*SURE Rapid Response*

# Are herbal photolarvicides efficient and safe to use in vector management?

May 2011

This rapid response was prepared by the Uganda country node of the Regional East African Community Health (REACH) Policy Initiative.

## Key messages

- Larvicides cut both the number of mosquitoes and the prevalence of malaria, and using larvicides has been found to be viable and cost-effective costing about US$0.5 cents to protect a person for a year compared with US$2 for Insect Treated Nets although the two approaches should be used in complement of each other.

- Recently a mechanism using a natural plant extract (photoactive porphyrine derivative) and mosquito larvae attractant (autolized yeast) packaged under the name Sunlight- Active Formulated Extract (SAFE) has been proposed for testing and use in several of these countries including Uganda.

- A number of studies have identified several plant extracts that have photolarvicidal activity and these include dictamine, khelin, angelicin, 8-methoxypsoralen, α-hipericin, α-terthienyl from plant species including Rutaceae, Umbeliferae, Asteraceae, Leguminosae, Solanaceae, Hypericaceae

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SURE
Supporting the Use of Research Evidence

REACH
Regional East African Community Health Policy Initiative

EVIPNet
EVIDENCE-INFORMED POLICY NETWORK

### Who requested this rapid response?

This document was prepared in response to a specific question from a policy maker in Uganda.

### This rapid response includes:

- **Key findings** from research
- **Considerations about the relevance** of this research for health system decisions in Uganda

### Not included:

- Policy or practice related recommendations
- Detailed descriptions

### What is SURE Rapid Response Service?

SURE Rapid Responses address the needs of policymakers and managers for research evidence that has been appraised and contextualised in a matter of hours or days, if it is going to be of value to them. The Responses address questions about arrangements for organising, financing and governing health systems, and strategies for implementing changes.

### What is SURE?

**SURE** – Supporting the Use of Research Evidence (SURE) for policy in African health systems - is a collaborative project that builds on and supports the Evidence-Informed Policy Network **(EVIPNet) in** Africa and the Regional East African Community Health **(REACH)** Policy Initiative (see back page). SURE is funded by the European Commission's 7th Framework Programme.
www.evipnet.org/sure

### Glossary

of terms used in this report:
www.evipnet.org/sure/rr/glossary

# Background

The use of larvicides had died out in developing countries with the introduction of the pesticide Dichloro-Diphenyl-Trichloroethane commonly known as DDT. However following a report by researchers in Tanzania that found that killing mosquitoes before adulthood could cut malaria infection rates in urban areas drastically, there is renewed interest in this mechanism of fighting malaria and other vector borne diseases (1). Larvicides cut both the number of mosquitoes and the prevalence of malaria, and using larvicides has been found to be viable and cost-effective costing about US$0.5 cents to protect a person for a year compared with US$2 for Insect Treated Nets although the two approaches should be used in complement of each other (2). Larvicides come in different forms, for example, chemical larvicides as in the case of DDT, biological ones like those used in the Tanzanian studies referred to earlier, and others. They also have varying mechanisms of action. Of recent interest are those that are photoactive (they may be natural or synthetic). In comparison to the traditional organophosphate and carbamate pesticides, photoactivatable larvicides (and insecticides) have several favorable features (3); they have been reported to be inexpensive, effective and also environmentally safe. The main classes of photodynamic sensitizers that have been used as photoinsecticides are xanthenes, porphyrins, phenothiazines, furanocoumarins, acridines, thiophenes, and polyacetylenes (4). The World Health Organization, amongst others, has in recent years advocated for the provision of financial and technical resources to advance the development of innovative, alternative but safe methods to eradicate malaria (5), contributing to the stimulation of the investigation of insecticidal properties of plant-derived extracts, among others. Recent studies in this field have concluded that these plant derivative extracts are actually environmentally safe, degradable, and target specific (6). This has further stimulated more research to identify as many plant extracts that have larvicidal activity, as possible. Herbal or plant extracts that are biological, degradable and target-specific can be used to kill mosquito larvae using several mechanisms, a few of which are listed below (7-9).

