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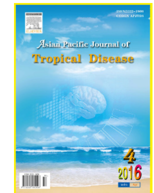

Zika research doi: 10.1016/S2222-1808(15)61025-8

The recent outbreaks of Zika virus: Mosquito control faces a further challenge

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ARTICLE INFO

Article history:
Received 15 Mar 2016
Received in revised form 28 Mar 2016
Accepted 10 Apr 2016
Available online 19 Apr 2016

Keywords:
Arbovirus
Asian tiger mosquito
Biological control agents
Biosafety
Culicidae
Dengue
Flavivirus
Green nanosynthesis
Integrated pest management

ABSTRACT

The recent outbreaks of Zika virus infection occurring in South America, Central America and the Caribbean, represent the most recent of four key arrivals of arboviruses in the Western Hemisphere over the last 20 years. Zika virus is mainly vectored by *Aedes* mosquitoes. The development of effective and eco-friendly mosquito control methods is required in order to minimize the negative effects of currently marketed synthetic pesticides, including multidrug resistance. In this scenario, natural product research can afford solutions as part of integrated pest management strategies. In this review, we focused on neem (*Azadirachta indica*) products as sources of cheap control tools of *Aedes* vectors. Current knowledge on the larvicidal, pupicidal, adulticidal and oviposition deterrent potential of neem-borne products against the arbovirus vectors *Aedes aegypti* and *Aedes albopictus* is reviewed. Furthermore, we considered the rising importance of neem extraction by-products as sources of bio-reducing agents for the synthesis of nanoformulated mosquitocides. The last section examined biosafety and non-target effects on neem-borne mosquitocides in the aquatic environment. Overall, we support the employ of neem-borne molecules as an advantageous alternative to build newer and safer *Aedes* control tools, in the framework of Zika virus outbreak prevention.

1. Introduction

Arthropods are dangerous vectors of deadly parasites and pathogens, which may hit as epidemics or pandemics in the increasing world population of humans and animals[1,2]. The scenario of arthropod-borne diseases, due to the spread of highly infective viruses or other microorganisms by arthropod vectors, is rapidly changing. Currently, they are far to be effectively controlled, and millions of humans and animals are yearly subjected to malaria, yellow fever, dengue, West Nile, chikungunya and filariasis[3,4].

The globalization process and climate changing often play a key role in the spreading of arbovirus infections. For instance, the emergence of blue tongue virus (BTV) in Europe is a noteworthy study case[5,6]. BTV is a devastating disease of ruminants causing more than one million of sheep deaths[7], with epidemic spread in the Mediterranean area and in particular in Sardinia island[8,9]. BTV replicates in different ruminant species, but the severe disease is mostly restricted to certain breeds of sheep, producing fine wool, with annual losses of US$ million[7]. BTV is transmitted among ruminants by *Culicoides* biting midges (Diptera: Ceratopogonidae[10,11]. BTV has historically made only brief and sporadic incursion in Europe, until 1998, when six strains of BTV have spread across 12 countries and 800 km, reaching the north of Europe, including UK and Scandinavia. Until 1997, the limit of *Culicoides imicola* was stable in the ordinary limit including Portugal, Southwestern Spain and some Greek islands[5]. From 1998, BTV epidemic began, following two routes: the first from Greek islands, close to the Turkish coast, spread to West and later to North, involving Bulgaria, Kosovo, Albania, Macedonia, Serbia, Montenegro and Croatia. The second route mainly occurred in 2000, spreading from Tunisia and Algeria into Sardinia, Sicilia and other

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Foundation Project: Supported by PROAPI (PRAF 2015) and University of Pisa, Department of Agriculture, Food and Environment (Grant ID: COFIN2015_22).
The journal implements double-blind peer review practiced by specially invited international editorial board members.

parts of Italy[10]. The hypothesis is that BTV was so far restricted to tropical and subtropical areas worldwide. Its entrance in Europe was probably due to the presence of disease-resistant host animals or by the dispersal of infected *Culicoides* midges, but transmission in Europe is considered a consequence of the climatic changes, being the BTV-*Culicoides* system exquisitely sensible to changes in climate. Notably, in Europe climate changes generated higher temperatures during the cold season, and fewer frost days during the winter. The BTV emergency in Europe was coincident with a period of warming (1976–2000). During incursions, only one or two countries were affected at a time and only a single BTV serotype was involved. Once the invasion reached the new habitat, its eradication is difficult to reach[11]. The migrations and evolution of serotypes, including the emergence of dangerous pathogen and parasite strains, were carefully monitored by the scientific community. Often, despite extensive advises from the scientific side, any invasion finds local authorities largely unprepared and first measures have to wait long periods. The story of the recent explosive epidemic spread of Zika virus is similar to BTV and other vector-borne diseases, such as the recent olive quick decline syndrome. In four years, the olive quick decline syndrome caused the death of thousands of olive trees in the South Italy and was menacing the survival of olive grown in all the Mediterranean area. The pathogenic agent, the bacterium *Xylella fastidiosa* (*X. fastidiosa*), was known from hundreds of years in America and at least from decades in Southern Italy, without any devastating effects[11].

