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Residual Effectiveness of Crude Ethanol Extracts of Mature Green Fruits of Endod *Phytolacca dodecandra* (L' Herit) against *An. gambiae* (Diptera: Culicidae) Larvae

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Abstract Mosquitoes are vectors of aborvirals and malaria and are a threat to public health. We evaluated residual effectiveness of crude ethanol extracts of Endod *Phytolacca dodecandra* (L' Herit) leaves and mature green fruits in water against *Anopheles gambiae* (Diptera: Culicidae) larvae to determine duration of protection of the extract against *An. gambiae* mosquitoes. Stock's solution of 80mg/100 mls of crude ethanol extract of mature green fruits and leaves of Endod were prepared using rain harvested water and left to stand. Six such stocks' solutions were prepared on six separate days. Each of the stock's solutions was serially diluted to 40, 20, 10, 5 and 2.5 mg/100 ml and poured in five sets of separate plastic containers. Each container measuring 6 cm top ×5.7 cm bottom ×3.5 cm height contained a solution of a particular concentration. Twenty L3s were exposed per container. Extracts of Neem leaves and deltamethrin were used as positive and rain water alone as negative control. WHO threshold of>80% mortality was used to assess residual effectiveness. Mortality was determined after 24 hours. Resudual effectiveness was dose dependent. As low as 10 mg/100 mls solutions of freshly to two day old preparations of crude ethanol extract of mature green fruits and leaves of Endod killed more than 80% larvae. The solutions remained effective for three day only. Effectiveness reduced rapidly after day four. Crude ethanol extracts of Endod mature green fruits and leaves have a residual period of three days in water against *An. gambiae* larvae.
Keywords *An. gambiae*; Endod; Ethanol; Neem; Deltamethrin; Residual effect

Background

Mosquitoes are vectors of aborvirals (Dupont-Rouzeyrol, 2012; Huang et al., 2014; Ghimire and Dhakal, 2015; Polwiang, 2015; Kindhauser et al., 2016) and malaria (Beck-Johnson et al., 2013; Kumar et al., 2013) and are a threat to public health. The first line management tool for mosquitoes is synthetic insecticides such as organophosphate, carbamate and pyrethroid insecticides (Tabti and Abdellaoui-Hassa ïne, 2013; A ïssaoui and Boudjelida, 2014; Hamaidia and Soltani, 2014).

Synthetic insecticides are often applied indoor on surfaces or outdoor on water bodies for the control of both adult (Deletre et al., 2013) and larval stages (Pravin et al., 2015) of mosquitoes respectively. However, the insecticides are not only costly but have several human (Cartilla and De la Cruz, 2012) as well as environmental concerns (Haouari-Abderrahim and Rehimi, 2014). These challenges have led to intense search and evaluation of ecologically safe and potentially effective control alternatives.

Plant extracts have demonstrated ecological safety in that the extracted compounds are biodegradable, environmentally friendly (Regnault-Roger et al., 2012), and are of low mammalian toxicity (Isman, 2000). In addition, the novel compounds are target specific. The compounds achieve specificity by acting on selective biochemical sites in insects such as chitin synthesis inhibitors, or juvenile hormone analogues and ecdysone agonists, which affect the hormonal regulation of different processes (Berghiche et al., 2008; Suman et al., 2013).

This has seen an intense extraction and screening of plant compounds against haematophagous disease vectors such as mosquitoes (Shaalam et al., 2005; Gleiser and Zygadlo, 2007). One of such plants is *Phytolacca dodecandra* (L. Herit) also known as Endod. Water and methanol extracts of Endod has been screened against

immature of *Culex quinquefasciatus* (Nurie et al., 2012) and *Anopheles gambiae* (Diptera: Culicidae) (Yugi et al., 2015a; 2015b; 2016) and found effective. However, the length of time the extracts last (residual effect) or remains effective to provide protection on any given medium is yet to be demonstrated.

Such information on duration (residual effect) of the extract in or on a medium is important as it will also inform on the periodicity of application. This is crucial for vector control, since it informs on the minimum interval between applications to maintain the sensitivity of the insecticide. According to WHO (1997; 2006), deltamethrin is recommended for indoor residual spray (IRS) at 20-25 mg a.i./m^2 for malaria vector control and its residual efficacy is estimated to be 3-6 months. Other insecticides have relatively shorter residual effect (pyrethroids: 4-6 months; organophosphates and carbamates: 2-6 months) (WHO, 2006).

