International Journal of Tropical Insect Science Vol. 28, No. 4, pp. 220–224, 2008 doi:10.1017/S1742758408108839

Growth disruption activity of polar extracts from *Kotschya uguenensis* (Fabaceae) against *Anopheles gambiae* s.s. (Diptera: Culicidae) larvae

Ester Innocent[1,2]*, Cosam C. Joseph[3], Nicholas K. Gikonyo[4], Mayunga H.H. Nkunya[3] and Ahmed Hassanali[2]

[1]Institute of Traditional Medicine, Muhimbili University of Health and Allied Sciences, PO Box 65001, Dar es Salaam, Tanzania: [2]*icipe* – African Insect Science for Food and Health, PO Box 30772-00100, Nairobi, Kenya: [3]Department of Chemistry, University of Dar es Salaam, PO Box 35061, Dar es Salaam, Tanzania, and [4]Department of Pharmacy & Complementary/Alternative Medicine, Kenyatta University, PO Box 43844-00100, Nairobi, Kenya

(Accepted 25 September 2008)

Abstract. Studies on the larvicidal properties of extracts and fractions from *Kotschya uguenensis* Verdc. were done by long-term exposure of *Anopheles gambiae* s.s. larvae. The cumulative mean percentage mortalities and deformities at different concentrations for the extracts and fractions were recorded after every 24 h. Treatment of *A. gambiae* s.s. larvae with *K. uguenensis* extracts showed growth disruption by forming elongated guts and resulting in eventual death. Larvae treated with the methanol extracts from the stem and root barks, and fractions of the former extract attained complete mortality in 6–8 days at 50 and 100 ppm. More deformed larvae were observed from the methanol extract of the stem bark (40%) and methanol-soluble fraction (88%) than those treated with water-soluble fraction (22%) and methanol extract of the root bark (5%). The growth disruption may be associated with constituents in the plant that interfere with the normal endocrine system functions.

Key words: *Kotschya uguenensis*, larvicide, larval growth disruption, elongated gut, long-term exposure, *Anopheles gambiae* s.s.

Introduction

Kotschya uguenensis Verdc. belongs to the family Fabaceae (initially Leguminosae). The family comprises three subfamilies, namely Mimosoideae, Caesalpinioideae and Papilionoideae (Verdcourt, 1971; Gillett *et al.*, 1971). About 440 genera and 12,000 species of Papilionoideae are widely distributed in most parts of the world, but with the greatest diversity in tropical and subtropical regions (Verdcourt, 1971; Gillett *et al.*, 1971). The genus *Kotschya* consists of about 30 species distributed in tropical Africa and Madagascar (Verdcourt, 1971; Gillett *et al.*, 1971). *K. uguenensis* is traditionally used to repel the chicken mite *Dermanyssus gallinae* DeGeer (Acarina: Dermanyssidae) in central Tanzania. Information on phytochemicals from *K. uguenensis* or its insecticidal properties against *Anopheles gambiae* s.s. Giles (Diptera: Culicidae) larvae is unavailable in the current literature.

*E-mail: einnocent@muhas.ac.tz

However, several plant species in the Fabaceae family are known to have insecticidal properties (Dev and Kaoul, 1997; Gusmão *et al.*, 2002). For example, rotenone, which is isolated from *Derris* spp., is a non-systemic contact insecticide and feeding repellent for many vegetable, fruit and cattle pests (Dev and Kaoul, 1997). To date, *D. elliptica* Juss is being cultivated in the tropics as its roots are a valuable source of rotenone (Dev and Kaoul, 1997). Another Fabaceae plant, *Derris urucu* (Killip & AC Smith) J.F. Macbr., modifies the peritrophic matrix structures of *Aedes aegypti* Linn. (Diptera: Culicidae) by weakening the interaction among peritrophic membrane proteins, chitin and/or proteoglycans (Gusmão *et al.*, 2002). In this study, we report on the disruption of growth and eventual death of *A. gambiae* s.s. larvae when exposed to methanol extracts of *K. uguenensis.*

Materials and methods

Plant material

Root and stem barks of *K. uguenensis* were collected near Ngwazi dam and Kisolombe farm in Mufindi district along Iringa-Mbeya road 12 km from Iringa town in Tanzania. The plant specimens were identified and deposited at the Department of Botany Herbarium, University of Dar es Salaam, Tanzania.