- Anti-feedant- some plant extracts may be used as anti-feedants, meaning that they cause the larvae to cease eating. Eventually they would starve and die.
- Reduced fecundity and induced sterility- There are plant extracts that have been identified and found not to kill the larvae outright but instead cause significant changes to their reproductive systems. For example, Neem oil has been shown to reduce fecundity or fertility in the larva's developing reproductive system, meaning that they will have fewer offspring when they become adult mosquitoes. Some mosquitoes have also been found to have been sterilized after introduction of appropriate plant extracts and those next-generation larvae that did hatch having a low survival rate.
- Growth retardation and morphogenetic effects: Plant oils have been shown to introduce biological and genetic modifications to the developing larvae. This makes them more

susceptible to disease and reduces their vitality. Furthermore it interferes with their physical development and they are unable to grow to the adult stage. In fact allelochemicals that are released in many plants to repel other plants and insects are now extracted from the oils and have shown potential as growth regulators and mutators in many insects and larvae.

- Direct larvicidal activity: Some phytochemicals from plant extracts are used as larvicides. For example, one of the most effective is the extract of neem oil, known as azadiractin; another is Ipomoea, a commonly grown hanging plant that has demonstrated a 100% mortality rate in several types of mosquito larvae; what is found in the most successful larvicides is a high concentration of methanol and ethanol.

### How this Response was prepared

After clarifying the question being asked, we searched for systematic reviews, local or national evidence from Uganda, and other relevant research. The methods used by the SURE Rapid Response Service to find, select and assess research evidence are described here:

www.evipnet.org/sure/rr/methods

Many developing countries are also involved in the research for these novel but safe methods. Recently a mechanism using a natural plant extract (photoactive porphyrine derivative) and mosquito larvae attractant (autolized yeast) packaged under the name Sunlight- Active Formulated Extract (SAFE) has been proposed for testing and use in several of these countries including Uganda (10). This paper will highlight some of the work that has been done on photo (bio) larviciding, and the results that have been realised.

# Summary of findings

During a number of studies that were motivated by a need to find new types of pesticides that are both efficacious and safe, it emerged that several types of parasites are affected by compounds acting as photo-pesticides such as arthropods, caterpillars, fruit fly larvae, mosquitoes and mosquito larvae among others (11). It was noted that compounds capable of causing photodynamic action are produced by the natural defence mechanisms of plants and microorganisms against parasites. Several plants have been subjected to extraction procedures in the search for these new photo sensitizers and they include but are not limited to those shown in the table below.

**Table 1: Photoactive compounds produced by plants**

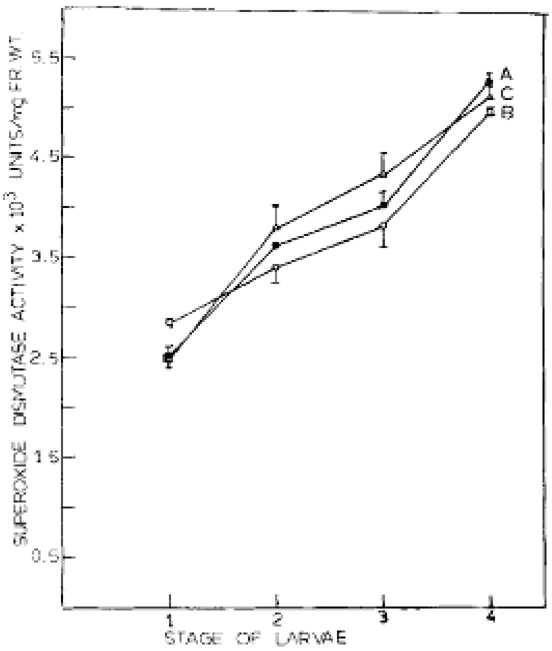
| Class of compounds | Substance | Bioactivity | Plant family |
|---|---|---|---|
| Alkaloids | Dictamine | Antifungal | Rutaceae |
| Furanochromones | Khelin | Antiviral<br>Bactericidal | Rutaceae<br>Umbelliferae |
| Coumarins | Angelicin<br><br>8-methoxypsoralen | **Larvicidal**<br><br>Antifungal | Asteraceae<br>Leguminosae<br>Solanaceae<br>Umbeliferae (Apiaceae) |
| Quinones | α-Hipericin | **Larvicidal**<br>Antiviral | Hypericaceae |
| Thiophenes | α-Terthienyl | **Larvicidal**<br>Insecticidal | Asteraceae |

*Adapted from Plant-derived antimycotics: current trends and future prospects(11)*

