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Fabrication of nano-mosquitocides using chitosan from crab shells: Impact on non-target organisms in the aquatic environment

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ABSTRACT

Mosquitoes are arthropods of huge medical and veterinary relevance, since they vector pathogens and parasites of public health importance, including malaria, dengue and Zika virus. Currently, nanotechnology is considered a potential eco-friendly approach in mosquito control research. We proposed a novel method of biofabrication of silver nanoparticles (AgNP) using chitosan (Ch) from crab shells. Ch-AgNP nanocomposite was characterized by UV–vis spectroscopy, FTIR, SEM, EDX and XRD. Ch-AgNP were tested against larvae and pupae of the malaria vector *Anopheles stephensi* obtaining LC_{50} ranging from 3.18 ppm (I) to 6.54 ppm (pupae). The antibacterial properties of Ch-AgNP were proved against *Bacillus subtilis, Klebsiella pneumoniae* and *Salmonella typhi*, while no growth inhibition was reported in assays conducted on *Proteus vulgaris*. Concerning non-target effects, in standard laboratory considtions the predation efficiency of *Danio rerio* zebrafishes was 68.8% and 61.6% against I and II instar larvae of *A. stephensi*, respectively. In a Ch-AgNP-contaminated environment, fish predation was boosted to 89.5% and 77.3%, respectively. Quantitative analysis of antioxidant enzymes SOD, CAT and LPO from hepatopancreas of fresh water crabs *Paratelphusa hydrodromous* exposed for 16 days to a Ch-AgNP-contaminated aquatic environment were conducted. Notably, deleterious effects of Ch-AgNP contaminating aquatic enviroment on the non-target crab *P. hydrodromous* were observed, particularly when doses higher than 8–10 ppm are tested. Overall, this research highlights the potential of Ch-AGNP for the development of newer control tools against young instar populations of malaria mosquitoes, also highlighting some risks concerned the employ of nanoparticles in aquatic environments.

1. Introduction

Arthropods are dangerous vectors of deadly diseases, which may hit as epidemics or pandemics in the increasing world population of humans and animals (Mehlhorn et al., 2012; Benelli et al., 2016a, 2016b, 2016c). Among them, mosquitoes (Diptera:

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Culicidae) act as vectors for a number of important pathogens and parasites, including malaria, avian malaria, yellow fever, dengue, chikungunya, Zika virus, Rift valley fever, Western equine encephalomyelitis, bancroftian and brugian filariae, canine heartworm disease (*Dirofilaria immitis*) and setariosis (*Setaria* spp.) (Rueda, 2008; Benelli, 2015a, 2016a, 2016b, 2016c). Malaria is caused by a parasite of the genus *Plasmodium* that is transmitted by female Anopheline mosquitoes. *Anopheles stephensi* is the leading vector of malaria in India, parts of Asia and the Middle East (Harris et al., 2010). There were about 198 million cases of malaria in 2013 and an estimated 584,000 deaths. However, malaria mortality rates have fallen by 47% globally since 2000 and by 54% in the African region. Most deaths occur among children living in Africa, where a child dies every minute from malaria. Malaria mortality rates among children in Africa have been reduced by an estimated 58% since 2000 (WHO, 2016). Besides the fall of malaria infection rates worldwide, with special reference to sub-Saharan Africa, 2015 was an *annus mirabilis* for malaria control, due to the Nobel Prize to the Chinese scientist Tu for the discovery of artemisinin and the development of the first vaccine against *Plasmodium falciparum* malaria. 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, and RTS,S vaccine does not offer protection against *Plasmodium vivax* malaria, which predominates in many countries outside of Africa (Benelli and Mehlhorn, 2016).

Mosquito young instars are attractive targets for control operation due to their low mobility in the breeding habitat (Howard et al., 2007; Benelli, 2015a). Mosquito control is facing a threat because of the emergence of resistance to synthetic insecticides (Naqqash et al., 2016). In this scenario, insecticides of botanical origin may serve as suitable alternative biocontrol techniques in the future (Benelli, 2015a). In particular, the concrete potential of screening plants and fungi as sources of metabolites for parasitological purposes, is worthy of attention, as elucidated by the recent Tu's example (Benelli and Mehlhorn, 2016). Cheap and eco-friendly mosquito control tools are required (Benelli et al., 2015a, 2015b, 2015c; Murugan et al., 2015a, 2015b, 2015c). Notably, plant-borne molecules are often effective at few parts per million against *Aedes, Anopheles* and *Culex* young instars (Pavela et al., 2015a,b; Benelli, 2015b, 2016a, 2016b).

