MOSQUITO LARVICIDAL BIOLOGICAL CONTROL AGENT FROM SRI LANKAN ISOLATE OF *Metarhizium anisopliae*

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Micro-organisms such as virus, bacteria and fungi have been shown to serve as a source of biological control agents against many pests including mosquitoes. However, most concern is the mosquito-transmitted diseases worldwide including Sri Lanka. Due to drawbacks of synthetic insecticides at present, the main focus is on biological control methods in mosquito control. In Sri Lanka, no biological control methods are available, to reduce the mosquito population.

Mosquito larvicidal activity of *Metarhizium anisopliae* has been previously reported for foreign isolates but not for local isolates. The objective of the present study is to study the liquid growing mediums for production of Sri Lankan isolates of *M. anisopliae* and to evaluate mosquito larvicidal activity against the local vector mosquitoes, *Aedes aegypti, Culex quinquefasciatus* and *Anopheles tessellatus* and to isolate and characterize the toxins from *M. anisopliae.*

Metarhizium anisopliae was isolated from the infected coconut beetles. Mosquito larvicidal activity of *M. anisopliae* was tested against *Cx. quinquefasciatus* and *Ae. aegypti* under laboratory conditions and bio-assay followed the WHO protocols. The fungus was cultured in Potato Dextrose Broth Yeast and Czapex-Dox culture media, in different incubation time periods 3, 5, 8, and 13 days to obtain mycelium cake, crude broth and spores.

Chromatographic techniques, Dry Column Flash Chromatography, Preparative Thin Layer Chromatography were extensively used in fractionation to isolate the compounds 1 to 11 from the ethyl acetate extract of the mycelium cake of *M. anisopliae*. Analytical TLC was used extensively to identify the purity of compounds and fractions. Structure elucidation of compound 6 was attempted by NMR spectroscopic techniques, ^{1}H, ^{13}C NMR, HMQC, HMBC and COSY.

The highest yield of mycelium and crude broth of *M. anisopliae* was obtained in Czapex-dox medium after 8 and 13 days of shaking at 26 ^{0}C. In Potato dextrose broth yeast medium, the highest mycelial yield was obtained after 13 days of incubation and the highest crude broth after 3 days of incubation. Highest yield of crude broth and mycelium were obtained after 13 days of the incubation in both CZ and PDBY mediums. The results suggested that CZ medium is more effective medium to obtain good yield of mycelia and crude broth for liquid culturing of *M. anisopliae.*

The highest larvicidal activity against *Cx. quinuefasciatus* has been shown in CZ medium spores (LC_{50} = 1.11 x 10^{4} spores/mL), crude broth (LC_{50} = 99.90 ppm), and mycelium cake (LC_{50} = 133.79 ppm) after 8 days. The mosquito larvicidal activity against *Ae. aegypti* has been shown in CZ medium after 8 days shaking for crude broth (LC_{50} = 50.73 ppm) and mycelium (LC_{50} = 121.19 ppm).

The fractionation of the ethyl acetate extract of the mycelium cake of *M. anisopliae* was resulted the isolation eleven compounds. Structure elucidation was attempted for compound 6. Compound 6 contains 15 carbons in 12 different environments, including four methane, six methylene, two methyls and three quaternary carbons and 22 hydrogens. Compound 6 also contains an ester group and three double bonds. NMR spectral data indicated the presence of benzene ring in the molecule which disudstituted.