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Insecticidal Effects of *Capsicum annuum* on Aquatic Stages of *Anopheles gambiae* Giles under Laboratory Conditions

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Abstract: The developing trend of resistance in *Anopheles gambiae* toward synthetic mosquitocidal agents makes their management extremely difficult. A new approach in the fight against malaria vectors consist of using native plants with insecticidal value. Two varieties of *Capsicum annuum* the red and the yellow varieties were evaluated for their toxicities to *An. gambiae*. Ovicidal, larvicidal and pupal mortality effects of *C. annuum* fruit have been noted. The results showed that for 60 eggs, embryonic mortality was 100% when treated with the yellow variety of *C. annuum*. In addition, this variety was also detrimental to the embryonic development of *An. gambiae*. In contrast, at a concentration of 1 g L^{-1} of the red variety of *C. annuum* 19% of egg-hatched and 93% of total mortality rate from eggs to emergence. At a concentration of 0. 25 g L^{-1} of the red variety of *C. annuum* 44% of egg-hatched and 67% of total mortality rate from eggs to emergence. The embryonic development duration and first instars larvae development duration were longer (82 and 77 h, respectively) in media containing 1 g L^{-1} of *C. annuum*. The total mortality rate and the quantity of *C. annuum* in breeding media were highly correlated ($r = 0.964$; $p<0.01$). The number of males, females and the number of eggs laid per female were negatively correlated with the quantity of *C. annuum* in the breeding medium. This study shows that *C. annuum* fruits are significantly toxic on eggs, larval and pupal stages of *An. gambiae*. Future tests in the field will help determine if *C. annuum* may be effective for malaria control in tropical region.

Key words: *Anopheles gambiae*, *Capsicum annuum*, developmental stages, insecticidal products

INTRODUCTION

The malaria morbidity and mortality rates in Africa began an upward trend in the 1970s probably due to an increasing of parasite resistance to chloroquine and resistance of the vector mosquitoes to chemical insecticides (Carter and Mendis, 2002; Korenromp *et al*., 2003; Terkuile *et al*., 2004). Most human infections of *Plasmodium* are due to the infecting sting of mosquito of the *Anopheles* genera. *Anopheles gambiae*, *Anopheles funestus* and *Anopheles arabiensis* are the most important vectors of malaria in Africa (Coluzzi, 1984; Fondjo *et al*., 1992; Mouchet and Carnevale, 1991). In Cameroon, *An. gambiae* is the main vector of malaria in the rural and urban regions (Manga *et al*., 1992). Currently, Insecticide-Treated Nets (ITNs) represent the most practical and economical methods of controlling vectors (Copeland *et al*., 1995).

Although the fight against malaria vectors is the most efficient preventative way to reduce the incidence of this endemic illness (Carnevale and Mouchet, 1990); chemical insecticides (e.g., DDT,

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dieldrine…) used in the past proved inefficient due to the detrimental effect to the environment, the development of pesticide resistant mosquitoes and the escalating cost of application. Products used were inefficient for the vector control in various regions of Africa South of Sahara. The control of populations of Anopheles by plants extract with insecticidal effects is one of the new tracks of research (Rey *et al*., 2001). Some plants are source of substances used to repulse or to kill mosquitoes and other insect pests. Odalo *et al.* (2005) showed that *Croton pseudopulchellus* Pax, *Mkilua fragrans* Verdc, (Annonaceae), *Endostemon tereticaulis* (Poir.) Ashby, *Ocimum forskolei* Benth., *Ocimum fischeri Guerke*, *Plectranthus longipes* Baker (Labiateae) and *Cymbopogon nardus* Melissa (Curtis *et al*., 1989) have been demonstrated to exhibit good repellent activities against *Anopheles gambiae*. The recent plant- insect interactions studies reveal that some secondary metabolites of plants could have an effect on the growth, the development and the behavior of larvae (Muller and Su, 1999). Among these, several phenolic compounds are known for their toxicity to insects (Mercer and Anderson, 1994) including carvacrol, 4-isopropylbenzenemethanol, phytol, thymol 1-methylpyrrole which exhibited high individual repellencies to *An. gambiae* (Odalo *et al*., 2005).

Capsicum annuum (Solanaceae) is cultivated in all regions of Cameroon. Its fruits that are used as spices are very rich in phenolic compounds (Diaz *et al*., 1998). The most abundant of these compounds are the capsaicinoids, including capsaicine and dihydrocapsaicine which represent 77 to 90% of secondary compounds. These compounds have been used to control bugs and birds (Diaz *et al*., 1998). In addition, these compounds are biodegradable without any harmful effect on the environment (Isman, 1999).

