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Mediators of arrested development as attractive malaria vector control tools: The *Azadirachta indica* and azadirachtin routes

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Abstract

Insecticides are utilized as key components in agriculture and public health tool-kits. Mosquitoes are targeted using out/indoor insecticide sprays and treated bed nets to reduce malaria. These practices are associated with increasing resistance in various species of mosquitoes, multiple insecticide use, environmental pollution, high cost and spread of malaria into regions where they did not exist before. This paper explores the possibility of developing a safer route to mosquito control through the use of plant secondary metabolites. Peer-reviewed literature on safe substances from biological sources with attractive potentials to mediate arrested development on water-based stages of mosquitoes were appraised. Google Scholar search engine was used to locate published works from reputable journals and institutions by feeding its dialogue search box with relevant key words. Insecticide use is bedeviled with lots of impediments which embolden malaria vectors to acquire resistance, destabilize the ecosystem besides causing public health problems. Plants such as *Citrullus colocynthi, Azadirachta indica,* etc. contain secondary metabolites that are effective in arresting eggs, larva and pupa in water. *Azadirachta indica* extract is reputed to have the highest activity against insect vectors. Its most active ingredient against vectors is azadirachtin. Its use in mosquito control will be sustainable as it is well endowed with both reduced cost, accessibility and effectiveness in small water bodies around human surroundings. Small- and large-scale production of azadirachtin can be done through plant tissue culture which is boosted with new editing tools in genetic engineering. Use of *A. Indica*'s azadirachtin is a safe malaria vector control that can be accomplished through imposition of arrested development on immature mosquitoes. Its role in deceiving egg laden mosquitoes deserve further investigations while funding agencies such as World Health Organization (WHO) and governments of Sub Sahara Africa should take advantage of this harmless route to eliminate malaria.

Keywords: arrested development, insecticide resistance, mosquito control, malaria control, *Azadirachta indica*, azadirachtin.

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INTRODUCTION

Some *Plasmodia* are transmitted through the bite of infected *Anopheles* mosquitoes to humans who suffer malaria among other diseases. Five *Plasmodium* species are involved, namely *P. falciparum*, *P. vivax, P. ovale, P. malariae and P. knowlesi.* Each may have a discrete medical picture and geographical distribution. However, *Plasmodium falciparum* is the cause of the most dreaded form of malaria worldwide (WHO, 2018). Malaria occurs in all six WHO regions and a recent report showed that 241 million cases of malaria occurred in 2020 globally causing 627,000 deaths with the WHO African Region accounting for 95% of all malaria cases and under-five-years children accounting for 77 % of all deaths (WHO, 2021). Mosquitoes are malaria vectors and genus such as *Anopheles* is important effective malaria vector. Though the main carrier is *A. gambiense* (Cissel *et al*, 2015), other species, such as *A. fenestus*, could have a localized transmission pre-eminence (Obach, 2016).

Insecticides are substances which deter or harm insects in various ways and man had utilized them to his own ends in health care and in agriculture more than any other sector. For mosquito control, the two most widely used are pyrethroids and dichlorodiphenyl trichloroethane (DDT), with the former used extensively in bed nets. The latter's continued use is justified because of its significance in saving dear lives but now being discontinued in agriculture because of its prolonged persistence in the environment, declines avian population, hinders plant growth and lethal to man/animals thereby lending to diseases (Li, 2022). Insects do not just fold their hands and suffer the deleterious effects of insecticides. Like every living animal, they respond to them biologically. Common means of resistance are by behavioural changes such as avoidance, physiological (like cuticular) changes, point mutation (such as the modification of the kdr gene) and modification of insecticides (using a host of enzymes such as the cytochrome oxidase P450 enzymes (Wood *et al*., 2010; Ingham, 2015; Awolola *et al*., 2018; WHO, 2018).

Attempts to challenge the course of insecticide resistance led to three unacceptable worse case scenarios: unrestrained overdose of insecticides (Sayono *et al*., 2019), use of more insecticides as when applied in blending two or more together (Hancock *et al*., 2020), alternations between types and mosaics of available ones (Tangena *et al*., 2020). These measures bear concomitant increasing costs and long-term risks to man, animals and the environment (Sayono *et al*., 2019; Aïzoun *et al*., 2022). If the same insecticide is used consistently for long period, the vector may not only develop resistance but may even progress to depend on such insecticide for dissemination of the disease to other locality where the vector is unknown hitherto (Wood *et al*., 2010). Over the years there is persisting struggles to develop more "effective" insecticides. Unfortunately, each insecticide appears to follow a common trajectory – if the concentration of insecticide that attain a desired effect is monitored over time, the effectiveness of each is only short lived, so high and higher dozes is required to obtain the same effect (Zhu *et al*., 2016). The next option is the use of multiple insecticides and upgrading of the undesirable consequences of insecticides uses (Tangena *et al*., 2020; Hancock *et al*., 2020). Secondly, applying genetically modified insects engender stiff opposition by "naturalists" whose genuine fear of possible dire consequences in future is founded (Bouwman and Van den Berg, 2011).

