Neem (Azadirachta indica): larvicidal properties - a review

Neem (Azadirachta indica): propriedade larvicidas – uma revisão

Lorena Sales Ferreira[1]; Stênio Nunes Alves[1]

[1.] Laboratório de Insetos Vetores de Doenças, Universidade Federal de São João del Rei - *Campus* Centro-Oeste Dona Lindu, Divinópolis, Minas Gerais, Brasil

Abstract

Introduction: Disease vector mosquitoes are a public health problems worldwide. However, controlling these insects is a challenge, so natural insecticides are a promising strategy due to their potential and low toxicity. Neem (Azadiractha indica A. Juss, Meliaceae) is a tree that has several bioactive compounds and a wide spectrum of action, including repellency and larvicide. **Objective:** This review aims to show the use of Neem-based products used against disease vectors. Several articles were analyzed and showed that different derived compounds are being evaluated, such as extracts, oils, neem cake (extraction by-product), in addition to synthesized nanoparticles. **Results:** Based on the results, we provide a list of compounds evaluated that have shown to be variable in relation to the LC_{50} values in relation to the three species of mosquitoes (Culex quinquefasciatus, Anopheles stephensi and Aedes aegypti). Conclusion: Prove the medical relevance of this tree in combating these disease vectors.

Keywords: Neem; Culicidae; Larvicidal; Insecticide; Resistance.

Autora correspondente:
Lorena Sales Ferreira
E-mail : lorenasalesf@hotmail.com

Recebido em: 20/06/2020
Revisado em: 25/06/2020
Aceito em: 08/02/2021
Publicado em: 08/07/2021

Resumo

Introdução**: Os mosquitos vetores de doenças são um problema de saúde pública em todo o mundo. Entretanto, controlar esses insetos é um desafio, e, assim, os inseticidas naturais são uma estratégia promissora devido seu potencial e baixa toxicidade. O neem (Azadiractha indica A. Juss, Meliaceae) é uma árvore que possui vários compostos bioativos e um amplo espectro de ação, incluindo repelência e larvicida.* ***Objetivos: *Esta revisão tem como objetivo mostrar o uso de produtos à base de Neem usados contra vetores de doenças.* ***Metodologia:*** *Vários artigos foram analisados e mostraram que diferentes compostos derivados têm sido avaliados, como extratos, óleos, bolo de neem (subproduto da extração), além de nanopartículas sintetizadas.* ***Resultados:*** *Com base nos resultados, forneceu-se uma lista de compostos avaliados que mostraram ser variáveis em relação aos valores de CL_{50} em relação a três espécies de mosquitos (Culex quinquefasciatus, Anopheles stephensi e Aedes aegypti).* ***Conclusão:*** *Comprovou-se a relevância médica dessa árvore no combate a esses vetores de doenças.*

Palavras-chave: *Neem; Culicidae; Larvicida; Insecticida; Resistência.*

Introduction

The Brazil is one of the largest consumers of pesticides in the world[1] and the excessive dependence on chemical insecticides used against agricultural pests and public and animal health can still present health and environmental risks [2-4]. However, even with various strategies for controlling insect vector vectors, the use of insecticides is still the main form of control of these vectors [5] and since 2012 World Health Organization has been working on a Global Plan for Insecticide Resistance Management, especially for mosquitoes[6].

These insects belong to the Culicidae family and are primarily responsible for the transmission of most vector-associated diseases[7]. Their larvae are aquatic and feed on particulate organic matter or detritus and biofilm[8]. The abundance of these insects is believed to be related to the availability of the host, climate and habitat of the larvae[9] as temperature and precipitation contribute to a faster development cycle[10].

Aedes aegypti and *A. albopictus* are the main transmitting species of dengue, Chikungunya, Zika and yellow fever viruses [11,12]. Larvae of *A. aegypti* predominate in highly urbanized habitats, while *A. albopictus* predominates in rural areas[13]. However, *A. albopictus* has been adapting to urban environments with larvae reproducing in artificial containers[14-16].

The genus Anopheles is the transmitter of the protozoan that causes malaria. This species has increased its geographical distribution but has not become as invasive as *Aedes* species[17,18]. Larvae of this mosquito genus are adapting to new environments created by urbanization [19,20].

Another species, *Culex quinquefasciatus*, in turn, is the vector of *Wuchereria bancrofti*, among other phylarids and different arboviruses[21], being more prevalent in the tropics and subtropics, but predicted to spread to climate countries temperate as Canada[22,23]. Their larvae require high nutrient content for development[24-26], and some species are able to sustain at high temperatures and extreme values pH[27].

Thus, glimpsing the combat of these insects and especially their larvae, without the use of chemical pesticides today, is still an attitude that human beings cannot overlook. However, the use of botanical insecticides has gained prominence, especially with the use of substances derived from the Neem tree (*Azadiractha indica A.* Juss, Meliaceae). From this tree, it is possible to generate products with a broad spectrum of activity against pests, and has been shown to be an alternative means of combating various insects of medical and veterinary importance, including mosquitoes and their larvae[28].

In this context, a bibliographic review was made about Neem tree and the activity of different neem-based products employed against Culicidae mosquito larvae, vectors of important diseases.

Methodology

Literature on the Neem tree and its insecticidal action was taken from indexed scientific journals and online databases: Science direct, Pubmed and Google Scholar. The terms used in the research were: "Neem (Azadirachta indica); larvicidal activity, mosquitoes". The research result was revised to identify relevant articles related to the plant and its activity against Culex, Aedes and Anopheles mosquitoes. Works available in English and Portuguese were included. The works were selected by reading titles and abstracts, where the inclusion criteria were the works published in the last 20 years, including scientific articles related to the proposed theme and excluding articles outside the time limit and outside the central theme of the research. . Information on characteristics, active ingredients, mode of action and insecticidal effect were collected in tabulated disease vectors.