In the study reported here, we determined the possible effects of *C. annuum* at varying concentrations on mosquito growth and development. We examined the inhibition effect of two varieties of *C. annuum* on egg-hatching, larval and pupal development of *An. gambiae* in an effort to find a biological control agent that is benign to the environment, but will effective by reduce the multiplication of the malaria vector.

MATERIALS AND METHODS

Mature fruits of *Capsicum annum* Linne, commonly called "thick pimento" were obtained from the Agronomic Research Institute for Development (Yaounde-Cameroon). Fruit of both the red and yellow varieties were obtained dried and crushed with a moulinex to form a fine powder. The resultant powder was added to the mosquito breeding medium.

Breeding of *An. gambiae*

Eggs were obtained from laboratory strain of *An. gambiae* maintained at ambient rearing condition. All the bioassays were conducted at 26-28°C, 70-80% RH, 12:12(L:D), in 2003 and in Yaoundé1 University (Yaoundé, Cameroon).

Sixty eggs of *An. gambiae* were added to each of three plastic trays (18 cm Ø, 5 cm deep) containing 1, 2 and 4 L of spring water from Simbock (locality near Yaounde) with a sufficient quantity of Tetrababy fish food "R" (0,3 g/100 larvae/d). This food is rich in proteins and usually used for the nutrition of young fishes of less than 1 cm of length.

Three rearing media were constituted containing the powder of *Capsicum annuum*:

- Medium 1: One liter of spring water containing 1 g of the powder
- Medium 2: Two liter of spring water containing 1 g of the powder
- Medium 3: Four liter of spring water containing 1 g of the powder

Control mediums consisted of (1, 2 and 4 L) of spring water without *C. annuum* powder. The number of eggs hatched was determined daily by counting the number of first instar larvae observed

in each tray until no hatching occurred for 10 days. The duration of embryonic development was determined using the method reported by Dempster (1961). Embryonic development was calculated by the time it takes for 2/3 of the eggs to become first instar larvae. The experiment took 20 days and was replicated 10 times in order to eliminate possible tray effects or cage effects.

Pupae and larvae of *An. gambiae* are counted daily. At emergence, adults were counted and their sex was determined by visual inspection. Sex ratio was determined (number of female per the number of male that emerged). A sugar solution (10%) was provided *ad libitum*. Three days after emergence, females were allowed to blood feed on a rabbit and kept under laboratory conditions to lay eggs. Eggs laid by females were counted and the average fecundity for each female was estimated (total number of eggs laid over the total number of females). The total duration of development (from eggs to adults) was determined by considering embryonic development duration, larval development duration and pupal development duration. Throughout our experiment, mortality rate was determined at each developmental stage (number of death over the total number of larvae or pupae at the developmental stage). Larvae were considered dead when they did not react to touching. Adult mortality rate was determined 5 days after emergence by divided number of death at the punter with the total number of adult recorded immediately after emergence.

Analyses of Data

Mean and standard errors of different parameters were calculated and compared using SPSS (SPSS, 1999; Tripathi *et al*., 2003) notably tests of comparison non parametric Z of Wilcoxon (1945), U of Mann Whitney (1947) and χ^2 of Kruskal Wallis (1952) and the test of interrelationship of Spearman.

RESULTS

Effect of *Capsicum annuum* on Egg-hatching, Larval and Pupal Development Duration

No egg hatching was recorded for each quantity of *C. annuum* (yellow variety) (1, 0.5, 0.25 g L^{-1}). This variety didn't permit us to obtain any larvae during the ten series of experiments in which 60 eggs were tested. Each experiment took 7 days.

The egg-hatching rate varied within the quantity of *C. annuum* of red variety. It could be seen from Table 1, that percentage of hatched eggs was 19.33% in the medium with 1 g L^{-1} of *C. annuum* (so called medium 1), 30.83% in the medium with 0.5 g L^{-1} of *C. annuum* (medium 2) and 44.33% in the medium with 0.25 g L^{-1} of *C. annuum* (medium 3). The embryonic development duration was about 81.6 h in the medium 1; 69.60 h in the medium 2 and 69.6 h in the medium 3.

The larval and pupal development duration was as followed: first instars larvae: 76.8 h in the medium 1; 45.6 h in the medium 2 and 45.6 h in the medium 3; second instars larvae: 24 h in medium 1; 48 h in medium 2 and 36 h in medium 3; third instars larvae: 33.6 h in medium 1; 40.8 h in medium 2 and 33.6 h in medium 3; fourth instars larvae: 28.8 h in medium 1; 43.2 h in medium 2 and 31.2 h in medium 3; pupa: 43.2 h in medium 1; 33.6 h in medium 2 and 33.6 h in medium 3. The pre-adult development duration was 12 days in medium 1; 11.7 days in medium 2 and 10.4 days in medium 3 (Table 1).

