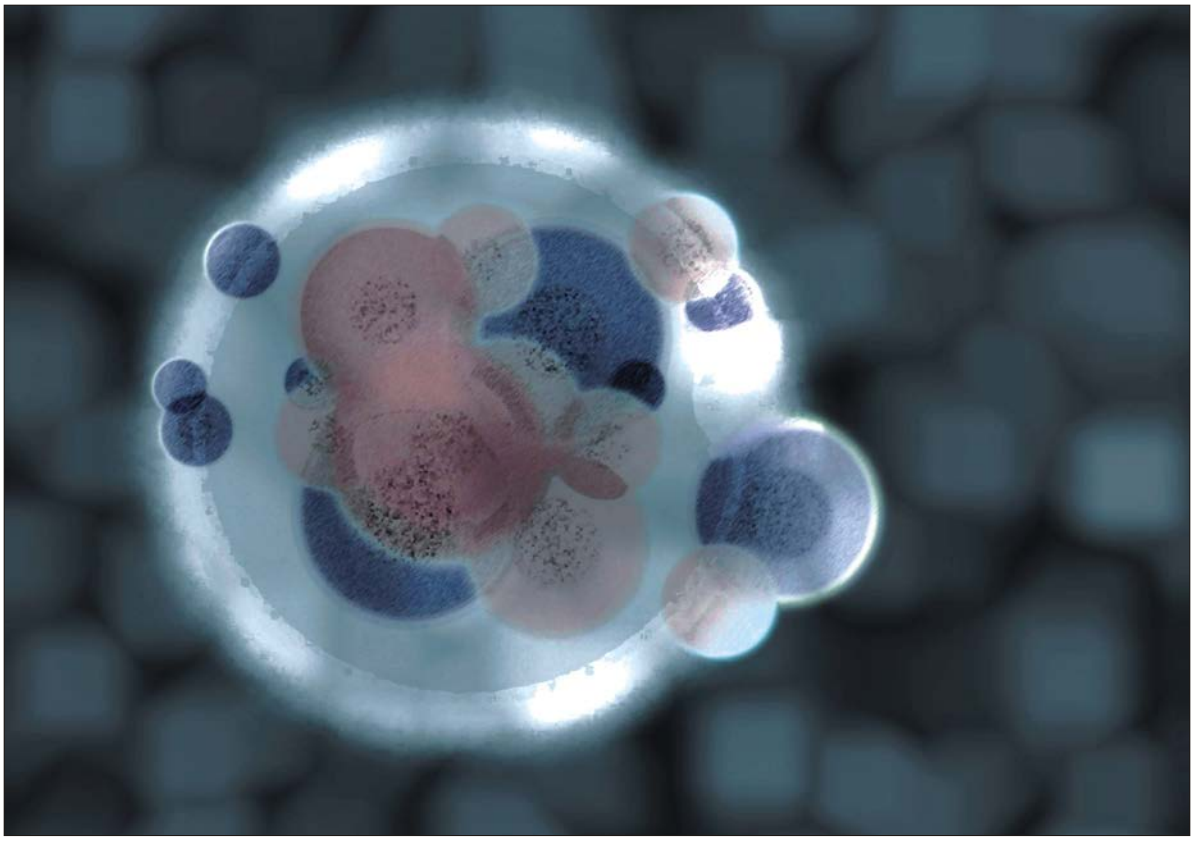

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Larvicidal Activity of *Piper guineense* and *Spilanthes mauritiana* Crude-Powder Against *Anopheles gambiae* and *Culex quinquefasciatus* in Kilifi District, Kenya