Results and discussion

Literature overview

Neem (*Azadirachta indica*)

Neem is a tree, widely cultivated in the Indian subcontinent, but rapidly expanding worldwide, including in subtropical regions of America[29]. Its use has, among other advantages, a broad spectrum of activity, including medicinal, antibacterial, antifungal and insecticidal properties, of medical relevance, low resistance induction and low mammalian toxicity[29,30].

Today, the United Nations recognizes the tree as the "21st Century Tree" [31,32]. Among its features is its fast and easy growth, reaching up to 40 meters in height. In addition, its bioactive potential and low toxicity make it a frequent target for medical and environmental studies, such as insertion in insect control programs[33]. Plant cultivation is already described in different countries in Asia, Africa and America, but India is still the main producer[31].

All parts of neem have phytochemicals with different action potentials, but commercial use is limited to removable parts such as leaves, fruits and especially seeds, which are one of the main materials used in the production of bioactive compounds[31]. More than 100 of these compounds have been described, being alkaloids, flavonoids, saponins, tannins, phenols, cardiac glycosides and terpenoids. Among terpenoids the main constituents are azadiractin, nimbine, nimbidine and nimbolides[34-36].

Azaractin is found in neem leaves and bark, but a greater amount of this biologically active substance is found in seeds and is considered to be the richest plant part of this molecule[36] Being the basis of many insecticides, it has hormonal regulatory properties, acting in various physiological processes in larvae and adults of disease vectors[38].

One product whose research has reported insecticidal action is nanoparticles based on Neem seed extract. This has gained prominence with studies on green nanoparticles with insecticidal properties, bioactivity synthesis, large scale production and the lowest environmental impact[39].

Recently, neem cake has also been researched, a reusable byproduct of the extraction of neem oil with bioactive property, having great prominence in the studies of this plant. India has an annual potential of 80.000 metric tons of plant oil and 330.000 metric tons of neem cake. The use of this abundant raw material in the production of ecological insecticides, makes *A. Indica* even more economically favorable[11,36].

All the different Neem products have the advantage of having multiple modes of action, which makes insect resistance difficult, which together with environmental safety characterize them as excellent candidates for insect control methods, especially mosquitoes[14].

Neem Activities

One of the activities of Neem based products is repellency. The use of repellents by the population is one of the most common and ancient practices in the protection of disease-carrying insect vector bites, but their application to the skin must be careful for the side effects they may cause[11].

Abiy et al. (2015)[40] reported that Neem was 100% effective for 3 consecutive hours in repelling *A. Arabiensis*, the main vector of malaria in Ethiopia. The repellent effect of Neem oil was also evaluated against *Culex*, with 12 hours of protection at 20% concentration. The study also indicated that protective activity time was directly proportional to concentration [41]. Benelli et al. (2014)[11] also reported that several neem cake fractions were able to exert good mosquito repellency (above 70%) at a concentration of 100 ppm.

Insecticidal activity however is still the main search for Neem based products. In this sense, several researchers have evaluated the potential, larvicide of *A. indica* in different formulations against mosquitoes of medical importance [31,42]. Given this, it can be seen in **TABLE 1** that there are different formulations and LC_{50} for mosquito species.

TABLE 1: Larvicidal activities (LC_{50}) off different nem products

Extracts	**Larvae**	**LC_{50} (ppm)**	**Reference**
Ethanol extract of endocarp fruits *A. indica*	*A. aegypti* No-fed Fed	0.044 (%g) 440 ppm 0.056 (%g) 560 ppm	Wandscheer et al., 2004[43]
Isolated extract of *A. indica* seeds	*A. stephensi*	290.3 ppm	Murugan et.al. 2016[44]
Methanolic extract *A. indica*	*C. quinquefasciatus*	74.04 ppm	Batabyal et al., 2009[41]
A. indica extract with carbon tetrachloride	*C. quinquefasciatus*	86.00 ppm	Batabyal et al., 2009[41]
A. indica extract with light petroleum	*C. quinquefasciatus*	79.17 ppm	Batabyal et al., 2009[41]

Extract de *A. indica*	*C. pipiens*	81.21 ppm	Shoukat et al., 2016[45]
Oil	**Larvae**	**LC_{50}**	**Reference**
Neem oil (leaves)	*A. aegypti*	0,05% (500 ppm)	Mukhtar et al., 2015[46]
Neem oil formulation (Neem oil with polyoxyethylene ether-sorbitan dioleate and peichlorhydrin)	*C. quinquefasciatus* *A. aegypti* *A. stephensi*	1.8 ppm 1.7 ppm 1.6 ppm	Dua et al., 2009[47]
Formulação de óleo de Neem (32%) (8 dias)	*An. gambiae*	10.7 ppm	Okumu et al., 2007[48]
Óleos de semente de Neem industriais NSO1- Indian Producer	*A. albopictus*	171.735 ppm	Benelli et al., 2015[33]
Commercial products	**Larvae**	**LC_{50}**	**Reference**
Neem extract (Neemarim)	*A. stephensi* *C. quinquefasciatus*	0.35 ppm 0.69 ppm	Vatandoost and Vaziri, 2004[49]
Neem extract (NeemAzal)	*A. stephensi* *A. aegypti* *C. quinquefasciatus*	1.923 ppm 8.416 ppm 15.866 ppm	Gunasekaran et al., 2009[50]
Cake of Neem	**Larvae**	**LC_{50}**	**Reference**

