

## **Mosquitocidal and repellent properties of plant extracts**

*Extrait de Artemisia Compendium. From polytherapy to prophylaxy :  
The power of the Artemisia plants*

Mosquitoes are progressively becoming resistant to industrial repellents and insecticides. This is the case for pyrethroids used on bednets.

Pyrethroids are toxic. Aquatic ecosystems are particularly vulnerable to the impact of permethrin. A fragile balance exists between the quality and quantity of insects and other invertebrates that serve as fish food. The 48-hour LC50 for rainbow trout is 0.0125 mg/L for 24 hours, and 0.0054 mg/L for 48 hours. The 48-hour LC50 in salmon is 0.0018 mg/L. As a group, synthetic pyrethroids were toxic to all estuarine species tested. They had a 96-hour LC50 of less than or equal to 0.0078 mg/L for these species.

Effects on other organisms: Permethrin is extremely toxic to bees. Severe losses may be expected if bees are present at treatment time, or within a day thereafter. Permethrin is also toxic to wildlife. It should not be applied, or allowed to drift, to crops or weeds in which active foraging takes place. Increased release of neurotransmitters disturbs synaptic transmission.

Most of the industrial pesticides are expensive and African households cannot afford their purchase.

Plants, their extracts and their essential oils have been used during centuries to fight aggressive mosquitoes responsible for malaria, dengue, sleeping sickness, but also insects acting as vectors for many other diseases.

Native plants have the advantage of low cost and local availability. Their action is related to several constituents working in synergy; the probability that they develop resistance is lower as for synthetic insecticides used in monotherapy.

The repellent and insecticidal potential of plant material has been exploited for thousands of years by man, most simply by hanging bruised plants in houses. Many plant volatiles are deterrent or repellent because they have high vapour toxicity to insects.

In China dried *Artemisia* were put under the beds to repel mosquitoes. In Africa some people slowly burn *Artemisia afra* leaves on charcoal to repel mosquitoes from their living quarters

*Artemisia annua* has insecticidal properties

*AK Tripathi, V Prajapati, KK Aggarwal, SPS Khanuja, S Kumar. Repellency and toxicity of oil from Artemisia annua to certain stored-product beetles. Journal of economic entomology 93 (1), 43-47*

Neem *Azadirachta indica* is famous for its insecticidal properties, but it operates more by starvation than by a knock-out effect.

*AN Kumar, K Murugan, P Madhiyazhagan, K Prabhu. Spinosad and neem seed kernel extract as bio-controlling agents for malarial vector, Anopheles stephensi and non-biting midge, Chironomus circumdatus. Asian Pacific journal of tropical medicine 4 (8), 614-618*

A study made in Italy on anti-plasmodial and insecticidal activities of essential oils of aromatic plants growing in the Mediterranean area showed that thymol from savory (*Satureja thymbra*) showed the highest larvicidal and adulticidal activities. In essential oil fractions obtained by hydrodistillation carvacrol and terpene-4-ol also showed some activity.

*Dell'Agli M, Sanna C, Rubiolo P, Anti-plasmodial and insecticidal activities of essential oils of aromatic plants growing in the Mediterranean area, Malaria Journal 11 :219*

Thymol appears to be a neuractive chemical which affects the flight motor activity and wing beat frequency in flies.

*Waliwitya R, Kennedy CJ, Lawenberger CA. ( 2009) Larvicidal and oviposition altering activity of monoterpenoids, trans- anethol and rosemary oil to the yellow fever mosquito Aedes aegypti (Diptera: Culicidae). Pest Manag Sci. 65: 241– 248*

An extensive study on the insecticidal properties of thymol is a thesis from the Iowa State University.

*Sang Kyun Lee. Insecticidal properties of monoterpenoids and their derivatives as a new natural means of crop protection. 1997 Iowa State University*

This thesis compares the mortality of adult *Musca domestica* (housefly) exposed to 3 phenols, 12 alcohols, 7 ketones, 4 acids and 7 monoterpenes. Thymol is the strongest and comes close to pyrethrins.

