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The potential of the extracts of *Tagetes minuta* Linnaeus (Asteraceae), *Acalypha fruticosa* Forssk (Euphorbiaceae) and *Tarchonanthus camphoratus* L. (Compositae) against *Phlebotomus duboscqi* Neveu Lemaire (Diptera: Psychodidae), the vector for *Leishmania major* Yakimoff and Schokhor

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Abstract

Background & objectives: Harmful effects of synthetic chemical insecticides including vector resistance, environmental pollution and health hazards have necessitated the current significance in the search for plant-based insecticide products that are environmentally safe and effective to leishmaniases control. The insecticidal activity of *Tagetes minuta* Linnaeus (Asteraceae), *Acalypha fruticosa* Forssk (Euphorbiaceae) and *Tarchonanthus camphoratus* L. (Compositae) extracts were investigated against *Phlebotomus duboscqi* Neveu Lemaire (Diptera: Psychodidae).

Methods: The extracts were prepared from dried aerial parts soaked in methanol and ethyl acetate twice until the filtrates became clear, filtered and dried out by rotary evaporation at 30–35°C. The solid extracts obtained were later prepared into 2.5, 5 and 10 mg/ml. Two millilitres of the solutions were blotted on filter papers, which were dried overnight and placed into jars where adult sandflies were aspirated. Males and females were assayed separately.

Results & conclusion: The extracts had significant mortality ($p<0.05$) in both males and females bioassays but were not significantly different between sexes. The extracts of *Acalypha fruticosa* and *Tagetes minuta* had significantly higher mortality rates than those of *Tarchonanthus camphoratus* and the different concentrations used showed significantly different mortality rates and 10 mg/ml was the most effective concentration. Cent percent mortality was obtained at 96 h of exposure to 5 and 10 mg/ml concentrations except for *Tarchonanthus camphoratus* which had a mortality of only 46.7% in 10 mg/ml bioassay. These extracts were found to be insecticidal to adult sandflies.

Key words *Acalypha fruticosa*; leishmaniases control; *Phlebotomus duboscqi;* plant extracts; *Tagetes minuta; Tarchonanthus camphoratus*

Introduction

Leishmaniases group of parasitic diseases are globally distributed, caused by more than 30 species of protozoans in the genus *Leishmania* Ross and actual transmission to humans is through the bite of approximately 30 species of phlebotomine sandflies[1,2]. They are endemic in more than 60 countries worldwide. At present successful measures to decrease the incidence of leishmaniases is by per-

sonal protection and indoor residual spraying. Residual synthetic insecticides currently in use are harmful to the environment and have caused vector resistance including sandflies and malaria vectors.

Natural insecticide products derived from plants have been used successfully since ancient times to control a variety of insect pests that directly or otherwise endanger human survival[3]. Interest in their use has been growing due to their safety and desirable properties[4]. Plant essential oils have been found useful in protection against sandfly bites[5]. The demand for new precautionary strategies and improved health education is overwhelming hence the supreme need for safe, efficient and cost-effective alternative approaches[6]. *Acalypha fruticosa, Tagetes minuta* and *Tarchonanthus camphoratus* plants abundantly available in Kenya are partially dried and hang indoors to repel biting flies in leishmaniases prevalent areas. Their extracts have been shown to be insecticidal to other arthropods by other research groups. This study, therefore, sort to determine the potential of *T. minuta, A. fruticosa*, and *T. camphoratus* extracts against *P. duboscqi* and add onto the current botanical products effective against phlebotomine sandflies, the vectors for leishmaniases.

Material & Methods

Plants collection and preparation: Floral and foliar parts of *T. camphoratus*, *A. fruticosa*, and *T. minuta* were collected from Baringo district in the Rift Valley Province of Kenya. These plants parts were dried under the shade before packaging in paper bags for transportation to the laboratory for further drying. Voucher specimens of the plant parts were taken to the National Museums of Kenya herbarium for identification, storage and referencing. Further drying was done under shade for a month until completely dry. The dry plant samples were ground to fine powder using laboratory waring blender. Each sample of 100 g was weighed and put in conical flasks. An amount of 300 ml of methanol and ethyl acetate each were added into two separate flasks of each plant sample and placed on a shaker and soaked for 48 h. The samples were soaked further with additional 300 ml of the solvents for 24 h until the filtrates remained clear. The extracts were then filtered using Whatman filter paper No. 1, concentrated and dried under vacuum using rotary evaporator at 30–35^{o}C[7]. The concentrates were then transferred to sample bottles and the weight of the dry extracts was recorded and samples were stored at –20°C until required.

