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Laboratory evaluation of some eastern African Meliaceae as sources of larvicidal botanicals for *Anopheles gambiae*

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Abstract. Root bark extracts of five Meliaceae species (*Turraea abyssinica* Hochst., *Turraea wakefeldii* Oliv., *Turraea mombassana* Hiern ex C.DC., *Trichilia roka* (Forsk) Chiov. and *Melia volkensii* Guerke.) and different fractions thereof (following chloroform extraction and column chromatographic separation) were compared for their immediate toxicity and long-term effects on *Anopheles gambiae* Giles *sensu stricto* (Diptera: Culicidae). Larvicidal effects of the extracts appeared to be largely associated with limonoids of medium polarity with *M. volkensii* and *T. mombassana* extracts being more potent than those of the other Meliaceae. Long-term (6–8 days post-exposure) observations at lower doses showed that 100% cumulative mortality can be achieved with some extracts (particularly those of *T. mombassana* and *M. volkensii*) with interesting growth-inhibition effects. Follow-up studies that are needed and practical implications of the results in terms of development of larval control strategies for African malaria vectors are discussed.

Key words: *Anopheles gambiae*, *Turraea abyssinica*, *Turraea wakefeldii*, *Turraea mombassana*, *Trichilia roka*, *Melia volkensii*, limonoids, larvicide, growth inhibition

Résumé. Des extraits d'écorce de racines de cinq espèces de Meliaceae (*Turraea abyssinica* Hochst., *Turraea wakefieldi* Oliv., *Turraea mombassana* Hiern ex C.DC., *Trichilia roka* (Forsk) Chiov. et *Melia volkensii* Guerke.) et différentes fractions de ces extraits (après extraction au chloroforme et séparation par chromatographie en colonne) ont été comparées en terme de toxicité immédiate et de leurs effets à long terme sur des larves d'*Anopheles gambiae* Giles *sensu stricto* (Diptera: Culicidae). Les effets larvicides des extraits semblent en grande partie associés aux limonoides de polarité moyenne des extraits de *M. volkensii* et *T. mombassana*, qui sont plus actifs que ceux des autres Meliaceae. A long terme (6–8 jours après exposition) les traitements à faibles doses peuvent *provoquer près de 100% de mortalité cumulée avec certains extraits* (en particulier ceux de *T. mombassana* et *M. volkensii*) avec des effets d'inhibition de la croissance intéressants. On discute des futures voies d'investigation et des implications pratiques de ses résultats dans le cadre du développement de stratégies de lutte larvaire contre les vecteurs de la malaria Africaine.

Mots clés: *Anopheles gambiae*, *Turraea mombassana*, *Turraea wakefieldi*, *Turraea mombassana*, *Trichilia roka*, *Melia volkensii*, limonoides, larvicide, inhibition de la croissance

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Introduction

Between 300 and 500 million people, primarily in tropical countries, suffer from malaria every year (Service, 1993). Africa accounts for 90% of reported cases, with over one million children under five years dying from the disease (Gilles, 1993). Development of drug resistance by the malaria parasites and insecticide resistance by the vectors, as well as environmental concerns associated with synthetic insecticides, have greatly hampered malaria control. Given the unique situation of malaria in Africa, the search for alternative strategies and measures for the control of the parasite and vectors has assumed a great deal of importance in the continent. Readily available traditional plant remedies for both the disease and vectors are seen as potential candidates in malaria control (Khalid *et al.*, 1989; Sukumar *et al.*, 1991; Van der Nat *et al.*, 1991; Mackinnon *et al.*, 1997).

Plant products may be used against malaria vectors in two principal ways: as sources of repellents to reduce human–vector contact, and in the control of mosquito larvae and/or adults. The use of plant repellents is widespread in different communities in Africa and the performance of many has been evaluated experimentally (Snow *et al.*, 1987; Curtis *et al.*, 1991; Kokwaro, 1993; Pålsson and Jaenson, 1999a,b; Seyoum *et al.*, 2002a,b). No related use of plant products targeted for vector control has been documented, although extracts or individual components of a number of plants have been shown to have potent toxic or growth-inhibitory activities against larvae of different species of mosquitoes (Bohlman *et al.*, 1974; Jondiko, 1986; Mwangi and Mukiama, 1988; Mwangi and Rembold, 1988; Jayaprakasha *et al.*, 1997; Mwangi, 1997). Larval source reduction by habitat modification or use of larval control agents is generally considered as a less costly and more effective approach to control mosquito populations, particularly, in community participation programmes (Zebitz, 1987; Becker and Margalit, 1993; Slooff, 1987). In the African situation, the availability of target-specific, environmentally benign botanicals could allow the incorporation of larval control into integrated malaria and vector management programmes.

We have targeted members of the Meliaceae family for discovering potentially useful botanicals for the control of both larval and adult malaria vectors. These plants are characterized by the presence of blends of tetranortriterpenoids (limonoids), which exhibit a number of interesting anti-insect properties including anti-oviposition, antifeedant, growth disruption and toxicity (Schmutterer and Ascher, 1984; Jones *et al.*, 1989; Champagne *et al.*, 1992; Isman, 1995). The family is well represented in eastern Africa with over 40 species in 11 genera (Blundell, 1987). In the present study, we selected five Meliaceae species that are found growing in different agro-ecological zones and different altitudes in Kenya for initial evaluation in the laboratory against *Anopheles gambiae* Giles *s.s.* The effects of crude rootbark extracts and limonoid-rich fractions of these on the larvae were compared.