1. Asteraceae

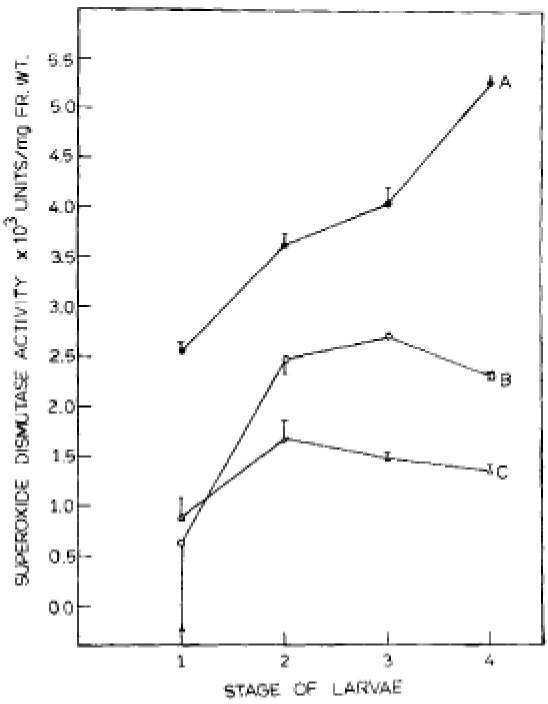
Many of the common plants of Asteraceae (Compositae) contain thiophenes, a group of biologically active secondary plant metabolites (12). These have been reported to be potent larvicides; for example α -terthienyl (terthiophene), a thiophene from marigold, has been shown to be effective against mosquito larvae, among other organisms and many other biomolecules including RNA and DNA are targets of this naturally occurring thiophene in presence of long-wave UV light (13). The toxicity of α-terthienyl increases when an organism treated with this compound is exposed to sunlight or long-wave ultraviolet light. For mosquito larvae, studies show that the thiophenes seem to affect superoxide dismutase, a chemical in their anal gills. A study done to establish this in the larvae of Aedes aegypti showed loss of the dismutase activity with the photosensitized excited state of α-terthienyl and the generation of a high flux of singlet oxygen appearing to be responsible for this inhibition (13). Graphs 1 and 2 below represent the findings from this study. These findings are in agreement with those from Arnason et al.'s study in which extracts from Asteraceae were screened for their near-UV-mediated larvicidal properties to the mosquito Aedes, aegypti (14). The effects of the radiation dose on larval survival were studied and the action spectra for the phototoxic effects of compounds on the larvae were obtained. These revealed the potential of these polyacetylene extracts as larvicides.

Graphs 1




**Graph 1**
A. Usual Superoxide dismutase in the anal gills of mosquito larvae
B. Effect of UV light
C. Effect of α-terthienyl in the dark

Graph 2




*Graphs adapted from Nivsarkar et al. (13)*

**Graph 2**
A. Superoxide dismutase in the anal gills of mosquito larvae in all four instars
B. Inhibition of superoxide dismutase by α-terthienyl in the presence of ultraviolet light in vivo
C. Inhibition of superoxide dismutase by α-terthienyl in the presence of ultraviolet light in vitro (C).

2. Apiaceae (Umbeliferae)

In a study carried out to find out the larvicidal, growth disruption and behavioural effects of the ethanol extract of the seed of celery, *A. graveolens* against dengue fever mosquito, *Aedes aegypti*, larvicidal activity of celery seed extract in ethanol against fourth instar *A. aegypti* larvae was demonstrated as is shown in the table below (15).

**Table 2: A potent mosquito larvicidal effect due to the toxicity of the phototoxin enriched ethanol extract derived from the seed of A. graveolens.**

| Exposure periods (hrs) | Test Conc. in ppm | % Larval mortality | Average Lethal Cconcentration$_{50}$ ($LC_{50}$) |
|---|---|---|---|
| 24 | 100 | 28 | 126.13 |
| | 200 | 88 | |
| | 300 | 99 | |
| | Control 1 (DW+DMSO) | 0 | |
| | Control 2 (DW) | 0 | |
| 48 | 100 | 37 | 112.53 |
| | 200 | 96 | |
| | 300 | 99 | |
| | Control 1 (DW+DMSO) | 0 | |
| | Control 2 (DW) | 0 | |
| The values above are based on three concentrations (100, 200, 300ppm) and five replications with 20 *A. aegypti* (4$^{th}$ stage) larvae each | | | |

*Table adapted from Khondkar Ehteshamul Kabir et al. (15)*

The susceptibility of the larvae to various concentrations of the seed extract was concentration and time-dependent. This study was also able to suggest mechanisms that these compounds use on the larvicides and other target organisms. These included the fact that the photoactivated cytotoxicity of some of the compounds in the extracts on treated fourth instar larvae possibly generated from the neuromuscular disturbance and subsequent cytological degenerations in the electrolytes control mechanisms located in the anal papillae of the mosquito larvae, probably further led to an interruption of the osmotic and ionic regulation and may be this phenomenon was intrinsically associated with the death of the mosquito larvae.

