Abstract

The problem of ever accumulating wastes and its inadequate management has made the God’s own country, a heaven for insects and pests and hell for human dwelling. Further, the special ecogeographical and climatic characteristics have afforded favourable and congenial conditions for the spread of insect–borne, particularly mosquito borne diseases. The fact that the hillock of wastes seen everywhere making this abode, a rendezvous for rats, snakes and the millipedes obviously aggravate the situation. The prevalent practice of ignition of coils and other repellents are appeared like sugar coated poisons. They help repel mosquitoes, but invariably bring many uninvited hazards. The apprehended depth of these hazards for health and environment still remains incomprehensible. Since the arthropods become easily resistant to pesticides, heavy and frequent application is required leading to problem of accumulation of toxic residues adversely affecting the environment particularly nontarget organisms. So before mosquitoes gain complete resistance, against the key weapons now used among them, the mosquito control personnel should initiate extensive research to explore and launch safe, effective and eco-friendly biomaterials for their control.

Another growing trend in South Indian scenario is the dwindling of paddy fields due to the reclamation for urbanisation. Though the staple food of Kerala is rice, the sad truth is that people of Kerala have forgotten the genuine taste of fresh rice, as the paddy cultivation has become neither attractive nor advisable. Further the agriculture has become non profitable and impracticable owing to the proliferation of pests and invincible might of the mites. So the people are forced to depend on rice transported after storage and packaging from the neighbouring states. Between the time of harvest and
transport the rice becomes a “non-vegetarian rice” as it becomes impregnated with weevils. The intensity of hazards caused by the use of pesticides in cereals and pulses can never be underestimated.

Combating the mosquitoes and the weevils by employing synthetic chemicals does not anyway appear feasible, because of the potential ability of target organisms for mutation thereby gaining tolerance and resistance against the chemicals. In view of all these in mind the present endeavour is to formulate eco-friendly phytochemicals for curbing the menace caused by mosquitoes and weevils, affording safe protection to life and environment.

To find out the plants that are traditionally used against mosquitoes, an ethno botanical survey was schemed among ethnic communities living in different parts of Kerala state. Thus Kumarakom of the coastal region, Manarcaud of central region and Vagamon of hilly region were selected. Based on the information collected, specific parts of twenty plants suggested by them were used for preparation of aqueous extracts. The larvicidal activity against *Culex quinquefasciatus* mosquito, at the 4th instar larval stage was checked and recorded. After primary screening *Gliricidia sepium* 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.

In the present study crude extracts were prepared from dried powdered leaves of *G. sepium* 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 carried out for separation of the compounds. Fractions were separated after vortexing and centrifuging using chloroform, diethyl ether, acetonitrile, acetone and methanol separately and, acetonitrile fraction was found to be the most effective. 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 8,11,14 –eicosatrienoic acid, 9,12 octadecadienoyl chloride, 14-methyl-8- hexadecyn -1-ol and n-oct decanal. Such a combination of compounds may be christened as “Glirine” for further reference. So far no report is published on the presence of these chemicals in Gliricidia sepium. Invariably this is the first report on the application of these chemicals for insecticidal activities. The biomolecule with maximum activity was found to be 8,11,14 -eicosatrienoic acid, a poly unsaturated fatty acid. Trivial name is Dihomo-g-linolenic acid, with molecular formula C$_{20}$H$_{34}$O$_{2}$ and molecular mass 306.48g/mol. Structure elucidation was carried out by GCMS and it was further confirmed by FTIR and NMR spectroscopy.

The MTT assay has proved that biochemical 8,11,14 –eicosatrienoic acid is safe up to 0.2 mg/ml for human as it showed cell activity upto 70%. Toxicity studies were also conducted in rats. Two branded mosquito coils in popular use were selected (Mortein, Good knight) and the toxic effect was compared with coils prepared by us, by employing eicosatrienoic acid from G.sepium. Rat pups while in their mother’s womb were also subjected to experiments with the newly made mosquito coil. Studies on the effects of the phytochemical on aquatic non-target organisms was also undertaken, on fish. Results clearly recommend the safety of eicosatrienoic acid as a new insecticide.
Application studies of the new compound were conducted on mosquitoes and weevils. Mosquitocidal activity was checked on larvae, pupae and adults of *Culex quinquefasciatus* and results confirmed the potency of the phytochemical. Contact toxicity on weevils was measured by applying the chemical directly to thorax while fumigant toxicity was checked using fumes in the glass vials. Results showed the promising effect of eicosatrienoic acid against *Sitophilus granarius*. This is the first report regarding the impact of eicosatrienoic acid, on both the mosquitoes and weevils.