



Potential Effects Of Pyrethrin Incorporated In Sucrose Baits Against *Phlebotomus duboscqi* Neveu Lemaire (Diptera: Psychodidae) In Leishmaniasis Control Strategies.

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None

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Abstract

Background: Due to challenges of controlling leishmaniasis in Kenya through the vector, the efficacy of pyrethrin EC (Pymos™ 0.6% W/V) a mosquito adulticide was evaluated against *Phlebotomus duboscqi* while incorporated in sucrose as a possible complementary method.

Methods: Pyrethrin was prepared into concentrations of 0.08, 0.25 and 0.5 mg/ml using 10% sucrose. Ten male and female sand flies were each aspirated into jars and fed separately with the prepared solutions using cotton wool pads placed atop experimental jars and mortality monitored each day. The experiments were replicated three times.

Results: Significant mortality ($P < 0.05$) for both sexes was observed. The concentration of 0.08mg/ml gave low adult mortality in 24 hours but amplified with time. In the 0.25 bioassay, mortality of females was 10 (33.3%) and males had 21 (70%) in 24 hours. The LD50 for females and males tied at 0.1 mg/ml in 48 hours of exposure. The concentration of 0.25 mg/ml was ideal at 48 hours of feeding where majority of the sand flies succumbed to the insecticide. There were significant mortality differences ($PP > 0.05$) was observed in male and female mortality at 48 hours of exposure however. Although we cannot rule out sand fly mortality due to contact with the test material, the results of this study however suggest that control strategies using low-dose pyrethrin-sugar combinations as baits may be effective against phlebotomine sand flies and other sugar feeding pests of medical importance.

Introduction

Phlebotomine sand flies of the genera *Phlebotomus* and *Lutzomyia* are the vectors of visceral and cutaneous leishmaniases; diseases caused by protozoa in the Genus *Leishmania* and are prevalent in about 88 tropical and sub-tropical countries worldwide. The diseases cause significant morbidity

and mortality [1] and afflict the poorest in the society [2]. Personal protection measures against the vector constitute the first line of defense against arthropod bites and arthropod-borne diseases [3]. Cutaneous leishmaniasis is the most widespread clinical form of the disease with characteristic sand fly vectors and mammalian hosts [4], and risks due to its infection are great [5]. In Kenya cutaneous leishmaniasis transmitted by *Phlebotomus duboscqi* [6] and diffuse cutaneous leishmaniasis caused by the bite of an infected female *P. pedifer* [7] do occur. *Phlebotomus martini* has been incriminated as a vector for visceral leishmaniasis in Kenya [8, 9]. Current strategies to control adult sand flies by use of conventional insecticides for indoor residual sprays and space spraying, use of repellents and long lasting treated nets have been met with several drawbacks including vector resistance, destruction of beneficial biota and pollution of ecosystem. New strategies to interrupt transmission through the vector as one of the crucial methods have been slow paced. Due to limited information on vector capability, species association, organization, seasonal population patterns and evolution designing new control strategies may entail vigorous studies to embrace the current trends [10]. Sugar baits using insecticidal materials against sand flies have been done previously [11]. Pyrethrins, complex esters extracted from two daisy-like flowers of *Chrysanthemum cinerariaefolium* and *Chrysanthemum cinereum* have been most commonly used to enhance household and garden insecticides since early part of the twentieth century [12] and are known to have low mammalian toxicity and non-persistence in the environment [13]. Pyrethrins kill insects by disrupting their nervous systems. Although pyrethrin resistance has been reported in about fifteen insect species, resistance ratios between resistant and susceptible hosts are often relatively low [13], except in four species, the German cockroach, the granary weevil, and the two house flies with ratios of above 100 [14]. Development of resistance to pyrethrin can be caused by repeated exposure to synthetic insecticides including pyrethroids. Pyrethrins have been used to control sand flies in methods including indoor residual

sprays, space spray in animal shelters, impregnation in dog collars, treating fabrics and bed nets [15] and have not been tried against sand flies in sugar baits. This study sought to evaluate the efficacy of natural pyrethrin currently being used as a mosquito adulticide in Kenya when incorporated in sugar baits against adult phlebotomine sand flies.

Materials and methods

Sand flies colony maintenance

Sand flies obtained from a colony of *Phlebotomus duboscqi* Neveu Lemaire (Diptera: Psychodidae) that were derived from Baringo district, Rift Valley, and were kept at the Centre Biotechnology Research and Development insectaries in Kenya Medical Research Institute were used. These were field-captured females which were sustained using methods previously described [16]. The female sand flies were fed on blood using Syrian golden hamsters for egg development and reared at $28 \pm 1^\circ\text{C}$, and an average RH of 85-95% and 12:12 (L: D) photoperiod. Corn syrup (Karo®) and slices of apple supplied on daily basis were used as sources of carbohydrates.

