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Date: 26-07-2019

CERTIFICATE

This is to certify that this thesis entitled **"Studies on Induced Sterility on FilarialVector, *Culexquinquefasciatus* Say, Using Plant Based Bio- active Compounds"** is an authentic work carried out by **Rahana, V K.**, in the Department of Zoology, University of Calicut, under my supervision and guidance for the full period prescribed under the Ph. D ordinance of University of Calicut. I further certify that no part thereof has been presented earlier for any other degree.

C. U. Campus

Dr. E. Pushpalatha

Studies on Induced Sterility on Filarial Vector, *Culex quinquefasciatus* Say, Using Plant based Bio- active compounds

Thesis submitted to the
University of Calicut in partial fulfilment of
the requirement for the degree of

DOCTOR OF PHILOSOPHY IN ZOOLOGY

Submitted by

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DECLARATION

I hereby declare that the work presented in the thesis entitled **"Studies on Induced Sterility on Filarial Vector, *Culex quinquefasciatus* Say, Using Plant based Bio- active compounds"**, is a genuine record of research work carried out by me under the guidance and supervision of **Dr. E. Pushpalatha**, Assistant Professor, Department of Zoology, University of Calicut.

To the best of my knowledge, no part of this thesis has been previously submitted for the award of any degree, diploma or associateship in any other University.

Calicut University **Rahana. V. K.**

07 September, 2018.

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Rahana, V. K

Dedicated to,

My Son Parthasarathy

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GENERAL INTRODUCTION

Insects cover more than ¾th of the entire world fauna and are the dominant animals of the present day with over 7,50,000 species grouped in 26 orders. They are highly specialized group of invertebrates belonging to the largest animal phyla, the Arthropoda, which are the joined legged animals. Insects appeared on the earth 350 million years ago, during the Mississippian period of the Palaeozoic era. Among these wonderful animals, a large number of them are harmless and they play important role in maintaining food chain and energy flow in the ecosystem. A large number of insect species are adapted to feed over a variety of plants and animals ruthlessly causing appreciable economic loss to our economy and even to the livestock and public health. Such destructive insects attain a status of pest, which has characteristics that are regarded as injurious or unwanted. This is most often because it causes damage to agriculture through feeding on crops or parasitizing livestock and cause illness in cattle and other agricultural animals and the most dangerously, as vectors which causes several dreadful human diseases.

Insects, which transmit the diseases are known as 'vectors', and the diseases are known as the "Vector- borne diseases" (WHO, 2014). One of the largest insect order, 'Dipterans'form a major group of pathogen vectors with 120,000 species that are relatively small, with two-winged, soft bodied insects, commonly called 'flies'. These insects, along with many scavenging flies are serious vectors of diseases, transmitting a huge number of pathogens and other pests of cultivated plants (CDC, 2016). Many such vectors are hematophagous, which feed on blood at some or all stages of their lives, which ingests disease producing microorganisms during a blood meal from an infected host and later inject it into a new organism during their subsequent blood meal.

Vector- borne infectious diseases cause significant fraction of the global infectious disease burden, nearly half of the World's population is infectious with at least one type of vector- borne diseases (WHO, 2004a). These diseases profoundly restrict socio-economic status and development in countries with the highest rates of infection, many of which are located in the tropics and subtropics.

Every year more than 1 billion cases and 1 million deaths from vector- borne diseases have been reported. Malaria, filariasis, dengue, schistosomiasis, Chagas disease, yellow fever, Onchocerciasis and Japanese Encephalitis are some of the dreadful diseases caused by the vector insects globally (WHO, 2017). Arthropods such as mosquitoes, ticks, flies, sand flies, fleas, triatomine bugs and some fresh water snails are the best- known diseases vectors. Among these, mosquito- borne diseases attained special attention due to the dreadful effect caused by the vector mosquitoes on human beings. The major vector mosquitoes are *Anopheles* Sp. causing Malaria, *Culex* Sp. causing Japanese Encephalitis, Lymphatic filariasis and West Nile Fever (WNV) and *Aedes* Sp. causing threatful diseases like Zika fever, Dengue fever, Yellow fever, Chikungunya and Rift Valley fever (WHO, 2014).

Over the past few decades, during which many mosquito-borne human illness were controlled in many areas through the use of habitat modification and with insecticides. Although, several diseases such as Malaria and Dengue fever have re-emerged in Asia and the Americas, West Nile Virus (WNV) had spreadrapidly throughout the United States and Chikungunya fever has resurged in Asia and Africa and emerged in Europe (Gubler, 1998, Yergolker *et al*., 2006).

Even though, India is also endemic for malaria, lymphatic filariasis and kala-azar (African sleeping sickness) (Dikid *et al*., 2013). Nowadays, outbreaks of Dengue, Chikungunya and Japanese Encephalitis occur every year in many

parts of the country. Prevalently, Dengue and Chikungunya are the two dreadful mosquito- borne diseases of great public health concern in India. National Vector-Borne Disease Control Programme (NVBDCP, 2014) reported more than12,250 cases of Chikungunya, across the country. There is an upsurge noted in the number of cases of some of the arboviral diseases like Dengue and Chikungunya, especially in the urban areas. Japanese Encephalitis (JE) outbreaks have been reported from different parts of the country periodically.

There is a constant threat of Malaria and other vector- borne diseases for spread into new areas as a result of climate change and various environmental factors (NVBDCP, 2005). More recently, Zika fever disease from local mosquito-borne Zika virus transmission has been reported in the Continental United States. Zika is spread mostly by the bite of an infected *Aedes* Sp. mosquito. The first documented outbreak occurred among people in the Federated States of Micronesia in 2007 (CDC, 2017). As of 2016, the disease has been reported in 20 regions of the Americas. It is also known to occur in Asia, Africa and Pacific regions (CDC, 2017). Due to an outbreak in Brazil in 2015, WHO declared it a Public Health Emergency of International Concern in February, 2016. There are no vaccines or medicines for Zika. In spite of some spectacular success against Malaria, we are still far from attaining freedom from all these dreadful diseases. It can be possible by maintenance of good hygiene, and proper vector control strategies. Moreover, individual participation of each and every one for the proper management of environment is the most important aspect of vector- control strategies.

The World Health Organization (WHO, 2004) states that control and prevention of vector-borne diseases are emphasizing “Integrated Vector Management (IVM)” programmes, which is an approach that links between health and environment, optimizing benefits to both. WHO (2004) issued the

Integrated Vector Management (IVM) strategy on the promise that effective vector control requires the collaboration of various public as well as private agencies and with community participation. Various key elements of IVM are advocacy, social mobilization, strengthening of regulatory and legislative controls for public health, empowerment communities, collaboration with health and other sectors in planning and decision-making, use of available resources for vector control, implementation of evidence- based strategies and capacity- building (Beier *et al*., 2008). The goal of IVM programmes is to make a significant contribution to the prevention and control of Vector-borne diseases based on global targets by making it more efficient, cost effective, ecologically sound and sustainable.

Vector control is the fundamental element of the existing global strategy to fight Vector-borne diseases. Such controls have been proven to successfully reduce or interrupt the contagious transmission, when coverage is sufficiently high. The core vector control interventions can be supplemented by certain methods, such as source reduction management and the scale- up of personal protection measures (WHO, 2004). At central level, Directorate, NVBDCP facilitate the framing of policies and strategic plans for implementation by States/ UTs (NVBDCP, 2005). However, there has to be a linkage of vector control and vector surveillance activities under Integrated Vector Management (IVM) at central and local levels.

Conventional approaches to the control of individual vector-borne diseases have almost always worked in isolation from one another. A variety of strategies were used in the vector control programmes, particularly the most frequent type of vector control, which are the mosquito control.

The Centres for Disease Control (CDC) and the Environmental Protection Agency (EPA) collaborate on mosquito control programmes throughout the world to control various vector-borne diseases (CDC, 2017). ‘Integrated

Mosquito Management' (IMM) is the vector control programme associated with the combination of methods to control and prevent the mosquito vectors that spread the protozoan parasites like *Plasmodium* Sp., *Wuchereria bancrofti* and *Brugia malayi*and viruses for Zika fever, Chikungunya, Dengue fever, West Nile Viruses etc. IMM is mainly based on an understanding of the mosquito's life cycle, mosquito biology and the parasitic transmission process. It is an integrated approach, utilizing larviciding, adulticiding and source reduction by incorporating with public education and various awareness programmes. A proper IMM programmes uses various techniques in order to reduce the vector mosquitoes maintaining a quality environment.

In the 20th century, large scale vector control programmes successfully brought vector-borne disease transmissions under control over huge areas by the use of broad spectrum conventional/ synthetic insecticides. IVM facilitated the elimination of *Anopheles gambiae* from Brazil around 1940 and elimination of *Aedes aegypti* for urban yellow fever from Americas by 1960. Cuba came close to eradicating *Ae. aegypti* in 1980's and Singapore has reduced the mosquito population down to very low level, though dengue incidence has recently increased (Alphey *et al*., 2010). These programmes were highly successful on the large scale, organized use of broad spectrum insecticides. However, these chemical controls have not generally produced sustainable control of vector population and it also generates various environmental issues. Therefore, disease transmission is likely to persist and the intensity of these programmes declines after its initial success. Besides all these conventional practices, features like more selective, less harmful and more compatible with biological controls, Insect Growth Regulators (IGRs) becomes an attractive alternative to broad- spectrum insecticides.

If IGRs are incorporated into IVM programme, considerations need to be given to the IGRs from natural sources as phytochemicals/ botanicals, which

can improve the quality of the environment by reducing the harmful effects caused by the synthetic insecticides, particularly on non- target species. Scientific literatures provide the information only about the efficacy of the synthetic IGRs, so attention need to be there for botanical IGRs.

Sterile Insect Technique (SIT) is another type of biological insect control method involving the mass- rearing and sterilization of a target pest/ vectors, followed by the systematic wide release of the sterile males and there by declining vector/ pest population (Dyck *et al*., 2005). It is the most environment-friendly, cost effective insectvector control method ever developed. SIT is also called the autocidal control, because it interrupts the insect's reproductive cycle, and it is species specific (Alphey *et al*., 2010).

SIT was first developed in USA and has been used successfully for more than 60 years (Knipling, 1955). Incorporating with other control methods, SIT has been successful in controlling a number of high-profile insect pests, including Mediterranean Fruitfly, tsetsefly, screwworm fly (Lindquist *et al*., 1992), pink boll worm, codling moth, cactus moth, Australian painted apple moth and "mosquitoes".

SIT is also known as 'Sterile Male technique', because the released insects are preferably male insects and a female that mates with a sterile male produce no younger generation, thereby reducing the vector population (Knipling, 1955). Interest in SIT for vector control has re-emerged recently, and it would be within an Integrated Vector Management (IVM), that used integrated control methods simultaneously. SIT can be the most effective control methods, especially for mosquito control, because of its species-specific property, efficacy and sustainability, also it is environmentally sound technique and more appropriate in some situations than other.

More recently, it has been proven that, SIT provides excellent tool for the population eradication of vector mosquitoes by highly sustainable and species-specific approach. On the other hand, SIT can be considered as a type of genetic control programme within the species, there may be mating barriers between different chromosomal forms or type of the species. Sterile males typically released periodically to maintain a permanent standing population of sterile males in the target area, so that there always have a high chance of mating of a female with a sterile male.

SIT mainly consists of irradiation, use of chemosterilants and an alternative method to exploit the natural phenomenon of Cytoplasmic Incompatibility (CI) (Lees *et al*., 2015). In most diploid-diploid species, CI is expressed as embryonic lethality after mating between infected males and uninfected females. Therefore, CI could be used to manage disease vectors through population suppression. CI based population suppression is known as Incompatible Insect Technique (IIT).

The joint FAO/ IAEA programme and their collaborators have been the main drivers for the development of the SIT for mosquitoes. Reviving the use of the SIT against mosquitoes was an Italian group, released around 1000 irradiated pupae of *Ae. albopictus* per hectare per week, induced up to 68% sterility in the target populations (NAFA, 2017). Releases of sterile males in the field were continued for 5 years, and they have succeeded to suppress *Ae. albopictus* population. Similar stage of advancement is also done in a project in South Africa, targeting *Anopheles arabiensis* (NAFA, 2017). FAO/ IAEA is limited a Coordinated Research Project (CRP), consisting mosquito handling, transparent release and male trapping methods to support these projects in developing and validating suitable methods for releasing Sterile male mosquitoes and surveying the target population before, during and after suppression trials.

During the last few years, there have been significant developments towards the use of Incompatible Insect Technique (IIT) for population suppression of mosquito vectors. New *Wolbachia* infection and CI types have been developed for one of the major dengue vectors, *Aedes albopictus* (Calvitti *et al*., 2012). Mating experiments have shown that *Wolbachia* infected males exhibited full CI with uninfected females. Thisanalysis proves the efficiency of the strain, suggesting that it could be principally used for population suppression. Growing interests and demand for the development and application of SIT, combination with the IIT against mosquito vectors, significant advancement had been made in developing protocols for rearing, sterilizing and assessing the quality of male *Aedes* and *Anopheles* mosquitoes (Baldini *et al*., 2014).

Among the various Sterile Insect Techniques (SIT), ionising radiation/ irradiation becomes the principal technique for sterilization, compared with the chemosterilants, because the amount of pollutants released in the environment is very low, even though, it can reduce competitiveness of the males more than chemosterilants.

Sterilization using ionising radiation has been extremely effective and applied successfully for population suppression or eradication of several major disease vectors (Andreasen and Curtis 2005). Irradiation process is generally carried out with gamma rays, due to their high energy and penetrating capacity. During the irradiation process, presence of the free radicals results in DNA damage, leading dominant lethality in the germ cells (Lachancce 1967 and Curtis 1971) and somatic damage can occur in cells undergoing mitosis. Moreover, damage induced by irradiation is greater with increasing dose and the competitiveness of irradiated one will be lower than the competitiveness of wild mosquitoes (Davis *et al*., 1959). In mosquitoes, both pupal as well as adult stages can be irradiated. Pupae are easier to handle for

irradiation than adults. Competitiveness loss is also considered greater in pupal stages than in the adult stages (Curtis, 1976).

Moreover, sexual sterilization in insects can be accomplished by irradiation or by Chemosterilization (Dame *et al*., 1964). Chemosterilants are the chemicals that interfere with the reproductive potential of an organism. Chemosterilants induce heritable changes in the insect's genome, so that it would not present any residual persistence in the environment. Combining insecticidal and sterilizing activity are the other characteristics of some chemosterilant compounds (White, 1966). Chemosterilants can be applied on both larval as well as pupal stage of an insect, which give rise to sterile adults with fertile insects producing non-viable eggs or no offspring. Chemosterilants may be used to control vectors by sterilizing the adult populations, particularly the male insects.

Apholate, tepa, metepa, hexamethylphosphoramide (hmpa), thiotepa, bisazir, and several other alkylating compounds and antimetabolites are well-known for their chemosterilant activity with anti-fertility effect, which could interrupt the reproductive phase of the vector organism. Population of several species of insects have been successfully controlled with the application of the chemosterilant apholate (Chamberlain, 1964). The wide diversity of the chemosterilant may alternately lead to sterility and reproductive dysfunction with extremely variable biochemical mechanisms nevertheless, very inadequately classified by their structural and chemical characteristics (Weidhaas, 1962).

Not only the chemosterilants, almost all the synthetic insecticides suffer from very great disadvantage, their danger to higher animals and human beings limit their use as much as possible. Synthetic insecticides mainly include organochlorines, organophosphates, carbamates and pyrethroids became successful in controlling the vectors. But it could not maintain the quality of

human life as well as the environment. One of the major negative impacts of synthetic insecticides includes their long-term effects, which may lead to chemical pollution. Studies have shown that the chemical residue found in the atmosphere as well as in the water bodies lead to bioaccumulation andeventually biomagnification of these chemical pollutants. The insecticides like DDT and some organochlorines are persistent organic pollutants (POPs) that resist degradation and thus remains in the environment for years. Some POPs have the ability to bioaccumulate and biomagnify and can concentrate upto 70,000 times their original concentration. It can also affect the non-target organisms including humans by disrupting several organ systems.

In these manners, synthetic insecticides considerably affect the natural biological equilibrium. It also diminishes the biodiversity by reducing nitrogen fixation, poses threat to fishes and destroys habitats of birds and other animals and also contributes the disappearance of pollinators. Risk of poisoning depends on toxicity, dose, period of exposure and sensitivity of synthetic insecticides; several millions of cases of pesticide poisonings are registered every year. Also, insecticide exposure is damaging the immune system and endocrine system, because many of the insecticides are endocrine disrupters and can produce detrimental effects up on hormonal balance not only for insects, but also for other animals.

Due to the possible health risks and all the drawbacks associated with the use of synthetic insecticides, the whole world put an initiative to implement an alternative strategy to control insect pests/ vectors by maintaining the natural balance of the ecosystem remaining fairly undisturbed. The most common and suitable alternative vector/ pest control method being the biological control.

In the past few decades, there is an urge to invent new alternatives to reduce the effects the synthetic insecticides have, on the environment. Nowadays,

IVM programmes employ chemical use only when other alternatives are ineffective. The focus is broader than on a specific vector, consider a wide range of control alternatives. To resolve the problems associated with the synthetic insecticides, the most promising alternatives used for vector control are the natural, inexpensive, organic insecticides derived from natural materials like microbes, plants and from certain minerals.

Biopesticides or natural products include several types of pest management intervention through predatory, parasitic or chemical relationships. Recently biopesticides are often important components of Integrated Vector Management (IVM) programmesand received much practical attention as substitutes to synthetic insecticides (WHO, 2012). Biopesticides are inherently less toxic and target- specific with broad spectrum approach. Recently, biopesticides offer powerful tool to produce a new generation of sustainable agricultural products, taking also into consideration, the pest resistance to the conventional insecticides.

Among these, biopesticides of plant origin have long been treated as an attractive alternative to synthetic insecticides for vector- management, because botanicals pose little threat to the environment or to human health (NPIC, 2016). The literatures surveyed the bioactivity of plant derivatives to many vector species, which is yet to be explored and investigated in a big way. Very few botanicals are currently used in the field of vector management and there are few prospects for commercial development of new botanical products. A number of plant products have been considered for use as repellents, antifeedants and toxicants. Pyrethrum and Neem are the well-established commercial products that have found a good position in the market place (Copping *et al*., 2009).

During the last three decades, there have been many reports on the botanicals exhibiting diverse biological efficacies. The insecticides of plant origin are

readily biodegradable and are quite safer for non- target human being and other animals and are well suitable for maintaining the quality of the environment. Slow resistance development, no known environmental hazards, less residual activity and are effective on the pretext of development of insecticide resistance in target organisms. Due to all these features, botanicals are considered as potent and reliable tool in Integrated Vector Management (IVM) programmes.

Phytochemicals are classified either as primary or secondary plant metabolites. Till date, about 2400 plant species have been reported to possess insecticidal properties, approximately more than 350 insecticidal compounds, >800 insect anti- feeding deterrents and quite a good number of Insect Growth Regulators (IGRs) and inhibitors, but apparently only few have achieved the commercial status (EPA, 2017). Currently marketed biopesticides in the world include pyrethrins, nicotine, rotenone, sabadilla and neem-based products. During the last few years, certain plant essential oils mono and sesquiterpenoids are developed as 'green pesticides', because these oils are well known insect toxins, repellents and deterrents.

Phytochemicals/ Secondary metabolites form large molecules with functional groups such as esters, fatty acids, phenols, aldehydes, ketones, hydrocarbons, alcohols, acetylenic compounds, alkaloids and coumarins. They are also considered as plant excretion products. Terpenes, phenols and nitrogenous compounds were the major secondary metabolites produced by several plant species.

However, reports have shown that, still there are barriers existing for the commercialization of phytochemicals such as scarcity of the botanical resources for large scale production, standardisation of extracts and quality control based on the active ingredients of phytochemicals. To isolate,

characterize and synthesis/ formulate the compounds of interest in vector/ pest control becomes a constant challenge.

Due to the increasing demands for chemical diversity in botanicals, screening programmes for active compounds seeking proper analytical methodologies, which include extraction, isolation, characterization and formulation of active ingredients from the natural products. The analysis of bioactive compounds present in the plant extracts involves the applications of several phytochemical screening assays, various chromatographic techniques, spectroscopic analysis and finally different formulation techniques.

The present study envisages a better means to bring about an effective reduction in the population of filarial vector *Culex quinquefasciatus* Say by inducing the phytosterilant effects using selected plant species *Sterculia guttata* Roxb., *Andrographis paniculata* (Burm.) Nees., *Bougainvillea spectabilis* Willd. and *Polyalthia longifolia* Sonn., without causing any environmental contamination.

1.2 Objectives of the present study

1. To screen different extracts of selected plant species for their sterility induction against *Culex quinquefasciatus*.
2. To study the efficiency of plant based bioactive compounds as sterilants in *Cx. quinquefasciatus*.
3. To identify, purify and isolate the plant based bioactive compounds, which have the potential to induce sterility in *Cx. quinquefasciatus*.
4. To make comparative assessment of sterility induction by synthetic chemosterilant versus plant based bioactive compounds.
5. To assess mating competitiveness of sterile males in the laboratory condition.
6. To prepare plant- based formulations and study the effects in the wild mosquito population.

REVIEW OF LITERATURE

Insect vectors are defined as the living organisms that transmit infectious diseases, both in animals as well as in human populations (WHO, 2003). Many of these vectors are blood- sucking organisms, which ingest disease causing pathogens during a blood meal from an infected host. WHO (1996) declared mosquito as "public enemy number one". According to Taubes (1997) and Pincheiro (1997) and WHO (2005), different species of mosquitoes represented significant threat to human health, because of their mode of habit and habitat particularly their ability to carry several disease-causing pathogens that afflict millions of people worldwide.

India is endemic for malaria, dengue fever, filariasis, chikungunya, Japanese encephalitis and visceral leishmaniasis (Mehdi *et al.,* 2012). WHO (2005) and Southgate (1984) reported more than two billion people live in tropical endemic regions with about one million deaths been claimed yearly from malaria and filariasis. Molavi (2003) analysed the Africa's malaria death and confirmed that malaria was the principle cause of at least one- fifth of all young child death in Africa. Service (1983), Gubler (1998), surveyed several species belonging to genera *Aedes, Anopheles* and *Culex* and were the vectors of serious human diseases like Dengue, Dengue Hemorrhagic fever, Japanese Encephalitis, malaria and filariasis. In Florida, major St. Louis encephalitis epidemics occurred in 1959, 1961, 1962, 1977, and 1990 (Day 1989, Gill *et al.* 2000 and Shroyer and Rey 2004). Other epidemics in the U.S. include Colorado 1985, Arkansas 1991, New York 1999, and Louisiana 2001 (CDC, 2007).

Recently, the world paid more attention towards one of the dreadful disease Zika fever, which was also transmitted by *Aedes* species mosquitoes. Zika can cause birth defects and is linked to Guillain- Barre syndrome. WHO (2016)

and CDC (2017) had surveillance system for collecting data on Zika virus, its vectors, transmission and its effects on peoples. WHO (2008) and NVBDCP (2014) conducted the surveillance study about the transmission of dengue fever. Approximately, two-fifth of the World's population are now at risk of dengue. Total of 28,292 cases and 110 deaths were reported in India at 2010.

Meenakshi *and* Jayapraksh (2014) presented an alarming data that billions of people in India is being affected by diseases transmitted by mosquitos every year. According to Ghosh *et al.,* (2012), among the threatening diseases caused by mosquitoes, malaria infects 1-2 million deaths annually and Lymphatic filariasis affected at least 120 million people in 73 countries including Africa, India, South East Asia and Pacific Islands. Bagavan and Rahuman(2010) reported Japanese Encephalitis annual incidence was 30,000-50,000 with mortality estimate d to be around 10,000.

Bernhard *et al.* (2003) mentioned *Culex quinquefasciatus* Say the filarial vector which causes Lymphatic filariasis. It is widely distributed in tropical zones with around 120 million people infected worldwide and 44 million people having common chronic manifestations. But despite its debilitating effects, lymphatic filariasis was given a very low control priority (Ramaiah *et al*., 2006).

WHO (2004) implemented an integrated vector control (IVC) strategy based on the principles of Integrated Pest Management in agriculture. IVM based on different combination approach that improves the efficacy, cost effectiveness, ecological soundness and sustainability of vector control interventions using available resources. NIMR (2013) launched an alternative strategy for malaria control based on non- insecticide components.

Sharma and Sharma (1989), Sharma *et al*., (1985b) and Sharma (1990) described the main components of the strategy for mosquito control,

comprised of reduction of mosquito breeding sites, biological control using larvivorous fishes, health education, community participation, early detection of infection and treatment, environmental improvement through social forestry and intersectoral co- ordination. Dua *et al*., (1991 and 2000) reported industrial malaria control in Bharat Heavy Electricals (BHEL) was began in July 1986 and later on extended to Indian Drugs and Pharmaceuticals Ltd (IDPL). Owing to implementing this IVM strategy, there was a major deduction in insecticides residues were also recorded (NIMR, 2013).

To bring together experts in national, regional and global levels to advance the development and promotion of IVM programs, WHO has organized a consultation meeting in Geneva (WHO, 2007) and consequently, WHO (2008) issued a position statement to IVM to support the advancement of the concept in vector- borne disease control. Regional profile of IVM implementation was first executed by WHO/AFRO (2003), initiated several strategies by re- orienting the Ministries of health to emphasize on IVM as a new strategic approach of vector control in African region. The African region was the first to develop an IVM framework for vector control in 2001 (WHO, 2001) as an integrated approach and it registered a significant decrease in malaria cases from 15,121 in 1998 to 4,996 in 2001(Chanon *et al.,* 2003). Moreover this achievement was realized without using DDT, and as a result of this IVM strategy, Mexico was able to abandon usage of DDT ahead of the scheduled time of 2002.

Matthews (2011) briefly discussed the IVM programme in the context of elimination and eradication of disease vectors. He focussed mainly on possible new tools such as biopesticides and the use of genetically engineered mosquitoes. Gosh *et al.,* (2012) described Integrated Mosquito Management (IMM) involves a combination of strategies for maintaining mosquito vectors under controlled condition.

Over the years, the synthetic pesticides used for mosquito control have varied greatlyin structure, toxicity, persistence and environmental impact. The use and success of chemicals drastically changed with the development of synthetic pesticide (FCCMC, 2009). But the over application and injudicious use of synthetic pesticides affects badly on nature that were not widely questioned until the early 1960s when Rachel Carson published the book Silent Spring (Richards, 1999).

DDT, BHC, Chordane, Hepatochlores, Aldrin and Dieldrin were widely used for mosquito control worldwide. Between 1973 and 1988 EPA banned the uses of these chemicals in U. S (Ware, 1994). White and Krynitsky (1986) found out that, in spite of the banning of these chemicals, many soils and rivers were still contaminated with persistent residues of these compounds and they continue to be detected in wild life. It was reported that organophosphates were generally less persistent than organochlorines (Pimentel and Lehman, 1993), but some had higher acute toxicity for other non- target organisms. WHO (1986a) recommended certain organic compounds including malathion (Fyfanon ®) and naled (Dibrom ®) as adulticides and Temephos (Abate ®) as larvicide. These compounds have relatively low mammalian toxicity and break down rapidly. WHO (1986a) also reported that the accidental discharge of these compounds into aquatic environments were hazardous and toxic to fishes and certain micro crustaceans.

Nowak *et al*.,2017 emphasized the use of pyrethroid insecticides. Pyrethroid insecticides became popularised in the commercial field in the 1990s as a replacement for the chemicals Diazinon and Dursban ® that were curtailed for various environmental and human health reasons. Pyrethroid insecticides were based on the chemical structure of naturally occurring compound pyrethrum, derived from flower of *Chrysanthemum Sp.* native to Africa.

Permethrin, Resmethrin and Sumethrin are the synthetic pyrethroids used for mosquito control (FCCMC, 2009). WHO (1989) reported that the persistency of pyrethroids were more than that of natural pyrethrins and in few cases it was often more persistent than organophosphates. Walker and Lynch (2007) stated that larval control of mosquitoes can be accomplished through the use of contact poisons, growth regulators, surface films, stomach poisons and by using certain biological agents such as fungi, nematodes, copepods and fishes.

Many synthetic compounds discovered as conventional insecticides include mercuric chloride (1860), Paris green (1865), phenol and cresols (1867), naphthalenes (1882), Bordeaux mixture (1883), Rosin- fish oil soap (1886), calcium arsenate (1907) and Nicotine sulphate (1909). The discovery of DDT as insecticide by Paul Muller in 1942 revolutionized the field of pest and vector control strategies. ICMR bulletin (2003) recommended for indoor residual sprays (IRS) for mosquito control, insecticides that could be used were DDT (WP, 50%), malathion (WP, 25%) as well as synthetic pyrethroids including deltamethrin (WP, 2.5%), cyfluthrin (WP, 10%), lambda cyhalothrin (WP, 10%) and alpha cypermethrin (WP, 5%). Insecticides like synthetic pyrethroids were also used for impregnation of bed nets.

ICMR (2002) recorded temephos 50% (EC) and fenthion 82.5% (EC) were used as larvicides for mosquito control. Distillate of crude oil and malaria larvicidal oil (MLO) were also applied as larvicides in mosquito control programmes. For adulticidal control, technical malathion and pyrethrum extract (WP, 2%) used as space sprays. Malathion, an organophosphate insecticide was introduced into the malaria control programme in Gujarat and Maharashtra in 1969 to control *Anopheles culicifacies*which was repeatedly resistant to DDT (Rahman *et al.,* 1959 and Luen and Shalby, 1962).

According to Ohio Vector News (1990), mosquito control also contributed to certain environmental problems, but when compared to agricultural methods,

pesticides used for mosquito controls were applied at lower dosages and in smaller amounts. But the difficulty with the use of pesticides on mosquito control were that, it often applied directly into residential areas and are also sensitive to natural environment (FCCMC, 2009). O' Brien, 1967and Azmi and Naqvi (2011) reported the negative impacts of synthetic pesticides on target and non- target organisms on soil, wildlife and water resources. Taking into concern all these, VDCI (2013) developed Integrated Mosquito Management (IMM) program including surveillance, source reduction, public education, habitat manipulation and use of larvicides and adulticides.

According to ICMR (2002) insecticidal resistance of the vector species remains one of the major drawbacks of using synthetic insecticides. The malarial vector *An. culicifacies* had developed resistance to all groups of insecticides such as DDT, HCH, malathion and deltamethrin. Mittal *et al.,*2002, and Singh *et al*., 2002mentioned the development of resistance to synthetic pyrethroids warrants a caution of the impending possibilities of widespread resistance to other compounds of this group of insecticides.

The massive use of conventional pesticides not only generated several environmental problems, but also the insects soon began to develop resistance against insecticides. To overcome these problems, new generation insecticides called bio- rational insecticides had been developed, which was based on understanding the physiological process specific for communication of insects. These products mainly included analogues of juvenile hormones and moulting hormones, pheromones, inhibitors of tissue formation and hatching and certain biological insecticides (Mamatha *et al.,* 2008). Klowden and Chambers, 1989 mentioned 'methoprene' a biorational insecticide with broad spectrum of activities that interferes with life cycle of a pest/ vector by preventing the reproductive stage. Kawada *et al.*, 2014 described that the

morphogenetic abnormalities were also resulted with the juvenile hormone analogues in larval stages, death in pupal stage and sterility effects in adults.

Iwanga and Kanda (1988) mentioned another bio-rational insecticide pyriproxyfen, which had been used as IGRs in mosquitoes and other insect pests, which caused reduction in blood feeding, number of sperms, mating activity and egg production. Aktar *et al*., 2009reported about the massive use of synthetic pesticides, which caused detrimental effects on the agro ecosystems and the development of resistance often lead to pest resurgence. It was found that resistance had developed in more than 84 species of mosquitoes for each of the groups of synthetic pesticides. Furthermore, the accumulation of insecticidal residues often ends up with water bodies which polluted the whole ecosystems.

According to Ranson *et al.,* (2010) most of the effective mosquito control program was hindered by the development of resistance of vectors, even in recently invaded regions also. Therefore, increasing demands for effective, more sustainable and environment friendly complimentary tactics was appreciated. Knipling (1960b) reported a novel approach to vector control/ pest control which may possibly interfere with the reproductive phase of a pest insect. That technique played a key role for the successful eradication of the screw worm, *Cochliomyia hominivorax* (Coquerel) from certain islands in the West Indies and from South Eastern United States. According to Borkovec (1962), subsequent research based on these accomplishments and by the concern for environmental protection identified three major sterility concepts: Sterile Male Technique by radiation or chemicals, the direct sterilization technique by chemosterilants and genetic technique, in which special mutant strains could be released to suppress natural populations. The concept of Sterile Male technique to insect control or eradication was credited

to Knipling (1955) however, Bushland and Hopkins (1951) reported the first laboratory studies based on this technique.

Lees *et al.,* (2015) observed Sterile Insect Technique (SIT) could be one such tactic implanted in the field of mosquito control programs. According to Knipling (1955, 1979, 1998), Krafsur (1998) and Dyck *et al.,* (2005a) SIT was a species specific and environmentally non-polluting method of insect pest control that relies on the release of large scale of sterile insects. Dyck *et al.,* (2005) reported sterilization using Ionizing radiation has been extremely effective and applied successfully for population suppression or eradication of several major insect pests/ vector species. Joshi *et al.,* (2014), McGraw and O' Neill (2013) and Bourtzis *et al.,* (2014) produced the proof - of-Concept, which has been provided that Cytoplasmic Incompatibility (CI) based population suppression or Incompatible Insect Technique (IIT) could be used to manage insect pests and disease vectors through population suppression or replacement approaches.

Klassen and Cartis (2005) reported, the use of sterile male release for mosquito control was largely abandoned in the 1960s to early 1980s. Whatever, the growing pressure from the proposed use of modern bio-technologies to sterilize or alter mosquitoes had led to revived interest in recent years. Lees *et al.,* (2015) mentioned the Joint FAO/ IAEA program and their collaborators had been the main drivers of the development of SIT for mosquito control for the last few decades. Many countries requested to develop and evaluate the SIT against mosquitoes which have spurred the development and ongoing validation of mass rearing equipment and diet and also protocols for mosquito control strategies. Reviving the use of SIT against mosquitoes was an Italian group (Bellini *et al.,* 2007). With these inspired demonstrations, several vector control groups supported by the FAO/ IAEA were included preparatory activities and initiated pilot trials that included SIT.

Brooke *et al.,* (2013) reviewed a project in South Africa targeting *Anopheles arabiensis* was at a similar stage of advancement. FAO/ IAEA ("Mosquito Handling, Transport, Release and Male Trapping Methods") initiated a Co-ordinate Research Project (CRP) to support these SIT programs, in developing and validating suitable methods for releasing sterile male mosquitoes and surveying the target populations before, during and after suppression trials (http:// www-naweb.iaea-org/nafalipe/ index. html). Lindquist *et al.,* (1992) reported the highly successful, area wide SIT programmes, which had eliminated the screw worm fly *Cochliomyia hominivorax* Coquerel from United States, Mexico and Central America and also from Libya, which suffered a series of outbreaks in 1989. According to Alphey *et al.,* (2010) another successful control of area wide SIT program included the Mediterranean fruit fly (Medfly) *Ceratitis capitata* and the pink boll worm *Pectinomorpha gossypiella* Saunders in the United States and Codling moth *Cydia pomonella* L. in Canada. These area-wide SIT programs succeeded on very large scale – primarily for use in California and Guatemala, the EI Pino facility in Gautemala alone produced around 2 billion sterile male medflies per week.

According to Knipling (1959), Borkovec (1966) and Smith *et al*., (1964) Sterility could be induced by irradiation and chemosterilization whereas, chemosterilization superceded on irradiation methods. Borkovec(1966) defined chemosterilants as chemical compounds that reduce or eliminate the reproductive capacity of the organism to which they are applied. Sterility principle for Insect control or eradication (1971) commented on considerable limitations of operational and economical factors on the apparent universalities, ample laboratory and field experiences to substantiate the practicability and effectiveness of Sterile Male Technique. However, within these limits acceptable chemosterilization have already been developed.

Many chemosterilants had been used to control various insect pests like Mexican Fruit Fly, *Anastrepha ludens* (Loew) (Steiner *et al*., 1965), the house fly, *Musca domestica* L. (Chang *et al.,* 1973), the boll weevil, *Anthonomus grandis* Boheman (Nelson *et al.,* 1972), the screw worm (Crystal, 1971) and several species of mosquitoes (Sharma, *et al*., 1973 and Grover *et al*., 1971). However, not on the basis of its sterilizing properties, but on the basis of its insecticidal properties which exhibited in immature stages of certain insects, these compounds were now being developed commercially. Mulla (1964), Das (1967), Pillai *et al.*, 1969 proved the effect of alkylating chemosterilants on *Cx. pipiens fatigans* by conducting several laboratory screening studies. Borkovec (1964 & 1966) and Chang and Borkovec (1966) commented that chemostrilants containing aziridinyl functional groups had been the most effective compounds and among them the aziridinyl phosphine oxides are particularly outstanding one. Borkovec (1969a & 1969b) reviewed the different classes of chemosterilants. Among these, the toxicological properties of alkylating agents made their direct application in the field undesirable. But in the genetic technique, the alkylating agents were clearly outstanding with its high effectiveness and apparently unlimited spectrum activity in insects and other animals particularly in the male organism. Chang (1973) experimented Pilot Boll Weevil irradiation experiment by using 1,4-butanediol dimethane sulfonate (busulfan), an alkylating agent as a chemosterilant.

With pupal treatment pupae were tolerant to high doses of the chemosterilants and sterility could be induced with shorter duration of treatment, therefore pupal treatment was more advantageous. Knippling (1968) opined that the chemosterilization with pupal treatment in mosquitoes were worth exploiting in sterilization programs. But Das (1967) observed pupal treatment did not effectively induced sterility in *Cx. p. fatigans*, whereas, Pillai and Grover (1969) reported the pupal treatment with the females of *Cx. p. fatigans*

required higher doses of chemosterilant to induce complete sterility. White (1966) also obtained similar results in *Ae. aegypti* and he suggested this was probably due to larger size of female pupae. Pillai and Grover (1969) commented that all the chemosterilants induced higher sterility in males than females both in larval and pupal treatments. The apholate treatment of larvae and adults produced more sterility in males and low fecundity in females.

Application of aziridinyl chemosterilants to natural breeding populations demanded special caution, since these were highly unstable and breakdown into non- sterilizing products in the presence of organic materials and high temperature. Furthermore, these compounds were carcinogenic as well as mutagenic. Krishnamurthy, Ray and Joshi (1962) attempted field-trails with release of sterilized males of *Cx. p. fatigans* into natural populations using irradiated males. Laven (1967) carried out similar experiments with incompatible males. Pal (1974) reviewed a series of release trails with chemosterilized and cytoplasmically incompatible male *Cx. p. fatigans* in villages of Delhi Union Territory during 1972-73. The result of these experiments successfully proved the incidence of egg-raft sterility observed in the villages were less than the proportion of sterile males established in the village populations. Berryman (1967), Haisch (1970) and Fried (1971) had used the term 'mating competitiveness', a parameter used for competitive mating ability of insects. Fried (1971) pointed out the competitiveness were expected to be constant at different sterile: fertile male ratios.

Several researchers had done considerable works on chemical sterilization of insects. La Brecque (1961), Chamberlain (1962) and Crystal (1963) were considered to be the pioneers of chemosterilization techniques. According to Siddall (1976), certain insect's hormones and their derivatives also disrupted the insect's normal development, therefore it is important to consider these compounds as chemosterilants as a perspective of the role of sterility and its

induction in new approaches to pest control. Weidhaas *et al.,* (1961) extended the studies of chemosterilants on mosquitoes.Apholate, Tepa, Metepa, Thiotepa and several other alkylating agents showed promising sterilizing properties among various insect species. Murray and Bickely (1964) reported the effects of apholate on *Cx. p. quinquefasciatus*. Auerbach (1947) observed mustard gas alone produced certain cytogenic effects like mutations and chromosomal breakage in *Drosophila*. Fahmy and Bird (1953), Fahmy and Fahmy (1954) reported a number of studies of the mode of action of "nitrogen mustards" and other alkylating agents on *Drosophila melanogaster*. Rai (1964a) studied thee cytogenic effects of the chemosterilant apholate induced aberrations in the somatic chromosomes of *Ae. aegypti* (L.). Rai (1963a) also reported various x-ray induced chromosomal aberrations in the brain tissue of fourth instar larvae of *Ae. aegypti* (L.). Akstein (1962) observed the presence of a secondary constriction in an arm of one of the larger pairs of chromosomes in *Ae. aegypti* (L.). Alexander (1960) also reported apholate induced cross linking of the two chromatids and the DNA chain of the chromosomes not only breaks but possibly interfere with normal replication as well.

Besides all these, the use of synthetic insecticide causes various environmental consequences (Casida*,* 2010a). Mosquitoes developed genetic resistance to synthetic insecticides (Wattal *et al.,* 1981) and the excessive application of synthetic organochloride, organophosphate and organocarbonate has resulted in development of genetic resistance in mosquito larvae (Sharma *et al.,* 1986). Resistance against DDT was reported in *Anopheles gambiae* earlier in Sudan (Abdalla *et al.,* 2014). The continued wide spread use of malathion on *Aedes aegypti* in Latin America had generated insecticide resistance on target mosquitoes. Insecticide resistance was reported in *Cx. quinquefasciatus*, *Aedes aegypti* and, *Aedes albopictus*

larvae against malathion, premethrin and temphos in Malaysia (Hamdan *et al*., 2005).

Plants produce numerous chemicals, many of which have insecticidal properties. More than 2000 plant species have been known to produce chemical factors and metabolites in value of pest control programs (Shaalan *et al.,* 2005). Russian weed *Anabasis* were reported to have high larvicidal activity against *Culex sp.* (Campbell *et al*., 1993; Thangam *et al*., 1993), because of the presence of several secondary metabolites such as alkaloids, nicotine, anabasine, methyl anabasine and lupinine. Pyrethrum extracted from the flower heads of *Chrysanthemum cinerariifolium* was well-known for its anti-larval activity (Kerkut and Lawrence 1985). Rhizome of the plant *Acoruscalamus*, bulbs of *Allium sativam*and dried exudates of *Gardenia gummifera* also proved its mosquitocidal activities against the larvae of *Cx. quinquefasciatus*. *A. calamus* and *G. gummifera*may be exploited commercially as larvicides (Suryadevara and Khanam,2002). *Sida acuta, Nepeta cataria* had insecticidal potencies, which have been reported by various authors (Adewole *et al*., 2013).

Larvicidal and ovicidal potential of crude hexane, benzene, chloroform, ethylacetate and methanol solvent extracts of the medicinal plant *Delonix elata* against *Anopheles stephensi* and *Aedes aegypti* were observed (Marimuthu *et al*.,2012). The larvicidal effect of aqueous extract of *Eclipta prostrata* leaves were tested against *Cx. quinquefasciatus* (Khanna and kannabiran 2007) and the insecticidal activity of aqueous, methyl alcohol, hexane and petroleum ether extracts of the aerial part of *Scrophularia carina* against the second and fourth instar larvae and adult female of *Cx. pipiens molestus* was investigated (Germinara *et al.,* 2011). Pavela (2008) demonstrated the larvicidal effects of various Euro-Asiatic plants against *Cx. quinquefasciatus*.

Saxena and Tikku (1990) had found, the plant alkaloids could affect the physiological systems in higher animals as well as in insects. A variety of plant species of different families had been also reported to exhibit insecticidal and other biological activities like sterilant, feeding and ovi-posit deterrency, repellency etc. (Jotwani and Srivastava, 1981, Banerji *et al.,* 1985, Kalyanasundaram and Das, 1985, Saxena and Sumithra 1985, Chavan and Nikan, 1988, Saxena and Saxena, 1992, and Saxena *et al.,* 1992).

Apart from these larvicidal efficacies, many of the plant secondary metabolites produced several deleterious effects on insects, which can be manifested in several manners including toxicity, mortality, antifeedant, growth inhibitors or suppression of reproductive behaviour, such as reduction in fecundity and fertility (Jbilou *et al*., 2006). Secondary metabolites also induced toxicity mainly on physiological and biochemical processes of certain Lepidopteran pests (Nathan, 2013). Some natural products consisting of the alkaloids, flavonoids and the phenolic acids exhibited antiviral, antibacterial and cytotoxicity effects (Özçelik *et al*., 2011). Secondary metabolites produced by the plants could alter an insect's several metabolic pathways. Certain alkaloids such as Pyrrolizidine alkaloids produced hepatotoxicity and mutagenicity among different organisms, and it showed antimitotic effects and it could cross the placental barrier. Several alkaloids could induce chromosomal aberrations and it can interact with DNA (Wink and Schimmer, 2009). Flavonoids also induced mutagenicity in both prokaryotic and eukaryotic organisms (Brown, 1980; Nago *et al.,* 1981 and Elliger *et al.,* 1984).

Literature surveys produce several demonstrations, which could establish the toxic properties of secondary metabolites derived from several plants (Rattan, 2010). Most of all the phytoconstituents might induce mutagenicity and genotoxicity with chromosomal aberrations, interfering with cytoskeleton by

altering cell stability, phagocytosis, cellular interactions and cell movements. Many of the phytochemicals might interfere with protein biosynthesis, which was essential for almost all the metabolic pathways in organisms. Most of the compounds substantially affect DNA and certain enzymes, which was influenced by translation processes. Besides these, many of the compounds strongly affect neuronal transmission, particularly on Ach receptor and Na+ - channels. Inhibition of all these metabolic pathways produced prominent malformations on target organisms, which might affect all the developmental processes of an organism. Therefore, usage of plant- derived compounds to regulate pest/vector population promises, development of a sustainable strategy adopted in the field of vector management as an eco-friendly approach without causing residual problems to the environment.

CHAPTER I

SCREENING AND EXTRACTION OF SELECTED PLANTS AND ITS EFFECT ON FRESHLY HATCHED I INSTAR LARVAE OF *CULEX QUINQUEFASCIATUS* SAY.

1.1 **INTRODUCTION**

Insecticides are the chemicals/ agents, of chemical or natural origin that are used to control harmful insects. These insecticides work in different ways and the controls might be to kill, repel, and harm the insects in one or other way or otherwise preventing it from behaviours deemed destruction. Insecticides may be natural or man-made and applied to the target organism through various formulations and delivery systems like baits, sprays, slow- release diffusion etc. (Salyani *et al*., 2006). Insecticides are commonly used in the field of agricultural, industrial applications and public health and are classified based on their structure and mode of action. Some insecticides disrupt the nervous system of insects, whereas others may damage the hormonal system, energy metabolism, reproductive system and sometimes repel them or control them by some other means (NPIC, 2016).

Owing to many reasons, insecticides which are obtained from plants and plant derived products attract more attention for Integrated Vector Management (IVM) programs. Plants have evolved for over 400 million years that have equipped them with plenty of chemical defense in the form of secondary metabolites to defend themselves from insect attack (Tehri and Singh, 2015). Till date, more than 2000 plant species have been known to have introduced in biological pest control programs and among these, the secondary metabolites/ products of some 344 species have been reported with significant activity against mosquitoes (Remia and Logaswamy, 2009). The plant families Asteraceae, Labiatae, Solanaceae, Oocystaceae, Cladophoraceae,

Rutaceae and Meliaceae are well known for their larvicidal, adulticidal and repellent activities against different species of mosquitoes (Shaalan *et al*., 2005). The plant- based compounds like phenolics, terpenoids and alkaloids have proved their activity as repellents, antifeedents, oviposition deterrents, growth inhibitors, moulting hormones, anti-moulting hormones, juvenile hormone mimics, as well as attractants, which are responsible for interfering with the biological activity of the target organisms (Rattan, 2010).

Isolation of secondary metabolites from plants involves subjecting different parts of the plants to various separation processes. Extractions are the crucial first step for separation, which mainly involved the separation of active ingredients of plant tissues from the inert components using selective solvents (Ncube *et al.,* 2008). The products obtained after extraction are relatively complex mixture of metabolites in liquid form or semi solid form or as dry powder form (Tiwari *et al.,* 2011). Polarity was one of the most important factors that had a high influence on the potency of extracted active biochemical from plants. Polar molecules are extracted with polar solvents and non- polar molecules with non- polar solvents. Polar solvents such as methanol, ethanol and ethyl acetate are used for the extraction of hydrophilic compounds, whereas, dichloromethane or dichloromethane/ methanol in ratio of 1:1 are used commonly for lipophilic compounds (Sasidharan *et al*., 2011). Maceration, sonification, heating under reflux and Soxhlet extraction are commonly used for the plant samples extraction. However, solvents with minimum polarity are extracted with hexane or petroleum ether and for maximum polarity with aqueous/ steam distillations. Successful elution of biologically active compounds from the plant material mainly depends on the type of solvents used, which affects the quantity of phytochemicals to be extracted, rate of extraction, diversity of compounds, toxicity of the compounds and subsequent handling of the extractants (Eloff, 1998).

The systematic screening of plant species with the view to discover new bioactive compounds of appropriate toxicity is a routine activity. Pre-extraction and extraction procedures are the important steps in the processing of phytoconstituents from the plant materials. Maceration and Soxhlet extraction are commonly used at the small research setting level. Significant advances have been made in the processing such as modern extraction methods; Microwave-Assisted Extraction (MAE), Ultrasound-Assisted Extraction (UAE) and Supercritical Fluid Extraction (SFE) to increase yield at lower cost (Azwanida, 2015). The quantitative and qualitative estimation of phytoconstituents from the plants are important for the exploration of new biomolecules to be used for synthesis of plant- based insecticides.

Rattan, 2010 reviewed the mechanism of action of plant secondary metabolites on insects. Several physiological and morphological disruptions were observed on insects by the use of botanical derivatives. Physiological disruptions such as inhibition of acetylcholinesterase (by essential oils), sodium and potassium ion exchange disruptions (by pyrethrum), inhibition of GABA- gated chloride channel (by thymol), and cellular respiration (by rotenone). Other mechanisms include blockage of nerve cell membrane action (by sabadilla), of calcium channels (by ryanodium), of octopamine receptors (by thymol), mitotic poisoning (by azadirachtin), disruption of hormonal balance and of the molecular events of morphogenesis and alteration in the behaviour and memory of cholinergic system (by essential oils) were also observed in insects subjected to botanical treatment. Thus, a wide array of biological disruptions is invoked by various plant derived components which promise the unlimited potential of the botanicals for effective use against harmful insects.

Among all insects that harm human health, mosquitoes form the major group of insects causing spread of many deadly diseases. *Culex quinquefasciatus* is known to be vector of many pathogens of humans, domestic and wild animals. Currently, worldwide there are approximately 120 million cases of lymphatic filariasis (WHO, 2000) reported. The mosquito picks up the microfilaria of *Wuchereria bancrofti* from an infected vertebrate. The microfilaria develops inside the mosquito, and is passed on to another vertebrate through the successive blood meal (Foster and Walker, 2002). It also transmits certain viruses, included West Nile virus (WNv), St. Louis Encephalitis virus (SLEv) and Western Equine Encephalitis virus (WEEv). *Cx. quinquefasciatus* is the principal vector of SLEv in the southern U.S.

Although *Cx. quinquefasciatus* is not considered the likely primary vector of WNV in Florida, it likely plays an important role in maintaining the virus within bird populations, and is capable to transmitting it to humans (Day and Stark, 2000). Outside the U.S, *Cx. quinquefasciatus* is responsible for transmitting the filarial nematode, *Wuchereria bancrofti* especially in the Tropical Africa and Southeast Asia, and Rift Valley fever virus (RVF) in Africa (Foster and Walker, 2002). Rift Valley fever has been responsible for major epidemics in Africa and Asia.

Lymphatic filariasis (LF) or elephantiasis is considered globally as a 'neglected tropical disease', transmitted by *Culex* sp. It is estimated that more than 1.3 billion people in 72 countries worldwide are at risk of the disease, and 30% of them live in Africa. Over 120 million people infected with LF, 40 million are incapacitated or disfigured by the disease. To overcome these global issues of vector borne disease outbreaks, proper control measures should incorporate to reduce the number of these causative organisms by using appropriate alternatives in the traditional control programs. Prevention

with synthetic insecticides is well suitable control measures to any of the nuisance vectors/ pests. Unfortunately, the impact of these synthetic insecticides into the entire ecosystem emphasized the search of new alternatives, which do not produce any residual effect on the ecosystem. For these, the best suitable alternatives are the phytoinsecticides, which could not produce any deleterious effect on environment and other non- target organisms.

The potential of plant derived products to target insects of veterinary and medical significance appears strong owing to many reasons like ill effects of the conventionally used chemicals and synthetic pesticides on both target and non-target organisms, to various environmental issues and development of resistance. Several phytochemicals are already widely used to this end, with the effectiveness of products such as pyrethrum and neem particularly well supported through a history of successful use against a range of insects in all sectors. Despite, a lot of plants and their derivatives are yet to be explored for their efficacy as vector control agents. Therefore, the present study also initiated a step towards the use of phytoinsecticides to the control the filarial vector mosquito *Cx. quinquefasciatus*.

The present study envisages to carry out search for the mosquitocidal/ phytosterilant activities of certain phytoconstituents, extracted from the seeds of *Sterculia guttata* Roxb. and *Polyalthia longifolia* Sonn. and also from the leaves of *Andrographis paniculate* Burm. and *Bougainvillea spectabilis* Wild. on the filarial vector *Culex quinquefasciatus* Say.

1.2 REVIEW OF LITERATURE

Mosquitoes are the most important insect vectors which spread major life-threatening diseases such as dengue, chikungunya, malaria, fialariasis, Japanese encephalitis etc. Continuous application of synthetic insecticides for the control of these insect vectors has resulted in different magnitudes such as development of resistance in target species, deposition of residues in the environment and has led to bio magnification and moreover, contributed to environmental pollution which ultimately affects public health. Hence the scientific world now focuses on a less toxic, environmentally safe and cost-effective alternatives in plant- based formulations for the control of these insect vectors. Plants have rich source of secondary metabolites which act against insects as self-defense mechanism to fight herbivorous insects. This property of plants can be explored for a safe alternative to control these dreadful insect vectors. Several plants have reported their potential insecticidal properties. According to Srivastava *et al*., (2012) it was essential to extract plant constituents to isolate biologically active compounds in understanding its toxicity effects as well. Tiwari *et al.,* (2011) reported that phytochemicals were secondary metabolites and naturally synthesized compounds found in all parts of the plant body. It was reported that successful determination of biologically active compounds from plant material was mainly dependent on the type of solvent used for extraction process. According to Remington (2010), the products obtained after extraction from plants were relatively complex mixture of metabolites as dry powder, in liquid or semi solid state. Such preparations included decoctions, infusions, fluid extracts, tinctures, powdered extracts, which had been popularly called 'galenicals'. Ncube *et al*., 2008 mentioned, during the extraction process, solvents diffused into the solid plant material and solubilized compounds with

similar polarity. Tiwari *et al*., (2011) reviewed the general techniques of plant extraction included maceration, infusion, percolation, digestion, decoction, Soxhlet extraction and Counter-current extraction. Handa *et al*., (2008) has reported some of the latest extraction methods for aromatic plants which included headspace trapping, solid phase micro extraction method, protoplast extraction, thermomicrodistillation, microdistillation and molecular distillation.

Campbell *et al.,* (1993) found plant alkaloids like nicotine, methyl anabasine, anabasine and lupinine extracted from the Russian weed *Anabasis aphylla*, killed larvae of *Culex pipiens* Linn., *Culex quinquefasciatus* Say, and *Culex territans* Walker. Pyrethrum and other organic as well as synthetic derivatives stand prominent as effective insecticides (Hartzell and Scudder, 1942). Extracts from Amur Cork tree fruit *Phellodendron amurense* yielded a quick acting mosquito larvicide (Haller, 1940). Hartzell and Wilcoxon (1941) evaluated the toxicity effects of 150 species of plants against mosquitoes and found several to possess very effective mosquitocidal properties. Wilcoxon *et al*., (1940) also reported the mosquitocidal activity of a toxic constituent 'filicin' a phloroglucinol propyl ketone, derived from a male fern *Aspidiumfilix*- mas, against *Cx. quinquefasciatus.*

Jacobson (1958) reviewed botanical derivatives of several phytochemicals against various species of mosquitoes, covering a period from 1941 to 1953. Effectiveness of the botanical derivatives often varies with different mosquito species, and also extracts from different parts of the same species of plants also exhibited various degrees of toxicity to mosquitoes.

According to Marcard *et al*., (1986), different plant parts from *Ajuga remota* and *Ajuga reptans* exerted different toxicity effects against the mosquito larvae of *Aedes aegypti* (Linn.), *Aedes togoi* (Theobald) and *Cx. quinquefasciatus* Say, such as larvicidal activity was decreased with roots >

leaves> shoots> flowers. Patterson *et al*., (1975) reported the activity of extracts from 325 wild growing plants of North Dakota and Western Minnesota were found varied larvicidal effects on *Aedes aegypti* by both plant species as well as with different plant parts of the same species. Minijas and Sarda (1986) reviewed the same phytochemicals from a single plant species exhibited various degrees of toxicity to different mosquito species. It was found that saponin isolated from the crude extract of the fruit pods of *Swartzia madagascariensis* produced higher mortality rate in larvae of *Anopheles gambiae* Giles than in larvae of *Ae. aegypti* and no mortality were observed in larvae of *Cx. quinquefasciatus*.

Sujatha *et al*., (1998) showed differential susceptibilities with petroleum ether extracts of six different plant species against larvae of three different species of mosquitoes. Among these extracts, *Acorus calamus* extract was the most effective against *Cx. quinquefasciatus*, while *Bambusa arundanasia* was toxic against *An. stephensi* Liston. *Citrus medica* extract was toxic only for *An. stephensi* larvae, whereas *Madhuca longifolia* extract had no effect on this species. Graham and Schooley (1984) observed the effect of the extracts of pond weeds *Myriophyllum* and *Potamogeton* against larvae of *Anopheles occidentalis* Dyar and Knab and *Culex pipiens*, in which *Cx. pipiens* showed more resistance to both extracts. Dhillon *et al.,* (1982) also noticed such a differential species susceptibility of certain algal toxins from *Rhizoctonium heirogliphicum* and *Chlorella ellipsoidea* against *Ae. aegypti, Cx. quinquefasciatus* and *Culiseta incidens* (Thomson). Among these, *Culiseta incidens* was found to be the most susceptible and *Cx. quinquefasciatus* the least susceptible to *Rhizoctonium* extracts. But the *Chlorella* extracts appeared to be more toxic to *Cx. quinquefasciatus* and *Culiseta incidens* than to *Ae. aegypti.*

Over years, observations also showed, certain developmental stages of mosquitoes were more susceptible to phytochemicals. It was found that the volatiles of lemongrass (*Cymbopogon citratus*), oil of linalool (*Bursera delpechiana*) and oil of geranium (*Pelargonium roseum*) were noticed poor ovicides and had no effect on first instar larvae, but had caused significant growth inhibition and mortality in later developmental stages of *Ae. aegypti* (Osmani and Sighamony, 1980). It was found that the butanol extract of Soapberry plant (*Phytolacca dodecandra*) on *Ae. aegypti, Cx. pipiens molestus* Forskal and *Anopheles quadrimaculatus* Say were very toxic to second and third instar larvae, but eggs and pupae were unaffected and adult mortality was observed only after ingestion of concentrated extract.

Plant chemicals with specific organic solvent extraction resulted great influence on the bioactivity of most of the insect species. The activity of certain phyto constituents was responsible in great measures only with certain solvents. Sheril and Hall (1985) proved the activities of water and other organic solvents produced higher mortality in *Cx. quinquefasciatus*. It could have been due to the polarity range of the solvents. Hartzell (1944) experimented on the activity of acetone extract and water extracts of certain plants against the larvae of *Cx. quinquefasciatus* and found great results with acetone extract. Chavan *et al*., (1979) observed rhizomes of *Acorus calamus*, extracted with different solvents like petroleum ether, chloroform, ether and alcohol against *Cx. quinquefasciatus* larvae and obtained the best results with extract of petroleum ether.

Besides all these, certain botanical derivatives showed enhanced action in the presence of light. Sukumar *et al*., (1991) obtained polyacetylenes and thiophenes from certain plants of the Asteraceae family, which exhibited the greatest potential as photoactive pest control agents. Arnason *et al.,* (1981) and Kagan *et al*., (1987) had proved the polyacetylene-alpha-terthienyl from

the roots of common marigold, *Tagetes* Sp. highly toxic to *Ae. aegypti* larvae. It was found that activity increased with light, which showed the phototoxic action of alpha- terthienyl. Philogene *et al.,* (1984) reported the fluorescent nature of berberine, an isoquinoline alkaloid present in many plant families. Berberine treatment against *Aedes atropalpus* (Coq.) affected the survival of larvae, pupae and adults by the activity of the alkaloid which increased after the exposure to light. Pimprikar *et al.,* (1979) observed the photosensitized oxidation reaction of Rose Bengal axanthenes – derivative, caused enhanced mortality in mosquito larvae, depends on the absorption of visible light energy, causing photooxidative toxicity.

Studies also had proved that the toxicity of plants might vary with different geographical distribution. Novak (1985) observed acetone and alcohol extracts of garlic and *Allium sativum* on *Aedes* larvae did not produce any toxic effect, when tested in Czechoslovakia. But Amonkar and Reever (1970) in U S A, assayed the extracted oil and crude methanolic extract of garlic against larval mosquitoes of five species, but the results showed the control of mosquito larvae were possible even at very low concentrations. The acetone extract of *Vetiveira zizanoides* roots from U S A failed to induce larval mortality in *Cx. quinquefasciatus* (Jacobson, 1958), but Murthy and Jamil (1987) observed the oil of Vetiveira roots from India was very effective against the larvae of *Cx. quinquefasciatus*.

Phytochemicals generally act as toxicants, however sometimes showed selective interference with growth and reproduction. Cupp *et al.,* (1977) reported Insect Growth Regulatory (IGR) activity of Precocene from *Ageratum* in mosquitoes. It prevented molting of pupa and adult emergence, by the exposure of newly hatched I instars and thus interfering with growth by transgressing certain stages of development. Kelly and Fuchs (1978) also noted the toxic effect of precocene with the female mosquitoes. When females

were treated with precocene after blood feeding it retarded ovarian maturation by inhibiting trypsin synthesis, resulted abnormal oviposition. Aristolochic acid from *Aristolochia bracteata* induced sterility in mosquitoes by inhibiting reproduction (Saxena *et al.,* 1979). Borkovec (1987) reviewed aflatoxin from *Aspergillus flavus*, pactamycin and porfiromycin from lower plants and biotin from certain plants had also sterilized different mosquito species. Present study also carried out to evaluate the larvicidal as well as anti-fertility effect of methanolic extracts of *Sterculia guttata* (Seed), *Andrographis paniculata* (Leaf), *Bougainvillea spectabilis* (Leaf) and *Polyalthia longifolia* (Seed) on filarial vector mosquito *Culex quinquefasciatus*.

1.3 MATERIALS AND METHODS

1.3.1 Collection and Screening of Plants

A total of fifteen plants belonging to different families and genera were collected from in and around Calicut University Campus and Thalassery, Kerala, India. The plants were selected based on available literature, abundant availability and insecticidal properties. Collected plants were taxonomically identified from the Department of Botany, University of Calicut. The list of plants utilised for the present study is provided in **Table 1**. Among these plants, *Sterculia guttata* Roxb., *Andrographis paniculata* (Burm.f.) Nees, *Bougainvillea spectabilis* Willd and *Polyalthia longifolia* Sonn. were selected for the present study after conducting screening tests against the larvae of *Culex quinquefasciatus*.

Table 1: Lists of plants collected from Calicut University Campus and Thalassery, Kerala, and screened against the larvae of *Cx. quinquefasciatus*.

SI No.	Botanical Name	Common Name	Family	Plant parts used
1.	*Mimusops elengi*	'Spanish Cherry'	Sapotaceae	Seed
2.	*Pongamia pinnata*	'Indian Beech Tree'	Fabaceae	Seed
3.	*Sterculia guttata*	'Spotted Sterculia'	Sterculaceae	Seed
4.	*Synedrella nodiflora*	'Cindrella weed'	Asteraceae	Leaf
5.	*Andrographis paniculata*	'King of Bitters'	Acanthaceae	Leaf
6.	*Tridax procumbens*	'Coat buttons'	Asteraceae	Leaf
7.	*Cyanthillium cinereum*	'Little Ironweed'	Asteraceae	Leaf
8.	*Bougainvillea spectabilis*	'Paper Flower'	Nyctaginaceae	Leaf
9.	*Erythrina variegata*	'Indian Coral Tree'	Fabaceae	Seed
10.	*Polyalthia longifolia*	'Buddha Tree'	Annonaceae	Seed
11.	*Croton hirtus*	'Hairy Cotton'	Euphorbiaceae	Leaf
12.	*Pogostemon quadrifolius*	'Bengal Pogostemon'	Lamiaceae	Leaf
13.	*Pogostemonpaniculatus*	'PanicledPogostemon'	Lamiaceae	Leaf
14.	*Gliricidia sepium*	'Quickstick'	Fabaceae	Leaf
15.	*Saraca asoca*	'Sorrowless Tree'	Fabaceae	Leaf

1.3.2 Plants selected for the study

a.*Sterculiaguttata*Roxb.

Kingdom : Plantae

Order : Malvales

Family : Malvaceae

Sub family : Sterculioideae

Genus : *Sterculia*

Species : *guttata*

Plate 1-*Sterculia guttata* Roxb.

The genus *Sterculia* was previously placed in the now obsolete Sterculaceae, which comprised approximately 200 species distributed mainly in tropical and subtropical regions. *Sterculia guttata* Roxb. **(Plate 1)**commonly called as 'Spotted Sterculia' and 'Kavalam, Pottakaavalam and Peenari' in Malayalam. It is a deciduous tree grow up to a height of 20 m and bole straight; the bark is 15-25 mm thick, greyish-brownish, smooth, exfoliating in thin scales; blaze pink, and radially streaked. Leaves are simple, alternate; stipules lateral, ensiform, cauducous; petiole 25-50 mm long stout, swollen at both ends, stellate-tomentose; lamina 12-25 x 6-15 cm, broadly ovate or broadly obovate-oblong, base obtuse, subcordate or truncate, apex acuminate or caudate-acuminate, margin entire, glabrous above, stellate-tomentose beneath, coriaceous; 3-5-ribbed from base, prominent, lateral nerves 5-7 pairs, pinnate, prominent, intercostate scalariform, prominent.

Flowers of *S. guttata* are polygamous, white, dotted with pink, arranged in simple cymes of 3, also on peduncle of short rusty-pubescent axillary racemes; bracts lanceolate; calyx greenish outside, reddish inside, campanulate, clothed with stellate hairs, united to middle; lobes 5, acute,

ultimately reflexed; petals absent; male flowers: staminal column recurved, anthers 10-12 arranged at tip, column hairy at apex; bisexual flowers: ovary 5, free, superior, globose, strigose with stellate hairs; gynophore stout, round; style is stout and deflexed.

Fruit is an aggregate of 1-5 radiating follicles, obovoid, red tomentose, smooth and pink within and seeds are ovoid, black, smooth, shining.

Most of the species of Sterculaceae family comprises medically important plants. *Sterculia guttata* is also known for its highest medicinal value. Its leaves and barks were used as folk medicines (Katade *et al*., 2006). Moreover, this plant is known 'faminefood'. The seeds were eaten raw or roasted by tribes, especially during food scarcity. Some of the species of the *Sterculia* are used for the production of timber and also cultivated as ornamentals. Literature survey revealed the presence of malvelic and sterculic (2.1 and 5.8%, respectively), hexadecanoic (palmitic), octadecanoic (stearic), 9, 12-octadecedienoic (Z, Z) (linoleic), 9-octadecenoic (Z) (oleic) and 9-hexadecenoic (Z) (palmitoleic) acids from the seeds of *Sterculia guttata.*

a. ***Andrographis paniculata*** **(Burm.f.) Wall. ex Nees.**

Kingdom : Plantae

Order : Lamiales

Family : Acanthaceae

Genus : *Andrographis*

Species : *paniculata*

Plate 2- ***Andrographis paniculata*****(Burm.f.) Wall. ex Nees**

Andrographis paniculate **(Plate 2)** comes under the family Acanthaceae which comprises about 40 species of plants. The plant is best known for its medicinal properties, which was traditionally used for the treatment of array of diseases such as diabetics, cancer, influenza, high blood pressure, flatulence, leprosy, skin diseases, colic, bronchitis dysentery, dyspepsia, ulcer, and malaria for centuries in Asia, America and Africa continents. It is an erect annual herb extremely bitter in taste in all parts of the plant body. As an Ayurveda herb, the plant is commonly known as "king of bitters", 'Kalamegha' meaning "dark cloud". It is also known as "neem of the ground" or "Nila- Vembu".

Andrographis paniculate is distributed in tropical Asian countries, often in isolated patches and also as plains, hillsides, coastlines, and disturbed and cultivated areas such as roadsides, farms, and wastelands. It is an annual herb grows erect to a height of 30–110 cm in moist and shady places. The stem is slender and dark green in colour, squared in cross-section with longitudinal furrows and wings along the angles. The lance-shaped leaves have hairless blades measuring up to 8 cm long. The flowers are small and borne in spreading racemes. The fruit is a capsule around 2 cm (0.79 in) long and a few millimeters wide. It contains many yellow-brown seeds.

A. paniculata is widely used in ayurvedic systems of medicines. Though it is used as a valuable medicinal plant, it also exerted insecticidal properties against certain insect pests.

b. ***Bougainvillea spectabilis*** **Willd.**

Kingdom : Plantae

Order : Caryophyllales

Family : Nyctaginacea

Genus : *Bougainvillea*

Species : *spectabilis*

Plate 3- ***Bougainvillea spectabilis*** **Willd.**

Bougainvillea spectabilis Willd. **(Plate 3)** is referred to as "Paper Flower" because its bracts are thin and papery with various colours range from purple or magenta and white to orange. It is native to Brazil, Argentina, Bolivia, Peru and Chubut Province. Literatures provided the information regarding the medicinal properties of *B. spectabilis* such as antidiabetic, antiviral, antibacterial, heptaoprotective and it possess insecticidal properties also. Traditionally it is used to cure the diseases like Diarrhoea, sore throat, cough, stomach disorder, and hepatitis.

The plant is a native to South America, Peru, Brazil and Argentina. It is a woody vine or shrub, reaching 15 to 40 feet. It has a thorny and pubescent stem. Leaves are heart-shaped and flowers are generally small, white, and inconspicuous, highlighted by several brightly coloured modified leaves called bracts with varying in colour, ranging from white, red, mauve, purple-red, or orange. Fruit is small, inconspicuous, dry and elongated achene.

c. ***Polyalthia longifolia*** **Sonn.**

Kingdom : Plantae

Order : Magnoliales

Family : Annonacea

Genus : *Polyalthia*

Species : *longifolia*

Plate 4-*Polyalthia longifolia* Sonn.

Polyalthia longifolia Sonn. **(Palte 4)** is a native in India and Sri Lanka and widely used in parts of Jakarta in Indonesia and the Caribbean islands of Trinidad and Tobago. The plant is commonly known as 'false ashoka', the 'Buddha tree', 'Indian mast tree', and 'Indian fir tree'. It is a lofty evergreen tree, commonly planted due to its effectiveness in alleviating noise pollution. 'Polyalthia' derived from Greek word, which means 'many cure' with reference to the medicinal properties of the tree. Different parts of the tree were used to cure the diseases like uterus ailment, gonorrhoea, leucorrhea, fever, and menorrhagia (Raghunathan and Mitra, 1982). Mouth ulcers were also cured by the decoction made from the bark of *P. longifolia* (Garg and Jain, 1999).

The plant has a symmetrical pyramidal growth with willowy weeping pendulous branches and long narrow lanceolate leaves with undulate margins and is known to grow up to 30ft in height. The stem is short, branches-undivided, glabrous and pendulous. Leaves are alternate, estipulate, shining, distichous, glabrous, narrowly lanceolate and mildly aromatic. Flowers are

delicate, star-like, pale green flowers. The flowers last for a short period, usually two to three weeks, are not conspicuous due to their colour. Fruits are born in clusters of 10-20, initially green but turning purple or black when ripe.

1.3.3 Test Organism- *Culex quinquefasciatus* Say

Kingdom : Animalia

Phylum : Arthropoda

Class : Insecta

Order : Diptera

Family : Culicidae

Genus : *Culex*

Species : *quinquefasciatus*

Culex quinquefasciatus taxonomically comes under the member of *Culex pipiens* species complex. It is commonly known as 'Southern House Mosquito', which is the major vector of Bancroftian malaria, avian malaria and arboviruses including Western Equine Encephalitis virus, St. Louis Encephalitis virus, West Nile virus and Zika virus. It is considered as the primary vector *Wuchereria bancrofti,* a nematode that causes lymphatic filariasis. It also transmits a malarial parasite of bird *Plasmodium relictum* and is the principle vector in Hawaii. Female *Cx. quinquefasciatus* is the definitive host for malarial parasite as it harbours the sexual cycle (Farajollahi, *et al*., 2011).

1.3.3.1 *Culex quinquefasciatus* Say- Reproductive Biology and Breeding Behaviour

*Culex quinquefasciatus*is a medium-sized, brown coloured mosquito,and the body is about 3.96 to 4.25 mm long. While the main body is brown, the proboscis, thorax, wings, and tarsi are darker than the rest of the body. Males can be differentiated from females in having large palps and feathery antennae. In optimum temperature and humidity, the lifecycle will be completed in approximately with seven days, passing through egg, larval, pupal, and adult stages.

Cx. quinquefasciatus usually selects organically rich and polluted surface waters and artificial containers for their breeding places. It was found in domestic collections of water and in places like flooded latrines, flooded open cement drains, kitchens overflow water from houses, as well as in ditches, ground pools and shallow wells. This species also breeds in a wide variety of sites, mostly characterized by coloured, foul water with high nutrient values and low dissolved oxygen content, such as canals, wastewater treatment ponds, septic tanks, rain pools, sewage overflows, paddy fields, cesspools, drains, vegetable trenches etc. (Matthys *et al*., 2006 and Opoku *et al*., 2007).

Females of *Cx. quinquefasciatus* mate within 2-6 days of emergence and may begin to seek hosts within 48 hours of emergence (Subra,1981). The duration of larval stages was 118 hours for males and 135 hours for females (Meillon *et al.*, 1967). *Cx.quinquefasciatus* is active and reproduces year- round, since this mosquito must require blood meal for reproduction and did not undergo a reproductive diapause. In India, during the hotter season, *Cx. quinquefasciatus* may complete 2-3 gonotropic cycles in a life time, while in the cooler season 4-8 cycles were observed.

Eggs

The eggs of *Cx. quinquefasciatus* was laid in oval rafts loosely cemented together with 100 or more in a raft and will normally hatch 24 to 30 hours being oviposited (Bates, 1949). Gravid female of *Cx. quinquefasciatus* lays single egg raft during each gonotropic cycle. Variation in the number of eggs mainly depends on the age of mosquito, blood source and blood volume (Reuben *et al.,* 1994) **(Plate 5)**.

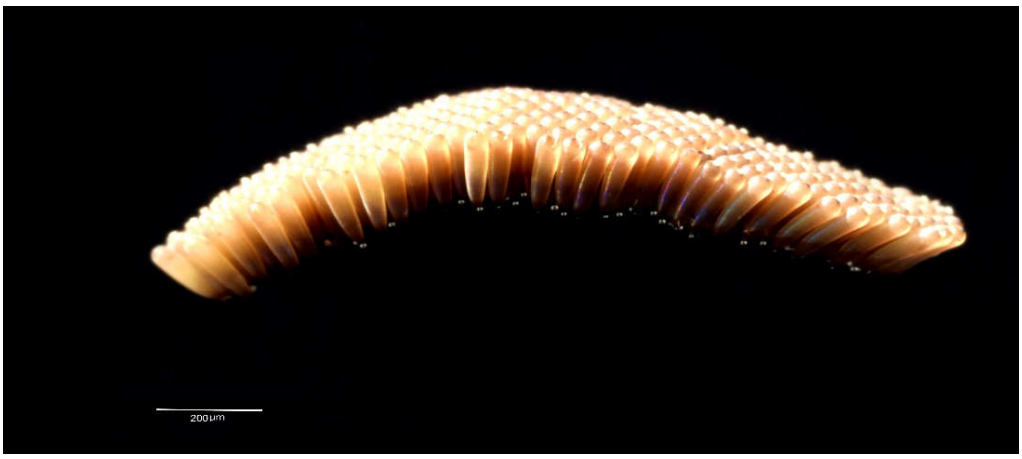

Plate 5- Egg raft of *Culex quinquefasciatus*

Larvae

Larval to adult developments are mainly depended on nutrition, temperature and population density and can be as short as 7 days under optimal temperature (Rueda *et al.,* 1990). The larvae hatch from the egg passes through four larval instars, and towards the end of the fourth instar, they stop eating and undergo moulting to give rise to pupae. Between each moulting they shed their rigid outer skin termed as 'exuvia' and increases the size. The larvae feed on organic material in the water bodies and require between five and eight days to complete their development at optimum temperature (Hill *et al*., 2009).