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Abstract: Field trials were conducted in Kilifi District, Kenya on the activity of *Piper guineense* and *Spilanthes mauritiana* powders against field populations of *Anopheles gambiae s.l.* and *Culex quinquefasciatus* larvae. Pools containing mosquito larvae were sampled and larval populations determined before and after application of plant powders. Four doses, 0.5, 1.0, 1.5 and 2.0 g L^{-1}, were used in the trials and larval mortality monitored after 24, 48 and 72 h. After 24 h, *P. guineense* powder at 0.5 g L^{-1} gave larval mortalities of 18.8 and 23.6% for *An. gambiae s.l.* and *Cx. quinquefasciatus*, respectively. At 2.0 g L^{-1}, mortalities at the same duration were 80.1 and 67.8% for *An. gambiae s.l.* and *Cx. quinquefasciatus*, respectively. In *S. mauritiana* treated larvae, in 24 h, mortality of 20% was obtained for both *An. gambiae s.l.* and *Cx. quinquefasciatus* at 0.5 g L^{-1}. For 2.0 g L^{-1}, at the same duration, mortalities of 98 and 100% for *An. gambiae s.l.* and *Cx. quinquefasciatus*, respectively, were recorded. After 72 h, at the highest dose, *S. mauritiana* and *P. guineense* powders induced larval mortalities of 100, 99.8%, in *An. gambiae s.l.* and 100, 97.7% in *Cx. quinquefasciatus*, respectively. At 24 h, the LD_{50} values were 0.98 and 0.76 g L^{-1} for *S. mauritiana* and *P. guineense*, respectively, for *An. gambiae s.l.* Similarly, LD_{50} of 0.85 and 0.68 g L^{-1}, respectively, for *Cx. quinquefasciatus* were obtained. *Piper guineense* and *S. mauritiana* derived powder yielded promising results and merit further study as potential larval control agents.

Key words: *Piper guineense*, *Spilanthes mauritiana*, *Anopheles gambiae s.l*, *Culex quinquefasciatus*, powder

INTRODUCTION

Insect-transmitted disease remains a major source of morbidity and mortality worldwide. Mosquito vectored pathogens infect more than 700 million people annually around the world through diseases such as malaria, filariasis, dengue, yellow fever, Rift valley fever and Japanese encephalitis (WHO, 2007). Malaria alone kills 3 million each year, including 1 child every 30 sec (WHO, 2005). Although mosquito-borne diseases currently represent a greater health problem in tropical and sub-tropical climates, no part of the world is immune to this risk (Fradin and Day, 2002). Control of such diseases is becoming increasingly difficult because of increasing resistance of mosquitoes to insecticides (Pates and Curtis, 2005; Senthil Nathan *et al*., 2005).

An alternative approach for mosquito control is the use of natural products of plant origin. The botanical insecticides are often active against a limited number of species including specific target insects, less expensive, easily biodegradable to non-toxic products and potentially suitable for use in integrated vector management programs (Alkofahi *et al*., 1989; Su and Mulla, 1999). In addition, most of the mosquito control programs by plant derived products target the larval stage in their breeding sites with larvicides, because adulticides may only reduce the adult population temporarily (El Hag *et al*., 2001).

Piper guineense Schum and Thonn is a dicotyledonous plant that belongs to the family Piperaceae. The insecticidal, larvicidal and repellent properties of *P. guineense* have recently been shown

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against the following insects, rust-red flour beetle *Tribolium castaneum* (Lale and Alaga, 2001), fish beetle *Dermestes maculatus* (Fasakin and Aberejo, 2002), cowpea weevil *Callosobuchus maculates* (Abdullahi and Muhammad, 2004) and banana weevil *Cosmopolites sordidus* (Inyang and Emosairue, 2005). Substantial evidence from chemical studies has shown that *P. guineense* contains naturally-occurring piperine-type alkaloids (Addae-Mensah *et al*., 1977). The alkaloids isolated from *P. guineense* were found to be very active on *Aedes aegypti* larvae (Addae-Mensah and Achieng, 1986). Consequently, under laboratory conditions, the same extracts have been shown to have high larvicidal activity against *An. gambiae s.l.* Giles (Okinyo, 2002).

