Adulticidal Effects of Essential Oils Extracts from *Capsicum annuum* (Solanaceae) *Piper nigrum* (Piperaceae) and *Zingiber officinale* (Zingiberaceae) on *Anopheles gambiae* (Diptera-Culicidae), Vector of Malaria

G.A. Foko Dadji, J.L. Tamesse and F. Fekam Boyom

University of Yaoundé I, Higher Teacher’s Training College, Laboratory of Zoology, P.O. Box 47 Yaounde, Cameroon

University of Yaoundé I, Faculty of Science, Laboratory of Biochemistry, P.O. Box 812, Yaounde, Cameroon

Corresponding Author: G.A. Foko Dadji, University of Yaoundé I, Higher Teacher’s Training College, Laboratory of Zoology, P.O. Box 47, Yaounde, Cameroon. Tel: (237) 7752 36 50

ABSTRACT

We have evaluated the effect of *Capsicum annuum*, *Piper nigrum* and *Zingiber officinale* essential oils on *Anopheles gambiae* adults. Volatile oils extracted from this tree plants species were evaluated toxicity on adults of *An. gambiae* under laboratory conditions using WHO cones covered with volatile oils treated nets. Oils dissolved in acetone, were tested at 7.8, 3.9, 2, 1 and 0.3% (w/v). The knockdown effect 3 min after contact with the adults of *An. gambiae* is superior to 95% for the oils extracted from *C. annuum* and *Z. officinale* at concentrations of 7.8, 3.9 and 70% with the *P. nigrum* oil at concentration of 7.8 and 3.9%. Sixty minutes after, the knockdown effect is superior to 90% for oils extracted from *C. annuum* and *Z. officinale* at concentration of 7.8%. This effect is of 65% with the *P. nigrum* oil. Sixty minutes after, the knockdown effect is superior to 80% for oils extracted from *C. annuum* and *Z. officinale* at concentration 3.9%. This effect is of 46% with the *P. nigrum* oil. Twenty four hours after, for the concentrations of 7.8 and 3.9%, the mortality rate of adults is in average 90% for the oils extracted from *C. annuum* and *Z. officinale*. This rate is in average 45% with *P. nigrum* oil. *C. annuum* and *Z. officinale* seem to be very useful for the control of adults of *An. gambiae*.

Key words: *Anopheles gambiae*, *Capsicum annuum*, *Piper nigrum*, *Zingiber officinale*, biopesticides

INTRODUCTION

Malaria threatens the lives and livelihoods of more than 500 million Africans and exerts such a huge public health burden that it has been considered as one of the factors involved in the continued under-development of the continent as a whole (Snow et al., 1999). Most of sub-Saharan Africa has stable endemic malaria because climatic conditions, which are ideal for the transmission, coincide with the ranges of *Anopheles gambiae* Giles, *An. arabiensis* Patton and *An. funestus* Giles, the most efficient vector mosquitoes in the world (Beier et al., 1999). In Cameroon, *An. gambiae* is the principal vector of malaria in rural and urban areas (Manga et al., 1992). Malaria has been identified as a key contributor to slow economic growth and investment in Africa, because it experiences the most intense malaria transmission in the world (Beier et al., 1999). This can be due to parasite resistance to chloroquine and vector resistance to chemical insecticides (Carter and Mendis, 2002; Korenromp et al., 2003; Ter Kuile et al., 2004).
Faced with the resurgence of malaria, new control methods are developed including the use of impregnated mosquito nets Insecticide-Treated Nets (ITNs). The ITNs represent the most practical and cheapest methods to control these vectors (Copeland et al., 1995). Even the most efficacious of these, pyrethroid-treated bed nets, have proven difficult to implement on a sustainable basis for reasons of availability, acceptability and high cost (Snow et al., 1999). However, with increasing problems of toxicity to non target organisms and the resistance of mosquitoes to synthetic insecticides, our interest in natural or botanical insecticides has been revived.

Phytoproduts, on account of minimal hazardous effect on the environment and wide range of availability, offer promises in future mosquito control programs. They have revolutionized the fields of vector control, as they possess different bioactive components and can be used as general toxicants against various larval stages of the mosquito (Mohan et al., 2005) and other insect vectors of human diseases. Also, these can be used as repellants (Omolo et al., 2004), ovicides (Thenmozhi and Kingsly, 2004), feeding deterents (Thicson et al., 2004) and adulticides (Yang et al., 2004).