The larvae of *Cx. quinquefasciatus* have a short and stout head becoming darker towards the base. The mouth consists of brushes have long yellow filaments used for filtering organic materials. The abdomen consists of eight segments, the siphon, and the saddle **(Plates6- a, b, c & d)**. Each segment has a unique setae pattern (Sirivanakam and White, 1978). Larvae breathe air from spiracles at the tail end of the body, generally through a structure termed a siphon. The siphon is on the dorsal side of the abdomen, and is four times longer than its breadth. The siphon has multiple setae tufts (Darsie and Morris, 2000). They hang below the water surface with only the tip of the siphon exposed to the air. They could remain motionless on the bottom for some time, but need to return to the surface for air to prevent suffocation. The saddle is barrel-shaped and located on the ventral side of the abdomen with four long anal papillae protruding from the posterior end (Sirivanakam and White, 1978).

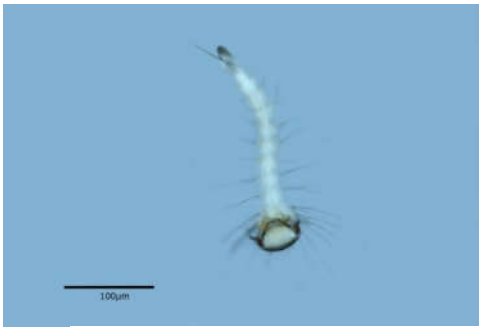

Plate 6- (a)- I instar larvae

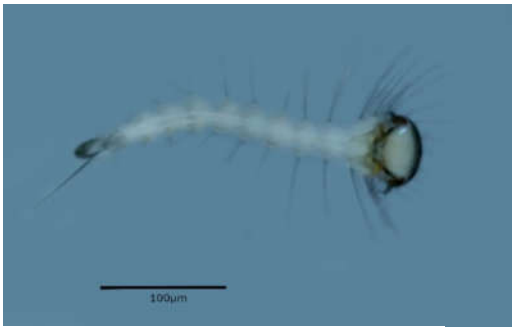

Plate 6- (b)- II instar larvae

Plate 6- (c)- III instar larvae

Plate 6- (d)- IV instar larvae

Pupae

Pupae of *Cx. quinquefasciatus* were 'comma' shaped and consist of cephalothorax (fused head and thorax) and abdomen. The colour of the cephalothorax varies with habitat and darkens on the posterior side. The pupa breathes through a pair of tube-like organs (trumpet) situated at the 'head' end of the comma-shaped body. The abdomen has eight segments. The first four segments are darkened, and the color lightens towards the posterior end **(Plate 7)**. The paddle, at the apex of the abdomen, is translucent and robust with two small setae on the posterior end (Sirivanakarn and White 1978). Duration of the pupal stage mainly depends on temperature and is generally completed in 2-3 days.

At the time for emergence, the pupa swims to the water surface and stretches itself out to full length and the pupal skins splits along the back and the adult mosquito emerges above the water surface. After emerging from the pupal casing, the adult mosquito rests on the water surface for a short time, to allow its wings and body to dry, before flying off in search of nourishment and a mate. Male mosquitoes developed faster than females, and are usually the first to emerge.

Plate 7- Pupae of *Culex quinquefasciatus*

Adults

Cx. quinquefasciatus is a medium-sized brown coloured mosquito **(Plate 8)**. The body is about 3.96 to 4.25 mm long and brown, the proboscis, thorax, wings, and tarsi are darker than the rest of the body. The antennae and the proboscis are about the same length. The flagellum has 13 segments that may have few or no scales. The scales of the thorax are narrow and curved. The abdomen has pale, narrow, rounded bands on the basal side of each tergite. The female is pale brown with darker brown thorax with paler markings. Males can be differentiated from females in having large palps and feathery antennae. Both male and female mosquitoes feed on plant juices, sugars from flowers and fruit nectars to replenish expended energy reserves. Female mosquitoes then mate with a male, usually near a breeding site at dusk. It can mate only once, as the sperm packet introduced by a male during the mating act is sufficient for the female to fertilize all batches of eggs subsequently produces. For the development of egg, female mosquitoes require protein via a blood meal. Ingested blood is necessary for the production of eggs. A single female can lay up to five rafts of eggs in a lifetime, with each raft containing 200-300 eggs. The exact number varies depending on climatic conditions.

In general terms, mosquitoes are attracted to a warm- blooded host by a combination of factors; carbon dioxide, a product of respiration is an important attractant and various body odours and chemicals such as lactic acid. These seem to be the longer- range attractants. At closer distances, temperature can be a factor, as can visual perception at very close proximities.

The life span of adult mosquitoes is not well known. Some species apparently live one or two months during the summer, although under unfavourable conditions this period may be greatly reduced. All stages in the life cycle of a mosquito are dependent upon a number of environmental factors for their survival and development.

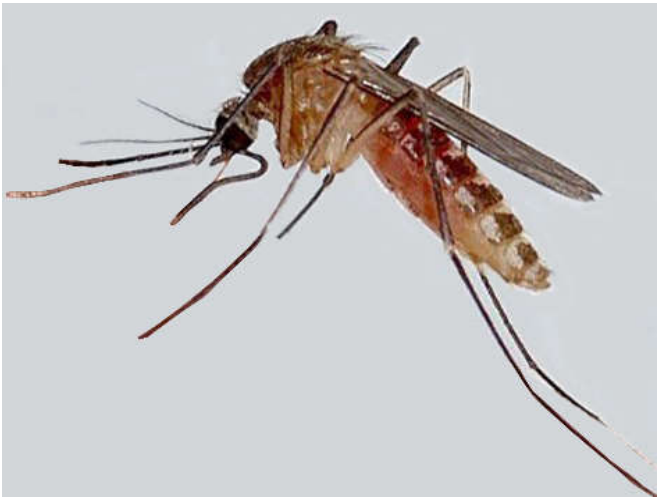

Plate 8- Adult *Culex quinquefasciatus*

1.3.4. Maintenance of Laboratory Culture of *Cx. quinquefasciatus* Say

Developmental stages of *Culex quinquefasciatus* were collected from open drains from Kalluthan Kadavu Colony **(Plates 9 & 10)**, Calicut, brought to the laboratory and maintained at 27±2°c and 75-85% relative humidity, fewer than 14:10h light and dark cycles. The larvae were kept in plastic or enamel trays containing tap water and fed by a diet of fine powder of dog biscuits and Brewer's yeast in the ratio of 3: 1 respectively. The pupae were kept inside the standard emergence cages. After emergence the mosquitoes were identified and species confirmed before rearing **(Plates 11& 12)**. The adults were fed by 10% sucrose solution and additional blood meal was provided (immobilized quail) to adult females to facilitate the development of egg. A bowl containing water kept in the emergence cages to facilitate oviposition. The eggs laid were removed from the cage and after hatching, the larvae were reared in the laboratory at room temperature.

Plates- 9 **Plate 10**

(Kalluthan Kadavu Colony, Calicut (Collection site)

Plate- 11 **Plate 12**

(Mosquito rearing cages)

1.3.5 Extraction of Plant Materials

Plant materials such as seeds of *S. guttata* and *P. longifolia* and leaves of *A. paniculata* and *B. spectabilis* were collected and brought to the laboratory, washed with dechlorinated water, shade dried under room temperature. The plant materials were powdered individually using an electric blender. 20gm

powder of each plant material was separately extracted with methanol and acetone by using Soxhlet apparatus. The solvent extraction was fixed for 12hrs. Distilled water was used to obtain aqueous extract; magnetic stirrer was used for a period of 24hrsfor proper mixing of the powder during aqueous extraction. The extracted content was then subjected to rotary vacuum evaporation until solvents were completely evaporated to get the solidified crude extracts. The crude extracts thus obtained was stored in sterilized amber coloured bottles and maintained at 4^0C in a refrigerator. Crude extract was dissolved in appropriate solvents for making 1% stock solution. From the stock solution, different concentrations were prepared and used for bioassays on larval instars of *Cx. quinquefasciatus*.

1.3.6 Percentage yield of Plant Extracts

Extraction yield/ Percentage yield of the individual plant material was calculated using the formula;

$$\textbf{Percentage yield} = \frac{\textbf{Mass of extract}}{\textbf{Mass of dry matter}}$$

1.3.7 Larvicidal Bioassay (Lc_{50})

Bioassay for the estimation of larvicidal activity using **WHO** protocol **(2005)** with slight modifications was adopted for the study. Larvicidal bioassay of individual plant extracts were tested against freshly hatched first instars larvae of *Cx. quinquefasciatus* using desired concentrations of the plant extracts **(Table 2)**. From the 1% stock solution, different concentrations were prepared and applied on the glass beakers containing 100ml of dechlorinated tap water.

Table 2- List of different extracts of the selected plants used for larvicidal bioassay

SI No.	Name of plants	Solvents used	Concentrations (ppm)
1.	*Sterculia guttata*	a. Acetone	0.1, 0.5, 1.0, 2.0 & 5.0
		b. Methanol	5.0, 10, 50, 70 & 100
		c. Aqueous	100, 300, 500, 700 & 1000
2.	*Andrographis paniculata*	a. Acetone	80, 100, 200, 300 & 400
		b. Methanol	1.0, 3.0, 5.0, 10 & 50
		c. Aqueous	200, 400, 600, 800 & 1000
3.	*Bougainvillea spectabilis*	a. Acetone	5.0, 10, 50, 100 & 150
		b. Methanol	100, 300, 500, 700 & 900
		c. Aqueous	100, 300, 500, 700 & 1000
4.	*Polyalthia longifolia*	a. Acetone	50, 100, 200, 400 & 600
		b. Methanol	200, 400, 600 800 & 1000
		c. Aqueous	200, 400, 600, 800 & 1000

Ten healthy,first instars larvae were released in each glass beakers containing 100ml tap water with respective extracts of desired concentrations and larval mortality was observed after 24hrs of exposure at each concentration. Triplicates for each dose and both methanol (+ve) and water controls (-ve) were maintained. The control mortality was corrected using **Abbott's formula (1987).**

$$\text{Percentmortality} = \frac{\%\,\text{mortalityin treated} - \%\,\text{mortalityin control}}{100 - \%\,\text{mortalityin control}} \text{X}100$$

Statistical analysis was done with Pearson's Correlation (1895) analysis to verify the associations between concentrations and percentage larval mortality.

1.3.8 Bioassay for estimation of Effective Concentration (Ec_{50})

It was carried out by using **WHO** protocol **(2005)** with slight modifications. Half of the lethal concentration was prepared from 1% stock solution and applied on the beakers containing 100ml of tap water. Ten freshly hatched first instars larvae were introduced in each beaker. Triplicates and both methanol and water controls were maintained. The emergence in treated and the controls were recorded and calculated Ec_{50} values.

The percentage emergence was corrected using **Abbott's formula (1987).**

$$\text{Percent emergence} = \frac{\text{\% emergence in treated - \% emergence in contro}}{\text{100 - \% emergence in control}} 1 \text{ X } 100$$

1.3.9 Fractionation of the crude extract using Column Chromatography.

Column chromatography was conducted according to the protocol **Harwood *et al.*, 1989** with slight modifications. Column of size 50cm x 2.5cm were used for the study. The bottom of the column was plugged with little cotton to prevent the adsorbent pass out, and prepared slurry of silica gel 60- 120 mesh size with n-hexane into a homogenous suspension (Stationary phase) and poured gently into the column, set aside for 10 minutes and used. Opened the stop cock and allow some solvent to drain out until the layer of solvent should cover the adsorbent.

The dry powder of crude methanolic seed extracts of *S. guttata* and *P. longifolia* and leaf extracts of *A. paniculata* and *B. spectabilis* was loaded on the top of silica slurry. The column was eluted with (Polar X Polar) solvents

like Methanol: Ethyl acetate (1:9, 2:8, 3:7, 4:6, 5:5, 6:4, 7:3, 8:2 and 9:1) and solvents (Non- polar X Polar) like n-hexane: Ethyl acetate (1:9, 2:8, 3:7, 4:6, 5:5, 6:4, 7:3, 8:2 and 9:1) with increasing polarity respectively for the selected plants. The coloured bands/ different fractions travel down the column as the compound was eluted. The eluted fractions were collected by changing the glass beakers according to the coloured bands. The different column fractions were evaporated to dryness and prepared 1% stock solution and stored in the refrigerator.

1.4 RESULTS

1.4.1 Percentage yield of plant extracts

Percentage yield of the extracts of *S. guttata, A. paniculata, B. spectabilis* and *P. longifolia* seeds and leaves were prepared by Soxhlet extraction methods using acetone, methanol and water are summarized in the **Table 3**. In reflux, percentage yield of different solvent extracts of *S. guttata* seed powder are 6%, 2.9% and 7% for acetone, methanol and aqueous extracts respectively. The leaf extract of *A. paniculata* yielded 0.2%, 3.6% and 6% for acetone, methanol and aqueous extracts respectively. *B. spectabilis* leaf extracts in acetone, methanol and aqueous yielded 9%, 0.85% and 9% respectively. The acetone, methanol and aqueous extracts of *P. longifolia* seed yielded 0.50%, 1.75%and 1.5% respectively.

Table 3 – Data on Percentage yield obtained for the different extracts of the selected plants

SI No.	Name of plants	Plant parts used	Solvents used	% Yield
1.	*Sterculia guttata*	Seeds	a. Acetone	6.00
			b. Methanol	2.90
			c. Aqueous	7.00
2.	*Andrographis paniculate*	Leaves	a. Acetone	0.20
			b. Methanol	3.60
			c. Aqueous	6.00
3.	*Bougainvillea spectabilis*	Leaves	a. Acetone	.00
			b. Methanol	0.85
			c. Aqueous	9.00
4.	*Polyalthia longifolia*	Seeds	a. Acetone	0.50
			b. Methanol	1.75
			c. Aqueous	1.50

1.4.2 Larvicidal Bioassays

In the preliminary screening, crude methanolic, acetonic and aqueous extracts of S. *guttata, A. paniculata, B. spectabilis* and *P. longifolia* were tested for their potent larvicidal toxicity on first instar larvae of *Cx. quinquefasciatus.* The results of the initial assay of extracts showed that crude methanolic extract of *S. guttata* induced 96.67% mortality at 100ppm, while acetonic extract showed 93.33% mortality at 5ppm **(Table 4)**. At 50ppm concentration, the toxicity was 90% on crude methanolic extract of *A. paniculata* and showed 100% mortality on acetonic extract **(Table 5).**

Crude methanolic extract of *B. spectabilis* exerted 100% larval mortality at 900ppm, and 96.67% mortality at 150ppm on acetonic extract **(Table 6).** Toxicity of *P. longifolia* was 100% at 1000ppm on methanolic extract and 96.67% at 600ppm on acetonic extract **(Table 7)**. Larval mortality was not observed in aqueous extracts of all the four plants even at 1000ppm concentration. No larval mortality was observed both in methanol and acetone (+ve) and water (–ve) control experiments.

Table 4 - Percent mortality observed after 24hrs treatment with the Crude Methanol and Acetone extracts of *Sterculia guttata* tested against the first instar larvae of *Cx. quinquefasciatus.*

SI No.	Extracts	Concentration (ppm)	Mortality (%)	Corrected %	P- Value	Controls	
						Methanol	Water
1	Methanol	5	23.33 ± 3.33	23.33	0.0001*	0.00±0.00	0.00±0.00
2		10	50.00 ±5.77	50.00			
3		50	73.33 ± 3.33	73.33			
4		70	90.00 ± 5.77	90.00			
5		100	96.67 ± 3.33	96.67			
1	Acetone	0.1	30.00± 0.00	30.00	0.0020*	Acetone	Water
2		0.2	36.67±3.33	36.67		10.00± 0.00	0.00±0.00
3		1.0	40.00 ± 0.00	40.00			
4		2.0	56.67 ±3.33	56.67			
5		5.0	93.33±3.33	93.33			

Note: 10 numbers of treated females and males were taken in each of the 3 replicates.
The values are expressed as mean ± SD for 10 animals (n=10) per group
* Significant at $P < 0.05$ with Control experiments.

Table 5 - Percent mortality of Crude Methanolic and Acetonic extracts of *Andrographis paniculata* against the first instar larvae of *Culex quinquefasciatus.*

SI No.	Extracts	Concentration (ppm)	Mortality (%)	Corrected %	P- Value	Controls	
						Methanol	Water
1	Methanol	1	20.00±0.00	20.00	0.0023*	0.00± 0.00	0.00±0.00
2		3	36.67±6.67	36.67			
3		5	53.33 ± 3.33	53.33			
4		10	70.00 ±5.77	70.00			
5		50	90.00±0.00	90.00			
1	Acetone	80	30.00± 0.00	30.00	0.0008*	Acetone	Water
2		100	46.67 ± 3.33	46.67		0.00±0.00	0.00± 0.00
3		200	66.67±3.33	66.67			
4		300	86.67 ±3.33	86.67			
5		400	100.00±0.00	100.00			

Note: 10 numbers of treated females and males were taken in each of the 3 replicates.
The values are expressed as mean ± SD for 10 animals (n=10) per group
* Statistically Significant at$P < 0.05$ with Control experiments.

Table 6 - Percent mortality of Crude Methanolic and Acetonic extracts of *Bougainvillea spectabilis* against the first instar larvae of *Culex quinquefasciatus.*

SI No.	Extracts	Concentration (ppm)	Mortality (%)	Corrected %	P- Value	Controls	
						Methanol	Water
1	Methanol	100	23.33±3.33	23.33	0.0069*	10.00± 0.00	0.00±0.00
2		300	40.00±5.77	40.00			
3		500	53.33 ±6.67	53.33			
4		700	96.67 ±3.33	96.67			
5		900	100.00 ±0.00	100.00			
1	Acetone	5.0	16.67±3.33	16.67	0.0065*	Acetone	Water
2		10	30.00±0.00	30.00		12.33±3.33	0.00±0.00
3		50	46.67 ± 3.33	46.67			
4		100	66.67 ± 3.33	66.67			
5		150	96.67± 3.33	96.67			

Note: 10 numbers of treated females and males were taken in each of the 3 replicates.
The values are expressed as mean $\pm$ SD for 10 animals (n=10) per group
* Statistically Significant at$P < 0.05$ with Control experiments.

Table 7 - Percent mortalty of Crude Methanolic and Acetonic extracts of *Polyalthia longifolia* against the first instar larvae of *Culex quinquefasciatus*

SI No.	Extracts	Concentration (ppm)	Mortality (%)	Corrected %	P- Value	Controls	
						Methanol	Water
1	Methanol	200	16.67 ±3.33	16.67	0.0028*	10.00±0.00	0.00±0.00
2		400	46.00 ±6.67	46.00			
3		600	73.33 ±6.67	73.33			
4		800	93.33 ± 3.33	93.33			
5		1000	100.00 ± 0.00	100.00			
1	Acetone	50	26.67 ±3.33	26.67	0.0014*	12.33± 3.33	0.00±0.00
2		100	40.00 ± 0.00	40.00			
3		200	63.33 ± 3.33	63.33			
4		400	83.33 ± 3.33	83.33			
5		600	96.67 ±3.33	96.67			

Note: 10 numbers of treated females and males were taken in each of the 3 replicates.

The values are expressed as mean $\pm$ SD for 10 animals (n=10) per group

* Statistically Significant at$P < 0.05$ with Control experiments.

On the basis of preliminary screening on larvicidal toxicity, the different plant extracts were subjected to detailed investigations and 24 hr LC_{50} and LC_{90} and the data are provided in **Table 8**. 24 hr LC_{50} of methanol extract of *S. guttata* seed is 24.02 ppm and that of acetone extract is 1.46 ppm. 24 hr LC_{50} values of methanol and acetone extracts of *A. paniculata* leaf are 67.3 and 134.5 ppm respectively. LC_{50} estimated for *B. spectabilis* leaf extracts when treated with I instar larvae of *Cx. quinquefasciatus* after 24 hrs exposure are 358.8 and 56.9 ppm for methanol and acetone extracts and that of *P. longifolia* seed are 431.7 and 161.9 ppm for methanol and acetone extracts respectively. Corresponding LC_{90} values of methanol and acetone extracts are 73.8 and 4.61 ppm, for *S. guttata* seed, 147. 4 and 301.3 ppm for *A. paniculata* leaf, 698.3 and 139.2 ppm for *B. spectabilis* leaf and 737.2 and 456.7 ppm for *P. longifolia* seed respectively **(Table 8).**

Table 8- 24hrs LC_{50} and LC_{90} (ppm) and related statistics of the selected plant extracts tested against first instar larvae of *Culex quinquefasciatus*

Plant/ parts	Extracts	24 hrs LC_{50} (LC_{90}) ppm	Lower Fiducial Limit (LFL)	Upper Fiducial Limit (UFL)	X^2	Regression	Significance
S. guttata seed	Methanol	24.02 (73.8)	22.02 (54.03)	38.82 (126.9)	11.3	y= 0.715x + 33.23 R^2 = 0.895	0.002*
	Acetone	1.46 (4.61)	1.17 (3.99)	1.77 (5.51)	0.73	Y= 12.55x + 30.49 R^2 = 0.989	0.865
A. paniculata leaf	Methanol	67.3 (147.4)	42.56 (109.61)	102.3 (249.3)	9.86	y= 0.427x+ 17.18 R^2 = 0.965	0.004*
	Acetone	134.5 (301.3)	80.75 (248.63)	174.35 (412.5)	6.269	y= 0.206x+ 21.33 R^2 = 0.961	0.099
B. spectabilis leaf	Methanol	358.8 (698.3)	66.46 (514.5)	559.4 (1540.9)	24.15	y= 0.105x + 10.11 R^2= 0.934	0.000*
	Acetone	56.9 (139.2)	31.14 (105.39)	84.26 (223.98)	9.663	y= 0.504x+ 19.53 R^2= 0.981	0.022*
P. longifolia Seed	Methanol	431.7 (737.2)	395.58 (690.1)	465.3 (797.28)	1.460	y= 0.126x – 2.377 R^2= 0.996	0.691
	Acetone	161.9 (456.7	130.19 (409.1)	191.01 (521.9)	3.178	y= 0.123x+ 28.72 R^2= 0.931	0.365

* Statistically Significant at $P < 0.05$

Pearson's Correlation analysis reflected the degree of linear relationship between concentrations and percentage larval mortality. Positive correlation was found between the concentrations and percentage larval mortality in methanolic and acetonic extracts of *S. guttata, A.paniculata, B. spectabilis* and *P. longifolia* except the methanolic extract of *P. longifolia* which was negatively correlated with larval mortality (**Figures– 1 a & b, 2 a & b, 3 a & b, 4 a & b**).

Multiple regression analysis of concentration over percentage larval mortality have been presented on **Figures- 1 a & b, 2 a & b, 3 a & b, 4 a & b**. In case of methanolic and acetonic extract of *S. guttata*, the Coefficient of determination (R^2) was 89.5% and 98.9% respectively ie., more than 89% and 98% of the larval mortality could be explained by the independent concentrations (**Figure 1 a & b**). The relationship between Concentrations and percentage mortality was positive. The Coefficient of determination (R^2) was 96.5% and 96.1% respectively for methanolic and acetonic extract of *A. paniculata,* which means more than 96% of larval mortality was explained by the independent concentrations (**Figures 2 a & b**). The relationship between concentrations and percentage mortality was found to be positive.

For *B. spectabilis* and *P. longifolia*, the Coefficient of determination (R^2) was 93.4% and 98.1% and 99.6% and 93.1% respectively for both methanolic and acetonic extracts ie., more than 90% of the larval mortality could be explained by the independent concentrations **(Figures 3 a & b and 4 a & b)**. The relationship between concentrations and percentage larval mortality was positive except the methanolic extract of *P. longifolia*, which was found to be negative.

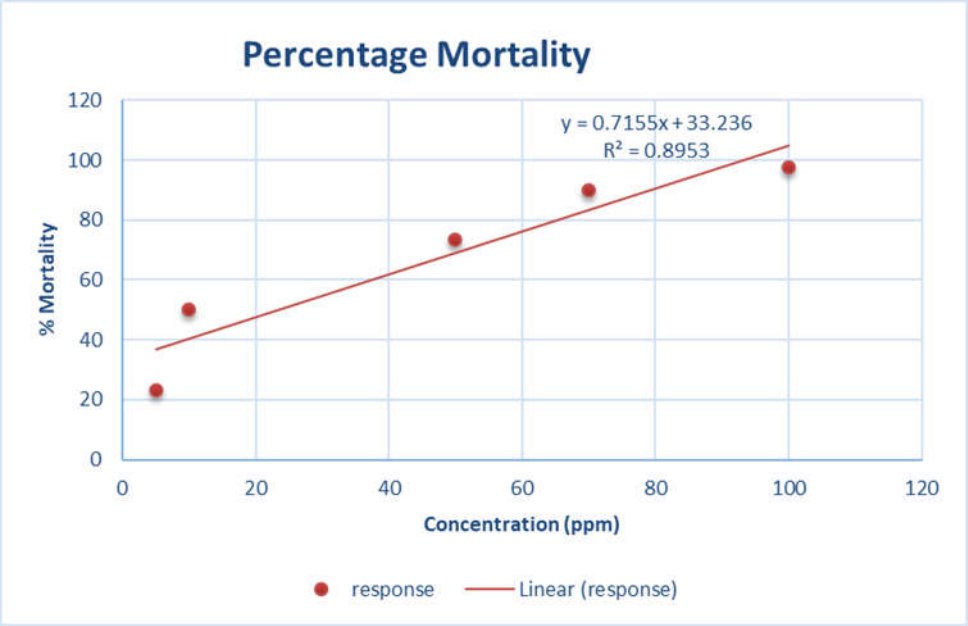

Figure 1 (a) – Methanol extract

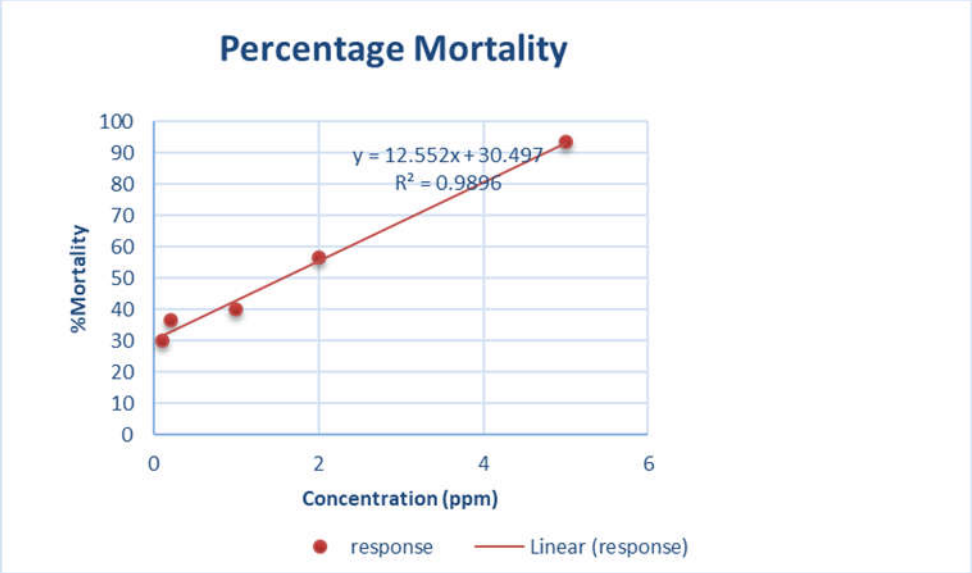

Figure 1 (b) – Acetone extract

Figure 1 a & b- Correlation and Regression analysis of Percentage mortality of methanol and acetone extracts of *Sterculia guttata* against the first instar larvae of *Culex quinquefasciatus.*

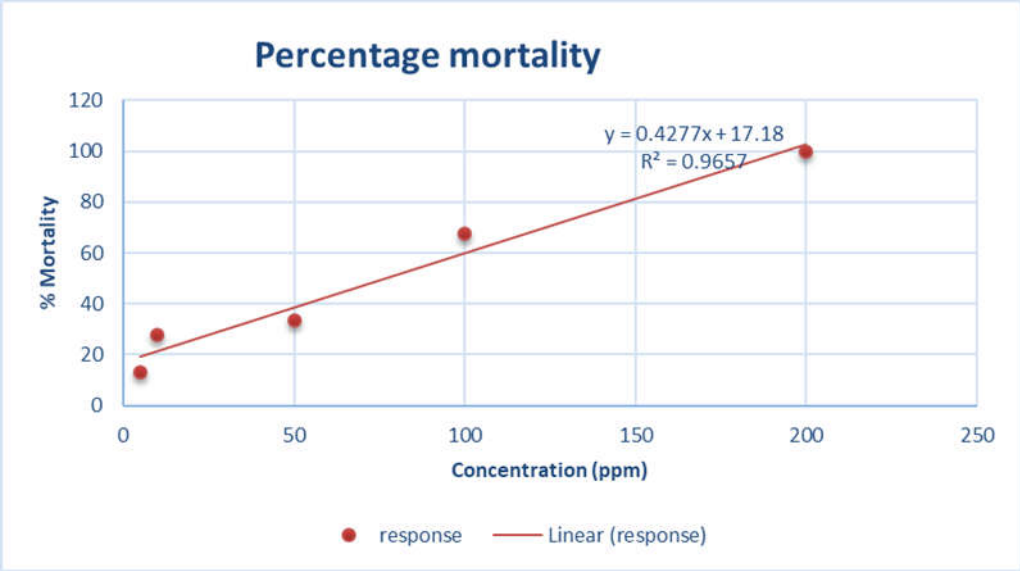

Figure 2 (a) – Methanol extract

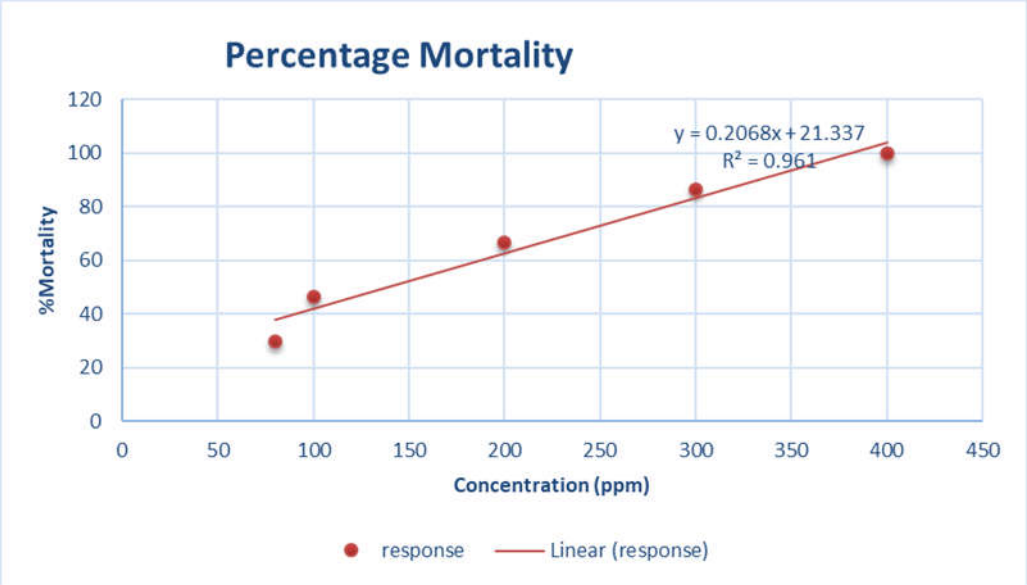

Figure 2 (b) – Acetone extract

Figure 2 a & b- Correlation and Regression analysis of Percentage mortality of methanol and acetone extracts of *Andrographis paniculata* when tested against the first instar larvae of *Culex quinquefasciatus.*

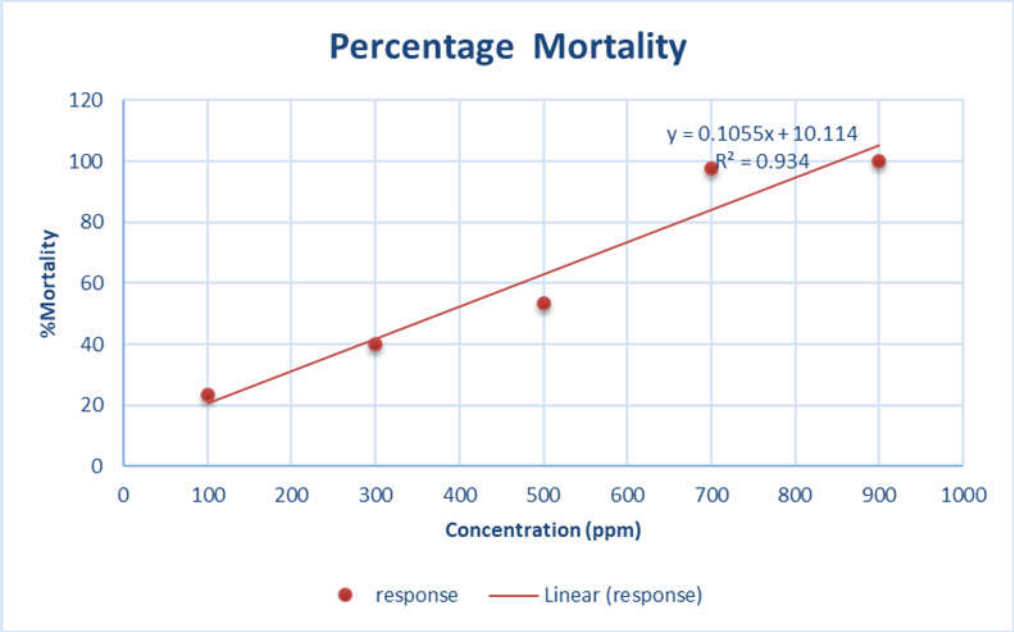

Figure 3 (a) – Methanol extract

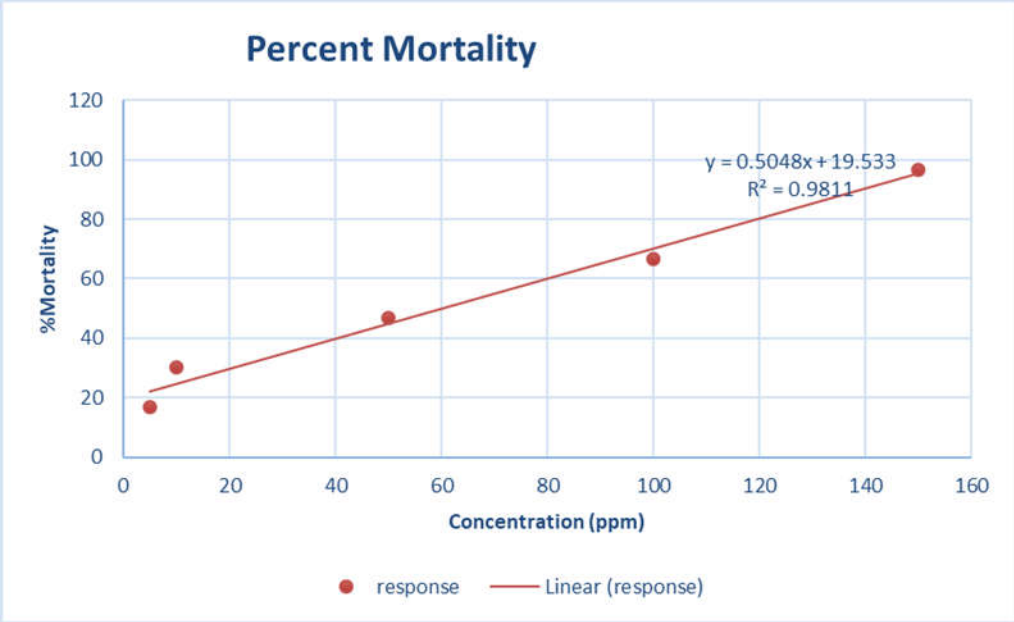

Figure 3 (b) – Acetone extract

Figure 3 a & b- Correlation and Regression analysis of Percentage mortality of methanol and acetone extracts of *Bougainvillea spectabilis* when tested against the first instar larvae of *Culex quinquefasciatus.*

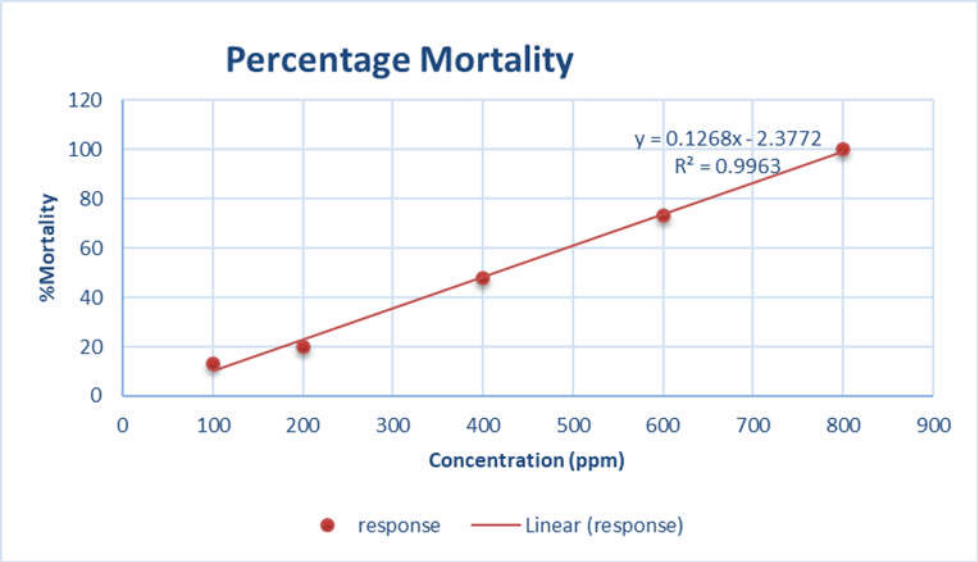

Figure 4 (a) – Methanol extract

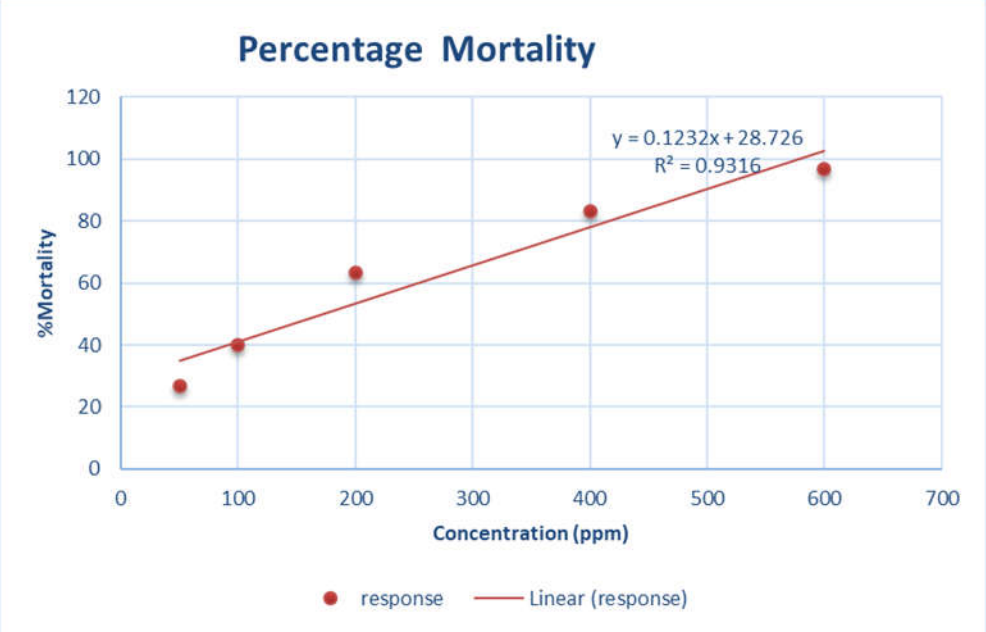

Figure 4 (b) – Acetone extract

Figure 4 a & b- Correlation and Regression analysis of Percentage mortality of methanolic and acetonic extracts of *Polyalthia longifolia* against the first instar larvae of *Culex quinquefasciatus.*

1.4.3 Effect of different Column fractions of selected plant extracts on I instar larvae of *Culex quinquefasciatus.*

Silica gel Column Fractionation of crude methanol extracts of *S. guttata, A. paniculata, B. spectabilis* and *P. longifolia* were tested for larvicidal activity and adult emergence with I instar larvae of *Cx. quinquefasciatus.* Apart from the nine gradients of methanol: Ethyl acetate (1:9, 2:8, 3:7, 4:6, 5:5, 6:4, 7:3, 8:2 and 9:1) column fractions of *S. guttata*, *A. paniculata and P. longifolia*, Methanol: Ethyl acetate (4:1) was the only fraction which produced 80.00%, 90.00% and 90.00% adult emergence with concentrations of 1ppm, 0.1ppm and 5ppm respectively. In the case of *B. spectabilis*, the column fraction n-Hexane: Ethyl acetate (5:5) is the only fraction showed 96.67% adult emergence larval mortality of 96.67% at 200ppm concentration.

Table 9 shows the percent mortality of the column fraction of Methanol: Ethyl acetate (4:1) of *S. guttata* tested against the first instar larvae of *Culex quinquefasciatus*. Treatment with 0.1, 0.3, 0.5, 10 and 50 ppm of the Methanol: Ethyl acetate (4:1) column fraction of ***S. guttata*** on I instar larvae of *Cx. quinquefasciatus* provided 20.00 ±0.00, 36.67 ± 6.67, 53.33 ±3.33, 70.00 ±0.00 and 90.00 ±0.00per cent mortality respectively after 24 hrs of exposure. Data on percent mortality of I instar larvae when treated with different concentrations of the column fraction Methanol: Ethyl acetate (4:1) of ***A. paniculata*** is provided in **Table 10**. Concentrations of 0.5, 1.0, 1.5, 2.0 and 25 ppm of the Methanol: Ethyl acetate (4:1) of ***A. paniculata*** column fraction produces 13.33 ± 3.33, 46.67 ±3.33,70.00± 0.00, 86.67 ±3.33 and 100± 0.00 % mortality after 24 hr exposure of the treatment with I instar larvae of *Cx. quinquefasciatus* (**Table 10**). **Table 11** provides data on percent mortality of n-Hexane: Ethyl acetate (5:5) column fraction of ***B. spectabilis*** treated against the I instar larvae of *Cx. quinquefasciatus*. At 25, 50, 100,150 and 200 ppm of the n-Hexane: Ethyl acetate (5:5) column fraction of *B.*

spectabilis produces26.67 ± 3.33, 36.67 ±3.33, 53.33 ±3.33, 76.67 ±3.33 and 96.67 ±3.33 % respectively after 24 hr of exposure **(Table 11)**. Methanol: Ethyl acetate (4:1) Column fraction of ***P. longifolia*** produced23.33 ±3.33, 43.33 ±3.33, 56.67 ± 3.33, 76.67 ± 3.33 and90.00 ± 0.00% mortality for 0.5, 10, 50, 100 and 200 ppm respectively after 24 hrs of exposure (**Table 12**).

Table 9: Percent mortality of column gradients of *S. guttata* (Methanol: Ethyl acetate, 4:1) tested against the first instar larvae of *Culex quinquefasciatus.*

SI No.	Concentration (ppm)	Mortality (Mean+ SE)	P- Value
1	0.1	20.00±0.00	0.0056*
2	0.3	36.67±6.67	
3	0.5	53.33±3.33	
4	10	70.00 ±0.00	
5	50	90.00 ±0.00	
6	Control	0.00±0.00	

Note: The values are expressed as mean ± SE for 10 animals (n=10) per group
* Statistically Significant at P < 0.05 with Control experiment.

Table 10: Percent mortality of column gradients of *A. paniculata* (Methanol: Ethyl acetate, 4:1) tested against the first instar larvae of *Culex quinquefasciatus.*

SI No.	Concentration (ppm)	Mortality (Mean+ SE)	P- Value
1	0.5	13.33±3.33	0.0439*
2	1.0	46.67±3.33	
3	1.5	70.00± 0.00	
4	2.0	86.67 ±3.33	
5	2.5	100.00 ±0.00	
6	Control	0.00±0.00	

Note: The values are expressed as mean ± SE for 10 animals (n=10) per group
* Statistically Significant at P < 0.05 with Control experiment.

Table 11 - Percent mortality of column gradients of *B. spectabilis* (Hexane: Ethyl acetate, 5:5) tested against the first instar larvae of *Culex quinquefasciatus.*

SI No.	Concentration (ppm)	Mortality (Mean± SE)	P- Value
1	25	26.67±3.33	0.0020*
2	50	36.67±3.33	
3	100	53.33±3.33	
4	150	76.67 ±3.33	
5	200	96.67 ±3.33	
6	Control	0.00±0.00	

Note: The values are expressed as mean ± SE for 10 animals (n=10) per group
* Statistically Significant at $P < 0.05$ with Control experiment.

Table 12: Percent mortality of column gradients of *P. longifolia* (Methanol: Ethyl acetate, 4:1) tested against the first instar larvae of *Culex quinquefasciatus.*

SI No.	Concentration (ppm)	Mortality (Mean± SE)	P- Value
1	0.5	23.33±3.33	0.0012*
2	10	43.33±3.33	
3	50	56.67±3.33	
4	100	76.67 ± 3.33	
5	200	90.00 ± 0.00	
6	Control	0.00± 0.00	

Note: The values are expressed as mean ± SE for 10 animals (n=10) per group
* Statistically Significant at $P < 0.05$ with Control experiment.

24 hrs LC_{50} and LC_{90} and associated statistics of the column fractions of the selected plants extracts are provided in **Table 13**. 24 hrs LC_{50} and LC_{90} of Methanol: Ethyl acetate, 4:1 column fraction of *Sterculia guttata*,

Andrographis paniculata, *Polyalthia longifolia* are 24.25, 3.01, 145.7 ppm and 94.1, 7.9, 177.5 ppm respectively. 24 hrLC_{50} of *Bougainvillea spectabilis* (n- Hexane: Ethyl acetate (5:5) column fraction tested against I instar larvae of *Cx. quinquefasciatus* is 221.38 ppm.

Table 13 - 24 hr LC_{50} and LC_{90} (ppm) and associated statistics of Methanol: Ethyl acetate, 4:1 column fraction of *Sterculia guttata*, *Andrographis paniculata, Polyalthia longifolia* and *Bougainvillea spectabilis* (n- Hexane: Ethyl acetate, 5:5) column fraction tested against I instar larvae of *Cx. quinquefasciatus*.

Plant / Column fraction	24 hrs LC_{50} (LC_{90})	Lower Fiducial Limit (LFL)	Upper Fiducial Limit (LFL)	X^2	Regression	Significance
S.guttata Methanol: Ethyl acetate (4:1)	24.254 (94.162)	-16.467 (58.973)	60.009 (317.420)	16.479	y= 1.111x + 38.65 R^2= 0.691	0.001*
A. paniculata Methanol: Ethyl acetate, 4:1	3.013 (7.989)	1.629 (6.113)	4.389 (12.532)	7.392	y= 7.995x + 22.15 R^2= 0.882	0.060
P. longifolia Methanol: Ethyl acetate, 4:1	145.675 (177.549)	-26.121 (119.128)	90.310 (436.597)	12.309	y= 0.303x + 35.85 R^2= 0.856	0.006*
B. spectabilis n- Hexane: Ethyl acetate, 5:5	221.386 (-125.522)	-107.532 (-6239.520)	632.533 (2.866)	19.648	y= -0.192x +72.11 R^2= 0.792	0.000*

* Statistically Significant at $P < 0.05$

1.4.4 Percent emergence of the first instar larvae of *Culex quinquefasciatus* using different Column fractions of the selected plant extracts

Sub lethal concentrations of the selected column fractions of different plant extracts were treated against first instar larvae of *Cx. quinquefasciatus* and percent emergence was observed and recorded (**Tables 14 to 17**). The

percent emergence of *Cx. quinquefasciatus* after treatment with 1, 5, 20, 40 and 60 ppm of the methanol: Ethyl acetate 4:1 column fraction of ***Sterculia guttata*** is 80.00 ± 5.77, 53.33 ±3.33, 40.00 ± 5.77, 10.00 ± 0.00 and 0.00 ±0.00% respectively (**Table 14**). There is 100% emergence recorded in control set. Percent emergence of *Cx. quinquefasciatus* observed after treatment with methanol: ethyl acetate 4:1 column fraction of ***Andrographis paniculata*** is 90.00 ±0.00, 70.00 ±0.00, 50.00 ±0.00, 30.00 ±0.00 and 13.33 ±3.33% for 0.1, 0.5, 1, 5 and 10 ppm respectively (**Table15**) whereas 10, 50, 80, 100 and 300 ppm of the n-Hexane: Ethyl acetate 5:5 column fraction of ***Bougainvillea spectabilis*** produces a percent emergence of 80.00 ±0.00, 70.00 ± 0.00, 50.00 ±0.00, 36.67 ± 3.33 and 20.00 ± 0.00 % respectively (**Tables 16 & 17**).The percent emergence of *Cx. quinquefasciatus* when treated with methanol: ethyl acetate 4:1 column fraction of ***Polyalthia longifolia*** are 90.00 ±0.00, 80.00 ±0.00, 66.67 ± 3.33, 53.33 ± 3.33 and 36.67 ±3.33% for 5, 10, 50, 70 and 90 ppm respectively

Table 14: Data on percent emergence of the column fraction Methanol: Ethyl acetate, 4:1 of *Sterculia guttata* seed extract. The experiment commenced from the first instar and observations were made till emergence.

Concentration (ppm)	Emergence (Mean±SE)	Corrected % (Mortality)	P-Value
1.0	80.00±5.77	20.00	0.0121*
5.0	53.33±3.33	46.67	
20	40.00±5.77	60.00	
40	10.00 ± 0.00	90.00	
60	0.00 ±0.00	100.00	
Control (+ve)	93.33±3.33	6.67	
Control (-ve)	96.67±0.00	3.33	

Note: The values are expressed as mean ± SE for 10 animals (n=10) per group
* Significant at $P < 0.05$.

Table 15: Data on percent emergence of the column fraction Methanol: Ethyl acetate, 4:1 of *Andrographis paniculate* leaf extract. The experiment commenced from the first instars and observations were made till emergence.

Concentration (ppm)	Emergence (Mean±SE)	Corrected % mortality	P- Value
0.1	90.00±0.00	10.00	0.0226*
0.5	70.00±0.00	30.00	
1.0	50.00±0.00	50.00	
5.0	30.00 ±0.00	70.00	
10	13.33 ±3.33	86.67	
Control (+ve)	90.00±0.00	10	
Control (-ve)	96.67± 0.00	3.33	

Note: The values are expressed as mean ± SE for 10 animals (n=10) per group
* Significant at $P < 0.05$.

Table 16: Data on percent emergence of the column fraction of *Bougainvillea spectabilis* (n- Hexane: Ethyl acetate, 5:5) *leaf* extract. The experiment commenced from the first instars and observations were made till emergence.

SI No.	Concentration (ppm)	Emergence (Mean±SE)	Corrected %mortalty	P-Value
1	10	80.00±0.00	20.00	0.0111*
2	50	70.00±0.00	30.00	
3	80	50.00±0.00	50.00	
4	100	36.67 ± 3.33	63.33	
5	300	20.00 ± 0.00	80.00	
6	Control (+ve)	90.00±0.00	10.00	
7	Control (-ve)	93.33±3.33	6.67	

Note: The values are expressed as mean ± SE for 10 animals (n=10) per group
* Significant at $P < 0.05$.

Table 17: Data on percent emergence of the column fraction of *Polyalthia longifolia* (Methanol: Ethyl acetate, 4:1) *leaf* extract. The experiment commenced from the first instars and observations were made till emergence.

SI No.	Concentration (ppm)	Emergence (Mean± SE)	Corrected% mortality	P-Value
1	5.0	90.00±0.00	10.00	
2	10	80.00±0.00	20.00	
3	50	66.67±3.33	33.33	0.0215*
4	70	53.33 ± 3.33	46.67	
5	90	36.67 ±3.33	63.33	
6	Control (+ve)	93.33± 3.33	6.67	
7	Control (-ve)	93.33± 3.33	6.67	

Note: The values are expressed as mean ± SE for 10 animals (n=10) per group
* Significant at $P < 0.05$.

Table 18: EC_{50}& EC_{90}ppm and associated statistics of the selected column fractions of the different plant extracts when tested against the 1 instar larvae of *Culex quinquefasciatus*.

Plant/ Column fraction	EC_{50} EC_{90})	Lower Fiducial Limit (LFL)	Upper Fiducial Limit (LFL)	X^2	Regression	Significance
S. guttata (MeOH: EA 4:1)	12.838 (-12.769)	1.899 (-47.932)	21.960 (-0.817)	11.047	y= -1.254x + 68.26 R^2= 0.912	0.011*
A. paniculata (MeOH: EA, 4:1)	1.050 (-3.453)	-5.258 (-191.544)	23.030 (0.492)	30.949	y= -6.626x + 73.33 R^2= 0.833	0.001*
P. longifolia (MeOH: EA, 4:1)	72.601 (-5.218)	64.679 (-23.637)	82.908 (7.088)	3.268	y= -0.560x + 90.56 R^2= 0.965	0.352
B. spectabilis Hex: EA, 5:5	110.693 (-125.522)	-107.532 (-6239.520)	632.533 (2.866)	19.648	y= -0.192x +72.11 R^2= 0.792	0.001*

* Significant at $P < 0.05$.

The Coefficient of determination (R^2) of column fraction Methanol: Ethyl acetate, 4:1 of *S. guttata, A. paniculata* and *P. longifolia* exhibited 91.2%, 83.3% and 96.5% of adult emergence respectively, which indicated more than 90% of adult emergence could be explained by the independent fractionation (**Figures 5, 6&7**). The relationship between concentrations and percentage adult emergence was positive. Whereas, in the case of *Bougainvillea spectabilis*, column fraction n- Hexane: Ethyl acetate showed 79.2% adult emergence and the relationship between concentrations and emergence was positive **(Figure 8)**.

Correlation and Regression analysis of Percentage emergence of different column gradients against the first instar larvae of *Culex quinquefaciatus.*

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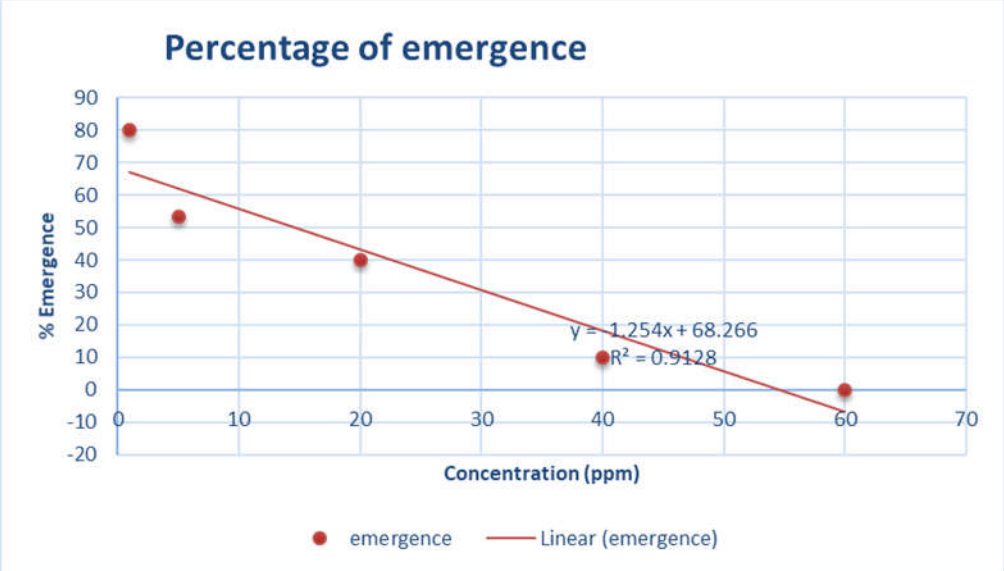


Figure 5- *Sterculia guttata* (Methanol: Ethyl acetate - 4:1

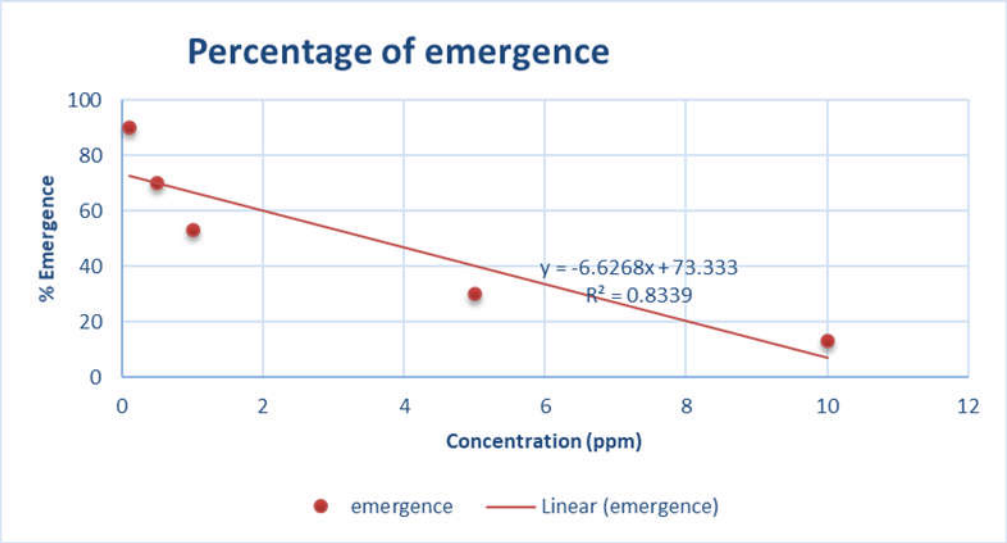


Figure 6– *Andrographis paniculata* (Methanol: Ethyl acetate – 4:1)

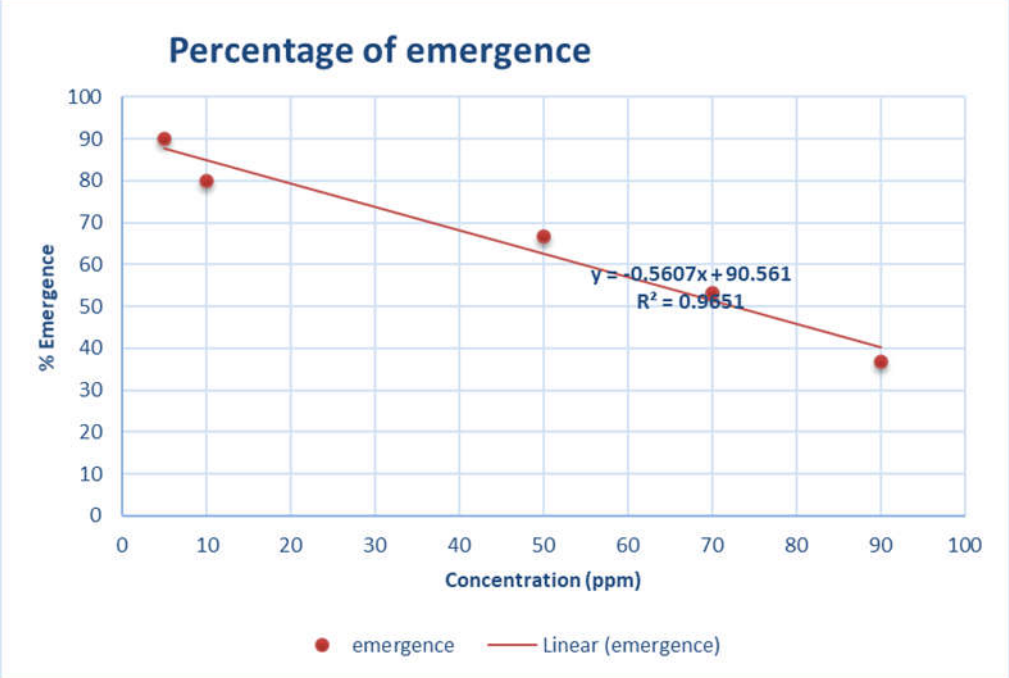


Figure 7– *Polyalthia longifolia* (Methanol: Ethyl acetate – 4:1)

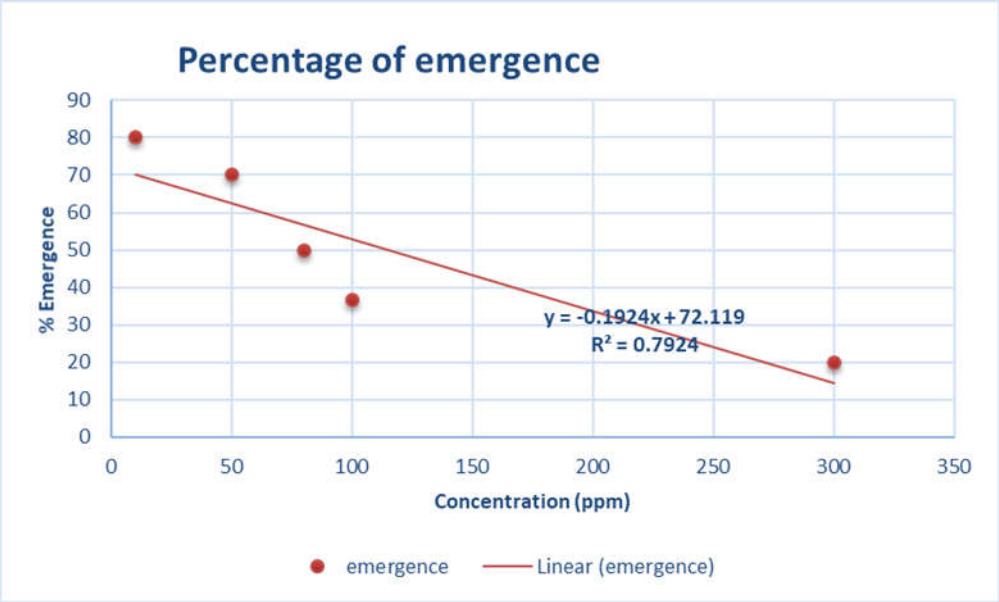


Figure 8– *Bougainvillea spectabilis* (n- Hexane: Ethyl acetate – 5:5)

The present study mainly focuses on the phytosterilant properties of the selected plant extracts on the filarial vector, *Cx. quinquefasciatus* and hence the column fraction of Methanol: Ethyl acetate (4:1) for *S. guttata, A. paniculata* and *P. longifolia* and n- Hexane: Ethyl acetate (5:5) for *B. spectabilis* were used further to investigate the phytosterilant properties because of the adult emergence efficacies of the selected column fractions.

1.5 DISCUSSION

Recently the mosquito control programs using synthetic insecticides faces set back due to the development of resistance in mosquitoes. The current vector control management programs mainly target on the elimination of larval instars as they are confined to the medium and cannot escape from their surroundings sites until the adult stage and also the overall usage of insecticides can be limited, thereby reducing the environmental pollution. The biopesticides are often considered to be important components of IVM strategies and have received much practical attention as an alternative to synthetic insecticides (Hancock, 2009). Phytochemicals are considered to be suitable alternatives to synthetic insecticides as they are relatively inexpensive, safe and are readily available in all parts of the world (Arivoli and Tennyson, 2011). More than two thousand plant species have been reported to have insecticidal properties and plant derived products have drawn increased attention as potential insect control agents and as a source of new insecticides for the pesticide industry in the last three decades (Sukumar *et al*., 1991).

The larvicidal efficacies of the plants *Sterculia guttata, Andrographis paniculata, Bougainvillea spectabilis* and *Polyalthia longifolia* in mosquitoes were not well established as plant species having insecticidal properties. All these plants were well- known for their medicinal properties. Present study investigated the larvicidal efficacies of separately extracted crude methanolic and acetonic extracts of seeds of *S.guttata and P. longifolia* and leaves of *A. paniculata* and *B. spectabilis* on I instar larvae of *Cx. quinquefasciatus*. It has been found to possess promising larvicidal activity with LC_{50} value 24.016ppm and 1.469ppm respectively for Crude methanolic and acetonic extract of *S. guttata* after 24hrs of exposure **(Table 8)**. The plant extracts caused larval mortality in a dose- dependent manner. The larval mortality was above 89% and 98% respectively for crude methanolic and acetonic extracts. It was observed that acetonic extract exhibited potent larvicidal activity with

LC_{50} value 1.469ppm. The percentage of larval mortality was found to be significantly different ($P < 0.05$, t- test) from that of control and untreated groups.

The larvicidal activity of seeds of *S. guttata* was also comparable to different solvent extracts on different species of mosquitoes (Katade *et al*., 2006). It was observed that ethanol, chloroform and hexane fractions of the seed extract of *S. guttata* against the IV instar larvae of *Aedes aegypti* and *Culex quinquefasciatus* exhibited promising larvicidal activity with ethanol fraction, with LC_{50} value 21.552ppm on *Cx. quinquefasciatus* and LC_{50} as 520ppm against *Ae. aegypti* after 24hrs of exposure. The present study corroborated with earlier findings, when LC_{50} values of ethanol extract of seeds of *S. guttata* were 21.552ppm against the IV instar larvae of *Cx. quinquefasciatus*. Contrarily, no further information regarding the larvicidal activity of seed extract of *S. guttata* was accessible yet.

On the other hand, both methanolic and acetonic leaf extracts of *A. paniculata* exhibited 96% larval mortality with LC_{50} value 67.240ppm and 134.496ppm after 24hrs of exposure respectively against I instar larvae of *Cx. quinquefasciatus* **(Table 8)**. These results were quite comparable to the previous reports on larvicidal efficacies of the plant *A.paniculata* against some mosquito species. Aqueous and petroleum ether leaf extracts of *A. paniculata* exhibited potent larvicidal activity against two mosquito species, *Ae. aegypti* and *Cx. quinquefasciatus* (Renugadevi *et al*., 2013). In a 24hrs bioassay experiments, highest mortality was observed in petroleum ether extract with LC_{50} value 200ppm on *Cx. quinquefasciatus*. 'Andrographolidae', a bio active compound isolated from leaves of *A. paniculata* exhibited highest larvicidal activity with lethal concentration (LC_{50}) value 12ppm and also showed ovi- position deterrent activity against the Dengue vector, *Ae. aegypti* (Edwin *et al*., 2016). The methanolic and

Ethyl acetate extract of *A. paniculate* produced 100% mortality at 200ppm against *Cx. quinquefasciatus* and at 250ppm on *Ae. aegypti* respectively. It also showed ovicidal activity in these two mosquito species to various concentrations ranging from 50- 300ppm (Govindarajan, 2011).

Gautam *et al*., 2013 reported the larvicidal efficacies of the flavonoid extract of whole aerial part of *A. paniculata* against late II or early IV instar larvae of *Ae. aegypti* and *An. stephensi*. It was found to be inactive even at 600ppm concentration against the selected larvae of *Ae. aegypti*, whereas, the extract caused 70% mortality in *An. stephensi* at 200ppm concentration. The toxicity of acetonic and ethanolic extract of *A. paniculata* on *Cx. quinquefasciatus* revealed highest mortality rate of 20% and 73.3% in 300ppm at 3hrs and 24hrs respectively (Sheeja, 2012). The larval toxicity was mainly dependent on the dose and time of exposure of the extract. The leaf extracts with various solvents of *A. paniculata* were found to be susceptible against the larvae of *Ae. aegypti, An. stephensi* and *Cx. quinquefasciatus* (Jeyasankar and Ramar, 2015). The highest larval mortality was detected in petroleum ether extract on *An. stephensi* with LC_{50} value 20.85ppm, *Ae. aegypti* with LC_{50} 31.54ppm and *Cx. quinquefasciatus* with LC_{50} of 43.0ppm, followed by chloroform and Ethyl acetate extract with LC_{50} values 33.6ppm, 42.76ppm, 51.25ppm, 81.04ppm, 63.62ppm and 104.43ppm respectively after 24hrs of exposure. Thangavel *et al*., 2015 evaluated the larvicidal effect of acetone extract of *A. paniculata* leaves on dengue vector, *Ae. aegypti*. The maximum toxic effects were noticed against I instar larvae > II > III > Pupae > IV instars respectively with LC_{50} values 113.661ppm, 149.068ppm, 162.731ppm, 216.888ppm and 93.958ppm.

Mosquitocidal properties like larvicidal, pupicidal and egg hatchability was evaluated by the acetonic leaf extract of *B. spectabilis* on *Ae. aegypti* (Rajmohan and Logankumar, 2012). It was observed that the percentage of

egg hatchability, larval and pupal mortality were found to increase with the dosage. The LC_{50} values of egg (31mg/l), I instars (59mg/l), II instars (231mg/l), III instars (606mg/l), IV instars (1578mg/l) and pupa (637mg/l) were observed.

The results obtained with the present study also strengthened the larvicidal efficacy of methanolic and acetonic leaf extracts of *B. spectabilis* on I instar larvae of *Cx. quinquefasciatus*. The crude methanolic extracts of *B. spectabilis* exhibited 100% larval mortality with LC_{50} value 358.816ppm and acetonic extract exerted 96.67% larval mortality with LC_{50} value 56.918ppm **(Table 8)**. Literature survey could not provide sufficient information regarding the mosquitocidal properties of leaves of *B. spectabilis*, it was observed that only one such study proved the mosquitocidal efficacy of *B. spectabilis* against *Ae. aegypti* (Rajmohan and Logankumar, 2012). Therefore, the present study furnishes certain details regarding the larvicidal efficacy of *B. spectabilis* leaf extracts on filarial vector, *Cx. quinquefasciatus*.

From the results, it was apparent that the crude acetonic leaf extract was the more potent larvicide than the crude methanolic leaf extract of *B. spectabilis* against I instar larvae of *Cx. quinquefasciatus*.

Mosquitocidal activities of the crude extracts of seeds of *P. longifolia* have been tested earlier by very few investigators. Chandra*et al*., 2016 studied the larvicidal activity of aqueous seed extract of *P. longifolia* against the filarial vector, *Cx. quinquefasciatus*. With respect to LC_{50} value 0.065%, aqueous seed extract of *P. longifolia* showed 100% mortality against III instar larvae of *Cx. quinquefasciatus* at 1% concentration within 72hrs of exposure. Rathy *et al*., 2015 conducted a preliminary study on plant diversity for mosquito control, which mentioned the larvicidal property of aqueous extract of leaf of *P. longifolia* against the larval instars of *Ae. aegypti*. It was found 100% mortality on larval instars at 0.5ml concentration after 48hrs of exposure.

Findings of the present study revealed the larval toxicity of crude acetonic and methanolic seed extracts of *P. longifolia* on I instar larvae of *Cx. quinquefasciatus,* which exhibited 96.67% and 100% mortality respectively with LC_{50} values 161.923ppm and 431.701ppm after 24hrs of exposure **(Table 8)**. The percentage mortality was also analyzed with t-test, compared both experimental and control groups, with a significance level established at $P< 0.05$. Co- efficient of determination (R^2) also showed above 96% mortality explained by the concentrations.

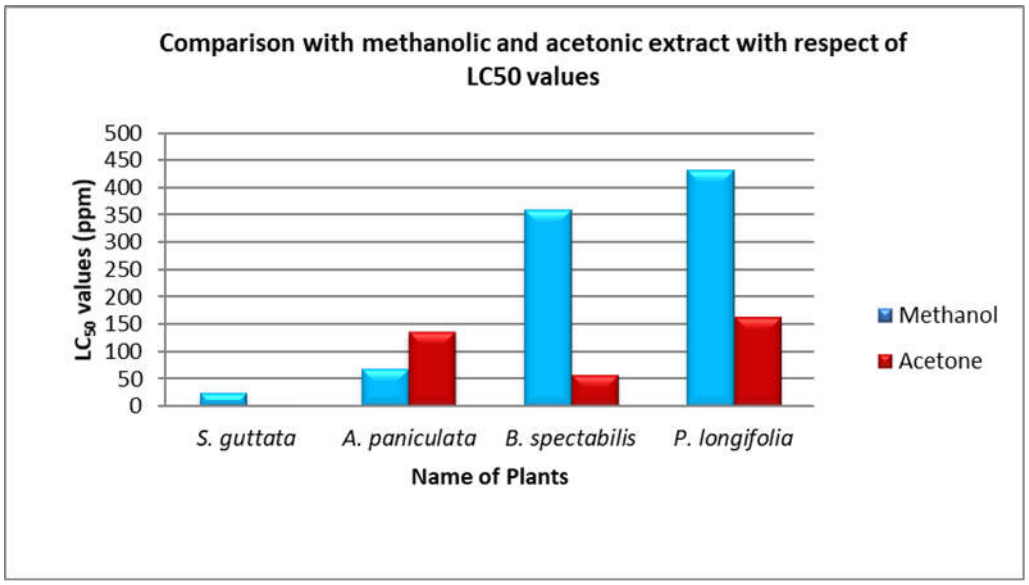


Figure 9-Comparison of LC_{50} values of Crude methanolic and acetonic extracts of selected plants on I instar larvae of *Cx. quinquefasciatus.*

When compared with the LC_{50} values of the selected plants, crude acetonic extract of *S. guttata*, *B. spectabilis* and *P. longifolia* exhibited larvicidal toxicity with least concentrations **(Figure 9)**. Whereas, only *A. paniculata* showed highest mortality with methanolic extract. Among these, acetonic seed extract of *S. guttata* and *P. longifolia* showed lowest activity with LC_{50}

value 161.923ppm. Not only the plant *S. guttata,* the other three plants *A. paniculata, B. spectabilis* and *P. longifolia* also exhibited promising larvicidal effect with crude acetone extracts (LC_{50} value 1.469ppm, 134.496ppm, 56.918ppm and 161.923ppm), which shows that these extracts produced larvicidal activity at concentrations below 200ppm. These results suggest that the acetonic extracts of seeds of *S. guttata* and *P. longifolia* and leaves of *A. paniculata* and *B. spectabilis*can be effectively used for the control of I larval instars of *Cx. quinquefasciatus*. Even though, acetonic extracts showed highest mortality with least LC_{50} values, it was desirable to select methanolic extracts of selected plants for sterility inducing effects in filarial vector, *Cx. quinquefasciatus*.

Critical concentrations of Ethyl acetate fractions of seeds of *Calophyllum inophyllum*, leaf of *Solanum surattense* and leaf of *Samadera indica* and the petroleum ether fraction of leaf of *Rhinacanthus nasutus*were reported to produce adult emergence in 50% of treated larvae (EC_{50}) of *Cx. quinquefasciatus, An. stephensi* and *Ae. aegypti* and significantly decreased the fecundity of mosquitoes and the hatchability of their eggs (Muthukrishnan and Pushpalatha, 2001). Arriving at the effective concentration (EC_{50}) for adult emergence is inevitable as the present study mainly focuses on the sterility inducing properties of selected plant species. Among the selected plants, only the crude methanolic extract of *B. spectabilis* exhibited 96.67% adult emergence with EC_{50} value 119.519ppm. However, the rest of the plants *S. guttata, A. paniculata* and *P. longifolia* showed 100% mortality during the developmental periods. When compared to crude acetonic extracts of selected plant species, methanolic extract possessed lower larvicidal toxicity against I instar larvae of *Cx. quinquefasciatus*. Therefore, the crude methanolic extract of *S. guttata, A. paniculata, B. spectabilis* and *P. longifolia* were subjected to fractionation with Column chromatography.

As a polar solvent, methanolic extract was found to possess significant pharmacological activity when compared to other solvents, and therefore it is

used for extraction and isolation of phytoconstituents. On the other hand, methanol is a solvent which is completely miscible with water (NCBI, 2008). Column fractionation was done with polar x polar solvents like Methanol: Ethyl acetate and with Non- polar x polar solvents like n- Hexane x Ethyl acetate with increasing polarity. The results obtained from the larvicidal bioassays carried out by using column fractions of crude methanolic extracts of *S. guttata, A. paniculata* and *P. longifolia* revealed that only the fraction of Methanol: Ethyl acetate – 4:1 could give rise to above 80% adult emergence from the treated larvae of *Cx. quinquefasciatus* **(Table 18)**. On the other hand, crude methanolic extract of *B. spectabilis* exhibited 80% adult emergence with the fraction n- Hexane: Ethyl acetate – 5:5. These fractions also exhibited significant larval mortality with LC_{50} values 24.254ppm, 3.013ppm, 110.693ppm and 145.675ppm respectively for *S. guttata* (MeOH: EA– 4:1), *A. paniculate* (MeOH: EA – 4:1), *B. spectabilis* (H: EA – 5:5) and *P. longifolia* (MeOH: EA – 4:1)**(Table 18)**.