Pyrethrin

The Pyrethrin 0.6% w/v emulsifiable concentrate (EC), Pymos™ registration number PCPB (T) 0225 was purchased from the Pyrethrum Board of Kenya, Nakuru. This is usually a domestic class mosquito adulticide used in spraying of dwelling houses and animal sheds.

Adulticidal bioassays

This was done in a similar technique as is normally used to feed sugar to contained insects [17], equivalent to the one [11] used to feed sand flies with *Bacillus sphaericus* in sugar baits. Preliminary tests were done using anthrone test to confirm that sand flies fed on the sucrose-pyrethrin mixtures. The sand flies were killed using ether, washed in normal saline to remove any sugar on the body of the insects and degutted. The guts were placed in micro well plates, ground using a glass rod and one or two drops of anthrone reagent added. It was let to stand for an hour and any colour change was noted. The test is highly sensitive and is based on the dehydration of monosaccharides to furfural derivatives, e.g. hydroxymethylfurfural which react with anthrone to form a deep blue-green color [18], shade of which depend on the amount of sugar present in the sand fly gut.

Adult *P. duboscqi* sand flies were carefully aspirated into plastic rearing jars partially filled with plaster of Paris and fitted with screen tops. 10% sucrose solution

was used to prepare 0.08, 0.25 and 0.5 mg/ml of 0.6 % pyrethrin by serial dilution and used in the feeding of the flies. Cotton wool pads were soaked in the preparations and placed on the screen tops. Two triplicate series with 10 flies each of *P. duboscqi* were used for each dilution. The first triplicate contained 10 females and the second triplicate contained 10 males in each jar. 60 specimens were assayed for each dilution and gender. Sand flies that fed on 10% sucrose solution soaked in cotton wool pads and placed onto the screen tops were used as controls. Males and females were tested differently and the lethal mean dosage, designated LD50 determined every 24 hours of exposure.

Data analysis

All experiments were done in triplicate, whereby mortality of between 10% and 90% were considered and data entered into Microsoft excel program. Control groups in the experimental bioassays with more than 20% mortality were repeated. Where mortality in the control groups fell between 5 and 20%, the observed percentage mortality was corrected using Abbott's formula [19]:

$$\text{Test\% mortality-control \% mortality} \\ \text{Observed \% mortality} = \frac{\text{Test\% mortality-control \% mortality}}{100 - \text{control \% mortality}} \times 100$$

Data on the dose-mortality effects of different extracts on both larvae and adults were subjected to computerized Probit analysis [20] for LD50 values for different concentrations of the most active extracts on all bioassays at alpha significance level of 95%. Sex variability was compared using ANOVA [21].

Results and discussions

When the sand flies were subjected to pyrethrin in 10% sucrose both male and female sand flies were killed after feeding on the solutions. There was no significant difference in male and female mortality ($F = 0.1$, $P = 0.812$). Males death recorded mean of 15.5 ± 6.396 while females death recorded a mean of 17.75 ± 6.396 (ANOVA). Probit analysis- females $\chi^2 = 18.9$, LD50= 0.1, Males $\chi^2 = 17.6$, LD50 = 0.11 mg/ml at 48 hours of exposure (Illustration 1). In this feeding technique and within 24 hours, the lowest concentration (0.08mg/ml) gave a mortality rate of 2 females (6.6%) and 1 male (3.3 %) while the highest (0.5mg/ml) had 26 (86.6%) females dead and 29 males (96.6%) dead. When comparing male to female mortality due to different concentrations used we

found out that mortality was concentration dependent for both sexes. The concentration which worked better was 0.25 mg/ml within 48 hours of feeding where more than 50% of the sand flies succumbed to the insecticide (Illustration 2). Although pyrethrum is the oldest insecticide known to man and well recognized for its low mammalian toxicity and non-persistent in the environment, it has only been used routinely to treat materials such as curtains and bed nets, and as indoor residual sprays [22]. Bioassay on the evaluation of the pyrethrum components, pyrethrins in sucrose against sand flies was carried out and revealed promising insight into a novel vector control strategy. Under laboratory conditions, *Phlebotomus duboscqi* males and females readily fed on the mixture of low-dose pyrethrin and sucrose solutions with significant mortality. Despite the very low concentrations used in this study, mortality was high and had low LD50 values during 48 hours of exposure depicting superior insecticidal properties. The concentration of 0.25mg/ml gave the best results with LD50 for both male and females as 0.1mg/ml, an appreciably low concentration. There are no records available on the use of pyrethrin products in arthropod feeding trials in the literature. The study described here was based on the hypothesis that vectors feed on plant secretions, juice and nectar and may therefore feed on the solutions used and is in agreement with a similar study that shown sand flies could feed on aqueous sucrose solution containing a larval toxicant *Bacillus sphaericus* Neide, when sprayed on vegetation cover near burrows and termite mould and had sand fly population drastically reduced [11].

