Research Article

Open Access

The Stressor Effect of Ethanol and Water Extracts of the Magical Herb *Phytolacca dodecandra* (L' Herit) on Life History of *Anopheles gambiae* (Diptera: Culicidae) Mosquitoes

Yugi J.O. ✉

School of Science and Technology, University of Kabianga, P.O. Box 2030-20200, Kericho, Kenya

✉ Corresponding author email: yugijared@gmail.com

Journal of Mosquito Research, 2019, Vol.9, No.2doi: [10.5376/jmr.2019.09.0002](https://doi.org/10.5376/jmr.2019.09.0002)

Received: 01 Mar., 2019

Accepted: 28 Apr., 2019

Published: 6 Jun., 2019

Copyright © 2019 Yugi, This is an open access article published under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

Preferred citation for this article:

Yugi J.O., 2019, The stressor effect of ethanol and water extracts of the magical herb *Phytolacca Dodecandra* (L' Herit) on life history of *Anopheles gambiae* (Diptera: Culicidae) mosquitoes, Journal of Mosquito Research, 9(2): 9-17 (doi:[10.5376/jmr.2019.09.0002](https://doi.org/10.5376/jmr.2019.09.0002))

Abstract In this study an informal “after only with control” experimental design was used to evaluate the impact of ethanol and water extracts of leaf and mature green fruits of *Phytolacca dodecandra* on the life history of *An. gambiae*. Eighty milligrams of crude extracts of mature green fruits of *P. dodecandra* was dissolved in 100 millilitres of rain water and the solution serially diluted to concentrations of 80, 40, 20, 10, 5, and 2.5 mg/100 mL. The diluents were then added into five groups of six sets of larval rearing trays each measuring 21 cm × 15 cm × 8 cm. This was repeated for leaves of *P. dodecandra*, *Azadirachta indica* leaves, and Deltamethrin. Each tray received 100 freshly laid *An. gambiae* eggs. Climatic conditions within insectaries were 29°C–30°C, 70%–80% relative humidity, and 12:12, L: D photoperiod. The experiments were replicated four times. The extracts impacted the life history of exposed mosquitoes irrespective of parts of *P. dodecandra* used or dose. Parts of plant used impacted mortality significantly ($p < 0.001$) compared to dose ($p > 0.05$). Water extract was more potent than ethanol extracts ($p < 0.05$). Though toxicity reduced with time, longevity of the extracts was dose dependent and significantly influenced mortality ($p < 0.001$). Lethal doses (LC₅₀) were relatively lower (doses of ≤ 4.6) with small lower and upper 95% CI. It is concluded that extracts of *P. dodecandra* impacts the life cycle of *An. gambiae* mosquitoes critically interfering with their development.

Keywords *An. gambiae*; *A. indica*; Ethanol; Longevity; *P. dodecandra*; Water

Background

Malaria is a global health problem with an estimate of 3.2 billion people at risk worldwide. Sub-Saharan Africa populace suffers the most with a record of 88% infections and 90% deaths (WHO, 2015). In Kenya, malaria is a major cause of morbidity and mortality with prevalence upward trend in terms of age, with children aged 11–14 years showing the highest prevalence (Marufa et al., 2017). Mosquitoes are vectors to a wide range of death causing human diseases such as filariasis, malaria, dengue, yellow fever, and Japanese encephalitis in addition to malaria (Pugazhvendan and Elumali, 2013).

Malaria vector control programmes mainly involve the use of pyrethroid based synthetic chemical in the form of insecticides treated nets and indoor residual sprays (Oss'e et al., 2013; Okia et al., 2013). While these strategies have been successful, overuse (Zhao et al., 2002) has led to emergence of mosquito (Ruikar et al., 2012; Chouaibou et al., 2012; Alex et al., 2012; Brown et al., 2013; Lo and Coetzee, 2013) and parasite (Checchi et al., 2006) resistance.

To ameliorate the above trend, scientist have turned to plants for new sources of products against malaria vector for the simple reason that plants produce a broad range of bioactive chemical compounds (Altemimi et al., 2017) many of which have promising mosquitocidal activities (Tennyson et al., 2012). Indeed some phytochemical have even proven to be more effective than synthetic insecticides (Olaitan and Abiodun, 2011). Besides being target-specific (Mann and Kaufman, 2012), the plant-derived insecticides also decompose quickly (Khater, 2012) do not biomagnify (Isman, 2006) and due to poor penetrance in ecosystems, do not affect large animals (Evergetis et al., 2012).

The extracts act as environmental stressor and initiate transient states involving change in physiological responses that bring an insects near to or over the edges of their “ecological niches” (Korsloot et al., 2004). The changes affect certain crucial aspects such as fertility, fecundity, development time, and longevity. These factors are referred to as life history parameters and are important determinants of an organisms’ fitness (Grech et al., 2007). Indeed the longer the insect takes to mature, the lower the probability of the larvae emerging into adults and the higher the probability of increased exposure of emerged adults to potential predation and human interference (Padmanabha et al., 2011). This in the end impacts fitness of adult emergence and subsequent biological life cycle (Kweka et al., 2012). On the other hand if the vector does not live long enough (vectors of diseases with intrinsic incubation period like malaria), the parasite might never be able to be transmitted, and the vector competence is therefore compromised (Garrett-Jones and Shidrawi, 1969; Muturi et al., 2011; Araujo et al., 2012; Takken et al., 2013; Lefèvre et al., 2013; Moller-Jacobs et al., 2014; Breaux et al., 2014).