Sandfly colony maintenance: Sandflies were obtained from a colony of *P. duboscqi* Neveu Lemaire that originated from Marigat Division, Baringo district, Rift Valley, and were maintained at the Centre for Biotechnology Research and Development insectaries in Kenya Medical Research Institute, Nairobi. The colony of *P. duboscqi* was established using field-captured females and was maintained according to the methods of Beach *et al*[8]. This colony was rejuvenated with fresh isolates from the same locality at an interval of one year. The female sandflies were fed on blood using Syrian golden hamsters anaesthetized with sodium pentobarbitone (Sagatal®). The hamsters were usually shaved using an electric shaver underneath for easy sandfly access. The sandflies were reared at 28 ± 1^{o}C, and an average RH of 85–95% and 12:12 h (light : dark) photoperiod in Perspex insect rearing cages. Sandflies were maintained using slices of apple supplied on daily basis as sources of carbohydrates.

Adulticidal bioassays: These were done according to methods previously described[9] but with little modifications. Briefly, filter papers measuring 5 x 8 cm were blotted with the extract concentrations of 2.5, 5 and 10 mg/ml and allowed to dry overnight at room temperature under shade. The filter papers were placed at the bottom of the plastic jars filled with an inch of Plaster of Paris and fitted with screen tops. The jars were wetted with distilled water to maintain the optimum relative humidity between 80 and 90%. A total of 10 *P. duboscqi* adult flies were gently aspirated into the plastic rearing jars using a mouth aspirator. Two triplicate series with 10 specimens of *P. duboscqi* were used for each plant extract at each dilution. The first triplicate series contained 10 males

and the second, 10 females in each jar. Therefore, at least 60 specimens (30 for males and 30 for females) were assayed for each plant extract and dilution. The same protocol was applied to negative control experiments in which sandflies were aspirated into jars containing filter papers soaked in distilled water and dried in the same condition as for the extracts. Mortality was recorded at 24 h intervals.

Data analysis: All experiments were done in triplicate, whereby mortality between 10 and 90% was considered and data entered into Microsoft Excel program. Control groups in the experimental bioassays with >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[10] as below:

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

Data on the dose-mortality effects of different extracts on adults were subjected to computerized Probit analysis[11] for LD_{50} values for different concentrations of the most active extracts on all bioassays. Variation effects of extracts and between males and females were compared using ANOVA. Values of ≤ 0.05 were considered significant.

Results & Discussion

The results of the evaluation of *T. minuta, A. fruticosa* and *T. camphoratus* extracts against adult *P. duboscqi* showed significant mortality rate. Each of the extracts evoked a significant mortality ($p < 0.05$) in bioassays with both male and female species. Using this technique *T. minuta* extracts showed a significant higher mortality to adult sandflies than other extracts with mean mortality of 18.63 (62.1%) and 19.25 (64.2%) in the methanol and ethyl acetate extract bioassays respectively (Fig. 1). Extracts of *T. minuta* and *A. fruticosa* showed a significantly higher mortality rates in adult sandflies and were more active than those of *T. camphoratus*. A comparison of