Findings from this study are in keeping with those from a study done by Kalu and colleagues in an attempt to find new larvicidal products from the extracts of garlic plants to control the filarial vector *Culex quinquefasciatus (16)*. The study which used standard World Health

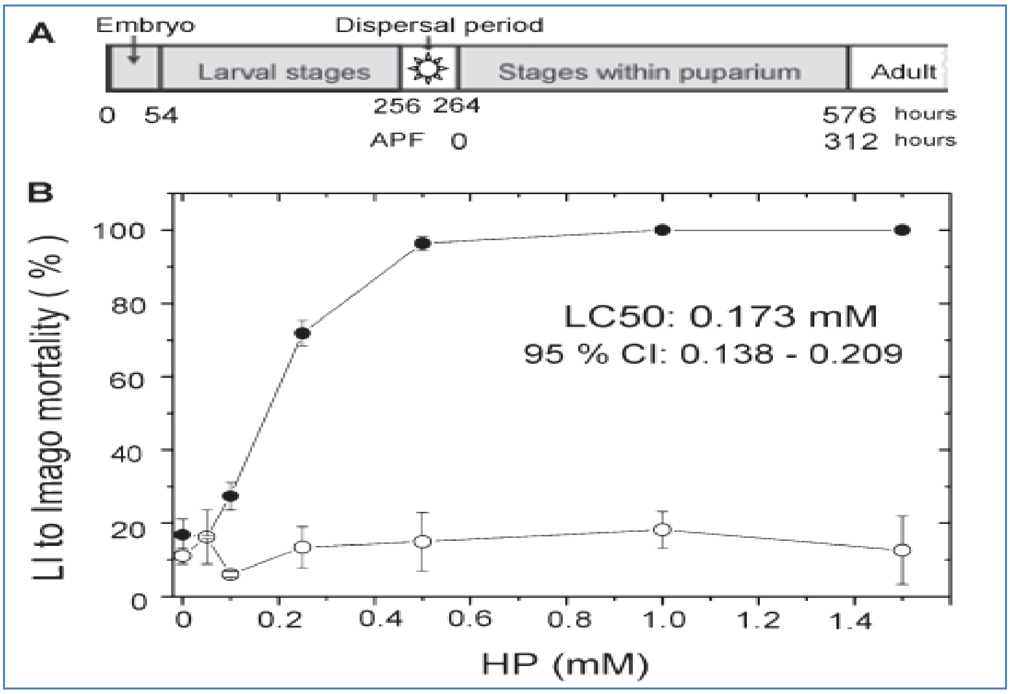
Organization (WHO) methods (17) noted toxicity of garlic bulb extracts to the second, third and fourth instar larvae of *C. quinquefasciatus* mosquitoes. Average lethal concentration $LC_{50}$ (ppm) was 144.54, 165.70, 184.18 for second, third and fourth instars respectively showing very effective larvicidal activity.

Similarly the larvicidal efficacy of the leaf extract of *C. pubescens* against late third instar larvae of *Anopheles stephensi*, *C. Quinquefasciatus* and *Aedes aegypti* have been reported as have the use of garlic and lemon peel extracts as *Culex pipens* larvacides in which the interaction and persistence of the extracts with the organophosphate resistance mechanism (18, 19). Despite these latter studies being similar to the former, they did not test for photoactivity.

3. Porphyrins and hematoporphyrins

In a study in which immature stages of *Ceratitis capitata* were tested as a model for hematoporphyrin IX (HP IX) phototoxicity, a direct correlation between light-dependent HP IX mortality, evidence of reactive oxygen species (ROS) and lipid peroxidation (conjugated dienes and thiobarbituric acid reactive substances) was established in *C. capitata* larvae (20). HP IX is a porphyrin; porphyrins are organic molecules that are structurally very similar to both chlorophyll in plants and hemoglobin in animal blood. They are classified as tetrapyrrole compounds and often contain metals such as nickel and vanadium. This study confirmed HP IX as a strong and fast photolarvicide against *C. capitata*.

**Figure 2: Ceratitis capitata mortality during post embryonic development until adult ecdysis.**



A: Experimental design indicating the span of the different stages and the 8 hr dispersal period subjected to light exposure.

B: Hematoporphyrin (HP) IX-dependent mortality. Black circles indicate larvae subjected to direct white light while open circles represent insects kept in the dark.