Monoterpenoids commonly found in plant essential oils were tested for acute toxicity via topical application to tobacco cutworms, a lepidopter. The most lethal among such compounds was thymol from garden thyme (*Thymus vulgaris*) Feeding deterrence was determined using a cabbage leaf disk choice test. Thymol again was the most efficient. Essential oil or aqueous leaf extract from *Azadirachta indica* (neem) are also excellent feeding deterrents.

Essential oils of thyme (*Thymus numidicus*), from Algeria were isolated by steam distillation. The major components were thymol (51.3%), carvacrol (9.4%), linalool (3.3%), caryophyllene, (2.7%). The fraction containing thymol (51%) and linalool (3.2%) showed the highest insecticidal activity against *Rhizoperta dominica*.

*Faiza Saidj, Sid-Ahmed Rezzoug, Fatiha Bentahar, Chahrazed Boutekdjiret. Chemical Composition and Insecticidal Properties of Thymus numidicus (Poiret) Essential Oil from Algeria. Journal of essential oil-bearing plants, 2008, 11 (4), pp.397-405*

The same insecticidal properties of thymol in the essential oil of *Trachyspermum ammi* against *Anopheles stephensi* have been documented.

*S. K. Pandey Shikha Upadhyay A. K. Tripath. Insecticidal and repellent activities of thymol from the essential oil of Trachyspermum ammi (Linn) Sprague seeds against Anopheles stephensi. Parasitology Research. July 2009, Volume 105, Issue 2, pp 507–512*

A study of monoterpenoids showed that for topical acute toxicity on larvae the most potent were thymol and carvacrol. The most profound synergistic effect was found in the mixture p-cymene, thymol, carvacrol.

*Roman Pavel, Insecticidal activity of certain medical plants, Fitoterapia. 75.2004*

The same author in another study found in laboratory conditions that the sublethal application of thyme oil to the adults of the housefly significantly reduced their longevity and the natality decreased by 80%, And even larvae which emerged from eggs from female flyies which had been treated, were non vital. In a third study the same author studied the insecticidal activity of 34 essential oils extracted from plants and found those from *Origanum vulgare, majorana, compactum, Ocimum basilicum, Thymus vulgaris* were lethal in doses in the microgram range. The advantage of these plants is that they are easy to grow in many countries and have well known nutritional values. Some of the terpenes, like

carvacrol, also have strong antiplasmodial properties. Thymol contributes to the acaricidal properties of *Ocimum gratissimum*.

Oregano oils are rich in thymol (38.8%), carvacrol (32.9%), p-cymene (7.9%). They have strong toxicities against *Rhizoperta dominica* by contact or by fumigation.

*O Khalfi, N Sahraoui, J Sc Food and Agricul, 2008, 9, 1562-66). Chemical composition and insecticidal properties of Origanum glandulosum (Desf.) essential oil from Algeria. Journal of the Science of Food and Agriculture. Volume 88, Issue 9. July 2008. Pages 1562–1566*

*Karpouhtsis I, Pardali E, Feggou E, Kokkini S, Scouras ZG, Mavragani-Tsipidou P. Insecticidal and genotoxic activities of oregano essential oils. Journal of Agricultural and Food Chemistry. 1998; 46: 1111-15.*

A thesis from the Kenyatta University evaluated the insecticidal and repellent properties of plants from Kenya. Among the plants studied *Artemisia afra* showed reasonable insecticidal activity. *A. afra* essential oil attained 100 % mortality at 8% after 4 hours and 86% at 2% concentration after 6 hours. This activity is possibly related to thujone.

*Simyiu Silas Khamala, Bioevaluation of insecticidal and repellent plants from Central Region of Kenya Kenyatta University April 2004*

Coumarins have a knock down effect, scopoletin being the most potent, but do not kill mosquitoes.

*Venugopala K. Narayanaswamy, Raquel M. Gleiser, Kabange Kasumbwe, Bandar E. Aldhubiab, Mahesh V. Attimarad, and Bharti Odhav Evaluation of Halogenated Coumarins for Antimosquito Properties, The Scientific World Journal, 2014. Volume 2014 (2014), Article ID 189824*

Saponins which are present in many plants have strong insecticidal properties. In fact, saponins protect plants from phytopathogenic microorganisms, phytophagous mammalian and insects.

