

## SCIENTIFIC NOTE

### Evaluation of the Potential Insecticide Activity of *Tagetes minuta* (Asteraceae) Essential Oil Against the Head Lice *Pediculus humanus capitis* (Phthiraptera: Pediculidae)

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Avaliação da Atividade Potencial Inseticida de Óleo Essencial de *Tagetes minuta* (Asteraceae) Contra *Pediculus humanus capitis* De Geer (Phthiraptera: Pediculidae)

**ABSTRACT** - Pediculosis is a worldwide head infestation caused by *Pediculus humanus capitis* De Geer. Resistance to chemical active ingredients shows the importance of new approaches for head lice control. The aim of the present work was to evaluate the potential insecticide activity of the medicinal plant *Tagetes minuta* (Asteraceae) against *P. humanus capitis* as well as to investigate histopathological aspects to find the target sites of the botanical extract in the insect. Lice were collected from heads of infested children 6-12 years old from Marília, São Paulo State, Brazil. All the procedures from the collection to the insecticide evaluation were done according to the World Health Organization protocols. Batches of 10 lice each were released onto a clean piece of filter paper in a petri dish. A 100 ppm concentration of essential oil impregnated filter papers were made by dropping 0.1 ml of *T. minuta* essential oil. Petri dishes were placed in a rearing chamber (30°C and 70% relative humidity) in the dark. Eight replicates were used for treatment and two replicates for control batches. The Median Lethal Time (LT<sub>50</sub>) was 16.4 ± 1.62 min. For histopathological investigation, twenty treated and untreated lice were fixed in 10% solution of formalin for 24h and processed according to classical histology techniques. Examined cross sections of treated adults showed disassembly of actin and myosin filaments arrangement when compared to untreated adults. This aspect allied to the LT<sub>50</sub> obtained suggests a toxic action of the *T. minuta* essential oil.

**KEY WORDS:** Insecta, pediculosis, botanical extract

**RESUMO** - De distribuição mundial, a pediculose é uma infestação na cabeça causada pelo piolho *Pediculus humanus capitis* De Geer. A resistência já verificada a diversos ingredientes ativos químicos demonstra a importância de serem avaliadas novas alternativas para o controle de piolhos. O objetivo do presente trabalho foi avaliar o potencial inseticida da planta medicinal *Tagetes minuta* (Asteraceae), conhecida como cravinho-de-defunto, contra *P. humanus capitis*, assim como investigar aspectos histopatológicos para se encontrarem os sítios de ação no inseto. Os piolhos foram coletados de crianças infestadas, de 6 a 12 anos de idade, no município de Marília, SP. Todos os procedimentos desde a coleta até a avaliação da atividade inseticida foram feitos de acordo com os protocolos da Organização Mundial de Saúde. Grupos de 10 piolhos foram colocados sobre círculos de papel de filtro, no fundo de placas de Petri. Os círculos foram impregnados com o óleo essencial de *T. minuta* gotejando 0,1 ml de solução do extrato bruto obtendo-se a concentração de 100 ppm do extrato bruto. As placas foram colocadas em estufas de criação (30 °C e 70% UR) no escuro. Oito repetições foram feitas para o tratamento e duas repetições para o controle. O tempo letal mediano para a concentração de 100 ppm do extrato bruto obtido foi de 16,4 ± 1,62 min. Para a investigação histológica, vinte piolhos tratados e não tratados foram fixados em formol 10% por 24h e processados pelas técnicas clássicas de histologia. O exame dos cortes revelou desarranjo dos filamentos de actina e miosina nos indivíduos tratados. Este aspecto aliado ao TL<sub>50</sub> obtido sugerem uma ação tóxica do óleo essencial de *T. minuta*.

**PALAVRAS-CHAVE:** Insecta, pediculose, piolho, extrato vegetal, cravinho-de-defunto

Pediculosis is a skin infestation caused by hematophagous lice, the most common of which is the head louse, *Pediculus humanus capitis* De Geer (= *Pediculus capitis* De Geer). Infestations of human lice are prevalent worldwide, especially among schoolchildren in both developed and developing countries (Gratz 1997). According to Meinking *et al.* (2002) the number of cases of head and body lice infestation has been estimated to be higher than 100 million. Although being not a major health hazard or disease vector, head lice can cause considerable distress due to scalp erythema, itching and potential secondary infections.

In order to produce a viable strategy for the future control of head lice, a wider range of alternatives should be evaluated. Botanical extracts may offer new options for head lice control. Extracts of *Tagetes minuta* (Asteraceae) is an option because *Tagetes* spp. have been shown effective against many microbial agents such as fungus (Bii *et al.* 2000), virus (Abad *et al.* 1999) and gram positive and negative bacteria (Tereschuk *et al.* 2003). Brazil is a major producer of *T. minuta* according to Craveiro *et al.* (1988).

