# Anophelinocidal Activity of Volatile Oil from *Tagetes minuta* L. (Asteraceae).

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Key words: Anophelinocidal activity - Flower extract - Volatile oil - *Tagetes minuta* - *Republic Democratic of Congo.*


## Summary

Tagetes minuta, *a common weed found throughout Republic Democratic of Congo is used by native population to drive away insects. Volatile oil from flowers extract is endowed with anophelinocidal activity which remains for about seven days. The LD90 and LD50 of the crude extract are respectively of 75 mg and 45.6 mg/ml.*

## Résumé

Tagetes minuta, *une herbe très répandue en République démocratique du Congo, est utilisée par la population locale pour chasser les insectes. L'huile volatile extraite de ses fleurs est dotée d'un pouvoir anophelinocidal qui agit pendant près de sept jours. Les LD90 et LD50 de l'extrait brut sont respectivement de 75 mg et 45,6 mg/ml.*


## Introduction

An entomological survey conducted at Lwiro (Kivu province, Eastern Republic Democratic of Congo, 1750 m of altitude) from 1988 to 1992 allowed to identify six species of the genus *Anopheles: A. gambiae, A. funestus, A. marshalli, A. demeillioni, A. coustani and A. christyi.* The two first species attain a prevalence rate of 97.5 % and are the major vectors of malaria in the study area where they are present throughout the year (1,7).

Malaria infection is a real problem for the public health in the tropics. It is the most devastating of the tropical diseases widespread in about 100 countries in which 270 millions of persons are infected and 2 millions die annually (7). In Republic Democratic of Congo, the malaria infection is endemic but the degree of endemicity varies from one region to another: at Lwiro is a mesoendemicity level of malaria according to the international classification observed (10), which is characterized by a parasite rate range between 11 % and 50 %.

The hypoendemic situation observed between 1960 and 1980 (parasite rate less than 10 %) has unfortunately evolved to a mesoendemic level and infants which age varied between 1 to 4 years are the most struck by the malaria infection due to the presence and predominance of *Plasmodium falciparum* (96.37 % among the positive cases), known to be the most human mortal plasmodial species (1). At Lwiro, malaria is the main cause of consultation where 20 % of hospitalization cases are caused by malaria infection among which a rate of 8% of deaths are observed annually (3).

To solve this problem integrated curative and preventive measures are needed. Moreover, plasmodium strains show resistance to common drugs such as chloroquine (16) and vectors are also resistant to insecticides which are considered as pollutants. Thus, one of the advisable methods of prevention of malaria infection consists in the reduction of manmosquito contact by using substances able to kill or to drive away vectors (7). Several of these substances are from plant origin (5, 6, 9 ).

With the aim of searching new, cheap and efficient drugs endowed with mortal activity against mosquitoes, a biological screening was undertaken on the chloroformic extract of flowers of *Tagetes minuta* L. (Asteraceae).

The present laboratory work deals with the in vitro anophelinocidal activity of the aforementioned plant.

*Tagetes minuta* L. is a weed of Bresilian origin introduced in Republic Democratic of Congo where it is now often seen, growing in uncultivated areas which are kept as fallow. Its aerial parts are used by native population for driving away insects (ants) out of dwelling-houses and are used for crop devastators control. In Malagassy, traditional healers prescribe the flowers decoction to treat orally splenomegaly and hepatic disorders due to malarial infection (2). It is also used in South Africa as parasiticide for cattles. *Tagetes* spp. exhale a characteristic odour due to their content in various oily and terpenic components. In fact, in previous works, it has been demonstrated that the *Tagetes* volatile oil contains carvone, linalol, limonene, ocimene, ß-myrcenon, aromaden-dron, α-terthienyl, quercetagetrin, flavonoids and phenols (12, 13, 14). Biopharmacological activity of *Tagetes minuta* volatile oil has been investigated. It has hypotensive, bronchodilatory, spasmolytic, digestive, vermifuge, cholagogue, sedative in gastric pain antiabortifacient and anti-inflammatory activities (2, 4).

