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Research Article

The effects of *Mundulea sericea* (Fabales: Fabaceae) water extracts on *Phlebotomus duboscqi* (Diptera: Psychodidae) eggs and larvae

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Background: The sand fly, *Phlebotomus duboscqi* is the vector for zoonotic cutaneous leishmaniasis caused by *Leishmania major*. No definite control method for *P. duboscqi* is known. Of the plants known for entomocidal properties, *Mundulea sericea* is one, with the toxic principle being rotenone. Dried crude *M. sericea* powder extract mixed with water is used by the Giriama people of Coastal region, Kenya, to kill cat fleas and bedbugs. A recent laboratory study using *M. sericea* non-polar and polar extracts displayed remarkable entomicidal potential. Against this backdrop, *M. sericea* was chosen for this study.

Objective: To evaluate water extracts of *Mundulea sericea* leaves for efficacy against *P. duboscqi* eggs and larvae.

Methodology: The plant leaves were ground into fine powder and extracted using water. 1 g leaf powder was extracted using 100 ml distilled water, and then filtered. The eggs were then exposed to 1 ml of the filtered extract. Further, 0.001 g of leaf powder was mixed with 1 g of larval food and fed to larvae. The eggs were incubated at 25 ^{0}C and 80% relative humidity and observed for eclosion, and larvae for hatching to adult stage.

Results: The filtered extracts did not inhibit eggs from hatching. Additionally, all the larvae that were fed on food mixed with ground *M. sericea* leaves survived.

Conclusion: These findings suggest that suggest that *M. sericea* water extracts do not penetrate the egg chorion, thus the lack of activity. Moreover, the inability of *P. duboscqi* to digest the chlorophyll of *M. sericea* so as to release rotenone could be responsible for absence of entomocidal effect.

Key words: *Mundulea sericea; Phlebotomus duboscqi; Leishmania major.*

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1. Introduction

The sand fly *Phlebotomus (Phlebotomus) duboscqi* (Diptera: Psychodidae) is the vector for zoonotic cutaneous leishmaniasis caused by *Leishmania (Leishmania) major* (Kinetoplastida: Trypanosomatidae) in Baringo District, Rift Valley Province, Kenya (Beach et al, 1984). This sand fly is a sylvatic species found mainly in animal burrows, where it rests and feeds on rodents such as *Arvicanthisniloticus, Taterarobusta, Aethomyskaiseri,*, *Taterillusemini* and *Mastomysnatalensis*, which have been incriminated as *L. major* reservoir hosts (Githureet al, 1984; Githureet al, 1986).

No definite control method for *P. duboscqi* is known. Biological control methods have been tested both in the laboratory and in the field. In a laboratory study,

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aqueous suspensions of *Bacillus sphaericus* (*B. sphaericus)* at concentrations as low as 0.05 and 0.11 mg/cm^2 were shown to inhibit hatching of *P. duboscqi* and *Sergentomyia schwetzi* by 95%. However, the same concentrations did not affect the ability of pupae to emerge as adults (Robert et al, 1998).*Bacillus sphaericus* has been tested in the control of *P. duboscqi* in Baringo with some success. Attempts to control *P. duboscqi* by spraying vegetation, animal burrows and eroded termite mounds with sucrose solution incorporated with *B. sphaericus* were made. Significant larval mortality was observed for 2-12 weeks post-treatment (Robert et al, 1997).

Of the plants known for entomocidal properties, *Munduleasericea* Willd (Fabales: Fabaceae) is one, with the toxic principle known as rotenone ($C_{23}H_{22}O_6$), an isoflavonoid (Verdcourt and Trump, 1967) which inhibits mitochondrial electron transport in insect tissues (Fakamiet al, 1967). Rotenone is soluble in chloroform. Further, solutions of rotenone in organic solvents decompose and oxidize upon exposure to light and air with color formation occurring (Dawson et al, 1986).

