

Larvicidal activity of *Kotschya uguenensis* plant powders and methanol extracts against *Anopheles gambiae* s.s. larvae in the laboratory and in simulated ponds

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ABSTRACT

Polar constituents of *Kotschya uguenensis* Verdc. (Fabaceae) do not exhibit acute toxicity but cause growth disruption of *Anopheles gambiae* s.s. Gile (Diptera: Culicidae) larvae with eventual death. Time-course larvicidal effects of powders of root and stem barks and their crude methanol extracts in form of emulsions were compared in the laboratory and in artificial semi-field ponds. *Kotschya uguenensis* powders of root and stem barks and emulsions of their crude methanol extracts were assayed against *An. gambiae* s.s according to protocols of WHO 1996 & 2005. All formulations were equally effective under laboratory conditions giving 100% larval mortality within three days at a dose of 50 µg/ml of the extracts or concentrations of powders corresponding to the same level of extractable material. Under semi-field conditions, suspensions of the powder materials appeared to perform better than emulsions of methanol extracts. Time taken to give 80% mortality (LT₈₀) of larvae and pupa at 0.1% w/v was 6.06 days for powders of root bark and 5.60 days for powders of stem bark. The LT₈₀ for the root bark extract at 200 µg/ml was 8.28 days while that for the stem bark methanol extract was 12.47 days. No residual effects of the test materials on the larvae or pupae were evident in semi-field ponds 14 days after the re-introduction of the test materials. Our results suggest that, for the control of anophelines in the field, a weekly application of appropriate amounts of powders of *K. uguenensis* may be effective.

INTRODUCTION

Few eco-friendly botanical insecticides have been developed and used when compared to synthetic insecticides (Silva-Aguayo, 2000). However, problems associated with synthetic insecticides like environmental contamination, residues in food and feed, and development of pest resistance necessitate the search and use of more eco-friendly alternatives such as botanical insecticides. In recent years, two plants *viz.* Neem and Pongam trees have been acknowledged as prominent sources of effective biopesticides in semi-purified forms. *Pongamia* extracts have been considered as good synergists of malathion and cypermethrin and have been combined with these insecticides for the control of several pests (Rao *et al.*, 1997; Parmar *et al.*, 1987; Narasimhan *et al.*, 1998; Pathak *et al.*, 1998).

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The neem tree extracts have been formulated into insecticides, repellents, larvicides and growth-regulating products (Koul *et al.*, 1990; Moore *et al.*, 2003). In the developing world, however, a new pesticide, natural or synthetic, must be readily accessible, easy to use, acceptable, affordable and with low mammalian and environmental toxicity. This is because most people in the rural communities are poor and illiterate. Therefore, simple, low-technology methods of harvesting and using bioactive agents that can be adopted by individuals and communities are likely to make greater impact on their lives. Traditionally, *Kotschya uguenensis* Verdc. (Fabaceae) is used to deter chicken mite, *Dermanyssus gallinae* DeGeer (Acarina: Dermanyssidae), from infesting their hosts in some parts of East Africa (Innocent *et al.*, 2008, 2009). In a recent study, we investigated the larvicidal and larval growth inhibitory activities of solvent extracts of this plant against *Anopheles gambiae* s.s. Gile larvae in the laboratory with promising results (Innocent *et al.*, 2008).

Since the use of powder plant materials may be a more cost-effective and practical method of controlling larvae in natural ponds, in the present study we compared the effects of suspensions of pulverized root and stem bark of *K. uguenensis* with solvent extracts under similar conditions.

MATERIALS AND METHODS

Collection, authentication and processing of Plant materials

Root and stem barks of *Kotschya uguenensis* were collected at Ngwazi dam in Mufindi district, Tanzania. The plant species (voucher specimens No. FMM 3292) was identified by Mr. Mbago, F. and deposited at the Department of Botany Herbarium, University of Dar-es-Salaam, Tanzania. The root and stem barks of *Kotschya uguenensis* were separately air-dried under roof, pulverized and one portion of each soaked sequentially in *n*-hexane, dichloromethane and methanol, twice in each solvent, each occasion lasting for 72 h.

Each solvent extract was filtered and concentrated *in vacuo* in a rotary evaporator (yields from stem and root bark were 0.95 and 0.4% with hexane, 0.45 and 0.3% with dichloromethane, and 13.8 and 5.4% with methanol, respectively). Only methanol extract from the root and stem bark were used during these investigations. Each of the root and stem barks portions were pulverized, sieved to 30-150 μ m powder, and stored in a cold room at 4°C.