Materials and methods

Plant materials

The root bark of *Turraea mombassana*, *T. wakefeldii* and *Trichilia roka* were collected from Shimba Hills National Park, Kwale, south coast of Kenya, while those of *Melia volkensii* and *Turraea abyssinica* were collected from Kabu in Kibwezi, and Kijabe Rift Valley Province of Kenya, respectively. Voucher specimens have been kept in the herbarium of the Botany Department of the University of Nairobi, Kenya.

Mosquito larvae

Anopheles gambiae s.s. were reared under standard laboratory conditions. This strain of mosquitoes originates from Njage village (70 km from Ifakara, south-east Tanzania), and has been reared under laboratory conditions since April 1996. Eggs were allowed to emerge in plastic containers filled with distilled water, and were transferred to larger pans ($37 \times 31 \times 6\,cm^3$) at densities of 200–300 at the L2 stage. Water temperature was kept at 29 ± 1°C in the bioassay room. Larvae were fed on Tetramin® fish food and were used for the experiments upon reaching the late L3 or early L4 developmental stage.

Extraction and fractionation

The root bark of the plants was air-dried for three weeks and ground into powder. The respective powders (1 kg each) were soaked separately in 4 l of methanol for three days at room temperature and filtered. The filtrate was concentrated *in vacuo* at 40 °C, using a rotary evaporator. The chloroform extracts were obtained by partitioning portions (22 g) of the respective methanol extracts between water (250 ml) and chloroform (200 ml × 3) and the latter concentrated in vacuum. The chloroform extracts (30 g) were chromatographed on silica gel (glass column 45 mm ID × 81 cm) using hexane–ethyl acetate gradient (Fig. 1). Separation was monitored by thin layer chromatography (TLC). The TLC plates were developed with hexane–ethyl acetate (2:1). The plates were sprayed with Ehrlich reagent (2% 4-dimethylaminobenzaldehyde in

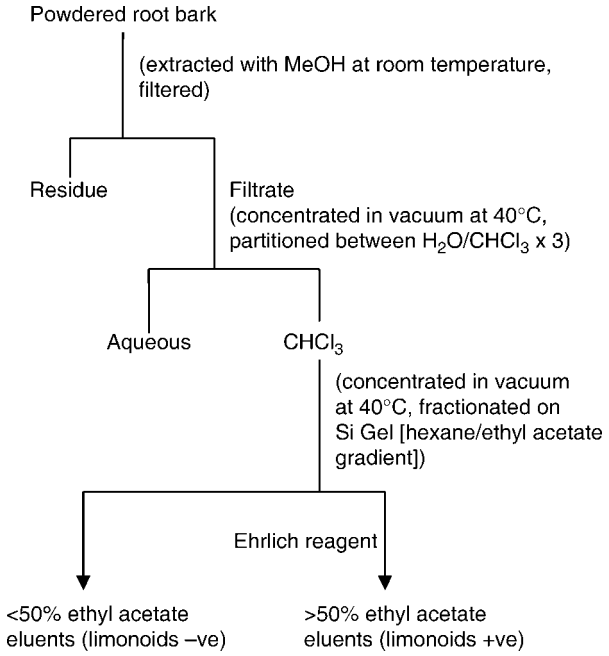

Fig. 1. Bioassay guided fractionation of the root bark extracts of five Meliaceae plants

ethanol) and developed in a hydrogen chloride gas chamber. Fractions that eluted with 55–100% ethyl acetate gave typical limonoid reactions (pinkish/reddish spots) (Torto *et al.*, 1996).

Bioassays

These were carried out in two ways: (i) the effects of different doses of the methanol and chloroform extracts, and the chromatographic fractions, on larval mortality after 24 h were determined, and LD_{50} values computed (since the non-limonoid fractions demonstrated relatively low activities, they were pooled and assayed as a single blend); and (ii) the long term effects of the lower doses (50–100 ppm) of the extracts and chromatographic fractions were monitored every 24 h until the emergence of the adults, if any. The standard WHO larvicidal assay procedure was used (WHO, 1996). Briefly, 1 ml of a standard w/v concentrate of each extract in acetone was made to 100 ml with distilled water in a 250 ml beaker. Twenty late L3 or young L4 instar larvae were used per beaker with five beakers per dose (water temperature 25 ± 1 °C). Tetramin® fishfood was added and, for long-term observations, this was supplemented every second day.

Data analysis

A two-way analysis of variance was run to evaluate the combined effects of the extracts and dose on the larval mortality. In addition to the single factors, that is, (extract, $F = 594.75$; df $= 11, 287$; $P < 0.0001$) and (dose, $F = 3232.90$; df $= 5287$; $P < 0.0001$), effect of interaction between the extracts and the doses ($F = 48.32$; df $= 55, 287$; $P < 0.0001$) was highly significant. Consequently, the mortality induced by each dose was evaluated for each extract. Means were Student-Newman-Keuls (SNK).