Knowledge of the malaria vector’s interaction with factors that influence important aspects in its life cycle such as insecticides and other environmental stressors can lead to the development of new vector control tools. In this study, the impact of ethanol and water extracts of mature green fruit and leaves of *P. dodecandra* on the life history of malaria vector, *Anopheles gambiae* (Diptera: Culicidae) mosquito eggs was evaluated.

1 Results

It was found that all the treatments impacted on the life history of the exposed mosquito population irrespective of parts of *P. dodecandra* used or dose. However, extracts from different parts of *P. dodecandra* significantly influenced mortality of the different life stages ($[p < 0.001]$ (Table 1) while dose did not ($[p > 0.05]$, Table 2). Water extract of *P. dodecandra* was observed to be relatively more potent than ethanol extracts though t and F (Levene’s statistics on equality of population variance) statistics ($p < 0.05$) showed that none of the solvent significantly influenced toxicity of the extracts on the life stages (Table 3).

Table 1 Percent mortality of *An. gambiae* mosquito life stages exposed to *P. dodecandra* fruit and leaf extracts compared to *Azadirachta indica* leaf extracts and Deltamethrin

Mosquito life history stage	<i>P. dodecandra</i>		Positive control		Negative control	df	F	P
	Fruit	Leaves	<i>A. indica</i>	Deltamethrin	Water			
L1	71.25±3.97	74.83±4.39	81.83±2.06	0.00±0.00	97.83±0.60	4	180.83	0.000
L2	25.50±9.41	72.08±5.01	73.58±2.22	0.00±0.00	94.75±0.79	4	63.91	0.000
L3	21.92±8.71	67.50±5.49	67.83±3.22	0.00±0.00	92.00±0.70	4	60.69	0.000
L4	21.08±8.51	64.25±5.31	66.67±3.21	0.00±0.00	90.58±0.58	4	61.24	0.000
Pupae	15.42±6.33	51.42±6.48	50.75±4.13	0.00±0.00	89.25±0.41	4	61.28	0.000
Adults	14.58±6.65	50.17±6.42	47.92±4.20	0.00±0.00	87.25±0.51	4	56.13	0.000

Note: 1: df: equals to degrees of freedom; 2: F is the F statistics; 3: P is the probability on level of significance. P was considered significant at $p < 0.05$

Table 2 Percent mortality of *An. gambiae* mosquito life stages exposed to different concentrations of *P. dodecandra* extracts

Mosquito life stage	Concentration of treatments (mg/100mls) used against <i>An. gambiae</i> life history stages						df	F	P
	2.5	5.0	10.0	20.0	40.0	80.0			
L1	71.70±12.15	69.30±11.86	67.60±11.78	66.00±11.63	60.50±11.11	55.80±10.76	5	0.26	0.931
L2	68.40±11.64	61.70±11.84	55.80±12.76	49.20±13.32	43.40±12.54	40.60±12.24	5	0.76	0.586
L3	66.10±11.35	58.00±11.59	52.20±12.29	46.40±12.99	40.00±11.91	36.40±11.57	5	0.87	0.504
L4	64.10±11.00	57.10±11.50	50.90±12.12	44.20±12.47	39.40±11.78	35.40±11.39	5	0.87	0.509
Pupae	58.50±10.65	49.90±10.67	41.80±11.05	36.70±11.53	32.30±10.99	29.00±11.05	5	1.03	0.408
Adults	58.60±10.33	47.70±10.67	40.10±11.13	35.20±11.13	31.20±10.46	27.10±10.88	5	1.16	0.342

Notes: 1: df: equals to degrees of freedom; 2: F is the F statistics; 3: P is the probability on level of significance. P was considered significant at $p < 0.05$

Table 3 Independent t-test results on contribution of solvent effect on toxicity of different concentrations of *P. dodecandra* extracts on *An. gambiae* life stages

Conc. of <i>P. dodecandra</i> extracts (mg/100 mL)	Solvent of extraction		t-test for equality of means		Levene's test for equality of variances	
	Ethanol	Water	t	p	F	p
2.5	69.63±4.068	65.76±4.208	0.661	0.255	0.555	0.457
5.0	63.39±4.281	60.63±4.159	0.462	0.323	0.059	0.809
10.0	58.34±4.581	57.04±4.172	0.210	0.417	1.744	0.189
20.0	53.96±4.801	54.06±4.292	-0.016	0.494	2.697	0.103
40.0	49.54±4.697	50.33±4.357	-0.123	0.452	1.109	0.294
80.0	46.33±4.733	44.96±4.694	0.206	0.419	0.037	0.847

Note: 1: df: equals to degrees of freedom; 2: F is the F statistics; 3: P is the probability on level of significance. P was considered significant at $p < 0.05$

When the residual effectiveness of the extracts in the environment was evaluated, it was found to be dose dependent but reduced with time (Table 4). Dose of treatment nonetheless impacted significantly on mortality of all life stages ($p < 0.001$). When the amount of extract needed to kill at least 50% of exposed immature stages (LC_{50}) was evaluated it was found that relatively lower doses (≤ 4.6) with small differences between the lower and upper 95% CI of log concentration of the extracts were needed to effect the observed mortalities (Table 4). Chi-square test statistics on relationship between considered concentrations showed an influence on mortality for the first larval instars (L1s) for ethanol extracts of *P. dodecandra* fruits but not for *P. dodecandra* leaves or *Azadirachta indica* leaves (Table 5). Chi-square test statistics on water extracts of *P. dodecandra* fruits and leaves as well as *Azadirachta indica* leaves showed no significant influence of dose on mortality of all life stages of *An. gambiae* mosquitoes (Table 6).

