ISSN 1927-646X (Online)

Journal of Mosquito Research

Mosquitoes, Flies Making Troubles to Humans

ISSN 1927-646X
14004
9 771927 646008 >

2014

JMR Vol 4

Publisher
Sophia Publishing Group

Edited by
Editorial Team of Journal of Mosquito Research
Email：edit@jmr.sophiapublisher.com
Website：http://jmr.sophiapublisher.com

Address:
11388 Stevenston Hwy,
PO Box 96016,
Richmond, V7A 5J5, British Columbia, Canada

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Journal of Mosquito Research, 2014, Vol. 4, No. 11

Journal of Mosquito Research, 2014, Vol. 4, No. 11, 1-6
ISSN 1927-646X
http://jmr.biopublisher.ca

Research Report **Open Access**

Toxicity and Fumigant Effect Of Powder and Oil Extracts of *Cleisthopholis Patens* (Benth) against Larvae and Adults *Anopheles* Mosquito

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Journal of Mosquito Research, 2014, Vol.4, No.11 doi: 10.5376/jmr.2014.04.0011
Received: 30 Jun., 2014
Accepted: 28 Jul., 2014
Published: 19 Aug., 2014

Preferred citation for this article:
Akinneye and Afolabi, 2014, Toxicity and Fumigant Effect Of Powder and Oil Extracts of *Cleisthopholis Patens* (Benth) against Larvae and Adults *Anopheles* Mosquito, Journal of Mosquito Research, Vol.4, No.11, 1-6 (doi: 10.5376/jmr.2014.04.0011)

Abstract The ethanolic and aqueous extracts of the stem-bark of *Cleisthopholis patens* (Benth) were tested against the larvae and adults of *Anopheles gambiae* at varying concentrations (10%, 20%, 30%, 40% and 50% for powder, 1%, 2%, 3%, 4% and 5% for oil extract and 10%, 20%, 30%, 40% and 50% for aqueous extract). The ethanolic oil extract of *C. patens* at 1-5% concentrations had significant effect on larvae and adult of *An. gambiae* with percentage mortality range of 83-100% within 90mins of exposure periods. The aqueous extract of *C. patens* was relatively ineffective against the larvae of *An. gambiae* at all levels of concentrations while the fumigant effect of the powder was slightly effective against adult *An. gambiae* which achieved 23.30 - 36.70% mortality of the insect. The results obtained showed significant difference when the value of ethanolic extract, the aqueous extract and the powder form were compared ($p < 0.05$). This shows that the plant oil (ethanolic extract) of *C. patens* is highly toxic at all concentrations (1-5%) to the larvae and adults of *An. gambiae* which resulted in the mortality effects.
Keywords *Anopheles* mosquito; Fumigant; *Cleisthopholis patens*; Mortality

Background

The control of mosquitoes is essential, as many species of mosquitoes are vectors of diseases. The female mosquitoes vector pathogens that transmit diseases such as malaria, yellow fever, dengue, filariasis etc. they also constitute intolerable biting nuisance to the human host (Collins and Paskewitz, 1995). Nigeria is located in malaria risk zones of sub Saharan Africa, being a highly populated country, her populace are at the highest risk of morbidity and mortality caused by the disease, which kills more than one million people annually (WHO 2010; RBM 2011). Many of this death are as a result of poverty, because several poor Africans may not be able to afford the cost of medication or good accommodation to screen out mosquitoes. Therefore, in line with Nigeria governments continued effort to eradicate malaria, researchers have routinely screened readily available plants for their bio efficacy against the vector of malaria pathogen (the *Anopheles* mosquito). Mosquitoes are estimated to transmit disease to more than 700 million people annually in Africa, South America, Central America, Mexico and much of Asia with millions of resulting deaths (Mark, 1998). The prevalence of malaria and other diseases which mosquitoes transmit can be largely attributed to the success of these dipterans in the tropical biome as well as their abilities to develop immunity and resistance against conventional method of control. Malaria is the most prevalent of mosquito borne disease being endemic in about 109 countries, infecting 190-330 million people and causing about 1 million deaths every year. With the increasing knowledge of these *Anopheles* mosquitoes, the disease and nuisance caused by these mosquitoes have led to the use of various methods of controlling or reducing their populations. Use of insecticides and natural enemies are the most common. Also, use of chemical such as diazon, Dichlorodiphenyltrichloroethane (DDT) and permetrin can reduce their population but these chemicals has limitations which include, their toxic effects on human, genetic resistant strains, workers safety, high cost, serious environment etc. (Shin-foon, 1984). This has led to the banning of these chemicals.