Essential oils from a large number of plants, including Ocimum sp. (Tawatsin et al., 2001), Cymbopogon sp. (Ansari and Razdan, 1995), Eucalyptus maculata citriodron (Collins and Brady, 1993), Pelargonium citrosum (Matsuda et al., 1996), Artemisia vulgaris (Hwang et al., 1985), Lantana camara (Seyoum et al., 2002a, b), Mentha piperita (Ansari et al., 2000), Vitex rotundifolia (Grayson, 2000), Curcuma sp. (Pitasawat et al., 2003), Conyza nevii, Plectranthus marrubioideus, Tetradenia riparia, Tarchonanthus camphoratus, Lippia javanica and L. ukambensis (Omolo et al., 2004), have been demonstrated to exhibit good repellent activities against mosquitoes.

Some of these plants are characterised as aromatic plants. One of the earliest reports on the use of plant extracts against mosquito larvae has been credited to Campbell et al. (1933), who found that plant alkaloids like nicotine, anabasine, methylanabasine and lupinine, extracted from the Russian weed Anabasis aphylla, killed Culex pipiens, Culex territans and Culex quinquefasciatus. Our previous study shows that Capsicum annuum (Solanaceae) powder have ovicidal, larvicidal and nymphicidal effect on An. gambiae and mortality rates (from eggs to adults) were 100% for Capsicum annuum yellow variety, 93% for Capsicum annuum red variety (Foko Dadjé et al., 2007). We didn't evaluate the adulticidal effects of Capsicum annuum on Anopheles gambiae.

Considering the chemical prospects, in addition to the primary compounds, the genus Capsicum contains various secondary metabolites such as alkaloids in the form of capsaicidine (Saber, 1976), capsaicin (Cordell and Araujo, 1993) and solanine (Newall et al., 1996), saponins (De Lucca et al., 2002), flavonoids and other phenolic compounds (Zang and Hamauru, 2003). Piper nigrum contains piperine and isobutyl amide compound, as major repellent properties because of its taste burning at high concentrations and after consumption, it affects the nervous system (Scott et al., 2002). According to Mercer and Anderson (1994), Z. officinale contains mainly the gingeberine. The primary and secondary metabolites found in these plants are likely to have insecticides effects on adults of An. gambiae.

The present study constitutes part of our bioprospecting project to screen African plants with mosquito repellent constituents. Due to this objective, we have evaluated the effect of Capsicum annuum, Piper nigrum and Zingiber officinale essential oils on An. gambiae adults.

MATERIALS AND METHODS
Mosquitoes: In 2005, Wild larvae (second and third instars) of Anopheles mosquitoes were collected by dipping on the field in Yaounde and carefully taken to the Laboratory at the University
of Yaoundé I. They were reared at room temperature (26-30°C), 70-80% RH in the spring water containing TetraMin® and pupae were put into 20 cm cubic cages. At emergence, adults were identified as An. gambiæ using Gilles and De Meillon’s (1968) key and put in separate cages. Eggs were collected on paper towels after blood feeding the females adults on rabbit of six months old. Adults were maintained in cages with a 10% glucose solution (Umar et al., 2008).

Plant material: Mature fruits of Capsicum annuum Linne (yellow and red varieties), seeds of Piper nigrum and root of Zingiber officinale were obtained from the Agronomic Research Institute for Development (Yaoundé-Cameroon).

Extraction of essential oils: Essential oils of our samples were obtained by the conventional method of hydrodistillation using Clevenger apparatus.

Matures fruits of C. annuum, roots of Z. officinale and seeds of P. nigrum are weighed and introduced into the ball with a quantity of water, approximately 3 to 5 times its weight. The set is brought to a boil on a hot cap. Water vapor in charge of oil rises in the column, condenses in the condenser (which circulates tap water continuously) and falls in the column to settle. It is formed of two phases:

- Aqueous-phase lower constantly recycled
- A higher organic phase consisting of fragrant essential oil, collected at the tap

The duration of distillation ranges from 5 to 12 h depending on the plant organ used. The traces of water are eliminated in the oil filter sodium sulfate anhydrous.

