



Research on Mosquitocidal Properties of Plants: A Call for Enduring Collaborative Bridge between the Scientific Laboratories and the Society

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Introduction

Insect-borne diseases remain to be potential threat to global public health, particularly in the resource-limited settings [1]. It has been estimated that nearly half the world's population is at risk and they account to over seventeen percent of all infectious diseases. They contribute to more than one billion infections and over one million deaths every year [2]. It indirectly affects human lives in more spheres and domains in terms of poverty, social debility and negative socio-economic development [3]. Majority of the victims often belong to the poorest section of the society, particularly where there is a lack of access to quality healthcare, adequate housing, safe potable-water and sanitation [2]. In order to emphasize the significance of vector control interventions, the WHO has highlighted the slogan called 'Small bite, big threat'.

Mosquitoes are considered as one of the most dangerous creatures on the planet because of their capability to transmit several dreadful diseases such as malaria, filariasis, dengue, yellow fever, Japanese encephalitis and other arbo-viral diseases (Table 1). Disease geographical distribution is often determined by a complex dynamic of environmental, behavioral and socio-economic factors, and in the recent years, the disease transmission patterns have significantly altered due to several concomitant factors like: (a) explosive global economic development, (b) increased international travel, (c) new water development projects, (d) global warming, (e) changes in agricultural practices, and (f) unplanned urbanization [4-6]. The impacts of bio-geographic factors and anthropogenic activities spurt the unprecedented major mosquito-borne disease outbreaks like dengue, chikungunya and West Nile virus (Table 1), even in the countries where they were previously unknown.

Mosquitoes: The Killer

Mosquitoes (Family: Culicidae) belong to the insects order known as Diptera [Spanish; (di-two and ptera-means wings)] and they are easily distinguishable from other group of insects through their long piercing and sucking tubular proboscis (mouthparts) for blood-feeding and hairy scales on their body. There are approximately 3500 mosquito specieses grouped into forty-one genera [6]. Mosquitoes are found on all continents except Antarctica. Anopheles [Greek anopheles-useless: an-without+ophelos-advantage] [7] is a genus of mosquito first described and named by Meigen (1818) [8], and about 460 Anopheles species are recognized while, only thirty-to-forty serve as human malaria vectors in the wide-range of endemic settings [9]. Nearly 550 and 700 of Culex and Aedes cosmo-tropical species are found worldwide, respectively (Table 1). It is important to note that the dominant vector species varies from region to region, and different species are observed to play different roles for sustaining the transmission of the pathogens.

Most mosquito species are either nocturnal or crepuscular both indoors (endophagic) and outdoors (exophagic) [10,11]. Typically, both male and female mosquitoes (phytophagous) feed on nectar and plant juices. However, in addition to nectar, female mosquitoes (hematophagous) require blood-meals to carry out egg production,

and such blood meals are the link between the human and the mosquito hosts in the digenetic parasite life cycle [6]. Majority of the Anopheles mosquito specieses are showed highly anthropophagic behavior (prefer to feed on human), while a few mosquitoes which feed upon animals (zoophagic) in order to obtain the blood meals. Subsequently, after feeding, the mosquito rests for two or three days for the digestion of a blood-meal and the simultaneous development of eggs. Depending on the species some mosquitoes prefer to rest indoors (endophilic) while others prefer outdoors (exophilic). A female mosquito chooses oviposition sites by a combination of visual and chemical cues.

The mosquito goes through four separate and distinct stages of its life cycle: egg, larva, pupa, and imago (adult) (Figure 1). The first three stages occur in water, but the adult is an active flying insect. Culex mosquito is widespread across urban and semi-urban areas; the Anopheles mainly in rural areas, and Aedes, mainly in and around urban areas [12]. Aedes mosquitoes are commonly known as 'container-breeder' as they preferred to breed in temporary manmade water containers as well as discarded tires, closely associated with domestic and peridomestic human settings and often indoors, whereas secondary vector *Ae. albopictus*, prefer water-filled natural habitats [13-22].

