C. papaya* against the *T. castaneum* adults after 6h of exposure

Dose	Ldos (+1)	#U	Kl	%Kill	Cr%	E Pr	Ex Pr	Wk Pro	Weght	F Pro
1.274	1.105	30	15	50.000	50	5.00	5.107	4.990	19.02	5.096
1.019	1.008	30	14	46.667	47	4.92	4.730	4.922	18.48	4.728
0.764	0.883	30	6	20.000	20	4.16	4.243	4.150	15.09	4.254

REGRESSION EQUATION:  $Y = 0.900 + 3.797X$

CHI-SQUARED IS 1.071 WITH 1 DEGREES OF FREEDOM

NO SIG HETEROGENEITY

LOG LD<sub>50</sub> IS 1.079mg cm<sup>-2</sup>

LD<sub>50</sub> IS 1.202mg cm<sup>-2</sup>

95% CONF LIMITS ARE 0.973 TO 1.485mg cm<sup>-2</sup>

**Appendix Table II:** Dose-mortality effect of leaf extract (Pet.E.) of *C. papaya* against *T. castaneum* adults after 12h of exposure

Dose	Ldos (+1)	#U	Kl	%Kill	Cr%	E Pr	Ex Pr	Wk Pro	Weght	F Pro
1.274	1.105	30	20	66.667	67	5.44	5.463	5.429	18.03	5.457
1.019	1.008	30	18	60.000	60	5.25	5.104	5.240	19.02	5.102
0.764	0.883	30	10	33.333	33	4.56	4.642	4.551	18.03	4.644
0.510	0.707	30	4	13.333	13	3.87	3.991	3.878	12.15	3.998
0.255	0.406	30	1	3.333	3	3.12	2.877	3.256	2.76	2.895

REGRESSION EQUATION:  $Y = 1.406 + 3.665X$

CHI-SQUARED IS 1.069 WITH 3 DEGREES OF FREEDOM

NO SIG HETEROGENEITY

LOG LD<sub>50</sub> IS 0.981mg cm<sup>-2</sup>

LD<sub>50</sub> IS 0.956mg cm<sup>-2</sup>

95% CONF LIMITS ARE 0.819 TO 1.116mg cm<sup>-2</sup>

**Appendix Table III:** Dose-mortality effect of leaf extract (Pet.E.) of *C. papaya* against *T. castaneum* after 24h of exposure

Dose	Ldos (+1)	#U	Kl	%Kill	Cr%	E Pr	Ex Pr	Wk Pro	Weght	F Pro
1.274	1.105	30	22	73.333	73	5.61	5.612	5.610	16.74	5.625
1.019	1.008	30	19	63.333	63	5.33	5.225	5.358	18.81	5.235
0.764	0.883	30	11	36.667	37	4.67	4.726	4.662	18.48	4.732
0.510	0.707	30	4	13.333	13	3.87	4.023	3.873	13.17	4.024
0.255	0.406	30	1	3.333	3	3.12	2.821	3.256	2.76	2.813

REGRESSION EQUATION:  $Y = 1.178 + 4.024X$

CHI-SQUARED IS 1.222 WITH 3 DEGREES OF FREEDOM

NO SIG HETEROGENEITY

LOG LD<sub>50</sub> IS 0.949mg cm<sup>-2</sup>

LD<sub>50</sub> IS 0.891mg cm<sup>-2</sup>

95% CONF LIMITS ARE 0.777 TO 1.021mg cm<sup>-2</sup>

**Appendix Table IV:** Dose-mortality effect of leaf extract (Pet.E.) of *C. papaya* against *T. castaneum* after 36h of exposure

Dose	Ldos (+1)	#U	Kl	%Kill	Cr%	E Pr	Ex Pr	Wk Pro	Weght	F Pro
1.274	1.105	30	26	86.667	87	6.13	6.087	6.087	13.17	6.084
1.019	1.008	30	23	76.667	77	5.74	5.628	5.730	16.74	5.627
0.764	0.883	30	15	50.000	50	5.00	5.036	5.000	19.11	5.037
0.510	0.707	30	5	16.667	17	4.05	4.202	4.048	15.09	4.207
0.255	0.406	30	1	3.333	3	3.12	2.776	3.379	2.28	2.786

REGRESSION EQUATION:  $Y = 0.869 + 4.719X$

CHI-SQUARED IS 1.385 WITH 3 DEGREES OF FREEDOM

NO SIG HETEROGENEITY

LOG LD<sub>50</sub> IS 0.875mg cm<sup>-2</sup>

LD<sub>50</sub> IS 0.751mg cm<sup>-2</sup>

95% CONF LIMITS ARE 0.666 TO 0.845mg cm<sup>-2</sup>

**Appendix Table V:** Dose-mortality effect of leaf extract (Pet.E.) of *C. papaya* against *T. castaneum* after 48h of exposure

Dose	Ldos (+1)	#U	Kl	%Kill	Cr%	E Pr	Ex Pr	Wk Pro	Weght	F Pro
1.274	1.105	30	28	93.333	93	6.48	6.351	6.424	10.08	6.278
1.019	1.008	30	26	86.667	87	6.13	6.001	6.087	13.17	5.931
0.764	0.883	30	22	73.333	73	5.61	5.549	5.584	17.43	5.484
0.510	0.707	30	7	23.333	23	4.26	4.913	4.315	19.02	4.855
0.255	0.406	30	6	20.000	20	4.16	3.825	4.230	11.10	3.778

REGRESSION EQUATION:  $Y = 2.326 + 3.576X$

CHI-SQUARED IS 8.516 WITH 3 DEGREES OF FREEDOM

VARIANCE HAS BEEN ADJUSTED FOR HETEROGENEITY

LOG LD<sub>50</sub> IS 0.748mg cm<sup>-2</sup>

LD<sub>50</sub> IS 0.559mg cm<sup>-2</sup>

95% CONF LIMITS ARE 0.429 TO 0.729mg cm<sup>-2</sup>



**Appendix Table VI:** Dose-mortality effect of stem extract (Pet.E.) of *C. papaya* against *T. castaneum* after 6h of exposure

Dose	Ldos (+1)	#U	Kl	%Kill	Cr%	E Pr	Ex Pr	Wk Pro	Weght	F Pro
2.038	1.309	30	24	80.000	80	5.85	5.528	5.780	17.43	5.536
1.783	1.251	30	15	50.000	50	5.00	5.204	5.020	18.81	5.211
1.529	1.184	30	15	50.000	50	5.00	4.830	5.020	18.81	4.835
1.274	1.105	30	4	13.333	13	3.87	4.388	3.946	15.96	4.391
1.019	1.008	30	4	13.333	13	3.87	3.847	3.873	11.10	3.847
0.764	0.883	30	2	6.667	7	3.52	3.149	3.724	4.62	3.146

REGRESSION EQUATION:  $Y = -1.809 + 5.609X$

CHI-SQUARED IS 7.072 WITH 4 DEGREES OF FREEDOM

NO SIG HETEROGENEITY

LOG LD<sub>50</sub> IS 1.214mg cm<sup>-2</sup>

LD<sub>50</sub> IS 1.636mg cm<sup>-2</sup>

95% CONF LIMITS ARE 1.492 TO 1.793mg cm<sup>-2</sup>

**Appendix Table VII:** Dose-mortality effect of stem extract (Pet.E.) of *C. papaya* against *T. castaneum* after 12h of exposure

Dose	Ldos (+1)	#U	Kl	%Kill	Cr%	E Pr	Ex Pr	Wk Pro	Weght	F Pro
2.038	1.309	30	27	90.000	90	6.28	6.085	6.210	13.17	6.038
1.783	1.251	30	23	76.667	77	5.74	5.784	5.734	15.96	5.741
1.529	1.184	30	20	66.667	67	5.44	5.438	5.429	18.03	5.397
1.274	1.105	30	12	40.000	40	4.75	5.028	4.750	19.11	4.991
1.019	1.008	30	9	30.000	30	4.48	4.526	4.460	17.43	4.494
0.764	0.883	30	5	16.667	17	4.05	3.879	4.077	11.10	3.854

REGRESSION EQUATION:  $Y = -0.676 + 5.128X$

CHI-SQUARED IS 2.096 WITH 4 DEGREES OF FREEDOM

NO SIG HETEROGENEITY

LOG LD<sub>50</sub> IS 1.107mg cm<sup>-2</sup>

LD<sub>50</sub> IS 1.279mg cm<sup>-2</sup>

95% CONF LIMITS ARE 1.167 TO 1.402mg cm<sup>-2</sup>

**Appendix Table VIII:** Dose-mortality effect of stem extract (Pet.E.) of *C. papaya* against *T. castaneum* after 24h of exposure

Dose	Ldos (+1)	#U	Kl	%Kill	Cr%	E Pr	Ex Pr	Wk Pro	Weght	F Pro
2.038	1.309	30	28	93.333	93	6.48	6.508	6.451	8.07	6.426
1.783	1.251	30	28	93.333	93	6.48	6.237	6.383	11.10	6.166
1.529	1.184	30	25	83.333	83	5.95	5.924	5.984	14.13	5.866
1.274	1.105	30	17	56.667	57	5.18	5.554	5.136	17.43	5.511
1.019	1.008	30	16	53.333	53	5.08	5.101	5.065	19.02	5.076
0.764	0.883	30	11	36.667	37	4.67	4.517	4.656	17.43	4.515

REGRESSION EQUATION:  $Y = 0.553 + 4.486X$

CHI-SQUARED IS 3.518 WITH 4 DEGREES OF FREEDOM

NO SIG HETEROGENEITY

LOG LD<sub>50</sub> IS 0.991mg cm<sup>-2</sup>

LD<sub>50</sub> IS 0.980mg cm<sup>-2</sup>

95% CONF LIMITS ARE 0.859 TO 1.119mg cm<sup>-2</sup>

**Appendix Table IX:** Dose-mortality effect of stem extract (Pet.E.) of *C. papaya* against *T. castaneum* after 36h of exposure

Dose	Ldos (+1)	#U	Kl	%Kill	Cr%	E Pr	Ex Pr	Wk Pro	Weght	F Pro
2.038	1.309	30	29	96.667	97	6.88	6.860	6.909	5.40	6.844
1.783	1.251	30	29	96.667	97	6.88	6.575	6.759	8.07	6.560
1.529	1.184	30	28	93.333	93	6.48	6.247	6.383	11.10	6.232
1.274	1.105	30	22	73.333	73	5.61	5.859	5.562	15.09	5.844
1.019	1.008	30	18	60.000	60	5.25	5.383	5.240	18.48	5.370
0.764	0.883	30	14	46.667	47	4.92	4.770	4.922	18.48	4.758

REGRESSION EQUATION:  $Y = 0.434 + 4.896X$

CHI-SQUARED IS 2.608 WITH 4 DEGREES OF FREEDOM

NO SIG HETEROGENEITY

LOG LD<sub>50</sub> IS 0.933mg cm<sup>-2</sup>

LD<sub>50</sub> IS 0.856mg cm<sup>-2</sup>

95% CONF LIMITS ARE 0.738 TO 0.993mg cm<sup>-2</sup>

**Appendix Table X:** Dose-mortality effect of stem extract (Pet.E.) of *C. papaya* against *T. castaneum* after 48h of exposure

Dose	Ldos (+1)	#U	Kl	%Kill	Cr%	E Pr	Ex Pr	Wk Pro	Weght	F Pro
1.529	1.184	30	28	93.333	93	6.48	6.273	6.383	11.10	6.178
1.274	1.105	30	23	76.667	77	5.74	5.953	5.756	14.13	5.890
1.019	1.008	30	20	66.667	67	5.44	5.560	5.416	17.43	5.538
0.764	0.883	30	17	56.667	57	5.18	5.054	5.175	19.11	5.084

REGRESSION EQUATION:  $Y = 1.875 + 3.634X$

CHI-SQUARED IS 1.139 WITH 2 DEGREES OF FREEDOM

NO SIG HETEROGENEITY

LOG LD<sub>50</sub> IS 0.860mg cm<sup>-2</sup>

LD<sub>50</sub> IS 0.725mg cm<sup>-2</sup>

95% CONF LIMITS ARE 0.547 TO 0.959mg cm<sup>-2</sup>

**Appendix Table XI:** Dose-mortality effect of root extract (Pet.E.) of *C. papaya* against *T. castaneum* after 6h of exposure

Dose	Ldos (+1)	#U	Kl	%Kill	Cr%	E Pr	Ex Pr	Wk Pro	Weght	F Pro
1.529	1.184	30	9	30.000	30	4.48	4.471	4.480	16.74	4.462
1.274	1.105	30	6	20.000	20	4.16	4.233	4.150	15.09	4.230
1.019	1.008	30	5	16.667	17	4.05	3.941	4.062	12.15	3.947
0.764	0.883	30	2	6.667	7	3.52	3.565	3.519	8.07	3.581

REGRESSION EQUATION:  $Y = 0.998 + 2.924X$

CHI-SQUARED IS 0.295 WITH 2 DEGREES OF FREEDOM

NO SIG HETEROGENEITY

LOG LD<sub>50</sub> IS 1.369mg cm<sup>-2</sup>

LD<sub>50</sub> IS 2.336 mg cm<sup>-2</sup>

95% CONF LIMITS ARE 1.229 TO 4.440 mg cm<sup>-2</sup>

**Appendix Table XII:** Dose-mortality effect of root extract (Pet.E.) of *C. papaya* against *T. castaneum* after 12h of exposure

Dose	Ldos (+1)	#U	Kl	%Kill	Cr%	E Pr	Ex Pr	Wk Pro	Weght	F Pro
1.529	1.184	30	17	56.667	57	5.18	5.057	5.175	19.11	5.069
1.274	1.105	30	13	43.333	43	4.82	4.889	4.838	18.81	4.895
1.019	1.008	30	11	36.667	37	4.67	4.683	4.659	18.03	4.683
0.764	0.883	30	7	23.333	23	4.26	4.418	4.270	16.74	4.409
0.510	0.707	30	6	20.000	20	4.16	4.044	4.160	13.17	4.023

REGRESSION EQUATION:  $Y = 2.473 + 2.192X$

CHI-SQUARED IS 0.857 WITH 3 DEGREES OF FREEDOM

NO SIG HETEROGENEITY

LOG LD<sub>50</sub> IS 1.153 mg cm<sup>-2</sup>

LD<sub>50</sub> IS 1.422 mg cm<sup>-2</sup>

95% CONF LIMITS ARE 1.0445 TO 1.936 mg cm<sup>-2</sup>

**Appendix Table XIII:** Dose-mortality effect of root extract (Pet.E.) of *C. papaya* against *T. castaneum* after 24h of exposure

Dose	Ldos (+1)	#U	Kl	%Kill	Cr%	E Pr	Ex Pr	Wk Pro	Weght	F Pro
1.529	1.184	30	23	76.667	77	5.74	5.673	5.730	16.74	5.676
1.274	1.105	30	22	73.333	73	5.61	5.466	5.591	18.03	5.465
1.019	1.008	30	16	53.333	53	5.08	5.212	5.098	18.81	5.206
0.764	0.883	30	12	40.000	40	4.75	4.884	4.760	18.81	4.873
0.510	0.707	30	8	26.667	27	4.39	4.422	4.390	16.74	4.403
0.255	0.406	30	3	10.000	10	3.72	3.633	3.730	9.06	3.600

REGRESSION EQUATION:  $Y = 2.516 + 2.668X$

CHI-SQUARED IS 0.952 WITH 4 DEGREES OF FREEDOM

NO SIG HETEROGENEITY

LOG LD<sub>50</sub> IS 0.931 mg cm<sup>-2</sup>

LD<sub>50</sub> IS 0.853 mg cm<sup>-2</sup>

95% CONF LIMITS ARE 0.719 TO 1.012 mg cm<sup>-2</sup>

**Appendix Table XIV:** Dose-mortality effect of root extract (Pet.E.) of *C. papaya* against *T. castaneum* after 36h of exposure

Dose	Ldos (+1)	#U	Kl	%Kill	Cr%	E Pr	Ex Pr	Wk Pro	Weght	F Pro
1.529	1.184	30	27	90.000	90	6.28	6.139	6.270	12.15	6.113
1.274	1.105	30	26	86.667	87	6.13	5.909	6.136	14.13	5.882
1.019	1.008	30	20	66.667	67	5.44	5.627	5.430	16.74	5.600
0.764	0.883	30	15	50.000	50	5.00	5.264	5.020	18.81	5.236
0.510	0.707	30	11	36.667	37	4.67	4.752	4.662	18.48	4.724
0.255	0.406	30	5	16.667	17	4.05	3.877	4.077	11.10	3.847

REGRESSION EQUATION:  $Y = 2.665 + 2.911X$

CHI-SQUARED IS 3.229 WITH 4 DEGREES OF FREEDOM

NO SIG HETEROGENEITY

LOG LD<sub>50</sub> IS 0.802 mg cm<sup>-2</sup>

LD<sub>50</sub> IS 0.634 mg cm<sup>-2</sup>

95% CONF LIMITS ARE 0.534 TO 0.753 mg cm<sup>-2</sup>

**Appendix Table XV:** Dose-mortality effect of root extract (Pet.E.) of *C. papaya* against *T. castaneum* after 48h of exposure

Dose	Ldos	#U	Kl	%Kill	Cr%	E Pr	Ex Pr	Wk Pro	Weght	F Pro
1.274	1.105	30	28	93.333	93	6.48	6.175	6.408	12.15	6.088
1.019	1.008	30	22	73.333	73	5.61	5.883	5.562	15.09	5.810
0.764	0.883	30	20	66.667	67	5.44	5.507	5.416	17.43	5.451
0.510	0.707	30	14	46.667	47	4.92	4.976	4.915	19.02	4.945
0.255	0.406	30	6	20.000	20	4.16	4.069	4.160	13.17	4.081

REGRESSION EQUATION:  $Y = 2.914 + 2.872X$

CHI-SQUARED IS 2.291 WITH 3 DEGREES OF FREEDOM

NO SIG HETEROGENEITY

LOG LD<sub>50</sub> IS 0.726 mg cm<sup>-2</sup>

LD<sub>50</sub> IS 0.532 mg cm<sup>-2</sup>

95% CONF LIMITS ARE 0.439 TO 0.646 mg cm<sup>-2</sup>

**Appendix Table XVI:** Dose-mortality effect of stem extract (CHCl<sub>3</sub>) of *C. papaya* against *T. castaneum* after 12h of exposure

Dose	Ldos	#U	Kl	%Kill	Cr%	E Pr	Ex Pr	Wk Pro	Weght	F Pro
4.076	0.610	30	14	46.667	47	4.92	4.928	4.915	19.02	4.914
3.057	0.485	30	12	40.000	40	4.75	4.760	4.740	18.48	4.750
2.038	0.309	30	9	30.000	30	4.48	4.522	4.460	17.43	4.519
1.529	0.184	30	9	30.000	30	4.48	4.354	4.490	15.96	4.355
1.019	0.008	30	5	16.667	17	4.05	4.116	4.056	14.13	4.124

REGRESSION EQUATION:  $Y = 4.114 + 1.311X$

CHI-SQUARED IS 0.418 WITH 3 DEGREES OF FREEDOM

NO SIG HETEROGENEITY

LOG LD<sub>50</sub> IS 0.676mg cm<sup>-2</sup>

LD<sub>50</sub> IS 4.745mg cm<sup>-2</sup>

95% CONF LIMITS ARE 2.348 TO 9.591mg cm<sup>-2</sup>

**Appendix Table XVII:** Dose-mortality effect of stem extract (CHCl<sub>3</sub>) of *C. papaya* against *T. castaneum* after 24h of exposure

Dose	Ldos	#U	Kl	%Kill	Cr%	E Pr	Ex Pr	Wk Pro	Weght	F Pro
4.076	0.610	30	16	53.333	53	5.08	5.060	5.075	19.11	5.049
3.057	0.485	30	13	43.333	43	4.82	4.902	4.815	19.02	4.894
2.038	0.309	30	12	40.000	40	4.75	4.681	4.740	18.03	4.675
1.529	0.184	30	10	33.333	33	4.56	4.524	4.544	17.43	4.520
1.019	0.008	30	7	23.333	23	4.26	4.303	4.266	15.96	4.301

REGRESSION EQUATION:  $Y = 4.291 + 1.243X$

CHI-SQUARED IS 0.237 WITH 3 DEGREES OF FREEDOM

NO SIG HETEROGENEITY

LOG LD<sub>50</sub> IS 0.571mg cm<sup>-2</sup>

LD<sub>50</sub> IS 3.719mg cm<sup>-2</sup>

95% CONF LIMITS ARE 2.087 TO 6.629mg cm<sup>-2</sup>

**Appendix Table XVIII:** Dose-mortality effect of stem extract (CHCl<sub>3</sub>) of *C. papaya* against *T. castaneum* after 36h of exposure

Dose	Ldos	#U	Kl	%Kill	Cr%	E Pr	Ex Pr	Wk Pro	Weght	F Pro
4.076	0.610	30	19	63.333	63	5.33	5.260	5.358	18.81	5.274
3.057	0.485	30	14	46.667	47	4.92	5.062	4.925	19.11	5.072
2.038	0.309	30	13	43.333	43	4.82	4.784	4.818	18.48	4.788
1.529	0.184	30	11	36.667	37	4.67	4.586	4.656	17.43	4.586
1.019	0.008	30	7	23.333	23	4.26	4.308	4.266	15.96	4.301

REGRESSION EQUATION:  $Y = 4.288 + 1.615X$

CHI-SQUARED IS 0.669 WITH 3 DEGREES OF FREEDOM

NO SIG HETEROGENEITY

LOG LD<sub>50</sub> IS 0.441mg cm<sup>-2</sup>

LD<sub>50</sub> IS 2.759mg cm<sup>-2</sup>

95% CONF LIMITS ARE 1.979 TO 3.846mg cm<sup>-2</sup>

**Appendix Table XIX:** Dose-mortality effect of stem extract (CHCl<sub>3</sub>) of *C. papaya* against *T. castaneum* after 48h of exposure

Dose	Ldos	#U	Kl	%Kill	Cr%	E Pr	Ex Pr	Wk Pro	Weght	F Pro
4.076	0.610	30	20	66.667	67	5.44	5.542	5.416	17.43	5.524
3.057	0.485	30	20	66.667	67	5.44	5.319	5.422	18.48	5.304
2.038	0.309	30	16	53.333	53	5.08	5.004	5.075	19.11	4.994
1.529	0.184	30	11	36.667	37	4.67	4.780	4.662	18.48	4.775
1.019	0.008	30	9	30.000	30	4.48	4.465	4.480	16.74	4.465

REGRESSION EQUATION:  $Y = 4.450 + 1.759X$

CHI-SQUARED IS 0.823 WITH 3 DEGREES OF FREEDOM

NO SIG HETEROGENEITY

LOG LD<sub>50</sub> IS 0.312mg cm<sup>-2</sup>

LD<sub>50</sub> IS 2.053mg cm<sup>-2</sup>

95% CONF LIMITS ARE 1.567 TO 2.690mg cm<sup>-2</sup>

**Appendix Table XX:** Dose-mortality effect of leaf extract (CH<sub>3</sub>OH) of *C. papaya* against *T. castaneum* after 6h of exposure

Dose	Ldos (+1)	#U	Kl	%Kill	Cr%	E Pr	Ex Pr	Wk Pro	Weght	F Pro
2.038	1.309	30	7	23.333	23	4.26	4.212	4.252	15.09	4.238
1.529	1.184	30	4	13.333	13	3.87	3.849	3.873	11.10	3.859
1.019	1.008	30	1	3.333	3	3.12	3.337	3.148	6.24	3.326
0.764	0.883	30	1	3.333	3	3.12	2.973	3.172	3.30	2.948

REGRESSION EQUATION:  $Y = 0.273 + 3.028X$

CHI-SQUARED IS 0.369 WITH 2 DEGREES OF FREEDOM

NO SIG HETEROGENEITY

LOG LD<sub>50</sub> IS 1.561mg cm<sup>-2</sup>

LD<sub>50</sub> IS 3.639mg cm<sup>-2</sup>

95% CONF LIMITS ARE 1.783 TO 7.431mg cm<sup>-2</sup>

**Appendix Table XXI:** Dose-mortality effect of leaf extract (CH<sub>3</sub>OH) of *C. papaya* against *T. castaneum* after 12h of exposure

Dose	Ldos (+1)	#U	Kl	%Kill	Cr%	E Pr	Ex Pr	Wk Pro	Weght	F Pro
2.038	1.309	30	19	63.333	63	5.33	5.305	5.318	18.48	5.289
1.529	1.184	30	18	60.000	60	5.25	4.989	5.240	19.02	4.977
1.019	1.008	30	10	33.333	33	4.56	4.543	4.544	17.43	4.536
0.764	0.883	30	3	10.000	10	3.72	4.227	3.810	15.09	4.224
0.510	0.707	30	2	6.667	7	3.52	3.782	3.546	10.08	3.783
0.255	0.406	30	1	3.333	3	3.12	3.020	3.135	3.93	3.031
0.127	0.105	30	1	3.333	3	3.12	2.258	5.669	0.75	2.278

REGRESSION EQUATION:  $Y = 2.015 + 2.501X$

CHI-SQUARED IS 13.155 WITH 5 DEGREES OF FREEDOM

VARIANCE HAS BEEN ADJUSTED FOR HETEROGENEITY

LOG LD<sub>50</sub> IS 1.194mg cm<sup>-2</sup>

LD<sub>50</sub> IS 1.562mg cm<sup>-2</sup>

95% CONF LIMITS ARE 1.062 TO 2.298mg cm<sup>-2</sup>

**Appendix Table XXII:** Dose-mortality effect of leaf extract (CH<sub>3</sub>OH) of *C. papaya* against *T. castaneum* after 24h of exposure

Dose	Ldos (+1)	#U	Kl	%Kill	Cr%	E Pr	Ex Pr	Wk Pro	Weght	F Pro
2.038	1.309	30	25	83.333	83	5.95	6.086	5.923	13.17	6.085
1.529	1.184	30	25	83.333	83	5.95	5.722	5.926	15.96	5.722
1.019	1.008	30	20	66.667	67	5.44	5.210	5.462	18.81	5.212
0.764	0.883	30	9	30.000	30	4.48	4.846	4.500	18.81	4.849
0.510	0.707	30	8	26.667	27	4.39	4.334	4.394	15.96	4.339
0.255	0.406	30	1	3.333	3	3.12	3.459	3.180	7.14	3.466
0.127	0.105	30	1	3.333	3	3.12	2.583	3.860	1.50	2.593

REGRESSION EQUATION:  $Y = 2.288 + 2.899X$

CHI-SQUARED IS 7.521 WITH 5 DEGREES OF FREEDOM

NO SIG HETEROGENEITY

LOG LD<sub>50</sub> IS 0.935mg cm<sup>-2</sup>

LD<sub>50</sub> IS 0.862mg cm<sup>-2</sup>

95% CONF LIMITS ARE 0.732 TO 1.014mg cm<sup>-2</sup>

**Appendix Table XXIII:** Dose-mortality effect of leaf extract (CH<sub>3</sub>OH) of *C. papaya* against *T. castaneum* after 36h of exposure

Dose	Ldos (+1)	#U	Kl	%Kill	Cr%	E Pr	Ex Pr	Wk Pro	Weght	F Pro
2.038	1.309	30	29	96.667	97	6.88	6.698	6.810	7.14	6.640
1.529	1.184	30	27	90.000	90	6.28	6.311	6.250	10.08	6.251
1.019	1.008	30	25	83.333	83	5.95	5.765	5.926	15.96	5.702
0.764	0.883	30	15	50.000	50	5.00	5.378	4.980	18.48	5.313
0.510	0.707	30	12	40.000	40	4.75	4.832	4.760	18.81	4.764
0.255	0.406	30	4	13.333	13	3.87	3.899	3.873	11.10	3.826
0.127	0.105	30	1	3.333	3	3.12	2.966	3.172	3.30	2.888

REGRESSION EQUATION:  $Y = 2.561 + 3.116X$

CHI-SQUARED IS 3.343 WITH 5 DEGREES OF FREEDOM

NO SIG HETEROGENEITY

LOG LD<sub>50</sub> IS 0.783mg cm<sup>-2</sup>

LD<sub>50</sub> IS 0.607mg cm<sup>-2</sup>

95% CONF LIMITS ARE 0.516 TO 0.712mg cm<sup>-2</sup>

**Appendix Table XXIV:** Dose-mortality effect of leaf extract (CH<sub>3</sub>OH) of *C. papaya* against *T. castaneum* after 48h of exposure

Dose	Ldos (+1)	#U	Kl	%Kill	Cr%	E Pr	Ex Pr	Wk Pro	Weght	F Pro
1.529	1.184	30	29	96.667	97	6.88	6.576	6.759	8.07	6.595
1.019	1.008	30	28	93.333	93	6.48	6.078	6.333	13.17	6.095
0.764	0.883	30	20	66.667	67	5.44	5.724	5.414	15.96	5.741
0.510	0.707	30	19	63.333	63	5.33	5.227	5.358	18.81	5.242
0.255	0.406	30	5	16.667	17	4.05	4.375	4.074	15.96	4.388
0.127	0.105	30	4	13.333	13	3.87	3.524	3.981	8.07	3.535

REGRESSION EQUATION:  $Y = 3.237 + 2.835X$

CHI-SQUARED IS 6.106 WITH 4 DEGREES OF FREEDOM

NO SIG HETEROGENEITY

LOG LD<sub>50</sub> IS 0.622mg cm<sup>-2</sup>

LD<sub>50</sub> IS 0.419mg cm<sup>-2</sup>

95% CONF LIMITS ARE 0.348 TO 0.504mg cm<sup>-2</sup>

**Appendix Table XXV:** Dose-mortality effect of stem extract (CH<sub>3</sub>OH) of *C. papaya* against *T. castaneum* after 6h of exposure

Dose	Ldos (+1)	#U	Kl	%Kill	Cr%	E Pr	Ex Pr	Wk Pro	Weght	F Pro
1.274	1.105	30	12	40.000	40	4.75	4.522	4.740	17.43	4.483
1.019	1.008	30	2	6.667	7	3.52	4.254	3.708	15.09	4.235
0.764	0.883	30	6	20.000	20	4.16	3.909	4.200	12.15	3.914

REGRESSION EQUATION:  $Y = 1.649 + 2.564X$

CHI-SQUARED IS 6.327 WITH 1 DEGREES OF FREEDOM

VARIANCE HAS BEEN ADJUSTED FOR HETEROGENEITY

LOG LD<sub>50</sub> IS 1.307mg cm<sup>-2</sup>

LD<sub>50</sub> IS 2.026mg cm<sup>-2</sup>

95% CONF LIMITS ARE 0.204 TO 20.079mg cm<sup>-2</sup>

**Appendix Table XXVI:** Dose-mortality effect of stem extract (CH<sub>3</sub>OH) of *C. papaya* against *T. castaneum* after 12h of exposure

Dose	Ldos (+1)	#U	Kl	%Kill	Cr%	E Pr	Ex Pr	Wk Pro	Weght	F Pro
1.274	1.105	30	23	76.667	77	5.74	5.567	5.696	17.43	5.523
1.019	1.008	30	13	43.333	43	4.82	5.159	4.815	19.02	5.132
0.764	0.883	30	13	43.333	43	4.82	4.633	4.821	18.03	4.627
0.510	0.707	30	4	13.333	13	3.87	3.892	3.873	11.10	3.916

REGRESSION EQUATION:  $Y = 1.060 + 4.038X$

CHI-SQUARED IS 3.128 WITH 2 DEGREES OF FREEDOM

NO SIG HETEROGENEITY

LOG LD<sub>50</sub> IS 0.976mg cm<sup>-2</sup>

LD<sub>50</sub> IS 0.945mg cm<sup>-2</sup>

95% CONF LIMITS ARE 0.821 TO 1.088mg cm<sup>-2</sup>

**Appendix Table XXVII:** Dose-mortality effect of stem extract (CH<sub>3</sub>OH) of *C. papaya* against *T. castaneum* after 24h of exposure

Dose	Ldos (+1)	#U	Kl	%Kill	Cr%	E Pr	Ex Pr	Wk Pro	Weght	F Pro
1.274	1.105	30	28	93.333	93	6.48	6.327	6.424	10.08	6.343
1.019	1.008	30	21	70.000	70	5.52	5.956	5.490	14.13	5.969
0.764	0.883	30	23	76.667	77	5.74	5.478	5.699	18.03	5.485
0.510	0.707	30	16	53.333	53	5.08	4.805	5.098	18.81	4.804
0.255	0.406	30	1	3.333	3	3.12	3.654	3.261	9.06	3.640

REGRESSION EQUATION:  $Y = 2.069 + 3.868X$

CHI-SQUARED IS 7.048 WITH 3 DEGREES OF FREEDOM

NO SIG HETEROGENEITY

LOG LD<sub>50</sub> IS 0.758mg cm<sup>-2</sup>

LD<sub>50</sub> IS 0.573mg cm<sup>-2</sup>

95% CONF LIMITS ARE 0.494 TO 0.664mg cm<sup>-2</sup>

**Appendix Table XXVIII:** Dose-mortality effect of stem extract (CH<sub>3</sub>OH) of *C. papaya* against *T. castaneum* after 36h of exposure

Dose	Ldos (+1)	#U	Kl	%Kill	Cr%	E Pr	Ex Pr	Wk Pro	Weght	F Pro
1.274	1.105	30	29	96.667	97	6.88	6.920	6.844	4.62	6.950
1.019	1.008	30	26	86.667	87	6.13	6.600	5.910	7.14	6.618
0.764	0.883	30	28	93.333	93	6.48	6.187	6.408	12.15	6.190
0.510	0.707	30	27	90.000	90	6.28	5.606	6.120	16.74	5.587
0.255	0.406	30	5	16.667	17	4.05	4.611	4.119	18.03	4.556
0.127	0.105	30	3	10.000	10	3.72	3.616	3.730	9.06	3.525

REGRESSION EQUATION:  $Y = 3.164 + 3.426X$

CHI-SQUARED IS 12.787 WITH 4 DEGREES OF FREEDOM

VARIANCE HAS BEEN ADJUSTED FOR HETEROGENEITY

LOG LD<sub>50</sub> IS 0.536mg cm<sup>-2</sup>

LD<sub>50</sub> IS 0.343mg cm<sup>-2</sup>

95% CONF LIMITS ARE 0.254 TO 0.464mg cm<sup>-2</sup>

**Appendix Table XXIX:** Dose-mortality effect of stem extract (CH<sub>3</sub>OH) of *C. papaya* against *T. castaneum* after 48h of exposure

Dose	Ldos (+1)	#U	Kl	%Kill	Cr%	E Pr	Ex Pr	Wk Pro	Weght	F Pro
1.274	1.105	30	29	96.667	97	6.88	6.964	6.844	4.62	6.982
1.019	1.008	30	27	90.000	90	6.28	6.679	6.180	7.14	6.694
0.764	0.883	30	28	93.333	93	6.48	6.311	6.424	10.08	6.322
0.510	0.707	30	29	96.667	97	6.88	5.794	6.374	15.96	5.799
0.255	0.406	30	9	30.000	30	4.48	4.909	4.490	19.02	4.904
0.127	0.105	30	6	20.000	20	4.16	4.024	4.160	13.17	4.009

REGRESSION EQUATION:  $Y = 3.697 + 2.973X$

CHI-SQUARED IS 10.914 WITH 4 DEGREES OF FREEDOM

VARIANCE HAS BEEN ADJUSTED FOR HETEROGENEITY

LOG LD<sub>50</sub> IS 0.438mg cm<sup>-2</sup>

LD<sub>50</sub> IS 0.274mg cm<sup>-2</sup>

95% CONF LIMITS ARE 0.197 TO 0.383mg cm<sup>-2</sup>



**Appendix Table XXX:** Dose-mortality effect of root extract (CH<sub>3</sub>OH) of *C. papaya* against *T. castaneum* after 6h of exposure

Dose	Ldos (+1)	#U	Kl	%Kill	Cr%	E Pr	Ex Pr	Wk Pro	Weght	F Pro
1.019	1.008	30	15	50.000	50	5.00	5.081	5.000	19.11	5.099
0.764	0.883	30	13	43.333	43	4.82	4.893	4.838	18.81	4.907
0.510	0.707	30	12	40.000	40	4.75	4.629	4.740	18.03	4.636
0.255	0.406	30	9	30.000	30	4.48	4.177	4.550	14.13	4.173
0.127	0.105	30	1	3.333	3	3.12	3.725	3.314	10.08	3.710