Exposure of *An. gambiae* eggs and different larvae development stages to *capsicum annuum* powder of red variety showed a significant difference compares to the results obtained from control media. Indeed, results obtained in medium 1 compared to those of control medium showed a significant difference for the egg-hatching rate ($Z = -2.16$; $p = 0.03$), embryonic development duration ($Z = -2.34$; $p = 0.03$), first instars larvae development duration ($Z = -2.56$; $p = 0.03$) and the total development duration ($Z = -2.207$; $p = 0.03$). The comparison of the developmental parameters of *An. gambiae* obtained in medium 2 and its control medium and also results from medium 3 and its control medium permitted us to reveled that only the hatching rate ($Z = -2.15$; $p = 0.03$) and the total development duration ($Z = -2.31$; $p = 0.03$, $Z = -2.34$; $p = 0.03$, respectively) showed the significant difference (Table 1).

Table 1: Effects of *C. annuum* (red variety) on the development and mortality of *An. gambiae* and comparison between experimental media and their corresponding controls (non parametric test of wilcoson; p<0.05)

Parameters	Medium 1 (1 g L^{-1})	Control 1 (without product)	Medium 1/ Control 1	Medium 2 (0.5 g L^{-1})	Control 2 (without product)
Average of hatching rates	19.33±3.62	81.7±2.4	Z = -2.16; p = 0.030	30.8±3.8	85.8±3.5
Embryonic duration (h)	81.6±12.4	48±0.0	Z = -2.34; p = 0.030	69.6±7.6	48±0.0
First instars larvae development duration (h)	76.8±10.1	36±17	Z = -2.56; p = 0.030	45.6±7.6	48±0.0
Second instars larvae Development duration (h)	24±0.0	24±0.0	Z = 0.00; p = 1.00	48.0±0.0	36±17
Third instars larvae development duration (h)	33.6 12.4	24±0.0	Z = -0.25; p = 0.909	40.8±11.6	24±0.0
Fourth instars larva Edevelopment duration (h)	28.8±10.1	36±17	Z = -0.66; p = 0.758	43.2±10.1	36±17
Pupal development duration (h)	43.2±10.1	24±0.0	Z = -2.09; p = 0.121	33.6±12.4	24±0.0
Total development duration (days)	12±1.1	8±0.0	Z = -2.20; p = 0.030	11.7±0.9	9±0.0
First instars mortality rate	13.1±4.5	3±1.4	Z = -2.15; p = 0.030	9.1±3.9	2.9±1.3
Second instars mortality rate	12.8±3.5	1.1±1.5	Z = -2.16; p = 0.030	9.1±3.9	4±0.1
Third instars mortality rate	20.9±8.9	3.2±1.5	Z = -2.05; p = 0.030	9.1±4.7	3.1±1.4
Fourth instars mortality rate	17.9±6.9	1.1±1.6	Z = -2.19; p = 0.030	8.8±5.3	2.1±0.0
Pupal mortality rate	21±6.6	5.6±1.6	Z = -2.17; p = 0.030	10.2±6.7	5.5±1.6
Adults mortality rate	33.9±12	8±1.1	Z = -2.17; p = 0.030	25.5±5.9	15.1±1.1
Total mortality rate	92.8±2.8	29.2±1.2	Z = -2.19; p = 0.030	81.7±4.1	28.3±2.4
Number of adults	4.4±1.8	42.5±0.7	Z = -2.17; p = 0.030	11.0±2.5	43±1.4
Number of males	1.8±0.8	20.5±0.7	Z = -2.23; p = 0.030	5.8±1.2	21.5±2.1
Number of females	2.6±1.2	22±0.0	Z = -2.18; p = 0.030	5.2±1.4	21.5±0.7
Number of eggs laid per females	67.1±11.2	136.5± 6.4	Z = -2.14; p = 0.030	81.5±4.7	150±1.4