Silver Nanoparticles (AgNPs) - Aqueous extract of leaves and bark	*A. stephensi* *C. quinquefasciatus*	2 ppm 10 ppm	Soni et al., 2014[51]
Neem oil cake	*C. quinquefasciatus* *A. aegypti* *A. stephensi*	5600 ppm (0.56%) 2900 ppm (0.29) 4500 ppm (0.45)	Shanmugasundaram et al., 2008[52]
Neem Cake Extract Methanolic Ethyl acetate	*An. culicifacies*	1.321 ppm 1.818 ppm	Benelli et al., 2017[53]
Neem Cake Extract Fractions: NTMeOH, NTAcOEt, NRAcOEt, NRBuOH NRH_2O	*An. culicifacies*	1.321ppm 1.504 ppm 1.818 ppm 1.950 ppm 2.545 pmm	Chandramohan et al., 2016[36]

Neem nanoparticles	**Larvae**	**LC_{50}**	**Reference**
Silver nanoparticles of aqueous extract of Neem leaves	*A. aegypti* *C. quinquefasciatus*	0.006 ppm 0.047 ppm	Poopathi et al., 2015[35]

Silver nanoparticles (AgNP) from *A. indica* seed extract	*A. stephensi*	5.6 ppm	Murugan et. al., 2016[44]
Neem-Urea Nanoemulsion (NUNE)	*A. aegypti* *C. tritaeniorhynchus*	99261 ppm 60847 ppm	Mishra et al., 2018[54]
Silver nanoparticles with Neem extract	*A. aegypti*	5.425 ppm	Chandramohan, 2016[36]
Nanoemulsion Neem Oil 31.03 nm 93.0 nm 251.43 nm	*C. quinquefasciatus*	11.75 ppm 25.99 ppm 62.89 ppm	Anjali et al, 2010[55]

LC_{50}: Median lethal concentration; ppm:part per million; nm:nanometers

As mentioned, several studies are conducted against the different neem formulations, but the extracts are the main ones in the tests of insecticidal activity. And as a way to improve the quality and protection of the active ingredients for a longer useful life, in one study the plant extracts were encapsulated by different materials, including alginate, which had a larval mortality of 98%. A. aegypti after 84 hours and 100% after 96 hours. There was no mortality in the control containers. One of the advantages of the emulsifier is biocompatibility, better dispersion in water and, consequently, causing the larvae to be exposed to higher concentrations of this substance. Despite the delayed effect, the authors observed that the alginate granule formulation showed potential against dengue mosquito larvae, and this fact was attributed to the alginate granule formulation which may have preserved the stability of the active ingredient.

As mentioned, several studies are conducted against the different formulations of neem but extracts are the primary in the insecticide activity tests. And as a way to improve the quality and protection of the active ingredients for a longer shelf life, in one study plant extracts were encapsulated by different materials, including alginate, which showed a larval mortality rate of 98% *A. aegypti* after 84 hours and 100% after 96 hours. There was no mortality in the control containers. One of the advantages of the emulsifier is biocompatibility, better dispersion in water and consequently, causing the larvae to be exposed to higher concentrations of this substance. Despite the late effect, the authors observed that the formulation of alginate beads showed potentiality against dengue mosquito larvae and this fact was attributed to the alginate granule formulation which may have preserved the stability of the active ingredient[56].

In turn, Anjali et al. (2012)[55] carried out a study with neem oil nanoemulsion, and observed that the size of the drop influences the effectiveness. The reduced size of nanoemulsion increased larvicidal efficacy, with lower LC_{50} value due to the greater dispersion of thin particles. Nanoemulsions have the advantage of being economically viable, less toxic when compared to synthetic insecticides and therefore an alternative to insect vector control[55].

Another strategic alternative to conventional pesticides is nanoparticle biosynthesis. The synthesis of nanoparticles is based on oxidation / reduction reactions and the appropriate choice of solvent. Plants have a wide variety of metabolites that assist in the reduction process. Neem is an example of this, in which terpenoids are reducing phytochemicals[57].

Extending studies of the larvicidal effect of neem, Imbahale and Mukabana (2015) studied population control of vector larvae in six different Anopheline and Culicini mosquito habitat types. In this research, pure splinters from neem stem were used as raw material. Regarding the larvae, 7782 Anopheline (93% late instars) and 11.590 Culicini (71% first instars) were sampled. With the study, it was observed that in habitats treated with Neem, larvae did not develop, suggesting that the evaluated compound discourages the development of resistance in the vectors since more evolved larval stages and pupae of both groups were outnumbered. In addition, it is also suggested that pregnant female mosquitoes still oviposited in the treated habitats[42].

The aqueous extract of neem fruit, bark and leaf was evaluated for the percentage of mortality of *C. quinquefasciatus* larvae after 24 hours of exposure, as well as larval susceptibility to raw neem leaf dust. In the study by Kudon et al (2011)[58], bark, fruit and leaf extract (0.1 g/mL) showed an average 24-hour larval mortality of 72.7%, 68.7% and 60% respectively. Leaf powder caused 100% mortality at a concentration of 1 g/mL. However, at a concentration of 0.1 g/mL, mortality was less than 20%. It is noteworthy that neem powder floats in water; thus, in larger doses, it may have acted as a barrier and not a pesticide that prevented the larva from breathing and resulting in death by suffocation; but also with an efficient effect on vector control.

Several neem oils, commercial or not, are also evaluated for larval insect susceptibility. Silapanuntakul et al, 2016[56] showed that Thai Neem Oil caused 97% mortality of *A. aegypti* larvae, while Thai Neem Alginate bead formulation resulted in a 62% mortality rate. Demonstrating the best oil efficiency, which is justified by easier and more uniform dispersion in water.

