Physical, chemical and biological strategies to combat malaria

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In sub-Saharan Africa, the entomological inoculation rates (the number of bites by infectious mosquitoes per person per unit time) can reach levels of more than a thousand infective bites per person per year. Research conducted by scientists at the University of Pretoria, under the auspices of the faculty research theme (FRT) Sustainable Malaria Control, focuses on the elimination of malaria and the promotion of health.

This FRT forms part of a process initiated by the University to identify the unique research strengths of the institution and to support the development of strong multidisciplinary research groups clustered around these identified strengths. These research themes, of which Sustainable Malaria Control is one, are designed to recognise and foster excellence in research. They are led by acknowledged international leaders in their specific disciplines across more than one faculty.

The researchers involved in investigating malaria vector control strategies are Mthokozisi Sibanda and Prof Walter Focke of the Department of Chemical Engineering, Heino Heyman of the Department of Plant Sciences, and Prof Lyn-Marie Birkholtz of the Department of Biochemistry. The latter two researchers are associated with the University's Faculty of Natural and Agricultural Sciences.

According to research, it is estimated that existing interventions can only reduce the annual inoculation rate by an order of magnitude, which suggests that the current interventions recommended by the World Health Organisation (WHO) might not suffice in achieving the goal of eliminating malaria in Africa. The problem is compounded by mosquito vectors that develop multiple-resistance mechanisms to pyrethroids. Effective vector control methods suppress vector populations to a bare minimum. This reduces the capacity of the mosquito to transmit the malaria parasite. The pesticide DDT has been shown to be an effective chemical in achieving this. The goal, however, is to find an alternative technology that is more cost-effective and efficient than DDT.

An integrated approach

Malaria is a parasitic disease that is mostly confined to tropical areas. It is transmitted by the female *anopheles* mosquito, and results in approximately 250 million clinical cases of malaria and nearly a million deaths annually. Malaria is particularly prevalent in sub-Saharan Africa, where it affects mostly pregnant women and children under five years of age. The WHO recommends an integrated approach that combines clinical control of the malaria parasite along with the use of long-life insecticide-treated nets and indoor residual spraying. Since mosquitoes are the vectors in malaria transmission, decreasing their population could lead to a reduction in malaria morbidity and mortality.

Vector elimination

Techniques currently in use to eliminate the mosquito vector include aerial spraying (ultra-low volume spraying), larviciding, the biological control of the mosquito ovum, larvae and adults, environmental control and sterilising the male mosquito.

Ultra-low volume spraying is a process of applying the minimum effective volume of an undiluted formulation of insecticide. Ultra-low volume is the favoured aerial spray method used worldwide for mosquito adulticide control. It is used in areas where the mosquitoes are predominantly exophilic. It is expensive to operate and the insecticides currently in use do not have a long residual period. In an article published in the *Journal of the American Mosquito Control Association* in 1996, authors Mount, Terry and Haile report that small-scale tests using a small, fixed-wing, single-engine aircraft equipped with high-volume spray systems that are modified to allow ultra-low volume applications showed satisfactory mortality rates of various mosquito species (89–100%).

The mosquito life cycle has four stages, of which three are spent in a stagnant aquatic environment. Destroying the larvae, either chemically or biologically, while in the aquatic environment is an effective vector control method. The earliest chemical larvicide, cupric acetoarsenite, was successfully used to eradicate *Anopheles gambiae* in Brazil before World War II. Temephos, an organophosphate larvicide, is much safer to use than arsenic but also kills mosquito predators.

Recently, insect growth regulators have been implemented as larvicides. Insect growth regulators work by binding onto larvae hormone receptors, preventing the insects from reaching the next stage of development. A significant advantage of an insect growth regulator is that it is target-specific and has shown a good margin of safety on non-target organisms. However, these agents may be toxic to immature stages of other aquatic insects.

Biological agents, such as parasites, pathogens and predators, can be used to target various life stages of the mosquito. Bacteria, fungi, viruses and fish have been employed to decrease the mosquito larvae populations. These agents are inexpensive to implement, and safe to humans and non-target organisms. They therefore provide a potentially environmentally friendly option. Fish feeding on mosquito larvae include *Gambus affinis* and *Gambus Holbrook*. They have been used for many generations in malaria-endemic regions.

According to an article in the *Journal of Vector Borne Diseases* in 2003, author PK Mittal reports that pathogenic bacteria, such as *Bacillus thuringiensis var. israelensis* (Bti) and *Bacillus sphaericus* (Bs) have been shown to be highly effective in controlling mosquito larvae at very low doses.