The fact is: man must control dangerous insects. More ways have to be found to complement, replace or modify existing malaria vector control. If the current practice of continually developing and deploying more chemical insecticides continues, they would soon be too many in the market thereby provoking choice related confusion among consumers. It would also simulate the scenario akin to the "arms race" which existed between the US and Soviet Union and now being renewed between US and Russia. Such situation is not sustainable. The solution is a kind of truce where man uses far less deleterious substances that is natural, environmentally friendly, could be used in small sized water bodies around our surroundings (Younoussa *et al*., 2016) and would not provoke undesirable counter reactions from malaria vectors. It therefore becomes expedient that we explore published works to know what options are available. In this paper, we identify arrested development as an ideal biological response which vectors may recourse to when provoked. We argue that if vectors are compelled to arrest, they would be on their way to attrition thereby obviating the risks of insecticide use such as insecticide resistance and concomitant pollution to the environment. We further posit that imposing arrested development on naïve vectors will incapacitate them from acquiring resistance. Lastly, we show that use of neem extract mediated arrested development of malaria vectors in their earlier stages in aquatic environment offers itself as simple, effective, accessible, cheap and sustainable control of mosquito/malaria as the extract is amenable to simple, small scale traditional and large scale state of the art industrial extraction that can meet all supply needs in both public health and agriculture.

REVIEW METHODOLOGY

The internet was used to search for appropriate texts which formed the major source of updated peer reviewed publications used in this work. Advanced Google Scholar search engine was employed to obtain relevant articles by typing key words as search items in appropriate text boxes and limiting searches within a range of time in years. The following words or combination of words were used for the search: arrested development, insecticide resistance, mosquito control, malaria control, *Azadirachta indica* and azadirachtin. Except for a few, mostly recent (2009–2022) publications from reputable journals were downloaded for study. Also utilized in this study were works from academic institutions that are published online. Papers that were excluded were those without depth and relevance.

RESULTS AND DISCUSSION

Arrested development in mosquito: Arrested development is the option available to animals when they are threatened (Ugwu and Ume, 2021). It is a period when environmental conditions are unpleasant for the animal and the animal is compelled to halt development (Lou *et al*., 2019). The threat to mosquitoes is occasioned by the knowledge that they are incriminated in malaria and other deadly diseases which humans and his domestic animals suffer and which are antithetical to their well-being. Man therefore must engage whatever it takes to eliminate or avert the danger by controlling mosquitoes. Ugwu and Onyali (2005), experimenting on *Ancylostoma caninum,* obtained paused development of the nematode at a particular point in its premature parasitic development, namely the third stage larvae. This is a state of life when metabolism is down regulated resulting in significant reduction in development or differentiation. Such organism would possess the ability to resist severe ecological changes. Ugwu and Ume (2021) were of the view that arrested development has inverse relationship with metabolism but direct proportionate relationship with the inducer up to a level which if exceeded, would result in the extinction of the organism. In temperate regions, anti-mosquito measures during arrested development period could lower subsequent mosquito intensity but will be unsuccessful to curtail the crest during the next abundance period (Lou *et al*., 2019). In mosquitoes, it is not clearly defined if there is a precise point in their development where arrest in any of its water development forms will occur. Rather, developmental arrest could be induced at all stages and adult mosquitoes can also arrest behaviourally such as hiding in obscure places and halting pre-feeding whining sounds if they realize the host is still awake. Imposing arrested development on mosquitoes during their normal growing season is relevant to significantly decrease mosquito population (Lou *et al*., 2019).