Extraction and fractionation

The plant materials were air-dried under a roof, pulverized and soaked sequentially in *n*-hexane, dichloromethane and methanol for 72 h and then filtered. Soaking was done twice for every solvent. The crude filtrates were concentrated *in vacuo* using a rotary evaporator, while maintaining the bath temperature at 40 °C. Partitioning of the methanol extract of the stem bark (80 g) between water and dichloromethane yielded three fractions that were coded KUSMC, KUSMM and KUSMH, representing the dichloromethane, methanol and water-soluble fractions, respectively. Analysis of the profiles resulting from high-performance liquid chromatography of the methanolic extracts from the root and stem barks, whose codes were KURM and KUSM, respectively, and the three fractions of the latter extract (KUSMC, KUSMM and KUSMH) showed slight quantitative and qualitative differences of the chemical constituents.

Mosquito larvae

Anopheles gambiae s.s. larvae were obtained from a colony maintained at the International Centre of Insect Physiology and Ecology (*icipe*) Nairobi insectaries. This strain originated from *icipe*'s Mbita Point Field Station near Lake Victoria in 2003. Eggs were allowed to hatch in plastic containers filled with distilled water. Batches of 200–300 second instar larvae were transferred to large plastic pans (37 × 31 × 6 cm). The larvae were fed on Tetramin® fish food (Tetra GmbH, Germany), and the water temperature was maintained at 28 ± 2 °C throughout the larval development.

Larvicidal and insect growth regulation assay

Preliminary assay to compare larvicidal activities of *K. uguenensis* extracts was carried out using *A. gambiae* s.s. larvae. Thus 20 late third or young fourth instar larvae were exposed to various concentrations (50, 100, 250 and 500 ppm) of the extracts in distilled water, obtained by adding known volume of the stock solution in beakers to make up 100 ml of water–sample solution (WHO, 2005). A further detailed bioassay of the extracts and fractions was carried out using lower doses (10, 50 and 100 ppm). Ethanol (99.5%) was used to dissolve samples and hence was used as the blank in the control experiment. The test was tripled from separately reared batches of larvae. Mortality delay was studied by recording cumulatively the number of dead larvae, deformities and emerged adults after every 24 h for 14 days. During the experiment, the room temperature was kept at 33 ± 2 °C, and water temperature at 28 ± 2 °C, and the larvae were fed on the fish food at 1 mg per beaker per day.

Data analysis

Data for mortality and deformities were subjected to ANOVA and the mean percentage mortality compared using Student–Newman–Keuls test of the SAS package (SAS Institute, 2000).

Results

During extraction, the yield of the methanol extract of the stem bark (KUSM, 13.8%) and root bark (KURM, 5.4%) was considerably larger compared with dichloromethane extracts of the stem bark (0.45%) and root bark (0.3%), and the *n*-hexane extracts of the stem bark (0.95%) and root bark (0.4%), respectively. On partitioning of the KUSM extract, the yield of the dichloromethane-soluble fraction (3.3%) was low in comparison with the methanol-soluble fraction (40.6%) and the water-soluble fraction (56%), implying that the plant materials contained polar compounds.