REGRESSION EQUATION:  $Y = 3.548 + 1.539X$

CHI-SQUARED IS 4.059 WITH 3 DEGREES OF FREEDOM

NO SIG HETEROGENEITY

LOG LD<sub>50</sub> IS 0.944 mg cm<sup>-2</sup>

LD<sub>50</sub> IS 0.878 mg cm<sup>-2</sup>

95% CONF LIMITS ARE 0.573 TO 1.347 mg cm<sup>-2</sup>

**Appendix Table XXXI:** Dose-mortality effect of root extract (CH<sub>3</sub>OH) of *C. papaya* against *T. castaneum* after 12h of exposure

Dose	Ldos (+1)	#U	Kl	%Kill	Cr%	E Pr	Ex Pr	Wk Pro	Weght	F Pro
1.019	1.008	30	22	73.333	73	5.61	5.923	5.604	14.13	5.909
0.764	0.883	30	24	80.000	80	5.85	5.618	5.820	16.74	5.618
0.510	0.707	30	18	60.000	60	5.25	5.188	5.240	19.02	5.209
0.255	0.406	30	11	36.667	37	4.67	4.453	4.690	16.74	4.510
0.127	0.105	30	2	6.667	7	3.52	3.717	3.546	10.08	3.811

REGRESSION EQUATION:  $Y = 3.567 + 2.323X$

CHI-SQUARED IS 3.258 WITH 3 DEGREES OF FREEDOM

NO SIG HETEROGENEITY

LOG LD<sub>50</sub> IS 0.617 mg cm<sup>-2</sup>

LD<sub>50</sub> IS 0.414 mg cm<sup>-2</sup>

95% CONF LIMITS ARE 0.331 TO 0.518 mg cm<sup>-2</sup>

**Appendix Table XXXII:** Dose-mortality effect of root extract (CH<sub>3</sub>OH) of *C. papaya* against *T. castaneum* after 24h of exposure

Dose	Ldos (+1)	#U	Kl	%Kill	Cr%	E Pr	Ex Pr	Wk Pro	Weght	F Pro
1.019	1.008	30	28	93.333	93	6.48	6.606	6.450	7.14	6.656
0.764	0.883	30	27	90.000	90	6.28	6.333	6.250	10.08	6.374
0.510	0.707	30	26	86.667	87	6.13	5.948	6.136	14.13	5.976
0.255	0.406	30	20	66.667	67	5.44	5.291	5.462	18.81	5.297
0.127	0.105	30	9	30.000	30	4.48	4.633	4.470	18.03	4.617

REGRESSION EQUATION:  $Y = 4.379 + 2.257X$

CHI-SQUARED IS 1.721 WITH 3 DEGREES OF FREEDOM

NO SIG HETEROGENEITY

LOG LD<sub>50</sub> IS 0.275 mg cm<sup>-2</sup>

LD<sub>50</sub> IS 0.188 mg cm<sup>-2</sup>

95% CONF LIMITS ARE 0.139 TO 0.256 mg cm<sup>-2</sup>

**Appendix Table XXXIII:** Dose-mortality effect of root extract (CH<sub>3</sub>OH) of *C. papaya* against *T. castaneum* after 36h of exposure

Dose	Ldos (+1)	#U	Kl	%Kill	Cr%	E Pr	Ex Pr	Wk Pro	Weght	F Pro
1.019	1.008	30	28	93.333	93	6.48	6.707	6.398	6.24	6.737
0.764	0.883	30	28	93.333	93	6.48	6.476	6.491	9.06	6.495
0.510	0.707	30	28	93.333	93	6.48	6.151	6.408	12.15	6.154
0.255	0.406	30	22	73.333	73	5.61	5.596	5.584	17.43	5.570
0.127	0.105	30	14	46.667	47	4.92	5.040	4.925	19.11	4.987

REGRESSION EQUATION:  $Y = 4.783 + 1.938X$

CHI-SQUARED IS 1.579 WITH 3 DEGREES OF FREEDOM

NO SIG HETEROGENEITY

LOG LD<sub>50</sub> IS 0.112 mg cm<sup>-2</sup>

LD<sub>50</sub> IS 0.129 mg cm<sup>-2</sup>

95% CONF LIMITS ARE 0.082 TO 0.204 mg cm<sup>-2</sup>

**Appendix Table XXXIV:** Dose-mortality effect of root extract (CH<sub>3</sub>OH) of *C. papaya* against *T. castaneum* after 48h of exposure

Dose	Ldos (+1)	#U	Kl	%Kill	Cr%	E Pr	Ex Pr	Wk Pro	Weght	F Pro
0.510	0.707	30	29	96.667	97	6.88	6.820	6.909	5.40	6.790
0.255	0.406	30	24	80.000	80	5.85	5.970	5.870	14.13	5.961
0.127	0.105	30	17	56.667	57	5.18	5.120	5.165	19.02	5.131

REGRESSION EQUATION:  $Y = 4.842 + 2.756X$

CHI-SQUARED IS 0.214 WITH 1 DEGREES OF FREEDOM

NO SIG HETEROGENEITY

LOG LD<sub>50</sub> IS 0.058 mg cm<sup>-2</sup>

LD<sub>50</sub> IS 0.114 mg cm<sup>-2</sup>

95% CONF LIMITS ARE 0.077 TO 0.169 mg cm<sup>-2</sup>

**Appendix Table XXXV:** Dose-mortality effect of stem bark extract (Pet.E) of *M. oleifera* against *T. castaneum* after 6h of exposure

Dose	Ldos (+1)	#U	Kl	%Kill	Cr%	E Pr	Ex Pr	Wk Pro	Weght	F Pro
1.529	1.184	30	12	40.000	40	4.75	4.793	4.740	18.48	4.779
1.274	1.105	30	11	36.667	37	4.67	4.645	4.659	18.03	4.635
1.019	1.008	30	9	30.000	30	4.48	4.463	4.480	16.74	4.458
0.764	0.883	30	7	23.333	23	4.26	4.229	4.252	15.09	4.231
0.510	0.707	30	4	13.333	13	3.87	3.900	3.873	11.10	3.910

REGRESSION EQUATION:  $Y = 2.622 + 1.821X$

CHI-SQUARED IS 0.069 WITH 3 DEGREES OF FREEDOM

NO SIG HETEROGENEITY

LOG LD<sub>50</sub> IS 1.306mg cm<sup>-2</sup>

LD<sub>50</sub> IS 2.022mg cm<sup>-2</sup>

95% CONF LIMITS ARE 1.113 TO 3.675mg cm<sup>-2</sup>

**Appendix Table XXXVI:** Dose-mortality effect of stem bark extract (Pet.E) of *M. oleifera* against *T. castaneum* after 12h of exposure

Dose	Ldos (+1)	#U	Kl	%Kill	Cr%	E Pr	Ex Pr	Wk Pro	Weght	F Pro
1.529	1.184	30	20	66.667	67	5.44	5.393	5.422	18.48	5.393
1.274	1.105	30	17	56.667	57	5.18	5.202	5.202	18.81	5.200
1.019	1.008	30	14	46.667	47	4.92	4.968	4.915	19.02	4.963
0.764	0.883	30	11	36.667	37	4.67	4.666	4.659	18.03	4.659
0.510	0.707	30	7	23.333	23	4.26	4.241	4.252	15.09	4.230

REGRESSION EQUATION:  $Y = 2.507 + 2.437X$

CHI-SQUARED IS 0.068 WITH 3 DEGREES OF FREEDOM

NO SIG HETEROGENEITY

LOG LD<sub>50</sub> IS 1.023mg cm<sup>-2</sup>

LD<sub>50</sub> IS 1.055mg cm<sup>-2</sup>

95% CONF LIMITS ARE 0.864 TO 1.289mg cm<sup>-2</sup>

**Appendix Table XXXVII:** Dose-mortality effect of stem bark extract (Pet.E) of *M. oleifera* against *T. castaneum* after 24h of exposure

Dose	Ldos (+1)	#U	Kl	%Kill	Cr%	E Pr	Ex Pr	Wk Pro	Weght	F Pro
1.529	1.184	30	22	73.333	73	5.61	5.623	5.610	16.74	5.609
1.274	1.105	30	20	66.667	67	5.44	5.442	5.429	18.03	5.434
1.019	1.008	30	16	53.333	53	5.08	5.221	5.098	18.81	5.219
0.764	0.883	30	15	50.000	50	5.00	4.935	4.990	19.02	4.942
0.510	0.707	30	12	40.000	40	4.75	4.533	4.740	17.43	4.552
0.255	0.406	30	3	10.000	10	3.72	3.846	3.720	11.10	3.885

REGRESSION EQUATION:  $Y = 2.985 + 2.215X$

CHI-SQUARED IS 1.237 WITH 4 DEGREES OF FREEDOM

NO SIG HETEROGENEITY

LOG LD<sub>50</sub> IS 0.909mg cm<sup>-2</sup>

LD<sub>50</sub> IS 0.812mg cm<sup>-2</sup>

95% CONF LIMITS ARE 0.663 TO 0.994mg cm<sup>-2</sup>

**Appendix Table XXXVIII:** Dose-mortality effect of stem bark extract (Pet.E) of *M. oleifera* against *T. castaneum* after 36h of exposure

Dose	Ldos (+1)	#U	Kl	%Kill	Cr%	E Pr	Ex Pr	Wk Pro	Weght	F Pro
1.529	1.184	30	25	83.333	83	5.95	6.013	5.923	13.17	5.981
1.274	1.105	30	24	80.000	80	5.85	5.803	5.800	15.09	5.780
1.019	1.008	30	21	70.000	70	5.52	5.547	5.500	17.43	5.533
0.764	0.883	30	17	56.667	57	5.18	5.217	5.202	18.81	5.216
0.510	0.707	30	14	46.667	47	4.92	4.752	4.922	18.48	4.769
0.255	0.406	30	4	13.333	13	3.87	3.957	3.878	12.15	4.004

REGRESSION EQUATION:  $Y = 2.972 + 2.540X$

CHI-SQUARED IS 0.701 WITH 4 DEGREES OF FREEDOM

NO SIG HETEROGENEITY

LOG LD<sub>50</sub> IS 0.798mg cm<sup>-2</sup>

LD<sub>50</sub> IS 0.628mg cm<sup>-2</sup>

95% CONF LIMITS ARE 0.517 TO 0.763mg cm<sup>-2</sup>

**Appendix Table XXXIX:** Dose-mortality effect of stem bark extract (Pet.E) of *M. oleifera* against *T. castaneum* after 48h of exposure

Dose	Ldos (+1)	#U	Kl	%Kill	Cr%	E Pr	Ex Pr	Wk Pro	Weght	F Pro
1.529	1.184	30	28	93.333	93	6.48	6.400	6.491	9.06	6.371
1.274	1.105	30	27	90.000	90	6.28	6.187	6.270	12.15	6.160
1.019	1.008	30	24	80.000	80	5.85	5.926	5.870	14.13	5.903
0.764	0.883	30	20	66.667	67	5.44	5.589	5.416	17.43	5.571
0.510	0.707	30	16	53.333	53	5.08	5.115	5.065	19.02	5.103
0.255	0.406	30	8	26.667	27	4.39	4.303	4.394	15.96	4.303

REGRESSION EQUATION:  $Y = 3.224 + 2.657X$

CHI-SQUARED IS 0.869 WITH 4 DEGREES OF FREEDOM

NO SIG HETEROGENEITY

LOG LD<sub>50</sub> IS 0.669mg cm<sup>-2</sup>

LD<sub>50</sub> IS 0.466mg cm<sup>-2</sup>

95% CONF LIMITS ARE 0.375 TO 0.580mg cm<sup>-2</sup>

**Appendix Table XL:** Dose-mortality effect of root bark extract (Pet.E) of *M. oleifera* against *T. castaneum* after ½h of exposure

Dose	Ldos (+1)	#U	Kl	%Kill	Cr%	E Pr	Ex Pr	Wk Pro	Weght	F Pro
1.529	1.184	30	13	43.333	43	4.82	4.836	4.838	18.81	4.830
1.274	1.105	30	11	36.667	37	4.67	4.687	4.659	18.03	4.682
1.019	1.008	30	9	30.000	30	4.48	4.504	4.460	17.43	4.502
0.764	0.883	30	8	26.667	27	4.39	4.268	4.388	15.09	4.270
0.510	0.707	30	4	13.333	13	3.87	3.936	3.878	12.15	3.943

REGRESSION EQUATION:  $Y = 2.628 + 1.859X$

CHI-SQUARED IS 0.303 WITH 3 DEGREES OF FREEDOM

NO SIG HETEROGENEITY

LOG LD<sub>50</sub> IS 1.276mg cm<sup>-2</sup>

LD<sub>50</sub> IS 1.888mg cm<sup>-2</sup>

95% CONF LIMITS ARE 1.108 TO 3.216mg cm<sup>-2</sup>

**Appendix Table XLI:** Dose-mortality effect of root bark extract (Pet.E.) of *M. oleifera* against *T. castaneum* after 6h of exposure

Dose	Ldos (+1)	#U	Kl	%Kill	Cr%	E Pr	Ex Pr	Wk Pro	Weght	F Pro
1.529	1.184	30	21	70.000	70	5.52	5.606	5.520	16.74	5.599
1.274	1.105	30	19	63.333	63	5.33	5.428	5.321	18.03	5.426
1.019	1.008	30	18	60.000	60	5.25	5.211	5.280	18.81	5.215
0.764	0.883	30	16	53.333	53	5.08	4.930	5.065	19.02	4.943
0.510	0.707	30	11	36.667	37	4.67	4.535	4.656	17.43	4.559
0.255	0.406	30	3	10.000	10	3.72	3.859	3.720	11.10	3.902

REGRESSION EQUATION:  $Y = 3.017 + 2.181X$

CHI-SQUARED IS 1.203 WITH 4 DEGREES OF FREEDOM

NO SIG HETEROGENEITY

LOG LD<sub>50</sub> IS 0.909mg cm<sup>-2</sup>

LD<sub>50</sub> IS 0.812mg cm<sup>-2</sup>

95% CONF LIMITS ARE 0.661 TO 0.998mg cm<sup>-2</sup>

**Appendix Table XLII:** Dose-mortality effect of root bark extract (Pet.E) of *M. oleifera* against *T. castaneum* after 12h of exposure

Dose	Ldos (+1)	#U	Kl	%Kill	Cr%	E Pr	Ex Pr	Wk Pro	Weght	F Pro
1.529	1.184	30	25	83.333	83	5.95	5.785	5.926	15.96	5.767
1.274	1.105	30	21	70.000	70	5.52	5.629	5.520	16.74	5.615
1.019	1.008	30	19	63.333	63	5.33	5.440	5.321	18.03	5.429
0.764	0.883	30	17	56.667	57	5.18	5.195	5.165	19.02	5.188
0.510	0.707	30	14	46.667	47	4.92	4.850	4.942	18.81	4.850
0.255	0.406	30	7	23.333	23	4.26	4.261	4.252	15.09	4.271

REGRESSION EQUATION:  $Y = 3.490 + 1.923X$

CHI-SQUARED IS 0.937 WITH 4 DEGREES OF FREEDOM

NO SIG HETEROGENEITY

LOG LD<sub>50</sub> IS 0.785mg cm<sup>-2</sup>

LD<sub>50</sub> IS 0.609mg cm<sup>-2</sup>

95% CONF LIMITS ARE 0.476 TO 0.782mg cm<sup>-2</sup>

**Appendix Table XLIII:** Dose-mortality effect of root bark extract (Pet.E) of *M. oleifera* against *T. castaneum* after 24h of exposure

Dose	Ldos (+1)	#U	Kl	%Kill	Cr%	E Pr	Ex Pr	Wk Pro	Weght	F Pro
1.529	1.184	30	26	86.667	87	6.13	5.946	6.136	14.13	5.923
1.274	1.105	30	23	76.667	77	5.74	5.783	5.734	15.96	5.763
1.019	1.008	30	20	66.667	67	5.44	5.584	5.416	17.43	5.566
0.764	0.883	30	18	60.000	60	5.25	5.327	5.240	18.48	5.313
0.510	0.707	30	15	50.000	50	5.00	4.965	4.990	19.02	4.956
0.255	0.406	30	8	26.667	27	4.39	4.345	4.394	15.96	4.346

REGRESSION EQUATION:  $Y = 3.523 + 2.027X$

CHI-SQUARED IS 1.204 WITH 4 DEGREES OF FREEDOM

NO SIG HETEROGENEITY

LOG LD<sub>50</sub> IS 0.729mg cm<sup>-2</sup>

LD<sub>50</sub> IS 0.536mg cm<sup>-2</sup>

95% CONF LIMITS ARE 0.415 TO 0.691mg cm<sup>-2</sup>

**Appendix Table XLIV:** Dose-mortality effect of root bark extract (Pet.E) of *M. oleifera* against *T. castaneum* after 36h of exposure

Dose	Ldos (+1)	#U	Kl	%Kill	Cr%	E Pr	Ex Pr	Wk Pro	Weght	F Pro
1.529	1.184	30	27	90.000	90	6.28	6.154	6.270	12.15	6.148
1.274	1.105	30	25	83.333	83	5.95	5.991	5.984	14.13	5.984
1.019	1.008	30	22	73.333	73	5.61	5.791	5.606	15.96	5.783
0.764	0.883	30	22	73.333	73	5.61	5.533	5.584	17.43	5.524
0.510	0.707	30	17	56.667	57	5.18	5.171	5.165	19.02	5.159
0.255	0.406	30	10	33.333	33	4.56	4.550	4.544	17.43	4.535

REGRESSION EQUATION:  $Y = 3.693 + 2.073X$

CHI-SQUARED IS 0.746 WITH 4 DEGREES OF FREEDOM

NO SIG HETEROGENEITY

LOG LD<sub>50</sub> IS 0.631mg cm<sup>-2</sup>

LD<sub>50</sub> IS 0.427mg cm<sup>-2</sup>

95% CONF LIMITS ARE 0.319 TO 0.572mg cm<sup>-2</sup>

**Appendix Table XLV:** Dose-mortality effect of root bark extract (Pet.E) of *M. oleifera* against *T. castaneum* after 48h of exposure

Dose	Ldos	#U	Kl	%Kill	Cr%	E Pr	Ex Pr	Wk Pro	Weght	F Pro
1.529	1.184	30	29	96.667	97	6.88	6.426	6.759	9.06	6.423
1.274	1.105	30	27	90.000	90	6.28	6.245	6.230	11.10	6.242
1.019	1.008	30	24	80.000	80	5.85	6.024	5.800	13.17	6.021
0.764	0.883	30	23	76.667	77	5.74	5.738	5.734	15.96	5.735
0.510	0.707	30	18	60.000	60	5.25	5.335	5.240	18.48	5.333
0.255	0.406	30	12	40.000	40	4.75	4.647	4.740	18.03	4.644

REGRESSION EQUATION:  $Y = 3.716 + 2.286X$

CHI-SQUARED IS 1.988 WITH 4 DEGREES OF FREEDOM

NO SIG HETEROGENEITY

LOG LD-50 IS 0.5617943

LD-50 IS 0.3645812

95% CONF LIMITS ARE 0.269 TO 0.493

**Appendix Table XLVI:** Dose-mortality effect of root wood extract (Pet.E) of *M. oleifera* against *T. castaneum* after 6h of exposure

Dose	Ldos	#U	Kl	%Kill	Cr%	E Pr	Ex Pr	Wk Pro	Weght	F Pro
1.783	0.251	30	5	16.667	17	4.05	4.085	4.037	13.17	4.071
1.529	0.184	30	4	13.333	13	3.87	3.824	3.873	11.10	3.818
1.274	0.105	30	2	6.667	7	3.52	3.515	3.519	8.07	3.519
1.019	0.008	30	1	3.333	3	3.12	3.136	3.116	4.62	3.153

REGRESSION EQUATION:  $Y = 3.122 + 3.778X$

CHI-SQUARED IS 0.055 WITH 2 DEGREES OF FREEDOM

NO SIG HETEROGENEITY

LOG LD<sub>50</sub> IS 0.497mg cm<sup>-2</sup>

LD<sub>50</sub> IS 3.142mg cm<sup>-2</sup>

95% CONF LIMITS ARE 1.387 TO 7.122mg cm<sup>-2</sup>

**Appendix Table XLVII:** Dose-mortality effect of root wood extract (Pet.E) of *M. oleifera* against *T. castaneum* after 12h of exposure

Dose	Ldos (+1)	#U	Kl	%Kill	Cr%	E Pr	Ex Pr	Wk Pro	Weght	F Pro
1.783	1.251	30	14	46.667	47	4.92	4.891	4.942	18.81	4.892
1.529	1.184	30	12	40.000	40	4.75	4.768	4.740	18.48	4.769
1.274	1.105	30	10	33.333	33	4.56	4.623	4.551	18.03	4.624
1.019	1.008	30	9	30.000	30	4.48	4.446	4.480	16.74	4.445
0.764	0.883	30	7	23.333	23	4.26	4.217	4.252	15.09	4.216
0.510	0.707	30	4	13.333	13	3.87	3.895	3.873	11.10	3.892

REGRESSION EQUATION:  $Y = 2.592 + 1.839X$

CHI-SQUARED IS 0.201 WITH 4 DEGREES OF FREEDOM

NO SIG HETEROGENEITY

LOG LD<sub>50</sub> IS 1.309mg cm<sup>-2</sup>

LD<sub>50</sub> IS 2.041mg cm<sup>-2</sup>

95% CONF LIMITS ARE 1.307 TO 3.188mg cm<sup>-2</sup>

**Appendix Table XLVIII:** Dose-mortality effect of root wood extract (Pet.E) of *M. oleifera* against *T. castaneum* after 24h of exposure

Dose	Ldos (+1)	#U	Kl	%Kill	Cr%	E Pr	Ex Pr	Wk Pro	Weght	F Pro
1.783	1.251	30	17	56.667	57	5.18	5.135	5.165	19.02	5.129
1.529	1.184	30	15	50.000	50	5.00	5.024	5.000	19.11	5.019
1.274	1.105	30	13	43.333	43	4.82	4.894	4.838	18.81	4.889
1.019	1.008	30	12	40.000	40	4.75	4.734	4.740	18.48	4.729
0.764	0.883	30	10	33.333	33	4.56	4.527	4.544	17.43	4.524
0.510	0.707	30	7	23.333	23	4.26	4.237	4.252	15.09	4.234
0.255	0.406	30	3	10.000	10	3.72	3.739	3.720	10.08	3.739

REGRESSION EQUATION:  $Y = 3.071 + 1.645X$ 

CHI-SQUARED IS 0.098 WITH 5 DEGREES OF FREEDOM

NO SIG HETEROGENEITY

LOG LD<sub>50</sub> IS 1.173mg cm<sup>-2</sup>LD<sub>50</sub> IS 1.489mg cm<sup>-2</sup>95% CONF LIMITS ARE 1.082 TO 2.049mg cm<sup>-2</sup>**Appendix Table XLIX:** Dose-mortality effect of root wood extract (Pet.E) of *M. oleifera* against *T. castaneum* after 36h of exposure

Dose	Ldos (+1)	#U	Kl	%Kill	Cr%	E Pr	Ex Pr	Wk Pro	Weght	F Pro
1.783	1.251	30	21	70.000	70	5.52	5.445	5.510	18.03	5.438
1.529	1.184	30	19	63.333	63	5.33	5.332	5.318	18.48	5.325
1.274	1.105	30	17	56.667	57	5.18	5.199	5.165	19.02	5.191
1.019	1.008	30	15	50.000	50	5.00	5.036	5.000	19.11	5.027
0.764	0.883	30	12	40.000	40	4.75	4.826	4.760	18.81	4.815
0.510	0.707	30	10	33.333	33	4.56	4.529	4.544	17.43	4.517
0.255	0.406	30	5	16.667	17	4.05	4.023	4.037	13.17	4.007

REGRESSION EQUATION:  $Y = 3.319 + 1.693X$ 

CHI-SQUARED IS 0.202 WITH 5 DEGREES OF FREEDOM

NO SIG HETEROGENEITY

LOG LD<sub>50</sub> IS 0.993mg cm<sup>-2</sup>LD<sub>50</sub> IS 0.983mg cm<sup>-2</sup>95% CONF LIMITS ARE 0.772 TO 1.251mg cm<sup>-2</sup>**Appendix Table L:** Dose-mortality effect of root wood extract (Pet.E) of *M. oleifera* against *T. castaneum* after 48h of exposure

Dose	Ldos (+1)	#U	Kl	%Kill	Cr%	E Pr	Ex Pr	Wk Pro	Weght	F Pro
1.783	1.251	30	26	86.667	87	6.13	5.902	6.136	14.13	5.887
1.529	1.184	30	23	76.667	77	5.74	5.771	5.734	15.96	5.756
1.274	1.105	30	21	70.000	70	5.52	5.615	5.520	16.74	5.601
1.019	1.008	30	20	66.667	67	5.44	5.425	5.429	18.03	5.411
0.764	0.883	30	15	50.000	50	5.00	5.180	4.990	19.02	5.166
0.510	0.707	30	12	40.000	40	4.75	4.834	4.760	18.81	4.821
0.255	0.406	30	8	26.667	27	4.39	4.243	4.388	15.09	4.231

REGRESSION EQUATION:  $Y = 3.435 + 1.959X$ 

CHI-SQUARED IS 2.029 WITH 5 DEGREES OF FREEDOM

NO SIG HETEROGENEITY

LOG LD<sub>50</sub> IS 0.799mg cm<sup>-2</sup>LD<sub>50</sub> IS 0.629mg cm<sup>-2</sup>95% CONF LIMITS ARE 0.496 TO 0.797mg cm<sup>-2</sup>

**Appendix Table LI:** Dose-mortality effect of fruit extract (CH<sub>3</sub>OH) of *M. oleifera* against *T. castaneum* after ½h of exposure

Dose	Ldos (+1)	#U	Kl	%Kill	Cr%	E Pr	Ex Pr	Wk Pro	Weght	F Pro
2.038	1.309	30	11	36.667	37	4.67	4.621	4.659	18.03	4.598
1.529	1.184	30	5	16.667	17	4.05	4.214	4.048	15.09	4.215
1.019	1.008	30	4	13.333	13	3.87	3.641	3.931	9.06	3.674
0.764	0.883	30	1	3.333	3	3.12	3.234	3.121	5.40	3.290

REGRESSION EQUATION:  $Y = 0.576 + 3.072X$

CHI-SQUARED IS 1.239 WITH 2 DEGREES OF FREEDOM

NO SIG HETEROGENEITY

LOG LD<sub>50</sub> IS 1.439mg cm<sup>-2</sup>

LD<sub>50</sub> IS 2.754mg cm<sup>-2</sup>

95% CONF LIMITS ARE 1.752 TO 4.329mg cm<sup>-2</sup>

**Appendix Table LII:** Dose-mortality effect of fruit extract (CH<sub>3</sub>OH) of *M. oleifera* against *T. castaneum* after 6h of exposure

Dose	Ldos (+1)	#U	Kl	%Kill	Cr%	E Pr	Ex Pr	Wk Pro	Weght	F Pro
2.038	1.309	30	24	80.000	80	5.85	5.731	5.830	15.96	5.706
1.529	1.184	30	20	66.667	67	5.44	5.492	5.429	18.03	5.471
1.019	1.008	30	15	50.000	50	5.00	5.156	4.990	19.02	5.140
0.764	0.883	30	14	46.667	47	4.92	4.918	4.915	19.02	4.905
0.510	0.707	30	11	36.667	37	4.67	4.582	4.656	17.43	4.573

REGRESSION EQUATION:  $Y = 3.242 + 1.883X$

CHI-SQUARED IS 0.825 WITH 3 DEGREES OF FREEDOM

NO SIG HETEROGENEITY

LOG LD<sub>50</sub> IS 0.934mg cm<sup>-2</sup>

LD<sub>50</sub> IS 0.859mg cm<sup>-2</sup>

95% CONF LIMITS ARE 0.655 TO 1.126mg cm<sup>-2</sup>

**Appendix Table LIII:** Dose-mortality effect of fruit extract (CH<sub>3</sub>OH) of *M. oleifera* against *T. castaneum* after 12h of exposure

Dose	Ldos (+1)	#U	Kl	%Kill	Cr%	E Pr	Ex Pr	Wk Pro	Weght	F Pro
2.038	1.309	30	26	86.667	87	6.13	6.046	6.087	13.17	5.986
1.529	1.184	30	24	80.000	80	5.85	5.795	5.830	15.96	5.752
1.019	1.008	30	18	60.000	60	5.25	5.441	5.240	18.03	5.422
0.764	0.883	30	16	53.333	53	5.08	5.191	5.065	19.02	5.188
0.510	0.707	30	15	50.000	50	5.00	4.837	5.020	18.81	4.858

REGRESSION EQUATION:  $Y = 3.534 + 1.872X$

CHI-SQUARED IS 1.609 WITH 3 DEGREES OF FREEDOM

NO SIG HETEROGENEITY

LOG LD<sub>50</sub> IS 0.783mg cm<sup>-2</sup>

LD<sub>50</sub> IS 0.607mg cm<sup>-2</sup>

95% CONF LIMITS ARE 0.418 TO 0.879mg cm<sup>-2</sup>



**Appendix Table LIV:** Dose-mortality effect of fruit extract (CH<sub>3</sub>OH) of *M. oleifera* against *T. castaneum* after 24h of exposure

Dose	Ldos (+1)	#U	Kl	%Kill	Cr%	E Pr	Ex Pr	Wk Pro	Weght	F Pro
2.038	1.309	30	27	90.000	90	6.28	6.156	6.270	12.15	6.144
1.529	1.184	30	24	80.000	80	5.85	5.940	5.870	14.13	5.931
1.019	1.008	30	21	70.000	70	5.52	5.636	5.520	16.74	5.630
0.764	0.883	30	20	66.667	67	5.44	5.421	5.429	18.03	5.416
0.510	0.707	30	17	56.667	57	5.18	5.117	5.165	19.02	5.116

REGRESSION EQUATION:  $Y = 3.908 + 1.708X$

CHI-SQUARED IS 0.496 WITH 3 DEGREES OF FREEDOM

NO SIG HETEROGENEITY

LOG LD<sub>50</sub> IS 0.639mg cm<sup>-2</sup>

LD<sub>50</sub> IS 0.436mg cm<sup>-2</sup>

95% CONF LIMITS ARE 0.246 TO 0.772mg cm<sup>-2</sup>

**Appendix Table LV:** Dose-mortality effect of fruit extract (CH<sub>3</sub>OH) of *M. oleifera* against *T. castaneum* after 36h of exposure

Dose	Ldos (+1)	#U	Kl	%Kill	Cr%	E Pr	Ex Pr	Wk Pro	Weght	F Pro
2.038	1.309	30	28	93.333	93	6.48	6.298	6.383	11.10	6.199
1.529	1.184	30	25	83.333	83	5.95	6.053	5.923	13.17	5.981
1.019	1.008	30	21	70.000	70	5.52	5.708	5.510	15.96	5.673
0.764	0.883	30	20	66.667	67	5.44	5.463	5.429	18.03	5.454
0.510	0.707	30	18	60.000	60	5.25	5.118	5.240	19.02	5.146

REGRESSION EQUATION:  $Y = 3.909 + 1.749X$

CHI-SQUARED IS 1.019 WITH 3 DEGREES OF FREEDOM

NO SIG HETEROGENEITY

LOG LD<sub>50</sub> IS 0.624mg cm<sup>-2</sup>

LD<sub>50</sub> IS 0.420mg cm<sup>-2</sup>

95% CONF LIMITS ARE 0.235 TO 0.751mg cm<sup>-2</sup>

**Appendix Table LVI:** Dose-mortality effect of fruit extract (CH<sub>3</sub>OH) of *M. oleifera* against *T. castaneum* after 48h of exposure

Dose	Ldos	#U	Kl	%Kill	Cr%	E Pr	Ex Pr	Wk Pro	Weght	F Pro
2.038	1.309	30	28	93.333	93	6.48	6.418	6.491	9.06	6.385
1.529	1.184	30	26	86.667	87	6.13	6.170	6.132	12.15	6.147
1.019	1.008	30	23	76.667	77	5.74	5.822	5.698	15.09	5.812
0.764	0.883	30	22	73.333	73	5.61	5.574	5.584	17.43	5.574
0.510	0.707	30	18	60.000	60	5.25	5.226	5.280	18.81	5.239

REGRESSION EQUATION:  $Y = 3.892 + 1.905X$

CHI-SQUARED IS .334 WITH 3 DEGREES OF FREEDOM

NO SIG HETEROGENEITY

LOG LD<sub>50</sub> IS 0.582mg cm<sup>-2</sup>

LD<sub>50</sub> IS 0.382mg cm<sup>-2</sup>

95% CONF LIMITS ARE 0.211 TO 0.690mg cm<sup>-2</sup>

**Appendix Table LVII:** Dose-mortality effect of stem bark extract (CH<sub>3</sub>OH) of *M. oleifera* against *T. castaneum* after 6h of exposure

Dose	Ldos (+1)	#U	Kl	%Kill	Cr%	E Pr	Ex Pr	Wk Pro	Weght	F Pro
1.274	1.105	30	13	43.333	43	4.82	4.916	4.815	19.02	4.892
1.019	1.008	30	11	36.667	37	4.67	4.592	4.656	17.43	4.587
0.764	0.883	30	7	23.333	23	4.26	4.175	4.284	14.13	4.195
0.510	0.707	30	2	6.667	7	3.52	3.586	3.519	8.07	3.642

REGRESSION EQUATION:  $Y = 1.421 + 3.141X$

CHI-SQUARED IS 0.428 WITH 2 DEGREES OF FREEDOM

NO SIG HETEROGENEITY

LOG LD<sub>50</sub> IS 1.139mg cm<sup>-2</sup>

LD<sub>50</sub> IS 1.379mg cm<sup>-2</sup>

95% CONF LIMITS ARE 1.015 TO 1.873mg cm<sup>-2</sup>

**Appendix Table LVIII:** Dose-mortality effect of stem bark extract (CH<sub>3</sub>OH) of *M. oleifera* against *T. castaneum* after 12h of exposure

Dose	Ldos (+1)	#U	Kl	%Kill	Cr%	E Pr	Ex Pr	Wk Pro	Weght	F Pro
1.274	1.105	30	24	80.000	80	5.85	5.835	5.800	15.09	5.812
1.019	1.008	30	19	63.333	63	5.33	5.456	5.321	18.03	5.436
0.764	0.883	30	17	56.667	57	5.18	4.968	5.165	19.02	4.953
0.510	0.707	30	6	20.000	20	4.16	4.279	4.150	15.09	4.271
0.255	0.406	30	1	3.333	3	3.12	3.102	3.116	4.62	3.106

REGRESSION EQUATION:  $Y = 1.533 + 3.872X$

CHI-SQUARED IS 1.321 WITH 3 DEGREES OF FREEDOM

NO SIG HETEROGENEITY

LOG LD<sub>50</sub> IS 0.896mg cm<sup>-2</sup>

LD<sub>50</sub> IS 0.786mg cm<sup>-2</sup>

95% CONF LIMITS ARE 0.685 TO 0.902mg cm<sup>-2</sup>

**Appendix Table LIX:** Dose-mortality effect of stem bark extract (CH<sub>3</sub>OH) of *M. oleifera* against *T. castaneum* after 24h of exposure

Dose	Ldos (+1)	#U	Kl	%Kill	Cr%	E Pr	Ex Pr	Wk Pro	Weght	F Pro
1.274	1.105	30	27	90.000	90	6.28	6.430	6.290	9.06	6.454
1.019	1.008	30	25	83.333	83	5.95	6.070	5.923	13.17	6.092
0.764	0.883	30	25	83.333	83	5.95	5.607	5.910	16.74	5.626
0.510	0.707	30	15	50.000	50	5.00	4.954	4.990	19.02	4.970
0.255	0.406	30	3	10.000	10	3.72	3.838	3.720	11.10	3.847

REGRESSION EQUATION:  $Y = 2.333 + 3.729X$

CHI-SQUARED IS 2.155 WITH 3 DEGREES OF FREEDOM

NO SIG HETEROGENEITY

LOG LD<sub>50</sub> IS 0.715mg cm<sup>-2</sup>

LD<sub>50</sub> IS 0.519mg cm<sup>-2</sup>

95% CONF LIMITS ARE 0.443 TO 0.608mg cm<sup>-2</sup>

**Appendix Table LX:** Dose-mortality effect of stem bark extract (CH<sub>3</sub>OH) of *M. oleifera* against *T. castaneum* after 36h of exposure

Dose	Ldos (+1)	#U	Kl	%Kill	Cr%	E Pr	Ex Pr	Wk Pro	Weght	F Pro
1.274	1.105	30	29	96.667	97	6.88	7.075	6.845	3.93	7.048
1.019	1.008	30	28	93.333	93	6.48	6.695	6.450	7.14	6.660
0.764	0.883	30	29	96.667	97	6.88	6.206	6.587	11.10	6.160
0.510	0.707	30	19	63.333	63	5.33	5.517	5.304	17.43	5.456
0.255	0.406	30	7	23.333	23	4.26	4.338	4.266	15.96	4.252