Oils are conserved in bottles, weighed and stored in a refrigerator at a temperature of around 4°C.

Test of essential oils on An. gambiæ adults: The mosquitoes used in this study were laboratory reared female Anopheles gambiæ. The insects were reared as describe above. The larvae were reared at 26-28°C and fed on TetraMin®. The adults were maintained on 10% glucose solution (Umar et al., 2008) and the females fed on rabbit blood thrice a week. Rearing temperatures and relative humidity in the adult insectaria were 26-28°C and 70-80%, respectively.

Impregnated mosquito nets: Twenty four pieces of mosquitoes nets (10×10 cm) were impregnated by day for a test. Each fragment is primarily spread separately in a petri dish smaller than for a good soak.

Five concentrations (7.8, 3.9, 2, 1 and 0.3% (w / v)) were used. In a test tube, we prepare dilutions oils with acetone concentrations for use. In the petri dish containing a piece of net, we impregnated gently tulle net until complete evaporation of acetone.

Test protocol: The fragment of nets is adjusted on the cone and maintained using the tape. Four cones are introduced as well prepared in the four holes in the Flexiglas and covered with a plexiglass plate thinner. The resulting device is maintained by clamps and placed on a wooden installation that is inclined at about 45°. Twenty mosquitoes are brought into contact with the cones, or 5 female mosquitoes per cone. The 3 min after contact, these mosquitoes are placed under observation in the cardboard cups covered with tulle non-impregnated mosquito nets. They are
observed every 10 min for 1 h to assess the effect of knockdown and then 24 h later we note the death of females. During this period, mosquitoes were fed with 10% sucrose solution. The experiment was repeated 6 times to minimize errors and have an effective reliable statistics. The control consists of 100 mosquitoes exposed to the tulle impregnated with only acetone and observed under the same conditions as those described above.

The test is conducted at a temperature of 26-28°C and a relative humidity of 70-80%.

**Data analysis:** To evaluate the effectiveness of essential oils on the mortality of female *An. gambiae*, we noted the following variables: Kd3, kd10, kd20, kd30, kd40, kd50 and kd60 and mortality after 24 h of observation for each concentration from the six replicates. The average ranking was done by the Scheffé test at the 5% interval. Dose response relationships were determine using tkd50 (time during which 50% of mosquitoes are knockdown) and tkd95 (time during which 95% of mosquitoes are knocked down) as expressed by the values obtained from the regression equations.

**Kd: knockdown:** (Kd3 = number of mosquitoes knockdown 3 min after exposure to the essential oil)

**RESULTS**

Sensitivity of *Anopheles gambiae* to essential oils extracted from red and yellow varieties of *Capsicum annuum*, *Piper nigrum* and *Zingiber officinale*.

To assess the effectiveness of essential oils on the mortality of females of *A. gambiae*, we noted the following: Kd3, kd10, kd20, kd30, kd40, kd50 and kd60 and mortality after 24 h of observation. The average ranking was done by the Scheffé test at the 5% threshold. From the rate of knockdown, a straight regression line is drawn and used to estimate the tkd50 (time at which 50% of mosquitoes are knocked down) and tkd 95 (time at which 95% of mosquitoes are knocked down).

**Sensitivity of Anopheles gambiae to essential oils extracted from red variety of Capsicum annuum:** The knockdown effect of mosquitoes after exposure to the essential oil was evaluated.

At 3 min of exposure, the percentage of mosquitoes knockdown varies from 25.8 at 0.3 to 100% at 7.8%. After 30 min of exposure, this percentage varies from 23% at 0.3 to 84 at 3.9%. Sixty min after exposure; it was between 23% at 0.3 and 92% at 7.9%. The mortality rate 24 h after exposure of mosquitoes to the essential oil of *C. annuum* red variety varies from 17% at 0.3 to 94% at 7.8% of essential oil (Table 1).