Dried crude *M. sericea* powder extract mixed with water is used by the Giriama people of Coastal region, Kenya, to kill cat fleas (*Ctenocephalides*), human fleas (*Pulex irritans*) and bedbugs (*Cimex lectularis*) (Luitgards-Moura et al, 2002). A recent study in our laboratory using *M. sericea* non-polar and polar extracts displayed remarkable entomicidal potential against *Aedes aegypti* larvae mosquito. The hexane extract of the root bark had an LC_{50} of 130 ppm, followed by 180 ppm for methanol extract and 450 ppm for dichloromethane extract. Comparatively, methanol extracts from the root bark had significantly higher activity than that of the seedpods. Indeed, the active ingredient, believed to be rotenone was extracted more by hexane, a non-polar solvent (Langat et al, 2012). This vindicates an earlier study that indeed rotenone is soluble in chloroform (non-polar solvent) (Dawson et al, 1986).

Other plants containing rotenone such as *Derris amazonica* (Papillionaceae), *Antonia ovata* (Loganaceae) have been shown to have entomocidal properties against the sand fly *Lutzomyia longipalpis*, the vector of *L. infantum chagasi*, and that as an insecticide, rotenone has a relatively long residual effect of up to six months (Luitgards-Mouraet al, 2002). It is for these reasons that *M. sericea* was chosen for this study.

2. Materials and methods

2.1 Plant collection and preparation

M. sericea leaves were harvested from Bamba, Kilifi county, Coast region, Kenya. The plant was positively identified at the Botany Department Herbarium, University of Nairobi (Voucher no: MS 002). Leaves were semi-dried under a shade for 48 hrs and then transported to Nairobi where they were further dried to a brittle state. They were ground to fine dust, weighed and 1 g leaf powder dissolved in 100 ml distilled water. It was left to stand at 4 ^{0}C to ensure maximum extraction of entomocidal constituents after which they were filtered. Before use, the samples were stored in a refrigerator at -4 °C until use in the bioassays.

2.2 Sand fly colony and maintenance

Laboratory bred *P. duboscqi* larvae and eggs were used. A *P. duboscqi*s and fly colony was set up at Kenya Medical Research Institute (KEMRI). Eggs for its initiation were obtained from Baringo district. The eggs were incubated at 25^{0}C and 80% relative humidity.

2.3 Bioassays

A total of 220 freshly laid *P. duboscqi* eggs on plaster of Paris were moistened with 1 ml of the filtered water extract on 0 day post-oviposition (DPO), and another group of 220 moistened with 1ml of distilled water, as negative controls. The eggs were incubated at 25^{0}C and 80% relative humidity and observed for eclosion. Additionally, 220 larvae were fed on food mixed with ground *M. sericea* leaves. Briefly, 0.001 g of leaf powder was mixed with 1g of food. The two were mixed thoroughly by triturating in a mortar. The larvae were then observed for mortality.

3. Results and discussion

When five eggs from each group (control and experimental) were microscopically examined at 6 DPO, they were seen to have a thin, smooth chorionic layer without any sculpturing, unlike normal untreated eggs as (**Figure 1**). When the treated eggs were again observed at 10 DPO, all had hatched. The filtered water extracts of *M. sericea* leaves did not inhibit hatching, but was only able to erode the chorionic sculpturing of the upper layers of the exochorion without inhibiting larval hatching. Being soluble in chloroform (Dawson et al, 1986), most rotenone would be extracted efficiently using chloroform. However, in our case we extracted it using water, as per the ethnopharmacological information from the Giriama people, from coastal region of Kenya (Luitgards-Moura et al, 2002). Inability of the water extracts to cross the egg membrane could explain the lack of activity.

Additionally, all the first instar larvae that were fed on food mixed with ground *M. sericea* leaves survived. Microscopic examination of freshly killed and mounted larvae revealed the presence of undigested chlorophyll in their guts as shown in the plate below (**Figure 2** and **Figure 3**). The same was observed when second and third instar larvae were examined. Throughout the pre-imaginal period, no deaths were observed in all instars. All the larvae completed the development into adults. Observation of undigested *M. sericea* in the guts of live larvae that were mounted is a clear indication that the plant was not able to kill them. The ability of *P. duboscqi* larvae to feed but not digest the chlorophyll of *M. sericea* without dying suggest that this sand fly species may not be susceptible to the killing effects of this plant. Moreover, digestion of the leaves indicates that all the constituents of the plant, including rotenone, would be released, absorbed across the gut, be in the tissues, inhibits mitochondrial electron transport in insect tissues (Fakami et al, 1967), and thus killing the insect.