Results

Larval mortality after 24 h

All the methanol and chloroform soluble extracts and column chromatography fractions thereof exhibited larvicidal activity against larvae of

Table 1. Mean[+] percent mortality (±SE) (24 h) of *Aropheles gambiae* larvae induced by different doses of the methanol (MeOH) and chloroform ($CHCl_3$) extracts of *Turraea asbynsinica*, *T. mombassana*, *T. wakefeldii*, *Trichilia roka* and *Melia volkensii*

	Dose ppm					
Extract	50	100	250	500	750	1000
MeOH						
T. abyssinica	0 ± 0.0^{eE}	0 ± 0.0^{eH}	5.0 ± 1.58^{dF}	18.0 ± 1.22^{cF}	25.0 ± 1.58^{bD}	38.0 ± 2.55^{aC}
T. roka	5 ± 1.58^{fDC}	21 ± 1.0^{eE}	55 ± 2.24^{dC}	69 ± 1.25^{cD}	78 ± 2.54^{bB}	100 ± 0.0^{aA}
T. wakefeldii	2 ± 1.22^{dDE}	11 ± 1.0^{cF}	15 ± 1.58^{cE}	15 ± 1.58^{cF}	60 ± 3.16^{bC}	93 ± 3.0^{aB}
M. volkensii	15 ± 1.58^{dB}	41 ± 1.0^{cB}	80 ± 0.0^{bA}	90 ± 1.58^{bC}	100 ± 0.0^{aA}	100 ± 0.0^{aA}
T. mombassana	0 ± 0.0^{eE}	0 ± 0.0^{eH}	4 ± 1.87^{dF}	45 ± 1.58^{cE}	62 ± 5.14^{bC}	100 ± 0.0^{aA}
$CHCl_3$						
T. abyssinica	0 ± 0.0^{dE}	2.0 ± 1.22^{dG}	53.0 ± 2.54^{cC}	90.0 ± 1.58^{bC}	98.0 ± 2.0^{aA}	100.0 ± 0.0^{aA}
T. roka	10 ± 1.58^{eBC}	35 ± 1.58^{dBC}	49 ± 1.87^{cC}	95 ± 1.58^{bB}	100 ± 0.0^{aA}	100 ± 0.0^{aA}
T. wakefeldii	5 ± 1.58^{dDE}	27 ± 1.22^{cD}	35 ± 1.58^{bD}	100 ± 0.0^{aA}	100 ± 0.0^{aA}	100 ± 0.0^{aA}
M. volkensii	40 ± 0^{eA}	67 ± 2.55^{dA}	81 ± 1.0^{cA}	100 ± 0.0^{aA}	100 ± 0.0^{aA}	100 ± 0.0^{aA}
T. mombassana	48 ± 2.0^{dA}	66 ± 1.0^{cA}	70 ± 1.58^{cB}	94 ± 1.87^{bBC}	100 ± 0.0^{aA}	100 ± 0.0^{aA}

[+] Means followed by the same capital letter within the same column and same small letter within the same row are not significantly different at 5% level (SNK).

Table 2. LD_{50} (ppm) (24 h) of the methanol and chloroform extracts and column chromatographic fractions of *Turraea abyssinica*, *T. mombassana*, *T. wakefeldii*, *Trichilia roka* and *Melia volkensii* against *Anopheles gambiae* larvae

Plant	MeOH extract	$CHCl_3$ extract	Pooled non-limonoid fractions	Limonoid rich fractions[+]				
				I	II	III	IV	V
T. abyssinica	1359.0	253.0	697.7	39.2	113.9	116.5	122.6	122.6
T. mombassana	555.6	63.0	422.9	50.7	62.9	175.3	[1]	[1]
T. roka	244.9	162.0	318.1	19.8	46.4	59.9	64.9	86.6
T. wakefeldii	579.8	192.0	567.0	48.2	51.4	53.7	59.1	78.3
M. volkensii	115.5	67.0	207.0	0.09	5.5	42.9	77.9	122.0

[+] Fractions I–V eluted with 65–100% ethyl acetate.
[1] Insufficient amount obtained for bioassay.

An. gambiae within the first 24 h (Tables 1 and 2). The methanol extract of *M. volkensii* was most potent at all treatment levels tested (LD_{50} 115.5 ppm); and that of *T. abyssinica* was least active with an LD_{50} of 1359 ppm. The chloroform soluble fractions were 1.5–9-fold more potent than the corresponding methanol extracts. Of the fractions obtained by column chromatography of the chloroform extracts, the non-limonoid fractions were much less active (LD_{50} 207–697 ppm) (Table 2). The more active fractions tested were all positive for limonoids (Ehrlich reagent). This was confirmed by comparing TLC stains of these fractions with those of limonoids previously characterized from *T. floribunda* (Torto *et al.*, 1996).