Table 4 Residual strength of different concentrations (Mg/100mls) of *P. dodecandra* extracts on life stages of *An. gambiae*

Conc. of <i>P. dodecandra</i> extracts	Longevity (days) of exposure of <i>An. gambiae</i> life history stages to <i>P. dodecandra</i> extracts							df	F	P
	1	3	5	7	9	11	13			
2.5	100.00±0.00	70.05±8.54	67.45±8.22	64.75±7.95	62.05±7.71	55.60±7.09	53.95±6.96	6	4.595	0.000
5.0	100.00±0.00	66.40±8.17	61.10±8.12	56.85±7.68	55.05±7.70	48.65±7.05	46.00±7.01	6	6.584	0.000
10.0	100.00±0.00	63.10±8.04	55.70±8.28	51.55±7.92	50.25±7.81	42.85±7.16	40.40±7.20	6	7.894	0.000
20.0	100.00±0.00	61.00±7.98	49.65±8.53	47.15±8.30	44.90±8.01	38.50±7.45	36.85±7.23	6	8.808	0.000
40.0	100.00±0.00	55.80±7.61	44.20±8.02	41.70±7.98	39.70±7.83	34.95±7.36	33.20±7.02	6	10.817	0.000
80.0	100.00±0.00	51.95±7.51	38.90±8.29	35.70±8.03	34.50±7.88	30.30±7.62	28.15±7.41	6	12.171	0.000

Notes: 1: df: equals to degrees of freedom; 2: F is the F statistics; 3: P is the probability on level of significance. P was considered significant at $p < 0.05$

2 Discussion

The present study has shown that exposure of *An. gambiae* mosquito eggs to water and ethanol extracts of leaf (shoot and midsection) and mature green fruits of *P. dodecandra*, affected the life history traits of this mosquito under laboratory conditions. For instance, hatchability of *An. gambiae* eggs to first larval instar (L1) and subsequent transformation to the various larval stages to pupae and then adult reduced greatly. That is fewer eggs hatched from experimental groups as opposed to control groups. Hatchability rate was also dose dependent with the higher the dose of treatment used the fewer the hatchlings. Additionally a small number of larvae hatching from treatment groups survived to adulthood with survival being greatly influenced by the dose to which the eggs the hatchlings emerged from were exposed.

Anopheles gambiae eggs are embryonated when laid. Indeed it had earlier been reported that *An. gambiae* eggs laid on damp or moist substrates hatch spontaneously even at a level of dampness as the first-instar larvae was observed to wait inside the cap-opened chorions, with only the head protruding, for water to arrive (Huang et al., 2006). If this is the case then in the present study it is apparent that the treatments adduced stress to the developing

embryo or the emerging larvae. This then either killed or affected the developing exposed embryo and explained the reasons for some eggs remaining unhatched (dead) or the larvae dying immediately thereafter or later on. However, when the embryo survived the onslaught, the period over which the larvae emerged into adults was greatly protracted.

Table 5 Estimated lethal concentration of ethanol extracts of parts of *P. dodecandra* and *Azandiracta indica* Leaves that kills 50% (LC₅₀) of exposed *An. gambiae* life stages. The estimated LC₅₀ are reported together with lower and upper 95% confident limits

Mosquito stage		Plant part extract used											
	df	<i>P. dodecandra</i> fruits				<i>P. dodecandra</i> leaves				<i>Azandiracta indica</i> Leaves			
		LC ₅₀ estimate	Lower upper	χ^2	p	LC ₅₀ estimate	Lower upper	χ^2	p	LC ₅₀ estimate	Lower upper	χ^2	p
Eggs	4	2.566 ^a	2.049 4.156	0.503	0.973	2.006 ^a	1.814 2.315	2.813	0.590	3.231 ^a	2.494 5.751	0.599	0.963
L1	4	1.264 ^b	0.933 1.683	18.717	0.001	3.048 ^a	2.485 4.967	3.089	0.543	3.231 ^a	2.494 5.751	0.599	0.963
L2	4	4.041 ^a	2.881 11.949	2.929	0.570	4.823 ^a	3.126 43.814	0.493	0.974	3.137 ^a	2.543 4.927	0.434	0.980
L3	4	4.026 ^a	-	1.367	0.850	3.515 ^a	2.668 7.793	0.845	0.932	4.306 ^a	-	0.837	0.933
L4	4	2.498 ^a	2.067 5.123	7.499	0.112	3.089 ^a	2.487 4.761	5.259	0.262	4.610 ^a	2.989 31.624	0.180	0.996
Pupae	4	2.648 ^a	2.272 4.098	1.730	0.785	7.033 ^a	-	0.901	0.924	4.675 ^a	3.049 52.802	1.465	0.833

Notes: 1: df = degrees of freedom (n-2); 2: χ^2 = chi-square test statistics of relationship between the considered factors; 3: p = level of significance. This was considered significant at $p < 0.05$; 4: Columns' having estimated LC₅₀ superscripted with different letters indicates a significant influence of dose on the *An. gambiae* life stage mortality