REGRESSION EQUATION:  $Y = 2.628 + 3.999X$

CHI-SQUARED IS 2.905 WITH 3 DEGREES OF FREEDOM

NO SIG HETEROGENEITY

LOG LD<sub>50</sub> IS 0.593mg cm<sup>-2</sup>

LD<sub>50</sub> IS 0.392mg cm<sup>-2</sup>

95% CONF LIMITS ARE 0.329 TO 0.466mg cm<sup>-2</sup>

**Appendix Table LXI:** Dose-mortality effect of stem bark extract (CH<sub>3</sub>OH) of *M. oleifera* against *T. castaneum* after 48h of exposure

Dose	Ldos (+1)	#U	Kl	%Kill	Cr%	E Pr	Ex Pr	Wk Pro	Weght	F Pro
0.510	0.707	30	23	76.667	77	5.74	5.695	5.730	16.74	5.684
0.255	0.406	30	11	36.667	37	4.67	4.760	4.662	18.48	4.744
0.127	0.105	30	4	13.333	13	3.87	3.825	3.873	11.10	3.804

REGRESSION EQUATION:  $Y = 3.476 + 3.123X$

CHI-SQUARED IS 0.213 WITH 1 DEGREES OF FREEDOM

NO SIG HETEROGENEITY

LOG LD<sub>50</sub> IS 0.488mg cm<sup>-2</sup>

LD<sub>50</sub> IS 0.308mg cm<sup>-2</sup>

95% CONF LIMITS ARE 0.248 TO 0.382mg cm<sup>-2</sup>

**Appendix Table LXII:** Dose-mortality effect of stem wood extract (CH<sub>3</sub>OH) of *M. oleifera* against *T. castaneum* after ½h of exposure

Dose	Ldos (+1)	#U	Kl	%Kill	Cr%	E Pr	Ex Pr	Wk Pro	Weght	F Pro
1.529	1.184	30	5	16.667	17	4.05	4.080	4.037	13.17	4.077
1.274	1.105	30	5	16.667	17	4.05	3.931	4.062	12.15	3.936
1.019	1.008	30	2	6.667	7	3.52	3.749	3.546	10.08	3.763
0.764	0.883	30	3	10.000	10	3.72	3.515	3.750	8.07	3.541
0.510	0.707	30	1	3.333	3	3.12	3.184	3.116	4.62	3.227

REGRESSION EQUATION:  $Y = 1.968 + 1.781X$

CHI-SQUARED IS 1.101 WITH 3 DEGREES OF FREEDOM

NO SIG HETEROGENEITY

LOG LD<sub>50</sub> IS 1.703mg cm<sup>-2</sup>

LD<sub>50</sub> IS 5.044mg cm<sup>-2</sup>

95% CONF LIMITS ARE 0.915 TO 27.816mg cm<sup>-2</sup>

**Appendix Table LXIII:** Dose-mortality effect of stem wood extract (CH<sub>3</sub>OH) of *M. oleifera* against *T. castaneum* after 6h of exposure

Dose	Ldos (+1)	#U	Kl	%Kill	Cr%	E Pr	Ex Pr	Wk Pro	Weght	F Pro
1.529	1.184	30	14	46.667	47	4.92	4.945	4.915	19.02	4.971
1.274	1.105	30	12	40.000	40	4.75	4.746	4.740	18.48	4.762
1.019	1.008	30	12	40.000	40	4.75	4.502	4.740	17.43	4.507
0.764	0.883	30	5	16.667	17	4.05	4.187	4.056	14.13	4.178
0.510	0.707	30	2	6.667	7	3.52	3.744	3.546	10.08	3.714
0.255	0.406	30	1	3.333	3	3.12	2.986	3.172	3.30	2.921

REGRESSION EQUATION:  $Y = 1.851 + 2.634X$

CHI-SQUARED IS 1.718 WITH 4 DEGREES OF FREEDOM

NO SIG HETEROGENEITY

LOG LD<sub>50</sub> IS 1.195mg cm<sup>-2</sup>

LD<sub>50</sub> IS 1.568mg cm<sup>-2</sup>

95% CONF LIMITS ARE 1.189 TO 2.069mg cm<sup>-2</sup>

**Appendix Table LXIV:** Dose-mortality effect of stem wood extract (CH<sub>3</sub>OH) of *M. oleifera* against *T. castaneum* after 12h of exposure

Dose	Ldos (+1)	#U	Kl	%Kill	Cr%	E Pr	Ex Pr	Wk Pro	Weght	F Pro
1.529	1.184	30	14	46.667	47	4.92	5.116	4.915	19.02	5.155
1.274	1.105	30	16	53.333	53	5.08	4.899	5.098	18.81	4.927
1.019	1.008	30	14	46.667	47	4.92	4.632	4.929	18.03	4.649
0.764	0.883	30	6	20.000	20	4.16	4.289	4.150	15.09	4.290
0.510	0.707	30	2	6.667	7	3.52	3.806	3.567	11.10	3.785
0.255	0.406	30	1	3.333	3	3.12	2.979	3.172	3.30	2.920

REGRESSION EQUATION:  $Y = 1.754 + 2.871X$

CHI-SQUARED IS 4.086 WITH 4 DEGREES OF FREEDOM

NO SIG HETEROGENEITY

LOG LD<sub>50</sub> IS 1.131mg cm<sup>-2</sup>

LD<sub>50</sub> IS 1.350mg cm<sup>-2</sup>

95% CONF LIMITS ARE 1.091 TO 1.672mg cm<sup>-2</sup>

**Appendix Table LXV:** Dose-mortality effect of stem wood extract (CH<sub>3</sub>OH) of *M. oleifera* against *T. castaneum* after 24h of exposure

Dose	Ldos (+1)	#U	Kl	%Kill	Cr%	E Pr	Ex Pr	Wk Pro	Weght	F Pro
1.529	1.184	30	21	70.000	70	5.52	5.459	5.510	18.03	5.499
1.274	1.105	30	20	66.667	67	5.44	5.256	5.462	18.81	5.282
1.019	1.008	30	15	50.000	50	5.00	5.008	5.000	19.11	5.016
0.764	0.883	30	10	33.333	33	4.56	4.687	4.551	18.03	4.674
0.510	0.707	30	4	13.333	13	3.87	4.236	3.912	15.09	4.191
0.255	0.406	30	3	10.000	10	3.72	3.463	3.810	7.14	3.366

REGRESSION EQUATION:  $Y = 2.253 + 2.741X$

CHI-SQUARED IS 3.477 WITH 4 DEGREES OF FREEDOM

NO SIG HETEROGENEITY

LOG LD<sub>50</sub> IS 1.002mg cm<sup>-2</sup>

LD<sub>50</sub> IS 1.005mg cm<sup>-2</sup>

95% CONF LIMITS ARE 0.845 TO 1.196mg cm<sup>-2</sup>

**Appendix Table LXVI:** Dose-mortality effect of stem wood extract (CH<sub>3</sub>OH) of *M. oleifera* against *T. castaneum* after 36h of exposure

Dose	Ldos (+1)	#U	Kl	%Kill	Cr%	E Pr	Ex Pr	Wk Pro	Weght	F Pro
1.529	1.184	30	26	86.667	87	6.13	6.137	6.132	12.15	6.107
1.274	1.105	30	24	80.000	80	5.85	5.884	5.800	15.09	5.853
1.019	1.008	30	24	80.000	80	5.85	5.574	5.780	17.43	5.542
0.764	0.883	30	14	46.667	47	4.92	5.174	4.915	19.02	5.141
0.510	0.707	30	10	33.333	33	4.56	4.612	4.551	18.03	4.576
0.255	0.406	30	3	10.000	10	3.72	3.649	3.730	9.06	3.611

REGRESSION EQUATION:  $Y = 2.308 + 3.208X$

CHI-SQUARED IS 2.150 WITH 4 DEGREES OF FREEDOM

NO SIG HETEROGENEITY

LOG LD<sub>50</sub> IS 0.839mg cm<sup>-2</sup>

LD<sub>50</sub> IS 0.691mg cm<sup>-2</sup>

95% CONF LIMITS ARE 0.593 TO 0.805mg cm<sup>-2</sup>

**Appendix Table LXVII:** Dose-mortality effect of stem wood extract (CH<sub>3</sub>OH) of *M. oleifera* against *T. castaneum* after 48h of exposure

Dose	Ldos	#U	Kl	%Kill	Cr%	E Pr	Ex Pr	Wk Pro	Weght	F Pro
1.529	1.184	30	28	93.333	93	6.48	6.601	6.450	7.14	6.564
1.274	1.105	30	28	93.333	93	6.48	6.325	6.424	10.08	6.293
1.019	1.008	30	26	86.667	87	6.13	5.987	6.136	14.13	5.960
0.764	0.883	30	18	60.000	60	5.25	5.551	5.220	17.43	5.531
0.510	0.707	30	16	53.333	53	5.08	4.938	5.065	19.02	4.926
0.255	0.406	30	4	13.333	13	3.87	3.889	3.873	11.10	3.893

REGRESSION EQUATION:  $Y = 2.498 + 3.433X$

CHI-SQUARED IS 2.760 WITH 4 DEGREES OF FREEDOM

NO SIG HETEROGENEITY

LOG LD<sub>50</sub> IS 0.729mg cm<sup>-2</sup>

LD<sub>50</sub> IS 0.535mg cm<sup>-2</sup>

95% CONF LIMITS ARE 0.453 TO 0.633mg cm<sup>-2</sup>

**Appendix Table LXVIII:** Dose-mortality effect of root bark extract (CH<sub>3</sub>OH) of *M. oleifera* against *T. castaneum* after 6h of exposure

Dose	Ldos (+1)	#U	Kl	%Kill	Cr%	E Pr	Ex Pr	Wk Pro	Weght	F Pro
1.274	1.105	30	20	66.667	67	5.44	5.397	5.422	18.48	5.408
1.019	1.008	30	19	63.333	63	5.33	5.226	5.358	18.81	5.233
0.764	0.883	30	15	50.000	50	5.00	5.006	5.000	19.11	5.007
0.510	0.707	30	9	30.000	30	4.48	4.697	4.470	18.03	4.690
0.255	0.406	30	6	20.000	20	4.16	4.167	4.170	14.13	4.147
0.127	0.105	30	3	10.000	10	3.72	3.638	3.730	9.06	3.603

REGRESSION EQUATION:  $Y = 3.414 + 1.804X$

CHI-SQUARED IS 1.323 WITH 4 DEGREES OF FREEDOM

NO SIG HETEROGENEITY

LOG LD<sub>50</sub> IS 0.879mg cm<sup>-2</sup>

LD<sub>50</sub> IS 0.757mg cm<sup>-2</sup>

95% CONF LIMITS ARE 0.579 TO 0.989mg cm<sup>-2</sup>

**Appendix Table LXIX:** Dose-mortality effect of root bark extract (CH<sub>3</sub>OH) of *M. oleifera* against *T. castaneum* after 12h of exposure

Dose	Ldos (+1)	#U	Kl	%Kill	Cr%	E Pr	Ex Pr	Wk Pro	Weght	F Pro
1.274	1.105	30	23	76.667	77	5.74	5.702	5.734	15.96	5.683
1.019	1.008	30	22	73.333	73	5.61	5.515	5.584	17.43	5.501
0.764	0.883	30	17	56.667	57	5.18	5.275	5.202	18.81	5.266
0.510	0.707	30	12	40.000	40	4.75	4.936	4.740	19.02	4.935
0.255	0.406	30	10	33.333	33	4.56	4.356	4.586	15.96	4.368
0.127	0.105	30	3	10.000	10	3.72	3.777	3.720	10.08	3.802

REGRESSION EQUATION:  $Y = 3.604 + 1.881X$

CHI-SQUARED IS 1.783 WITH 4 DEGREES OF FREEDOM

NO SIG HETEROGENEITY

LOG LD<sub>50</sub> IS 0.742mg cm<sup>-2</sup>

LD<sub>50</sub> IS 0.552mg cm<sup>-2</sup>

95% CONF LIMITS ARE 0.433 TO 0.704mg cm<sup>-2</sup>

**Appendix Tale LXX:** Dose-mortality effect of root bark extract (CH<sub>3</sub>OH) of *M. oleifera* against *T. castaneum* after 24h of exposure

Dose	Ldos (+1)	#U	Kl	%Kill	Cr%	E Pr	Ex Pr	Wk Pro	Weght	F Pro
1.274	1.105	30	26	86.667	87	6.13	6.027	6.087	13.17	5.982
1.019	1.008	30	24	80.000	80	5.85	5.832	5.800	15.09	5.792
0.764	0.883	30	21	70.000	70	5.52	5.580	5.500	17.43	5.546
0.510	0.707	30	16	53.333	53	5.08	5.226	5.098	18.81	5.200
0.255	0.406	30	11	36.667	37	4.67	4.620	4.659	18.03	4.608
0.127	0.105	30	5	16.667	17	4.05	4.015	4.037	13.17	4.016

REGRESSION EQUATION:  $Y = 3.809 + 1.967X$

CHI-SQUARED IS 0.429 WITH 4 DEGREES OF FREEDOM

NO SIG HETEROGENEITY

LOG LD<sub>50</sub> IS 0.606mg cm<sup>-2</sup>

LD<sub>50</sub> IS 0.403mg cm<sup>-2</sup>

95% CONF LIMITS ARE 0.316 TO 0.515mg cm<sup>-2</sup>

**Appendix Table LXXI:** Dose-mortality effect of root bark extract (CH<sub>3</sub>OH) of *M. oleifera* against *T. castaneum* after 36h of exposure

Dose	Ldos (+1)	#U	Kl	%Kill	Cr%	E Pr	Ex Pr	Wk Pro	Weght	F Pro
1.274	1.105	30	27	90.000	90	6.28	6.263	6.230	11.10	6.225
1.019	1.008	30	25	83.333	83	5.95	6.063	5.923	13.17	6.030
0.764	0.883	30	24	80.000	80	5.85	5.804	5.800	15.09	5.780
0.510	0.707	30	21	70.000	70	5.52	5.440	5.510	18.03	5.427
0.255	0.406	30	13	43.333	43	4.82	4.817	4.838	18.81	4.824
0.127	0.105	30	6	20.000	20	4.16	4.194	4.170	14.13	4.220

REGRESSION EQUATION:  $Y = 4.009 + 2.004X$

CHI-SQUARED IS 0.322 WITH 4 DEGREES OF FREEDOM

NO SIG HETEROGENEITY

LOG LD<sub>50</sub> IS 0.494mg cm<sup>-2</sup>

LD<sub>50</sub> IS 0.312mg cm<sup>-2</sup>

95% CONF LIMITS ARE 0.239 TO 0.408mg cm<sup>-2</sup>

**Appendix Table LXXII:** Dose-mortality effect of root bark extract (CH<sub>3</sub>OH) of *M. oleifera* against *T. castaneum* after 48h of exposure

Dose	Ldos (+1)	#U	Kl	%Kill	Cr%	E Pr	Ex Pr	Wk Pro	Weght	F Pro
1.274	1.105	30	29	96.667	97	6.88	6.671	6.810	7.14	6.627
1.019	1.008	30	27	90.000	90	6.28	6.426	6.290	9.06	6.390
0.764	0.883	30	25	83.333	83	5.95	6.111	5.948	12.15	6.083
0.510	0.707	30	23	76.667	77	5.74	5.667	5.730	16.74	5.652
0.255	0.406	30	14	46.667	47	4.92	4.907	4.915	19.02	4.914
0.127	0.105	30	6	20.000	20	4.16	4.148	4.170	14.13	4.176

REGRESSION EQUATION:  $Y = 3.918 + 2.452X$

CHI-SQUARED IS 0.654 WITH 4 DEGREES OF FREEDOM

NO SIG HETEROGENEITY

LOG LD<sub>50</sub> IS 0.441mg cm<sup>-2</sup>

LD<sub>50</sub> IS 0.276mg cm<sup>-2</sup>

95% CONF LIMITS ARE 0.218 TO 0.350mg cm<sup>-2</sup>

**Appendix Table LXXIII:** Dose-mortality effect of root wood extract (CH<sub>3</sub>OH) of *M. oleifera* against *T. castaneum* after 6h of exposure

Dose	Ldos (+1)	#U	Kl	%Kill	Cr%	E Pr	Ex Pr	Wk Pro	Weght	F Pro
1.529	1.184	30	22	73.333	73	5.61	5.556	5.584	17.43	5.533
1.274	1.105	30	20	66.667	67	5.44	5.315	5.422	18.48	5.295
1.019	1.008	30	12	40.000	40	4.75	5.019	4.750	19.11	5.003
0.764	0.883	30	11	36.667	37	4.67	4.638	4.659	18.03	4.627
0.510	0.707	30	6	20.000	20	4.16	4.101	4.170	14.13	4.097

REGRESSION EQUATION:  $Y = 1.968 + 3.011X$

CHI-SQUARED IS 1.662 WITH 3 DEGREES OF FREEDOM

NO SIG HETEROGENEITY

LOG LD<sub>50</sub> IS 1.007mg cm<sup>-2</sup>

LD<sub>50</sub> IS 1.017mg cm<sup>-2</sup>

95% CONF LIMITS ARE 0.865 TO 1.195mg cm<sup>-2</sup>

**Appendix Table LXXIV:** Dose-mortality effect of root wood extract (CH<sub>3</sub>OH) of *M. oleifera* against *T. castaneum* after 12h of exposure

Dose	Ldos (+1)	#U	Kl	%Kill	Cr%	E Pr	Ex Pr	Wk Pro	Weght	F Pro
1.529	1.184	30	27	90.000	90	6.28	6.019	6.210	13.17	5.970
1.274	1.105	30	23	76.667	77	5.74	5.718	5.734	15.96	5.671
1.019	1.008	30	15	50.000	50	5.00	5.349	4.980	18.48	5.304
0.764	0.883	30	13	43.333	43	4.82	4.873	4.838	18.81	4.832
0.510	0.707	30	7	23.333	23	4.26	4.203	4.252	15.09	4.166
0.255	0.406	30	1	3.333	3	3.12	3.057	3.135	3.93	3.029

REGRESSION EQUATION:  $Y = 1.493 + 3.780X$

CHI-SQUARED IS 2.922 WITH 4 DEGREES OF FREEDOM

NO SIG HETEROGENEITY

LOG LD<sub>50</sub> IS 0.928mg cm<sup>-2</sup>

LD<sub>50</sub> IS 0.847mg cm<sup>-2</sup>

95% CONF LIMITS ARE 0.744 TO 0.964mg cm<sup>-2</sup>

**Appendix Table LXXV:** Dose-mortality effect of root wood extract (CH<sub>3</sub>OH) of *M. oleifera* against *T. castaneum* after 24h of exposure

Dose	Ldos (+1)	#U	Kl	%Kill	Cr%	E Pr	Ex Pr	Wk Pro	Weght	F Pro
1.529	1.184	30	29	96.667	97	6.88	6.292	6.587	11.10	6.261
1.274	1.105	30	27	90.000	90	6.28	6.033	6.210	13.17	6.007
1.019	1.008	30	19	63.333	63	5.33	5.716	5.286	15.96	5.697
0.764	0.883	30	17	56.667	57	5.18	5.308	5.162	18.48	5.298
0.510	0.707	30	13	43.333	43	4.82	4.732	4.818	18.48	4.734
0.255	0.406	30	4	13.333	13	3.87	3.748	3.894	10.08	3.771

REGRESSION EQUATION:  $Y = 2.471 + 3.199X$

CHI-SQUARED IS 5.043 WITH 4 DEGREES OF FREEDOM

NO SIG HETEROGENEITY

LOG LD<sub>50</sub> IS 0.790mg cm<sup>-2</sup>

LD<sub>50</sub> IS 0.617mg cm<sup>-2</sup>

95% CONF LIMITS ARE 0.524 TO 0.726mg cm<sup>-2</sup>

**Appendix Table LXXVI:** Dose-mortality effect of root wood extract (CH<sub>3</sub>OH) of *M. oleifera* against *T. castaneum* after 36h of exposure

Dose	Ldos (+1)	#U	Kl	%Kill	Cr%	E Pr	Ex Pr	Wk Pro	Weght	F Pro
1.529	1.184	30	29	96.667	97	6.88	6.789	6.822	6.24	6.808
1.274	1.105	30	29	96.667	97	6.88	6.509	6.759	8.07	6.527
1.019	1.008	30	27	90.000	90	6.28	6.167	6.270	12.15	6.183
0.764	0.883	30	21	70.000	70	5.52	5.725	5.510	15.96	5.739
0.510	0.707	30	16	53.333	53	5.08	5.102	5.065	19.02	5.113
0.255	0.406	30	6	20.000	20	4.16	4.038	4.160	13.17	4.043

REGRESSION EQUATION:  $Y = 2.599 + 3.554X$

CHI-SQUARED IS 1.586 WITH 4 DEGREES OF FREEDOM

NO SIG HETEROGENEITY

LOG LD<sub>50</sub> IS 0.676mg cm<sup>-2</sup>

LD<sub>50</sub> IS 0.474mg cm<sup>-2</sup>

95% CONF LIMITS ARE 0.399 TO 0.563mg cm<sup>-2</sup>

**Appendix Table LXXVII:** Dose-mortality effect of root wood extract (CH<sub>3</sub>OH) of *M. oleifera* against *T. castaneum* after 48h of exposure

Dose	Ldos (+1)	#U	Kl	%Kill	Cr%	E Pr	Ex Pr	Wk Pro	Weght	F Pro
1.019	1.008	30	28	93.333	93	6.48	6.354	6.424	10.08	6.309
0.764	0.883	30	23	76.667	77	5.74	5.945	5.756	14.13	5.914
0.510	0.707	30	20	66.667	67	5.44	5.369	5.422	18.48	5.356
0.255	0.406	30	8	26.667	27	4.39	4.383	4.394	15.96	4.403

REGRESSION EQUATION:  $Y = 3.117 + 3.166X$

CHI-SQUARED IS 0.565 WITH 2 DEGREES OF FREEDOM

NO SIG HETEROGENEITY

LOG LD<sub>50</sub> IS 0.595mg cm<sup>-2</sup>

LD<sub>50</sub> IS 0.393mg cm<sup>-2</sup>

95% CONF LIMITS ARE 0.317 TO 0.487mg cm<sup>-2</sup>



**Appendix Table LXXVIII:** Dose-mortality effect of leaf extract (Pet.E.) of *Mu. sapientum* against *T. castaneum* after 6h of exposure

Dose	Ldos (+1)	#U	Kl	%Kill	Cr%	E Pr	Ex Pr	Wk Pro	Weght	F Pro
2.038	1.309	30	7	23.333	23	4.26	4.161	4.284	14.13	4.189
1.783	1.251	30	5	16.667	17	4.05	4.016	4.037	13.17	4.036
1.529	1.184	30	3	10.000	10	3.72	3.850	3.720	11.10	3.859
1.274	1.105	30	2	6.667	7	3.52	3.652	3.529	9.06	3.650
1.019	1.008	30	2	6.667	7	3.52	3.411	3.540	7.14	3.394
0.764	0.883	30	1	3.333	3	3.12	3.100	3.116	4.62	3.064

REGRESSION EQUATION:  $Y = 0.732 + 2.641X$

CHI-SQUARED IS 0.639 WITH 4 DEGREES OF FREEDOM

NO SIG HETEROGENEITY

LOG LD<sub>50</sub> IS 1.616mg cm<sup>-2</sup>

LD<sub>50</sub> IS 4.134mg cm<sup>-2</sup>

95% CONF LIMITS ARE 1.852 TO 9.229mg cm<sup>-2</sup>

**Appendix Table LXXIX:** Dose-mortality effect of leaf extract (Pet.E.) of *Mu. sapientum* against *T. castaneum* after 12h of exposure

Dose	Ldos (+1)	#U	Kl	%Kill	Cr%	E Pr	Ex Pr	Wk Pro	Weght	F Pro
2.038	1.309	30	13	43.333	43	4.82	4.592	4.824	17.43	4.605
1.783	1.251	30	7	23.333	23	4.26	4.443	4.270	16.74	4.450
1.529	1.184	30	6	20.000	20	4.16	4.270	4.150	15.09	4.271
1.274	1.105	30	5	16.667	17	4.05	4.066	4.037	13.17	4.060
1.019	1.008	30	4	13.333	13	3.87	3.816	3.873	11.10	3.801
0.764	0.883	30	2	6.667	7	3.52	3.494	3.540	7.14	3.467

REGRESSION EQUATION:  $Y = 1.107 + 2.672X$

CHI-SQUARED IS 1.704 WITH 4 DEGREES OF FREEDOM

NO SIG HETEROGENEITY

LOG LD<sub>50</sub> IS 1.457mg cm<sup>-2</sup>

LD<sub>50</sub> IS 2.865mg cm<sup>-2</sup>

95% CONF LIMITS ARE 1.799 TO 4.561mg cm<sup>-2</sup>

**Appendix Table LXXX:** Dose-mortality effect of leaf extract (Pet.E.) of *Mu. sapientum* against *T. castaneum* after 24h of exposure

Dose	Ldos (+1)	#U	Kl	%Kill	Cr%	E Pr	Ex Pr	Wk Pro	Weght	F Pro
2.038	1.309	30	17	56.667	57	5.18	5.104	5.165	19.02	5.090
1.783	1.251	30	15	50.000	50	5.00	4.928	4.990	19.02	4.920
1.529	1.184	30	8	26.667	27	4.39	4.725	4.402	18.48	4.724
1.274	1.105	30	10	33.333	33	4.56	4.484	4.570	16.74	4.492
1.019	1.008	30	8	26.667	27	4.39	4.189	4.436	14.13	4.208
0.764	0.883	30	3	10.000	10	3.72	3.810	3.720	11.10	3.841

REGRESSION EQUATION:  $Y = 1.252 + 2.932X$

CHI-SQUARED IS 3.117 WITH 4 DEGREES OF FREEDOM

NO SIG HETEROGENEITY

LOG LD<sub>50</sub> IS 1.279mg cm<sup>-2</sup>

LD<sub>50</sub> IS 1.899mg cm<sup>-2</sup>

95% CONF LIMITS ARE 1.532 TO 2.354mg cm<sup>-2</sup>

**Appendix Table LXXXI:** Dose-mortality effect of leaf extract (Pet.E.) of *Mu. sapientum* against *T. castaneum* after 36h of exposure

Dose	Ldos (+1)	#U	Kl	%Kill	Cr%	E Pr	Ex Pr	Wk Pro	Weght	F Pro
2.038	1.309	30	21	70.000	70	5.52	5.460	5.510	18.03	5.456
1.783	1.251	30	18	60.000	60	5.25	5.262	5.280	18.81	5.262
1.529	1.184	30	13	43.333	43	4.82	5.033	4.825	19.11	5.038
1.274	1.105	30	13	43.333	43	4.82	4.762	4.818	18.48	4.773
1.019	1.008	30	11	36.667	37	4.67	4.430	4.690	16.74	4.449
0.764	0.883	30	4	13.333	13	3.87	4.003	3.873	13.17	4.032

REGRESSION EQUATION:  $Y = 1.079 + 3.343X$

CHI-SQUARED IS 2.265 WITH 4 DEGREES OF FREEDOM

NO SIG HETEROGENEITY

LOG LD<sub>50</sub> IS 1.173mg cm<sup>-2</sup>

LD<sub>50</sub> IS 1.489mg cm<sup>-2</sup>

95% CONF LIMITS ARE 1.299 TO 1.707mg cm<sup>-2</sup>

**Appendix Table LXXXII:** Dose-mortality effect of leaf extract (Pet.E.) of *Mu. sapientum* against *T. castaneum* after 48h of exposure

Dose	Ldos (+1)	#U	Kl	%Kill	Cr%	E Pr	Ex Pr	Wk Pro	Weght	F Pro
2.038	1.309	30	25	83.333	83	5.95	5.949	5.984	14.13	5.946
1.783	1.251	30	23	76.667	77	5.74	5.709	5.734	15.96	5.709
1.529	1.184	30	18	60.000	60	5.25	5.433	5.240	18.03	5.436
1.274	1.105	30	17	56.667	57	5.18	5.105	5.165	19.02	5.113
1.019	1.008	30	14	46.667	47	4.92	4.705	4.922	18.48	4.718
0.764	0.883	30	5	16.667	17	4.05	4.188	4.056	14.13	4.208

REGRESSION EQUATION:  $Y = 0.606 + 4.079X$

CHI-SQUARED IS 1.873 WITH 4 DEGREES OF FREEDOM

NO SIG HETEROGENEITY

LOG LD<sub>50</sub> IS 1.077mg cm<sup>-2</sup>

LD<sub>50</sub> IS 1.195mg cm<sup>-2</sup>

95% CONF LIMITS ARE 1.063 TO 1.343mg cm<sup>-2</sup>

**Appendix Table LXXXIII:** Dose-mortality effect of stem extract (Pet.E.) of *Mu. sapientum* against *T. castaneum* after 1/2h of exposure

Dose	Ldos (+1)	#U	Kl	%Kill	Cr%	E Pr	Ex Pr	Wk Pro	Weght	F Pro
2.038	0.309	30	3	10.000	10	3.72	3.816	3.720	11.10	3.797
1.529	0.184	30	3	10.000	10	3.72	3.556	3.750	8.07	3.569
1.019	0.008	30	1	3.333	3	3.12	3.188	3.116	4.62	3.247

REGRESSION EQUATION:  $Y = 3.232 + 1.826X$

CHI-SQUARED IS 0.410 WITH 1 DEGREES OF FREEDOM

NO SIG HETEROGENEITY

LOG LD<sub>50</sub> IS 0.968mg cm<sup>-2</sup>

LD<sub>50</sub> IS 9.289mg cm<sup>-2</sup>

95% CONF LIMITS ARE 0.295 TO 292.334mg cm<sup>-2</sup>

**Appendix Table LXXXIV:** Dose-mortality effect of stem extract (Pet.E.) of *Mu. sapientum* against *T. castaneum* after 6h of exposure

Dose	Ldos (+1)	#U	Kl	%Kill	Cr%	E Pr	Ex Pr	Wk Pro	Weght	F Pro
2.038	1.309	30	22	73.333	73	5.61	5.529	5.584	17.43	5.507
1.529	1.184	30	17	56.667	57	5.18	5.205	5.202	18.81	5.192
1.019	1.008	30	10	33.333	33	4.56	4.747	4.558	18.48	4.749
0.510	0.707	30	6	20.000	20	4.16	3.966	4.200	12.15	3.991
0.255	0.406	30	1	3.333	3	3.12	3.184	3.116	4.62	3.233

REGRESSION EQUATION:  $Y = 2.211 + 2.518X$

CHI-SQUARED IS 1.373 WITH 3 DEGREES OF FREEDOM

NO SIG HETEROGENEITY

LOG LD<sub>50</sub> IS 1.108mg cm<sup>-2</sup>

LD<sub>50</sub> IS 1.282mg cm<sup>-2</sup>

95% CONF LIMITS ARE 1.029 TO 1.597mg cm<sup>-2</sup>

**Appendix Table LXXXV:** Dose-mortality effect of stem extract (Pet.E.) of *Mu. sapientum* against *T. castaneum* after 12h of exposure

Dose	Ldos (+1)	#U	Kl	%Kill	Cr%	E Pr	Ex Pr	Wk Pro	Weght	F Pro
2.038	1.309	30	25	83.333	83	5.95	5.838	5.902	15.09	5.809
1.529	1.184	30	20	66.667	67	5.44	5.507	5.416	17.43	5.478
1.019	1.008	30	15	50.000	50	5.00	5.040	5.000	19.11	5.012
0.510	0.707	30	6	20.000	20	4.16	4.242	4.150	15.09	4.215
0.255	0.406	30	2	6.667	7	3.52	3.443	3.540	7.14	3.418

REGRESSION EQUATION:  $Y = 2.342 + 2.648X$

CHI-SQUARED IS 0.371 WITH 3 DEGREES OF FREEDOM

NO SIG HETEROGENEITY

LOG LD<sub>50</sub> IS 1.004mg cm<sup>-2</sup>

LD<sub>50</sub> IS 1.009mg cm<sup>-2</sup>

95% CONF LIMITS ARE 0.827 TO 1.230mg cm<sup>-2</sup>

**Appendix Table LXXXVI:** Dose-mortality effect of stem extract (Pet.E.) of *Mu. sapientum* against *T. castaneum* after 24h of exposure

Dose	Ldos (+1)	#U	Kl	%Kill	Cr%	E Pr	Ex Pr	Wk Pro	Weght	F Pro
2.038	1.309	30	26	86.667	87	6.13	6.120	6.132	12.15	6.120
1.529	1.184	30	23	76.667	77	5.74	5.766	5.734	15.96	5.770
1.019	1.008	30	18	60.000	60	5.25	5.266	5.280	18.81	5.275
0.510	0.707	30	9	30.000	30	4.48	4.411	4.480	16.74	4.429
0.255	0.406	30	2	6.667	7	3.52	3.557	3.519	8.07	3.584

REGRESSION EQUATION:  $Y = 2.442 + 2.809X$

CHI-SQUARED IS 0.099 WITH 3 DEGREES OF FREEDOM

NO SIG HETEROGENEITY

LOG LD<sub>50</sub> IS 0.910mg cm<sup>-2</sup>

LD<sub>50</sub> IS 0.814mg cm<sup>-2</sup>

95% CONF LIMITS ARE 0.671 TO 0.986mg cm<sup>-2</sup>

**Appendix Table LXXXVII:** Dose-mortality effect of stem extract (Pet.E.) of *Mu. sapientum* against *T. castaneum* after 36h of exposure

Dose	Ldos (+1)	#U	Kl	%Kill	Cr%	E Pr	Ex Pr	Wk Pro	Weght	F Pro
2.038	1.309	30	27	90.000	90	6.28	6.347	6.250	10.08	6.329
1.529	1.184	30	26	86.667	87	6.13	5.959	6.136	14.13	5.946
1.019	1.008	30	18	60.000	60	5.25	5.412	5.240	18.03	5.406
0.510	0.707	30	10	33.333	33	4.56	4.478	4.570	16.74	4.484
0.255	0.406	30	2	6.667	7	3.52	3.544	3.519	8.07	3.561

REGRESSION EQUATION:  $Y = 2.316 + 3.066X$

CHI-SQUARED IS 1.209 WITH 3 DEGREES OF FREEDOM

NO SIG HETEROGENEITY

LOG LD<sub>50</sub> IS 0.876mg cm<sup>-2</sup>

LD<sub>50</sub> IS 0.751mg cm<sup>-2</sup>

95% CONF LIMITS ARE 0.624 TO 0.904mg cm<sup>-2</sup>

**Appendix Table LXXXVIII:** Dose-mortality effect of stem extract (Pet.E.) of *Mu. sapientum* against *T. castaneum* after 48h of exposure

Dose	Ldos	#U	Kl	%Kill	Cr%	E Pr	Ex Pr	Wk Pro	Weght	F Pro
2.038	1.309	30	28	93.333	93	6.48	6.470	6.491	9.06	6.450
1.529	1.184	30	27	90.000	90	6.28	6.136	6.270	12.15	6.117
1.019	1.008	30	21	70.000	70	5.52	5.666	5.520	16.74	5.648
0.510	0.707	30	12	40.000	40	4.75	4.861	4.760	18.81	4.846
0.255	0.406	30	6	20.000	20	4.16	4.057	4.160	13.17	4.044

REGRESSION EQUATION:  $Y = 2.961 + 2.665X$

CHI-SQUARED IS 0.889 WITH 3 DEGREES OF FREEDOM

NO SIG HETEROGENEITY

LOG LD<sub>50</sub> IS 0.765mg cm<sup>-2</sup>

LD<sub>50</sub> IS 0.582mg cm<sup>-2</sup>

95% CONF LIMITS ARE 0.469 TO 0.723mg cm<sup>-2</sup>

**Appendix Table LXXXIX:** Dose-mortality effect of root extract (Pet.E.) of *Mu. sapientum* against *T. castaneum* after 6h of exposure

Dose	Ldos	#U	Kl	%Kill	Cr%	E Pr	Ex Pr	Wk Pro	Weght	F Pro
1.783	0.251	30	8	26.667	27	4.39	4.407	4.390	16.74	4.392
1.529	0.184	30	5	16.667	17	4.05	4.062	4.037	13.17	4.058
1.274	0.105	30	3	10.000	10	3.72	3.655	3.730	9.06	3.663
1.019	0.008	30	1	3.333	3	3.12	3.156	3.116	4.62	3.179