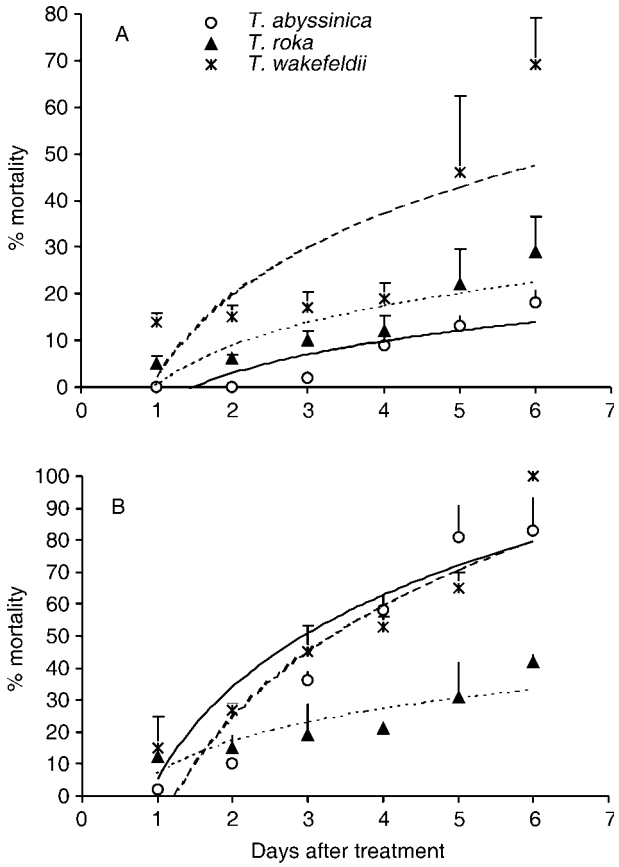

Fig. 2. Cumulative mean % larval mortality of *Anopheles gambiae* larvae in rearing water treated with 50 ppm (A) and 100 ppm (B) of the chloroform extracts of *Turraea abyssinica*, *T. wakefeldii* and *Trichilia roka*

Longer term effects of lower doses

The effects of lower doses of crude extracts of the five Meliaceae plants are shown in Figs 2–5. In all cases, the larval stage (L4) was prolonged (6–8 days post-treatment) compared to that of controls (4 days). Delayed mortality of the larvae treated with chloroform extracts of *Turraea abyssinica*, *T. wakefeldii* and *Trichilia roka* at 50 and 100 ppm was significantly different ($P < 0.0001$) (Fig. 2). At 250 ppm, mean daily mortality due to the extracts during the 6-day period was not significantly different ($P > 0.0001$) (results not included). At both 50 and 100 ppm, all surviving larvae pupated and attained adulthood. However, at 100 ppm, the adults resulting from surviving larvae were relatively small compared to those from the control and showed some morphogenetic aberrations. The mouthparts and legs were stuck to the body and the wings could not expand for

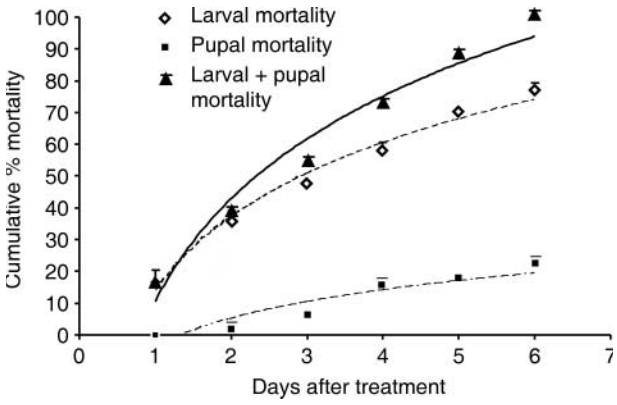

Fig. 3. Cumulative percent larval and pupal mortality in water treated with methanol extract of *Turraea mombassana* at 100 ppm

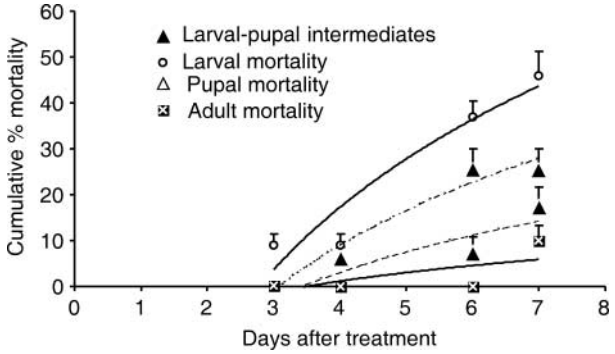

Fig. 4. Cumulative % larval–pupal intermediates, larval, pupal and adult mortality in rearing water treated with 50 ppm methanol extract of *Turraea mombassana*

flight. These adults died on the water surface. These morphogenetic aberrations were ascribed to growth-inhibitory effects of the limonoid constituents. The crude methanol extracts of the three plants were much less potent relative to the respective chloroform extracts, and no special effects were observed at different doses tested.