Spilanthes mauritiana Rich belongs to the family Asteraceae. The plant has been reported to have many medicinal properties (Fabry *et al*., 1996a, b, 1998). The plant owes its activity to the antiseptic alkaloid spilanthol and immune-stimulating alkylamides (Fabry *et al*., 1998). *Spilanthes mauritiana* extracts, as potential insecticides, have not been extensively studied. However, hexane extracts of *S. mauritiana* were shown to be active against *Ae. aegypti* larvae and *Helicoverpa zea* neonates (Ramsewak *et al*., 1999). Jondiko (1989) also reported its larvicidal properties under laboratory conditions. The activity was found to be due to long chain fatty amides such as N-isobuyl-2E, 4E, 8Z, 10Z-dodeca-2,4,8,10-tetraenamide. These identified extracts demonstrated good activity against mosquitoes, but they were only evaluated under laboratory conditions. However, the evaluation of the activity should also preferably be carried out under field conditions using natural populations. A comparison for the activity of the larvicides under laboratory and natural conditions will provide a stronger basis for their use in mosquito control programmes. The objective of this study was to investigate the larvicidal activity of crude powder derived from the plants, *P. guineense* and *S. mauritiana*, against mosquitoes under field conditions.

MATERIALS AND METHODS

Study site: The study was conducted along Jaribuni stream (03° 36.81°S and 03° 949.28°E) of Jaribuni village in Kilifi District, Kenya. The selection of this site was dependent on 3 factors; known aquatic habitats of *An. gambiae s.l.* and *Cx. quinquefasciatus* larvae in the area, presence of a relatively high larval and adult populations of mosquitoes and the relative permanence of aquatic habitats in the area.

Plant collection and preparation: Green leaves of *P. guineense* and *S. mauritiana* were collected from Kakamega forest in Western Kenya and dried under shade for 30 days. The dry dark leaves were separated from the leaf petioles and ground into a fine powder by motor driven hammer mills. The powdered material was further filtered through a series of sieves with small (1 μm) mesh sizes to give the final material for bioassay.

Larval sampling and assays: Mosquito larvae were collected from aquatic habitats along Jaribuni stream pool in Kilifi District. Each habitat was first inspected for the presence of mosquito larvae. The mosquito larvae when present were sampled by standard dipping technique as described by Service (1993). A total of 36 circular pools of 35 cm in diameter and depth of 15 cm were dug 1 m from the edge of the stream. All the pools flooded with water during the study period were considered incomplete experiments and thus were not included in the analysis. Thirty six plastic wash basins (35×13 cm) with a capacity of 3500 mL smeared with mud to mimic the natural aquatic mosquito larval soil habitats found in the area were inserted into each pool. Water (3500 mL) from the river was introduced into each pool and 24 of the pools treated with a known amount of the plant-derived powder and 12 used as untreated controls. Into each of the artificial habitats, a known number (50-100) of *An. gambiae s.l.* and *Cx. quinquefasciatus* larvae of various instars were introduced. To 3500 mL water in each basin containing mosquito larvae, a known amount of the plant powder was added giving a known dose. The doses used were 0.5, 1.0, 1.5 and 2.0 g L^{-1}, respectively. Larval mortalities were monitored after 24, 48 and 72 h.

To determine the Lethal Doses (LD) for each powder, acute toxicity data were analyzed by Probit analysis (Finney, 1981). In all tests, percentage reduction of larvae was determined and the percentage mortality calculated indirectly using Abbott's formula, taking into account mortality in the controls (Abbott, 1925).

$$P_T = \frac{P_O - P_C}{100 - P_C} \times 100$$

Where:

P_T = Corrected % mortality
P_O = Observed % mortality
P_C = Control % mortality

Percentage data were transformed using square root (x + 1) prior to analysis of variance (ANOVA). Treatment means were compared and separated by Least Significant Difference (LSD) test at p = 0.05. Statistical analyses were performed using the statistical package SAS and Microsoft© Excel 2000.