Molecular mechanisms during diapause in mosquitoes

A state of dormancy or retarded metabolic activities in living organisms is referred to as diapause (Martins *et al*., 2000), is a synonym for arrested development. Specific genes are known to code for activities in living things. In mosquito's diapause, *Aedes albopictus* and *Culex pipiens* were considered model organisms studied to better understand gene expressions during diapause (Diniz *et al*., 2017). A set of forty genes were reportedly noted to express differentially in *Cx. pipiens* when suppressive subtractive hybridization was adopted during changes in environmental stress (Robich *et al*., 2007). They further explained that during unfavorable environmental conditions such as desiccation or cold, that a heat shock protein called HSP70 was reportedly expressed. HSP70 was revealed to function by preventing protein folding abnormalities during unfavorable conditions. Similarly, some metabolic related genes in *Cx. Pipiens* such as methylmalonate-semialdehyde dehydrogenase (mmd) and cytochrome oxidase genes (cox) were expressed during severe cold. Owing to increased stress, the mosquito experiences slowed metabolism with expressions of genes that play different roles in initiation of diapause. Prior to diapause in mosquitoes for instance, a protein coding gene called actin was noted to be overexpressed adding to the actin cytoskeletal framework in the cell including high flying potentials before dormancy sets in (Poelchau *et al*., 2013; Diniz *et al*., 2017). In addition, Poelchau *et al*. (2013) further reported actin gene and ribosomal genes (*s3*A, *rps*24 and *rpsb*) knock down as the diapause mode was experienced by the mosquito. In as much as unfavorable environmental conditions can cause down regulation/knock down of actin gene and ribosomal genes (*s3*A, *rps*24 and *rpsb*) to encourage diapause in mosquitoes, therefore knocking off of the above mentioned genes in mosquitoes is expected to specifically slowdown metabolic activities and encourage inactive state or diapause mode in mosquito. This implies that the mosquito remains alive but inactive to transmit diseases. The effects of some bioinsecticides on aquatic stage of mosquitoes are shown in Table 1.

Insecticide impasse

Mosquitoes are typical pathogenic insect which attract insecticide use to minimize the harm they cause as they bite both animals and humans causing intense irritations and transmission of not only malaria but other parasitic diseases which include but not limited to protozoan and helminthic diseases. Insecticides are chemical substances which control insects by countering their biological processes to make them unfit for living. They could be killed, inactivated or rendered incapable of further development. Today, the main application route indoor is via long lasting insecticidal nets (LLINs) which has the thumb print of the World Health Organization (WHO) (Djègbè *et al*., 2018). Indoor residual spraying is the second most popular indoor use of insecticide (Okumu, 2020). Currently, the only class of insecticide approved for LLINs is pyrethroids and had been widely applied in Sub Sahara Africa since 2005 as a result of campaigns including those from the Roll Back Malaria program (Hancock *et al*., 2020). Pyrethroids are a group of insecticide which include but not limited deltamethrin, permethrin, lambdacyhalothrin, cyfluthrin etc. Other groups of insecticides in use include organophosphates (fenitrothion and malathion), carbamates (bendiocarb and propoxur) and organochlorines (dichlorodiphenyltrichloroethane, DDT) (Aïzoun *et al*., 2022). Insecticide use in agriculture is said to be the major source of insecticide contamination to the environment, although its use for public health may also contribute to a lesser degree as when applied for indoor residual sprays (IRS) and LLIN (Hancock *et al*., 2020). Pyrethroids are less costly; therefore, its use is more pervasive. As a result, insects develop resistance to it faster. To counter this scenario, there are many ways of combining different insecticides. Combination of a pyrethroid and a pyrrole, an insect growth controller, enhance the inhibition of a resistant mechanism intrinsic in mosquitoes (Hancock *et al*., 2020). Insecticides may be applied singly, in combination such as neonicotinoid clothianidin and the pyrethroid deltamethrin or in rotation (Zho *et al*., 2016; WHO, 2018). Insects are animals and as such change their behaviours in response to environmental demands. When repelled with indoor insecticides, mosquitoes divert to feeding outdoors. There are diverse ways insecticide act on their target – an exposed insect may impede *Plasmodium* development in its gut (Minetti *et al*., 2020). Where behavioural evasion strategies cannot apply, insects adopt other innate ways to circumvent the deleterious effects of insecticides by recourse to metabolites *in-vivo* or gene modifications. There have been increasing reports of reduction of *mosquito* susceptibility to insecticides such as organophosphates, organochlorines and carbamates (Cissel *et al*., 2015; Awolola *et al*., 2018; WHO, 2018; Hancock *et al*., 2020) and *Anopheles* had been found to be resistant to all insecticide classes in West Africa (Djègbè *et al*., 2018; WHO, 2018). In some places within Sub Sahara Africa, it was observed that between 2005 and 2017 that the mean mortality after insects were exposed to insecticide declined from complete mortality to 30% (Hancock *et al*., 2020). Resistance of *Anopheles* to pyrethroid insecticides is now widespread in Sub Sahara Africa (Awolola *et al*., 2018). Drivers of insecticide resistance depend on geography, population structure, mosquito species, ecological factors, blood feeding choices (Hancock *et al*., 2020). However, it is reiterated that combination of insecticides are employed to discourage acquisition of resistance (Hancock *et al*., 2020; Zoh *et al*. 2021).