## Material and Methods

### 1. Plant material and preparation of extract

Plant samples of *Tagetes minuta* L. (Asteraceae) were collected at Lwiro Station (Kivu province, Eastern Republic Democratic of Congo, 1750 m of altitude) in August 1993. The plant was identified by reference to the Herbarium of the Botany Laboratory and voucher specimens are preserved in the Laboratory of Medicinal Plants, Department of Biology at Lwiro, Republic Democratic of Congo.

Twenty gram of dried flowers were submitted without grinding to steam distillation (8). The volatile oil was collected under refrigeration in a glass beaker containing cold water The supernatant oil was then exhaustively extracted with chloroform and separated from the aqueous phase by decantation. The chloroformic phase was evaporated to dryness and a yellow oily residue was obtained (yield: 5 %).

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Serial dilutions in chloroform were prepared: 100, 75, 50, 25, 10, 5 and 1 mg/ml, afterwards chloroform was evaporated by a spin dryer

**2. Evaluation of the anophelinocidal activity**

Adult female mosquitoes of both malaria vector species (*A. gambiae* and *A. funestus)* were collected early in the morning between 5 and 6 H inside the dwelling houses under torch light at Lwiro Station. The identification of specimen was made by one of the authors (K. B.) by referring to the entomological collection of the Medical Entomology Laboratory where voucher specimens are preserved at Lwiro.

Transparent cylindric tubes of 125 mm in length and 44 mm in diameter (WHO label) were used for exposure (15). Fifteen adult mosquitoes were exposed into such a tube where was set out a piece of Schleicher & Schull filter paper n° 589 (30 $cm^2$) impregnated with the prepared dose. The exposure time was one hour and afterwards mosquitoes were transferred to another tube containing a not impregnated paper and kept during 24 hours for observation in a shaded place. Mosquitoes were fed on a cotton saturated with sweetened water. Mortality counts were done at the end of the observation time and affected mosquitoes unable to walk were taken into account as recommended by WHO's procedure (15). Each test was repeated four times with a control test using a tube where impregnated paper was replaced by a sheet of clean filter paper. When control mortality rate was ranged between 5 and 20 %, the percentage of test mortality was corrected by Abbott's formula ( 11).

$$\% \text{ Mortality corrected} = \frac{\% \text{ test mortality} - \% \text{ control mortality}}{100 - \% \text{ control mortality}} \times 100$$

Test for which the mortality rate was more than 20 % was omitted and the test re-evaluated.

## Results and Discussion

Anophelinocidal activity of the volatile oil extracted from *Tagetes minuta* flowers are shown in Table 1. The volatile oil obtained by steam distillation has a real killing effect on adult mosquitoes caught inside the dwelling houses (*A. gambiae* and *A. funestus).*

The LD90, LD50 and LDOO are respectively 75 mg, 45.6 mg and 5 mg/ml. The LD50 was calculated by the regression line Y = 11.45 x + 2.92, established by the results obtained after exposure. Trials in the laboratory conditions are in progress to evaluate the degree of the volatile oil, to determine the lethal dose depending - time, to isolate and to purify the active principles. However, in the nearest future it is intended to propose ointments and serpentine smoke producing devices from oil of the studied plant with aim at protecting people against mosquito stings.

**Table 1 : Effect of *Tagetes minuta* volatile oil on anophelins Mortality rate after one hour exposure and 24 hours of recovery time.**

| Concentration mg/ml | Test mortality rate | Control mortality rate | Corrected test mortality rate |
|---|---|---|---|
| 1 | 6.67 | 8.33 | 0.00 |
| 5 | 10.00 | 8.33 | 1.82 |
| 10 | 18.33 | 10.00 | 9.26 |
| 25 | 18.33 | 10.00 | 9.26 |
| 30 | 36.67 | 18.33 | 22.46 |
| 40 | 56.67 | 15.00 | 49.02 |
| 50 | 81.67 | 13.33 | 78.93 |
| 75 | 88.33 | 3.33 | 88.33 |
| 100 | 100.00 | 10.00 | 100.00 |

## Literature


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