Table 6 Estimated lethal concentration of water extracts of *P. dodecandra* and *Azandiracta indica* that kills 50% (LC₅₀) of exposed *An. gambiae* life stages. The estimated LC₅₀ are reported together with lower and upper 95% confident limits

Mosquito stage		Plant part extract used											
	df	<i>Phytolacca dodecandra</i> fruits				<i>Phytolacca dodecandra</i> leaves				<i>Azandiracta indica</i> Leaves			
		LC ₅₀ estimate	Lower upper	χ^2	p	LC ₅₀ estimate	Lower upper	χ^2	p	LC ₅₀ estimate	Lower upper	χ^2	p
Eggs	4	0.985 ^a	0.408 1.336	0.327	0.988	2.232 ^a	1.957 2.747	3.021	0.554	1.575 ^a	1.437 1.762	5.556	0.235
L1	4	2.286 ^a	2.051 2.696	2.688	0.611	3.185 ^a	-	1.608	0.807	3.844 ^a	2.824 9.097	1.958	0.744
L2	4	3.375 ^a	2.631 6.338	1.004	0.909	3.438 ^a	2.654 6.779	3.217	0.522	3.674 ^a	2.766 7.579	0.245	0.993
L3	4	3.558 ^a	2.703 7.466	0.757	0.944	3.085 ^a	2.498 5.180	2.420	0.659	5.195 ^a	-	0.304	0.990
L4	4	4.406 ^a	-	5.183	0.269	2.945 ^a	2.391 4.417	1.183	0.881	3.745 ^a	2.796 7.886	5.181	0.269
Pupae	4	3.626 ^a	2.738 7.613	1.160	0.885	4.315 ^a	2.982 15.452	0.299	0.990	4.444 ^a	-	0.843	0.933

Notes: 1: df = degrees of freedom (n-2); 2: χ^2 = chi-square test statistics of relationship between the considered factors; 3: p = level of significance. This was considered significant at $p < 0.05$; 4: Columns' having estimated LC₅₀ superscripted with similar letters indicates no significant influence of dose on *An. gambiae* stage mortality

Stress element introduced by treatments to the exposed *An. gambiae* eggs in the present study indeed enhanced development time of the strain. It is speculated that if extracts of *P. dodecandra* are to be used in nature, exposed *An. gambiae* mosquitoes are likely to suffer the consequences of running the risk of exposure to potential predators and human interference as observed in *Aedes aegypti* (Padmanabha et al., 2011). This is likely to alter the female mosquito fertility and fecundity (Zuharah et al., 2016), crucial vectorial traits necessary for survival. It is also likely to affect epidemiologically relevant mosquito disease transmission traits such as longevity or vector competence (Beldomenico and Begon, 2010; Muturi et al., 2011; Takken et al., 2013; Moller-Jacobs et al., 2014). Indeed it was reported that when exposed to insecticides containing temephos and propoxur, *Anopheles stephensi* females produced less viable eggs (Sanil and Shetty, 2012).

It is speculated that chemical constituents of *P. dodecandra* could have caused abnormalities in the developing embryo and this had a direct effect on the larvae and some fundamental aspects related to the larval development (Promsiri et al., 2006). Similar findings were reported in fertility studies of *Stegomyia albopicta*, in which the percentage of their egg hatching was reduced after sublethal exposure of females to boric acid sugar bait (Ali et al., 2006) and *Cx. quinquefasciatus* and *Ae. aegypti*, after exposure to leaf extracts of *Calophyllum inophyllum* and *Rhinocanthus nasutus* (Muthukrishnan and Pushpalatha, 2001).

It has been observed that an insect's immune system is susceptible to different factors including but not limited to insecticides or plant-derived compounds (Zibae and Bandani, 2010; James and Xub, 2012). The suppression of this system upon exposure to biopesticides arrests the insect's cell cycle and induces apoptotic effects in insect cell lines (Huang et al., 2011; Shu et al., 2015) and also makes the insects more susceptible to infection (Zibae et al., 2012) and other negative environmental factors. Although the physiological effect of exposure to extracts of *P. dodecandra* on exposed *An. gambiae* mosquitoes was not assessed in the present study, the fact that the embryos died and larvae either died immediately after hatching or had a protracted larval development period suggests that exposure to extracts of *P. dodecandra* could have impaired the exposed *An. gambiae* embryo's immune system interfering with their humoral and cellular immunity responses. This then could have made them vulnerable to infections and other environmental stressors that could have interfered with their development patterns. In this regard, the finding of this study is similar to an observation reported for *Rhodnius prolixus* Stal (Hemiptera: Reduviidae) (Figueiredo et al., 2006), *Spodoptera litura* Fabricius (Lepidoptera: Noctuidae) (Sharma et al., 2003) and *Spodoptera littoralis* Boisduval (Lepidoptera: Noctuidae) (Shaurub et al., 2014) when they were exposed to azadirachtin an active chemical present in extracts of *Azadirachta indica* leaves.

3 Material and Methods

3.1 Study area and experimental mosquitoes

The experiments were conducted in the Entomology laboratory at the Centre for Global Health Research/Kenya Medical Research Institute (CGHR/KEMRI). *An. gambiae* mosquitoes maintained at the laboratories and reared following standard techniques (Das et al., 2007; Parekh et al., 2005) were used. Climatic conditions within insectaries were 29°C~30°C, 70%~80% relative humidity, and 12:12, L:D photoperiod (Yugi et al., 2014).