Chitosan (Ch), a polysaccharide of animal origin (Bittelli et al., 2001), is a natural, biodegradable polysaccharide polymer, which is the major structural component in the exoskeleton of crabs, lobsters, shrimps, prawns, crayfish and insects. It is also found to lesser extents in other animals, plants, fungi and bacteria (Lee et al., 2009). A major source for the large-scale production of chitin and chitosan are crustacean processing wastes (Cahu et al., 2012). Chitosan is a semi-crystalline polymer and crystallinity plays an important role in adsorption efficiency (Trung et al., 2006). The reactive functional groups of chitosan include an amino group and both primary and secondary hydroxyl groups at C-2, C-3, and C-6 positions, respectively. The amino contents of chitosan are the main factors influencing their structures and physicochemical properties and are correlated with their chelation, flocculation, and biological functions (Xia, 2003). Chitosan has attracted considerable interest, not only as an underutilized byproduct, but also for its biological properties, which include antioxidant, antimicrobial, hypocholesterolemic, immunity enhancing and antitumor activity, drug delivery, and its capacity to accelerate Ca and Fe absorption (Ting and Shen, 2005; Trang and Huynh, 2015). We hypothesize that the combination of chitosan with a noble metal, such as silver, may improve its potential against parasites and vectors.

Nanotechnology opens newer pathways for a wide array of eco-friendly and cheap applications in the fields of biomedical, sensors, *anti*microbials, catalysts, electronics, optical fibers, agricultural and bio-labeling (Salam et al., 2012), drug delivery, gene delivery, artificial implants, tissue engineering, and parasitology (Morones et al., 2005; Benelli, 2016a, 2016b). Among different nanoparticles investigated, silver ones are the most promising and are used in the field of nanomedicine for their antimicrobial activity against different microbes (Rai and Bai, 2011). The use of silver nanoparticles (AgNP) as drug carriers is a promising method for the treatment of a wide variety of diseases (Benelli et al., 2016a,2016b,2016c). Hence, AgNP have emerged with diverse medical applications, including silver based dressings and silver coated medicinal devices, such as nano-gels and nano-lotions, as well as nanocomposite for mosquitocidal purposes (Singh et al., 2010; Murugan et al., 2015a, 2015a, 2015b; Benelli, 2016a, 2016b; Govindarajan et al., 2016a, 2016b, 2016c).

Predation is an important factor for the maintenance of trophic equilibrium of ecological communities, including aquatic ones (Sih et al., 1985). Good examples are odonate young instars, water bugs, tadpoles, fishes, crabs, and copepods (Bowatte et al., 2013; Kalimuthu et al., 2014; Murugan et al., 2015a, 2015a, 2015a, 2015a). The zebrafish, *Danio rerio*, is a tropical freshwater fish belonging to the minnow family (Cyprinidae). *D. rerio* is omnivorous, its natural diet primarily consisting of zooplankton and insects, although phytoplankton, filamentous algae and vascular plant material, spores and invertebrate eggs, fish scales, arachnids, detritus, sand and mud have also been reported from gut content analysis (Spence et al., 2007)**.** To the best of our knowledge, no evidences are available about how Ch-AgNP affect the predatory efficiency of *D. rerio* predating mosquito young instars (Subramaniam et al., 2016a, 2016b).

Aquatic organisms, in general, are found to accumulate exceptionally high levels of heavy metals in tissues without showing any discernible biochemical stress. This clearly suggests that these organisms have evolved some kind of intrinsic mechanism to detoxify the impact of heavy metals. The freshwater crab, *Paratelphusa hydrodromous*, has the capability of accumulating heavy metals (Reinecke et al., 2003), making it a suitable bioindicator for environmental contamination (Schuwerack et al., 2001). Hepatopancreas is one of the most important crab organs involved in metal detoxification (Gibson and Barker, 1979). It has antioxidant defense mechanisms that scavenge ROS (lipid peroxidation) or prevent ROS-mediated cellular damage (Valavanidis et al., 2006), including enzymes sensitive to free radical proliferation such as superoxide dismutase (SOD) and catalase (CAT) (Livingstone, 2001).

In this study, we developed a new nanocomposite for mosquito control based on chitosan-mediated hydrothermal synthesis of silver nanoparticles. The process exploited the cheap shell powder of vent crabs, *Xenograpsus testudinatus,* as a source of chitosan. Biofabricated Ch-AgNP were characterized by UV–visualization spectroscopy, Fourier Transform Infrared Spectroscopy (FTIR), X-ray diffraction analysis (XRD), EDAX and SEM analysis. Then, the following issues has been investigated: (*i*) impact of biosynthesized Ch-AgNP against young instars of the malaria vector *A. stephensi*, (*ii*) antioxidant activity, (*iii*) antibacterial activity against four bacteria species, (*iv*) predation efficiency of zebra fishes, *D. rerio*, on larvae of *A. stephensi* in a Ch-AgNP contaminated aquatic environment; (*v*) impact of Ch-AgNP on antioxidant enzymes superoxide dismutase (SOD), catalase (CAT), lipid peroxidation (LPO) in the hepatopancreas of non-target freshwater crabs *P. hydrodromous*.