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 vectorborne 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; Si and Muller, 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 Thorn is a dicotyledonous plant that belongs to the family Piperaceae. The insecticidal, larvicidal and repellent properties of *P. guineense* have recently been shown
against the following insects, rust-red flour beetle Tribolium castaneum (Lale and Alaga, 2001), fish beetle Dermestes maculatus (Fasakin and Aberjeo, 2002), cowpea weevil Callosobruchus maculate (Abdullahi and Muhammad, 2004) and banana weevil Cosmopolites sordidus (Inyang and Emodaire, 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. (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 splanchthol 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-isobutyyl-2E, 4E, 8Z, 10Z-dodeca-2,4,8,10-tetranamide. 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’ N and 03° 94.28’ 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).

\[
P_r = \frac{P_O - P_C}{100 - P_C} \times 100
\]

Where:
- \(P_r\) = 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 6 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* powder 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 LD₉₀ and LD₄₀ are also shown. At 24 h, LD₉₀ values of 0.76 and 0.68 g L⁻¹ for *An. gambiae s.l.* and *Cx. quinquefasciatus*, respectively, were recorded. The LD₉₀ values at the same

<table>
<thead>
<tr>
<th>Mosquito species</th>
<th>Dose (g L⁻¹)</th>
<th>24</th>
<th>48</th>
<th>72</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>An. gambiae s.l.</em></td>
<td>0.5</td>
<td>18.80±1.44a</td>
<td>55.80±1.31a</td>
<td>80.10±1.17a</td>
</tr>
<tr>
<td></td>
<td>1.0</td>
<td>86.00±0.37b</td>
<td>87.60±0.29b</td>
<td>92.30±0.59b</td>
</tr>
<tr>
<td></td>
<td>1.5</td>
<td>97.60±1.40c</td>
<td>97.20±1.02c</td>
<td>99.40±0.49b</td>
</tr>
<tr>
<td></td>
<td>2.0</td>
<td>97.60±1.27c</td>
<td>98.80±0.90c</td>
<td>99.80±1.1b</td>
</tr>
<tr>
<td><em>Cx. quinquefasciatus</em></td>
<td>0.5</td>
<td>23.60±1.17a</td>
<td>54.20±1.27a</td>
<td>67.80±1.69a</td>
</tr>
<tr>
<td></td>
<td>1.0</td>
<td>92.00±1.75b</td>
<td>83.00±1.20b</td>
<td>91.70±1.33b</td>
</tr>
<tr>
<td></td>
<td>1.5</td>
<td>99.00±0.44b</td>
<td>85.00±1.44b</td>
<td>93.60±1.63b</td>
</tr>
<tr>
<td></td>
<td>2.0</td>
<td>99.00±0.17b</td>
<td>97.50±0.41c</td>
<td>97.70±1.29b</td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>Mosquito species</th>
<th>Dose (g L⁻¹)</th>
<th>24</th>
<th>48</th>
<th>72</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>An. gambiae s.l.</em></td>
<td>0.5</td>
<td>20.30±1.85a</td>
<td>60.70±1.04a</td>
<td>89.50±1.66a</td>
</tr>
<tr>
<td></td>
<td>1.0</td>
<td>47.30±1.56b</td>
<td>94.10±1.03b</td>
<td>98.80±1.62b</td>
</tr>
<tr>
<td></td>
<td>1.5</td>
<td>89.30±1.55c</td>
<td>99.10±0.26b</td>
<td>100.00±0.06b</td>
</tr>
<tr>
<td></td>
<td>2.0</td>
<td>98.20±1.05d</td>
<td>99.50±0.54b</td>
<td>100.00±0.08b</td>
</tr>
<tr>
<td><em>Cx. quinquefasciatus</em></td>
<td>0.5</td>
<td>20.10±1.98a</td>
<td>60.80±1.69a</td>
<td>85.90±1.33a</td>
</tr>
<tr>
<td></td>
<td>1.0</td>
<td>63.80±1.57b</td>
<td>84.90±1.38b</td>
<td>90.00±1.44a</td>
</tr>
<tr>
<td></td>
<td>1.5</td>
<td>96.80±1.52c</td>
<td>97.40±0.45c</td>
<td>98.30±0.76b</td>
</tr>
<tr>
<td></td>
<td>2.0</td>
<td>100.00±0.00c</td>
<td>100.00±0.00c</td>
<td>100.00±0.00b</td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>Mosquito species</th>
<th>Time (h)</th>
<th>LD₅₀</th>
<th>95% CL</th>
<th>LD₉₀</th>
<th>95% CL</th>
<th>Slope</th>
<th>SE</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>An. gambiae s.l.</em></td>
<td>24</td>
<td>0.76</td>
<td>0.47-0.95</td>
<td>1.27</td>
<td>1.05-1.75</td>
<td>2.5±0.57</td>
<td></td>
</tr>
<tr>
<td></td>
<td>48</td>
<td>0.19</td>
<td>0.06-0.52</td>
<td>1.38</td>
<td>1.14-1.83</td>
<td>1.08±0.22</td>
<td></td>
</tr>
<tr>
<td></td>
<td>72</td>
<td>0.36</td>
<td>0.19-0.48</td>
<td>0.83</td>
<td>0.73-0.92</td>
<td>1.31±0.16</td>
<td></td>
</tr>
<tr>
<td><em>Cx. quinquefasciatus</em></td>
<td>24</td>
<td>0.68</td>
<td>0.44-0.79</td>
<td>1.12</td>
<td>0.90-1.32</td>
<td>3.0±0.27</td>
<td></td>
</tr>
<tr>
<td></td>
<td>48</td>
<td>0.35</td>
<td>0.21-0.47</td>
<td>1.33</td>
<td>1.14-1.66</td>
<td>1.08±0.09</td>
<td></td>
</tr>
<tr>
<td></td>
<td>72</td>
<td>0.23</td>
<td>0.02-0.37</td>
<td>1.19</td>
<td>1.0-1.31</td>
<td>1.08±0.10</td>
<td></td>
</tr>
</tbody>
</table>

