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Jared Owiti Yugi
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Effective Dose of Crude Ethanol and Aqueous Extracts of *Phytolacca Dodecandra* (L' Herit) on *Anopheles Gambiae* (Diptera: Culicidae) Larvae

Yugi J.O.[1 ✉], Okeyo-Owour J B.[2], Were-Kogogo P.[3], Luvaha G.M.[1], Okwiri L.O.[1], Rono T.M.[4], Vulule M.J.[5]

1. Department of Applied Sciences, Kisumu Polytechnic, P. O. Box 143-40100, Kisumu, Kenya
2. School of Agriculture, Natural resource and Environmental Studies, Rongo University College, P.O. Box 103-40404, Rongo-Kenya
3. School of Biological and Physical Sciences, Jaramogi Oginga Odinga Technical University, P. O. Box Bondo, Kenya
4. University of Eldoret, P. O. Box 1125-30100. Eldoret, Kenya
5. Kenya Medical research Institute, Centre for Global Health Research, P. O. Box 1578-4100. Kisumu Kenya

✉ current address: Department of Biological Sciences, University of Kabianga, P. O. Box 2030-20200, Kericho, Kenya

✉ Corresponding author's email: yugijared@gmail.com

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Abstract Objective: malaria vector control is either by adult or larvae management. This study reports on the effective dose of crude ethanol and aqueous extracts of leaf and mature green fruits of Endod; *Phytolacca dodecandra* (L' Herit) as a larvicide against *Anopheles gambiae* (Diptera: Culicidae) mosquitoes.

Material & Methods: Batches of twenty freshly hatched or moulted larvae (1st, 2nd, 3rd and 4th instars) of *An. gambiae* were transferred using droppers into plastic containers measuring 6 cm mouth and 5.7 cm base diameter by 3.5 cm height arranged in sets. Each container contained an approximate of 33 millilitre solution of a particular concentration of leaf (shoot or midsection) or fruit extracts of Endod. Endod plants were sourced from Nyando and Eldoret. The solutions were a serial dilution of 40, 20, 10, 5 and 5mg/100mls of the crude extracts. Solutions of Neem and deltamethrin were used as positive control and that of water only as negative control. Three replicates were set up for each concentration. WHO threshold of > 80 % mortality was used as standard to assess effectiveness of the larvicide.

Results: Over 80% of all larvae (L1s, L2s, L3s and L4s) died from exposure to concentrations of 20mg and higher of ethanol extracts of mature green fruits of Endod irrespective of source. Only 40 mg/100 mls of aqueous extracts of Endod leaves of the shoot sourced from Eldoret killed > 80% of exposed L1s. Higher mortalities were observed for extracts from leaves of the shoot of Endod than those from the midsection.

Conclusion: Higher doses of ethanol and water extracts of Endod leaves and fruits were lethal to all stages of *An. gambiae* larvae.
Keywords *Anopheles gambiae*; Larvae; *Phytolacca dodecandra*; Neem; Deltamethrin

Introduction

Synthetic insecticides (Poopathi and Archana, 2010) have for a long time been used to manage extensive morbidity and mortality within disease-endemic countries (Boutayeb, 2006) due to Mosquito-borne diseases. Though effective this strategy is accompanied with serious harm to human and other animals in the environment (Cartilla and De la Cruz, 2012). In addition continued use of synthetic insecticides has been demonstrated to lead to reduced control due to development of resistant in insect populations (Charles and Nielsen-LeRoux, 2000).

The increasing negative effect of synthetic insecticides has made scientists seek for alternative eco-friendly pest control methods (Poopathi and Archana, 2010; Montasser et al., 2011). Botanicals such as *Phytolacca dodecandra* here after Endod have been found promising as alternative larvicidals against *Culex quinquefasciatus* (Misganaw et al., 2012) and *An. gambiae* (Yugi et al., 2015) and pupicidals against *Culex quinquefasciatus* (Misganaw et al., 2012). There is also evidence of demonstrable microbial activity (Joshi et al., 2011). Though efficacy of Endod extracts on *An. gambiae* larvae has been demonstrated, no information exists on the dose or amount of the extracts

that are effective on each larval stage. It is in line with this that this study was designed to study and report on the effective dose of crude ethanol and water extracts of mature green fruits and leaves of Endod on larvae of *An. gambiae* in a laboratory set up.