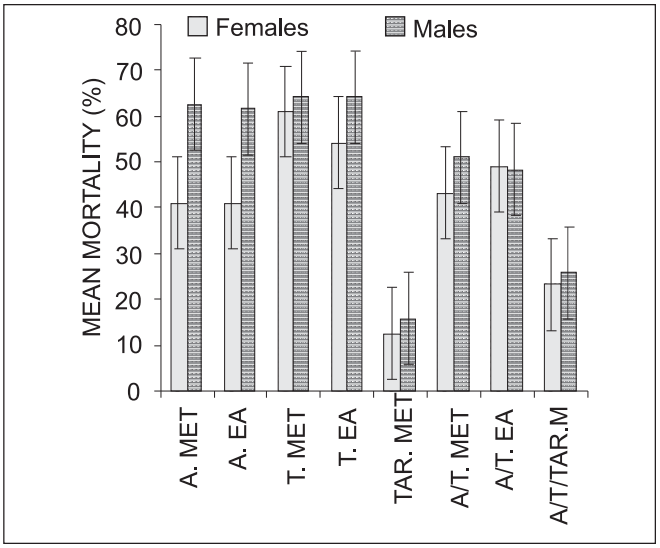

Fig. 1: Mean mortality of adult *P. duboscqi* when exposed to the extracts of *A. fruticosa*, *T. minuta* and *T. camphoratus* at 48 h of exposure. At least 30 sandflies were used in each extract bioassay (A. MET= *Acalypha* methanol; A. EA= *Acalypha* ethyl acetate; T. MET = *Tagetes* methanol; T. EA = *Tagetes* ethyl acetate; TAR. MET = *Tarchonanthus* methanol; A/T. MET = *Acalypha/Tagetes* methanol; A/T. EA= *Acalypha/Tagetes* ethyl acetate; A/T/TAR. M = *Acalypha/Tagetes/Tarchonanthus* methanol extracts; Mean mortality was computed on the product of the mortality among 0, 2.5, 5 and 10 mg/ml).

this study with the outcome of other similar studies on the efficacy of these extracts against phlebotomine sandflies is difficult since only scanty information in the literature is available. This contact method gave 90–100% mortality by the 96th hour in 5 mg/ml and above. Lower concentrations gave low mortality even after 96 h of exposure. Literature on insecticidal evaluation on *A. fruticosa* is scanty. In the present study, its extracts exhibited good insecticidal properties. However, there were no significant mortality difference between the extracts (Fig. 2). The LD_{50} for the females was 8.95 mg/ml ($\chi^2 = 39.4$) at 48 h of exposure while the LD_{50} for the males at the same exposure time was 3.26 mg/ml ($\chi^2 = 7.3$). *A. fruticosa* has been used extensively for its medicinal value, food, fodder and repellent activities against insects and dried leaves could be powdered, soaked in water and the solution applied on animal skin and wound as a repellent or insecticide against ectoparasites and flies with appreciable results[12].

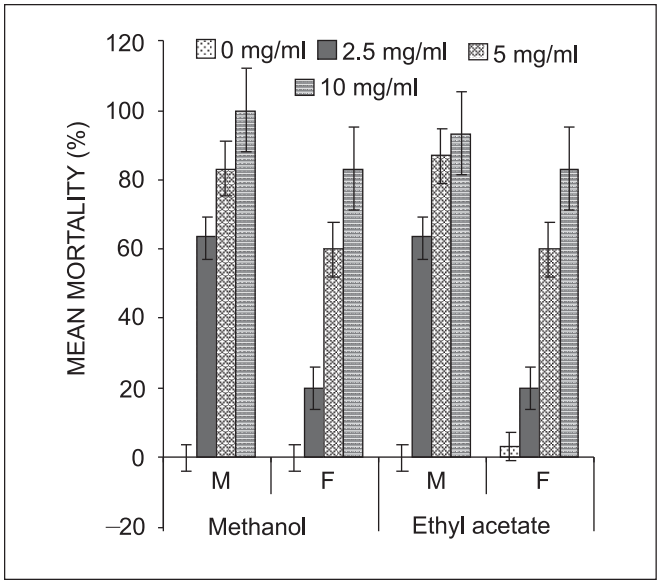

Fig. 2: Mortality rates of male and female sandflies exposed to *A. fruticosa* extracts incorporated in filter papers and dried under shade (M=Male; F=Female).