Interesting effects were also observed with the crude methanol extract of *Turraea mombassana*. Although the extract was weakly active at 50 and 100 ppm as a larvicide (<10% mortality in 24 h), long term observations showed 100% cumulative mortality at both the doses (Figs 3 and 4). At 50 ppm, an average of 46% of population treated died during ecdysis; about 26% failed to ecdyse to normal pupae, producing larval–pupal intermediates (Fig. 5) reminiscent of a previous report with a sublethal dose of *Bacillus sphaericus* (Mulla *et al.*, 1991), which were short-lived; and although about 18% moulted successfully, the resulting pupae died. An average of 10% of larvae attained adulthood, but were unable to expand their wings, and died on the water surface. At the higher dose (100 ppm), about 78% of the population treated died at the larval stage during the 8-day period; and about 22% at the pupal stage. Neither the chloroform extract of *T. mombassana*, nor the chromatographic fractions of this extract demonstrated the above effects, suggesting that the presence of a polar component (or components) associated with the crude methanol extract is (are) important for these activities.

Melia volkensii extracts were more potent as longer term larvicides than those of the other Meliaceae tested in this study. Delayed mortality in surviving larvae treated with 50 ppm of methanol and chloroform extracts of *M. volkensii* was observed. Overall mortality of 100% of the population treated was observed 4 and 8 days post treatment with 50 ppm of chloroform and methanol extracts, respectively (Fig. 6A). The most potent limonoid-rich chromatography fractions

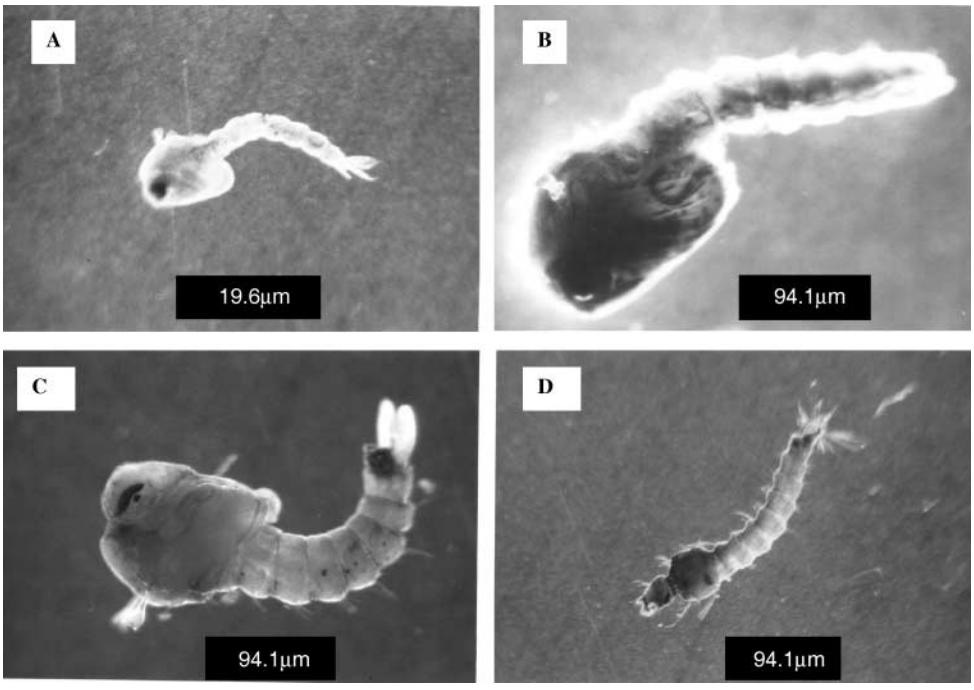

Fig. 5. A. Pupa of *Anopheles gambiae* developing from surviving larvae treated with sublethal dose of methanol extract of *Turraea mombassana*. The pupa died soon after moulting due to incomplete melanization or hardening; B. Dead partially formed pupa. The pupa had a transparent jelly-like film around it that could not harden; C. Larval–pupal intermediate that was short-lived; D. Dead larvae possessing normal shape but jet black in colour at the head region

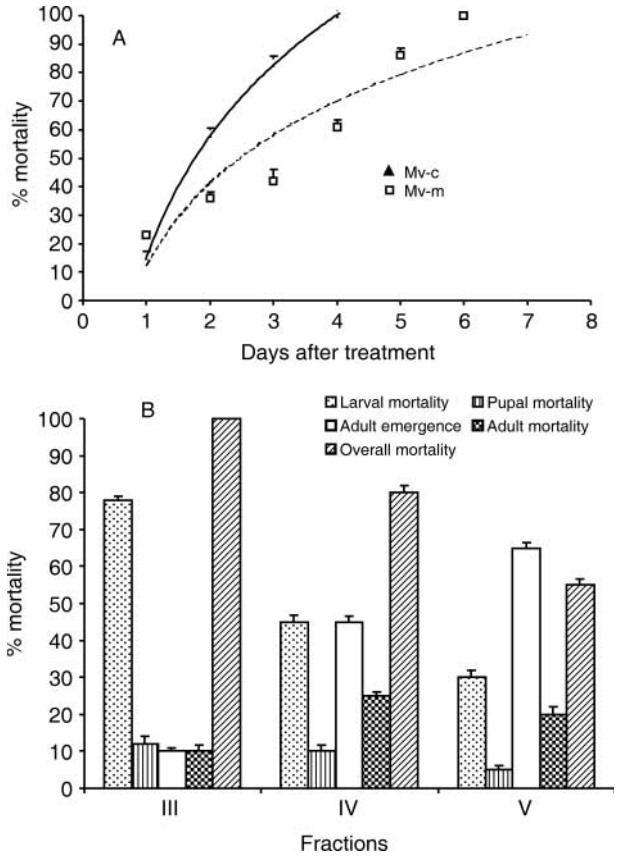

Fig. 6. Cumulative mean larval- and pupal-mortality and adult emergence (%) in rearing water treated with 50 ppm of methanol and chloroform extracts (A) and column chromatography fractions (B) from *Melia volkensii*