1 Results

The experiment was conducted for a period of eight months using a total of 84,240 larvae (L1s, L2s, L3s and L4s). WHO threshold of >80% mortality was used as standard to decide on the effectiveness of the various doses against the exposed larvae. For L1s, concentration of 20 mgs and 40 mgs of ethanol extracts of mature green fruits of Endod from Eldoret and Nyando respectively killed over 80% of the exposed larvae. For aqueous extracts of Endod mature green fruits and leaves of the shoot, mortality of over 80% was observed for concentrations of 40mgs only irrespective of source (Figure 1).

For L2s, concentrations of 20 mg and 40 mg of ethanol extracts of mature green fruits from Eldoret and Nyando killed over 80% of exposed L1s respectively. Mortality of over 80% was observed for concentrations of 40 mgs of extracts of leaves of the shoot and midsection of Endod from Nyando and Eldoret respectively. Concentrations of 20 mg and 40 mgs of aqueous extracts of mature green fruits from Eldoret and Nyando killed over 80% of exposed L2s respectively (Figure 2).

For L3s, concentrations of 10 mg and 20 mg and above of ethanol extracts of mature green fruits and leaves of the shoot of Endod from Eldoret and Nyando respectively killed over 80% of exposed L3s. The same was observed for concentration of 40 mgs of ethanol extracts of leaves of the midsection of Endod (Figure 3). For the L4s, more than 80% mortality of exposed larvae was observed for concentration of 10mgs and above for ethanol extracts of mature green fruits irrespective of source (Figure 4).

Mortality of over 80% was observed for L1s and L3s for ethanol and aqueous extracts respectively for Neem while Deltamethrin killed all exposed larvae irrespective of stage or concentration. The effects of the extracts were considered significantly different at $p < 0.05$ (Table 1) for all exposed larval stages.

2 Discussions

Ethanol extracts of mature green fruits of Endod at higher concentrations killed all exposed aquatic stages of *An. gambiae*. Mortality percentages however, reduced with continued dilution. This trend was similar to Ethanol extracts of Endod leaves although observed mortalities were lower than that of fruit extracts. Earlier the same trend had been reported for powdered extracts of Endod on *An. gambiae* larvae (Were, 2008). This demonstrates that extracts of ethanol and water extracts of Endod have similar efficacies as *Chenopodium ambrosioides* Linn (Jude et al., 2013) and *Jatropha curcas* L (Zewdneh et al., 2011) against *An. gambiae* and *Anopheles arabiensis* larvae respectively.

Extracts from both mature green fruits and leaves of Endod were potent against *An gambiae* larvae though potency of leaf extracts were lower than that of fruits. Potency of the extracts also differed with the vertical position of leaves on the Endod plant such that ethanol extracts of leaves found at the shoot were more potent than those found at the midsection of the plant. This showed that the probable distribution of the bioactive on Endod plant are such that fruits > leaves at the shoot > leaves at the midsection.

Ethanol extracts of Endod parts were more toxic than aqueous extracts of the same parts for all concentrations against *An. gambiae* larvae. Solvent type used in extraction had been demonstrated to determine potency of extracted bioactives (Anupam et al., 2012) as well as larviciding potency. This had been demonstrated for *Solanum* xanthocarpum (Mohan et al., 2006), *Euphorbia tirucalli* (Singh et al., 2007), *Eucalyptus* globules (Maurya et al., 2007), *Citrullus colocynthis* (Sakthivadivel and Daniel, 2008), *Azadirechta indica* (Mgbemena, 2010) and *Solanum nigrum* (Raghavendra et al., 2009).

Ethanol extracts of Endod parts sourced from Eldoret were more potent than of the same parts sourced from Nyando. This showed that geographical origin of the plants played a role in the concentration and distribution of bioactives within the plants (Anupam et al., 2012). A similar observation had been made on Endod parts (Were, 2008), *Citrus* sp, *Ocimum sanctum* and *Azadirechta indica* (Mgbemena, 2010) and *Jatropha* sp (Sakthivadivel and Daniel, 2008) just to mention but a few.