The analysis test of Scheffé shows that the knockdown rate of mosquitoes is roughly constant over time (90%) for 7.8 and 3.9% concentration oil where this Kd rate is less than 80% for concentration 2%. At the concentrations 1%, the knockdown rate of mosquitoes decreases over time ranging from 77 to 40%. At 0.3%, the knockdown rate of mosquitoes fairly decreases from 29 to 17% (Table 1). The exploitation of the regression line of the kd percentage over time and depending on the concentration of the product lets show the slope of different lines representing the ratio of the rate of mosquitoes knocked down on the time and revealed the time required for 95 and 50% knockdown of the mosquitoes in the presence of the oil extracted from *C. annuum* red variety. At 7.8 and 3.9%, the slope were low, respectively 0.188 and 0.233. 95% of the mosquitoes are knocked down (kd95), after 6 min 23 sec and 4 min 46 sec, respectively for the concentration of
Table 1: Effects of essential oil extracted from Capsicum annuum red variety on Anopheles gambiae

<table>
<thead>
<tr>
<th>Time</th>
<th>7.8%</th>
<th>3.9%</th>
<th>2%</th>
<th>1%</th>
<th>0.3%</th>
</tr>
</thead>
<tbody>
<tr>
<td>3 min</td>
<td>100±0.0 a</td>
<td>99.1±0.49 a</td>
<td>87.5±1.22 a</td>
<td>76.6±0.81 a</td>
<td>25.8±0.98 a</td>
</tr>
<tr>
<td>10 min</td>
<td>91.6±1.21 a</td>
<td>90.6±0.89 b</td>
<td>83.3±1.03 b</td>
<td>68.3±1.86 b</td>
<td>20.1±1.16 b</td>
</tr>
<tr>
<td>20 min</td>
<td>90.0±1.20 a</td>
<td>85.8±1.16 b</td>
<td>79.1±1.16 c</td>
<td>59.1±1.94 c</td>
<td>23.3±1.36 c</td>
</tr>
<tr>
<td>30 min</td>
<td>80.8±0.98 a</td>
<td>83.3±0.51 b</td>
<td>71.6±1.03 d</td>
<td>53.3±1.96 e</td>
<td>23.3±1.21 e</td>
</tr>
<tr>
<td>40 min</td>
<td>90.8±0.98 a</td>
<td>83.3±1.03 b</td>
<td>68.3±0.81 d</td>
<td>48.3±0.81 d</td>
<td>26.6±1.63 e</td>
</tr>
<tr>
<td>50 min</td>
<td>77.6±0.68 a</td>
<td>82.5±1.04 b</td>
<td>65.8±0.75 c</td>
<td>47.5±0.83 d</td>
<td>25.8±1.72 e</td>
</tr>
<tr>
<td>60 min</td>
<td>90.8±0.98 a</td>
<td>83.3±1.21 b</td>
<td>61.5±0.36 b</td>
<td>47.5±1.22 d</td>
<td>22.5±1.22 e</td>
</tr>
<tr>
<td>24 h</td>
<td>94.1±1.32 a</td>
<td>80.8±1.16 b</td>
<td>61.5±1.21 b</td>
<td>40.0±0.89 b</td>
<td>16.6±1.21 b</td>
</tr>
</tbody>
</table>

Within the same column, averages weighted by the same lowercase letter do not differ statistically between them (Scheffé test) (p<0.05)

Fig. 1: The regression line of the effects of essential oil from Capsicum annuum red variety on Anopheles gambiae

7.8 and 3.9%. The value of the slope was 0.510 at 1% concentration and 50% of mosquitoes are knocked down (kd50) after 44 min 39 sec. (Fig. 1).

Sensitivity of Anopheles gambiae to essential oils extracted from the yellow variety of Capsicum annuum: The knockdown effect of mosquitoes (kd) after exposure to the essential oil of C. annuum yellow variety varies with the concentration and the duration of exposure.

Three minutes after exposure, the average percentage of mosquitoes’ knockdown varies from 67% at 0.3 to 100% at 7.8%, of essential oil concentration. Thirty minutes after exposure, the average percentage of mosquitoes knockdown was between 56% at 0.3 and 98% at 7.8% concentration of essential oil. Sixty minutes after exposure, the average rate of mosquitoes knockdown varies from 45% at 0.3 to 98% at 7.8%, concentration of essential oil. (Table 2). The mortality rate 24 h after exposure of mosquitoes to the essential oil of C. annuum yellow variety varies from 18% at 0.3 to 92% at 7.8%, concentration of essential oil. The analysis test of Scheffé shows that the knockdown rate of mosquitoes varies with the percentage of essential oil and the duration of exposure. This percentage is almost constant and equal to 98% at 7.8% of essential oil.