For Endod, this information is now available courtesy of the present study and will inform on frequency of application, cost and effect on the environment. The present study seeks to inform on the residual effect (duration of effectiveness) of crude water and ethanol extract of Endod in water in the laboratory.

1 Results

It was observed in general that extracts of Endod remained effective for three days killing more than 80% of exposed third larvae instars (L3s) of *An. gambiae*. Effectiveness of the solutions reduced rapidly after day 4. In particular it was found that concentrations of 10mg and higher for freshly prepared (Figure 1), 20 mg/ml and higher for day two old (Figure 2) and 40 mgs for day three old (Figure 3) solutions of ethanol extracts of Endod leaves and mature green fruits of Endod killed more than 80% of the exposed L3s respectively. Preparations of deltamethrin killed an equal number of exposed L3s while preparations of Neem leaf extracts were lower.

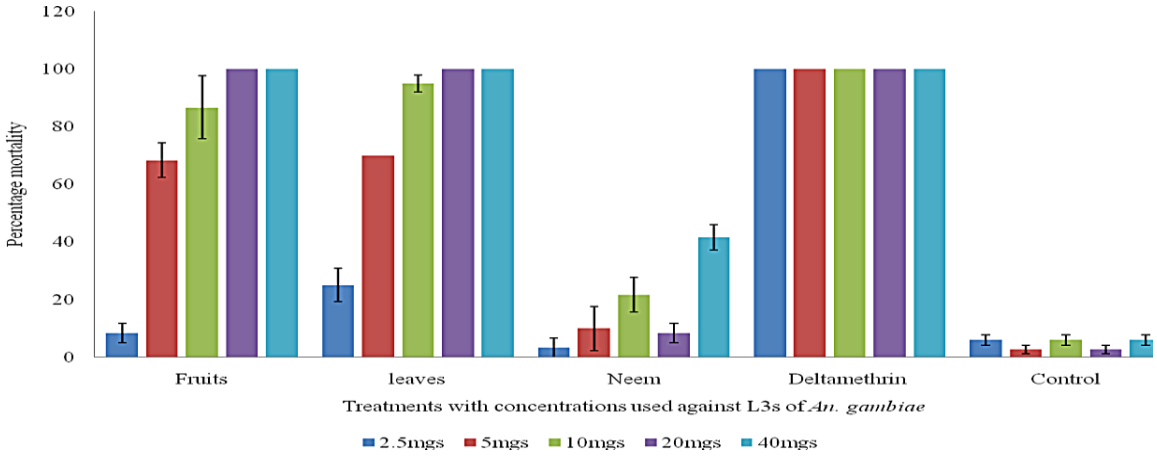

Figure 1 Percent mortality of *An. gambiae* L3s from freshly prepared solutions. Error bars represents standard error of means

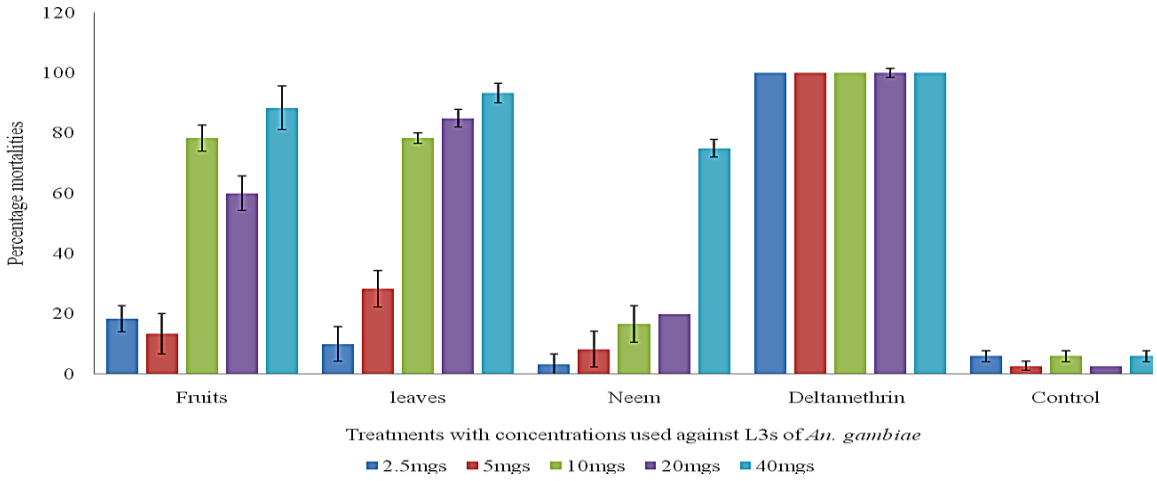

Figure 2 Percent mortality of *An. gambiae* L3s from two day old solutions. Error bars represents standard error of means

