2014

Larvicidal potential of wild mushroom extracts against Culex quinquefasciatus Say, Aedes aegypti and Anopheles gambiae Giles S.S

Chelela, Baraka

American Journal of Research Communication

Larvicidal potential of wild mushroom extracts against *Culex quinquefasciatus* Say, *Aedes aegypti* and *Anopheles gambiae* Giles S.S

Baraka Luca Chelela, Musa Chacha, Athanasia Matemu*

The School of Life Sciences and Bio-Engineering,
Nelson Mandela African Institute of Science and Technology (NM-AIST),
P. o. Box 447, Arusha Tanzania
*Author to whom correspondence should be addressed: athyone@yahoo.com

ABSTRACT

The crude extracts from six wild mushrooms namely *Lactarius densifolius, Lactarius gymnocarpoides, Russula cellulata, Russula kivuensis, Amanita phalloides and Boletus species* were evaluated for larvicidal activity against *Anopheles gambiae, Aedes aegypti* and *Culex quinquefasciatus* larvae. Generally, the crude mushroom extracts demonstrated low to high larvicidal activities against all tested mosquito larvae. The *L. gymnocarpoides* ethanol extract (BM2E) exhibited the highest activity against *A. aegypti* with LC_{50} of 10.75 µg/mL after 72 h of exposure. *Lactarius densifolius* chloroform extract (BM8C) was effective against *A. gambiae* (LC_{50} = 91.33 µg/mL) and moderate effective against *C. quinquefasciatus* (LC_{50} = 181.16 µg/mL) respectively. Therefore, wild mushrooms can be a potential source of bio-insecticides for commercial mosquito vector management especially in aquatic ecosystems.

Key words: *Anopheles gambiae, Aedes aegypti, Culex quinquefasciatus,* Larvicidal activity, wild mushroom extracts

Running title: Larvicidal activity of wild mushroom extracts

{Citation: Baraka Luca Chelela, Musa Chacha, Athanasia Matemu. Larvicidal potential of wild mushroom extracts against *Culex quinquefasciatus* Say, *Aedes aegypti* and *Anopheles gambiae* Giles S.S. American Journal of Research Communication, 2014, 2(8): 105-114} www.usa-journals.com, ISSN: 2325-4076.

INTRODUCTION

Mosquito-borne diseases like malaria, filariasis, Japan encephalitis (JE), dengue fever, chikungunya, dengue hemorrhagic fever (DHF) and yellow fever has created huge impact on humans over the whole world (Mandal, 2012; Borah *et al.*, 2010). Poor drainage system, especially during rainy seasons, the existence of many fish ponds, irrigation channels and the rice fields provide abundant mosquito breeding places (Prabhu *et al.*, 2011). Malaria, dengue fever and other vector-borne diseases contribute to the major disease burden in Tanzania and they cause allergic responses to humans, which include local skin reaction and systemic reactions such as angioedema and urticarial (Osunga, 2013). In recent years, *Aedes aegypti* (Diptera: Culicidae) spread the virus of dengue fever and chikungunya which affect most part of the coastal areas of Tanzania (Moi, 2010; Hertz, 2012) hence increasing burden to the heath sector. The recurrence of these diseases is due to high number of breeding places and the increasing resistance of mosquitoes to the use of commercial insecticides (Borah *et al.*, 2010; Cowdhury *et al.*, 2008). The most successful method of minimizing the incidence of mosquito-borne diseases is to eradicate and control the mosquito vectors. The main effective control remains to be the chemical insecticides (Mandal, 2012; Njogu*et al.*, 2009) which are not safe for humans and environment. Synthetic insecticides such as organochlorine, organophosphourous, carbamates, pyrethrins and pyrethroids are commonly used for controlling the ever increasing population of vectors. The over use of these chemical insecticides is not safe due to environment hazard caused by their potential toxicity to non-target organisms and has resulted in development of resistance among the vectors (Omondi and Omondi, 2013; Chirchir *et al*., 2013). Also, high operational costs and community acceptance is additional short falls of chemical pesticides (Mandal, 2012; Mohamed *et al.*, 2013; Omondi and Omondi, 2013). Therefore, bioactive compounds extracted from wild mushrooms can be alternative bio-pesticides to reduce stress put to humans and environments through the use of chemical insecticides.