Neem oil toxicity against *A. aegypti* was also evaluated by Gomes et al. (2016)[59], who observed a mortality rate of 82% after 7 days and resulted in a 50% reduction in survival rates over 2 days, with a concentration of 1% . Compared to the 0.001% neem concentration, 77% of the larvae survived after the seventh day of evaluation, similar to the control (86%). However, survival rates were significantly lower when applied the combination of neem and the fungus *Metarhizium anisopliae* agains vector of dengue, proposing a reduction in the chance of developing resistance.[59].

The synergism of natural products in turn is also a strategic alternative to further enhance Neem's pesticidal activity. Assifuah-Hasford (2018)[60] showed that neem oil and fresh extracts of sweet orange (*Citrus sinensis*), pennyroyal leaves (*Mentha pulegium*) and garlic (*Allium sativum*) have insecticidal properties similar to chemical pesticides. The 100% efficacy in larvicidal activity was also observed in the tests performed against *A. stephensi* and *C. quinquefasciatus* using PONNEEM at 0.1 ppm.

PONNEEM is a biopesticide prepared from oils of *A. indica* and *Pongamia glabra*. The synergism of phytoconstituents was a crucial factor as it caused restlessness, slowness and convulsions that triggered insect death[61].

A large number of published studies on green nanoparticle synthesis show their potential for controlling disease vectors. More than 5.600 publications in the SCOPUS database were retrieved in a survey by Mirsha et al. (2018)[62]. In 2017, using as keywords "mosquito nanoparticles" more than 200 published articles were surveyed, reporting the effectiveness of plant-synthesized nanoparticles as pesticides.

Regarding the studies of Neem's silver-based nanoparticles (AgNPs), Poopathi et al. (2015)[35] synthesized AgNPs from Neem leaf extract, showing activity against LC_{50} and LC_{90} against *A. aegypti* and *C. quinquefasciatus* (**TABLE 1**). In contrast, silver nanoparticles ($AgNO_3$ - 1Mm) used as control showed no larvicidal activity. Particle size may be directly associated with the mechanism of action. Nanoparticles with a size between 41 and 60 nm allow the passage through the insect cuticle and individual cells, where they interfere with molting as well as other physiological processes.

In another study, silver nanoparticles (AgNP) were synthesized using neem cake and larvicidal activity evaluated against *A. aegypti*. The study showed efficacy at low doses for vector control. In this paper, the authors report a higher toxicity of AgNPs compared to Neem bolus. The biosynthesized particles presented LC_{50} about 27 times lower than the extract alone[36].

Similarly, studies of the efficacy of AgNPs produced with Neem bark and leaf extracts were carried out for the different developmental stages of *C. quinquefasciatus* and *A. Stephensi*, noting the close relationship between mortality and life stages. Aqueous extracts of leaves and bark of *A. indica* were used in the synthesis, forming spherical AgNPs of varying size. Soni et al. (2014)[52], testaram diferentes concentrações e observaram um melhor efeito sobre o estágio larval de 1° e 4º instares de *C. quiquefasciatus* e 1 e 2º instar de *A. stephensi* com extrato de folhas. Larvae had a 100% mortality rate after the first hours of exposure (15 min and 1h: 30 min / 1h respectively). On the other hand, the effectiveness of the nanoparticles against the 2[th] and 3[th] instars of *C. quinquefasciatus* were verified after this exposure period. The 2[nd] instar larvae presented LC_{50} 6, LC_{90} 12 and LC_{99} 14 ppm; while the third instar larvae LC_{50} 10, LC_{90} 18, and LC_{99} equal to 20 ppm. For *A. Stephensi*, the LC_{50} for 3[th] and 4[th] instar larvae was determined after 12h and 17h respectively, showing a lower susceptibility of the larvae. In relation to nanoparticles synthesized with neem bark extract, again the larval stage was more susceptible. The first three stages presented 100% mortality for *C. quiquefasciatus* and the fourth presented an LC_{50} of 2 ppm, half of that observed for pupae. For *A. Stephensi*, 1[th] and 2[th] instar larvae were 100% killed after 1h of exposure to nanoparticles[52].

As shown, larvae are the main insect elimination strategy as they cannot dissipate from their breeding grounds and control them more effectively. However, studies on other evolutionary stages of insects are performed. Authors describe the mosquito activity for Aedes, most effectively in later hours. After 24 hours of exposure to extracts of *A. indica*, they had a mortality ranging from 26.6 to 48.9%, much lower than the mortality observed after 48h, which reached 100%, with a lower LC_{50}, passing from 1.17 mL to 0.09 mL[63].

The effect of azadirachtin was studied against *C. pipiens*, showing remarkable mosquito control. Again, time and dose were determining factors. At concentrations of 24 and 48 mg/ L the maximum value in mortality rate was evidenced, oviposition dropped from 91 in the control to 53 and 39 at concentrations of 3 and 6 mg/L respectively. In addition, a reduction in male fertility and increased male sterility has been observed[64].

Compared to *A. sthephensi*, in a study by Nathan et al (2005)[65], after 24h of azadirachtin (neem limonoid) exposure, the mortality rate was 69.3% (0.025 ppm), 80.4%. (0.05 ppm) and 95.4% (0.1 ppm).

It is noteworthy that, in addition to its activity against biological pest control, studies have shown that azadiractin has cellular action, with relevant cytotoxicity, as well as apoptotic induction in Sf9 cells (Leptoptera cells), as demonstrated by the study by Huang et al. (2013)[66].

It should be noted that, in addition to its activity against biological pest control, studies have shown that azadiractin has cellular action, with relevant cytotoxicity, as well as apoptotic induction in Sf9 cells (Leptoptera cells), as demonstrated by the study by Huang et al. (2013).