The anophelocidic activity of the aqueous, terpenic and steroidal extracts of *Artemisia annua* were studied at the laboratory of Lwiro in R.D. Congo.

*Bertin Zawadi Musaka, Janvier Bandibabone Balikubiri, Jean Augustin Rubabura Kituka, Chihire Barhahakana, Josué Fikiri Kwigonda, and Luc Ombeni Bashwira. Anophelocide activity of the aqueous, terpenic and steroidal extract of the plants Mentha aquatica and Artemisia annua. International J Innovation and Scientific Research, 2014, 10-2, 318-323*

The highest mortality is noticed for the aqueous and steroidal extracts, much more than for the terpenic components. Wormwood *Artemisia absinthium* wetted in seawater and sprayed kills insects in living quarters:

*CRA Goodfray, Agrochemicals from Natural Products. 1985 NY, Dekker ed*

*Artemisia absinthium* has strong acaricidal properties and could be useful in controlling *R. microplus* cattle ticks.

*S. Parveen, R. Godara, R. Katoch, A. Yadav, P. K. Verma, M. Katoch, and N. K. Singh. In Vitro Evaluation of Ethanolic Extracts of Ageratum conyzoides and Artemisia absinthium against Cattle Tick, Rhipicephalus microplus. The Scientific World Journal. Volume 2014 (2014), Article ID 858973, <http://dx.doi.org/10.1155/2014/858973>*

Thymol, saponins and carvacrol are FDA approved and are included by the Council of Europe in the list of chemical flavourings.

## **Repellency**

The insect repellent diethyltoluamide (DEET) is among the most frequently detected organic chemical contaminants in water across a wide range of geographies from around the world. These observations are raising critical questions and increasing concerns regarding potential environmental relevance, particularly when the emergence of severe neurological conditions attributed to the Zika virus has increased the use of insect repellents. After dermal application, DEET is washed from the skin when bathing and enters the municipal sewer system before discharge into the environment.

*Merel S, Snyder SA. Critical assessment of the ubiquitous occurrence and fate of the insect repellent N,N-diethyl-m-toluamide in water. Environ Int. 2016 Nov;96:98-117. doi: 10.1016/j.envint.2016.09.004.*

The association ACECI in Burundi ([www.aceci.org](http://www.aceci.org)) has developed a mosquito repellent based on *Nepeta cataria* (catnip in english, cataire en français, Katzenminze auf deutsch). The study by local students in medicine in collaboration with Montreal's Ecole Polytechnique, together with the Government of Burundi, showed that catnip oil reduced the number of bites from mosquitoes by 91.7%. The trial involved 60 volunteers. The results and the analytical data have been submitted for publication.

Trials NCT01160809 in Ethiopia, were financed by the U.S. National Institute of Health. Daily application of mosquito repellent during the evening followed by the use of LLINs during bedtime at community level has significantly reduced malaria infection. The finding has strong implication particularly in areas where malaria vectors feed mainly in the evening

before bedtime. The topical repellent used was Buzz-Off® petroleum jelly and essential oil blend obtained from the GREEN PLC in Addis Ababa,

The University at Nairobi has published a well documented paper on the repellent activity of several plants.

*Michael A. Birkett, Ahmed Hassanali, Solveig Høglund, Jan Pettersson, John A. Pickett* Repellent activity of catmint, *Nepeta cataria*, and iridoid nepetalactone isomers against Afro-tropical mosquitoes, ixodid ticks and red poultry mites. *Phytochemistry*, 2011, 72, 109-114

*Nepeta cataria* was in many aspects (spatial and contact repellency, knockdown) equivalent to the synthetic DEET. A study made by USDA-ARS finds that mosquitoes land less on surfaces covered with nepetalactone than on a surface covered with DEET.