REGRESSION EQUATION:  $Y = 3.138 + 4.994X$

CHI-SQUARED IS 0.065 WITH 2 DEGREES OF FREEDOM

NO SIG HETEROGENEITY

LOG LD<sub>50</sub> IS 0.373mg cm<sup>-2</sup>

LD<sub>50</sub> IS 2.359mg cm<sup>-2</sup>

95% CONF LIMITS ARE 1.630 TO 3.416mg cm<sup>-2</sup>

**Appendix Table XC:** Dose-mortality effect of root extract (Pet.E.) of *Mu. sapientum* against *T. castaneum* after 12h of exposure

Dose	Ldos (+1)	#U	Kl	%Kill	Cr%	E Pr	Ex Pr	Wk Pro	Weght	F Pro
1.783	1.251	30	19	63.333	63	5.33	5.148	5.315	19.02	5.150
1.529	1.184	30	14	46.667	47	4.92	4.974	4.915	19.02	4.971
1.274	1.105	30	11	36.667	37	4.67	4.767	4.662	18.48	4.759
1.019	1.008	30	9	30.000	30	4.48	4.514	4.460	17.43	4.501
0.764	0.883	30	5	16.667	17	4.05	4.188	4.056	14.13	4.167
0.510	0.707	30	4	13.333	13	3.87	3.729	3.894	10.08	3.697

REGRESSION EQUATION:  $Y = 1.808 + 2.671X$

CHI-SQUARED IS 1.349 WITH 4 DEGREES OF FREEDOM

NO SIG HETEROGENEITY

LOG LD<sub>50</sub> IS 1.195mg cm<sup>-2</sup>

LD<sub>50</sub> IS 1.568mg cm<sup>-2</sup>

95% CONF LIMITS ARE 1.258 TO 1.953mg cm<sup>-2</sup>

**Appendix Table XCI:** Dose-mortality effect of root extract (Pet.E.) of *Mu. sapientum* against *T. castaneum* after 24h of exposure

Dose	Ldos (+1)	#U	Kl	%Kill	Cr%	E Pr	Ex Pr	Wk Pro	Weght	F Pro
1.783	1.251	30	22	73.333	73	5.61	5.532	5.584	17.43	5.522
1.529	1.184	30	20	66.667	67	5.44	5.340	5.422	18.48	5.328
1.274	1.105	30	15	50.000	50	5.00	5.112	4.990	19.02	5.100
1.019	1.008	30	12	40.000	40	4.75	4.833	4.760	18.81	4.819
0.764	0.883	30	8	26.667	27	4.39	4.474	4.390	16.74	4.458
0.510	0.707	30	5	16.667	17	4.05	3.967	4.062	12.15	3.950
0.255	0.406	30	1	3.333	3	3.12	3.101	3.116	4.62	3.080

REGRESSION EQUATION:  $Y = 1.906 + 2.889X$

CHI-SQUARED IS 0.762 WITH 5 DEGREES OF FREEDOM

NO SIG HETEROGENEITY

LOG LD<sub>50</sub> IS 1.071mg cm<sup>-2</sup>

LD<sub>50</sub> IS 1.177mg cm<sup>-2</sup>

95% CONF LIMITS ARE 1.007 TO 1.375mg cm<sup>-2</sup>

**Appendix Table XCII:** Dose-mortality effect of root extract (Pet.E.) of *Mu. sapientum* against *T. castaneum* after 36h of exposure

Dose	Ldos (+1)	#U	Kl	%Kill	Cr%	E Pr	Ex Pr	Wk Pro	Weght	F Pro
1.783	1.251	30	28	93.333	93	6.48	5.998	6.364	14.13	5.934
1.529	1.184	30	24	80.000	80	5.85	5.804	5.800	15.09	5.740
1.274	1.105	30	20	66.667	67	5.44	5.574	5.416	17.43	5.511
1.019	1.008	30	15	50.000	50	5.00	5.293	5.020	18.81	5.230
0.764	0.883	30	11	36.667	37	4.67	4.931	4.665	19.02	4.868
0.510	0.707	30	7	23.333	23	4.26	4.421	4.270	16.74	4.358
0.255	0.406	30	4	13.333	13	3.87	3.549	3.981	8.07	3.486

REGRESSION EQUATION:  $Y = 2.309 + 2.897X$

CHI-SQUARED IS 6.543 WITH 5 DEGREES OF FREEDOM

NO SIG HETEROGENEITY

LOG LD<sub>50</sub> IS 0.929mg cm<sup>-2</sup>

LD<sub>50</sub> IS 0.849mg cm<sup>-2</sup>

95% CONF LIMITS ARE 0.729 TO 0.987mg cm<sup>-2</sup>

**Appendix Table XCIII:** Dose-mortality effect of root extract (Pet.E.) of *Mu. sapientum* against *T. castaneum* after 48h of exposure

Dose	Ldos (+1)	#U	Kl	%Kill	Cr%	E Pr	Ex Pr	Wk Pro	Weght	F Pro
1.783	1.251	30	29	96.667	97	6.88	6.239	6.587	11.10	6.212
1.529	1.184	30	27	90.000	90	6.28	6.030	6.210	13.17	6.006
1.274	1.105	30	24	80.000	80	5.85	5.782	5.830	15.96	5.763
1.019	1.008	30	18	60.000	60	5.25	5.479	5.240	18.03	5.466
0.764	0.883	30	12	40.000	40	4.75	5.088	4.750	19.11	5.082
0.510	0.707	30	8	26.667	27	4.39	4.538	4.376	17.43	4.542
0.255	0.406	30	5	16.667	17	4.05	3.596	4.289	8.07	3.619

REGRESSION EQUATION:  $Y = 2.373 + 3.068X$

CHI-SQUARED IS 9.323 WITH 5 DEGREES OF FREEDOM

NO SIG HETEROGENEITY

LOG LD<sub>50</sub> IS 0.856mg cm<sup>-2</sup>

LD<sub>50</sub> IS 0.718mg cm<sup>-2</sup>

95% CONF LIMITS ARE 0.615 TO 0.615mg cm<sup>-2</sup>

**Appendix Table XCIV:** Dose-mortality effect of leaf extract (CH<sub>3</sub>OH) of *Mu. sapientum* against *T. castaneum* after 12h of exposure

Dose	Ldos (+1)	#U	Kl	%Kill	Cr%	E Pr	Ex Pr	Wk Pro	Weght	F Pro
1.529	1.184	30	8	26.667	27	4.39	4.375	4.394	15.96	4.371
1.274	1.105	30	6	20.000	20	4.16	4.207	4.150	15.09	4.203
1.019	1.008	30	5	16.667	17	4.05	4.001	4.037	13.17	3.996
0.764	0.883	30	3	10.000	10	3.72	3.736	3.720	10.08	3.730

REGRESSION EQUATION:  $Y = 1.850 + 2.129X$

CHI-SQUARED IS 0.073 WITH 2 DEGREES OF FREEDOM

NO SIG HETEROGENEITY

LOG LD<sub>50</sub> IS 1.479mg cm<sup>-2</sup>

LD<sub>50</sub> IS 3.018mg cm<sup>-2</sup>

95% CONF LIMITS ARE 0.955 TO 9.534mg cm<sup>-2</sup>

**Appendix Table XCV:** Dose-mortality effect of leaf extract (CH<sub>3</sub>OH) of *Mu. sapientum* against *T. castaneum* after 24h of exposure

Dose	Ldos (+1)	#U	Kl	%Kill	Cr%	E Pr	Ex Pr	Wk Pro	Weght	F Pro
1.529	1.184	30	13	43.333	43	4.82	4.859	4.838	18.81	4.848
1.274	1.105	30	11	36.667	37	4.67	4.687	4.659	18.03	4.681
1.019	1.008	30	9	30.000	30	4.48	4.477	4.480	16.74	4.476
0.764	0.883	30	7	23.333	23	4.26	4.205	4.252	15.09	4.213
0.510	0.707	30	4	13.333	13	3.87	3.823	3.873	11.10	3.841
0.255	0.406	30	1	3.333	3	3.12	3.169	3.116	4.62	3.206

REGRESSION EQUATION:  $Y = 2.349 + 2.109X$

CHI-SQUARED IS 0.083 WITH 4 DEGREES OF FREEDOM

NO SIG HETEROGENEITY

LOG LD<sub>50</sub> IS 1.256mg cm<sup>-2</sup>

LD<sub>50</sub> IS 1.805mg cm<sup>-2</sup>

95% CONF LIMITS ARE 1.214 TO 2.682mg cm<sup>-2</sup>

**Appendix Table XCVI:** Dose-mortality effect of leaf extract (CH<sub>3</sub>OH) of *Mu. sapientum* against *T. castaneum* after 36h of exposure

Dose	Ldos (+1)	#U	Kl	%Kill	Cr%	E Pr	Ex Pr	Wk Pro	Weght	F Pro
1.529	1.184	30	25	83.333	83	5.95	5.740	5.926	15.96	5.713
1.274	1.105	30	23	76.667	77	5.74	5.594	5.696	17.43	5.567
1.019	1.008	30	19	63.333	63	5.33	5.415	5.321	18.03	5.388
0.764	0.883	30	15	50.000	50	5.00	5.184	4.990	19.02	5.158
0.510	0.707	30	10	33.333	33	4.56	4.859	4.578	18.81	4.833
0.255	0.406	30	8	26.667	27	4.39	4.303	4.394	15.96	4.278
0.127	0.105	30	4	13.333	13	3.87	3.747	3.894	10.08	3.724

REGRESSION EQUATION:  $Y = 3.529 + 1.844X$

CHI-SQUARED IS 3.366 WITH 5 DEGREES OF FREEDOM

NO SIG HETEROGENEITY

LOG LD<sub>50</sub> IS 0.798mg cm<sup>-2</sup>

LD<sub>50</sub> IS 0.628mg cm<sup>-2</sup>

95% CONF LIMITS ARE 0.499 TO 0.788mg cm<sup>-2</sup>

**Appendix Table XCVII:** Dose-mortality effect of leaf extract (CH<sub>3</sub>OH) of *Mu. sapientum* against *T. castaneum* after 48h of exposure

Dose	Ldos (+1)	#U	Kl	%Kill	Cr%	E Pr	Ex Pr	Wk Pro	Weght	F Pro
1.529	1.184	30	29	96.667	97	6.88	6.465	6.759	9.06	6.487
1.274	1.105	30	28	93.333	93	6.48	6.328	6.424	10.08	6.350
1.019	1.008	30	27	90.000	90	6.28	6.162	6.270	12.15	6.181
0.764	0.883	30	24	80.000	80	5.85	5.947	5.870	14.13	5.964
0.510	0.707	30	19	63.333	63	5.33	5.644	5.310	16.74	5.658
0.255	0.406	30	17	56.667	57	5.18	5.126	5.165	19.02	5.135
0.127	0.105	30	12	40.000	40	4.75	4.608	4.740	18.03	4.612

REGRESSION EQUATION:  $Y = 4.429 + 1.738X$

CHI-SQUARED IS 3.287 WITH 5 DEGREES OF FREEDOM

NO SIG HETEROGENEITY

LOG LD<sub>50</sub> IS 0.329mg cm<sup>-2</sup>

LD<sub>50</sub> IS 0.213mg cm<sup>-2</sup>

95% CONF LIMITS ARE 0.148 TO 0.307mg cm<sup>-2</sup>

**Appendix Table XCVIII:** Dose-mortality effect of stem extract (CH<sub>3</sub>OH) of *Mu. sapientum* against *T. castaneum* after 12h of exposure

Dose	Ldos (+1)	#U	Kl	%Kill	Cr%	E Pr	Ex Pr	Wk Pro	Weght	F Pro
0.510	0.707	30	10	33.333	33	4.56	4.737	4.558	18.48	4.723
0.382	0.582	30	11	36.667	37	4.67	4.367	4.714	15.96	4.388
0.255	0.406	30	3	10.000	10	3.72	3.846	3.720	11.10	3.915

REGRESSION EQUATION:  $Y = 2.824 + 2.685X$

CHI-SQUARED IS 2.625 WITH 1 DEGREES OF FREEDOM

NO SIG HETEROGENEITY

LOG LD50 IS 0.810mg cm<sup>-2</sup>

LD50 IS 0.646mg cm<sup>-2</sup>

95% CONF LIMITS ARE 0.381 TO 1.097mg cm<sup>-2</sup>

**Appendix Table XCIX:** Dose-mortality effect of stem extract (CH<sub>3</sub>OH) of *Mu. sapientum* against *T. castaneum* after 24h of exposure

Dose	Ldos (+1)	#U	Kl	%Kill	Cr%	E Pr	Ex Pr	Wk Pro	Weght	F Pro
0.510	1.707	30	24	80.000	80	5.85	5.787	5.830	15.96	5.772
0.382	1.582	30	20	66.667	67	5.44	5.315	5.422	18.48	5.302
0.255	1.406	30	9	30.000	30	4.48	4.650	4.470	18.03	4.639
0.127	1.105	30	1	3.333	3	3.12	3.513	3.211	8.07	3.506
0.064	0.804	30	1	3.333	3	3.12	2.375	4.847	0.93	2.373

REGRESSION EQUATION:  $Y = -0.653 + 3.764X$

CHI-SQUARED IS 7.230 WITH 3 DEGREES OF FREEDOM

NO SIG HETEROGENEITY

LOG LD50 IS 1.502mg cm<sup>-2</sup>

LD50 IS 0.318mg cm<sup>-2</sup>

95% CONF LIMITS ARE 0.273 TO 0.370mg cm<sup>-2</sup>

**Appendix Table C:** Dose-mortality effect of stem extract (CH<sub>3</sub>OH) of *Mu. sapientum* against *T. castaneum* after 36h of exposure

Dose	Ldos (+2)	#U	Kl	%Kill	Cr%	E Pr	Ex Pr	Wk Pro	Weght	F Pro
0.510	1.707	30	28	93.333	93	6.48	6.463	6.491	9.06	6.503
0.382	1.582	30	28	93.333	93	6.48	5.999	6.364	14.13	6.031
0.255	1.406	30	15	50.000	50	5.00	5.345	4.980	18.48	5.366
0.127	1.105	30	8	26.667	27	4.39	4.227	4.388	15.09	4.228
0.064	0.804	30	1	3.333	3	3.12	3.110	3.116	4.62	3.091

REGRESSION EQUATION:  $Y = 0.052 + 3.778X$

CHI-SQUARED IS 4.705 WITH 3 DEGREES OF FREEDOM

NO SIG HETEROGENEITY

LOG LD50 IS 1.309mg cm<sup>-2</sup>

LD50 IS 0.204mg cm<sup>-2</sup>

95% CONF LIMITS ARE 0.174 TO 0.239mg cm<sup>-2</sup>

**Appendix Table CI:** Dose-mortality effect of stem extract (CH<sub>3</sub>OH) of *Mu. sapientum* against *T. castaneum* after 48h of exposure

Dose	Ldos (+2)	#U	Kl	%Kill	Cr%	E Pr	Ex Pr	Wk Pro	Weght	F Pro
0.510	1.707	30	29	96.667	97	6.88	7.125	6.789	3.30	7.073
0.382	1.582	30	29	96.667	97	6.88	6.585	6.759	8.07	6.549
0.255	1.406	30	23	76.667	77	5.74	5.826	5.698	15.09	5.811
0.127	1.105	30	11	36.667	37	4.67	4.527	4.656	17.43	4.550
0.064	0.804	30	1	3.333	3	3.12	3.228	3.121	5.40	3.288

REGRESSION EQUATION:  $Y = -0.083 + 4.191X$

CHI-SQUARED IS 1.162 WITH 3 DEGREES OF FREEDOM

NO SIG HETEROGENEITY

LOG LD50 IS 1.213mg cm<sup>-2</sup>

LD50 IS 0.163mg cm<sup>-2</sup>

95% CONF LIMITS ARE 0.139 TO 0.191mg cm<sup>-2</sup>



**Appendix Table CII:** Dose-mortality effect of root extract (CH<sub>3</sub>OH) of *Mu. sapientum* against *T. castaneum* after 12h of exposure

Dose	Ldos (+1)	#U	Kl	%Kill	Cr%	E Pr	Ex Pr	Wk Pro	Weght	F Pro
0.764	0.883	30	7	23.333	23	4.26	4.270	4.252	15.09	4.272
0.637	0.804	30	6	20.000	20	4.16	4.132	4.170	14.13	4.129
0.510	0.707	30	3	10.000	10	3.72	3.962	3.740	12.15	3.955
0.382	0.582	30	5	16.667	17	4.05	3.744	4.126	10.08	3.729
0.255	0.406	30	1	3.333	3	3.12	3.435	3.180	7.14	3.412

REGRESSION EQUATION:  $Y = 2.679 + 1.804X$

CHI-SQUARED IS 2.559 WITH 3 DEGREES OF FREEDOM

NO SIG HETEROGENEITY

LOG LD<sub>50</sub> IS 1.287mg cm<sup>-2</sup>

LD<sub>50</sub> IS 1.935mg cm<sup>-2</sup>

95% CONF LIMITS ARE 0.556 TO 6.734mg cm<sup>-2</sup>

**Appendix Table CIII:** Dose-mortality effect of root extract (CH<sub>3</sub>OH) of *Mu. sapientum* against *T. castaneum* after 24h of exposure

Dose	Ldos (+1)	#U	Kl	%Kill	Cr%	E Pr	Ex Pr	Wk Pro	Weght	F Pro
0.764	0.883	30	20	66.667	67	5.44	5.305	5.422	18.48	5.302
0.637	0.804	30	17	56.667	57	5.18	5.216	5.202	18.81	5.212
0.510	0.707	30	14	46.667	47	4.92	5.107	4.915	19.02	5.103
0.382	0.582	30	15	50.000	50	5.00	4.966	4.990	19.02	4.962
0.255	0.406	30	13	43.333	43	4.82	4.767	4.818	18.48	4.763

REGRESSION EQUATION:  $Y = 4.304 + 1.129X$

CHI-SQUARED IS 1.012 WITH 3 DEGREES OF FREEDOM

NO SIG HETEROGENEITY

LOG LD<sub>50</sub> IS 0.616mg cm<sup>-2</sup>

LD<sub>50</sub> IS 0.413mg cm<sup>-2</sup>

95% CONF LIMITS ARE 0.266 TO 0.641mg cm<sup>-2</sup>

**Appendix Table CIV:** Dose-mortality effect of root extract (CH<sub>3</sub>OH) of *Mu. sapientum* against *T. castaneum* after 36h of exposure

Dose	Ldos (+1)	#U	Kl	%Kill	Cr%	E Pr	Ex Pr	Wk Pro	Weght	F Pro
0.764	0.883	30	28	93.333	93	6.48	6.363	6.424	10.08	6.310
0.637	0.804	30	26	86.667	87	6.13	6.249	6.077	11.10	6.209
0.510	0.707	30	26	86.667	87	6.13	6.110	6.132	12.15	6.085
0.382	0.582	30	24	80.000	80	5.85	5.930	5.870	14.13	5.925
0.255	0.406	30	23	76.667	77	5.74	5.677	5.730	16.74	5.699

REGRESSION EQUATION:  $Y = 5.178 + 1.282X$

CHI-SQUARED IS 0.408 WITH 3 DEGREES OF FREEDOM

NO SIG HETEROGENEITY

LOG LD<sub>50</sub> IS -0.139mg cm<sup>-2</sup>

LD<sub>50</sub> IS 0.073mg cm<sup>-2</sup>

95% CONF LIMITS ARE 0.009 TO 0.562mg cm<sup>-2</sup>

**Appendix Table CV:** Dose-mortality effects of root extract (CH<sub>3</sub>OH) of *Mu. sapientum* against *T. castaneum* after 48h of exposure

Dose	Ldos (+1)	#U	Kl	%Kill	Cr%	E Pr	Ex Pr	Wk Pro	Weght	F Pro
0.510	0.707	30	28	93.333	93	6.48	6.998	6.236	4.62	7.000
0.382	0.582	30	27	90.000	90	6.28	6.363	6.250	10.08	6.367
0.255	0.406	30	26	86.667	87	6.13	5.467	5.969	18.03	5.474
0.127	0.105	30	2	6.667	7	3.52	3.935	3.602	12.15	3.949

REGRESSION EQUATION:  $Y = 3.416 + 5.067X$

CHI-SQUARED IS 8.706 WITH 2 DEGREES OF FREEDOM

VARIANCE HAS BEEN ADJUSTED FOR HETEROGENEITY

LOG LD<sub>50</sub> IS 0.313mg cm<sup>-2</sup>

LD<sub>50</sub> IS 0.205mg cm<sup>-2</sup>

95% CONF LIMITS ARE 0.152 TO 0.277mg cm<sup>-2</sup>

**Appendix Table CVI:** Lethal effects of leaf extract (Pet.E.) of *C. papaya* against *A. salina* nauplii after 6h of exposure

Dose	Ldos	#U	Kl	%Kill	Cr%	E Pr	Ex Pr	Wk Pro	Weght	F Pro
250.000	2.398	30	3	10.000	10	3.72	3.553	3.750	8.07	3.610
200.000	2.301	30	1	3.333	3	3.12	3.454	3.180	7.14	3.499
150.000	2.176	30	2	6.667	7	3.52	3.326	3.572	6.24	3.357
100.000	2.000	30	1	3.333	3	3.12	3.147	3.116	4.62	3.157

REGRESSION EQUATION:  $Y = 0.884 + 1.137X$

CHI-SQUARED IS 1.183 WITH 2 DEGREES OF FREEDOM

NO SIG HETEROGENEITY

LOG LC<sub>50</sub> IS 3.621ppm

LC<sub>50</sub> IS 4180.528ppm

95% CONF LIMITS ARE 2.055 TO 8503968ppm

**Appendix Table CVII:** Lethal effects of leaf extract (Pet.E.) of *C. papaya* against *A. salina* nauplii after 12h of exposure

Dose	Ldos	#U	Kl	%Kill	Cr%	E Pr	Ex Pr	Wk Pro	Weght	F Pro
250.000	2.398	30	5	16.667	17	4.05	3.908	4.062	12.15	3.892
200.000	2.301	30	1	3.333	3	3.12	3.727	3.314	10.08	3.717
150.000	2.176	30	3	10.000	10	3.72	3.494	3.810	7.14	3.492
100.000	2.000	30	1	3.333	3	3.12	3.166	3.116	4.62	3.175

REGRESSION EQUATION:  $Y = -0.424 + 1.799X$

CHI-SQUARED IS 2.728 WITH 2 DEGREES OF FREEDOM

NO SIG HETEROGENEITY

LOG LC<sub>50</sub> IS 3.014ppm

LC<sub>50</sub> IS 1032.428ppm

95% CONF LIMITS ARE 90.426 TO 11787.660ppm

**Appendix Table CVIII:** Lethal effects of leaf extract (Pet.E.) of *C. papaya* against *A. salina* nauplii after 18h of exposure

Dose	Ldos	#U	Kl	%Kill	Cr%	E Pr	Ex Pr	Wk Pro	Weght	F Pro
250.000	2.398	30	14	46.667	47	4.92	4.647	4.929	18.03	4.679
200.000	2.301	30	6	20.000	20	4.16	4.471	4.180	16.74	4.488
150.000	2.176	30	6	20.000	20	4.16	4.245	4.150	15.09	4.242
100.000	2.000	30	5	16.667	17	4.05	3.927	4.062	12.15	3.895

REGRESSION EQUATION:  $Y = -0.044 + 1.969X$

CHI-SQUARED IS 3.182 WITH 2 DEGREES OF FREEDOM

NO SIG HETEROGENEITY

LOG LC<sub>50</sub> IS 2.561ppm

LC<sub>50</sub> IS 363.954ppm

95% CONF LIMITS ARE 178.161 TO 743.498ppm

**Appendix Table CIX:** Lethal effects of leaf extract (Pet.E.) of *C. papaya* against *A. salina* nauplii after 24h of exposure

Dose	Ldos	#U	Kl	%Kill	Cr%	E Pr	Ex Pr	Wk Pro	Weght	F Pro
250.000	2.398	30	26	86.667	87	6.13	6.018	6.087	13.17	5.978
200.000	2.301	30	22	73.333	73	5.61	5.762	5.606	15.96	5.733
150.000	2.176	30	20	66.667	67	5.44	5.432	5.429	18.03	5.417
100.000	2.000	30	15	50.000	50	5.00	4.967	4.990	19.02	4.971

REGRESSION EQUATION:  $Y = -0.094 + 2.532X$

CHI-SQUARED IS 0.423 WITH 2 DEGREES OF FREEDOM

NO SIG HETEROGENEITY

LOG LC<sub>50</sub> IS 2.012ppm

LC<sub>50</sub> IS 102.701ppm

95% CONF LIMITS ARE 72.057 TO 146.376ppm

**Appendix Table CX:** Lethal effects of stem extract (Pet.E.) of *C. papaya* against *A. salina* nauplii after 6h of exposure

Dose	Ldos	#U	Kl	%Kill	Cr%	E Pr	Ex Pr	Wk Pro	Weght	F Pro
75.000	1.875	30	5	16.667	17	4.05	3.871	4.077	11.10	3.902
50.000	1.699	30	2	6.667	7	3.52	3.757	3.546	10.08	3.783
25.000	1.398	30	2	6.667	7	3.52	3.562	3.519	8.07	3.580
12.500	1.097	30	2	6.667	7	3.52	3.367	3.572	6.24	3.377
6.250	0.796	30	1	3.333	3	3.12	3.173	3.116	4.62	3.174

REGRESSION EQUATION:  $Y = 2.637 + 0.675X$

CHI-SQUARED IS 1.189 WITH 3 DEGREES OF FREEDOM

NO SIG HETEROGENEITY

LOG LC<sub>50</sub> IS 3.502ppm

LC<sub>50</sub> IS 3173.579ppm

95% CONF LIMITS ARE 8.563 TO 1176216ppm

**Appendix Table CXI:** Lethal effects of stem extract (Pet.E.) of *C. papaya* against *A. salina* nauplii after 12h of exposure

Dose	Ldos	#U	Kl	%Kill	Cr%	E Pr	Ex Pr	Wk Pro	Weght	F Pro
75.000	1.875	30	15	50.000	50	5.00	4.726	5.000	18.48	4.757
50.000	1.699	30	7	23.333	23	4.26	4.510	4.264	17.43	4.527
25.000	1.398	30	5	16.667	17	4.05	4.141	4.056	14.13	4.133
12.500	1.097	30	3	10.000	10	3.72	3.771	3.720	10.08	3.740
6.250	0.796	30	2	6.667	7	3.52	3.402	3.540	7.14	3.347

REGRESSION EQUATION:  $Y = 2.308 + 1.306X$

CHI-SQUARED IS 2.651 WITH 3 DEGREES OF FREEDOM

NO SIG HETEROGENEITY

LOG LC<sub>50</sub> IS 2.061ppm

LC<sub>50</sub> IS 115.179ppm

95% CONF LIMITS ARE 52.497 TO 252.707ppm

**Appendix Table CXII:** Lethal effects of stem extract (Pet.E.) of *C. papaya* against *A. salina* nauplii after 18h of exposure

Dose	Ldos	#U	Kl	%Kill	Cr%	E Pr	Ex Pr	Wk Pro	Weght	F Pro
75.000	1.875	30	21	70.000	70	5.52	5.386	5.500	18.48	5.379
50.000	1.699	30	16	53.333	53	5.08	5.101	5.065	19.02	5.090
25.000	1.398	30	8	26.667	27	4.39	4.614	4.389	18.03	4.597
12.500	1.097	30	6	20.000	20	4.16	4.128	4.170	14.13	4.105
6.250	0.796	30	3	10.000	10	3.72	3.641	3.730	9.06	3.612

REGRESSION EQUATION:  $Y = 2.308 + 1.638X$

CHI-SQUARED IS 1.255 WITH 3 DEGREES OF FREEDOM

NO SIG HETEROGENEITY

LOG LC<sub>50</sub> IS 1.644ppm

LC<sub>50</sub> IS 44.033ppm

95% CONF LIMITS ARE 31.059 TO 62.426ppm

**Appendix Table CXIII:** Lethal effects of stem extract (Pet.E.) of *C. papaya* against *A. salina* nauplii after 24h of exposure

Dose	Ldos	#U	Kl	%Kill	Cr%	E Pr	Ex Pr	Wk Pro	Weght	F Pro
75.000	1.875	30	28	93.333	93	6.48	6.409	6.491	9.06	6.383
50.000	1.699	30	25	83.333	83	5.95	6.024	5.923	13.17	6.002
25.000	1.398	30	19	63.333	63	5.33	5.367	5.318	18.48	5.350
12.500	1.097	30	12	40.000	40	4.75	4.709	4.740	18.48	4.698
6.250	0.796	30	5	16.667	17	4.05	4.051	4.037	13.17	4.047

REGRESSION EQUATION:  $Y = 2.324 + 2.165X$

CHI-SQUARED IS 0.239 WITH 3 DEGREES OF FREEDOM

NO SIG HETEROGENEITY

LOG LC<sub>50</sub> IS 1.236ppm

LC<sub>50</sub> IS 17.229ppm

95% CONF LIMITS ARE 13.384 TO 22.181ppm

**Appendix Table CXIV:** Lethal effects of root extract (Pet.E.) of *C. papaya* against *A. salina* nauplii after 6h of exposure

Dose	Ldos	#U	Kl	%Kill	Cr%	E Pr	Ex Pr	Wk Pro	Weght	F Pro
100.000	2.000	30	3	10.000	10	3.72	3.737	3.720	10.08	3.763
80.000	1.903	30	3	10.000	10	3.72	3.566	3.750	8.07	3.585
60.000	1.778	30	1	3.333	3	3.12	3.344	3.148	6.24	3.356
40.000	1.602	30	1	3.333	3	3.12	3.032	3.135	3.93	3.033

REGRESSION EQUATION:  $Y = 0.096 + 1.833X$

CHI-SQUARED IS 0.549 WITH 2 DEGREES OF FREEDOM

NO SIG HETEROGENEITY

LOG LC<sub>50</sub> IS 2.675ppm

LC<sub>50</sub> IS 472.899ppm

95% CONF LIMITS ARE 28.534 TO 7837.606ppm

**Appendix Table CXV:** Lethal effects of root extract (Pet.E.) of *C. papaya* against *A. salina* nauplii after 12h of exposure

Dose	Ldos	#U	Kl	%Kill	Cr%	E Pr	Ex Pr	Wk Pro	Weght	F Pro
100.000	2.000	30	10	33.333	33	4.56	4.527	4.544	17.43	4.518
80.000	1.903	30	9	30.000	30	4.48	4.357	4.490	15.96	4.348
60.000	1.778	30	2	6.667	7	3.52	4.138	3.676	14.13	4.128
40.000	1.602	30	6	20.000	20	4.16	3.829	4.230	11.10	3.819
20.000	1.301	30	1	3.333	3	3.12	3.302	3.148	6.24	3.290

REGRESSION EQUATION:  $Y = 1.002 + 1.758X$

CHI-SQUARED IS 5.227 WITH 3 DEGREES OF FREEDOM

NO SIG HETEROGENEITY

LOG LC<sub>50</sub> IS 2.274ppm

LC<sub>50</sub> IS 187.898ppm

95% CONF LIMITS ARE 84.636 TO 417.149ppm

**Appendix Table CXVI:** Lethal effects of root extract (Pet.E.) of *C. papaya* against *A. salina* nauplii after 18h of exposure

Dose	Ldos	#U	Kl	%Kill	Cr%	E Pr	Ex Pr	Wk Pro	Weght	F Pro
100.000	2.000	30	19	63.333	63	5.33	5.221	5.358	18.81	5.243
80.000	1.903	30	17	56.667	57	5.18	4.991	5.165	19.02	5.014
60.000	1.778	30	5	16.667	17	4.05	4.695	4.119	18.03	4.719
40.000	1.602	30	11	36.667	37	4.67	4.278	4.728	15.09	4.304
20.000	1.301	30	2	6.667	7	3.52	3.565	3.519	8.07	3.594

REGRESSION EQUATION:  $Y = 0.524 + 2.359X$

CHI-SQUARED IS 9.939 WITH 3 DEGREES OF FREEDOM

VARIANCE HAS BEEN ADJUSTED FOR HETEROGENEITY

LOG LC<sub>50</sub> IS 1.897ppm

LC<sub>50</sub> IS 78.891ppm

95% CONF LIMITS ARE 50.355 TO 123.598ppm

**Appendix Table CXVII:** Lethal effects of root extract (Pet.E.) of *C. papaya* against *A. salina* nauplii after 24h of exposure

Dose	Ldos	#U	Kl	%Kill	Cr%	E Pr	Ex Pr	Wk Pro	Weght	F Pro
100.000	2.000	30	26	86.667	87	6.13	6.126	6.132	12.15	6.051
80.000	1.903	30	27	90.000	90	6.28	5.757	6.150	15.96	5.690
60.000	1.778	30	9	30.000	30	4.48	5.280	4.500	18.81	5.223
40.000	1.602	30	13	43.333	43	4.82	4.608	4.821	18.03	4.567
20.000	1.301	30	2	6.667	7	3.52	3.459	3.540	7.14	3.444

REGRESSION EQUATION:  $Y = -1.409 + 3.731X$

CHI-SQUARED IS 14.542 WITH 3 DEGREES OF FREEDOM  
VARIANCE HAS BEEN ADJUSTED FOR HETEROGENEITY

LOG LC<sub>50</sub> IS 1.718ppm

LC<sub>50</sub> IS 52.268ppm

95% CONF LIMITS ARE 38.031 TO 71.833ppm

**Appendix Table CXVIII:** Lethal effects of leaf extract (CHCl<sub>3</sub>) of *C. papaya* against *A. salina* nauplii after 12h of exposure

Dose	Ldos	#U	Kl	%Kill	Cr%	E Pr	Ex Pr	Wk Pro	Weght	F Pro
200.000	2.301	30	8	26.667	27	4.39	4.342	4.394	15.96	4.366
150.000	2.176	30	5	16.667	17	4.05	4.058	4.037	13.17	4.073
100.000	2.000	30	3	10.000	10	3.72	3.657	3.730	9.06	3.659
75.000	1.875	30	1	3.333	3	3.12	3.372	3.148	6.24	3.365
50.000	1.699	30	1	3.333	3	3.12	2.972	3.172	3.30	2.951

REGRESSION EQUATION:  $Y = -1.043 + 2.351X$

CHI-SQUARED IS 0.530 WITH 3 DEGREES OF FREEDOM  
NO SIG HETEROGENEITY

LOG LC<sub>50</sub> IS 2.571ppm

LC<sub>50</sub> IS 372.025ppm

95% CONF LIMITS ARE 177.651 TO 779.069ppm

**Appendix Table CXIX:** Lethal effects of leaf extract (CHCl<sub>3</sub>) of *C. papaya* against *A. salina* nauplii after 18h of exposure

Dose	Ldos	#U	Kl	%Kill	Cr%	E Pr	Ex Pr	Wk Pro	Weght	F Pro
200.000	2.301	30	20	66.667	67	5.44	5.436	5.429	18.03	5.439
150.000	2.176	30	17	56.667	57	5.18	5.259	5.202	18.81	5.261
100.000	2.000	30	17	56.667	57	5.18	5.009	5.175	19.11	5.011
75.000	1.875	30	12	40.000	40	4.75	4.831	4.760	18.81	4.833
50.000	1.699	30	10	33.333	33	4.56	4.581	4.544	17.43	4.583
25.000	1.398	30	6	20.000	20	4.16	4.154	4.170	14.13	4.155

REGRESSION EQUATION:  $Y = 2.168 + 1.422X$

CHI-SQUARED IS 0.713 WITH 4 DEGREES OF FREEDOM  
NO SIG HETEROGENEITY

LOG LC<sub>50</sub> IS 1.992ppm

LC<sub>50</sub> IS 98.248ppm

95% CONF LIMITS ARE 71.704 TO 134.619ppm

**Appendix Table CXX:** Lethal effects of leaf extract (CHCl<sub>3</sub>) of *C. papaya* against *A. salina* nauplii after 24h of exposure

Dose	Ldos	#U	Kl	%Kill	Cr%	E Pr	Ex Pr	Wk Pro	Weght	F Pro
100.000	2.000	30	28	93.333	93	6.48	6.426	6.491	9.06	6.405
75.000	1.875	30	27	90.000	90	6.28	6.321	6.250	10.08	6.311
50.000	1.699	30	26	86.667	87	6.13	6.173	6.132	12.15	6.180
25.000	1.398	30	25	83.333	83	5.95	5.920	5.984	14.13	5.954

REGRESSION EQUATION:  $Y = 4.908 + 0.748X$

CHI-SQUARED IS 0.145 WITH 2 DEGREES OF FREEDOM

NO SIG HETEROGENEITY

LOG LC<sub>50</sub> IS 0.122ppm

LC<sub>50</sub> IS 1.326ppm

95% CONF LIMITS ARE 0.003 TO 658.439ppm

**Appendix Table CXXI:** Lethal effects of stem extract (CHCl<sub>3</sub>) of *C. papaya* against *A. salina* nauplii after 6h of exposure