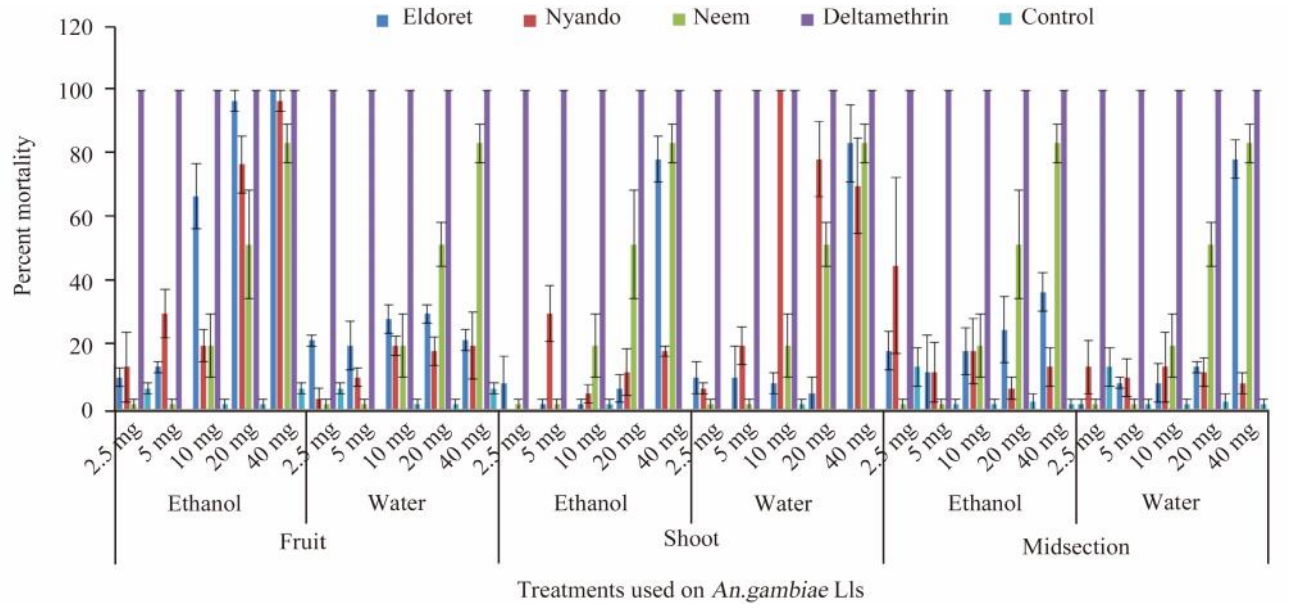

Figure 1 Mortality of *An. gambiae* first instars (L1) exposed to different concentrations of crude ethanol and water extract of Endod. Error bars represents standard error of means

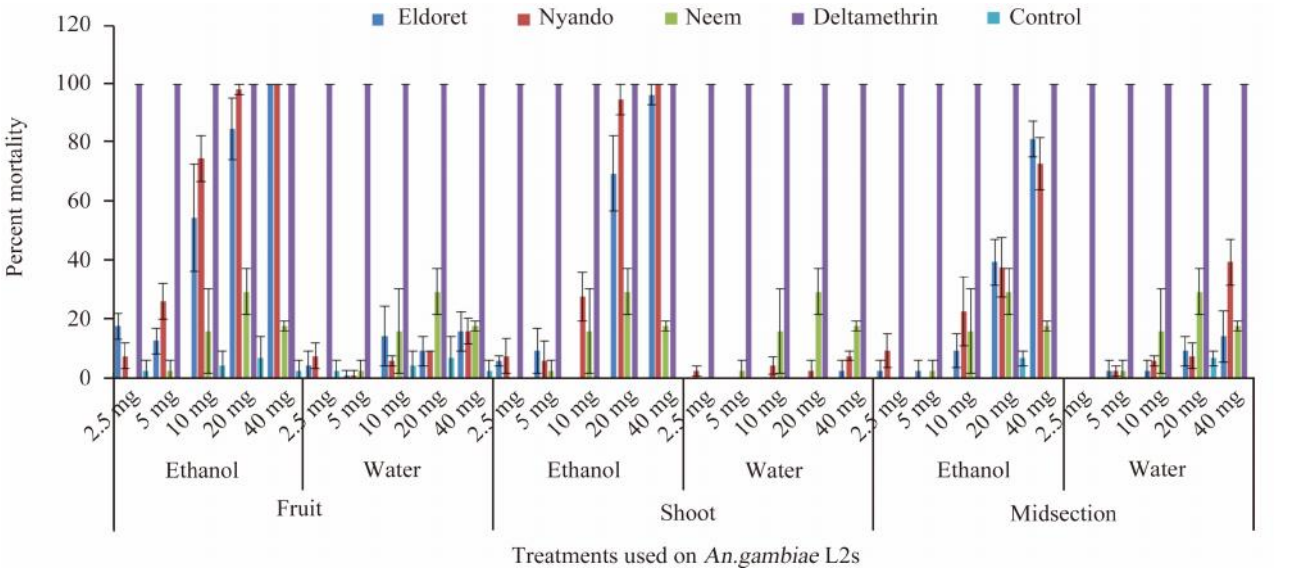

Figure 2 Mortality of *An. gambiae* second instars (L2) exposed to different concentrations of crude ethanol and water Endod extract. Error bars represents standard error of means

This study demonstrates that crude ethanol and water extracts of Endod are potent against *An. gambiae* larvae at high doses. It also demonstrates that solvent type used in the extraction influences activity of extracts from Endod irrespective of parts or geographical origin.