This analysis shows a significant decrease in the percentage of mosquito knockdown between the 3rd and 60th minute after exposure.
Table 2: Effects of essential oil extracted from Capsicum annuum yellow variety on Anopheles gambiae

<table>
<thead>
<tr>
<th>Time</th>
<th>7.8%</th>
<th>3.9%</th>
<th>2%</th>
<th>1%</th>
<th>0.3%</th>
</tr>
</thead>
<tbody>
<tr>
<td>3 min</td>
<td>100.0±0.0⁸</td>
<td>90.15±0.40⁸</td>
<td>91.65±0.81⁹</td>
<td>79.15±0.5⁹</td>
<td>67.50±1.37⁹</td>
</tr>
<tr>
<td>10 min</td>
<td>100.0±0.0⁸</td>
<td>90.15±0.40⁸</td>
<td>93.30±1.03⁹</td>
<td>76.65±1.36⁹</td>
<td>67.50±1.51⁹</td>
</tr>
<tr>
<td>20 min</td>
<td>97.50±0.54⁸</td>
<td>97.50±0.83⁸</td>
<td>86.65±0.81⁹</td>
<td>73.30±0.81⁹</td>
<td>6415.00±0.98⁸</td>
</tr>
<tr>
<td>30 min</td>
<td>98.30±0.81⁹</td>
<td>95.00±0.63⁸</td>
<td>86.65±1.5⁹</td>
<td>74.15±1.72⁹</td>
<td>55.80±0.98⁹</td>
</tr>
<tr>
<td>40 min</td>
<td>95.00±1.29⁹</td>
<td>91.65±1.03⁸</td>
<td>78.30±1.75⁸</td>
<td>66.65±2.58⁸</td>
<td>53.30±1.21⁸</td>
</tr>
<tr>
<td>50 min</td>
<td>96.65±0.81⁹</td>
<td>85.80±0.98⁸</td>
<td>73.30±1.21⁸</td>
<td>60.65±2.28⁸</td>
<td>49.15±0.98⁹</td>
</tr>
<tr>
<td>60 min</td>
<td>93.30±1.5⁹</td>
<td>85.80±1.16⁸</td>
<td>69.15±2.13⁸</td>
<td>55.00±2.09⁸</td>
<td>45.00±0.63⁹</td>
</tr>
<tr>
<td>24 h</td>
<td>91.65±1.5⁹</td>
<td>82.50±1.2⁸</td>
<td>48.30±0.81⁸</td>
<td>44.15±0.98⁹</td>
<td>18.30±0.81⁸</td>
</tr>
</tbody>
</table>

Within the same column, averages weighted by the same lowercase letter do not differ statistically between them (Scheffé test) (p<0.05)

Fig. 2: The regression line of the effects of essential oil from Capsicum annuum yellow variety on Anopheles gambiae

For the concentrations of 3.94, 2, 1 and 0.3% of the essential oil we noted that at 3.9% the percentage of mosquitoes knocked down decrease from 99 to 86%. At 2% this kd rate decreases from 92 to 69%. At 1%, this kd percentage decreases from 79 to 55%. At 0.3% concentration it decreases from 67 to 45 % (Table 2).

The regression lines of the effect of essential oil of C. annuum yellow variety shows that at 7.8% and 3.9% the slope were low, respectively 0.107 and 0.269. Ninety five percent of mosquitoes are knocked down (kd95) after 51 min 20 sec and 24 min 40 sec, respectively for the concentration of 7.8 and 3.9%. The value of the slope was 0.416 at 0.3% concentration and 50% of the mosquitoes are knocked down (kd50) after 48 min 1 sec (Fig. 2).

Sensitivity of Anopheles gambiae to essential oils extracted from Piper nigrum: The knockdown (kd) effect of mosquitoes after exposure to essential oil of P. nigrum varies with the concentration and the duration of exposure.

Three min after exposure, the average kd percentage of mosquitoes varies from 33% at 0.3% to 82% at 7.8%.

At 30 min after exposure, the average kd rate of mosquitoes knocked down was between 19% at 0.3% and 65% at 7.8%, of essential oil.
At 60 min after exposure, the average number of mosquitoes knockdown varies from 12% at 0.3% to 63% at 7.8%, of essential oil (Table 3).