Preliminary biological evaluation showed that compounds from the KUSM and KURM extracts

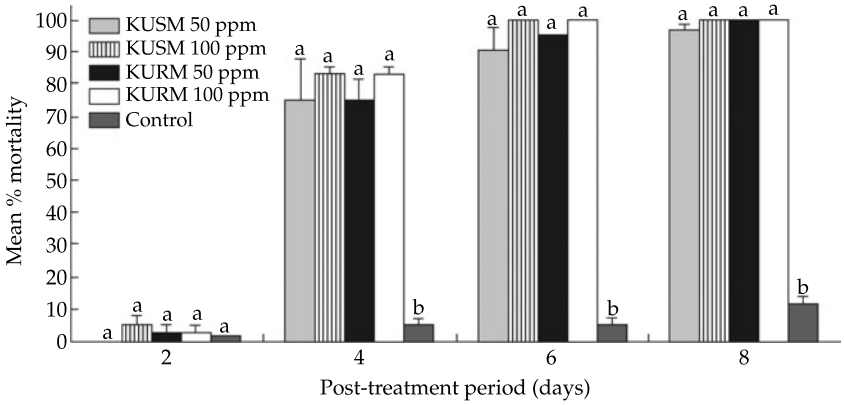

Fig. 1. Cumulative mean percentage mortality (±SE) of *Anopheles gambiae* s.s. larvae in rearing water treated with methanolic extracts of the stem and root bark of *Kotschya uguenensis* at 50 and 100 ppm. KUSM 50 ppm, methanol extract of the stem bark of *Kotschya uguenensis* at 50 ppm; KUSM 100 ppm, methanol extract of the stem bark of *Kotschya uguenensis* at 100 ppm; KURM 50 ppm, methanol extract of the root bark of *Kotschya uguenensis* at 50 ppm; KURM 100 ppm, methanol extract of the root bark of *Kotschya uguenensis* at 100 ppm; columns with the same letter at a particular period of exposure are not significantly different at $P < 0.05$.

did not exhibit an acute effect (Fig. 1), but caused larval growth disruption with mosquitoes dying at the larval stage or before completing moulting (Fig. 2A–E). The KUSM and KURM extracts had an effect of elongating the gut and extruding some materials through the anal cavity, thus causing much of the peritrophic matrix to come out, thus weakening the gut of the mosquito larva and causing death in 6–8 days (Fig. 1). Abnormalities were observed 24 h post-exposure of larvae to the extracts. The larval abdomen elongated, forming tail-like structures that attained dark brown colouration in 48–72 h. When viewed under a microscope, the tail-like structures were identified as parts of the gut being extruded through the anal canal (Fig. 2B and C). Larva–pupa intermediates and pupa–adult intermediates were observed, but only for brief periods before they died (Fig. 2D and E). There were no significant differences ($P < 0.05$) in mortalities between the two concentration levels (50 and 100 ppm) of the two extracts (KUSM and KURM) after a particular period of exposure, but there was a significant difference with the control (Fig. 1). Mortality in the control experiment was up to 11.3% on the eighth day, while the rest of the larvae successfully emerged to adults. Detailed assay of the methanol extract of the stem and root bark and the subsequent fractions of the methanol extract of the stem bark showed substantial numbers of larvae with elongated guts (Fig. 3). At 100 ppm, larvae with elongated guts constituted 40% in the methanol extract of the stem bark treatment, while in the methanol-soluble fraction treatment, they constituted 88% (Fig. 3). This observation showed that the compound(s) contributing to larval deformities were present in large amounts in these treatments. Although the methanol extract of the root bark resulted in few deformed larvae (5%), it was equally active, attaining complete larval mortality at 100 ppm (Fig. 3).