Conclusions

Although it is possible that some of the sand fly died due to contact with the test material, the results of this study however suggest that control strategies using low-dose pyrethrin-sugar combinations as baits may be effective against phlebotomine sand flies and other sugar feeding pests of medical importance. Preliminary bioassays with low dose concentrations on blood fed females showed considerable reduction of viable eggs. There are still no records available on the use of pyrethrin products in arthropod feeding trials in the literature. Other chemicals used in the control of other pests and are known to reduce sand fly populations [23] have not also been evaluated in feeding bioassays as food baits. Studies therefore entailing effects of low-dose pyrethrin on the adult fecundity, capacity to support parasite propagation and competence, and possible interference in

developmental time of larvae in nature are considered necessary.

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Illustrations

Illustration 1

Insecticidal activity of Pyrethrin in 10% sucrose solution when fed to male and female sand flies in 24, 48 and 72 hours.

Illustration 1. Insecticidal activity of Pyrethrin (0.6% EC) dilutions (0, 0.08, 0.25 and 0.5mg/ml) in 10% sucrose solution when fed to male and female sand flies in 24, 48 and 72 hours.

24 hours of exposure

sex	LD ₅₀ ¹	95% CL ²	X ²	df	P
F	0.30	0.21 – 0.54	19.8	5	0.002
M	0.28	0.19 – 0.45	19.8	5	0.001

48 hours

F	0.10	0.05 – 0.22	18.9	5	0.001
M	0.11	0.09 – 0.24	17.6	5	0.001

72hours

F	0.04	-0.01 – 0.07	13.2	5	0.022
M	0.03	0.02 – 0.04	12.8	5	0.011

¹LD₅₀ – expressed in mg/ml, ²Confidence limits for effective dose at 95% probability level

F= female M= male

Illustration 2

Adult sand fly mortality due to various concentrations of pyrethrin (Pymos)

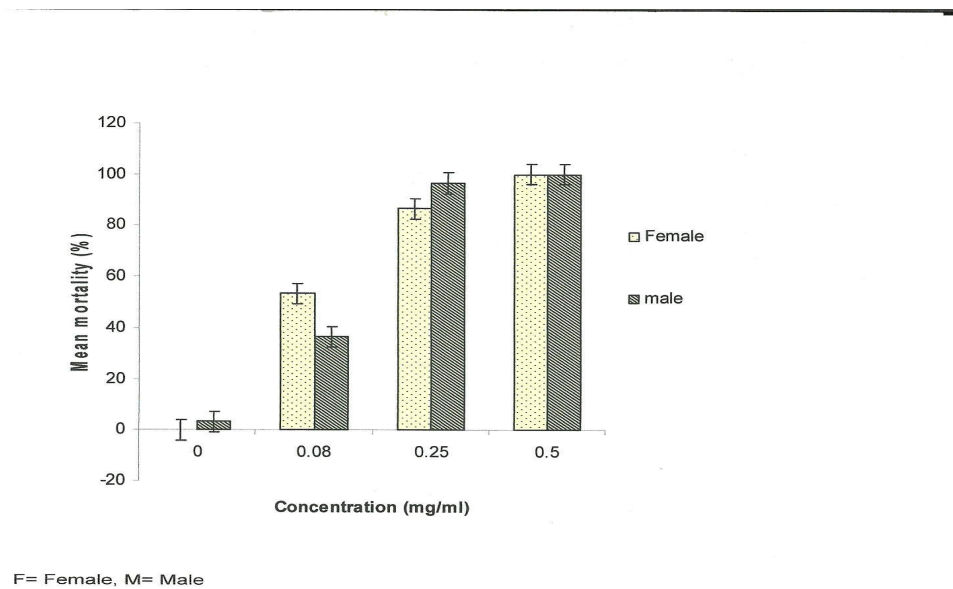


Illustration 2: Adult sand fly mortality due to various concentrations of pyrethrin (Pymos™) fed while in 10% sucrose solution at 48 hours of exposure. A total of 30 sand flies were used in each concentration and sex.

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