Preparation of emulsions of methanol extracts

Different solvents were initially tested to see which one was giving a homogeneous mixture and methanol was found to be the best. Stock solution of 200 mg/ml of each of the stem and root bark (8 g each) extracts were made in methanol (40 ml). About 5% w/w (0.4 g) of Tween 80 (Polyoxyethylene (20) sorbitan monooleate with 65 hydroxyl number and 45.55 saponification value) was added to homogenize the solution. Previously, Liu *et al.*, 2003 reported that methanol do permeate *Anopheles gambiae* peritrophic membrane; the small amount swallowed by larvae in the process of feeding is therefore easily extruded from the gut. Tween 80 is unlikely to be toxic to humans at the concentration used since it is used in pharmacological preparation of most drugs (Ten Tije *et al.*, 2003).

Mosquito larvae

Anopheles gambiae s.s. larvae were obtained from a colony maintained at the International Center of Insect Physiology and Ecology (ICIPE) insectary. This strain originated from Mbita-ICIPE station in western Kenya and it has been reared since 2003 at ICIPE-Nairobi insectary. Eggs were allowed to emerge in plastic containers filled with distilled water, and were transferred to large plastic pans (37 x 31 x 6 cm) at densities of 200-300 at the second instar stage. Larvae were fed on tetramin[®] fish food, and water temperature maintained at 26±2 °C throughout larval development.

Laboratory evaluation of powders and extracts

Growth inhibitory effect of *Kotschya uguenensis* powders and extracts were carried out in the laboratory using *Anopheles gambiae s.s.* larvae. 20 late 3rd or young 4th instar larvae in 100 ml of distilled water were exposed to various concentrations (10, 50 and 100 μ g/ml for the extracts, and 0.01, 0.05 and 0.1 % w/v of powdered suspensions; WHO, 1996; 2005). An equal proportion of Tween 80 was added to the control set of emulsions. For the experiment involving powdered plant materials, distilled water was used as blank. The test was triplicated from separately reared batches of larvae. Numbers of alive larvae and pupae were recorded every 24 h and any reduction in larval and pupal density calculated using Mulla's formula (Mulla *et al.*, 1971):

$$\% \text{ reduction} = 100 - (C_1 T_2 / C_2 T_1) \times 100\%$$

Whereby C_1 = pre-treatment larval density in controls,

C_2 = post-treatment larval and pupal density in controls

T_1 = pre-treatment larval density in treatments

T_2 = post-treatment larval and pupal density in treatments

During the experiment larvae were fed on Tetramin[®] fish food at 1 mg per beaker per day (water temperature 26 ± 2 °C). Results from laboratory investigations formed the basis of selecting the levels of concentrations of the emulsions or powders used at semi-field experiments.

Determination of efficacy of the four formulations in simulated ponds

The trial was carried out in a screen house (7 x 3.5 x 2 m) built on a pesticide free area at the ICIPE ground. The walls of the screen house were made of netting material from the ground up to 1 m high and the roof was covered with a polyethene sheet (λ_{max} = 205 nm). A total of 36 circular pools were dug in the ground of the screen house to fit large cylindrical plastic dishes of 50 cm diameter and 20 cm height. The dishes were filled with 3 litres of spring water collected from Githurai River, Nairobi. The inside of the dishes was smeared with a thin layer of mud from the floor of the screen house to mimic the environment of natural aquatic mosquito ponds. Early third instar *An. gambiae s.s.* Giles mosquito larvae (100 in number) were introduced into each of the artificial habitats and left for one hour to allow them to get acclimatized before introducing test samples. Based on the preliminary laboratory evaluation of these formulations, three concentrations of methanolic extracts (50, 100 and 200 μ g/ml) and powders of plant materials (750, 1500 and 3000 mg) were selected to be investigated at semi-field level. The ponds were covered with modified 'Saliternick' mosquito cages (50 x 50 x 50 cm) which were made from iron rods and covered with a net to prevent the escape of emerging adults. Larval and pupal densities and emergence of the adults was monitored after every 24 h. Standard dipping technique with an enamel bowl (400 ml) was used to sample larvae and pupae. This technique involves immersing a mosquito dipper (enamel bowl, with a long handle) in ponds at an angle of 45° (WHO, 2005). The test organisms that flowed into the bowl were picked by pipettes, counted and returned into the pond.