Parameters	Medium 2/Control 2	Medium 3 (0.25 g L^{-1})	Control 3 (without product)	Medium 3/Control 3
Average of hatching rates	Z = -2.15; p = 0.030	44.3±4.3	84.2±3.5	Z = -2.15; p = 0.030
Embryonic duration (h)	Z = -2.56; p = 0.061	69.6±7.6	48±0.0	Z = -2.56; p = 0.061
First instars larvae development duration (h)	Z = -0.44; p = 0.909	45.6±7.6	48±0.0	Z = -1.32; p = 0.485
Second instars larvae development duration (h)	Z = -2.23; p = 0.364	36±12.7	24±0.0	Z = -1.25; p = 0.364
Third instars larvae development duration (h)	Z = -1.75; p = 0.182	33.6±12.4	36±17	Z = -1.04; p = 0.485
Fourth instars larvae development duration (h)	Z = -0.85; p = 0.606	31.2±11.6	24±0.0	Z = -0.52; p = 0.758
Pupal development duration (h)	Z = -1.04; p = 0.485	33.6±12.4	24±0.0	Z = -1.04; p = 0.485
Total development duration (days)	Z = -2.31; p = 0.030	10.4±0.5	8.5±0.7	Z = -2.34; p = 0.030
First instars mortality rate	Z = -2.14; p = 0.030	8±3.2	4±0.2	Z = -1.93; p = 0.061
Second instars mortality rate	Z = -1.71; p = 0.121	7.1±2.4	4.2±3.1	Z = -2.15; p = 0.030
Third instars mortality rate	Z = -2.14; p = 0.030	5.9±2.5	5.6±5	Z = -1.40; p = 0.182
Fourth instars mortality rate	Z = -1.29; p = 0.273	5.3±3.7	4.4±2.7	Z = -1.51; p = 0.182
Pupal mortality rate	Z = -1.51; p = 0.182	5.4±4	3.5±1.3	Z = 0.00; p = 1.00
Adults mortality rate	Z = -2.14; p = 0.030	14.8±4.5	13.6±0.7	Z = -1.71; p = 0.120
Total mortality rate	Z = -2.15; p = 0.030	67.7±5.6	32.5±5.9	Z = -2.15; p = 0.030
Number of adults	Z = -2.15; p = 0.030	19.4±3.4	40.5±3.5	Z = -2.15; p = 0.030
Number of males	Z = -2.15; p = 0.030	10±1.7	20±0.0	Z = -2.17; p = 0.030
Number of females	Z = -2.20; p = 0.030	9.4±2.5	20.5±3.5	Z = -2.17; p = 0.030
Number of eggs laid per females	Z = -2.15; p = 0.030	92.8±6.5	174±14.1	Z = -2.14; p = 0.030

When comparing the effect of different quantities of *Capsicum annuum* of red variety on egg-hatching and development duration of larval and pupal stages until the adult reared in medium with those reared in the medium 2, we noted a significant difference: egg-hatching rate (Z = -3.75; p = 0.000); first instars larvae development duration (Z = -4.11; p = 0.000); second instars larvae development duration (Z = -4.35; p = 0.000); fourth instars larvae development duration (Z = -2.61; p = 0.023). The comparison of results obtain from medium 1 to those of medium 3 shows a significant difference for egg-hatching rate (Z = -3.78; p = 0.000), first instars larvae development duration (Z = -4.1; p = 0.000) and total development duration (Z = -3.33; p = 0.001). On the other hand, when we compared the results gotten in medium 2 to those of medium 3, only the egg-hatching rate (Z = -3.78; p = 0.000) and the pre-adult development duration (Z = -3.12; p = 0.003) showed a significant difference. Comparison between parameters of the development of this insect in the three media (medium 1, medium 2, medium 3) showed that only the third instars larvae development duration and the pupal development duration showed a non significant difference (Table 2).

The interrelationship test of Spearman achieved between the eggs hatching, the development duration of different larval stages and the quantity of *Capsicum annuum* of red variety in the rearing medium revealed a significant correlation between the hatching rate (r = -0.961; p <0,01), the embryonic development duration (r = 0.714; p = 0.01), the first instars larvae development duration (r = 0.754; p <0.05), the pre-adult development duration (r = 0.808; p <0.01) and the quantity of *Capsicum annuum* in the rearing medium (Table 3).