Dose	Ldos	#U	Kl	%Kill	Cr%	E Pr	Ex Pr	Wk Pro	Weght	F Pro
75.000	1.875	30	8	26.667	27	4.39	4.307	4.394	15.96	4.339
50.000	1.699	30	3	10.000	10	3.72	3.851	3.720	11.10	3.846
25.000	1.398	30	1	3.333	3	3.12	3.072	3.135	3.93	3.004

REGRESSION EQUATION:  $Y = -0.908 + 2.798X$

CHI-SQUARED IS 0.293 WITH 1 DEGREES OF FREEDOM

NO SIG HETEROGENEITY

LOG LC<sub>50</sub> IS 2.111ppm

LC<sub>50</sub> IS 129.233ppm

95% CONF LIMITS ARE 62.656 TO 266.551ppm

**Appendix Table CXXII:** Lethal effects of stem extract (CHCl<sub>3</sub>) of *C. papaya* against *A. salina* nauplii after 12h of exposure

Dose	Ldos	#U	Kl	%Kill	Cr%	E Pr	Ex Pr	Wk Pro	Weght	F Pro
75.000	1.875	30	17	56.667	57	5.18	4.965	5.165	19.02	4.990
50.000	1.699	30	10	33.333	33	4.56	4.692	4.551	18.03	4.703
25.000	1.398	30	6	20.000	20	4.16	4.227	4.150	15.09	4.213
12.500	1.097	30	2	6.667	7	3.52	3.761	3.546	10.08	3.722
6.250	0.796	30	2	6.667	7	3.52	3.295	3.629	5.40	3.231

REGRESSION EQUATION:  $Y = 1.934 + 1.629X$

CHI-SQUARED IS 2.224 WITH 3 DEGREES OF FREEDOM

NO SIG HETEROGENEITY

LOG LC<sub>50</sub> IS 1.881ppm

LC<sub>50</sub> IS 76.026ppm

95% CONF LIMITS ARE 46.519 TO 124.249ppm

**Appendix Table CXXIII:** Lethal effects of stem extract (CHCl<sub>3</sub>) of *C. papaya* against *A. salina* nauplii after 18h of exposure

Dose	Ldos	#U	Kl	%Kill	Cr%	E Pr	Ex Pr	Wk Pro	Weght	F Pro
75.000	1.875	30	22	73.333	73	5.61	5.507	5.584	17.43	5.511
50.000	1.699	30	17	56.667	57	5.18	5.215	5.202	18.81	5.214
25.000	1.398	30	11	36.667	37	4.67	4.717	4.662	18.48	4.706
12.500	1.097	30	5	16.667	17	4.05	4.219	4.048	15.09	4.199
6.250	0.796	30	4	13.333	13	3.87	3.721	3.894	10.08	3.692

REGRESSION EQUATION:  $Y = 2.349 + 1.686X$

CHI-SQUARED IS 0.890 WITH 3 DEGREES OF FREEDOM

NO SIG HETEROGENEITY

LOG LC<sub>50</sub> IS 1.572ppm

LC<sub>50</sub> IS 37.335ppm

95% CONF LIMITS ARE 27.146 TO 51.348ppm

**Appendix Table CXXIV:** Lethal effects of stem extract (CHCl<sub>3</sub>) of *C. papaya* against *A. salina* nauplii after 24h of exposure

Dose	Ldos	#U	Kl	%Kill	Cr%	E Pr	Ex Pr	Wk Pro	Weght	F Pro
50.000	1.699	30	26	86.667	87	6.13	6.410	6.089	9.06	6.433
25.000	1.398	30	27	90.000	90	6.28	5.630	6.120	16.74	5.622
12.500	1.097	30	8	26.667	27	4.39	4.850	4.422	18.81	4.811
6.250	0.796	30	6	20.000	20	4.16	4.070	4.160	13.17	4.000

REGRESSION EQUATION:  $Y = 1.857 + 2.694X$

CHI-SQUARED IS 8.407 WITH 2 DEGREES OF FREEDOM

VARIANCE HAS BEEN ADJUSTED FOR HETEROGENEITY

LOG LC<sub>50</sub> IS 1.167ppm

LC<sub>50</sub> IS 14.689ppm

95% CONF LIMITS ARE 9.306 TO 23.186ppm

**Appendix Table CXXV:** Lethal effects of root extract (CHCl<sub>3</sub>) of *C. papaya* against *A. salina* nauplii after 12h of exposure

Dose	Ldos	#U	Kl	%Kill	Cr%	E Pr	Ex Pr	Wk Pro	Weght	F Pro
100.000	2.000	30	10	33.333	33	4.56	4.574	4.544	17.43	4.555
75.000	1.875	30	9	30.000	30	4.48	4.433	4.480	16.74	4.420
50.000	1.699	30	6	20.000	20	4.16	4.235	4.150	15.09	4.229
25.000	1.398	30	4	13.333	13	3.87	3.895	3.873	11.10	3.903
12.500	1.097	30	3	10.000	10	3.72	3.556	3.750	8.07	3.576
6.250	0.796	30	1	3.333	3	3.12	3.217	3.121	5.40	3.250

REGRESSION EQUATION:  $Y = 2.387 + 1.084X$

CHI-SQUARED IS 0.499 WITH 4 DEGREES OF FREEDOM

NO SIG HETEROGENEITY

LOG LC<sub>50</sub> IS 2.410ppm

LC<sub>50</sub> IS 257.124ppm

95% CONF LIMITS ARE 83.311 TO 793.571ppm



**Appendix Table CXXVI:** Lethal effects of root extract (CHCl<sub>3</sub>) of *C. papaya* against *A. salina* nauplii after 18h of exposure

Dose	Ldos	#U	Kl	%Kill	Cr%	E Pr	Ex Pr	Wk Pro	Weght	F Pro
100.000	2.000	30	12	40.000	40	4.75	4.781	4.740	18.48	4.762
75.000	1.875	30	11	36.667	37	4.67	4.621	4.659	18.03	4.609
50.000	1.699	30	8	26.667	27	4.39	4.396	4.394	15.96	4.394
25.000	1.398	30	4	13.333	13	3.87	4.010	3.873	13.17	4.027
12.500	1.097	30	4	13.333	13	3.87	3.624	3.931	9.06	3.659
6.250	0.796	30	1	3.333	3	3.12	3.238	3.121	5.40	3.292

REGRESSION EQUATION:  $Y = 2.321 + 1.221X$

CHI-SQUARED IS 1.191 WITH 4 DEGREES OF FREEDOM

NO SIG HETEROGENEITY

LOG LC<sub>50</sub> IS 2.195ppm

LC<sub>50</sub> IS 156.739ppm

95% CONF LIMITS ARE 73.267 TO 335.307ppm

**Appendix Table CXXVII:** Lethal effects of root extract (CHCl<sub>3</sub>) of *C. papaya* against *A. salina* nauplii after 24h of exposure

Dose	Ldos	#U	Kl	%Kill	Cr%	E Pr	Ex Pr	Wk Pro	Weght	F Pro
100.000	2.000	30	27	90.000	90	6.28	6.012	6.210	13.17	5.967
75.000	1.875	30	23	76.667	77	5.74	5.708	5.734	15.96	5.662
50.000	1.699	30	15	50.000	50	5.00	5.280	5.020	18.81	5.234
25.000	1.398	30	7	23.333	23	4.26	4.548	4.264	17.43	4.501
12.500	1.097	30	5	16.667	17	4.05	3.816	4.077	11.10	3.769
6.250	0.796	30	1	3.333	3	3.12	3.084	3.135	3.93	3.036

REGRESSION EQUATION:  $Y = 1.099 + 2.434X$

CHI-SQUARED IS 3.798 WITH 4 DEGREES OF FREEDOM

NO SIG HETEROGENEITY

LOG LC<sub>50</sub> IS 1.603ppm

LC<sub>50</sub> IS 40.072ppm

95% CONF LIMITS ARE 32.581 TO 49.285ppm

**Appendix Table CXXVIII:** Lethal effects of leaf extract (CH<sub>3</sub>OH) of *C. papaya* against *A. salina* nauplii after 6h of exposure

Dose	Ldos	#U	Kl	%Kill	Cr%	E Pr	Ex Pr	Wk Pro	Weght	F Pro
100.000	2.000	30	4	13.333	13	3.87	3.883	3.873	11.10	3.878
80.000	1.903	30	3	10.000	10	3.72	3.782	3.720	10.08	3.783
60.000	1.778	30	3	10.000	10	3.72	3.653	3.730	9.06	3.659
40.000	1.602	30	2	6.667	7	3.52	3.471	3.540	7.14	3.486
20.000	1.301	30	1	3.333	3	3.12	3.160	3.116	4.62	3.189

REGRESSION EQUATION:  $Y = 1.905 + 0.987X$

CHI-SQUARED IS 0.131 WITH 3 DEGREES OF FREEDOM

NO SIG HETEROGENEITY

LOG LC<sub>50</sub> IS 3.137ppm

LC<sub>50</sub> IS 1370.555ppm

95% CONF LIMITS ARE 15.869 TO 118373.200ppm

**Appendix Table CXXIX:** Lethal effects of leaf extract (CH<sub>3</sub>OH) of *C. papaya* against *A. salina* nauplii after 12h of exposure

Dose	Ldos	#U	Kl	%Kill	Cr%	E Pr	Ex Pr	Wk Pro	Weght	F Pro
100.000	2.000	30	6	20.000	20	4.16	4.047	4.160	13.17	4.065
80.000	1.903	30	5	16.667	17	4.05	3.988	4.062	12.15	4.001
60.000	1.778	30	3	10.000	10	3.72	3.911	3.740	12.15	3.919
40.000	1.602	30	3	10.000	10	3.72	3.804	3.720	11.10	3.804
20.000	1.301	30	3	10.000	10	3.72	3.620	3.730	9.06	3.606

REGRESSION EQUATION:  $Y = 2.753 + 0.656X$

CHI-SQUARED IS 0.771 WITH 3 DEGREES OF FREEDOM

NO SIG HETEROGENEITY

LOG LC<sub>50</sub> IS 3.426ppm

LC<sub>50</sub> IS 2665.860ppm

95% CONF LIMITS ARE 3.857 TO 1842682ppm

**Appendix Table CXXX:** Lethal effects of leaf extract (CH<sub>3</sub>OH) of *C. papaya* against *A. salina* nauplii after 18h of exposure

Dose	Ldos	#U	Kl	%Kill	Cr%	E Pr	Ex Pr	Wk Pro	Weght	F Pro
100.000	2.000	30	8	26.667	27	4.39	4.324	4.394	15.96	4.357
80.000	1.903	30	9	30.000	30	4.48	4.236	4.490	15.09	4.264
60.000	1.778	30	4	13.333	13	3.87	4.122	3.904	14.13	4.144
40.000	1.602	30	3	10.000	10	3.72	3.961	3.740	12.15	3.975
20.000	1.301	30	4	13.333	13	3.87	3.687	3.931	9.06	3.686

REGRESSION EQUATION:  $Y = 2.436 + 0.960X$

CHI-SQUARED IS 2.822 WITH 3 DEGREES OF FREEDOM

NO SIG HETEROGENEITY

LOG LC<sub>50</sub> IS 2.670ppm

LC<sub>50</sub> IS 467.792ppm

95% CONF LIMITS ARE 43.733 TO 003.815ppm

**Appendix Table CXXXI:** Lethal effects of leaf extract (CH<sub>3</sub>OH) of *C. papaya* against *A. salina* nauplii after 24h of exposure

Dose	Ldos	#U	Kl	%Kill	Cr%	E Pr	Ex Pr	Wk Pro	Weght	F Pro
100.000	2.000	30	12	40.000	40	4.75	4.676	4.740	18.03	4.685
80.000	1.903	30	11	36.667	37	4.67	4.563	4.656	17.43	4.570
60.000	1.778	30	8	26.667	27	4.39	4.417	4.390	16.74	4.420
40.000	1.602	30	4	13.333	13	3.87	4.212	3.912	15.09	4.210
20.000	1.301	30	5	16.667	17	4.05	3.861	4.077	11.10	3.851

REGRESSION EQUATION:  $Y = 2.297 + 1.194X$

CHI-SQUARED IS 2.109 WITH 3 DEGREES OF FREEDOM

NO SIG HETEROGENEITY

LOG LC<sub>50</sub> IS 2.264ppm

LC<sub>50</sub> IS 183.443ppm

95% CONF LIMITS ARE 64.947 TO 518.135ppm

**Appendix Table CXXXII:** Lethal effects of stem extract (CH<sub>3</sub>OH) of *C. papaya* against *A. salina* nauplii after 6h of exposure

Dose	Ldos	#U	Kl	%Kill	Cr%	E Pr	Ex Pr	Wk Pro	Weght	F Pro
200.000	2.301	30	21	70.000	70	5.52	5.540	5.500	17.43	5.519
150.000	2.176	30	20	66.667	67	5.44	5.388	5.422	18.48	5.372
100.000	2.000	30	16	53.333	53	5.08	5.174	5.065	19.02	5.165
50.000	1.699	30	14	46.667	47	4.92	4.807	4.942	18.81	4.811
25.000	1.398	30	8	26.667	27	4.39	4.441	4.390	16.74	4.457

REGRESSION EQUATION:  $Y = 2.814 + 1.176X$

CHI-SQUARED IS 0.640 WITH 3 DEGREES OF FREEDOM

NO SIG HETEROGENEITY

LOG LC<sub>50</sub> IS 1.859ppm

LC<sub>50</sub> IS 72.338ppm

95% CONF LIMITS ARE 47.978 TO 109.066ppm

**Appendix Table CXXXIII:** Lethal effects of stem extract (CH<sub>3</sub>OH) of *C. papaya* against *A. salina* nauplii after 12h of exposure

Dose	Ldos	#U	Kl	%Kill	Cr%	E Pr	Ex Pr	Wk Pro	Weght	F Pro
200.000	2.301	30	21	70.000	70	5.52	5.852	5.460	15.09	5.830
150.000	2.176	30	25	83.333	83	5.95	5.688	5.910	16.74	5.668
100.000	2.000	30	21	70.000	70	5.52	5.456	5.510	18.03	5.441
50.000	1.699	30	18	60.000	60	5.25	5.060	5.250	19.11	5.052
25.000	1.398	30	9	30.000	30	4.48	4.664	4.470	18.03	4.664

REGRESSION EQUATION:  $Y = 2.859 + 1.291X$

CHI-SQUARED IS 4.548 WITH 3 DEGREES OF FREEDOM

NO SIG HETEROGENEITY

LOG LC<sub>50</sub> IS 1.658ppm

LC<sub>50</sub> IS 45.542ppm

95% CONF LIMITS ARE 28.645 TO 72.406ppm

**Appendix Table CXXXIV:** Lethal effects of stem extract (CH<sub>3</sub>OH) of *C. papaya* against *A. salina* nauplii after 18h of exposure

Dose	Ldos	#U	Kl	%Kill	Cr%	E Pr	Ex Pr	Wk Pro	Weght	F Pro
200.000	2.301	30	27	90.000	90	6.28	6.650	6.180	7.14	6.676
150.000	2.176	30	28	93.333	93	6.48	6.403	6.491	9.06	6.417
100.000	2.000	30	28	93.333	93	6.48	6.055	6.333	13.17	6.052
50.000	1.699	30	21	70.000	70	5.52	5.459	5.510	18.03	5.428
25.000	1.398	30	11	36.667	37	4.67	4.863	4.682	18.81	4.805

REGRESSION EQUATION:  $Y = 1.909 + 2.072X$

CHI-SQUARED IS 3.247 WITH 3 DEGREES OF FREEDOM

NO SIG HETEROGENEITY

LOG LC<sub>50</sub> IS 1.492ppm

LC<sub>50</sub> IS 31.059ppm

95% CONF LIMITS ARE 21.390 TO 45.098ppm

**Appendix Table CXXXV:** Lethal effects of stem extract (CH<sub>3</sub>OH) of *C. papaya* against *A. salina* nauplii after 24h of exposure

Dose	Ldos	#U	Kl	%Kill	Cr%	E Pr	Ex Pr	Wk Pro	Weght	F Pro
100.000	2.000	30	29	96.667	97	6.88	6.674	6.810	7.14	6.679
50.000	1.699	30	22	73.333	73	5.61	5.721	5.606	15.96	5.723
25.000	1.398	30	13	43.333	43	4.82	4.768	4.818	18.48	4.767

REGRESSION EQUATION:  $Y = 0.329 + 3.175X$

CHI-SQUARED IS 0.389 WITH 1 DEGREES OF FREEDOM

NO SIG HETEROGENEITY

LOG LC<sub>50</sub> IS 1.471ppm

LC<sub>50</sub> IS 29.593ppm

95% CONF LIMITS ARE 22.731 TO 38.526ppm

**Appendix Table CXXXVI:** Lethal effects of root extract (CH<sub>3</sub>OH) of *C. papaya* against *A. salina* nauplii after 6h of exposure

Dose	Ldos	#U	Kl	%Kill	Cr%	E Pr	Ex Pr	Wk Pro	Weght	F Pro
200.000	2.301	30	22	73.333	73	5.61	5.759	5.606	15.96	5.757
150.000	2.176	30	22	73.333	73	5.61	5.641	5.610	16.74	5.635
100.000	2.000	30	23	76.667	77	5.74	5.473	5.699	18.03	5.465
50.000	1.699	30	17	56.667	57	5.18	5.187	5.165	19.02	5.173
25.000	1.398	30	13	43.333	43	4.82	4.900	4.815	19.02	4.881

REGRESSION EQUATION:  $Y = 3.525 + 0.969X$

CHI-SQUARED IS 1.446 WITH 3 DEGREES OF FREEDOM

NO SIG HETEROGENEITY

LOG LC<sub>50</sub> IS 1.521ppm

LC<sub>50</sub> IS 33.176ppm

95% CONF LIMITS ARE 15.713 TO 70.047ppm

**Appendix Table CXXXVII:** Lethal effects of root extract (CH<sub>3</sub>OH) of *C. papaya* against *A. salina* nauplii after 12h of exposure

Dose	Ldos	#U	Kl	%Kill	Cr%	E Pr	Ex Pr	Wk Pro	Weght	F Pro
200.000	2.301	30	25	83.333	83	5.95	5.959	5.984	14.13	5.953
150.000	2.176	30	23	76.667	77	5.74	5.835	5.698	15.09	5.827
100.000	2.000	30	24	80.000	80	5.85	5.659	5.820	16.74	5.650
50.000	1.699	30	18	60.000	60	5.25	5.359	5.240	18.48	5.346
25.000	1.398	30	16	53.333	53	5.08	5.058	5.075	19.11	5.042

REGRESSION EQUATION:  $Y = 3.632 + 1.009X$

CHI-SQUARED IS 0.979 WITH 3 DEGREES OF FREEDOM

NO SIG HETEROGENEITY

LOG LC<sub>50</sub> IS 1.356ppm

LC<sub>50</sub> IS 22.699ppm

95% CONF LIMITS ARE 9.084 TO 56.720ppm

**Appendix Table CXXXVIII:** Lethal effects of root extract (CH<sub>3</sub>OH) of *C. papaya* against *A. salina* nauplii after 18h of exposure

Dose	Ldos	#U	Kl	%Kill	Cr%	E Pr	Ex Pr	Wk Pro	Weght	F Pro
200.000	2.301	30	27	90.000	90	6.28	6.211	6.230	11.10	6.157
150.000	2.176	30	25	83.333	83	5.95	6.058	5.923	13.17	6.011
100.000	2.000	30	25	83.333	83	5.95	5.842	5.902	15.09	5.805
50.000	1.699	30	19	63.333	63	5.33	5.474	5.321	18.03	5.452
25.000	1.398	30	17	56.667	57	5.18	5.105	5.165	19.02	5.099

REGRESSION EQUATION:  $Y = 3.462 + 1.171X$

CHI-SQUARED IS 0.695 WITH 3 DEGREES OF FREEDOM

NO SIG HETEROGENEITY

LOG LC<sub>50</sub> IS 1.313ppm

LC<sub>50</sub> IS 20.559ppm

95% CONF LIMITS ARE 8.819 TO 47.929ppm

**Appendix Table CXXXIX:** Lethal effects of root extract (CH<sub>3</sub>OH) of *C. papaya* against *A. salina* nauplii after 24h of exposure

Dose	Ldos	#U	Kl	%Kill	Cr%	E Pr	Ex Pr	Wk Pro	Weght	F Pro
150.000	2.176	30	25	83.333	83	5.95	6.087	5.923	13.17	6.025
100.000	2.000	30	26	86.667	87	6.13	5.893	6.038	15.09	5.848
50.000	1.699	30	20	66.667	67	5.44	5.561	5.416	17.43	5.545
25.000	1.398	30	18	60.000	60	5.25	5.229	5.280	18.81	5.241

REGRESSION EQUATION:  $Y = 3.833 + 1.008X$

CHI-SQUARED IS 0.999 WITH 2 DEGREES OF FREEDOM

NO SIG HETEROGENEITY

LOG LC<sub>50</sub> IS 1.158ppm

LC<sub>50</sub> IS 14.402ppm

95% CONF LIMITS ARE 3.945 TO 52.573ppm

**Appendix Table CXL:** Lethal effects of fruit extract (Pet.E.) of *M. oleifera* against *A. salina* nauplii after 6h of exposure

Dose	Ldos	#U	Kl	%Kill	Cr%	E Pr	Ex Pr	Wk Pro	Weght	F Pro
80.000	1.903	30	16	53.333	53	5.08	4.884	5.098	18.81	4.938
70.000	1.845	30	5	16.667	17	4.05	4.372	4.074	15.96	4.387
60.000	1.778	30	4	13.333	13	3.87	3.781	3.894	10.08	3.751
50.000	1.699	30	1	3.333	3	3.12	3.083	3.135	3.93	2.999

REGRESSION EQUATION:  $Y = -13.141 + 9.499X$

CHI-SQUARED IS 2.324 WITH 2 DEGREES OF FREEDOM

NO SIG HETEROGENEITY

LOG LC<sub>50</sub> IS 1.909ppm

LC<sub>50</sub> IS 81.213ppm

95% CONF LIMITS ARE 73.472 TO 89.769ppm

**Appendix Table CXLI:** Lethal effects of fruit extract (Pet.E.) of *M. oleifera* against *A. salina* nauplii after 12h of exposure

Dose	Ldos	#U	Kl	%Kill	Cr%	E Pr	Ex Pr	Wk Pro	Weght	F Pro
80.000	1.903	30	24	80.000	80	5.85	5.575	5.780	17.43	5.586
70.000	1.845	30	19	63.333	63	5.33	5.214	5.358	18.81	5.223
60.000	1.778	30	10	33.333	33	4.56	4.796	4.558	18.48	4.803
50.000	1.699	30	5	16.667	17	4.05	4.303	4.074	15.96	4.307
40.000	1.602	30	2	6.667	7	3.52	3.698	3.529	9.06	3.699
30.000	1.477	30	2	6.667	7	3.52	2.919	4.082	3.30	2.916

REGRESSION EQUATION:  $Y = -6.344 + 6.269X$

CHI-SQUARED IS 7.718 WITH 4 DEGREES OF FREEDOM

NO SIG HETEROGENEITY

LOG LC<sub>50</sub> IS 1.809ppm

LC<sub>50</sub> IS 64.503ppm

95% CONF LIMITS ARE 59.359 TO 70.093ppm

**Appendix Table CXLII:** Lethal effects of fruit extract (Pet.E.) of *M. oleifera* against *A. salina* nauplii after 18h of exposure

Dose	Ldos	#U	Kl	%Kill	Cr%	E Pr	Ex Pr	Wk Pro	Weght	F Pro
80.000	1.903	30	25	83.333	83	5.95	5.579	5.864	17.43	5.592
70.000	1.845	30	20	66.667	67	5.44	5.296	5.462	18.81	5.300
60.000	1.778	30	13	43.333	43	4.82	4.969	4.815	19.02	4.962
50.000	1.699	30	5	16.667	17	4.05	4.582	4.096	17.43	4.562
40.000	1.602	30	3	10.000	10	3.72	4.108	3.790	14.13	4.073
30.000	1.477	30	5	16.667	17	4.05	3.497	4.440	7.14	3.443

REGRESSION EQUATION:  $Y = -4.012 + 5.047X$

CHI-SQUARED IS 14.216 WITH 4 DEGREES OF FREEDOM

VARIANCE HAS BEEN ADJUSTED FOR HETEROGENEITY

LOG LC<sub>50</sub> IS 1.786ppm

LC<sub>50</sub> IS 61.055ppm

95% CONF LIMITS ARE 50.968 TO 73.138ppm

**Appendix Table CXLIII:** Lethal effects of fruit extract (Pet.E.) of *M. oleifera* against *A. salina* nauplii after 24h of exposure

Dose	Ldos	#U	Kl	%Kill	Cr%	E Pr	Ex Pr	Wk Pro	Weght	F Pro
80.000	1.903	30	25	83.333	83	5.95	5.647	5.910	16.74	5.639
70.000	1.845	30	22	73.333	73	5.61	5.385	5.578	18.48	5.370
60.000	1.778	30	13	43.333	43	4.82	5.083	4.825	19.11	5.060
50.000	1.699	30	7	23.333	23	4.26	4.725	4.298	18.48	4.693
40.000	1.602	30	5	16.667	17	4.05	4.287	4.048	15.09	4.245
30.000	1.477	30	6	20.000	20	4.16	3.723	4.300	10.08	3.666

REGRESSION EQUATION:  $Y = -3.177 + 4.632X$

CHI-SQUARED IS 10.611 WITH 4 DEGREES OF FREEDOM

VARIANCE HAS BEEN ADJUSTED FOR HETEROGENEITY

LOG LC<sub>50</sub> IS 1.765ppm

LC<sub>50</sub> IS 58.228ppm

95% CONF LIMITS ARE 49.449 TO 68.566ppm

**Appendix Table CXLIV:** Lethal effects of leaf extract (Pet.E.) of *M. oleifera* against *A. salina* nauplii after 6h of exposure

Dose	Ldos	#U	Kl	%Kill	Cr%	E Pr	Ex Pr	Wk Pro	Weght	F Pro
200.000	2.301	30	10	33.333	33	4.56	4.274	4.592	15.09	4.334
150.000	2.176	30	4	13.333	13	3.87	4.108	3.904	14.13	4.149
100.000	2.000	30	3	10.000	10	3.72	3.874	3.720	11.10	3.888
75.000	1.875	30	3	10.000	10	3.72	3.708	3.720	10.08	3.703
50.000	1.699	30	2	6.667	7	3.52	3.473	3.540	7.14	3.442
25.000	1.398	30	1	3.333	3	3.12	3.073	3.135	3.93	2.995

REGRESSION EQUATION:  $Y = 0.924 + 1.482X$

CHI-SQUARED IS 2.313 WITH 4 DEGREES OF FREEDOM

NO SIG HETEROGENEITY

LOG LC<sub>50</sub> IS 2.750ppm

LC<sub>50</sub> IS 562.916ppm

95% CONF LIMITS ARE 173.615 TO 1825.159ppm

**Appendix Table CXLV:** Lethal effects of leaf extract (Pet.E.) of *M. oleifera* against *A. salina* nauplii after 12h of exposure

Dose	Ldos	#U	Kl	%Kill	Cr%	E Pr	Ex Pr	Wk Pro	Weght	F Pro
200.000	2.301	30	14	46.667	47	4.92	4.752	4.922	18.48	4.770
150.000	2.176	30	8	26.667	27	4.39	4.523	4.376	17.43	4.532
100.000	2.000	30	6	20.000	20	4.16	4.199	4.170	14.13	4.197
75.000	1.875	30	4	13.333	13	3.87	3.969	3.878	12.15	3.959
50.000	1.699	30	3	10.000	10	3.72	3.645	3.730	9.06	3.624
25.000	1.398	30	1	3.333	3	3.12	3.092	3.135	3.93	3.051

REGRESSION EQUATION:  $Y = 0.389 + 1.904X$

CHI-SQUARED IS 1.072 WITH 4 DEGREES OF FREEDOM

NO SIG HETEROGENEITY

LOG LC<sub>50</sub> IS 2.422ppm

LC<sub>50</sub> IS 264.065ppm

95% CONF LIMITS ARE 157.754 TO 442.018ppm

**Appendix Table CXLVI:** Lethal effects of leaf extract (Pet.E.) of *M. oleifera* against *A. salina* nauplii after 18h of exposure

Dose	Ldos	#U	Kl	%Kill	Cr%	E Pr	Ex Pr	Wk Pro	Weght	F Pro
200.000	2.301	30	17	56.667	57	5.18	4.875	5.202	18.81	4.915
150.000	2.176	30	10	33.333	33	4.56	4.704	4.558	18.48	4.731
100.000	2.000	30	8	26.667	27	4.39	4.464	4.390	16.74	4.470
75.000	1.875	30	6	20.000	20	4.16	4.293	4.150	15.09	4.285
50.000	1.699	30	4	13.333	13	3.87	4.052	3.873	13.17	4.025
25.000	1.398	30	4	13.333	13	3.87	3.641	3.931	9.06	3.579

REGRESSION EQUATION:  $Y = 1.511 + 1.479X$

CHI-SQUARED IS 3.902 WITH 4 DEGREES OF FREEDOM

NO SIG HETEROGENEITY

LOG LC<sub>50</sub> IS 2.358ppm

LC<sub>50</sub> IS 228.101ppm

95% CONF LIMITS ARE 131.874 TO 394.545ppm

**Appendix Table CXLVII:** Lethal effects of leaf extract (Pet.E.) of *M. oleifera* against *A. salina* nauplii after 24h of exposure

Dose	Ldos	#U	Kl	%Kill	Cr%	E Pr	Ex Pr	Wk Pro	Weght	F Pro
200.000	2.301	30	24	80.000	80	5.85	5.450	5.780	18.03	5.442
150.000	2.176	30	16	53.333	53	5.08	5.195	5.065	19.02	5.178
100.000	2.000	30	11	36.667	37	4.67	4.836	4.682	18.81	4.807
75.000	1.875	30	7	23.333	23	4.26	4.581	4.264	17.43	4.543
50.000	1.699	30	6	20.000	20	4.16	4.221	4.150	15.09	4.171
25.000	1.398	30	4	13.333	13	3.87	3.607	3.931	9.06	3.536

REGRESSION EQUATION:  $Y = 0.584 + 2.111X$   
 CHI-SQUARED IS 5.374 WITH 4 DEGREES OF FREEDOM  
 NO SIG HETEROGENEITY  
 LOG LC<sub>50</sub> IS 2.092ppm  
 LC<sub>50</sub> IS 123.482ppm  
 95% CONF LIMITS ARE 97.195 TO 156.878ppm

**Appendix Table CXLVIII:** Lethal effects of stem wood extract (Pet.E.) of *M. oleifera* against *A. salina* nauplii after 12h of exposure

Dose	Ldos	#U	Kl	%Kill	Cr%	E Pr	Ex Pr	Wk Pro	Weght	F Pro
80.000	1.903	30	19	63.333	63	5.33	5.224	5.358	18.81	5.246
70.000	1.845	30	11	36.667	37	4.67	4.898	4.682	18.81	4.908
60.000	1.778	30	11	36.667	37	4.67	4.522	4.656	17.43	4.518
50.000	1.699	30	5	16.667	17	4.05	4.076	4.037	13.17	4.057

REGRESSION EQUATION:  $Y = -5.838 + 5.824X$   
 CHI-SQUARED IS 1.534 WITH 2 DEGREES OF FREEDOM  
 NO SIG HETEROGENEITY  
 LOG LC<sub>50</sub> IS 1.861ppm  
 LC<sub>50</sub> IS 72.589ppm  
 95% CONF LIMITS ARE 65.010 TO 81.052ppm

**Appendix Table CXLIX:** Lethal effects of stem wood extract (Pet.E.) of *M. oleifera* against *A. salina* nauplii after 18h of exposure

Dose	Ldos	#U	Kl	%Kill	Cr%	E Pr	Ex Pr	Wk Pro	Weght	F Pro
80.000	1.903	30	19	63.333	63	5.33	5.250	5.358	18.81	5.264
70.000	1.845	30	14	46.667	47	4.92	5.067	4.925	19.11	5.080
60.000	1.778	30	14	46.667	47	4.92	4.856	4.942	18.81	4.867
50.000	1.699	30	10	33.333	33	4.56	4.607	4.551	18.03	4.616
40.000	1.602	30	8	26.667	27	4.39	4.302	4.394	15.96	4.308
30.000	1.477	30	4	13.333	13	3.87	3.908	3.878	12.15	3.912

REGRESSION EQUATION:  $Y = -0.776 + 3.174X$   
 CHI-SQUARED IS 0.936 WITH 4 DEGREES OF FREEDOM  
 NO SIG HETEROGENEITY  
 LOG LC<sub>50</sub> IS 1.819ppm  
 LC<sub>50</sub> IS 66.061ppm  
 95% CONF LIMITS ARE 56.052 TO 77.858ppm



**Appendix Table CL:** Lethal effects of stem wood extract (Pet.E.) of *M. oleifera* against *A. salina* nauplii after 24h of exposure

Dose	Ldos	#U	Kl	%Kill	Cr%	E Pr	Ex Pr	Wk Pro	Weght	F Pro
80.000	1.903	30	21	70.000	70	5.52	5.350	5.500	18.48	5.345
70.000	1.845	30	16	53.333	53	5.08	5.240	5.098	18.81	5.234
60.000	1.778	30	16	53.333	53	5.08	5.112	5.065	19.02	5.106
50.000	1.699	30	14	46.667	47	4.92	4.962	4.915	19.02	4.954
40.000	1.602	30	13	43.333	43	4.82	4.777	4.818	18.48	4.768
30.000	1.477	30	10	33.333	33	4.56	4.539	4.544	17.43	4.528

REGRESSION EQUATION:  $Y = 1.697 + 1.917X$   
 CHI-SQUARED IS 0.902 WITH 4 DEGREES OF FREEDOM  
 NO SIG HETEROGENEITY  
 LOG LC<sub>50</sub> IS 1.723ppm  
 LC<sub>50</sub> IS 52.857ppm  
 95% CONF LIMITS ARE 42.282 TO 66.077ppm

**Appendix Table CLI:** Lethal effects of root bark extract (Pet.E.) of *M. oleifera* against *A. salina* nauplii after 12h of exposure

Dose	Ldos	#U	Kl	%Kill	Cr%	E Pr	Ex Pr	Wk Pro	Weght	F Pro
100.000	2.000	30	18	60.000	60	5.25	5.331	5.240	18.48	5.328
90.000	1.954	30	16	53.333	53	5.08	5.075	5.075	19.11	5.075
80.000	1.903	30	14	46.667	47	4.92	4.789	4.922	18.48	4.792
70.000	1.845	30	10	33.333	33	4.56	4.465	4.570	16.74	4.472
60.000	1.778	30	4	13.333	13	3.87	4.091	3.873	13.17	4.102
50.000	1.699	30	3	10.000	10	3.72	3.649	3.730	9.06	3.664

REGRESSION EQUATION:  $Y = -5.729 + 5.529X$   
 CHI-SQUARED IS 1.344 WITH 4 DEGREES OF FREEDOM  
 NO SIG HETEROGENEITY  
 LOG LC<sub>50</sub> IS 1.941ppm  
 LC<sub>50</sub> IS 87.223ppm  
 95% CONF LIMITS ARE 79.136 TO 96.136ppm

**Appendix Table CLII:** Lethal effects of root bark extract (Pet.E.) of *M. oleifera* against *A. salina* nauplii after 18h of exposure

Dose	Ldos	#U	Kl	%Kill	Cr%	E Pr	Ex Pr	Wk Pro	Weght	F Pro
100.000	2.000	30	23	76.667	77	5.74	5.740	5.734	15.96	5.738
90.000	1.954	30	21	70.000	70	5.52	5.459	5.510	18.03	5.454
80.000	1.903	30	17	56.667	57	5.18	5.145	5.165	19.02	5.137
70.000	1.845	30	12	40.000	40	4.75	4.788	4.740	18.48	4.777
60.000	1.778	30	6	20.000	20	4.16	4.377	4.170	15.96	4.362
50.000	1.699	30	5	16.667	17	4.05	3.891	4.077	11.10	3.871

REGRESSION EQUATION:  $Y = -6.669 + 6.204X$   
 CHI-SQUARED IS 1.158 WITH 4 DEGREES OF FREEDOM  
 NO SIG HETEROGENEITY  
 LOG LC<sub>50</sub> IS 1.881ppm  
 LC<sub>50</sub> IS 76.028ppm  
 95% CONF LIMITS ARE 70.642 TO 81.825ppm

**Appendix Table CLIII:** Lethal effects of root bark extract (Pet.E.) of *M. oleifera* against *A. salina* nauplii after 24h of exposure