Some fungi have been used to control mosquito larvae and adults. Such entomopathogenic fungi (for example, the aquatic *Coelomomyces* sp. and soil fungi *Beauveria bassiana*) can kill *Anopheles gambiae* through tarsal contact. Unlike other mosquito biocontrol agents, such as bacteria, microsporidia and viruses, the fungi can infect and kill without being ingested. The spores germinate on the insect cuticle, and this is followed by penetration and growth into the mosquito hemolymph within one to two weeks. Very low concentrations of spores suffice. This has been shown to reduce malaria transmission by up to 90% as soon as the mosquitoes become infected with the fungus after ingesting *Plasmodia*. Transgenic fungi that can produce antimalarial peptides in the mid-gut of the mosquito have also been reported.

The basic rationale of environmental management is to eliminate all possible mosquito breeding sites. Environmental management was used extensively before the advent of DDT and provided a single effective way of fighting malaria. Environmental management has been applied in four distinct epidemiological settings: malaria in forest areas, rural malaria attributable to water resources development and management, rural malaria attributable to wetlands, rivers, coastlands and non-agricultural man-made habitats, and urban areas. Research by Keiser, Singer and Utzinger, published in *Lancet Infectious Diseases*, shows that environmental control can reduce the risk of malaria transmission by 88%.

When a female mosquito mates with an infertile male, she becomes infertile for the rest of her lifespan. Sterilisation of male mosquitoes is achieved by exposing male pupae-stage or adult mosquitoes to high-energy ionising radiation. This causes damage to germ cells making them sterile. Sustained release of large numbers of sterile males (outnumbering the fertile native males) over a period of time that covers several generations of the target population, will lead to a gradual decrease in the productive capacity of the mosquitoes. Eventually, so few fertile insects remain that no fecund mating events occur and the population is eliminated. Sterile insect technique (SIT) is usually initiated when the number of mosquitoes reaches a seasonal minimum, for example, at the end of winter.

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Preventing mosquito bites

Preventative measures to combat malaria include the use of long-life insecticide-treated nets (LLITNs), the use of repellents and attractants, and the careful design of human settlements.

African countries favour LLITN programmes, as they are inexpensive and, unlike indoor residual spraying (IRS) programmes, are easy to implement. The downside of LLITN is that protection is only offered during sleeping time. Washing gradually diminishes the insecticidal activity of the nets. The effectiveness of LLITNs is thus limited by the need to wash the nets from time to time.

Before the advent of DDT and pyrethroids in the 1950s and 1970s respectively, researchers focused on diverting the female mosquitoes from human beings. This could be done by attracting or repelling them. With the initial perceived success of DDT and pyrethroid insecticides, research in this field became subdued. However, recently, with the controversy arising from the use of DDT and the emergence of resistance to pyrethroids, there is renewed interest in alternative control methods.

The mechanisms whereby repellents function are not clearly understood. They include chemical products with an offensive smell or taste to mosquitoes. Plant-derived compounds with repellence properties are most likely chemicals that are produced in defence against insects that pose a threat to the plant itself. These chemicals can be grouped into different categories, based on the functional groups present. They include nitrogen-containing compounds, terpenoids, phenolics, proteinase inhibitors and growth regulators. These compounds are generally produced to fight off a broad spectrum of insects, and are thus also effective against mosquitoes. Plants with better repellent properties fall into distinct families, with the *Poaceae* family (citronella-based) being the pre-eminent one. Species of *Lamiaceae*, *Fabaceae* and *Asteraceae* also show promising results. The future potential is thus very clear as these families cover large numbers of plant species, many of which have not yet been researched.

Prior to the extensive use of synthetically produced repellents, aromatic/essential oils were commonly used. The military was a significant consumer of these oils. Soldiers were issued with creams containing citronella, camphor and paraffin. Numerous essential oil-producing plants from the *Lamiaceae*, *Poaceae*, *Rutaceae* and *Myrtaceae* families have very well-known repellent activity. Synthetic products that have been used as repellents include Indalone, dimethylpthalate 2-ethyl-1, 3-hexane diol (Rutgers 612) and *N*,*N*-diethyl-*m*-toluamide (DEET). DEET is by far the most effective and widely used repellent.

Attractants present an option for controlling mosquito vector populations via mosquito traps. Host-seeking female mosquitoes are guided by attractant odours released by their target. In an article published in *PLoS ONE*, the open access peer-reviewed journal of the Public Library of Science, in 2010, authors Okumu et al. state that *Anopheles gambiae* is attracted to the ammonia, lactic acid and other carboxylic acids naturally present in the body odour and sweat produced by warm-blooded animals.

