ABSTRACT

The control of mosquito vectors by utilizing plant-based eco-friendly nano-larvicides is pivotal in suppressing the spread of mosquito borne diseases with minimum environmental toxicity. The current study was designed to evaluate the larvicidal activity of essential oils (EOs) of *Myristica fragrans* Houtt., *Citrus limon* L. and *Salvia rosmarinus* (Spenn 1836) against *Aedes aegypti* L. and *Aedes albopictus* (Skuse 1894) mosquitoes. The EOs were extracted by steam distillation and their component analysis was performed. The larvicidal activities were assessed by exposing fourth instar larvae to various concentrations of EOs for 24 hours. The mode of action of EOs was assessed by analyzing their inhibitory activities against key mosquito enzymes including acetylcholinesterase and α-amylase. Morphological abnormalities in treated mosquito larvae were also observed. Nanoliposomes and yeast microcapsules were prepared, characterized and their larvicidal activities were analyzed against 4th instar *Aedes* larvae.

The percentage yield of EO extracted from seeds of *M. fragrans* by steam distillation was highest (2.18%) followed by *S. rosmarinus* (1.88%) and *C. limon* EOs (1.64%) respectively. As evidenced by the GCMS analyses, the EOs of *M. fragrans*, *S. rosmarinus* and *C. limon* were composed of a diverse array of chemical compounds including 1,8-cineole, α-terpineol, camphor, α-pinene, β-pinene, linalool, sabinene, isopulegol, β-phellandrene, γ-terpinene and myristicin. The findings of larvicidal activities revealed that the EOs of *M. fragrans*, *S. rosmarinus* and *C. limon* possess significant larval toxicity ($P<0.0001$) against fourth instar *Ae aegypti* and *Ae. albopictus* larvae. The highest percentage mortality at 60 mg/L was exhibited by the *M. fragrans* EO against both the *Ae aegypti* (98.33 %) and *Ae. albopictus* larvae (88.33 %) followed by the group exposed to *C. limon* EO and *S. rosmarinus* EO. Similarly, lowest LC_{50} values against *Ae aegypti* and *Ae. albopictus* larvae respectively were observed for *M. fragrans* EO (33.3 and 39.13 mg/L), followed by *C. limon* EO (33.43 and 38.01 mg/L) and *S. rosmarinus* EO (44.96 and 49.53 mg/L). The larvae exposed to EOs of *M. fragrans*, *S. rosmarinus* and *C. limon* showed distinct physical changes including gut disintegration and leakage of gut content, loss of pigmentation and damaged cuticles. The results of *in-vitro* enzyme inhibition assays showed that all these plants EOs possess significant inhibitory activities against both the α-amylase and acetylcholinesterase enzymes. The docking scores obtained by *in-silico* studies further confirmed that all the

major constituents of plant EOs possess high binding affinities against acetylcholinesterase and α-amylase.

M. fragrans EOs were selected for the encapsulation process and were successfully encapsulated in nanoliposomes (MF-lipo), and yeast microcapsules (MF-YMC) with the encapsulation efficacy ranging from 78-82%. The LC_{50} values of MF-lipo (39.92 and 42.79 mg/L) and MF-YMC (41.36 and 43.18 mg/L) against both the *Ae aegypti* and *Ae. albopictus* were comparable to that of *M. fragrans* EO. The peaks in FTIR absorption spectra of nanoliposomes and yeast microcapsules were comparable to the FTIR spectra of pure *M. fragrans* essential oil, implying that these nanocarriers were prepared precisely, and the essential oil having diverse chemical compositions was successfully encapsulated. The hydrodynamic diameter of experimental nanoliposomes was in the range of 140-155 nm. While the hydrodynamic diameter of yeast microcapsules was in the range of 8-8.5 μm. The polydispersity index (PDI) values were less than 0.2, confirming the homogenous distribution of the particles. The Zeta potential values of the prepared nanoliposomes and yeast microcapsules were less than -28 mV, confirming their colloidal stability. Based on these results, it is concluded that EOs of *C. limon*, *S. rosmarinus* and *M. fragrans* EOs are rich in phytochemicals and possess significant larvicidal activities against *Ae. aegypti* and *Ae. albopictus* larvae. The nanoliposomes and yeast microcapsules of *M. fragrans* EO were stable, homogenous, and these have further improved the efficacy of EOs.

Keywords: Mosquito control; acetylcholinesterase; α-amylase; plant essential oils; nanoliposomes; yeast microcapsules; larvicides