3.2 Plant extracts preparation

Fresh leaves (shoot and midsection) and mature green fruits of *P. dodecandra* were collected from the field near Moi Girls High School, Eldoret [+0.518829°N, 35.284927°E], and Kanyagwal, Nyando [-0.250393°N, 34.870190°E]. Fresh leaves of *Azadirachta indica* (Neem) were also collected from Kanyagwal, Nyando [-0.250393°N, 34.870190°E]. The plant parts were identified by Mr. Patrick Mutiso of the School of Biological Sciences, University of Nairobi, and voucher specimen number JOY2012/001 for *P. dodecandra* and JOY2012/002 for *Azadirachta indica* issued and thereafter deposited in the herbarium at the School. The plants were prepared and extracted using standard methods (Das et al., 2007) and the extract kept in airtight glass bottles to serve as stock quantity.

3.3 *An. gambiae* life history bioassay

An informal ‘after-only with control’ experimental research design (Kothari, 2004) was used to investigate influence of ethanol and water extracts of mature green fruit and leaves of *P. dodecandra* as environmental stressor on the development time and survival rate of *An. gambiae* mosquito life stages to adulthood. Eighty milligrams of stock’s crude ethanol extracts of mature green fruits of *P. dodecandra* was weighed and dissolved in 100 millilitres of rain water and the solution serially diluted to different concentrations of 80, 40, 20, 10, 5, and 2.5 mg/100 mL and then added into five groups of six sets of larval rearing trays. Each tray measured 21 cm × 15 cm × 8 cm and contained 100 mL of a particular concentration. The procedure was repeated for ethanol extracts of leaves of *P. dodecandra*, and *Azadirachta indica* and for water extracts of similar parts of *P. dodecandra* and *Azadirachta indica*. Similar concentrations of Deltamethrin were used together with ethanol and water extracts of the plant parts as comparison. Concentrations of extracts of *Azadirachta indica* and deltamethrin and rain water only were used as positive and negative control respectively. To each of the trays was added 100 freshly laid *An. gambiae* eggs and the set ups left to stand in the laboratory at temperatures of 29°C~30°C. *An. gambiae* mosquitoes were exposed to the treatments at the egg stage only. The experiments were replicated four times.

The eggs were left to incubate in the treated solutions and observed on the 3rd, 5th, 7th, 9th, 11th, and 13th day to access and count emerged larval instars 1 (L1s), larval instars 2 (L2s), larval instars 3 (L3s), larval instars 4 (L4s), pupae and emerged adults respectively. Periodic observations were made to determine the number of individuals of each *An. gambiae* immature stage that died or survived. Rearing water was changed twice; at the L2s, and the L4 stage respectively. Feeding was done once every two days at a rate of 0.03 milligrams of TetraMin[®] Baby fish food per larva. Pupae developing from L4s were collected put in collection plastic cups and placed inside adult mosquito holding paper cups with mouths covered in mosquito netting to prevent the emerged adults from escape. The paper cups were labeled with the type of treatment used against the larvae and provided with 10% sugar solution soaked in cotton wool placed on the netting cover to feed the emerged adults. The emerged adults were counted and noted for each treatment and concentration and thereafter the experiment stopped.

Toxicological activities of the extracts were tested in accordance with the WHO procedure (WHO, 1997). Records of the immature mortalities were taken at the end of observation activities and for every set up, moribund, and dead larvae were collected and disposed off in a septic tank. Mortality rate was calculated for each concentration using the formula:

$$\% \text{ Mortality} = \frac{\text{Number of dead immature stage}}{\text{Total number of } An. gambiae \text{ eggs exposed}} \times 100$$

Standard WHO procedures were used to assess effectiveness of the extracts as larvicide at a mortality rate of > 80% (WHO, 2005).

3.4 Data analysis

Data was entered in excel spreadsheets and the relationship between larvicidal effect of the extracts with part of *P. dodecandra* plant used and concentration determined using descriptive statistics. One way analysis of variance (ANOVA) was used to determine the level of significance of the effects of treatments on pupae mortality. Regression (probit) analysis was used to calculate the lethal concentration (LC₅₀) and χ^2 statistics of the extracts used. Results on concentrations of extracts were expressed as mean ± SD and those on effect of dose expressed as mean ± SE of three replicates in each treatment. *P*-values were considered significant at *p* < 0.05. All statistical analysis was performed using Statistical Package for Social Scientist (SPSS) version 16.

Acknowledgements

The author wishes to sincerely thank Richard Amoto, Charles Owaga, Trevor Omondi, and Harnel Owiti for culturing the experimental mosquitoes, Centre for Global Health Research (CGHR) for laboratory space and mosquitoes. This study was funded by the National Commission for Science Technology and Innovation [(NACOSTI) Grant contract # NCST/5/003/3rd Call PhD/056].

