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Insecticidal Bioassays for Mosquitoes and Houseflies

Mosquitoes and houseflies cause serious diseases of humans and animals. The presence of houseflies in the home and environment indicates unhygienic conditions that lead to the transmission of bacterial and viral infections. Mosquitoes transmit some of the world's worst life threatening parasitic and viral diseases including dengue fever, dengue hemorrhagic fever (DHF), yellow fever, chikangungya (*Aedes aegypti*), malaria (*Anopheles culicifacies*), filariasis and Japanese encephalitis (*Culex quinquefasciatus*).

Chemical pesticides are generally used to control mosquitoes and houseflies. Their use has led to the development of resistance and adverse environmental effects. Therefore new alternatives for their control from new sources are in demand. Insecticidal bio-assays (mosquito and housefly assays) play a crucial role in finding new sources for insecticidal compounds. The mosquito and housefly assays described below targets control of either adult or larval stage of the insect life cycle.

Mosquitocidal Bioassay

Three days old adult females of *C. quinquefasciatus*, *A. aegypti* and *A. tessellatus* are used to test the sample for knock-down and mortality. The mosquitoes, obtained from the laboratory colonies maintained at the Medical Research Institute (MRI), were reared under laboratory conditions at 27 °C and 80% relative humidity.

The bioassay is conducted in an experimental kit consisting of two cylindrical plastic tubes both measuring 125×44 mm following the WHO standard method for adult mosquito susceptibility test using paper impregnated with the several concentrations of the sample. One tube serves to expose the mosquitoes to the test sample - labeled in red colour. Another tube is used to hold the mosquitoes before and after the exposure periods and this tube is labeled in green.

Prepare different concentrations of test samples in ethanol and impregnate the papers (140×120 mm) with 1.2 mL of this test solution. Air-dry the impregnated papers at room temperature. Roll the impregnated papers and place them in the exposure tube. Use a blank paper impregnated with only ethanol as a control. Close each tube at one end with 16 mesh size wire screen. Introduce fifteen glucose-fed adult female mosquitoes in to the holding tube and expose them to the impregnated papers. Record the number of mosquitoes knocked-down after a one hour exposure period. Transfer the mosquitoes in to the holding tube and keep for 24 h under laboratory conditions. Place a cotton pad soaked in 20% glucose solution in the tube during the holding period of 24 h. Record mortality of the mosquitoes after 24 h. Carry out a control experiment simultaneously with the test and carry out each test in triplicate.

Mosquito Larvicidal Bioassay

The mosquito larvicidal bioassay follows the WHO standard method for the determination of susceptibility or resistance of mosquito larvae for insecticides.

Weigh sample (e.g. 100 mg) and placed in a volumetric flask (100 mL). Add sterilized distilled water (100 mL) and mix on a vortex agitator for 5 min to obtain the stock solution (1000 ppm). Any necessary subsequent dilutions are prepared from the stock solution by serial dilution. Fill plastic cups (10 cm diameter, 5 cm height) with distilled water (250 mL) and add twenty-five late third or early fourth larvae to each cup. Use micropipettes and add 1000 μ L, 750 μ L, 500 μ L, 250 μ L, 125 μ L and 62.5 μ L respectively of a given sample stock solution (1000 ppm) to the cups and mix to produce final concentrations of 4, 3, 2, 1, 0.5 and 0.25 ppm. Use three replicate cups for each concentration. Set up the control, distilled water (250 mL), under similar conditions. Carry out the bioassays at 25 - 26 °C and record mortality after 24 and 48 h treatment.

Housefly Bioassay- Topical Application Method

Collect the houseflies gently using an aspirator, transfer to paper cups and anaesthetize carefully with diethyl ether. Dose eight days old female houseflies (15 per concentration) on the thorax with 1 μ l of the test solution. Maintain the treated flies at laboratory conditions and feed on a cotton wool plug soaked in a glucose and milk solution. After 1 h record the number of flies that are knocked down and assess the percentage mortality after 24h. Use five different concentrations from the sample with three replicates of 15 flies for each. Calculate the KD_{50} values in micrograms of test sample per fly.