Due to the fact that application of synthetic insecticides has poisoned the environment as well as non- target organisms, these problems brought about a search for safer alternative control measure for mosquito which led to the use of natural products of plant origin with insecticidal properties (Sharma *et al.*, 1990). It has been found that medicinal plant extracts are one the safer alternative method of controlling mosquitoes. One of the early reports on the use of plant extracts against mosquito larvae was that of Campbell *et al* (1993) where it was discovered that plant alkaloids like nicotine, anabasine, methyl anabasine, and lumpinin extracted from the Russian weed, *Anabasis aphylla*, killed the larvae of *Culex sp*. Monzon *et al* (1994) reported that some medicinal plants containing natural toxins were effective against mosquito larvae. Not only can medicinal plant extracts be effective but also they may greatly reduce the risk of adverse ecological effects and they do not induce pesticide resistance in mosquitoes. Since these chemicals are taken from medicinal plants, they are expected to have less toxicity on human (that is if they are toxic at all), and they should be highly biodegradable (Choochote *et al*., 1999.) *Cleisthopholis patens* have been selected as potential candidate against the larvae and adults of *Anopheles* mosquito. This research is sought to evaluate the toxicity of ethanolic oil extract, aqueous extract and powder of *C. patens* against the larvae and adult of *Anopheles gambiae.*

Follow the instructions regarding references in the text.

1 Results

1.1 Contact Effect of Oil Extract on Mortality of Mosquito Larvae

The contact effect of oil extract on mosquito larvae is presented in Table 1. At all levels of oil concentration no larvae mortality was recorded at 30 minutes post-treatment periods. All concentrations of the plant oil were able to cause 43.30%~70.00% larvae mortality at an application period of one hour (1 hr). The 0.4 mL and 0.5 mL of the oil extract concentrations were the earliest to attain 66.7% and 70.00% larvae mortality respectively within 1hr post treatment period and they differ significantly from others ($p<0.05$). These same concentration 0.4 mL and 0.5 mL caused 100% larvae mortality at 1 hr 30mins exposure period and no concentration rate of the plant oil was able to achieve this level of larvae mortality and were significantly different ($p<0.05$) from other except for 0.3 mL oil extract which had 96.70% larval mortality.

Table 1 Contact effect of oil extract of *C. patens* on the mortality of mosquito larvae

Plant oil extracts Conc. (%)	% Mortality after		
	30 mins	1 h	1 h 30 mins
1	0.00±0.00^{a}	43.3±00.33^{b}	83.30±0.33^{b}
2	0.00±0.00^{a}	53.3±00.33^{c}	90.00±0.00^{c}
3	0.00±0.00^{a}	60.0±00.00^{bc}	96.70±0.33^{d}
4	0.00±0.00^{a}	66.70±0.33^{cd}	100.0±00.00^{d}
5	0.00±0.00^{a}	70.00±0.00^{d}	100.0±00.00^{d}
Control	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}

Note: Each value is the mean + standard error of 3 replicates. Mean followed by the same letters are not significantly ($P>0.05$) different from each other using New Duncan's Multiple Range Test

1.2 Contact Effect of Aqueous Extract on Mortality of Mosquito Larvae

The contact effect of powder on mortality of mosquito larval is presented in Table 2. At all levels of concentrations, no larva mortality was recorded within 30 minutes of exposure periods. Meanwhile, 40% and 50% concentrations were the earliest to cause 10% larva mortality at 1 hour of exposure periods, and there were significant difference ($p<0.05$) when compared to other concentrations (10%, 20% & 30%) and the control. At 1 hr 30 mins exposure no concentration rate of the plant powders attained >26.70% larvae mortality and were significantly different ($P<0.05$) from control except for 10% which had 3.30%.