According to an article published in the journal *Chemical Senses* in 2001 by authors Smallegange, Qiu, Van Loon and Takken, blends of these compounds attract more mosquitoes than individual compounds applied in isolation, suggesting synergistic interactions. Blends of lactic acid, carboxylic acids and ammonia were shown to be attractive in laboratory tests and field tests. However, relative to human sweat, these blends are still less attractive. This implies that there are additional compounds, yet to be discovered, with synergistic activity for attracting mosquitoes. Research conducted by Mboera, Takken and Sambu, published in the *Bulletin of Entomological Research* in 2000, shows that carbon dioxide also plays an important role in mosquito host-seeking behaviour. In field tests, mosquito trap catches increased with the addition of CO_2 to the odour blend. *Anopheles gambiae* apparently prefers to attack its victims on the feet and ankles. It is attracted to this particular area because of the odour emitted by the feet. This odour is produced by *Brevibacterium linens*, which survives in the humid and warm clefts between the toes. The odour resembles that of limburger cheese. Bart Knols, in his book *Cheese, dogs and pills to end malaria* (2012), confirmed the attractiveness of limburger cheese to *Anopheles gambiae*.

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An alternative technique that prevents a mosquito from locating its blood host is to disable its odour receptors. Mark Stopfer, in his book *Malaria: Mosquitoes bamboozled* (2011), described three classes of odour receptor disruptors. Inhibitors such as hexanol and butanol have the ability to block CO_2 receptors in mosquitoes. Secondly, irritators such as 2-butanone can be used to simulate CO_2, and thus has the ability to distract mosquitoes from human beings. Lastly, blinders such as 2,3-butanedione cause prolonged activation of CO_2 sensory neurons, thereby disabling the ability of the mosquito to sense CO_2 coming from humans.

According to an article published in *Trends in Parasitology* in 2002, authors Lindsay, Emmerson and Charlwood found that the careful design of human settlements effectively reduces the spread of malaria. In malaria-endemic areas, transmission was reduced by up to 96% by covering the windows and doors of human dwellings with thin muslin. The most efficient vectors in Africa are superbly adapted to feeding on human beings and are able to enter a human dwelling in search of a blood meal. *Anopheles gambiae* is attracted to the human odour coming from an abode. It flies upwards as soon as it meets an obstacle such as the wall of a dwelling.

If there is an opening in the roof, such as open eaves, the vector will use this as an entry point. Closing the eaves and installing a ceiling or a net screen is beneficial, as it reduces the house entry of mosquitoes. In an article published in the journal *Tropical Medicine and International Health* in 2003, authors Lindsay, Jawara, Paine, Pinder, Walraven and Emerson show that house entry by mosquitoes was reduced by 59% with a ceiling and 37% when the eaves were closed.

Killing mosquitoes after they have bitten

Strategies are also implemented to combat malaria by killing mosquitoes after they have bitten. Such techniques include the use of indoor residual spray and insecticide-treated wall linings. The WHO promotes the use of these two methods as a primary operational vector control intervention to prevent malaria transmission. Indoor residual spray is deemed particularly effective and is widely applied in southern Africa. The WHO has approved 12 insecticides for use in indoor residual spraying. Six of the insecticides are classified as pyrethroids (alphacypermethrin, betacyfluthrin, bifenthrin, deltamethrin, etofenprox and lamdacyhalothrin), three as organophosphates (malathion, fenitrothion and pirimiphos-methyl), two as carbamates (propoxur and bendiocarb). The last one is DDT.