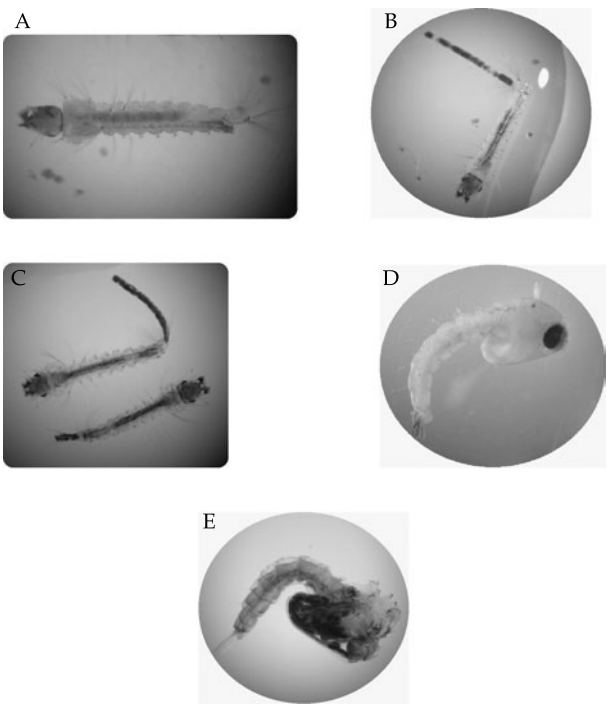

Fig. 2. Morphology of *Anopheles gambiae* s.s. immatures showing (A) a normal larval abdomen from the control experiment; (B) affected larval abdomen; (C) larvae with elongated guts after treatment with methanolic extracts of *Kotschya uguenensis*; (D) larva–pupa intermediate; (E) pupa–adult intermediate.

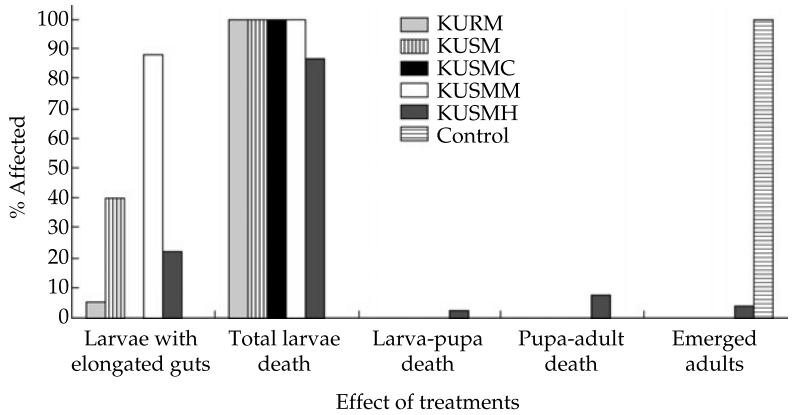

Fig. 3. Proportion of larval deformity and mortality in *Anopheles gambiae* s.s. upon exposure to methanolic extracts of the stem and root barks of *Kotschya uguenensis* and the subsequent fractions of the methanol extract of the stem bark at 100 ppm for 14 days ($n = 60$). KURM, methanol extract of the root bark of *Kotschya uguenensis*; KUSM, methanol extract of the stem bark of *Kotschya uguenensis*; KUSMC, dichloromethane-soluble fraction; KUSMM, methanol-soluble fraction; and KUSMH, water-soluble fraction.

Discussion

Information on the phytochemical and insecticidal properties of *K. uguenensis* using *A. gambiae* s.s. larvae is unavailable from the current literature. Results from this study revealed that, although polar compounds from the methanol extracts of the stem and root barks did not exhibit acute effects, most of the tested larvae died at larval stage or before completing moulting. Similar studies of methanolic extracts of the root bark of *Turraea mombassana* Hiern ex C. DC. (Meliaceae) resulted in larval and pupal deformities in *A. gambiae* s.s. due to incomplete melanization. The deformities were due to blends of several limonoids of medium polarity (Ndung'u *et al.*, 2004). In another study, the growth inhibition of *A. aegypti* mosquito larvae treated with acetone extracts of *Polygonum senegalense* (Meissn.) (Polygonaceae) was more potent than its constituent, quercetin. Larvae treated with quercetin attained reddish colouration in the thorax and abdomen which disappeared from surviving larvae in 3–5 days. The decolourization was thought to be due to detoxification and was consequently excreted as coloured substances (Gikonyo *et al.*, 1998). In this investigation, it is not yet known what exactly caused the elongated guts, but two reasons are likely. First, the extracts are not absorbed in the larval intestine thus causing muscular disturbances and are excreted together with the peritrophic matrix. Second, the extracts contain some compounds, which interfere with the endocrine system, preventing the release of the digestive enzymes, and this disturbance leads to the death of the treated mosquito larvae. In any case, since the peritrophic matrices are extruded in large amounts, the larva is left unprotected and any invasion of a compound or a pathogen can cause its death.