Journal of Mosquito Research, 2014, Vol. 4, No. 11, 1-6
ISSN 1927-646X
http://jmr.biopublisher.ca

Table 2 Contact effect of aqueous extract of *C. patens* on the mortality of mosquito larvae

Plant powder Conc. (%)	% Mortality after		
	30 mins	1 h	1 h 30 mins
10	0.00±0.00^{a}	0.00±0.00^{a}	3.30±0.33^{ab}
20	0.00±0.00^{a}	0.00±0.00^{a}	10.00±0.00^{ab}
30	0.00±0.00^{a}	3.30±0.33a	16.70±0.33^{cd}
40	0.00±0.00^{a}	10.00±0.00^{b}	23.30±0.33^{de}
50	0.00±0.00^{a}	10.00±0.00b	26.70±0.33^{e}
Control	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}

Note: Each value is the mean + standard error of 3 replicates. Mean followed by the same letters are not significantly (P >0.05) different from each other using New Duncan's Multiple Range Test

1.3 Fumigant Effect of Powder on Adult Mosquitoes

The fumigant effect of powder on mortality of adult mosquito is presented in Table 3. At all grams of the powder (1.0 g, 2.0 g, 3.0 g, 4.0 g and 5.0 g), there was no adult mortality at 30minutes post-treatment periods. At one hour post-treatment period 3.0 g, 4.0 g and 5.0 g powder connections were able to cause 13.30 to 16.70% adult mortality at 30 minutes and these were significantly different (P<0.05) from the mortality at 1.0 g, 2.0 g and the control. The 1.0 g and 2.0 g of the powder caused 23.30% adult mortality and were not significantly different from one another (P>0.05). The other concentrations (3.0 g, 4.0 g and 5.0 g) attained 30.00 to 36.70% adult mortality and were not significantly different (P>0.05) from the others but significantly different (P<0.05) from the control.

Table 3 Fumigant effect of powder of C. patens on the mortality of Adult mosquito

Plant powder W/w (g)	% Mortality after		
	30 mins	1 h	1 h 30 mins
1.0	0.00±0.00^{a}	0.00±0.00^{a}	23.30±0.33^{b}
2.0	0.00±0.00^{a}	3.30±0.33^{a}	23.30±0.33^{b}
3.0	0.00±0.00^{a}	13.30±0.33^{b}	30.00±0.33^{c}
4.0	0.00±0.00^{a}	16.70±0.33^{b}	33.00±0.33^{c}
5.0	0.00±0.00^{a}	16.70±0.33^{b}	36.70±0.33^{c}
Control	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}

Note: Each value is the mean + standard error of 3 replicates. Mean followed by the same letters are not significantly (P >0.05) different from each other using New Duncan's Multiple Range Test

Table 4 Fumigant effect of oil extract of *C. patens* on the mortality of Adult mosquito

Plant oil extract Conc. (%)	% Mortality after		
	30 mins	1 h	1 h 30 mins
10	36.70±0.33^{b}	73.30±0.33^{b}	93.30±0.33^{b}
15	43.30±0.33^{b}	80.00±0.00^{c}	100.00±0.00^{b}
20	53.30±0.33^{c}	90.00±0.00^{d}	100.00±0.00^{c}
25	66.70±0.33^{d}	90.00±0.00^{d}	100.00±0.00^{c}
30	70.00±0.00^{d}	90.00±0.00^{d}	100.00±0.00^{c}
Control	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}

Note: Each value is the mean + standard error of 3 replicates. Mean followed by the same letters are not significantly (P >0.05) different from each other using New Duncan's Multiple Range Test

1.4 Fumigant Effect of Oil on Adult Mortality of Mosquitoes

The Fumigant effect of oil on adult mortality of mosquito is presented in Table 4. At 30 minutes post-treatment, all concentrations of the plant oil (10%, 15%, 25%, and 30%) caused 36.70~70% adult mortality. The plant oil at 20~30% was the earliest to cause 90% mortality at 1 hour post-treatment period, whereas, the 10-15% attained 73.30~80.00% adult mortality at 1 hour post-treatment period. At 1 hr 30 mins post-treatment period, all concentrations of the plant oil (10%, 15%, 25%, and 30%) caused

93.30~100.00% adult mortality and were not significantly different ($p>0.05$) from one another except for 1.0 mL which had 93.30% adult mortality but are all different from the control.