RESULTS

The larvicidal activity of *P. guineense* powder to *An. gambiae s.l.* and *Cx. quinquefasciatus* mosquitoes are presented in Table 1. At 24 h, for 0.5 g L^{-1}, mortalities of 18.8 and 23.7% were obtained for *An. gambiae s.l.* and *Cx. quinquefasciatus*, respectively, at 72 h, this mortality increased to 80.1 and 67.8%, for the two species of larvae, respectively. At the highest dose, mortalities of 94.7 and 99.7% for *An. gambiae s.l.* and *Cx. quinquefasciatus*, respectively, were observed at 24 h. There was a reduction in *Cx. quinquefasciatus* larval mortality at 48 h compared to 24 h at doses 1.0, 1.5 and 2.0 g L^{-1}. The percentage mortality for the *An. gambiae s.l.* and *Cx. quinquefasciatus* exposed to *S. mauritiana* powder is shown in Table 2. At 24 h, for 0.5 g L^{-1}, mortality of 20% was recorded for both *An. gambiae s.l.* and *Cx. quinquefasciatus*, this increased at 72 h to 89.5 and 85.9% for the 2 species of larvae, respectively. For the highest dose, at 24 h, mortalities of 98.2 and 100% were recorded for *An. gambiae s.l.* and *Cx. quinquefasciatus*, respectively. At the highest dose, *Cx. quinquefasciatus* treated with *S. mauritiana* posted mortality of 100% at 24 h, however, 100% mortality was realized by *An. gambiae s.l.* after 72 h.

The results of the acute toxicity of *P. guineense* to the larvae of *An. gambiae s.l.* and *Cx. quinquefasciatus* are presented in Table 3. The slope from probit analysis and the lower and upper confidence limits of the LD_{50} and LD_{90} are also shown. At 24 h, LD_{50} values of 0.76 and 0.68 g L^{-1} for *An. gambiae s.l.* and *Cx. quinquefasciatus*, respectively, were recorded. The LD_{90} values at the same

Table 1: Percent mean larval mortality (±SE) of *Anopheles gambiae s.l.* and *Culex quinquefasciatus* introduced into artificial pools treated with *Piper guineeuse* powder for the duration 24, 48 and 72 h

		Time (h)		
Mosquito species	Dose (g L^{-1})	24	48	72
An. gambiae s.l.				
	0.5	18.80±1.44a	55.80±1.31a	80.10±1.17a
	1.0	86.20±1.37b	87.60±0.29b	92.30±0.59b
	1.5	97.90±1.04c	97.20±1.02c	99.40±1.04b
	2.0	97.40±1.27c	98.80±0.60c	99.80±0.14b
Cx. quinquefasciatus				
	0.5	23.60±1.17a	54.20±1.27a	67.80±1.69a
	1.0	92.40±1.75b	83.00±1.20b	91.70±1.33b
	1.5	99.00±0.44b	85.00±1.44b	93.60±1.63b
	2.0	99.70±0.17b	97.50±0.41c	97.70±1.29b

Values in column for each species followed by different letter(s) are significantly different (p>0.05, LSD test)

Table 2: Percent mean larval mortality (±SE) of *Anopheles gambiae s.l.* and *Culex quinquefasciatus* introduced into artificial pools treated with *Spilanthes mauritiana* powder for the duration 24, 48 and 72 h

		Time (h)		
Mosquito species	Dose (g L^{-1})	24	48	72
An. gambiae s.l.				
	0.5	20.30±1.86a	60.70±1.04a	89.50±1.66a
	1.0	47.30±1.56b	94.10±1.03b	98.80±1.62b
	1.5	89.20±1.59c	99.10±0.26b	100.00±0.00b
	2.0	98.20±1.05d	99.50±0.54b	100.00±0.00b
Cx. quinquefasciatus				
	0.5	20.10±1.98a	60.80±1.69a	85.90±1.33a
	1.0	63.80±1.57b	84.90±1.38b	90.00±1.44a
	1.5	96.80±1.52c	97.40±0.45c	98.30±0.76b
	2.0	100.00±0.0c	100.00±0.00c	100.00±0.00b

Values in column for each species followed by different letter(s) are significantly different (p>0.05, LSD test)

Table 3: Toxicity of *Piper guineense* powder to *Anopheles gambiae s.l.* and *Culex quinquefasciatus* larvae introduced into artificial pools for the duration 24, 48 and 72 h