The study aimed to further investigate the mechanism of azadirachtin induced apoptosis in Lepidopteran. It was rshowed that the release of cytochrome c into the cytosol is an important event during lepidopteran apoptosis, followed by the cytochrome c, the activation of initiator and effector caspases (caspase-9 and -3) also was detected. However, most importantly, in apoptosis induced by azadiractin, the released cytochrome c is accompanied by the generation of ROS (intracellular oxygen) [66]. In this study, after 12h and 48h of exposure, it was observed the formation of apoptotic bodies, as well as a significant increase in the reactive species of intracellular oxygen (ROS) after exposure to the active principle of Neem.

Conclusion

From this review, we can conclude that different by-products derived from the Neem tree have been studied and shown potential and effective effects in combating insect vectors of diseases. In addition, its importance is still high, since phytochemicals are promising because of their relative safety and low toxicity to the environment.

The vast activity of this plant makes it a great control tool. Its multiple modes of action, coupled with its different metabolites, means that a substantial number of studies are and are being conducted each year. Their goal is unique, it is common, to seek more and more information to complement the findings and enhance the effects on reducing mosquito vectors and diseases caused or not, with less damage to human health, animals and the environment.

Conflicts of interest

The authors declare no conflicts of interest.

Acknowledgments

We would like to thank Uiversidade Federal de São João del-Rei, Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) and Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) by financial support.

References

1. SOARES, J., Brasil é o maior consumidor de agrotóxico do mundo**. Jornal da USP. [Journal of internet]** 2018. Available from: https://jornal.usp.br/atualidades/brasil-e-o-maior-consumidor-de-agrotoxico-do-mundo/ Accessed 03/25/2020.

2. BEKETOV, M. A.; KEFFORD, B. J.; SCHÄFER, R. B.; Liess, M. Pesticides reduce regional biodiversity of stream invertebrates. **Proceedings of the National Academy of Sciences**, v.110, p.11039-11043, 2013.

3. MALAJ, E.; PETER, C.; GROTE, M.; KÜHNE, R.; MONDY, C. P.; USSEGLIO-POLATERA, P.; BRACK W.; SCHÄFER, R. B. Organic chemicals jeopardize the health of freshwater ecosystems on the continental scale. **Proceedings of the National Academy Sciences**, v.111, p.9549-9554, 2014.

4. STEHLE, S.; SCHULZ, R. Agricultural insecticides threaten surface waters at the globalscale. **Proceedings of the National Academy of Sciences,** v.112, p.5750–5755, 2015.

5. MOREIRA, M.F., MANSUR, J.F., FIGUEIRA-MANSUR, J.. Resitência e Inseticidas. Estratégias, Desafios e Perspectivas no controle de Insetos . **2012Tópicos avançados em Entomologia. Molecular Instituto Nacional de Ciência e Tecnologia em Entomologia Molecular**. Rio de Janeiro. INCT-EM. Available from: http://www.inctem.bioqmed.ufrj.br/images/documentos/biblioteca/Capitulo_15_Resistencia_a_Inseticidas Estrategias_Desafios_e_Perspectivas_no_Controle_de_Insetos.pdf. Accessed 03/25/2020.

6. WHO, 2019. **Core vector control methods**. Available from:

https://www.who.int/malaria/areas/vector_control/core_methods/en/. Accessed 03/25/2020.

7. HUANG, Y-J.S.; HIGGS, S.; VANLANDINGHAM, D.L. Arbovirus-Mosquito Vector-Host Interactions and the Impact on Transmission and Disease Pathogenesis of Arboviruses. **Frontiers in Microbiology.** v.10, p.1-14, 2019.

8. BRIEGEL, H. Physiological bases of mosquito ecology. **Journal of Vector Ecology**, v.28, p.1-11, 2003.

9. MCCLURE, K. M.; LAWRENCE, C.; KILPATRICK, A. M. Land Use and Larval Habitat Increase *Aedes albopictus* (Diptera: Culicidae) and *Culex quinquefasciatus* (Diptera: Culicidae) Abundance in Lowland Hawaii. **Journal of Medical Entomology**, v.55, p.1509-1516, 2018.

10. RUYBAL, J. E.; KRAMER, L. D.; KILPATRICK, A. M. Geographic variation in the response of *Culex pipiens* life history traits to temperature. **Parasites Vectors**, v.9, p.1-9, 2016.

11. BENELLI, G.; CONTI, B.; GARREFFA, R.; NICOLETTI, M. Shedding light on bioactivity of botanical by-products: Neem cake compounds deter oviposition of the arbovirus vector *Aedes albopictus* (Diptera: Culicidae) in the field. **Parasitology Research,** v.113, p.933–940, 2014.

12. GOMES, S.A., PAULA, A.R., RIBEIRO, A., MORAES, C.O.P., SANTOS, J.W.A.B., SILVA, C.P., SAMUELS, R.I. Neem oil increases the efficiency of the entomopathogenic fungus *Metarhizium anisopliae* for the control of *Aedes aegypti* (Diptera: Culicidae) larvae. **Parasites Vectors**, v.8, p.1-8, 2015.

13. BRAKS, M. A. H.; HONÓRIO, N. A.; LOURENÇO, DE-O. R.; JULIANO, S. A.; LOUNIBOS L. P. Convergent habitat segregation of *Aedes aegypti* and *Aedes albopictus* (Diptera: Culicidae) in southeastern Brazil and Florida. **Journal of Medical Entomology**, v.40, p.785-794. 2003.

14. HIGA, Y. Dengue Vectors and their Spatial Distribution. **Tropical Medicine International Health.** v.39, p.17–27, 2011.