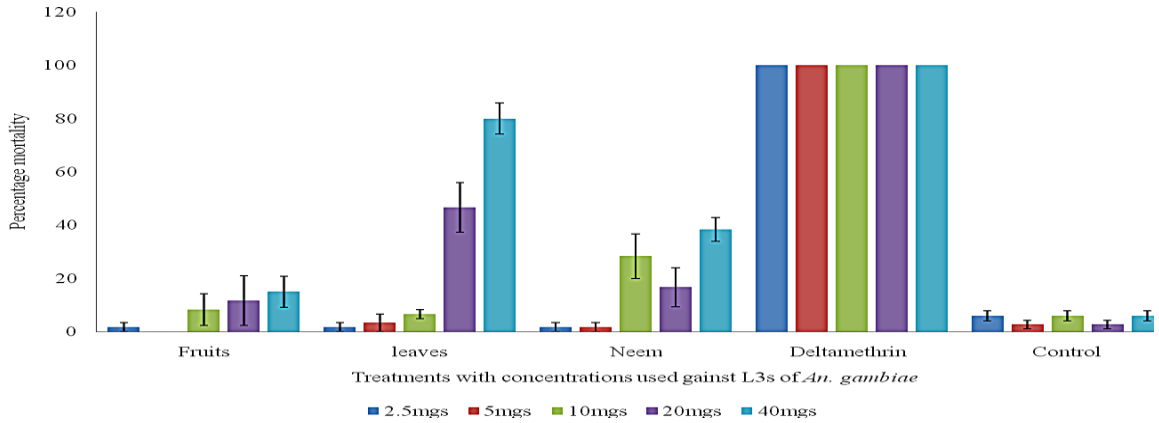

Figure 3 Percent mortality of *An. gambiae* L3s from three day old solutions. Error bars represents standard error of means

For four (Figure 4) and five (Figure 5) days old solutions, extracts of mature green fruits and leaves of Endod killed less than the WHO >80% mortality threshold and were thus adjudged ineffective as insecticides. All preparations of deltamethrin of the same age however, killed all exposed larvae. Duncan's Multiple Range test and one way analysis of variance (ANOVA) found mortality of the exposed larvae due to all concentrations of freshly and one, two, three, four and five day old solutions significantly different at $p < 0.05$ (Table 1).

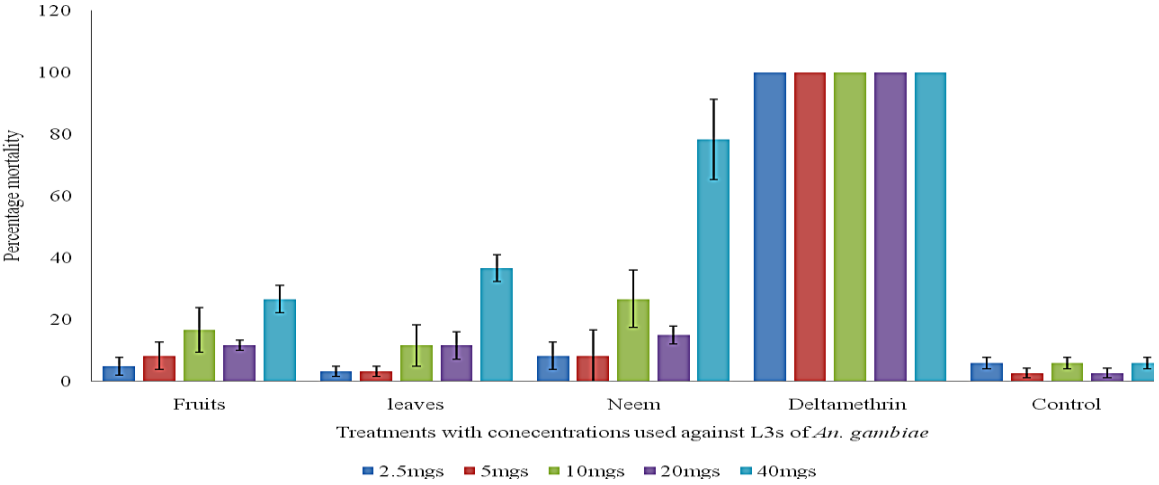

Figure 4 Percent mortality of *An. gambiae* L3s from four day old solutions. Error bars represents standard error of means