An interval of 2-3 minutes between each dip was used to allow larvae and pupae to return to the surface. In every pond, larval and pupal densities in five dips were counted and recorded. The pH and temperature of water in the ponds were also recorded everyday evening. Larvae were provided with Tetramin® fish food. Each test was replicated five times from separately reared batches of larvae. Percentage reduction of larval and pupal density was calculated using the Mulla's formula (Mulla *et al.*, 1971). Follow up tests to check for any residual efficacy of the test materials was carried out from the 14th day using 100 fresh early third instar *An. gambiae* s.s. larvae. The test solutions were not changed in the experimental ponds.

Study design and statistical analysis

Randomized block design was used in semi-field studies with two formulations in three concentration levels and their control, each arranged in five replicates. Means of larval and pupal densities in the five replicates were obtained after dipping five times per each replicate. Regression relationship between percentage reduction in larval and pupal density and post-treatment days were plotted and time taken to give 50% and 80% mortality (LT₅₀ and LT₈₀) calculated.

RESULTS

Performance of methanol extracts

The emulsions of the stem and root bark extracts of *K. uguenensis* gave complete larval mortality at a concentration of 50 µg/ml within 3 days when tested under laboratory conditions (Fig 1; Table 1). On the other hand, slightly different results were obtained when the assay was done under semi-field conditions where the activities of the extracts took several days (Fig 1; Table 1). At concentration of 200 µg/ml, the time taken to kill 80% (LT₈₀) of larvae and pupae for the root bark extract was 8.28 days and that for the stem bark extract was 12.47 days (Fig 1; Table 1) and both extracts prolonged the time of larval development relative to the control. For example, until day 8, larvae in the control ponds had all pupated while no pupa was emerged in the ponds treated with root bark extracts. Neither extract caused significant larval and pupal mortality below 200 µg/ml and after 14 days post treatment.

Performance of powdered plant materials

The powders of the stem and root barks of *K. uguenensis* gave comparable results under similar experimental conditions (Fig 2; Table 1). High mortality was recorded within a short time (~3 days) under laboratory conditions similar to the effects of methanol extracts (Fig 2; Table 1). Neither formulation gave any noticeable mortality below 0.05% w/v in semi-field experiments. Tests in simulated ponds at 0.1% w/v gave LT₈₀ value of 5.60 days for stem bark powder and 6.06 days for root bark powder. Re-introduction of larvae by 14th day post treatment into the ponds showed no significant larval or pupal mortality.

DISCUSSION AND CONCLUSIONS

Methanol extracts and plant powders performed better in the laboratory than in the artificial ponds under semi-field conditions. This may have been due to relatively high afternoon temperatures in the screen house and/or due to exposure of the active constituents to microbial populations in artificial ponds, which may have affected their biodegradation. Interestingly, the two plant powders performed better than methanol extracts in screen house experiments. More detailed time-course comparison of constituents of aqueous substrates associated with powder suspensions and solvent extracts may help to throw some light on this difference. From a practical perspective, pulverized root and stem barks of *K. uguenensis* constitute a safe and more readily accessible material for mosquito larval control by rural communities. The plant's constituents tend to disrupt larval development and prolonging the duration of different developmental stages and thus causing their eventual death. Similar growth-disrupting observations have been reported when immature stages of mosquitoes are exposed to tetranortriterpenoid (limonoid) constituents of *Azadirachta indica* A. Juss as well as those from other species of Meliaceae, Rutaceae, Cneoraceae, and Simaroubaceae family (Schmutterer, 1990). Studies involving these plant constituents indicate that much of their effects are due to their growth regulating properties rather than to their direct toxicity (Moore *et al.*, 2003). The major constituents encountered in the root and stem barks of *K. uguenensis* are glycoside terpenoids (Innocent, 2007). It would be interesting to isolate and elucidate their mode of action.

Table 1: Physico-chemical parameter and Lethal time (LT) of larvae and pupa population due to treatment with emulsion and powders of plant materials of *Kotschyia uguenensis* at laboratory and semi field conditions.