Median lethal dose of *S. guttata, A. paniculata* and *P. longifolia* (MeOH: EA– 4:1) column fraction exhibited 91.2% adult emergence with EC_{50} value 12.838ppm, 83.3% with EC_{50} value 1.050ppm and 96.5% emergence with EC_{50} value 72.601ppm respectively. *B. spectabilis* (H: EA– 5:5) fraction showed 79.2% adult emergence with EC_{50} value 110.693ppm **(Tables 14, 15, 16 & 17).**

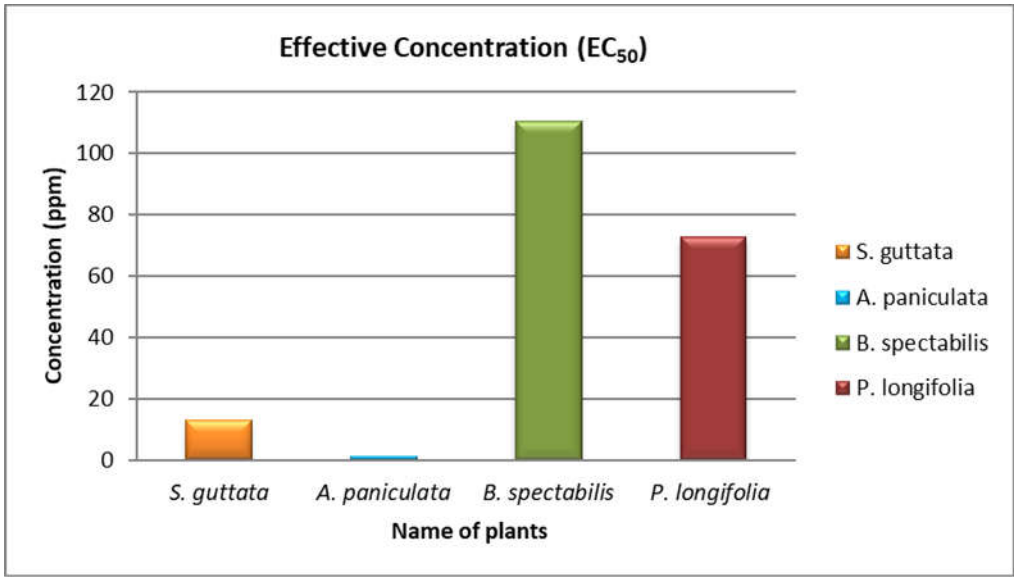


Figure 10 -Comparison with different column fractions of selected plants with respect of EC_{50} value on I instar larvae of *Cx. quinquefasciatus.*

When compared with the EC_{50} values of column fractions of selected plants, *A. paniculata* (MeOH: EA – 4:1) exhibited least EC_{50} value 1.050ppm < *S. guttata* (MeOH: EA – 4:1) with 12.838ppm <*P. longifolia* (MeOH: EA – 4:1) with 72.601ppm and *B. spectabilis* (H: EA – 5:5) 110.693ppm respectively **(Figure 10)**. Treatment of the larvae with 50% EC_{50} of the column fractions produced 91.2%, 83.3%, 79.2% and 96.5% adult emergence respectively for *S. guttata* (MeOH: EA – 4:1), *A. paniculata* (MeOH: EA – 4:1), *B. spectabilis* (H: EA – 5:5), *P. longifolia* (MeOH: EA – 4:1) in the progeny of *Cx. quinquefasciatus*.

The findings of the present study clearly demonstrated that the crude acetonic extracts of the selected plants *S. guttata* (seeds), *A. paniculata* (leaves), *B. spectabilis* (leaves) and *P. longifolia* (seeds) exhibited promising larval toxicity on I instar larvae of *Cx. quinquefasciatus*, as in agreement with the above mentioned literatures. Moreover, the crude methanolic extract also showed comparable larvicidal effects on larvae of *Cx quinquefasciatus*.

Column fractions, except the Methanol: Ethyl acetate –4:1 for *S. guttata, A. paniculata* and *P. longfolia* and n Hexane: Ethyl acetate – 5:5 for *B. spectabilis* found to possess highest larvicidal effects on I instar larvae of *Cx. quinquefasciatus*. Since a large population was threatened by the filarial vector *Cx. quinquefasciatus*, the results of the present study using plant derived compounds could help to control these vector mosquitoes and offer immense opportunities for those plant derived compounds to be utilised in integrated vector management programmes world over as these plants tested are readily available around the world. The highly bioactive compounds present in the Seeds of *S. guttata* and *P. longifolia* and leaves of *A. paniculata* and *B. spectabilis*thus offer an opportunity for developing better alternatives to rather expensive and environmentally hazardous synthetic insecticides.

2.1 INTRODUCTION

The control of filarial vector *Cx. quinquefasciatus* population is very important as far as global and national public health is concerned. Current methods of use of synthetic insecticides result in an increasingly unacceptable rate of unwanted environmental effects. As an alternative strategy, the sterile-male technique (SIT) has been considered to be a promising tool for the control of insect pests/ vectors (Pillai and Grover, 1969). There is an increased interest in applying this approach to vector control, because SIT is species specific, environmentally sound and effective technique to control an insect population (Alphey, 2010). Sterilization can be induced by irradiation or chemosterilization methods, which can induce toxicity on the reproductive cells of the insects (Andrea, 2013).

Goldsmith *et al*., 1948 is credited with the pioneering research with chemosterilants as they found out that certain chemicals could induce subsequent sterilization of the female sex and also showed retardation of ovarian development in insects. Later, La Breque (1961) experimented with certain group of chemicals which were sterilizing both male and female mosquitoes, house flies and stable flies. Knipling (1955, 1959, 1962, 1963 & 1968), Serebrovsky (1969) and Murdie and Campion (1972) elaborated the potentialities of chemosterilization for the control of insect pests. A number of reviews have appeared dealing with the various aspects of chemosterilants (Weidhaas and Mc Duffie, 1963., Aacher, 1964 & 1969., Zakharova, 1966., Kilgore, 1967., White, 1967., La Breque and Smith, 1968., Stuben, 1969., Morgan and Mandava, 1987) based on the chemistry of chemosterilants, its classifications, mode of actions, species specificity, effect on the male and female reproductive systems and their efficacy in inducing sterility in both sexes.

According to Kilgore (1967) and chemosterilants can be classified into-biological alkylating agents, antimetabolites and miscellaneous compounds.

The most significant aspect of the chemosterilants in mosquito control was its efficacy, either in the larval or adult stages (Dame and Ford, 1964). Irradiation is the most commonly used sterilization technique, in which pupal stage is exposed to gamma rays, which induces dominant lethal mutations in the sperm of the male mosquitoes. Higher doses induced complete sterilization with damaging the other cells of male reproductive organs, which decreases the fitness and competitiveness of male mosquitoes (Helinski and Knols, 2009). 1960s-70s research identified thousands of potential chemosterilants against several insect pests. Unfortunately, the effort of implication of chemosterilants in field population among insects being largely abandoned due to some external factors as well as concerns regarding toxicity (Baxter, 2017). However, in the past fifteen years, great innovations have been made in the genetics and molecular analysis of mosquitoes and other disease vectors.

A perusal of the literature reveals that the chemosterilants have long been used in sterilization programs for managing insect pests and disease- causing vectors. But adverse effects such as development of resistance among insect population, toxic effect of non- target animals and other environmental issues, there is a felt necessity for specific alternatives for use as sterilants. As the chemical mode of controlling measures invoke undesirable effects in target and non-target organisms and also causes ill effects on the environment. Thus, there is an impetus on developing better controlling agents from natural source.

Several botanical pesticides exhibited deleterious effects on the growth and development of insects, by reducing the survival rates of larvae and pupae as well as lengthening the developmental stages and inhibited the adult

emergence (Koul, *et al*., 2008). According to Asawalam and Adesiyan (2001) and Shaalan (2005), certain plant parts, oils, extracts and plant powder could reduce insect oviposition, egg hatchability, postembryonic and progeny development. Saxena *et al.,* 1993 evaluated the chemosterilant activity of alkaloids from *Annona squamosa* against *Anopheles stephensi.* Treatment with the alkaloids produced significant effect on emergence and reproductive physiology of *An. stephensi.* The larval instars of *Cx. quinquefasciatus* exposed to alkaloid isolated from *Ageratum conyzoides* showed growth regulating and chemosterilant activities such as shortening of total developmental periods, reduced fecundity and fertility in female mosquitoes (Arya and Sahai, 2014).

Present study was carried out to determine the biosterilant properties of selected plant extracts, *S. guttata*, *A. paniculata, B. spectabilis* and *P. longifolia* on the vector mosquito *Cx. quinquefasciatus*. Following parameters are important to understand the mode of action of phytochemicals on the reproductive system of mosquitoes, which can contribute phytosterilant properties like mating inhibition, reproductive suppression, and low survival and reduced fecundity rate.

Reproductive Biology of the Vector species *Culex quinquefasciatus* Say

In insects, sexual dimorphism is common, where the male and female sexes are mostly separate. Besides such diversity occurring in the mode of reproduction, most of the insects possess well- defined internal organs for reproduction, which mainly consists of paired gonads, genital ducts leading to a gonopore and some accessory structures.

Reproduction- Female

In the past 50 years, female reproduction has been one of the most intensively studied aspects of insect biology. In female insects, reproduction mainly involves producing eggs, receive sperm, store sperm, and manipulate sperm from different males and laying fertilized eggs. Reproductive systems of female *Cx. quinquefasciatus* are made up of a pair of ovaries, accessory glands, three spermathecae and various connecting ducts. Ovaries make eggs, which are made up of number of egg tubes called ovarioles. The number of ovarioles varies with each female mosquito. Accessory glands produce a variety of substances for sperm maintenance, transport and fertilization as well as protection of eggs. They could produce glue like substance for coating eggs or tough coverings for a batch of eggs called oothecae. Spermathecae are tubes or sacs in which sperm can be stored between the time of mating and an egg is fertilized. As all Diptera, the ovaries of *Cx. quinquefasciatus* are of polytrophic type and each ovary is surrounded by two sheaths (the ovarian sheath and ovariolar sheath). Each ovariole consist of four distinct partitions: terminal filament, germarium, follicle and pedicel.

The following account is based on the descriptions of the reproductive organs in *Culex* sp. by Nicholson (1921), Curtin and Jones (1961) and Bertram (1961). The two ovaries lie dorso- laterally in the posterior portion of the 6-7 abdominal segments and are connected by lateral oviducts to a common oviduct, which opens by the primary genital opening, the gonopore into a genital chamber. The number of the ovarioles in the two ovaries ranges from 150-200 varying with the size of the female mosquito (Colless and Challapah, 1960).

The ovarioles are short and extremely numerous, radiating about an extension of each lateral oviduct called the calyx. Each ovariole consists of an anterior germarium, a posterior vitellarium and two sheaths, the tunica propria and the ovariole sheaths. The germarium and vitellarium are closely bounded by a very fine elastic membrane, the tunica propria and less closely by the ovariole sheath. The germarium consists of a central mass of large nuclei belonging to oogonia, oocytes and nurse cells (trophocytes), and a peripheral layer containing smaller nuclei which give rise to the follicular epithelium. The vitellarium comprises 2 or 3 follicles, each consist of an oocyte and 7 nurse cells surrounded by follicular epithelium. The follicles are connected to one another to the germarium and to the calyx by narrow, tubular regions of the tunica propria called funicles. At the junction with the calyx the posterior funicle is called as pedicel, is surrounded by a sphincter of 6-8 muscle fibres. Tracheae arising from the 3rd & 4th or 4th and 5th abdominal spiracles pass to the ovaries and profusely branched before penetrating the ovarioles as tracheoles.

Spermathecae, an accessory gland and bursa copulatrix are open into the genital chamber through a small structure, the dorsal plate, in its dorsal surface. Spermatheca of *Cx. quinquefasciatus* consists of a large median with its own duct and 2 smaller lateral spermathecae with fused ducts with sclerotized shell, which in inseminated females contain sperm, surrounded by a layer of cells, which in turn possibly nourishes the sperm with its secretions (Polovodova, 1947). The accessory gland is a globular structure of variable size filled with secretions. The bursa copulatrix is a large, distensible sac like organ, into which sperms are deposited or it act as reservoir that is adjacent to and attaches at the base of the common oviduct (Degner and Harrington, 2016).

The female terminalia consist of the gonotreme or opening of the genital chamber behind the 8th sternite, in front of it there often lie 2 sclerites, the sigma and insula and behind it another, the cowl. Behind the gonotreme is a moderate- sized sclerite, the postgenital plate and the 10th segment bears the cerci and anus (Christophers, 1923).

Growth of the Egg and Follicle

A group of 8 large cells at the inner end of the germarium, consisting of an oocyte and 7 nurse cells formed from a single oogonium, becomes surrounded by the smaller, outer cells of the germarium and separates forming a follicle. In the adult mosquitoes, each ovarioles contains 2 or 3 follicles and as the female passes through a gonotropic cycle the posterior follicles in each ovariole develops to maturity. Females of *Cx. quinquefasciatus* in nature need blood meals to develop their first batch of eggs. After the mosquito has taken the blood meal the oocyte at the base of each ovariole grows rapidly forming a large amount of yolk and elongating to the shape of mature eggs. Meanwhile, the follicular epithelium secretes the chorion and micropyle apparatus. The chorion or egg shell consists of 2 layers, the endochorion and exochorion. The endochorion remains soft until sometime after the egg has been laid, when it hardens to form a thin dark membrane.

Hormonal control of reproduction in females

A series of endocrine events are initiated by the blood ingestion in female mosquitoes, which is dominated by the effects on Juvenile Hormone (JH), Ecdysteroids and several other peptide hormones, resulting in the maturation of a batch of eggs. The development of the follicle is stimulated by a gonadotropic hormone secreted by the Corpora allata after the female mosquitoes have taken the blood meal. Ecdysone and Juvenile Hormone (JH) are the two major hormones that control female reproduction and also control

pre- adult development and metamorphosis (Wheeler and Nijout, 1982). In adult females, ecdysone is produced by the ovaries, while JH is produced by a pair of glands called Corpora allata in both pre- adult and adult stages. Juvenile Hormone (JH) is considered as a key regulator of development and reproduction in mosquitoes (Zhu and Noriega, 2016). During development, JH plays an antimetamorphic role to maintaining the larval stages and preventing immature insects from being turned into adults (Jindra *et al*., 2013). JH titres are high while in the larval stages, but drop to permit metamorphosis (Goodman and Cusson, 2012). The functions of JH during immature stages in mosquitoes were well studied by using JH or its analogues (JHA). Methoprene and Pyriproxifen have been widely used as synthetic IGRs for mosquito control, as they affect the development of mosquitoes by blocking the embryonic development and inhibit egg hatching in *Ae. aegypti* (Spielman and Williams, 1966). It also interfered with metamorphosis and prevent the emergence of adults.

Majority of the methoprene treated larvae died during the pupal stage (Henrick, 2007). JHA has influence on the metamorphic midgut remodelling and adult cuticle formation in mosquitoes after the larval exposure (Spielman and Williams, 1966, Spielman and Skaff, 1967., Nishiura *et al*., 2003 and Wu *et al*., 2006). The pupae developed from methoprene treated larvae contain two midgut epithelial layers, larval midgut and the pupal/ adult midgut (Wu *et al*., 2006).

Three major stages are identified in the development of the ovaries during a gonotropic cycle; previtellogenesis (PVG), ovarian resting stage (ORS) and vitellogenesis (VG) (Klowden, 1997). Soon after the emergence of adult females, the previtellogenic stages begin with JH secretion from the Corpora allata (CA). JH stimulates previtellogenic growth of ovaries, makes female receptive to mating and prepares the fat body to be competent for responding

to later hormones and secretion of vitellogenins (Nation, 2008). Under the influence of residual 20-hydroxyecdysone remaining from the pupal to adult transformation, follicles begin to separate from the growing previtellogenic ovary (Whisenton *et al*., 1989). After emergence, female seek blood meal, which provides proteins and nutrients and initiates the vitellogenic stage. With availability of nutrients, ovaries release Corpora cardiaca stimulating factor (CCSF) by the stimulation of JH. CCSF is a neuropeptide probably produced in the young ovary before blood meal (Nation, 2008) and its target cells are in the corpora cardiaca (CC), which release Egg Development Neurohormone (EDNH) (Hagedorn *et al*., 1979), recently named as Ovarian ecdysteroidogenic Hormone I (OEH). In mosquitoes, neurosecretion (Ovarian ecdysteroidogenic Hormone, OEH) is released for only the first few hours after the blood meal.

Follicular epithelium cells in the ovary respond to OEH by producing and releasing Ecdysone into the circulating haemolymph. Ecdysone is converted rapidly into 20-hydroxyecdysone by many types of the cells including the target fat body cells. Fat body cells respond to 20-hydroxyecdysone by synthesizing vitellogenins (Nation, 2015). The post vitellogenic stage terminates vitellogenin production in the fat body, when the primary oocytes have reached maturity (one mature egg per ovariole). After maturation, the ovaries release an oostatic hormone (OSH) (Klowden, 1997), which stops the uptake of yolk by secondary oocytes and thus stopping their growth until the primary set of eggs are laid. The overall functions of the OSH is to keep the ovary and abdomen from becoming over distended by too many eggs growing to maturity at the same time (Nation, 2008). Now the females seek an appropriate place for oviposition.

Oviposition

Mosquitoes exhibited a remarkable diversity of oviposition behaviours, which ensures the successful oviposition site for larval development and emergence of next generation. In mosquitoes' oviposition may be varied according to the climatic conditions also with several other factors. Generally, female mosquitoes lay their eggs shortly after maturation, but it might be delayed or prevented by cold (Nicholson, 1921 and Mayne, 1926a), absence of water Wokw *et al*., 1956) or by failure to mate (Mer, 1936., Tate and Vincent, 1936).

According to Clements, 1963, ovulation and oviposition were affected principally by the movement of the lateral oviducts and abdomen as a whole, which was controlled by the nervous system. Gravid females (sometimes containing 250 eggs or more) initiate oviposition searching flights with their normal daily activity periods (Day, 2016). Generally, warm, moist, humid and calm conditions favor flight and oviposition and gravid female rely on visual and olfactory cues to identify potential oviposition sites (Bidlingmayer, 1974).

Cx. quinquefasciatus is an urban mosquito, which oviposits in reclaimed water storage ponds, poorly maintained swimming pools, gutters, catch basins, ornamental ponds and storm drains (Bheehler *et al*., 1993). Several factors have been identified in regard to the choice of oviposition habitat such as from chemical cues (Dadd and Kleinjan 1974, Ikeshoji, 1966, Bently and Day, 1989, Beehler *et al*., 1994b, Millar *et al*., 1994 and Braks *et al*., 2007) to quantity and quality of oviposition medium (Reisen and Meyer, 1990 and Harrington *et al*., 2008). Bently and Day, 1989 and Silver, 2008 reported elevated nutrients increased the number of egg rafts oviposited by *Cx. quinquefasciatus*.

Fecundity

The number of eggs laid in a batch during first gonotropic cycle varies greatly between species. In the first gonotropic cycle, *Cx. quinquefasciatus* lays up to 200-300 eggs. A single female could lay up to five rafts of eggs in a lifetime (Gerberg *et al*., 1994). Several factors might affect the size of the egg batches such as size of the female mosquito (Roy, 1936., Shannon and Hadjinicalao, 1941., Detinove, 1955), amount of blood ingested (Roy, 1936., Woke *et al.,* 1956 and Colless and Chellapah, 1960) and the number of ovarioles also showed a positive correlation with the size of the female (Colless and Chellapah, 1960). Roubaud, 1934 and Putnam and Shannon, 1934 reported that in all mosquitoes, number of eggs laid in each batch decreases with successive gonotropic cycles. Seasonal variations also affected the size of egg batches (Mer, 1931., Shannon and Hadjinicalao, 1941 and Detinova, 1955). Detiniova (1955) showed the progressive decline in size of egg batches with increasing age affected at various seasons. From the literature, it is also seen that the conditions of temperature and nourishment during larval development affect the size of the female mosquitoes and consequently the size of the egg batches and thus decreasing the fecundity rate in mosquitoes.

Reproduction- Male

The male reproductive system of insects produces haploid gametes, and the spermatozoa, which fertilizes the haploid female gamete, an oocyte. The basic component of a male reproductive system consists of paired testes, suspended in the body cavity by tracheae and fat body (Jones and Wheeler, 1965), an anterior portion of the reproductive ducts vasa efferentia and the posterior portion vasa defferentia, which opens into paired seminal vesicles (Hodapp and Jones, 1961), a pair of accessory glands flanks the seminal vesicles and an ejaculatory duct which penetrates the aedeagus flanked by a pair of parameres, which takes the form of claspers (Snodgrass, 1957).

The testes are situated dorso-laterally in the 5th and 6th abdominal segments, pear shaped, elongate body covered with fat body, their anterior ends pointed and ending in terminal filaments of connective tissue which are attached to the heart and alary muscles (Clements, 1963). Each testis consists of a single follicle enclosed in an investing sheath, which is divided into cysts and germ cells in various stages of development. The posterior cyst contains spermatozoa, which opens in to the reproductive duct of its side.

The vasa efferentia is a thin- walled tubes without muscle fibres, whereas, the vasa defferentia are thick- walled and muscular. The seminal vesicles are distensible regions of the efferent ducts, which could store spermatozoa (Clement, 1963). Accessory glands consist of columnar cells packed with secretions and surrounding a central duct. Large granules secreted by the anterior regions of each gland with the fluid component of the accessory gland secretion accompany the sperm at copulation. Ejaculatory ducts, which penetrates the aedeagus is lined with cuticle and contain muscle fibre (Clement, 1963).

The male genitalia originated on the 9th abdominal segment and consist of intermittent organ, the aedeagus, which is flanked by a pair of parameres or claspers, being divided in to a large basimere and a slender telomere. Both segments are independently movable by strong antagonistic muscles (Snodgrass, 1959 and Christophers, 1960). The semen flows through the ejaculatory duct, which leads directly to the genital pore located near the base of the aedaegus, and contractions of the male AG provide pressure for the sperm transfer (Spielman, 1964). Semen transfer itself requires only a few seconds (Lum, 1961a).

Mating Behaviour

Considering the mosquito life strategy, mating is probably least understood and most understudied (Takken *et al.*, 1998). Males of many mosquito species required several days to mature, because male accessory glands mature during the first few days of adult life and this was needed before the sperm can be successfully transferred (Clements, 1999). One of the major characteristics of culicine species during mating was that they mate in swarms, in which male mosquitoes aggregate in large numbers, forming nearly- cylindrical swarms of several metres height (Takken *et al*., 1998). It is unknown how males aggregate and how females locate male swarms. Single females fly in to the swarm and are detected by their lower wing- beat frequency (Belton, 1994; Clements, 1999). Takken (1999) and Takken and Knols (1999) suggested that swarm findings in females were directed by olfactory cues, chemical cues (pheromones) and by some visual cues. Mating in mosquitoes usually takes place in the air. The claspers of the male mosquitoes used to grab on to the female and the aedaegus ejects and extends into the female vagina and is widened to enlarge the opening in to the burs inseminalis (Spielman, 1964). Males transfer a mixture of sperms and accessory glands secretions. After termination of the coitus, atrium of the female regains its precopulatory appearance and the sperms begin to concentrate close to the spermathecal eminence (Spielman, 1964). The accessory gland (AG) secretions along with the sperm provide a liquid medium for the movement of sperms and play a role in the migration of sperms to the spermathecae. Jones and Wheeler (1965a) reported that one third of the sperm cells transferred to the Bursa Inseminalis (BI) did not reach the sperathecae and the sperm that failed to reach the spermathecae become compressed into the posterior end of the BI, lose their motility by the second day after copulation, and are then digested and absorbed in the BI (Spielman, 1964).

Sperm Morphology

Sperm of mosquitoes are long and slender. There are significant variations in length of the sperm from 100 μm (Klowden and Chambers 2004) to 570 μm (Breland *et al*., 1968) within different species (Klowden and Chambers, 2004). Average sperm lengths varied with body size of the mosquitoes (Voordouw *et al*., 2008). Klowden and Chambers, 2004 suggested that even short sperm are also capable for fertilization because all sperm contains nuclear DNA. Mosquito sperm heads possess diameter of 0.5–0.6 μm and are as wide as the tail (Clements and Potter 1967 and Tongu 1968). The head contains the nucleus and are identifiable with an undulating flagellum, which makes its rigidity. The flagellum consists of two mitochondrial derivatives, which extends the length of the flagellum and a microtubular structure named as axoneme, which was responsible for sperm motility (Clements and Potter 1967; Bao *et al*., 1992).

The chemicals contained in the seminal fluid exerts certain effects on the female, such as inducing to lay eggs and take larger amount of blood meals. Most of the female mosquitoes will mate just once and they store for the rest of their lives and typically produce one batch of eggs for every blood meal they have taken.

Hormonal control of reproduction in male

In male mosquitoes the production of sperm may begin early during the larval and pupal stages and often continues throughout the adult life (Klowden, 2002). During metamorphosis, the testes development respond to the hormone 20-hydroxyecdysone (20HE). Regulation of spermatozoa within the testes has been implicated by both 20HE and Juvenile Hormone (JH). High levels of 20HE increase the rate at which the spermatogonia undergo mitotic divisions to form spermatocysts and it was abolished by high levels of JH (Klowden,

2007). A peak of 20HE occurs before the end of the larval periods that allow the larva to find a spot in which to pupate, while a postwandering peak of 20HE unblock the meiotic division of the spermatocytes and allow the cells to proceed to metaphase (Klowden, 2007). JH also accelerates spermatogenesis. JH and 20HE interaction during post embryonic development regulates the development and differentiation of the accessory glands. JH alone might control the synthesis of some specific proteins, which are transferred to the female.

Hormonal Control of Molting and Metamorphosis

Most of the insects consist of rigid exoskeleton and therefore an insect can only grow by periodically shedding their exoskeleton, and the process is known as molting. Molting occurs repeatedly during larval development and the adult emerges at the final molt (Tembhare, 1997). Molting is possible in insects due to the integration of several neuroendocrine factors. When an immature insect grown sufficiently and require a larger exoskeleton, certain sensory input from the body activates some neurosecretory cells in the brain, which respond by secreting certain brain hormone that triggers the Corpora cardiaca to release the 'Prothoracicotropic hormone' (PTTH) into the circulatory system, which stimulates the Prothoracic glands to secrete the molting hormone 'Ecdysteroids (Ecdysone)' (Nijhout, 1994). This ecdysone stimulates a series of physiological events (Apolysis) that lead to synthesis of a new exoskeleton. Ecdysteroid concentration falls towards the end of apolysis and the neurosecretory cells in the ventral ganglia begin to secreting 'eclosion hormone', which triggers 'ecdysis' or shedding the old exoskeleton. On the other hand, a rising concentration of eclosion hormone stimulates the cells in the ventral ganglia to secrete 'bursicon', a hormone that hardens and darkens the integument (Wigglesworth, 1954).

Wigglesworth (1934) mentioned Juvenile hormone (JH) secreted by the Corpora allata also plays a key role in the process of metamorphosis. JH inhibits the genes that promote development of adult characteristics such as wings, external genitalia and reproductive organs and causing the insects to remain as immature (larva). During the last larval instars, the Corpora allata become atrophied and stop producing JH secretion, which promote the development of adult characteristics and causes the insect to molt into an adult (Wigglesworth, 1954). But whenever the adult insect attains sexual maturity, the brain neurosecretory cells release certain brain hormones that reactivate the corpora allata, which stimulates the renewed production of JH. In adult females, JH stimulates vitellogenesis whereas, in adult males, it stimulates the accessory glands to produce proteins needed for seminal fluid and the case of the spermatophore. In the absence of normal JH production, the adult insects remain sexually sterile (Wigglesworth, 1939).

Present study also made a comparison of the phytosterilant properties of the selected plant extracts with a chemosterilant Hexamethylphosphoramide (HMPA), to understand the potentialities of phytosterilant derived from the selected plants, *S. guttata, A. paniculata, B. spectabilis* and *P. longifolia*.

Hexamethylphosphoramide (HMPA)

According to Knipling (1955 & 1959), sterilization of insects were ultimately more efficient than killing as a method of controlling insect population. A number of chemosterilants especially alkylating agents possessed promising sexual sterility in insects. Hayes (1964) reviewed the chemosterilant activity of some chemical compounds such as alkylating agents like Metepa, Tepa, Morzid, Apholate, Tretamine and CB1506, antimetabolites like Aminopterin, Methotrexate, 2- Thiouracil and Fluoroorotic acid and Miscellaneous compound like Thiourea.

Non- aziridine alkylating agents like Hexamethylphosphoramide (HMPA) could be used as a complete substitution of aziridines in tepa (Baxter 2016). Its low mammalian toxicity and less harmful effects on environment compared to aziridines, HMPA was initially considered advantageous and it was used in the field tests to control *Musca domestica* (Chang *et al.*, 1964). Glancey (1965) investigated the chemosterilant activity in yellow Fever Mosquito *Ae. aegypti* using Hexamethylphosphoramide (HMPA). The literature regarding acute and chronic influence of HMPA in human reproductive, developmental and carcinogenic effects are sparse. But an increased incidence of nasal tumors from inhalation exposure to HMPA was reported in rats. EPA has not classified HMPA as a carcinogen, however, the International Agency for Research on Cancer (IARC) has been classified HMPA as a group of 2B, possible human carcinogen. HMPA is a chemosterilant and is anticipated to be carcinogen (NCBI). Sittig (1985), U. S. EPA (1982) and IARC (1987) reviewed that certain animal studies reported reproductive effects, reduced fertility, reduction in sperm count and significantly reduced testicular weights from oral exposure to HMPA.

2.2 REVIEW OF LITERATURE

Nowadays, as an alternative strategy, pest/ vector management program had conceived an approach to insect control in which the natural reproductive process of an insect is disrupted by chemical or physical mechanisms thus rendering the insect sterile. These sterile insects are released in to the target areas in order to mate with the native population and thereby reducing the number of next generation of pest/ vectors. These Sterile Insect Technique (SIT) proved to be an extensively successful strategy for the control of insect pests/ vectors.

Much progress has been made around the world in recent years towards developing the Sterile Insect Technique (SIT) to bring about mosquito population suppression using sterility aspect (Lees *et al.,* 2015). Bellini *et al.*, (2007) released around 1000 irradiated *Ae. albopictus* pupae per hectare per week, inducing up to 68% sterility in the target population in three pilot sites having area between 16 and 45ha. Since, five years of release of sterile males continued and demonstrated the potential of sterile males to suppress an *Ae. albopictus* population. Lees *et al*., (2015) and Ageep *et al.,* (2014) reviewed semi-field and field experiments that have demonstrated selected radiation dose could have been given sufficient sterility without significantly impacting competitiveness.

Krishnamurthy *et al.,* (1962), Morlan *et al*., (1962), Weidhaas *et al*., (1962) and Laven (1967) reviewed the spectacular success of the control of filarial vector *Culex pipiens fatigans* Weidemann in Okpo, Burma by the release of incompatible males into the natural population once again reinforced the faith in the potentialities of the sterile male- release technique for control of mosquito vectors, wherever earlier attempts of these SITs were not well encouraged.

Borkovec (1968) initiated a research program directed towards discovering chemosterilants and since then 10,000 compounds were synthesized and screened for sterilant properties in various laboratories. Juvenile hormones and their analogs or mimics and specific growth regulators were also developed independently without direct ties to chemosterilant research. Weidhaas *et al.,* (1961) reported chemosterilant activity in mosquitoes by application in the larval rearing media. Serebrovsky (1969) suggested the use of mutant strains of insects for control purposes, and later it was rediscovered independently by Curtis (1976). But the basic idea was that of Sterile Male release method by Knipling (1955). According to Borkovec (1968) the mutant strain was derived from a single insect that had been treated by a mutagenic agent and in most instances it was by lower doses of ionizing radiations.

Chang and Borkovec (1964) observed 'tepa' a chemosterilant was 13 times more active in sterilizing male houseflies than its methyl homologue 'metepa'. Mulla (1964) found 'Apholate' to be superior to tepa and metepa on *Cx. p. quinquefasciatus* Say with the treatment in larvae or adults. Mc Cray and Schoof (1967) also proved apholate to produce more sterility in adults of *Cx. p. quinquefasciatus* Say than that of tepa using mist application. Murrey and Bickley (1964) observed that the larval treatment of apholate produced high sterility rate with *Cx. p. quinquefasciatus* Say. Das (1967) reported apholate induced high sterility with treatment of larvae or adults in *Cx. p. fatigans* Weidemann. But tepa and apholate were equally inducing sterility in *Ae. aegypti* (L.) after larval treatment, whereas, after adult treatment apholate was more effective (Weidhaas 1962 and Dame, Woodward and Ford (1964).

Weidhaas, Schmidt and Seabrook (1962) proved the effect of apholate and tepa was equally good enough after adult treatment in *Anopheles quadrimaculatus* Say. However, treatment with tepa was more effective than with apholate in adult houseflies (Labrecque, 1961). Pillai and Grover (1969)

reported among the diaziridinyl compounds, methyl ester was more effective than tepa for larval treatment, because these compounds caused moderate toxicity in *Cx. p. fatigans*. Borkovec *et al*., (1966) found all substitutions at the aziridinyl carbon atom of the tepa molecule decreased the sterilizing activity of the parent compound in houseflies. Pillai and Grover (1969) commented tepa was found to be better with pupal treatment as a non- toxic dose which induced about 99% sterility, but even higher doses of apholate and metepa could not produce as much sterility as tepa in *Cx. p. fatigans*. They also observed among the diaziridinyl esters, the isopropyl and ethyl ester produced about 100% and 91% sterility after pupal treatment.

White (1966) Grover *et al*., (1971) obtained high sterility effects with pupal treatment in *Ae. aegypti* and *Cx. p. fatigans* respectively. Chamberlain and Barrett (1968) observed the alkylating chemosterilants could inhibit DNA synthesis in the nuclei of follicular cells and nurse cells of the ovary in Stable fly. It was also observed that DNA- dependent protein synthesis during the gonadotrophic cycle was inhibited and thus prevented the egg formation in female mosquitoes. Auerbach (1958) found treatment with tepa and apholate produced molting and structural abnormalities, whereas, these anomalies might be due to the mutagenic properties of these chemosterilants. Henneberry *et al*., (1966) reported similar effects in male cabbage looper, in which tepa had been shown to cause copulatory aberrations and affected longevity also. Bertram (1959) and White (1966) found similar structural abnormalities in eggs of *Ae. aegypti* after pupal and adult treatment with thiotepa. The mode of action of aziridinyl compounds in the chemosterilants was not well understood. According to Borkovec (1966), the alkylation of chromatin material in the sperm nucleus of males, but in females the mode of action was uncertain. Rai (1964) observed visible chromosomal aberrations such as breaks, deletion and clumping of chromatin in the somatic and reproductive organs of *Ae. aegypti* by the apholate treatment. There was a

reduction in the size of the testes in *Cx. p. quinquefasciatus* treated with apholate (Murray and Bickley, 1964).

Sharma and Rai (1967) reported sperm depletion in case of apholate sterilized males of *Ae. aegypti*. DNA synthesis was inhibited in eggs of houseflies which were treated by apholate and thiotepa (Kilgore and Painter 1964, and Painter and Kilgoore 1967). Pillai and Grover (1969) observed the dissection of non- viable eggs of *Cx. p. fatigans* showed death of embryos at an early stage of development from a cross involving an apholate- treated male. But the effect was delayed in crosses with treated females. Fahmy and Fahmy (1954) were also observed similar results in Drosophila and Screw- worm flies respectively, using tetramine.

Borkovec (1974) mentioned non- alkylating phosphorus amides, particularly 'HMPA' (Hexamethyl Phosphoramide) as a potentially useful group of chemosterilants. Shott *et al*., (1971) reported the toxicological effect of hempa, the data showed only moderate mammalian toxicity, but the compound or its metabolites were mutagenic. Post and Vincent (1973) reported another chemosterilant 1- (5-chlorophenyl) -3- (2, 6-difluorobenzoyl) urea (TH 6040) that could inhibit the chitin synthesis in developing insects. According to them, the mechanism of action of this material was apparently entirely different from that of the mutagenic sterilants. However, TH 6040 toxicity to immature stages were much more pronounced than its sterilizing activity, so the compounds come under the group of insecticides rather than a chemosterilant.

Saxena *et al*., 1993 studied the larvicidal and chemosterilant activity of *Annona squamosa* alkaloids against *An. stephensi*. Spielman and Skaff (1967) found the butanol extract of *Phytolacca dodecandra* (Soapberry) induced certain morphogenetic aberrations in *Cx. pipiens, Ae. aegypti* (Linn.) and *Anopheles quadrimaculatus* Say. Supavaran *et al*., (1974) reported the

extension of larval duration in *Ae. aegypti* with the methanol extract of 17 families from different plant species which not only caused acute toxicity, but also significantly lengthened the larval periods, which was due to the interference with normal hormonal activity.

Sujatha *et al*., (1998) also described some morphogenetic abnormalities in *Ae. aegypti, Cx. quinquefasciatus* and *An. stephensi*, when treated with the plant extracts of *Moringa longifolia, Acorus calamus* and *Ageratum conyzoides,* which not only produced larval- pupal intermediates but also, decolourized and extended pupae and incompletely emerged adults. Saxena and Sumithra (1985) observed the effects of *Ipomea cornea* extract on the growth and development of Cx. *quinquefasciatus and An. stephensi*, in which the growth index was shorter than the control and untreated groups. According to Saxena *et al*., (1993), plant alkaloids resulted in significant loss in fecundity and fertility in the adult of mosquitoes from treated larvae as compared to the control. Ferrari and Georghion (1981) observed reduced egg production in *Cx. quinquefasciatus*, when exposed to sublethal concentration of botanical insecticides.

Zebits (1984) indicated that azadirachtin from *Azadirachta indica* (neem) seed kernals acted as anti ecdysteroid, which affected the neuroendocrine control of the ecdysteroids. The unique mode of its action on hormones and its favourable toxicological and selective properties from various ecological perspectives provided a promising phytochemical in mosquito control programmes. Several plants of North Dakota exhibited ecdysones and juvenile hormone mimics in insects and the activities varied according to the plant parts used for extraction (Patterson *et al.,* 1975). These growth inhibitors also found to be species specific. Sujatha *et al*., (1998) found the extracts of *Acorus calamus* and *Madhuca longifolia* induced malformations and growth inhibition on *An. stephensi* and *Cx. quinquefasciatus* to a greater extend.

Dhillon *et al*., (1982) observed the methanol eluted fraction of petroleum ether extracts from the filamentous algae *Rhizoclonium heiroglyphisum* exhibited growth inhibition and various structural abnormalities in eclosion in adults of *Ae. aegypti, Cx. quinquefasciatus* and *Culisetaincidens*.

In addition to growth inhibition, certain phytochemicals influenced the oviposition behaviour of mosquitoes. Consoli *et al*., (1989) observed ethanolic, hexanic and lypophilized extracts of *Anacardium occidentale, Allium sativum, Bidens segetum, Caesalpinia peltophoroide, Jatropha curcas, Mikania schenkii, Poinciana regia* and *Spatodea campanulata* showed oviposition deterrency in *Aedes fluviatilis* (Lutz). Sharma *et al*., (1985) reported the acetone extracts of four species of Labiatae plant family for its ovipositional deterrents to *Ae. aegypti*. Ovipositional deterrence from phytochemicals depends mainly on the factors of species specificity and the solvent selection for the extraction process. Judd and Borden (1980) proved aqueous extracts of *Lemna minor* significantly deterred oviposition in *Ae. aegypti*, but had no effect on *Cx. pipiens*. Several botanical derivatives offered great promise as better source of phytochemicals for mosquito control.

2.3 MATERIALS AND METHODS

2.3.1 Insect Growth Regulating Activity

Selected Column fractions of the different plant extracts were tested for Insect Growth Regulating (IGR) activity according to the protocol of **Mehdi *et al.* (2012)** with slight modifications against freshly hatched I instar larvae of *Cx. quinquefasciatus*. Fifty numbers of I instar larvae were transferred to a beaker containing 500ml of 0.08% saline solution. Desired sub lethal concentrations of the different column fractions of the selected plant extracts like *S. guttata* (MeOH: EA- 4:1), *A. paniculata* (MeOH: EA- 4:1), *B. spectabilis* (H: EA- 5:5) and *P. longifolia* (MeOH: EA- 4:1) were tested for the estimation of Effective concentration (EC_{50}) to allow emergence of 50% of the treated larval population. Triplicates and controls were also maintained for each experiment. For the accurate determination of IGR activity, extension of total developmental duration, deformities and mortality rates were observed and recorded every day until adult emergence occur. Larvae were provided with fine powder of dog biscuits and yeast in the ratio 3:1respectively at fixed intervals during the observation period. Incidences of malformations were recorded as larval-larval intermediates, larval-pupal intermediates and pupal-adult intermediates.

Data Management and Statistical analysis

Mortality was corrected according to Abbott's formula (1925). The LC_{50} values and Chi- square tests were calculated according to Finney's Probit analysis (1971). P value was calculated using t- test and $p < 0.05$ were considered to be statistically significant.

2.3.2 Fecundity and Fertility experiments and mating competitiveness of adult *Cx. quinquefasciatus* treated with different column fractions of selected plant extracts

Fertility and fecundity effects of selected plant extracts on the adult males and females of *Cx. quinquefasciatus* were analyzed according to the protocol of Saxena *et al*. (1993)**.** These experiments were conducted using 10 numbers of males and 10 numbers of females from the treated and untreated sets and placed in the standard mating cages (20 x 20 x 20cm) in to the following groups to assess mating competitiveness of both male and female *Cx. quinquefasciatus*.

i. Treated females X Treated males **(T ♀X T ♂)**

ii. Treated females X Untreated males **(T♀ X UT ♂)**

iii. Untreated females X Treated males **(UT♀X T♂)**

iv. Untreated females X Untreated males **(UT ♀x UT ♂)**

Three days after the blood meal, eggs were collected daily from the ovitraps from the cages. Freshly hatched larvae were transferred to the glass beaker containing 0.08% saline solution. Adult mortality rates, oviposition day after blood meal, number of eggs, and percentage of hatching and larval mortality were observed regularly. Total Developmental periods, fecundity rate, fertility rate, % Control of reproduction, Growth Index (GI) and Sterility Index (SI) were calculated using the following formulas;

$$\text{Growth Index (GI)} = \frac{\%\text{ of Adult Emergence}}{\text{Average developmental period}}$$

1. $$\text{Sterility Index (SI)} = 100 - \frac{\text{Treated no. of eggs x \% of hatching}}{\text{Control no. of eggs x \% of hatching}} \times 100$$

2. % Control of reproduction = $\frac{V1 - V2}{V1} \times 100$

Where, V1= No. of viable eggs/ female in control
V2= No. of viable eggs/ female in test

3. Fecundity rate = $\frac{\text{No. of eggs}}{\text{Total no. of females}}$

4. Fertility (%) = $\frac{\text{No. of eggs treated}}{\text{No. of eggs in control}} \times 100$

2.3.3 Determination of Oviposition Activity Index (OAI)

The comparative oviposition preference and attractiveness was expressed by Oviposition Activity Index (OAI), which was calculated according to Kramer and Mulla (1979).

Oviposition Activity Index (OAI) = (NT-NS)/ (NT+ NS)

Where, NT = Number of eggs laid in the test
NS = Number of eggs laid in the control

Index value ranges from +1 to -1, with a positive value indicating more of oviposition attractant than the control and a negative value indicating oviposition repellent.

Statistical analysis

Statistical evaluations of the data were carried out by Probit analysis (Finney, 1971) and level of significance by Duncan's (1963) Multiple Range Test.

2.3.4 Method employed for the study of post-treatment effects on reproductive organs

Dissections

To study the effect of selected plant extracts on ovarian and testicular development, the reproductive system of the adult males and females of *Cx. quinquefasciatus* were carefully dissected out after removal of fat bodies and was stained with eosin. The following observations were recorded;

a. Total size of ovaries and testes

Total size of ovaries and testes were measured by placing the tissues on a clean glass slide kept under a Trinocular Stereo Zoom microscope to measure the dimensions of length and breadth of each ovaries and testes.

b. Total number of ovarioles

The number of ovarioles per ovary was measured by placing the tissues on a clean glass slide kept under a stereomicroscope and counted the number of each ovarioles.

2.3.5 Comparative assessment of sterility induction by synthetic chemosterilant Hexamethylphosphoramide (HMPA) and selected plant extracts.

2.3.5.1 Larvicidal Bioassay

Larvicidal bioassay was carried out according to **WHO** protocol **(1996)** with slight modifications. Using 1% stock solution of the different column fractions, different concentrations were prepared in glass beakers containing 100ml of saline solution. Ten, freshly hatched I instar larvae were introduced in each medium. Triplicates for each concentration and both +ve(Solvent) and –ve(Water) controls were maintained. The larval mortality at different doses

and in control was recorded after 24 hours of exposure. The control mortality was corrected using **Abbott's formula (1925).**

$$\text{Percent mortality} = \frac{\%\text{ mortality in treated} - \%\text{ mortality in control}}{100 - \%\text{ mortality in control}} \times 100$$

2.3.5.2 Bioassay for effective Concentration

The experiments for the estimation of effective concentration to allow 50% of the adult emergence were carried out using **WHO** protocol (1996) with slight modifications. Deferent sub lethal concentrations were prepared from 1% stock solution of the column fraction in glass beakers containing 100ml of 0.08% saline solution. Ten, freshly hatched I instar larvae were introduced in each beaker and triplicates were maintained for each set. Both +ve and –ve controls were also maintained in triplicates. The emergence in treated and the controls were recorded. The percentage emergence was corrected using **Abbott's** formula (1925).

$$\text{Percent emergence} = \frac{\%\text{emergence in treated} - \%\text{emergence in control}}{100 - \%\text{ emergence in control}} \times 100$$

2.3.5.3 Fecundity, Fertility and mating competitiveness Experiments with HMPA

Fecundity experiments were carried out according to the protocol of **Saxena *et al.,*** (1993). Mating competitiveness of both males and females of *Cx. quinquefasciatus* from the treated and untreated sets were conducted by taking 10 numbers of males and females as the following groups;

1. Treated females X Treated males **(T ♀X T♂)**

2. Treated females X Untreated males **(T♀ X UT ♂)**

3. Untreated females X Treated males **(UT ♀X T ♂)**

4. Untreated females X Untreated males **(UT ♀X UT ♂)**

Three days after blood meal, eggs were collected daily from the plastic bowls. Just after hatching, the larvae were transferred to the enamel trays containing 0.08% saline solution. Larval mortality, developmental period, Growth Index (GI) and Sterility Index (SI) were recorded until adult emergence. Percentage of hatching and effect upon metamorphosis were also assessed.

1. Growth Index (GI) = $\frac{\%\text{ of adult Emergence}}{\text{Average developmental period}}$

2. % Control of reproduction = $\frac{V1 - V2}{V1} \times 100$

V1= No. of viable eggs/ female in control
V2= No. of viable eggs/ female in test

3. Fecundity rate = $\frac{\text{No. of eggs}}{\text{Total no. of females}}$

4. Fertility (%) = $\frac{\text{No. of eggs treated}}{\text{No. of eggs in control}} \times 100$

5. Sterility Index (SI) = $100 - \frac{\text{Treated no. of eggs x \% of hatching}}{\text{Control no. of eggs x \% of hatching}} \times 100$

2.4 RESULTS

2.4.1 Insect Growth Regulating Activity

Time taken for total larval and pupal periods (in days) during developmental metamorphosis were observed and recorded. The experimental results revealed that the selected plant extracts could induce prolonged larval and pupal periods, when compared to control (**Table 19)**. Larval duration significantly increased in the treatment of *S. guttata* (MeOH: EA- 4:1) as 17.00±0.00 days and total developmental period extended to 21.33± 0.33 days at 6.419ppm concentration. Whereas, in control total developmental duration was 13.67± 0.33 days and larval duration was 10.67±0.33 days. While, in *A. paniculata* (MeOH: EA- 4:1) the larval period lasted up to 18.00± 0.00 days and 22.00± 0.58 days extended for total development at 0.525ppm. In control, larval period lasted to 11.00± 0.00 days and total developmental days lasted to 14.67±0.33 days.

Treatment with column fraction of *B. spectabilis* (H: EA- 5:5) and *P. longifolia* (MeOH: EA- 4:1), larval duration was extended to 19.33±0.33 days and 14.00±0.00 days and the total developmental days extended to 23.67±0.33 days and 17.00±0.58 days respectively at concentrations 55.346ppm and 36.300ppm. Whereas, in control, total period lasted to 15.67±0.33 days and 16.33±0.33 days and larval duration was 12.67±0.33 days and 13.33±0.33 days respectively.

Table 19- Data on larval and total developmental duration of *Cx. quinquefasciatus* when treated with different concentrations of column fraction of the selected plant extracts

SI No.	Name of the plant/ Column gradient	Conc. (ppm)	Extension of larval duration (days)				Extension of Pupal duration (days)	Total developmental duration (days)	P Value
			I instar	II instar	III instar	IV instar			
1.	*S. guttata* MeOH: EA- 4:1	6.419	4.33± 0.33	4.00± 0.58	4.00± 0.00	4.67± 0.33	4.33±0.88	21.33± 0.33	0.0001*
	Control	--------	2.67± 0.33	3.00± 0.00	2.33± 0.33	2.67± 0.00	3.00± 0.00	13.67±0.33	
2.	*A. paniculata* MeOH: EA- 4:1	0.525	5.33± 0.33	4.00± 0.58	5.00± 0.00	3.67±0.33	4.00±0.58	22.00± 0.58	0.0057*
	Control	---------	3.00± 0.33	2.33± 0.33	3.00± 0.33	2.67±0.33	3.67± 0.33	14.67± 0.33	
3.	*B. spectabilis* H: EA- 5:5	55.346	5.00± 0.00	5.33± 0.33	4.67± 0.33	4.33± 0.33	4.33± 0.33	23.67± 0.33	0.0003*
	Control	--------	2.67± 0.33	3.00± 0.00	3.33± 0.33	3.67± 0.00	3.00± 0.00	15.67±0.33	
4.	*P. longifolia* MeOH: EA- 4:1	36.300	4.33± 0.33	3.00± 0.58	3.00± 0.00	3.67±0.33	3.00±0.58	17.00± 0.58	0.6608
	Control	--------	3.00± 0.33	3.33± 0.33	3.33± 0.33	3.67 ±0.33	3.00± 0.33	16.33± 0.33	

Note: Number of individuals per sample= 50
*Significant at the level P < 0.005

2.4.2 Effect on growth and metamorphosis of *Cx. quinquefasciatus*after the exposure of fractionated column extracts of selected plants

Adult emergence against *Cx. quinquefasciatus* at 6.419ppm concentration of *S. guttata* (MeOH: EA- 4:1) recorded as 38.00% with 63.32% larval mortality, 6.66% pupal mortality and 4% adult mortality (Table 20), compared with control adult emergence as 88.60%. Whereas, *A. paniculata* (MeOH: EA- 4:1) treatment with a concentration of 0.525 ppm exhibited 43.32% adult emergence with 37.34% larval mortality, 9.34% pupal mortality and 10.00% adult mortality (**Table 20**), whereas in control, adult emergence was 70.00%. *B. spectabilis* (H: EA- 5:5) produced larval mortality 30.00%, pupal mortality 7.34% and 62.00% adult emergence without any adult mortality at 55.346ppm and in control it was 78.00%. 32.66% adult emergence was showed in *P.longifolia* (MeOH: EA- 4:1) at 36.30ppm concentration with 58.02% larval mortality, 4.66% pupal mortality and 4.66% adult mortality with 80.66% control adult emergence (**Table 20**).

Table 20- Effect of Column fractions of selected plant extracts on the Growth and metamorphosis of *Cx. quinquefasciatus*

SI No.	Name of the plant	Conc. (ppm)	Larval mortality (Mean± SE)	Pupal mortality (Mean± SE)	Adult mortality (Mean± SE)	% adult emergence (a)	Total developmental periods (days) (b)	Growth Index (GI) (a/b)
1	*S. guttata* MeOH: EA- 4:1	6.419	31.65± 1.20 (63.32%)	3.33±0.88 (6.66 %)	2.00±0.00 (4.00%)	19.00±1.15 (38.00 %)	21.33± 0.33	1.78
	Control	0	5.00± 1.15 (10.00 %)	0.67± 0.33 (1.34 %)	0.00+ 0.00 (0.00%)	44.33±1.20 (88.60 %)	13.67±0.33	6.48
2	*A. paniculata* MeOH: EA- 4:1	0.525	18.67±0.88 (37.34%)	4.67±0.33 (9.34%)	5.00± 0.00 (10.00%)	21.66±0.88 (43.32%)	22.00± 0.58	1.97
	Control	-------	12.00± 0.58 (25.40%)	2.33± 0.33 (4.60%)	0.00+ 0.00 (0.00%)	35.00± 0.58 (70.00%)	14.67± 0.33	4.71
3	*B. spectabilis* H: EA- 5:5	55.346	15.00± 0.58 (30.00%)	3.00± 0.58 (6.00 %)	0.00+ 0.00 (0.00%)	32.00±1.45 (64.00 %)	19.67±0.33	3.25
	Control	-------	7.33±0.33 (14.66%)	2.67±0.33 (5.34%)	0.00+ 0.00 (0.00%)	39.00±0.58 (78.00%)	15.67±0.33	4.97
4	*P. longifolia* MeOH: EA- 4:1	36.300	29.01± 0.58 (58.02%)	2.33± 0.33 (4.66%)	2.33±0.33 (4.66%)	16.33+ 0.88 (32.66%)	17.00± 0.58	1.92
	Control	-------	7.67± 0.33 (15.34%)	2.67± 0.33 (5.34%)	0.00+ 0.00 (0.00%)	40.33± 0.88 (80.66%)	16.33± 0.33	4.93

Note: Number of individuals per sample= 50

2.4.3 Morphogenetic deformities associated with *Cx. quinquefasciatus* larvae exposed to the selected plant materials.

Sub lethal concentrations of the silica gel column fractions of the selected plant extracts tested against the larvae of *Cx. quinquefasciatus* caused several developmental defects such as changes in the morphological features of larvae, pupae and adult, deformed wings, mobility, inhibition of adult emergence, flying nature, longevity and other behavioral aspects. The metamorphic abnormalities such as larval inability to moult in to next stage (Larval- larval intermediates), Larval- pupal intermediates and Pupal- adult intermediates were noticed, recorded and provided in the table 21. It was observed that the treatment with of *S. guttata* column fraction (MeOH: EA- 4:1) exhibited 15.34% larval- larval intermediates, 12.00% larval- pupal intermediates and 2.66% pupal- adult intermediates (**Table 21**).

Microscopic examination of dead larvae revealed certain morphological deformities like scleratisation of larval cuticle, which might be characteristic features of pupal cuticle. Changes in morphological features of larval-larval intermediates consists ecdysial suture. Even though, the presence of ecdysial suture, the larvae could not molt into next instars **(Plates 13, 14 & 15)**. The dead pupa on the other hand, showed a variety of malformations like partially demelanized pupae with straight abdomen like larval forms (Larval- pupal intermediates) **(Plate 16)** and partly emerged pupae with attached head capsule (Pupal- adult intermediates) (**Plates 17&18**) and melanized pupae with straight abdomen and head capsule with antennae, which is a characteristic feature of adult mosquitoes.

Morphogenetic deformities associated with the exposure of column fractionated seed extract of *S. guttata* (M: EA- 4:1) on freshly hatched I instars larvae of *Cx. quinquefasciatus* at half of the median lethal dose (6.419ppm) treatment (Plates-13, 14, 15, 16, 17& 18).

Plate 13-Larval-larval intermediates

Plate14- Larval- larval intermediates

(Larval moulting arrested with ecdysial suture)

Plate 15- Deformed larvawith demelanized abdomen

Plate 16- Larval- pupal intermediate (Pupa with straight abdomen)

Plate 17- Pupal- adult intermediate (Partially emerged adult with attached head capsule with antennae)

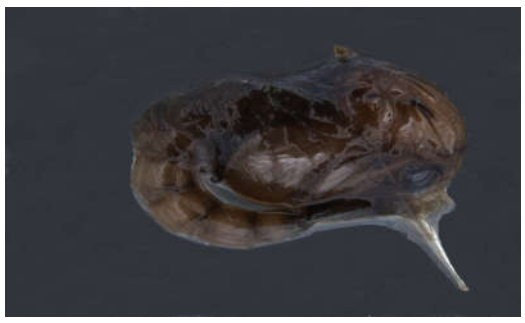


Plate 18-Pupal- adult intermediate (Partially developed adult with head capsule enclosed within the pupal case)

The IGR effects of *A. paniculata* (MeOH: EA- 4:1) on *Cx. quinquefasciatus* after the exposure of half of the sublethal dose exhibited with the appearance of 10.66% larval- pupal intermediates and 14.66% of pupal- adult intermediates **(Table 21)**. In case of larval- pupal intermediates, death has occurred at an early stage of pupation, demelanized pupa with straight abdomen and dwarf pupa with retarded abdomen **(Plates 19, 20, 21& 22)**. Whereas, in pupal- adult intermediates, death occurred after complete molting from pupal skin, but some parts remained attached to the pupal exuvia **(Plates 23& 24)** and inability of the pupae to develop completely as an adult, in which the head and the appendages of adults were formed but it was enclosed within the pupal case with extended abdomen.

Morphogenetic deformities exhibited after the exposure of *A. paniculata*(M: EA- 4:1)at half of the median lethal dose (0.525ppm)on freshly hatched I instars larvae of *Cx. quinquefasciatus*

Plate 19-Larval- pupal intermediate (Deformed pupa with straight abdomen)

Plate 20-Larval- pupal intermediate (Dwarf pupa with straight abdomen)

Plate 21Larval- pupal intermediate (Dwarf pupa withretarded abdomen)

Plate 22-Larval- pupal intermediate (Dwarf pupa with retarded abdomen)

Plate 23- Pupal- adult intermediate (Deformed adult enclosed within the pupal exuvia)

Plate 24- Pupal- adult intermediate (Fully developed adult enclosed within the pupal case)

The morphogenetic anomalies, during the development of *Cx. quinquefasciatus*, after the exposure to column fraction of *B. spectabilis* (H: EA- 5:5) exhibited the formation of 7.34% larval-pupal intermediates and 8.66% pupal- adult intermediates **(Table 21)**. Larval- pupal intermediates mainly consist of partly emerged pupae with straight abdomen and dwarf pupae with retarded abdomen **(Plates 25 & 26)**. In pupal- adult intermediates, completely developed adults could not detach from the pupal exuvia **(Plates 27, 28, 29 & 30).**

Morphogenetic deformities associated with the exposure of column fractionated leaf extract of *B. spectabilis* (H: EA- 5:5) on freshly hatched I instars larvae of *Cx. quinquefasciatus*at half of the median lethal dose (55.346ppm) treatment.

Plate 25- Pupal- adult intermediate (Pupa with straight abdomen)

Plate 26- Larval- pupal intermediate (Pupa with straight abdomen)

Plate 27- Pupal- adult intermediate (Adult with pupal exuvia)

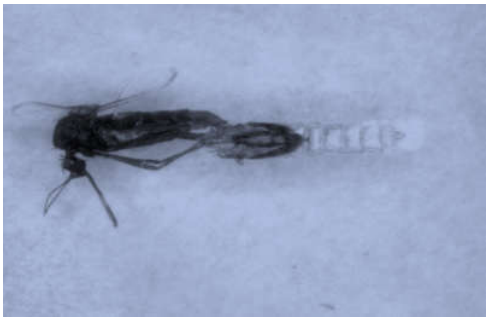


Plate 28- Pupal- adult intermediate (Adult with pupal exuvia)

Plate 29- Pupal- adult intermediate (Adult with pupal exuvia)

Plate 30- Pupal- adult intermediate (Adult with pupal exuvia)

Fractionated column seed extract of *P. longifolia* (MeOH: EA- 4:1) on larval instars of *Cx. quinquefasciatus* exhibited a variety of malformations like larval-larval intermediates as 6.00%, larval- pupal intermediates as 12.00% and pupal- adult intermediates as 8.66% **(Table 21).** In the case of larval-larval intermediates, malformations like partly melanized larvae with ecdysial sutures and scleratization of larval cuticle were observed **(Plates 31 & 32)**. Whereas, in larval- pupal intermediates, observations mainly included dwarf pupa with retarded abdomen, melanized pupa with extended abdomen and partly emerged pupae with attached head capsule **(Plates 33 & 34)**. Pupal-adult intermediates resulted in appearance of partly emerged adults with extended abdomen and complete molting of adults with pupal exuvia remained attached to the adults **(Plates 35 & 36)**.

Morphological deformities after the exposure of *P. longifolia* (MeOH: EA-- 4:1) at half of the median lethal dose (36.300ppm) on the freshly hatched I instars larvae of *Cx. quinquefasciatus*

Plate 31- Larval- larval intermediate (Partially melanized larvae with ecdysial suture)

Plate 32- Larval- larval intermediate (Scleratisation of larval cuticle)

Plate 33- Larval- pupal intermediate (Melanized pupa with extended abdomen)

Plate 34- Larval- pupal intermediate (Dwarf pupa with retarded abdomen)

Plate 35- Pupal- adult intermediate (Partially emerged adult with extended abdomen)

Plate 36- Pupal- adult intermediate (Complete adult molt with pupal exuvia)

Table 21- Data on Morphological deformities of mosquito larvae exposed to different concentrations of the fractionated column extracts of the selected plants.

SI No.	Name of the plants (Column fractions)	Conc. (ppm)	Larval- larval intermediate (Mean± SE)	Larval-pupal intermediate (Mean± SE)	Pupal-adult intermediate (Mean± SE)
1	*S. guttata* MeOH: EA- 4:1	6.419	7.67±0.88 (15.34%)	6.00± 1.15 (12.00%)	1.33± 0.33 (2.66%)
2	*A. paniculata* MeOH: EA- 4:1	0.525	0.00± 0.00 (0.00%)	5.33± 1.20 (10.66%)	7.33± 0.67 (14.66%)
3	*B. spectabilis* H: EA- 5:5	55.346	0.00± 0.00 (0.00%)	3.67± 0.88 (7.34%)	4.33± 0.67 (8.66%)
4	*P. longifolia* MeOH: EA- 4:1	36.300	3.00±0.58 (6.00%)	6.00±0.58 (12.00%)	4.33± 1.20 (8.66%)
5	Control	--------	0.00± 0.00 (0.00%)	0.00± 0.00 (0.00%)	0.00± 0.00 (0.00%)

Note: Number of individuals per sample= 50

2.4.4 Effect of selected plant extracts on oviposition, egg hatchability, Controlreproduction and mating competitiveness of *Cx.quinquefasciatus* adults

The column fraction of the selected plant extracts, *S. guttata* (MeOH: EA- 4:1), *A. paniculata* (MeOH: EA- 4:1), *B. spectabilis* (H: EA- 5:5) and *P. longifolia* (MeOH: EA- 4:1) exhibited significant effects on oviposition

day, number of egg rafts and eggs, egg hatchability, percentage control of reproduction and mating competitiveness of treated adults of *Cx. quinquefasciatus*. **Table 22** shows the gonotropic cycle, number of egg rafts and number of eggs laid by each gravid female, percentage hatchability of eggs and percentage of control of reproduction induced by MeOH: EA- 4:1column fraction of *S. guttata* on **T ♂X T ♀, T ♂X UT ♀, T ♀X UT♂, UT ♂X UT ♀**of *Cx. quinquefasciatus*. Results showed that the gonotropic cycles of **T ♂X T ♀, T ♂X UT ♀, T ♀X UT♂, UT ♂X UT ♀** consisted 5.33±0.33, 4.33±0.33, 10.33± 0.33 and 2.67± 0.33 days respectively after successive blood meal. The number of egg rafts laid by each gravid female in treated groups **T ♂X T ♀, T ♂X UT ♀ & T ♀X UT♂** resulted 4.33± 0.33, 3.33± 0.33 and 1.67± 0.33 egg rafts whereas, **UT ♂X UT ♀**showed7.67± 0.33 egg rafts, respectively. The number of eggs counted from the collected egg rafts for each group of **T♂ x T ♀, T ♂ x UT ♀, T ♀ x UT♂, UT ♂ x UT ♀** were obtained as 545.33± 63.05, 232.72±17.41, 174.46± 17.76 and 1514.00±0.00 respectively. Oviposition Activity Index (OAI) of each groups obtained as -0.47, -0.73, -0.73 and 0.00 for **T ♂ x T ♀, T ♂ x UT ♀, T ♀ x UT♂, UT ♂ x UT ♀**. Eggs hatching were observed to take place on different days in each group of **T♂ x T ♀, T ♂ x UT ♀, T ♀ x UT♂, UT ♂ x UT ♀** such as3.33±0.33, 2.67±0.33, 3.33±0.33 & 2.00±0.00 days respectively. Whereas, percentage of eggs hatchability in **UT ♂ x UT ♀** were recorded as 83.70% and the other groups percentage hatchability were varied with treated groups **T ♂ x T ♀, T ♂ x UT ♀, T ♀ x UT♂** such as 67.76%, 56.34% and 61.0%respectively.Percentage Control of reproduction were also observed as 31.09%, 66.88%, 78.14% and 00.00% respectively for **T ♂ x T ♀, T ♂ x UT ♀, T ♀ x UT♂, UT ♂ x UT ♀** groups.

Table 22-: Data on oviposition, gonotropic cycle, egg hatchability and control of reproduction of adult *Cx. quinquefasciatus*when treated with column fraction (MeOH: EA- 4:1) of *S. guttata*at half of the median lethal dose (6.419ppm)

SI No.	Treated groups	Gonotropic cycle (days)	No. of egg rafts laid (per 10 number of females)	Average no. of eggs (per 10 number of females)	Oviposition Activity Index (OAI)	No. of days taken for egg hatching	% of hatch	% of Control of reproduction
1	a. (T ♂ x T ♀)	5.33±0.33	4.33±0.33	545.33±63.05	-0.47	3.33± 0.33	67.76	31.09
2	b. (T ♂ x UT ♀)	4.33±0.33	3.33±0.33	232.72±17.41	-0.73	2.67±0.33	56.34	66.88
3	c. (T ♀ x UT♂)	10.33±0.33	1.67±0.33	174.46± 17.76	-0.73	3.33± 0.33	61.00	78.14
4	d. (UT ♂ x UT ♀)	2.67±0.33	7.67± 0.33	1514.00±0.00	0.00	2.00±0.00	83.70	00.00

Note: 10 numbers of treated females and males were taken in each of the 3 replicates

Column fraction of *A. paniculate* (MeOH: EA- 4:1) adversely effected **(Table 23)** on the gonotropic cycle of the treated groups **T ♂ x T ♀, T ♂ x UT ♀& T ♀ x UT♂,** of adult *Cx. quinquefasciatus* and were recorded as 3.33±0.33, 3.67± 1.86 and 4.33±0.33 days, whereby, **UT ♂ x UT ♀** groups showed 3.33± 0.33 days for oviposition. The number of egg rafts laid after mating was also varied according to the groups, such as 3.33±0.33, 0.67±0.33, 5.00± 0.58 and 8.67± 0.33 respectively for **T ♂ x T ♀, T ♂ x UT ♀, T ♀ x UT♂, UT ♂ x UT ♀.** Eggs enclosed in each egg rafts were counted and recorded for all the groups as 371.60± 38.86, 46.67±26.17, 569.00±26.21 and 1142.00±67.99 for **T ♂ x T ♀, T ♂ x UT ♀, T ♀ x UT♂, UT ♂ x UT ♀** respectively. Oviposition Activity Index (OAI) was noted as -0.5, -0.92, -0.33 for treated groups **T ♂ x T ♀, T ♂ x UT ♀, T ♀ x UT♂** and 0.00 for **UT ♂ x UT ♀.** Number of days and percentage of eggs hatching were also observed as 3.33±0.33, 4.33±0.33, 2.67±0.33 and 2.00± 0.00 days and 77.65%, 44.27%, 59.16% and 87.27% respectively for **T ♂ x T ♀, T ♂ x UT ♀, T ♀ x UT♂, UT ♂ x UT ♀** groups. Percentage of control of reproduction was obtained as 70.87%, 97.90%, 66.60% and 00.00% for each treated **T ♂ x T ♀, T ♂ x UT ♀, T ♀ x UT♂** and untreated **UT ♂ x UT ♀** groups respectively.

Table 23- Effect of column fraction of *A. paniculata* (MeOH: EA- 4:1) at half of the median lethal dose (0.525ppm) on oviposition, gonotropic cycle, egg hatchability and control of reproduction of adult *Cx. quinquefasciatus*.

SI No.	Treated groups	Gonotropic cycle (days)	No. of egg rafts laid	Average no. of eggs obtained	Oviposition Activity Index (OAI)	No. of days taken for egg hatching	% of hatch	% of Control of reproduction
1	a. (T ♂ x T ♀)	3.33±0.33	3.33±0.33	371.60±38.86	-0.5	3.33±0.33	77.65	70.87
2	b. (T ♂ x UT ♀)	3.67±1.86	0.67± 0.33	46.67±26.17	-0.92	4.33± 0.33	44.27	97.90
3	c. (T ♀ x UT♂)	4.33± 0.33	5.00±0.58	569.00± 26.21	-0.33	2.67±0.33	59.16	66.60
4	d. (UT ♂ x UT ♀)	3.33±0.33	8.67± 0.33	1142.00± 67.99	0.00	2.00± 0.00	87.27	00.00

Note: 10 numbers of treated females and males were taken in each of the 3 replicates

B. spectabilis (H: EA- 5:5) fraction resulted considerable changes in the gonotropic cycle, egg hatchability and percentage of control of reproduction in treated and untreated groups of adult *Cx. quinquefasciatus* (**Table 24**). It has been observed that the gonotropic cycle varied as 7.67± 0.88, 6.67± 0.33, 5.33± 0.33 and 2.67± 0.33 days for **T ♂ x T ♀, T ♂ x UT ♀, T ♀ x UT♂, UT ♂ x UT ♀** groups respectively. Egg rafts laid by females from each group was also recorded as 4.67±0.33, 2.33±0.33, 7.67±0.58 and 8.67±0.33 respectively (**Table 24**). The number of eggs obtained from each egg raft was counted for treated groups **T ♂ x T ♀, T ♂ x UT ♀, T ♀ x UT♂** as424.33± 32.77, 82.67±17.32, 1312.00±179.52 and **UT ♂ x UT ♀** as 1384.00± 53.15 respectively. Oviposition Active Index (OAI) was varied with each of the treated groups as -0.53, -0.88 and -0.02 respectively. Percentage and number of days for egg hatching was also calculated and presented in **Table 24**. 2.33± 0.33, 4.67±0.33, 2.67±0.33, 2.00±0.00 days were taken for eggs hatching in **T ♂ x T ♀, T ♂ x UT ♀, T ♀ x UT♂, UT ♂ x UT ♀** groups. Percentage of eggs hatching observed as 69.59%, 66.58%, 78.60% and 84.85% and control of reproduction were 73.25%, 95.08%, 83.72% and 00.00% respectively for **T ♂ x T ♀, T ♂ x UT ♀, T ♀ x UT♂, UT ♂ x UT ♀** groups.

Table 24- Effect of column fraction of *B. spectabilis* (H: EA- 4:1) at half of the median lethal dose (55.346ppm) on oviposition, gonotropic cycle, egg hatchability and control of reproduction of adult *Cx. quinquefasciatus*.

SI No.	Treated groups	Gonotropic cycle (days)	No. of egg rafts laid (per 10 number of females)	Average no. of eggs obtained (per 10 number of femlaes)	Oviposition Activity Index (OAI)	No. of days taken for egg hatching	% of hatch	% of Control of reproduction
1	a. (T ♂ x T ♀)	7.67±0.88	4.67±0.33	424.33±32.77	-0.53	2.33±0.33	69.59	73.25
2	b. (T ♂ x UT ♀)	6.67±0.33	2.33±0.33	82.67±17.32	-0.88	4.67±0.33	66.58	95.08
3	c. (T ♀ x UT♂)	5.33±0.33	7.67± 0.58	1312.00±179.52	-0.02	2.67±0.33	78.60	83.72
4	d. (UT ♂ x UT ♀)	2.67± 0.33	8.67± 0.33	1384.00±53.15	0.00	2.00± 0.00	84.85	00.00

Note: 10 numbers of treated females and males were taken in each of the 3 replicates

The column fraction of *P. longifolia* (MeOH: EA- 4:1) exhibited lengthening of gonotropic cycle as 4.33±0.33, 5**.**67± 0.33, 4.33±0.33 days for treated groups **T ♂ x T ♀, T ♂ x UT ♀, T ♀ x UT♂** and untreated group **UT ♂ x UT ♀** as2.33±0.33 days. The number of egg rafts collected after oviposition were 6.00±0.58, 3.67±0.33, 6.67±0.58 and 9.00±0.00 each for **T ♂ x T ♀, T ♂ x UT ♀, T ♀ x UT♂, UT ♂ x UT ♀** groups. Number of eggs were counted from each egg rafts and recorded as 701.33± 94.01, 231.00± 56.72, 872.00±32.70 for treated groups **T ♂ x T ♀, T ♂ x UT ♀, T ♀ x UT♂** and 1554.33±71.79 for **UT ♂ x UT ♀** group. Oviposition Activity Index (OAI) were calculated and recorded as -0.37, -0.74 and -0.28 respective for treated groups. Number of days for egg hatching and percentage of control were noted as 2.67±0.33, 3.67± 0.33, 2.33±0.33 and 2.00± 0.00 and 49.81%, 52.38%, 74.09% and 85.56% respectively for **T ♂ x T ♀, T ♂ x UT ♀, T ♀ x UT♂, UT ♂ x UT ♀** groups. Whereby, percentage control of reproduction was exhibited as 70.52%, 90.69%, 57.02% and 00.00% for each groups **T ♂ x T ♀, T ♂ x UT ♀, T ♀ x UT♂, UT ♂ x UT ♀ (Table 25).**

Table 25- Effect of column fraction of *P. longifolia* (MeOH: EA- 4:1) at half of the median lethal dose (36.300ppm) on oviposition, gonotropic cycle, egg hatchability and control of reproduction of adult *Cx. quinquefasciatus*.

SI No.	Treated groups	Gonotropic cycle (days)	No. of egg rafts laid (per 10 number of females)	Average no. of eggs (per 10 number of females)	Oviposition Activity Index (OAI)	No. of days taken for egg hatching	% of hatch	% of Control of reproduction
1	a. (T ♂ x T ♀)	4.33±0.33	6.00±0.58	701.33± 94.01	-0.37	2.67±0.33	49.81	70.52
2	b. (T ♂ x UT ♀)	5.67±0.33	3.67±0.33	231.00± 56.72	-0.74	3.67± 0.33	52.38	90.69
3	c. (T ♀ x UT♂)	4.33±0.33	6.67±0.58	872.00±32.70	-0.28	2.33±0.33	74.09	57.02
4	d. (UT ♂ x UT ♀)	2.33±0.33	9.00±0.00	1554.33±71.79	0.00	2.00± 0.00	85.56	00.00

Note: 10 numbers of treated females and males were taken in each of the 3 replicates

2.4.5 Effect of column fractionated extracts of selected plants on fecundity, fertility and sterility in adults of *Cx. quinquefasciatus*

The column fractions of selected plant extracts resulted in a significant loss in fecundity and fertility in adults of treated larvae from the untreated larvae. The effect of *S. guttata* (MeOH: EA- 4:1) on fecundity and fertility of different treated groups **T ♂ x T ♀, T ♂ x UT ♀, T ♀ x UT♂** and untreated **UT ♂ x UT ♀** exhibited36.02%, 15.37% and 11.52%and 100.00% of fertility respectively for treated and untreated groups and 54.53, 23.27, 17.45 and 151.4 respectively as fecundity rate**.** When compared with untreated group **UT ♂ x UT ♀** (0.00), the Sterility Index (SI) was found to be higher in treated groups **T ♂ x T ♀, T ♂ x UT ♀, T ♀ x UT♂** as 70.84, 89.66 and 91.61, when mated with equal number of males and females **(Table 26)**.

Table 26-Data on Fecundity rate, fertility percentage and Sterility Index of the adults of *Cx. quinquefasciatus,* experiment commenced from the first instar larvae with the exposure of fractionated column seed extract of *S. guttata* (MeOH: EA- 4:1) at half of the median lethal dose (6.419ppm)

SI NO.	Treated Groups	Fecundity rate	Fertility (%)	Sterility Index (SI)	P-Value
1	a. (T ♂ x T ♀)	36.02	54.53	70.84	0.5463
	b. (T ♂ x UT ♀)	15.37	23.27	89.66	0.4605
	c. (T ♀ x UT♂)	11.52	17.45	91.61	0.4438
	d. (UT ♂ x UT ♀)	151.4	100.00	0.00	1.000

* Statistically Significant at $P < 0.05$

Note: 10 numbers of treated females and males were taken in each of the 3 replicates.

A. paniculata (MeOH: EA- 4:1) reduced the fecundity and fertility rate of adult *Cx. quinquefasciatus* and also exhibited higher Sterility Index with all the treated groups **(Table 27)** from the untreated groups. Treated groups such as **T ♂ x T ♀, T ♂ x UT ♀, T ♀ x UT♂** showed 32.53%, 4.08% and 49.82% fertility with 100.00% for **UT ♂ x UT ♀**groups**.** The fecundity rate was also reduced as 37.16, 4.67 and 56.90 for treated groups and 114.2 for untreated group and the Sterility Index observed as 71.05, 97.93 and 66.23 for treated groups **T ♂ x T ♀, T ♂ x UT ♀, T ♀ x UT♂** respectively with untreated group **UT ♂ x UT ♀** as 0.00**.**

Table 27-Data on Fecundity rate, fertility percentage and Sterility Index of the adults of *Cx. quinquefasciatus,* experiment commenced from the first instar larvae with the exposure of fractionated column seed extract of *A. paniculata* (MeOH: EA- 4:1) at half of the median lethal dose (0.525ppm).

SI NO.	Treated Groups	Fecundity rate	Fertility (%)	Sterility Index (SI)	P- Value
1	a. (T ♂ x T ♀)	32.53	37.16	71.05	0.5537
	b. (T ♂ x UT ♀)	4.08	4.67	97.93	0.4932
	c. (T ♀ x UT♂)	49.82	56.90	66.23	0.7237
	d. (UT ♂ x UT ♀)	114.2	100.00	0.00	1.000

* Statistically Significant at $P < 0.05$

Note: 10 numbers of treated females and males were taken in each of the 3 replicates

Effects on Fecundity, fertility and sterility of *B. spectabilis* (H: EA- 5:5) on adults of *Cx. quinquefasciatus* were also observed **(Table 28)**. The treated groups **T ♂ x T ♀, T ♂ x UT ♀, T ♀ x UT♂** exhibited 30.65%, 5.97% and 94.79% fertility and untreated **UT ♂ x UT ♀** as 100% fertility**.** Fecundity rate was also reduced as 42.43, 8.27, 131.20 and 138.40 respectively for **T ♂ x T ♀, T ♂ x UT ♀, T ♀ x UT♂, UT ♂ x UT ♀.** Sterility Index observed for the treated groups **T ♂ x T ♀, T ♂ x UT ♀, T ♀ x UT♂ are** 74.86, 95.31 and 12.19 respectively when compared with control.