References

- Alex A., Raphael N.G., Martin A., Chris C., and Mark R., 2012, Loss of household protection from use of insecticide-treated nets against pyrethroid-resistant mosquitoes, Benin. *Emerging Infectious Diseases*, 18(7): 1101
<https://doi.org/10.3201/eid1807.120218>
- Ali A., Xue R.D., and Barnard D.R., 2006, Effects of sublethal exposure to boric acid sugar bait on adult survival, host-seeking, blood feeding behavior, and reproduction of *Stegomyia albopicta*. *Journal of American Mosquito Control Association*, 22(3): 464-468
[https://doi.org/10.2987/8756-971X\(2006\)22\[464:EOSETB\]2.0.CO;2](https://doi.org/10.2987/8756-971X(2006)22[464:EOSETB]2.0.CO;2)
- Altemimi A., Lakhssassi N., Baharlouei A., Watson D.G., and Lightfoot D.A., 2017, Phytochemicals: Extraction, Isolation, and Identification of Bioactive Compounds from Plant Extracts. *Plants*, 6, 42
- Araujo M.S., Gil L.H., and de Almeida e Silva A., 2012, Larval food quantity affects development time, survival and adult biological traits that influence the vectorial capacity of *Anopheles darlingi* under laboratory conditions, *Malaria Journal*, 11(1): 261
<https://doi.org/10.1186/1475-2875-11-261>
- Beldomenico P.M., and Begon M., 2010, Disease spread, susceptibility and infection intensity: vicious circles? *Trends in Ecological Evolution*, 25(1): 21-27
<https://doi.org/10.1016/j.tree.2009.06.015>
- Berenbaum M.R., and Johnson R.M., 2015, Xenobiotic detoxification pathways in honeybees, *Current Opinion in Insect Science*, 10: 51-58
<https://doi.org/10.1016/j.cois.2015.03.005>
- Breaux J.A., Schumacher M.K., and Juliano S.A., 2014, What does not kill them makes them stronger: larval environment and infectious dose alter mosquito potential to transmit filarial worms, *Proceedings of Royal Society of Biology*, 281(1786): 20140459
<https://doi.org/10.1098/rspb.2014.0459>
- Brown Z.S., Dickinson K.L., and Kramer R.A., 2013, "Insecticide resistance and malaria vector control: the importance of fitness cost mechanisms in determining economically optimal control trajectories," *Journal of Ecological Entomology*, 106(1): 366-374
<https://doi.org/10.1603/EC11365>
- Checchi F., Cox J., Balkan S., Tamrat A., Priotto G., Alberti K., Zurovac D., and Guthmann J., 2006, Malaria epidemics and interventions, Kenya, Burundi, Southern Sudan, and Ethiopia, *Emerging Infectious Diseases*, 12(10): 1477-1485
<https://doi.org/10.3201/eid1210.060540>
- Che-Mendoza A., Penilla R.P., and Rodriguez D.A., 2009, Insecticide resistance and glutathione S-transferases in mosquitoes: A review, *African Journal of Biotechnology*, 8(8): 1386-1397
- Chouaibou M.S., Chabi J., Bingham G.V., Knox T.B., N'Dri L., Kesse N.B., Bonfoh B., and Jamet H.V.P., 2012, Increase in susceptibility to insecticides with aging of wild *Anopheles gambiae* mosquitoes from Côte d'Ivoire, *BMC Infectious Diseases*, 12(1): 214
<https://doi.org/10.1186/1471-2334-12-214>
- Das N.G., Goswami D., and Rabha B., 2007, Preliminary evaluation of mosquito larvicidal efficacy of plant extracts, *Journal of Vector Borne Diseases*, 44(2): 145-148
- Evergetis E., Michaelakis A., and Haroutounian S.A., 2012, Essential oils of Umbelliferae (Apiaceae) family taxa as emerging potent agents for mosquito control, In: Soloneski S, editor. *Integrated pest management and pest control: current and future tactics*. Rijeca: InTechnology; pp.613-638
<https://doi.org/10.5772/31777>
- Figueiredo M.B., Castro D.P., Nogueira N.F.S., Garcia E.S., and Azambuja P., 2006, Cellular immune response in *Rhodnius prolixus*: Role of ecdysone in hemocyte phagocytosis, *Journal of Insect Physiology*, 52(7): 711-716
<https://doi.org/10.1016/j.jinsphys.2006.03.011>
- Garrett-Jones C., and Shidrawi G., 1969, Malaria vectorial capacity of a population of *Anopheles gambiae*: an exercise in epidemiological entomology. *Bulletin of World Health Organization*, 40(4): 531-545
- Goldstone J.V., Hamdoun A., Cole B.J., Howard-Ashby M., Nebert D.W., Scallie M., Deane M., Epel D., Hahna M.E., and Stegeman J.J., 2006, The chemical defense: Environmental sensing and response genes in the *Strongylocentrotus purpuratus* genome, *Developmental Biology*, 300(1): 366-384
<https://doi.org/10.1016/j.ydbio.2006.08.066>
- Grech K., Maung L.A., and Read A.F., 2007, The effect of parental rearing conditions on offspring life history in *Anopheles stephensi*, *Malaria Journal*, 6(1): 130
<https://doi.org/10.1186/1475-2875-6-130>
- Huang J.F., Shui K.J., Li H.Y., Hu M.Y., and Zhong G.H., 2011, Antiproliferative effect of azadirachtin A on *Spodoptera litura* SI-1 cell line through cell cycle arrest and apoptosis induced by up-regulation of p53, *Pesticide Biochemistry and Physiology*, 99(1): 16-24
<https://doi.org/10.1016/j.pestbp.2010.08.002>
- Isman M.B., 2006, Botanical insecticides, deterrents, and repellents in modern agriculture and an increasingly regulated world, *Annual Review of Entomology*, 51: 45-66
<https://doi.org/10.1146/annurev.ento.51.110104.151146>
- James R.R., and Xub J., 2012, Mechanisms by which pesticides affect insect immunity, *Journal of Invertebrate Pathology*, 109(2): 175-182
<https://doi.org/10.1016/j.jip.2011.12.005>
- Khater H.F., 2012, Prospects of botanical biopesticides in insect pest management, *Pharmacologia*, 3(12): 641-56
<https://doi.org/10.5567/pharmacologia.2012.641.656>
- Korsloot A., Van Gestel C.A.M., and Van Straalen N.M., 2004, *Environmental stress and cellular response in arthropods*, Boca Raton, Florida, CR Press
<https://doi.org/10.1201/9781420023336>