Secondary metabolites derived from plant and fungi can act as larvicides, insect growth controllers, repellents and ovipositional attractants with deterrent or restrictive activities. An effective repellent will be useful in reducing man vector contact and in interjecting disease spread (Prabhu *et al.*, 2011). The bioactive compounds synthesized by mushrooms have reported to demonstrate a broad spectrum of bioactivity varying from neurologically activity in humans to nematicidal and insecticidal in lower form of animals (Mohamed *et al*., 2013). Bio-pesticides is currently gaining acceptance among vector and pest control strategies which includes microbial control agents, phytochemicals with insecticidal activity, toxins produced by fungi and organisms (WHO 1998; WHO 1996). Emphasis is now focusing on extracting, and screening of bioactive compounds from plants and higher fungi for insecticidal activity. The botanicals

might be used as an alternative to other insecticides for the control of mosquito and thus mosquito borne diseases (Mandal, 2012). Many studies on larvicidal activity of plants have been reported (Shaalan *et al.*, 2005; Njogu *et al.*, 2009) but little has been done on wild mushrooms. Despite fungi being known as prolific producers of novel biologically active molecules with some prominent applications as pharmaceuticals, nutraceuticals and agrochemicals (McCartney *et al*., 2007; Njogu *et al*., 2009), fungi specially mushrooms have been under-studied as larvicidal agents in the control of mosquito. Therefore, this study explores the potential of wild mushrooms as bio-pesticides giving an insight for the search of new and better ways to develop fungi-based anti-mosquito agents for vectors control with little or no risks to humans or environment.

MATERIALS AND METHODS

Materials

Fruiting bodies of wild mushrooms both edible (*Lactarius densifolius* and *Russula cellulata*) and inedible (*Lactarius gymnocarpoides, Russula kivuensis, Amanita phalloides and Boletus species*) were collected in Njombe and Mufindi districts - Tanzania in January, 2014. The mushroom samples were identified in the Department of Molecular Biology and Biotechnology, University of Dar-Es-Salaam. *Anopheles gambiae s.s, Culex quinquefasciatus* Say, and *Aedes aegypti* larvae were obtained from the Tropical Pest Research Institute (TPRI) in Arusha, Tanzania. All other chemicals and reagents used were of analytical grade.

Preparation of wild mushroom extracts

Twenty five grams of fresh mushroom (entire mushroom) were ground in a blender (Singsung - Singapore) and extracted using 250 mL of ethanol and chloroform separately for 48 h. The wild mushroom extracts were concentrated in a rotary evaporator (Heildolph, Germany) and the collected crude extracts were stored in refrigerator for further bioassays.

Larvicidal assay

Larvicidal assay was carried out by exposing 10 late 3rd instar into crude wild mushroom extracts of various concentrations (50, 100, 250, 500 and 800 µg/mL) according to (WHO, 1996; Nondo *et al.*, 2011) with minor modifications. The crude extracts of known concentration were then added into beakers containing 10

late 3rd instar. Water was used to prepare samples but in some cases dimethyl sulphoxide (DMSO) 50% v/v was used. In each case negative control or blank was included. The beakers were then covered by mosquito nylon mesh to prevent other mosquitoes or insects from laying eggs or to avoid falling debris (Andemo *et al.*, 2014). The room temperature was maintained at 26 ± 2 °C during the experiment and larvae were fed on Tetramin fish food at 1.0 mg per beaker per day (Innocent *et al.*, 2009). Larvae were confirmed dead when they failed to move after probing them with a needle (Andemo *et al.*, 2014). The numbers of dead larvae were recorded after 24, 48 and 72 h of exposure and the mean percentage mortalities for each concentration were calculated according to Nondo *et al.*, (2011).

Data analysis

For each assay, the whole mushroom specie was used and the results represent means of triplicates determinations as calculated using Microsoft Excel (2013). The concentrations killing fifty percent of the larvae (LC_{50}) was calculated from the regression equations obtained from the graphs. Regression equations obtained from the graphs were used to obtain LC_{16}, LC_{50}, LC_{84} and the 95% Confidence Interval values.