Mosquito Control

Over decades, vector control remains to be a cornerstone to prevent outbreaks as well as to minimize the vector-borne disease associated illness and deaths worldwide [23]. Vector control strategies rely heavily on use of synthetic insecticides [24], particularly pyrethroids through Insecticide-Treated Nets (ITNs) and Indoor Residual Spraying (IRS) as a mainstay. Besides, larvicides are also used to eliminate larvae stages in the breeding sites, whereas repellents are applied to drive-away mosquitoes from the source in order to minimize the man-vector-contact. Currently WHO has recommended only twelve insecticides for IRS, but they belong to only four chemical classes viz.; organochlorines, organophosphates, carbamates and pyrethroids [25].

The injudicious and indiscriminate usage of pesticides, chiefly pyrethroids have led to the emergence of resistant mosquito strains via natural selection and it undermines the potentialities of chemical control interventions. Multiple and cross-resistance have also been

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| S. No. | Major disease | Important mosquito genus | Parasite(s) | Distribution and global disease burden | References |
|--------|---|--|--|---|---|
| | Malaria | <i>Anopheles</i> | <i>Plasmodium</i> species | Nearly 3.2 billion people-almost half of the world's population-are at risk of malaria. 97 countries and territories had ongoing malaria transmission. The latest WHO estimates, there were 214 million cases of malaria in 2015 and 438 000 deaths. | WHO 2015 [13] |
| | West Nile virus | <i>Culex</i> | West Nile virus (WNV) | It has been estimated that more than three million persons in the USA have been infected with the virus up to 2010. | Petersen 2013 [14] |
| 3. | Japanese encephalitis | <i>Culex tritaeniorhynchus</i> and similar species that lay eggs in rice paddies and other open water sources. | <i>Japanese encephalitis virus (JEV)</i> | Many countries of Asia with nearly 68 000 clinical cases every year. In rural and suburban areas where rice culture and pig farming coexist. | WHO 2014 [15] |
| 4. | St. Louis Encephalitis | <i>Culex sp. and Mansonia pseudotitillans</i> | SLEV | SLEV was first identified as the cause of human disease in North America after a large urban epidemic in St. Louis, Missouri, during the summer of 1933. Since then, numerous outbreaks of St. Louis encephalitis have occurred throughout the continent. In south Florida, a 1990 epidemic lasted from August 1990 through January 1991 and resulted in 226 clinical cases and 11 deaths in 28 counties. | Day 2001 [16] |
| 5. | Western and Eastern Equine Encephalitis | <i>Culex sp.</i> | WEEV and EEEV | Human symptomatic cases of EEE exhibit a case fatality rate of roughly 50%, and many survivors suffer residual neurological sequelae | Calisher et al. 1986; Martin et al. 2000 [17, 18] |
| 6. | Rift valley fever | <i>Culex sp.</i> | RVF | In 1997-98, a major outbreak occurred in Kenya, Somalia and Tanzania and in September 2000, RVF cases were confirmed in Saudi Arabia and Yemen, marking the first reported occurrence of the disease outside the African continent and raising concerns that it could extend to other parts of Asia and Europe | WHO 2010 [19] |
| 7. | Bancroftian filariasis/ Elephantiasis | <i>Culex quinquefasciatus</i> and <i>Culex pipiens</i> | <i>Wuchereria bancrofti</i> and <i>Brugia timori</i> | About 1.23 billion people in 58 countries worldwide are threatened by lymphatic filariasis. Over 120 million people are infected, with about 40 million disfigured and incapacitated by the disease. | WHO 2015 [12] |
| 8. | Chikungunya | <i>Aedes sp.</i> | Chikungunya virus | The disease occurs in Africa, Asia and the Indian subcontinent. In recent decades mosquito vectors of chikungunya have spread to Europe and the Americas. In 2007, disease transmission was reported for the first time in a localized outbreak in north-eastern Italy. Outbreaks have since been recorded in France and Croatia. | WHO 2015 [20] |
| 9. | Yellow fever | <i>Aedesaegypti</i> and <i>Aedes albopictus</i> | Yellow fever virus | There are an estimated 200 000 cases of yellow fever, causing 30 000 deaths, worldwide each year, with 90% occurring in Africa. The virus is endemic in tropical areas of Africa and Latin America, with a combined population of over 900 million people. | WHO 2014 [21] |
| 10. | Dengue fever | <i>Aedes aegypti</i> and <i>Aedes albopictus</i> | There are 4 distinct serotypes of the virus (DEN-1, DEN-2, DEN-3 and DEN-4). | now found in 100 countries, putting more than 2.5 billion people - over 40% of the world's population - at risk | WHO 2015 [12] |
| 11. | Brugian (Malayan) filariasis | <i>Mansonia</i> and <i>Aedes</i> | <i>Brugia malayi</i> | It is found in rural areas of Asia, in addition to isolated pockets in countries extending from the west coast of India to New Guinea, the Philippines and Japan. Cases are concentrated in Asia, including South China, India, Indonesia, Thailand, Vietnam, Malaysia, the Philippines, and South Korea | Edington and Gilles 1969 [22] |
| 12. | Saint Louis encephalitis | <i>Mansonia pseudotitillans</i> | SLEV | NA | NA |

