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Larviciding potency of water and ethanol extracts of *Phytolacca dodecandra* (L' Herit) on *Anopheles gambiae* (Diptera: Culicidae)

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Abstract Introduction: Plant extracts are an attractive target for search of effective malaria vector control agents. The reason for this is that they present a cost effective, target specific and bio-degradable insecticides. The other reason is that they possess varied phytochemical contents that vectors are unlikely to develop resistance to very soon. In this study, we report on effectiveness of ethanol and water extracts of *Phytolacca dodecandra* (L' Herit) against *Anopheles gambiae* (Diptera: Culicidae) larvae. Methods: Crude ethanol and water extracts of leaves (shoot and midsection) and mature green fruits of *P. dodecandra* were scrutinized for larvicidal activity against 1st to 4th instar larvae of *An. gambiae*. Larvicidal bioassays were conducted and effectiveness evaluated using the >80% as per the WHO methods and threshold respectively. ANOVA analyses were performed for statistical justifications of the larvicidal property with *P* considered significant at $p < 0.05$. The effects of the extracts were evaluated under laboratory conditions. Result: Ethanol extracts were more potent than water extracts of *P. dodecandra* as larvicide. The highest mortality (56%) was recorded for L4s for ethanol extracts of mature green fruits of Endod sourced from Eldoret. Water extracts of Neem leaves killed 31% L3s while deltamethrin killed over 80% of all exposed larvae. Conclusion: Ethanol extracts *P. dodecandra* killed more of the exposed *An. gambiae* larvae than water extracts. Recorded mortalities due to exposure to the extracts were less than the WHO threshold of >80%. We recommend that additional refinement and tests need to be done before commercial exploitation as a malaria vector larvicide.

Keywords *Anopheles gambiae*, *Phytolacca dodecandra*, *Azadirachta indica*, Deltamethrin, Ethanol

Introduction

Anopheles gambiae Giles s.s. is one of the most prolific malaria vector in Africa (Coetzee et al., 2000). It undergoes a complete life cycle that consists of eggs-larvae-pupae and adults. The eggs hatch to give rise to larvae that develop through four larval instars (L1 -L4). The L4s coil up to become pupae which after a day split dorsally to release adult mosquitoes (Gwadz and Collins, 1996). The larvae occupy temporary aquatic habitats (Minakawa et al., 2004) that are most often small flooded open and sunlight depressions (Adeleke et al., 2008) with mostly clean water.

Management of these aquatic habitats is crucial in the fight against mosquito vectors. Targeting the larvae may be challenging due the fact that the habitats are many and different. However, it has been done by

employing source management procedures (Fillinger and Lindsay, 2011) using synthetic larvicides. The problem with this method has been the indiscriminate use of the larvicides and this has led to build up of insecticide pressure resulting in resistance (Devine, 2007) by the target larvae. In addition, the insecticides have been found to be non-selective, non-biodegradable and harmful both to the environment and human (Lee et al., 2001; Cartilla and De la Cruz, 2012).

This has led to intensified search for tools that demonstrate eco-friendliness and target specificity and this has been found with plant extracts otherwise known as botanicals (Chowdhury et al., 2009; Rawani et al., 2009; Chakraborty et al., 2013). Many of these plant extracts have demonstrated mosquito larvicidal effect. For example leaf extracts of *Holoptelea*

integrifolia against *Culex vishuni* (Singha et al., 2012), root extracts of *Tragia involucrata* L. (Euphorbiaceae) against *Culex quinquefasciatus* (Bhattacharya and Chandra, 2014), *Nelumbo nucifera* against *Anopheles stephensi* (Ray et al., 2014) and extracts from berries of *Phytolacca dodecandra* against immature of Filarial Vector *Culex quinquefasciatus* (Misganaw et al., 2012). Other plant constituents have also demonstrated pupicidal effects (Rawani et al., 2012), knockdown on *An. gambiae* adults (Yugi et al., 2014), repellency as well as adulticidal (Singha et al., 2011, Chowdhury et al., 2007) toxicity against different mosquito species. In this study we demonstrate larvicidal effect of ethanol and water extracts of *Phytolacca dodecandra* (L' Herit) or Endod on *An. gambiae* larvae under laboratory conditions.

1 Results

This experiment was conducted for a period of eight months. A total of 84, 240 larvae (L1s, L2s, L3s and L4s) of laboratory cultured *An. gambiae* mosquitoes were used. It was found that ethanol extracts of Endod plant parts killed the largest percentage of all exposed larval instars compared to water extracts of the same parts.