as: LD₅₀ and LD₉₀ 95% CL not available
Table 4: Toxicity of Sphingites mauritianus powder to Anopheles gambiae s.l. and Culex quinquefasciatus larvae introduced into artificial pools for the duration 24, 48 and 72 h

<table>
<thead>
<tr>
<th>Mosquito species</th>
<th>Time (h)</th>
<th>LD₅₀ (g L⁻¹)</th>
<th>95% CL</th>
<th>LD₉₀ (g L⁻¹)</th>
<th>95% CL</th>
<th>Slope±SE</th>
</tr>
</thead>
<tbody>
<tr>
<td>An. gambiae s.l.</td>
<td>24</td>
<td>0.98</td>
<td>0.93-1.02</td>
<td>1.62</td>
<td>1.55-1.70</td>
<td>1.99±0.09</td>
</tr>
<tr>
<td></td>
<td>48</td>
<td>0.26</td>
<td>0.09-0.45</td>
<td>1.09</td>
<td>0.94-1.30</td>
<td>1.55±0.24</td>
</tr>
<tr>
<td></td>
<td>72</td>
<td>0.05</td>
<td>0.04-0.33</td>
<td>0.51</td>
<td>0.16-0.69</td>
<td>1.34±0.30</td>
</tr>
<tr>
<td>Cx. quinquefasciatus</td>
<td>24</td>
<td>0.85</td>
<td>0.75-0.95</td>
<td>1.41</td>
<td>1.29-1.58</td>
<td>2.20±0.23</td>
</tr>
<tr>
<td></td>
<td>48</td>
<td>0.33</td>
<td>0.07-0.49</td>
<td>1.19</td>
<td>1.06-1.37</td>
<td>1.49±0.19</td>
</tr>
<tr>
<td></td>
<td>72</td>
<td>0.27</td>
<td>0.05-0.42</td>
<td>0.78</td>
<td>0.67-0.88</td>
<td>1.21±0.16</td>
</tr>
</tbody>
</table>

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 LD₅₀ 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 LD₅₀ 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, LD₅₀ 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, LD₉₀ 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 alopecuroëdes and Azadirachta indica increased with time of exposure and concentration (Obomanu et al., 2006). Similarly, crude extracts of Neorautamnia 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 madagascariensis 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 Haplophyllum tuberculatum did not have any ovicidal effect but killed first instar larvae of Cx. quinquefasciatus. However, essential oils extracted from dried leaves of Cymbopogon proximus, Lippia multiforma 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 (Dytiscidae), 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.

ACKNOWLEDGMENTS

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