Table 1: Major mosquito-borne diseases and the important mosquito genus with distribution and global disease burden.

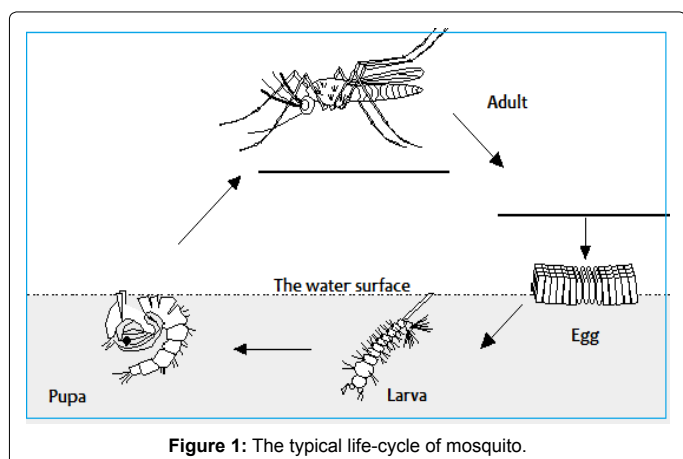


Figure 1: The typical life-cycle of mosquito.

reported in widerange of settings and therefore it is regarded as a potential threat to the global public health [24]. It makes the existing insecticides feckless and limits the available mosquito control options [26]. Although it is an evolutionary phenomenon, it can be tackled judiciously through comprehensive resistance monitoring and evaluation mechanisms prescribed within the WHO global strategic framework of integrated vector management [24].

Source reduction (larval control) is an ideal approach, as the target is very specific. However, application of conventional insecticides into the mosquitoes' breeding sites could lead to undesirable effects in the aquatic ecosystem [1]. The repeated use of persistent pesticides in agriculture, public health, and industrial sectors has indeed resulted with 'biomagnification'. It has led to pesticide residues in foods, vegetables, fruits and even in mothers' breast milk [27]. Though it is a global concern, it has serious negative implications in the developing and impoverished countries due to the higher prevalence of residues

in the foodstuff, exceeding the Maximum Residue Levels (MRL). In the light of current knowledge, looking for alternative potential new classes of plant-based insecticides for public health purpose could be a practicable and viable solution to address this catastrophe [25,27,28] and to sustain the current scaling-up efforts of preventing avoidable morbidity and mortality [25].