The highest mortality of exposed larvae was recorded for ethanol extracts of Endod parts sourced from Eldoret compared to ethanol extracts of the same parts of Endod sourced from Nyando. Though ethanol extracts of mature green fruits killed the highest number of exposed larvae (L4s), mortality from

exposure to extracts of mature green fruits sourced from Eldoret was almost the same as that of the same parts sourced from Nyando. Ethanol extracts from Neem leaves killed 30% of L3s (Table 1).

The L1s were the most sensitive to water extracts of Endod parts with the highest number of mortality being observed for both extracts of Endod leaves of the shoot sourced from Eldoret and Nyando. L3s were more sensitive to water extracts of Neem leaves (Table 2). Preparations of deltamethrin killed over 80% of all exposed larval instars.

2 Discussions

Inability to mount an effective control of mosquito borne diseases remains a big challenge even today. Though many methods are available for use to protect against mosquito bites, there is room for further advice and even tools to control the menace. The best approach to reduce mosquito population is to employ cost-effective, biodegradable natural insecticides from botanicals. This is because aside from being readily available and effective, their chemical components are varied and therefore the target insects might never develop resistance to their use (Miresmailli et al., 2006).

In this study it was observed that extracts of both mature green fruits and leaves of Endod were potent against larval stages of *An gambiae* though percent mortality did not meet the WHO threshold. The fourth larval instars were more sensitive to ethanol extracts from mature green fruits of Endod that resulted in

Table 1 Percentage mean mortality of larval stages of *An. gambiae* exposed to crude ethanol extracts of Endod parts

Stage	N	Source and parts of Endod used						Neem	Deltamethrin
		Eldoret			Nyando				
		Fruits	Shoot	Midsection	Fruits	Shoot	Midsection		
L1	18	48.89±9.91	16.11±7.00	20.56±3.47	39.72±8.76	11.11±3.04	16.11±5.57	26.67±8.05	83.61±8.89
L2	18	45.83±9.46	30.56±9.52	23.06±7.34	52.22±9.91	39.72±10.30	24.17±6.78	11.39±3.57	83.33±9.04
L3	18	53.33±11.11	37.22±10.57	41.11±8.18	41.94±10.16	55.56±10.49	32.22±8.76	30.00±7.95	83.61±8.90
L4	18	56.11±10.17	49.44±8.50	37.50±8.61	48.33±11.53	46.67±9.97	22.22±6.93	6.67±1.98	84.17±8.60

Notes: Each value is a mean ± standard error of mean of three replicates

Table 2 Percentage mean mortality of larval stages of *An. gambiae* from exposure to crude water extracts of parts of Endod

Stage	N	Treatments							
		Source and parts of Endod used						Neem	Deltamethrin
		Eldoret			Nyando				
		Fruits	Shoot	Midsection	Fruits	Shoot	Midsection		
L1	60	20.28±3.19	20.59±7.75	18.61±6.68	12.22±2.56	45.83±9.74	10.29±2.52	28.24±8.38	83.61±8.89
L2	60	8.06±2.50	0.56±0.56	5.28±2.04	8.53±1.65	3.33±1.00	11.18±3.68	12.06±3.71	83.33±9.04
L3	60	6.67±1.14	3.89±1.03	2.94±0.86	6.11±1.43	4.12±0.98	3.06±1.00	31.18±8.30	83.61±8.90
L4	60	5.56±1.14	2.06±0.75	4.72±1.03	10.29±4.42	2.65±0.87	6.39±1.61	6.67±1.98	84.17±8.60

Notes: Each value is a mean ± standard error of mean of three replicate

over 55% deaths. This observation was consistent with that of essential oils of *Plectranthus glandulosus* and *Callistemon rigidus* extracted from their Leaves and used against L4s of *Ae. aegypti*, *An. gambiae* and *Cx. Quinquefasciatus* (Pierre et al., 2014) and *Anacardium occidentale*, *Afromomum melegueta*, *Garcinia kola* and *Citrus sinensis* against the developmental stages of mosquito, *An. gambiae* Giles (Ileke et al., 2014).

Ethanol extracts of all parts of Endod were more potent than water extracts of similar parts. This demonstrated that the type of solvent used in the extraction of the phytochemical determined the potency of the extracted bioactive botanicals (Anupam et al., 2012). Ethanol is less polar (polarity index of 0.1) than water (polarity index of 10.2). It extracted biochemicals with lower molecular weight as compared to those extracted by water. Biochemicals of high molecular weights such as proteins, glycans are of low potency and this explains the difference in potency between ethanol and water extracts.