The aim of the present work was to evaluate the potential insecticide activity of the medicinal plant *T. minuta* against *P. humanus capitis* as well as to investigate histopathological aspects to find the target sites of the botanical extract in the insect.

Lice were collected from heads of infested children 6-12 years old from nurseries and day care institutions in different areas of Marília, São Paulo State, Brazil. The insects were collected by combing the children scalps and transferred immediately to a plastic pill box containing a few strands of human hair. Head lice collected within a 2h period were transported to the laboratory. The lice were examined and damaged specimens were discarded according to WHO (1981). All lice used in bioassay were tested within 3h after collection and were protected from sunlight and heat.

Batches of 10 lice each were released onto a clean piece of Whatman n° 1 filter paper (7 cm diameter) that had been placed at the bottom of a petri dish. The petri dishes were placed in a rearing chamber (30°C and 70% relative humidity) in the dark.

For extract preparation the aerial parts of *T. minuta* were air-dried under the shade, ground in a knife mill and extracted by rotary evaporation under increasing temperature over 100°C. The resulting crude extract (essential oil) was stored in the dark.

The concentration of 100 ppm of the essential oil was prepared by dropping 0.1 ml of *T. minuta* essential oil on the filter papers. Adult lice of unknown age and sex were used to establish the  $LT_{50}$ . Eight replicates were used for treatment and two replicates for control batches. The bioassay was conducted at 30°C and 70% relative humidity. Mortality was established as the inability of the lice to walk on the filter paper.

Insecticide activity of *T. minuta* essential oil against *P. humanus capitis* was evaluated by estimating the Median Lethal Time ( $LT_{50}$ ) with a linear regression model (Statistica® Software).

For histopathological investigation, twenty treated and untreated lice were fixed in 10% solution of formalin for 24h,

dehydrated in increasing alcohol concentrations, cleared in xylol and embedded in paraffin. Five micrometer-thick sections were stained with Ehrlich's haematoxylin and eosin.

The Median Lethal Time estimated was  $16.4 \pm 1.62$  min (100 ppm of essential oil) (line equation:  $y = -76.124 + 7.674x$ ; 5 degrees of freedom) suggests a toxic action of the essential oil as demonstrated by histological analysis. The use of the concentration of 100 ppm of essential oil of *T. minuta* is according to the median lethal concentrations reported for mosquitoes studies (Green *et al.* 1991, Macedo *et al.* 1997).

Examined cross sections of treated adults showed disassembly of actin and myosin filaments and consequent fiber dissociation when compared to untreated adults (Fig. 1). In fact, *T. minuta* is rich in many secondary compounds including monoterpenes, sesquiterpenes, flavonoids and thiophenes (Zygadlo *et al.* 1990, Garcia *et al.* 1995). Oil components could have partitioned into the lipid bilayer,

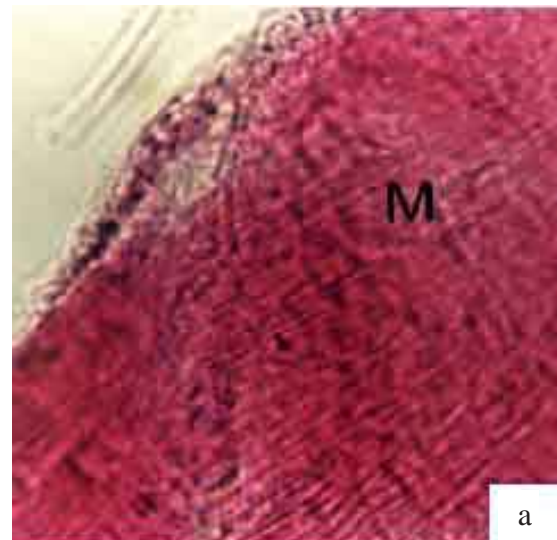


Figure 1. Cross sections of adults lice untreated (a) and treated (b) with *T. minuta* oil showing disassembly of muscular filaments (M). (a): 400 x ; (b): 200x

increasing membrane fluidity and permeability of muscular cells causing movement reduction of the legs and antennae and death. According to Perich *et al.* (1995) the *T. minuta* oil essential terpenes are responsible for the toxic effects reported in mosquitoes. The examination of cross sections of treated adults showed no histological alteration in any other tissue. The most distinctive symptom of treated adults was a gradual movement reduction of legs and antennae even under stimulation.

Insecticide activity of *Tagetes* species has been reported against stored products pests (Keita *et al.* 2000, Sarin 2004) and mosquitoes (Green *et al.* 1991, Saxena *et al.* 1992, Macedo *et al.* 1997, Seyoun *et al.* 2002). The results obtained in the present work increase the range of insect pests that could be controlled by *Tagetes* spp. extracts.

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