Table 28-Data on Fecundity rate, fertility percentage and Sterility Index of the adults of *Cx. quinquefasciatus,* experiment commenced from the first instar larvae with the exposure of fractionated column seed extract of *B. spectabilis* (H: EA- 5:5) at half of the median lethal dose (55.346ppm)

SI NO.	Treated Groups	Fecundity rate	Fertility (%)	Sterility Index (SI)	P- Value
1	a. (T ♂ x T ♀)	30.65	42.43	74.86	0.5247
	b. (T ♂ x UT ♀)	5.97	8.27	95.31	0.4443
	c. (T ♀ x UT♂)	131.20	94.79	12.19	0.9990
	d. (UT ♂ x UT ♀)	138.40	100.00	0.00	1.000

* Statistically Significant at $P < 0.05$

Note: 10 numbers of treated females and males were taken in each of the 3 replicates

Treatmentwith *P. longifolia* column fraction (MeOH: EA- 4:1) provided significant reduction in the fecundity and fertility rate **(Table 29)**. The fertility percentage was lower in treated groups **T ♂ x T ♀, T ♂ x UT ♀, T ♀ x UT ♂** as 45.12%, 14.86% and 56.10%, with untreated group **UT ♂ x UT ♀** as

100.0%**.** The fecundity rate was observed as 70.13, 23.10, 87.20 and 155.43 respectively for **T ♂ x T ♀, T ♂ x UT ♀, T ♀ x UT♂, UT ♂ x UT ♀** groups. Whereas, the sterility index were found to be higher in treated groups and was exhibited as 73.74, 90.91 and 51.42 for **T ♂ x T ♀, T ♂ x UT ♀, T ♀ x UT ♂** and 0.00 for **UT ♂ x UT ♀** groups **(Table 29)**.

Table 29-Data on Fecundity rate, fertility percentage and Sterility Index of the adults of *Cx. quinquefasciatus,* experiment commenced from the first instar larvae with the exposure of fractionated column seed extract of *P. longifolia* (MeOH: EA- 4:1) at half of the median lethal dose (36.300ppm)

SI NO.	Treated Groups	Fecundity rate	Fertility (%)	Sterility Index (SI)	P- Value
1	a. (T ♂ x T ♀)	45.12	70.13	73.74	0.6578
	b. (T ♂ x UT ♀)	14.86	23.10	90.91	0.4584
	c. (T ♀ x UT♂)	56.10	87.20	51.42	0.6880
	d. (UT ♂ x UT ♀)	155.43	100.00	0.00	1.000

***** Statistically Significant at $P < 0.05$

Note: 10 numbers of treated females and males were taken in each of the 3 replicates

2.4.6 Morphogenetic changes of the reproductive organs of the adults of *Cx. quinquefasciatus* after the exposure of freshly hatched I instar larvae to the selected plant extracts.

Generally, reproductive system of a normal mature male mosquito consists of a pair of testes, in which sperm tubes are enclosed within a membranous sac. Two tubular vasa deferentia connect to a median ejaculatory duct and a portion of vas deferense enlarged to form the seminal vesicle, which stores the sperm and also secrete nutrients for nourishment and maintenance of the sperm **(Plate 37)**. Whereas, the reproductive system of a female mosquito consists of a pair of ovaries with ovarioles, oviducts, accessory glands, bursa inseminalis and three spermathecae **(Plate 38)**.

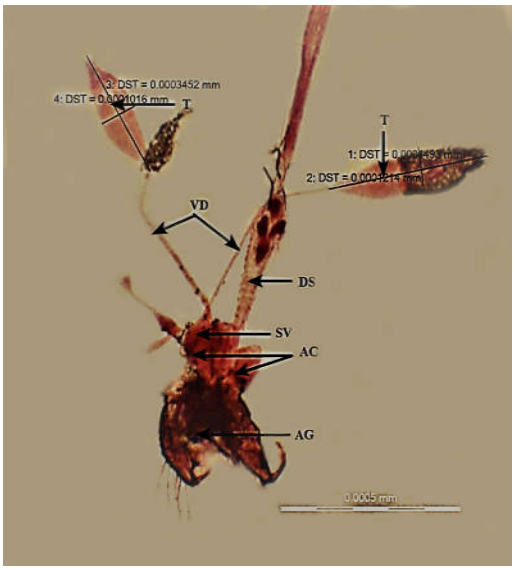


Plate 37- Normal mature male reproductive

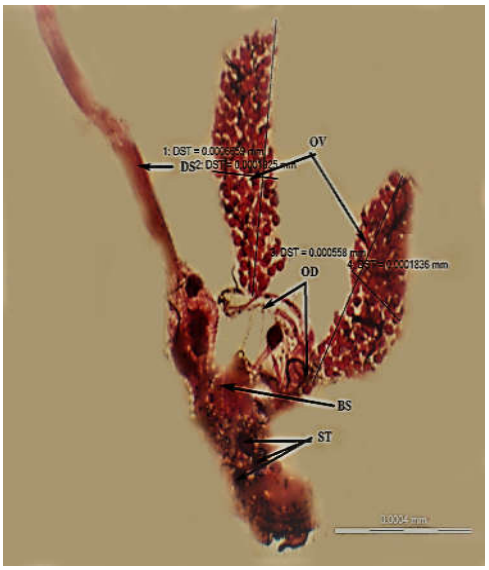


Plate 38- Normal mature female reproductive system

(T- testes, VD- Vas Deference, system SV- Seminal Vesicle, AC- Accessory Glands, AG- Aedeges, ED- Ejaculatory Duct, DS- digestive system)

(OV- Ovaries, OD- Oviducts, OVL- Ovariole, ST- Spermathecae, BS- Bursa Inseminalis, DS- Digestive System)

The adults emerged from the sub lethal concentrations and control sets were subjected to dissection and removed the reproductive organs for further studies on the morphogenetic changes. The dissections revealed substantial morphogenetic changes of the reproductive organs of the adults of treated *Cx. quinquefasciatus*. The column fraction of *S. guttata* (MeOH: EA- 4:1) treated with I instar larvae inhibited mating, apart from affecting the morphological features of the reproductive organs. **Plates 40 & 42)** displayed the ovarian and testicular abnormalities of the treated *Cx. quinquefasciatus.* **Plate 42- a, b & c** showed degeneration of the ovaries, oviducts and bursa inseminalis. In most of the cases length and size of the ovaries and ovarioles varied **(Plates 42- d & e).** Number of ovarioles also varied **(Table 30)**. Similarly, the testicular abnormalities mainly included the degeneration of testes **(Plates 40- a & b)** and the variation among the length and size of testes **(Plates 40- c & d) (Table 30)** when compared with normal mature male **(Plate 39)** and female **(Plate 41)** reproductive system.

Morphogenetic changes of the reproductive organs of the adults of *Cx. quinquefasciatus* after the exposure of freshly hatched I instar larvae to *S. guttata* (MeOH: EA- 4:1) at half of the median lethal dose (6.419ppm)

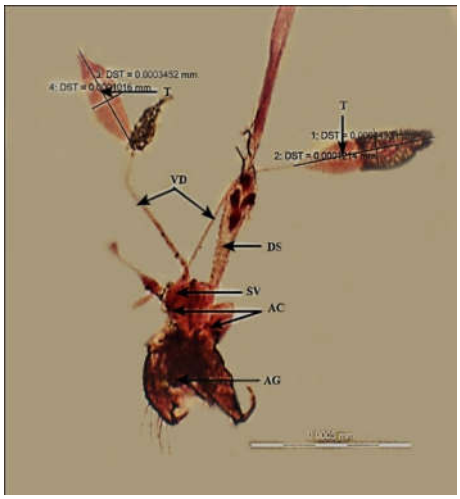


Plate 39- Normal mature male reproductive system

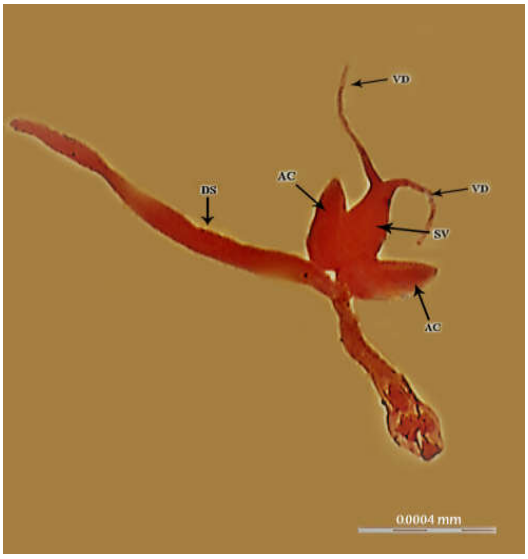


Plate 40 (a)

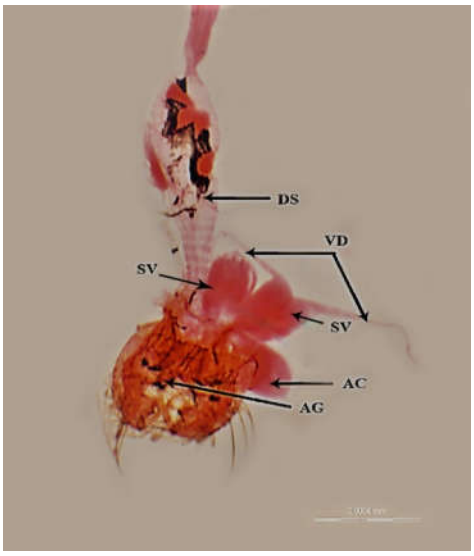


Plate 40 (b)

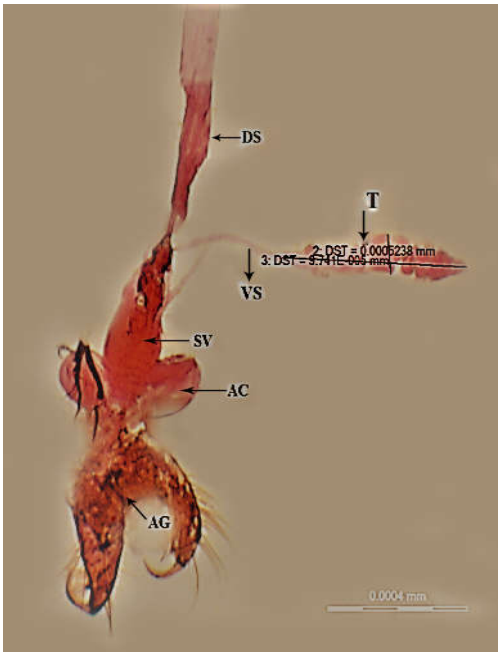


Plate 40 (c)

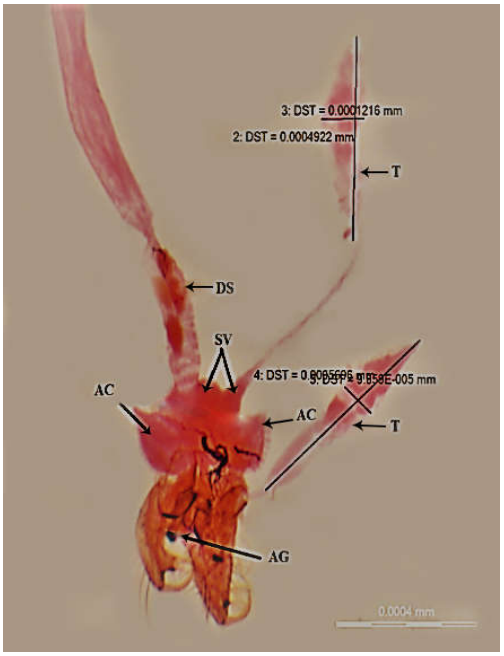


Plate 40 (d)

Plates 40- a, b, c & d- Male reproductive systems with degenerated tested and with figure h & I displayed varied length and size of the testes.

(T- testes, VD- Vas Deferrence, SV- Seminal Vesicle, AC- Accessory Glands, AG- Aedeges , ED- Ejaculatory Duct, DS- Digestive System)

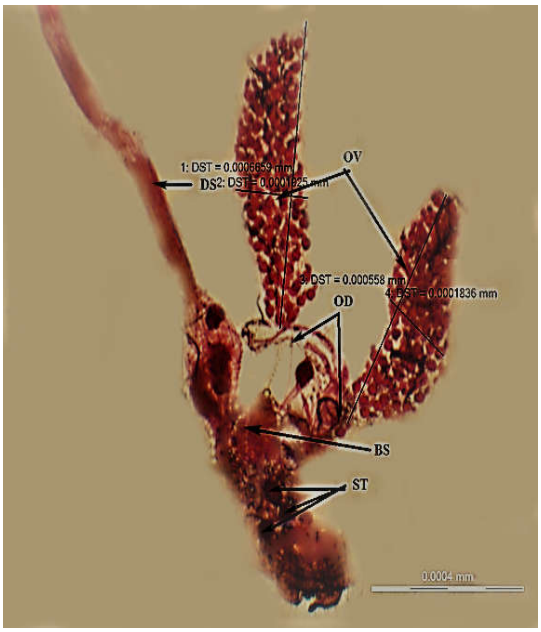


Plate 41- Normal Mature female reproductive system

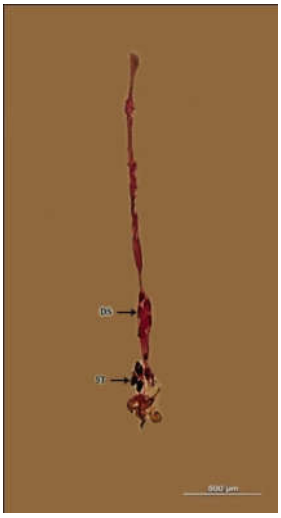


Plate 42 (a)

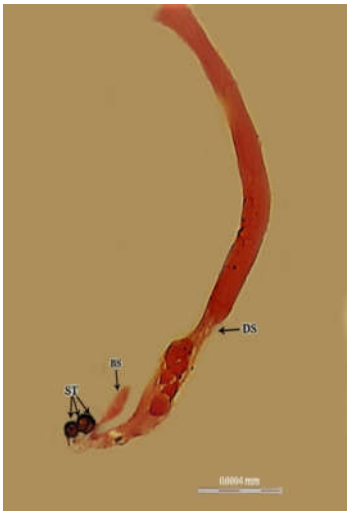


Plate 42 (b)

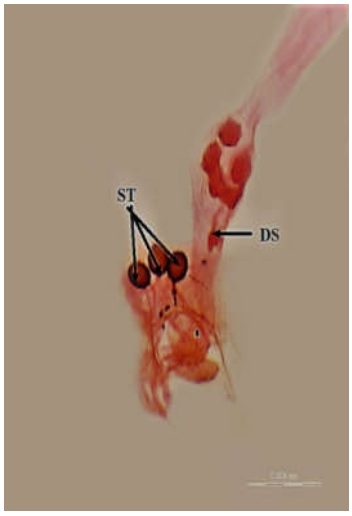


Plate 42 (c)

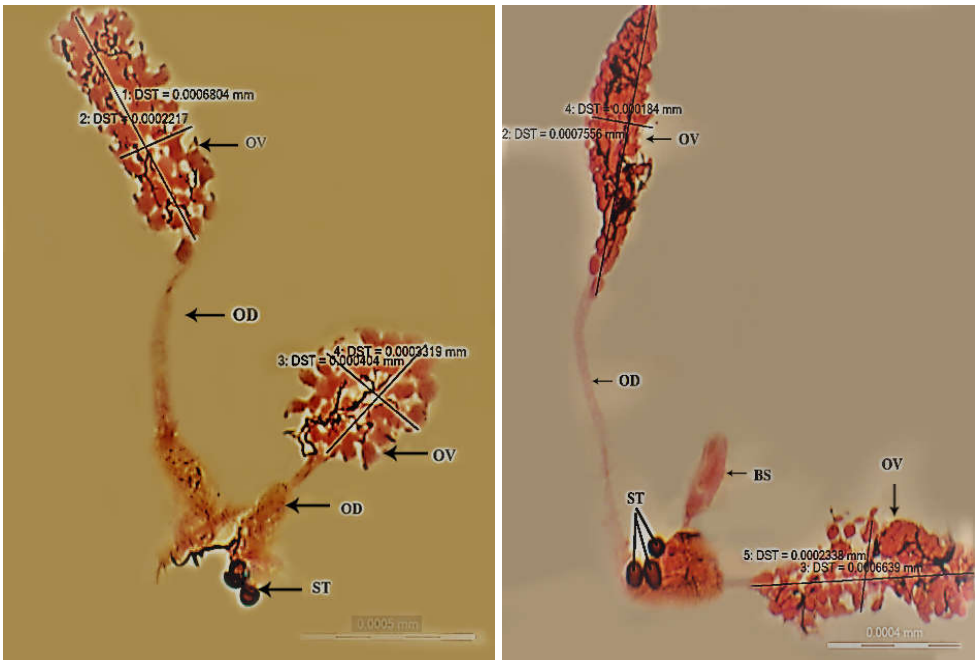


Plate 42 (d) **Plate 42 (e)**

Plates 42- a, b, c, d & e- Female reproductive systems with degenerated ovaries, oviducts and ovarioles and a & c with degenerated Bursa Inseminalis and d & e with undeveloped/ atrophied ovaries which varied in length and size with disintegrated ovarioles.

(OV- Ovaries, OD- Ovi Duct, OVL- Ovariole, ST- Spermatheca, BS- Bursa Inseminalis, DS- Digestive System**)**

Testicular development was drastically affected in *A. paniculata* (MeOH: EA- 4:1) treated resultant adults of *Cx. quinquefasciatus*. The testes and ovaries of resultant abnormal adults and few treated resultant morphologically normal adults showed variation in the length and size of the testes and ovaries, number of ovarioles **(Table 30)** and degeneration of testes **(Plates 44- a, b & c)**. It was also observed atrophied testes and ovaries **(Plate 46- a)** compared with mature male and female reproductive system **(Plates 43 & 45)**.

Morphogenetic changes of the reproductive organs of the adults of *Cx. quinquefasciatus* after the exposure of freshly hatched I instar larvae to *A. paniculate* (MeOH: EA- 4:1) at half of the median lethal dose (0.525ppm)

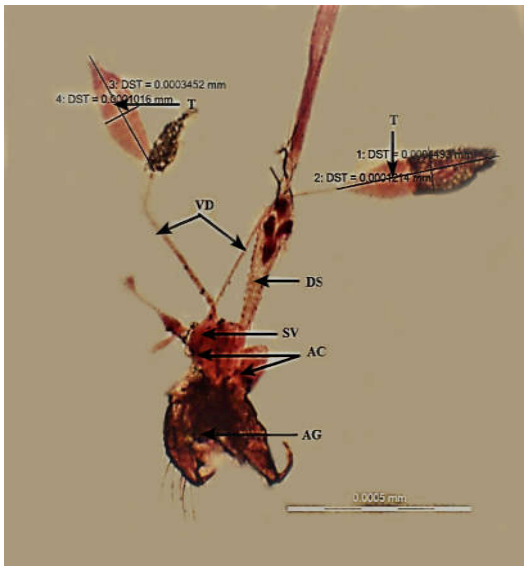


Plate 43- Normal mature male reproductive system

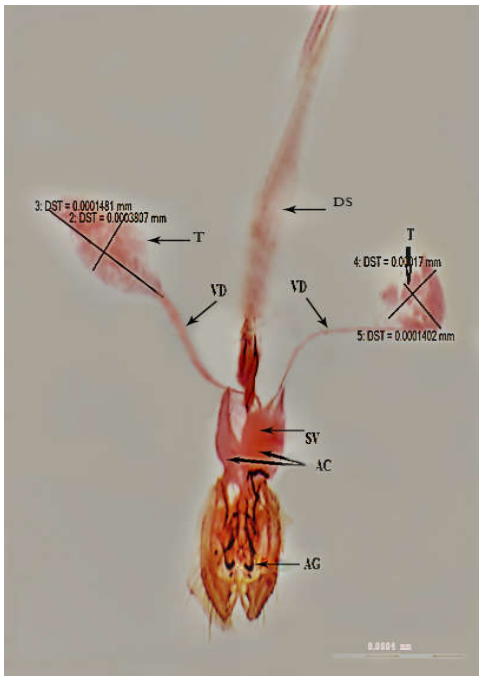


Plate 44 (a)

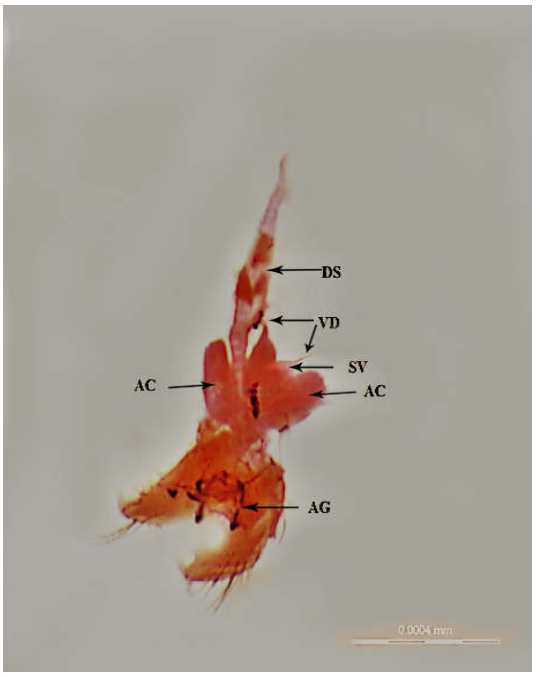
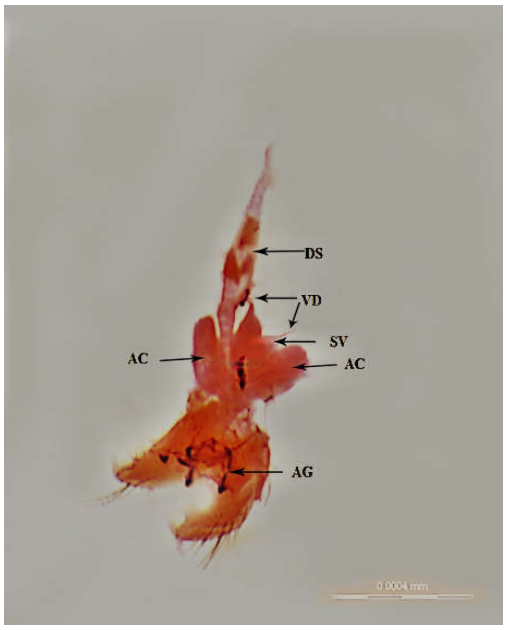


Plate 44 (b)



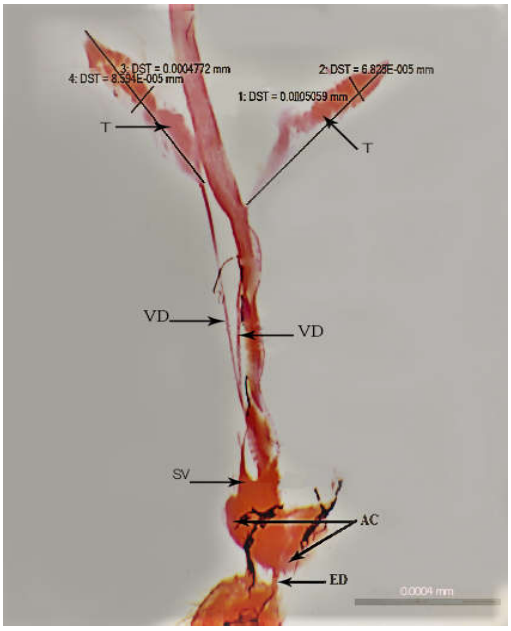


Plate 44 (c) **Plate 44 (d)**

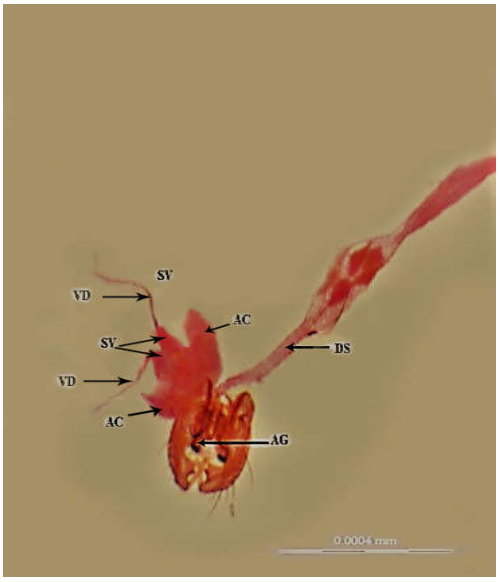


Plate 44 (e)

Plates 44- (a, b, c, d & e) Male reproductive systems with atrophied testes displayed varied in length and size of the testes and degenerated testes.

(T- testes, VD- Vas Deference, SV- Seminal Vesicle, AC- Accessory Glands, AG- Aedeges, ED- Ejaculatory Duct, DS- Digestive System)

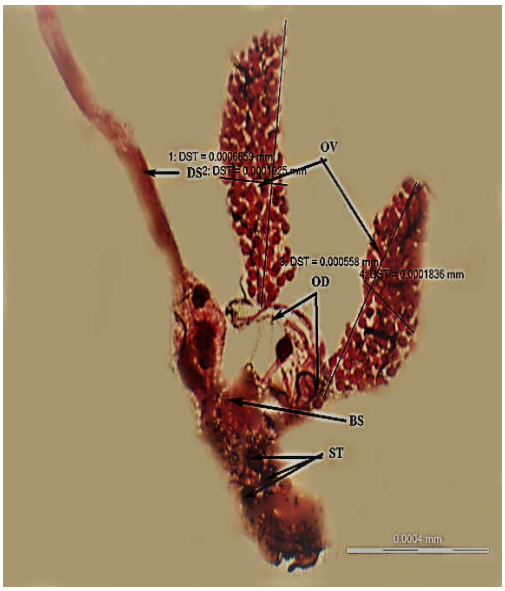


Plate 45- Normal mature female reproductive system

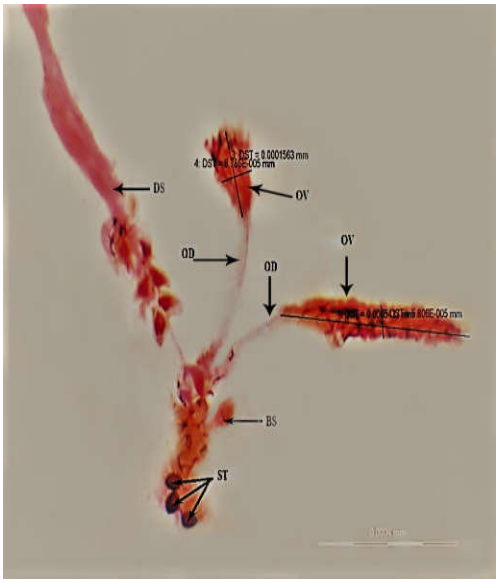


Plate 46 (a)

Plate 46- a- Female reproductive system with degenerated ovaries, ovarioles and atrophied ovaries varied in length and size with disintegrated ovarioles.

(OV- Ovaries, OD- Ovi Duct, OVL- Ovariole, ST- Spermatheca, BS- Bursa Inseminalis, DS- Digestive System)

The extract of *B. spectabilis* (n- Hexane: EA- 5:5) when treated with the larvae of *Cx. quinquefasciatus* inhibited ovarian and testicular development. The dissections revealed substantial morphological changes of the reproductive organs such as degeneration of ovaries and testes **(Plates 48- a, b & c)**, disintegration of ovarioles **(Plate 50- a, b & c),** and size of thereproductive organs **(Table 30),** when compared with normal mature male **(Plate 47)** and female **(Plate 49)** reproductive system.

Morphogenetic changes of the reproductive organs of the adults of *Cx. quinquefasciatus* after the exposure of freshly hatched I instar larvae to *B. spectabilis* (H: EA- 5:5)at half of the median lethal dose (55.346ppm)

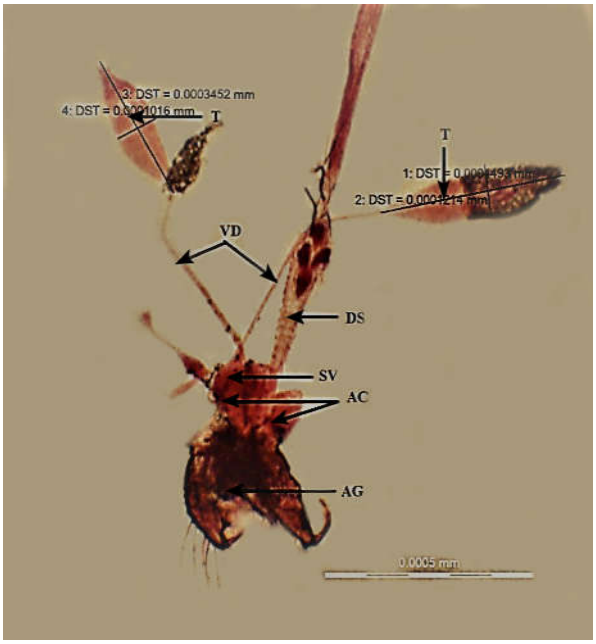


Plate 47- Normal mature male reproductive system

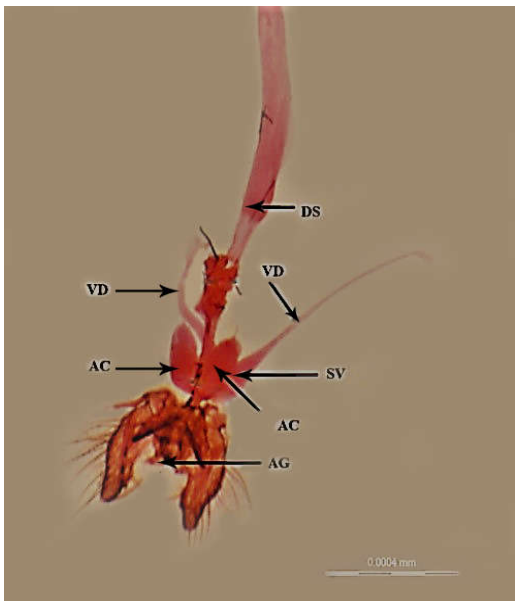


Plate 48 (a)

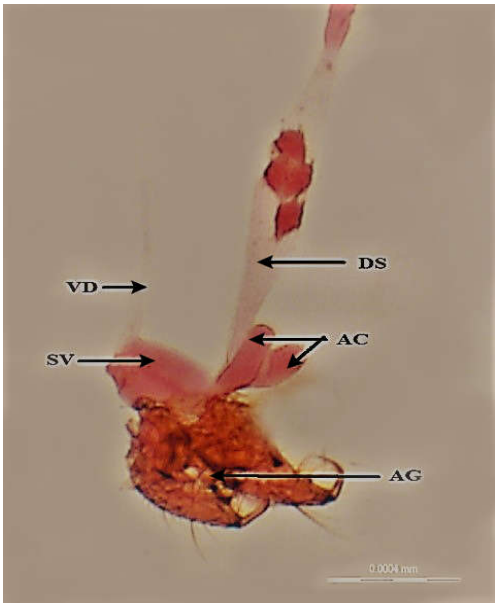


Plate 48 (b)

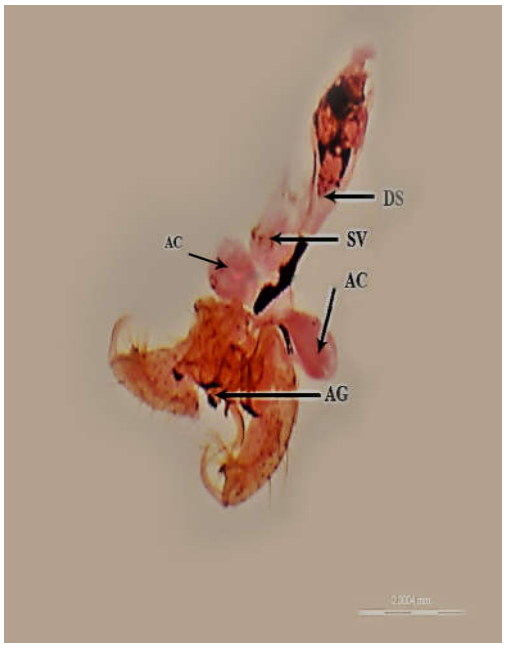


Plate 48 (c)

Plates 48- a, b & c- Male reproductive systems with degenerated testes and disintegrated vas deference.

(T- testes, VD- Vas Deference, SV- Seminal Vesicle, AC- Accessory Glands, AG- Aedeges, ED- Ejaculatory Duct, DS- Digestive System)

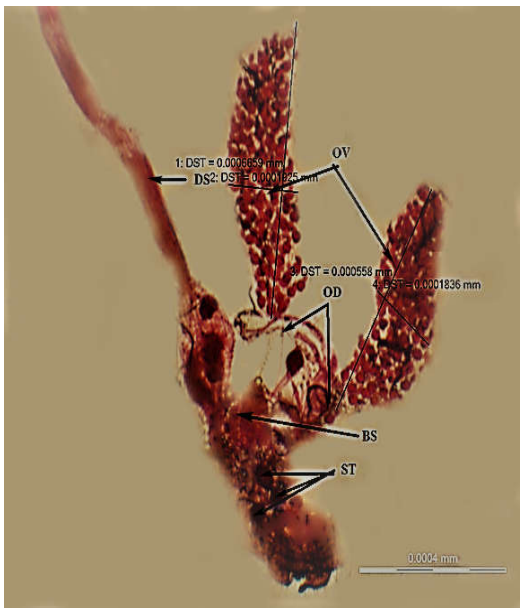


Plate 49- Normal mature female reproductive system

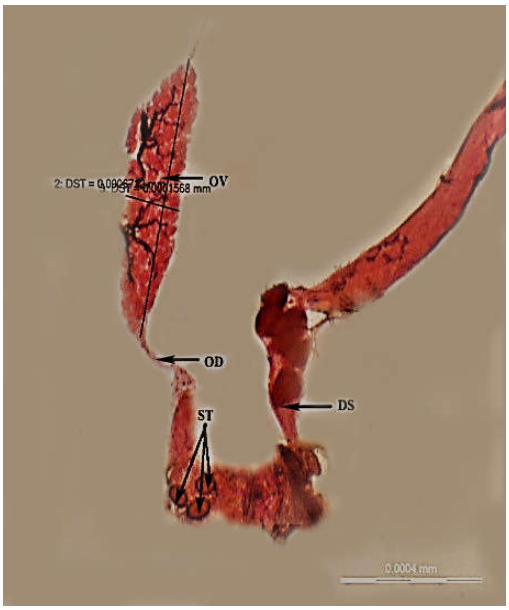


Plate 50 (a)

Plate 50 (b)

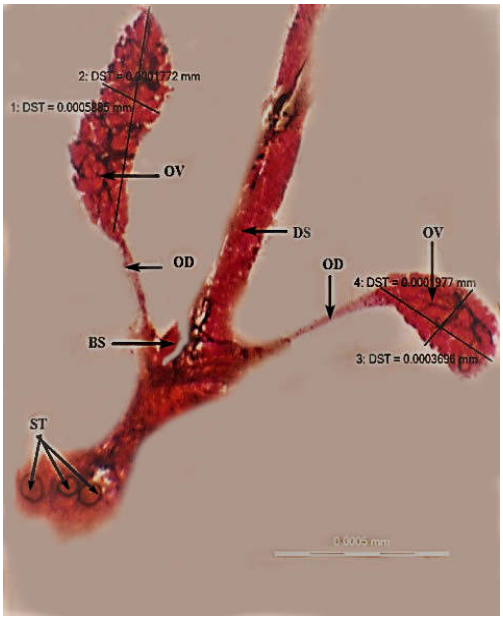


Plate 50 (c)

Plate 50- a, b&c- Female reproductive system with degenerated ovaries, ovarioles and atrophied ovaries varied in length and size with disintegrated ovarioles.

(OV- Ovaries, OD- Ovi Duct, OVL- Ovariole, ST- Spermatheca, BS- Bursa Inseminalis, DS- Digestive System)

The reproductive toxicity of *P. longifolia* **(MeOH: EA- 4:1)** to adults of *Cx. quinquefasciatus* was exhibited as malfunctioning of testes and ovaries, mainly degeneration of testes **(Plates 52- a & b)** and ovaries, disintegration of ovaries **(Plates 54- a & b),** variation in the number of ovarioles and variation in the sizes of ovaries compared with control **(Table 30)** compared with normal mature male **(Plate 51)** and female **(Plate 53)** reproductive system.

Morphogenetic changes of the reproductive organs of the adults of *Cx. quinquefasciatus* after the exposure of freshly hatched I instar larvae to *P. longifolia* (MeOH: EA- 4:1) at half of the median lethal dose (36. 300ppm)

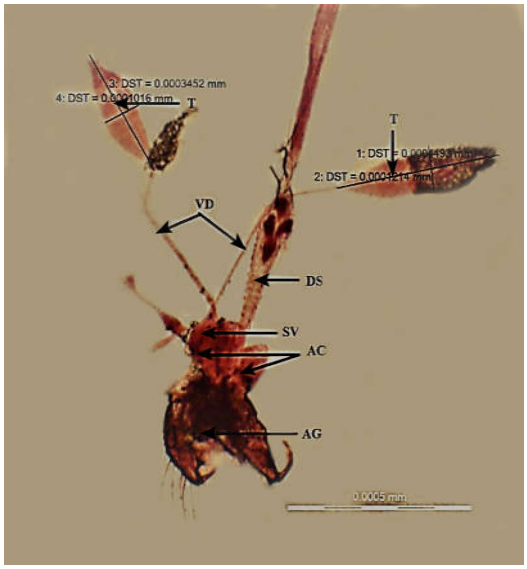


Plate 51- Normal mature male reproductive system

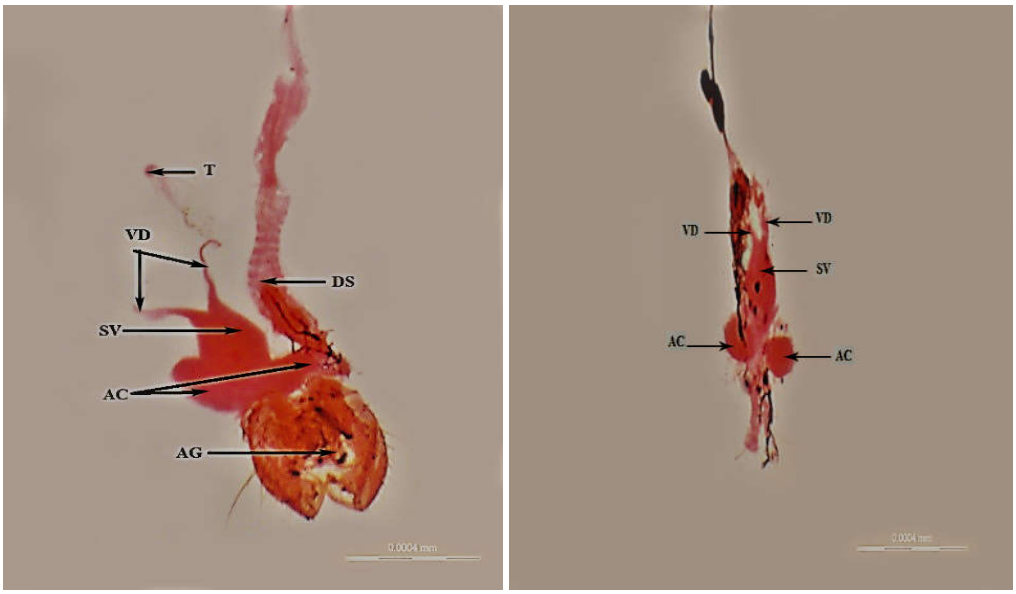


Plate 52 (a) **Plate 52 (b)**

Plates 52- a& b)- Male reproductive systems with degenerated testes and disintegrate vas deference

(T- testes, VD- Vas Deference, SV- Seminal Vesicle, AC- Accessory Glands, AG- Aedeges, ED- Ejaculatory Duct, DS- Digestive System)

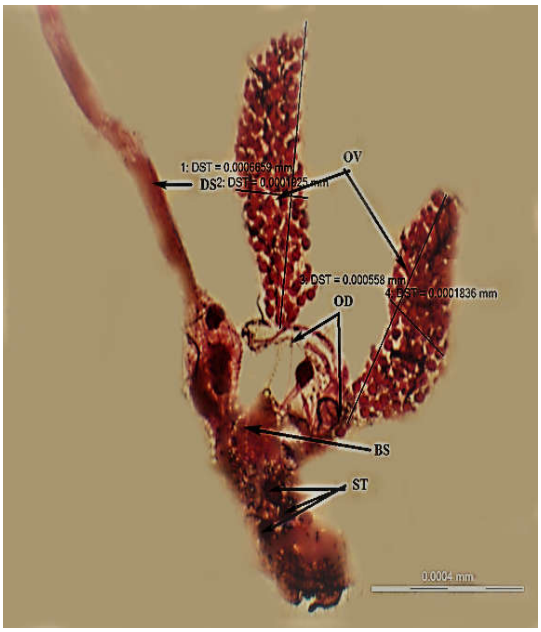


Plate 53- Normal mature female reproductive system

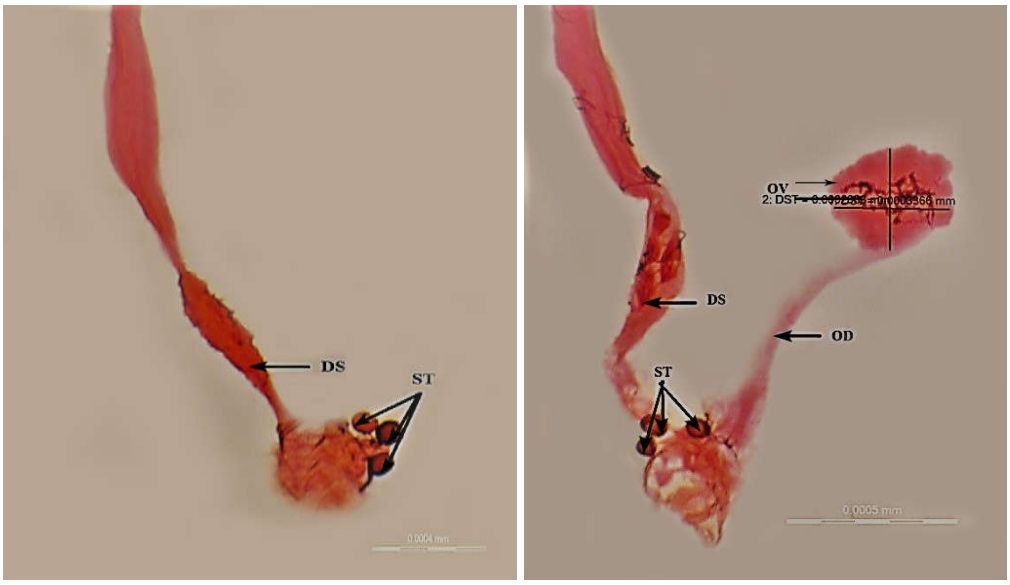


Plate 54 (a) **Plate 54 (b)**

Plate 54- a &b- Female reproductive system with degenerated ovaries, ovarioles and atrophied ovaries varied in length and size with disintegrated ovarioles.

(OV- Ovaries, OD- Ovi Duct, OVL- Ovariole, ST- Spermatheca, BS- Bursa Inseminalis, DS- Digestive System)

Table 41compares the sizes of normal mature ovaries with treated ovaries. The average length and breadth of a normal ovary was 0.0009521±0.0003100mm and 0.0002587±0.0005974mmrespectively, while that of a *S. guttata* (MeOH: EA- 4:1) treated ovary exhibited 0.0002775±0.0000347mm length and 0.0001206± 0.0000383mm breadth. It also showed variation in number of ovarioles such as 106.70± 4.62 for normal mature ovaries and 24.40±4.90 for treated ovaries. Whereas, *A. paniculata* (MeOH: EA- 4:1) treated ovaries were found to be 0.0001658±0.0000321mm length and 0.0000150±0.0000521mmbreadth respectively and showed 37.49± 3.67 number of ovarioles. Treated ovaries of *B. spectabilis* (H: EA- 5:5) and *P. longifolia* (MeOH: EA- 4:1) exhibited 0.0000102± 0.00004761mm, 0.0000652± 0.0000158mm length and 0.000100± 0.0000419mm and 0.0000213± 0.0000165mm breadth respectively. The number of ovarioles was also varied respectively as 28.67±2.84 and 39.85±5.67.

Table 30-Post-treatment effect on female reproductive organs of adult *Cx. quinquefasciatus* after the exposure of selected plant extracts on freshly hatched I instars larvae at half of the median lethal dose concentrations

SI No.	Name of plants	Normal mature ovaries		Treated ovaries		No. of ovarioles	
		Length(mm)	Breadth(mm)	Length (mm)	Breadth(mm)	Treated	Normal
1	*S. guttata* MeOH: EA- 4:1	0.0008653± 0.00001674	0.0002154± 0.0001520	0.0002775± 0.0000347	0.0001206± 0.0000383	24.40±4.90*	91.90± 2.33
2	*A. paniculata* MeOH: EA- 4:1	0.0007604± 0.0001243	0.0002654± 0.0001402	0.0001658± 0.0000321	0.0000150± 0.0000021	37.49±3.67*	106.70±4.62
3	*B. spectabilis* H: EA- 5:5	0.0007985± 0.0004621	0.0001986± 0.0000216	0.0000102± 0.00000761	0.000100± 0.000041	28.67±2.84*	91.37± 4.21
4	*P. longifolia* MeOH: EA- 4:1	0.0009521± 0.0003100	0.0002587± 0.0001974	0.0000652± 0.0000158	0.0000213± 0.0000165	39.85±5.67*	89.78± 3.65

Note: 10 numbers of treated females and males were taken in each of the 3 replicates.

* Significant at P< 0.0001

The extracts of *S. guttata* (MeOH: EA- 4:1), *A. paniculata* ((MeOH: EA- 4:1), *B. spectabilis* (H: EA- 5:5) and *P. longifolia* (MeOH: EA- 4:1) were showed considerable difference in the length and breadth of treated testes when compared with that of control. The average length of *S. guttata* (MeOH: EA- 4:1) treated testes exhibited 0.0006206± 0.0000194mm length and 0.0001285± 0.0000067mm breadth compared with control 0.0009690± 0.0004537mm length and 0.0002101±0.0004312mm breadth.Similarly,*A. paniculata*, *B. spectabilis* and *P. longifolia* were also found to be 0.0002025±0.0000426mm, 0.0000113± 0.0000325mm, 0.0000170± 0.0002790mm length and 00.0000706± 0.0000196mm, 0.0000101± 0.0000325mm and 0.0000112± 0.00002467mm breadth respectively **(Table 31)**.

Table 31- Post- treatment effect on male reproductive organs of adult *Cx. quinquefasciatus* after the exposure of selected plant extracts on freshly hatched I instars larvae at half of the median lethal dose concentrations

SI No.	Name of plants	Normal mature testes		Treated testes	
		Length (mm)	Breadth (mm)	Length (mm)	Breadth (mm)
1	*S. Guttata* MeOH: EA- 4:1	0.0009245± 0.0004533	0.0001697± 0.0000108	0.0006206± 0.0000194	0.0001285± 0.0000067
2	*A. paniculata* MeOH: EA- 4:1	0.0008765± 0.0002163	0.0001321± 0.0000261	0.0002025± 0.0000426	0.0000706± 0.0000196
3	*B. Spectabilis* H: EA- 5:5	0.0009542± 0.0003165	0.0002101± 0.0001312	0.0000113± 0.0000325	0.0000101± 0.0000325
4	*P. longifolia* MeOH: EA- 4:1	0.0009690± 0.0004537	0.0001980± 0.0000534	0.0000170± 0.0000090	0.0000112± 0.0000046

2.4.7 Fecundity, Fertility and mating competitiveness of a synthetic chemosterilant Hexamethylphosphoramide (HMPA)

i. Effect of HMPA on percentage mortality of I instar larvae of *Culex quinquefasciatus*.

Larval mortality was observed after the treatment of HMPA was found to be 100% at 100ppm concentration **(Table 32)**. On the basis of larval toxicity, LC_{50} value expressed as 36.963ppm, it was highly significant when compared with control, because there was no mortality observed on control experiment **(Table 33)**.

Table 32- Percentage mortality of HMPA against I instar larvae of *Culex quinquefasciatus.*

SI No.	Concentration (ppm)	Mortality (%)	Corrected %	P-Value
1	1.00	0.00+ 0.00	0.00	0.0811*
2	5.00	13.33 ± 3.33	13.33	
3	10.00	33.33 ± 3.33	33.33	
4	50.00	60.00 ± 5.77	60.00	
5	100.00	100.00± 0.00	100.00	

Note: The values are expressed as mean ± SE for 10 animals (n=10) per group

* Statistically Significant at $P < 0.05$ with Control experiment.

Table 33-Probit analysis of larvicidal efficacies of HMPA against I instar larvae of *Culex quinquefasciatus.*

SI No	24 hrs LC_{50} (LC_{90})	Lower Fiducial Limit (LFL)	Upper Fiducial Limit (LFL)	X^2	Regression	Significance
1.	36.963 (73.569)	12.884 (45.077)	131.279(371.435)	33.714	y = 0.886x +13.90 R^2 = 0.892	0.000*

* Statistically Significant at P < 0.05

Pearson's Correlation analysis reflected positive correlation between concentrations and percentage larval mortality has been presented on **Figure 11**. Multiple regression analysis of concentration over percentage larval mortality also exhibited the Coefficient of determination (R^2) as 89.2% explained by the independent concentration.

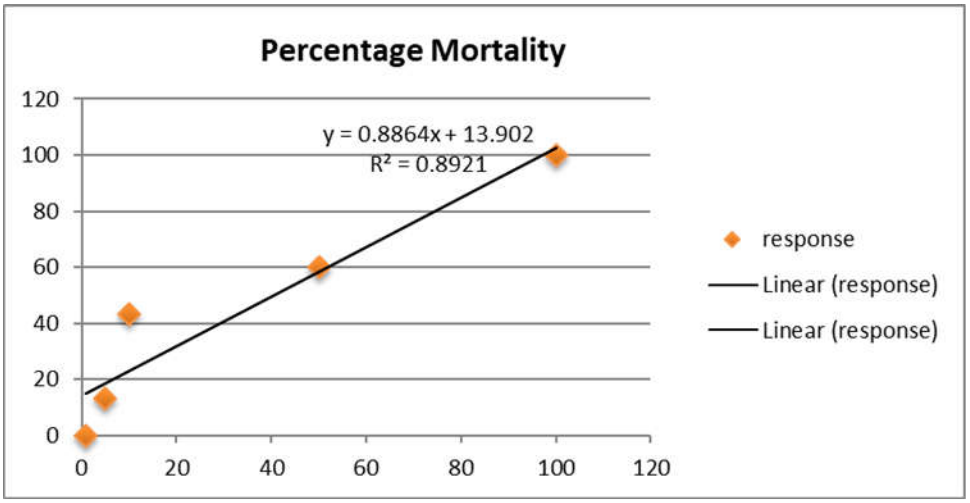


Figure 11- Correlation and Regression analysis of Percentage mortality and Percentage emergence of HMPA against the first instar larvae of *Culex quinquefasciatus.*

ii. Effect of HMPA on Maximal Effective Concentration of I instar larvae of *Culex quinquefasciatus.*

The sub lethal dose-response bioassay of HMPA exhibited 80.00% adult emergence with EC_{50} value 7.371ppm **(Tables 34 & 35).**

Table 34- Percentage emergence of HMPA against the first instar larvae of *Culex quinquefasciatus.*

SI No.	Concentration (ppm)	Emergence (%)	Corrected %	P-Value
1	1.0	80.00 ± 0.00	20.00	0.0065*
2	5.0	43.33 ± 0.33	56.67	
3	10.0	30.00 ± 0.00	70.00	
4	20.0	10.00 ± 0.00	90.00	
5	30.0	0.00 ± 0.00	100.00	

Note: The values are expressed as mean ± SE for 10 animals (n=10) per group

* Statistically Significant at $P < 0.05$ with Control experiment.

Table 35-Probit analysis of the efficient concentration of HMPA against the I instar larvae of *Culex quinquefasciatus*.

SI No	24 hrs LC_{50} (LC_{90})	Lower Fiducial Limit (LFL)	Upper Fiducial Limit (LFL)	X^2	Regression	Significance
1.	7.371 (-4.185)	6.102 (-7.166)	8.587 (-2.006)	4.148	y = -2.655x + 71.71 R^2 = 0.926	0.000*

* Statistically Significant at $P < 0.05$

The Coefficientof determination (R^2) showed 92.6% adult emergence and the relationship between concentration and adult emergence was positive **(Figure 12)**.

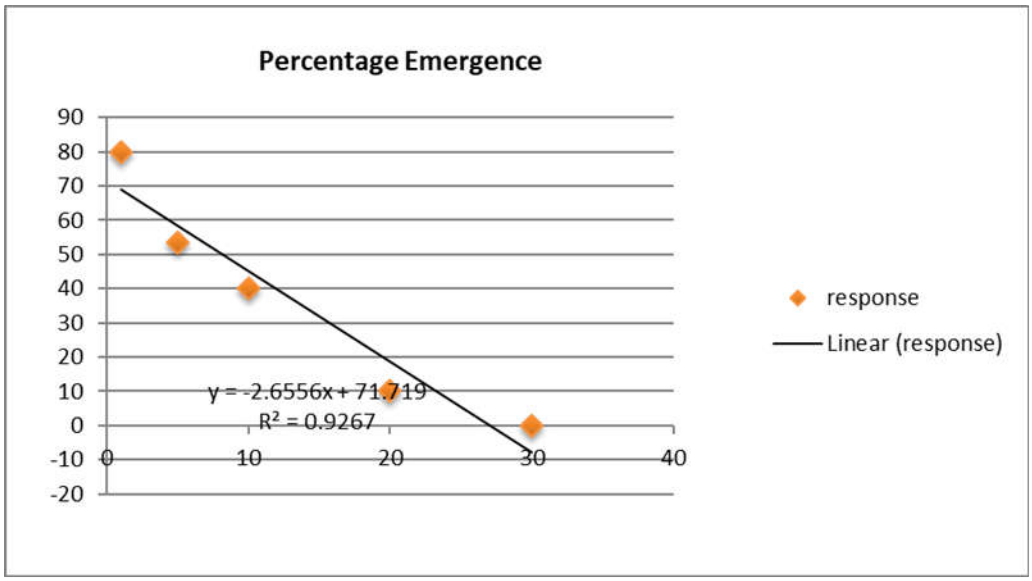


Figure 12- Correlation and Regression analysis of Percentage emergence of first instar larvae of *Culex quinquefasciatus* on HMPA.

iii. Effect of HMPA on developmental duration of *Cx. quinquefasciatus.*

During developmental progress, the larval and pupal periods were observed and compared with that of control. In the case of HMPA treated on freshly hatched I instar larvae exhibited 21.33±0.33 days larval period extension and 2.67±0.33dayshave been taken for pupal periods. Total developmental period extended up to 24.67±0.33days **(Table 36)** when compared with control as 10.67±0.33 days forl arval periods, 2.67±0.00days for pupal periods and 13.67±0.33days for total developmental periods.

Table 36- Extension of total developmental duration

Sl No.	Conc. (ppm)	Extension of larval duration (days)				Extension of Pupal duration (days)	Total developmental duration (days)
		I instar	II instar	III instar	IV instar		
1.	3.685	5.33± 0.33	6.00±0.00	4.33± 0.33	5.67± 0.33	2.67±0.33	24.67± 0.33
	Control	2.67± 0.33	3.00± 0.00	2.33± 0.33	2.67± 0.00	3.00± 0.00	13.67±0.33

iv. Effect of HMPA on growth and metamorphosis of *Cx. quinquefasciatus.*

The activity of HMPA at 3.685ppm concentration exerted 57.50%larval mortality, 13.25% pupal mortality and 29.25% adult emergence **(Table 37)** whereas, in control larval mortality was observed as 8.00%, 1.34% pupal mortality and 90.66% adult emergence. There was a remarkable difference between the Growth index (GI) associated with HMPA and control such as 1.19 and 7.28 respectively.

Table 37- Effect of HMPA on growth and metamorphosis of *Cx. quinquefasciatus.*

SI No.	Conc. (ppm)	Larval mortality (Mean±SE)	Pupal mortality (Mean± SE)	Adult mortality (Mean±SE)	% adult emergence (a)	Total developmental periods (days) (b)	Growth Index (GI) (a/b)
1	3.685	28.75± 1.15 (57.50%)	6.62± 0.67 (13.25%)	0.00±0.00 (0.00%)	14.62± 1.45 (29.25%)	24.67± 0.33	1.19
	Control	4.00± 1.15 (8.00 %)	0.67± 0.33 (1.34 %)	0.00+ 0.00 (0.00%)	45.33±0.67 (90.66 %)	13.00± 0.58	7.28

Note: Number of individuals per sample= 50

v. Morphogenetic deformities associated with exposure of *Cx. quinquefasciatus* larvae to HMPA.

The metamorphic abnormalities observed on the treatment of HMPA on *Cx. quinquefasciatus* exhibited 32.00% larval- larval intermediates, 9.34% larval-pupal intermediates and 7.34% pupal- adult intermediates **(Table 38)**. In the case of larval- larval intermediates and larval- pupal intermediates, death has occurred at an early stage of development. In larval- pupal intermediates, the abdomen retracted to halfway along the larval abdominal skin and adopted the pupal characteristics, (**Plates 55 & 56)**. Whereas, in pupal- adult intermediates, death occurred after complete molting from pupal skin but some parts remained attached to pupal exuviae (**Plates 57 & 58**).

Table 38 -Data on Morphological deformities of mosquito larvae exposed to different concentrations of the chemosterilant HMPA.

SI No.	Conc. (ppm)	Larval- larval intermediate (Mean±SE)	Larval- pupal intermediate (Mean±SE)	Pupal-adult intermediate (Mean± SE)
1	3.685	16.00 ± 1.15 (32.00%)	4.67 ± 0.67 (9.34%)	3.67 ± 0.33 (7.34%)
5	Control	0.00± 0.00 (0.00%)	2.33± 0.33 (4.66%)	0.00± 0.00 (0.00%)

Morphogenetic deformities associated with the exposure at half of the median lethal dose (3.685ppm) of chemosterilant HMPA on freshly hatched I instars larvae of *Cx. quinquefasciatus.*

Plate 55- Larval- pupal intermediate

Plate 56- Larval- pupal intermediate

(Abdomen retracted to halfway along the larval abdominal skin and adopted the pupal characteristics)

Plate 57- Pupal- adult intermediate Plate 58- Pupal- adult intermediate (Complete moult of the adult, but remain attached with pupal exuvia)

vi. Effect of exposure to HMPA on oviposition, egg hatchability and control of reproduction of *Cx. quinquefasciatus*

Treatment with sub lethal concentration of HMPA exhibited considerable effects on gonotropic cycles, oviposition, percentage of hatchability and control of reproduction on the treated groups **T ♂ x T ♀, T ♂ x UT ♀, T ♀ x UT♂, UT ♂ x UT ♀**of *Cx. quinquefasciatus* **(Table 39)**. It was observed that the gonotropic cycles of **T ♂ x T ♀, T ♂ x UT ♀, T ♀ x UT♂, UT ♂ x UT ♀** showed 9.33± 0.33, 6.33±0.33, 5.33± 0.33 and 2.67± 0.33 days respectively, The number of egg rafts laid by gravid female of each treated groups showed1.33± 0.33, 1.33± 0.33 and 2.67± 0.33 respectively for **T ♂ x T ♀, T ♂ x UT ♀ & T ♀ x UT♂,** which was lower when compared with control **UT ♂ x UT ♀** as 7.67± 0.33. The number of eggs obtained after oviposition were counted as 74.50± 11.50, 68.67± 6.49 and 98.33± 13.54 for treated groups **T ♂ x T ♀, T ♂ x UT ♀, T ♀ x UT♂,** and for **UT ♂ x UT ♀** it was 1654.00±0.00**.** Oviposition Activity Index (OAI) of each treated groups **T ♂ x T ♀, T ♂ x UT ♀, T ♀ x UT♂, T ♂ x T ♀,** and **UT ♂ x UT ♀** groups exhibited -0.91, -0.92, -0.88 and 0.00 respectively. Percentage egg hatchability was calculated as 58.04%, 53.00%, 49.63% and 83.97% for respective groups **T ♂ x T ♀, T ♂ x UT ♀, T ♀ x UT♂, T ♂ x T ♀,** and **UT ♂ x UT ♀.** Percentage control of reproduction was also noted as 97.84%, 98.31%, 96.76% and 00.00% respectively for **T ♂ x T ♀, T ♂ x UT ♀, T ♀ x UT♂, T ♂ x T ♀,** and **UT ♂ x UT ♀.**

Table 39- Effect of exposure to HMPA on oviposition, gonotropic cycles, egg hatchability and control of reproduction of adults of *Cx. quinquefasciatus* at half of the median lethal dose (3.685ppm)

SI No.	Treated groups	Gonotropic cycle (days)	No. of egg rafts laid	Average no. of eggs obtained	Oviposition Activity Index (OAI)	No. of days taken for egg hatching	% of hatch	% of Control of reproduction
1	a. (T ♂ x T ♀)	9.33± 0.33	1.33± 0.33	74.50± 11.50	-0.91	3.33± 0.33	58.04	97.84
2	b. (T ♂ x UT ♀)	6.33±0.33	1.33± 0.33	68.67± 6.49	-0.92	3.67± 0.33	53.00	98.31
3	c. (T ♀ x UT♂)	5.33± 0.33	2.67± 0.33	98.33± 13.54	-0.88	3.33± 0.33	49.63	96.76
4	d. (UT ♂ x UT ♀)	2.67± 0.33	7.67± 0.33	1654.00±0.00	0.00	2.00± 0.00	83.97	00.00

Note: 10 numbers of treated females and males were taken in each of the 3 replicates.

vii. Fecundity and fertility effects of exposure to HMPA on the adults of *Cx. quinquefasciatus*fromtreated larvae.

HMPA on *Cx. quinquefasciatus* resulted in significant loss in the fertility and fecundity rate as well as on Sterility Index also (SI) **(Table 40)**. It was observed that treated groups **T ♂ x T ♀, T ♂ x UT ♀, T ♀ x UT♂** exhibited4.5%, 4.2% and 5.9% and untreated **UT ♂ x UT ♀** as 100.00% fertility percentage. The fecundity rate was also reduced as 7.45, 6.86 and 9.83 for treated groups **T ♂ x T ♀, T ♂ x UT ♀, T ♀ x UT♂** and 165.4for untreated group **UT ♂ x UT ♀.** Sterility Index resulted for treated groups as higher when compared with untreated groups, such as 97.83, 98.32 and 96.77 for **T ♂ x T ♀, T ♂ x UT ♀, T ♀ x UT♂** and 0.00 for **UT ♂ x UT ♀.**

Table 40- Fecundity and fertility effects of HMPA on the adults of *Cx. quinquefasciatus* from treated larvae at half of the median lethal dose (3.685ppm)

SI NO.	Treated Groups	Fecundity rate	Fertility (%)	Sterility Index (SI)
1	a. (T ♂ x T ♀)	7.45	4.5	97.83
	b. (T ♂ x UT ♀)	6.86	4.5	98.32
	c. (T ♀ x UT♂)	9.83	5.9	96.77
	d. (UT ♂ x UT ♀)	165.4	100.00	0.00

Note: 10 numbers of treated females and males were taken in each of the 3 replicates.

viii. Morphogenetic changes of the reproductive organs of the adults of *Cx. quinquefasciatus* after the exposure of freshly hatched I instar larvae to HMPA

The dissections of both treated and untreated adult males and females of *Cx. quinquefasciatus* revealed significant changes in the reproductive organs. **Plates 60 a & b and 62- a & b** showed various ovarian and testicular abnormalities on treated individuals, whereas, normal development was obtained in untreated adults. Ovarian abnormalities mainly consisted of undeveloped ovaries, variation in length and sizes of ovaries, variation in number of ovarioles, disintegration of ovarioles, and ovariole degeneration **(Plates 62- a & b)**, whereas, the testicular abnormalities were observed as formation of poorly developed testes, variation in length and size of testes and disintegration of testes and vas deference **(Plates 60- a & b)** compared with normal mature male **(Plate 59)** and female **(Plate 61)** reproductive system.

Morphogenetic deformities associated with the reproductive organs of the adults of *Cx. quinquefasciatus* after the exposure of freshly hatched I instar larvae to HMPA at half of the median lethal dose (3.685ppm)

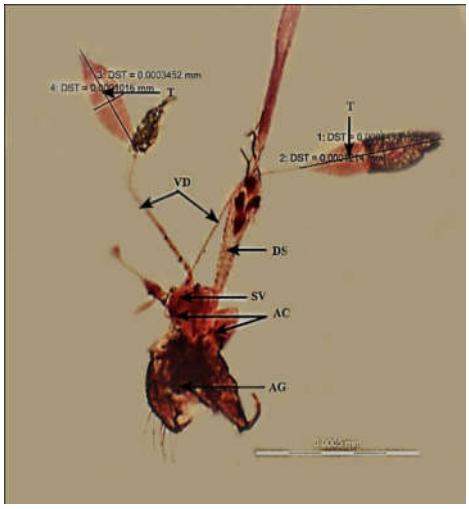


Plate 59- Normal mature female reproductive system

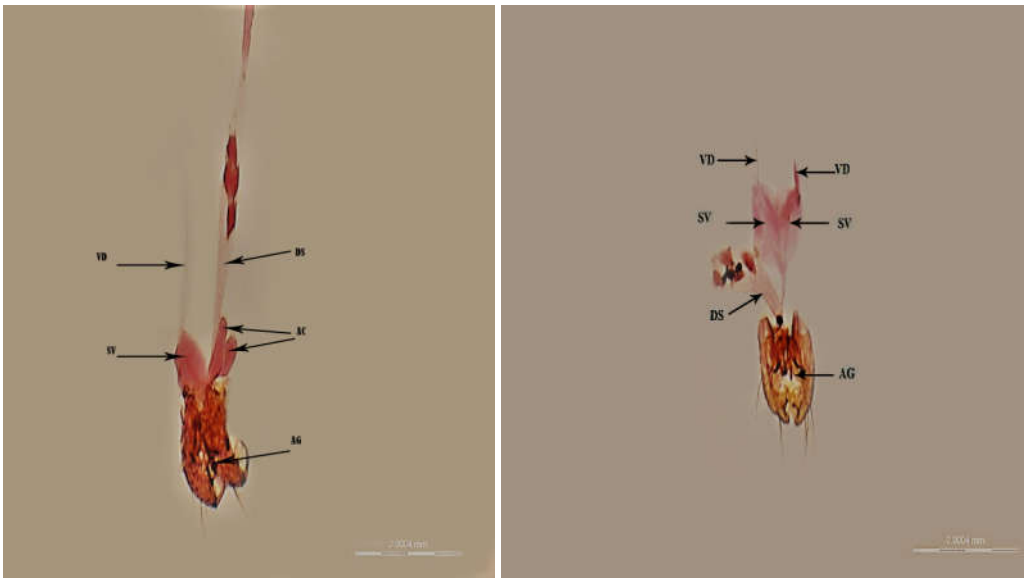


Plate 60 (a) **Plate 60 (b)**

Plates 50 & 51- Male reproductive systems with atrophied testes displayed varied in length and size of the testes and degenerated testes.

(T- testes, VD- Vas Deference, SV- Seminal Vesicle, AC- Accessory Glands, AG- Aedeges, ED- Ejaculatory Duct, DS- Digestive System)

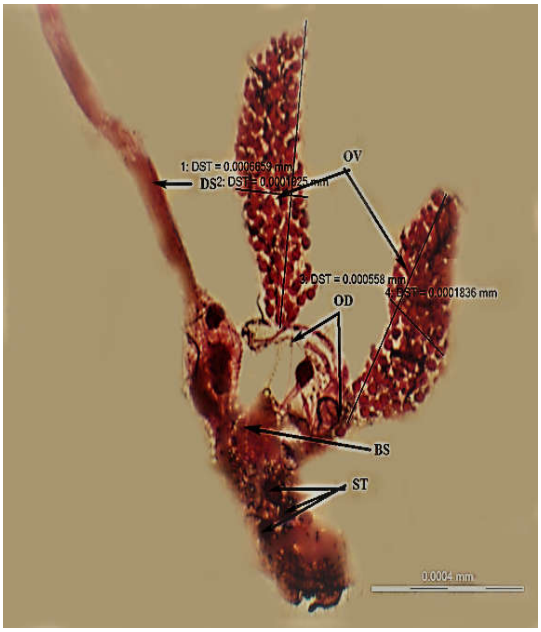


Plate 61- Normal mature female reproductive system

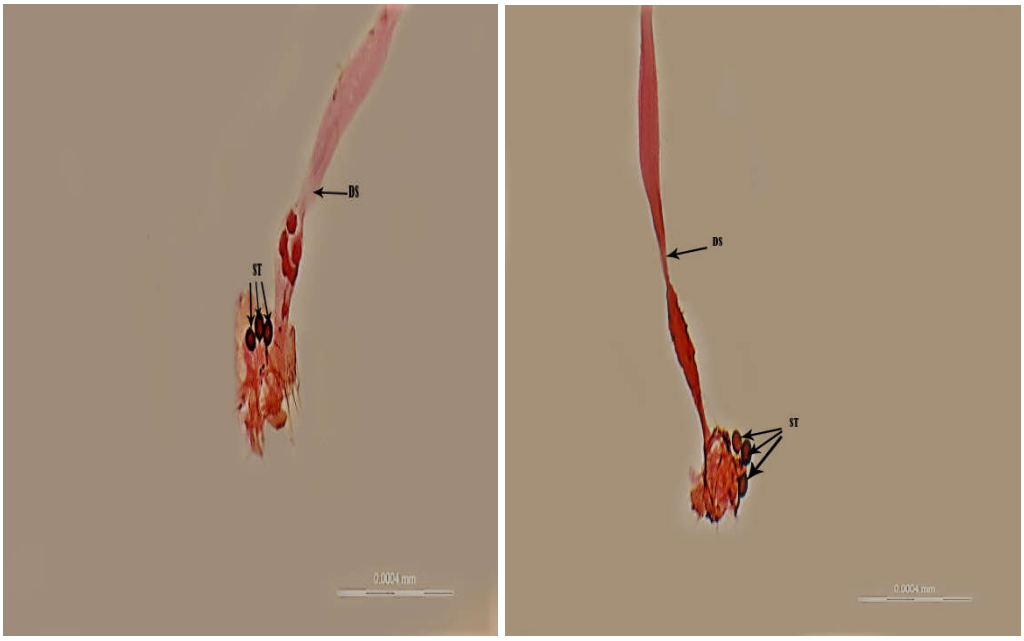


Plate 62 (a) **Plate62 (b)**

Plates 62- a & b- Female reproductive systems with degenerated ovaries, oviducts and ovarioles with degenerated Bursa Inseminalis and with undeveloped/ atrophied ovaries.

(OV- Ovaries, OD- Ovi Duct, OVL- Ovariole, ST- Spermatheca, BS- Bursa Inseminalis, DS- Digestive System)

Treated ovaries showed 0.0000112±0.00002341mm length and 0.0000203± 0.0000124mm breadth, compared with normal ovaries such as 0.0009730±0.0002200mm length and 0.0002465±0.00053742mm breadth **(Table 41)**. The number of ovarioles also varied as 35.82±3.64 and 128.70±3.42 respectively for treated and untreated ovaries. Similarly, testes also showed variation in length and breadth as 0.0004106± 0.0000165mm and 0.0001285±0.0000067mm respectively with control 0.0009329± 0.0003341mm length and 0.0002101±0.0004312mm breadth.

Table 41- Post- treatment effect on reproductive organs of adult *Cx. quinquefasciatus* after the exposure of HMPA on freshly hatched I instars larvae at half of the median lethal dose (6.419ppm)

Sl No.	Normal mature ovary		Treated ovary		No. of ovarioles		Normal mature testes		Treated testes	
	Length (mm)	Breadth (mm)	Length (mm)	Breadth (mm)	Treated	Control	Length (mm)	Breadth (mm)	Length (mm)	Breadth (mm)
1	0.0009730±0.0002200	0.0002465± 0.00013742	0.0000112± 0.00001341	0.0000203± 0.0000124	35.82 ± 3.64	128.70± 3.42	0.0009329±0.0003341	0.0002101±0.0001312	0.0004106± 0.0000165	0.0001285± 0.0000067

Note: 10 numbers of treated females and males were taken.

2.4.9 Comparative assessment of sterility induction by plant-based bio-active compounds versus synthetic chemosterilant (HMPA) with respect to control

When compared with the Growth Index (GI) and Sterility Index (SI) of all the selected plant extracts and HMPA exhibited highly significant effects (P< 0.000) with respect to control. HMPA showed, above 95% sterility in all type of crosses, but the plant extracts *A. paniculata* (MeOH: EA- 4:1), *B. spectabilis* (H: EA- 5:5), *P. longifolia* (MeOH: EA- 4:1) exhibited above 90% sterility only in **T ♂ x UT ♀** and *S. guttata* (MeOH: EA- 4:1) with 91.61% in **T ♀ x UT♂,** whereas, the control showed 0.00% sterility. Fertility percentage was also lowered when compared to control. *S. guttata* (MeOH: EA- 4:1), *A. paniculata* (MeOH: EA- 4:1), *B. spectabilis* (H: EA- 5:5) and *P. longifolia* (MeOH: EA- 4:1) exhibited lowest fertility percentage 11.52% for **T ♀ x UT♂** groups of *S. guttata***,** whereas, **T ♂ x UT ♀** groups of *A. paniculata* (MeOH: EA- 4:1), *B. spectabilis* (H: EA- 5:5) and *P. longifolia* (MeOH: EA- 4:1) showed 4.08%, 5.97% and 14.86% respectively, which was considerably lower than the untreated groups **UT ♂ x UT ♀** as 100.00%. Similarly, the fertility percentage of HMPA was found to be lowest as 4.5%, 4.5% and 5.9% for all the treated groups **T ♂ x T ♀, T ♂ x UT ♀, T ♀ x UT♂ (Table 42)**.

Table 42- Data on comparison of Growth Index, Fertility percentage and Sterility Index by fractionated column extracts of selected plants versus synthetic chemosterilant (HMPA) with respect to control

SI NO.	Name of the selected compounds	Growth Index (GI)	Fertility (%)	Sterility Index (SI)
1.	*S. guttata* (T ♀ x UT♂) CH3OH : C4H8O2- 4:1	1.78	11.52	91.61
2.	*A. paniculata* (T ♂ x UT ♀) CH3OH : C4H8O2- 4:1	1.97	4.08	97.93
3.	*B. Specatbilis* (T ♂ x UT ♀) C6H12: C4H8O2- 5:5	3.25	5.97	95.31
4.	*P. longifolia* (T ♂ x UT ♀) CH3OH: C4H8O2- 4:1	1.92	14.86	90.91
5.	HMPA	1.19	4.5	95.00
6.	Control	7.28	100.00	0.00

Table 43 showed remarkable significant difference between fractionated column extracts of selected plants as well as the chemosterilant HMPA with respect to control associated with percent mortality, percent emergence, Growth Index (GI) and Sterility Index (SI) among treated adults of *Cx. quinquefasciatus*.

Table 43- Data on significance of Percent mortality, Percent emergence, Growth Index (GI) and Sterility Index (SI) associated with the exposure of fractionated column extracts of selected plants and synthetic chemosterilant (HMPA) with respect to control

SI No.	Name of plants	Percent mortality		Percent emergence		Growth Index (GI)		Sterility Index (SI)	
		F	Sig	F	Sig	F	Sig	F	Sig
1	*S. guttata* CH3OH: C4H8O2- 4:1	5.114	0.025*	10.486	0.002*	164.771	0.005*	190.399	0.000*
2	*A. paniculata* CH3OH: C4H8O2- 4:1	5.610	0.019*	9.489	0.003*	7.354	0.252	82.333	0.000*
3	*B. Specatbilis* C6H12: C4H8O2- 5:5	5.550	0.020*	11.489	0.002*	16.084	0.174	11.660	0.009*
4	*P. longifolia* CH3OH: C4H8O2- 4:1	5.855	0.017*	11.866	0.001*	18.075	0.164	58.732	0.000*

* Statistically Significant at P < 0.05

2.5 DISCUSSION

The environmental safety of an insecticide is considered to be of prominent importance as the insecticides used for public health programmes are selective toxicants with low mammalian toxicity in addition to causing environmental issues. The improper usage of insecticides more or less modifies the environmental conditions and some of the most widely sprayed insecticides are chemically very stable and sustain in the environment for a very long period. Current public concern about some chemicals in vector management causing health and environmental problems, and the requirements of some overseas countries for no chemical residues and has reinforced the need to develop techniques to eliminate or reduce the present level of chemical inputs in vector management. However, most of the products have not been made to test their field efficiency and viability. This asserts the necessity for developing a new cost effective, eco-friendly alternative insecticides to keep the vector population under control.