Solvent types were also seen influencing larvicidal potency of *Solanum xanthocarpum* (Mohan et al., 2006), *Euphorbia tirucalli* (Singh et al., 2007), *Eucalyptus globules* (Maurya et al., 2007; Singh et al., 2007), *Citrullus colocynthis* (Sakthivadivel et al., 2008), *Azadirachta indica* (Mgbemena et al., 2010) and *Solanum nigrum* (Raghavendra et al., 2009). It is probable that ethanol extracts were superior to water

extracts of the parts of Endod by causing the highest mortality of the exposed larvae by blocking more efficiently the spiracles through which the larvae breathe (Kaufmann and Briegel, 2004). This then leads to asphyxiation and subsequent death (Ileke and Oni, 2011; Ileke and Olotuah, 2012) of the larvae.

Ethanol extracts of bioactive phytochemicals from Endod parts sourced from Eldoret were more potent than ethanol extracts of the same parts sourced from Nyando. This showed that the geography of the source of the plants played a role in the concentration and distribution of phytochemical in the plant. This finding was similar to an earlier one that found variation in phytochemical activity from Endod (Were, 2008), *Citrus* sp, *Ocimum sanctum* and *Azadirachta indica* (Mgbemena et al., 2010) and *Jatropha* sp (Sakthivadivel and Daniel, 2008) based on geographical source (Anupam et al., 2012).

This study concludes that the phytoextracts from Endod has activity on all larval instars of *An. gambiae*. However, before commercial exploitation there is a need for the components of the larvicidal extracts to be isolated, processed further and exposed to further tests under laboratory and field conditions.

3 Materials and methods

3.1 Study area, experimental mosquitoes and study design

Eggs used for the experiments were of *An. gambiae* mosquitoes kept at the insectary of the Entomological laboratory at the Centre for Global Health Research/Kenya Medical Research Institute (CGHR/KEMRI) and reared following standard techniques (Das et al., 2007), following procedures and conditions described in Yugi et al., (2014). A completely randomized informal 'after-only with control' experimental design (Kothari 2004) was used to investigate the ovicidal effect of crude ethanol and water extracts of Endod on *An. gambiae* mosquito larvae.

3.2 Plant materials

Fresh leaves (shoot and midsection) and mature green fruits of *Phytolacca dodecandra* (Endod) 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) collected from Kanyagwal, Nyando [-0.250393°N, 34.870190°E]. The plant parts were collected on 3rd and 4th May 2012, thereafter 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 *A. indica* deposited in the herbarium at the School.

3.3 Plant extract and deltamethrin preparation

Leaves (shoot and midsection) and mature green fruits of Endod and the leaves of Neem were used to obtain plant extracts. Ethanol and water extracts were obtained from the plant parts, freeze dried, preserved and serially diluted as described elsewhere (Yugi et al., 2014). Deltamethrin was obtained and similarly prepared as described elsewhere (Yugi et al., 2014).

3.4 Larvicidal bioassays

Larvicidal activities were conducted in accordance to the WHO method (WHO, 1996). Batches of twenty freshly hatched or moulted larvae (1st, 2nd, 3rd and 4th instars) of *An. gambiae* were transferred by means of the dropper to plastic containers measuring 6 cm mouth and 5.7 cm base diameter by 3.5 cm height arranged in sets according to the concentrations of the extracts used. Each set was made up of three plastic containers and each container received approximately 33 millilitres of a particular concentration of treated harvested rain aqueous. Appropriate volume of stock

solutions of each treatment of crude ethanol and aqueous extracts were added to 100 ml aqueous in the glass beakers and then serially diluted to obtain 40, 20, 10, 5 and 2.5mg/100mls of the crude extracts. Three replicates were set up for each concentration including two positive (Neem and deltamethrin) and one negative control (untreated harvested rain aqueous).

The exposed larval stages of *An. gambiae* were left to stay in the treatments on experimental tables overnight. Mortality rate were registered after 24 hour exposure period. Standard WHO procedures were used to assess the level of toxicity (larvicidal effectiveness) at a mortality rate of > 80% (WHO, 2005). All exposed larvae were collected in a pail of hot aqueous at the end of the experiments and disposed off in a septic tank.

3.5 Data analysis

Data obtained from the bioassays was entered in excel spreadsheets and the relationship between larvicidal effect of the extracts with part of Endod 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 larvae mortality. All statistical analysis was performed using SAS statistical package version 20.

Authors' contributions

YJO conceived the idea, sourced for funds, conducted the experiments and wrote the manuscript. YJO and O-OJB designed the experiments and sourced for funds. O-OJB, WKP and VJM supervised and guided the experiments. YJO, ACA and JJI extracted phytochemicals from the plants and sourced for wild *An. gambiae* mosquitoes. All authors read and corrected the manuscript.

Competing interest

The authors declare that they have no competing interest.

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