Dose	Ldos	#U	Kl	%Kill	Cr%	E Pr	Ex Pr	Wk Pro	Weght	F Pro
100.000	2.000	30	26	86.667	87	6.13	5.959	6.136	14.13	5.940
90.000	1.954	30	23	76.667	77	5.74	5.697	5.730	16.74	5.679
80.000	1.903	30	19	63.333	63	5.33	5.405	5.321	18.03	5.386
70.000	1.845	30	13	43.333	43	4.82	5.073	4.825	19.11	5.054
60.000	1.778	30	10	33.333	33	4.56	4.690	4.551	18.03	4.671
50.000	1.699	30	9	30.000	30	4.48	4.237	4.490	15.09	4.218

REGRESSION EQUATION:  $Y = -5.502 + 5.721X$

CHI-SQUARED IS 3.039 WITH 4 DEGREES OF FREEDOM

NO SIG HETEROGENEITY

LOG LC<sub>50</sub> IS 1.836ppm

LC<sub>50</sub> IS 68.485ppm

95% CONF LIMITS ARE 63.149 TO 74.271ppm

**Appendix Table CLIV:** Lethal effects of root wood extract (Pet.E.) of *M. oleifera* against *A. salina* nauplii after 6h of exposure

Dose	Ldos	#U	Kl	%Kill	Cr%	E Pr	Ex Pr	Wk Pro	Weght	F Pro
100.000	2.000	30	14	46.667	47	4.92	4.788	4.922	18.48	4.815
90.000	1.954	30	5	16.667	17	4.05	4.299	4.048	15.09	4.297
80.000	1.903	30	4	13.333	13	3.87	3.752	3.894	10.08	3.718

REGRESSION EQUATION:  $Y = -17.811 + 11.313X$

CHI-SQUARED IS 1.462 WITH 1 DEGREES OF FREEDOM

NO SIG HETEROGENEITY

LOG LC<sub>50</sub> IS 2.016ppm

LC<sub>50</sub> IS 103.841ppm

95% CONF LIMITS ARE 93.403 TO 115.445ppm

**Appendix Table CLV:** Lethal effects of root wood extract (Pet.E.) of *M. oleifera* against *A. salina* nauplii after 12h of exposure

Dose	Ldos	#U	Kl	%Kill	Cr%	E Pr	Ex Pr	Wk Pro	Weght	F Pro
100.000	2.000	30	16	53.333	53	5.08	4.971	5.065	19.02	4.968
90.000	1.954	30	12	40.000	40	4.75	4.790	4.740	18.48	4.784
80.000	1.903	30	10	33.333	33	4.56	4.586	4.544	17.43	4.579
70.000	1.845	30	7	23.333	23	4.26	4.356	4.266	15.96	4.346
60.000	1.778	30	5	16.667	17	4.05	4.090	4.037	13.17	4.077
50.000	1.699	30	4	13.333	13	3.87	3.776	3.894	10.08	3.759

REGRESSION EQUATION:  $Y = -3.062 + 4.015X$

CHI-SQUARED IS 0.543 WITH 4 DEGREES OF FREEDOM

NO SIG HETEROGENEITY

LOG LC<sub>50</sub> IS 2.008ppm

LC<sub>50</sub> IS 101.871ppm

95% CONF LIMITS ARE 84.278 TO 123.136ppm

**Appendix Table CLVI:** Lethal effects of root wood extract (Pet.E.) of *M. oleifera* against *A. salina* nauplii after 18h of exposure

Dose	Ldos	#U	Kl	%Kill	Cr%	E Pr	Ex Pr	Wk Pro	Weght	F Pro
100.000	2.000	30	17	56.667	57	5.18	5.154	5.165	19.02	5.151
90.000	1.954	30	15	50.000	50	5.00	4.990	4.990	19.02	4.986
80.000	1.903	30	13	43.333	43	4.82	4.806	4.838	18.81	4.802
70.000	1.845	30	10	33.333	33	4.56	4.598	4.544	17.43	4.593
60.000	1.778	30	7	23.333	23	4.26	4.358	4.266	15.96	4.352
50.000	1.699	30	6	20.000	20	4.16	4.074	4.160	13.17	4.067

REGRESSION EQUATION:  $Y = -2.052 + 3.602X$

CHI-SQUARED IS 0.304 WITH 4 DEGREES OF FREEDOM

NO SIG HETEROGENEITY

LOG LC<sub>50</sub> IS 1.958ppm

LC<sub>50</sub> IS 90.784ppm

95% CONF LIMITS ARE 77.293 TO 106.629ppm

**Appendix Table CLVII:** Lethal effects of root wood extract (Pet.E.) of *M. oleifera* against *A. salina* nauplii after 24h of exposure

Dose	Ldos	#U	Kl	%Kill	Cr%	E Pr	Ex Pr	Wk Pro	Weght	F Pro
100.000	2.000	30	21	70.000	70	5.52	5.468	5.510	18.03	5.472
90.000	1.954	30	18	60.000	60	5.25	5.264	5.280	18.81	5.268
80.000	1.903	30	15	50.000	50	5.00	5.037	5.000	19.11	5.039
70.000	1.845	30	12	40.000	40	4.75	4.779	4.740	18.48	4.780
60.000	1.778	30	9	30.000	30	4.48	4.482	4.480	16.74	4.481
50.000	1.699	30	6	20.000	20	4.16	4.130	4.170	14.13	4.127

REGRESSION EQUATION:  $Y = -3.463 + 4.468X$

CHI-SQUARED IS 0.114 WITH 4 DEGREES OF FREEDOM

NO SIG HETEROGENEITY

LOG LC<sub>50</sub> IS 1.894ppm

LC<sub>50</sub> IS 78.393ppm

95% CONF LIMITS ARE 70.858 TO 86.729ppm

**Appendix Table CLVIII:** Lethal effects of fruit extract (CHCl<sub>3</sub>) of *M. oleifera* against *A. salina* nauplii after 6h of exposure

Dose	Ldos	#U	Kl	%Kill	Cr%	E Pr	Ex Pr	Wk Pro	Weght	F Pro
200.000	2.301	30	13	43.333	43	4.82	4.819	4.838	18.81	4.816
150.000	2.176	30	10	33.333	33	4.56	4.509	4.544	17.43	4.508
100.000	2.000	30	7	23.333	23	4.26	4.073	4.283	13.17	4.074
75.000	1.875	30	1	3.333	3	3.12	3.763	3.314	10.08	3.766
50.000	1.699	30	1	3.333	3	3.12	3.327	3.148	6.24	3.332
25.000	1.398	30	1	3.333	3	3.12	2.581	3.860	1.50	2.589

REGRESSION EQUATION:  $Y = -0.858 + 2.466X$

CHI-SQUARED IS 5.298 WITH 4 DEGREES OF FREEDOM

NO SIG HETEROGENEITY

LOG LC<sub>50</sub> IS 2.376ppm

LC<sub>50</sub> IS 237.446ppm

95% CONF LIMITS ARE 161.576 TO 348.943ppm

**Appendix Table CLIX:** Lethal effects of fruit extract (CHCl<sub>3</sub>) of *M. oleifera* against *A. salina* nauplii after 12h of exposure

Dose	Ldos	#U	Kl	%Kill	Cr%	E Pr	Ex Pr	Wk Pro	Weght	F Pro
200.000	2.301	30	22	73.333	73	5.61	5.541	5.584	17.43	5.558
150.000	2.176	30	15	50.000	50	5.00	5.170	4.990	19.02	5.192
100.000	2.000	30	14	46.667	47	4.92	4.648	4.929	18.03	4.676
75.000	1.875	30	9	30.000	30	4.48	4.277	4.490	15.09	4.310
50.000	1.699	30	1	3.333	3	3.12	3.754	3.314	10.08	3.794
25.000	1.398	30	1	3.333	3	3.12	2.860	3.256	2.76	2.911

REGRESSION EQUATION:  $Y = -1.187 + 2.931X$   
 CHI-SQUARED IS 5.079 WITH 4 DEGREES OF FREEDOM  
 NO SIG HETEROGENEITY  
 LOG LC<sub>50</sub> IS 2.111ppm  
 LC<sub>50</sub> IS 128.977ppm  
 95% CONF LIMITS ARE 107.588 TO 154.619ppm

**Appendix Table CLX:** Lethal effects of fruit extract (CHCl<sub>3</sub>) of *M. oleifera* against *A. salina* nauplii after 18h of exposure

Dose	Ldos	#U	Kl	%Kill	Cr%	E Pr	Ex Pr	Wk Pro	Weght	F Pro
200.000	2.301	30	26	86.667	87	6.13	6.075	6.087	13.17	6.078
150.000	2.176	30	25	83.333	83	5.95	5.712	5.926	15.96	5.703
100.000	2.000	30	16	53.333	53	5.08	5.200	5.098	18.81	5.175
75.000	1.875	30	11	36.667	37	4.67	4.837	4.682	18.81	4.800
50.000	1.699	30	5	16.667	17	4.05	4.325	4.074	15.96	4.272
25.000	1.398	30	3	10.000	10	3.72	3.451	3.810	7.14	3.369

REGRESSION EQUATION:  $Y = -0.823 + 2.999X$   
 CHI-SQUARED IS 3.182 WITH 4 DEGREES OF FREEDOM  
 NO SIG HETEROGENEITY  
 LOG LC<sub>50</sub> IS 1.942ppm  
 LC<sub>50</sub> IS 87.433ppm  
 95% CONF LIMITS ARE 74.593 TO 102.482ppm

**Appendix Table CLXI:** Lethal effects of fruit extract (CHCl<sub>3</sub>) of *M. oleifera* against *A. salina* nauplii after 24h of exposure

Dose	Ldos	#U	Kl	%Kill	Cr%	E Pr	Ex Pr	Wk Pro	Weght	F Pro
200.000	2.301	30	27	90.000	90	6.28	6.265	6.230	11.10	6.242
150.000	2.176	30	26	86.667	87	6.13	5.927	6.136	14.13	5.900
100.000	2.000	30	20	66.667	67	5.44	5.450	5.429	18.03	5.418
75.000	1.875	30	14	46.667	47	4.92	5.112	4.915	19.02	5.077
50.000	1.699	30	8	26.667	27	4.39	4.635	4.389	18.03	4.595
25.000	1.398	30	5	16.667	17	4.05	3.821	4.077	11.10	3.771

REGRESSION EQUATION:  $Y = -0.055 + 2.737X$   
 CHI-SQUARED IS 3.088 WITH 4 DEGREES OF FREEDOM  
 NO SIG HETEROGENEITY  
 LOG LC<sub>50</sub> IS 1.847ppm  
 LC<sub>50</sub> IS 70.318ppm  
 95% CONF LIMITS ARE 58.939 TO 83.892ppm

**Appendix Table CLXII:** Lethal effects of stem bark extract (CHCl<sub>3</sub>) of *M. oleifera* against *A. salina* nauplii after 6h of exposure

Dose	Ldos	#U	Kl	%Kill	Cr%	E Pr	Ex Pr	Wk Pro	Weght	F Pro
100.000	2.000	30	21	70.000	70	5.52	5.264	5.540	18.81	5.262
75.000	1.875	30	10	33.333	33	4.56	4.834	4.578	18.81	4.835
50.000	1.699	30	5	16.667	17	4.05	4.229	4.048	15.09	4.233
25.000	1.398	30	2	6.667	7	3.52	3.193	3.724	4.62	3.204

REGRESSION EQUATION:  $Y = -1.573 + 3.418X$

CHI-SQUARED IS 4.461 WITH 2 DEGREES OF FREEDOM

NO SIG HETEROGENEITY

LOG LC<sub>50</sub> IS 1.923ppm

LC<sub>50</sub> IS 83.813ppm

95% CONF LIMITS ARE 68.777 TO 102.137ppm

**Appendix Table CLXIII:** Lethal effects of stem bark extract (CHCl<sub>3</sub>) of *M. oleifera* against *A. salina* nauplii after 12h of exposure

Dose	Ldos	#U	Kl	%Kill	Cr%	E Pr	Ex Pr	Wk Pro	Weght	F Pro
100.000	2.000	30	21	70.000	70	5.52	5.173	5.490	19.02	5.163
75.000	1.875	30	13	43.333	43	4.82	4.902	4.815	19.02	4.893
50.000	1.699	30	6	20.000	20	4.16	4.518	4.180	17.43	4.512
25.000	1.398	30	2	6.667	7	3.52	3.864	3.567	11.10	3.862
12.500	1.097	30	3	10.000	10	3.72	3.209	4.010	5.40	3.211

REGRESSION EQUATION:  $Y = 0.840 + 2.161X$

CHI-SQUARED IS 8.485 WITH 3 DEGREES OF FREEDOM

VARIANCE HAS BEEN ADJUSTED FOR HETEROGENEITY

LOG LC<sub>50</sub> IS 1.925ppm

LC<sub>50</sub> IS 84.075ppm

95% CONF LIMITS ARE 50.578 TO 139.757ppm

**Appendix Table CLXIV:** Lethal effects of stem bark extract (CHCl<sub>3</sub>) of *M. oleifera* against *A. salina* nauplii after 18h of exposure

Dose	Ldos	#U	Kl	%Kill	Cr%	E Pr	Ex Pr	Wk Pro	Weght	F Pro
100.000	2.000	30	22	73.333	73	5.61	5.260	5.618	18.81	5.291
75.000	1.875	30	15	50.000	50	5.00	5.070	5.000	19.11	5.091
50.000	1.699	30	10	33.333	33	4.56	4.802	4.578	18.81	4.808
25.000	1.398	30	5	16.667	17	4.05	4.344	4.074	15.96	4.325
12.500	1.097	30	5	16.667	17	4.05	3.886	4.077	11.10	3.841
6.250	0.796	30	2	6.667	7	3.52	3.428	3.540	7.14	3.358

REGRESSION EQUATION:  $Y = 2.080 + 1.606X$

CHI-SQUARED IS 5.016 WITH 4 DEGREES OF FREEDOM

NO SIG HETEROGENEITY

LOG LC<sub>50</sub> IS 1.819ppm

LC<sub>50</sub> IS 65.847ppm

95% CONF LIMITS ARE 46.821 TO 92.606ppm

**Appendix Table CLXV:** Lethal effects of stem bark extract (CHCl<sub>3</sub>) of *M. oleifera* against *A. salina* nauplii after 24h of exposure

Dose	Ldos	#U	Kl	%Kill	Cr%	E Pr	Ex Pr	Wk Pro	Weght	F Pro
100.000	2.000	30	23	76.667	77	5.74	5.407	5.699	18.03	5.404
75.000	1.875	30	16	53.333	53	5.08	5.221	5.098	18.81	5.214
50.000	1.699	30	13	43.333	43	4.82	4.959	4.815	19.02	4.945
25.000	1.398	30	7	23.333	23	4.26	4.512	4.264	17.43	4.486
12.500	1.097	30	6	20.000	20	4.16	4.064	4.160	13.17	4.027
6.250	0.796	30	3	10.000	10	3.72	3.617	3.730	9.06	3.568

REGRESSION EQUATION:  $Y = 2.354 + 1.525X$

CHI-SQUARED IS 3.471 WITH 4 DEGREES OF FREEDOM

NO SIG HETEROGENEITY

LOG LC<sub>50</sub> IS 1.735ppm

LC<sub>50</sub> IS 54.302ppm

95% CONF LIMITS ARE 39.036 TO 75.536ppm

**Appendix Table CLXVI:** Lethal effects of stem wood extract (CHCl<sub>3</sub>) of *M. oleifera* against *A. salina* nauplii after 6h of exposure

Dose	Ldos	#U	Kl	%Kill	Cr%	E Pr	Ex Pr	Wk Pro	Weght	F Pro
75.000	1.875	30	18	60.000	60	5.25	5.235	5.280	18.81	5.308
50.000	1.699	30	14	46.667	47	4.92	4.849	4.942	18.81	4.902
25.000	1.398	30	7	23.333	23	4.26	4.189	4.284	14.13	4.210
12.500	1.097	30	1	3.333	3	3.12	3.529	3.211	8.07	3.517
6.250	0.796	30	1	3.333	3	3.12	2.868	3.256	2.76	2.824

REGRESSION EQUATION:  $Y = 0.992 + 2.302X$

CHI-SQUARED IS 1.392 WITH 3 DEGREES OF FREEDOM

NO SIG HETEROGENEITY

LOG LC<sub>50</sub> IS 1.741ppm

LC<sub>50</sub> IS 55.125ppm

95% CONF LIMITS ARE 41.454 TO 73.304ppm

**Appendix Table CLXVII:** Lethal effects of stem wood extract (CHCl<sub>3</sub>) of *M. oleifera* against *A. salina* nauplii after 12h of exposure

Dose	Ldos	#U	Kl	%Kill	Cr%	E Pr	Ex Pr	Wk Pro	Weght	F Pro
75.000	1.875	30	25	83.333	83	5.95	5.774	5.926	15.96	5.773
50.000	1.699	30	19	63.333	63	5.33	5.347	5.318	18.48	5.346
25.000	1.398	30	9	30.000	30	4.48	4.617	4.470	18.03	4.617
12.500	1.097	30	3	10.000	10	3.72	3.887	3.720	11.10	3.888
6.250	0.796	30	2	6.667	7	3.52	3.157	3.724	4.62	3.159

REGRESSION EQUATION:  $Y = 1.232 + 2.422X$

CHI-SQUARED IS 2.568 WITH 3 DEGREES OF FREEDOM

NO SIG HETEROGENEITY

LOG LC<sub>50</sub> IS 1.556ppm

LC<sub>50</sub> IS 35.967ppm

95% CONF LIMITS ARE 28.611 TO 45.214ppm

**Appendix Table CLXVIII:** Lethal effects of stem wood extract (CHCl<sub>3</sub>) of *M. oleifera* against *A. salina* nauplii after 18h of exposure

Dose	Ldos	#U	Kl	%Kill	Cr%	E Pr	Ex Pr	Wk Pro	Weght	F Pro
75.000	1.875	30	27	90.000	90	6.28	5.979	6.250	14.13	5.974
50.000	1.699	30	21	70.000	70	5.52	5.526	5.500	17.43	5.522
25.000	1.398	30	9	30.000	30	4.48	4.751	4.480	18.48	4.750
12.500	1.097	30	3	10.000	10	3.72	3.976	3.740	12.15	3.977
6.250	0.796	30	3	10.000	10	3.72	3.201	4.010	5.40	3.205

REGRESSION EQUATION:  $Y = 1.164 + 2.565X$

CHI-SQUARED IS 6.614 WITH 3 DEGREES OF FREEDOM

NO SIG HETEROGENEITY

LOG LC<sub>50</sub> IS 1.496ppm

LC<sub>50</sub> IS 31.297ppm

95% CONF LIMITS ARE 25.256 TO 38.783ppm

**Appendix Table CLXIX:** Lethal effects of stem wood extract (CHCl<sub>3</sub>) of *M. oleifera* against *A. salina* nauplii after 24h of exposure

Dose	Ldos	#U	Kl	%Kill	Cr%	E Pr	Ex Pr	Wk Pro	Weght	F Pro
75.000	1.875	30	28	93.333	93	6.48	6.116	6.408	12.15	6.028
50.000	1.699	30	22	73.333	73	5.61	5.730	5.606	15.96	5.652
25.000	1.398	30	11	36.667	37	4.67	5.069	4.675	19.11	5.008
12.500	1.097	30	7	23.333	23	4.26	4.408	4.270	16.74	4.365
6.250	0.796	30	5	16.667	17	4.05	3.747	4.126	10.08	3.721

REGRESSION EQUATION:  $Y = 2.016 + 2.138X$

CHI-SQUARED IS 5.710 WITH 3 DEGREES OF FREEDOM

NO SIG HETEROGENEITY

LOG LC<sub>50</sub> IS 1.394ppm

LC<sub>50</sub> IS 24.774ppm

95% CONF LIMITS ARE 19.385 TO 31.663ppm

**Appendix Table CLXX:** Lethal effects of root bark extract (CHCl<sub>3</sub>) of *M. oleifera* against *A. salina* nauplii after 6h of exposure

Dose	Ldos	#U	Kl	%Kill	Cr%	E Pr	Ex Pr	Wk Pro	Weght	F Pro
50.000	1.699	30	9	30.000	30	4.48	4.463	4.480	16.74	4.464
25.000	1.398	30	5	16.667	17	4.05	4.083	4.037	13.17	4.078
12.500	1.097	30	3	10.000	10	3.72	3.703	3.720	10.08	3.693

REGRESSION EQUATION:  $Y = 2.289 + 1.280X$

CHI-SQUARED IS 0.034 WITH 1 DEGREES OF FREEDOM

NO SIG HETEROGENEITY

LOG LC<sub>50</sub> IS 2.118ppm

LC<sub>50</sub> IS 131.175ppm

95% CONF LIMITS ARE 25.336 TO 679.159ppm

**Appendix Table CLXXI:** Lethal effects of root bark extract (CHCl<sub>3</sub>) of *M. oleifera* against *A. salina* nauplii after 12h of exposure

Dose	Ldos	#U	Kl	%Kill	Cr%	E Pr	Ex Pr	Wk Pro	Weght	F Pro
50.000	1.699	30	14	46.667	47	4.92	4.878	4.942	18.81	4.892
25.000	1.398	30	13	43.333	43	4.82	4.744	4.818	18.48	4.752
12.500	1.097	30	9	30.000	30	4.48	4.610	4.470	18.03	4.613
6.250	0.796	30	8	26.667	27	4.39	4.476	4.390	16.74	4.474
3.125	0.495	30	8	26.667	27	4.39	4.343	4.394	15.96	4.334
1.563	0.194	30	7	23.333	23	4.26	4.209	4.252	15.09	4.195

REGRESSION EQUATION:  $Y = 4.105 + 0.463X$   
 CHI-SQUARED IS 0.719 WITH 4 DEGREES OF FREEDOM  
 NO SIG HETEROGENEITY  
 LOG LC<sub>50</sub> IS 1.933ppm  
 LC<sub>50</sub> IS 85.637ppm  
 95% CONF LIMITS ARE 11.337 TO 646.899ppm

**Appendix Table CLXXII:** Lethal effects of root bark extract (CHCl<sub>3</sub>) of *M. oleifera* against *A. salina* nauplii after 18h of exposure

Dose	Ldos	#U	Kl	%Kill	Cr%	E Pr	Ex Pr	Wk Pro	Weght	F Pro
50.000	1.699	30	19	63.333	63	5.33	5.333	5.318	18.48	5.318
25.000	1.398	30	20	66.667	67	5.44	5.161	5.415	19.02	5.149
12.500	1.097	30	12	40.000	40	4.75	4.989	4.740	19.02	4.980
6.250	0.796	30	11	36.667	37	4.67	4.817	4.682	18.81	4.812
3.125	0.495	30	10	33.333	33	4.56	4.645	4.551	18.03	4.643
1.563	0.194	30	11	36.667	37	4.67	4.473	4.690	16.74	4.474

REGRESSION EQUATION:  $Y = 4.366 + 0.560X$   
 CHI-SQUARED IS 3.692 WITH 4 DEGREES OF FREEDOM  
 NO SIG HETEROGENEITY  
 LOG LC<sub>50</sub> IS 1.132ppm  
 LC<sub>50</sub> IS 13.548ppm  
 95% CONF LIMITS ARE 6.032 TO 30.432ppm

**Appendix Table CLXXIII:** Lethal effects of root bark extract (CHCl<sub>3</sub>) of *M. oleifera* against *A. salina* nauplii after 24h of exposure

Dose	Ldos	#U	Kl	%Kill	Cr%	E Pr	Ex Pr	Wk Pro	Weght	F Pro
50.000	1.699	30	26	86.667	87	6.13	6.048	6.087	13.17	5.988
25.000	1.398	30	25	83.333	83	5.95	5.758	5.926	15.96	5.711
12.500	1.097	30	17	56.667	57	5.18	5.467	5.159	18.03	5.435
6.250	0.796	30	15	50.000	50	5.00	5.176	4.990	19.02	5.159
3.125	0.495	30	14	46.667	47	4.92	4.886	4.942	18.81	4.882
1.563	0.194	30	12	40.000	40	4.75	4.595	4.740	17.43	4.606

REGRESSION EQUATION:  $Y = 4.428 + 0.918X$   
 CHI-SQUARED IS 3.159 WITH 4 DEGREES OF FREEDOM  
 NO SIG HETEROGENEITY  
 LOG LC<sub>50</sub> IS 0.623ppm  
 LC<sub>50</sub> IS 4.197ppm  
 95% CONF LIMITS ARE 2.404 TO 7.328ppm



**Appendix Table CLXXIV:** Lethal effects of root wood extract (CHCl<sub>3</sub>) of *M. oleifera* against *A. salina* nauplii after 6h of exposure

Dose	Ldos	#U	Kl	%Kill	Cr%	E Pr	Ex Pr	Wk Pro	Weght	F Pro
200.000	2.301	30	7	23.333	23	4.26	4.171	4.284	14.13	4.164
150.000	2.176	30	5	16.667	17	4.05	4.027	4.037	13.17	4.021
100.000	2.000	30	3	10.000	10	3.72	3.824	3.720	11.10	3.819
50.000	1.699	30	1	3.333	3	3.12	3.476	3.180	7.14	3.475
25.000	1.398	30	1	3.333	3	3.12	3.128	3.116	4.62	3.130
12.500	1.097	30	1	3.333	3	3.12	2.780	3.379	2.28	2.786

REGRESSION EQUATION:  $Y = 1.530 + 1.145X$   
 CHI-SQUARED IS 1.741 WITH 4 DEGREES OF FREEDOM  
 NO SIG HETEROGENEITY  
 LOG LC<sub>50</sub> IS 3.032ppm  
 LC<sub>50</sub> IS 1075.790ppm  
 95% CONF LIMITS ARE 181.773 TO 6366.888ppm

**Appendix Table CLXXV:** Lethal effects of root wood extract (CHCl<sub>3</sub>) of *M. oleifera* against *A. salina* nauplii after 12h of exposure

Dose	Ldos	#U	Kl	%Kill	Cr%	E Pr	Ex Pr	Wk Pro	Weght	F Pro
200.000	2.301	30	10	33.333	33	4.56	4.565	4.544	17.43	4.545
150.000	2.176	30	9	30.000	30	4.48	4.522	4.460	17.43	4.505
100.000	2.000	30	8	26.667	27	4.39	4.460	4.390	16.74	4.449
50.000	1.699	30	8	26.667	27	4.39	4.355	4.394	15.96	4.353
25.000	1.398	30	8	26.667	27	4.39	4.251	4.388	15.09	4.258
12.500	1.097	30	7	23.333	23	4.26	4.146	4.284	14.13	4.162
6.250	0.796	30	4	13.333	13	3.87	4.041	3.873	13.17	4.067

REGRESSION EQUATION:  $Y = 3.814 + 0.318X$   
 CHI-SQUARED IS 1.079 WITH 5 DEGREES OF FREEDOM  
 NO SIG HETEROGENEITY  
 LOG LC<sub>50</sub> IS 3.735ppm  
 LC<sub>50</sub> IS 5437.378ppm  
 95% CONF LIMITS ARE 20.780 TO 1422742ppm

**Appendix Table CLXXVI:** Lethal effects of root wood extract (CHCl<sub>3</sub>) of *M. oleifera* against *A. salina* nauplii after 18h of exposure

Dose	Ldos	#U	Kl	%Kill	Cr%	E Pr	Ex Pr	Wk Pro	Weght	F Pro
200.000	2.301	30	16	53.333	53	5.08	5.118	5.065	19.02	5.104
150.000	2.176	30	15	50.000	50	5.00	5.045	5.000	19.11	5.033
100.000	2.000	30	14	46.667	47	4.92	4.941	4.915	19.02	4.933
50.000	1.699	30	13	43.333	43	4.82	4.764	4.818	18.48	4.761
25.000	1.398	30	11	36.667	37	4.67	4.587	4.656	17.43	4.590
12.500	1.097	30	10	33.333	33	4.56	4.410	4.570	16.74	4.419
6.250	0.796	30	5	16.667	17	4.05	4.233	4.048	15.09	4.248

REGRESSION EQUATION:  $Y = 3.795 + 0.569X$   
 CHI-SQUARED IS 1.173 WITH 5 DEGREES OF FREEDOM  
 NO SIG HETEROGENEITY  
 LOG LC<sub>50</sub> IS 2.118ppm  
 LC<sub>50</sub> IS 131.251ppm  
 95% CONF LIMITS ARE 51.512 TO 334.423ppm

**Appendix Table CLXXVII:** Lethal effects of root wood extract (CHCl<sub>3</sub>) of *M. oleifera* against *A. salina* nauplii after 24h of exposure

Dose	Ldos	#U	Kl	%Kill	Cr%	E Pr	Ex Pr	Wk Pro	Weght	F Pro
200.000	2.301	30	23	76.667	77	5.74	5.636	5.730	16.74	5.614
150.000	2.176	30	20	66.667	67	5.44	5.518	5.416	17.43	5.500
100.000	2.000	30	18	60.000	60	5.25	5.352	5.240	18.48	5.339
50.000	1.699	30	15	50.000	50	5.00	5.067	5.000	19.11	5.064
25.000	1.398	30	14	46.667	47	4.92	4.783	4.922	18.48	4.789
12.500	1.097	30	11	36.667	37	4.67	4.499	4.690	16.74	4.513
6.250	0.796	30	5	16.667	17	4.05	4.215	4.048	15.09	4.238

REGRESSION EQUATION:  $Y = 3.511 + 0.914X$

CHI-SQUARED IS 2.003 WITH 5 DEGREES OF FREEDOM

NO SIG HETEROGENEITY

LOG LC<sub>50</sub> IS 1.629ppm

LC<sub>50</sub> IS 42.595ppm

95% CONF LIMITS ARE 27.225 TO 66.642ppm

**Appendix Table CLXXVIII:** Lethal effects of fruit extract (CH<sub>3</sub>OH) of *M. oleifera* against *A. salina* nauplii after 6h of exposure

Dose	Ldos	#U	Kl	%Kill	Cr%	E Pr	Ex Pr	Wk Pro	Weght	F Pro
200.000	2.301	30	5	16.667	17	4.05	4.112	4.056	14.13	4.113
150.000	2.176	30	5	16.667	17	4.05	3.996	4.062	12.15	3.999
100.000	2.000	30	4	13.333	13	3.87	3.831	3.873	11.10	3.838
50.000	1.699	30	2	6.667	7	3.52	3.551	3.519	8.07	3.563

REGRESSION EQUATION:  $Y = 2.011 + 0.914X$

CHI-SQUARED IS 0.124 WITH 2 DEGREES OF FREEDOM

NO SIG HETEROGENEITY

LOG LC<sub>50</sub> IS 3.272ppm

LC<sub>50</sub> IS 1871.084ppm

95% CONF LIMITS ARE 29.182 TO 119968.400ppm

**Appendix Table CLXXIX:** Lethal effects of fruit extract (CH<sub>3</sub>OH) of *M. oleifera* against *A. salina* nauplii after 12h of exposure

Dose	Ldos	#U	Kl	%Kill	Cr%	E Pr	Ex Pr	Wk Pro	Weght	F Pro
200.000	2.301	30	11	36.667	37	4.67	4.589	4.656	17.43	4.584
150.000	2.176	30	8	26.667	27	4.39	4.438	4.390	16.74	4.433
100.000	2.000	30	6	20.000	20	4.16	4.225	4.150	15.09	4.220
50.000	1.699	30	4	13.333	13	3.87	3.861	3.873	11.10	3.857
25.000	1.398	30	2	6.667	7	3.52	3.498	3.540	7.14	3.493

REGRESSION EQUATION:  $Y = 1.806 + 1.207X$

CHI-SQUARED IS 0.215 WITH 3 DEGREES OF FREEDOM

NO SIG HETEROGENEITY

LOG LC<sub>50</sub> IS 2.646ppm

LC<sub>50</sub> IS 442.596ppm

95% CONF LIMITS ARE 147.538 TO 1327.737ppm

**Appendix Table CLXXX:** Lethal effects of fruit extract (CH<sub>3</sub>OH) of *M. oleifera* against *A. salina* nauplii after 18h of exposure

Dose	Ldos	#U	Kl	%Kill	Cr%	E Pr	Ex Pr	Wk Pro	Weght	F Pro
200.000	2.301	30	17	56.667	57	5.18	5.086	5.175	19.11	5.089
150.000	2.176	30	13	43.333	43	4.82	4.899	4.838	18.81	4.902
100.000	2.000	30	10	33.333	33	4.56	4.636	4.551	18.03	4.639
50.000	1.699	30	7	23.333	23	4.26	4.185	4.284	14.13	4.189
25.000	1.398	30	3	10.000	10	3.72	3.734	3.720	10.08	3.740

REGRESSION EQUATION:  $Y = 1.651 + 1.494X$

CHI-SQUARED IS 0.489 WITH 3 DEGREES OF FREEDOM

NO SIG HETEROGENEITY

LOG LC<sub>50</sub> IS 2.242ppm

LC<sub>50</sub> IS 174.437ppm

95% CONF LIMITS ARE 111.856 TO 272.031ppm

**Appendix Table CLXXXI:** Lethal effects of fruit extract (CH<sub>3</sub>OH) of *M. oleifera* against *A. salina* nauplii after 24h of exposure

Dose	Ldos	#U	Kl	%Kill	Cr%	E Pr	Ex Pr	Wk Pro	Weght	F Pro
200.000	2.301	30	22	73.333	73	5.61	5.537	5.584	17.43	5.520
150.000	2.176	30	19	63.333	63	5.33	5.324	5.318	18.48	5.308
100.000	2.000	30	14	46.667	47	4.92	5.023	4.925	19.11	5.008
50.000	1.699	30	9	30.000	30	4.48	4.510	4.460	17.43	4.497
25.000	1.398	30	5	16.667	17	4.05	3.997	4.062	12.15	3.985

REGRESSION EQUATION:  $Y = 1.609 + 1.699X$

CHI-SQUARED IS 0.301 WITH 3 DEGREES OF FREEDOM

NO SIG HETEROGENEITY

LOG LC<sub>50</sub> IS 1.995ppm

LC<sub>50</sub> IS 98.856ppm

95% CONF LIMITS ARE 73.858 TO 132.315ppm

**Appendix Table CLXXXII:** Lethal effects of leaf extract (CH<sub>3</sub>OH) of *M. oleifera* against *A. salina* nauplii after 6h of exposure

Dose	Ldos	#U	Kl	%Kill	Cr%	E Pr	Ex Pr	Wk Pro	Weght	F Pro
200.000	2.301	30	8	26.667	27	4.39	4.248	4.388	15.09	4.265
150.000	2.176	30	3	10.000	10	3.72	4.097	3.750	13.17	4.117
100.000	2.000	30	6	20.000	20	4.16	3.885	4.230	11.10	3.908
50.000	1.699	30	2	6.667	7	3.52	3.522	3.519	8.07	3.550
25.000	1.398	30	1	3.333	3	3.12	3.158	3.116	4.62	3.193

REGRESSION EQUATION:  $Y = 1.534 + 1.187X$

CHI-SQUARED IS 3.188 WITH 3 DEGREES OF FREEDOM

NO SIG HETEROGENEITY

LOG LC<sub>50</sub> IS 2.921ppm

LC<sub>50</sub> IS 832.692ppm

95% CONF LIMITS ARE 145.449 TO 4767.125ppm

**Appendix Table CLXXXIII:** Lethal effects of leaf extract (CH<sub>3</sub>OH) of *M. oleifera* against *A. salina* nauplii after 12h of exposure

Dose	Ldos	#U	Kl	%Kill	Cr%	E Pr	Ex Pr	Wk Pro	Weght	F Pro
200.000	2.301	30	11	36.667	37	4.67	4.467	4.690	16.74	4.508
150.000	2.176	30	5	16.667	17	4.05	4.317	4.074	15.96	4.351
100.000	2.000	30	7	23.333	23	4.26	4.106	4.284	14.13	4.129
50.000	1.699	30	2	6.667	7	3.52	3.745	3.546	10.08	3.750
25.000	1.398	30	2	6.667	7	3.52	3.384	3.572	6.24	3.371

REGRESSION EQUATION:  $Y = 1.610 + 1.259X$

CHI-SQUARED IS 2.788 WITH 3 DEGREES OF FREEDOM

NO SIG HETEROGENEITY

LOG LC<sub>50</sub> IS 2.691ppm

LC<sub>50</sub> IS 491.176ppm

95% CONF LIMITS ARE 154.813 TO 1558.362ppm

**Appendix Table CLXXXIV:** Lethal effects of leaf extract (CH<sub>3</sub>OH) of *M. oleifera* against *A. salina* nauplii after 18h of exposure

