

Abstract

Mosquito-borne diseases cause numerous deaths globally, including in Pakistan. There is no effective vaccine or drug available therefore, vector control is the only option to control the disease. The growing resistance of mosquitoes to synthetic insecticides necessitated for innovative and eco-friendly alternatives. Essential oils are promising option due to their low toxicity to mammals, rapid environmental degradation, and complex mixtures of bioactive components that effectively target mosquito populations. The main objective of the current study was to evaluate the larvicidal activity of *Syzygium aromaticum* essential oil based nanoemulsions against laboratory reared early fourth/late third instars *Aedes aegypti*. Essential oil was extracted from clove flower buds by hydrodistillation. The chemical composition of the essential oil was analyzed using Fourier transform infrared (FTIR) spectroscopy while the physical characteristics (droplet size and surface charge) of nanoemulsions were analyzed using the dynamic light scattering technique (DLS). Initially bioassays were performed in five different concentrations (25, 50, 75, 100, 200ppm) of essential oil in distilled water (1:1). LC50 (44 ppm) of essential oil concentrations was used to prepare nanoemulsions using acetone as solvent and Tween 20 as a surfactant. Both low energy (magnetic stirrer mixing) and high energy (sonication) methods were employed to prepare the different ratios (1:1, 1:2, 1:3, 1:4, 1:5) of essential oil based nanoemulsions from stock solution (with constant essential oil concentration; 44 ppm). FTIR result indicated 27 and 16 different components of bulk essential oil and stock solution (acetone+ distilled water) of essential oil respectively. The main active components present in essential oil were eugenol, eugenol acetate and beta caryophyllene. The zeta potential of nanoemulsions prepared with sonication mixing with 1:4 concentration was -35.4 mV with smallest droplet size of 176 d.nm indicating most stable concentration. The results indicated 100% larval mortality against 200 and 100 ppm of essential oil stock solution after 24-48 hours exposure respectively. The estimated LC50 and LC90 values after 24 hours of larval exposure with the essential oil stock solution were 44.08 and 110.12 ppm while the estimated LC50 of various

ratios of nanoemulsions dropped 29.16 (65%) and 4.24 (91%) ppm with spontaneous and sonication mixing methods respectively. The residual activity of essential oil based nanoemulsions indicated 100% larval mortality post 72 hours exposure with sonication method, was most effective as compared to spontaneous method where mortality dropped from 100 to 90% post 96 hours exposure with 1:5 ratio. Overall, sonication and the surfactant seem to be the most important parameters affecting the physical characteristics of the nanoemulsions. In current study sonication played a key role in reducing the droplet size, zeta potential and polydispersity index (PdI) values resulting in more stable nanoemulsions. Furthermore, 1:4 ratio of essential oil: surfactant nanoemulsion was found the most stable nanoemulsion. In conclusion current study indicated that *Syzygium aromaticum* essential oil based nanoemulsions provide novel insights that can be used in the development of safer, natural alternatives to synthetic insecticides for the control of *Aedes aegypti* vector populations. The current research requires further investigations under natural/field conditions to assess its potential and efficiency against the wild population of major dengue vector, *Aedes aegypti*.