Although several control measures for mosquito vectors have been practiced, the efforts are futile mainly due to the development of resistance against the synthetic insecticides and also environmental and health hazards. Insect Growth Regulators (IGRs) and Chemosterilants offer considerable potential for the control of insect pests/ vectors (Arivoli and Tennyson, 2011). IGRs have lower mammalian toxicities and do not appear to cause spontaneous genetic mutation and these materials are relatively short lived in the environment which reduces the potential for contamination. So, it is the need to inculcate the newer strategies to incorporating IGRs into vector control programmes. Regarding the use of Insect Growth Regulators (IGRs) as biosterilants, Azadirachtin is the most widely used botanical insect growth regulators (Hoffman et al., 1998). Since IGRs are chemical pesticides, certain JH agonists and Chitin synthesis Inhibitor have the potential to cause damage

if they get into aquatic systems. All these issues lead to the need for an alternative source to produce a newer, natural friendly, cost effective biosterilant to eradicate the vector population.

Despite the toxic effects and reduction in adult emergence, Insect Growth Regulators (IGRs) also exhibited combined effects on the developmental period and adult emergence with extending to the developmental duration of exposed larvae (Kamalakannan *et al*., 2015).

Present study also inculcated the proof of IGR activity exerted by the plant extracts on *Cx. quinquefasciatus* based on the developmental process. During the developmental progress of *Cx. quinquefasciatus* exposed to selected plant extracts exhibited prolonged larval and pupal periods, when compared with control. Larval and pupal period was significantly increased ($P< 0.005$) with the treatment of *S. guttata* (MeOH: EA- 4:1), *A. paniculata* (MeOH: EA- 4:1), *B. spectabilis* (H: EA- 5:5) and *P.longifolia* (MeOH: EA- 4:1) on freshly hatched I instar larvae **(Figure 13)**. Total developmental period was also significantly extended, compared with control. The higher extension of developmental duration was observed in *B. spectabilis* (H: EA- 5:5) as 23.67± 0.33days**,** whereas, normal developmental duration was noted as 13.67±0.33days in control. But *P. longifolia* exhibited only slight variation as 17.00±0.58days compared with control. *S. guttata* and *A. paniculata* also showed significant changes in the larval duration with a total developmental period extended up to 21.33± 0.33days and22.00±0.58days respectively**.** All the selected plants except *P. longifolia* (MeOH: EA- 4:1) exhibited significant extension ($P<0.005$) of developmental duration, compared with control.

The current results are in conformity with other research findings, showed growthregulatory responses in the dengue vector *Aedes albopictu s*Skuse on the treatment of three indigenous plant extracts *Mimusops elengi*, *Pongamia pinnata* and *Erythrina variegata* (Rahana *et al*., 2016). It was observed that

total developmental duration was extended to 39.20±0.73, 49.21±1.11and36.98±1.33days respectively compared with control as 21.00±2.00days. At sublethal doses of 50ppm and 100ppm of crude methanolic extract of *Ageratum conyzoides* on *Anopheles gambiae sensustricto* and *Anopheles arabiensis* greatly affected the development in the immature stages such as prolongation of larval instars, pupal durations, inhibition of larval and pupal molting, morphological abnormalities and mortality especially during molting and melanization processes (Muema *et al*., 2016). According to Kuwano *et al*., (2008) pyriproxifen extended the length of larval developmental period, may be due to the presence of JH in the haemolymph of insect larval stage. Mwangi and Mukiama (1988) recorded an extension of larval periods and inhibition of pupal duration in *An. Arabiensis* treated with *Melia volkensii.*

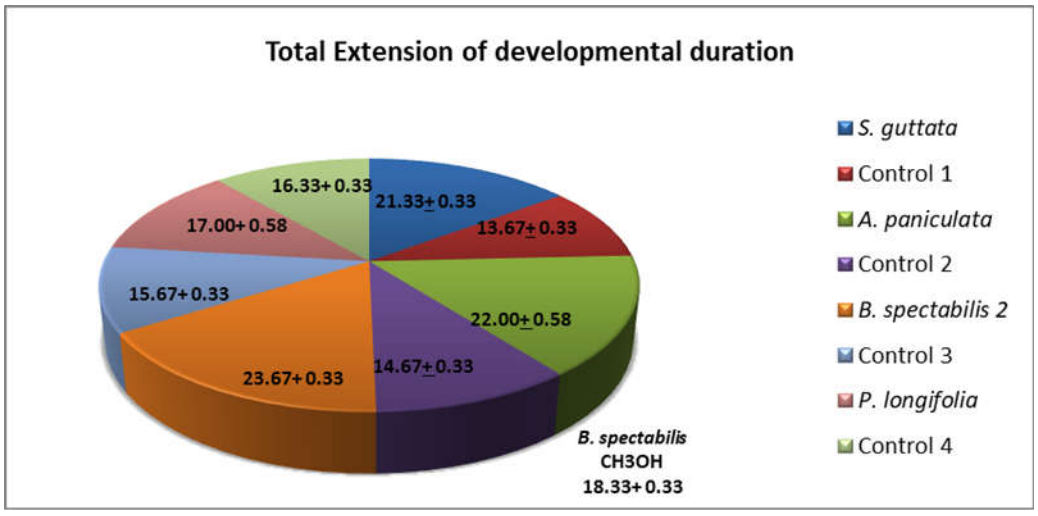


Figure 13- Total extension of developmental duration

A number of synthetic compounds and plant derivatives were found to possess adult emergence inhibition activities against vector mosquitoes. It was observed that *S. guttata (*MeOH: EA- 4:1) exhibited highest larval mortality, compared with control and other plant extracts **(Figure 14)**. *P. longifolia*

(MeOH: EA- 4:1) also showed significantly higher larval mortality during the developmental period. Whereas, in pupal mortality, *A. paniculate* (MeOH: EA- 4:1) exhibited higher pupal mortality **(Figure 15)** compared with control and other selected plants during the developmental periods from freshly hatched I instars to Pupae. However, in the case of adult mortality *A. paniculate* (MeOH: EA- 4:1) showed significantly higher adult mortality compared with *S. guttata* and *P. longifolia*, whereas control and *B. spectabilis* (H: EA- 5:5) exhibited 0.00% adult mortality **(Figure 16)**.

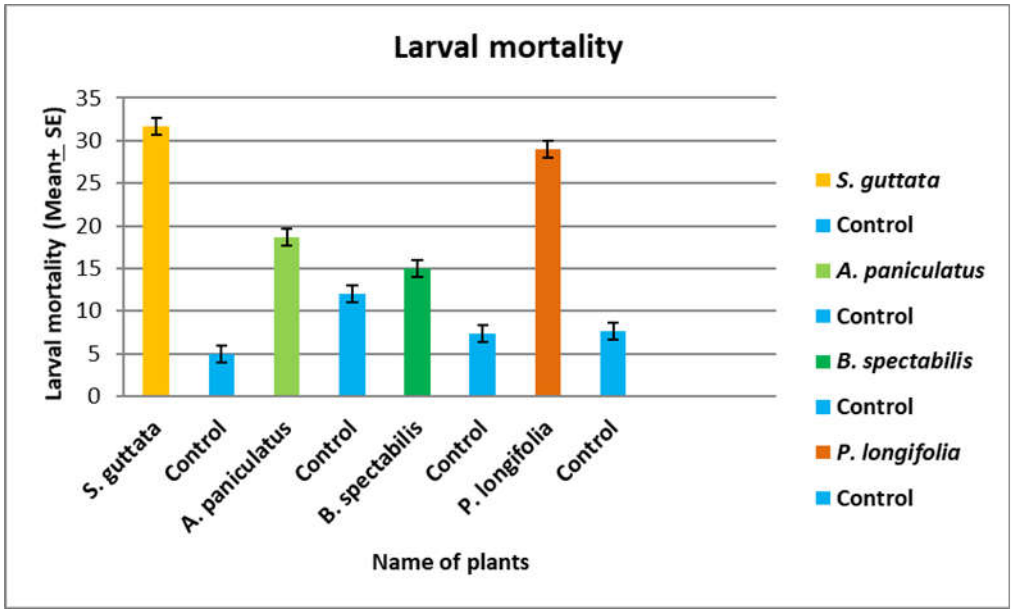


Figure 14- Effect of Column fractions of *S. guttata* (MeOH: EA- 4:1), *A. paniculata* (MeOH: EA- 4:1), *B. spectabilis* (H: EA- 5:5) and *P. longifolia* (MeOH: NEA- 4:1) on the larval mortality of *Cx. quinquefasciatus* during developmental process.

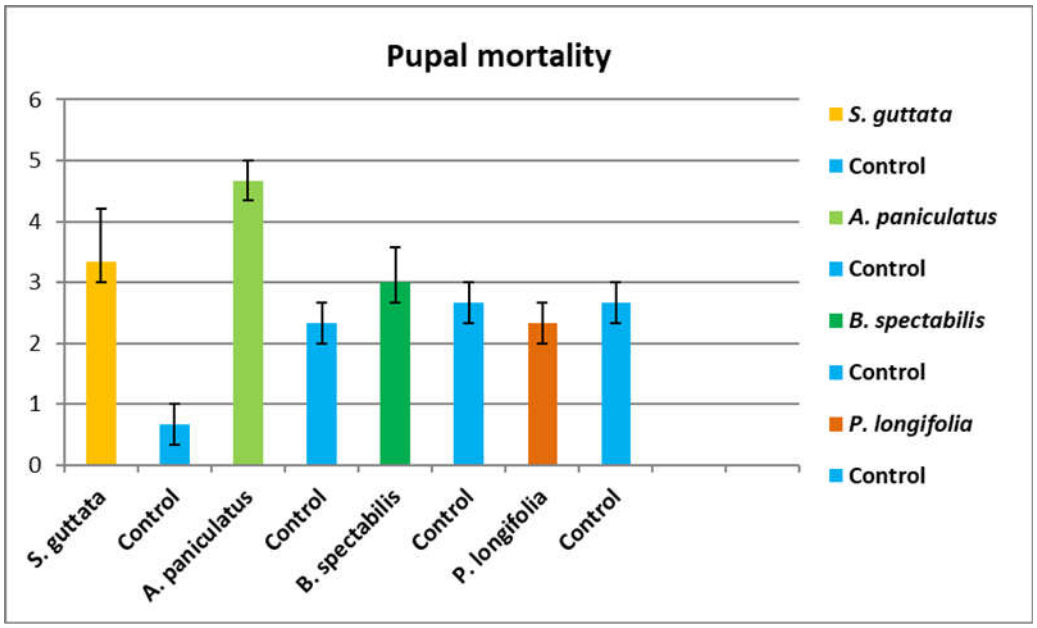


Figure 15- Effect of Column fractions of *S. guttata* (MeOH: EA- 4:1), *A. paniculata* (MeOH: EA- 4:1), *B. spectabilis* (H: EA- 5:5) and *P. longifolia* (MeOH: NEA- 4:1) on the Pupal mortality of *Cx. quinquefasciatus* during developmental process.

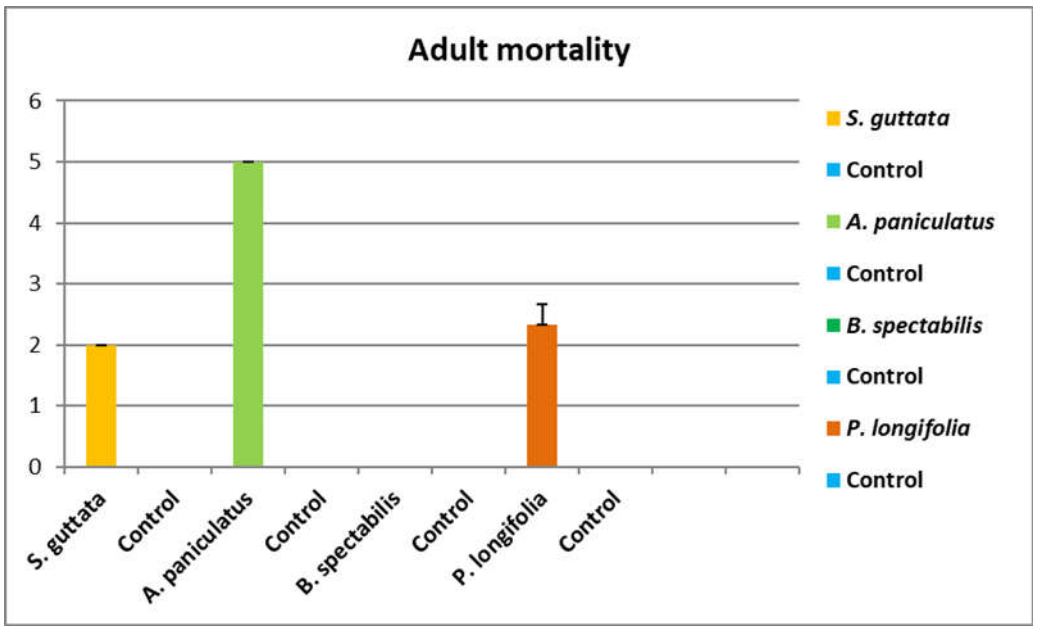


Figure 16- Effect of Column fractions of *S. guttata* (MeOH: EA- 4:1), *A. paniculata* (MeOH: EA- 4:1), *B. spectabilis* (H: EA- 5:5) and *P. longifolia*

(MeOH: NEA- 4:1) on theAdult mortality of *Cx. quinquefasciatus* during developmental process.

The selected plant extracts thus contributed to produce adult emergence inhibition activity on *Cx. quinquefasciatus*. The sub- lethal dose treatments inhibited growth and caused mortality in a dose- dependent manner and also presented growth inhibiting effects on the various developmental stages. The results obtained with the present study enriched the knowledge on the adult emergence inhibition activity of the plants *S. guttata* (MeOH: EA- 4:1), *A. paniculata* (MeOH: EA- 4:1), *B. spectabilis* (H: EA- 5:5) and *P. longifolia* (MeOH: EA- 4:1). **Figure 17** compares the percentage of adult emergence of selected plants with control. Among the four plants selected, ***P. longifolia* (MeOH: EA- 4:1)** exhibited highest percentage of adult emergence inhibition, whereas, ***B. spectabilis* (H: EA- 5:5)** showed lowest percentage of adult emergence inhibition, compared with control. The adult emergence inhibition activities of selected plants were also comparable to different species of plant extracts in different families (Pushpalatha and Muthukrishnan, 1999; Muthukrishnan *et al*., 1999). The crude methanolic extracts of leaves of *Abutilon indicum* found to possessed adult emergence inhibition activity against *Ae. aegypti, An. Stephensi* and *Cx. quinquefasciatus* (Arivoli and Tennyson, 2011). The literature search proved that adult emergence inhibition activity of selected plants *S. guttata, A. paniculata, B. spectabilis* and *P. longifolia*has not been reported before and thus the present results throws light on the adult emergence inhibition activity of these plants tested against the filarial vector *Cx. quinquefasciatus*.

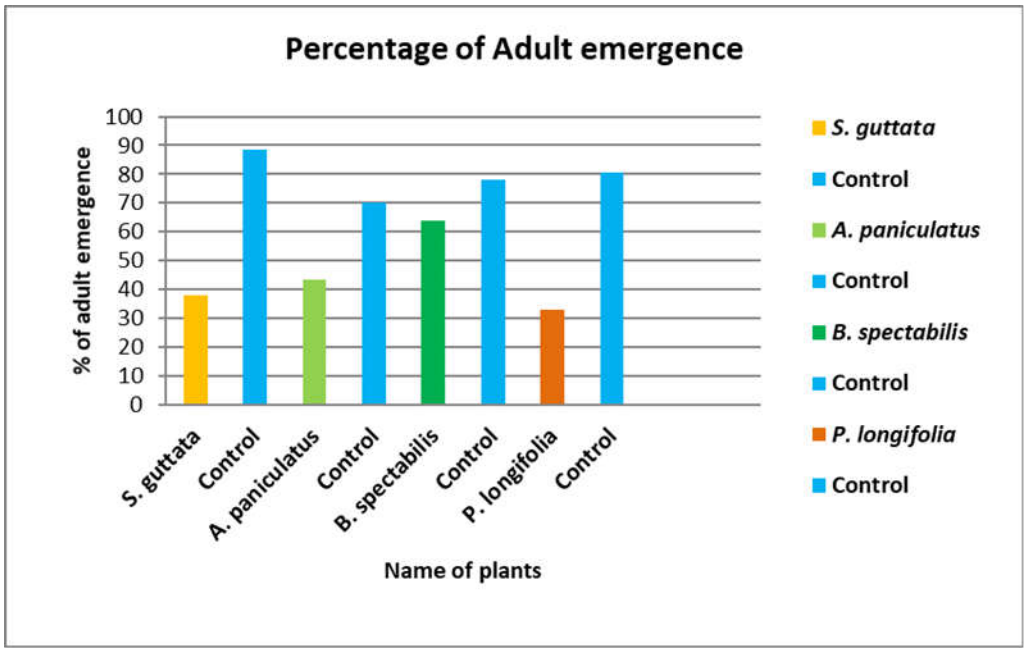


Figure 17- Effect of Column fractions of *S. guttata* (MeOH: EA- 4:1), *A. paniculata* (MeOH: EA- 4:1), *B. spectabilis* (H: EA- 5:5) and *P. longifolia* (MeOH: NEA- 4:1) on adult emergence of *Cx. quinquefasciatus* during developmental process.

The growth index (GI) was considerably reduced at treatment of selected plant extracts on *Cx. quinquefasciatus*, signifying the anti- juvenile effect of the extracts. *S. guttata* (MeOH: EA- 4:1) exhibited lowest Growth Index (1.78), compared with other three selected plants and with control (6.48). *A. paniculata* (MeOH: EA- 4:1) (1.97) and *P. longifolia* (MeOH: EA- 4:1) (1.92) also showed lower Growth Index, compared with control, whereas, *B. spectabilis* (H: EA- 5:5) was found to induce slight variation in the Growth Index (3.25) compared with control (4.97) **(Figure 18)**. Growth Index was significantly reduced on the activity of *Annona squamosa* alkaloids against *An. stephensi* (Saxena *et al*., 1993). Chloroform extract of *Ageratum*

conyzoides on *Cx. quinquefasciatus* also reduced the growth index 1.14 significantly at 250ppm concentration (Arya and Sahai, 2014).

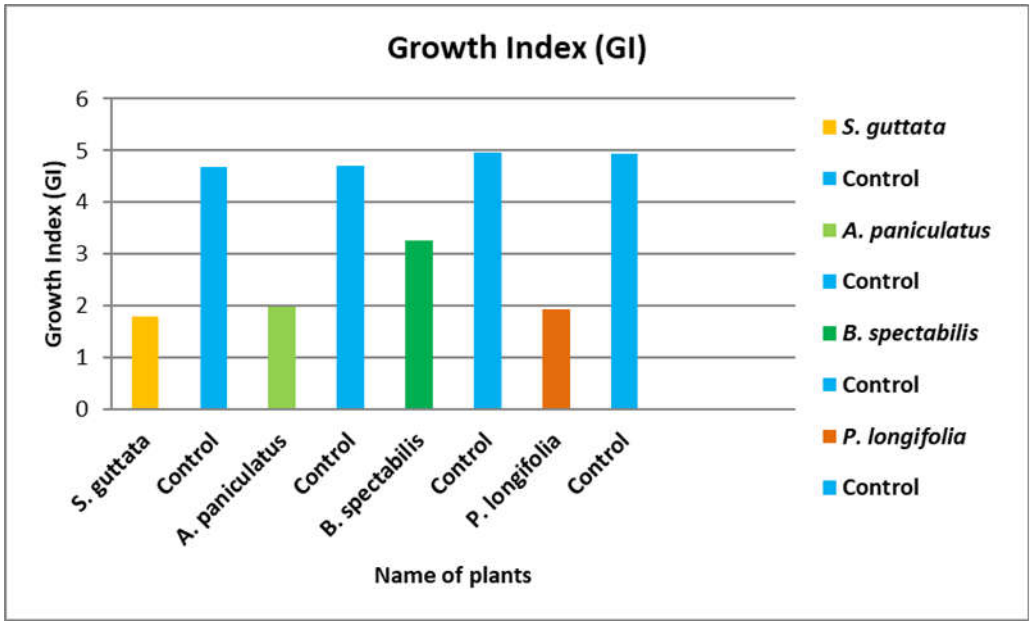


Figure 18- Effect of Column fractions of *S. guttata* (MeOH: EA- 4:1), *A. paniculata* (MeOH: EA- 4:1), *B. spectabilis* (H: EA- 5:5) and *P. longifolia* (MeOH: EA- 4:1) on Growth Index (GI) of *Cx. quinquefasciatus* during developmental process.

Furthermore, the developmental progress was affected by showing several deformities including moulting inhibition, morphological abnormalities and mortality especially during moulting and melanisation processes. Deformities that developed in the treatment of *S. guttata* (MeOH: EA- 4:1), *A. paniculata* (MeOH: EA- 4:1), B. *spectabilis* (H: EA- 5:5) and *P. longifolia* (MeOH: EA- 4:1) attributed to the dechitinizing effect of extracts with the formation of Larval- larval intermediates, larval- pupal intermediates and Pupal- adult intermediates. Sub- lethal doses of *S. guttata* (MeOH: EA- 4:1) resulted in appearance of highest number of larval- larval intermediates, larval- pupal intermediates and lowest number of Pupal- adult intermediates **(Figures 19, 20 & 21)**. *A. paniculata* (MeOH: EA- 4:1) extract also caused higher number

of Pupal- adult intermediates and Larval- pupal intermediates, but formation of larval- larval intermediates were not observed. Whereas, in the case of *B. spectabilis* (H: EA- 5:5) and *P. longifolia* (MeOH: EA- 4:1) treatment exhibited by the appearance of Larval- pupal intermediates and Pupal- adult intermediates and larval- larval intermediates were also observed in *P. longifolia* (MeOH: EA- 4:1), but was not occurred in *B. spectabilis* (H: EA- 5:5).

In the case of larval- larval intermediates, death has occurred with molting sutures on head of the larvae, therefore it could not molt into next instars whereas, the formation of larval- pupal intermediates resulting in death of treated larvae at an early stage of pupation. It was observed that there was an alteration in shapes of head and abdomen. The abdomen has also retracted along the larval skin and adopted the characteristic of pupal shape. The pupal abdomen has stretched like larvae with pupal head. Whereas, in the case of Pupal- adult intermediates, death occurred after complete molting, but some parts remain attached with pupal exuviae.

Based on aforementioned data, the column fractions of *S. guttata* (MeOH: EA- 4:1), *A. paniculata* (MeOH: EA- 4:1), *B. spectabilis* (H: EA- 5:5) and *P. longifolia* (MeOH: EA- 4:1) seems to have IGR activity, as it caused more profound harmful effects in larvae and pupae during molting.

Hormonal control of molting and metamorphosis has been well studied. Molting and pupation was under the control of two hormones Prothoracicotropic Hormone (PTTH) and Ecdysone. Acting together PTTH and ecdysone trigger larva- to- larva molt as well as Pupa- to- adult molt (Nijhout, 1994). The JH analogues induced mortality in the pupal stage and emerging adults while the chitin inhibitors produce mortality during ecdysis as well as metamorphosis (Mulla, 1991). Juvenile Hormone (JH) also contributed the larval- larval molting. If JH is enough, ecdysone promotes

larva- to- larva molt (Wigglesworth, 1934), therefore in the case of larval-larval intermediates the larvae could not molt in to next the instars, because there might be apparent decrease in the level of ecdysone and JH. But with lower amounts of JH, ecdysone promotes pupation (Wigglesworth, 1934). So in larval- pupal intermediates, the molted pupa could not attain the pupal characteristics such as the coma shape or curved abdomen, but it appeared as stretched as larval abdomen, this was because of the presence of JH in pupae. Similarly, in the case of pupal- adult intermediates, the adult which was emerged from the pupae existed with pupal exuviae, these might be due to the presence of increasing amount of JH and decreasing amount of ecdysone on the adult mosquitoes, because only the complete absence of JH results in formation of the adults (Wigglesworth, 19334). From these results, it was clear that the selected plant extracts *S. guttata* (MeOH: EA- 4:1), *A. paniculata* (MeOH: EA- 4:1), *B. spectabilis* (H: EA- 5:5) and *P. longifolia* (MeOH: EA- 4:1) might be acting as JH analogue and as well as ecdysone agonist, because the application of these plant extracts inhibited cuticle elaboration, sclerotization and ecdysis and induced developmental arrest in various developmental stages of *Cx. quinquefasciatus*.

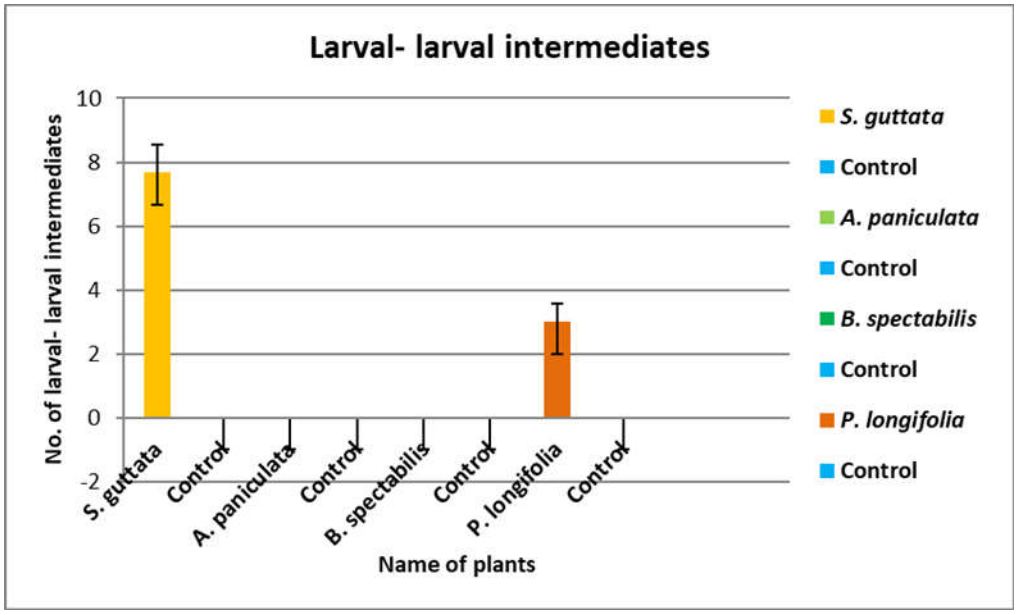


Figure 19- Data on comparison of morphological deformities as larval- larval intermediates associated with the exposure of fractionated column extracts of selected plants on *Cx. quinquefasciatus*.

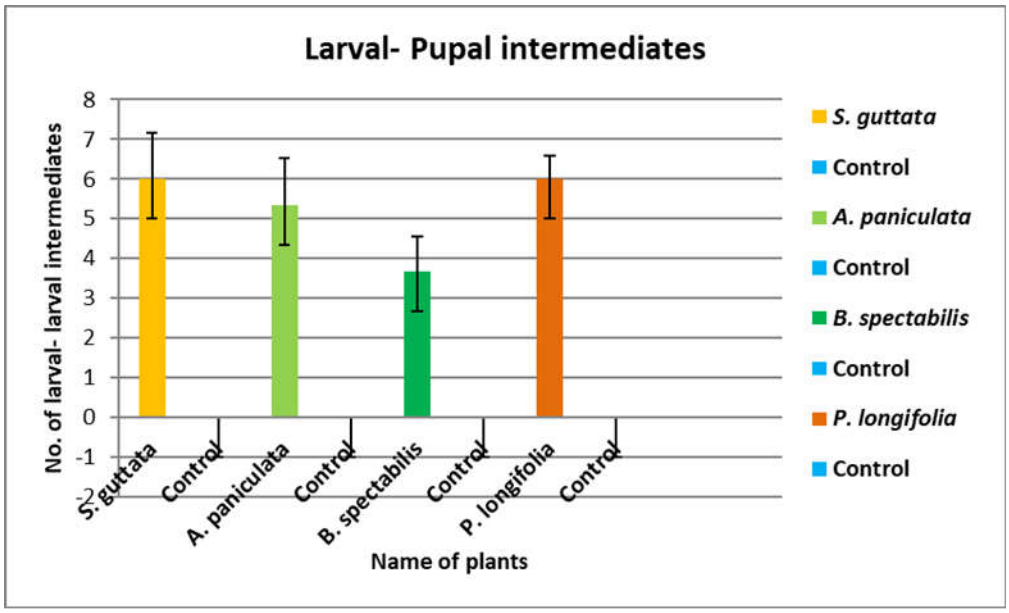


Figure 20- Data on comparison of morphological deformities as larval- pupal intermediates associated with the exposure of fractionated column extracts of selected plants on *Cx. quinquefasciatus*.

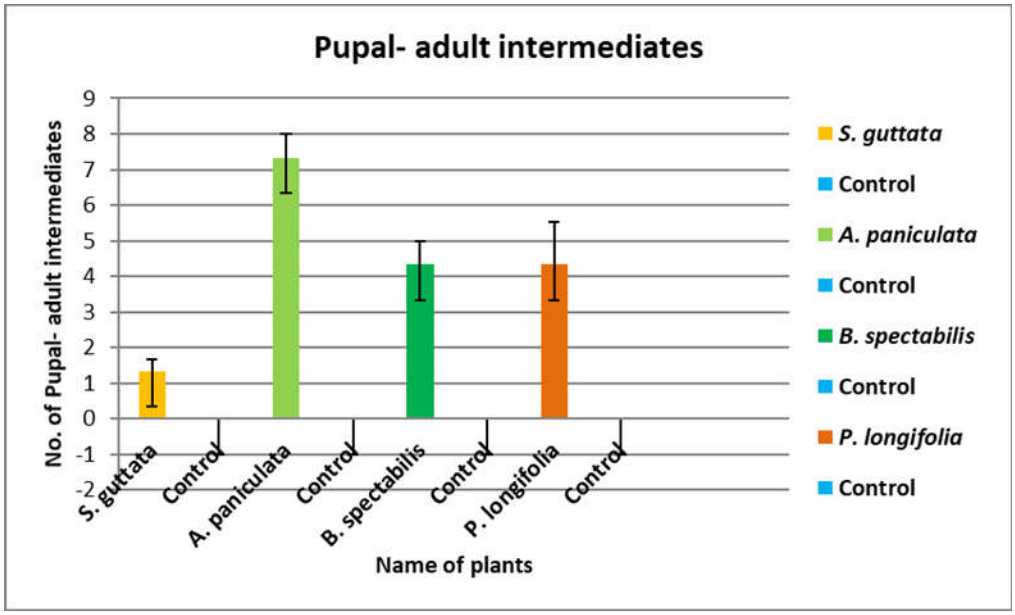


Figure 21- Data on comparison of morphological deformities as pupal- adult intermediates associated with the exposure of fractionated column extracts of selected plants on *Cx. quinquefasciatus*.

The chemosterilant effects of selected plant extracts of *S. guttata* (MeOH: EA- 4:1), *A. paniculata* (MeOH: EA- 4:1), *B. spectabilis* (H: EA- 5:5) and *P. longifolia* (MeOH: EA- 4:1) applied at I instar larvae of *Cx. quinquefasciatus* included induction of ovicidal activity, reduction in number of egg rafts, hatchability of eggs, and percentage control of reproduction, compared with control. It was observed when treated males and treated females T ♂ x T ♀of *S. guttata* (MeOH: EA- 4:1), *A. paniculata* (MeOH: EA- 4:1), *B. spectabilis* (H: EA- 5:5) and *P. longifolia* (MeOH: EA- 4:1) crossed together, there was little variation in gonotropic cycles, compared with control **(Table 44)** showed, all the treated groups of *S. guttata* (MeOH: EA- 4:1) and *A. paniculata* (MeOH: EA- 4:1) exhibited statistical significance ($P<0.05$) on number of egg rafts and eggs laid by the treated female, number of days taken for egg hatching, Oviposition Activity Index (OAI), percentage of hatching and control of reproduction, compared with untreated groups. Similarly, *B.*

spectabilis (H: EA- 5:5) and *P. longifolia* (MeOH: EA- 4:1) showed significant difference ($P<0.05$) on gonotropic cycles, percentage of egg hatching and control of reproduction. But in the case of *P. longifolia* (MeOH: EA- 4:1), it was found to be statistical significance on number of egg rafts laid by the treated females and Oviposition Activity Index (OAI), when compared with untreated groups. The resulted Oviposition Activity Index (OAI) of all the selected plants obtained was negative values **(Table 44)**, which indicated that the test waters were less oviposition attractant, suggesting that the test waters acted as a repellent for oviposition (Chakraborty and Chatterjee, 2015).

Table 44- Statistical evaluation of Efficacy of all the treated groups of *S. guttata* (MeOH: EA- 4:1), *A. paniculata* (MeOH: EA- 4:1), *B. spectabilis* (H: EA- 5:5) and *P. longifolia* (MeOH: EA- 4:1) on oviposition, gonotropic cycle, egg hatchability and control of reproduction of adult *Cx. quinquefasciatus*, compared with Untreated groups.

SI No.	Treated Plants	Gonotropic cycle (days)	No. of egg rafts laid	Average no. of eggs obtained	Oviposition Activity Index (OAI)	No. of days taken for egg hatching	% of hatch	% of Control of reproduction
1	*S. guttata*	0.0978	0.0042*	0.0005*	0.0018*	0.0073*	0.0027*	0.0144*
2	*A. paniculate*	0.2028	0.0108*	0.0059*	0.0292*	0.0403*	0.0495*	0.0013*
3	*B. specatbilis*	0.0046*	0.0708	0.1010	0.1289	0.1691	0.0214*	0.0002*
4	*P. longifolia*	0.0054*	0.0174*	0.0760	0.302*	0.0913	0.0253*	0.0017*

*Statistically significant at P< 0.05

The mating competitiveness of treated groups and untreated groups under field cage conditions were observed and evaluated. Among the larval treatments with the selected plant extracts of *S. guttata* (MeOH: EA- 4:1), *A. paniculata* (MeOH: EA- 4:1), *B. spectabilis* (H: EA- 5:5) and *P. longifolia* (MeOH: EA- 4:1), followed by crosses between treated males and treated females **T ♂ x T♀,** *S. guttata* (MeOH: EA- 4:1), *A. paniculata* (MeOH: EA- 4:1) and *B. spectabilis* (H: EA- 5:5) produced 32.53%, 36.01% and 30.65% of number of eggs, compared with untreated groups (100%) **(Figure 22)**. Whereas, *P. longifolia* (MeOH: EA- 4:1) showed 45.13% of number of eggs laid by the treated females, compared with control (100%).

In the case of crosses between treated males and untreated females **T ♂ x UT♀ (Figure 23),** *A. paniculata* (MeOH: EA- 4:1) was found to be the most effective, due to considerable decrease in the number of eggs as 4.08% laid by the females, whereas, *B. spectabilis* (H: EA- 5:5) and *P. longifolia* (MeOH: EA- 4:1) also showed production of lowest number of eggs as 5.97% and 14.86% , compared with control (100%). But in the case of *S. guttata* (MeOH: EA-4:1) exhibited slight increase in the number of eggs as 15.37%.

The number of eggs were also varied, when the crosses are between treated females and untreated males **T ♀ x UT♂ (Figure 24).** In these crosses, *S. guttata* (MeOH: EA-4:1) exhibited production of least number of eggs as 11.52%, while *A. paniculata* (MeOH: EA- 4:1) *P. longifolia* (MeOH: EA- 4:1) and *B. spectabilis* (H: EA- 5:5) showed 49.82%, 56.11% and 94.79% respectively, compared with control (100%).

The above discussed data revealed the efficacy of the selected plant extracts on the ovipositional and egg laying activity of *Cx. quinquefasciatus*. From the data, it was clear that *S. guttata* (MeOH: EA-4:1) exerted highest activity on the egg laying process in crosses between **T ♀ x UT♂,** whereas,*A. paniculata* (MeOH: EA- 4:1) *B. spectabilis* (H: EA- 5:5) and *P. longifolia* (MeOH: EA-

4:1) was found to be most effective on **T ♂ x UT♀** groups**.** Therefore,treatment of *S. guttata* (MeOH: EA-4:1) was more effective in treated females than treated males. On the other hand, *A. paniculata* (MeOH: EA- 4:1) *B. spectabilis* (H: EA- 5:5) and *P. longifolia* (MeOH: EA- 4:1) showed higher activity in treated males than treated females.

Similar results were obtained in percentage control of reproduction **(Figure 25)** such as highest percentage control of reproduction was obtained on **T ♀ x UT♂** groups of *S. guttata* (MeOH: EA-4:1) and **T ♂ x UT♀** groups of *A.paniculata* (MeOH: EA- 4:1) *B. spectabilis* (H: EA- 5:5) and *P. longifolia* (MeOH: EA- 4:1), compared with untreated groups **UT ♂ x UT♀**. Not only the treated females of *S. guttatta* (MeOH: EA- 4:1) and treated males of *A. paniculata* (MeOH: EA- 4:1) *B. spectabilis* (H: EA- 5:5) and *P. longifolia* (MeOH: EA- 4:1) both treated males and females also exhibited higher efficacy against the egg laying capacity of *Cx. quinquefasciatus*. These results also incorporated in the fertility efficacy of *Cx. quinquefasciatus*, which means all the selected plants are found to be capable of inducing sterility in *Cx. quinquefasciatus*.

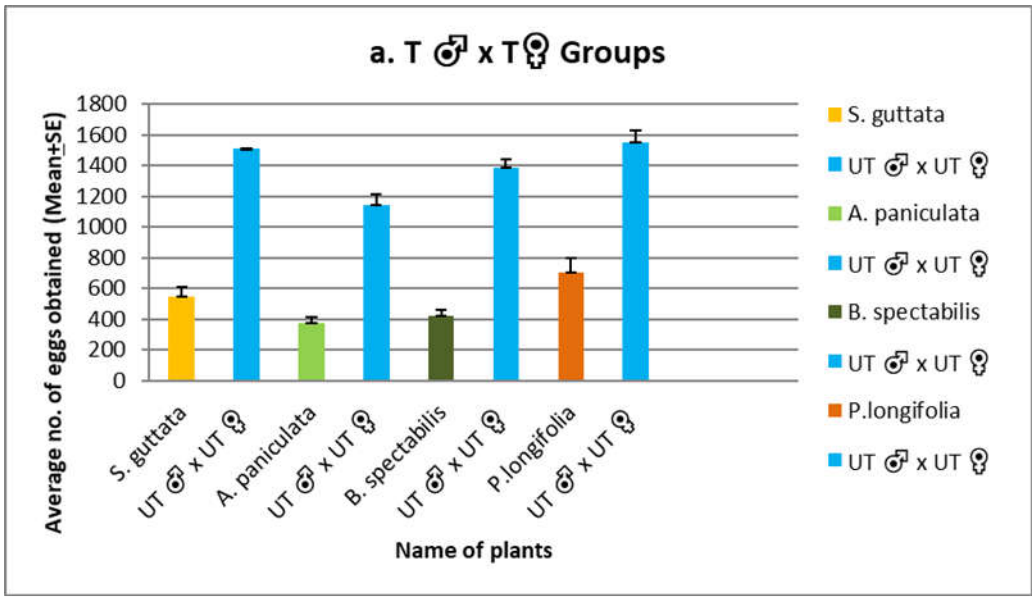


Figure 22-Mating competitiveness and effect of treated groups T ♂ x T♀on number of eggs of *Cx. quinquefasciatus* adults exposed as newly emerged I instar larvae.

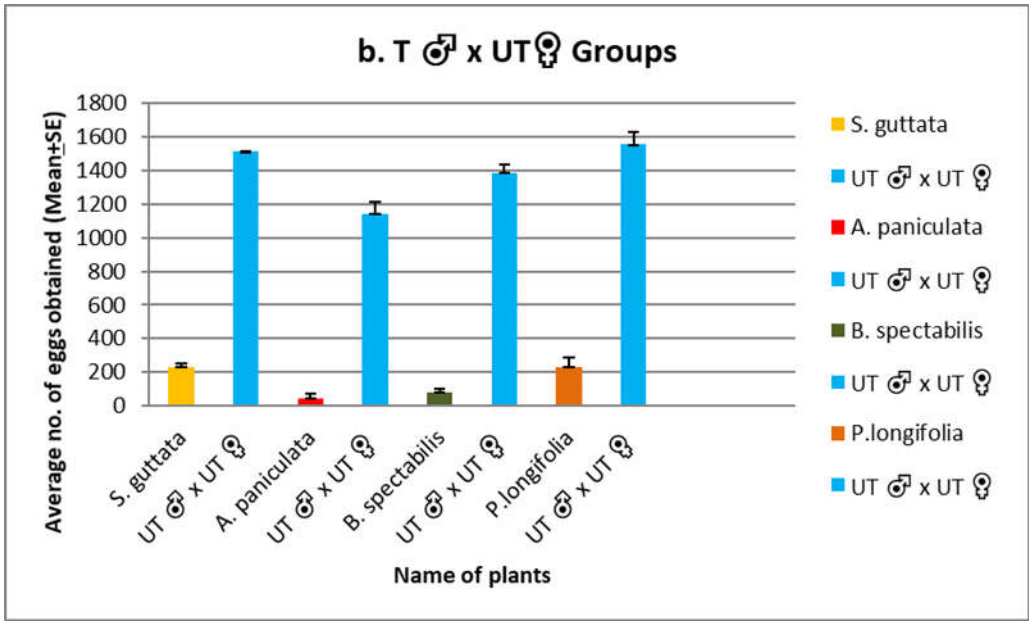


Figure 23-Mating competitiveness and effect of treated groups T ♂ x UT♀on number of eggs of *Cx. quinquefasciatus* adults exposed as newly emerged I instar larvae.

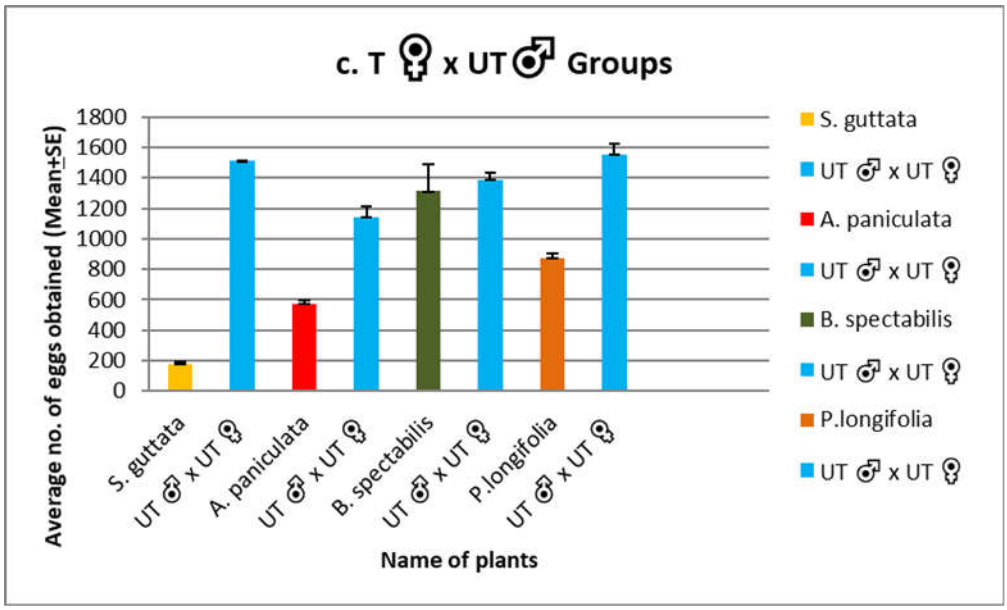


Figure 24-Mating competitiveness and effect of treated groups T ♀x UT♂on number of eggs of *Cx. quinquefasciatus* adults exposed as newly emerged I instar larvae.

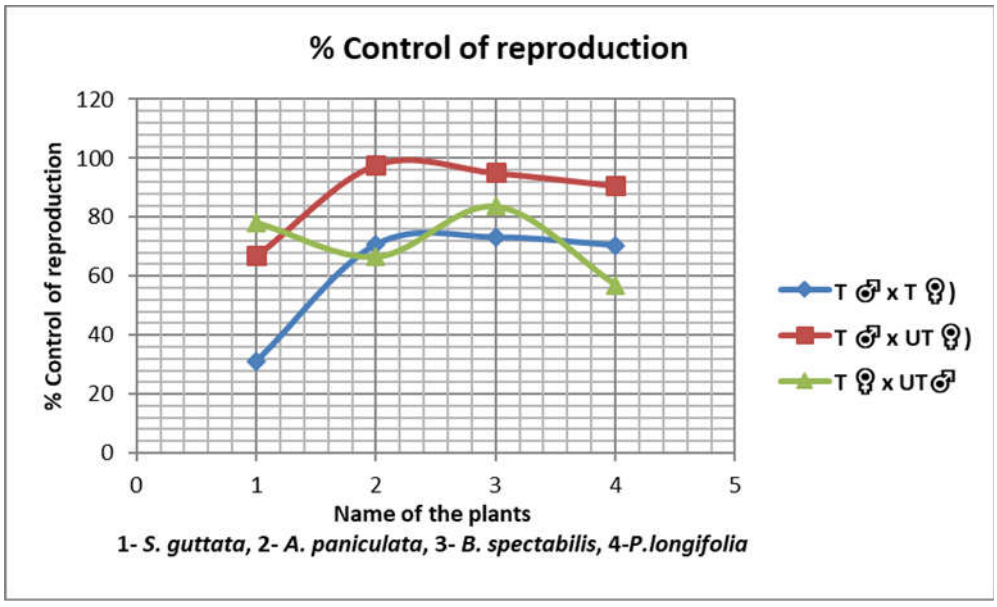


Table 25- Effect of selected plant extracts on percentage control of reproduction of *Cx. quinquefasciatus* adults exposed as newly emerged I instar larvae.

Due to the combined effects of mating inhibition, reproductive suppression and low survival, fecundity realization was not possible for *Cx. quinquefasciatus* in a normal way. But, if the treated mosquitoes survived longer and mating was possible, then only low fecundity realization could have been noted. The selected plants, *S. guttata* (MeOH: EA- 4:1), *A. paniculata* (MeOH: EA- 4:1), *B. spectabilis* (H: EA- 5:5) and *P. longifolia* (MeOH: EA- 4:1) resulted in a significant reduction in fecundity and fertility in the adults from treated larvae as compared to the untreated larvae. Similar results were obtained with the treatment of *Annona squamosa* alkaloids against *Anopheles stephensi* (Saxena *et al*., 1993). Sublethal concentration of alkaloids of *A. squamosa* against the larvae of *An. stephensi* caused developmental defects as well as larval mortality and was found to cause significant reduction in Sterility Index. Ferrari and Georghiou, 1981 and Robert and Olson, 1989 also investigated reduced egg production at the exposure of sublethal concentrations of insecticides in *Cx. quinquefasciatus*.

Females obtained from the treated larvae of *Cx. quinquefasciatus* showed loss in fecundity and fertility. Fecundity rate, fertility percentage and Sterility Index (SI) were significantly reduced ($P< 0.05$) in the crosses between **T ♀ x UT♂** in *S. guttata* (MeOH: EA- 4:1) **(Table 45) (Figures 26, 27 & 28).** Whereas,in the case of *A. paniculata* (MeOH: EA- 4:1), *B. spectabilis* (H: EA- 5:5) and *P. longifolia* (MeOH: EA- 4:1), fecundity, fertility and Sterility Index was considerably reduced in the treated crosses **T ♂ x UT ♀ (Figures 26, 27 & 28)** compared with other groups and untreated groups.

From the above- mentioned data **(Table 44)**, it was inferred that the frequency of sterile eggs were greater with treated female with treated male in the treatment of *S. guttata* (MeOH: EA- 4:1), but in the case of *A. paniculata* (MeOH: EA- 4:1), *B. spectabilis* (H: EA- 5:5) and *P. longifolia* (MeOH: EA-

4:1), sterile egg frequency increased with crossing of treated males and untreated females. Among these selected plants, *A. paniculata* (MeOH: EA- 4:1) **T ♂ x UT ♀** groups were found to be the most effective phytosterilant. It produced about 97.93% sterility and 97.90% control of reproduction with 0.5ppm concentration. *B. spectabilis* (H: EA- 5:5) **T ♂ x UT ♀** groups exhibited 95.31% sterility and 95.08% control of reproduction with 21.49ppm concentration, *S. guttata* (MeOH: EA- 4:1) **T♀ x UT ♂** groups showed 91.61% sterility and 78.04% control of reproduction at 6.41ppm concentration and finally *P. longifolia* (MeOH: EA- 4:1) **T ♂ x UT ♀** group was found to induce 90.91% sterility and 90.69% control of reproduction at 36.30ppm concentration.

From data presented in **Table 45** it was observed that except *S. guttata* (MeOH: EA- 4:1), other three plants *A. paniculata* (MeOH: EA- 4:1), *B. spectabilis* (H: EA- 5:5) and *P. longifolia* (MeOH: EA- 4:1) induced higher sterility in treated males than treated females, whereas, *S. guttata* (MeOH: EA- 4:1) exhibited higher sterility in treated females than treated males. However, in all these selected plants, both treated males and females could also induced sterility, but in some to a lower extend when compared with untreated groups. On the other hand, the number of egg rafts, oviposition and percentage hatch of abnormal eggs were considerably lower when compared to untreated eggs. Dissection of sterile eggs showed fully developed larvae in a few eggs, many of which failed to hatch. In summary, *A. paniculata* (MeOH: EA- 4:1), *B. spectabilis* (H: EA- 5:5) and *P. longifolia* (MeOH: EA- 4:1) on *Cx. quinquefasciatus* exhibited male sterility and *S. guttata* (MeOH: EA- 4:1) induced female sterility. Therefore, these selected plants could be considered as potent phytosterilants in the field of mosquito eradication process.

Table 45- Comparative assessment of Sterility Index (SI) by different plant extracts with respect to control based on t- test analysis.

SI No.	*S. guttata* (MeOH: EA- 4:1)	*A. paniculata* (MeOH: EA- 4:1)	*B. specatbilis* (H: EA- 4:1)	*P. longifolia* (MeOH: EA- 4:1)	P value
1	T ♂ x T ♀	T ♂ x T ♀	T ♂ x T ♀	T ♂ x T ♀	0.0001*
2	T ♂ x UT ♀	T ♂ x UT ♀	T ♂ x UT ♀	T ♂ x UT ♀	0.0001*
3	T ♀ x UT♂	T ♀ x UT♂	T ♀ x UT♂	T ♀ x UT♂	0.0117*

*Significant at $P < 0.05$

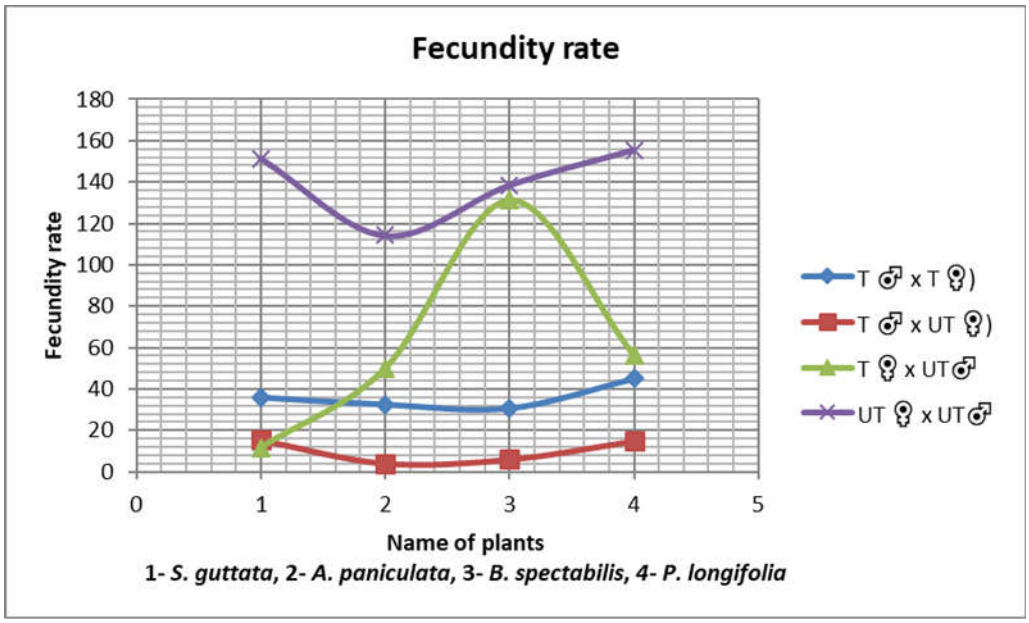


Figure 26- Fecundity effects of selected plant extracts on the adults of *Cx. quinquefasciatus* from treated larvae

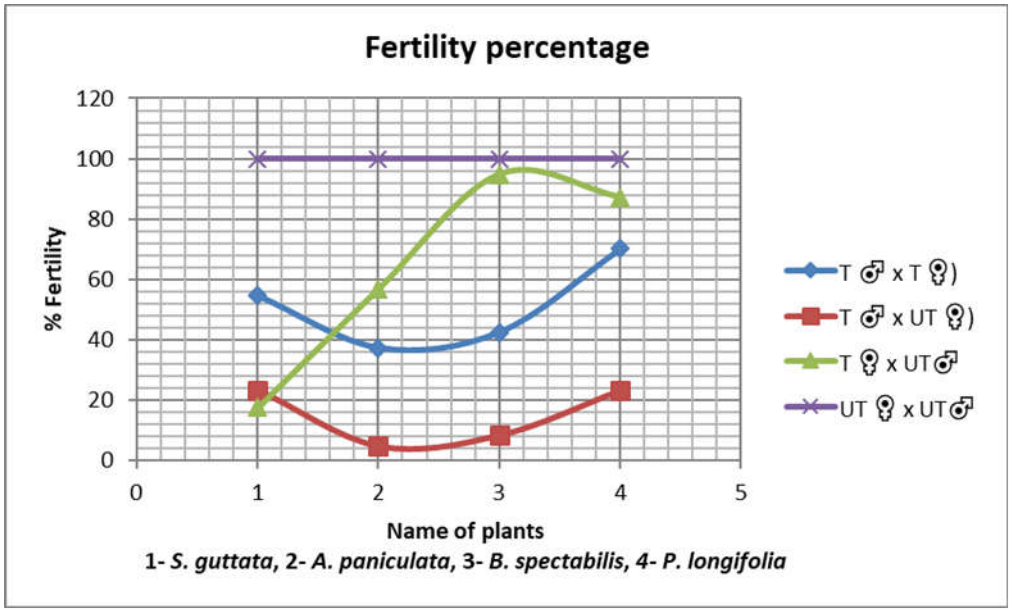


Figure 27- Fertility percentage of selected plant extracts on the adults of *Cx. quinquefasciatus* from treated larvae.

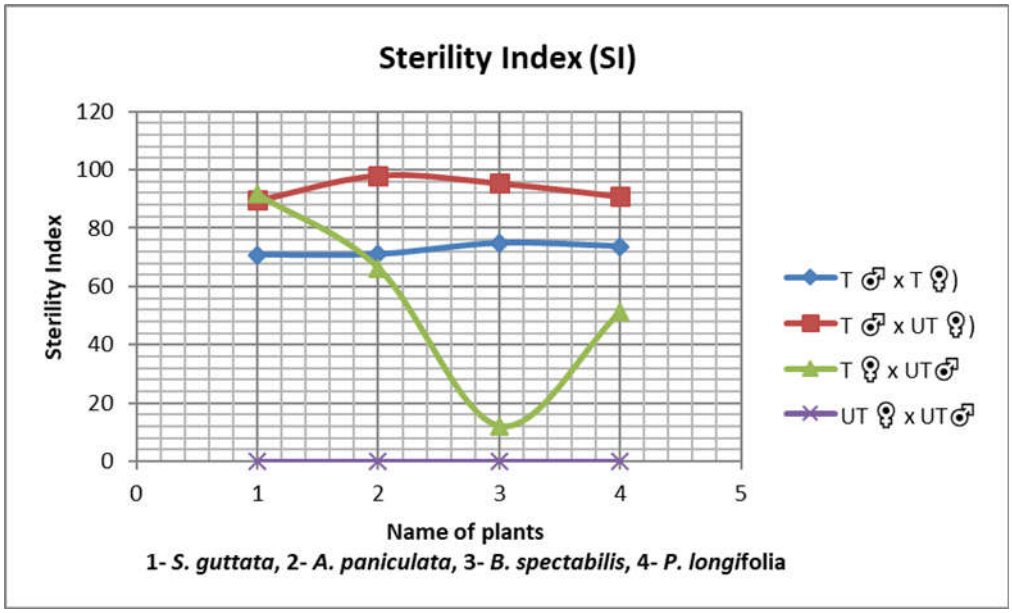


Figure 28- Sterility Index (SI) of selected plant extracts on the adults of *Cx. quinquefasciatus* from treated larvae

The dissections revealed substantial morphometric changes of the reproductive organs of the treated mosquitoes. Ovarian development was drastically affected in *S. guttata* (MeOH: EA- 4:1) treated female adult *Cx. quinquefasciatus*. Ovarian development was drastically affected in *S. guttata* (MeOH: EA- 4:1) treated resultant adults of *Cx. quinquefasciatus*. *A. paniculata* (MeOH: EA- 4:1), *B. spectabilis* (H: EA- 5:5) and *P. longifolia* (MeOH: EA- 4:1) also exhibited moderate effects on ovarian development. The ovaries of treated resultant adults showed variation in length and size of ovaries, number of ovarioles and degeneration, oocyte degeneration, resorption and inhibition of oviposition. Application of extracts on larvae of *Cx. quinquefasciatus* prevented ovarian development by inhibiting vitellogenesis and oocyte development. It was also observed many treated resultant mosquitos exhibited single ovary and degeneration of bursa inseminalis.

The effects of *A. paniculata* (MeOH: EA- 4:1), *B. spectabilis* (H: EA- 5:5) and *P. longifolia* (MeOH: EA- 4:1) on treated larvae interfered with the male reproductive systems, with lack of testes and also varying the size and shape, compared to untreated. But aedegus, seminal vesicles and accessory glands did not have significant abnormalities. Thus, due to the combined effects of low survival and longevity, mating inhibition and reproductive suppression and also fecundity realization was not possible for the mosquitoes in a normal way.

Furthermore, the extracts of the selected plants when treated with the freshly hatched I instars larvae of *Cx. quinquefasciatus* inhibited mating, apart from affecting the size of reproductive organs. This could be due to hormonal imbalance caused by the effect of the treated extracts on the reproductive organs. The continuous intake of low doses also caused early death of larval

instars as compared to normal untreated ones. In males, sterility was caused due to the malfunctioning of interstitial cells, which leads changes in spermatogenesis, secretions of accessory glands and to the Activation of sperm. The embryo development was also affected by the alkylating agents such as apholate and tepa during the initial phase of development and cleavage division, whereas, the antimetablolite 6- azauridine affected the organogenesis in developed embryo, which appears to affect egg metabolism ((Landaand Matolin, 1971). Thus, due to the combined effects on ovaries and oogenesis, on testes and spermatogenesis and on embryogenesis together exerted sterilizing effects of selected plants with juvenile- hormone activity. Either depends on larvicidal activity or sterizing effects such as low fecundity realization, it could be stated that the selected plants *S. guttata* (MeOH: EA- 4:1), *A. paniculata* (MeOH: EA- 4:1), *B. spectabilis* (H: EA- 5:5) and *P. longifolia* (MeOH: EA- 4:1) had a strong phytosterilant effect on both sexes of *Cx. quinquefasciatus*.

When compared with the phytosterilant effects of selected plants with a chemosterilant Hexamethyl Phosphoramide (HMPA) revealed highly significant effects ($P< 0.001$) with respect to control. HMPA exhibited potent larvicidal activity with an LC_{50} value 36.963ppm and effective concentration with EC_{50} value 7.371ppm **(Figures 29 & 30)**. In the case of selected plants, *S. guttata* (MeOH: EA- 4:1) and *A. paniculata* (MeOH: EA- 4:1) also exhibited significantly lower LC_{50} values as 24.254ppm and 3.013ppm respectively (**Figures 29 & 30).**

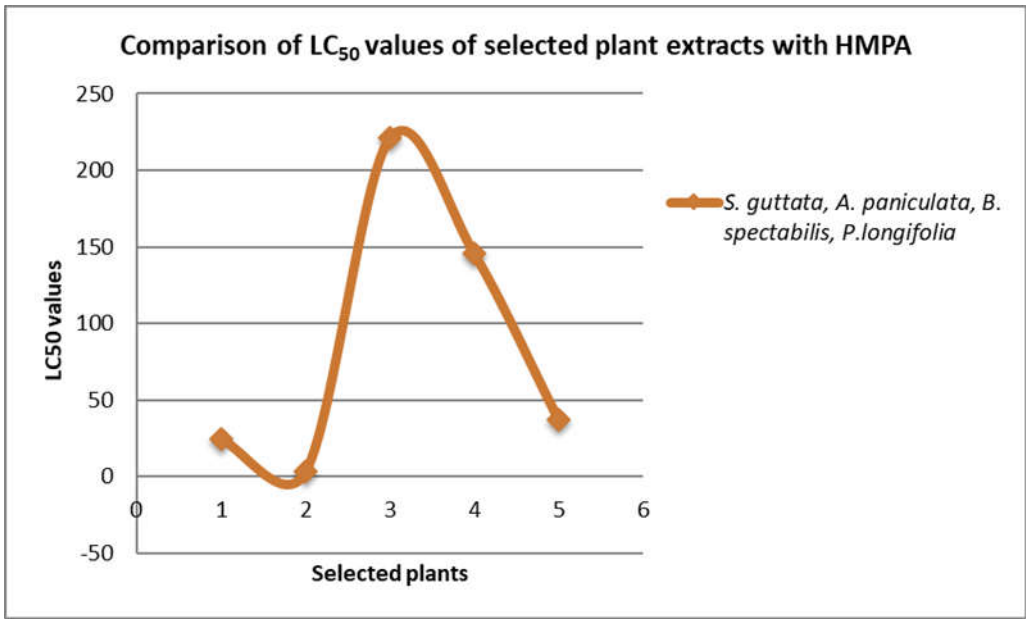


Figure 29- Comparison of LC_{50} values of selected plant extracts with HMPA

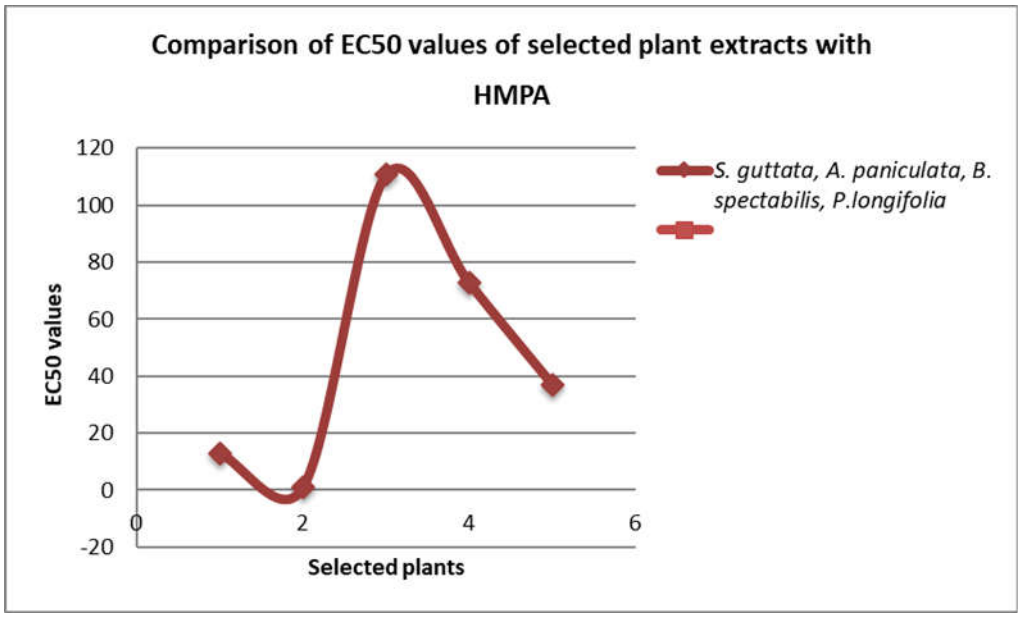


Figure 30- Comparison of EC_{50} values of selected plant extracts with HMPA

HMPA extended total developmental duration of *Cx. quinquefasciatus* significantly higher when compared with control, whereas, *B. spectabilis*

(MeOH: EA- 4:1) and other plants exhibited slight variation in the developmental duration, compared with HMPA and control (**Figure 31**).

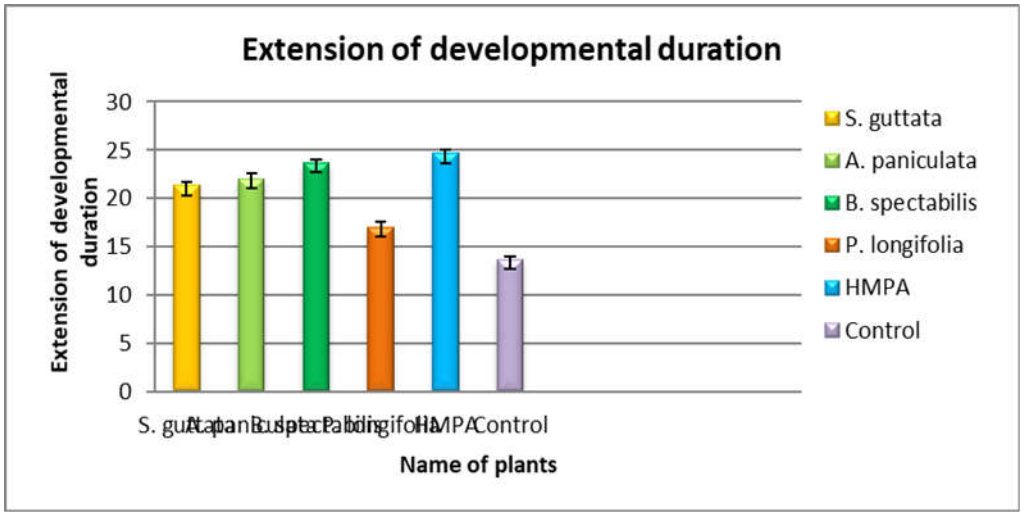


Figure 31- Comparison of extension of developmental duration of selected plant extracts with HMPA

The Growth Index (GI) was found to be significantly reduced ($P < 0.001$) in the case of HMPA and selected plant extracts, compared to control. Among these, HMPA and *S. guttata* (MeOH: EA- 4:1) exhibited significant reduction in Growth Index (GI), while *A. paniculata* (MeOH: EA- 4:1), *P. longifolia* (MeOH: EA- 4:1) and *B. spectabilis* also showed reduced GI, compared with control (**Figure 32**). HMPA exhibited various morphogenetic deformities such as larval- larval intermediates, larval- pupal intermediates and pupal-adult intermediates. It has greatly influenced the gonotropic cycles, oviposition and control of reproduction on all the treated groups of *Cx. quinquefasciatus*. The percentage of egg hatching was also reduced, compared with control. Successive inhibition of all these combined effects along with high mortality rates at larval and pupal stages reduced the adult emergence. HMPA also exhibited morphogenetic deformities associated with the reproductive organs of adult *Cx. quinquefasciatus*. Both ovarian and testicular development was drastically affected such as disintegration of

ovaries and testes, degeneration of ovarioles and testes, oocyte degeneration and inhibition of oviposition.

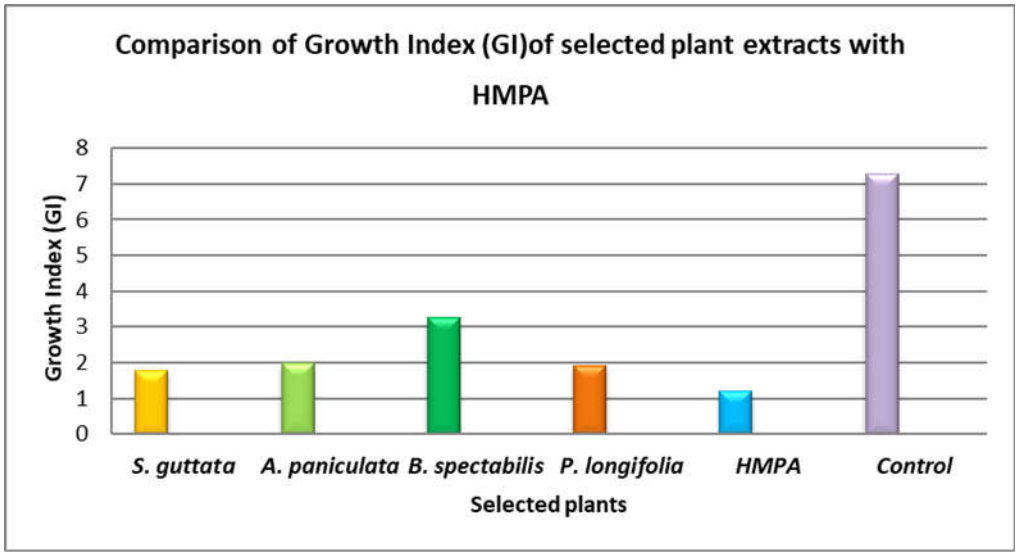


Figure 32- Comparison of Growth Index (GI) of selected plant extracts with HMPA

HMPA and the selected plant extracts resulted in a significant loss in fecundity and fertility in the adults of *Cx. quinquefasciatus* as compared to control. The Sterility Index (SI) was found to be significantly reduced ($P < 0.001$) in the case of HMPA and the selected plant extracts (**Table 46**). But considering all the treated groups the selected plant extracts showed slightly lower SI, compared with HMPA **(Figure 33)**. HMPA exhibited above 95% Sterility Index (SI) for all the treated groups, whereas selected plant extracts, *S. guttata* (MeOH: EA- 4:1) showed 91.61% on **T ♀ x UT♂** groups and *A. paniculata* (MeOH: EA- 4:1), *B. spectabilis* (H: EA- 4:1) and *P. longifolia* (MeOH: EA- 4:1) exhibited above 90% Sterility Index (SI) on **T ♂ x UT ♀** groups**.** Based on these inferences it was confirmed that the selected plant extracts could be used as potent phytosterilant, because these plants could exhibit sterilizing effects to the same extend as exerted by the universally accepted chemosterilant HMPA.

Table 46- Comparative assessment of sterility induction by plant-based bio-active compounds versus synthetic chemosterilant (HMPA) with respect to control based on one - way ANOVA (Duncan's Multiple range of comparisons. *Significant at P < 0.05).

SI No.	Name of plants	Percent mortality		Percent emergence		Growth Index (GI)		Sterility Index (SI)	
		F	Sig	F	Sig	F	Sig	F	Sig
1	*S. guttata* CH3OH: C4H8O2- 4:1	5.114	0.025*	10.486	0.002*	164.771	0.005*	190.399	0.001*
2	*A. paniculata* CH3OH: C4H8O2- 4:1	5.610	0.019*	9.489	0.003*	7.354	0.252	82.333	0.001*
3	*B. Specatbilis* C6H12: C4H8O2- 5:5	5.550	0.020*	11.489	0.002*	16.084	0.174	11.660	0.009*
4	*P. longifolia* CH3OH: C4H8O2- 4:1	5.855	0.017*	11.866	0.001*	18.075	0.164	58.732	0.001*

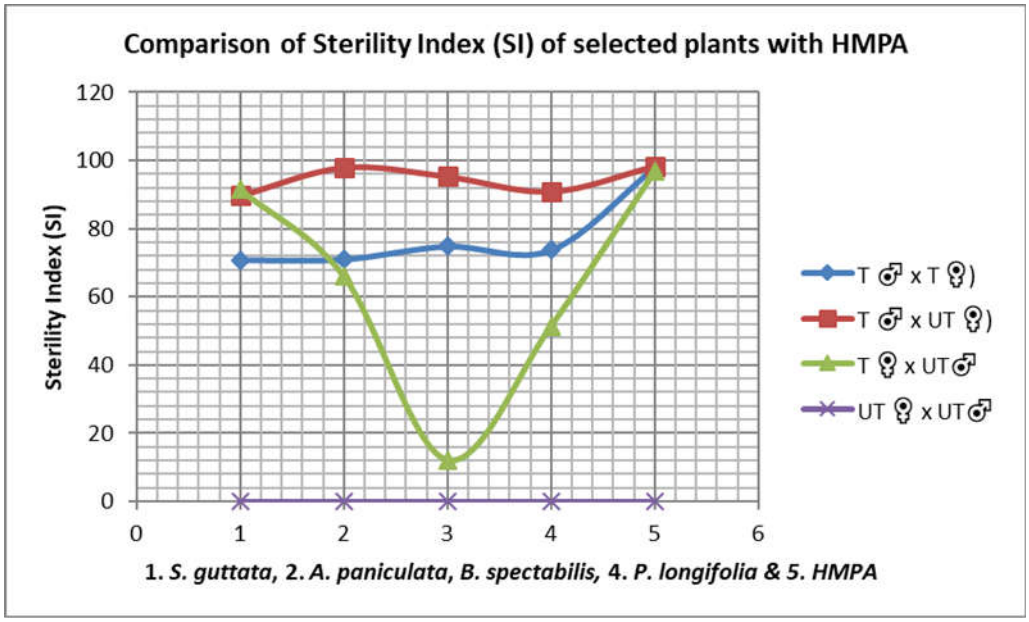


Figure 33- Comparison of Sterility Index (SI) of selected plant extracts with HMPA

Based on these findings it was clearly indicated that the selected plant extracts *S. guttata* (MeOH: EA- 4:1), *A. paniculata* (MeOH: EA- 4:1), *B. spectabilis* (H: EA- 5:5) and *P. longifolia* (MeOH: EA- 4:1) employed are very promising in sterilizing *Cx. quinquefasciatus* and are also competent with the chemosterilant Hexamethyl Phosphoramide (HMPA). These plants also exhibited potent larvicidal efficacies in higher concentrations along with reproductive toxicity, which mainly included mating inhibition, reproductive suppression, low survival, extended developmental duration and gonotropic cycles and low fecundity rate. Moreover, the dissections of reproductive organs revealed the proof of reproductive toxicity of the selected plants on the adults of *Cx. quinquefasciatus*. Among these plants, *S. guttata* (MeOH: EA- 4:1) inhibited ovarian development, affecting the fertility and female reproductive potentiality by reducing the number of ovarioles and sometimes appeared to cause degenerated ovaries and bursa inseminalis whereas, *A. paniculata* (MeOH: EA- 4:1), *B. spectabilis* (H: EA- 5:5) and *P. longifolia*

(MeOH: EA- 4:1) disrupted the male reproductive system. In most of the cases, testes varied in length and size and exhibited degeneration.

Dealing with mosquito-borne diseases is a major global challenge faced by the public health sector and demand targeted management efforts, with mosquito control represents the most crucial strate yet to be employed around the world. Normally mosquito repellents make a major share of all the mosquito control products across the world. Analysis of the market trends and increasing environmental concern strongly predicts a definite growth and expansion and diversification to innovative product formulations using phytosterilants for mosquito control fora paradigm shift from the conventional chemical-based formulations to herbal and organic products for better mosquito control regimes. Now the results obtained from the present investigation and the inferences, it may be emphasized that the above mentioned four plants viz; ***S. guttata*, *A. paniculata*, *B. spectabilis* and *P. longifolia*** can be used as potent phytosterilants to have an effective control of the menacing filarial vector *Cx. quinquefasciatus* population.

3.1 INTRODUCTION

In complex multicellular organisms like plants and animals, metabolism, growth and development are coordinated by various metabolites such as enzymes, proteins, sugars, hormones and several other signal compounds. Like higher animals, plants can also synthesize a variety of compounds as primary metabolites and secondary metabolites. Primary metabolites are essential for the survival of plants, which mainly include proteins, sugars and amino acids, whereas, secondary metabolites are not directly involved in growth and development of a plant species, but they play a major role in plant defense mechanism against herbivores, which also include destructive insects/ pests (Bennett and Wallsgrove, 1994). The secondary metabolites, which are derived from any part of the plants like leaves, flowers, fruits, roots, seeds and barks constitute phenolics, terpenoids, alkaloids, phytosterols and other compounds, which act as antifeedants, moulting hormones, repellents, attractants, oviposition deterrents, growth inhibitors, juvenile hormone mimic as well as antimoulting hormones (Jacobson, 1975). Identifying these phytochemicals which are interfering with the insect physiology such as insect behaviour, development and reproduction might contribute evolution of alternative strategies for synthetic pesticides.

Collectively more than 10,000 secondary metabolites are known for their insecticidal activities, which included phenols, flavonoids, coumarines, tannins, quinones, phenolpropanoids derivatives, isoprene derivatives, diterpenes, triterpenes, saponins, steroids, alkaloid, kinolene alkaloids, sesquiterpenes, indole, glycosides, ethereal oils and gums are examples of such compounds. Based on biosynthetic origin, secondary metabolites are classified into phenols, terpenoids and nitrogenous alkaloid compounds (Taiz and Zeiger, 2010).

Phenolic compounds or polyphenols are one of the most numerous groups of substances with more than 8,000 phenolic structures widely distributed among the plant kingdom (Harborne, 1993). Phenolic compounds are chemically a heterogenous group, which are very unstable, easily oxidized and susceptible to degradation and are classified into several types such as phenolic acids, coumarines, flavonoids, xanthones, anthocyanins, lignan polymers and tannins (Carvalho *et al.,* 2007). Among the phenolic compounds, flavonoids and isoflavones commonly occurs as esters, ether or glycosides derivatives (Brito *et al.*, 2001). Phenolic compounds exhibited several biological activities like antioxidants, antimicrobials, photoreceptors, visual appeal and as a repellent.