Insecticides use had altered the environment for mosquito vectors as well as threaten their mode of tracking host and successes in blood feeding (Diop *et al*., 2021). It has also impacted on mosquitoes in curious ways – bigger mosquitoes are more likely to overcome insecticide treatment (Owusu *et al*, 2017) as well as possession of thicker cuticles especially by female mosquitoes (Wood *et al*., 2010). Mosquitoes have the capacity to bite through nets, whether insecticide treated or not: Houser *et al*. (2019) noted that 71% of sensitive mosquitoes still fed through insecticide net whereas almost all mosquitoes will feed through untreated nets. Unfortunately for man, insecticide use has also unveiled a can of worms that appears to counter the initial euphoria when it was introduced in both the health sector and in agriculture. It has been noted that in the presence of insecticide net treated with permethrin, kdr gene mutation increased the success rate of blood meals (Diop *et al*., 2021). Zoh *et al*. (2021) found that kdr mutation in mosquito is enhanced with continued use of deltamethrin; and consistent use of clothianidin also elevated transcription of P450s. Also, blood meals confer on mosquitoes the added ability to resist insecticide (Hauser *et al*., 2019). Induction of resistance among target organisms and creation of ecological incongruity pose grievous danger to man, animals and non-target insects or other organisms (Arivoli and Tennyson, 2011A). Khan *et al* (2020) agree that anti mosquito schemes are limited because of environmental pollution. Fang *et al* (2019) appear to be comfortable with cessation of insecticide use altogether where there is lofty occurrence of kdr (target-site) mutations as in Shanghai where they recommend other alternative mosquito control methods. Insecticide use provokes a lot of debates between those who are for, in-between or against their use (O'Shaughnessy, 2008; Bouwman and Van den Berg, 2011). What remains unanswered is why DDT advocates, the World Health Organization (O'Shaughnessy, 2008), would want its use to be continued (especially in Sub Sahara Africa) when those who actually manufacture it banned its use since the early 1970s. Some plant extracts appear to inhibit mosquito development by interfering with their growth enzymes. Muema *et al*. (2017) indicated that induced hormonal changes were responsible for abnormalities observed in their experiment with larval forms. According to Nathan *et al*. (2005), Azadirachtin is by far the most potent enzyme inhibitor among all the limonoids from neem tree, *Azadirachta indica.* Also, according to Arivoli and Tennyson (2011A & B) and Njom *et al*. (2011), mosquito larva and pupa can be arrested and they will result in reduced transition from larva to pupa and also reduced emergence of the adult from the pupa. Arivoli and Tennyson, (2011A) noted that activity of the extract they used had pronounced effect on *Anopheles stepehensi* growth and should be employed to control mosquitoes in small volume aquatic habitats or breeding sites around homes. Substances that induce arrest in organism that are plant products could have egg laying inhibition, anti-larvicidal, growth regulatory and repellence may be lethal if certain levels of the concentration in vivo are exceeded as Njom *et al*. (2011) observed that mortality to vector was concentration dependent. They showed that at 1 % concentration, few adults emerged whereas methanolic extract killed all larva suggesting that lower insecticide concentration in certain solvent push insects to arrest, that is, they could still survive to resume development when the activity of the insecticide wane. It seems that *A. indica* aqueous extract might be useful in deceiving mosquitoes – Howard *et al* (2011) found that 1 % concentration had a marginal attraction for gravid mosquitoes to oviposit. This same concentration would arrest their development or kill developing larva as noted above (Njom *et al*., 2011).

Criteria for selection of agents capable of inducing arrested mosquito development in water