Dose	Ldos	#U	Kl	%Kill	Cr%	E Pr	Ex Pr	Wk Pro	Weght	F Pro
200.000	2.301	30	14	46.667	47	4.92	4.702	4.922	18.48	4.718
150.000	2.176	30	6	20.000	20	4.16	4.537	4.180	17.43	4.550
100.000	2.000	30	9	30.000	30	4.48	4.303	4.490	15.96	4.312
50.000	1.699	30	4	13.333	13	3.87	3.904	3.878	12.15	3.905
25.000	1.398	30	2	6.667	7	3.52	3.504	3.519	8.07	3.499

REGRESSION EQUATION:  $Y = 1.611 + 1.351X$

CHI-SQUARED IS 3.667 WITH 3 DEGREES OF FREEDOM

NO SIG HETEROGENEITY

LOG LC<sub>50</sub> IS 2.509ppm

LC<sub>50</sub> IS 323.301ppm

95% CONF LIMITS ARE 147.414 TO 709.045ppm

**Appendix Table CLXXXV:** Lethal effects of leaf extract (CH<sub>3</sub>OH) of *M. oleifera* against *A. salina* nauplii after 24h of exposure

Dose	Ldos	#U	Kl	%Kill	Cr%	E Pr	Ex Pr	Wk Pro	Weght	F Pro
200.000	2.301	30	15	50.000	50	5.00	4.912	4.990	19.02	4.905
150.000	2.176	30	11	36.667	37	4.67	4.741	4.662	18.48	4.732
100.000	2.000	30	9	30.000	30	4.48	4.500	4.460	17.43	4.489
50.000	1.699	30	5	16.667	17	4.05	4.089	4.037	13.17	4.073
25.000	1.398	30	3	10.000	10	3.72	3.677	3.730	9.06	3.657

REGRESSION EQUATION:  $Y = 1.725 + 1.382X$

CHI-SQUARED IS 0.309 WITH 3 DEGREES OF FREEDOM

NO SIG HETEROGENEITY

LOG LC<sub>50</sub> IS 2.369ppm

LC<sub>50</sub> IS 234.246ppm

95% CONF LIMITS ARE 128.558 TO 426.819ppm

**Appendix Table CLXXXVI:** Lethal effects of stem bark extract (CH<sub>3</sub>OH) of *M. oleifera* against *A. salina* nauplii after 6h of exposure

Dose	Ldos	#U	Kl	%Kill	Cr%	E Pr	Ex Pr	Wk Pro	Weght	F Pro
90.000	1.954	30	7	23.333	23	4.26	4.300	4.252	15.09	4.295
70.000	1.845	30	6	20.000	20	4.16	4.167	4.170	14.13	4.165
50.000	1.699	30	5	16.667	17	4.05	3.989	4.062	12.15	3.991
30.000	1.477	30	3	10.000	10	3.72	3.718	3.720	10.08	3.727
10.000	1.000	30	1	3.333	3	3.12	3.137	3.116	4.62	3.159

REGRESSION EQUATION:  $Y = 1.969 + 1.190X$

CHI-SQUARED IS 0.098 WITH 3 DEGREES OF FREEDOM

NO SIG HETEROGENEITY

LOG LC<sub>50</sub> IS 2.546ppm

LC<sub>50</sub> IS 351.887ppm

95% CONF LIMITS ARE 66.673 TO 1857.190ppm

**Appendix Table CLXXXVII:** Lethal effects of stem bark extract (CH<sub>3</sub>OH) of *M. oleifera* against *A. salina* nauplii after 12h of exposure

Dose	Ldos	#U	Kl	%Kill	Cr%	E Pr	Ex Pr	Wk Pro	Weght	F Pro
90.000	1.954	30	8	26.667	27	4.39	4.370	4.394	15.96	4.362
70.000	1.845	30	6	20.000	20	4.16	4.281	4.150	15.09	4.279
50.000	1.699	30	6	20.000	20	4.16	4.162	4.170	14.13	4.169
30.000	1.477	30	6	20.000	20	4.16	3.982	4.200	12.15	4.002
10.000	1.000	30	2	6.667	7	3.52	3.594	3.519	8.07	3.641

REGRESSION EQUATION:  $Y = 2.886 + 0.755X$

CHI-SQUARED IS 0.869 WITH 3 DEGREES OF FREEDOM

NO SIG HETEROGENEITY

LOG LC<sub>50</sub> IS 2.799ppm

LC<sub>50</sub> IS 629.882ppm

95% CONF LIMITS ARE 34.830 TO 11390.930ppm

**Appendix Table CLXXXVIII:** Lethal effects of stem bark extract (CH<sub>3</sub>OH) of *M. oleifera* against *A. salina* nauplii after 18h of exposure

Dose	Ldos	#U	Kl	%Kill	Cr%	E Pr	Ex Pr	Wk Pro	Weght	F Pro
90.000	1.954	30	13	43.333	43	4.82	4.662	4.821	18.03	4.659
70.000	1.845	30	7	23.333	23	4.26	4.537	4.264	17.43	4.538
50.000	1.699	30	8	26.667	27	4.39	4.369	4.394	15.96	4.375
30.000	1.477	30	7	23.333	23	4.26	4.115	4.284	14.13	4.128
10.000	1.000	30	2	6.667	7	3.52	3.567	3.519	8.07	3.597

REGRESSION EQUATION:  $Y = 2.484 + 1.113X$

CHI-SQUARED IS 2.176 WITH 3 DEGREES OF FREEDOM

NO SIG HETEROGENEITY

LOG LC<sub>50</sub> IS 2.260ppm

LC<sub>50</sub> IS 182.039ppm

95% CONF LIMITS ARE 62.790 TO 527.766ppm

**Appendix Table CLXXXIX:** Lethal effects of stem bark extract (CH<sub>3</sub>OH) of *M. oleifera* against *A. salina* nauplii after 24h of exposure

Dose	Ldos	#U	Kl	%Kill	Cr%	E Pr	Ex Pr	Wk Pro	Weght	F Pro
90.000	1.954	30	20	66.667	67	5.44	4.922	5.415	19.02	4.929
70.000	1.845	30	10	33.333	33	4.56	4.840	4.578	18.81	4.842
50.000	1.699	30	9	30.000	30	4.48	4.730	4.480	18.48	4.726
30.000	1.477	30	8	26.667	27	4.39	4.563	4.376	17.43	4.549
10.000	1.000	30	8	26.667	27	4.39	4.204	4.388	15.09	4.170

REGRESSION EQUATION:  $Y = 3.374 + 0.796X$   
 CHI-SQUARED IS 8.165 WITH 3 DEGREES OF FREEDOM  
 VARIANCE HAS BEEN ADJUSTED FOR HETEROGENEITY  
 LOG LC<sub>50</sub> IS 2.043ppm  
 LC<sub>50</sub> IS 110.484ppm  
 95% CONF LIMITS ARE 21.730 TO 561.736ppm

**Appendix Table CXC:** Lethal effects of stem wood extract (CH<sub>3</sub>OH) of *M. oleifera* against *A. salina* nauplii after 6h of exposure

Dose	Ldos	#U	Kl	%Kill	Cr%	E Pr	Ex Pr	Wk Pro	Weght	F Pro
250.000	2.398	30	11	36.667	37	4.67	4.571	4.656	17.43	4.566
200.000	2.301	30	10	33.333	33	4.56	4.513	4.544	17.43	4.510
150.000	2.176	30	8	26.667	27	4.39	4.439	4.390	16.74	4.436
100.000	2.000	30	6	20.000	20	4.16	4.334	4.170	15.96	4.333
50.000	1.699	30	6	20.000	20	4.16	4.156	4.170	14.13	4.156
25.000	1.398	30	5	16.667	17	4.05	3.977	4.062	12.15	3.979

REGRESSION EQUATION:  $Y = 3.158 + 0.587X$   
 CHI-SQUARED IS 0.705 WITH 4 DEGREES OF FREEDOM  
 NO SIG HETEROGENEITY  
 LOG LC<sub>50</sub> IS 3.136ppm  
 LC<sub>50</sub> IS 1368.165ppm  
 95% CONF LIMITS ARE 88.930 TO 21048.870ppm

**Appendix Table CXCI:** Lethal effects of stem wood extract (CH<sub>3</sub>OH) of *M. oleifera* against *A. salina* nauplii after 12h of exposure

Dose	Ldos	#U	Kl	%Kill	Cr%	E Pr	Ex Pr	Wk Pro	Weght	F Pro
250.000	2.398	30	12	40.000	40	4.75	4.714	4.740	18.48	4.703
200.000	2.301	30	11	36.667	37	4.67	4.643	4.659	18.03	4.634
150.000	2.176	30	10	33.333	33	4.56	4.551	4.544	17.43	4.543
100.000	2.000	30	7	23.333	23	4.26	4.422	4.270	16.74	4.416
50.000	1.699	30	7	23.333	23	4.26	4.201	4.252	15.09	4.199
25.000	1.398	30	5	16.667	17	4.05	3.980	4.062	12.15	3.982
12.500	1.097	30	3	10.000	10	3.72	3.759	3.720	10.08	3.765

REGRESSION EQUATION:  $Y = 2.974 + 0.721X$   
 CHI-SQUARED IS 0.535 WITH 5 DEGREES OF FREEDOM  
 NO SIG HETEROGENEITY  
 LOG LC<sub>50</sub> IS 2.809ppm  
 LC<sub>50</sub> IS 644.586ppm  
 95% CONF LIMITS ARE 163.154 TO 2546.632ppm

**Appendix Table CXCI:** Lethal effects of stem wood extract (CH<sub>3</sub>OH) of *M. oleifera* against *A. salina* nauplii after 18h of exposure

Dose	Ldos	#U	Kl	%Kill	Cr%	E Pr	Ex Pr	Wk Pro	Weght	F Pro
250.000	2.398	30	24	80.000	80	5.85	6.021	5.800	13.17	5.992
200.000	2.301	30	23	76.667	77	5.74	5.874	5.698	15.09	5.853
150.000	2.176	30	23	76.667	77	5.74	5.683	5.730	16.74	5.673
100.000	2.000	30	21	70.000	70	5.52	5.415	5.510	18.03	5.419
50.000	1.699	30	18	60.000	60	5.25	4.957	5.240	19.02	4.986
25.000	1.398	30	11	36.667	37	4.67	4.499	4.690	16.74	4.552
12.500	1.097	30	3	10.000	10	3.72	4.041	3.750	13.17	4.119

REGRESSION EQUATION:  $Y = 2.539 + 1.440X$   
 CHI-SQUARED IS 4.389 WITH 5 DEGREES OF FREEDOM  
 NO SIG HETEROGENEITY  
 LOG LC<sub>50</sub> IS 1.709ppm  
 LC<sub>50</sub> IS 51.143ppm  
 95% CONF LIMITS ARE 37.301 TO 70.121ppm

**Appendix Table CXCI:** Lethal effects of stem wood extract (CH<sub>3</sub>OH) of *M. oleifera* against *A. salina* nauplii after 24h of exposure

Dose	Ldos	#U	Kl	%Kill	Cr%	E Pr	Ex Pr	Wk Pro	Weght	F Pro
250.000	2.398	30	27	90.000	90	6.28	6.350	6.250	10.08	6.372
200.000	2.301	30	26	86.667	87	6.13	6.193	6.132	12.15	6.214
150.000	2.176	30	24	80.000	80	5.85	5.991	5.870	14.13	6.011
100.000	2.000	30	24	80.000	80	5.85	5.705	5.830	15.96	5.724
50.000	1.699	30	21	70.000	70	5.52	5.218	5.540	18.81	5.235
25.000	1.398	30	12	40.000	40	4.75	4.730	4.740	18.48	4.745
12.500	1.097	30	5	16.667	17	4.05	4.243	4.048	15.09	4.255

REGRESSION EQUATION:  $Y = 2.469 + 1.627X$   
 CHI-SQUARED IS 3.091 WITH 5 DEGREES OF FREEDOM  
 NO SIG HETEROGENEITY  
 LOG LC<sub>50</sub> IS 1.555ppm  
 LC<sub>50</sub> IS 35.877ppm  
 95% CONF LIMITS ARE 26.237 TO 49.057ppm

**Appendix Table CXCI:** Lethal effects of root bark extract (CH<sub>3</sub>OH) of *M. oleifera* against *A. salina* nauplii after 6h of exposure

Dose	Ldos	#U	Kl	%Kill	Cr%	E Pr	Ex Pr	Wk Pro	Weght	F Pro
100.000	2.000	30	13	43.333	43	4.82	4.855	4.838	18.81	4.870
50.000	1.699	30	9	30.000	30	4.48	4.595	4.460	17.43	4.599
25.000	1.398	30	8	26.667	27	4.39	4.336	4.394	15.96	4.328
12.500	1.097	30	8	26.667	27	4.39	4.076	4.447	13.17	4.056
6.250	0.796	30	3	10.000	10	3.72	3.816	3.720	11.10	3.785
3.125	0.495	30	1	3.333	3	3.12	3.556	3.211	8.07	3.514

REGRESSION EQUATION:  $Y = 3.068 + 0.901X$   
 CHI-SQUARED IS 3.223 WITH 4 DEGREES OF FREEDOM  
 NO SIG HETEROGENEITY  
 LOG LC<sub>50</sub> IS 2.144ppm  
 LC<sub>50</sub> IS 139.279ppm  
 95% CONF LIMITS ARE 50.763 TO 382.145ppm

**Appendix Table CXCIV:** Lethal effects of root bark extract (CH<sub>3</sub>OH) of *M. oleifera* against *A. salina* nauplii after 12h of exposure

Dose	Ldos	#U	Kl	%Kill	Cr%	E Pr	Ex Pr	Wk Pro	Weght	F Pro
100.000	2.000	30	15	50.000	50	5.00	5.078	5.000	19.11	5.050
50.000	1.699	30	12	40.000	40	4.75	4.804	4.760	18.81	4.793
25.000	1.398	30	10	33.333	33	4.56	4.530	4.544	17.43	4.536
12.500	1.097	30	9	30.000	30	4.48	4.256	4.490	15.09	4.279
6.250	0.796	30	5	16.667	17	4.05	3.983	4.062	12.15	4.023
3.125	0.495	30	2	6.667	7	3.52	3.709	3.546	10.08	3.766

REGRESSION EQUATION:  $Y = 3.344 + 0.853X$   
 CHI-SQUARED IS 1.244 WITH 4 DEGREES OF FREEDOM  
 NO SIG HETEROGENEITY  
 LOG LC<sub>50</sub> IS 1.942ppm  
 LC<sub>50</sub> IS 87.429ppm  
 95% CONF LIMITS ARE 37.246 TO 205.225ppm

**Appendix Table CXCVI:** Lethal effects of root bark extract (CH<sub>3</sub>OH) of *M. oleifera* against *A. salina* nauplii after 18h of exposure

Dose	Ldos	#U	Kl	%Kill	Cr%	E Pr	Ex Pr	Wk Pro	Weght	F Pro
100.000	2.000	30	18	60.000	60	5.25	5.327	5.240	18.48	5.311
50.000	1.699	30	16	53.333	53	5.08	5.052	5.075	19.11	5.042
25.000	1.398	30	13	43.333	43	4.82	4.777	4.818	18.48	4.773
12.500	1.097	30	10	33.333	33	4.56	4.503	4.544	17.43	4.504
6.250	0.796	30	7	23.333	23	4.26	4.228	4.252	15.09	4.235
3.125	0.495	30	4	13.333	13	3.87	3.953	3.878	12.15	3.966

REGRESSION EQUATION:  $Y = 3.524 + 0.894X$   
 CHI-SQUARED IS 0.278 WITH 4 DEGREES OF FREEDOM  
 NO SIG HETEROGENEITY  
 LOG LC<sub>50</sub> IS 1.652ppm  
 LC<sub>50</sub> IS 44.824ppm  
 95% CONF LIMITS ARE 24.386 TO 82.391ppm

**Appendix Table CXCVII:** Lethal effects of root bark extract (CH<sub>3</sub>OH) of *M. oleifera* against *A. salina* nauplii after 24h of exposure

Dose	Ldos	#U	Kl	%Kill	Cr%	E Pr	Ex Pr	Wk Pro	Weght	F Pro
100.000	2.000	30	22	73.333	73	5.61	5.576	5.584	17.43	5.570
50.000	1.699	30	18	60.000	60	5.25	5.295	5.280	18.81	5.292
25.000	1.398	30	15	50.000	50	5.00	5.015	5.000	19.11	5.014
12.500	1.097	30	12	40.000	40	4.75	4.735	4.740	18.48	4.737
6.250	0.796	30	9	30.000	30	4.48	4.455	4.480	16.74	4.459
3.125	0.495	30	6	20.000	20	4.16	4.174	4.170	14.13	4.181

REGRESSION EQUATION:  $Y = 3.725 + 0.922X$   
 CHI-SQUARED IS 0.019 WITH 4 DEGREES OF FREEDOM  
 NO SIG HETEROGENEITY  
 LOG LC<sub>50</sub> IS 1.382ppm  
 LC<sub>50</sub> IS 24.119ppm  
 95% CONF LIMITS ARE 14.805 TO 39.295ppm



**Appendix Table CXC VIII:** Lethal effects of root wood extract (CH<sub>3</sub>OH) of *M. oleifera* against *A. salina* nauplii after 6h of exposure

Dose	Ldos	#U	Kl	%Kill	Cr%	E Pr	Ex Pr	Wk Pro	Weght	F Pro
300.000	2.477	30	7	23.333	23	4.26	4.135	4.284	14.13	4.149
250.000	2.398	30	5	16.667	17	4.05	4.077	4.037	13.17	4.088
200.000	2.301	30	4	13.333	13	3.87	4.006	3.873	13.17	4.014
150.000	2.176	30	4	13.333	13	3.87	3.915	3.878	12.15	3.919
100.000	2.000	30	4	13.333	13	3.87	3.787	3.894	10.08	3.784

REGRESSION EQUATION:  $Y = 2.256 + 0.764X$

CHI-SQUARED IS 0.697 WITH 3 DEGREES OF FREEDOM

NO SIG HETEROGENEITY

LOG LC<sub>50</sub> IS 3.592ppm

LC<sub>50</sub> IS 3906.864ppm

95% CONF LIMITS ARE 9.375 TO 1628051ppm

**Appendix Table CXC IX:** Lethal effects of root wood extract (CH<sub>3</sub>OH) of *M. oleifera* against *A. salina* nauplii after 12h of exposure

Dose	Ldos	#U	Kl	%Kill	Cr%	E Pr	Ex Pr	Wk Pro	Weght	F Pro
300.000	2.477	30	10	33.333	33	4.56	4.568	4.544	17.43	4.554
250.000	2.398	30	9	30.000	30	4.48	4.492	4.480	16.74	4.483
200.000	2.301	30	7	23.333	23	4.26	4.399	4.266	15.96	4.396
150.000	2.176	30	8	26.667	27	4.39	4.279	4.388	15.09	4.284
100.000	2.000	30	7	23.333	23	4.26	4.110	4.284	14.13	4.126
50.000	1.699	30	3	10.000	10	3.72	3.822	3.720	11.10	3.855

REGRESSION EQUATION:  $Y = 2.330 + 0.898X$

CHI-SQUARED IS 0.993 WITH 4 DEGREES OF FREEDOM

NO SIG HETEROGENEITY

LOG LC<sub>50</sub> IS 2.974ppm

LC<sub>50</sub> IS 941.192ppm

95% CONF LIMITS ARE 169.069 TO 5239.537ppm

**Appendix Table CC:** Lethal effects of root wood extract (CH<sub>3</sub>OH) of *M. oleifera* against *A. salina* nauplii after 18h of exposure

Dose	Ldos	#U	Kl	%Kill	Cr%	E Pr	Ex Pr	Wk Pro	Weght	F Pro
300.000	2.477	30	15	50.000	50	5.00	4.968	4.990	19.02	4.961
250.000	2.398	30	13	43.333	43	4.82	4.882	4.838	18.81	4.878
200.000	2.301	30	11	36.667	37	4.67	4.778	4.662	18.48	4.776
150.000	2.176	30	12	40.000	40	4.75	4.643	4.740	18.03	4.645
100.000	2.000	30	10	33.333	33	4.56	4.452	4.570	16.74	4.460
50.000	1.699	30	5	16.667	17	4.05	4.127	4.056	14.13	4.144

REGRESSION EQUATION:  $Y = 2.359 + 1.051X$

CHI-SQUARED IS 0.761 WITH 4 DEGREES OF FREEDOM

NO SIG HETEROGENEITY

LOG LC<sub>50</sub> IS 2.514ppm

LC<sub>50</sub> IS 326.569ppm

95% CONF LIMITS ARE 166.925 TO 638.894ppm

**Appendix Table CCI:** Lethal effects of root wood extract (CH<sub>3</sub>OH) of *M. oleifera* against *A. salina* nauplii after 24h of exposure

Dose	Ldos	#U	Kl	%Kill	Cr%	E Pr	Ex Pr	Wk Pro	Weght	F Pro
300.000	2.477	30	20	66.667	67	5.44	5.285	5.462	18.81	5.288
250.000	2.398	30	17	56.667	57	5.18	5.185	5.165	19.02	5.186
200.000	2.301	30	15	50.000	50	5.00	5.063	5.000	19.11	5.062
150.000	2.176	30	12	40.000	40	4.75	4.906	4.740	19.02	4.901
100.000	2.000	30	11	36.667	37	4.67	4.685	4.659	18.03	4.675
50.000	1.699	30	8	26.667	27	4.39	4.306	4.394	15.96	4.289

REGRESSION EQUATION:  $Y = 2.108 + 1.284X$   
 CHI-SQUARED IS 1.329 WITH 4 DEGREES OF FREEDOM  
 NO SIG HETEROGENEITY  
 LOG LC<sub>50</sub> IS 2.253ppm  
 LC<sub>50</sub> IS 178.993ppm  
 95% CONF LIMITS ARE 126.707 TO 252.856ppm

**Appendix Table CCII:** Lethal effects of leaf extract (Pet.E.) of *Mu. sapientum* against *A. salina* after 6h of exposure

Dose	Ldos	#U	Kl	%Kill	Cr%	E Pr	Ex Pr	Wk Pro	Weght	F Pro
200.000	2.301	30	10	33.333	33	4.56	4.388	4.586	15.96	4.433
150.000	2.176	30	5	16.667	17	4.05	4.202	4.048	15.09	4.233
100.000	2.000	30	5	16.667	17	4.05	3.940	4.062	12.15	3.951
75.000	1.875	30	2	6.667	7	3.52	3.754	3.546	10.08	3.751
50.000	1.699	30	2	6.667	7	3.52	3.492	3.540	7.14	3.469
25.000	1.398	30	1	3.333	3	3.12	3.044	3.135	3.93	2.988

REGRESSION EQUATION:  $Y = 0.749 + 1.601X$   
 CHI-SQUARED IS 1.585 WITH 4 DEGREES OF FREEDOM  
 NO SIG HETEROGENEITY  
 LOG LC<sub>50</sub> IS 2.655ppm  
 LC<sub>50</sub> IS 451.924ppm  
 95% CONF LIMITS ARE 175.893 TO 1161.131ppm

**Appendix Table CCIII:** Lethal effects of leaf extract (Pet.E.) of *Mu. sapientum* against *A. salina* after 12h of exposure

Dose	Ldos	#U	Kl	%Kill	Cr%	E Pr	Ex Pr	Wk Pro	Weght	F Pro
200.000	2.301	30	17	56.667	57	5.18	4.912	5.165	19.02	4.891
150.000	2.176	30	9	30.000	30	4.48	4.673	4.470	18.03	4.657
100.000	2.000	30	7	23.333	23	4.26	4.335	4.266	15.96	4.327
75.000	1.875	30	4	13.333	13	3.87	4.095	3.873	13.17	4.092
50.000	1.699	30	3	10.000	10	3.72	3.757	3.720	10.08	3.762
25.000	1.398	30	2	6.667	7	3.52	3.180	3.724	4.62	3.198

REGRESSION EQUATION:  $Y = 0.576 + 1.875X$   
 CHI-SQUARED IS 4.046 WITH 4 DEGREES OF FREEDOM  
 NO SIG HETEROGENEITY  
 LOG LC<sub>50</sub> IS 2.359ppm  
 LC<sub>50</sub> IS 228.629ppm  
 95% CONF LIMITS ARE 145.336 TO 359.656ppm

**Appendix Table CCIV:** Lethal effects of leaf extract (Pet.E.) of *Mu. sapientum* against *A. salina* after 18h of exposure

Dose	Ldos	#U	Kl	%Kill	Cr%	E Pr	Ex Pr	Wk Pro	Weght	F Pro
200.000	2.301	30	19	63.333	63	5.33	5.018	5.325	19.11	5.049
150.000	2.176	30	11	36.667	37	4.67	4.826	4.682	18.81	4.844
100.000	2.000	30	9	30.000	30	4.48	4.555	4.460	17.43	4.555
75.000	1.875	30	7	23.333	23	4.26	4.362	4.266	15.96	4.351
50.000	1.699	30	4	13.333	13	3.87	4.091	3.873	13.17	4.062
25.000	1.398	30	4	13.333	13	3.87	3.628	3.931	9.06	3.569

REGRESSION EQUATION:  $Y = 1.279 + 1.279X$

CHI-SQUARED IS 3.885 WITH 4 DEGREES OF FREEDOM

NO SIG HETEROGENEITY

LOG LC<sub>50</sub> IS 2.271ppm

LC<sub>50</sub> IS 186.776ppm

95% CONF LIMITS ARE 122.682 TO 284.354ppm

**Appendix Table CCV:** Lethal effects of leaf extract (Pet.E.) of *Mu. sapientum* against *A. salina* after 24h of exposure

Dose	Ldos	#U	Kl	%Kill	Cr%	E Pr	Ex Pr	Wk Pro	Weght	F Pro
200.000	2.301	30	24	80.000	80	5.85	5.377	5.760	18.48	5.360
150.000	2.176	30	15	50.000	50	5.00	5.152	4.990	19.02	5.130
100.000	2.000	30	10	33.333	33	4.56	4.834	4.578	18.81	4.805
75.000	1.875	30	8	26.667	27	4.39	4.609	4.389	18.03	4.574
50.000	1.699	30	6	20.000	20	4.16	4.291	4.150	15.09	4.249
25.000	1.398	30	5	16.667	17	4.05	3.747	4.126	10.08	3.694

REGRESSION EQUATION:  $Y = 1.114 + 1.845X$

CHI-SQUARED IS 6.942 WITH 4 DEGREES OF FREEDOM

NO SIG HETEROGENEITY

LOG LC<sub>50</sub> IS 2.106ppm

LC<sub>50</sub> IS 127.604ppm

95% CONF LIMITS ARE 96.646 TO 168.478ppm

**Appendix Table CCVI:** Lethal effects of stem extract (Pet.E.) of *Mu. sapientum* against *A. salina* after 6h of exposure

Dose	Ldos	#U	Kl	%Kill	Cr%	E Pr	Ex Pr	Wk Pro	Weght	F Pro
90.000	1.954	30	14	46.667	47	4.92	4.569	4.936	17.43	4.530
70.000	1.845	30	2	6.667	7	3.52	4.348	3.754	15.96	4.317
50.000	1.699	30	7	23.333	23	4.26	4.052	4.283	13.17	4.032
30.000	1.477	30	1	3.333	3	3.12	3.603	3.261	9.06	3.599
10.000	1.000	30	1	3.333	3	3.12	2.637	3.568	1.86	2.668

REGRESSION EQUATION:  $Y = 0.716 + 1.952X$

CHI-SQUARED IS 11.304 WITH 3 DEGREES OF FREEDOM

VARIANCE HAS BEEN ADJUSTED FOR HETEROGENEITY

LOG LC<sub>50</sub> IS 2.195ppm

LC<sub>50</sub> IS 156.611ppm

95% CONF LIMITS ARE 40.829 TO 600.719ppm

**Appendix Table CCVII:** Lethal effects of stem extract (Pet.E.) of *Mu. sapientum* against *A. salina* after 12h of exposure

Dose	Ldos	#U	Kl	%Kill	Cr%	E Pr	Ex Pr	Wk Pro	Weght	F Pro
90.000	1.954	30	17	56.667	57	5.18	5.066	5.175	19.11	5.080
70.000	1.845	30	8	26.667	27	4.39	4.842	4.422	18.81	4.857
50.000	1.699	30	16	53.333	53	5.08	4.542	5.104	17.43	4.559
30.000	1.477	30	4	13.333	13	3.87	4.085	3.873	13.17	4.106
10.000	1.000	30	1	3.333	3	3.12	3.105	3.116	4.62	3.132

REGRESSION EQUATION:  $Y = 1.091 + 2.041X$

CHI-SQUARED IS 9.630 WITH 3 DEGREES OF FREEDOM

VARIANCE HAS BEEN ADJUSTED FOR HETEROGENEITY

LOG LC<sub>50</sub> IS 1.915ppm

LC<sub>50</sub> IS 82.246ppm

95% CONF LIMITS ARE 45.604 TO 148.327ppm

**Appendix Table CCVIII:** Lethal effects of stem extract (Pet.E.) of *Mu. sapientum* against *A. salina* after 18h of exposure

Dose	Ldos	#U	Kl	%Kill	Cr%	E Pr	Ex Pr	Wk Pro	Weght	F Pro
90.000	1.954	30	17	56.667	57	5.18	5.166	5.165	19.02	5.165
70.000	1.845	30	11	36.667	37	4.67	4.929	4.665	19.02	4.932
50.000	1.699	30	16	53.333	53	5.08	4.610	5.091	18.03	4.620
30.000	1.477	30	4	13.333	13	3.87	4.127	3.904	14.13	4.147
10.000	1.000	30	1	3.333	3	3.12	3.088	3.135	3.93	3.128

REGRESSION EQUATION:  $Y = 0.994 + 2.134X$

CHI-SQUARED IS 6.186 WITH 3 DEGREES OF FREEDOM

NO SIG HETEROGENEITY

LOG LC<sub>50</sub> IS 1.877ppm

LC<sub>50</sub> IS 75.313ppm

95% CONF LIMITS ARE 56.216 TO 100.898ppm

**Appendix Table CCIX:** Lethal effects of stem extract (Pet.E.) of *Mu. sapientum* against *A. salina* after 24h of exposure

Dose	Ldos	#U	Kl	%Kill	Cr%	E Pr	Ex Pr	Wk Pro	Weght	F Pro
90.000	1.954	30	22	73.333	73	5.61	5.578	5.584	17.43	5.578
70.000	1.845	30	16	53.333	53	5.08	5.294	5.098	18.81	5.295
50.000	1.699	30	18	60.000	60	5.25	4.914	5.240	19.02	4.915
30.000	1.477	30	6	20.000	20	4.16	4.337	4.170	15.96	4.340
10.000	1.000	30	1	3.333	3	3.12	3.097	3.135	3.93	3.102

REGRESSION EQUATION:  $Y = 0.508 + 2.594X$

CHI-SQUARED IS 3.196 WITH 3 DEGREES OF FREEDOM

NO SIG HETEROGENEITY

LOG LC<sub>50</sub> IS 1.732ppm

LC<sub>50</sub> IS 53.893ppm

95% CONF LIMITS ARE 44.060 TO 65.919ppm

**Appendix Table CCX:** Lethal effects of root extract (Pet.E.) of *Mu. sapientum* against *A. salina* after 6h of exposure

Dose	Ldos	#U	Kl	%Kill	Cr%	E Pr	Ex Pr	Wk Pro	Weght	F Pro
100.000	2.000	30	14	46.667	47	4.92	4.919	4.915	19.02	4.974
80.000	1.903	30	13	43.333	43	4.82	4.527	4.824	17.43	4.561
60.000	1.778	30	2	6.667	7	3.52	4.023	3.627	13.17	4.029
40.000	1.602	30	2	6.667	7	3.52	3.311	3.572	6.24	3.278

REGRESSION EQUATION:  $Y = -3.549 + 4.262X$

CHI-SQUARED IS 3.934 WITH 2 DEGREES OF FREEDOM

NO SIG HETEROGENEITY

LOG LC<sub>50</sub> IS 2.006ppm

LC<sub>50</sub> IS 101.402ppm

95% CONF LIMITS ARE 82.580 TO 124.513ppm

**Appendix Table CCXI:** Lethal effects of root extract (Pet.E.) of *Mu. sapientum* against *A. salina* after 12h of exposure

Dose	Ldos	#U	Kl	%Kill	Cr%	E Pr	Ex Pr	Wk Pro	Weght	F Pro
100.000	2.000	30	22	73.333	73	5.61	5.415	5.591	18.03	5.413
80.000	1.903	30	21	70.000	70	5.52	5.130	5.490	19.02	5.129
60.000	1.778	30	4	13.333	13	3.87	4.763	4.038	18.48	4.763
40.000	1.602	30	7	23.333	23	4.26	4.245	4.252	15.09	4.248
20.000	1.301	30	3	10.000	10	3.72	3.360	3.890	6.24	3.366

REGRESSION EQUATION:  $Y = -0.443 + 2.928X$

CHI-SQUARED IS 14.483 WITH 3 DEGREES OF FREEDOM

VARIANCE HAS BEEN ADJUSTED FOR HETEROGENEITY

LOG LC<sub>50</sub> IS 1.859ppm

LC<sub>50</sub> IS 72.269ppm

95% CONF LIMITS ARE 47.957 TO 108.909ppm

**Appendix Table CCXII:** Lethal effects of root extract (Pet.E.) of *Mu. sapientum* against *A. salina* after 18h of exposure

Dose	Ldos	#U	Kl	%Kill	Cr%	E Pr	Ex Pr	Wk Pro	Weght	F Pro
100.000	2.000	30	26	86.667	87	6.13	5.770	6.054	15.96	5.767
80.000	1.903	30	25	83.333	83	5.95	5.430	5.861	18.03	5.429
60.000	1.778	30	4	13.333	13	3.87	4.993	4.065	19.02	4.993
40.000	1.602	30	9	30.000	30	4.48	4.376	4.490	15.96	4.378
20.000	1.301	30	3	10.000	10	3.72	3.321	3.890	6.24	3.328

REGRESSION EQUATION:  $Y = -1.213 + 3.490X$

CHI-SQUARED IS 23.227 WITH 3 DEGREES OF FREEDOM

VARIANCE HAS BEEN ADJUSTED FOR HETEROGENEITY

LOG LC<sub>50</sub> IS 1.780ppm

LC<sub>50</sub> IS 60.274ppm

95% CONF LIMITS ARE 39.806 TO 91.265ppm

**Appendix Table CCXIII:** Lethal effects of root extract (Pet.E.) of *Mu. sapientum* against *A. salina* after 24h of exposure

Dose	Ldos	#U	Kl	%Kill	Cr%	E Pr	Ex Pr	Wk Pro	Weght	F Pro
100.000	2.000	30	27	90.000	90	6.28	5.970	6.250	14.13	5.875
80.000	1.903	30	26	86.667	87	6.13	5.681	6.030	16.74	5.593
60.000	1.778	30	9	30.000	30	4.48	5.309	4.460	18.48	5.230
40.000	1.602	30	9	30.000	30	4.48	4.784	4.480	18.48	4.718
20.000	1.301	30	7	23.333	23	4.26	3.886	4.383	11.10	3.842

REGRESSION EQUATION:  $Y = 0.059 + 2.908X$

CHI-SQUARED IS 20.423 WITH 3 DEGREES OF FREEDOM

VARIANCE HAS BEEN ADJUSTED FOR HETEROGENEITY

LOG LC<sub>50</sub> IS 1.699ppm

LC<sub>50</sub> IS 50.018ppm

95% CONF LIMITS ARE 31.512 TO 79.392ppm

**Appendix Table CCXIV:** Lethal effects of leaf extract (CHCl<sub>3</sub>) of *Mu. sapientum* against *A. salina* after 6h of exposure

Dose	Ldos	#U	Kl	%Kill	Cr%	E Pr	Ex Pr	Wk Pro	Weght	F Pro
100.000	2.000	30	14	46.667	47	4.92	4.751	4.922	18.48	4.761
75.000	1.875	30	10	33.333	33	4.56	4.636	4.551	18.03	4.642
50.000	1.699	30	8	26.667	27	4.39	4.473	4.390	16.74	4.475
25.000	1.398	30	5	16.667	17	4.05	4.194	4.056	14.13	4.188
12.500	1.097	30	5	16.667	17	4.05	3.916	4.062	12.15	3.902

REGRESSION EQUATION:  $Y = 2.859 + 0.951X$

CHI-SQUARED IS 1.307 WITH 3 DEGREES OF FREEDOM

NO SIG HETEROGENEITY

LOG LC<sub>50</sub> IS 2.252

LC<sub>50</sub> IS 178.457

95% CONF LIMITS ARE 57.571 TO 553.175

**Appendix Table CCXV:** Lethal effects of leaf extract (CHCl<sub>3</sub>) of *Mu. sapientum* against *A. salina* after 12h of exposure

