SYNOPSIS

"Studies on the efficacy of deploying phytochemicals against pests and insects"

(Submitted to Mahatma Gandhi University Kottayam)

The 20th century has witnessed a hi-tech production of synthetic chemicals as wellness agents, spuriously branded as herbal medicines with a myriad of refreshing rewards and attracting offers, aiming at uncontrolled use and consumption. Despite all these, pharmacognosic significance of secondary metabolites and the interest in indigenous knowhow in pursuit of high therapeutic value, have reached high amplitudes. Gautam et al., 2007 have thus branded about 2500 plants as ethnomedicinal. Traditionally used medicinal plants produce a torrent of compounds of known therapeutic properties. Presently the importance of ethnobotanic studies in health care and conservation programmes have overwhelmed all boundaries as it is centred on interaction between plants and man over time and space.

By the advent of 21st century Kerala has recognised that waste management is an insurmountable problem. The accumulation of waste materials like the effluents from industrial and agricultural fields, as well as effusion from the markets and the restaurants, coupled with an inordinate inadequacy in the disposal programme have made Kerala a favourite abode for the mosquitoes. Other refuses which are not biodegradable such as tins, cans, shells, jars, tyres, coconut shells etc. would collect rain water affording conditions congenial for the growth and multiplication of mosquitoes. Thus Kerala has become the paradise of mosquitoes due to the characteristic eco-geographical landscape of the region rich on lakes and backwaters coupled with heavy rainfall, by both Northeast and Southwest monsoon. Several surveys revealed that over one million people die from mosquito borne diseases every year. Most common among the different types of mosquitoes in the southern peninsula are culex, anopheles and aedes, all well known as vectors inflicting the humans and the domestic animals. Very significant among the diseases are Chikungunya, Malaria, Yellow fever, Dengue, Filariasis, Encephalitis etc. The recent outrage of Chikungunya is sufficient enough to establish mosquitoes cause more sufferings to the humans than any other organisms.

Another breath taking problem evolved in last century is finding measures for preventing deterioration in quality, during the transit and the storage of agricultural products-mainly due to the storage pests viz rice weevils (Sitophilus oryza), granary weevils(

Sitophilus granarius), and maize weevils (Sitophilus zeamais). Control of these insects relies heavily on the use of synthetic insecticides and fumigants, which has led to problems such as disturbances of the environment, increasing costs of application, pest resurgence, pest resistance to pesticides and lethal effects on non-target organisms in addition to direct toxicity to users (Jembere et al., 1995; Okonkwo and Okoye, 1996).

Chemical Control of pests and insects is perhaps the most widely used method. Even from time immemorial, humans have utilized pesticides and insecticides to protect themselves as well as their cash crops. By the advent of 15th century systematic practices, like fumigation and poisoning, with compounds containing arsenics, lead etc. have become prevalent. The application of synthetic insecticides, as a pest control method, with a view to increase the target of yield, and to enhance marketing, would surely pose environmental hazards on one hand and resistance in the target organism on the other. They are extremely toxic, affecting the environment adversely (Hayes & Laws, 1991). According to Lamiri et al., (2001) the residues and leftovers of the insecticides are very dangerous for the entire biosphere. Thus it has become immensely necessary to formulate precautions and prophylactic measures against such hazards.

Tolerance and resistance developing against insecticides, is another stumbling block in the war against vector-transmitted-etiologies. Recent studies all over the globe have confirmed the development of this phenomenon in mosquitoes as well as in weevils too. As the resistance against traditional chemical pesticides increases, the concern about the potential health and environmental risk should also increase *at par*. Plant based drugs which have been proved to possess only little side effects (Goglay et al., 2002; Teixeira, 2006) warrant the exploitation of better and powerful drugs with larvicidal and insectisidal activities yet with minimum side effects. Herbal insecticides have long been mooted as an attractive alternative to synthetic chemical insecticides for pest management and protection of human health. This is true as the phytochemicals have reputedly pose only very little threat (Isman, 2006).