Synthetic insecticides (organochlorines, organophosphates, pyrethroids and carbamates) have prohibitive cost, very low biodegradability indices, induce resistance and toxic to non-target organism (Aïzoun *et al*., 2022). These constitute the reasons for the clamour to halt their usage entirely. Njom *et al* (2011) listed criteria for effective mosquito control to be those measures that are of epidemiological relevance to contain disease transmission, must be specific for the vector, must be simple in concept and application, cheap and locally available, amenable to local customs with no complicated skill requirement as well as being environmentally safe. Surely, the way to go is via use of source reduction via non-environmental toxicity based larval control. Substances that should be involved in any of such form of mosquito control should be environmentally friendly and not cause any form of toxicity to other living things. It should also not be toxic beyond the targeted organism. This is because of food cycle/food chain where a killed mosquito would be ingested by say termites and the toxic agent could be eventually bioconcentrated or accumulated in man. Chemical insecticides are toxicants and also carcinogenic. Where people have cultural affinity to eat particular food grown with insecticides, they stand prone to risks because they could easily exceed the critical threshold for that insecticide (Dao *et al*., 2020). Usable insecticide should also be biodegradable. The foregoing is a tall order for synthetic chemicals which is the preferred option for large scale manufacturers that offer investors quick turn-over and fast recovery of investment capital. Dealing with this problem can be conveniently tackled when agents that would be used in this way are of biological origin. Muema *et al*, (2017) noted that using biochemicals from plants to relay information that alter mosquito vector behavior but which result in stopping their transmission is gaining popularity. Mafara and Bukura (2021) concur asserting that natural products from plant are considered the best contender to reverse the ills of chemical insecticides. Table 1 shows studies involving use of plant-based extracts that can be applied in this direction.On the contrary, chemical substances often become a cause for worries, apprehension and resentment from well-meaning advocates who are concerned about the preservation of species of value to man. The anecdote from our research center could be of relevance at this juncture. There was this great desire to launch modern bee keeping within the university community since 2005 in more than 3 occasions but they failed resoundingly despite huge investment in human and material resources. A number of experts had been employed, hired, accommodated and dearly paid to achieve this project as well. We later rationalized the cause of failure to be over-use of insecticide from the agricultural sector around the campus. Safe biological sources that can be used to provide for a sustainable vector control via arrested development must be dependent on use of natural products. For initial malaria treatment needs, between 50 – 80% of people in developing countries rely on extract and concoctions obtained from plant sources (Sarkar *et al* (2021). Neem and other plants have products which fulfill this aspiration because they are eco-friendly, have no non-target effects, have multiple modes of action, cheap, easy to generate, and increasingly being recommended for newer and safer arthropod control as well as its disposition to synergize with other control measures (Muema *et al*., 2017; Mafara and Bukura, 2021). In a related study, leaf extracts of *Boswellia dalzielii* was found to be active against all water stages of *Anopheles gambiae* and *Culex quinquefasciatus* (Younoussa *et al*., 2016).

Azadirachta indica tree (neem)

This is a tree of Indian origin which had been grown worldwide and particularly in Sub Sahara Africa where it can easily be grown naturally from seeds (Howard *et al*., 2009). Taxonomical classification and botanical description are provided by Hashmat *et al* (2012 and Sarkar *et al*. (2021). The entire *A. indica* – leaf, bark, wood, root – is effective source of active ingredients against insect vectors and also used for ethnomedicine (Howard *et al*., 2011; Hashmat *et al*., 2012). Neem as ethnobotanical is very successful because it is endowed with rich reservoir of diterpenes, saponin, phytosterols, glycoside, alkaloids, proteins and limonoids. Limonoids are insecticidal and those associated with neem tree are nimbin, salannin, meliantriol, azadirachtin (Hashmat *et al*., 2012; Selvam *et al*., 2018; Eleta *et al*., 2021) and other active volatile oils (Hashmat *et al*., 2012). List of phenolic compounds from *A. indica* are up to 32 (Sarkar *et al*, 2021). Selvam *et al* (2018) did a phytochemical analysis on 12 plants and confirmed that *A. indica* aqueous extract possesses the highest amounts of listed compounds. The seed kernel is where most of the active ingredients are located and may contain azadirachtin up to 5 mg/g of kernel (Njom *et al*., 2011). Neem oil and cake are other by-products that have manifold action mechanism against arthropod pests especially mosquitoes (Mafara and Bukura, 2021). In-vitro and in-vivo experiments show that neem extracts have strong influence on biological activities (Sarkar *et al*., 2021); however, caution may be taken as they are known emmenagogue and had been implicated in abortions (Hashmat *et al*., 2012). Neem extracts have essential functions in the regulation of several biological pathways like mitigating the effects of reactive oxygen species, reduction of release of cytokines that promote inflammation, raising lymphocyte counts (CD8+T-cells and CD4+), regulating apoptosis among others (Sarkar *et al*., 2021).