*U. Bernier, K Furman DL Kline, S Allan, D Barnard.* Comparison of Contact and Spatial Repellency of Catnip Oil and *N,N*-Diethyl-3-methylbenzamide (Deet) Against Mosquitoes *J Med Entomol* 42, 306-11, 2005

A study from Thailand found that mosquitoes try to escape from a chamber containing catnip oil

*S Polsomboon, J Grieco, N Achee, KR Chauhan,* Behavioral responses of catnip *Nepeta cataria* on two species of mosquitoes, *Aedes aegyptii* and *Anopheles* .*J Am Mosquito Control Assoc.* 2008, 24, 513-19

A study from India found that nepetalactone repels mosquitoes ten times more than DEET.

*O Koul, S Wali, GS Dhaliwal.* Essential Oils as Green Pesticides: Potential and Constraints. *Biopestic. Int.* 4(1): 63–84 (2008)

Another study from the Dupont de Nemours company documents the repellency of catnip oil formulations against black flies and mosquitoes in the field. Hydrogenated catmint oil is registered as biopesticide by EPA. *Nepeta* oils have a longlasting effect.

*Spero NC, Gonzalez YI, Scialdone MA, Hallahan DL.* Repellency of hydrogenated catmint oil formulations to black flies and mosquitoes in the field. *J Med Entomol.* 2008 Nov; 45(6): 1080-6.

Evaluations of catnip oil toxicity were conducted to provide a broad-spectrum safety profile of catnip oil use as a potential biting and nuisance insect repellent in urban settings. Acute oral, dermal, inhalation, primary dermal and eye irritation toxicity tests were performed and were found to be extremely low. The acute oral LD(50) of catnip oil was found to be 3160 mg/kg body weight (BW) and 2710 mg/kg BW in female and male rats, respectively. The

acute dermal LD50 was >5000 mg/kg BW. The acute inhalation LD50 was observed to be > 10,000 mg/m<sup>3</sup>.

*Zhu JJ, Berkebile DR, Dunlap CA, Zhang A, Boxler D, Tangtrakulwanich K, Behle RW, Baxendale F, Brewer G. Nepetalactones from essential oil of Nepeta cataria represent a stable fly feeding and oviposition repellent. Med Vet Entomol. 2012 Jun;26(2):131-8. doi: 10.1111/j.1365-2915.2011.00972.x.*

When comparing with pyrethroids or malathion this is very encouraging because these synthetic repellents applied on bed nets or on indoor walls are toxic for pregnant women and children, and are carcinogenic.

*Shukla Y, Yadav A, Arora A. Carcinogenic and cocarcinogenic potential of cypermethrin on mouse skin. Cancer. Lett., 182: 33-41.*

*Shukla Y, Taneja P2002. Mutagenic potential of cypermethrin in mouse dominant lethal assay. Environ. Pathol. Toxicol. Oncol., 21: 259-65.*

A study from India compared the action of essential oils from 23 plants. Top ranking in repellency and knockdown effect were catnip, basil, thyme, citronella.

*A. Uniyal, S. N. Tikar, R. Singh, Shakti V. Shukla, O. P. Agrawal, Vijay Veer and D. Sukumaran. Repellent effect, knockdown study and electrophysiological responses of essential oils against Aedes aegypti J Entomology and Zoology Studies, 2014, 2, 351-357*

Some of them were almost equivalent to malathion, without creating the adverse effects like irritation, rashes and allergic reactions.

Ocimum and Moringa are also well known for their repellent properties.

*K Prabhu, K Murugan, A Nareshkumar, N Ramasubramanian Larvicidal and repellent potential of Moringa oleifera against malarial vector, Anopheles stephensi Liston (Insecta: Diptera: Culicidae). Asian Pacific journal of tropical biomedicine 1 (2), 124-129*

*Ocimum americanum* potted plants are often used as repellents inside houses. *Ocimum gratissimum* and *Ocimum basilicum* have recently been compared to other plant repellents and generally are top ranking compared to *Azadirachta indica*, *Citrus lemon*, *Jatropha curcas*.