- Kweka E.J., Zhou G., Beilhe L.B., Dixit A., Afrane Y., Gilbreath III T.M., Munga S., Nyindo M., Githeko A.K., and Yan G., 2012, "Effects of co-habitation between *Anopheles gambiae* s.s. and *Culex quinquefasciatus* aquatic stages on life history traits", *Parasite & Vectors*, 5(1): 33
<https://doi.org/10.1186/1756-3305-5-33>
- Lefèvre T., Vantaux A., Dabiré R.K., Mouline K., and Cohuet A., 2013, Non-genetic determinants of mosquito competence for malaria parasites, *PLoS Pathology*, 9(6): e1003365
<https://doi.org/10.1371/journal.ppat.1003365>
- Liu N., 2015, Insecticide resistance in mosquitoes: impact, mechanisms and research directions, *Annual Review of Entomology*, 60: 537-559
<https://doi.org/10.1146/annurev-ento-010814-020828>
- Lo T.M., and Coetzee M., 2013, "Marked biological differences between insecticide resistant and susceptible strains of *Anopheles funestus* infected with the murine parasite *Plasmodium berghei*," *Parasite & Vectors*, 6(1): 184
<https://doi.org/10.1186/1756-3305-6-184>
- Mann R.S., and Kaufman P.E., 2010, Natural product pesticides: their development, delivery and use against insect vectors, *Mini Review of Organic Chemistry*, 9(2): 185-202
<https://doi.org/10.2174/157019312800604733>
- Marufa S., Nurnabi S., Rashidul A.M., Tania J., Ziaul I., and Abdur R.S., 2017, Prevalence and associated determinants of malaria parasites among Kenyan children. *Tropical Medicine and Health*, 45:25
 DOI 10.1186/s41182-017-0066-5
- Moller-Jacobs L., Murdock C., and Thomas M., 2014, Capacity of mosquitoes to transmit malaria depends on larval environment, *Parasite & Vectors*, 7(1): 593
<https://doi.org/10.1186/PREACCEPT-1428945905137097>
- Muthukrishnan J., and Pushpalatha E., 2001, Effects of plant extracts on fecundity and fertility of mosquitoes, *Journal of Applied Entomology*, 125(1-2): 31- 35
<https://doi.org/10.1046/j.1439-0418.2001.00503.x>
- Muturi E.J., Kim C.H., Alto B.W., Berenbaum M.R., and Schuler M.A., 2011, Larval environmental stress alters *Aedes aegypti* competence for Sindbis virus, *Tropical and Medical Institute of Health*, 16(8): 955-964
<https://doi.org/10.1111/j.1365-3156.2011.02796.x>
- Okia M., Ndyomugenyi R., Kirunda J., Byaruhanga A., Adibaku S., Lwamafa D.K., and Kironde F., 2013, "Bioefficacy of long-lasting insecticidal nets against pyrethroid-resistant populations of *Anopheles gambiae* s.s. from different malaria transmission zones in Uganda," *Parasite & Vectors*, 6(1): 130
<https://doi.org/10.1186/1756-3305-6-130>
- Olaitan A., and Abiodun A., 2011, Comparative toxicity of botanical and synthetic insecticides against major field insect pests of Cowpea (*Vigna unguiculata* (L) Walp), *Journal of Natural Products and Plant Resources*, 1(3): 86-95
- Ossè R.A., Aikpon R., Gbédjissi G.L., Nguenon V., Sèzonlin M., Govoétchan R., Sovi A., Oussou O., Oké-Agbo F., and Akogbéto M., 2013, "A shift from Indoor Residual Spraying (IRS) with bendiocarb to Long-Lasting Insecticidal (mosquito)Nets (LLINs) associated with changes in malaria transmission indicators in pyrethroid resistance areas in Benin," *Parasite & Vectors*, 6(1): 73
<https://doi.org/10.1186/1756-3305-6-73>
- Padmanabha H., Bolker B., Lord C.C., Rubio C., and Lounibos L.P., 2011, Food availability alters the effects of larval temperature on *Aedes aegypti* growth, *Journal and Medical Entomology*, 48(5): 974-984
<https://doi.org/10.1603/ME11020>
- Parekh J., Jadeja D., and Chanda S., 2005, Efficacy of aqueous and methanol extracts of some medicinal plants for potential antibacterial activity, *Turkish Journal of Biology*, 29(4): 203-210
- Promsiri S., Naksathit A., Kruatrachue M., and Thavara U., 2006, Evaluations of larvicidal activity of medicinal plant extracts to *Aedes aegypti* (Diptera: Culicidae) and other effects on a non target fish, *Insect Science*, 13(3): 179-188
<https://doi.org/10.1111/j.1744-7917.2006.00080.x>
- Pugazhvendan S.R., and Elumali K., 2013, Larvicidal activity of selected plant essential oil against important vector mosquitoes: dengue vector, *Aedes aegypti* (L.), malarial vector, *Anopheles stephensi* (Liston) and filarial vector, *Culex quinquefasciatus* (Say) (Diptera: Culicidae), *Middle East Journal of Science Reviews*, 18(1): 91-95
- Ruikar A.D., Pawar P.V., Sen A., Phalgune U.D., Puranik V.G., and Deshpande N.R., 2012, Larvicidal potential of *Mimusops elengi* against *Aedes aegypti* (L) and *Culex quinquefasciatus* (Say), *Journal of Vector Borne Diseases*, 49(2): 111-113
- Sanil D., and Shetty N.J., 2012, The effect of sublethal exposure to temephos and propoxur on reproductive fitness and its influence on circadian rhythms of pupation and adult emergence in *Anopheles stephensi* Liston-a malaria vector, *Parasite Resources*, 111(1): 423-432
<https://doi.org/10.1007/s00436-012-2857-2>
- Sharma P.R., Sharma O.P., and Saxena B.P., 2003, Effect of Neem gold on hemocytes of the tobacco armyworm, *Spodoptera litura* (Fabricius) (Lepidoptera: Noctuidae), *Current Science, India*, 84: 690-695
- Shaurub E.H., Abd El-Meguid A., and Abd El-Aziz N.M., 2014, Quantitative and ultrastructural changes in the haemocytes of *Spodoptera littoralis* (Boisd.) treated individually or in combination with *Spodoptera littoralis*, Multicapsid nucleopolyhedrovirus (SpliMNPV) and azadirachtin, *Micron*, 65: 62-68
<https://doi.org/10.1016/j.micron.2014.04.010>
- Shu B., Wang W., Hu Q., Huang J., Hu M., and Zhong G., 2015, A comprehensive study on apoptosis induction by azadirachtin in *Spodoptera frugiperda* cultured cell line Sf9, *Arch Insect Biochemistry*, 89(3): 153-168
<https://doi.org/10.1002/arch.21233>