2 Discussion

The problem of high cost and development of resistance in many vector mosquito species to several of the synthetic insecticides have received interest in exploring the pest control potential of plants (Grainge and Ahmed, 1988). Also economic and environmental concerns have encouraged a tendency recently toward the use of 'soft' pesticide (Awad, 2003). The assessment of botanicals for the oil extract of *Cleisthopholis patens* shows that at all concentrations (1%, 2%, 3%, 4% and 5%) achieved 83~100% larvae mortality within 1hr 30mins exposure periods. This observation was similar to the report of Oke *et al*, (2011) that hexanolic extract of *Piper guineense* killed both 77 and 95% of *Aedes aegypti* larvae within 1~24hrs, respectively. The mortality of the larvae in this present study could be due to strong pungent odour of the plant oil. A number of plants with high pungency have been reported for their bioactivity against insect pests (Dupriez and De-Leener, 1998). Oils are commonly used in insect control because they are relatively bioactive against virtually all life stages of insects (Adedire 2003; Aranilewa *et al*. 2006). The fumigant effect of the ethanolic oil extract against the adult *An. gambiae* in this study achieved 93~100% mortality within 1hr 30mins post-treatment. This observation is in accordance with the findings of Akinkurolere *et al.* (2011) that the extract of *Xylopia aethiopica* is highly toxic to the larvae and adults of *An. gambiae* due to its strong pungent odour. The aqueous extract of *Cleisthopholis patens* was relatively ineffective against the larvae of *Anopheles gambiae*. The ineffectiveness of aqueous extract of *C. patens* against *Anopheles gambiae* larvae may be due to fact that water used for the extraction is a polar solvent. Also Fafioye *et al*. (2004) reported that the ethanolic oil extract of *Parkia biglobosa* and *Raphia vinifera* were more potent against the juvenile of *Clarias gariepinus* than the aqueous forms. This is due to the polarity, volatility and its power (ethanol) to dissolve more of the active ingredients. The fumigant effect of powder on adults of *Anopheles gambiae* achieved 23.30~36.70% mortality of the insect. Hence, they are slightly effective against the adult insect. The slight effect of the powder against the adult mosquito may be attributed to the choking nature of the plant powder; this may inhibit gaseous exchange between the adult insect and the microclimate environment within the container. The effectiveness of the oil extract might also be attributed to the presence of active ingredients such as tannin, saponin and alkaloid in the plant which has been reported to have antioxidant effect on the aquatic stages of mosquitoes (Akinkurolere *al*. 2011).

Results from this study are in agreement with previous findings where varying degrees of efficacy of plant materials against mosquito species were reported (Al Dakhil and Morsy, 1999; Amusan and Okorie, 2002; El- Bokl 2003; Nathan *et al*. 2005; Promsiri *et al.* 2006; Singh *et al*. 2006).

The plant material used (*Cleisthopholis patens*) in this study was highly effective against *Anopheles gambiae* and could be included in the management strategies of mosquitoes. In addition to their environmental friendliness, this plant is readily available, cheap, and affordable to the resource- poor persons in Nigeria.

3 Materials and Methods

3.1 Insect Culture

Stagnant water was collected in a plastic bowl and placed in a shady area at the back of Biology Laboratory, School of Science, Federal University of Technology Akure, Ondo State, Nigeria. After a few days (about 7days) mosquito larvae were observed to be emerging from the eggs which have been laid by the adult mosquitoes. These larvae were taken into the Biology laboratory for the experiment. In the laboratory, the larvae were transferred into another plastic container containing water; this was in turn transferred into a wooden net cage (of dimension 80×60×50 cm). Inside the container, the larvae were fed with yeast. The adult mosquitoes that emerged after a few days were blood fed on a live rat. The culture was maintained at (28±2)℃, (75±2) % photoperiod of 12 h light followed by 12 h dark (12L:12D).

What are the temperature, photoperiod and relative humidity rates?

3.2 Preparation of Plant Materials

3.2.1 Plant Powder

The stem-bark of *Cleisthopholis patens* (Benth) was obtained from a river bank at Modebiayo Camp in Ondo West Local Government Area of Ondo State. The stem- bark was cut into small pieces and air dried for 30 days in the laboratory before pounding using mortar and pestle. These materials were pulverized in to fine powder with the aid of an industrial electric pulverizing machine at the Department of Animal Production and Health Laboratory FUTA and sieved into fine powder. The powder was kept in an air tight plastic container and stored at ambient temperature (28±2)℃ until when needed.