Table 2: Effects of *C. annuum* (red variety) on the development and mortality of *An. gambiae* and comparison between several experimental media containing different quantities of powder. (non parametric tests of wilcoson. Mann whitney and Kruskal wallis; p<0.05)

Parameters	Medium 1/Medium 2	Medium 1/Medium 3	Medium 2/Medium 3	Medium 1/Medium 2/Medium 3
Average of egg-hatching rate	Z = -3.75; p = 0.000	Z = -3.78; p = 0.000	Z = -3.78; p = 0.000	χ^2 = 25.73; p = 0.000
Embryonic duration (h)	Z = -2.30; p = 0.089	Z = -2.30; p = 0.089	Z = 0.00; p = 1.000	χ^2 = 8.42; p = 0.015
First instars larvae development duration (h)	Z = -4.11; p = 0.000	Z = -4.10; p = 0.000	Z = 0.00; p = 1.000	χ^2 = 2.29; p = 0.000
Second instar larvae development duration (h)	Z = -4.35; p = 0.000	Z = -2.57; p = 0.063	Z = -2.51; p = 0.063	χ^2 = 19.33; p = 0.000
Third instars larvae development duration (h)	Z = -1.34; p = 0.280	Z = 0.00; p = 1.000	Z = -1.31; p = 0.280	χ^2 = 2.32; p = 0.313
Fourth instars larvae development duration (h)	Z = -2.61; p = 0.023	Z = -0.50; p = 0.739	Z = -2.19; p = 0.063	χ^2 = 8.13; p = 0.017
Pupal development duration (h)	Z = -1.78; p = 0.143	Z = -1.78; p = 0.143	Z = 0.00; p = 1.000	χ^2 = 4.14; p = 0.126
Total development duration (days)	Z = -0.69; p = 0.529	Z = -3.33; p = 0.001	Z = -3.12; p = 0.003	χ^2 = 14.32; p = 0.001
First instars larvae mortality rate	Z = -1.89; p = 0.063	Z = -2.68; p = 0.005	Z = -0.34; p = 0.739	χ^2 = 7.33; p = 0.026
Second instars larvae mortality rate	Z = -1.74; p = 0.089	Z = -3.37; p = 0.000	Z = -1.40; p= 0.165	χ^2 = 11.09; p = 0.004
Third instars larvae mortality rate	Z = -2.98; p = 0.002	Z = -3.63; p = 0.000	Z = -2.08; p = 0.035	χ^2 = 17.43; p = 0.000
Fourth instars larvae mortality rate	Z = -2.73; p = 0.005	Z = -3.79; p = 0.000	Z = -1.74; p = 0.089	χ^2 = 16.70; p = 0.000
Pupal mortality rate	Z = -3.00; p = 0.002	Z = -3.79; p = 0.000	Z = -1.97; p = 0.052	χ^2 = 18.11; p = 0.000
Adults mortality rate	Z = -1.89; p = 0.063	Z = -3.03; p = 0.002	Z = -3.40; p = 0.000	χ^2 = 15.33; p = 0.000
Total mortality rate	Z = -3.80; p = 0.000	Z = -3.80; p = 0.000	Z = -3.70; p = 0.000	χ^2 = 25.77; p = 0.000
Number of adults	Z = -3.79; p = 0.000	Z = -3.79; p = 0.000	Z = -3.74; p = 0.000	χ^2 = 25.74; p = 0.000
Number of males	Z = -3.82; p = 0.000	Z = -3.82; p = 0.000	Z = -3.67; p = 0.000	χ^2 = 25.50; p = 0.000
Number of females	Z = -3.41; p = 0.000	Z = -3.80; p = 0.000	Z = -3.43; p = 0.000	χ^2 = 23.44; p = 0.000
Number of eggs laid per females	Z = -2.92; p = 0.002	Z = -3.70; p = 0.000	Z = -3.25; p = 0.000	χ^2 = 20.86; p = 0,000

Table 3: Interrelationship test of Spearman between egg-hatching rate, development duration, mortality rate of developmental stages of *An. gambiae* and the quantities of *C. annuum* powder in breeding media ($p<0.05$)

Parameters	Coefficients of interrelationship (r)
Average of egg-hatching rate	-0.961**
Embryonic development duration (h)	0.714**
First instars larvae development duration (h)	0.754**
Second instars larvae development duration (h)	0.111*
Third instars larvae development duration (h)	0.178*
Fourth instars larvae development duration (h)	0.170*
Pupal development duration (h)	0.489**
Total development duration (days)	0.808**
First instars larvae mortality rate	0.692**
Second instars larvae mortality rate	0.741**
Third instars larvae mortality rate	0.803**
Fourth instars larvae mortality rate	0.776**
Pupal mortality rate	0.776**
Adults mortality rate	0.784**
Total mortality rate	0.964**
Number of adults	-0.963**
Number of males	-0.963**
Number of females	-0.935**
Number of eggs laid per females	-0.905**