Plant-based Mosquitocidal Agents

Since the prehistoric ages, mankind has exploited the wild plants for his food, clothing, housing, healthcare, insecticidal purposes and day-to-day basic amenities [29,30]. Prior to the advent of DDT (and other organic pesticides), our ancestors have been using only plant-based products as insecticidal agents against various annoying and blood-sucking insects, especially mosquitoes. Plants are known to synthesis various alkaloids, phenolics, oils and several secondary metabolites. These biological constituents possesses strong insecticidal properties and they were used by Romans and Chinese's in the ancient days [31]. Even in the era of computational-bio-chemical, people use several plants as repellents and insecticidal agents in the form of smoke and crude extracts, respectively [32-36].

Though numerous studies have been conducted to establish and authenticate the potentiality of phytochemicals as insecticidal agents, particularly mosquitoes worldwide, only a few but notable studies are included in the Table 2. These studies indicate that plant kingdom remains as a major repository for the several potential phyto-toxicological agents, particularly insecticides (Table 2), These can be widely used in the place of synthetic insecticides [37] as potential larvicidal, insect growth regulators, repellents and ovipositional

attractant agents [38]. Domestic insecticide products (mosquito paper, mat, coil, stick, lotions and vaporizer) are extremely inevitable in the tropical and sub-tropical settings, where both mosquitoes and mosquito-borne diseases are widespread.

Consequently, the health-conscious consumers prefer plant-derived insecticides due to their low mammalian and non-target toxicity [32,35] than their synthetic counterparts. In the past decades numerous researchers have evaluated over thousands of potential insecticidal plants for their mosquitocidal, larvicidal, antifeedant, repellent, oviposition deterrent, and growth regulatory activities [29]. Till today only a few of them like neem, lemon grass and pyrethrum have demonstrated their broad effectiveness against various insects [39]. Table 2 shows that plant-extracts could serve as potent larvicidal agents against mosquitoes and that it could considerably minimize the dependency on synthetic insecticides [23]. Therefore, there is an enduring demand for developing new pest control tools in terms of green pesticides or reduced-risk of pesticides by means of unique novel modes of action [29,40]. Nevertheless, in the majority of the studies, the mode of actions and their larvicidal and repellency potency is not well-studied under the field conditions. It is important to note that there are key challenges and issues that lie ahead for the advancement of the ideal plant-based insecticides [26,41-47].

Need of Collaborative Bridge Between the Scientific Laboratories and the Society

Today, majority of the existing commercialized mosquito repellent's active ingredients are derived from the well-known pyrethrum plant (Golden flower) (Asteraceae; Chrysanthemum

| S. No | Country | Scientific name | Name of mosquito species | Mosquitocidal | | Reference |
|-------|-----------------|---------------------------------------|---------------------------------|---------------|--------------------------|------------------------------------|
| | | | | Larvicide | Repellent | |
| 1 | India | <i>Vitex negundo</i> | <i>Culex tritaeniorhynchus</i> | √ | √ | Karunamoorthi et al. 2008 [34] |
| 2 | Ethiopia | <i>Silene macroserene</i> | <i>Anopheles arabiensis</i> | | √ | Karunamoorthi et al. 2008 [39] |
| | | <i>Ostostegia integrifolia</i> | | | √ | |
| | | <i>Olea europaea</i> | | | √ | |
| | | <i>Echinop sp.</i> | | | √ | |
| 3 | South Africa | <i>Lippia javanica</i> | <i>Anopheles arabiensis</i> | | √ | Govere et al. 2001 [40] |
| | | <i>Pelargonium reniforme</i> | | | √ | |
| | | <i>Cymbopogon excavatus</i> | | | √ | |
| 4 | Bolivian Amazon | <i>Attalea princeps</i> | <i>Anopheles darlingi</i> | | Husks burned on charcoal | Moore et al. 2007 [41] |
| | | | <i>Mansonia spp</i> | | Husks burned on charcoal | |
| 5 | Ethiopia | <i>Cymbopogon citratus</i> | <i>Anopheles arabiensis</i> | √ | √ | Karunamoorthi and Illango 2010 [1] |
| | | <i>Croton macrostachyus</i> | <i>Anopheles arabiensis</i> | √ | √ | |
| 6 | Nigeria | <i>Ocimum gratissimum</i> | <i>Aedes aegypti</i> | √ | | Sosan et al. 2001 [42] |
| | | <i>Cymbopogon citratus (DC) Stapf</i> | | √ | | |
| | | <i>Ageratum conyzoides</i> | | √ | | |
| 7 | Cameroon | <i>Cymbopogon citratus</i> | <i>Anopheles gambiae</i> Giles. | √ | | Tchoumboung et al. 2009 [43] |
| | | <i>Ocimum canum</i> | | √ | | |
| | | <i>Ocimum gratissimum.</i> | | √ | | |
| | | <i>Thymus vulgaris.</i> | | √ | | |
| 8 | Sudan | <i>Randia nilotica</i> | | √ | | Zarroug et al. 1988 [44] |
| | | <i>Gardenia lutea</i> | | √ | | |
| | | <i>Balanites aegyptiaca</i> | | √ | | |
| 9 | India | <i>Delonix elata</i> | <i>Aedes aegypti</i> | | √ | Govindarajan et al. 2015 [45] |
| 10 | India | <i>Artemisia nilagirica</i> | <i>Anopheles stephensi</i> | √ | √ | Panneerselvam et al. 2012 [46] |
| | | | <i>Aedes aegypti</i> | √ | √ | |
| 11 | India | <i>Hyptis suaveolens</i> | <i>Aedes albopictus</i> | √ | √ | Conti et al. 2013 [47] |