Terpenes constitute the largest group of secondary metabolites with more than 40,000 different molecules (Garcia and Carril, 2009). Terpenes generally act as defense against herbivore and having the same molecular structure of the molting hormone of insects to interrupt the process of molting and making complex sterol precursors of animal hormones (Taiz and Ziger, 2013). Several monoterpenes such as α-pinene, β-pinene, 3-carene, limonene, myrcene, a-terpinene and camphene were isolated and evaluated for their toxicity to different insect species (Juniour, 2003). The insecticidal activities of these compounds were mainly due to the inhibition of acetylcholinesterase, which might also cause certain effects such as appetite suppressants, retardation or growth inhibitor and reduction of reproductive capacity in certain insects (Marangoni *et al.*, 2012).

Nitrogenous compounds mainly consisted alkaloids and cyanogenic glycosides, which have toxic and medicinal effects on humans (Ziger and Taiz, 2013). Nitrogenous compounds like nicotine and nor-nicotine are the most important alkaloids used to control variety of pest species. Several researchers have found most of the secondary metabolites were responsible

for a variety of biological activities among tested organisms. Winterstein and Tier, 1910) opinioned that alkaloid, a secondary metabolite could produce greater or lesser toxicity, which acts primarily on the central nervous system (CNS) and the basic character of a chemical constitution. The most important point about the secondary metabolites is that, these chemicals are active at different cellular levels of organisms and they take part in many of the biological processes of an organism. Therefore, it is important to isolate the phytoconstituents from the plant species to analyze the specificity and mode of action of phytoinsecticides on target insect species.

The isolation and identification of phytoconstituents from different parts of the plants involved the application of several techniques, which mainly include extraction, common phytochemical screening and various chromatographic and non-chromatographic techniques and the detection of molecular structure of the compounds is also possible through Nuclear Magnetic Resonance (NMR), Fourier Transform Infra- Red spectroscopy and Mass Spectrometry (FTIR and MS) analysis.

Crude plant extracts usually occur as a combination of various types of phytoconstituents with different polarities. Therefore, isolation of these phytochemicals involves a number of separation techniques, mainly the chromatographic techniques to obtain pure compounds. Pure compounds are then subjected to the determination of structure and biological activity. Chromatography is the widely used separation technique, in which factors affecting on this separation process include molecular characteristics related to adsorption (liquid-solid), partition (liquid-solid) and affinity or differences among the molecular weights of the compounds (Cuatrecasas *et al*., 1968). Among the various Chromatographic techniques, partition chromatography is very effective on separation and identification of molecules like amino acids, fatty acids and carbohydrates, whereas, affinity chromatography (ion-

exchange chromatography) is used in the separation of macromolecules like nucleic acids and proteins. Gas- liquid chromatography is utilized in the separation of esters, alcohols, amino groups and lipids and observations of enzymatic reactions. The basis of chromatographic technique is the stationary phase, mobile phase and separation molecules. On a column (stationary phase), samples to be applied and separated, then wash with buffer (mobile phase). The eluted samples are accumulated at the bottom of the device and collected in a time- and volume- dependent manner (Das and Dasgupta, 1998).

Mass spectrometry (MS) is another tool used in the analytical technique to quantify known materials to identify unknown compounds within a sample and the structural elucidation and analysis of chemical properties of different molecule present in the sample. The basic principles involved is the conversion of sample molecules into gaseous ions with or without fragmentation and are then characterized by their mass - to- charge ratios (m/z) and relative abundance (Sparkman *et al*., 2011). A mass spectrum of the molecule is produced and ions provided the information concerning the nature and the structure of their precursor molecule. In a spectrum, the molecular ion appears at the highest value of m/z and gives the molecular mass of the compound. Different software also supports multi stage mass spectrometry data analysis, which enables structural elucidation and identification of fragmentation pathways thus identifying the unknown compounds from the samples (Downard and Kevin, 2004).

An important enhancement to the mass spectrometry is using it in tandem with certain chromatographic and other separation techniques. In a Gas chromatography-mass spectrometry (GC/MS or GC-MS), separated compounds is fed in to the ion source, a metallic filament to which voltage is applied and this filament emit electrons, which ionizes the compounds. The

ions can further fragment and pass in to the mass spectrometer's analyzer and are eventually detected (Eiceman, 2000). Liquid Chromatography- Mass spectrometry (LC/MS or LC-MS) also separates compounds chromatographically, but usually it consists of a mixture of water and organic solvents instead of gas and an electrospray ionization source is also commonly used in LC/MS (Sparkman *et al*., 2011). Data interpretation of mass spectra requires combined use of various techniques such as, for identifying unknown compound is to compare its mass spectrum against a library of mass spectra or manual interpretation or software assisted interpretation.

Nuclear Magnetic Resonance (NMR) spectroscopy also became an indispensable analytical tool for the determination of molecular structure, molecular dynamics and characterization of organic compounds (Pople *et al*., 1957). NMR uses a component of electromagnetic radiation (radio frequency waves) to promote transitions between nuclear energy levels (resonance). The commonly used NMR techniques are proton (^{1}H) and carbon -13 (^{13}C) NMR spectroscopy. NMR spectrometers are relatively strong, large and expensive liquid helium- cooled superconducting magnet, because the use of higher strength magnetic fields result in clear resolution of the peaks (Paudler, 1971). Energy of absorption, the resonance frequency and intensity of the signal are proportional to the strength of the magnetic field. Deuterated solvent systems are used in NMR, in which deuterated benzene is the common, whereas, the most used deuterated solvent for hydrophilic analyses is deuterochloroform ($CDCl_3$), although deuterium oxide (D_2O) and deuterated DMSO (DMSO-d_6). The chemical shift provides information regarding the structure of the molecule (Aue *et al*., 1976). Chemical shift is the difference in parts per million (ppm) between the resonance frequency of the observed proton and tetramethylsilanehydrogens (TMS, most common reference compound used in NMR analysis). The shape and area of peaks also serve as indicators of

chemical structure too. J-coupling or Scalar coupling (spin-spin coupling) between NMR active nuclei provides most useful information for structural determination in a one- dimensional NMR spectrum (Wemmer and David, 2000).

After the identification of an isolated compound, it can be modified as various insecticidal formulations through which, quick control of pest/ vector insects can be achieved. Insecticidal formulations typically consist of an active ingredient and several inactive materials, the adjuvants or additives, which increases the effectiveness of the active ingredients (Ware, 1994). The active ingredients of synthetic insecticides are not suitable in their unformulated forms, but in the case of phyto- insecticides, active ingredients are derived from plant parts, which might be useful for easy handling, storage, transport and stability or efficacy of formulations (Bohmont, 1990). Some of the most common kinds of insecticidal formulations are dusts (D), Granules (G), Aerosols, Wettable Powders (WP), Emulsifiable Concentrate (EC), Flowable liquids (F), Water soluble Concentrate (WC), Encapsulated pesticides, Soluble Powders (SP), Dry flowables and Poisonous baits (Knowles, 1998). In recent years, ultra- low volume aerosols of concentrated insecticides were used in mosquito control. These concentrates contain above 80% of active ingredients and these sprays are more effective as adulticides than fog application to control mosquitoes. Whereas, liquid formulations are best suitable for larval mosquitoes and they are prepared by dissolving the technical grade insecticides in a liquid solvent. Most of the technical grade insecticides are dissolved in water or one of the organic solvents.

Crude petroleum oils are commonly used as diluents for mosquito control insecticides. The choice of solvents mainly depends on the solubility of the insecticides and cost of the solvents. Solvents such as acetone, cyclohexanone, or heavy aromatic naphthalene (HAN) are also commonly

used (Rathburn, 1985). Liquid formulations may be applied as sprays, mists and aerosols depending upon the desired droplet size needed for the control of mosquitoes. Fine sprays, mists and aerosols are more effective against adult mosquitoes, whereas, medium to coarse sprays are most useful as larvicides. Although, dust formulations are highly toxic to mosquitoes as they are more difficult to apply than sprays or granules. Primarily, dusts were used as larvicides to treat large breeding areas or the larvicide that remained on the surface for long periods was needed. Also, sometimes they were preferred for control of adult mosquitoes in heavily vegetated areas. In place of sprays and dusts, granular formulations are now used extensively as mosquito larvicides (Rathburn, 1985). Granular formulations give greater penetration of vegetation and as a result their efficacy is also greatly increased. Granules are formed by impregnating coarse material with an insecticide and the carriers used mostly are celatom, pyrophyllite and sand usually between 16 and 60 meshes in size. There are many other types of special formulations necessitated by formulating problems of certain insecticides or by need of particular application.

The present study comprised purification, isolation and identification of biosterilant compounds from the seed extract of *Sterculia guttata* and *Polyalthia longifolia* and also leaf extracts of *Andrographis paniculata* and *Bougainvillea spectabilis,* using various chromatographic techniques described elsewhere and formulations like water soluble granules (WSG) and Syrups were prepared from the selected plant parts of the above mentioned plants and were tested on the filarial vector *Culex quinquefasciatus* by its natural breeding sites to induce sterility and thereby reduce the number of vector population.

3.2 REVIEW OF LITERATURE

Bio active components, derived from plants are attracting the interest of scientific fraternity nowadays, owing to their versatile applications. Plants are the richest bio-resource with chemical entities for synthetic drugs and insecticides (Ncube *et al.,* 2008). To obtain the active ingredients from the plants, several separation techniques were performed (Tiwari *et al*., 2011). Among these separation techniques first and foremost was the extraction, in which separation was possible by using selective solvents (Ncube *et al*., 2008). The choice of solvent was highly influenced by the successful determination of biologically active ingredients from different plant parts, which mainly affected the rate of extraction, percentage yield of extractants, diversity of the phytocompounds, ease of handling of extracts and toxicities of the solvents (Eloff, 1998).

The chemical composition of plant species have received much attention of the distribution of a wide range of secondary metabolites like alkaloids, flavonoids, phenols, steroids, terpenes, glycosides, tannins, fatty acids, proteins and amino acids, phytosterols and some sugars (Sanjay *et al*., 2013). Raghavendra *et al*., 2006 observed the presence of phenols, glycosides, carbohydrates, phytosterols and tannins from the powdered leaf material of *Oxalis corniculata* with different solvents. From *Pycanthu sangolensis*, Onwukaeme *et al*., 2007 detected reducing sugars, phenols, tannins and flavonoids. Vaghasiya and Chanda, 2007 evaluated the methanolic and acetonic extracts of 14 plants belonging to different families for phytochemical analysis, which revealed the presence of tannins, cardiac glycosides, steroids and saponins. Falodun *et al*., 2006 examined the aqueous and methanolic extracts of *Euphorbia heterophylla* and found the

occurrence of flavonoids, saponins, diterpenes and phorbol estersin in the extract.

Secondary metabolites are responsible for many of the biological activities of the plants, when it is exposed to other organisms. Terpenoids, phenloic compounds and alkaloids the major secondary metabolites produced by most of the plants. According to Linne, (2007) alkaloid could alter DNA, selectively deform cells and caused locoism and most of them acted as narcotics. They displayed antimicrobial and anti-parasitic properties also. Adeyemi, 2011 reviewed alkaloids such as, caffeine, cocaine, morphine, and nicotine and amino acids like aspartate, lysine, tyrosine and tryptophan had powerful effects on biological processes of certain insect pests. Caffeine, an alkaloid found in plants such as coffee (*Coffea arabica*), tea (*Camellia sinensis*), and cocoa (*Theobroma cacao*) were toxic to both insects and fungi. Freeman and Beattie, 2008 reported the toxicity of Cyanogenic glycosides, toxic class of nitrogenous compounds that break down to produce hydrogen cyanide (HCN), a lethal chemical that halts cellular respiration in aerobic organisms.

Phenolics, another large group of secondary metabolites consists a wide variety of defense-related compounds including flavonoids, anthocyanins, phytoalexins, tannins, lignin and furanocoumarins. Phytoalexins are isoflavonoids, which exhibited potent antibiotic and antifungal properties that are produced in response to pathogen attack by disrupting pathogen metabolism or cellular structure, but are often pathogen specific in their toxicity (Adeyemi, 2011). Medicarpin produced by alfalfa (*Medicago sativa*), rishitin produced by both tomatoes and potatoes (the Solanaceae family) and camalexin, produced by *Arabidopsis thaliana* were reported by Freeman and Beattie, 2008. Morimoto *et al.,* 2006 reported Pterocarpans, an isoflavones

isolated from the heartwood of *Pterocarpus macrocarpus* Kruz. showed deterrent activity against *Spodoptera litura*.

Adeyemi, 2011reviewed the toxic effect of several secondary metabolites, in which tannins induced toxicity to insects by binding with salivary proteins and digestive enzymes including trypsin and chymotrypsin resulting in protein inactivation. Freeman and Beattie, 2008 observed toxicity of Furanocoumarins, another phenolic compounds, which were activated by ultraviolet light and can be highly toxic to certain vertebrate and invertebrate herbivores due to their integration into DNA, which contributes to rapid cell death.

The largest class of secondary metabolites terpenes/ terpenoids also induced different types of toxicities on target organisms. Asawalam and Hassanali, 2006; Rozman*et al*., 2007 reported Eucalyptol (1,8 - Cineole), isolated from the leaves of *Vernonia amygdalina* essential oil showed significant level of reproductive toxicity and repellency towards *Sitophilus zeamais* weevil. Monoterpenoid esters like Pyrethrins produced by *Chrysanthemum* plants acted as insect neurotoxins (Isman and Machial, 2006). Monoterpenoids alpha and beta pinene isolated from Pine tree were potent insect repellents (Adeyemi, 2011). Phytoecdysones, a triterpene mimics as insect molting hormones, which could disrupt larval development and increase insect mortality (Adeyemi, 2011). Another class of triterpenoids called limonoidsare also well- known for its insect repellent property (Defago *et al*., 2006; Kumar *et al*., 2003). Citronella, an essential oil isolated from lemon grass, *Cymbopogon citratus* contains high limonoid levels and has become a popular insect repellent in the United States due to its low toxicity in humans and biodegradable properties (Adeyemi, 2011). Glycosylated triterpenoids and saponins, which are present in the cell membranes of many plants, could disrupt the cell membranes of invading fungal pathogens. *Gaeumanno*

mycesgraminis, the wheat pathogen was unable to infect oats that contain avenacins, a class of triterpenoid saponin (Freeman and Beattie, 2008).

Pino *et al*., 2013 reviewed some commercially available natural products, which consists of several secondary metabolites such as, pyrethrins I and II, cinerins I and II, jasmolins I and II (Pyrethrum), which acted as axonic poisons (sodium channels agonists) (Isman and Machial 2006; Copping and Duke 2007; Dayan *et al*., 2009). Azadirachtin, dihydroazadirachtin, variety of triterpenoids (nimbin and salannin), from neem plants, acted as moulting inhibitors, ecdysone antagonists, antifeedant/ repellent, physical smothering and desiccation (Isman and Machial, 2006; Copping and Duke 2007; Dayan *et al.,* 2009). Rotenone and deguelin (isoflavonoids) from *Derris, Lonchocarpus*and *Tephrosia* species acted as mitochondrial cytotoxin (Isman, and Machial, 2006; Copping and Duke 2007). (S)- isomer, (RS)-isomers, and (S)-isomer of nicotine sulphate from the plant *Nicotiana* Spp. also found as a neurotoxin (acetylcholine agonist) (Isman and Machial, 2006; Copping and Duke 2007; Dayan *et al*., 2009). Ryanodine, ryania, 9, 21-didehydroryanodine (alkaloids) obtained from the plant *Ryania* spp. (*Ryaniaspeciosa*Vahl), was a neuromuscular poison (calcium channel agonist) (Isman and Machial, 2006; Copping and Duke 2007; Dayan *et al*., 2009). Cevadine, veratridine mixture of alkaloids from the plant *Schoenocaulon*s pp. acted as axonic poisons (sodium channels agonists, heart and skeletal muscle cell membranes) (Isman, 2006; Copping and Duke 2007; Dayan *et al*., 2009). Cinnamaldehyde obtained from *Cassia tora* L., and *Cassia obtusifolia* was found to disrupt fungal membranes, repellent and attractant (Copping and Duke 2007 and Dayan *et al*., 2009). Karanjin, isolated from *Derris indica* (Lam.) Bennet acted as antifeedant/repellent and insect growth regulator (Copping and Duke 2007). Phenethyl propionate component of peppermint oil (*Mentha piperita* (L.) and peanut oil was found as repellent (Isman and Machial, 2006; Copping and Duke 2007 and Dayan *et al.,* 2009). Straight-chain wax esters from

Simmondsia californica Nutt., and *S.chinensis* Link was found to be as suffocation (eggs and immature life stages), repellent, blocking access to oxygen (Copping and Duke 2007 and Dayan *et al*., 2009). Eugenol (mixture of several predominantly terpenoid compounds) isolated from *Syzygium aromaticum* and *Eugenia caryophyllus* Spreng found as neurotoxic, interfering with the neuromodulator octopamine (Isman and Machial, 2006; Copping and Duke 2007; Dayan *et al*., 2009). Thymol and carvacrol obtained from *Thymus vulgaris* L., *Thymus* spp. acted as neurotoxic, interfering with GABA-gated chloride channels (Isman and Machial, 2006; Copping and Duke 2007). 1,8-cineole (borneol, camphor, monoterpenoids) from the plants *Rosmarinus officinalis* was found to be octopamine antagonists; membrane disruptors (Isman and Machial, 2006; Copping and Duke 2007). Cinnamaldehyde from *Cinnamomum zeylanicum*acted as octopamine antagonist and membrane disruptors (Copping and Duke 2007). Citronellal and citral obtained from *Cymbopogon nardus*, *Cymbopogon citratus* Stapf., *Cymbopogon flexuosus* D.C was found to be octopamine antagonists; membrane disruptors (Copping and Duke 2007).

From the literature reviews, it has been cleared that all most all the secondary metabolites derived from plants could induce toxicity on target species one or the other way. Present study also focused on the phytosterilant toxicity of the secondary metabolites which could be obtained from the selected plant extracts of *Sterculia guttata*, *Andrographis paniculata*, *Bougainvillea spectabilis* and *Polyalthia longifolia* on the filarial vector mosquito *Culex quinquefasciatus*.

3.3 MATERIALS AND METHODS

3.3.1 Phytochemical Screening

Phytochemical examinations were carried out for all the extracts as per the standard method of Tiwari *et al*., 2011. The methanolic crude extracts of *S. guttata* (Seed), *A. paniculata* (Leaf), *B. spectabilis* (Leaf) and *P. longifolia* (Seed) were subjected to following experiments;

1. Detection of alkaloids:

Extracts were dissolved individually in dilute Hydrochloric acid and filtered.

a. Mayer's Test:

Filtrates were treated with Mayer's reagent (Potassium Mercuric Iodide). Formation of a yellow coloured precipitate indicated the presence of alkaloids.

b. Wagner's Test:

Filtrates were treated with Wagner's reagent (Iodine in Potassium Iodide). Formation of brown/ reddish precipitate indicated the presence of alkaloids.

2. Detection of Carbohydrates:

Extracts were dissolved individually in 5ml distilled water and filtered. The filtrates were used to test for the presence of carbohydrates.

a. Molisch's Test:

Filtrates were treated with 2 drops of alcoholic α- naphthol solution in a test tube. Formation of the violet ring at the junction indicated the presence of carbohydrates.

b. Benedict's Test:

Filtrates were treated with Benedict's reagent and heated gently. Orange red precipitate indicated the presence of reducing sugars.

3. Detection of Glycosides:

5gm of extract was hydrolized with concentrated hydrochloric acid for 2hrs on a water bath, filtered and the hydrolysate is subjected to the following test.

a. Borntrager's Test:

To 2ml of filtered hydrolysate, 3ml of $CHCl_3$ was added and shaken, $CHCl_3$ layer is separated and 10% NH_3 solution was added to it. Pink colour indicated the presence of glycosides.

b. Legal's Test:

50mg of extract was dissolved in pyridine: sodium nitroprusside solution was added and made alkaline using 10% NaOH. The presence of glycosides indicated the formation of pink colour.

4. Detection of Saponins:

a. Froth Test:

Crude methanolic extracts of selected plants *S. guttata* (Seed), *A. paniculata* (Leaf), *B. spectabilis* (Leaf) and *P. longifolia* were diluted with distilled water to 20ml and this was shaken in a graduated cylinder for 15minutes. Formation of 1cm layer of foam indicated the presence of saponins.

b. Foam Test:

0.5gm of extract was shaken with 2ml of water. If foam produced persisted for ten minutes it indicated the presence of saponins.

5. Detection of phytosterols:

a. Salkowski's Test:

Extracts were treated with chloroform and filtered. The filtrates were treated with few drops of Conc. Sulfuric acid (H_2SO_4), shaken and allowed to stand. Appearance of golden yellow colour indicated the presence of triterpenes.

b. Libermann Burchard's Test:

Extracts were treated with chloroform and filtered. The filtrates were treated with few drops of acetic anhydride, boiled and cooled. Conc. H_2SO_4 was added. Formation of brown ring at the junction indicated the presence of phytosterols.

6. Detection of Phenols:

a. Ferric Chloride Test:

Extracts were treated with 3-4 drops of ferric chloride solution. Formation of bluish black colour indicated the presence of phenols.

7. Detection of Tannins:

a. Gelatin Test:

To the extract, 1% gelatin solution containing sodium chloride was added. Formation of white precipitate indicated the presence of tannins.

8. Detection of Flavonoids:

a. Alkaline Reagent Test:

Extracts were treated with few drops of sodium hydroxide solution. Formation of intense yellow colour, which becomes colourless on addition of dilute acid, indicated the presence of flavonoids.

b. Lead Acetate Test:

Extracts were treated with few drops of lead acetate solution. Formation of yellow colour precipitate indicated the presence of flavonoids.

9. Detection of Proteins and Amino Acids:

a. Xanthoproteic Test:

The extracts were treated with few drops of Conc. Nitric acid. Formation of yellow colour indicated the presence of proteins.

b. Ninhydrin Test:

To the extract, 0.25% w/v ninhydrin was added and boiled for few minutes. Formation of blue colour indicated the presence of aminoacids.

10. Detection Diterpenes:

a. Copper Acetate Test:

Extracts were dissolved in water and treated with 3-4 drops of copper acetate solution. Formation of emerald green colour indicated the presence of diterpenes.

11. Detection of Terpenoids:

a. Salkoeki's Test:

1ml of chloroform was added to 2ml of each extract followed by a few drops of Conc. H_2SO_4. A reddish- brown precipitate produced immediately indicated the presence of terpenoids.

12. Detection of Quinones:

A small amount of extract was treated with Conc. HCl and observed for the formation of yellow precipitate or colouration.

13. Detection of Phlobatannins:

A. Precipitate Test:

Deposition of a red precipitate when 2ml of extract was boiled with 1ml of 1% aqueous hydrochloric acid indicated the presence of phlobatannins.

14. Detection of Fixed Oils and Fats:

a. Spot Test:

Press a small quantity of extract separately between two filter papers. Oil stains on the paper indicated the presence of fixed oils.

b. Saponification Test:

Add a few drops of 0.5N alcoholic KOH to a small quantity of extract along with a drop of phenolphthalein. Heat the mixture on water bath for 1- 2hrs. Formation of soap or partial neutralization of alkali indicated the presence of fixed oils and fats.

15. Detection of Gums and Mucilage:

100mg extract was dissolved in 10ml of distilled water and to this 2ml of absolute alcohol was added with constant stirring. White or cloudy precipitate indicated the presence of gums and mucilage.

3.3.2 Qualitative Analysis by Thin Layer Chromatography (TLC)

Thin layer Chromatography (TLC) was carried out according to the protocol of Chakraborty *et al*., 2010, to confirm the principle components present in the selected plant extracts *S. guttata, A. paniculata, B. spectabilis* and *P. longifolia*. Different solvent systems with different polarities were prepared and TLC studies were carried out.

a. Preparation of TLC plates:

15gms of silica gel (mesh size) was weighed and made to a homogenous suspension with 30ml methanol for two minutes. The suspension was uniformly distributed over the plates and air dried until the transparency of the layer disappeared. The plates were dried in hot air oven at 110^0C for 30 minutes.

b. Preparation of Sample solution:

Samples were dissolved in a suitable solvent and then spotted on pre-coated plates 2cm above from its bottom, using capillary tubes and developed in a TLC chamber using suitable mobile phase.

c. Development of Chromatogram:

After the application of the sample, TLC plates were kept in the TLC chamber and allowed the mobile solvents to move through adsorbent phase up to ¾th of the plate and different coloured spots were obtained. The developed TLC plates were air dried and observed under ultra violet light UV at 254nm-366nm.They were also sprayed with different spraying reagents for few minutes for the development of colour in separated bands. The movement of analyte was expressed by its Retention Factor (R*f*) and R*f* values for different spots were calculated and determined.

$$R_f = \frac{\text{Distance travel by the solute}}{\text{Distance travel by the solvent}}$$

3.3.3 GC/MS (Gas Chromatography- Mass Spectrometry) analysis

GC/MS analysis was carried out in Care Keralam Pvt. Ltd., Kinfra Park, Koratty, Thrissur, Kerala, India. The phytochemical investigation of column gradients of selected plants such as *S. guttata* (MeOH: EA- 4:1), *A. paniculata* (MeOH: EA- 4:1), *B. spectabilis* (H: EA- 5:5) and *P. longifolia*

(MeOH: EA- 4:1) were performed on a GC/MS equipment- Agilent, GC – 7890A, MS – 5975C. Experimental conditions of GC/MS system were used as follows: DB – 5MS capillary standard non-polar column, dimension– 30Mts, ID- 0.25mm, film thickness 0.25um. Flow rate of mobile phase-carrier gas- He was set at 1.0 ml/min. In the gas chromatography part, temperature program (Oven temperature) was 40^0 C for 5 min. – 5 ^{0} C/min to 280 ^{0} C- hold for 10 min and injection volume was 1.0 ul. Sample preparation was done by 50 ul diluted to 1.5 ml with Methanol and were run fully at a range of 50- 650m/z and the results were compared by using spectral library search program.

3.3.4 LC-Q-TOF-MS analysis

LC-Q-TOF-MS analysis was carried out in the Department of Environmental Science, M G University, Kottayam, Kerala, India. The column gradients of selected plant extracts were subjected to Liquid Chromatography/ quadrupole time - of- flight mass spectrometry *(LC-Q-TOF-MS)* for metabolite profiling of the phytochemicals presented on *S. guttata* (Seed), *A. paniculata* (Leaf), *B. spectabilis* (Leaf) and *P. longifolia* (Seed) using the solvent gradient elution method. LC-Q-TOF-MS was conducted by using an Acquity H class (Waters) system coupled to an Ultra Performance Liquid Chromatography system. The instrument conditions are shown below:

LC run Conditions

Column (BEH C18 column (50 mm × 2.1 mm × 1.7 μm)

Mobile phase	LC-MS grade solvents	
Time (min)	Water+0.1% formic acid	Methanol
0.1	95	5
6	5	95
6.50	5	95
8	95	5
9	95	5

Total run time	9 minutes
Flow rate	0.3 ml/min

Q- TOF MS conditions

Ion mode	ESI Positive mode
Capillary Voltage	3 kV
Sample cone	30V
Extraction cone	1V
Source temperature	135^0 C
Desolvation temperature	350^0 C
Gas flow	Nitrogen Gas
Cone Gas	50 (L/H)
Desolvation gas	900 (L/H)

An overview of tentatively identified compound was highlighting retention time, precursor ion, elemental composition, exact mass, m/z, error and identification of metabolites.

3.3.5 NMR (Nuclear Magnetic Resonance) Spectroscopy analysis

The NMR studies were carried out in STIC, CUSAT, Cochin, Kerala, India. The purified samples were placed in an inert solvent (deuterated solvent) and the solution was placed between the poles of a powerful magnet. The different chemical shifts of the proton according to their molecular environments within the molecule were measured in the NMR apparatus relative to a standard, tetramethyl silane (TMS).

The NMR spectra were recorded on a Bruker AVANCE-III 400 spectrometer with Me4Si (TMS) as internal standard. Two to five milligrams of samples were dissolved in deuterated solvent in NMR tube and sonicated for two minutes. The 400 MHz 1H NMR spectra were recorded from a CDCI3 solution of sample at 25^0C, 3.9846387s acquisition time and 20ppm Spectral width using Standard Bruker Procedures. The 100 MHz 13C NMR spectra were recorded from a CDCI3 solution of sample using 1H decoupling,1.3631988s acquisition time and 240ppm Spectral width. 1H NMR and 13C NMR chemical shifts were referred to CHCI3 & CDCI3 (δ=7.26 ppm, δ=77.2 ppm respectively). Chemical shift were reported in ppm (δ), and the signals described as s (singlet), d (doublet), t (triplet), q(quartet), m (multiplet), b (broad), dd (doublet of doublet), dt (doublet of triplet).

3.3.6 Formulation

Formulation of the selected plant extracts for field application was carried out according to the protocol from Manual on Phyto- Insecticide Research, 2016. Syrup formulations of fractionated column extracts of selected plants were prepared for field application to induce sterility among the filarial vector *Cx. quinquefasciatus*. The preparation of the formulations is shown below:

Syrup Formulation

One liter of distilled water added with 850g of sucrose in a conical flask was mixed thoroughly and then added with 100ml of selected plant extracts and prepared syrup.

3.3.7 Small Scale Field trails

The efficacy and the phytosterilant activity of different plant extracts against natural populations of *Cx. quinquefasciatus*were evaluated in two sites with triplicates. Simulated Small- scale field trials were carried out according to the protocol of **WHO (2005) (Plates 63 & 64)**.

Plate- 63 **Plate- 64**

(Simulated Small Scaled Field Trials)

Three replicates of each type of habitats were randomly selected for each dosage of formulation, with an equal number of controls. The sizes of the plots were recorded taking into account on the surface area and depth. Treatment concentrations were calculated on the basis of surface area of the habitat. Appropriate numbers of samples were taken from each habitat based on the type and size of the habitat. Larval instars and pupae from each sample

were counted and recorded. Syrup formulation from each of the plant extracts were applied using measuring cylinder. Post- treatment immature abundance were monitored weekly until the density of the pupae in the treated habitats reached a level compared to that in control. Data were recorded. The pupae were collected and brought to the laboratory for adult emergence. The adults were maintained in the laboratory for fecundity and fertility experiments.

a. Fecundity and Fertility Experiments

These experiments were carried out According to the protocol of **Saxena *et al.* (1993),** conducted by taking equal numbers of male (10) and female (10) adult mosquitoes from the treated and untreated sets and placed in the mating cages (20 x 20 x 20 cm) into the following groups;

i. Treated females X Treated males **(T ♀ x T♂)**

ii. Treated females X Untreated males **(T♀ x UT)**

iii. Untreated females X Treated males **(UT ♀ x T ♂)**

iv. Untreated females X Untreated males **(UT♀xUT ♂)**

Three days after the blood meal, eggs were collected daily from the plastic bowls.Just after hatching, larvae were transferred to the enamel trays containing saline solution. Larval mortality, developmental period, Growth Index (GI) and Sterility Index (SI) were recorded by using the following formulas.

i. Growth Index (GI) = $\frac{\text{\% of adult Emergence}}{\text{Average developmental period}}$

ii. % Control of reproduction = $\frac{V1 - V2}{V1} \times 100$

V1= No. of viable eggs/ female in control

V2= No. of viable eggs/ female in test

iii. Fecundity rate = $\frac{\text{No. of eggs}}{\text{Total no. of females}}$

iv. Fertility (%) = $\frac{\text{No. of eggs treated}}{\text{No. of eggs in control}} \times 100$

v. Sterility Index (SI) = $100 - \frac{\text{Treated no. of eggs x \% of hatching}}{\text{Control no. of eggs x \% of hatching}} \times 100$

3.4 RESULTS

3.4.1 Phytochemical Screening

Results obtained for qualitative screening of phytochemicals in the selected plant extracts such as *S. guttata*, *A. paniculata*, *B. spectabilis* and *P. longifolia* are presented on the **Table 47.** Of the fifteen phytochemicals screened, some of them are absent in certain plants. In the case of *S. guttata* methanolic seed extract, presence of alkaloids, carbohydrates, glycosides, saponins, phytosterols, tannins, flavonoids, proteins and amino acids, diterpenes, quinones, oils and fats and gums and mucilage were confirmed. But phenols, terpenoids and phlobatannins were absent. Whereas, methanolic leaf extract of *A. paniculata* possessed alkaloids, carbohydrates, glycosides, phytosterols, phenols, tannins, flavonoids, diterpenes, terpenoids, phlobatannins, gums and mucilage. Saponins, proteins and amino acids, quinones and oils and fats were found to be absent. Methanolic leaf extract of *B. spectabilis* consisted most of the phytochemicals such as alkaloids, carbohydrates, glycosides, saponins, phytosterols, phenols, tannins, flavonoids, diterpenes, terpenoids, phlobatannins and gums and mucilage, but proteins and amino acids, quinones and fixed oils and fats were absent. Of the fifteen phytochemicals, eight were found to be present in methanolic seed extract of *P. longifolia*. They are alkaloids, glycosides, saponins, proteins and amino acids, diterpenes, phlobatannins, fixed oils and fats and gums and mucilage. Whereas, carbohydrates, phytosterols, phenols, tannins, flavonoids, terpenoids and quinones were found to be absent. This suggested that the seed extract of *S. guttata* and *P. longifolia* and leaf extracts of *A. paniculate* and *B. spectabilis* offer a wide array of phytochemicals.

Table 47- Phytochemical Screening of the selected plant extracts

SI No.	Name of Tests	*S. guttata*	*A. paniculata*	*B. spectabilis*	*P. longifolia*
1.	**Detection of alkaloids**				
	a. Mayer's Test	**+ ve**	**+ ve**	**+ ve**	**+ ve**
	b. Wagner's Test	**+ ve**	**+ ve**	**+ ve**	**+ ve**
2.	**Detection of Carbohydrates**				
	a. Molisch's Test	**+ ve**	**+ ve**	**+ ve**	**- ve**
	b. Benedict's Test	**+ ve**	**+ ve**	**+ ve**	**- ve**
3.	**Detection of Glycosides**				
	a. Bontrager's Test	**+ ve**	**+ ve**	**+ ve**	**+ ve**
	b. Legal's Test	**+ ve**	**+ ve**	**+ ve**	**+ ve**
4.	**Detection of Sapononis**				
	a. Froth Test	**+ ve**	**- ve**	**+ ve**	**+ ve**
	b. Foam Test	**+ ve**	**- ve**	**+ ve**	**+ ve**
5.	**Detection of Phytosterols**				
	a. Salkowski's Test	**+ ve**	**+ ve**	**+ ve**	**- ve**
	b. Libermann Burchard's Test	**+ ve**	**+ ve**	**+ ve**	**- ve**
6.	**Detection of Phenols**				
	a. Ferric- Chloride Test	**+ ve**	**+ ve**	**+ ve**	**+ ve**
7.	**Detection of Tannins**				
	a. Gelatin Test	**+ ve**	**+ ve**	**+ ve**	**- ve**

8.	**Detection of Flavonoids** **a. Alkaline Reagent Test**	**+ ve**	**+ ve**	**+ ve**	**- ve**
	b. Lead Acetate Test	**+ ve**	**+ ve**	**+ ve**	**- ve**
9.	**Detection of Proteins and Amino acids** **a. Biuret Test**	**+ ve**	**- ve**	**- ve**	**+ ve**
	b. Ninhydrin Test	**+ ve**	**- ve**	**- ve**	**+ ve**
10.	**Detection of Diterpenes** **a. Copper Acetate Test**	**+ ve**	**+ ve**	**+ ve**	**+ ve**
11.	**Detection of Terpenoids** **a. Salkowski's Test**	**- ve**	**+ ve**	**+ ve**	**+ ve**
12.	**Detection of Quinones**	**+ ve**	**- ve**	**- ve**	**- ve**
13.	**Detection of Phlobatannins** **a. Precipitate Test**	**- ve**	**+ ve**	**+ ve**	**+ ve**
14.	**Detection of Fixed Oils and Fats** **a. Spot Test**	**+ ve**	**- ve**	**- ve**	**+ ve**
	b. Saponification Test	**+ ve**	**- ve**	**- ve**	**+ ve**
15.	**Detection of Gums and Mucilage**	**+ ve**	**+ ve**	**+ ve**	**+ ve**

+ ve = Present, - ve = Absent

3.4.2 Qualitative analysis by Thin Layer Chromatography (TLC)

TLC profiling of the methanolic extract of selected plants confirmed the presence of various phytochemicals **(Tables 48, 49, 50 & 51)**.

a. TLC for Alkaloids

TLC of methanolic seed extract of *S. guttata*, leaf extracts of *A. paniculata* and *B. spectabilis* and seed extract of *P. longifolia* revealed the presence of alkaloid compounds having R_f values 0.35, 0.95, 0.94 and 0.90 respectively, when a solvent phase of Acetone: Ethyl acetate (4:6) was used **(Tables 48, 49, 50 & 51) (Plate 65)**.

b. TLC for Phenolic compounds

TLC profiling of the selected plant extracts confirmed the presence of phenolic compounds with R_f values 0.87, 0.80, 0.96 and 0.82 respectively for *S. guttata*, *A. paniculata*, *B. spectabilis* and *P. longifolia* with a solvent phase Methanol: Water (3:6) **(Tables 48, 49, 50 & 51) (Plate67)**.

c. TLC for Phytosterols

Presence of Phytosterols in the selected plants, *S. guttata, A. paniculate* and *B. spectabilis* confirmed by TLC profiling indicated, bands with R_f values 0.51, 0.37 and 0.63 and 0.84 respectively. No bands were noticed in the case *P. longifolia*. The solvent phase used was Hexane: Ethyl acetate (5:2) **(Tables 48, 49, 50 & 51) (Plate 68)**.

d. TLC for Flavonoids

Flavonoids were confirmed with R_f values 0.95, 0.93 and 0.79 and 0.19 respectively for *S. guttata, A. paniculata* and *B. spectabilis*. No bands were detected in TLC profiling of *P. longifolia*, indicated the absence of flavonoids with a solvent phase n-butanol: Acetic acid: Water (3:6:9) **(Tables 48, 49, 50 & 51) (Plate 66)**.

e. TLC for Saponins

TLC profiling carried out to detect the presence of Saponins for the selected plant extracts revealed the confirmation with R_f values 0.14, 0.28 and 0.61, 0.81 and 0.97 and 0.52 and 0.75 respectively for *S. guttata, B. spectabilis* and *P. longifolia*. But *A.paniculata* could not produce bands, indicated the absence of saponins, with a solvent phase Chloroform: Glacial acetic acid: Methanol: Water (3: 1: 0.5: 0.5) **(Tables 48, 49, 50 & 51) (Plate 69)**.

f. TLC for Terpenoids

TLC profiling for Terpenoids present in the selected plant extracts revealed the presence of the compounds with R_f values 0.20, 0.40 and 0.81, 1.0 and 0.90 for *A. paniculata, B. spectabilis* and *P. longifolia* respectively. No bands were detected in the leaf extract of *A. paniculata*, which indicated the absence of terpenoids in *A. paniculata*. The solvent phase used was Benzene: ethyl acetate (1:1) **(Tables 48, 49, 50 & 51) (Plate 70)**.

Table 48- TLC analysis of the phytochemicals presented in the seed extract of *S. guttata*

SI No.	Compounds	Mobile Phase	Ratio	R_f value
1	Alkaloids	Acetone:ethyl acetate	2:3	0.35
2	Phenolic compounds	Methanol: water	1.5: 3	0.87
3	Phytosterols	Hexane: ethyl acetate	2.5: 1	0.51
4	Flavanoids	n-butanol: Acetic acid: Water	3:6:9	0.95
5	Saponins	Chloroform: glacial acetic acid: methanol: water	3: 1: 0.5: 0.5	0.14 0.28 0.61
6	Terpenoids	Benzene: ethyl acetae	1: 1	-------

Table 49- TLC analysis of the phytochemicals presented in the leaf extract of *A. paniculata*

SI No.	Compounds	Mobile Phase	Ratio	R_f value
1	Alkaloids	Acetone:ethyl acetate	2:3	0.95
2	Phenolic compounds	Methanol: water	1.5: 3	0.80
3	Phytosterols	Hexane: ethyl acetate	2.5: 1	0.37 0.63
4	Flavanoids	n-butanol: Acetic acid: Water	3:6:9	0.79 0.93
5	Saponins	Chloroform: glacial acetic acid: methanol: water	3: 1: 0.5: 0.5	-----
6	Terpenoids	Benzene: ethyl acetae	1: 1	0.20 0.40 0.82

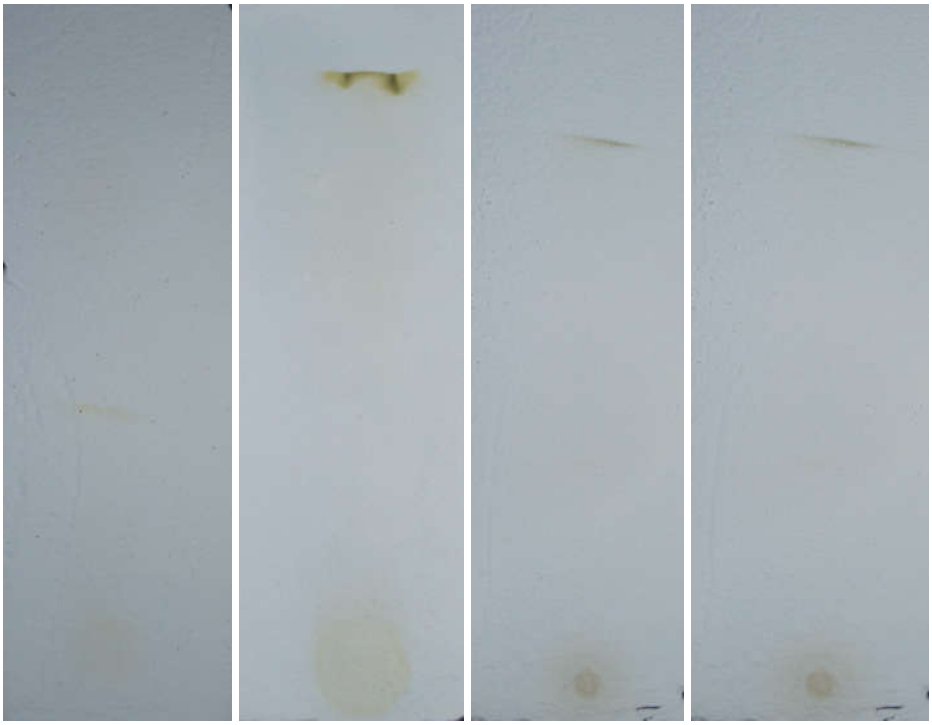
Table 50- TLC analysis of the phytochemicals presented in the leaf extract of *B. spectabilis*

SI No.	Compounds	Mobile Phase	Ratio	R_f value
1	Alkaloids	Acetone:ethyl acetate	2:3	0.94
2	Phenolic compounds	Methanol: water	1.5: 3	0.96
3	Phytosterols	Hexane: ethyl acetate	2.5: 1	0.84
4	Flavanoids	n-butanol: Acetic acid: Water	3:6:9	0.19
5	Saponins	Chloroform: glacial acetic acid: methanol: water	3: 1: 0.5: 0.5	0.81 0.97
6	Terpenoids	Benzene: ethyl acetae	1: 1	1.0

Table 51- TLC analysis of the phytochemicals presented in the seed extract of *P. longifolia*

SI No.	Compounds	Mobile Phase	Ratio	R_f value
1	Alkaloids	Acetone:ethyl acetate	2:3	0.90
2	Phenolic compounds	Methanol: water	1.5: 3	0.82
3	Phytosterols	Hexane: ethyl acetate	2.5: 1	------
4	Flavanoids	n-butanol: Acetic acid: Water	3:6:9	------
5	Saponins	Chloroform: glacial acetic acid: methanol: water	3: 1: 0.5: 0.5	0.52 0.75
6	Terpenoids	Benzene: ethyl acetae	1: 1	0.90

Photo Plates of TLC profiling of selected plants *S. guttata*, A. *paniculata, B. spectabilis* and *P. longifolia.*

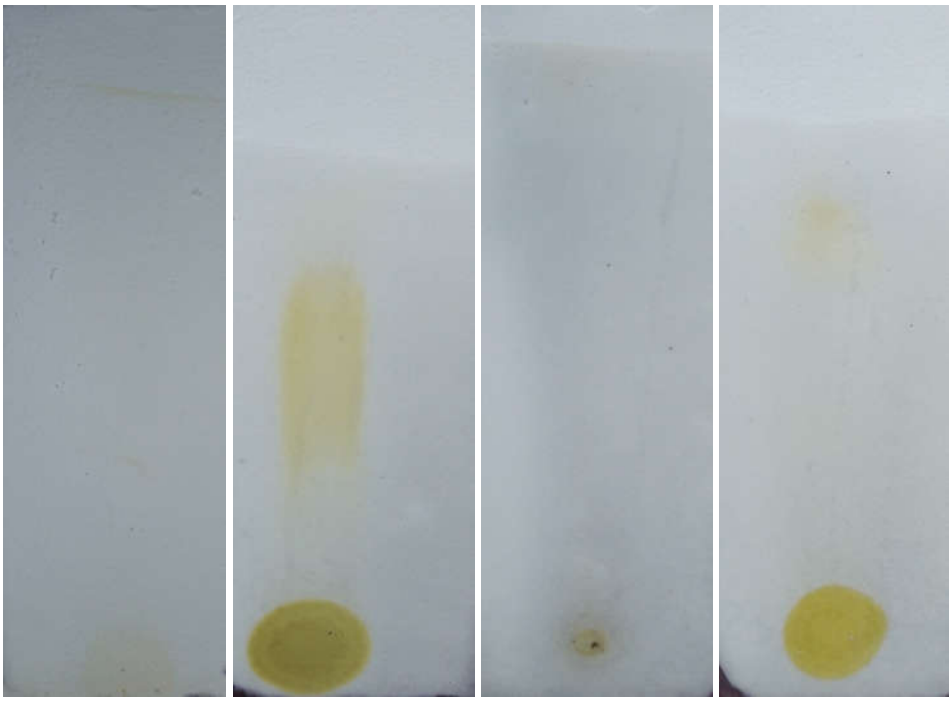


(a) (b) (c) (d)

Plate 65- Photo plates of TLC of Alkaloids (a. *S. guttata*, b. *A. paniculata*, c. *B. spectabilis* and d. *P. logifolia*).

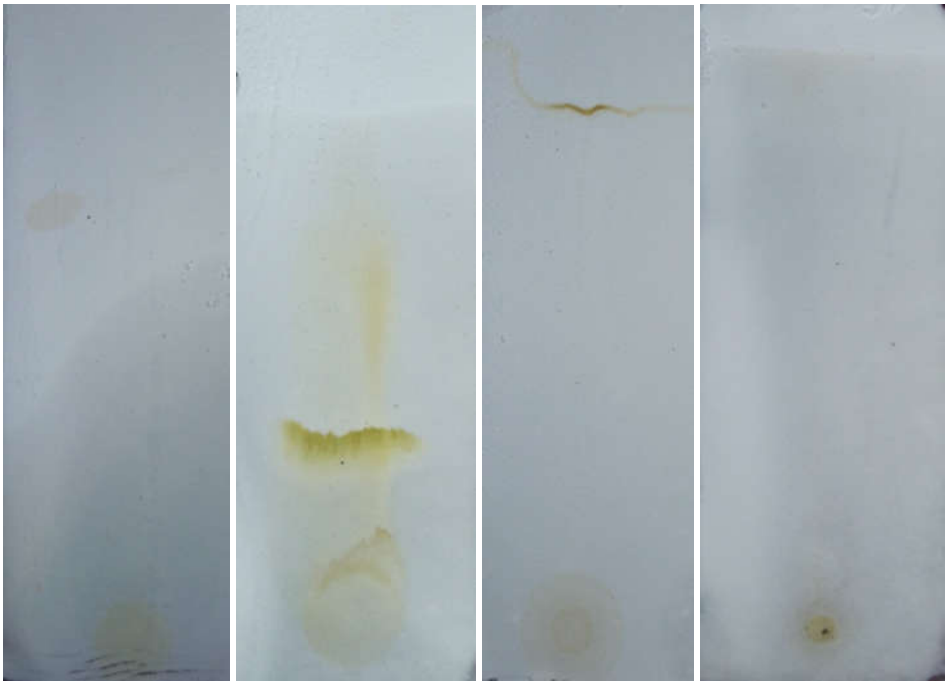
(a) **(b)** **(c)** **(d)**

Plate 66- Photo plates of TLC of Flavonoids (a. *S. guttata*, b. *A. paniculata*, c. *B. spectabilis* and d. *P. logifolia*).



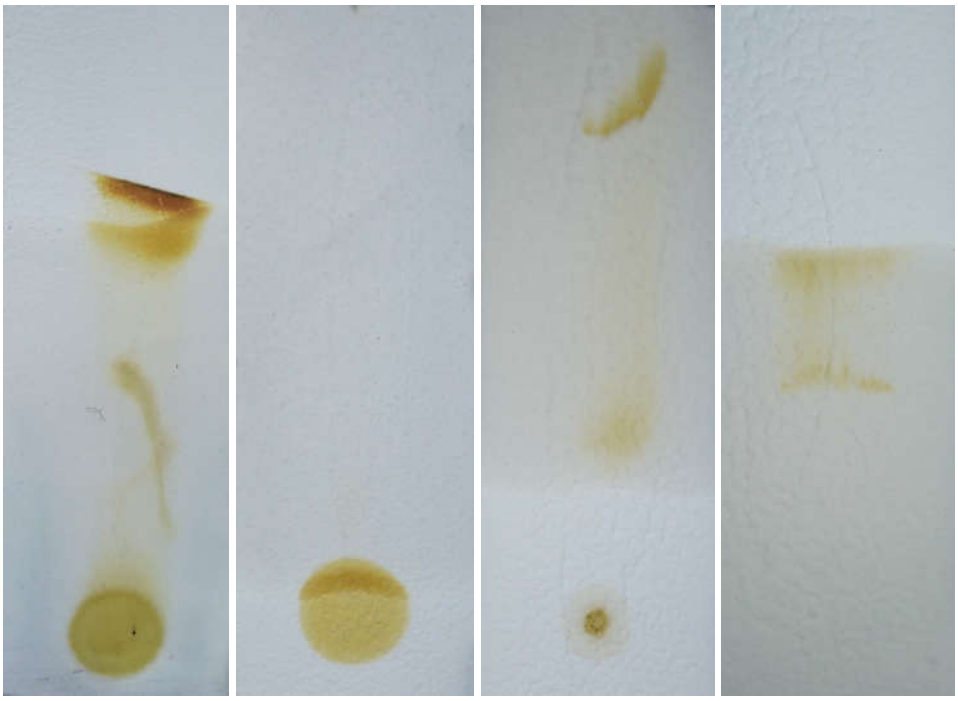
(a) **(b)** **(c)** **(d)**

Plate 67- Photo plates of TLC of Phenolic compounds (a. *S. guttata*, b. *A. paniculata*, c. *B. spectabilis* and d. *P. logifolia*)



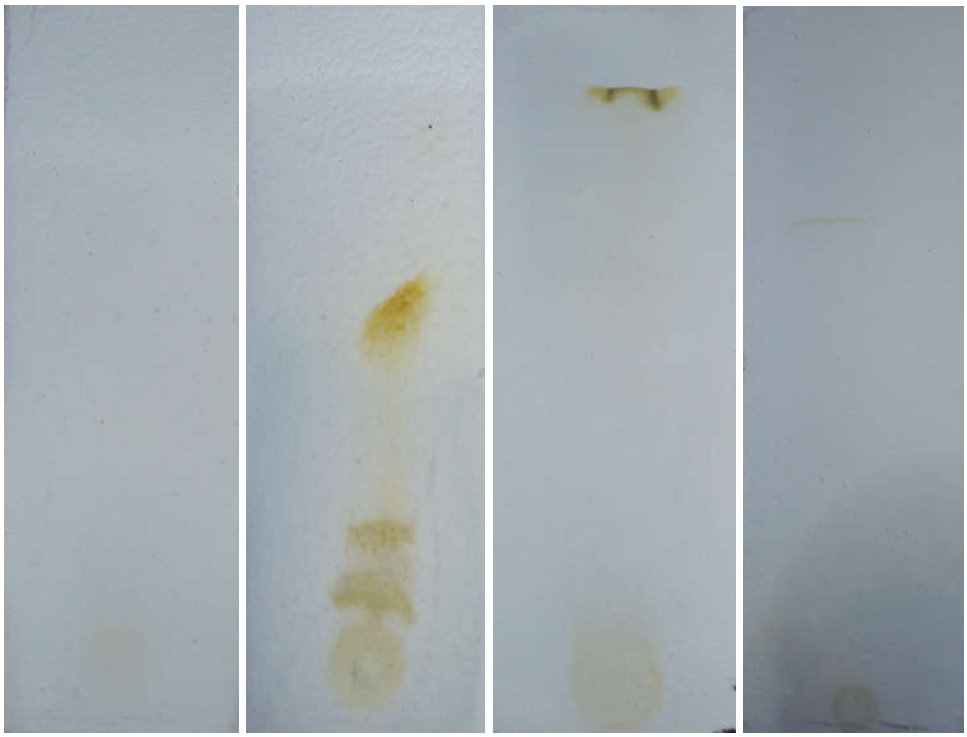
(a) **(b)** **(c)** **(d)**

Plate 68- Photo plates of TLC of Phytosterols (a. *S. guttata*, b. *A. paniculata*, c. *B. spectabilis* and d. *P. logifolia*)



(a) **(b)** **(c)** **(d)**

Plate 69- Photo plates of TLC of Saponins (a. *S. guttata*, b. *A. paniculata*, c. *B. spectabilis* and d. *P. logifolia*)



(a) **(b)** **(c)** **(d)**

Plate 70- Photo plates of TLC of Terpenoids (a. *S. guttata*, b. *A. paniculata*, c. *B. spectabilis* and d. *P. logifolia*)

3.4.3 GC/MS analysis of column fractions of selected plant extracts

Gas Chromatography Mass Spectroscopy (GC/MS) analysis of selected plant extracts were carried out and the GC/MS profile of the compounds identified is given in the **Figures 34, 35, 36 & 37 and Tables 52, 53, 54 & 55**respectively**.** The peaks in the chromatogram were integrated and were compared with the database of spectrum of known components stored in the GC/MS library. The total ion Chromatogram (TIC) of *S. guttata* (MeOH: EA-4:1) is shown in the **Figure- 34** and the detailed tabulations of GC/MS

analysis of the extracts are given in the **Table- 42**respectively. GC/MS profile revealed the presence of various phytochemicals such as fatty acids, heterocyclic compounds etc.

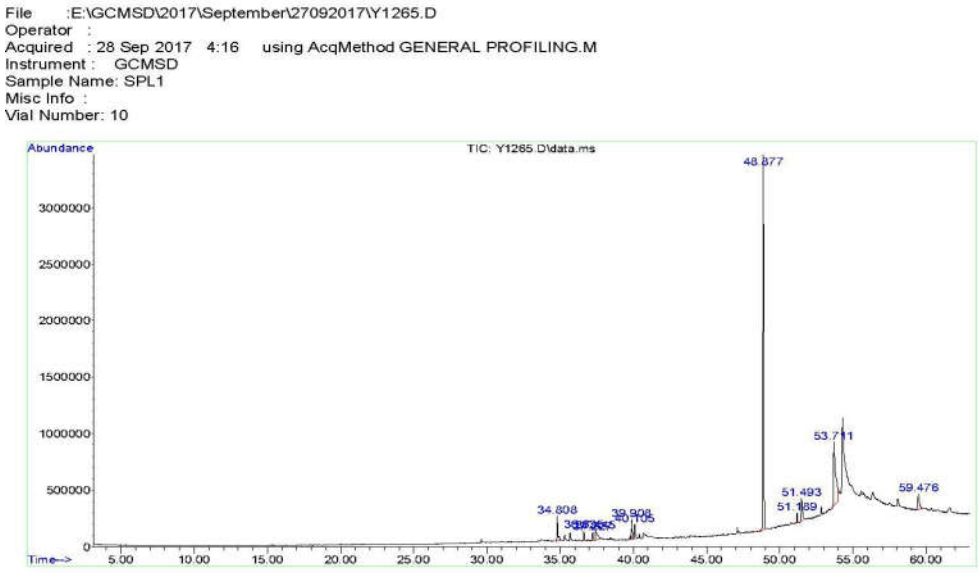
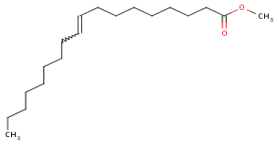
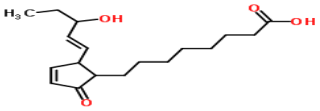


Figure 34- Total Ion Chromatogram (TIC) of seed extract of *S. guttata* (MeOH: EA- 4:1)

Table 52- Phytocomponents identified in the seed extract of *S. guttata* (MeOH: EA- 4:1) by GC/MS Peak report TIC

Peak	Retention Time (RT)	Area (%)	IUPAC Name, Chemical Formula, Common Name	Chemical Structure	Nature and Uses
1		70.76	Methyl hexadecanoate (Hexadecanoic acid, methyl ester) $C_{17}H_{34}O_2$ Palmitic acid		Saturated fatty acid, Food additives as flavouring agents.
	26.167	5.91	Methyl octadecanoate (Octadecanoic acid, methyl ester) $C_{19}H_{38}O_2$ Stearic acid		
2	30.385	6.61	Methyl(Z)-octadec-9-enoate (9- Octadecanoic acid, methyl ester) $C_{19}H_{36}O_2$ Oleic acid (Methyl oleate)		Antifoaming agent and fermentation nutrient. Clear to amber liquid, Food additives, flavouring agent.
3					
	30.774	4.45	8-(2-octylcyclopropen-1-yl)octanoic acid (Methyl 9,10-methylene-octadec-9-enoate) $C_{19}H_{34}O_2$ Sterculic acid		Cyclopropenic Fatty acid, Cyclopropene ring causes certain abnormalities in animals.
	31.429				
4	31.764				
	32.276	5.87	9, 12-octadecadienoic acid (z-z)-methyl ester $C_{19}H_{34}O_2$ Linoleic acid		Used as a flavouring ingredient.
6					
7		6.45	7-(2-octylcyclopropen-1-yl)heptanoic acid (Methyl 2-octylcycloprpene-1-heptanoate) $C_{18}H_{32}O_2$ Malvalic acid		Used as an edible oil

Methanol Soxhlet extract of *S. guttata* seed powder yielded as brownish yellow viscous oil. The seed oil was fractionated by Column Chromatography using silica gel as an adsorbent and was subjected to GC/MS analysis to detect the compounds **(Table 52)**. The compounds were found to be hexadecanoic acid methyl ester (Palmitic acid), octadecanoic acid methyl ester, methyl 9,10-methylene-octadec-9-enoate (Sterculic acid), 9, 12-octadecadienoic acid (z-z)-methyl ester (Linoleic acid), Methyl 2-octylcyclopropene 1-heptanoate (Malvalic acid), n-hexadecanoic acid, 9-octadecanoic acid (z)- methyl ester and heptadecanoic acid methyl ester. Confirmation of the compounds carried out by comparing it with established spectral libraries. Percent area indicated the abundance of the particular compounds and it showed higher quantity of Palmitic acid (70.76%) from the five compounds. From these, it could be suggested that the GC/MS analysis of the seed extract of *S. guttata* revealed the presence of these compounds, which might have contributed to the phytosterilant activity of the plant on filarial vector *Cx. quinquefasciatus*.

The phytoconstituents presented in column fractionated leaf extracts of *A. paniculata* was identified by GC/ MS analysis**.** The total ion Chromatogram (TIC) of *A. paniculata* (MeOH: EA- 4:1) is shown in the **Figure 35.** The active components with their retention time, percent area, IUPAC name and nature and activity are presented on **Table 53.** Five compounds were identified and among these 'Squalane' (Pentadecane, 2,6,10,14-tetramethyl-74.13%) was found to be highest concentration and Bicyclo[3.1.1]heptanes, 2,6,6-trimethyl-, (1.alpha., 2.beta., 5.alpha.,), 2- Ethylacridine, Phytoland 1,2,3-Propatriol, 1-indol-4-yl(ether) were presented in lower concentration.

File :E:\GCMSD\2017\September\27092017\Y1265.D
Operator :
Acquired : 28 Sep 2017 4:16 using AcqMethod GENERAL PROFILING.M
Instrument : GCMSD
Sample Name: SPL1
Misc Info :
Vial Number: 10

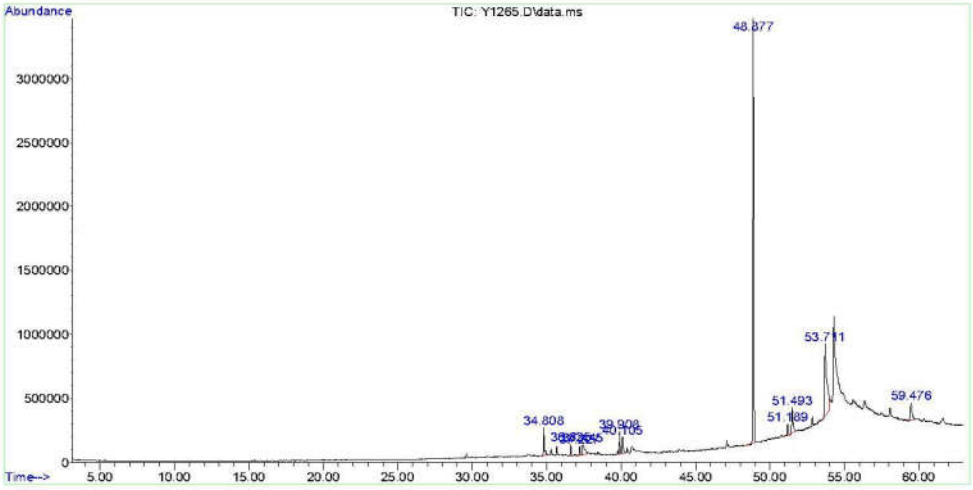
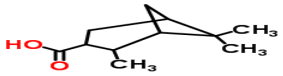
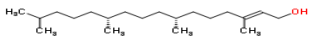
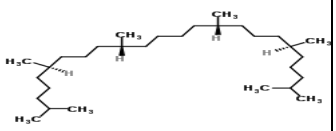
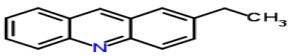
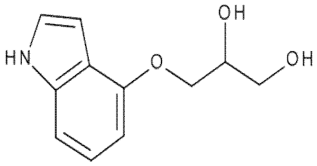


Figure 35- Total Ion Chromatogram (TIC) of leaf extract of *A. paniculata* (MeOH: EA- 4:1)

Table 53- Phytocomponents identified in the leaf extract of *A. paniculata* (MeOH: EA- 4:1) by GC/MS Peak report TIC

Peak	Retention Time (RT)	Area (%)	IUPAC Name, Chemical Formula, Common Name	Chemical Structure	Nature and Uses
1	34.808	5.55	4,6,6-trimethylbicyclo[3.1.1]heptanes (Bicyclo[3.1.1]heptanes, 2,6,6-trimethyl-, (1.alpha., 2.beta., 5.alpha.,) $C_{10}H_{18}$ Pinane		Clear colourless liquid with lint- like particles. Used as in termediates and odor agents.
2	40.105	6.10	Phytol $C_{20}H_{14}O$		Used as a precursor for the manufacture of synthetic forms of vitamin E and vitamin K1 and to modulate transcription in cells.
3	48.877	74.13	2,6,10,15,19,23-hexamethyltetracosane $C_{30}H_{62}$ Squalene		
4	51.493	4.29	2- Ethylacridine $C_{15}H_{13}N$		Liquid, Emollient, Skin Conditioning agenets.
5	53.711	9.93	3-(1H-indol-4-yloxy)propane-1,2-diol (1,2,3-Propatriol, 1-indol-4-yl (ether)) $C_{11}H_{13}NO_3$		A chemically novel antitumour agent which is thought to interact with DNA topoisomerase. Dietary supplements.

Phytochemical profiling of the mass spectra of column fractionated leaf extract of *B. spectabilis* (MeOH: EA- 5:5) **(Figure 36)** exhibited the presence of active compounds Thiophene, tetrahydro-2-methyl-, Methyl 4-0- methyl-d-arabinopyranose, Tetradecanoic acid (Myristic acid), Pentadecane, 2,6,10,14-tetramethyl (Pristane) and Indolizine, 2-(4-methylphenyl)-with their retention time, percent area, common name and nature and uses are presented in **Table 54**.76.47% abundance was observed with Tetradecanoic acid (Myristic).

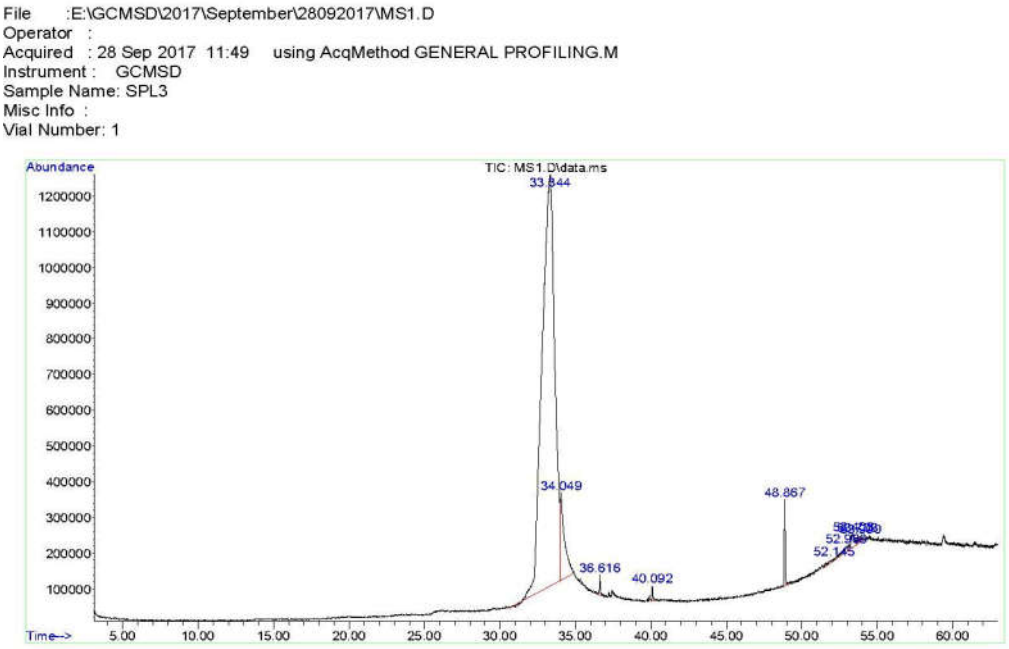
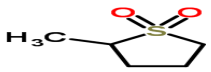
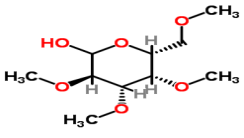
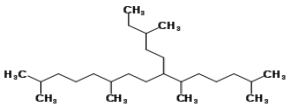
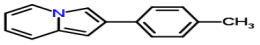


Figure 36- Total Ion Chromatogram (TIC) of leaf extract of *B. spectabilis*(H: EA- 5:5)

Table 54- Phytocomponents identified in the leaf extract of *B. spectabilis* (H: EA- 4:1) by GC/MS Peak report TIC

Peak	Retention Time (RT)	Area (%)	IUPAC Name, Chemical Formula, Common Name	Chemical Structure	Nature and Uses
1	33.344	9.47	2-methylthiolane 1,1-dioxide (Thiophene, tetrahydro-2-methyl-) $C_5H_{10}O_2S$		Heterocyclic compounds that are widely used as building blocks in many agrochemicals and pharmaceuticals
2	34.049	9.36	Methyl 4-0-methyl-d-arabinopyranose $C_{10}H_{20}O_6$		Not known
3	36.616	76.47	Tetradecanoic acid $C_{14}H_{28}O_2$ Myristic acid		Commonly added co-translationally to the penultimate, nitrogen-terminus, glycine in receptor-associated kinases to confer the membrane localization of the enzyme
4	48.867	4.23	Pentadecane, 2,6,10,14-tetramethyl $C_{19}H_{40}$ Pristane		Used as a lubricant, a transformer oil, an immunologic adjuvant, and an anti-corrosion agent, biological marker, plasmocytomas inducer and in production of monoclonal antibodies.
5	52.145	6.47	Indolizine, 2-(4-methylphenyl)- $C_{15}H_{13}N$		Used in the treatment of physiological disturbances consequent upon an excess of uric acid, upon disorders of the immunization system and as antiparasitic agents

The results pertaining to GC/MS analysis of the column fractionated seed extract of *P. longifolia* (MeOH: EA- 4:1) lead to the identification of a number of compounds **(Figure 37)**. 9-Octadecanoic acid, methyl ester (E) (Methyl Oleate), Cyclododecanone, 10, 13-octadecanoic acid, methyl ester (Methyl stearate), Methyl 8-(2-octylcyclopropen-1-yl) octanoate (Methyl sterculate) and Phenol, 2,4 -bis(1,1-dimethylethyl) were identified as active phytoconstituents. Among these, Phenol, 2,4-bis(1,1-dimethylethyl) were observed as the most abundant compound (69.23%). The GC/MS spectrum confirmed the presence of these compounds with different retention times and abundance of these compounds in the seed extract of *P. longifolia* was also recorded as percent area **(Table 55)** and the large compound fragments into small compounds giving rise to appearance of peaks at different m/z ratios.

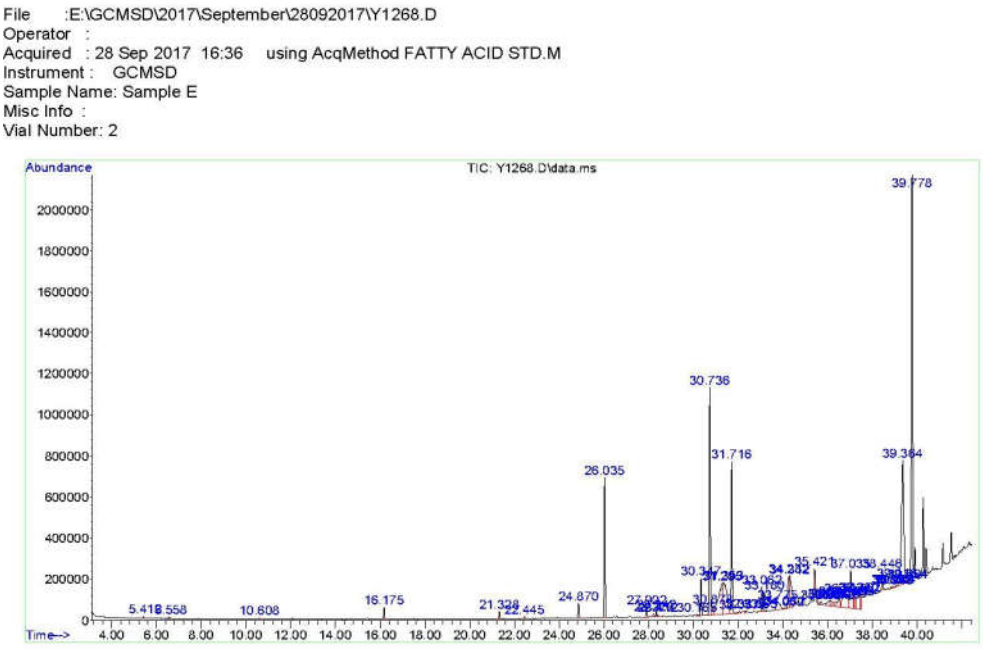
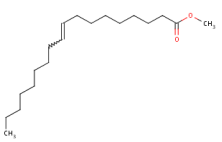
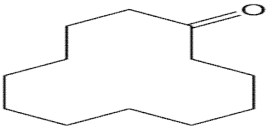
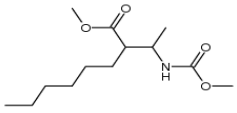
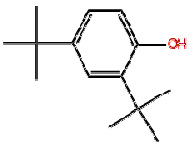


Figure 37- Total Ion Chromatogram (TIC) of seed extract of *P. longifolia* (MeOH: EA- 4:1)

Table 55- Phytocomponents identified in the seed extract of *P. longifolia* (MeOH: EA- 4:1) by GC/MS Peak report TIC

Peak	Retention Time (RT)	Area (%)	IUPAC Name, Chemical Formula, Common Name	Chemical Structure	Nature and Uses
1	30.736	7.11	Methyl(Z)-octadec-9-enoate (9-Octadecanoic acid, methyl ester (E)) $C_{19}H_{36}O_2$ Methyl Oleate		Clear to amber liquid. Flavouring agents.
2	31.353	6.04	Cyclododecanone $C_{12}H_{22}O$		Precursors to certain specialized nylons. Small amounts are also converted to cyclohexadecanone, which is used in some fragrances Antifoaming agent and fermentation nutrient.
3	31.716	11.15	10, 13-octadecanoic acid, methyl ester $C_{19}H_{38}O_2$ Methyl stearate		Antioxidant, Intermediates, Process regulators and solvents for cleaning or dressing.
4	39.364	6.47	Methyl 8-(2-octylcyclopropen-1-yl) octanoate $C_{20}H_{36}O_2$ Methyl sterculate		Used industrially as UV stabilizers and antioxidants for hydrocarbon-based products
5	39.778	69.23	2,4-ditert-butylphenol (Phenol, 2,4-bis(1,1-dimethylethyl) $C_{14}H_{22}O$		

GC/MS analysis of column fractionated extracts of *S. guttata* (seed), *A. paniculata* (leaf), *B. spectabilis* (leaf) and *P. longifolia* (seed) exhibited the presence of various phytoconstituents which might be responsible for phytosterilant activities on adults of the filarial vector *Cx. quinquefasciatus*.

The major phytoconstituents present in the seed extract of *S. guttata* was Methyl hexadecanoate (Palmitic acid) (62.76%). Similarly, leaf extract of *A. paniculata* possessed 2,6,10,15,19,23-hexamethyltetracosane (Squalane) (74.13%), leaf extract of *B. spectabilis* showed the presence of tetradecanoic acid (Myristic acid) (69.47%) and seed extract of *P. longifolia* exhibited the abundance of the phytocomponent 2,4-ditert-butylphenol (Phenol, 2,4-bis(1,1-dimethylethyl) (69.21%). Along with these phytoconstituents, all these plant extracts also possessed various other compounds in trace amounts.

3.4.4 LC-Q-TOF-MS analysis

LC-Q-TOF-MS analysis was performed in positive mode to obtain maximum information on the composition of the phytoconstituents present in the selected plant extracts. Compounds obtained were characterised based on their mass spectra, using the precursor ion, fragment ions and comparison of the fragmentation patterns with molecules described in the literature. Column fractionated extracts of *S. guttata* showed different peaks **(Figures 38 & 39, (Table 56)** on extracting the total ion chromatogram (TIC) into base peak chromatogram. At 8.2 minute, the highest peak corresponding to m/z [M+ H] + 271. 0606, was identified as Methyl Palmitate (Palmitic acid), a fatty acid with the fragmentation pattern m/z 270.26, 271.26 and 272.26. Fraction of *A. paniculata* yielded several peaks on extracting the base peak chromatogram **(Figures 40 & 41, Table 56),** at 5.4 minute, a peak with m/z [M+ H] + 420.3088 was identified with the fragmentation pattern of Squalane m/z 422.49, 423.49 and 424.49. Peak at 7.5 minute identified tetradecanoic acid at m/z [M+ H] + 228.2307 with fragmentation patterns 228.21, 229.21 and 230.22 on column fractionated leaf extract of *B. spectabilis* **(Figures 42 & 43, Table 56)**. The peak at 5.6minute, m/z [M+ H] + 207.1742 was identified as Phenol, 2, 4-bis (1-1 dimethyl ether) as it presented on column fractionated

seed extract of *P. longifolia* with fragmentation patterns 206.17, 206.32 and 207.28 **(Figures 44 & 45, Table 56)**.