*Figure adapted from Pujol-Lereis et al. (20)*

The findings in this study tally with those from another study in which the authors chose the hematoporphyrin dimethyl ether (HPde) as a representative of dicarboxylic porphyrins (21). They found this was more effective and chemically homogeneous than the clinically established agent photofrin. HP was found to be highly toxic for the aquatic larvae of mosquitoes; when 0.1 mM HP IX was applied to the fourth instar of C. pipiens, 100% mortality was recorded after 30 min of light exposure.

4. Hypericaceae

*Chrysolina* spp. (Coleoptera: Chrysomelidae) larvae have been shown to be susceptible to phototoxicity of the *Hypericum perforatum* spp. (L. Hypericaceae). These larvae, to avoid death, have learnt to feed on it at dawn and hide during the day so as to avoid the lethal sun activation (11, 22). Similarly the *Chrysolina* adults avoid phototoxicity by the presence of opaque cuticles that block the sunlight. Furthermore it has been shown that first-instar larvae of *Anaitis plagiata* (L.) (Lepidoptera: Geometridae) avoid feeding on the glands that contain the phototoxin. Later instar larvae feed on the entire leaf, but are not susceptible to phototoxicity, indicating that they have biochemical defenses against photo-induced damage.

5. K-01

A photolarvicide has been identified under the laboratory name K-01. In a study to evaluate its toxicity to the larvae of Aedes albopictus, it was noted that it is an effective photoactivated larvicide and that the maximum larvae-killing effect is achieved with 50mg/ml of it applied under sunlight (23). 100% mortality of larvae is achieved within 24hrs of its application. There is limitation to information about K-01 as much of the work done on it and publications about it are limited to the Chinese language.

Other larvicides with photoactivation potential still under study include:

6. Azadirachtin (Neem tree/Neem oil)

The neem tree *Azadirachta indica,* is in the *Meliaceae* plant family which is known to have a variety of compounds that show insecticidal, antifeedant, growth-regulating, and development-modifying properties (24). A recent study was conducted to study the effect of

Azadirachtin, a neem tree extract, against larvae and pupae of *Culex pipiens* mosquitoes in east of the Republic of Algeria (25). An insecticide containing azadirachtin, was tested against *Culex pipiens* mosquito larvae and under laboratory conditions. The results showed markedly reduced adult mosquito fecundity and increased sterility was after treatment of the fourth instar and pupal stage. Furthermore the treatment prolonged the duration of the larval stage. The results show that the Azadirachtin is promising as a larvicidal agent against *Culex pipiens*. A similar study was done in Iran, but this time both efficacy and efficiency were noted, that is, it was tested in both laboratory and field conditions (26). Although mortality was noted in both larval and pupal stages, that in the pupal stage was significantly higher. In field trials, mortality of Anopheles spp. larvae was also higher than Culex spp. This compound proved useful especially in the prevention of adult mosquitoes emerging and the maximum time of efficacy was 7 days at the highest concentration of larvicide.

7. Eucalyptus extract

Eucalyptus plants especially E. globulus are used for many functions including anesthetic, anodyne, antiseptic, astringent, deodorant, diaphoretic, disinfectant, expectorant, among others. They are also a folk remedy for several ailments including abscess, arthritis, asthma, burns, cancer, diabetes, diphtheria, dysentery, encephalitis, enteritis, erysipelas, fever, flu, inflammation, laryngalgia, laryngitis, leprosy, malaria (27). It is also popular in veterinary practice, where Eucalyptus oil is administered to horses in influenza, to dogs in distemper, to all animals in septicaemia. The most important constituent is Eucalyptol which makes up, up to 70% of *E. globulus*. A study done to test the effect of crude extract of *Eucalyptus* oil on the larvae of *Culex* mosquito, found the mortality of the larvae exposed to the extract was significantly higher than the control with distilled water and that the larval mortality increased with dose and time (28). Several parts were tested but seed extract appeared most lethal. This agreed with earlier work done by Brooker and Kleinig (29).

## Conclusion

This paper has shown from the literature that several photolarvicides gotten from plant extracts do exist and have been identified. It also notes this process is on-going having been stimulated by the recent renewed interest in these as inexpensive, safe and efficient methods of fighting vector borne diseases.

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**Conflicts of interest**

None known.

**This Rapid Response should be cited as**

Rhona Mijumbi, MPH, MSc; Anthony Batte MBChB. **Are herbal photolarvicides efficient and safe to use in vector management?** A SURE Rapid Response. May 2011.

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