From these results, it can be concluded that methanolic extracts of *K. uguenensis* are good alternatives to synthetic larvicides, such as organochlorine, organophosphorus, carbamate and pyrethroid compounds, which have adverse environmental effects and high levels of multi-resistance (WHO, 1996; Najera and Zaim, 2002). Furthermore, active constituents of *K. uguenensis* are polar, and so therefore they can be slowly released in water when used in the natural form or ground in powder for managing mosquito breeding sites. Characterization of the constituents in the extracts and quality-controlled small-scale semi-field experiments to evaluate the effects of the pulverized plant materials and emulsion are underway. This will establish the best method of deployment of these active substances for large-scale application in the field.

Acknowledgements

This study was supported by *icipe* through a grant from the WHO/TDR Special Programme for Research and Training in Tropical Diseases and the Singenberg Foundation. We thank Mr F.M. Mbago from the Herbarium of the Botany Department at the University of Dar es Salaam, Tanzania, for the identification of the investigated plant species.

References

Dev S. and Kaoul O. (1997) *Insecticides of Natural Origin*. Harwood Academic Publishers, New Delhi, India. pp. 15–19.

Gikonyo N. K., Mwangi R. W. and Midiwo J. O. (1998) Toxicity and growth-inhibitory activity of *Polygonum*

senegalense (Meissn.) surface exudates against *Aedes aegypti* larvae. *Insect Science and Its Application* 18, 229–234.

Gillett J. B., Milne-Redhead E. and Polhill R. M. (1971) *Flora of Tropical East Africa; Papilionoideae*. Crown Agents, London. 414 pp.

Gusmão D. S., Páscoa V., Mathias L., Vieira I. J. C., Braz-Filho R. and Lemos F. J. A. (2002) *Derris (Lonchocarpus) urucu* (Leguminosae) extract, modifies the peritrophic matrix structure of *Aedes aegypti* (Diptera: Culidae). *Memórias do Instituto Oswaldo Cruz* 97, 371–375.

Najera J. A. and Zaim M. (2002) *Malaria Vector Control: Decision Making Criteria and Procedures for Judicious Use of Insecticides.* Ref: WHO/CDS/WHOPES/2002.5 Rev. 1. WHO, Geneva.

Ndung'u M., Torto B., Knols B. G. J. and Hassanali A. (2004) Laboratory evaluation of some eastern African Meliaceae as sources of botanicals for *Anopheles gambiae*. *International Journal of Tropical Insect Science* 24, 311–318.

SAS Institute, (2000) *SAS Procedure Guide for Personal Computers,* version 8.01. SAS Institute, Cary, NC.

Verdcourt B. (1971) *Flora of Tropical East Africa; Leguminosae Parts 3&4; Papilionoideae Parts 1&2*. Rotterdam, Netherlands. pp. 410–415.

WHO (World Health Organization) (1996) Protocols for Laboratory and Field Evaluation of Insecticides and Repellents: Report of the WHO Informal Consultation on the Evaluation and Testing of Insecticides. WHO, Geneva, 7–11 October 1996. Ref: CTD/WHOPES/IC/96.1.

WHO (World Health Organization) (2005) Guidelines for Laboratory and Field Testing of Mosquito Larvicides. WHO, Geneva. Ref: WHO/CDS/WHOPES/GCDPP/2005.13.