3.2.2 Plant Oil

One hundred and fifty grams of the pulverized material was weighed into thimble and extracted using ethanol in a soxhlet extractor. Thereafter, the thimble was removed from the unit and the ethanol was recovered by redistilling the contents using rotary evaporator and then air dried to remove any trace of solvent. The oil was kept in a plastic bottle till when needed.

3.3 Toxicity Test

3.3.1 Effect of Ethanolic Oil Extracts on Mosquito Larvae

Different concentrations of the plant extracts; 1%, 2%, 3%, 4% and 5% were measured with the aid of syringe and poured into10 mL of water inside Petri dishes of 9 cm diameter and 3 cm depth. The 3rd instar larvae of mosquitoes were then collected from the breeding cage and introduced into the treated water inside the Petri dishes. Larval mortality was then counted after 30 minutes, 1hour and 1hour 30 minutes. The experiment was replicated three times and one untreated control.

3.3.2 Effect of Aqueous Extract on Mosquito Larvae

The following concentrations, 1 g, 2 g, 3 g, 4 g, and 5 g were soaked into 100 mL of distilled water for 24 hours and then filtered separately using Whatman's No. 1 filter paper. The filtrate was collected into a flask and corked. 10 mL was taken from each concentration prepared with the aid of syringe and emptied into Petri dishes of 9 cm diameter and 3 cm depth. Ten (10) 3rd instar larvae of mosquito were introduced into the treated water and mortality were counted after 30 mins, 1 hour, and 1 hour 30 minutes. Each concentration was replicated three times and untreated control. Larval mortality was calculated as number of dead larvae divided by total number of larvae (10) multiplied by 100.

3.3.3 Effect of Powder and Oil Extracts on Adult Mosquitoes

Different grams (1 g, 2 g, 3 g, 4 g and 5 g) of the plant powder were put in muslin cloths sown into 3 cm × 2 cm dimension and suspended with the aid of thread at a distance of 6 cm from the lid of plastic containers of dimension 13 cm depth 12 cm diameter. Ten (0~24 hrs old) adult mosquitoes were introduced into the plastic containers each containing the suspended bags of plant powders. The experiment was replicated three times and adult mortality was counted after 30 minutes, 1 hour and 24 hours.

The fumigant effect of the plant extract on adult *Anopheles* mosquitoes was tested by measuring the following of the raw extract 1 mL, 1.5 mL, 2.0 mL, 2.5 mL, and 3.0 mL of the oil extract and pouring it on 2 cm× 2 cm Whatman's No. 1 filter paper strips. These strips of filter paper were allowed to dry for 2 hours thereafter placed in plastic containers of dimension 13 cm diameter and 12 cm depth. Ten (0~24 hrs old) adult mosquitoes were introduced and the containers were covered with the lids. The experiment was replicated three times and adult mortality was counted after 30 minutes, 1 hour and 1 hour 30 minutes.

3.4 Statistical Analysis

All data were analysed using Analysis of Variance (ANOVA) and Duncan Multiple range was used to separate the means. All analyses were carried out at 95% confidence level using the Statistical Package for Social Sciences (SPSS) for windows version 16.0.

References

Adedire C.O., 2003, Use of Nutmeg *Myristica Fragans* (Houtt) powder and oil for the control of cowpea storage bruchid,

Callosobruchus maculatus (F). Journal of Plant Disease and Protection 109 (2) 193-199

Akinkulolere R.O., Adedire C.O., Odeyemi O.O., Raji j., and Owoeye J.A., 2011, Bioefficacy of extracts of some indigenous Nigerian plants on the developmental stages of mosquito *(Anopheles gambiae).* Jordan Journal of Biological Sciences. Vol.4, 237-242

Al Dakhil M.A., and Morsy T.A., 1999, The larvicidal activities of the peels oil of three citrus fruits against *C.pipiens.* Journal of Egypt Soc. Parasitol, 29:347-352

Arannilewa S.T., Ekrekene T and Akinneye J.O., 2006, Laboratory evaluation of four medicinal plants as protectants against the maize weevil, *Sitophilus zeamais (Mot.)* African Journal of Biotechnology, 5 (21): 2032-2036