Table 1: Possible sources of agents for inducing arrested development

Stage/Species of vector	Agent	Method of application	Observed Outcome	References
Eggs, early third instars larvae of *Anopheles stephensi, Aedes aegypti* and *Culex quinquefasciatus*	The whole plant crude extracts of *Citrullus colocynthis*	In solution	▪ Larval and pupa development was arrested resulting in decreased pupa transformation and adult emergence ▪ Adult emergence inhibition activity was more pronounced in *Anopheles stepehensi*. ▪ Hatching was delayed and its rate was reduced	Arivoli and Tennyson, *et al*., (2011A).
All four larval instars (hereafter called LI, LII, LIII, and LIV as appropriate) plus pupae used were *Anopheles gambiaes*.	Aqueous extracts of *Azadirachta indica* A. Juss (the neem tree) wood and bark chippings.	Neem chippings in 1 liter of distilled water	▪ Larvicide and growth disruptor ▪ Fifty percent inhibition of adult emergence (IE50) of all larval instars ▪ Inhibition of pupation was seen with some larvae staying as LIVs for 9 d before dying. ▪ Reduced reaction by larvae to visual and mechanical stimuli observed at higher neem concentrations may make them more `susceptible to natural predators.	Howard *et al*., (2009).
Eggs, larval and pupa of *Anopheles stephensi, Aedes aegypti* and *Culex quinquefasciatus*	Hexane, diethyl ether, dichloromethane and ethyl acetate crude extracts of *Abutilon indicum*	In solution	▪ Larval and pupa development was arrested ▪ Decreased pupa transformation and adult emergence. ▪ Appearance of larval–pupal and pupal–adult intermediates. ▪ Increase in the developmental period. ▪ Hatching was delayed and its rate was reduced compared to control. ▪ Presence of disrupted eggs. ▪ Shells and dechitinized body walls were observed, ▪ Growth index was considerably reduced.	Arivoli and Tennyson, (2011B).
Eggs, larvae and pupa of *Anopheles gambiae* and *Culex quinquefasciatus*.	Methanolic crude extract; n-hexane fraction; chloroform fraction; ethylacetate fraction; methanol fraction of green leaves of *Boswellia dalzielii.*	In solution	▪ The egg hatchability percentage decreased with ascending concentration of the botanicals. ▪ Hatchability of *A. gambiae* eggs was reduced to 5% with n-hexane	Younoussa *et al*., (2016).
Anopheles stephensi Liston eggs, 1st – 5th instars larvae, pupae	*Purified Azadirachta indica* limonoids–azadirachtin, salannin, deacetylgedunin, gedunin, 17-hydroxyazadiradione and deacetylnimbin.	In solution	▪ Significantly reduced oviposition ▪ Duration of larval instars and the total developmental time was prolonged up to 19 days. ▪ Slower movement in the water. ▪ Gradual increase in the pupal duration and reduced emergence. ▪ Larvae deformed with impeded development.	Nathan *et al*., (2005).
Late 3rd stage instars of *A. gambiae* and *A. arabiensis.*	Methanolic extract of *Agerantum conyzoides.*	In solution of ethanol-water.	▪ *A. gambiae* s.s. larvae demelanized. ▪ *A. gambiae* s. s. larval-pupal intermediate abnormal ▪ *A. gambiae* adult emergence arrested. ▪ *A. arabiensis* larval-pupal intermediate abnormal. ▪ Adult emergence in *A. arabiensis* failed.	Muema *et al*., (2017).

A. indica is a metaphor for accessibility. Utilizing the benefits ascribed to the tree appear to satisfy all concerned because whatever your status, you could access its active ingredients. Application of neem as pesticide can be applied in various forms such as aqueous extract of leaves, bark, seed, or chunk. The extract possesses egg laying inhibition, lethal to 3rd insta larva, arrest pupa (Njom *et al*., 2011). Its bark, wood, roots, kernel or leaves can be chewed raw, soaked in water or in organic solvent such as alcohol, acetone, etc. to release its contents. Biologically active compounds from neem do control many biological processes (Sarkar *et al*, 2021). For the foregoing sterling qualities, neem had been proposed to be beneficial option to develop safer tool for targeted arthropod control (Benelli *et al*., 2017).

Azadirachtin

This is a secondary metabolite (Shama *et al*., 2020). A biological product is said to be a secondary metabolite when that product is not needed for the development or multiplication of the producer but this product will interfere with the biological well-being of another organism which is rendered incompetent to challenge the producer. Such a product confers on the producer advantages which helps it to establish in that environment. Common examples of secondary metabolites are antibiotics such as penicillin or gentamycin produced by *Penicillum notatum* and *Streptomyces cerevisae* respectively (Paul and Joshi, 2022). Azadirachtin consist of 7 biologically dynamic isomeric compound designated as azadirachtin A – G although azadirachtin E is said to be more extra active (Hashmat *et al*., 2012). Azadirachtin is a tetracyclic triterpenoid that is structured like steroid hormone (Zhao *et al* 2022). It is the active insecticide against pathological insects and those of food grain storage importance. Aziz (2021) noted that it is effective against *Aedes aegypti*, *Anopheles stephensi, Culex quinquefasciatus* and *C. pipiens* mosquitoes. This bioactive organic compound is a versatile agent that is effective for not only control of mosquitoes but also the parasites itself which account for its use worldwide to treat malaria (Nathan *et al*., 2005; Agbo *et al*., 2019). Nathen *et al*. (2005) observed that at a concentration of 1 part per million, azadirachtin was active against all stages of water stage mosquitoes. Phytopesticide like azadirachtin is a convenient route to mosquito control because there is no apprehensions attached to it as it is safe for the environment, biodegradable and active only against targeted pest as well as being simple, accessible and affordable (Howard *et al*., 2009; Agbo *et al*., 2019, Wahedi *et al*., 2020). Muema *et al* (2017) noted that mechanism of action of limonoids are not well understood, nevertheless they mentioned the following: low dose caused arrested development and compromised fertility while high doses are deleterious interfering with endocrine balance, transmission of impulses in cholinergic nerves, neuromuscular coordination, polymerization of tubulin and cell cycle, apoptotic signals, cuticle melanization and chitinization. Semi-purified organic solvent constitutes another level of use of this natural pesticide while highly purified forms are sourced from high-tech laboratories.