RESULTS

The results of larvicidal activities of crude extracts from six wild mushroom species (*Lactarius densifolius, Lactarius gymnocarpoides, Russula cellulata, Russula kivuensis, Amanita phalloides and Boletus species*) collected in the Southern highlands of Tanzania against *Anopheles gambiae s.s* (Table 1), *Aedes aegypti* (Table 2), and *Culex quinquefasciatus Say* (Table 3) are as shown. According to Komalamisra *et al.* (2005) and Bucker *et al.* (2013), classification of plant larvicidal activities is considered as nontoxic when the LC_{50} is greater than 750 µg/mL, weakly effective (LC_{50} is between 200 to 750 µg/mL), moderate (LC_{50} is between 100 to 200 µg/mL), effective (LC_{50} is between 50 to 100 µg/mL), and highly effective (LC_{50} is less than 50 µg/mL). Ethanol extract of *L. gymnocarpoides* (BM2E) was highly effective against *A. aegypti* (LC_{50} = 10.75 µg/mL) (Table 2) and inactive against *C. quinquefasciatus* (LC_{50} = 1378.10 µg/mL) (Table 3). Chloroform extract of *L. densifolius* (BM8C) was effective against *A. gambiae* (LC_{50} = 91.33 µg/mL) (Table 1) and moderate effective against *C. quinquefasciatus* (LC_{50} = 181.16 µg/mL) (Table 3). Ethanol extract of *R. cellulata* (BM4E) was moderately effective (LC_{50} = 141.46 µg/mL) against *A. gambiae* (Table 1) and effective against *A. aegypti* (LC_{50} = 81.59 µg/mL) (Table 2), however, it was inactive against *C.*

quinquefasciatus (LC_{50} = 3943.03 µg/mL) (Table 3). On the other hand, *R. kivuensis* chloroform extract (MS4C) was weakly effective (LC_{50} = 230.17 µg/mL) against *A. aegypti* (Table 2). *Boletus sp.* chloroform extract (BS2C) was moderately effective against *A. gambiae* (LC_{50} = 175.30 µg/mL) (Table 1), whereas *L. densifolius* ethanol extract (BM8E) was inactive against *A. aegypti* (LC_{50} = 933.95 µg/mL) (Table 2).

Table 1: Larvicidal activity (LC_{50}) of extracts from *L. densifolius, Boletus sp* and *R. cellulata* mushroom species against *A. gambiae*

Extracts	Time (h)	LC_{50} (µg/mL)	95% Confidence Interval (µg/mL)	Regression equation	Regression coefficient (R^2)
BM8C	24	174.93	136.723-223.80	Y=82.54x-135.13	0.99
	48	112.85	82.31-154.72	Y=76.82x-107.67	0.92
	72	91.33	64.00-130.33	Y=68.18x-83.68	0.92
BM4E	24	283.96	208.74-386.31	Y=70.45x-122.84	0.99
	48	177.63	134.34-234.89	Y=77.62x-124.60	0.97
	72	141.46	103.52-193.29	Y=77.65x-117.00	0.95
BS2C	24	235.50	186.55-297.29	Y=93.04x-170.69	0.92
	48	205.73	160.79-263.23	Y=98.35x-177.52	0.92
	72	175.30	131.21-234.20	Y=83.66x-137.77	0.89

Table 2: Larvicidal activities (LC_{50}) of extracts from *L. densifolius, R. cellulata, R. kivuensis* and *L. gymnocarpoides* mushroom species against *A. aegypti*

Extracts	Time (h)	LC_{50} (µg/mL)	95% Confidence Interval (µg/mL)	Regression equation	Regression coefficient (R^2)
BM2E	24	445.61	227.91-871.26	Y=32.34x-35.67	0.82
	48	36.85	18.31-74.18	Y=30.97x+1.45	0.97
	72	10.75	4.89-23.64	Y=27.51x+21.62	0.96
BM4E	24	3943.03	1010.02-15393.18	Y=15.92x-7.25	0.99
	48	151.31	92.21-248.29	Y=43.77x-45.45	0.90
	72	81.59	48.71-136.65	Y=42.05x-30.37	0.89
MS4C	24	935.26	455.07-1922.15	Y=30.10x-39.43	0.91
	48	400.70	226.50-708.88	Y=38.01x-48.94	0.94
	72	230.17	141.97-373.15	Y=44.88x-56.00	0.95
BM8E	24	5047.46	2168.90-11746.46	Y=25.67x-45.07	0.99
	48	4270.23	1571.55-11603.06	Y=21.69x-28.75	0.87
	72	933.95	397.36-2195.16	Y=25.38x-25.37	0.94