15. CAPUTO, B.; IENCO, A.; CIANCI, D.; POMBI, M.; PETRARCA, V.; BASEGGIO, A.; DEVINE, G. J.; DELLA TORRE A. The ‘‘auto-dissemination’’ approach: a novel concept to fight *Aedes albopictus* in urban areas. **PLoS Neglected Tropical Diseases**, v.6, p.1-8. 2012.

16. BAGNY, L.; DELATTE, H.; ELISSA, N.; QUILICI, S.; FONTENILLE, D. *Aedes* (Diptera: Culicidae) vectors of arboviruses in Mayotte (Indian Ocean): distribution area and larval habitats. **Journal of Medical Entomology**, v.46, p.198–207. 2009.

17. DONNELLY, M.J.; LICHT, M.C.; LEHMANN, T. Evidence for a recent population expansion in the malaria vectors *Anopheles arabiensis* and *Anopheles gambiae*. **Molecular Biology Evolution**, v.18, p.1353–1364. 2001.

18. ONYABE, D.Y.; CONN, J.E. Population genetic structure of the malaria mosquito *Anopheles arabiensis* across Nigeria suggests range expansion. **Molecular Ecology**, v.10, p.2577–2591, 2001.

19. SATTLER, M.A.; MTASIWA, D.; KIAMA, M.; PREMJI, Z.; TANNER, M.; KILLEEN, G. F.; LENGELER, C.Habitat characterization and spatial distribution of *Anopheles sp*. mosquito larvae in Dar es Salaam (Tanzania) during an extended dry period. **Malaria Journal.** v.4, p.1-1, 2005.

20. ARCOS, A. N.; FERREIRA, F. A. S.; CUNHA, H. B.; TADEI, W. P. Characterization of

artificial larval habitats of *Anopheles darlingi* (Diptera: Culicidae) in the Brazilian Central Amazon. **Revista Brasileira de Entomologia,** v.62, p.267-274, 2018.

21. BHATTACHARYA, S.; BASU, P. The Southern House Mosquito, *Culex quinquefasciatus*: profile of a smart vector. **Journal of Entomology and Zoology Studies**, v.4, p.73-81, 2016.

22. HONGOH, V.; BERRANG-FORD, L.; SCOTT, M.E.; LINDSAY, L.R. Expanding geographical distribution of the mosquito, *Culex pipiens*, in Canada under climate change. **Applied. Geograph**. v.33, p.53–62. 2012.

23. SAMY, A. M.; ELAAGIP, A. H.; KENAWY, M. A.; AYRES, C. F. J.; PETERSON, A. T.; SOLIMAN, D. E. Climate change influences on the global potential distribution of the mosquito *Culex quinquefasciatus*, vector of West Nile virus and lymphatic filariasis. **PLoS ONE**. v.11, p.1-13, 2016.

24. FARAJOLLAHI, A., FONSECA, D.M., KRAMER, L.D., MARM KILPATRICK, A. "bird biting" mosquitoes and human disease: a review of the role of *Culex pipiens* complex mosquitoes in epidemiology. **Infection, Genetics and Evolution**, v.11, p.1577–1585, 2011.

25. 2YOSHIOKA, M.; COURET, J.; KIM, F.; MCMILLAN, J. R.; BURKOT, T. R.; DOTSON, E. M.; KITRON, U.; VAZQUEZ-PROKOPEC, G. M. Diet and density dependent competition affect larval performance and oviposition site selection in the mosquito species *Aedes albopictus* (Diptera: Culicidae). **Parasites Vectors**, v.5, p.1-11. 2012.

26. KOVAL, W., VAZQUEZ-PROKOPEC, G.M. Environmental stochasticity and intraspecific competition influence the population dynamics of *Culex quinquefasciatus* (Diptera: Culicidae). **Parasites Vectors**,11, 1-10, 2018.

27. Vinogradova, E.B. *Culex pipiens* pipiens mosquitoes: taxonomy, distribution, ecology, physiology, genetics, applied importance and control. **Sofia: Pensoft**. 250p. 2000. Available from: https://www.nhbs.com/culex-pipiens-pipiens-mosquitoes-taxonomy-distribution-ecology-physiology-genetics-applied-importance-and-control-book Accessed 03/25/2020.

28. BENELLI, G., MURUGAN, K., PANNEERSELVAM, C., MADHIYAZHAGAN, P., CONTI, B., NICOLETTI, M., 2015. Old ingredients for a new recipe? Neem cake, a low-cost botanical by-product in the fight against mosquito-borne diseases. **Parasitology Research,** v.114, p.391–397.2015.

29. ALI, E. O. M.; SHAKIL, N. A.; RANA, V. S.; SARKAR, D. J.; MAJUMDER, S.; KAUSHIK, P.; SINGH, B. B.; KUMAR, J. Antifungal activity of nano emulsions of Neem and citronella oils against phytopathogenic fungi, *Rhizoctonia solani* and Sclerotium rolfsii. **Industrial Crops and Products**, v.108, p. 379–387, 2017.

30. LENCIONI, V.; GRAZIOLI, V.; ROSSARO, B.; BERNABÒ, P. Transcriptional profiling induced by pesticides employed in organic agriculture in a wild population of Chironomus riparius under laboratory conditions. **Science of the Total Environment**. v.557–558, 183–191. 2016.

31. NICOLETTI, M.; MURUGAN, K.; SERRONE, P. D. Current mosquito-borne disease emergencies in Italy and climate changes. The Neem opportunity. **Trends in Vector Research and Parasitology**, v.1, p.1-9, 2014.

32. TIWARI, R.; VERMA, A. K.; DHAMA, K.; SINGH, S. V. Neem (*Azadirachta indica*) and its

Potential for Safeguarding Health of Animals and Humans: A Review. **Journal of Biological Sciences**, v.14, p.110-123, 2014.