The analysis of the variation in the kd rate of mosquitoes over time shows that this percentage decreases statistically between 3 and 60 min after contact with essential oil at all concentrations. The mortality rate of Anopheles 24 h after contact with essential oil of *Piper nigrum* varies from 10% at 0.3 to 63% at 7.8%, (Table 3).

The regression lines of the effect of the essential oil of *P. nigrum* show that at 7.8% the slope was 0.280, the value of the slope were 0.446 and 0.437, respectively at 3.9 and 2% concentration and 50% of the mosquitoes are knocked down (kd50) after 42 min 6 sec and 12 min 35 sec, respectively at 3.9 and 2% concentration (Fig. 3).

**Sensitivity of Anopheles gambiae to essential oils extracted from Zingiber officinale:**
The knockdown effect of mosquitoes (kd) after exposure to the essential oil of *Z. officinal* varies with the concentration and the duration of exposure. At 3 min after exposure, the average kd percentage of mosquitoes varies from 12.5% at 0.3 to 100% at 7.8% concentration of essential oil. Thirty minutes after exposure, the average knockdown percentage was between 11% at 0.3 and 99% at 7.8% concentration of essential oil. Sixty minutes after exposure, the average knockdown

<table>
<thead>
<tr>
<th>Time</th>
<th>7.8%</th>
<th>3.9%</th>
<th>2%</th>
<th>1%</th>
<th>0.3%</th>
</tr>
</thead>
<tbody>
<tr>
<td>3 min</td>
<td>81.65±0.81&lt;sup&gt;a&lt;/sup&gt;</td>
<td>72.50±0.83&lt;sup&gt;a&lt;/sup&gt;</td>
<td>57.50±1.04&lt;sup&gt;a&lt;/sup&gt;</td>
<td>46.65±0.83&lt;sup&gt;a&lt;/sup&gt;</td>
<td>33.30±1.36&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>10 min</td>
<td>74.15±1.83&lt;sup&gt;b&lt;/sup&gt;</td>
<td>66.65±1.39&lt;sup&gt;b&lt;/sup&gt;</td>
<td>52.50±1.51&lt;sup&gt;b&lt;/sup&gt;</td>
<td>43.30±1.36&lt;sup&gt;b&lt;/sup&gt;</td>
<td>28.30±1.98&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>20 min</td>
<td>66.65±1.89&lt;sup&gt;c&lt;/sup&gt;</td>
<td>53.30±1.39&lt;sup&gt;c&lt;/sup&gt;</td>
<td>41.65±1.21&lt;sup&gt;c&lt;/sup&gt;</td>
<td>34.15±1.16&lt;sup&gt;c&lt;/sup&gt;</td>
<td>22.50±1.37&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>30 min</td>
<td>65.00±1.67&lt;sup&gt;d&lt;/sup&gt;</td>
<td>51.65±1.21&lt;sup&gt;d&lt;/sup&gt;</td>
<td>40.80±0.98&lt;sup&gt;d&lt;/sup&gt;</td>
<td>35.80±1.47&lt;sup&gt;d&lt;/sup&gt;</td>
<td>19.15±0.98&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
<tr>
<td>40 min</td>
<td>65.80±1.47&lt;sup&gt;e&lt;/sup&gt;</td>
<td>50.00±0.89&lt;sup&gt;e&lt;/sup&gt;</td>
<td>37.50±1.64&lt;sup&gt;e&lt;/sup&gt;</td>
<td>24.15±1.47&lt;sup&gt;e&lt;/sup&gt;</td>
<td>20.00±1.67&lt;sup&gt;e&lt;/sup&gt;</td>
</tr>
<tr>
<td>50 min</td>
<td>63.30±1.21&lt;sup&gt;f&lt;/sup&gt;</td>
<td>45.80±0.98&lt;sup&gt;f&lt;/sup&gt;</td>
<td>30.80±1.32&lt;sup&gt;f&lt;/sup&gt;</td>
<td>22.50±1.76&lt;sup&gt;f&lt;/sup&gt;</td>
<td>12.50±1.04&lt;sup&gt;f&lt;/sup&gt;</td>
</tr>
<tr>
<td>60 min</td>
<td>63.30±1.21&lt;sup&gt;g&lt;/sup&gt;</td>
<td>46.65±1.21&lt;sup&gt;g&lt;/sup&gt;</td>
<td>30.80±1.47&lt;sup&gt;g&lt;/sup&gt;</td>
<td>23.30±1.63&lt;sup&gt;g&lt;/sup&gt;</td>
<td>10.00±1.26&lt;sup&gt;g&lt;/sup&gt;</td>
</tr>
<tr>
<td>24 h</td>
<td>58.30±1.21&lt;sup&gt;h&lt;/sup&gt;</td>
<td>44.15±1.32&lt;sup&gt;h&lt;/sup&gt;</td>
<td>26.65±1.89&lt;sup&gt;h&lt;/sup&gt;</td>
<td>19.15±1.47&lt;sup&gt;h&lt;/sup&gt;</td>
<td>5.00±0.65&lt;sup&gt;h&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

