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Larvicidal and pupicidal activity of *Trichoderma longibrachiatum* (Tl-AgNPs) and *Trichoderma viride* (Tv-AgNPs) mediated silver nanoparticles against dengue vector *Aedes aegypti*

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Dengue and dengue hemorrhagic fever play major public health problems in Sri Lanka, being endemic within the country since 1960s*. Aedes aegypti* vectors transmit dreadful diseases like Dengue, Chikungunya, Yellow fever and Zika causing millions of deaths every year throughout the world. An efficient biosynthesis process for the rapid production of nanoparticles would enable the development of a "microbial nanotechnology" for mass-scale production. In the present study, biological silver nanoparticle synthesis using the endo-lichenic fungus, *Trichoderma longibrachiatum* and *Trichoderma viride*, where the cell filtrate of the fungus will be used as a reducing and stabilizing agent in the process of nanoparticle synthesis and the filtrate is mixed with silver nitrate solutions to obtain nanoparticles. Fungi are attractive agents for biogenic synthesis of silver nanoparticles because they offer high tolerance to metals and are easy to handle. They also secrete large quantities of extracellular proteins that contribute to the stability of the nanoparticles. They also provide good biomass production and do not require additional steps to extract the filtrate. The mycelial mass of fungi is more resistant to agitation and pressure, so it is more suitable for large-scale syntheses. In the present study, larvicidal and pupicidal effects of *T. longibrachiatum* and *T. viride* mediated silver nanoparticles (Tl-AgNPs and Tv-AgNPs) against third instar stage *Ae. aegypti* larvae and pupae were investigated. The fungi were grown in Richards' broth containing glucose, agar, potassium nitrate, potassium dihydrogen phosphate, magnesium sulphate and ferric chloride. Fungal biomass filtrates obtained after keeping 48 hours with deionized water and the desired weight(10mg) of fungal mass, were mixed with $AgNO_3$ solution. Synthesized Tl-AgNPs and Tv-AgNPs were characterized by UV-Vis spectroscopy with maximum absorption at 445nm for *T. viride* and 448nm for *T. longibrachiatum.* Transmission Electron Microscopy (TEM) showing the formation of monodispersed spherical shaped particles with a mean diameter ranging from 15 to 25 nm. The color changes from pale yellow to dark brown in the solutions with time indicated the formation of nanoparticles initially. Larval and pupal toxicity tests were assessed according to WHO standard protocol. Data were analyzed using IBM SPSS version 20 software. Probit analysis was carried out to determine the LC_{50} and LC_{90} of larvicida and pupicidal effect of Tl-AgNPs and Tv-AgNPs for *Ae. aegypti* after 24 hours and 48 hours of exposure. Study revealed that LC_{50} for Tl-AgNPs for 24 hours(2.607mg/L) and 48 hours (2.174 mg/L) were higher than LC_{50} for Tv-AgNPS 24 hours (2.153 mg/L) and 48 hours (1.842 mg/L) exposure time for *Ae. aegypti* larvae. In addition, LC_{50} value obtained in pupal toxicity tests revealed Tl-AgNps (4.92 mg/L) is higher than that of Tv-AgNPs(4.79 mg/L) for 24 hour exposure time. It concludes that both Tl-AgNPs and Tv-AgNPs could be used as potential larvicidal and pupicidal agents and Tv-AgNPs are more efficient in controlling *Ae. aegypti* larvae and pupae.

Keywords: Ae.aegypti, Trichoderma longibrachiatum, Trichoderma viride, Nanoparticles (NPs), Larvicide, Pupicide

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