In view of all these in mind the present endeavour is to formulate eco-friendly phytochemicals for curbing the menace caused by mosquitoes and storage weevils, affording protection to life and environment. Hence an attempt is schemed for launching a new phytochemical which can act as a double edged sword, both as a larvicide and pesticide into the market. To find out the plants that are traditionally used against mosquitoes, an ethno

botanical survey was conducted among ethnic communities living in different parts of Kerala state. Thus Kumarakom of the coastal region, Manarcaud of central region and Melukavu of hilly region were selected. Based on informations collected, specific parts of twenty plants suggested by them were used for preparation of aqueous extracts and, larvicidal activity against *culex quinquefasciatus* mosquito, at the 4^{th} instar larval stage was checked and recorded. After primary screening *Gliricidia* belonging to Fabaceae, was selected for further study since it is considered as multipurpose tree in the light of various reports published. Easy availability and high larvicidal activity were also factors for selection. Mosquito species was selected based on the preponderance in Vazhappally area of Changanacherry.

In the present study crude extracts were prepared from dried powdered leaves of *Gliricidia* using petroleum ether, hexane, acetone, methanol and water successively, in Soxhlet apparatus and their mosquitocidal activity was examined. Petroleum ether extract was the best exhibiting maximum efficiency in larvicidal activity. So it was selected for further work on isolation and characterisation of the particular toxic compound. Thin layer chromatography was used for separation of the compounds. Fractions were separated after votexing and centrifuging using chloroform, diethyl ether, acetonitrile, acetone and methanol separately and, most active one was selected. Further purification was made using column chromatography. Out of the different methods deployed best separation with highly active compounds were obtained in acetone: methanol solvents after eluting the extract with chloroform. Isolated chemicals are Eicosatrienoic acid, Octadecadienoyl chloride, 14-Methyl-8- hexadecyn 1-0l and Octa decanal. So far no report is published on the presence of these chemicals in *Gliricidia*. Invariably this is the first report on the application of these chemicals for insecticidal activities. The chemical with maximum activity is Eicosatrienoic acid, a poly unsaturated fatty acid. Trivial name is Dihomo-g-linolenicacid, with molecular mass 306.48 and molecular formula $C_{20}H_{34}\ O_2$. It is a colourless liquid with fishy odour. Structure elucidation was carried out by GCMS and it was further verified by FTIR, HPLC, and NMR spectroscopy.

The MTT assay was performed to measure the metabolic activity of cells to reduce yellow colored tetrazolium salt 3-(4,5-Dimethyl thiazol -2-yl)-2,5- diphenyltetrazolium bromide to purple coloured Formazan. Different dilutions of extracts of test sample, negative control, reagent control and positive control in triplicate were placed on subconfluent monolayer of L-929 cells. Experiment proved the safety of the chemical Eicosatrienoic acid upto 0.2 mg/ml for human as it made destruction below 70%. Toxicity studies were

conducted in rats by observing biochemical changes occurring on fumigation. Histological studies were also conducted by examining the changes in Heart, Lungs, Liver, Kidney and Spleen. Two branded mosquito coils in popular use were selected (Mortein, Good knight) to get recommendative information. Subsequently the results were compared and contrasted with manually made new coils employing the newly detected mosquitocidal compound Eicosatrienoic acid from *Gliricidia*. Similar experiments were conducted with rats under gestation and, histological and biochemical studies have made in the newborns. Results clearly recommend the safety of Eicosatrienoic acid as a new insecticide.

Application studies of the new compound were conducted in *culex quinquefasciatus* mosquitoes and *Sitophilus granarius* weevils. The toxic activity of this phytochemical extracted from *Gliricidia* was determined by repellency test using filter paper and exposure of weevil adults. Contact toxicity was measured by applying the chemical directly to thorax while fumigant toxicity was checked using fumes in the glass vials. Results show the promising effect of Eicosatrienoic acid against *Sitophilus*. Mosquitocidal activity was checked, using 4th instar larvae of *Culex quinquefasciatus*. Various concentrations of the chemical were made and observation on mortality of the larvae was recorded after 24 hours of continuous exposure.

The results have already instigated the investigation towards developing devices for safe eradication of mosquitoes and weevils using the new biomolecule.