Amusan A.A.S., and Okorie T.G., 2002, The use of *Piper guineense* fruit oil (PFO) as Protectant of dried fish against *Dermestes maculatus* (Degeer) infection. Global Journal of Pure & Applied Science, 8(2): 197-201

Awad O.M., 2003, Operational use of neem oil as an alternative anopheline larvicide. WHO Health Journal pp.24

Campbell F.L., Sullivan W.W., and Smith L.N., 1933, The relative toxicity of nicotine, anabasine, methyl anabasine and lumpinin for Culine Mosquito larvae. Journal of Economic Entomology, 26: 505-509

Choochote W., Kanjanapothi, D., Panthong A., Taesoticul T.; Jitpakdi A., Chaithong U. and Pitasawat B., 1999, Larvicidal, adulticidal and repellent effects of *Kaempferia galanga*. South East Asian Journal of Tropical Medicine and Public health, 30: 470-476

Collins F.H., Paskewitz S.M., 1995**,** Malaria current and future prospects for control. Ann. Rev.Entomology. 40: 195-219 http://dx.doi.org/10.1146/annurev.en.40.010195.001211

Dupriez H., and De Leneer P., 1998, African gardens and orchards - growing vegetables and fruits. Macmillian Publishers Ltd, London. pp.333

EL-Bokl M.M., 2003, Latent toxicity of azadirachtin treatment on *C. pipens (Diptera: Culicidae).* Journal of Egypt Academic Social Environmental Development, 3**:** 63-74

Fafioye O.O., Adebisi A.A., and Fayode S.O., 2004, Toxicity of *Parkia biglobosa* and *Raphia vinifera* extracts on *Clarias gariepinus* juveniles. African Journal of Biotechnology, 3 (10)**:** 627-630

Grainge M., and Ahmed S., 1988, Handbook of plants with pest control properties. John Wiley and sons, New York, pp.41

Mark S. F., 1998, Mosquitoes and mosquito repellents: A clinician's guide by: Annals of Internal Medicine

Monzon R.B., Alvior J.P., Luczon L.L., Morales A.S., and Muctuc F.E., 1994, Larvicidal potentials of five Philippine plants against *Aedes aegypti* (Linnaeus) and *Culex quiguefasciatus* (say). South East Asian Journal of Tropical Medicine and Public Health, 25: 755-759

Nathan S.S., Kalaivani K., and Murugan K., 2005, Effects of neem limonoids on the malaria vector *Anopheles stephensi* Liston (Diptera: Culicidae). Act Trop, 96:47-55 http://dx.doi.org/10.1016/j.actatropica.2005.07.002

Oke O.A., Anyale O.O., Amusan A.A.S., and Okorie T.G., 2011, Toxicity of hexanolic extract of *Piper guineense Schum* and *Thunn (Piperaceae)* seed oil to larvae of *Aedes aegypti* (L). European Journal of Science Research, 18 (1): 6-11

Promsiri S., Naksathit, Kruatrachue M., and Thavara U., 2006, Evaluation of larvicidal activity of medicinal plant extracts to *Aedes aegypti* (Diptera: Culicidae) and other effects on a non-target fish. Insect Science, 13:179-188 http://dx.doi.org/10.1111/j.1744-7917.2006.00080.x

RBM (Roll Back Malaria. Malaria) key facts, January 2011, (Accessed July 20th, 2012, at http://www.rollbackmalaria.org/key facts.html)

Sharma R.N., Tera V.S., and Despandes S.G., 1990, New chemicals natural products and their permutation and combination to combat insect pest. In: Impact of environment on animals and aquaculture, Edited By G.K. Manna and B.B. Jain. 97-100

Shin-foon C., 1984, The active principle and insecticidal properties of some Chinese plants with special reference to Meliceae. 21: 255-262

Singh R.K., Dhiman R.C., and Mittal P.K., 2006, Mosquito larvicidal properties of *Momordica charantia* Linn (Family: Cucurbitaceae). Journal of Vector Borne Disease. 43:88-91

WHO 2010, World Health Organization, Malaria: Fact sheets. No 94, April 2010, (Accessed July 20th 2012, at http://www.who.Int/mediacentre/factsheets/fs094/enl)