

ABSTRACT

Dengue is an important arboviral diseases of humans in the tropical/subtropical areas of the world. Pakistan has seen yearly dengue epidemics since 2006. The disease is seasonal with the peak occurring during the post-monsoon involving approximately 70,000 confirmed dengue cases and more than 550 mortalities till 2022. Moreover, elevated temperatures due to climate change and precipitation as well as unchecked urbanization favors growth of *Ae. aegypti*, a major dengue vector in Pakistan. The unavailability of effective vaccines and antiviral drug along with the use of traditional insecticides caused biomagnification, resistance in vectors, and toxic effects on the environment diverts attention to biological control methods. The current study demonstrated a novel method to target the dengue vector population by reproductive manipulations using *Wolbachia* based bio-control agent. The intracellular, maternally transmitted α -proteobacterium, *Wolbachia pipientis* is known to infect over two-thirds of insects worldwide with a prevalence of 76 percent but not naturally found in *Ae. aegypti*, therefore, need to be induced by embryonic microinjection in the vector. The major objective of the current study was to evaluate the effect of wMel native *Wolbachia* strain in induced *Ae. aegypti* to control dengue in Pakistan. For this purpose, initially, the isolation and molecular characterization of the wMel *Wolbachia* strain from locally collected *Drosophila* using the *wsp* (*Wolbachia* surface protein) gene primers by PCR (polymerase chain reaction) was carried out. The wMel *Wolbachia* was successfully transfected in the laboratory-reared *Ae. aegypti* via embryonic microinjection. The impact of wMel was observed in transfected females as reproductive fitness (fecundity and fertility), larval development time, longevity/survival, and cytoplasmic incompatibility. The results indicated that reproductive fitness as fecundity was not significantly ($P=0.069$) affected in *Wolbachia* transfected females as compared to wild-type while, Fertility was significantly ($P=0.05$) reduced in transfected females than wild-type females. Moreover, no significant ($P=0.064$) difference was found in larval developmental time to transform into pupae. The female longevity/survival was significantly ($P=0.029$) decreased in *Wolbachia* transfected females as compared to non-infected females while no significant ($P= 0.06$) difference was observed in *Wolbachia* infected males. Complete cytoplasmic incompatibility with a 0% egg hatch rate ($P=0.001$) was observed in crosses between wMel-infected males and uninfected wild-type females indicated an 100% inhibition of eggs hatch rate (population suppressed). In conclusion, the wMel *Wolbachia* strain with complete (100%) CI has the potential in the laboratory to alter the host physiology and also induce reproductive manipulations to suppress *Ae. aegypti* dengue vector population indirectly reduced transmission of disease to humans. Further research on the establishment of wMel colony and mass rearing of *Wolbachia* infected males required to apply in nature.