

***In Vitro* Efficacy of Four Insecticides Against Eggs of *Tunga penetrans* (Siphonaptera)**

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Abstract: Systematic assessments of control measures against the jigger flea *Tunga penetrans* are scarce, and there are no published data available on the efficacy of environmental insecticides against immature stages. We tested four environmental contact insecticides used by Brazilian authorities for disease control (deltamethrin, bifenthrin, dichlorvos and etofenprox) against *T. penetrans* eggs. Eggs were reared *in vitro*. Hatch rates were observed under standardized conditions and compared to a control group (40 eggs in each group). No larvae hatched after treatment with the organophosphate dichlorvos (100% efficacy). The efficacies of the other products tested varied between 17% and 57%. The data show that the organophosphate dichlorvos had a good *in vitro* efficacy. The use of dichlorvos can be directed to typical spots where early stages of *T. penetrans* are expected, considering its toxicity. Disease control should also consist of prevention measures concerning housing and environmental conditions, veterinary and human health measures.

Keywords: chigoe flea, efficacy, insecticides, prevention, *Tunga penetrans*.

INTRODUCTION

Infestation with the jigger flea *Tunga penetrans* (tungiasis) is a Neglected Tropical Disease causing substantial health burden in endemic areas [1]. Though affecting many people in resource-poor communities in sub-Saharan Africa, the Caribbean and Latin America, systematic evaluations of control measures against tungiasis are scarce [2-4].

Control of tungiasis is complex and would need an interdisciplinary approach [5]. The prevalence in humans in endemic regions can easily reach more than 50% and cause considerable morbidity [6-12]. Domestic animals such as pigs and dogs are the main animal reservoirs, and control measures thus would need to consider domestic animals [4, 5, 13]. In addition, immature stages are found in the environment where they may survive for a prolonged period

[14]. For emergency control measures, eggs may be treated with environmental insecticides, but there are no efficacy data available on these insecticides against immature stages of *T. penetrans*. For example, in Rio Grande do Sul State in the extreme south of Brazil, the State Health Secretariat regularly sprays compounds of affected houses with alphacypermethrin without any evidence.

To provide evidence for control measures, we tested four insecticides used in disease control programs in Brazil against *T. penetrans* eggs. Eggs were reared *in vitro*, and hatch rates were observed under standardized conditions.

MATERIALS AND METHODS

The study was carried out in the city of Uberlândia (Minas Gerais State), situated in Brazil's savannah region. To obtain fertile *T. penetrans* eggs, laboratory-raised Wistar rats were exposed according to procedures described previously on compounds in the outskirts of Uberlândia where tungiasis occurs [15-17].

From September to October 2010, a total of 25 rats were exposed at 11 different locations. Two in two days, the infestation status and general status of animals were

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assessed. The rats were transported after 5 to 7 days to the laboratory of the Federal University of Uberlândia for a thorough examination. The lesions were counted and classified according to the Fortaleza Classification [18]. Infested rats with the embedded fleas expelling eggs (stage 3 according to Fortaleza Classification; Fig. 1) were held over black cardboard for 3 to 5 hours to facilitate detection and collection of deposited eggs. Eggs were examined for physical integrity, counted and transferred to Petri dishes.



Fig. (1). Rat infested with *T. penetrans* (stage III according to Fortaleza Classification).

Environmental insecticides tested are given in Table 1. All insecticides are registered by the Brazilian Ministry of Health for peridomestic and environmental spraying. The concentration was chosen as recommended in the product information for the control of small insects (Table 1). These concentrations are also used by the centers for zoonosis control throughout Brazil. In each insecticide and control group, 40 eggs were tested. Batches of eggs were pooled and then randomized into one of the four treatment or control groups. 175 μ l of the insecticide were applied from 5 cm distance using a standardized hand pump spray bottle. That equals an application of about 0.022 l/m². In the control group tap water was used.

The Petri dishes were placed in a polystyrene box and incubated at environmental temperature (24.7-27.8°C). To establish consistent humidity, damp cloth was placed inside the boxes. Relative humidity varied from 50.4% to 71%. After 3, 5 and 7 days the eggs were examined for hatching, and larvae counted. All procedures and examinations were

conducted by a single observer to prevent inter-observer variation.

Data were entered using Excel spread sheets and checked for entry errors. Corrected hatch rates were calculated as: (crude hatch rate in test group) / (crude hatch rate in untreated control group). Efficacy of a product was defined as: (1 - corrected hatch rate). Negative values (hatch rate in intervention group higher than in control group) were considered as 0% efficacy. 95% confidence intervals for efficacy were calculated according to an asymptotic formula, as described by Rosenheim and Hoy [19]. Calculated values below 0% or above 100% in confidence intervals were set to 0% or 100%, respectively. Relative frequencies between groups were compared applying Fisher's exact test to evaluate statistical significance. Analysis was done using SAS (version 9.2, SAS Institute Inc., Cary, USA).

The study was approved by the Ethical Review Board for Animals (*Comissão de Ética na Utilização de Animais - CEUA*) of the Universidade Federal de Uberlândia, protocol no. CEUA/UFU 092/10.

RESULTS

The 25 rats were exposed to households expected to be infested with the jigger fleas twice or three times. In total, data of 67 exposures were collected. In eight cases (11.9%), tungiasis lesions were found. The number of lesions per rat ranged between 1 and 18 (mean: 8.5).