3 Materials and Methods

3.1 Study area, experimental mosquitoes and study design

The experiments were conducted in the Entomology laboratory at Centre for Global research Institute/ Kenya medical research Institute (CGHR/KEMRI). Pink eyed *An. gambiae* mosquitoes maintained at the laboratories and reared following standard techniques (Das et al., 2007) were used in the experiments. A completely randomized informal 'after-only with control' experimental design (Kothari, 2004) was used to investigate the larvicidal effect of crude ethanol and water extracts of Endod on the mosquito larvae. The condition in the insectary was as described elsewhere (Yugi et al., 2014).

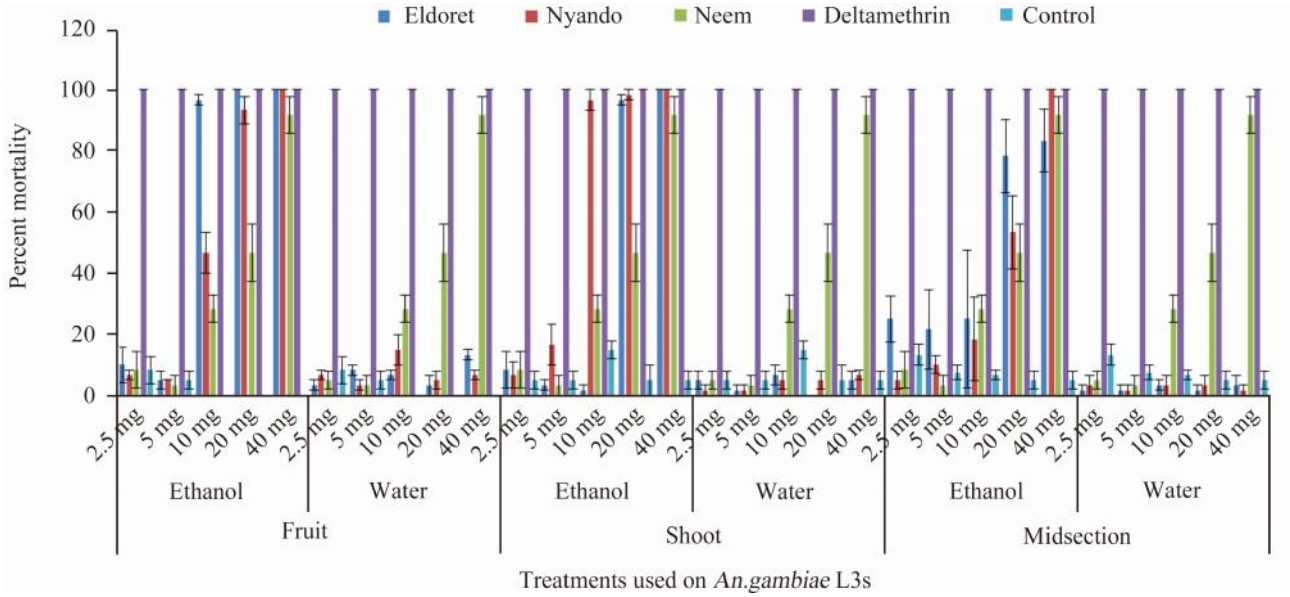

Figure 3 Mortality of *An. gambiae* third instars (L3) exposed to different concentrations of crude ethanol and water Endod extracts. Error bars represents standard error of means