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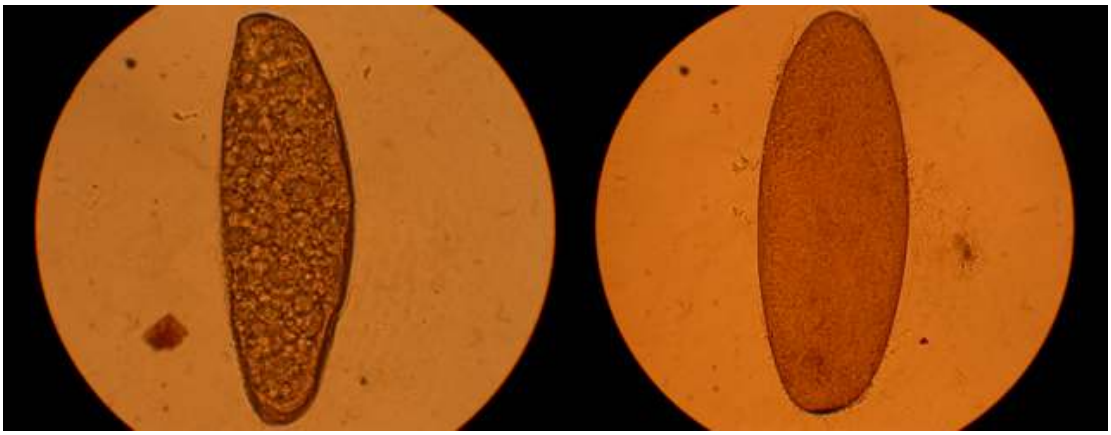

Figure 1: *P duboscqi*control egg left) and the *M. sericea* treated egg (right) x 400 magnification.

Figure 2: *P.duboscqi* third instar larva with *M. sericea* in the gut (Magnification x 200).

Figure 3: *P. duboscqi* third instar control larva fed on normal larval food (Magnification x 200)

The susceptibility to rotenone killing has been described as consistently good, variable or poor (Roark, 1942, 1944). When used against the melon aphid, *Aphis gossypii* Glover (Hemiptera: Aphididae) and the asparagus beetle, *Croceris asparagi* L (Coleoptera: Chrysomelidae), the action is considered, consistently good. Onion thrips, *Thrips tabaci* Lindeman (Thysanoptera: Thripidae) and cucurbits bugs, *Anasa tristis* De Geer show variable results whereas the cabbage aphid, *Brevicoryne brassicae* L (Hemiptera: Aphididae) and the corn earworm, *Helicoverpa zea* Boddie (Lepidoptera: Noctuidae) display poor results (McIndoo, 1947).

Dried crude *M. sericea* powder extract mixed with water is used by the Giriama people of Coast Province, Kenya to kill cat fleas, *Ctenocephalides,* human fleas, *Pulex irritans* L, (Siphonoptera: Pulicidae), bedbugs, and *Cimex lectularis* (Hemiptera: Cimicidae) (Luitgards-Mouraet al, 2002). This is taken to mean that *Ctenocephalides, Pulex irritans* and *Cimex lectularis* are endowed with a digestive mechanism that releases rotenone in the gut, at lethal concentrations, absorbed to the system, and thus occasioning their deaths.

4. Conclusion

Our findings indicate that the toxic components responsible for larvicidal effects, rotenone may not have been extracted sufficiently using water. Further, any polar active ingredients in the plant may not have been able to cross the membranes of the eggs and gut and thus the absence of entomicidal activity. Results from our study revealed that the lack of larvicidal potential of *M. sericea* water extracts may be attributed to inability of the larvae to digest the leaves, and release the toxic chemicals, responsible for activity.

Conflict of Interest declaration

The authors declare no conflict of interest

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