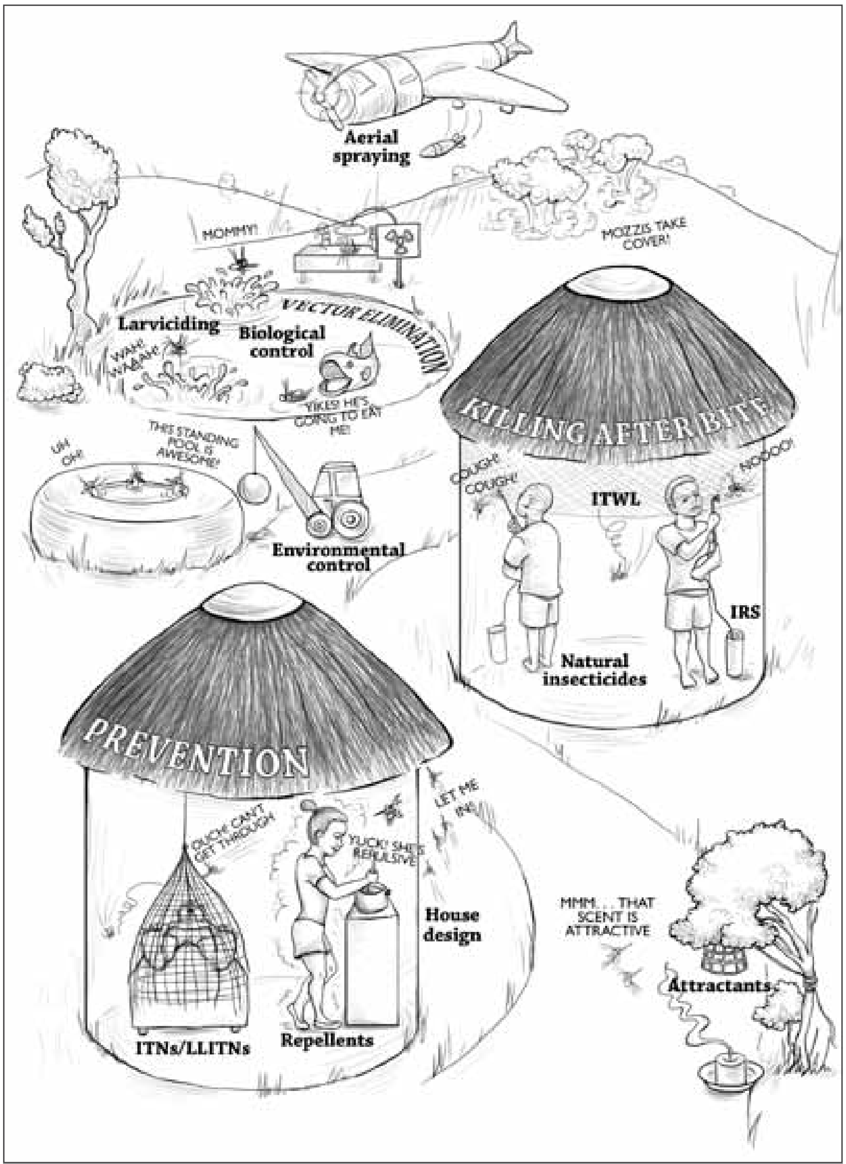
DDT is the most preferred pesticide for indoor residual spraying because it has an efficacy of 12 months or more, while the rest of the insecticides have an efficacy of up to six months. The longer-lasting efficacy of DDT provides a low-cost option, as one spray cycle per annum is sufficient, while two or more spray cycles may be required for the alternative insecticides.

In South Africa, DDT was temporarily replaced with deltamethrin between 1996 and 1999. However, DDT was reintroduced in 2000 when malaria transmission reached epidemic proportions. The failure of the pyrethroid was attributed to the return of *Anopheles funestus* strains. This major mosquito vector showed resistance to pyrethroids, but remained fully susceptible to DDT. The stabilisation of DDT alternatives for indoor residual spraying includes formulations based on micro-encapsulated insecticides that have been tested with great success. These results show that shielding the insecticides from the outside environment stabilises them against premature degradation.

However, the higher costs associated with such formulations may limit their widespread implementation as replacements for DDT. Recent work suggests that the precipitation of insecticides on low-cost phosphogypsum and the co-intercalation of the insecticides in organo-clays can provide significantly improved stabilisation of selected WHO-approved insecticides. Replacing synthetic with natural insecticides offers advantages and novel possibilities. These include different modes of action and the prevention of rapid degradation in the environment. Research into natural insecticides subsided during the early 1970s with the successful synthesis of pyrethroids (synthetic equivalent of the natural insecticide pyrethrum). Pyrethrum itself is a potent natural insecticide, but is unstable in ultraviolet light and at high temperatures.

However, there is renewed interest in natural insecticides as green alternatives. Research into natural mosquito adulticides has been limited and the commercial exploitation of natural insecticides is uncommon in the market.

