ARTICLE IN PRESS

Journal of King Saud University – Science (2016) xxx, xxx–xxx

King Saud University
Journal of King Saud University – Science
www.ksu.edu.sa
www.sciencedirect.com

Nanoparticles for mosquito control: Challenges and constraints

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Received 6 July 2016; accepted 20 August 2016

KEYWORDS

Aedes;
Anopheles;
Dengue;
Malaria;
Green nanosynthesis;
Zika virus

Abstract Mosquito control programs are facing important and timely challenges, including the recent outbreaks of novel arbovirus, the development of resistance in several Culicidae species, and the rapid spreading of highly invasive mosquitoes worldwide. Current control tools mainly rely on the employment of (*i*) synthetic or microbial pesticides, (*ii*) insecticide-treated bed nets, (*iii*) adult repellents, (*iv*) biological control agents against mosquito young instars (mainly fishes, amphibians and copepods) (*v*) Sterile Insect Technique (SIT), (*vi*) "boosted SIT", (*vii*) symbiont-based methods and (*viii*) transgenic mosquitoes. Currently, none of these single strategies is fully successful. Novel eco-friendly strategies to manage mosquito vectors are urgently needed. The plant-mediated fabrication of nanoparticles is advantageous over chemical and physical methods, since it is cheap, single-step, and does not require high pressure, energy, temperature, or the use of highly toxic chemicals. In the latest years, a growing number of plant-borne compounds have been proposed for efficient and rapid extracellular synthesis of metal nanoparticles effective against mosquitoes at very low doses (i.e. 1–30 ppm). In this review, we focused on the promising potential of green-fabricated nanoparticles as toxic agents against mosquito young instars, and as adult oviposition deterrents. Furthermore, we analyzed current evidences about non-target effects of these nanocomposites used for mosquito control, pointing out their moderate acute toxicity for non-target aquatic organisms, absence of genotoxicity at the doses tested against mosquitoes, and the possibility to boost the predation rates of biological control agents against mosquitoes treating the aquatic environment with ultra-low doses (e.g. 1–3 ppm) of green-synthesized nanoparticles, which reduce the motility of mosquito larvae. Challenges for future research should shed light on (*i*) the precise mechanism(s) of action of green-fabricated metal nanoparticles, (*ii*) their fate in the aquatic environment, and (*iii*) the possible toxicity of residual silver ions in the aquatic ecosystems, (*iv*)

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Peer review under responsibility of King Saud University.

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http://dx.doi.org/10.1016/j.jksus.2016.08.006
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the standardization of chemical composition of botanical products used as sources of reducing and capping metabolites, (*v*) the optimization of the green nanosynthetic routes, in order to develop large-scale production of eco-friendly nanomosquitocides.

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1. Introduction

Neglected tropical diseases are the most common infections of the poorest people in the world. They include ancient scourges such as hookworm and other soil-transmitted helminth infections, Chagas disease, amoebiasis, schistosomiasis, leishmaniasis, and dengue. Together, neglected tropical diseases produce a burden of disease that in certain regions even exceeds HIV/AIDS, while simultaneously trapping "bottom billion" in poverty through their deleterious effects on child physical and intellectual development, pregnancy outcome, and worker productivity (WHO, 2016a).

Arthropods are extremely dangerous vectors of pathogens and parasites, which may hit as epidemics or pandemics in the increasing world population of humans and animals (Bonizzoni et al., 2013; Mehlhorn, 2015; Mehlhorn et al., 2012; Benelli et al., 2016a). Among them, mosquitoes (Diptera: Culicidae) represent a huge threat for millions of people worldwide, vectoring important diseases, including malaria, dengue, yellow fever, filariasis, Japanese encephalitis and Zika virus (Jensen and Mehlhorn, 2009; Benelli and Mehlhorn, 2016; Pastula et al., 2016; Saxena et al., 2016). Furthermore, Culicidae transmit key pathogens and parasites that dogs and horses are very susceptible to, including dog heartworm, West Nile virus, and Eastern equine encephalitis (WHO, 2012; Mehlhorn, 2015). Unfortunately, no treatment is available for most of the arboviruses vectored by mosquitoes, with special reference to dengue. In addition, even for other mosquito-borne diseases, such as malaria, there are significant challenges that still preclude their successful management (see Benelli and Mehlhorn, 2016).