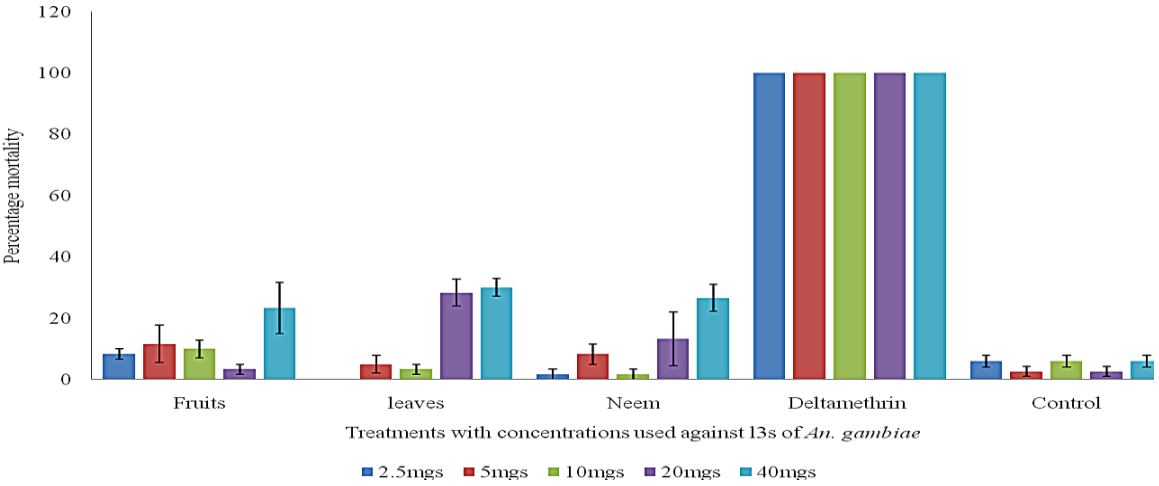

Figure 5 Percent mortality of *An. gambiae* L3s from five day old solutions. Error bars represents standard error of means

Table 1 Duncan's multiple range statistics on levels of the residual effectiveness of crude ethanol extracts from fruits and leaves of Endod in harvested rain water

Conc.	**df**	**Days for which the treatments were left to stand before use against third larval instars of *An. gambiae* mosquitoes**													
		1			**2**			**3**			**4**			**5**		
		R^2	**F**	**P**	**R^2**	**F**	**P**	**R^2**	**F**	**P**	**R^2**	**F**	**P**	**R^2**	**F**	**P**
2.5mg	4	0.982	144.87	0 0001	0.980	127.58	0.0001	0.998	1160.33	0.0001	0.991	292.48	0.0001	0.998	1691.3	0.0001
5mg	4	0.957	60.03	0.0001	0.959	62.00	0.0001	0.996	696.73	0.0001	0.973	95.27	0.0001	0.983	151.78	0.0001
10mg	4	0.924	32.56	0.0001	0.971	88.51	0.0001	0.964	71.15	0.0001	0.933	37.23	0.0001	0.996	653.53	0.0001
20mg	4	0.996	756.25	0.0001	0.973	97.83	0.0001	0.916	29.25	0.0001	0.989	249.30	0.0001	0.966	76.47	0.0001
40mg	4	0.985	175.00	0.0001	0.699	6.21	0.0175	0.963	69.42	0.0001	0.896	22.98	0.0003	0.954	55.62	0.0001

Note: 1. R^2 stands for the value of squares of the sample correlation; 2. F stands for value of F test; 3. Conc. Stands for concentration; 4. P stands for probability for level of significance. P value was considered significant at $p < 0.05$; 5. df stands for degrees of freedom

2 Discussion

Ethanol and water extracts of Endod were found effective against larvae of *An. gambiae* mosquitoes. The effectiveness was dose dependent and for only three days (WHO, 2005). It was also observed that activity of the treatments rapidly reduced with extension of exposure period from day four onwards, a trend similar to one observed earlier (Amal et al., 2013; Abdalla and Amal, 2015).

It was observed that fruit extracts generally showed better results than leaf extracts of Endod and that the effectiveness of the extracts progressively decreased as the residual durations increased. These findings were similar to that of Abdalla and Amal (2015), that tested water, ethanol and petroleum ether extracts of leaves and fruits of *Citrullus colocynthis* against larvae of *Anopheles arabiensis* Patton. They also confirmed the fact that insecticidal activities of plant extracts depended on plant parts extracted (Mullal et al., 2008) and reduced with time.