Zika was discovered in Uganda in 1947 during mosquito and primate surveillance, and remained for long time an obscure virus confined to a narrow equatorial belt, running across Africa and into Asia. In that period, Zika was predominantly confined in wild primates and arboreal mosquitoes, such as *Aedes africanus*. Rarely, it caused recognized "spillover" infections in humans, even in highly enzootic areas. A peculiar and remarkable aspect of the emergence of Zika virus infection is the tendency to follow *Aedes*-transmitted epizootics and epidemics, as highlighted for the United States current scenario. Zika is the most recent of four unexpected arrivals of important arthropod-borne viral diseases in the Western Hemisphere over the past 20 years. It followed dengue, which entered this hemisphere stealthily over decades and then more aggressively in the 1990s. Later, West Nile virus emerged in 1999, and chikungunya emerged in 2013. An analogous pattern began in 2013, when chikungunya spread pandemically from west to east, and Zika later followed. Zika has now circled the globe, arriving not only in the Americas but also in the country of Cape Verde in West Africa, near its presumed ancient ancestral home[2,12].

Unfortunately, as already known for other arboviruses such as dengue, West Nile and chikungunya, no vaccines or other specific treatments are available for Zika virus infection, and avoidance of mosquito bites remains the best strategy[13]. Besides territorial control, the development of vaccines and further research on Zika virus potential complications, the attention of researches focused on developing solutions for effective control of *Aedes* vectors (Figure 1). Behaviour-based control tools and the sterile insect technique recently received renewed attention[3,4,14]. However, current mosquito control in tropical and subtropical areas worldwide is still based on the application of mosquito ovicides, larvicides, pupicides and adulticides, as well as the employ of repellents applied on bed nets and uncovered body parts[2].

Synthetic insecticides are often harmful for human health and the environment, and lead to the development of resistance in the targeted pest populations[15,16]. Therefore, it may be helpful to consider natural products as suitable sources of eco-friendly mosquitocides[11,17], as key part in control of pests. Among the natural insecticides, a prominent place can be assigned to the neem [*Azadirachta indica* (*A. indica*)] seed oil[18], corresponding to several of the characters before reported. However, the cost of neem oil is actually quite high, limiting its use on large scale. A solution could be the use of neem cake, which still contains most of the activity of the neem oil, but is a cheap by-product[19,20], being obtained as waste during the neem kernels expression. Furthermore, a key problem in the utilization of neem-borne products is the variability among production sites and the rapid degradation and loss of efficacy in the field, due to photodecomposition of limonoids azadirachtin, the main active constituents[21]. The first problem could be solved with the utilization of high performance thin-layer chromatography, while the second may benefit by the employ of green nanotechnologies[11,22-25].

The development of effective and eco-friendly Culicidae control methods is required in order to minimize negative effects of currently marketed synthetic pesticides. In this scenario, natural product research can afford solutions as part of integrated pest management strategies. Here, we focused on neem products as sources of cheap control tools of *Aedes* vectors. Current knowledge on the larvicidal, pupicidal, adulticidal and oviposition deterrent potential of neem-borne products against the arbovirus vectors *Aedes aegypti* (*Ae. aegypti*) and *Aedes albopictus* (*Ae. albopictus*) is reviewed. Furthermore, we considered the rising importance of neem extraction by-products as sources of bio-reducing agents for the synthesis of nanoformulated mosquitocides. The last section deals with biosafety and non-target effects on neem-borne mosquitocides in the aquatic environment.

2. Neem-borne compounds as eco-friendly tools against *Aedes* mosquitoes

The neem insecticidal activity has been reported in about one hundred published researches (*e.g.*[26-30]; see[31] for a recent review), reporting insecticidal activity against more than 400 species. The neemome is particularly complex, with more than 100 biologically active compounds, and many formulations deriving from them showed antifeedancy, fecundity suppression, ovicidal and larvicidal activity, insect growth regulation and/or repellence against a wide range of arthropod pests of public health importance including ticks, house dust mites, cockroaches, raptor bugs, cat fleas, bed bugs, biting and bloodsucking lice, *Sarcoptes scabiei* mites infesting dogs, poultry mites, beetle larvae parasitizing the plumage of poultry and sand flies[30,32-40], as well as mosquito vectors[19,31,41-45].

Neem oil is toxic towards the larvae of several Culicidae species, including the Zika vectors belonging to the genus *Aedes*. For instance, applications of 10% emulsion in desert coolers against *Ae. aegypti* at dosages ranging from 40 to 80 mL/cooler resulted in complete inhibition of pupal production[46]. The application of 5% neem oil-water emulsion at 50 mL/m^2 in pools leads to 100% and 51.6% reduction of third and fourth instar larvae of *Anopheles stephensi* (*An. stephensi*) and *Culex quinquefasciatus* (*Cx. quinquefasciatus*), after 24 h[46]. The LC_{50} of neem oil co-formulated with polyoxyethylene ether, sorbitan dioleate and epichlorohydrin against *Ae. aegypti* larvae was 1.7 ppm, and similar results were obtained also against malaria vectors (*An. stephensi*, LC_{50} = 1.6 ppm) and filariasis vectors (*Cx. quinquefasciatus*, LC_{50} = 1.8 ppm[47],