Delivery of azadirachtin

Phytopesticide are believed to be insect growth regulators. Evidence of phytoinsecticide activities is shown as egg endosmosis, postponements in larval growth and prolonged pupations, inhibition of moults, structural aberration, melaninization interference, chitin dissolution and death especially during moulting (Arivoli and Tennyson, 2011A & B). The traditional means of delivering this pesticide to susceptible organism is via solvents. In vivo and in-vitro delivery of this bio-insecticide will continue to be water based so long as researchers have ordinary folks in mind as beneficiary of their field endeavours. This dependence is logical because even the target organisms themselves are also largely made of water. The implication is that diffusion into target organism would be fast and uptake and interactions at the cellular levels will also be prompt. However, we are not oblivious of the fact that the active agents consist of limonoids that are known to show dissolution preference for organic solvents. So, when researchers wish to use high doses of the active substances, organic solvents are preferred for extraction. Natives in this locality use palm wine, an alcoholic beverage, to decoct neem leaves, barks, wood or seed when they wish to treat malaria. Organic solvents used in phytometabolite extractions include ethyl acetate, dichloromethane, diethyl ether, methanol, chloroform, n-hexane or n-hexane (Arivoli and Tennyson, 2011B; Younoussa *et al*., 2016). Ejeta *et al*., (2021) report that ethanol extract of *A. indica* had a profound larvicidal effect on *A arabiensi*. Ethanol extract demonstrate enormous advantages in managing *Anopheles* larvae because total mortality under 24 is obtainable after exposure (Aïzoun *et al*., 2022). Eleta *et al*. (2021) observed that ethanol leaf extract was better than methanolic leaf extract because up to 95% of mosquito larva was killed compared to the 65% of that of methanol extract. Njom *et al*., (2011) obtained methanolic extract that had the same inhibitory effect like the aqueous extract at half the time it took the watery extract. Selvam *et al* (2018) curiously had water extract performing better than acetone or ethyl alcohol extracts meaning that environmental and other unknown factors may have some role to play in neem solubility and activities. Younoussa *et al*. (2016) found n-hexane fractions of *B. dalzielii* had the most ovicidal, larvicidal, and pupicidal effects on *A. gambiae* and *C. quinquefasciatus.* Presumably, organic solvent may have additive, or synergist effects along with serving as carrier system. Boiled neem extract is said to limit the activity of the active ingredients meaning that cold extract is preferable (Agbo *et al*., 2019).

The foregoing suggests that bioinsecticide such as azadirachtin can be delivered in much more concentrated forms in aqueous or organic solvents and added to water bodies around houses, on/in tree trunks that habour small water collections and even banana/plantain stalks. Extract could be presented to target sites with foliar sprays (Agbo *et al*., 2019); efficiency of delivery could be enhanced by using pressurized mechanized sprayer. Where the foregoing are not possible, other natural green sources of insecticides (Zhao *et al*., 2022) or *A. indica* leaves, bark, wood chippings, seeds can be placed in open water bodies. Extracts in oils and solvents can be added directly on water bodies. Like other natural substances, azadirachtin are labile so degenerate when exposed to irradiation, so slow-release technology such as microencapsulation is being applied to promote longevity (Muema *et al* 2017). Recent advances involve the delivery of biopesticides using silver nanoparticles of *A. indica* which is more effective in controlling both larval and adult forms of pathogenic mosquitoes than alcoholic extracts thus making it an attractive option to replace chemical pesticides (Aziz, 2021).