33. BENELLI, G.; BEDINI, S.; COSCI, F.; TONIOLO, C., CONTI, B., NICOLETTI, M. Larvicidal and ovideterrent properties of Neem oil and fractions against the filariasis vector *Aedes albopictus* (Diptera: Culicidae): a bioactivity survey across production sites. **Parasitology Research**, v.114, p.227–236. 2015.

34. IYAMAH, P. C.; IDU, M. Ethnomedicinal survey of plants used in the treatment of malaria in Southern Nigeria. **Journal of Ethnopharmacology**, v.173, p.287–302, 2015.

35. POOPATHI, S.; DE BRITTO, L. J.; PRABA, L.; MANI, C.; PRAVEEN, M. Synthesis of silver nanoparticles from *Azadirachta indica*—a most effective method for mosquito control. **Environmental Science and Pollution Research**, v.22, p.2956–2963, 2015.

36. CHANDRAMOHAN, B.; MURUGAN, K.; PANNEERSELVAM, C.; MADHIYAZHAGAN, P.; CHANDIRASEKAR, R.; DINESH, D.; KUMAR, P. M.; KOVENDAN, K.; SURESH, U.; SUBRAMANIAM, J.; RAJAGANESH, R.; AZIZ, A. T.; SYUHEI, B.; ALSALHI, M.S.; DEVANESAN S.; NICOLETTI, M.; WEI, H.; BENELLI, G. Characterization and mosquitocidal potential of Neem cake-synthesized silver nanoparticles: genotoxicity and impact on predation efficiency of mosquito natural enemies. **Parasitology Research**, v.115, p.1015–1025, 2016.

37. MORGAN, E.D. Azadirachtin, a scientific gold mine. **Bioorganic Medicinal Chemistry**, v.17, p.4096–4105, 2009.

38. DEMBO, E. G.; ABAY, S. M.; DAHIYA, N. D.; OGBOI, J. S.; CHRISTOPHIDES, G. K.; LUPIDI, G.; CHIANESE. G.; LUCANTONI, L.; HABLUETZEL, A. Impact of repeated NeemAzal®-treated blood meals on the fitness of *Anopheles stephensi* mosquitoes, **Parasites Vectors**, v.8, p.1-13. 2015.

39. Benelli G. Research in mosquito control: current challenges for a brighter future. **Parasitology Research**, v.114, p.2801–2805. 2015.

40. ABIY, E. ; GEBRE-MICHAEL, T.; BALKEW, M.; MEDHIN, G. Repellent efficacy of DEET, MyggA, Neem (*Azadirachta indica*) oil and chinaberry (*Melia azedarach*) oil against *Anopheles arabiensis*, the principal malaria vector in Ethiopia. **Malaria Journal**, v. 201514, p. 1-6, 2015.

41. BATABYAL, L.; SHARMA, P.; MOHAN, L.; MAURYA, P.; SRIVASTAVA, C. N. Relative toxicity of neem fruit, bitter gourd, and castor seed extracts against the larvae of filaria vector, *Culex quinquefasciatus* (Say). **Parasitology Research**, v.105, p.1205–1210, 2009.

42. IMBAHALE, S. S.; MUKABANA, W. R. Efficacy of Neem chippings for mosquito larval control under field conditions. **BMC Ecology**, v.15, p.1-8, 2015.

43. WANDSCHEER, C. B.; DUQUE, J. E.; DA SILVA, M. A. N.; FUKUYAMA, Y.; WOHLKE, J. L.; ADELMANN, J.; FONTANA, J. D. Larvicidal action of ethanolic extracts from fruit endocarps of *Melia azedarach* and *Azadirachta indica* against the dengue mosquito *Aedes aegypti*. **Toxicon**, v.44, p.829-835. 2004.

44. MURUGAN, K.; PANNEERSELVAM, C.; SAMIDON, C. M.; MADHIYAZHOGAN, P.; SURESH, U.; RONI, M.; CHANDRAMOHAN, B.; SUBRAMANIAM, J.; DINESH, D.; RAJAGANESH, R.; PAULPANDI, M.; WEI, H.; AZIZ, A.T.; ALSALHI, M.S.; DEVANESAN, S.; NICOLETTI, M.; PAVELA,

R.; CANALE, A.; BENELLI, G. In vivo and in vitro effectivess of *Azadirachta indica*-synthesized silver nanocrystals against *Plasmodium berghei* and *Plasmadium falciparum*, and their potential against malaria mosquitoes. **Research in Veterinary Science**. v.106, p.14-22, 2016.

45. SHOUKAT, R. F.; FREED, S.; AHMAD, K. W. Evaluation of binary mixtures of entomogenous fungi and botanicals on biological parameters of *Culex pipiens* (Diptera: Culicidae) under laboratory and field conditions. **International Journal Mosquito Research**, v.3, p.17-24, 2016.

46. MUKHTAR, M.U.; MUSHTAQ, S.; ARSLAN, A.; AYUB, A. B. Z.; HAMMAD, M.; BHATTI, A. Laboratory study on larvicidal activity of different plant extracts against *Aedes aegypti*. **International Journal of Mosquito Research**, v.2, p.127-130, 2015.

47. DUA, V.K.; PANDEY, A.C.; RAGHAVENDRA, K.; GUPTA, A.; SHARMA, T.; DASH, A.P. Larvicidal activity of neem oil (*Azadirachta indica*) formulation against mosquitoes. **Malaria Journal**, v.8, p.1-6. 2009.

48. OKUMU, F. O.; KNOLS, B. G. J.; FILLINGER, U. Larvicidal effects of a neem (*Azadirachta indica*) oil formulation on the malaria vector *Anopheles gambiae*. **Malaria Journal**, v.6, p.1-8, 2007.