- Steinberg C.E., 2012, Arms race between plants and animals: biotransformation system, In Stress Ecology, Springer, Dordrecht, pp. 61-106
https://doi.org/10.1007/978-94-007-2072-5_4
- Takken W., Smallegange R., Vigneau A., Johnston V., Brown M., Mordue-Luntz A., and Billingsley P.F., 2013, Larval nutrition differentially affects adult fitness and *Plasmodium* development in the malaria vectors *Anopheles gambiae* and *Anopheles stephensi*, Parasite & Vectors, 6(1): 345
<https://doi.org/10.1186/1756-3305-6-345>
- Tennyson S., Ravindran K.J., and Arivoli S., 2012, Screening of twenty five plant extracts for larvicidal activity against *Culex quinquefasciatus* Say (Diptera: Culicidae). Asian Pacific Journal of Tropical Biomedicine, 2Suppl: S1130-4
[https://doi.org/10.1016/S2221-1691\(12\)60372-4](https://doi.org/10.1016/S2221-1691(12)60372-4)
- Vantaux A., Ouattara I., Lefèvre T., and Dabiré K.R., 2016, Effects of larvicidal and larval nutritional stresses on *Anopheles gambiae* development, survival and competence for *Plasmodium falciparum*, Parasite & Vectors, 9(1): 226
<https://doi.org/10.1186/s13071-016-1514-5>
- World Health Organization (WHO), 1997, Chemical methods for the control of vectors and pests of public health importance, In: Chavasse DC, Yap HH, editors. Geneva: World Health Organization, WHO/CTD/WHOPES/1997.2
- World Health Organization (WHO), 2005, Global Malaria Programme, Indoor residual spraying: use of indoor residual spraying for scaling up global malaria control and elimination, Geneva: World Health Organization, WHO/HTM/MAL. p.1112.
- WHO, 2015, WHO Global Malaria Programme: World Malaria Report, World Health Organization 2015
- Zhao J.Z., Li Y.X., Collins H.L., Gusukuma-Minuto L., Mau R.F., Thompson G.D., and Shelto A.M., 2002, Monitoring and characterization of diamondback moth (Lepidoptera: Plutellidae) resistance to spinosad, Journal of Economic Entomology, 95(2): 430-436
<https://doi.org/10.1603/0022-0493-95.2.430>
- Zibace A., and Bandani A.R., 2010, Effects of *Artemisia annua* L. (Asteracea) on the digestive enzymatic profiles and the cellular immune reactions of the Sunn pest, *Eurygaster integriceps* (Heteroptera: Scutellaridae), against *Beauveria bassiana*, Bulletin of Entomological Resources, 100(1): 185-196
<https://doi.org/10.1017/S0007485309990149>
- Zibace A., Bandani A.R., and Malagoli D., 2012, Methoxyfenozide and pyriproxifen alter the cellular immune reactions of *Eurygasterintegriceps* Puton (Hemiptera: Scutelleridae) against *Beauveria bassiana*, Pesticide Biochemistry and Physiology, 102(1): 30-37
<https://doi.org/10.1016/j.pestbp.2011.10.006>
- Zuharah W.F., Ahbirami R., Dieng H., Thiagaletchumi M., and Fadzly N., 2016, Evaluation of Sub-lethal Effects of *Ipomoea cairica* Linn., Extract on Life History Traits of Dengue Vectors. Review of Institute of Tropical Medicine, Sao Paulo, 58: 44
<https://doi.org/10.1590/S1678-9946201658044>