* Non significant, **: Significant

Effect of *Capsicum annuum* on Larval and Pupal Mortality

The mortality rate of *An. gambiae* larvae and pupae have been evaluated in the different rearing media. The number of males, females and the number of eggs laid a female who survived from pupae was also evaluated. The mortality rate of larvae was as followed: first instars larvae, 13.1% in medium 1, 9.1% in medium 2 and 8% in medium 3; second instars larvae, 12.8% in medium 1, 9.1% in medium 2 and 7.1% in medium 3; third instars larvae, 20.9 in medium 1; 9.1 in medium 2 and 5.9% in medium 3; fourth instars larvae, 17.9 in the medium 1, 8.8 in medium 2 and 5.3 in medium 3. The mortality rate of pupae was as follow: 21% in medium 1, 10.2% in medium 2 and 5.4% in the medium 3. The adult's mortality rate was 33.9% in the medium 1, 25.5% in medium 2 and 14.8% in medium 3. The total mortality rate at emergence, known as the number of adults compared to the number of eggs at the beginning of each experiment, was 92.8; 81.7 and 67.7% in media 1, 2 and 3, respectively. Thus, the mortality rate is higher in medium 1 follow-up with medium 2 and then medium 3. At the end of the experiment the number of adults were 1.8 males and 2.6 females in medium 1; 5.8 males and 5.2 females in medium 2; 10 males and 9.4 females in medium 3. The number of eggs laid a female was, respectively 67.1; 81.5 and 92.8 in media 1, 2 and 3 (Table 1).

The mortality rate of the different development instars, the number of males and females and the number of eggs laid per female of *An. gambiae* showed a significant difference when we compared the results gotten in medium 1 to those of the control. In the other hand, between medium 2 and the control, we noted a significant difference for the first instars larvae mortality rate, the third instars larvae mortality rate, the adult mortality rate, the total mortality rate, the number of males and females and the number of eggs laid per female. Between the medium 3 and control we noted a significant difference for second instars larvae mortality rate, the total mortality rate, the number of males and females and the number of eggs laid per female (Table 1).

The comparison between the mortality rate of mosquitoes larvae and pupae reared in medium 1 to those observed in medium 2, showed a significant difference for the third instars ($Z = -2.98$; $p = 0.002$), fourth instars ($Z = -2.73$; $p = 0.005$), pupae ($Z = -3.00$; $p = 0.002$) and total mortality rate of adults at emergence ($Z = -3.8$; $p = 0.000$). Mortalities rates observed in medium 1 are meaningfully different from those observed in medium 3 ($p = 0.000$). The comparison between the mortality rate of mosquitoes larvae, pupae and adults reared in medium 3 to those of medium 2, show a significant difference for the third instars larvae ($Z = -2.08$; $p = 0.03$), adults ($Z = -3.40$; $p = 0.000$)

and the total mortality rate of adults at emergence (Z = -3.7; p = 0.000). The comparison of mortalities rates is meaningfully different in the three media (p = 0.001). The number of adults, (males and females) and the number of eggs laid per female were meaningfully different from one medium to another (Table 2).

The interrelationship test of Spearman between the *An. gambiae* mortality rate and the quantity of *C. annuum* in the rearing medium was meaningfully significant for the first instars larvae mortality rate (r = 0.692; p = 0.001), the second instars larvae mortality rate (r = 0.741; p = 0.01), the third instars larvae mortality rate (r = 0.803; p = 0.01), the fourth instars larvae mortality rate (r = 0.776; p = 0.01), the pupal mortality rate (r = 0.776; p = 0.01), the adults mortality rate (r = 0.784; p = 0.01) and the total mortality rate of adults at emergence (r = 0.964; p = 0.01) (Table 3).

DISCUSSION

Protection against mosquitoes is generally obtained by the use of synthetic chemical products with the culminating problems associated with the development of resistance in mosquitoes and toxicity to man and its environment (Tripathi *et al*., 2003). However, natural product provided useful future alternative means for mosquito control. Some plant extracts or phytochemicals products are known to possess ovicidal, larvicidal, repellent, antifeeding and insecticidal activities against various insect species (Isman, 1999). Results of the current study revealed that egg-hatching rate decrease with the concentration of *Capsicum annuum* in the medium, meanwhile the total larval development duration increase with the concentration of *C. annuum* in the medium.

Secondary plant metabolic compounds (polyphenols) are known to have adverse effects on the midgut epithelial barrier of Lepidoptera and Orthoptera larvae (Barbehenn and Martin, 1994). Phenolic components are known to have ovocidal and insecticidal properties against different species of insects (Isman, 1999). Yang *et al*. (2003) showed that terpenoids and alkaloids destroy eggs and females of *P. humanus* capitis.