Table 56- Metabolites identified from the fractionated extracts of selected plants using LC-Q-TOF-MS analysis

Selected Plants	R_t	Elemental Composition	m/z [M+ H]+ (experimental)	m/z [M+ H]+ (calculation)	Error (ppm)	Tendative Identification
S. guttata	8.2	$C_{17}H_{34}O_2$	271.0606	270.260	0.8006	Methyl Palmitate
A. apniculata	5.1	$C_{30}H_{62}$	420.3088	422.490	-2.1812	Squalane
B. specatbilis	7.5	$C_{14}H_{28}O_2$	228.2307	228.376	-0.1453	Myristic acid
P. longifolia	5.6	$C_{14}H_{22}O$	207.1742	206.170	1.0042	Phenol 2,4- bis (1-1 dimethyl ether)

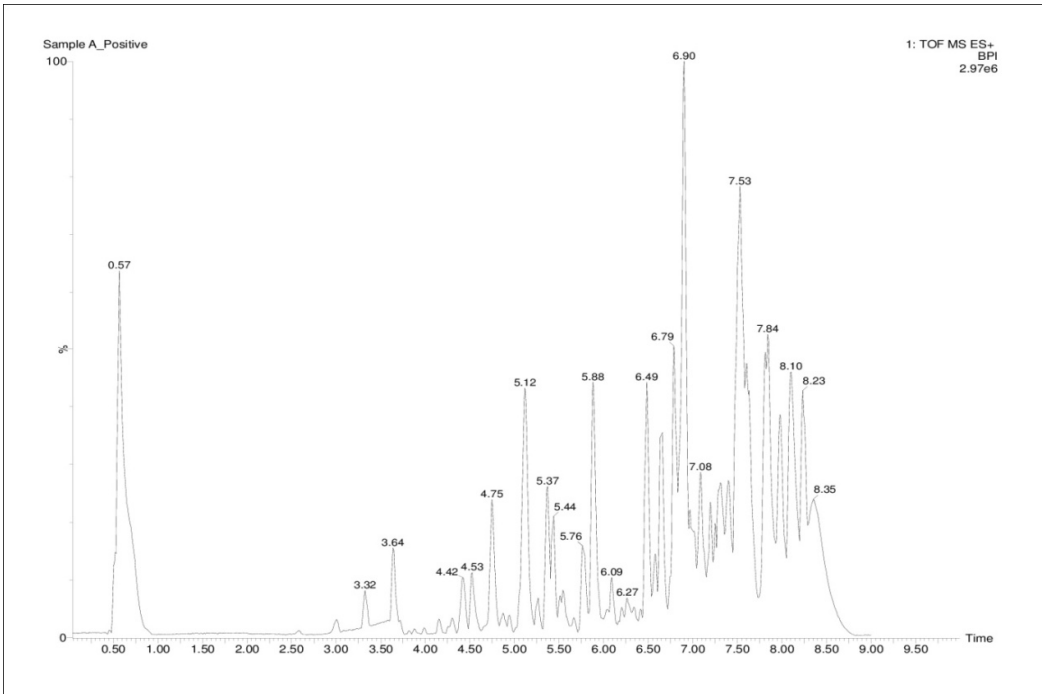


Figure 38- Total Ion Chromatogram (TIC) from LC-Q-TOF-MS analysis of fractionated *S. guttata* seed extract.

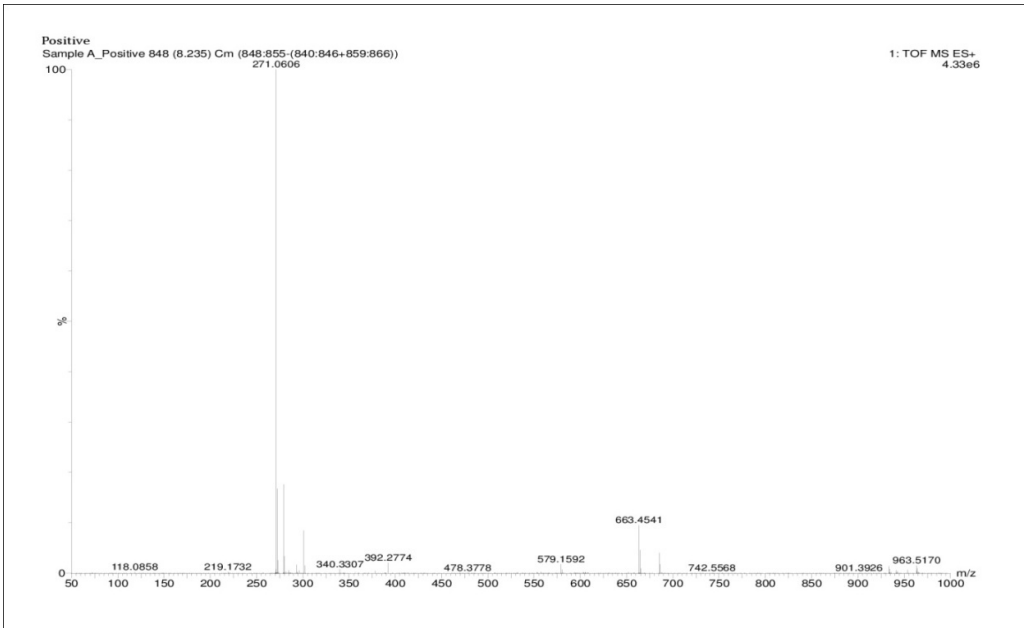


Figure 39- MS/MS spectrum at 8.2 min, scaled to highlight the peak at m/z [M+ H] + 271. 0606.

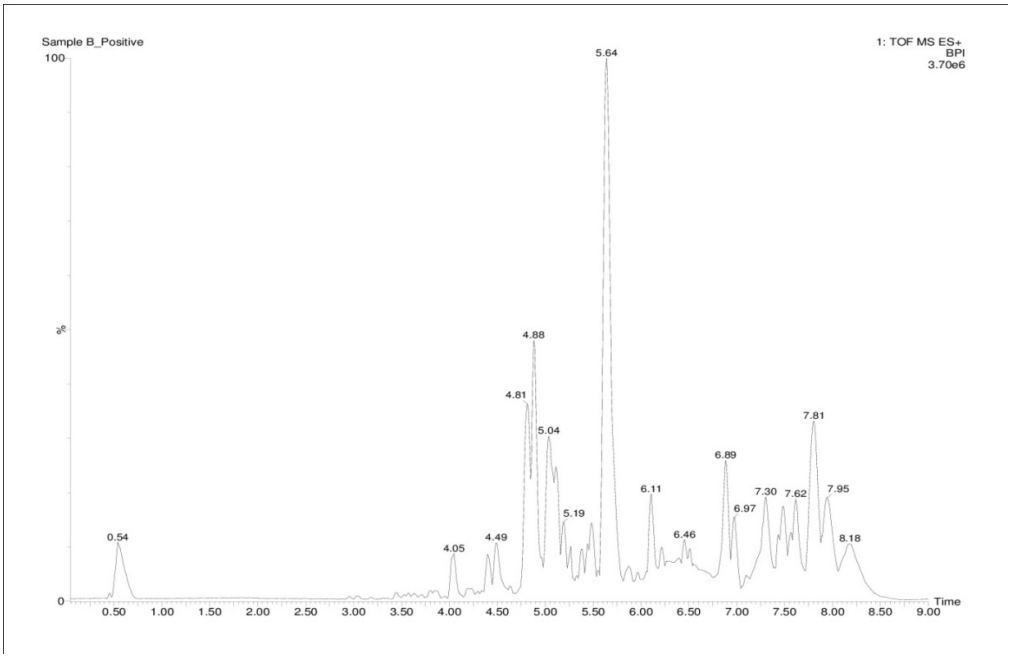


Figure 40- Total Ion Chromatogram (TIC) from LC-Q-TOF-MS analysis of fractionated *A. paniculata* leaf extract.

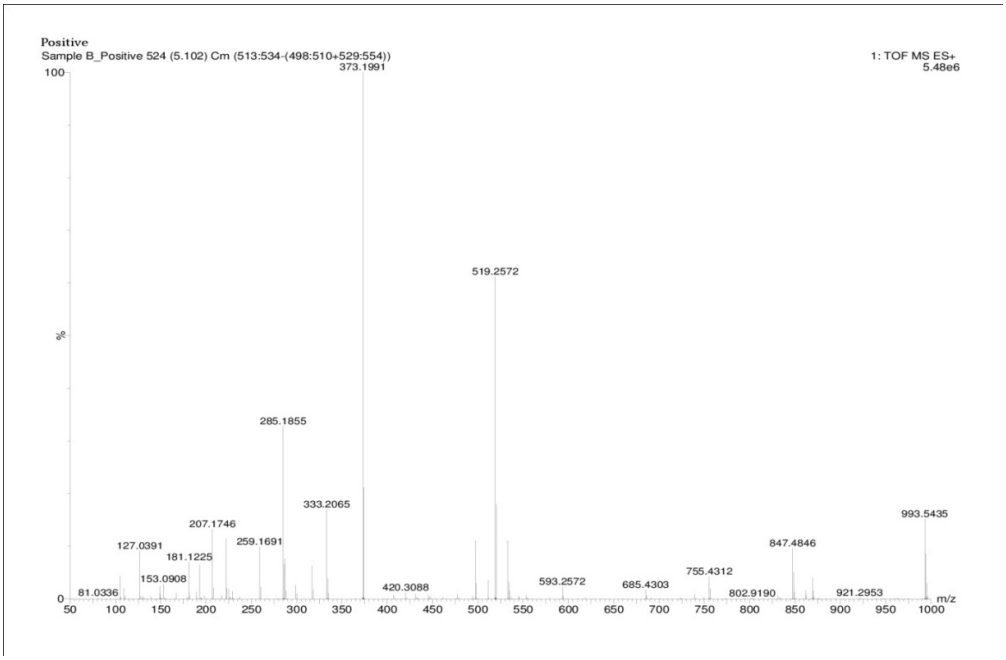


Figure 41- MS/MS spectrum at 5.1 min, scaled to highlight the peak at m/z [M+ H] + 420.3088.

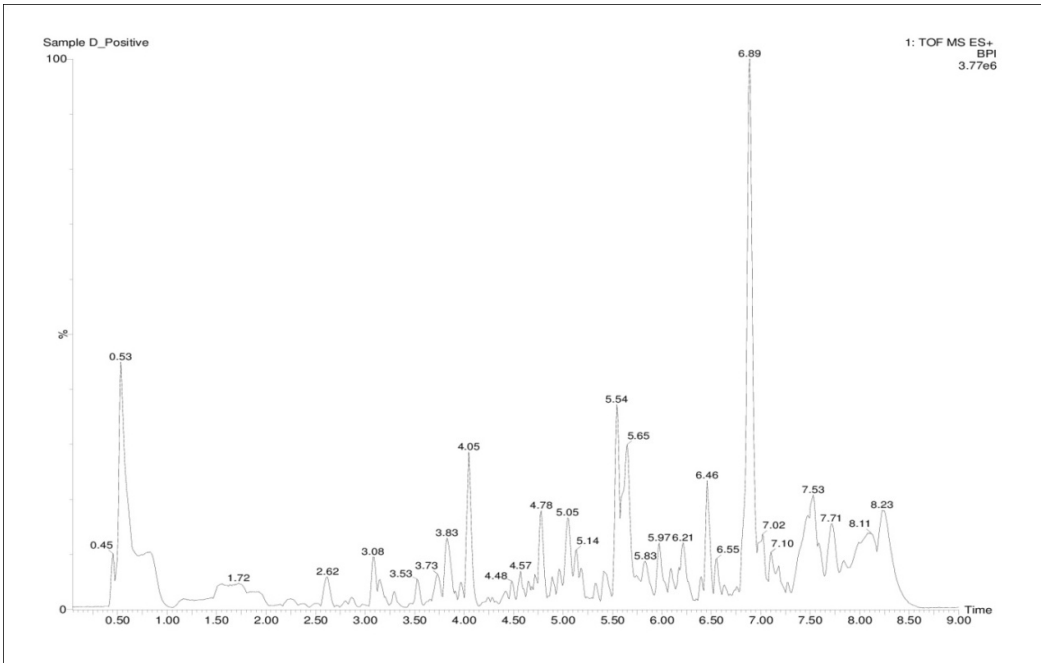


Figure 42- Total Ion Chromatogram (TIC) from LC-Q-TOF-MS analysis of fractionated *B. spectabilis* leaf extract.

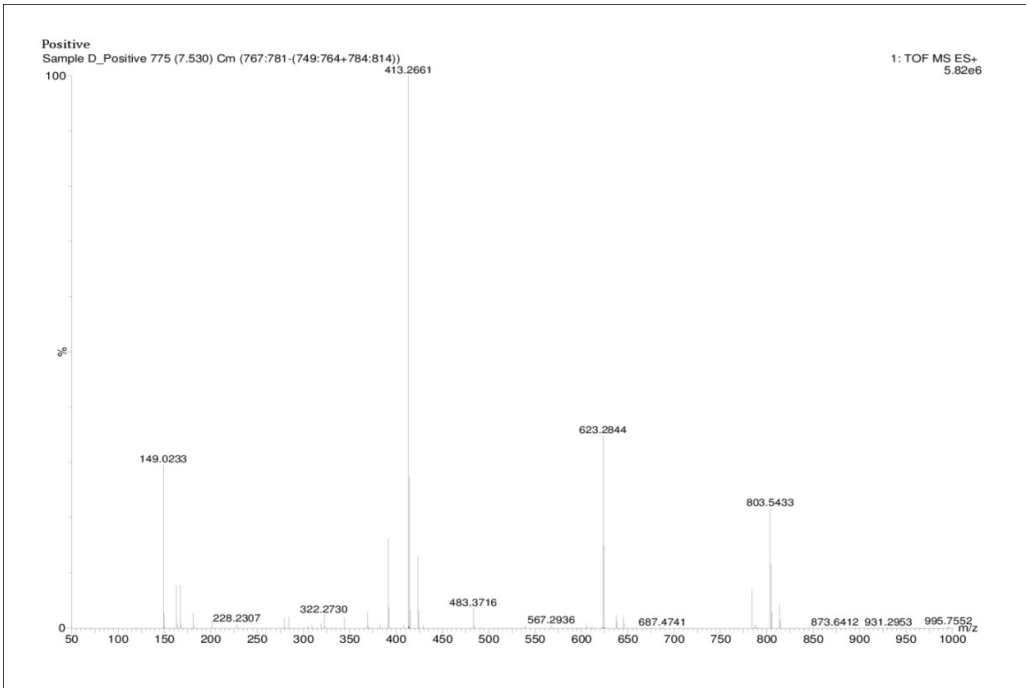


Figure 43- MS/MS spectrum at 7.5 min, scaled to highlight the peak at m/z [M+ H] + 228.2307.

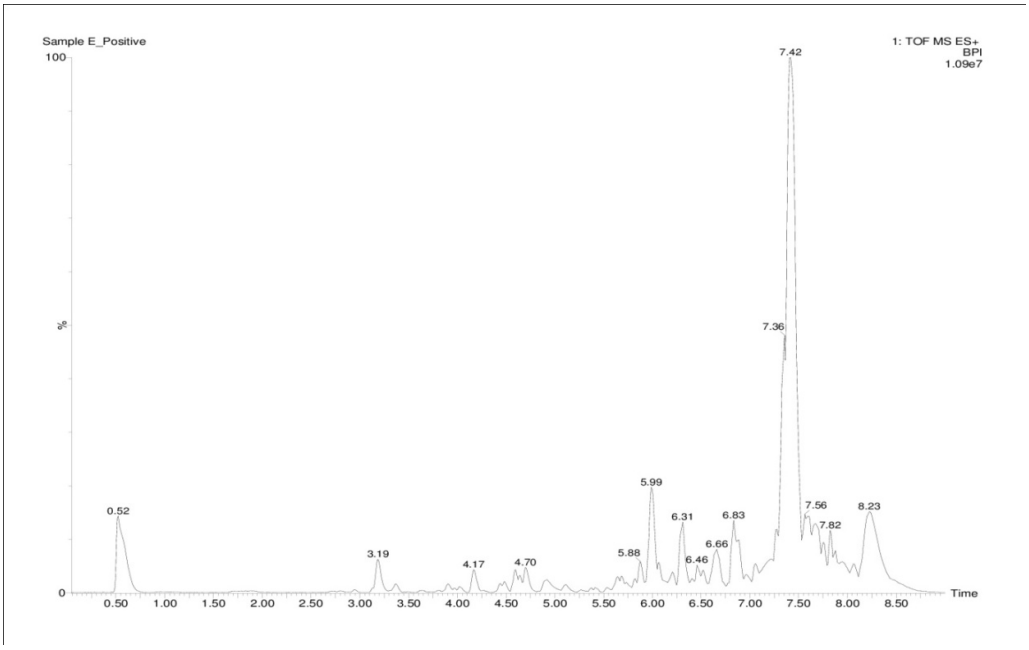


Figure 44- Total Ion Chromatogram (TIC) from LC-Q-TOF-MS analysis of fractionated *P. longifolia* seed extract.

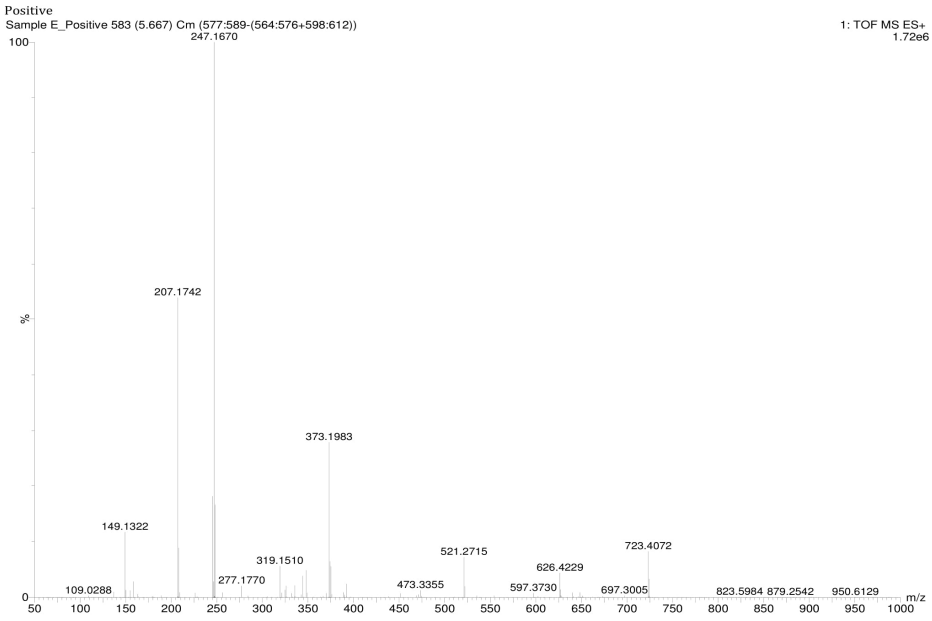


Figure 45- MS/MS spectrum at 5.6 min, scaled to highlight the peak at m/z [M+ H] + 207.1742.

3.4.5 NMR Analysis

Profiling of the bioactive principles of selected plant extracts by the 400MHz ^{1}H NMR were recorded from a $CDCl_3$ solution of sample at 25^0 C, 3.9846387s acquisition time and 20ppm spectral width using Standard Bruker Procedures. The 100 MHz ^{13}C NMR spectra were recorded from a $CDCl_3$ solution of sample using ^{1}H decoupling, 1.3631988s acquisition time and 240ppm spectral width (**Figures 46, 47, 48 & 49**). ^{1}H NMR and ^{13}C NMR chemical shifts were referred to $CHCl_3$ and $CDCl_3$ (δ 7.26ppm, δ 77.2ppm) respectively.

^{1}H NMR spectrum of Column fractionated seed extract of *S. guttata* showed the presence of Methyl hexadecanoate/ methyl palmitate (Palmitic acid), in which the compounds showing chemical shift values as multiplet at 5.351 with 2 hydrogen, singlet at 3.663 with 3 hydrogen, triplet at 2.770 with a coupling constant 6 and 2 hydrogen, multiplet at 2.334 with 4 hydrogen, triplet at 2.040 with a coupling constant 8 with 2 hydrogen, multiplet at 1.554 with 4 hydrogen, multiplet at 1.53 with 14 hydrogen and triplet at 0.880 with 3 hydrogen. In ^{13}C NMR spectra, chemical shift value were reported in δ 77.2ppm with δ 179.51, δ 34.32, δ 31.00 δ 29.6, δ 29.5, δ 29.4, δ 29.3, δ 29.2, δ 29.1, δ 27.4, δ 27.2, δ 25.9, δ 25.6, δ 24.7, δ 24.6, δ 22.7, δ 14.7 (**Table 57**).

Table 57- ^{1}H NMR and ^{13}C NMR spectral assignments for *S. guttata* (MeOH: EA- 4:1)

Compund	Observed NMR spectral assignments
^{1}H NMR(δ)	CDCl3):- 5.351 (m, 2H), 3.663 (s,3H); 2.770 (t, J=6,2H); 2.334 (m,4H); 2.040 (t, J=8,2H); 1.554 (m, 4H); 1.53 (m,14H); 0.880 (t, 3H).
^{13}C NMR(δ)	CDCI3: δ 179.51, δ 34.32, δ 31.00 δ 29.6, δ 29.5, δ 29.4, δ 29.3, δ 29.2, δ 29.1, δ 27.4, δ 27.2, δ 25.9, δ 25.6, δ 24.7, δ 24.6, δ 22.7, δ 14.7.

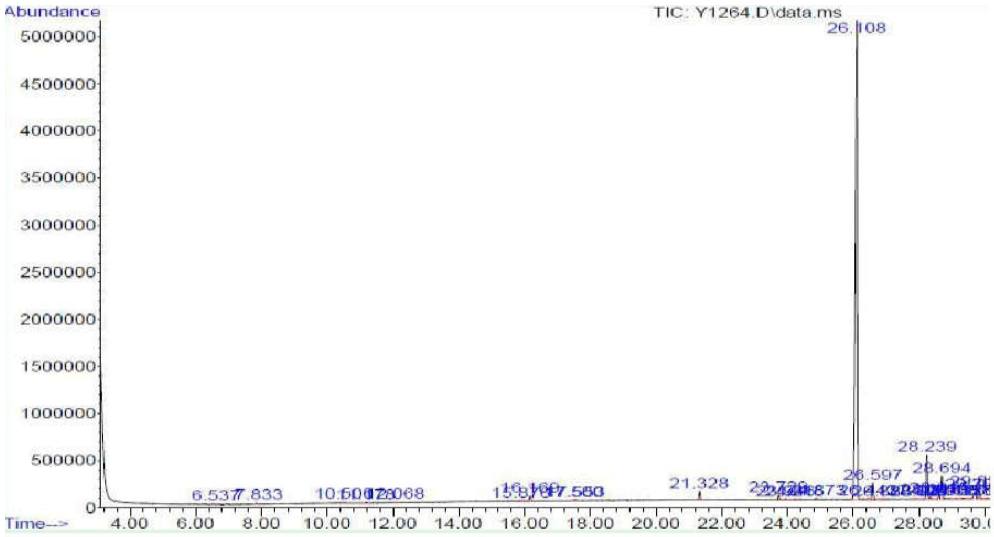


Figure 46- TIC of NMR Spectrum of *S. guttata* (MeOH: EA- 4:1)

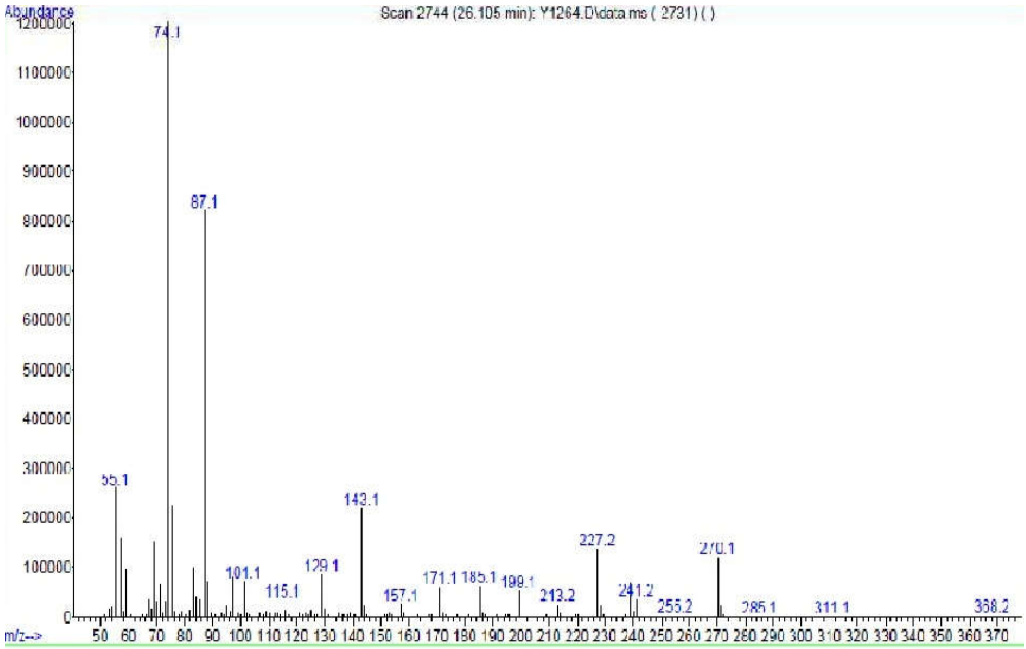


Figure 47- ^{1}H NMR Spectrum of *S. gutttata* (MeOH: EA- 4:1)

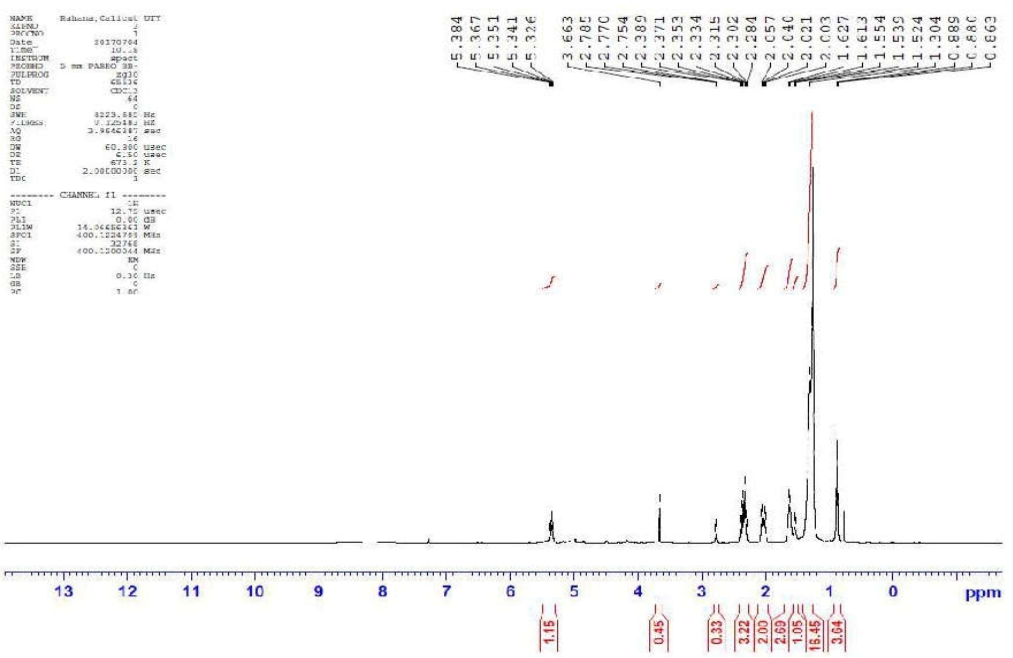
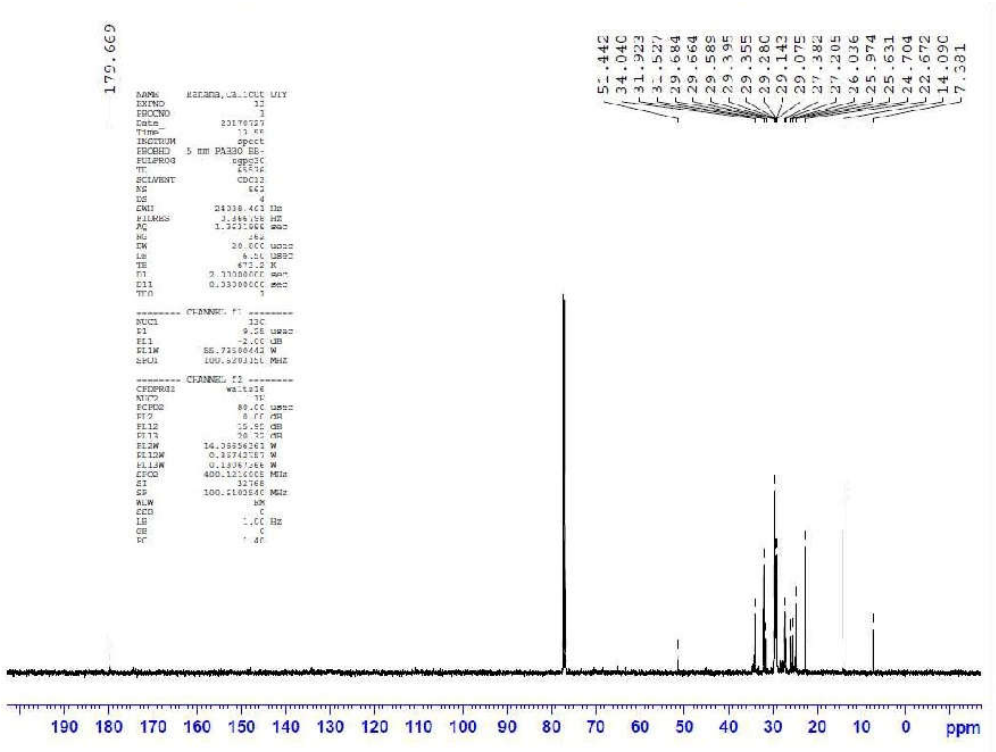


Figure 48- ^{13}C NMR Spectra of *S. guttata* (MeOH: EA).



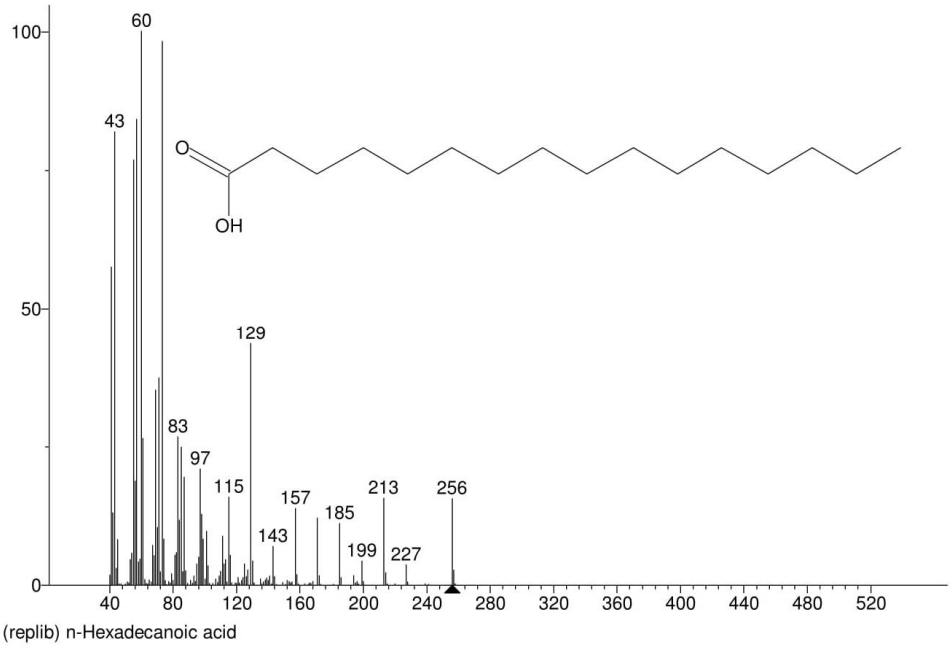
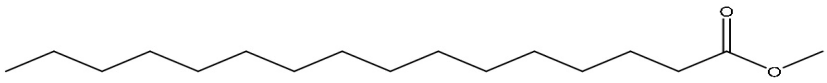


Figure 49- Structure of Methyl Palmitate (Palmitic acid)



Chemical Formula: $C_{17}H_{34}O2$

Exact Mass: 270.26

Molecular Weight: 270.45

m/z: 270.26 (100.0%), 271.26 (18.9%), 27.26 (2.0%)

Elemental Analysis: C, 75.50; H, 12.67; O, 11.83

Common Name: Palmitic acid

The elucidation of active compounds from the column fractionated leaf extract of *A. paniculata* was achieved by ^{1}H NMR and ^{13}C NMR

spectroscopy. ^{1}H NMR spectrum exhibiting the presence of 2,6,10,15,19,23-hexamethyltetracosane (Squalane) with chemical shift values δ doublet at 4.870 with a coupling constant 7 with 1 hydrogen, doublet at 4.798 with coupling constant 8 and 2 hydrogen, doublet at 3.475 with coupling constant 3 with 2 hydrogen, triplet at 2.333 with coupling constant 8 and 2 hydrogen, doublet at 2.806 with coupling constant 4 with 2 hydrogen, doublet at 2.504 with coupling constant 7 and 2 hydrogen, doublet at 2.017 with coupling constant 7 with 2 hydrogen, doublet at 1.785 with coupling constant 3 with 3 hydrogen, doublet at 2.017 with coupling constant 7 with 2 hydrogen, doublet at 2.017 with coupling constant 7 with 2 hydrogen, multiplet at 1.599 with 1 hydrogen, multiplet at 1.533 with 1 hydrogen, multiplet at 0.988 with 1 hydrogen, multiplet at 0.880 with 3 hydrogen, multiplet at 0.815 with 2 hydrogen. ^{13}C NMR spectra showed the chemical shift values as δ 80.9, δ 70, δ 69, δ 64, δ 61, δ 54, δ 47, δ 43, δ 38.8, δ 38, δ 36, δ 29.6, δ 29, δ 28, δ 23.8, δ 23, δ 22 and δ 16 (**Figures 50, 51, 52 & 53**) (**Table 58**).

Table 58- ^{1}H NMR and ^{13}C NMR spectral assignments for *A. paniculata* (MeOH: EA- 4:1)

Compund	Observed NMR spectral assignments
^{1}H NMR(δ)	CDCl3):- 4.870 (d, J= 7Hz, 1H), 4.798 (d, J= 8Hz, 2H); 3.475 (d, J=3Hz, 2H); 2.333 (t, J= 8Hz, 2H); 2.806 (d, J=4, 2H); 2.504 (d, J= 7Hz, 2H); 2.017 (d, J= 7Hz, 2H); 1.785 (d, J= 3Hz, 3H); 1.785(d, J= 3Hz, 3H); 1.733 (m, 2H); 1.599 (m, 1H), 1.533 (m, 1H); 0.988 (m, 1H); 0.880 (m, 3H), 0.815(m, 2H).
^{13}C NMR(δ)	CDCI3: δ 80.9, δ 70, δ 69, δ 64, δ 61, δ 54, δ 47, δ 43, δ 38.8, δ 38, δ 36, δ 29.6, δ 29, δ 28, δ 23.8, δ 23, δ 22, δ 16.

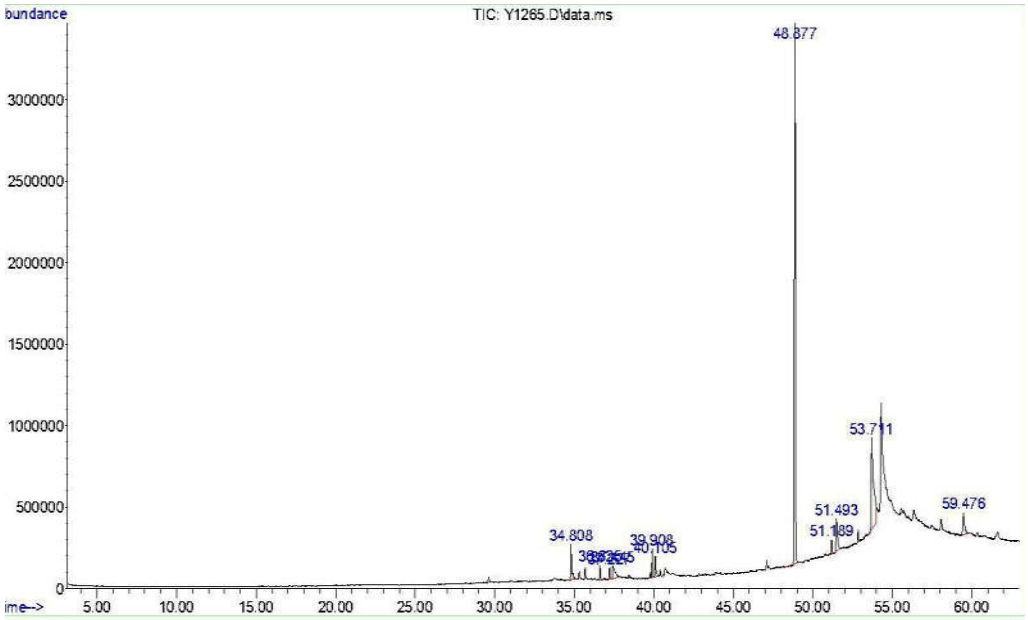


Figure 50- TIC of NMR Spectrum of *A. paniculata*(MeOH: EA- 4:1)

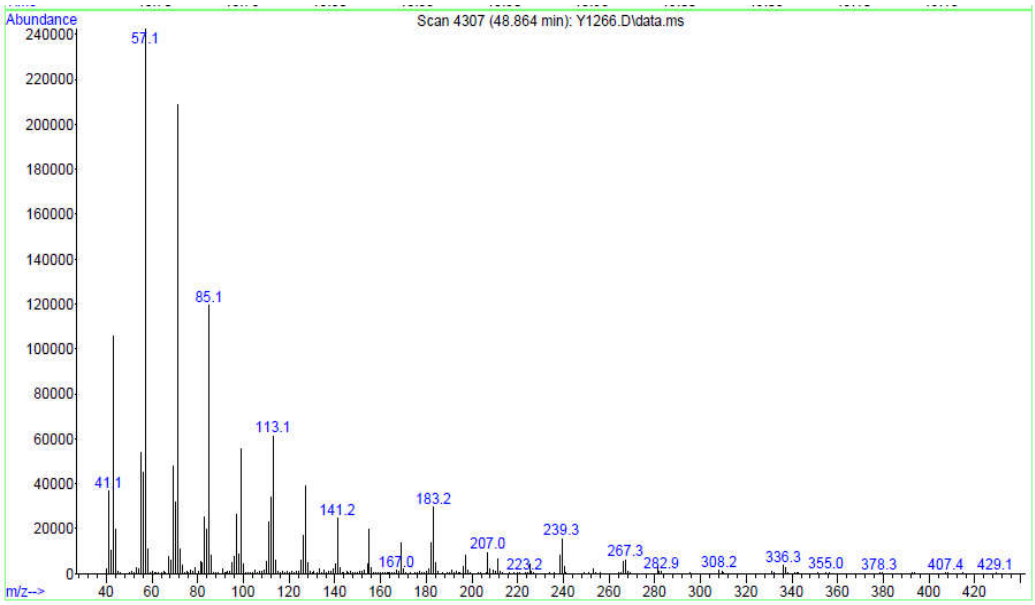


Figure 51- ^{1}H NMR Spectrum of *A. paniculata* (MeOH: EA- 4:1)

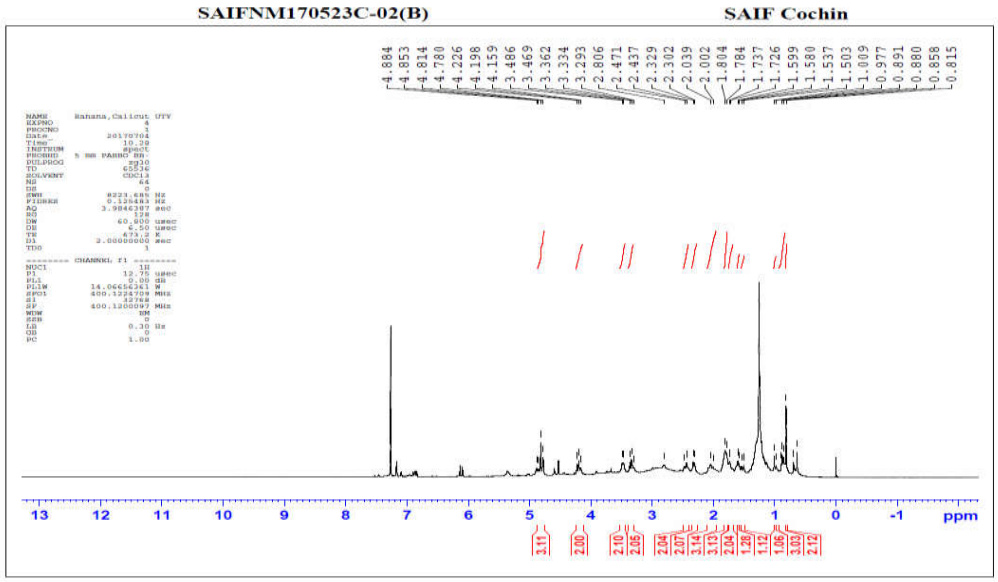
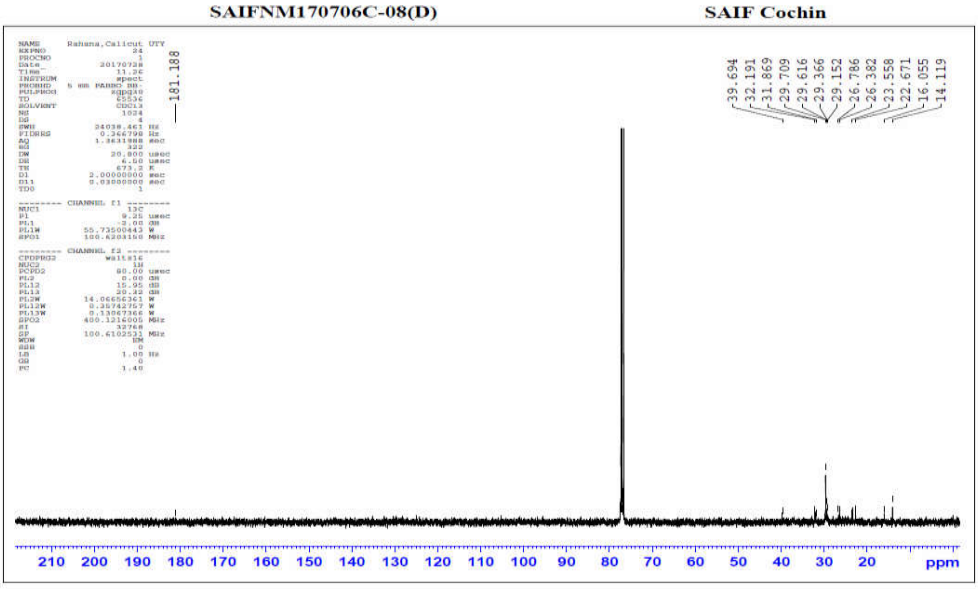


Figure 52- ^{13}C NMR Spectrum of *A. paniculata* (MeOH: EA- 4:1)



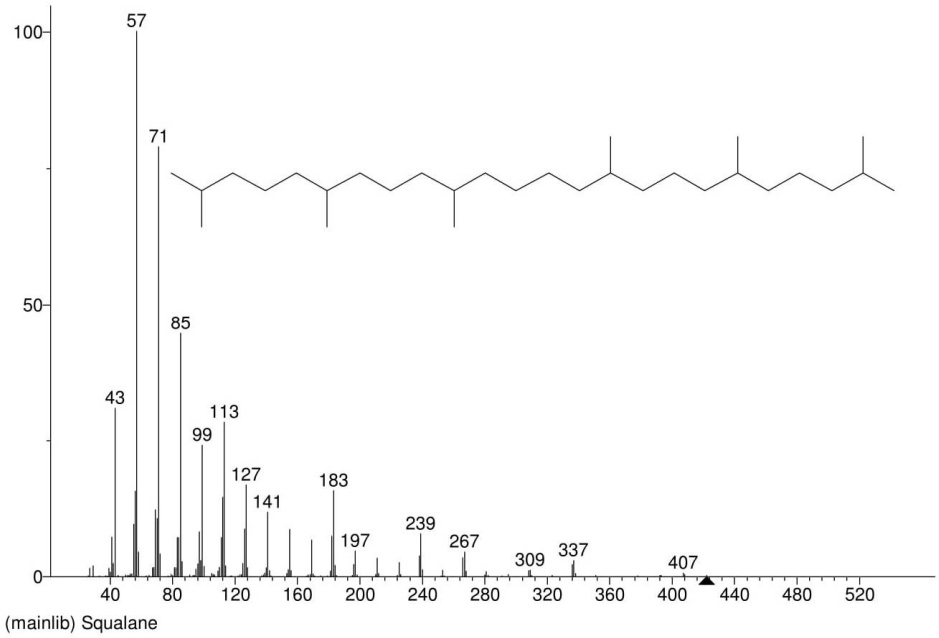
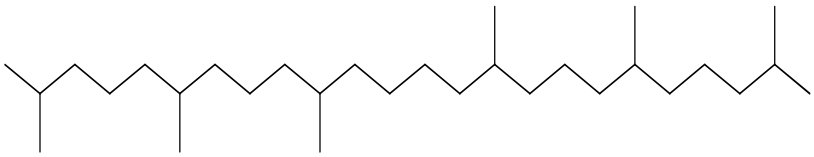


Figure 53- Structure of 2,6,10,15,19,23- hexamethyltetracosane (Squalane)



Chemical Formula: $C_{30}H_{62}$

Exact Mass: 422.49

Molecular Weight: 422.81

m/z: 422.49 (100.0%), 423.49 (33.2%), 424.49 (5.3%)

Elemental Analysis: C, 85.22; H, 14.78

Common Name: Sqaulane

^{1}H NMR and ^{13}C NMR spectra of column fractionated leaf extract of *B. spectabilis* revealed several signals as shown in the **Figures 54, 55, 56 & 57** respectively. The bioactive compounds obtained was tetradecanoic acid (Myristic acid) were individually characterised by ^{1}H NMR with their respective chemical shift values as broad at 5.350 with 1 hydrogen, triplet at 5.123 with coupling constant 8 and 2 hydrogen, multiplet at 3.320 with 2 hydrogen, multiplet at 1.679 with 4 hydrogen, multiplet at 1.590 with 2 hydrogen, multiplet at 1.304 with 4 hydrogen, multiplet 1.254 with 6 hydrogen and triplet at 0.874 with coupling constant 8 with 3 hydrogen. ^{13}C NMR spectra exhibited the chemical shift values δ 181, δ 39, δ 32, δ 31.8, δ 29.7, δ 29.3, δ 29.1, δ 26.8, δ 26.4, δ 23, δ 22, δ 16, δ 14 (**Table 59**).

Table 59- ^{1}H NMR and ^{13}C NMR spectral assignments for *B. Spectabilis* (H: EA- 5:5)

Compund	Observed NMR spectral assignments
^{1}H NMR(δ)	CDCl3):- 5.350 (b,1H),5.123 (t, J=8 Hz,2H); 3.320 (m, 2H); 1.679(m, 4H); 1.590 (m, 2H); 1.304 (m, 4H); 1.254 (m, 6H); 0.874 (t, J=8 Hz,3H).
^{13}C NMR(δ)	CDCI3: δ 80.9, δ 70, δ 69, δ 64, δ 61, δ 54, δ 47, δ 43, δ 38.8, δ 38, δ 36, δ 29.6, δ 29, δ 28, δ 23.8, δ 23, δ 22, δ 16.

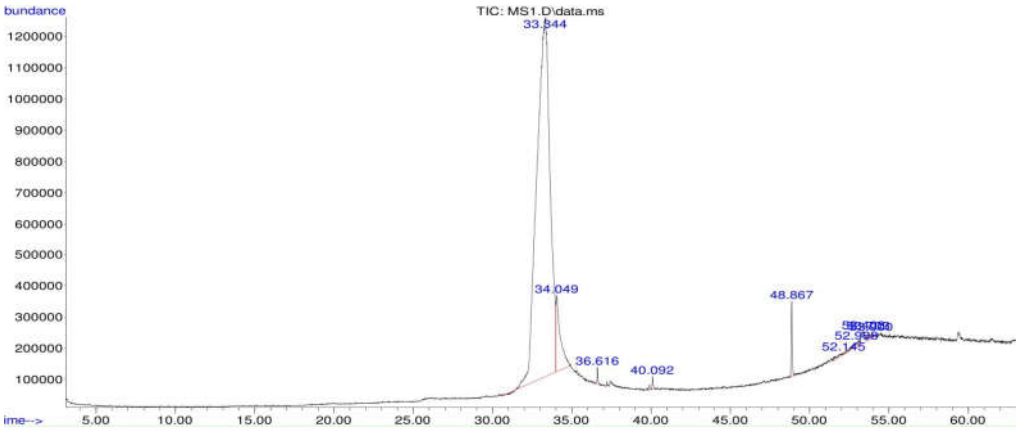


Figure 54- TIC of NMR Spectrum of *B. spectabilis* (H: EA- 5:5)

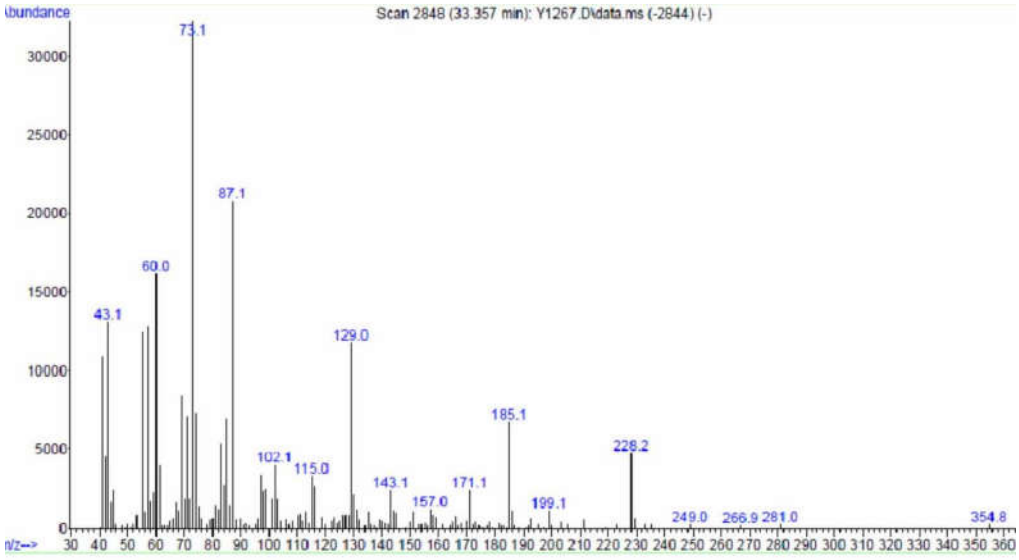


Figure 55- ^{1}H NMR Spectrum of *B. spectabilis* (MeOH: EA- 4:1)

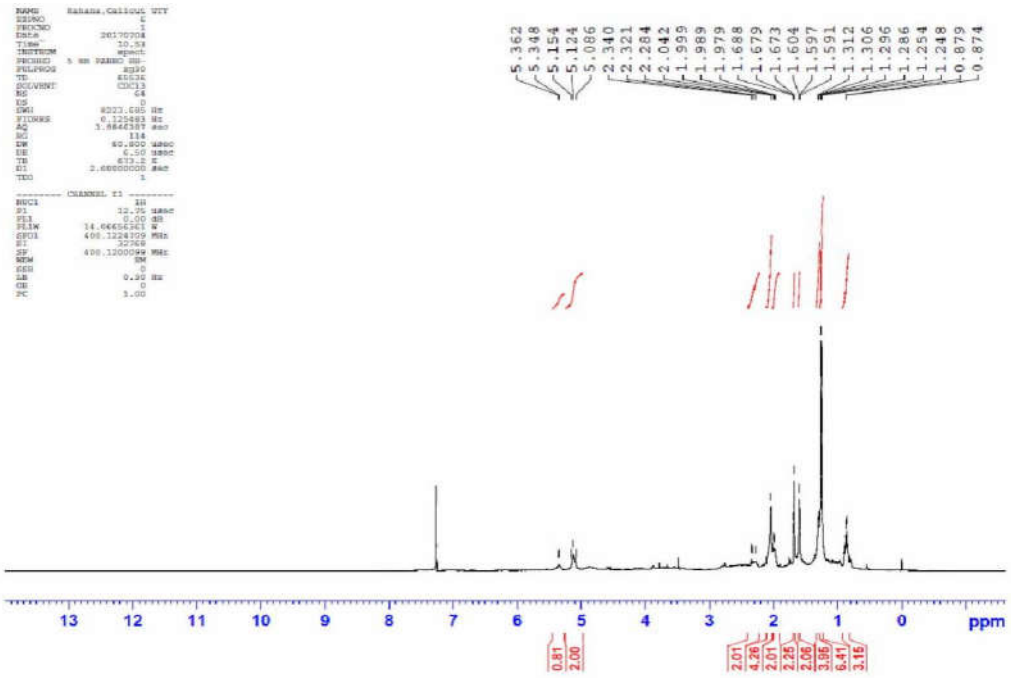
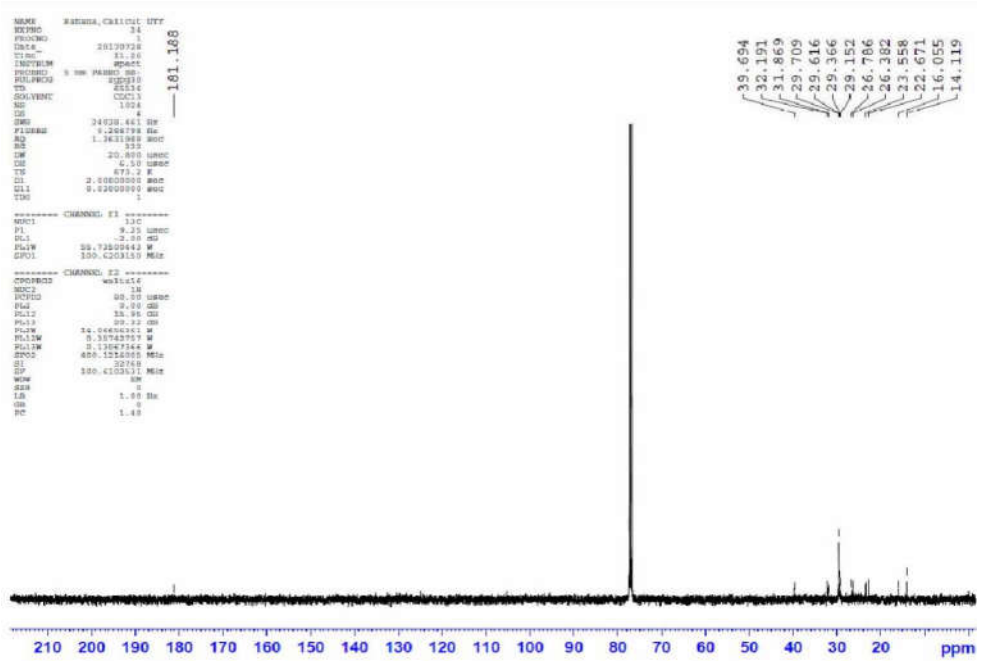


Figure 56- ^{13}C NMR Spectrum of *B. spectabilis* (MeOH: EA- 4:1)



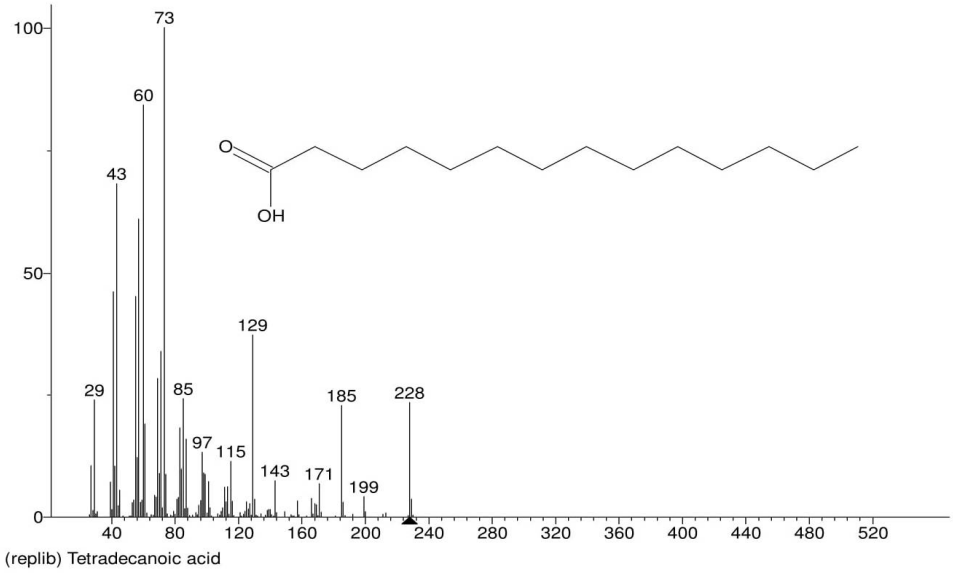
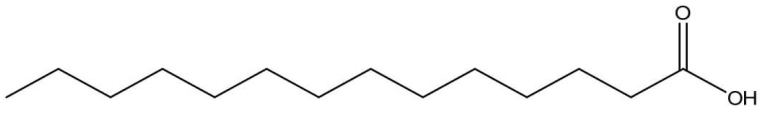


Figure 57- Structure of tetradecanoic acid (Myristic acid)



Chemical Formula: $C_{14}H_{28}O_2$

Exact Mass: 228.21

Molecular Weight: 228.37

m/z: 228.21 (100.0%), 229.21 (15.2%), 230.22 (1.1%)

Elemental Analysis: C, 73.63; H, 12.36; O, 14.1

Common Name: Myristic acid

Based on the NMR spectral data, the tentative structure of the compound presented in the column fractionated seed extract of *P. longifolia* is proposed as shown in the **Figures 58, 59, 60 & 61** respectively. The NMR data

exhibited the presence of the phytocompound Phenol, 2,4- bis (1-1 dimethylethyl) with ^{1}H NMR Spectrum with chemical shift values singlet at 0.99 with 9 hydrogen, singlet at 1.32 with 9 hydrogen, singlet at 1.46 with 9 hydrogen, doublet of doublet at 2.94 with coupling constant 9.52 and 2.71 with 1 hydrogen, doublet of doublet at 3.76 with coupling constant 11.05 and 9.52 with 1 hydrogen, doublet of doublet at 3.93 with coupling constant 11.05 and 2.71 with 1 hydrogen, doublet at 7.15 with coupling constant 2.44 with 1 hydrogen, doublet at 7.42 with coupling constant 2.44 with 1 hydrogen, singlet at 8.37 with 1 hydrogen and singlet at 13.59 with 1 hydrogen. ^{13}C NMR spectra showing the chemical shift values δ 15.1, δ 27.0, δ 29.5, δ 31.6, δ 34.0, δ 34.3, δ 35.2, δ 63.6, δ 69.8, δ 118.2, δ 122.5, δ 127.0, δ 137.7, δ 137.9, δ 161.0, δ 173.1 (**Table 60**)

Table 60- ^{1}H NMR and ^{13}C NMR spectral assignments for *P. longifolia* (MeOH: 4:1)

Compund	Observed NMR spectral assignments
^{1}H NMR(δ)	CDCl3):- 0.99 (s, 9H), 1.32 (s, 9H), 1.46 (s, 9H), 2.94 (dd, *J* = 9.52 Hz, 2.71 Hz, 1H), 3.76 (dd, *J* = 11.05 Hz, 9.52 Hz, 1H), 3.93 (dd, *J* = 11.05 Hz, 2.71 Hz, 1H), 7.15(d, *J* = 2.44 Hz, 1H), 7.42 (d, *J* = 2.44 Hz, 1H), 8.37 (s, 1H), 13.59 (s, 1H).
^{13}C NMR(δ)	CDCI3: δ 15.1, δ 27.0, δ 29.5, δ 31.6, δ 34.0, δ 34.3, δ 35.2, δ 63.6, δ 69.8, δ 118.2, δ 122.5, δ 127.0, δ 137.7, δ 137.9, δ 161.0, δ 173.1 .

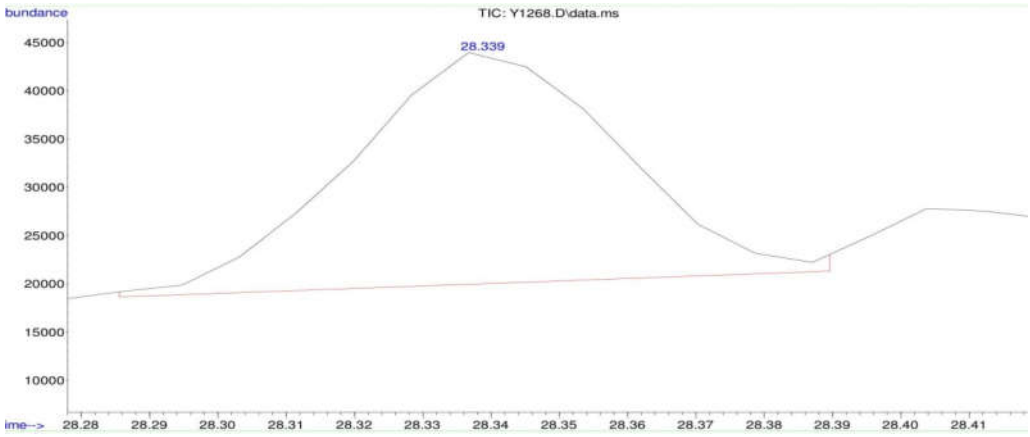


Figure 58- TIC of NMR Spectrum of *P. longifolia* (MeOH: EA- 5:5)

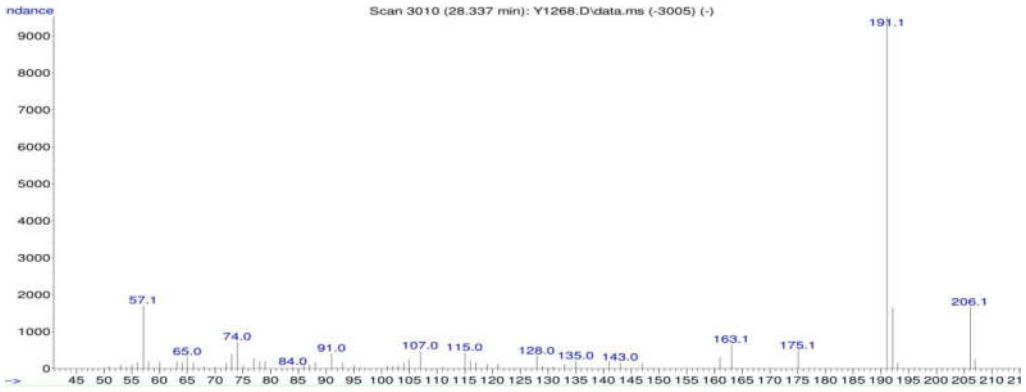


Figure 59- ^{1}H NMR Spectrum of *P. longifolia* (MeOH: EA- 4:1)

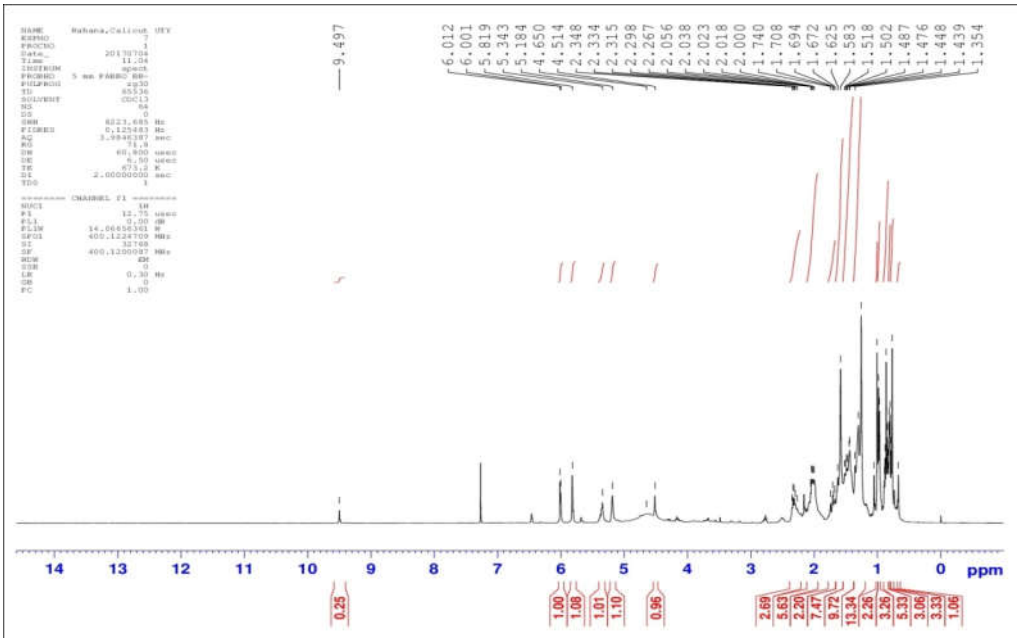
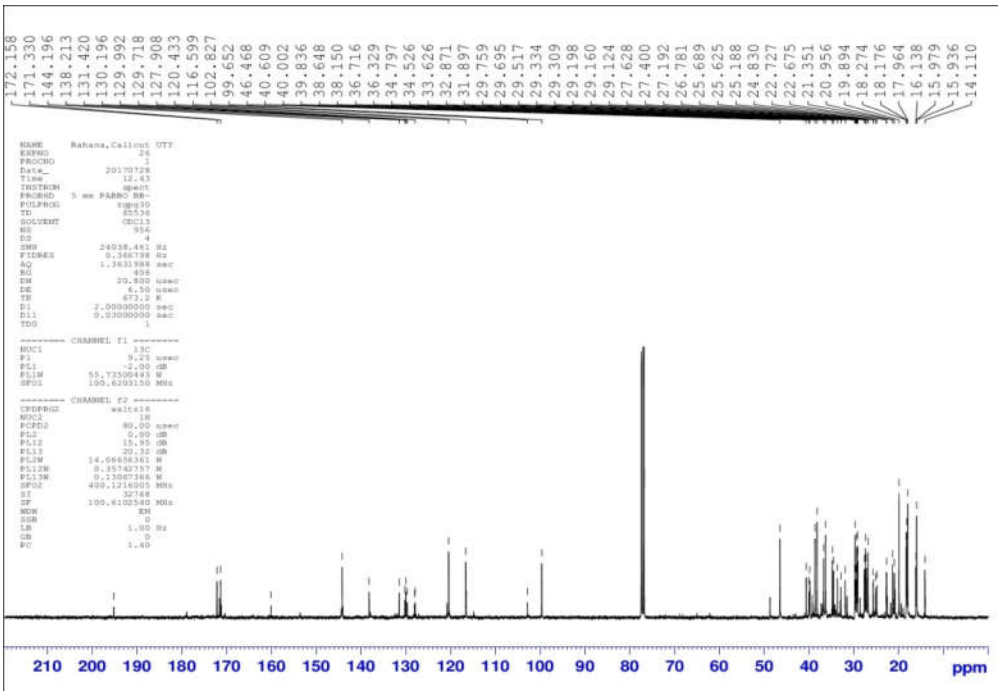


Figure 60- ^{13}C NMR Spectrum of *P. longifolia* (MeOH: EA- 4:1)



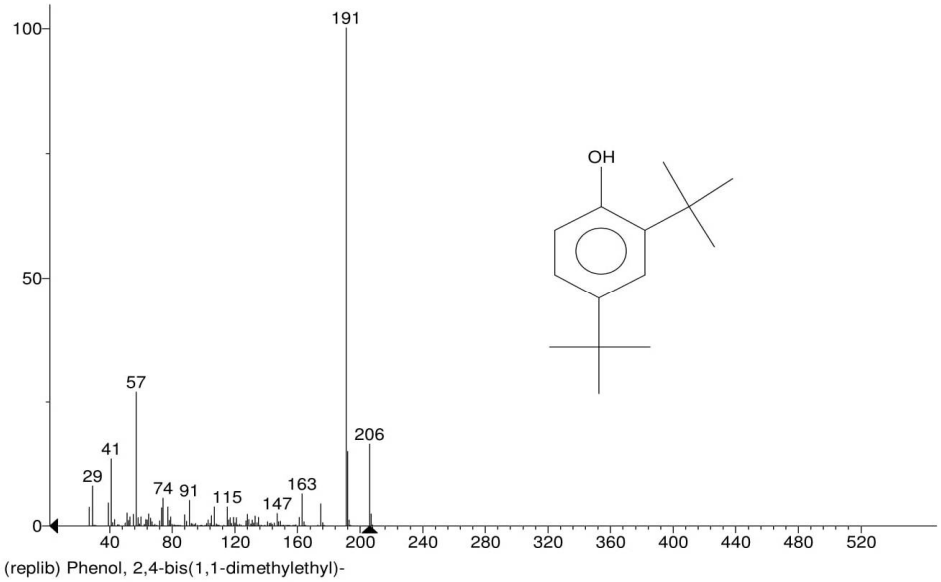
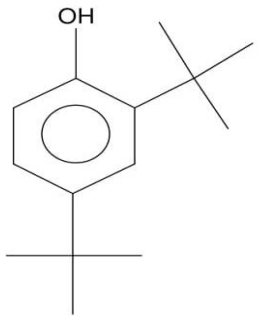


Figure 61- Structure of Phenol, 2, 4 - bis (1-1 dimethylethyl)



Chemical Formula: $C_4H_{22}O$

Exact Mass: 206.17

Molecular Weight: 206.32

m/z: 206.17 (100.0%), 207.21 (14.2%), 208.23 (2.1%)

Common Name: Phenol, 2, 4- bis (1-1 dimethylethyl)

3.4.6 Formulation

The phytosterilant activities of the selected plant extracts were confirmed and the phytoconstituents have been isolated and characterised from different chromatographic and spectroscopic techniques. Bioefficacies as phytosterilant activities of fractionated extracts of selected plants *S. guttata, A. paniculata, B. spectabilis* and *P. longifolia* were examined and prepared to make pesticide formulations. Presently, syrup formulations of selected plants were prepared, in which the active constituent was isolated from purified fractions of selected plant extracts **(Plate 71).**

Plate 71- Syrup formulations prepared from column fractionated seed extract of S. *guttata*, leaf extracts of *A. paniculata*, *B. spectabilis* and seed extract of *P. longifolia*.

3.4.7 Small Scale Field Trials

Field trials of formulated products were performed on a small scale against *Cx. quinquefasciatus* in simulated field trials. The formulations were tested at half of the efficient concentrations with three replicates with an equal number of controls. The sizes of the plots were recorded, taking account of surface area and depth. Pre-treatment immature abundance was recorded in both experimental and control sites. Post- treatment abundance was monitored until the density of pupae in the treated habitats and controls. The dosage of

formulations was prepared according to the surface area of the sites **(Table 61)**.

Table 61- Surface area and volume of the natural breeding sites.

SI No.	Area/ Field	Length (inch)	Width (inch)	Depth (inch)	Volume (L)	Syrup (ml)			
						S. guttata	*A. paniculata*	*B. spectabilis*	*P. longifolia*
1	Field I*	39.00	14.00	11.00	98.42	59.05	4.92	541.31	354.31
2	Field II*	39.00	17.00	8.00	86.92	52.15	4.34	478.06	312.91
3	Filed III*	39.00	15.00	10.00	95.86	57.52	4.79	527.23	345.09
4	Control	39.00	15.00	10.00	97.14	-----	-----	-----	-----

*Three replicates

The pupae collected from the treated and control sites were carried to the laboratory and maintained for adult emergence. The assessment of phytosterilantefficacy of Syrup formulation of *S. gutatta* (MeOH: EA- 4:1) on ovi- position, egg hatchability and control of reproduction were recorded and compared with control. **Table 62** shows the number of egg rafts laid by each gravid female, percentage hatchability and control of reproduction induced by the column fractionated seed extract of *S. guttata* on **T ♂ x T ♀, T ♂ x UT ♀, T ♀ x UT♂, UT ♂ x UT ♀**of *Cx. quinquefasciatus.* The gonotropic cycles observed on **T ♂ x T ♀, T ♂ x UT ♀, T ♀ x UT♂, UT ♂ x UT ♀** consisted 5.33± 0.33, 4.33±0.33, 6.33± 0.33 and 2.67± 0.33 days respectively after blood meal. The number of egg rafts for treated groups **T ♂ x T ♀, T ♂ x UT ♀ & T ♀ x UT♂** were lower when compared with **UT ♂ x UT ♀,** which showed7.67± 0.33 egg rafts, whereas, treated groups resulted 5.33±0.33, 4.33±0.33 and 4.67±0.33 egg rafts respectively. The collected egg rafts for each group of **T♂ x T ♀, T ♂ x UT ♀, T ♀ x UT♂, UT ♂ x UT ♀** consisted 64.50±11.50,

146.33±9.67, 89.33±13.54 and 1613.00±0.00 respectively. Percentage hatchability and control of reproduction was varied according to the treated groups **T ♂ x T ♀, T ♂ x UT ♀, T ♀ x UT♂** and it was observed as45.21%,56.67%, 36.27% and 89.48% hatchability and 78.35%, 72.85%, 87.92% and 0.00% control of reproduction respectively.

Table 62- Effect of syrup formulations of *S. guttata* (MeOH: EA- 4:1) on ovi- position, egg hatchability and control of reproduction of *Cx. quinquefasciatus* adults exposed as natural breeding places.

SI No.	Treated groups	Ovi- position day after blood meal	No. of egg rafts laid	Average no. of eggs obtained	% Hatch	% Control reproduction
1	T ♂ x T ♀	5.33±0.33	5.33±0.33	454.50± 11.50	35.21	78.35
2	T ♂ x UT ♀	4.33±0.33	4.33±0.33	646.33± 9.67	46.67	72.85
3	T ♀ x UT♂	6.33±0.33	4.67±0.33	269.33± 13.54	26.27	87.92
4	UT ♂ x UT ♀	2.67±0.33	7.67±0.33	1613.00±0.00	89.48	00.00

Note: 10 numbers of treated females and males were taken in each of the 3 replicates

Syrup formulation of column fractionated leaf extract of *A. paniculata* exhibited comparable changes on number of egg rafts, number of eggs, percentage hatchability and control of reproduction. The number of egg rafts obtained was 4.33± 0.33, 2.33± 0.33, 5.67± 0.58 and 7.67± 0.33 respectively for **T ♂ x T ♀, T ♂ x UT ♀, T ♀ x UT♂, UT ♂ x UT ♀** groups. The number of eggs and percentage of hatching also resulted in considerable changes when compared with control **(Table 63)**. Percentage control of reproduction

was calculated as 74.07%, 75.41%, 70.97% and 0.00% respectively for **T ♂ x T ♀, T ♂ x UT ♀, T ♀ x UT♂, UT ♂ x UT ♀** groups.

Table 63- Effect of syrup formulations of *A. paniculata* (MeOH: EA- 4:1) on ovi- position, egg hatchability and control of reproduction of *Cx. quinquefasciatus* adults exposed as natural breeding places.

SI No.	Treated groups	Ovi- position day after blood meal	No. of egg rafts laid	Average no. of eggs obtained	% Hatch	% Control reproduction
1	T ♂ x T ♀	5.33±0.33	4.33±0.33	524.50± 11.50	39.33	74.07
2	T ♂ x UT ♀	6.33±0.33	2.33±0.33	287.00±7.76	36.40	75.41
3	T ♀ x UT♂	4.33±0.33	5.67±0.33	778.33± 16.57	48..74	70.97
4	UT ♂ x UT ♀	2.67±0.33	7.67±0.33	1657.00±0.00	89.69	00.00

Note: 10 numbers of treated females and males were taken in each of the 3 replicates

The column fraction of *B. spectabilis* (H: EA- 4:1) exhibited comparable decrease of the number of egg rafts collected after oviposition as 3.33± 0.33, 5.33± 0.33, 3.67± 0.33 and 8.67± 0.33 each for **T ♂ x T ♀, T ♂ x UT ♀, T ♀ x UT♂, UT ♂ x UT ♀** groups. Number of eggs were counted from each egg rafts and recorded as 144.50± 11.50, 398.67± 6.49, 101.33± 13.54 and 1779.00±0.00 respectively for **T ♂ x T ♀, T ♂ x UT ♀, T ♀ x UT♂, UT ♂ x UT ♀** groups. Percentage control of reproduction was also showed as 65.86%, 65.67%, 66.78% and 0.00% for each groups **T ♂ x T ♀, T ♂ x UT ♀, T ♀ x UT♂, UT ♂ x UT ♀ (Table 64).**

Table 64- Effect of syrup formulations of *B. spectabilis* (H: EA- 5: 5) on ovi- position, egg hatchability and control of reproduction of adult *Cx. quinquefasciatus.*

SI No.	Treated groups	Ovi- position day after blood meal	No. of egg rafts laid	Average no. of eggs obtained	% Hatch	% Control reproduction
1	T ♂ x T ♀	6.33±0.33	3.33±0.33	444.50± 11.50	43.24	65.86
2	T ♂ x UT ♀	5.33±0.33	3.33±0.33	398.67± 6.49	54.40	65.67
3	T ♀ x UT♂	6.33±0.33	5.67±0.33	601.33± 13.54	48.34	66.78
4	UT ♂ x UT ♀	2.67±0.33	8.67±0.33	1779.00±0.00	85.78	00.00

Note: 10 numbers of treated females and males were taken in each of the 3 replicates

Formulated fraction of *P. longifolia* (MeOH: EA- 4:1) effected on the oviposition day of the treated groups **T ♂ x T ♀, T ♂ x UT ♀& T ♀ x UT♂** as 5.33± 0.33, 5.33± 0.33 and 4.33+ 0.33 days, whereby, **UT ♂ x UT ♀** groups showed 2.67+ 0.33 days. The number of eggs enclosed in each egg rafts were counted and recorded for all the groups as 296.50± 16.50, 295.67± 6.98, 388.33± 23.24 and 2003.00±0.00for **T ♂ x T ♀, T ♂ x UT ♀, T ♀ x UT♂, UT ♂ x UT ♀** respectively. Percentage control of reproduction was obtained as71.66%, 73.94%, 67.33% and 0.00%for each treated **T ♂ x T ♀, T ♂ x UT ♀, T ♀ x UT♂** and untreated **UT ♂ x UT ♀** groups **(Table 65)**.