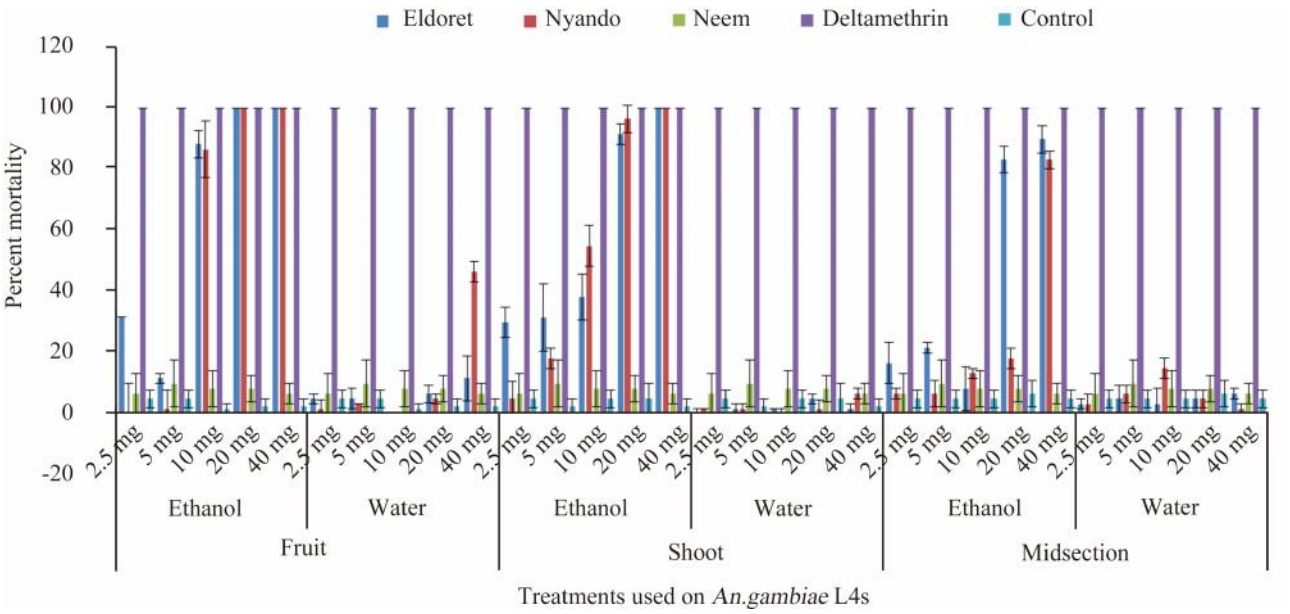

Figure 4 Mortality of *An. gambiae* fourth instars (L4s) exposed to different concentrations of crude ethanol and water Endod extract. Error bars represents standard error of means

Table 1 Duncan's statistics on mortalities due to exposure of *An. gambiae* larvae to crude extracts of parts of Endod

Stage	df	Treatments							
		Parts of Endod used						Neem	Deltamethrin
		Eldoret			Nyando				
		Fruits	Shoot	Midsection	Fruits	Shoot	Midsection		
L1	4	0.0128	0.0001	0.0001	0.0145	0.1037	0.3729	0.0001	-
L2	4	0.0449	0.0229	0.0015	0.0660	0.0296	0.0001	0.0008	-
L3	4	0.0603	0.0234	0.2632	0.0399	0.0830	0.0490	0.0001	-
L4	4	0.1679	0.2245	0.0401	0.0037	0.0867	0.0356	0.9642	-

Note: 1 P is the probability for the level of significance. P was considered significant at $p < 0.05$

2 df is the degree of freedom of the considered units

3.2 Deltamethrin (KOTab 1-2-3®) and plant materials acquisition and preparation

Deltamethrin (KOTab 1-2-3®) was acquired and prepared as described (Yugi et al., 2015). Fresh leaves (shoot and midsection) and mature green fruits of Endod and Fresh leaves of *Azadirachta indica* (Neem), were acquired, identified and voucher specimen deposited as described elsewhere (Yugi et al., 2015). The plant parts were dried in a shade at room temperature, grounded and extracts obtained using ethanol and water as described in details elsewhere (Tilahun et al., 2003; Parekh et al., 2005; Das et al., 2010; Yugi et al., 2015).

The extracts were concentrated by freeze drying using a rotary vacuum evaporator at 40-42°C to obtain essential oil that was then kept in airtight glass bottles to serve as stock quantity. From the freeze-dried stock, 80mg were weighed and serial dilutions made to obtain different concentrations of 40, 20, 10, 10, 5 and 2.5 mg in 100mls of rain harvested water.

3.3 Larvicidal bioassays

Larvicidal activities were tested in accordance with the WHO procedure (WHO, 1996) and as described elsewhere (Yugi et al., 2015). Mortality rate were registered after 24 hour exposure period and larval mortality calculated for each concentration using the formula;

$$\% \text{ Mortality} = \frac{\text{Number of dead larvae}}{\text{Total number of larvae introduced}} \times 100$$

Standard WHO procedures were used to assess effectiveness of the extracts as larvicide at a mortality rate of > 80% (WHO, 2005). Moribund and dead larvae were collected and disposed off in a septic tank.

3.4 Data analysis

Data obtained from the bioassays was entered in excel spreadsheets for ease of handling. The relationship between the effective doses of the extracts with respect to parts of Endod used was determined using descriptive statistics. One way analysis of variance (ANOVA) was used to assess the level of significance of the various doses on larvae mortality. All statistical analysis was performed using SAS statistical package version 20.

Competing interest

The authors declare that they have no competing interest.

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