Although *T. minuta* is perceived to have insecticidal activities, its action against phlebotomine sandflies had not been evaluated earlier. In this study, its activities against male and female sandflies caused significant mortality (p <0.05). It was found that in the first 24 h of exposure only 10 mg/ml and above had >15 (50%) mortality for both sexes in bioassays with methanol extract. The results at 48 h were comparable to those of *A. fruticosa.* LD_{50} for the females was 1.6 mg/ml (χ^2= 17.7) and that of males at the same time was 1.2 mg/ml (χ^2= 26.5) experiment with methanolic extract (Table 1). The LD_{50} at 48 h for ethyl acetate extract for female bioassay was 3.99 mg/ml (χ^2=19.1) and male was 2.44 mg/ml (χ^2=4.4), showing that these extracts were more effective than those of *A. fruticosa*. No significant mortality was observed between the extracts (Fig. 3). *Tagetes* species essential oil has been described by other research groups as insecticidal and some of its active derivatives were identified. Crude extracts from *T. minuta* aerial parts were described as effective larvicides to mosquito larvae with LC_{50} and LC_{90} as 1.5 mg/l and 1.0 mg/l respectively[13]. Insecticidal activity of *Tagetes* species against *Anopheles gambiae*, the vector for malaria was also illustrated[14]. Successful

Table 1. Response of adult *P. duboscqi* when exposed to different extracts in filter papers at 48 h of exposure (n = 30)

Plant species	Extract	Sex	LD_{50}[1]	χ^2	*p*-value
T. minuta	Methanol	Male	1.2	26.5	0.001
		Female	1.6	17.7	0.007
	Ethyl acetate	Male	2.44	4.4	0.487
		Female	3.99	19.1	0.002
A. fruticosa	Methanol	Male	3.26	7.3	0.202
		Female	8.95	39.4	0.001
	Ethyl acetate	Male	2.56	18.4	0.002
		Female	3.5	12.7	0.026
T. camphoratus	Methanol	Male	24.5	7.19	0.207
		Female	49.9	3.09	0.685
Acalypha/Tagetes	Methanol	Male	9.93	27.1	0.001
		Female	9.47	23.8	0.001
	Ethyl acetate	Male	4.88	32.3	0.001
		Female	4.23	60.7	0.001
Acalypha/Tagetes/ Tarchonanthus	Methanol	Male	19.5	12.6	0.026
		Female	18.8	3.8	0.575

[1]LD_{50}= mg/ml; Sex variation F = 1.00, $p > 0.05$; Concentration variation F= 33.7, $p < 0.05$.

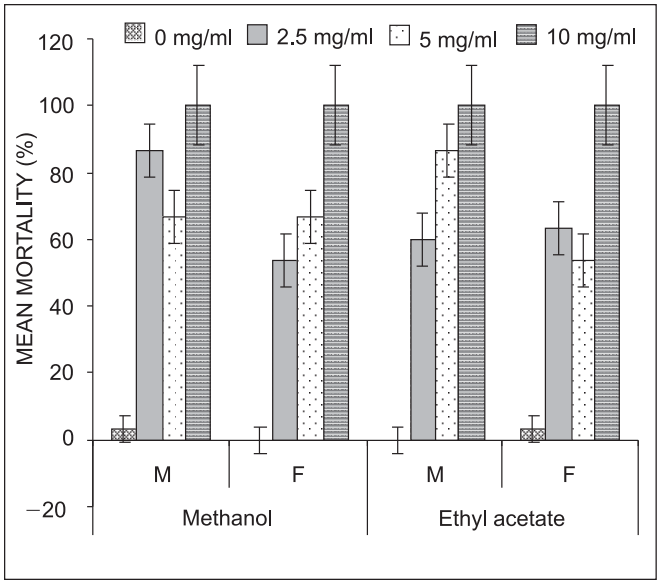

Fig. 3: Mortality rates of adult *P. duboscqi* exposed to *T. minuta* extracts incorporated in filter papers at 48 h of exposure (M=Male; F=Female).