Table 3: Larvicidal activities (LC_{50}) of extracts from *L. densifolius, R. cellulata, L. gymnocarpoides* and *A. phalloides* mushroom species against *C. quinquefasciatus*

Extracts	Time (h)	LC_{50} (µg/mL)	95% Confidence Interval (µg/mL)	Regression equation	Regression coefficient (R^2)
BM8C	24	445.61	227.91-871.27	Y=32.34x-35.67	0.82
	48	327.28	190.21-563.18	Y=39.95x-50.48	0.90
	72	181.16	116.34-282.10	Y=48.958x-60.55	0.84
MS2E	24	685.89	429.49-1095.37	Y=46.32x-81.38	0.98
	48	685.89	429.49-1095.37	Y=46.32x-81.38	0.98
	72	505.91	326.18-784.66	Y=49.41x-83.60	0.94
BM2E	24	71115.76	18216.40-277628.80	Y=15.92x-27.25	0.99
	48	3717.00	3428.34-4029.97	Y=26.82x-45.75	0.96
	72	1378.10	1298.14-1462.99	Y=36.27x-63.87	0.94
BM4E	24	12766.66	4533.70-35948.36	Y=20.95x-36.01	0.99
	48	7614.58	2403.82-24120.71	Y=18.81x-23.00	0.93
	72	3943.03	1010.02-15393.18	Y=15.92x-7.25	0.99

Key: BM8C = *Lactarius densifolius* chloroform extract, BS2C = *Boletus sp.* chloroform extract, BM4E = *Russula cellulata* ethanol extract, MS4C = *Russula kivuensis* chloroform extract, BM8E = *Lactarius densifolius* ethanol extract, BM4E = *Russula cellulata* ethanol extract, BM2E = *Lactarius gymnocarpoides* ethanol extract and MS2E = *Amanita phalloides* ethanol extract.

DISCUSSION

In the present study, most of the wild mushroom extracts exhibited moderate larvicidal activities with few extracts showing no activity against tested mosquito larvae. The larvicidal effect of wild mushroom extracts against *A. gambiae s.s, C. quinquefasciatus Say* and *A. aegypti* was dose dependent. Similar findings were also reported by Bucker *et al.* (2013) where larvicidal activities from basidiomycete's extracts of *Pestalotiopsis virgulata* ethyl acetate mycelia and *Pycnoporus sanguineus* ethyl acetate mycelia exhibited LC_{50} of 101.80 µg/mL and 156.80 µg/mL respectively against *A. aegypti.* Baraza *et al.* (2007) reported lethality effect of *Agaricus* sp. aff. *Arvensis* ethanol extract against *A. gambiae* mosquito larvae, with

moderate activity (LC_{50} = 150 µg/mL) after 72 h exposure. From the study, ethanol extracts of *A. phalloides*, *R. cellulata*, *L. gymnocarpoides* and *L. densifolius* exhibited weak or no activity against *C. quinquefasciatus* and *A. aegypti* (Table 2 & 3). Low larvicidal activities suggest presence of many compounds which may provoke each other and reduce the activity as established by Suay *et al.* (2000). According to Chirchir *et al*. (2013), mosquito larvicidal efficacies of the purified compounds were observed to be relatively higher than that of the crude extracts. This proposes that purification of the extracts is important to enhance the larvicidal activity of compounds. This indicates that bioactivity guided fractionation and purification may lead to the isolation of active chemical compounds responsible for the larvicidal activity. Therefore, wild mushroom species might be considered as a potential source of bio-insecticides compounds hence further studies to isolate and purify larvicidal active compounds is crucial.

Variability in larvicidal activities between edible and inedible wild mushroom species was also observed. Some extracts from edible mushroom species were effective and/or inactive against some of mosquito larvae, similar variations were also observed in inedible mushroom species (Table 1-3). Despite of *A. phalloides* being a deadly poisonous mushroom, its larvicidal activity was very weak (LC_{50} = 505.91 µg/mL) against *C. quinquefasciatus* (Table 3). This may be due to low affinity and intrinsic activities of compounds on mosquito larvae receptor sites.