*T. C. Kazembe and M. Chaibva. Mosquito Repellency of Whole Extracts and Volatile oils of Ocimum americanum, Jatropha curcas and Citrus limon. Bull Envir Pharmacol and Life Sciences, 2012, 8, 65,71*

In Tanzania trials were run with the essential oil of *Ocimum suave* against *Anopheles gambiae* and *Culex quinquefasciatus* and found that this oil provided personal protection of 90% comparable to DEET.

*Kweka EJ, Munga S, Mahande AM, Msangi S, Mazigo HD, Adrias AQ, Matias JR. Protective efficacy of menthol propylene glycol carbonate compared to N, N-diethyl-methylbenzamide against mosquito bites in Northern Tanzania. Parasit Vectors. 2012 Sep 5;5:189.*

Citronella *Cymbopogon citratus* oils also have good repellent properties but they are not long lasting

*Artemisia vulgaris* contains insect repellents which can be released from the plant by combustion. When tested against *Aedes aegyptii*, myrcene, beta-pinene, limonene, 1,8-cineole were identified as repellents, but the strongest was terpin-4-ol. In the study of Roman Pavela described in the previous section for acute fumigant toxicity the most potent terpenes were alpha-terpinene, p-cymene, 1,8-cineole. These are present in *Artemisia annua*, a plant well known in China for its fumigant properties.

In South-America, Ethiopia, Kenya, China several trials have been run with the essential oil of *Eucalyptus maculata citriodora* alias *Corymbia citriodora* leaves and give excellent results equivalent to DEET

*Ephrem Abiy Teshome Gebre-Michael, Meshesha Balkew and Girmay Medhin. 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 2015;14:187 DOI: 10.1186/s12936-015-0705-4*

*N Hill, A Lenglet, M Arnez, I Carneiro, Plant based insect repellent and insecticide treated bed nets to protect against malaria in areas of early evening biting vectors: double blind randomised placebo controlled clinical trial in the Bolivian Amazon. BMJ, Aug 2007*

*Sarah J Moore, Samuel T Darling, Moisés Sihuíncha, Norma Padilla and Gregor J Devine. A low-cost repellent for malaria vectors in the Americas: results of two field trials in Guatemala and Peru. Malaria Journal 2007;6:101 DOI: 10.1186/1475-2875-6-101*

The essential oil of the lemon-scented gum mainly consists of citronellal (80%), produced largely in Brazil and China. While unrefined oil from the lemon eucalyptus tree is used in perfumery, a refined form of this oil is used in insect repellents. The refined oil's citronellal content is turned into cis- and trans- isomers of p-menthane-3,8-diol (PMD), a process which occurs naturally as the eucalyptus leaves age. This refined oil, which includes related

compounds from the essential corymbia citriodora, is known widely by its registered tradename, "Citriodiol", but also by generic names which vary by country or continent: "oil of lemon eucalyptus" or "OLE"(USA); "PMD rich botanic oil" or "PMDRBO"(Europe); "PMD and related oil of lemon eucalyptus compounds" (Canada) ; in Scandinavia « MyggA »; Extract of Lemon Eucalyptus in Australia. Pure PMD is synthesized for commercial production from synthetic citronellal.

The essential oil from *Hyptis suaveolens*, bush mint, is as efficient as DEET and provides long lasting repellency

*A.Z. Abagli and T.B.C. Alavo. Essential Oil from Bush Mint, Hyptis suaveolens, is as Effective as DEET for Personal Protection against Mosquito Bites. The Open Entomology Journal, 2011, 5, 45-48 45*

Strong repellent properties of piperitenone from *Mentha spicata* have also been noticed against *Anopheles stephensi* (AK Tripathi et al., 2004, 41. 691-698). This plant is growing abundantly in Burundi.

All the trials run with plant based topical repellents used in the early evening followed by the use of insecticide treated nets during the night give impressive results of 90% malaria reduction, compared to 40% with the use of bed nets only. The recommendation of the London School of Hygiene and Tropical Diseases strongly recommends that treated bed nets should not be used as the only means of preventing malaria in areas where vectors feed mainly in the evening.

Further field tests with these plants extracts should urgently be run. Native plants have the advantage of low cost and local availability and it is important for African countries to develop their own technology in this fight, rather than importing molecules like pyrethroids which initially were of African origin.