The capsaicin, major phenolic components contained in the fruits of *C annuum* although its insecticide activity have not yet been well defined, it has been suggested that it would inhibit the growth of insects and would be implied in certain reactions of oxidization (Diaz *et al*., 1998). In this study, pre-adult development duration is positively correlated to the quantity of *C. annuum* in the medium (r = 0.808). Our results showed that *C. annuum* contained the poisonous compounds responsible for the retardation of egg-hatching, larval and pupal development of *An. gambiae*. It can therefore be use for larval control. However this approach was neglected and malaria control policy shifted toward domestic adulticid methods. Nevertheless, it is important to remind that Brazil, Egypt an Zambian have successfully suppressed malaria for over 10 years by using larval control program (Killeen *et al*., 2002).

The mortality rate of *An. gambiae* at different developmental stages was higher when the concentration of *C. annuum* in the medium was 1 g L^{-1} and low when this concentration was 0.25 g L^{-1}. The mortality rate thus recorded should be due to the chemical compounds of the *C. annuum* fruits. Rey *et al*. (2001) showed that this plant could produce few polyphenol and mainly capsaicinoids. Capsaicinoids are implied in the biosynthesis of the lignin (polyphenol). Larvae raised in a medium containing polyphenol ingest these poisonous compounds that accumulate in their intestine and destroy their partition epithelial (David *et al*., 2000b). The plant polyphenol showed a more elevated insecticide activity compared to the conventional insecticides (Rey *et al*., 2001; David *et al*., 2000a). This statement can explain the increase of their mortality rate and the affect of the punter of females (Tuno *et al*., 2004). Larvae of *An. gambiae* used to swim on to surface; when the medium is deep, they are obliged to flood.

With the yellow variety of *C. annuum*, we got 0% of egg-hatching rate. The main characteristic of pepper is their pungent flavor due to capsaicin and a related molecules, capsaicinoid. The quantity of these compounds varied within varieties of *C. annuum*, 1 to 4% (Rey *et al*., 2001). So the most efficient varieties are those, which have a high rate of capsaicinoids that would be the case of the yellow variety of *C. annuum*.

The total mortality is greatly correlated to the quantity of *C. annuum* in the medium of breeding. Although the World Health Organization considers that the larval control in Africa should have an applicability limited in Africa. Strategies of control of aquatic stages of *An. gambiae* have been the biologic struggle. Then for this control we recommend that the drainage or the drying up of larva habitats and or the immersion in these larval resting places of poisonous compounds of plant origins as *C. annuum*. According to our result *C. annuum* can be considered as a good material for the control of malaria vectors with no poisonous effect on human being and the environment.

CONCLUSIONS

The study of the development duration and the mortality rate of *An. gambiae* in a medium containing the powder of *Capsicum annuum* permitted to note that the development duration were longer when higher quantity of *C. annuum* were added in the breeding medium. The mortality rate was positively correlated to the concentration of *C. annuum*. Therefore, the immersion of powder of this plant in the resting places would be an efficient method for malaria vector control, particularly in the developing countries where mosquitoes larvae developed resistances facing the chemical insecticides. Larval control methods should now be reprioritized for research, development and implementation as an additional way to roll back malaria.

REFERENCES

Barbehenn, R.V. and M.M. Martin, 1994. Tannin sensitivity in larvae of *Malacosoma disstria* (Lepidoptera): Roles of the peritrophic envelope and midgut oxidation. J. Chem. Ecol., 20: 1985-2001.

Carnevale, P. and J. Mouchet, 1990. Vector control and malaria comtrol. Médecine Tropicale., 50: 391-398.

Carter, R. and K. Mendis, 2002. Evolutiony and historical aspects of the burden of malaria. Clin. Microiol. Rev., 15: 564-594.

Coluzzi, M., 1984. Heterogeneities of malaria vectorial system in the tropical Africa and their significance in malaria epidemiology and control. Bul. WHO., 62: 107-113.

Copeland, R.E., T.W. Walker, L.L. Robert, J.I. Githure, R.A. Wirtz and T.A. Klein, 1995. Response of wild *Anopheles funestus* to repellent-protected volunteers is unaffected by malaria infection of the vector. J. Am. Mosquito Contr. Assoc., 15: 342-347.

Curtis, C.F., J.D. Lines, Lu Baolin and A. Renz, 1989. Natural and synthetic repellents. Med. Vet. Entomol., 1: 109-119.

David, J.P., D. Rey, M.P. Pautou and J.C. Meryran, 2000a. Differential toxicity of environmental vegetation on some aquatic dipteran larvae of mosquito breeding sites. J. Invert. Pathol., 75: 9-18.

David, J.P., D. Rey, G. Marigo and J.C. Meryran, 2000b. Larvicidal effect of a cell-wall fraction isolated from alder decaying leaves. J. Chem. Ecol., 26: 901-913.