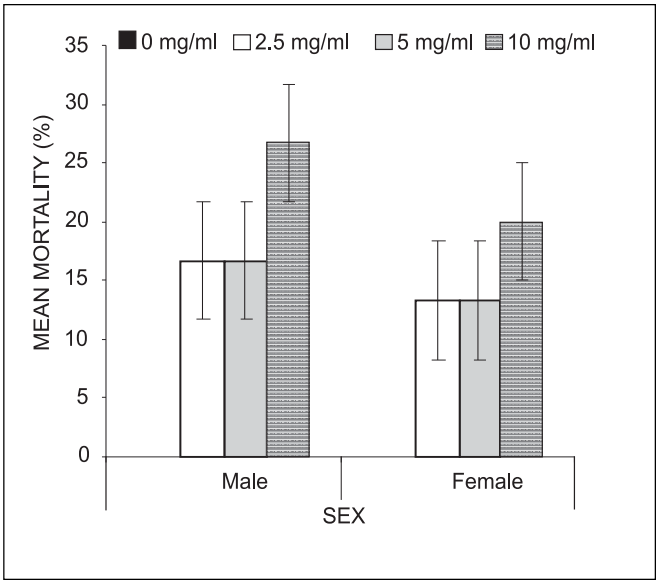

Fig. 4: Mortality rates of adult *P. duboscqi* exposed to the methanol extract of *T. camphoratus* incorporated in filter papers at 48 h of exposure (M=Male; F=Female).

evaluation of the same was done against stored product pests[15]. Cestari *et al*[16] evaluated the potential of 100 ppm of *T. minuta* essential oil against head lice *Pediculus humanus capitis* (Phthiraptera: Pediculidae) and obtained a lethal time (LT_{50}) of 16.4 ± 1.62 min denoting toxicity of the essential oil. *Tegetes minuta* oil essential terpenes were found to be responsible for the toxic effects reported in dipterans[17] and possibly in the present study. Only the methanol extract of *T. camphoratus* was insecticidal in the bioassays but there was no significant mortality between the sexes (Fig. 4). The ethyl acetate extract of *T. camphoratus* had virtually no insecticidal activity against *P. duboscqi* males and females and was hence excluded from the experiments. *Tarchonanthus camphoratus* methanol extract had higher LD_{50} values depicting inferior insecticidal activity against *P. duboscqi* to those of *T. minuta* and *A. fruticosa.* The LD_{50} for females at 48 h of exposure was 49.9 mg/ml ($\chi^2 = 3.09$) while males had a LD_{50} of 24.5 mg/ml ($\chi^2 = 7.19$) (Table 1). At 72 h of exposure 10 mg/ml gave a mortality of 10 (33.3%) for females and 12 (40%) for males, 96 h yielded a mortality of 15 (50%) females and 14 (46.7%) of males. Cent percent mortality was gained after 168 h of exposure to 20 mg/ml of the methanol extract. Combination of the methanol extracts of *T. minuta, A. fruticosa* and *T. camphoratus,* however, gave no synergistic properties and actually yielded weaker insecticidal properties to those of individual extracts. Generally there was no significant difference in the mortality between the extracts ($F = 1.4$, $p = 0.240$). Mortality of both male and female adult sandflies were not significantly different ($F = 1.00$, $p = 0.318$). Mean mortality at 48 h of exposure for females was 12.19 ± 1.846 and that of males was 14.81 ± 1.846. This showed that mortality of both female and male sandflies was similar due to the extracts used. When filter paper technique was used, the study found that concentration of the solutions used were significantly different in causing mortality to these sandflies ($F = 33.7$, $p < 0.05$). The most effective concentration was 10 mg/ml (mean mortality 23.19 ± 1.632) in all the extracts used. Concentration of 5 mg/ml had a mortality of 17.56 ± 1.632; Concentration of 2.5 mg/ml had a mortality of 12.31 ± 1.632 while control had a mean mortality of 0.937 ± 1.632.

In similar studies on *T. camphoratus,* it was eminent that derivatives and formulations of this plant have

repellent activities and medicinal uses[18]. Van Wyk and Van Wyk[19] observed that wild animals browse the leaves to keep off biting insects probably due to insecticidal effects as revealed in this study. The low values obtained in our study could be attributed probably to the composition of the active ingredients, method of extraction and the sandfly species used. It was observed that application of essential oils including lemon oil and 2% neem oil mixed in coconut or mustard oil provided appreciable protection against sandfly bites[5]. Although appreciable mortality was realized in the bioassays, studies are needed to establish the repellency effects of these extracts for complementing other personal protection measures since chemical impregnated clothing cannot offer 100% protection against leishmaniasis in the field conditions[20].

In conclusion, we suggest that the plant products evaluated here can be harnessed and used as promising insecticides in vector control programs. More studies should be extended to other medicinal plants to identify more potent extracts in sandfly control since plant-derived substances are potential sources of new insecticides that may play a more prominent role in integrated pest management programs and remains the only ideal option due to their safety in nature.

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