Dose	Ldos	#U	Kl	%Kill	Cr%	E Pr	Ex Pr	Wk Pro	Weght	F Pro
100.000	2.000	30	20	66.667	67	5.44	5.192	5.415	19.02	5.188
75.000	1.875	30	14	46.667	47	4.92	5.044	4.925	19.11	5.041
50.000	1.699	30	11	36.667	37	4.67	4.836	4.682	18.81	4.834
25.000	1.398	30	8	26.667	27	4.39	4.481	4.390	16.74	4.479
12.500	1.097	30	7	23.333	23	4.26	4.126	4.284	14.13	4.125

REGRESSION EQUATION:  $Y = 2.833 + 1.178X$

CHI-SQUARED IS 2.161 WITH 3 DEGREES OF FREEDOM

NO SIG HETEROGENEITY

LOG LC<sub>50</sub> IS 1.840ppm

LC<sub>50</sub> IS 69.203ppm

95% CONF LIMITS ARE 42.879 TO 111.688ppm

**Appendix Table CCXVI:** Lethal effects of leaf extract (CHCl<sub>3</sub>) of *Mu. sapientum* against *A. salina* after 18h of exposure

Dose	Ldos	#U	Kl	%Kill	Cr%	E Pr	Ex Pr	Wk Pro	Weght	F Pro
100.000	2.000	30	23	76.667	77	5.74	5.628	5.730	16.74	5.600
75.000	1.875	30	19	63.333	63	5.33	5.419	5.321	18.03	5.398
50.000	1.699	30	15	50.000	50	5.00	5.124	4.990	19.02	5.113
25.000	1.398	30	11	36.667	37	4.67	4.620	4.659	18.03	4.626
12.500	1.097	30	7	23.333	23	4.26	4.116	4.284	14.13	4.139
6.250	0.796	30	2	6.667	7	3.52	3.612	3.529	9.06	3.652

REGRESSION EQUATION:  $Y = 2.364 + 1.618X$

CHI-SQUARED IS 1.131 WITH 4 DEGREES OF FREEDOM

NO SIG HETEROGENEITY

LOG LC<sub>50</sub> IS 1.629ppm

LC<sub>50</sub> IS 42.582ppm

95% CONF LIMITS ARE 31.807 TO 57.007ppm

**Appendix Table CCXVII:** Lethal effects of leaf extract (CHCl<sub>3</sub>) of *Mu. sapientum* against *A. salina* after 24h of exposure

Dose	Ldos	#U	Kl	%Kill	Cr%	E Pr	Ex Pr	Wk Pro	Weght	F Pro
100.000	2.000	30	26	86.667	87	6.13	5.990	6.136	14.13	5.975
75.000	1.875	30	23	76.667	77	5.74	5.755	5.734	15.96	5.743
50.000	1.699	30	18	60.000	60	5.25	5.425	5.240	18.03	5.415
25.000	1.398	30	13	43.333	43	4.82	4.859	4.838	18.81	4.854
12.500	1.097	30	8	26.667	27	4.39	4.293	4.388	15.09	4.293
6.250	0.796	30	3	10.000	10	3.72	3.728	3.720	10.08	3.732

REGRESSION EQUATION:  $Y = 2.249 + 1.863X$

CHI-SQUARED IS 1.058 WITH 4 DEGREES OF FREEDOM

NO SIG HETEROGENEITY

LOG LC<sub>50</sub> IS 1.476ppm

LC<sub>50</sub> IS 29.951ppm

95% CONF LIMITS ARE 23.238 TO 38.605ppm

**Appendix Table CCXVIII:** Lethal effects of stem extract (CHCl<sub>3</sub>) of *Mu. sapientum* against *A. salina* after 6h of exposure

Dose	Ldos	#U	Kl	%Kill	Cr%	E Pr	Ex Pr	Wk Pro	Weght	F Pro
50.000	1.699	30	7	23.333	23	4.26	4.335	4.266	15.96	4.328
40.000	1.602	30	7	23.333	23	4.26	4.265	4.252	15.09	4.264
30.000	1.477	30	6	20.000	20	4.16	4.176	4.170	14.13	4.180
20.000	1.301	30	7	23.333	23	4.26	4.050	4.283	13.17	4.063
10.000	1.000	30	3	10.000	10	3.72	3.834	3.720	11.10	3.862

REGRESSION EQUATION:  $Y = 3.195 + 0.667X$

CHI-SQUARED IS 0.928 WITH 3 DEGREES OF FREEDOM

NO SIG HETEROGENEITY

LOG LC<sub>50</sub> IS 2.706ppm

LC<sub>50</sub> IS 508.222ppm

95% CONF LIMITS ARE 6.228 TO 41472.810ppm

**Appendix Table CCXIX:** Lethal effects of stem extract (CHCl<sub>3</sub>) of *Mu. sapientum* against *A. salina* after 12h of exposure

Dose	Ldos	#U	Kl	%Kill	Cr%	E Pr	Ex Pr	Wk Pro	Weght	F Pro
50.000	1.699	30	11	36.667	37	4.67	4.649	4.659	18.03	4.637
40.000	1.602	30	10	33.333	33	4.56	4.582	4.544	17.43	4.573
30.000	1.477	30	9	30.000	30	4.48	4.495	4.480	16.74	4.490
20.000	1.301	30	8	26.667	27	4.39	4.372	4.394	15.96	4.374
10.000	1.000	30	6	20.000	20	4.16	4.163	4.170	14.13	4.175

REGRESSION EQUATION:  $Y = 3.513 + 0.661X$

CHI-SQUARED IS 0.032 WITH 3 DEGREES OF FREEDOM

NO SIG HETEROGENEITY

LOG LC<sub>50</sub> IS 2.249ppm

LC<sub>50</sub> IS 177.202ppm

95% CONF LIMITS ARE 12.424 TO 2527.360ppm

**Appendix Table CCXX:** Lethal effects of stem extract (CHCl<sub>3</sub>) of *Mu. sapientum* against *A. salina* after 18h of exposure

Dose	Ldos	#U	Kl	%Kill	Cr%	E Pr	Ex Pr	Wk Pro	Weght	F Pro
50.000	1.699	30	14	46.667	47	4.92	4.859	4.942	18.81	4.865
40.000	1.602	30	11	36.667	37	4.67	4.789	4.662	18.48	4.793
30.000	1.477	30	13	43.333	43	4.82	4.700	4.821	18.03	4.701
20.000	1.301	30	9	30.000	30	4.48	4.574	4.460	17.43	4.570
10.000	1.000	30	8	26.667	27	4.39	4.358	4.394	15.96	4.347

REGRESSION EQUATION:  $Y = 3.606 + 0.741X$

CHI-SQUARED IS 0.938 WITH 3 DEGREES OF FREEDOM

NO SIG HETEROGENEITY

LOG LC<sub>50</sub> IS 1.881ppm

LC<sub>50</sub> IS 75.989ppm

95% CONF LIMITS ARE 19.343 TO 298.529ppm

**Appendix Table CCXXI:** Lethal effects of stem extract (CHCl<sub>3</sub>) of *Mu. sapientum* against *A. salina* after 24h of exposure

Dose	Ldos	#U	Kl	%Kill	Cr%	E Pr	Ex Pr	Wk Pro	Weght	F Pro
50.000	1.699	30	18	60.000	60	5.25	5.120	5.240	19.02	5.115
40.000	1.602	30	14	46.667	47	4.92	5.030	4.925	19.11	5.026
30.000	1.477	30	13	43.333	43	4.82	4.915	4.815	19.02	4.911
20.000	1.301	30	13	43.333	43	4.82	4.752	4.818	18.48	4.749
10.000	1.000	30	9	30.000	30	4.48	4.473	4.480	16.74	4.472

REGRESSION EQUATION:  $Y = 3.553 + 0.919X$

CHI-SQUARED IS 0.756 WITH 3 DEGREES OF FREEDOM

NO SIG HETEROGENEITY

LOG LC<sub>50</sub> IS 1.574ppm

LC<sub>50</sub> IS 37.456ppm

95% CONF LIMITS ARE 20.615 TO 68.055ppm



**Appendix Table CCXXII:** Lethal effects of root extract (CHCl<sub>3</sub>) of *Mu. sapientum* against *A. salina* after 6h of exposure

Dose	Ldos	#U	Kl	%Kill	Cr%	E Pr	Ex Pr	Wk Pro	Weght	F Pro
80.000	1.903	30	18	60.000	60	5.25	4.872	5.280	18.81	4.910
70.000	1.845	30	9	30.000	30	4.48	4.708	4.480	18.48	4.730
60.000	1.778	30	7	23.333	23	4.26	4.518	4.264	17.43	4.522
50.000	1.699	30	6	20.000	20	4.16	4.293	4.150	15.09	4.276
40.000	1.602	30	7	23.333	23	4.26	4.019	4.283	13.17	3.976

REGRESSION EQUATION:  $Y = -0.994 + 3.102X$

CHI-SQUARED IS 6.377 WITH 3 DEGREES OF FREEDOM

NO SIG HETEROGENEITY

LOG LC<sub>50</sub> IS 1.932ppm

LC<sub>50</sub> IS 85.551ppm

95% CONF LIMITS ARE 64.249 TO 113.915ppm

**Appendix Table CCXXIII:** Lethal effects of root extract (CHCl<sub>3</sub>) of *Mu. sapientum* against *A. salina* after 12h of exposure

Dose	Ldos	#U	Kl	%Kill	Cr%	E Pr	Ex Pr	Wk Pro	Weght	F Pro
80.000	1.903	30	19	63.333	63	5.33	5.044	5.325	19.11	5.043
70.000	1.845	30	12	40.000	40	4.75	4.907	4.740	19.02	4.903
60.000	1.778	30	9	30.000	30	4.48	4.750	4.480	18.48	4.743
50.000	1.699	30	10	33.333	33	4.56	4.563	4.544	17.43	4.553
40.000	1.602	30	9	30.000	30	4.48	4.335	4.490	15.96	4.320

REGRESSION EQUATION:  $Y = 0.474 + 2.401X$

CHI-SQUARED IS 3.769 WITH 3 DEGREES OF FREEDOM

NO SIG HETEROGENEITY

LOG LC<sub>50</sub> IS 1.885ppm

LC<sub>50</sub> IS 76.794ppm

95% CONF LIMITS ARE 57.357 TO 102.816ppm

**Appendix Table CCXXIV:** Lethal effects of root extract (CHCl<sub>3</sub>) of *Mu. sapientum* against *A. salina* after 18h of exposure

Dose	Ldos	#U	Kl	%Kill	Cr%	E Pr	Ex Pr	Wk Pro	Weght	F Pro
80.000	1.903	30	21	70.000	70	5.52	5.295	5.540	18.81	5.299
70.000	1.845	30	15	50.000	50	5.00	5.128	4.990	19.02	5.129
60.000	1.778	30	12	40.000	40	4.75	4.936	4.740	19.02	4.934
50.000	1.699	30	11	36.667	37	4.67	4.709	4.662	18.48	4.702
40.000	1.602	30	10	33.333	33	4.56	4.431	4.570	16.74	4.418

REGRESSION EQUATION:  $Y = -0.269 + 2.926X$

CHI-SQUARED IS 2.588 WITH 3 DEGREES OF FREEDOM

NO SIG HETEROGENEITY

LOG LC<sub>50</sub> IS 1.801ppm

LC<sub>50</sub> IS 63.219ppm

95% CONF LIMITS ARE 53.453 TO 74.768ppm

**Appendix Table CCXXV:** Lethal effects of root extract (CHCl<sub>3</sub>) of *Mu. sapientum* against *A. salina* after 24h of exposure

Dose	Ldos	#U	Kl	%Kill	Cr%	E Pr	Ex Pr	Wk Pro	Weght	F Pro
80.000	1.903	30	23	76.667	77	5.74	5.534	5.696	17.43	5.503
70.000	1.845	30	18	60.000	60	5.25	5.343	5.240	18.48	5.318
60.000	1.778	30	14	46.667	47	4.92	5.122	4.915	19.02	5.105
50.000	1.699	30	13	43.333	43	4.82	4.861	4.838	18.81	4.852
40.000	1.602	30	11	36.667	37	4.67	4.541	4.656	17.43	4.544

REGRESSION EQUATION:  $Y = -0.562 + 3.187X$

CHI-SQUARED IS 1.672 WITH 3 DEGREES OF FREEDOM

NO SIG HETEROGENEITY

LOG LC<sub>50</sub> IS 1.745ppm

LC<sub>50</sub> IS 55.624ppm

95% CONF LIMITS ARE 47.824 TO 64.695ppm

**Appendix Table CCXXVI:** Lethal effects of leaf extract (CH<sub>3</sub>OH) of *Mu. sapientum* against *A. salina* after 6h of exposure

Dose	Ldos	#U	Kl	%Kill	Cr%	E Pr	Ex Pr	Wk Pro	Weght	F Pro
200.000	2.301	30	17	56.667	57	5.18	5.083	5.175	19.11	5.078
150.000	2.176	30	12	40.000	40	4.75	4.983	4.740	19.02	4.979
100.000	2.000	30	14	46.667	47	4.92	4.841	4.942	18.81	4.841
50.000	1.699	30	12	40.000	40	4.75	4.598	4.740	17.43	4.603
25.000	1.398	30	7	23.333	23	4.26	4.356	4.266	15.96	4.366

REGRESSION EQUATION:  $Y = 3.264 + 0.788X$

CHI-SQUARED IS 1.949 WITH 3 DEGREES OF FREEDOM

NO SIG HETEROGENEITY

LOG LC<sub>50</sub> IS 2.202ppm

LC<sub>50</sub> IS 159.309ppm

95% CONF LIMITS ARE 72.879 TO 348.243ppm

**Appendix Table CCXXVII:** Lethal effects of leaf extract (CH<sub>3</sub>OH) of *Mu. sapientum* against *A. salina* after 12h of exposure

Dose	Ldos	#U	Kl	%Kill	Cr%	E Pr	Ex Pr	Wk Pro	Weght	F Pro
200.000	2.301	30	18	60.000	60	5.25	5.289	5.280	18.81	5.311
150.000	2.176	30	17	56.667	57	5.18	5.205	5.202	18.81	5.224
100.000	2.000	30	16	53.333	53	5.08	5.087	5.075	19.11	5.102
50.000	1.699	30	16	53.333	53	5.08	4.885	5.098	18.81	4.892
25.000	1.398	30	10	33.333	33	4.56	4.684	4.551	18.03	4.682

REGRESSION EQUATION:  $Y = 3.707 + 0.697X$

CHI-SQUARED IS 1.151 WITH 3 DEGREES OF FREEDOM

NO SIG HETEROGENEITY

LOG LC<sub>50</sub> IS 1.854ppm

LC<sub>50</sub> IS 71.492ppm

95% CONF LIMITS ARE 36.132 TO 141.459ppm

**Appendix Table CCXXVIII:** Lethal effects of leaf extract (CH<sub>3</sub>OH) of *Mu. sapientum* against *A. salina* after 18h of exposure

Dose	Ldos	#U	Kl	%Kill	Cr%	E Pr	Ex Pr	Wk Pro	Weght	F Pro
200.000	2.301	30	20	66.667	67	5.44	5.427	5.429	18.03	5.424
150.000	2.176	30	19	63.333	63	5.33	5.355	5.318	18.48	5.353
100.000	2.000	30	17	56.667	57	5.18	5.253	5.202	18.81	5.252
50.000	1.699	30	18	60.000	60	5.25	5.080	5.250	19.11	5.080
25.000	1.398	30	13	43.333	43	4.82	4.906	4.815	19.02	4.908

REGRESSION EQUATION:  $Y = 4.109 + 0.571X$

CHI-SQUARED IS 0.786 WITH 3 DEGREES OF FREEDOM

NO SIG HETEROGENEITY

LOG LC<sub>50</sub> IS 1.559ppm

LC<sub>50</sub> IS 36.238ppm

95% CONF LIMITS ARE 10.979 TO 119.599ppm

**Appendix Table CCXXIX:** Lethal effects of leaf extract (CH<sub>3</sub>OH) of *Mu. sapientum* against *A. salina* after 24h of exposure

Dose	Ldos	#U	Kl	%Kill	Cr%	E Pr	Ex Pr	Wk Pro	Weght	F Pro
200.000	2.301	30	24	80.000	80	5.85	5.841	5.800	15.09	5.798
150.000	2.176	30	24	80.000	80	5.85	5.729	5.830	15.96	5.692
100.000	2.000	30	19	63.333	63	5.33	5.571	5.304	17.43	5.542
50.000	1.699	30	20	66.667	67	5.44	5.300	5.422	18.48	5.287
25.000	1.398	30	15	50.000	50	5.00	5.030	5.000	19.11	5.031

REGRESSION EQUATION:  $Y = 3.844 + 0.849X$

CHI-SQUARED IS 1.651 WITH 3 DEGREES OF FREEDOM

NO SIG HETEROGENEITY

LOG LC<sub>50</sub> IS 1.362ppm

LC<sub>50</sub> IS 22.991ppm

95% CONF LIMITS ARE 7.828 TO 67.523ppm

**Appendix Table CCXXX:** Lethal effects of stem extract (CH<sub>3</sub>OH) of *Mu. sapientum* against *A. salina* after 6h of exposure

Dose	Ldos	#U	Kl	%Kill	Cr%	E Pr	Ex Pr	Wk Pro	Weght	F Pro
150.000	2.176	30	12	40.000	40	4.75	4.712	4.740	18.48	4.695
100.000	2.000	30	6	20.000	20	4.16	4.366	4.170	15.96	4.368
75.000	1.875	30	7	23.333	23	4.26	4.121	4.284	14.13	4.136
50.000	1.699	30	4	13.333	13	3.87	3.776	3.894	10.08	3.809
25.000	1.398	30	1	3.333	3	3.12	3.185	3.116	4.62	3.250

REGRESSION EQUATION:  $Y = 0.654 + 1.857X$

CHI-SQUARED IS 1.128 WITH 3 DEGREES OF FREEDOM

NO SIG HETEROGENEITY

LOG LC<sub>50</sub> IS 2.340ppm

LC<sub>50</sub> IS 218.929ppm

95% CONF LIMITS ARE 115.126 TO 416.325ppm

**Appendix Table CCXXXI:** Lethal effects of stem extract (CH<sub>3</sub>OH) of *Mu. sapientum* against *A. salina* after 12h of exposure

Dose	Ldos	#U	Kl	%Kill	Cr%	E Pr	Ex Pr	Wk Pro	Weght	F Pro
150.000	2.176	30	13	43.333	43	4.82	4.551	4.824	17.43	4.578
100.000	2.000	30	6	20.000	20	4.16	4.413	4.180	16.74	4.435
75.000	1.875	30	8	26.667	27	4.39	4.316	4.394	15.96	4.334
50.000	1.699	30	4	13.333	13	3.87	4.178	3.904	14.13	4.191
25.000	1.398	30	6	20.000	20	4.16	3.943	4.200	12.15	3.946

REGRESSION EQUATION:  $Y = 2.812 + 0.812X$

CHI-SQUARED IS 4.145 WITH 3 DEGREES OF FREEDOM

NO SIG HETEROGENEITY

LOG LC<sub>50</sub> IS 2.696ppm

LC<sub>50</sub> IS 496.209ppm

95% CONF LIMITS ARE 57.228 TO 4302.476ppm

**Appendix Table CCXXXII:** Lethal effects of stem extract (CH<sub>3</sub>OH) of *Mu. sapientum* against *A. salina* after 18h of exposure

Dose	Ldos	#U	Kl	%Kill	Cr%	E Pr	Ex Pr	Wk Pro	Weght	F Pro
150.000	2.176	30	14	46.667	47	4.92	4.681	4.929	18.03	4.693
100.000	2.000	30	8	26.667	27	4.39	4.573	4.376	17.43	4.579
75.000	1.875	30	9	30.000	30	4.48	4.496	4.480	16.74	4.498
50.000	1.699	30	6	20.000	20	4.16	4.388	4.170	15.96	4.384
25.000	1.398	30	8	26.667	27	4.39	4.203	4.388	15.09	4.188

REGRESSION EQUATION:  $Y = 3.281 + 0.649X$

CHI-SQUARED IS 3.056 WITH 3 DEGREES OF FREEDOM

NO SIG HETEROGENEITY

LOG LC<sub>50</sub> IS 2.648ppm

LC<sub>50</sub> IS 444.961ppm

95% CONF LIMITS ARE 38.604 TO 5128.763ppm

**Appendix Table CCXXXIII:** Lethal effects of stem extract (CH<sub>3</sub>OH) of *Mu. sapientum* against *A. salina* after 24h of exposure

Dose	Ldos	#U	Kl	%Kill	Cr%	E Pr	Ex Pr	Wk Pro	Weght	F Pro
150.000	2.176	30	22	73.333	73	5.61	5.137	5.565	19.02	5.126
100.000	2.000	30	10	33.333	33	4.56	4.934	4.565	19.02	4.919
75.000	1.875	30	10	33.333	33	4.56	4.790	4.558	18.48	4.772
50.000	1.699	30	9	30.000	30	4.48	4.587	4.460	17.43	4.566
25.000	1.398	30	9	30.000	30	4.48	4.240	4.490	15.09	4.212

REGRESSION EQUATION:  $Y = 2.572 + 1.174X$

CHI-SQUARED IS 8.261 WITH 3 DEGREES OF FREEDOM

VARIANCE HAS BEEN ADJUSTED FOR HETEROGENEITY

LOG LC<sub>50</sub> IS 2.069ppm

LC<sub>50</sub> IS 117.196ppm

95% CONF LIMITS ARE 48.361 TO 284.009ppm

**Appendix Table CCXXXIV:** Lethal effects of root extract (CH<sub>3</sub>OH) of *Mu. sapientum* against *A. salina* after 6h of exposure

Dose	Ldos	#U	Kl	%Kill	Cr%	E Pr	Ex Pr	Wk Pro	Weght	F Pro
200.000	2.301	30	17	56.667	57	5.18	5.279	5.202	18.81	5.293
150.000	2.176	30	18	60.000	60	5.25	5.065	5.250	19.11	5.074
100.000	2.000	30	11	36.667	37	4.67	4.765	4.662	18.48	4.764
50.000	1.699	30	7	23.333	23	4.26	4.251	4.252	15.09	4.236

REGRESSION EQUATION:  $Y = 1.250 + 1.757X$

CHI-SQUARED IS 0.948 WITH 2 DEGREES OF FREEDOM

NO SIG HETEROGENEITY

LOG LC<sub>50</sub> IS 2.134ppm

LC<sub>50</sub> IS 136.148ppm

95% CONF LIMITS ARE 98.898 TO 187.429ppm

**Appendix Table CCXXXV:** Lethal effects of root extract (CH<sub>3</sub>OH) of *Mu. sapientum* against *A. salina* after 12h of exposure

Dose	Ldos	#U	Kl	%Kill	Cr%	E Pr	Ex Pr	Wk Pro	Weght	F Pro
200.000	2.301	30	18	60.000	60	5.25	5.433	5.240	18.03	5.406
150.000	2.176	30	20	66.667	67	5.44	5.187	5.415	19.02	5.173
100.000	2.000	30	11	36.667	37	4.67	4.840	4.682	18.81	4.844
50.000	1.699	30	9	30.000	30	4.48	4.247	4.490	15.09	4.281
25.000	1.398	30	2	6.667	7	3.52	3.654	3.529	9.06	3.719

REGRESSION EQUATION:  $Y = 1.108 + 1.868X$

CHI-SQUARED IS 3.090 WITH 3 DEGREES OF FREEDOM

NO SIG HETEROGENEITY

LOG LC<sub>50</sub> IS 2.084ppm

LC<sub>50</sub> IS 121.240ppm

95% CONF LIMITS ARE 91.165 TO 161.238ppm

**Appendix Table CCXXXVI:** Lethal effects of root extract (CH<sub>3</sub>OH) of *Mu. sapientum* against *A. salina* after 18h of exposure

Dose	Ldos	#U	Kl	%Kill	Cr%	E Pr	Ex Pr	Wk Pro	Weght	F Pro
200.000	2.301	30	19	63.333	63	5.33	5.495	5.321	18.03	5.482
150.000	2.176	30	20	66.667	67	5.44	5.245	5.462	18.81	5.245
100.000	2.000	30	12	40.000	40	4.75	4.891	4.760	18.81	4.911
50.000	1.699	30	10	33.333	33	4.56	4.287	4.592	15.09	4.339
25.000	1.398	30	2	6.667	7	3.52	3.682	3.529	9.06	3.768

REGRESSION EQUATION:  $Y = 1.114 + 1.898X$

CHI-SQUARED IS 3.262 WITH 3 DEGREES OF FREEDOM

NO SIG HETEROGENEITY

LOG LC<sub>50</sub> IS 2.047ppm

LC<sub>50</sub> IS 111.449ppm

95% CONF LIMITS ARE 84.889 TO 146.319ppm

**Appendix Table CCXXXVII:** Lethal effects of root extract (CH<sub>3</sub>OH) of *Mu. sapientum* against *A. salina* after 24h of exposure

Dose	Ldos	#U	Kl	%Kill	Cr%	E Pr	Ex Pr	Wk Pro	Weght	F Pro
200.000	2.301	30	21	70.000	70	5.52	5.635	5.520	16.74	5.610
150.000	2.176	30	21	70.000	70	5.52	5.361	5.500	18.48	5.350
100.000	2.000	30	13	43.333	43	4.82	4.975	4.815	19.02	4.982
50.000	1.699	30	10	33.333	33	4.56	4.314	4.586	15.96	4.354
25.000	1.398	30	2	6.667	7	3.52	3.654	3.529	9.06	3.726

REGRESSION EQUATION:  $Y = 0.811 + 2.086X$

CHI-SQUARED IS 2.296 WITH 3 DEGREES OF FREEDOM

NO SIG HETEROGENEITY

LOG LC<sub>50</sub> IS 2.009ppm

LC<sub>50</sub> IS 101.977ppm

95% CONF LIMITS ARE 79.848 TO 130.238ppm



জীবনের জন্য বিজ্ঞান

বিসিএসআইআর গবেষণাগার, ঢাকা  
BCSIR LABORATORIES, DHAKA  
বাংলাদেশ বিজ্ঞান ও শিল্প গবেষণা পরিষদ

Bangladesh Council of Scientific and Industrial Research (BCSIR)

### Analytical Report

Referred by : Dr. M. Sarwar Jahan  
Work Order details : Analysis report of the supplied samples (as supplied).  
Sample supplied by : Rukhsana Shalim, (Supervisor Dr. Md. Nurul Islam, RU)  
Type of Sample : Waste Water  
Quantity of sample : About 500 ml.  
Packing and Marking : Plastic Bottle.

#### **Analytical Result:**

Sl. No.	Parameters	Results		
		S1	S2	S3
01	pH	7.24	7.29	8.74
02	EC	5.38mS/cm	5.46mS/cm	14.83 mS/cm
03	TDS	2.69g/L	2.73g/L	7.41 g/L
04	Fluoride	22.933ppm	20.288ppm	54.217 ppm
05	Chloride	1400.938ppm	1338.380ppm	1958.919 ppm
06	Nitrite	--	--	--
07	Nitrate	1.921ppm	2.835ppm	3.494 ppm
08	Bromide	---	---	4.613 ppm
09	Phosphate	---	---	15.600 ppm
10	Sulfate	425.12ppm	16.542ppm	---
11	BOD	286.00ppm	1874.00ppm	1768.00ppm
12	COD	320.00ppm	1998.00ppm	1854.00ppm

Dr. Qudrat-I-Khuda Road, Dhanmondi, Dhaka-1205, Bangladesh  
Phone: 88-02-8621741, Fax: 88-02-8613022 Email: bcsir@bangla.net

**Detection of metal ions (Arsenic, Cadmium, Cobalt, Chromium, Copper, Iron, Potassium, Manganese, Lead, Zinc) in the tannery effluent. [scanned copy]**

**Arsenic**

Sl. No.	Action	Sample ID	X	True Value (ppb)	Conc. (ppb)	Abs.	Pos.	%RSD
45	UNK11-AV	011			✓ 1.7449	0.0190	11	5.5971
46	UNK12-1	012			4.6379	0.0505	12	
47	UNK12-2	012			5.0787	0.0553	12	
48	UNK12-AV	012			✓ 4.8583	0.0529	12	6.4161

**Cadmium**

Sl. No.	Action	Sample ID	X	True Value (ppm)	Conc. (ppm)	- Abs.	Pos.	%RSD
60	UNK13-2	IES-1	X		0.0098	0.0063	28	
61	UNK13-3	IES-1			0.0145	0.0093	28	
✓62	UNK13-AV	IES-1			✓ 0.0163	0.0104	28	14.9580
63	UNK14-1	IES-2			0.0083	0.0053	29	
64	UNK14-2	IES-2	X		0.0111	0.0071	29	
65	UNK14-3	IES-2			0.0086	0.0055	29	
✓66	UNK14-AV	IES-2			✓ 0.0084	0.0054	29	2.6189
67	UNK15-1	IES-3	X		0.0108	0.0069	30	
68	UNK15-2	IES-3			0.0084	0.0054	30	
69	UNK15-3	IES-3			0.0083	0.0053	30	
✓70	UNK15-AV	IES-3			✓ 0.0084	0.0054	30	1.3217

**Cobalt**

Sl. No.	Action	Sample ID	X	True Value (ppm)	Conc. (ppm)	- Abs.	Pos.	%RSD
19	UNK1-1	001	X		-0.0037	0.0023	14	
20	UNK1-2	001			-0.0066	0.0020	14	
21	UNK1-3	001			-0.0086	0.0018	14	
22	UNK1-AV	001			✓ -0.0076	0.0019	14	7.4432
23	UNK2-1	002			-0.0017	0.0025	15	
24	UNK2-2	002			-0.0007	0.0026	15	
25	UNK2-AV	002			✓ -0.0007	0.0026	15	2.7730
26	UNK3-1	003			-0.0076	0.0019	15	
27	UNK3-2	003			-0.0056	0.0021	16	
28	UNK3-3	003	X		-0.0096	0.0017	16	
29	UNK3-AV	003			✓ -0.0066	0.0020	16	7.0711



## Chromium

	Action	Sample ID	X	True Value (ppm)	Conc. (ppm)	- Abs.	Pos.	%RSD
54	UNK14-1	IES-1			0.4765	0.0676	28	
55	UNK14-2	IES-1			0.4674	0.0663	28	
56	UNK14-AV	IES-1			✓ 0.4723	0.0670	28	1.3730
57	UNK15-1	IES-2			0.1600	0.0227	29	
58	UNK15-2	IES-2			0.1607	0.0228	29	
59	UNK15-AV	IES-2			✓ 0.1607	0.0228	29	0.3108
60	UNK16-1	IES-3			0.5985	0.0849	30	
61	UNK16-2	IES-3			0.5971	0.0847	30	
62	UNK16-AV	IES-3			✓ 0.5978	0.0848	30	0.1668
63	UNK17-1	1			0.0016	0.0008	1	

## Copper

	Action	Sample ID	X	True Value (ppm)	Conc. (ppm)	- Abs.	Pos.	%RSD
19	UNK1-1	001			-0.0037	0.0023	14	
20	UNK1-2	001			-0.0066	0.0020	14	
21	UNK1-3	001			-0.0086	0.0018	14	
22	UNK1-AV	001			✓ -0.0076	0.0019	14	7.4432
23	UNK2-1	002			-0.0017	0.0025	15	
24	UNK2-2	002			-0.0007	0.0026	15	
25	UNK2-AV	002			✓ -0.0007	0.0026	15	2.7730
26	UNK3-1	003			-0.0076	0.0019	16	
27	UNK3-2	003			-0.0056	0.0021	16	
28	UNK3-3	003			-0.0096	0.0017	16	
29	UNK3-AV	003			✓ -0.0066	0.0020	16	7.0711

## Iron

	Action	Sample ID	X	True Value (ppm)	Conc. (ppm)	- Abs.	Pos.	%RSD
55	UNK14-2	014			0.0207	0.0013	14	
56	UNK14-3	014			0.0080	0.0005	14	
57	UNK14-AV	014			✓ 0.0064	0.0004	14	35.3553
58	UNK15-1	015			0.0095	0.0006	15	
59	UNK15-2	015			0.0111	0.0007	15	
60	UNK15-3	015			0.0114	0.0026	15	
61	UNK15-AV	015			✓ 0.0095	0.0006	15	10.8786
62	UNK16-1	016			0.1575	0.0099	16	
63	UNK16-2	016			0.1559	0.0098	16	
64	UNK16-AV	016			✓ 0.1559	0.0098	16	0.7179

### Potassium

	Action	Sample ID	X	True Value (ppm)	Conc. (ppm)	- Abs.	Pos.	%RSD
12	STD-AV	STD 3		0.8000		0.4832	R4	0.5121
13	UNK1-1	001*10			0.3326	0.2122	14	
14	UNK1-2	001*10			0.3126	0.1995	14	
15	UNK1-AV	001*10			✓ 0.3225	0.2058	14	4.3625
16	UNK2-1	002*10			0.3443	0.2197	15	
17	UNK2-2	002*10			0.3277	0.2091	15	
18	UNK2-AV	002*10			✓ 0.3360	0.2144	15	3.4960
19	UNK3-1	003*10			0.3459	0.2207	16	
20	UNK3-2	003*10			0.3656	0.2333	16	
21	UNK3-AV	003*10			✓ 0.3557	0.2270	16	3.9249

### Manganese

	Action	Sample ID	X	True Value (ppm)	Conc. (ppm)	- Abs.	Pos.	%RSD
16	UNK2-1	1			0.2488	0.0321	14	
17	UNK2-2	1			0.2713	0.0350	14	
✓18	UNK2-AV	1			✓ 0.2604	0.0336	14	6.1121
19	UNK3-1	2			0.2287	0.0295	15	
20	UNK3-2	2			0.2139	0.0276	15	
✓21	UNK3-AV	2			✓ 0.2217	0.0286	15	4.7058
22	UNK4-1	3			0.0605	0.0078	16	
23	UNK4-2	3			0.0628	0.0081	16	
✓24	UNK4-AV	3			✓ 0.0620	0.0080	16	2.6683

### Lead

	Action	Sample ID	X	True Value (ppm)	Conc. (ppm)	- Abs.	Pos.	%RSD
50	UNK13-1	013			1.2716	0.0049	13	
51	UNK13-2	013			1.1419	0.0044	13	
52	UNK13-3	013			1.1678	0.0045	13	
✓53	UNK13-AV	013			✓ 1.1419	0.0044	13	1.5890
54	UNK14-1	014			1.1419	0.0044	14	
55	UNK14-2	014			1.2197	0.0047	14	
✓56	UNK14-AV	014			✓ 1.1938	0.0046	14	4.6622
57	UNK15-1	015			1.0381	0.0040	15	
58	UNK15-2	015			0.9343	0.0036	15	
59	UNK15-3	015			1.0381	0.0040	15	
✓60	UNK15-AV	015			✓ 1.0381	0.0040	15	0.0000

**Zinc**

	Action	Sample ID	X	True Value (ppm)	Conc. (ppm)	- Abs.	Pos.	%RSD
45	UNK11-2	011			0.3319	0.2015	11	
46	UNK11-AV	011			<b>0.3351</b>	<b>0.2034</b>	<b>11</b>	<b>1.2866</b>
47	UNK12-1	012			0.3618	0.2196	12	
48	UNK12-2	012			0.3637	0.2208	12	
49	UNK12-AV	012			<b>0.3627</b>	<b>0.2202</b>	<b>12</b>	<b>0.3853</b>
50	UNK13-1	013			0.0440	0.0267	13	
51	UNK13-2	013			0.0438	0.0266	13	
52	UNK13-AV	013			<b>0.0438</b>	<b>0.0266</b>	<b>13</b>	<b>0.2653</b>
53	UNK14-1	IES 01			0.0018	0.0011	14	
54	UNK14-2	IES 01			0.0020	0.0012	14	
55	UNK14-3	IES 01	X		0.0000	0.0000	14	
56	UNK14-AV	IES 01			✓ 0.0020	<b>0.0012</b>	<b>14</b>	<b>6.1488</b>
57	UNK15-1	IES 02			0.0056	0.0034	15	
58	UNK15-2	IES 02	X		0.0038	0.0023	15	
59	UNK15-3	IES 02			0.0058	0.0035	15	
60	UNK15-AV	IES 02			✓ 0.0056	<b>0.0034</b>	<b>15</b>	<b>2.0496</b>
61	UNK16-1	IES 03	X		0.0155	0.0094	16	
62	UNK16-2	IES 03			0.0132	0.0080	16	
63	UNK16-3	IES 03			0.0133	0.0081	16	
64	UNK16-AV	IES 03			✓ 0.0132	<b>0.0080</b>	<b>16</b>	<b>0.8784</b>