(I and II) of *M. volkensii* (Table 1) were not evaluated for long-term effects at sub-lethal doses. The more polar and less potent (as larvicides) fractions (III, IV and V) showed some growth-disrupting effects over 8-day exposure at 50 ppm (Fig. 6B). Of the surviving larvae (22% of the treated population in fraction III treatment), further mortality (12%) occurred in pupae and (10%) in partially emerged adults. Overall mortality rate was 100% (Fig. 6B). When larvae were treated with fraction IV at 50 ppm, 45% of the population treated died at L4 stage, 10% at the pupal stage while 45% emerged as adults. However, 25% (of treated population) of emerged adults had poorly developed wings that could not expand, and were not able to fly off and died on the water surface. Overall mortality rate was 80% for the 50 ppm treatment (Fig. 6B). Reduced delayed mortality also occurred in the 50 ppm treatment with fraction V in both pupae and adult stages. Overall, 5% of the treated population died at the pupal stage, 20% at the adult stage, giving overall mortality of 55% (30% of the treated population died at the larval stage) (Fig. 6B).

Discussion

In the present study, the effects of root bark extracts of five Meliaceae species on *An. gambiae* s.s. larvae were compared to allow an initial assessment to be made on their relative potential as sources of botanicals for the control of malaria vectors. Short-term (24 h) toxicity effects were found to be associated largely with non-hydrophilic limonoids of medium polarity. This is reflected in 1.5–9-fold increase in larvicidal activities (LD_{50}) of the chloroform-soluble fractions compared to the crude methanolic extracts, and in higher relative activities of some chromatographic fractions that eluted earlier from the normal-phase silica column (e.g. fractions I and II of *M. volkensii* and fractions I of *T. abyssinica* and *T. roka*, Table 1).

Long term observations of the effects of lower doses of chloroform extracts or fractions indicate that toxic effects are gradual (Figs 2–4, 6), suggesting that they are unlikely to be neurotoxic, similar to previously studied limonoids from *Azadirachta indica* (Rembold, 1995). Of particular interest is the growth disruptant effect of *T. mombassana* and *M. volkensii* extracts. Extracts of *M. volkensii* fruits have previously been found to disrupt the growth of nymphs of *Schistocerca gregaria* (Forskål) (Mwangi, 1982), *An. arabiensis* Patton larvae (Mwangi and Mukiama, 1988) and *Aedes aegypti* (Linnaeus) (Mwangi, 1997). Some limonoids and limonoids-containing extracts of Meliaceae, such as *A. indica*, *Melia azedarach* (Lee *et al.*, 1991; Rembold, 1995), *Trichilia harvanensis* (Lopez-Olquin *et al.*, 1997) and *T. pallida* (Roel *et al.*, 2000), have been implicated as possible disruptants of the endocrine system of insects. Because of their structural similarity to ecdysteroids, limonoids have been specifically considered as potential ecdysteroid agonists or antagonists, and two limonoids from *Turraea obtusifolia* have been shown to antagonize 20-hydroxyecdysone action in a *Drosophila* cell line (Sarker *et al.*, 1997).

Of the root bark extracts of the five Meliaceae tested in the present study, the extract of *M. volkensii* was the most potent larvicide. Previously, the root bark extract of this plant was investigated for potential anti-cancer constituents, and a series of limonoids have been characterized (Zeng *et al.*, 1995; Rogers *et al.*, 1998). It remains to be seen which of these, if any, may contribute to the observed effects of the extracts in the present study. The extracts of the seed kernels of the plant have also been investigated against several species of mosquito larvae (Mwangi and Mukiama, 1988; Mwangi and Rembold, 1988; Al-Sharook *et al.*, 1991). Two groups of constituents have been implicated, one toxic and the other growth-inhibitory. Although a number of limonoids from the seed kernel have

been chemically characterized (Rajab and Bentley, 1988; Rajab *et al.*, 1988), the specific components or blend of these associated with the observed effects on mosquito larvae are unknown.

From a practical standpoint, the present study shows that, in addition to *M. volkensii* (Mwangi, 1997), *T. mombassana* also represents a promising source of a botanical agent for the control of malaria vectors. Unlike *M. volkensii*, a tall tree (~30 ft) found mainly in the dry savannah areas of eastern Africa, *T. mombassana* is a fast growing shrub (up to 10 ft) common in areas with medium and high precipitation, from the coastal region to the highlands up to 6000 ft. In the present study, the crude methanol extract of the root bark was more potent in disrupting the development of treated insects and in causing their overall mortality than any of the enriched fractions, suggesting that the physiological effects observed may be due to a blend effect of limonoids of medium polarity as well as polar constituents associated with the crude methanol extract of the root bark. A previous phytochemical study on the root bark extract of the plant led to the isolation and identification of several prieurianin-type limonoids of medium polarity (Adul *et al.*, 1993). Interestingly, the two limonoids from *Turraea obtusifolia*, shown to act as antagonists of ecdysone action also had preurianin-type structures (Sarker *et al.*, 1997). Identification of key constituents in the effective blend associated with the crude methanol extract will help to throw further light on its mode of action on mosquito larvae and facilitate the development of an optimum recovery process and formulation for its practical deployment.