		Lethal doses (g L^{-1})				
Mosquito species	Time (h)	LD_{50}	95% CL	LD_{90}	95% CL	Slope±SE
An. gambiae s.l.						
	24	0.76	0.47-0.95	1.27	1.05-1.75	2.51±0.57
	48	0.19	0.06-0.52	1.38	1.14-1.83	1.08±0.22
	72	0.36	0.19-0.48	0.83	0.73-0.92	1.31±0.16
Cx. quinquefasciatus						
	24	0.68	aa	1.12	aa	3.01±2.73
	48	0.35	0.21-0.47	1.53	1.43-1.66	1.08±0.09
	72	0.23	0.02-0.37	1.19	1.10-1.31	1.06±0.10

aa: LD_{50} and LD_{90} 95% CL not available

Table 4: Toxicity of *Spilanthes mauritiana* powder to *Anopheles gambiae s.l.* and *Culex quinquefasciatus* larvae introduced into artificial pools for the duration 24, 48 and 72 h

		Lethal doses (g L^{-1})				
Mosquito species	Time (h)	LD_{50}	95% CL	LD_{90}	95% CL	Slope±SE
An. gambiae s.l.						
	24	0.98	0.93-1.02	1.62	1.55-1.70	1.99±0.09
	48	0.26	0.09-0.45	1.09	0.94-1.30	1.55±0.24
	72	0.05	0.04-0.33	0.51	0.16-0.69	1.34±0.30
Cx. quinquefasciatus						
	24	0.85	0.75-0.95	1.41	1.29-1.58	2.29±0.23
	48	0.33	0.07-0.49	1.19	1.06-1.37	1.49±0.19
	72	0.27	0.05-0.42	0.78	0.67-0.88	1.21±0.16

duration were 1.27 and 1.12 g L^{-1} for the two species of larvae, respectively. For *P. guineense* treated *An. gambiae s.l.* and *Cx. quinquefasciatus*, there was an increase in LD_{90} at 48 h compared to 24 h, this was followed by a decrease at 72 h. While for *P. guineense* treated *An. gambiae s.l.*, the LD_{50} values were higher at 48 h than at 72 h. The lethal dose values observed with *S. mauritiana* treated larvae are presented in Table 4. For *S. mauritiana* treated larvae, at 24 h, LD_{50} values of 0.98 and 0.85 g L^{-1} were recorded for *An. gambiae s.l.* and *Cx. quinquefasciatus*, respectively. At the same duration, LD_{90} values obtained were 1.62 and 1.41 g L^{-1} for the two groups of larvae, respectively. As expected, in the *S. mauritiana* treated *An. gambiae s.l.* and *Cx. quinquefasciatus*, there was a decrease in lethal dose values with time.

DISCUSSION

The findings of the present investigation indicate larvicidal properties in the powders of *P. guineense* and *S. mauritiana* against two mosquito species *An. gambiae s.l.* and *Cx. quinquefasciatus*. Some plant crude extracts have also been studied for their efficacy to kill larvae of these mosquitoes. Larval mortality of *An. gambiae* and *Cx. quinquefasciatus* exposed to crude extracts of *Lepidagathis alopecuroides* and *Azadirachta indica* increased with time of exposure and concentration (Obomanu *et al*., 2006). Similarly, crude extracts of *Neorautanenia mitis* (Joseph *et al*., 2004) and *Cussonia barteri* (Diallo *et al*., 2001) exhibited larvicidal activity against both species. Crude extracts of *Turraea wakefieldii* and *Turraea floribunda* were also found to exert larvicidal activity against third-instar larvae of *An. gambiae s.s.* (Ndungu *et al*., 2004).