within the same column, averages weighted by the same lowercase letter do not differ statistically between them (Scheffe test) (p<0.05)

![Fig. 3: The regression line of the effects of essential oil from *Piper nigrum* on *Anopheles gambiae*](image.png)
rate of mosquitoes varies from 9% at 0.3 to 99% at 7.8%, concentration of Z. officinalis essential oil (Table 4). Twenty four hours after contact between mosquitoes and the essential oil of Zingiber officinale one record mortality rate between 9% at 0.3 and 99% at 7.8% concentration of essential oil.

The analysis of the variation in the percentage of mosquitoes' knockdown during the exposure is almost constant for concentrations 7.8, 2, 1 and 0.3%. The value was 99% at 7.8%, 75% at 2%, 48% at 1 and 10% at 0.3%.

At 3.9%, the percentage of mosquitoes knockdown significantly decreased between 3 to 60 min. The exploitation of the regression line of kd percentage over time and depending on the concentration of the product lets show the slope of different lines representing the ratio of the rate of mosquitoes knocked down on the time and reveal the time required for 95 and 50% knockdown of mosquitoes in the presence of the oil extracted from Z. Officinale. At 7.8 and 3.9%, the slope were low, respectively 0.027 and 0.130. 95% of the mosquitoes are knocked down (kd95) after 45 min 54 sec at 3.9% concentration. The value of the slope was 0.293 at 1% concentration and 50% of mosquitoes are knocked down (kd50) after 25 min 57 sec (Fig. 4).

<table>
<thead>
<tr>
<th>Table 4: Effects of essential oil extracted from Zingiber officinale on Anopheles gambiae</th>
</tr>
</thead>
<tbody>
<tr>
<td>Concentrations (w/v)</td>
</tr>
<tr>
<td>Time</td>
</tr>
<tr>
<td>3 min</td>
</tr>
<tr>
<td>10 min</td>
</tr>
<tr>
<td>20 min</td>
</tr>
<tr>
<td>30 min</td>
</tr>
<tr>
<td>40 min</td>
</tr>
<tr>
<td>50 min</td>
</tr>
<tr>
<td>60 min</td>
</tr>
<tr>
<td>24 h</td>
</tr>
</tbody>
</table>

Within the same column, averages weighted by the same lowercase letter do not differ statistically between them (Sheffé test) (p<0.05)

![Regression line of the effects of essential oil from zingiber Officinale on Anopheles gambiae](image)

**Fig. 4:** The regression line of the effects of essential oil from zingiber Officinale on Anopheles gambiae
DISCUSSION

The extracts of a considerable number of plants are now known to have an effect on anopheles. Some extracts have a broad spectrum toxicity in short, medium and long term (Tripathi et al., 2004). Very few studies have examined the ability of essential oils to kill adult mosquitoes upon direct contact. Our study has shown that An. gambiae is sensitive to concentrations 7.8, 3.9 and 2% of essentials oils of C. annuum red and yellow varieties and concentrations 7.8 and 3.8% of essentials oils of Z. officinalis. Extracts of the fruits of these plants contain alkaloids derivatives, capsaicin in C. annuum and gingerine in Z. officinalis. In addition there was a stronger sensitivity of An. gambiae to C. annuum yellow variety extract, which contains high concentration of capsaicin. Madhumathy et al. (2007) was reported toxicity effect of Capsicum annum ethanol extract on Cx. quinquefasciatus and An. stephensi larvae. It has been suggested that capsaicin, the major component content in fruits of C. annum, although, its insecticidal activity has not yet been well defined, inhibits the development of insects and is involved in certain oxidative reactions (Diaz et al., 1998). What is the active ingredient in other components used in your analysis.