The highest larvicidal activity exhibited by *L. gymnocarpoides* ethanol extract (BM2E) against *A. aegypti*, suggest its potential application as an insecticidal agent. High larvicidal activity of *Pezicula livida* against *A. aegypti* was found to be strongly active, with 100% mortality observed at a concentration of 20 µg/mL after 2 h and its LC_{50} and LC_{90} values of 3.0 µg/mL and 59.0 µg/mL respectively Kendagor, (2013). From this study, it is clear that extracts of *L. gymnocarpoides* and *R. cellulata* can be useful as anti-mosquito agents as they were substantially effective against *A. gambiae* and *A. aegypti* respectively.

CONCLUSION

Wild mushrooms can be a potential source of insecticides for mosquito vector management. Variation in larvicidal activities of wild mushroom species against *A. aegypti, A. gambiae* and *C. quinquefasciatus* were observed. *Lactarius gymnocarpoides* ethanol extract exhibited the highest larvicidal activity against *A. aegypti* giving an insight on the possibility of commercial application as mosquito repellent against dengue, yellow fever and chikungunya vector (*A. aegypti*). The wild mushrooms are plenty available, inexpensive and eco-friendly providing an opportunity for developing bio-insecticides. Further studies on isolation of

bioactive compounds and synergistic combinations from wild mushrooms may lead into development of mosquito repellants and its application for mosquito control.

ACKNOWLEDGEMENTS

The authors are grateful to the Commission for Science and Technology (COSTECH) through the Nelson Mandela Africa Institute of Science and Technology (NM-AIST) for financial support. Also, thankful to Dr. Donatha Tibuhwa of Department of Molecular Biology and Biotechnology of the University of Dar-Es-Salaam for identification of wild mushroom species and Abdul Kidukuli of The Institute of Traditional Medicine, Muhimbili University of Health and Allied Sciences (MUHAS), Dar-Es-Salaam for his technical support.

REFERENCES

Andemo, A., D. Yewhalaw, B. Alemayehu and A. Ambelu, 2014: Evaluation of the mosquitocidal effect of Birbira (Mellitia ferruginea) seed extract against *Anopheles arabiensis* (Diptera: Culicidae) from Ethiopia. *Acta Tropica*. http://dx.doi.org/10.1016/j.actatropica.2014.04.016, 1-6.

Baraza, L., C. Joseph, M. Moshi and M. Nkunya, 2007*:* Chemical constituents and biological activity of three Tanzanian wild mushroom species. *Tanzania Journal of Science, 33, 1-7.*

Borah, R., M. Kalita, A. Kar and A. Talukdar, 2010: Larvicidal efficacy of *Toddalia asiatica* (Linn.) Lam against two mosquito vectors *Aedes aegypti* and *Culex quinquefasciatus*. *African Journal of Biotechnology*, 9, 2527-2530.

Bucker, A., N. C. F. Bucker, A. Q. L. d. Souza, A. M. d. Gama, E. Rodrigues-Filho, F. M. d. Costa, C. V. Nunez and W. P. Tadei, 2013*:*Larvicidal effects of endophytic and basidiomycete fungus extracts on Aedes and Anopheles larvae (Diptera, Culicidae). *Revista da Sociedade Brasileira de Medicina Tropical*, 46, 411-419.

Chowdhury, N., S. Laskar and G. Chandra, 2008: Mosquito larvicidal and antimicrobial activity of protein of *Solanum villosum* leaves. *BMC complementary and alternative medicine,* 8, 62-68.

Chirchir, D. K., Ouma, R. B. O., Cheplogoi, P. K., and Omolo, J. O., 2013: Larvicidal activity of extracellular secondary metabolites from a *Stereum species* Hill ex Pers.(JO5289) against the dengue fever mosquito, *Aedes aegypti* (Linn) (Diptera: Culicidae). *African Journal of Biotechnology*, 12, 6302-6309.

Hertz, J. T., Munishi, O. M., Ooi, E. E., Howe, S., Lim, W. Y., Chow, A., Morrissey, A, B., Bartlett, J, A., Onyango, J, J., Maro, V. P., Kinabo, G, D., Saganda, W., Gubler, D. J. and Crump, J, A. (2012): Chikungunya and dengue fever among hospitalized febrile patients in northern Tanzania. *The American journal of tropical medicine and hygiene, 86*, 171-177.

Innocent, E., Cosam, J., Gikonyo, N., Nkunya, M., and Hassanali, A., 2009: Larvicidal properties of some Tanzanian plant species against *Anopheles gambiae s.s Gile* (Diptera: Culicidae) mosquitoes. *International Journal of Biological and Chemical Sciences*, 3, 266-270.