Dempster, J.P., 1961. The analysis of data obtained regular sampling of insect population. J. Anim. Ecol., 30: 429-432.

Diaz, J., A. Bernal, F. Merino and A. Ros Barcelo, 1998. Phenolic metabolism in *Capsicum annuum* L. Recent Res. Devel. Phytochem, 2: 155-169.

Fondjo, E., V. Robert, G. LeGoff, J.C. Toto and P. Carnevale, 1992. The urban malaria in Yaoundé (Cameroon): 2-entomological study in two little urbanized districts. Bull. Soc. Path. Ex., 85: 57-63.

Isman, M.B., 1999. Pesticides based on plant essential oils: Pestic. Outlook, 2: 68-72.

Killeen, G.F., U. Fillinger, I. Kiche, L.C. Gouagna and G.J. Knols, 2002. Eradication of Anopheles gambiae from Brazil: Lessons for malaria control in Africa? Infect. Dis., 2: 618-627.

Korenromp, E.L., B.G. Williams, E.C. Gouws, C. Dey and R.W. Snow, 2003. Measurement of drends in childhood malaria mortality in Africa: An assessment of progress toward targets based on verbal autopsy. Lancet Infect. Dis., 3: 349-358.

Kruskal, W.H. and W.A. Wallis, 1952. Use of ranks in one-criterion variance analysis. J. Am. Statistical Assoc., 47: 583-621.

Manga, L., V. Robert, J. Messi, M. Desfontaines and P. Carnevale, 1992. The human malaria in Yaoundé (Cameroon). Entomological study in two central districts. Mem. Soc. Belge. Entomol., 35: 155-162.

Mann, H.B. and D.R. Whitney, 1947. On a test of whether one of 2 random variables is stochastically larger than the other. Ann. Mathematical Statistics, 18: 50-60.

Mercer, D.R. and I.R. Anderson, 1994. Tannins in treehole habitats and their effects on *Aedes sierrensis* (Diptera: Culicidae) production and parasitism by *Lambornella clarckis* (Ciliophora: Tetrahymenidae). Ann. Entomol. Soc. Am., 31: 159-167.

Mouchet, J. and P. Carnevale, 1991. Vectors and Transmission. In: Malaria, M. Danis and J. Mouchet (Eds.), Ellipses/UREF, Paris, pp: 35-59.

Muller, M.S. and T. Su, 1999. Activity and biological effects of neem products against arthropods of medical and veterinary importance. J. Am. Mosquito Contr. Assoc., 15: 133-152.

Odalo, J.O., M.O. Omolo. H. Malebo, J. Angira, P.M. Njeru, I.O. Ndiege and A. Hassanali, 2005. Repellency of essential oils of some plants from the Kenyan coast against *Anopheles gambiae*. Acta Trop., 95: 210-218.

Rey, D., J.P. David, G. Besnard, J.L. Jullien, C. Lagneau and J.C. Meyran, 2001. Comparative sensitivity of larval mosquotoes to vegetable polyphenols versus conventional insecticides. Entomol. Exp. Appl., 98: 361-367.

SPSS., 1999. SPSS for Windows, version 9.01. SPSS Chicago, IL.

Ter Kuile, F.O., M.E. Parise, F.H. Verhoeff, V. Udhaya kumar, R.D. newman, A.M. Van Eijk., S.J. Rogerson and R.W. Steketee, 2004. The burden of co-infection with human immunodeficiency virus type 1 and malaria in pregnant woman in sub-Sahara Africa. Am. J. Trop. Med. Hyg., 71: 41-54.

Tripathi, A.K., V. Prajapati, A. Ahmad, K.K. Aggarwal and S.P.S. Khanuja, 2003. Piperitenone Oxide as Toxic, Repellent and Reproduction Retardant Toward Malarial Vector Anopheles stephensi (Diptera: Anophelinae). J. Med. Ent., 41: 691-698.

Tuno, N., K. Miki, N. Minakawa, A. Githeko, G. Yan and M. Takagi, 2004. Diving ability of *Anopheles gambiae* (Diptera: Culicidae) Larvae. J. Med. Entomol., 41: 810-812.

Wilcoxon, F., 1945. Individual comparisons by ranking methods. Biometr. Bull., 1: 80-83.

Yang, Y.C., H.S. Lee, J.M. Clark and Y.J. Ahn, 2003. Insecticidal Activity of plant essential oils *Pediculus humanus capitis* (Anoplura; Pediculidae). J. Med. Entomol., 41: 699-704.