Although not significant, *Cx. quinquefasciatus* were more susceptible to *P. guineense* powder than the *An. gambiae s.l.* Unlike in the present study, some previous studies had shown that anophelines were more susceptible to plant extracts than culicines. Mwangi and Rembold (1988) reported that *An. arabiensis* larvae were more susceptible to *Melia volkensii* extracts than *Ae. aegypti*. Extracts of *Swartzia madagariensis* were more toxic to *An. gambiae s.l.* than *Ae. aegypti* and *Cx. quinquefasciatus* (Minjas and Sarda, 1986). The essential oil of *Pimpinella anisum* also showed higher toxicity against 4th instar larvae of *An. stephensi* than *Cx. quinquefasciatus* (Prajapati *et al*., 2005). Diallo *et al.* (2001) also observed that crude extracts of *Cussonia barteri* were more toxic to *An. gambiae* than *Cx. quinquefasciatus*. Similar trend have recently been reported by Obomanu *et al.* (2006). The difference in susceptibility could be attributed to the difference in the mode of feeding and physiological characteristics between the two groups of mosquitoes. *Anopheles gambiae* larvae are filter feeders, mainly ingesting food particles floating at the air-water interface (Clements, 1999). *Culex quinquefasciatus* feed below the water surface with their heads hanging down and the siphons anchored to the air water interface. In the larvae treated with *S. mauritiana* material, increasing exposure time resulted in asymptotic increase in larval mortality.

The high larvicidal activity against *Cx. quinquefasciatus* is advantageous since the culicines are vectors of filariasis amongst other diseases in sub-Saharan Africa. The asymptotic increase of mortality with time suggests that the larvae were feeding on the toxins continuously over time without any inhibition. However, in the pools treated with *P. guineense* material, for the doses, 1.0, 1.5 and 2.0 g L^{-1}, there were high mortalities after 24 h for first instars, which reduced at 48 h but increased after 72 h of exposure. This may suggest that, during larval sampling, mosquito eggs may have been collected together with the larvae. These eggs successfully hatched in the pools to first instars thus increasing the number of larvae after 48 h well above the initial sampled population at 24 h. However, the larvae often died 24 h after hatching suggesting that the powders are not ovicidal. These results support previous data by Mohsen *et al*. (1989) and Osmani and Sighamony (1980) who reported that ethanolic extracts of *Haplophylum tuberculatum* did not have any ovicidal effect but killed first instar larvae of *Cx. quinquefaciatus*. However, essential oils extracted from dried leaves of *Cymbopogon proximus*, *Lippia multiflora* and *Ocimum canum*, exhibited both larvicidal and ovicidal activity

against 3rd and 4th instar larvae of field-collected *Ae. aegypti*, *An. arabiensis* and *An. gambiae* (Bassole *et al*., 2003). Extracts of *Atriplex canescens* and *Artemisia annua* have also been shown to exert both ovicidal and larvicidal activity against *Cx. quinquefasciatus* (Ouda *et al*., 1998) and *An. stephensi* (Sharma *et al*., 2006), respectively. While the extracts of *Solanum trilobatum* and *Allium sativum* (garlic) were found to only possess ovicidal activity against *Cx. quinquefasciatus* (Rajkumar and Jebanesan, 2004) and *Ae. aegypti* (Jarial, 2001), respectively.

Studies conducted on the toxicity of powders from *P. guineense* and *S. mauritiana* on aquatic macro-invertebrates and two vertebrates revealed that, the powders are less toxic to some of the non-target aquatic organisms (Ohaga *et al*., 2007). The powders were less toxic to damselfly nymph (Gomphilidae), dragonfly nymph (Coenagrionidae), macro-dytiscids, micro-dytiscids (Dystiscidae), notonectids (backswimmers) (Notonectidae), freshwater shrimps (Palaemonidae), tadpoles (Ranidae) and tilapia fish (Cichlidae). This suggests that the plant powders could be used in mosquito breeding habitats co-inhabited by these predators complementing their roles towards population regulation of mosquitoes in integrated vector control (IVM).

To our knowledge, previous larvicidal experiments involved use of plant extracts and not crude-powder. The use of plant crude-powder reduces the cost of extraction and thus would make the larvicides more accessible to the resource poor rural farming communities, especially in irrigation schemes. In conclusion, the *P. guineense* and *S. mauritiana* derived materials could be useful for managing field populations of mosquitoes. Further studies on the insecticidal mode of action of these products, their possible effects on the environment and formulations for improving the potency and stability are needed for their practical use as naturally occurring mosquito larval control agents.

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