The numbers of hatched eggs and efficacies of the tested insecticides are shown in Table 2. No larvae hatched after treatment with the organophosphate dichlorvos. The efficacies of the other products tested varied between 17% and 57%. Dichlorvos showed significantly higher efficacy than the other insecticides tested ($p < 0.0001$). On day three, 21/29 (72%) larvae in the deltamethrin group showed spontaneous movement. No other larvae in the intervention groups showed vital signs. In the control group, all 35 hatched larvae were fully active.

DISCUSSION

This is the first systematic study on the efficacy of insecticides against immature stages of *T. penetrans*. The data show that at a concentration commonly used by Brazilian control programs, only the organophosphate dichlorvos had an acceptable ovicidal efficacy. As with the exception of deltamethrin, hatched larvae did not show vital signs for the other products, a residual larvicidal effect is possible.

The use of insecticides has been considered one of the means in an integrated approach to control tungiasis in affected regions [5]. In this context, environmental

Table 1. Details of Insecticides Tested Against Eggs of *Tunga penetrans*

Product	Producer	Active Ingredient	Tested Concentration
Deltametrina 25 ce	Fersol, Mairinque, SP, Brazil	25g/L Deltamethrin (type II Pyrethroid)	0.25g/L (5.5 mg/m ²)
Bifentol 200 sc	ChemoNE, Bezerros, PE, Brazil	200g/L Bifenthrin (type I Pyrethroid)	0.6g/L (13.2 mg/m ²)
DDVP 1000 ce	Fersol, Mairinque, SP, Brazil	825 g/L Dichlorvos	4.125g/L (90.75 mg/m ²)
Vectron 10 sc	Iharabras SA, Sorocaba, SP, Brazil	100 g/L Etofenprox	2 g/L (44 mg/m ²)

Table 2. Efficacy of Four Insecticides Tested Against Eggs of *Tunga penetrans*

Insecticide	Number of Eggs	Number of Larvae (= Hatched Eggs)	Crude Hatch Rate	Corrected Hatch Rate	Efficacy (95% Confidence Interval)
Dichlorvos	40	0	0%	0%	100% (92.1-100)
Etofenprox	40	15	37.5%	42.9%	57.1% (29.3-75.1)
Bifenthrin	40	18	45.0%	51.4%	48.6% (30.0-67.2)
Deltamethrin	40	29	72.5%	82.9%	17.1% 0-35.7
Control	40	35	87.5%	100%	0% (0-16.6)

insecticide spraying should be considered an emergency intervention and would need integration with other more sustainable measures. In fact, recent studies have shown that intermittent application of a plant-based repellent reduced morbidity significantly, but that transmission and subsequently morbidity increased rapidly after interruption of this intervention [20]. Control ideally should consist of individual therapeutic measures, and interventions concerning improved housing and environmental conditions, and veterinary measures to reduce animal reservoirs such as cats, dogs, pigs and rats [4, 21]. As off-host life stages of *T. penetrans* can be found indoors and/or outdoors (which may vary from setting to setting), the use of an efficacious insecticide such as dichlorvos may be performed in addition to these control measures, directed to typical areas where early stages are expected [14, 22, 23].

One matter of concern is the mammalian toxicity of dichlorvos, an organophosphate that is absorbed through skin, gastrointestinal tract and the respiratory system. The insecticide is highly volatile with a vapor pressure of 0.012 mmHg at 20°C [24], thus reasonable precautions have to be taken when used indoors. Organophosphates irreversibly inactivate the acetylcholine esterase. Overexposure may cause a variety of symptoms, according to the route of exposure. Whereas inhalation may cause ocular and respiratory symptoms, ingestion causes gastrointestinal discomfort. Dichlorvos may also lead to allergic contact dermatitis. In severe cases, paralysis of respiratory muscles can lead to death. Low-dose long-term effects are still discussed due to uncertainties in the study designs concerning dose and insecticide mixture [25].

Synthetic pyrethroids (deltamethrin, bifenthrin) are usually considered less toxic, but were less efficacious against eggs in our study. They block the voltage-dependent sodium channel resulting in a stable hyperexcitable state of tissues. Symptoms of acute pyrethroid intoxication include paraesthesia, nausea, headache, muscle fasciculation, dizziness, fatigue and convulsions. Only a few studies have been conducted concerning long-term toxicity of pyrethroids. The results support that there are limited effects in humans exposed to pyrethroids [26, 27].

Etofenprox is a non-ester synthetic pyrethroid with a similar mode of action. Yet its toxicity to humans and mammals is lower [28]. The main targets of acute intoxication are liver, kidney, thyroid and the hematopoietic system. The International Programme on Chemical Safety found no evidence for geno-toxicity [29]. The USEPA classified etofenprox as a possible human carcinogen. Mid-

and long term data as to the chronic impact on humans still need to be evaluated.

As larvae hatched but did not show vital signs on day three in both, the Etofenprox and Bifenthrin group, a residual larvicidal effect of these compounds is possible.

In conclusion, we have shown that control of immature stages of *T. penetrans* with environmental insecticides is feasible in the context of an integrated control approach, at a concentration used currently by Brazilian authorities for the control of different insect species. Control measures against other endemic diseases using these insecticides may thus be integrated with tungiasis control in endemic settings. Further studies are needed to assess the efficacy of environmental insecticides of low toxicity under laboratory and field conditions.

CONFLICT OF INTEREST

The authors confirm that this article content has no conflict of interest.

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