Kendagor, A. C., Langat, M. K., Cheplogoi, P. K., & Omolo, J. O. (2013). Larvicidal activity of mellein from cultures of an ascomycete Pezicula livida against Aedes aegypti. *Internation Journal of Life Sciences Biotechnology and Pharma Research, 2*, 70-80.

Komalamisra, N., Y. Trongtokit, Y. Rongsriyam and C. Apiwathnasorn, 2005: Screening for larvicidal activity in some Thai plants against four mosquito vector species. *South East Asian Journal of Tropical Medical and Public Health*, 36, 1412-22.

Mandal, S., 2012: Mosquito vector management with botanicals: The most effective weapons in controlling mosquito-borne diseases. *Asian Pacific Journal of Tropical Biomedicine*, 2, 336-336.

McCartney, C., P. Mercer, L. Cooke and B. Fraaije, 2007: Effects of a strobilurin-based spray programme on disease control, green leaf area, yield and development of fungicide-resistance in *Mycosphaerella graminicola* in Northern Ireland. *Crop protection*, 26, 1272-1280.

Mohamed Yacoob Syed Ali, S. R. a. J. M. B., 2013: Mosquito larvicidal activity of seaweeds extracts against *Anopheles stephensi, Aedesaegypti* and *Culex quinquefasciatusAsian. Pacific Journal of Tropical Diseases*, 3, 196-201.

Moi, M. L., Takasaki, T., Kotaki, A., Tajima, S., Lim, C. K., Sakamoto, Iwagoe, H., Kobayashi, K., M. and Kurane, I. (2010). Importation of dengue virus type 3 to Japan from Tanzania and Côte d'Ivoire. *Emerging infectious diseases, 16*, 1770-1772.

Njogu, E. M., A. Njue, J. O. Omolo and P. K. Cheplogoi, 2009: Larvicidal activity of (oxiran-2-yl) methylpentanoate extracted from mushroom *Cyptotramaasprata* against mosquito *Aedesaegypti*. *International Journal of Biological and Chemical Sciences*, 3, 1203-1211.

Nondo, R. S., Z. H. Mbwambo, A. W. Kidukuli, E. M. Innocent, M. J. Mihale, P. Erasto and M. J. Moshi, 2011: Larvicidal, antimicrobial and brine shrimp activities of extracts from *Cissampelosmucronata* and Tephrosiavillosa from coast region, Tanzania. *BMC complementary and alternative medicine*, 11, 33-40.

Omondi, O. R. B. and R. B. Omondi, 2013: Characterization of Mosquito Larvicidal Compounds from Cultures of a Basidiomycetes (*Coriolushirsutus)*. *A Thesis for the Degree of Master of Science in Chemistry of Egerton University.*

Osunga, S. A., 2013: Purification and Chemical Characterization of Larvicidal Compound (s) of Extracts Prepared from Submerged Cultures of Basidiomycetes against *Aedes aegypti*. *A Thesis for the Degree of Master of Science in Chemistry of Egerton University.*

Prabhu, K., K. Murugan, A. Nareshkumar, N. Ramasubramanian and S. Bragadeeswaran, 2011: Larvicidal and Repellent Potential of Moringa oleifera against Malarial vector, Anopheles stephensi Liston (Insecta: Diptera: Culicidae). *Asian Pacific journal of tropical biomedicine*, 1, 124-129

Shaalan, E. A.-S., D. Canyon, M. W. F. Younes, H. Abdel-Wahab and A.-H. Mansour, 2005: A review of botanical phytochemicals with mosquitocidal potential. *Environment international*, 31, 1149-1166.

Suay, I., Arenal, F., Asensio, F.J., Basilio, A., Cabello, M.A., Díez, M.T., García J.B., Val, A.G., Gorrochategui, J., Hernández, P., Peláez, F., Vicente, M.F. (2000). Screening of basidiomycetes from antimicrobial activities. Antonie van Leeuwenhoek, 78, 129-139.

WHO, 1996: Report of the WHO Informal Consultation on the Evaluation and Testing of Insecticides. WHO, Geneva, Switzerland, 7-11 October 1996.

WHO, 1998: Report of the WHO Informal consultation: Test procedures for Insecticide Resistance Monitoring in Malaria Vectors, Bio-Efficacies and persistence of insecticides on treated surfaces. WHO, Geneva, Switzerland, 28-30 September 1998.