Table 2: Some of the notable studies that evaluated various insecticidal plants to authenticate and validate their mosquitocidal properties against important vector mosquitoes.

cinerariaefolium) and citronella plant (Poeacea; *Cymbopogon nardus*) [48,29]. In Africa and rest of the world various indigenous peoples and ethnic groups are using numerous mosquito repellent plants without knowing their mode of action and their possible repell mechanisms (Table 2). Therefore, it demands for furthermore scientific analysis and experimentation in order to validate their insecticidal activity and their repellency. In general, green pesticides are target-specific and can be non-toxic. However, the following issues have to be considered in order to formulate novel potential green pesticides/reduced-risk pesticides in the near future [26,29],

- Though several plant species exhibit their insecticidal properties, there is a necessity of a well-advanced technological drive and analysis.
- Identification and isolation of novel bio-active molecules as well as their potentialities in terms of their repellent and insecticidal properties
- Analysis of the molecular basis of insecticidal specificity against particular insect groups.
- Evaluation of their non-target toxicity and environmental hazard.
- Establishment of sophisticated laboratory services with adequate skilled personals and funds at the regional and national level, are required.

Conclusions

To date, only a few plant-based insecticidal agents viz.: pyrethrum, citronella and neem have been successfully commercialized and have reached the market. In the past three decades, there have been quite a huge number of applied research conducted in the developing countries than ever before and it is an appreciable positive sign too. The primary objectives of these research were to assess the mosquitocidal properties of locally known insecticidal plants, eventually to identify and synthesize ideal green pesticides. It shows that we're on the right track and are expected to keep moving ahead to device the innovative, scalable, sustainable and cost-effective pesticidal interventions.

There are a vast number of literature available on the mosquitocidal properties of various plants which is quite promising. However we have miserably failed to construct the ever-lasting strong inter-linking bridges among the scientific laboratories, manufacturing industries and of course the poor and needy society. Therefore, it calls for a strong workable collaboration between the research institutes, particularly, higher learning institutions and the insecticide manufacturing industries to evolve from active ingredients to new public health pesticides. The policy-makers need to play a crucial role in order to bringing all the relevant stakeholders in the common platform to identify new-classes of insecticides from the plant kingdom. It can be achieved by providing the conducive environment in all the possible way to build the everlasting bridge between laboratories and societies. The future in fact has a very bright for the synthesis of risk-reduced-pesticides and eventually to attain meaningful vector-borne diseases free world in the near future.

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