Alkaloids are known to have toxic effects in contact with the embryos, through contact and/or ingestion by adult mosquitoes (Gakuru and Foua, 1996). Flavonoids and saponins currently present in plants extract are also toxic to the mosquito (Bouchelta et al., 2005). Thus the combined action of flavonoids and saponins can increase the level of toxicity of essential oils extracted from C. annum and reduce the potential development of resistance. The variety of toxic compounds involves different mechanisms of action. This limits the effectiveness of the mechanism of detoxification of these compounds (Shama and Dhiman, 1993; Corbel et al., 2004).

Essential oils are plant natural products for potential control of An. gambiae because some of them are selective, have small effects or no effect on non-target and can be used like other conventional insecticides (Morsy et al., 2000). Thus the use of biopesticides is an alternative way to circumvent the resistance of mosquitoes to pyrethroid, organophosphate, carbamate and DDT which are chemical insecticides currently recommended (Zaim et al., 2000). Like all current insecticides approved for ITNs, our essentials oils are extremely rapidly after contact and may reduce mosquito lifetime reproductive success.

Our previous work showed a decrease in eggs hatching rate, a high mortality rate at different development stages and a long duration of different development stages of An. gambiae rearing medium containing red and yellow varieties of C. annum, powder (Foko Dadji et al., 2007).

The knockdown effect, a key factor in preventing mosquito bites, was more than 95%; 3 min after the mosquitoes were in contact with the extracts of C. annum and Z. officinalis for concentrations of 7.8 and 3.9%. Moreover, the mortality rate after 24 h is about 90% which shows the sensitivity of An. gambiae to these products. The mosquitoes affected will not survive in a hostile environment such as natural mosquitoes because their activities (jumping, flying) will be reduced and they will be easily captured by their natural predators. Present results are in conformity with those of Kang et al. (2009), who obtain adulticidal activities ranging from 68 to 98% at 1hr after treatment on Culex pipiens pallens adults with the application of the essentials oils of cardamon, coriander, rosemary and sandalwood.

Present result indicate strong adulticidal activity of Z. officinale against An. gambiae which is confor to Agarwal et al. (2001) results who reported insect growth inhibitor and antifeeding activity against Spilarctia oblique in the same oil. Whereas, Prajapati et al. (2005) has reported ovicidal activity but not adulticidal activity in essential of Z. officinale against An. stephensi.

Extract of Piper nigrum fruits exhibited potent knockdown and lethal activity against An. gambiae adults. Park et al. (2002) observed the same effect on Culex pipiens pallens, Aedes
aegypti and Aedes togoi. These authors showed that the insecticidal activity of Piper nigrum extract is due to isobutylamides compounds not to piperine, principal component of extract.

Present results showed that essential oil of Piper nigrum had less effect on An. gambiae compared to oils of Capsicum annum and Zingiber officinalis. Although an alkaloid, piperine is the main compound. This low activity of piperine on An. gambiae can be due to rapid detoxification of the compound or a lack of specific receptors in the insect.

Protection against mosquitoes is achieved by the use of synthetic chemicals which led to the development of resistant mosquitoes which are associated with environmental toxicity (Tripathi et al., 2004). However, the product provides natural alternative means of mosquito control. Some plant extracts or phytochemicals are known to possess ovicidal, larvicidal, repellent and insecticide properties against several species of insects (Isman, 1999).

CONCLUSIONS

This study examined the effect of essential oils extracted from fruits of Capsicum annum, seeds of Piper nigrum and Zingiber officinale roots on An. gambiae adult reared in our laboratory.

The results show a high mortality rate in An. gambiae after 24 h exposure to the product. This rate is more significant at high concentrations of essential oils extracted from C. annum and Z. officinale. The plant extract investigated has an adulticidal effect on adults of An. gambiae principal vector of smalaria in Cameroon.

In the light of a widespread use of testing insecticidal essential oils of our plants will be conducted in homes.

REFERENCES