Formulation/ Treatment	Space Temp (°C)		Water Temp	pH	Regression equations	Lethal time (days)	
	Afternoon	Evening				LT ₅₀	LT ₈₀
CEL	32.22±1.30	23.75±1.16	23.87±0.40	8.25±0.22	y= 7.22ln (x) - 4.90	1999	127,299
REL				7.74±0.11	y= 91.49ln(x) + 0.72	1.74	2.38
SEL				7.78±0.09	y= 78.02ln (x)- 3.10	1.98	2.90
RPL				7.32±0.07	y= 73.92ln (x) + 9.49	1.74	2.59
SPL				7.46±0.18	y= 86.75ln (x) - 6.61	1.92	2.71
CPS	35.00±1.55	24.00±0.87	23.73±0.54	8.44±0.37	y= 10.62ln (x) - 2.62	142	2,397
RES				7.74±0.06	y= 43.91ln (x) - 12.81	4.18	8.28
SES				7.80±0.07	y= 33.54ln (x) - 4.63	5.01	12.47
SPS				7.32±0.11	y= 48.30ln (x) - 3.18	3.01	5.60
RPS				7.46±0.09	y= 46.06ln (x) - 2.87	3.15	6.06

REL and SEL (50 µg/ml) represent solvent extract of the root and stem barks, respectively, under laboratory conditions; RES and SES (200 µg/ml) represent solvent extract of the root and stem barks, respectively, under semi-field conditions; RPL and SPL represent plant powders of the root and stem bark, respectively, under laboratory conditions (0.1% w/v); RPS and SPS represent plant powders of the root and stem bark, respectively, under semi-field conditions (0.1% w/v); CPS and CEL represent control experiments for the plant powders and solvent extract respectively, under semi-field and laboratory conditions.

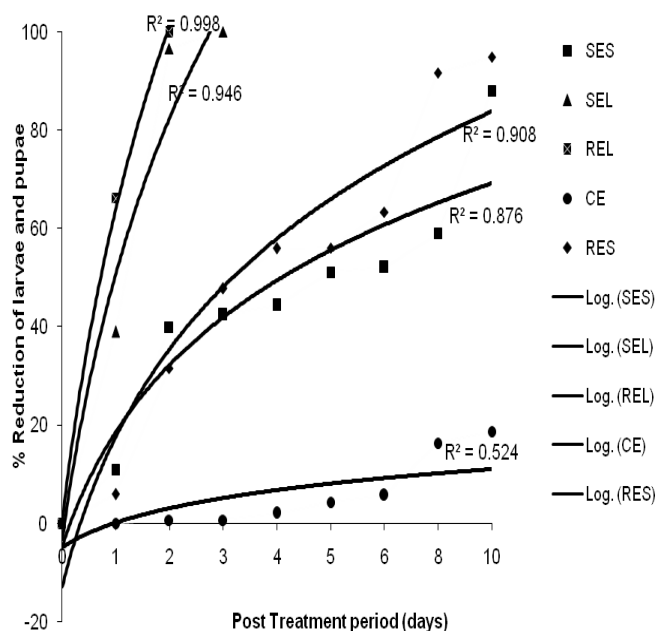


Fig. 1: Mean percentage reduction of larvae and pupae due to treatment with solvent extract of the root and stem bark under laboratory and semi-field conditions. REL and SEL (50 µg/ml) represent solvent extract of the root and stem barks, respectively, under laboratory conditions; RES and SES (200 µg/ml) represent solvent extract of the root and stem barks, respectively, under semi-field conditions; CE represents the control treatment.

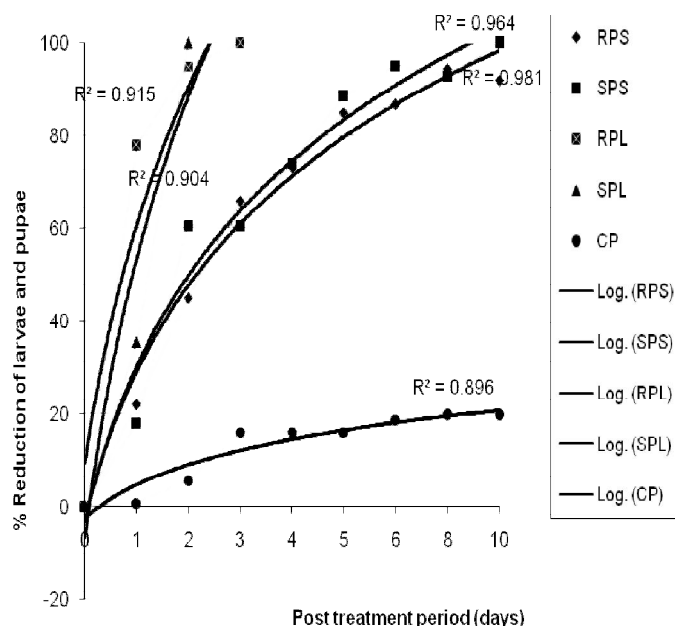


Fig. 2: Mean percentage reduction of larvae and pupae exposed to plant powders suspension under laboratory and semi-field conditions. RPL and SPL represent plant powders of the root and stem bark, respectively, under laboratory conditions (0.1% w/v); RPS and SPS represent plant powders of the root and stem bark, respectively, under semi-field conditions (0.1% w/v); CP represents the control treatment.