A problem is posed by the fact that the residual effect of these botanicals tends to be very short. For example, in the case of azadirachtin, it only lasts between four and eight days in the field. The essential oils of five plant species – caraway, celery, Chinese star anise, long pepper and zedoary – all showed significant effectiveness against pyrethroid-resistant *Anopheles aegypti* strains. The effectiveness of these oils against the pyrethroid-resistant strains is a major discovery, as it highlighted the potential utility of essential oils in prone areas that display a resistance to insecticides. Indoor residual spray depends heavily on the use of DDT. In addition to this, insecticide dusting is a problem in indoor residual spraying. Insecticide-treated wall linings (ITWLs) have



Alternative techniques to prevent malaria

The scientists involved in research on physical, chemical and biological strategies to combat malaria (from left) are Heino Heyman, Prof Lyn-Marie Birkholtz, Prof Walter Focke and Mthokozisi Sibanda.

been proposed as a safer alternative to indoor residual spraying. So far, the most advanced product is based on woven shade cloth made from polyethylene with 50% shading and treated with deltamethrin 4.4 g a.i./kg material. The type of treatment is similar to Type 2 LLITNs (the insecticide being included in the fibre itself). This product was developed and manufactured by Durable Activated Residual Textiles South Africa (DART).

Field test results show that ITWL nets are generally acceptable in a range of community settings. However, these wall linings were produced using labour intensive fabric weaving or knitting methods. Prof Focke and PhD student Mthokozisi Sibanda have developed inexpensive linings that are produced by extruding insecticide impregnated polyethylene directly into a net format. The results of preliminary field trials on these nets have been presented at two international malaria conferences.

Results indicate that the use of these pyrethroid-impregnated indoor linings is potentially as successful as indoor residual spraying. This new and potentially safe method might be a more sustainable approach for vector control.

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References

Keiser, J, Singer, B and Utzinger, J. 2005. Reducing the burden of malaria in different eco-epidemiological settings with environmental management: a systematic review. *Lancet Infectious Diseases*, 5:595–648.

Knols, B. 2012. Cheese, dogs and pills to end malaria. [Online]. Available at: http://www.ted.com/talks/bart_knols_cheese_dogs_and_pills_to_end_malaria.html?utm_source=newsletter_weekly. Accessed in May 2012.

Kruger, T, De Jager, C, Focke, W, Sibanda, M, Bornman, R. Slow release pyrethroid-impregnated indoor linings as a new malaria vector control method. Environment and Health – Bridging South, North, East and West. Conference of ISEE, ISES and ISIAQ. Basel, Switzerland 19 – 23 August 2013. [Online]. Available at: http://ehp.niehs.nih.gov/ehbasel13/p-3-23-24/

Kruger, T, De Jager, C, Focke, W, Sibanda, M, Bornman, R. Slow release pyrethroid-impregnated indoor linings as a new malaria vector control method. 6th MIM Pan-African Malaria Conference, 6 – 11 October 2013. International Convention Centre, Durban, South Africa.

Lindsay, S, Emmerson, P and Charlwood, J. 2002. Reducing malaria by mosquito-proofing houses. *Trends in Parasitology*, 18(11):510–514.

Lindsay, S, Jawara, M, Paine, K, Pinder, M, Walraven, G and Emerson, P. 2003. Changes in house design reduce exposure to malaria mosquitoes. *Tropical Medicine and International Health*, 8(6):512–517.

Mboera L, Takken W, and Sambu E. 2000. The response of *Culex quinquefasciatus* (Diptera: Culicidae) to traps baited with carbon dioxide, i-octen-3-ol, aceptone, butyric acid and human foot odour in Tanzania. *Bulletin of Entomological Research*, 90:155–159.

Mittal, P. 2003. Biolarvicides in vector control: challenges and prospects. *Journal of Vector Borne Diseases*, 40:20–32.

Mount, G, Terry, L and Haile, D. 1996. A review of ultralow-volume aerial sprays of insecticide for mosquito control. *Journal of the American Mosquito Control Association*, 12(4):601–618.

Okumu, F, Killeen, G, Ogoma, S, Biswaro, L, Smallegange, R and Mbeyela, E. 2010. Development and field evaluation of a synthetic mosquito lure that is more attractive than humans. *PLoS ONE*, 5(e8951).

Sibanda, M, Focke, W. Development of an insecticide impregnated wall lining. 6th MIM Pan-African Malaria Conference, 6 – 11 October 2013. International Convention Centre, Durban. [Online]. Available at: http://web.up.ac.za/sitefiles/file/44/1026/2063/18361/Sibanda%20MIMS%202013%20WWF.pdf

Smallegange, R, Qiu, Y, Van Loon, J, and Takken, W. 2001. Synergism between ammonia, lactic acid and carboxylic acids as kairomones in host-seeking behaviour of the malaria mosquito *Anopheles gambiae sensu stricto* (Diptera: Culicidae). *Chemistry Senses*, 30:140–152.

Stopfer, M. 2011. Malaria: Mosquitoes bamboozled. *Nature*, 474:40–41.

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