Though the present study did not test residual effectiveness of active phytochemicals of Endod, the fact that the crude extracts were active for only three days could be concluded to be similar with findings by Molgaard et al., (2000) and Misganaw et al., (2012). These researchers observed that saponin, the active phytochemical in Endod (*Phytolacca dodecandra*) was only stable for 2 days and thereafter biodegraded rapidly. These findings are also similar to other findings that found degradation of natural products to be of shorter period (Schmutterer, 1990) than formulated synthetic chemicals. This explains the reason as to why plant extracts are less likely to persists, pollute and inflict harm to life in water as opposed to conventional synthetic pesticides (Amer and Melhorn, 2006).

The present study concludes that ethanol extracts of Endod are effective against *An. gambiae* larvae, but that the residual period is three days. It also notes that the fact that the extracts degrades quickly indicates that they are not only life-saving but are unlikely to persists, pollute and inflict harm to the environment.

3 Materials and methods

3.1 Study area, experimental mosquitoes and study design

Mosquitoes larvae used for the experiments were of *An. gambiae* raised at the insectary of the Entomological laboratory at the Centre for Global Health Research/Kenya Medical Research Institute (CGHR/KEMRI). The mosquitoes were cultured following standard procedures (Parekh et al., 2005; Das et al., 2007) and conditions describe in Yugi et al. (2014). A completely randomized informal 'after-only with control' experimental research design (Kothari, 2004) was used to investigate the lethal effect of crude ethanol extracts of Endod on the exposed *An. gambiae* mosquito larvae.

3.2 Deltamethrin and plant materials, extraction and preparation

Deltamethrin was obtained in the form of synthetic KOTab 1-2-3® tablet, weighing 1.6 g and containing 0.4 g deltamethrin [pyrethroid (250 g/kg)]. Leaves and mature green fruits of Endod and leaves of Neem, *Azadirachta indica* used to obtain plant extracts were sourced identified and vouchers deposited as described (Yugi et al., 2015). In all cases 80 mgs of ethanol and water extracts obtained from the plant parts as well as deltamethrin were aliquoted, wrapped in pieces of aluminium foil and refrigerated at 4°C.

3.3 Residual bioassay

Eighty milligrams of stocks crude extracts of Endod derived from mature green fruits and leaves were dissolved in 100 mls of harvested rain water and the stocks solution left to stand. This was repeated for six days with each day a solution being made and left to stand. All the solutions were then serially diluted to different concentrations of 80, 40, 20, 10, 5 and 2.5 mg/100 ml and added into a set of six plastic containers measuring 6 cm top ×5.7 cm bottom × 3.5 cm height. Each set contained a solution of a particular concentration. Third larval instars (L3s) were picked from the rearing trays using a dropper and exposed in groups of twenty to the prepared solutions to test on potency. Two assumptions were made on the preparation. First was that if a solution prepared six days earlier could kill after

that long then it had the ability to remain potent for a period (residual period) similar to the same number of days it was left to stand. Second was that the number of days a solution was left to stand represent the duration such a solution would have taken on applied surface or media. The larvae were left exposed for 24 h, after which they were transferred to distilled water for another 24 h, to check for signs of recovery.

Larvicidal activities were tested in accordance with the WHO procedure (WHO, 1997). Daily records of larval mortalities were taken until the end of the study period and for every set up, moribund and dead larvae were collected and disposed off in a septic tank. The set ups were replicated five times

Larvae mortality rate were registered after 48 hours and 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).

3.4 Data analysis

Data obtained from the bioassays was entered in excel spreadsheets for ease of handling. The data was subjected to statistical analysis based on the applied design with relationship between the residual effects of Endod extracts with respect to parts of Endod and solvent used being determined using descriptive statistics. The means were compared using Duncan's Multiple Range test and one way analysis of variance (ANOVA) to determine the level of significance of larvae mortality with respect to residual period of exposure. All statistical analysis was performed using SAS statistical package version 20.

Authors' contributions

YJO conceived the idea, extracted phytochemicals from the plants, sourced for wild *An. gambiae* mosquitoes and cultured the mosquitoes, conducted the experiments, wrote the manuscript and sourced for funds. YJO, ADC and OHA designed the experiments, read and corrected the manuscript.

Competing interest

The authors declare that they have no competing interest.

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