Malaria is caused by *Plasmodium* parasites, which are vectored to people and animals through the bites of infected *Anopheles* mosquitoes (Fig. 1a). 2015 was an extraordinary year for malaria control, due to three hot news: the Nobel Prize to Y. Tu for the discovery of artemisinin, the development of the first vaccine against *Plasmodium falciparum* malaria [i.e. RTS, S/AS01 (RTS,S)], and the fall of malaria infection rates worldwide, with special reference to sub-Saharan Africa (White, 2015; WHO, 2015a; Benelli and Mehlhorn, 2016). However, there are major challenges that still deserve attention, in order to boost malaria prevention and control. Indeed, parasite strains resistant to artemisinin have been detected (WHO, 2015b), the RTS,S vaccine does not offer protection against *Plasmodium vivax* malaria, which predominates in many countries outside of Africa, and a number of malaria prevention and control tools currently available are quite expensive, thus not readily available for poor and marginalized populations in tropical and subtropical areas worldwide (Benelli and Mehlhorn, 2016).

Filariasis is caused by nematodes that in vertebrate hosts act as parasites of blood or the lymphatic system, muscles, and connective tissue. Main species include *Wuchereria bancrofti*, *Brugia malayi* and *Brugia timori*. They are mainly transmitted by the mosquito *Culex quinquefasciatus*. The adult nematodes obstruct the flow in lymphatic system causing firstly the inflammation of lymphatic vessel, and elephantasis. Filariasis is concentrated in Africa and India, but can be also be found in China, Japan, Sri Lanka and various Pacific islands. WHO has launched its "Global Programme to Eliminate Lymphatic Filariasis" in 2000. In 2012, the WHO neglected tropical diseases roadmap reconfirmed the target date for achieving elimination by 2020. In this framework, besides preventive chemotherapy and morbidity management, vector control in select settings contributed to the elimination of lymphatic filariasis (WHO, 2014).

Among arboviruses, a major role is played by dengue, a viral infection that slyly arrived in the Western Hemisphere over decades and became more aggressive in the 1990s, becoming a major international public health concern. Dengue is mainly vectored by *Aedes* mosquitoes (i.e. *Aedes aegypti* and, to a lesser extent, *Aedes albopictus*). The actual numbers of dengue cases are underreported and many cases are misclassified. 3900 million people, in 128 countries, are at risk of infection with dengue viruses (Becker et al., 2012; Brady et al., 2012; Bhatt et al., 2013). Four distinct, but closely related, serotypes of the virus cause dengue (DEN-1, DEN-2, DEN-3, and DEN-4). Recovery from infection by one provides lifelong immunity against that particular serotype. However, cross-immunity to the other serotypes after recovery is only partial and temporary. Currently, there is no specific treatment for dengue (WHO, 2015c; Sujitha et al., 2015).

Yellow fever is an acute viral hemorrhagic disease endemic in tropical areas of Africa and Central and South America (WHO, 2016b). It is transmitted by infected mosquitoes belonging to the genus *Aedes*, with special reference to *A. aegypti*. Symptoms of yellow fever include fever, headache, jaundice, muscle pain, nausea, vomiting and fatigue. A small proportion of patients who contract the virus develop severe symptoms and approximately half of those die within 7–10 days. Yellow fever is prevented by an extremely effective vaccine, which is safe and affordable. A single dose of yellow fever vaccine is sufficient to confer sustained immunity and life-long protection against yellow fever disease. The vaccine provides effective immunity within 30 days for 99% of persons vaccinated. There is currently no specific anti-viral drug for yellow fever treatment (WHO, 2016b).

The recent outbreaks of Zika virus infections, occurring in South America, Central America, and the Caribbean, represent the most recent of four arrivals of important arboviruses in the Western Hemisphere, over the last 20 years (Fauci and Morens, 2016). Zika virus follows dengue, West Nile virus (emerged in 1999), and chikungunya (emerged in 2013) (Attar, 2016; Benelli and Mehlhorn, 2016; WHO, 2016c). Even if Zika symptoms last only a few days in adult persons and are similar to other arbovirus infections, such as dengue (fever, skin rashes, conjunctivitis, muscle and joint pain, malaise,

Please cite this article in press as: Benelli, G. et al., Nanoparticles for mosquito control: Challenges and constraints. Journal of King Saud University – Science (2016), http://dx.doi.org/10.1016/j.jksus.2016.08.006

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