Sourcing azadirachtin

We have shown the source of azadirachtin to be natural plants. Owing to the quantum of biopepticide that will be required to produce enough for farmers and medical use, depending on cutting down the trees to obtain the extract will result in overharvesting, capacity reduction or loss of habitat (Lakshmaiah *et al*, 2022). This unpopular exercise will still not be able to meet global demand. So doing with time will eventually create monumental environmental hazard because all such plants will be wiped out entirely. It was *apro pos* that researchers had since taken up the challenge. Consequently, biotechnological techniques are evolving rapidly to meet this challenge especially when chemical manufacture is not an option and when it cannot be provided naturally (Sharma *et al*., 2020). Plant tissue culture techniques have sprung up as the best option to meet azadiraachtin needs. The outcome fortunately has resulted in a number of techniques that are useful in creating sources of secondary metabolites generally. The traditional method had been plant multiplication from seeds and budding and they are still being used to source neem product in rural settings.

Plant tissue culture procedures likes micropropagation, leaf, hairy root, calli and shoots have been adopted to enhance secondary metabolite production, azadirachtin inclusive (Sharma *et al*., 2020; Lakshmaiah *et al*., 2022;). The beauty of this approach is that both small scale and large-scale production of the product are at the disposal of big and small investors. These modern techniques depend on synthetic media consisting of complex mixtures. Glucose and phosphates are the principal limiting factors in *A. indica* cell suspension culture (Prakash and Srivastava, 2007). It can also be multiplied using the synseed approach in Murashige and Skoog media that yield regenerants that are genetically stable as the parent stock (Kader *et al*., 2022). Srivastava and Sricastava (2012) used *A. indica* hairy cultivation from leaf explants that was transformed with a certain strain of *Agrobacterium rhizogenes* to mass produce azadirachtin in an adapted Murashige and Skoog medium employing permeabilizing agent, growth regulator and a biotic elicitor. Improvement in the art is directed at raising the quantity of extracts obtained – Srivastava and Sricastava (2012) obtained 113mg/l of azadirachtin compared to 71 mg/l of Prakash and Srivastava (2007). Similarly, large scale production in bioreactors have had bright prospects: bimodal impellers stirred tank bioreactor raised azadirachtin production of *A. indica* suspension culture because of improved oxygen transfer and less medium shear force (Prakash and Srivastava, 2007). Also, with improved growth regulators such as auxin and cytokinins, callus induction mediated cell culture yielded production of azadirachtin of up to 285.64 mg/l (Farjaminezhad and Garoosi 2019). Molecular cloning techniques which involve precise gene editing like clustered regulatory interspaced short palindromic repeat (CRISPR), transcription activator-like effector nuclease (TALEN) or Zinc-finger nuclease (ZFN) have been evolving to select genes which produce enriched terpenoids, over-express them or silence competing ones through RNA interference technique (Lakshmaiah *et al*., 2022 ; Chowdhary *et al*., 2022; Zhao *et al*., 2022). Holowach-Keller and Patterson (1997) developed bioreactor capable of generating at large scale predictable high quality tetranortriterpenoid azadirachtin continuously that is independent of the environment factors, devoid of pathogens and their toxic products. Khan *et al*. (2019) provided a review of bioreactor design for hairy root culture system with low shear stress highly improved oxygen circulation and which is adjudged to have stable production capability of azadirachtin.

CONCLUSION AND RECOMMENDATION

Phytoinsecticides are alternative safe mosquito/malaria controls that have little or no deleterious effects on non-target insects. On target insects, they are effective when used against their water stages because, at that phase of their development, mosquitoes are either killed quickly or forced to arrest. These two outcomes reduce vector fitness and capacity thus making them incapable of mustering resistance such as avoidance or target-site modifications. This control will be ideal for fast growing slums in urban areas of Nigeria and other Sub Sahara countries where water collections are “few, fixed and findable” according to Muema *et al* (2017). The tree, *A indica* produce secondary metabolites that are versatile anti-mosquito and anti-parasite control agent. The tree fulfils the aphorism that God places the solution beside every problem: the plant is readily available throughout the epicenter of mosquito/malaria menace and beyond. Its most active bioinsecticidal ingredient is azadirachtin. Azadirachtin functions like a molecular deception tool because gravid mosquitoes do not avoid it during oviposition but end up losing their progenies because of the selective toxicity of the substance to water stages of mosquitoes. This idea merits further investigations to confirm the possibility of maximizing the utility of this control tool. Investors in Sub Sahara Africa should be conversant with opportunities inherent in azadirachtin production. Peasants suffer most from the vagaries of mosquitoes and malaria and so must be educated to be in the front lines to profit from the health/economic benefits of growing whole trees and utilizing them.

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Author’s contributions

Ugwu, designed the study and wrote the introduction; both authors did the literature review and reported the findings. Oyeagu fact-checked study findings.

Conflict of interest

There is no conflict of interest.

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