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DECLARATION OF INTEREST

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

REFERENCES

- Awad OM, Shimaili A. Operational use of neem oil as an alternative anopheline larvicide. Part A: Laboratory and field efficacy. *Eastern Mediterranean Health J.* 2003; 9: 637-645.
- Batra CP, Mittal PK, Sharma VP. Efficacy of neem oil-water emulsion against mosquito immatures. *Indian J Malariol.* 1998; 35:15-21.
- Gillett JB, Redhead M, Polhill RM. *Flora of tropical East Africa: Papilionoideae*, London, Crown Agents, (1971) 414.
- Innocent E, Joseph CC, Gikonyo NK, Nkunya MHH, Hassanali A. Growth disruption activities of polar extracts from *Kotschy uguensis* (Fabaceae) against *Anopheles gambiae* s.s. (Diptera: Culicidae) larvae. *Int J Trop Ins Sci.* 2008; 20: 220-224.
- Innocent E. *Antimosquito terpenoids and other constituents of selected Tanzanian Plants*. University of Dar es Salaam, Tanzania, *Thesis*, (2007) 200-215.
- Koul O, Isman MB, Ketkar CM. Properties and use of neem, *Azadirachta indica*. *Can. J Bot.* 1990; 68: 1-11.

- Liu X, Pan H, Mazur P. Permeation and toxicity of ethyl glycol and methanol in larvae of *Anopheles gambiae*. *J Exp Biol.* 2003; 206: 2221-2228
- Moore S, Camoron M, Hill N. Low technology approaches of the use of neem extract for mosquito control. In the Cole M. and Strang, R. (eds), *Science and application of neem*. Neemco, Irvine, (2003) 40-42.
- Mulla MS, Norland RL, Fanara DM, Darwazch HA, Makean D. Control of chironomid midges in recreational lakes. *J Econ Entomol.* 1971; 64: 300-307.
- Narasimhan V, Rajappan K, Ushamalani C, Kareem AA. Efficacy of new EC formulations of neem oil and pongam oil for the management of sheath rot disease of rice. *Phytoparasitica* 1998; 26: 301-306.
- Parmar BS, Dutta S. Evaluation of some oils as malathion synergists. *Intl J Trop Agric.* 1987; 5: 223-226.
- Pathak KML, Shukla RC. Efficacy of AV/EPP/14 (herbal ectoparasiticide) against canine demodicosis. *J Vet Parasitol.* 1998; 12: 50-1.
- Rao GR, Dhingra S. Synergistic activity of some vegetable oils in mixed formulations with cypermethrin against different instars of *Spodoptera litura* (Fabricius). *J Entomol Res.* 1997; 21: 153-60.
- Ten Tije AJ, Verweij J, Loos WJ, Sparreboom A. Pharmacological effects of formulation vehicles: Implications for cancer chemotherapy. *Clin Pharmacokinet.* 2003; 42: 665-685
- Sagar SK, Sehgal SS. Effect of aqueous extract of deoiled neem (*Azadirachta indica* A. juss.) seed kernel and Karanja (*Pongamia glabra* vent) seed kernel against *Culex quinquefasciatus*. *J Comm Dis.* 1996; 28: 260-269.
- Schmutterer H. Properties and potential of natural pesticides from the Neem tree, *Azadirachta indica*. *Ann Rev Entomol.* 1990; 35: 271-279.
- Silva-Aguayo G. (2000). Botanical insecticides. In: E. B. Radcliffe, W. D. Hutchison & R. E. Cancelado eds. *Radcliffe's IPM World Textbook*, University of Minnesota, St. Paul, MN [Online] <http://ipmworld.umn.edu/chapters/SilviaAguayo.htm>, Accessed on 10/12/2012.

World Health Organization. *Protocols for laboratory and field evaluation of insecticides and repellents*: Report of the WHO informal consultation on the evaluation and testing of insecticides, Ref: CTD/WHOPES/IC/96.1. WHO, Geneva, (1996) 37.

World Health Organization. *Guidelines for laboratory and field testing of mosquito larvicides*. Ref: WHO/CDS/WHOPES/GCDPP/2005.13. WHO, Geneva, (2005) 9.

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