49. VATANDOOST, H.; VAZIRI, V. M. Larvicidal activity of a neem tree extract (Neemarin) against mosquito larvae in the Islamic Republic of Iran. **Eastern Mediterranean Health Journal**, v.10, p.573-581, 2004.

50. GUNASEKARAN, K.; VIJAYAKUMAR, T.; KALYANASUNDARAM, M. Larvicidal & emergence inhibitory activities of NeemAzal T/S 1.2 per cent EC against vectors of malaria, filariasis & dengue. **The Indian Journal of Medical Research**. v.130, p.138-145, 2009.

51. SONI, N.; PRAKASH, S. Silver nanoparticles: a possibility for malarial and filarial vector control technology. **Parasitology Research**, v.113, p.4015–4022, 2014.

52. SHANMUGASUNDARAM, R.; JEYALAKSHMI, T.; SUNIL DUTT, M.; MURTHY, P.B. Larvicidal activity of Neem and karanja oil cakes against mosquito vectors, *Culex quinquefasciatus* (Say), *Aedes aegypti* (L.) and *Anopheles stephensi* (L.). **Journal Environmental Biology**, v.29, p.43–45, 2008.

53. BENELLI, G.; CHANDRAMOHAN, B.; MURUGAN, K.; MADHIYAZHAGAN, P.; KOVEDAN, K.; PANNEERSELVAM, C.; DINESH, D.; GOVINDARAJAN, M.; HIGUCHI, A.; TONIOLO, C.; CANALE, A.; NICOLETTI, M. Neem cake as a promising larvicide and adulticide against the rural malaria vector *Anopheles culicifacies* (Diptera: Culicidae): a HPTLC fingerprinting approach. **Natural Product Research**, v.31, p. 1185-1190, 2017.

54. MISHRA, P.; SAMUEL, M.K.; REDDY, R.; TYAGI, B. K.; MUKHERJEE, A.; CHANDRASEKARAN, N. Environmentally benign nanometric neem-laced urea emulsion for controlling mosquito population in environment. **Environmental Science and Pollution Research**, v.25, p.2211–2230, 2018.

55. ANJALI, C. H.; SHARMA, Y.; MUKHERJEE, A.; CHANDRASEKARAM, N. Neem oil (*Azadirachta indica*) nanoemulsion a poten larvicidal agent against *Culex quinquefasciatus*. **Pest Management Science**, v.68, p.158-163, 2012.

56. SILAPANUNTAKUL, S.; KEANJOOM, R.; WONGDYAN, P.; BOONCHUEN, S.;

SOMBATSIRI, K. Efficacy of thai Neem oil against *Aedes aegypti* (L.) larvae. **Southeast Asian Journal of Tropical Medicine and Public Health**, v.47, p.410-415, 2016.

57. AL-SAMARRAI, A. M. Nanoparticles as Alternative to Pesticides in Management Plant Diseases - A Review. **International Journal of Scientific Research Publications**, v.2, p.1-4, 2012.

58. KUDOM, A. A.; MENSAHB, B. A.; BOTCHEYC, M. A. Aqueous neem extract versus neem powder on *Culex quinquefasciatus*: Implications for control in anthropogenic habitats. **Journal of Insect Science**, v.11, p.1-14, 2011.

59. MONTEIRO, E. R.; LACERDA, J. T. Promoção do uso racional Gomes, S.A.; Paula, A.R.; Ribeiro, A.; Moraes, C.O.P.; Santos, J.W.A.B.; Silva, C.P.; Samuels, R.I. Neem oil increases the efficiency of the entomopathogenic fungus Metarhizium anisopliae for the control of *Aedes aegypti* (Diptera: Culicidae) larvae.

60. ASSIFUAH-HASFORD, K. A.; IMORO, Z. A.; COBBINA, J. S. Synthesis of Insecticides from Selected Plant Materials. **Journal of Applied Sciences and Environmental Manage**, v.22, p. 362–367, 2018.

61. MAHESWARAN, R.; IGNACIMUTHU, S. A novel herbal formulation against dengue vector mosquitoes *Aedes aegypti* and *Aedes albopictus*. **Parasitology Research**, v.110, p.1801–1813. 2012.

62. MISHRA P, TYAGI BK, CHANDRASEKARAN N. MUKHERJEE A. Biological nanopesticides: a greener approach towards the mosquito vector control. **Environmental Science and Pollution Research**, v. 25, p. 10151–10163, 2018

63. ZAKI, A. B.; AHMED, N.; KHAN, I. A.; SHAH, B.; KHAN, A.; RASHEED, M.T.; ADNAN, M.; JUNAID, K.; HUMA, Z.; AHMED, S. Adulticidal efficacy of *Azadirachta indica* (Neem tree), *Sesamum indicum* (til) and *Pinus sabinaena* (pine tree) extracts against *Aedes aegypti* under laboratory conditions. **Journal of Entomology Zoology Studies**, v.3, p.112-116, 2015.

64. MERABTI, B.; LEBOUZ, I.; OUKID, M.L. Larvicidal Activity and Influence of Azadirachtin (Neem Tree Extract) on the Longevity and Fecundity of Mosquito Species. **Acta Zoologica Bulgarica**, v.69, p.429-435, 2017.

65. NATHAN, S.S.; KANDASWAMY, K.; MURUGAN, K. Effects of neem limonoids on the malaria vector *Anopheles stephensi* Liston (Diptera: Culicidae). **Acta Tropica**, v.96, p.47-55, 2005.

66. HUANG, J.; LV, C.; HU, M.; ZHONG, G. The Mitochondria-Mediate Apoptosis of Lepidopteran Cells Induced by Azadirachtin. **Plos One**, v.8, p.1-15, 2013.