Table 65- Effect of syrup formulations of *P. longifolia* (MeOH: EA- 4:1) on ovi- position, egg hatchability and control of reproduction of *Cx. quinquefasciatus* adults exposed as natural breeding places.

SI No.	Treated groups	Ovi-position day after blood meal	No. of egg rafts laid	Average no. of eggs obtained	% Hatch	% Control reproduction
1	T ♂ x T ♀	5.33±0.33	4.33±0.33	596.50± 16.50	50.12	71.66
2	T ♂ x UT ♀	5.33±0.33	3.33±0.33	495.67± 6.98	36.40	73.94
3	T ♀ x UT♂	4.33±0.33	6.67±0.33	988.33± 23.24	58..81	67.33
4	UT ♂ x UT ♀	2.67±0.33	8.67±0.33	2003.00±0.00	89.72	00.00

Note: 10 numbers of treated females and males were taken in each of the 3 replicates

The syrup formulation of column fractionated extract of *S. guttata* (MeOH: EA- 4:1) resulted a significant loss in fecundity and fertility of different treated groups **T ♂ x T ♀, T ♂ x UT ♀, T ♀ x UT♂** and untreated **UT ♂ x UT ♀** exhibited 28.17%, 40.07%, 16.69% and 100.00% of fertility respectively and 45.45, 64.63, 26.93 and 161.3 as fecundity rate**.** When compared with untreated group **UT ♂ x UT ♀** (0.00), the Sterility Index (SI) was found to be higher in treated groups **T ♂ x T ♀, T ♂ x UT ♀, T ♀ x UT♂** as 88.92, 79.11 and 95.10 when mated with equal number of males and females **(Table 66)**.

Table 66- Fecundity, fertility and Sterility effects of syrup formulation prepared by *S. guttata* (MeOH: EA- 4:1) on the adults of *Cx. quinquefasciatus.*

SI NO.	Treated Groups	Fecundity rate	Fertility (%)	Sterility Index (SI)
1	a. (T ♂ x T ♀)	45.45	28.17	88.92
	b. (T ♂ x UT ♀)	64.63	40.07	79.11
	c. (T ♀ x UT♂)	26.93	16.69	95.10
	d. (UT ♂ x UT ♀)	161.3	100.00	0.00

Note: 10 numbers of treated females and males were taken in each of the 3 replicates

The treated groups of *A.paniculata* **T ♂ x T ♀, T ♂ x UT ♀, T ♀ x UT♂** exhibited 31.65%, 17.32% and 46.97% fertility and untreated **UT ♂ x UT ♀** as 100.00% fertility**.** Fecundity rate was also reduced as 52.45, 28.70, 77.83 and 165.70 respectively for **T ♂ x T ♀, T ♂ x UT ♀, T ♀ x UT♂, UT ♂ x UT ♀.** Sterility Index (SI) was found to be higher, when compared with **UT ♂ x UT ♀** group consisted of 0.00 and the treated groups **T ♂ x T ♀, T ♂ x UT ♀, T ♀ x UT♂** showed 86.12, 92.98 and 74.48 Sterility respectively **(Table 67)**.

Table 67- Fecundity, fertility and Sterility effects of syrup formulation prepared by *A. paniculata* (MeOH: EA- 4:1) on the adults of *Cx. quinquefasciatus.*

SI NO.	Treated Groups	Fecundity rate	Fertility (%)	Sterility Index (SI)
1	a. (T ♂ x T ♀)	52.45	31.65	86.12
	b. (T ♂ x UT ♀)	28.70	17.32	92.98
	c. (T ♀ x UT♂)	77.83	46.97	74.48
	d. (UT ♂ x UT ♀)	165.70	100.00	0.00

Note: 10 numbers of treated females and males were taken in each of the 3 replicates

The fertility percentage of syrup formulation of *B. spectabilis* (H: EA- 5:5) was lower in treated groups **T ♂ x T ♀, T ♂ x UT ♀, T ♀ x UT ♂** as 8.12%, 22.40% and 5.6%, compared with untreated group **UT ♂ x UT ♀** as 100.00%**.** The fecundity rate was observed as 14.45, 39.86, 10.13 and 177.90 respectively for **T ♂ x T ♀, T ♂ x UT ♀, T ♀ x UT♂, UT ♂ x UT ♀** groups. Whereas, thesterility index were found to be higher in treated groups as compared with untreated group. It was exhibited as 96.00, 85.80 and 91.61 for **T ♂ x T ♀, T ♂ x UT ♀, T ♀ x UT ♂** and 0.00 for **UT ♂ x UT ♀** groups **(Table 68)**.

Table 68- Fecundity, fertility and Sterility effects of syrup formulation prepared by *B. spectabilis* (H: EA- 5:5) on the adults of *Cx. quinquefasciatus.*

SI NO.	Treated Groups	Fecundity rate	Fertility (%)	Sterility Index (SI)
1	a. (T ♂ x T ♀)	44.45	24.98	87.50
	b. (T ♂ x UT ♀)	39.86	22.40	85.79
	c. (T ♀ x UT♂)	60.13	33.78	80.96
	d. (UT ♂ x UT ♀)	177.9	100.00	0.00

Note: 10 numbers of treated females and males were taken in each of the 3 replicates

Syrup formulation of seed extract of *P. longifolia* (MeOH: EA- 4:1) reduced the fecundity and fertility rate of adult *Cx. quinquefasciatus* and also exhibited comparatively higher Sterility Index with all the treated groups **(Table 69)** from the untreated groups. Treated groups such as **T ♂ x T ♀, T ♂ x UT ♀, T ♀ x UT♂** showed 14.80%, 14.76% and 19.38% fertility as compared with 100.00% for **UT ♂ x UT ♀** and 29.65, 29.56 and 38.83 for treated groups and 200.30 for untreated group respectively as fecundity rate. Whereas, the Sterility Index (SI) was higher as 91.80, 94.10 and 87.80 for treated groups **T ♂ x T ♀, T ♂ x UT ♀, T ♀ x UT♂,** when compared with untreated group **UT ♂ x UT ♀** as 0.00**.**

Table 69- Fecundity, fertility and Sterility effects of syrup formulation prepared by *P. longifolia* (MeOH: EA- 4:1*)* on the adults of *Cx. quinquefasciatus.*

SI NO.	Treated Groups	Fecundity rate	Fertility (%)	Sterility Index (SI)
1	a. (T ♂ x T ♀)	59.65	29.78	83.39
	b. (T ♂ x UT ♀)	49.56	24.74	89.97
	c. (T ♀ x UT♂)	98.83	49.34	67.66
	d. (UT ♂ x UT ♀)	200.3	100.00	0.00

Note: 10 numbers of treated females and males were taken in each of the 3 replicates

3.9 DISCUSSION

State or local level mosquito control programs always used almost all applicators for synthetic pesticide applications which, is hazardous to non-target organisms. For mosquito control, pesticide application is more restricted among residential areas, where human exposure is common and also application of synthetic larvicides and adulticides are accumulated the pesticide residues over water bodies as well as wind drift may also carry the pesticide over agricultural crops and other non- targeted areas. Vector resistance to certain larvicides and adulticides are the other major threats related with synthetic insecticides. Therefore, sustained integrated vector management requires alternatives for synthetic insecticides.

Plants, the richest source of phytochemicals could be trapped for use as insecticides (Jacobson, 1982). They play an important role as larvicides, adulticides, feeding deterrents, oviposition deterrents, insect growth regulators (IGRs) and also act as pheromones, attractants and as repellents (Sukumar *et al*., 1991). Most common secondary metabolite compounds obtained from different plant parts were isoprenoids, alkaloids, phenyl propanoids and glucosinolates. Different types of alkaloids, more than 6000 alkaloids, 4000 amino acids, 3000 terpenoids, 1000 flavonoids, thousands of phenyl terpenoids, 650 polyacyl and 500 quinones have been elucidated from different species of plants (Jebanesan, 2013).

Searching for phytochemicals that are capable for being used to develop phytoinsecticides against filarial vector mosquito, *Cx. quinquefasciatus* from the selected plant species revealed the presence of several phytoconstituents. In the present study, the presence of phytochemicals from the methanolic seed extract of *S. guttata* determined certain important secondary metabolites such

as alkaloids, carbohydrates, glycosides, saponins, phytosterols, phenols, tannins, flavonoids, proteins and amino acids, diterpenes, quinones, fixed oils and fats and gums and mucilage. Few studies have been also carried out on the phytoconstituents of seeds of *S. guttata*. Shanthasubitha and Saravanababu, 2016 carried out a study on pharmacognostical and fatty acids investigation on another species of *Sterculia (Sterculia foetida)* and confirmed the presence phytoconstituents like flavonoids, alkaloids, tannins, phenols, saponins, ascorbic acid, fixed oils, proteins, carbohydrates, phytosterols gums and mucilaginous substances. The chemical composition of *Sterculia* species have received much attention due to the distribution of a wide range of flavonoid constituents, mostly as flavones and flavonol glycosides, two major classes of phenylpropanoids: cinnamic acids and coumarines, limited terpenoids and some miscellaneous compounds such as sterculine I and II isolated from seeds of *S. lychnophora* and also confirmed the presence of several fatty acids such as linoleic, oleic, malvalic, palmitic and sterculic acids (Sherei *et al*., 2016). Therefore, present study and the literature surveys proved the presence of above said secondary metabolites from the seed methanolic extract of *S. guttata*.

The result of the phytochemical analysis of methanolic leaf extract of *A. paniculate* correlated with earlier findings. The methanolic leaf extract of *A. paniculata* exhibited the presence of secondary metabolites alkaloids, carbohydrates, glycosides, phytosterols, phenols, flavonoids, diterpenes, terpenoids, phlobatannins, gums and mucilage. Phytochemical screening of leaf extracts of *A. paniculata* showed the presence of flavonoids, glycosides, phenols, tannins, steroids and diterpenes on methanol extract. Whereas, only phenols, tannins and steroids were presented on ethanol extract and flavonoids, saponins, phenols and amino acids were presented in aqueous extract (Lalitha *et al*., 2015). Phytochemical analysis of *A. paniculata* using petroleum ether and chloroform extracts exhibited the presence of phenolic

compounds, flavonoids, alkaloid, steroids saponins and tannins, while, phytochemical screening with acetone leaf extract produced no any other reactions, which indicated the absence of all these secondary metabolites (Sithara *et al.*, 2016).The methanolic leaf extract of *A. paniculata* also confirmed the presence of secondary metabolite alkaloids, triterpenoids, steroids, flavonoids, tannins, quinine, sugars and gums (Dwivedi *et al*., 2015).

Determination of secondary metabolites present in the methanolic leaf extract of *B. spectabilis* by the present study revealed the presence of alkaloids, carbohydrates, glycosides, saponins, phytosterols, phenols, tannins, flavonoids, diterpenes, terpenoids, phlobatannins, mucilage and gums. It was quite similar with the earlier reports that the acetone, chloroform, ethanol, methanol and aqueous leaf extracts of *B. spectabilis* confirmed the presence of alkaloids, flavonoids, glycosides, phlobatannins, saponins, steroids, tannins and terpenoids (Rashid *et al*., 2013). *Bougainvillea glabra*, another species of *Bougainvillea* extracted with hydroalcoholic solvent extract also showed the presence of a number of secondary metabolites such as proteins, steroids, glycosides, flavonoids, alkaloids, phenolic compounds and tannins (Sahu and Saxena, 2012).

Alkaloids, saponins, glycosides, phenols, tannins, proteins and amino acids, diterpenes, phlobatannins, fixed oils and fats, gums and mucilage are the secondary metabolites obtained on the phytochemical screening of methanolic seed extract of *P. longifolia*. Phytochemical screening of different extracts of seeds of *P. longifolia* were confirmed the presence of various compounds such as steroids, tannins, phenols and flavonoids for hexane, ethyl acetate, acetone, ethyl alcohol and methyl alcohol extracts (Kavitha, *et al*., 2013).

The literature search provided the information regarding the presence of secondary metabolites found on the different parts of the plants *S. guttata, A.*

paniculata, B. spectabilis and *P. longifolia* which strongly support the observations made in the present study.

Several investigations also confirmed significant biological activities of the secondary metabolites presented in the genus *Sterculia,* which performed antibacterial, antifungal and antioxidant activities on acetone and chloroform extracts, because these extracts are found to be rich in phytochemical constituents, responsible for their potent biological activities (Shanthasubitha and Saravanababu*,* 2016). This genus is also well- known for anticancer activity. The leaves of *Sterculia* genus also showed high cytotoxic effects in almost all tests (Chinsembu and Hedimbi, 2010) and the ethanolic leaf extract had moderate activity against BGC-823, Bel-7402 and HCT- 8 cell lines (Xia *et al*., 2009). Similarly, *A. paniculata* also possessed a number of phytoconstituents, which were also responsible for pharmacological properties like anticancer, anti-hepatotoxocity, anti- diabetic and anti-inflammation (Bisht *et al*., 2011). *B. spectabilis* also comprises vast number of phytoconstituents, which had potent antioxidant and anti-hyperlipidemic activities. The genus *Polyalthia* consisted several phytoconstituents, which might be responsible for anticancer, antimicrobial, anti-inflammatory, hypotensive, anti- ulcer, hypoglycaemic and antioxidant activities (Katkar *et al*., 2010). Present study also proved potent larvicidal properties of crude methanolic extracts of the selected plants, *S. guttata*(seed)*, A. paniculata*(leaf)*, B. spectabilis*(leaf)and *P. longifolia*(seed), which might be due to the presence of the secondary metabolites produced by the plants.

Most of the phytoconstituents directly or indirectly produced some amount of toxicity to insect pests/ vectors after their exposure. Many of the secondary metabolites present in plants have significant ecological functions. Most of the nitrogenous secondary metabolites are synthesized from common amino acids, by which several plant proteins like protease inhibitors, had been

reported to cause effect on growth and development of the target insect (Manual on Phyto- insecticide research, 2016).

Alkaloids are the first organic compounds isolated from plants and mostly occur as combined plant acids. They possess pronounced pharmacological and toxic properties, such as high larvicidal activity in *Lycopersi conesculentum* and larval growth inhibition among *L. decemlineata* (Manual on Phyto- insecticide Research, 2016). Certain alkaloids can produce developmental defects in animals such as lambs born by sheep had serious facial deformations, which had consumed alkaloid treated plant (Thomas *et al*., 2004). Certain ornithine derived alkaloids caused acute and chronic liver toxicity in humans and other animals (Koleva *et al*., 2012). Highly carcinogenic toxicity and genotoxic effects of several alkaloids were also reported (Shimshoni *et al*., 2015). Insect pollinators exposed to a diverse array of alkaloids which had some negative consequences like reduced mobility, survivorship and reduced ovary development among various insect species (Matsuura et al., 2014). Furthermore, plant alkaloid toxicity often involves neurotoxicity and cell signaling disruption, which inhibits choline acetyltranferase and several other neurotransmitters and affects neurotransmission (Mithofer *et al*., 2012).

Cyanogenic glycosides themselves are not toxic but are broken down to give off poisons, because it can be activated by enzyme hydrolysis in the cytoplasm, which removes the sugar part of the molecule and release toxic hydrogen cyanide (Manual on Phyto-Insecticide Research, 2016). Glycosides are proposed to evolve as specific toxins toward enzymes of primary digestive metabolism (Spencer *et al*., 2009). Therefore, certain glycosides have been shown to act feeding deterrents among insects and they also possess cytotoxic activities like inhibition of respiration and produce certain neurotoxins (Ressler *et al*., 1969 and Nahrstedt, 1985).

Phytosterols, similar to cholesterol vary only in carbon side chains and presence or absence of double bond. Phytosterols modulate the activity of membrane- bound enzymes (De Smet *et al*., 2012). For the regulation of moulting process in insects, steroid metabolism should exploit two unique target areas (a) disrupting cholesterol production through inhibition of dealkylation and conversion of certain phytosterols to cholesterol, thereby reducing the amount of available moulting hormone precursor and (b) by blocking any of the biosynthetic steps between cholesterol and ecdysone (Horn, 1989). Therefore, effectively blocking the early larval stages is easily possible by successful disruption of these biochemical pathways.

Phenolic compounds serve as defenses against many of the herbivores and pathogens. A phenolic polymer with high defensive properties, are the 'tannins' (Manual on Phyto- Insecticide Research, 2016). Toxicity of the tannins mainly affects the growth and survival of the herbivores, which were consumed. Certain feeding assays also showed that phenolic compounds exerted inhibitory effects on insect growth and development (Rehr *et al*., 1973; Ananthakrishnan, 1997; Kubo *et al*., 2008; Huang *et al*., 2011 and Mithofer *et al*., 2012).

Terpenes are polymeric isoprene derivatives and the largest class of secondary metabolites being structurally identified. Pyrethroids are esters of monoterpes, showed striking insecticidal activities. Limnoids, a group of triterpenoids are well- known feeding deterrent. Another triterpene, saponins isolated from the plants *Glycin max* and *Pisum sativum* caused larval mortalities and it also inhibited larval growth in *Callosobruchus chinensis* (L.) (Manual on Phyto-Insecticide Research, 2016).

Plants synthesize a huge variety of fatty acids, consist of long hydrophobic, often un-branched chains of hydrocarbons, with hydrophilic carboxylic acid groups at one end. Insecticidal activity of fatty acids, oleic, linoleic and lauric

acids against *Callosobruchus maculatus* on cowpea was investigated (Pedro, 1990). Based on the investigations, it was found that lauric acid had no effect on progeny development, but oily oleic and linoleic acids were reduced progeny development and ovicidal action. Toxicity of fatty acid salts to German and American Cockroaches had also been evaluated (Baldwin *et al*., 2008). Potassium and Sodium laurate caused 95%- 100% mortality respectively for German and American cockroaches. Potassium fatty acids/ insecticidal soaps were used to control many of the pest insects (Cranshaw, 2008). These fatty acids might disrupt cell membranes, resulting in cytolysis. It also interferes with growth hormones and affect insect metabolism.

Plant steroids constitute a diverse group of natural products and serves as analogues to hormones, such as moulting hormone ecdysone, juvenile hormones and neurotransmitters. Phytoecdysteroids are polyhydroxylated steroid hormones. One of the most well- known examples is 20-hydroxyecdysone (20E), which regulates the moulting process as larval moult, onset of pupa formation and metamorphosis in insects (Lafont, 2002), also reproduction and other physiological processes such as embryonic development (Riddiford *et al*., 2000 and Spindler *et al*., 2001). Ecdysteroids act as hormones, pheromones or insect deterrents (Nijhout, 1994).

Present study confirmed the presence of these secondary metabolites in the crude extract of selected plants, such as seed extract of *S. guttata*, leaf extracts of *A. paniculate* and *B. spectabilis* and seed extract of *P. longifolia*. These plant extracts exhibited potent larvicidal activities on highest lethal doses of crude methanolic extracts (**Tables 8, 9, 10 & 11**), whereas, it also showed sterilant activities on filarial vector *Cx. quinquefasciatus* on the exposure of median lethal doses of column fractionated gradients (**Tables 24, 25, 26 & 27**). Literature surveys also put forth the proof of several biological activities of various secondary metabolites, which could alter insect metabolic

pathways. From these, it could be established that the larvicidal and phytosterilant properties of these selected plant extracts might be due to the activity of the secondary metabolites either alone or synergistically.

TLC profiling of all the selected plant extracts gives impressive results, which could confirm the presence of various secondary metabolites in the selected plant extracts. The secondary metabolites showed different R_f values in different solvent phases, which provides a very important information in understanding their polarities, because low polarity/ non- polar compounds have higher R_f values than higher polarity compounds. Based on the TLC profiling of column fractionated seed extract of *S. guttata*, alkaloids exhibited to be more polar with R_f value 0.35 (**Table 44**), leaf extract of *A. paniculata* resulted in higher polarity to Phytosterols with R_f value 0.37 (**Table 45**), leaf extract of *B. spectabilis* provided flavonoids as high polar compound with R_f value 0.19 (**Table 46**) and seed extract of *P. longifolia* exhibited saponins as polar compound with R_f values 0. 52 and 0.75 (**Table 47**).

Column fractionated seed extract of *S. guttata* (MeOH: EA- 4:1) was subjected to GC/ MS analysis and eight compounds were identified and found to be hexadecanoic acid methyl ester (Palmitic acid), octadecanoic acid methyl ester, methyl 9,10-methylene-octadec-9-enoate (Sterculic acid), 9, 12-octadecadienoic acid (z-z)-methyl ester (Linoleic acid), Methyl 2-octylcyclopropene 1-heptanoate (Malvelic acid), n-hexadecanoic acid, 9-octadecanoic acid (z)- methyl ester and heptadecanoic acid methyl ester (**Table 48**) . This result was correlated with the previous studies, which revealed the detection of hydrocarbons from the seeds of *S. guttata* as sterculic acid, malvelic acid, palmitic acid, octadecanoic, Stearic acid, linoleic acid, oleic and palmitoleic. Total Ion Chromatogram (TIC) of *S. guttata* showed several peaks, in which the major peak was observed at retention time 26.108 was identified as hexadecanoic acid, methyl ester (Palmitic acid)

(**Figure 34**). The structure was supported by LC-Q-TOF-MS analysis of TIC at 8.2 minute with high molecular ion peak at m/z [M+ H] + 271. 0606 with fragmentation pattern m/z 270.26, 271.26 and 272.26 (**Figures 38 & 39**). Palmitic acid is a long- chain saturated fatty acid with a 16- carbon backbone and 32- hydrogen and the structure elucidated with NMR spectroscopy also resembles the structure of methyl palmitate with ^{1}H NMR with chemical shift values 5.351 (m, 2H), 3.663 (s, 3H); 2.770 (t, J=6,2H); 2.334 (m, 4H); 2.040 (t, J=8,2H); 1.554 (m, 4H); 1.53 (m, 14H); 0.880 (t, 3H) and ^{13}C NMR with chemical shift values δ 179.51, δ 34.32, δ 31.00 δ 29.6, δ 29.5, δ 29.4, δ 29.3, δ 29.2, δ 29.1, δ 27.4, δ 27.2, δ 25.9, δ 25.6, δ 24.7, δ 24.6, δ 22.7, δ 14.7. All data pointing towards the confirmation of presence of hexadecanoic acid, methyl ester (Palmitic acid) **(Figure 62)** in the seeds of *S. guttata*, which might be the cause of phytosterilant properties of seed extract of *S. guttata* on *Cx. quinquefasciatus*.

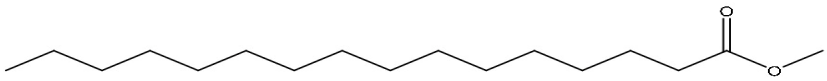


Figure 62- Structure of Palmitic acid

The toxicity studies related with Palmitic acid had been carried out by several researchers on several organisms. Benoit *et al*., 2010, investigated palmitic acid, mediated certain deleterious effects on hypothalamic insulin activity in rodents, which mainly affects Central Nervous System, specifically the neuropeptides. Hexadecanoic acid, methyl ester/ Methyl palmitate/ Palmitic acid is a saturated fatty acid with chemical formula $CH_3(CH_2)_{14}COOH$ with 16- carbon backbone. Palmitic acid is naturally produced by a wide range of plants and animals and it is the first product of fatty acid synthesis (Linstrom *et al*., 2014). Palmitate is the salt and esters of palmitic acid, which negatively feedback on the enzyme acetyl-CoA carboxylase, which was responsible for converting acetyl-CoA to malonyl-CoA, which in turn was used to add the

growing acyl chain, which prevents further palmitate production (Fatty acid Biosynthesis, Reference Pathway, 2015). Pascual *et al*., 2017, reported that Palmitic acid boosts the metastatic potential of $CD36^{+}$ metastasis-initiating cells in a CD36-dependent manner, on mouse models of human oral cancer. Lima *et al*., 2002, ranked polyunsaturated and volatile fatty acids, based on the toxicity on T-lymphocytes and B- lymphocytes. It was found that several fatty acids could cause cell death via apoptosis or, when concentrations are greater, necrosis. Zhang *et al*., 2004 also reported that Palmitic acid induced apoptosis/ inhibition of cell growth in a dose and time-dependent manner in human hepatoma cell line, HepG2 cells. Siegel *et al*., 1986, investigated that Palmitic acid and other free fatty acids could inhibit the sperm motility and spontaneous agglutination of sperm cells in whole semen among humans. Mu *et al*., 2001 demonstrated that saturated free fatty acid like Palmitic acid and Stearic acid remarkably suppressed the granulosa cell survival in a time- and dose- dependent manner in human granulosa cells. The suppressive effect on cell survival caused by apoptosis, which interfered by the metabolism of the respective acylcoenzyme A form**.** Inhibition of cell survival of granulosa cells might be responsible for causing reproductive abnormalities.

Ulloth and Casino*,* 2003 also demonstrated apoptotic cell death caused by Palmitic and Stearic acids in cultured model, which induced caspase-dependent and independent cell death in nerve growth factor differentiated PC12 cells. Ayvaz *et al*., 2002, found that, several free fatty acids in higher concentrations significantly lower insulin secretion from pancreas islet beta-cells, even in the presence of high levels of D- glucose. Dyntar, *et al.,* 2001 investigated that in adult rat, Palmitic acid induced apoptosis via de novo ceramide formation and activation of the apoptotic mitochondrial pathway, when exposed with the cardiomycetes of rats with higher concentration of Palmitic acid.

The toxicity of Palmitic acid on human beings and other organisms were widely studied. But insecticidal activities of Plamitic acid were studied by very few researchers. It was found that the contact toxicity of lauric, myristic and palmitic acid isolated from these plants caused high mortality on *Sitophilus granarius.* Insecticidal activities of certain other fatty acids were studied in various mosquito species and in some other insects. Ramsewak *et al*., 2001 found out insecticidal and insectistatic activities of linoleic acid against *Aedes aegypti*, *Helicoverpazea, Lymantria dispar, Malacosoma disstria*and *Orgyia leucostigma*. Rahuman *et al*., 2008 and Kannathansan *et al*., 2008 reported that oleic acid exerted insecticidal activity against *A. aegyptii, Anopheles stephensi* and *Culex quinquefasciatus.* Green, 2011 and Adebowale and Adedire, 2016 studied activity of linolenic acid against *Liposcelis bostrychophila* and *Callosobruchus maculates*. Brito *et al*., 2006 reported that linoleic acid, palmitic acid, and stearic acid exhibited potentpotent insecticidal activity against *S. frugiperda*.

Literature surveys confirmed the toxicity of Hexadecanoic acid, methyl ester (Palmitic acid) on humans and other organisms and also on several insect species. From these literatures, it has been cleared that Palmitic acid could induce cellular apoptosis, inhibition of neurotransmitter, several other deleterious effect on DNA and reproductive toxicity among several organisms. Besides these, larvicidal, adulticidal and other insecticidal activities were also observed on various insect species, when exposed to these compounds.

Present study also confirmed antifertility efficacies of column fractionated seed extract of *S. guttata* on *Cx. quinquefasciatus***(Table 8)**, which might be relied on Hexadecanoic acid, methyl ester (Palmitic acid), which was present abundantly on the seeds of *S. guttata*, was identified through GC/MS, LC-Q-TOF and NMR analysis.

Several species of *Sterculia* genus showed significant antimicrobial, antioxidant, anti- inflammatory and cytotoxic activities against variety of organisms (Sherei *et al*., 2016). But the mosquitocidal activity of seeds of *S. guttata* was restricted a little. Larvicidal activity of seeds of *S. guttata* on dengue vector *Aedes aegypti* and filarial vector *Cx. quinquefasciatus* were investigated (Katade *et al*., 2006) and it was found that ethanol, chloroform and hexane extracts exhibited 100% larval mortality within 24hrs of exposure at 500ppm concentration. Present study also exhibited potent larvicidal activity against *Cx. quinquefasciatus*, when higher dosage of crude acetonic extract of *S. guttata* with LC_{50} value 1.469ppm.

GC/ MS analysis of column fractionated leaf extract of *A. paniculata* revealed the presence of various phytocompounds such as pentadecane, 2,6,10,14-tetramethyl (Squalane), Bicyclo [3.1.1]heptanes, 2,6,6-trimethyl-, (1.alpha., 2.beta., 5.alpha.,hexadecanoic acid, methyl ester, phytol and 2- Ethylacridine, 1,2,3- Propatriol, 1-indole-4-yl(ether) (**Table 50 & Figure 35**). Kalaivani *et al*., 2012 also performed GC/ MS analysis of chloroform leaf extract of *A. paniculata*, which determined the presence of thirteen different compounds namely, tetradecanoic acid, 1,1,3-triethoxy-propane, phytol, 9,12-octadecadienoic acid, 3,7,11,15-tetramethly-2-hexadecene-1-ol, 1,2-benzenedicarboxylic acid, diisooctyl ester, retinoic acid methyl ester, squalene, 9,12,5- octadecatrienoic acid (Z,Z,Z), 5(a) and B-sitosterol and androstan-17-one,3-ethyl-3 hydroxy-.

Previous studies determined the chemical profile of acetonic extracts of *A. paniculata* leaves using GC-MS analysis (Thangavel *et al*., 2015). Nineteen components identified were ß terpinolene, terpinene, Ethyl iso-allocholate, 2-Propenamide, 2-methyl-N-phenyl-, Nonane, 1-chloro-, 1- Hexadecanol, Dotriacontane, Phenol, 2,4-bis(1,1-dimethylethyl)-, Cyclopentanetridecanoic acid, methyl ester, Dodecanoic acid, Ethyl Ester of Docosanoic Acid, Ethyl

Ester of Docosanoic Acid, Neophytadiene, D-glucose 6 O- D galactopyranosyl, Oxirane, hexadecyl-, 9-Eicosyne, Isochiapin B, ß pinene, 1,2-Benzenedicarboxylic acid, dioctyl ester and Naphthalene, 1,2,3,5,6,8a-hexahydro-4,7-dimethyl-1-(1- methylethyl)-, (1S-cis)-.

Sivakumar and Gayathri, 2015 identified 11 compounds from ethanolic leaf extract of *A. paniculata* namely, 3,7,11,15-tetramethyl-2-hexadecen-1-ol, phytol, 2,6,10,14,18,22-tetracosahexaene, 2,6,10,15,19,23-hexamethyl-, (all-e), heptacosane, 2,4,4-trimethyl -3-hydroxymethyl-5a-(3-methyl-but-2-enyl)-cyclohexene, 10,12-tricosadiynoic acid, trimethylsilyl ester, tetratetracontane, 3beta.-hydroxyguaia-4(15),10(14),11(13)-trien-6,12-olide 8-(.alpha.,.beta.-dihydroxybutyrate), dl -.alpha.-tocopherol, pentatriacontane, and beta.-sitosterol.

Chandrasekaran *et al*., 2009 isolated the therapeutically important active principle andrographolide from the aerial part of *A. paniculata*. Shen et al. (2006) reported six entlabdane diterpenoids from the aerial part of the plant i.e. 3-O-beta-D-glucopyranosyl-14, 19- dideoxyandrographolide, 14-deoxy-17- hydroxyandrographolide, 19-O-(beta-D- apiofuransy (1-2)-beta-D,glucopyranoyl)-3, 14-dideoxyandrographolide, 3-O-beta-D- glucopyranosyl andrographolide, 12S- hydroxyandrographolide and andragraphatoside.

The TIC GC/MS of *A. paniculata* exhibited the major peak at retention time 48.877 with TIC of LC-Q-TOF-MS analysis with molecular ion peak at 5.1 minute at m/z [M+ H] + 420.3088, was identified with fragmentation pattern m/z 422.49, 423.49 and 424.49. The identified compound was 2,6,10,15,19,23- hexamethyltetracosane (Squalene) **(Figures 40 & 41)**and the structure was supported by NMR spectroscopy with ^{1}H NMR chemical shift values 4.870 (d, J= 7Hz, 1H), 4.798 (d, J= 8Hz, 2H); 3.475 (d, J=3Hz, 2H); 2.333 (t, J= 8Hz, 2H); 2.806 (d, J=4, 2H); 2.504 (d, J= 7Hz, 2H); 2.017 (d, J= 7Hz, 2H); 1.785 (d, J= 3Hz, 3H); 1.785 (d, J= 3Hz, 3H); 1.733 (m, 2H); 1.599

(m, 1H), 1.533 (m, 1H); 0.988 (m, 1H); 0.880 (m, 3H), 0.815(m, 2H) and ^{13}C NMR with as δ 80.9, δ 70, δ 69, δ 64, δ 61, δ 54, δ 47, δ 43, δ 38.8, δ 38, δ 36, δ 29.6, δ 29, δ 28, δ 23.8, δ 23, δ 22, δ 16, resembles the 30- carbon and 50-hydrogen isoprenoid compound Squalene (**Figures 50, 51, 52 & 53**).

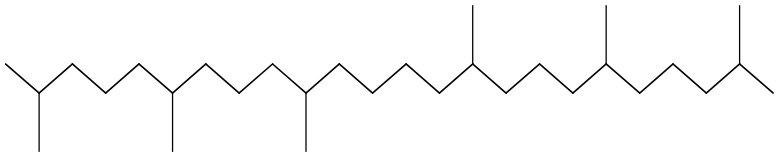


Figure 63- 2,6,10,15,19,23 - hexamethyltetracosane (Sqalane)

Squalene, **(Figure 63)** which was obtained from leaf extract of *A. paniculata* is a triterpene with 30- carbon organic compound and is a precursor of all plant and animal sterols. It is also present in shark liver oil as well as many other plants, including Amaranthus seed, rice bran, wheat gram and olive, which was commercially, used for fermenting the genetically engineered microorganisms as synthetic 'Sqalane'. Squalene is used as an adjuvant in vaccines, which is added to influenza vaccines to help stimulate the human body's immune response through production of CD4 memory cells (WHO, 2008).

Literature reviews informed Squalene did not produce much toxic effect on humans and other organisms. But, a few literatures provide certain information regarding the insecticidal activity of Squalene containing plant species. Chauhan *et al*., 2015 reported that the plant *Jatropha curcas* induced toxicity on different developmental stages of the house fly, *Musca domestica* were attributed to the presence of certain phytochemicals like transphytol, squalene and nonadecanone. Farag *et al*., 2012 evaluated the insecticide and repellent activity of acetonic extract and oils from the leaves of *Pelargonium hortorum.* It was observed that both the extract and oil showed high repellent

activities, but oil showed lowest insecticidal and extracts showed high insecticidal activities. They reported that the activity was mainly due to the highest percentage of volatile constituents such as, Squalene and Cyclooctacosan. Several researchers had been reported the relationships between insects and monoterpenoids, which showed efficacy and their modes of action on target insects (Karr and Coats 1988, Karr *et al*., 1990, Sharma and Saxena 1974, Harwood *et al*., 1990, Karr and Coats 1992, Khoshkhoo*et al*., 1993, Gunderson *et al*., 1985, Everaerts *et al*., 1988, Rice and Coats 1994). Fahmy, 1986 reported that terpenoid derivatives or analogs could possess many advantages over the parent compounds in biological activity, selectivity, stability, safety, phytotoxicity, and plant translocation.

Present study also identified the compound, Squalene from leaf extract of *A. paniculata,* which might be responsible for phytosterilant activity on *Cx. quinquefasciatus* **(Table 25)**. Literatures also strengthened the information regarding the insecticidal properties of Squalene as well as the plant *A. paniculata.*

Many of the researchers have suggested leaves of *A. paniculata* could be used as fungicide, nematicide and pesticide and also it is well- known for its medical properties, such as antibacterial, antiseptic, antioxidant, immunostimulant, cancer preventive, choleretic, contraceptive, hepatoprotective, antispermigenic, hypocholesterolemic, and sedative properties (Kalaivani *et al*., 2012). Leaf extracts of *A. paniculata* is also well-known for their insecticidal properties against various insect pests. Larvicidal activity of *Andrographis paniculata* leaf extracts against *Aedes aegypti, Anopheles stephensi* and *Culex quinquefasciatus* was determined with LC_{50} value 20.85 with petroleum ether leaf extract (Jeyasankar and Ramar, 2015). Aqueous and petroleum ether extract of *A. paniculata* and *A. lineata*nees exerted high larvicidal activity against *Ae. aegypti* and *Cx. quinquefasciatus* at

200ppm concentration individually, while synergistic effect produced 100% larval mortality at 150ppm concentration (Renugadevi *et al*., 2013). Flavonoid extracts from different parts of *A. paniculata* and *Vitex negundo* (L.) possessed high larvicidal activity on the late III or early IV instar larvae of *Aedes aegypti* and *Anopheles stephensi* (Liston) (Gautam *et al*., 2013). Five different solvents like benzene, hexane, ethyl acetate, methanol and chloroform extracts of *A. paniculata* and *Eclipta alba* exhibited significant adulticidal and repellent activities on *An. Stephensi* (Govindarajan *et al*., 2011). Besides these larvicidal efficacies, insect growth regulatory activities of ethanolic and methanolic leaf extracts of *A. paniculata* like prolonged larval duration and development, decrease in female longevity and fecundity, suppression of pupation and adult emergence were induced by the ethanolic and methanolic leaf extract of *A. paniculata* on *An. stephensi* was reported by Kuppusamy *et al*., 2010. The result of the present study is comparable with the larvicidal and IGR efficacies of leaf extract of *A. paniculata*, which were mentioned by several literatures. Methanolic leaf extract of *A. paniculata* possessed highest larval mortality at 67.240ppm (LC_{50}). Similarly, it produced several malformations during the development of filarial vector *Cx. quinquefasciatus*, like larval- larval, larval- pupal and pupal- adult intermediates with various morphological deformities. Present study also confirmed the potent larvicidal **(Table 9)** as well as IGR efficacies on the filarial vector *Cx. quinquefasciatus* at the exposure of minimum lethal dose and half of the median lethal dose respectively.

Six compounds were identified by GC/ MS analysis of column fractionated leaf extract of *B. spectabilis* namely, tetradecanoic acid (Myristic acid), thiophene, tetrahydro-2-methyl-, D- Fructose, 3-0-methyl-), methyl 4-0-methyl-d-arabinopyranose, pentadecane and indolizine, 2-(4-methylphenyl) **(Table 50 & Figure 36)**. The TIC of GC/MS analysis showed a major peak with retention time 33.344, which was supported by TIC of LC-Q-TOF-MS

analysis with molecular ion peak at 7.5 minute with m/z [M+ H] + 228.2307with fragmentation patterns m/z 228.21, 229.21 and 230.22 and the compound identified as tetradecanoic acid (Myristic acid) **(Figures 42 & 43)**. The structural elucidation by NMR analysis also supported the presence of tetradecanoic acid **(Figures 54, 55, 56 & 57)** with 14- carbon and 28-hydrogen with ^{1}H NMR chemical shift values 5.350 (b,1H),5.123 (t, J=8 Hz,2H); 3.320 (m, 2H); 1.679(m, 4H); 1.590 (m, 2H); 1.304 (m, 4H); 1.254 (m, 6H); 0.874 (t, J=8 Hz,3H) and ^{13}C NMR chemical shift values 181,39,32,31.8,29.7,29.3,29.1,26.8,26.4,23,22,16,14.

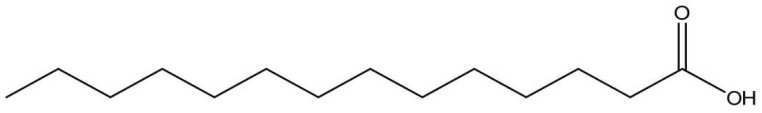


Figure 64-tetradecanoic acid (Myristic acid)

Tetradecanoic acid (Myristic acid) **(Figure 64)** was the compound isolated from the column fractionated leaf extract of *B. spectabilis*, which exerted phytosterilant activity on male *Cx. quinquefasciatus*. Myristic acid is a saturatedfatty acid with chemical formula $CH_3(CH_2)_{12}COOH$ with 14- carbon backbone. Burdock and Carabin, 2017 reported that Myristic acid showed low order of acute oral toxicity in rodents. Tetradecanoic acid (Myristic acid) was the compound isolated from the column fractionated leaf extract of *B. spectabilis*, which exerted phytosterilant activity on male *Cx. quinquefasciatus*. Myristic acid is a saturated fatty acid with chemical formula $CH_3(CH_2)_{12}COOH$ with 14- carbon backbone. Burdock and Carabin, 2007 reported that Myristic acid showed low order of acute oral toxicity in rodents. Abay *et al*., 2013, reported the insecticidal activity of various fatty acids isolated from Turkish mosses *Dicranum scoparium*, *Hypnum cupressiforme*, *Polytrichas trumformosum, Homalothecium lutescens* and the Turkish liverwort *Conocephalum conicum* against *Sitophilus granarius*. Among those

several fatty acids, highest mortality was obtained from Myristic acid. It was observed that very few studies have been carried out to determine the toxicity of Myristic acid on the basis of insecticidal activities. Therefore, this current study provides an initial stage of information regarding the phytosterilant as well as larvicidal efficacy of leaf extract of *B. spectabilis*, **(Tables 10 & 26)**, which consists of high amount of Myristic acid, which was identified by various spectroscopic analysis.

Guerrero, *et al*., 2017 identified 7 compounds from GC/ MS analysis of *Bougainvillea x buttiana* (var. Rose) Holttum and Standl including 2-Propenoic acid, 3-(2-hydroxyphenyl)-, (E)-, 2-Methoxy-4-vinylphenol, 3-O-Methyl-d-glucose, n-Hexadecanoic acid, Hexadecanoic acid, ethyl ester, 9,12-octadecadienoic acid, ethyl ester, and 9,12,15-Octadecatrienoic acid, ethyl ester (Z,Z,Z). Methanolic, ethanolic, acetonic and aqueous extract of *Bougainvillea x buttiana* also exhibited the following prevailing compounds such as 4H-pyran-4-one, 2,3-dihydro-3, 5-dihydroxy-6-methyl (2), 2-propenoic acid, 3-(2-hydrophenyl)- (E)- (3), and 3-O-methyl-D-glucose (6) (Abarca-Vargas *et al*., 2016). Several studies for isolation of phytochemicals from different parts of the plant *B. spectabilis* had been carried out by several researchers. Ethanolic and ethyl acetate extracts of stem indicated the presence of flavonoids viz. myricetin, formononetin- 7-O-rutinoside and genestein 7-O-rutinoside. Ethanolic and ethyl acetate leaf extracts considered as a rich source of polyphenolic compounds, omega-6 fatty acid and pinitol. Polyphenols from ethyl acetate stem extracts identified two compounds as hesperidin and orobol 7-O-glucoside and three compounds were isolated from the ethyl acetate flowers extract such as kaempferol 7-O-rhamopyranoside, kaempferol 7-O- rutinoside and kaempferol.

Heuer *et al.,* 1994, isolated and identified different compounds from the purple bract and white bract of *B. spectabilis*. Betacyanins such as

bougainvillein-r-l, isobougainvillein-r-1 and gomphrenin I was obtained from purple bracts, while flavonoids, such as isorhamnetin and quercetin were isolated from white bracts. Several biological studies were performed on the plant genus *Bougainvillea*, such as anti-diabetic (Jawla *et al*., 2012), anti-hyperlipidemic (Saikia and Lama, 2011), anti-microbial (Edwin *et al*., 2007).

Present study also identified the presence of various phytocompounds of the leaf extract of *B. spectabilis*, using GC/ MS analysis, LC-Q- TOF and NMR analysis, which was accordance with the earlier findings and the column fractionated leaf extract is also proved highest larvicidal as well as antifertility effect upon *Cx. quinquefasciatus* at minimal lethal dose **(Tables 10 & 26)**.

Column fractionated seed extract of *P. longifolia* was screened by GC/ MS analysis to identify the phytocomponents. The extract showed several peaks along with other very small peaks. Six compounds were identified namely, Phenol, 2,4-bis (1,1-dimethylethyl), hexadecanoic acid, 9-Octadecanoic acid, methyl ester, (E), cyclododecanone, 10, 13-octadecanoic acid, methyl ester and Methyl-2-octylcyclopropene-1-octanoate with a major peak noticed at retention time 29.339 **(Table 51 & Figure 37)** with TIC of GC/ MS and molecular ion peak at retention time 5.6 minute with LC-Q-TOF-MS analysis m/z [M+ H] + 207.1742 with fragmentation patterns m/z 206.17, 206.32 and 207.28 and the compound identified was Phenol, 2,4-bis (1,1-dimethylethyl) **(Figures 44 & 45)**. The NMR spectroscopy supported the structure with 14-carbon and 22 hydrogen with ^{1}H NMR chemical shift values **δ** 0.99 (s, 2H), 1.32 (s, 2H), 1.46 (s, 3H), 2.94 (dd, J = 9.52 Hz, 2.71 Hz, 1H), 3.76 (dd, J = 11.05 Hz, 9.52 Hz, 1H), 3.93 (dd, J = 11.05 Hz, 2.71 Hz, 1H), 7.15(d, J = 2.44 Hz, 1H), 7.42 (d, J = 2.44 Hz, 1H), 8.37 (s, 1H), 13.59 (s, 1H) and ^{13}C NMR chemical shift values δ 15.1, 27.0, 29.5, 31.6, 34.0, 34.3, 35.2, 63.6, 69.8, 118.2, 122.5, 127.0, 137.7, 137.9 **(Figures 58, 59, 60 & 61)**.

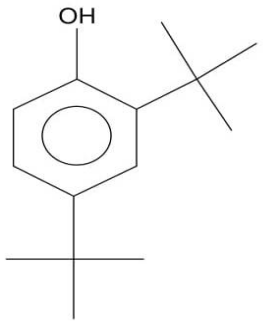


Figure 65- Phenol, 2,4 -bis (1,1-dimethylethyl)

The spectroscopic analysis identified the compound present in the seed extract of *P. longifolia* were Phenol, 2,4- bis (1-1 dimethylethyl) **(Figure 65)**, which might be responsible for the toxicity of the seed extract against *Cx. quinquefasciatus*. Phenol, 2,4- bis (1-1 dimethylethyl) is also known as 2,4-Di-tert-butylphenol, which was determined as an endocrine- disruptive phenols by manual shaking-enhanced, ultrasound-assisted emulsification microextraction (MS-USAEME) combined with ultraperformance liquid chromatography (UPLC) with UV detection from seawater samples and detergent samples (Shu *et al*., 2012). Salem *et al*., 2017, investigated the repellent and fumigant efficacies of the essential oils obtained from the plant *Ricinus communis* and *Mentha pulegium* on *Tribolium castaneumand Lasioderma serricorna*. Therefore, it could be possible to suggest phenol also exhibit insecticidal properties. Senthilkumar *et al*., 2012 identified certain phytochemicals from the methanolic leaf extract of *Trichilia connaroide,* which indicated the presence of richness of phenolics, fatty acids, flavonoids and antioxidants. It was observed that these compounds possessed antimicrobial, antioxidant, anticancer, antiulcerogenic and anti- insect activities.

Literature surveys provided very few information regarding the identification of phytocompounds from the seed extract of *P. longifolia*. Osuntokun *et al*., were reported on the identification of three compounds from the leaf essential oil, which were subjected to GC/ MS analysis and the main constituents obtained were 6-octadecenoic acid, Diisooctyl phthalate and 1,1,6- trimethyl-3-methylene. Azafluorene alkaloids and cytotoxic aporphine alkaloids from the leaves of *Polyalthia longifolia* were isolated by Wu *et al*., 1990. Jain and Sharma, 2011, isolated the compounds from column fractionated leaf extract of *P. longifolia*, it consisted of six compounds such as, caryophyllene, aromadendrene, viridiflorene, longifolene-(V4), α-guaiene and β-chamigrene. Ethanolic leaf extract of *P. longifolia* also revealed the presence of several phytocompounds such as Caryophyllene, Oxirane tetradecyl, 5-(7a-Isoprenyl-4,5-dimethyl-octahydroinden-4-yl)-3-methyl-pent-2-en-1-ol, Cedrandiol (8S,14) and γ-Gurjunenepoxide-(2), when subjected to GC/ MS analysis.

Literature survey also revealed a number of biological activities of the isolated compounds from the plant *P. longifolia*, such as antibacterial (Jayaveera *et al*., 2010), antifungal (Marthanda *et al*., 2005), anticancer (Verma *et al*., 2008), hypotensive (Saleem *et al*., 2005), fungicides (Shivpuri *et al*., 2001) and analgesic (Malairajan *et al*., 2008). Hence, the insecticidal activities, particularly, mosquitocidal activities of the plant *P. longifolia* had not well been established. Singha *et al*., 2013, demonstrated smoke toxicity effect of mosquito coil prepared by the leaves of *P. longifolia* against the filarial vector, *Cx. quinquefasciatus,* which caused 51%adult mortality after 24hrs of exposure. Leaf extract of *P. longifolia* also showed potent effect on larvae of *Cx. quinquefasciatus* of different habitat like laboratory, cesspit, cement drain and U drain. Larvicidal efficacy of leaf extract of *P. longifolia* was also observed on *Aedes* mosquitoes with 0.5ml concentration, which exhibited 100% mortality after 48hrs of exposure (Rathy *et al*., 2015).

From these, it has been cleared that the present study took an initiative step regarding the identification of phytocompounds from the seeds of *P. longifolia* by identifying six compounds (**Table 51 & Figure 37**), which might be due to the potent larvicidal and antifertility efficacies of the seed extract of *P. longifolia* on filarial vector *Cx. quinquefasciatus* (**Table 11 & 27**) at minimal lethal dose.

Laboratory studies proved promising larvicidal and antifertility efficacies of the selected plant extracts, *S. guttata* (Seed), *A. paniculate* (Leaf), *B. spectabilis* (Leaf) and *P. longifolia* (Seed) on the filarial vector *Cx. quinquefasciatus*. But the reliability of the extract should be confirmed by the application of field trials. Therefore, phytoformulation like Syrup formulation of selected plant extracts were prepared and applied on simulated field conditions. The field trials also showed promising phytosterilant property on adult *Cx. quinquefasciatus*. The field efficacies of the formulations were determined by its effect on oviposition, number of eggs, percentage of hatching, percentage of control reproduction, fecundity rate, fertility percentage and Sterility Index (SI) on **T ♂ x T ♀, T ♂ x UT ♀, T ♀ x UT♂** groups**.** The percentage control of reproduction was comparable with laboratory studies. When compared with field testing and laboratory testing, variations were observed among different treated groups, which exhibited comparatively higher percentage control of reproduction and Sterility Index (SI) with lower fecundity rate and fertility percentage among field populations compared with laboratory populations. When compared with Syrup formulation on field and laboratory tests with *S. guttata* (MeOH: EA- 4:1) and with chemosterilant HMPA exerted approximately same rate of Sterility Index in **T ♀ x UT♂** groups **(Figure 66).**While in the case *A. paniculata, B. spectabilis* and *P. longifolia,* both laboratory tests with column fractionated gradient and HMPA and field tests with syrup formulation provided nearly similar values for Sterility Index (SI) with **T ♂ x UT ♀** groups **(Figures 67,**

68 & 69). Among these, HMPA provided relatively higher Sterility Index (SI), when compared with Syrup formulations as well as column fractionated extracts of all the selected plants **(Figures 66, 67, 68 & 69)**.

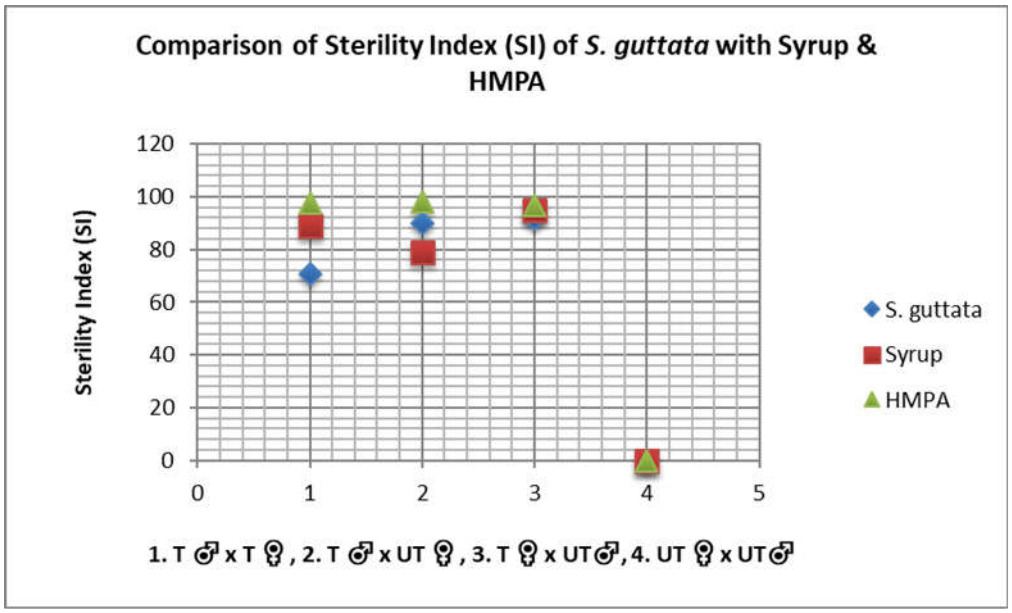


Figure 66- Comparison of Sterility Index (SI) of *S. guttata* with Syrup & HMPA

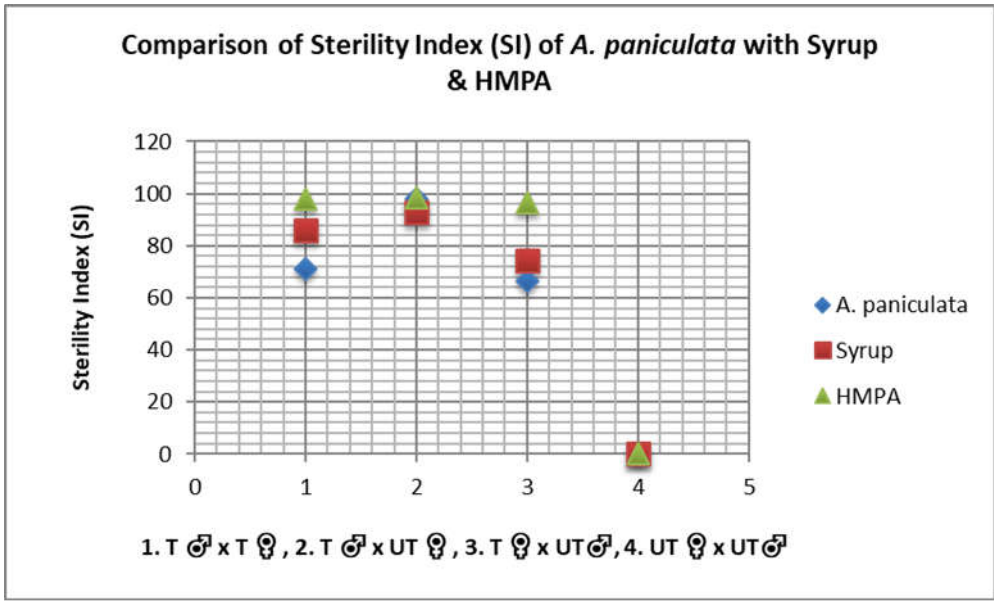


Figure 67- Comparison of Sterility Index (SI) of *A. paniculate* with Syrup & HMPA

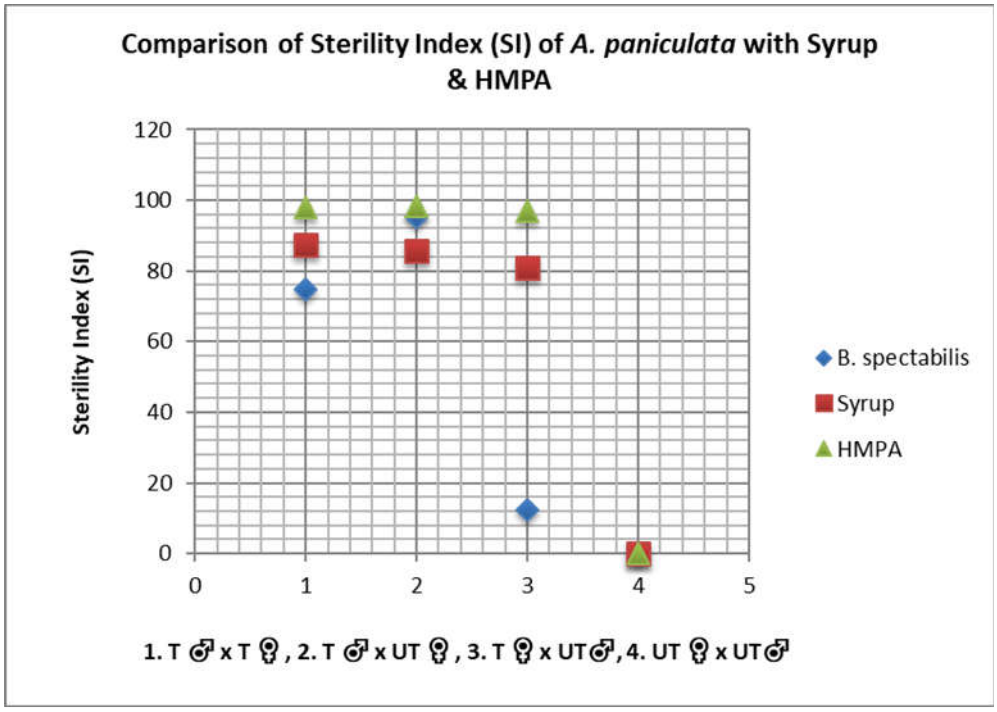


Figure 68- Comparison of Sterility Index (SI) of *B. spectabilis* with Syrup & HMPA

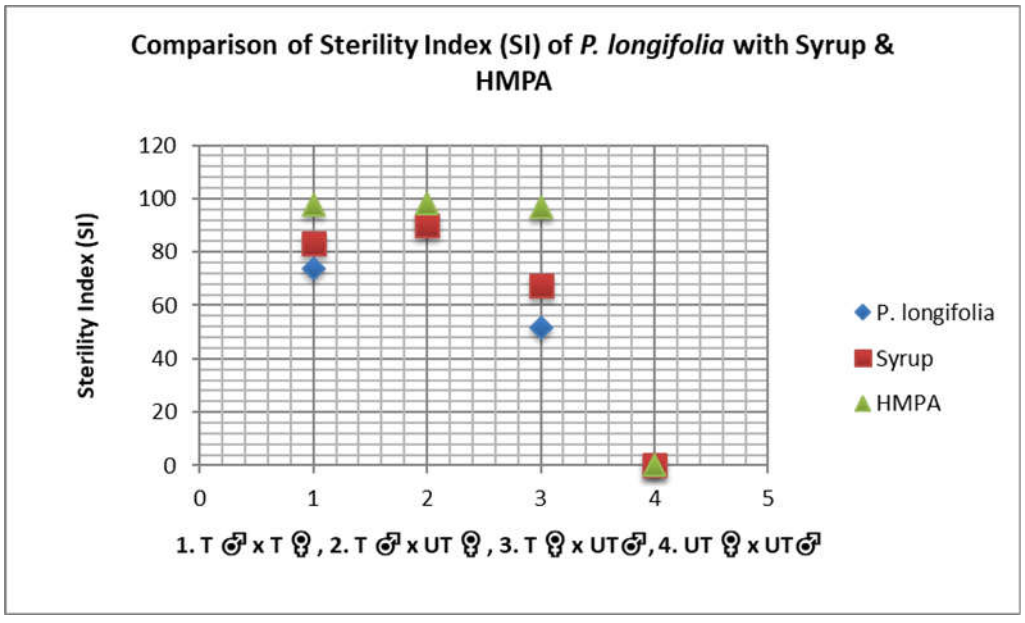


Figure 69- Comparison of Sterility Index (SI) of *P. longifolia* with Syrup & HMPA

From the observations, it has been clear that the syrup formulation of all the selected plants, *Sterculia guttata, Andrographis paniculata, Bougainvillea spectabilis* and *Polyalthia longifolia* might have the potency to induce sterility on filarial vector mosquito, *Culex quinquefasciatus* without burden the ecosystem. The significance of the study lies in the fact that column fractionated extracts of *A. paniculata, B. spectabilis* and *P. longifolia* could induce sterility among male mosquitoes, whereas extract of *S. guttata* induced sterility on female *Cx. quinquefasciatus* mosquitoes. These phytosterilant activities observed could be attributed to the presence of various phytochemicals detected by several isolation processes, which indicated the presence of Hexadecanoic acid methyl ester (Palmitic acid) in the seed extract of *S. guttata,* 2,6,10,15,19,23- hexamethyltetracosane (Squalane) from leaf extract of *A. paniculata*, Tetradecanoic acid (Myristic acid) from leaf extract of *B. spectabilis* and Phenol, 2,4- bis (1-1 dimethylethyl) from seed extract of *P. longifolia.*

From all these observations, it has become clear that the compounds identified from the selected plant extracts of *S. guttata, A. paniculata, B. spectabilis* and *P. longifolia* were considered to be potent insecticidal compounds, which affect the entire physiological process of an insect one or other way. The present study exhibited high larvicidal and phytosterilant efficacies of the selected plant extracts against the filarial vector *Cx. quinquefasciatus,* which might be possible because of the presence of these phytochemicals, which might be act alone or synergistically.

SUMMARY

Insects, form the largest fauna of all living organisms, which equally dominate the water, land and air, in which many of them are dangerous disease transmitting agents not only for human beings, but for other animals also. Among the Insect- borne diseases, Mosquito- borne diseases are more common, because mosquitoes are unevenly distributed in the human habitats and are always exposed to them. This unique relationship between humans and mosquitoes lead to the direct and indirect transmission of several dreadful mosquito- borne diseases like Dengue fever, Zika fever, yellow fever, West Nile Encephalitis, Chikunguniya, Malaria and Filariasis. Having spread to almost every country, these diseases became a huge burden globally, because at least 100 million cases occur annually, which also directly or indirectly affect the economy of a Nation.

Over the past few decades, scientists were exploring various methods to tackle threats from mosquito borne diseases and the exploitation of synthetic insecticides overwhelmed the major issues and has been used as major tool in mosquito control operations. However, the extensive and repeated use of synthetic insecticides disrupted natural systems heading to resistance and resurgence in target populations and destruction of non- target beneficial fauna including environment and human health concern. Therefore, since early times, many herbal products have been evaluated and used as natural insecticides. Since then, efforts have been done to determine the structure of different compounds and the commercial production of phyto- insecticide has been initiated as a part of Integrated Vector/ Pest Management (IVM).

The present study took an effort to control or reducing the mosquito population of *Culex quinquefasciatus* Say, which causesdreadful disease

'Filariasis', by exploiting certain botanicals, which has been derived from the plants, *Sterculiaguttata*Roxb., *Andrographis paniculata*(Burm. F.) Nees., *Bougainvillea spectabilis*Wild. and *Polyalthialongifolia*Sonn.

The investigations mainly focussed on the phytosterilant properties of the selected plants, which is more reliable to control the mosquito population of *Cx. quinquefasciatus*, becauseinducing sterility among these organisms not only affect the reproductive capacity but also influenced survival and longevity of these vector populations. For the experiments, mosquitoes were collected from Kalluthan Kadavu Colony, Calicut, brought to laboratory and reared and the bioassays were conducted to determine the insecticidal properties of the selected plants*S. guttata, A. paniculata, B. spectabilis* and *P. longifolia*. The crude methanolic and acetonic seed extracts of *S. guttata* and *P. longifolia* and leaf extracts of *A. paniculata* and *B. spectabilis*were prepared by using Soxhlet extraction procedure and bioassays for larvicidal efficacies were carried out by using these plant extracts.

The findings of the experimental bioassays revealed the larvicidal efficacies of the selected plant extracts on I instar larvae of *Cx. quinquefasciatus*. The crude methanolic and acetonic seed and leaf extracts of *S. guttata, A. paniculata, B. spectabilis* and *P. longifolia* had been found to possess efficient larvicidal activities against I instar larvae of *Cx. quinquefasciatus*. Maximum larvicidal activities were observed with crude acetonic extracts, whencompared with crude methanolic extracts of the selected plants after 24 hrs of exposure in a dose- dependent manner. The mortality values were significantly greater than that of control. The higher dose treatments caused mortality in a dose-dependent manner and it also possessed various growth inhibiting effects on the developmental stages when, exposed at lower dose treatments. It was undoubtedly proved that crude acetonic extracts of selected plants exhibited promising larvicidal efficacies with least LC_{50} values,

whereas, methanolic extracts also exhibited satisfactory larvicidal effects on I instar larvae of *Cx. quinquefasciatus*. Even though, crude acetonic extracts showed highest mortality with least LC_{50} values, it was desirable to select methanolic extracts for sterility experiments.The crude methanolic extracts of selected plants were purified by using Column Chromatography techniques to obtain most suitable fraction, which could induce sterility on *Cx. quinquefasciatus*. Among the different column fractions, Methanol: Ethyl Acetate- 4:1 fractions of *S. guttata, A. paniculata* and *P. longifolia*exhibited greater percentage emergence whereas, *B. spectabilis*showed highest percentage emergence on Hexane: Ethyl Acetate-5:5 fraction. Therefore, these column fractions were prepared and used for further sterility induction experiments.

During the developmental metamorphosis, treated larvae exhibited extension of total developmental duration, such as prolonged larval and pupal periods when compared with control. Larval progress was affected by induction ofextended larval durations with several morphogenetic deformities, including dechitinized body wall, alteration in head and abdomen, physically deformed pupae remained trapped in pupal eclusion and reduced body length compared with controls.Morphological deformities associated with developmental progresses included the appearance of larval-larval intermediates, larval-pupal intermediates and pupal-adult intermediates.In case of larval-larval and larval-pupal intermediates, mortality had occurred at an early stage of development**.**At the time of pupation, moulting started at the mid-thoracic region and the pupae thus emerged with a larval head and pupal abdomen**.** Microscopic examination of dead larvaerevealed that larval cuticle had scleratization, which was a characteristic feature of pupal cuticle. The dead pupae sometimes showed less scleratization of the cuticle and some partly emerged pupae with attached head capsule**.** The majority of dead pupae also exhibited a variety of malformations like completely demelanized pupa with

straight abdomen, dwarf pupa with retarded abdomen and partly melanized pupa with extended abdomen**.** In pupal-adult intermediates, sometimes death occurred after complete moulting from pupal skin butsome parts remained attached to the pupal exuvia**.**Those adults derived from treated larvae also showed deformed thorax and abdomen, reduced or deformed wings, curved and weak legs, and antennae twisted or sometimes without bristles**.** It was observed that, the larvae treated with *S. guttata, A. paniculata, B. spectabilis* and *P. longifolia*exhibited highest number of larval-larval intermediates, pupal-adult intermediates and maximum number of larval-pupal intermediates respectively. Growth Index (GI) was also found to be significantly lower than that of the controls.

Column fractionated extracts of the selected plants *S. guttata* (MeOH: EA- 4:1), *A. paniculata* (MeOH: EA- 4:1), *B. spectabilis* (H: EA- 5:5) and *P. longifolia* (MeOH: EA- 4:1) also exhibited significant reproductive toxicity on the adults of *Cx. quinquefasciatus*.The reproductive toxicity mainly included mating inhibition, reproductive suppression, low survival, extended oviposition period, low fecundity rate and high percentage control reproduction and sterility. Moreover,the dissections revealed substantial morphometric changes of the reproductive organs of the treated mosquitoes.The average sizes of the treated ovaries and testes were found to be significantly reduced when compared to untreated. Ovarian development was drastically affected in *S. guttata* treated resultant adults of *Cx. quinquefasciatus*. *A. paniculata*, *B. spectabilis* and *P. longifolia*also exhibited moderate effects on ovarian development. The ovaries of treated resultant adults showed variation in length and size of the ovarioles, ovariole degeneration, oocyte degeneration, resorption and inhibition of oviposition. Application of extracts on larvae of *Cx. quinquefasciatus*resulted in prevention of ovarian development by inhibiting vitellogenesis and oocyte development. It was observed that many treated resultants also exhibited

single ovary and degeneration of bursa inseminalis. On the other hands, theeffects of *A. paniculata*(MeOH: EA- 4:1), *B. spectabilis* (H: EA - 5:5) and *P. longifolia*(MeOH: EA- 4:1) on treated larvae interfered with the male reproductive organs.In most of the cases, degenerated or undeveloped testes were observed. But theaedegus, seminal vesicles and accessory glands did not have significant abnormalities. Thus, due to the combined effect of low survival & longevity, mating inhibition and reproductive suppression and also reduced fecundity realization was possible on filarial vector *Cx. quinquefasciatus* by using the purified fractions of selected plant extracts. From these results, it is apparent that the purified fractions of these selected plant extracts from*S. guttata, A. paniculata, B. spectabilis* and *P. longifolia*exhibited promising phytosterilant activities on *Cx. quinquefasciatus* by exhibiting significant reduction in percentage fertility and producing higher Sterility Index (SI), when compared with controls.

Comparative assessment of the phytosterilant properties of selected plant extracts with a potent chemosterilant Hexamethyl Phosphoramide (HMPA) also revealed that the Growth Index (GI) and Sterility Index (SI) exhibited by the plant extracts *S. guttata, A. paniculata, B. spectabilis* and *P. longifolia*tends to be in correlation with the results showed by HMPA on *Cx. quinquefasciatus*. However, HMPA showed, above 95% sterility in all type of crosses such as **T** ♂ **x T** ♀, **T** ♂ **x UT** ♀ and**T** ♀ **x UT**♂**,**but the plant extracts *A. paniculata*(MeOH: EA- 4:1), *B. spectabilis* (H: EA- 5:5) and *P. longifolia*(MeOH: EA- 4:1) exerted above 90% sterility only in **T** ♂ **x UT** ♀ and*S. guttata*(MeOH: EA- 4:1) with 91.61% in **T** ♀ **x UT**♂**,** when compared with control. Therefore, these observations clearly indicated that the selected plant extracts could induce promising sterilization effects on *Cx. quinquefasciatus* and also these plants proved to be competent with the chemosterilant HMPA by producing higher levels of reproductive toxicity on the adult vector mosquitoes *Cx. quinquefasciatus*.

In order to suggest a promising plant, it is very important to detect the nature of phytoconstituents present in the plants. Phytochemical screening is one of the most useful methods for the detection of phytoconstituents individually. Therefore, the crude methanolic extracts of the seeds and leaves of the plants *S. guttata, A. paniculata, B. spectabilis* and *P. longifolia* were subjected to phytochemical screening to identify the phytocompounds. The methanolic seed extract of *S. guttata* showed the presence of alkaloids, carbohydrates, glycosides, saponins,phytosterols, tannins, flavonoids, diterpenes, proteins and amino acids, quinones, oils & fats, gums & mucilage.*A. paniculata* revealed the presence of alkaloids, carbohydrates, glycosides, phytosterols, phenols, tannins, flavonoids, diterpenes, terpenoids,phlobatannins, gums and mucilage.*B. spectabilis* showed the presence of alkaloids, carbohydrates, glycosides, saponins, phytosterols, phenols, tannins, flavonoids, diterpenes, terpenoids, phlobatannins, gums and mucilage.*P. longifolia* exhibited the presence of alkaloids, glycosides, saponins, phenols, proteins and amino acids, diterpenes, phlobatannins, oils and fats , gums and mucilage.

GC-MS analysis, LC-QToF and NMR analysis were also performed to identify the phytocompounds, which could induce sterility on *Cx. quinquefasciatus*. GC/ MS analysis of column fractionated extract of*S. guttata* indicated the presence of eight compounds, which included Hexadecanoic acid methyl ester (Palmitic acid), octadecanoic acid methyl ester, methyl 9,10-methylene-octadec-9-enoate (Sterculic acid), 9, 12-octadecadienoic acid (z-z)-methyl ester (Linoleic acid), Methyl 2-octylcyclopropene 1-heptanoate (Malvelic acid), n-hexadecanoic acid, 9- octadecanoic acid (z)- methyl ester and heptadecanoic acid methyl ester, in which a major peak was identified as Hexadecanoic acid methyl ester (Palmitic acid). The structure was supported by LC-Q-TOF-MSanalysis with high molecular ion peak at m/z [M+ H] + 271.0606 with fragmentation pattern m/z 270.26, 271.26 and 272.26 and

NMR analysis with 16- carbon backbone and 32- hydrogen with ^{1}H NMR and ^{13}C NMR.

Column fractionated leaf extract of *A. paniculata* also revealed the presence of various phytoconstituents when subjected with GC-MS analysis, which mainly included the compounds likepentadecane, 2,6,10,14-tetramethyl (Squalene), Bicyclo[3.1.1]heptanes, 2,6,6-trimethyl-, (1.alpha., 2.beta., 5.alpha., hexadecanoic acid, methyl ester, phytol, 2- Ethylacridine and 1,2,3-Propatriol, 1-indole-4-yl(ether) with a major peak and was identified as 2,6,10,15,19,23- hexamethyltetracosane (Sqalene) with fragmentation pattern m/z 422.49, 423.49 and 424.49 in LC-Q-TOF-MSanalysis. NMR analysis supported by this structure with ^{1}H NMR and ^{13}C NMR by showing 30-carbon and 50- hydrogen.

When subjected toGC-MS analysis, column fractionated leaf extract of *B. spectabilis*,showed the presence of six compounds namely, tetradecanoic acid (Myristic acid), thiophene, tetrahydro-2-methyl-, D- Fructose, 3-0-methyl-), methyl 4-0- methyl-d-arabinopyranose, pentadecane and indolizine, 2-(4-methylphenyl)-. TIC of GC-MS analysis showed a major peak, which was identified as tetradecanoic acid (Myristic acid) with m/z [M+ H] + 228.2307with fragmentation patterns m/z 228.21, 229.21 and 230.22 with LC-Q-TOF-MSanalysis and the structure was supported by NMR analysis with 14-carbon and 28-hydrogen with ^{1}H NMR and ^{13}C NMR analysis.

GC-MS analysis of column fractionated seed extract of *P. longifolia* exhibited the presence of six compounds and were identified as Phenol, 2,4-bis (1,1-dimethylethyl), hexadecanoic acid, 9-Octadecanoic acid, methyl ester, (E), cyclododecanone, 10, 13-octadecanoic acid, methyl ester and Methyl2-octylcyclopropene-1-octanoate with a major peak noticed as Phenol, 2,4-bis (1,1-dimethylethyl), which was supported by LC-Q-TOF-MSanalysis with m/z [M+ H] + 207.1742 with fragmentation patterns m/z 206.17, 206.32 and

207.28. NMR spectroscopy also supported the structure with 14-carbon and 22-hydrogen with ^{1}H NMR and ^{13}C NMRanalysis.

The field efficacies of the purified column fractionated seed and leaf extracts of *S. guttata, A. paniculata, B. spectabilis* and *P. longifolia* were evaluated against *Cx. quinquefasciatus* in their natural breeding sites under simulated field conditions by preparing syrup formulation from these purified plant extracts. Weight uniformity test and Solubility tests were conducted to analyze the quality, reliability and stability of the formulations. Field trials also exhibited potent phytosterilant effects, followed by crosses between treated males (T ♂) and treated females (T ♀). Oviposition was extended and percentage hatch of abnormal eggs were considerably lower when compared to untreated eggs.Italso exhibited lower percentage of fertility and fecundity rates as well as number of egg rafts were also lower in all the treated females. However, field trials showed above 95% control of reproduction and sterility on syrup formulations of column fractionated extracts of *A. paniculata***,** *B. spectabilis* and *P. longifolia,* in treated males of *Cx. quinquefasciatus*than treated females. On the otherhand, *S. guttata* induced sterility in treated females considerably than in treated males. From these, it is clear that small scale field trials also confirmed the phytosterilant efficacies of these plant extracts with high percentage control of reproduction and Sterility Index on filarial vector, *Cx. quinquefasciatus*.

Regarding the sterilization techniques in insects, major cytogenetic effects of chemosterilants includedseveral morphogenetic and chromosomal aberrations, which mainly induced reproductive toxicity or sexual sterility in targeted insects. It has been confirmed that several hormones play major roles in the process of molting and metamorphosis in insects. Among these, Juvenile Hormones (JH), a group of structurally related sesquiterpenes regulates development and reproductive maturation in insects. Molting and pupation in

insects are also under the control of another hormone, Prothoracicotropic Hormone (PTTH), secrete a steroid hormone Ecdysone, which is acting together trigger every molt in an insect's metamorphosis. Therefore, interruption of JH biosynthesis and PTTH disrupted the normal development of an insect.

The present study demonstrated phytosterilant properties of selected plant extracts derived from the seeds of *Sterculia guttata* and *Polyalthia longifolia* and leaf extracts of *Andrographis paniculata* and *Bougainvillea spectabilis*, which could induce sexual sterility on *Culex quinquefasciatus* by exhibiting several developmental anomalies in each molt as well as morphogenetic abnormalities on reproductive structures of both male and females of adult *Cx. quinquefasciatus*, which might be possible because of the presence of the identified phytocompounds like Methyl Palmitate (Palmitic acid) in seeds of *Sterculiaguttata*, Squalene in *Andrographis paniculata*, Tetradecanoic acid (Myristic acid) in *Bougainvillea spectabilis* and Phenol, 2,4- bis (1,1-dimethylethyl)- in *Polyalthia longifolia*.

From these, it is clear that the selected plant extracts might act as Juvenile Hormone analogues, which could interfere with the life cycle of filarial vector *Cx. quinquefasciatus*. Therefore, the present study suggested that the purified seed extracts of *Sterculia guttata* and *Polyalthia longifolia* and the leaf extracts of *Andrographis paniculata* and *Bougainvillea spectabilis* can be used to control the wild population of the 'Filarial vector mosquito', *Culex quinquefasciatus*as a natural, biodegradable and cost- effective material without causing any environmental hazards.

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