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References

Adul G. G., Bentley M. D., Benson B. W., Huang F. Y., Gelbaum L. and Hassanali A. (1993) Two new prieurianin-class limonoids from *Turraea mombassana*. *J. Nat. Products* 56, 1414–1417.

Al-Sharook Z., Balan K., Jiang Y. and Rembold H. (1991) Insect growth inhibitors from two tropical Meliaceae: effect of crude seed extracts on mosquito larvae. *J. Appl. Entomol.* 11, 425–430.

Becker N. and Margalit J. (1993) Use of *Bacillus thuringiensis israelensis* against mosquitoes and blackflies, pp. 147–170. In Bacillus thuringiensis, *An Environmental Biopesticide: Theory and Practice* (Edited by P. F. Entwistle, J. S. Cory, M. J. Bailey and S. Higgs). John Wiley and Sons Ltd.

Blundell M. (1987) *Wild Flowers of East Africa*. William Collins Sons and Co Ltd., London. 135 pp.

Bohlmann F., Zdero C. H. and Suwita A. (1974) Weitare amide aus de tribus authemidaea. *Chem. Rev.* 107, 1038.

Champagne D. E., Koul O., Isman M. B., Scudder G. G. E. and Towers G. H. N. (1992) Biological activity of limonoids from the Rutales. *Phytochemistry* 31, 377–394.

Curtis C. F., Lines J. D., Baolins L. and Renz A. (1991) Natural and synthetic repellents, pp. 75–92. In *Control of Disease Vectors in The Community* (Edited by C. F. Curtis). Wolfe Publishing, London.

Gilles H. M. (1993) Historical outline, pp. 1–10. In *Bruce-Chwatts, Essential Malariology* (Edited by H. M. Gilles and D. A. Warrel). Little Brown and Company, Boston, Massachusetts.

Isman M. B. (1995) Leads and prospects for the development of new botanical insecticides. A review. *Pest. Toxicol.* 3, 1–20.

Jayaprakasha G. K., Singh R. P., Pereira J. and Sakariah K. K. (1997) Limonoids form *Citrus reticulata* and their moult inhibiting activity against *Culex quinquefasciatus* larvae. *Phytochemistry* 44, 843–846.

Jondiko I. J. O. (1986) A mosquito larvicide in *Spilanthes mauritania*. *Phytochemistry* 25 (10), 2289–2290.

Jones P. S., Ley S. V., Morgan E. D. and Santafanios D. (1989) The chemistry of the neem tree, pp 19–45. In *1988 Focus on Phytochemical Pesticides* (Edited by D. L. Jacobson). Vol. 1. CRC Press, Boca Raton, Florida.

Khalid S. A., Duddect H. and Gonzalez-Sierra M. (1989) Isolation and characterisation of an antimalarial agent from the neem tree, *Azadirachta indica*. *J. Nat. Products* 52, 922–927.

Kokwaro J. O. (1993) *Medicinal Plants of East Africa*. East African Literature Bureau, Nairobi, Kenya. 157 pp.

Lee S. M., Klocke J. A., Barnaby M. A., Yamasaki R. B. and Balandrin M. F. (1991) Insecticidal constituents of *Azadirachta indica* and *Melia azedarach* (Meliaceae), pp 293–300. In *Naturally Occurring Pest Bioregulators* ACS symposium series No. 449 (Edited by P. A. Hedin). American Chemical Society, Washington.

Lopez-Olquin J. F., Budia F., Chestnut S. P. and Vinvella E. (1997) Activity of *Trichilia havanensis* on larvae of *Spodoptera littoralis*. *Bull. Vegetal Health Plagues* 23, 3–10.

Mackinnon S., Durst T. and Arnason J. T. (1997) Antimalarial activity of tropical Meliaceae extracts and gedunin derivatives. *J. Nat. Products* 60, 336–341.

Mulla M. S., Singh N. and Darwazeh H. A. (1991) Delayed mortality and morphogenetic anomalies induced in *Culex quiquefasciatus* by the microbial control agent *Bacillus sphaericus*. *J. Am. Mosq. Cont. Assoc.* 7, 412–419.

Mwangi R. W. (1982) Locust antifeedant activity in fruits of *Melia volkensii*. *Entomol. Exp. Appl.* 32, 277–280.

Mwangi R. W. (1997) Studies of insecticidal activity in extract fractions of *Melia volkensii* (Gurke). *Discovery and Innovation* 9, 33–38.

Mwangi R. W. and Mukiama T. K. (1988) Evaluation of *Melia volkensii* extract fractions as mosquito larvicides. *J. Am. Mosq. Cont. Assoc.* 4, 442–447.

Mwangi R. W. and Rembold H. (1988) Growth inhibiting and larvicidal effects of *Melia volkensii* extracts on *Aedes aegypti* larvae. *Entomol. Exp. Appl.* 46, 103–108.

Pålsson K. and Jaenson T. G. T. (1999a) Comparison of plant products and pyrethroid- treated bednets for protection against mosquitoes (Diptera: Culicidae) in Guinea-Bissau, West Africa. *J. Med. Entomol.* 36, 144–148.

Pålsson K. and Jaenson T. G. T. (1999b) Plant products used as mosquito repellents in Guinea Bissau, West Africa. *Acta Trop.* 72, 39–52.

Rajab M. S. and Bentley M. D. (1988) Tetranortriterpenes from *Melia volkensii*. *J. Nat. Products* 53, 840–844.

Rajab M. S., Bentley M. D., Alford A. R. and Mendel M. J. (1988) A new limonoid insect antifeedant from the fruit of *Melia volkensii*. *J. Nat. Products* 51, 168–171.

Rembold H. (1995) Biological effects of neem and their modes of action: growth and metamorphosis, pp. 177–178. In *The Neem Tree* (Edited by H. Schumutterer). VCH Publishers, New York.

Roel A. R., Vendramim J. D., Frighetto R. T. and Frighetto N. (2000) Efeito do extrato acetato de etila de *Trichilia pallida* Swartz (Meliaceae) no desenvolvimento e sobrevivência da lagarta-do-cartucho. *Bragantia* 59, 53–58.

Rogers L. L., Lu Zeng and Mclaughlin J. L. (1998) New bioactive steroids from *Melia volkensii*. *J. Org. Chem.* 63, 3781–3785.

Sarker S. D., Savchenko T., Whiting P., Sik V. and Dinan L. N. (1997) Two limonoids from *Turraea obtusifolia* (Meliaceae), prieurianin and rohitukin, antagonise 20-hydroxyecdysone action in a *Drosophila* cell line. *Arch. Insect Biochem. Physiol*. 35, 211–217.

Schmutterer H. and Ascher K. R. S. (1984) Natural pesticides from the neem tree (*Azadirachta indica* A. Juss) and other tropical plants. *Proceedings, International Neem Conference (Ranischholzhausen, 1983)* GTZ, Eschborn, Germany. 583 pp.

Service M. W. (1993) The *Anopheles* vector, pp. 97–124. In *Bruce Chwatts Essential Malariology* (Edited by H. M. Gilles and D. A. Warrel). Little Brown and Company, Boston, Massachusetts.

Seyoum A., Palsson K., Kung'a S., Kabiru E. W., Lwande W., Killeen G. F., Hassanali A. and Knols B. G. J. (2002a) Traditional use of mosquito- repellent plants in western Kenya and their evaluation in semi-field experimental huts against *Anopheles gambiae*: Ethnobotanical studies and application by thermal expulsion and direct burning. *Trans. Roy. Soc. Trop. Med. Hyg.* 96, 225–231.

Seyoum A., Kabiru E. W., Lwande W., Killeen G. F., Hassanali A. and Knols B. G. J. (2002b) Repellency of live potted plants against *Anopheles gambiae* from human baits in semi-field experimental huts. *Am. J. Trop. Med. Hyg.* 67, 191–195.

Slooff R. (1987) The control of malaria vectors in the context of the health for all by the year 2000 global strategy, pp. 551–555. In *Proceedings of the 52nd Annual Meeting of the American Mosquito Control Association, Seattle, Washington*.

Snow R. W., Bradley A. K., Hayes R., Byass P. and Greenwood B. M. (1987) Does woodsmoke protect against malaria? *Ann. Trop. Med. Parasitol.* 81, 449–451.

Sukumar K., Perich M. J. and Boobar L. R. (1991) Botanical derivatives in mosquito control. A review. *J. of Am. Mosquito Control Assoc.* 7, 210–237.

Torto B., Hassanali A., Nyandat E. and Bentley M. D. (1996) A limonoid from *Turraea floribunda*. *Phytochemistry* 42, 1235–1237.

Van der Nat J. M., Van der Sluis W. G., 'Thart L. A., Van Djik H., De Silva K. T. D. and Labadie R. P. (1991) Activity-guided isolation and identification of *Azadirachta indica* A. Juss. (Meliaceae) bark extract constituents, which specifically inhibit luminol-dependent chemiluminescence by activated human polymorphonuclear leukocytes. *Planta Medica* 57, 65–68.

WHO (1996) *Report of the WHO Informal Consultation On the Evaluation and Testing of Insecticides*. WHO, Geneva. 37 pp.

Zebitz C. P. W. (1987) Potential of neem seed kernel extracts in mosquito control, pp. 555–573. In *Natural Pesticides from Neem Tree and Other Tropical Plants*. Proceedings of the 3rd neem conference (Nairobi, Kenya, 1986) (Edited by H. Schumutterer and K. R. S. Ascher).

Zeng L., Gu Z. M., Fang X. P., Fanwick P. E., Chang C. J., Smith D. L. and McLaughlin J. L. (1995) Two new bioactive triterpenoids from *Melia volkensii* (Meliaceae). *Tetrahedron* 51, 2477–2488.