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Larvicidal Effect of *Ipomoea stolonifera* on Laboratory-Reared and Field-Collected *Aedes aegypti* Mosquitoes in Enugu, Nigeria

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Authors' Contribution
GIN and FCO conceived and designed the study. GIN, MIN and AOA conducted the study. GIN, AOA and CDN collated the data and did the statistical analysis. GIN, FCO, AOA and CDN wrote the manuscript. FCO supervised the study.

Key words
***Aedes aegypti*, Larvae, *Ipomoea stolonifera*, Larvicidal effect**

ABSTRACT

Comparative study of the effects of methanolic crude extracts of *Ipomoea stolonifera* on 4th instars larvae of both laboratory-reared and field-collected *Aedes aegypti* were carried out. The 4th instars larvae of laboratory-reared and the field-collected *A. aegypti* mosquitoes were exposed to 5000mg/L, 2000 mg/L, 1000 mg/L, 500 mg/L, 100 mg/L, 50 mg/L of the extract. The solvent for the extract, dimethyl sulphoxide (DMSO) was used as control. The results indicate concentration dependent mortalities and significant differences (P<0.05) in the mean percentage mortality between the laboratory-reared and field-collected Aedes mosquitoes. The mortality rate due to the extract was found to be less in the field collected *A. aegypti* (LC_{50} = 2.81 ± 0.14; LCI = 2.55; UCI = 3.11) than the laboratory-reared ones (LC_{50}=1.24±0.09; LCI = 1.07; UCI = 1.43) after 24 h exposure. The median larvicidal concentration LC_{50} values of the extract were 1.24 ± 0.09g/L and 2.81 ± 0.14g/L for 4th instars larvae of both laboratory-reared and field-collected mosquito larvae respectively. Further research is recommended on the isolation of the active ingredients of *I. stolonifera* for use in effective control of mosquito.

INTRODUCTION

Vector-borne diseases affect over a billion people and lead to the death of more than one million of their victims annually (WHO, 2014). Mosquito-borne diseases are the most common constituent of these vector borne diseases (Burkett-Cadena and Vittor, 2018). Aedes mosquitoes are, in the meantime, the culprits for the transmission of various arboviral diseases. *Aedes aegypti*, found in Africa, Asia and Central and South America particularly transmit yellow fever, dengue and, of recent interest Chikungunya and Zika viruses, in different parts of the world (Ali *et al*., 2012; Paixão *et al*., 2017). These diseases have heightened over the years, principally due to insecticide and drug resistance developed by the mosquito vectors and the pathogens (Liu, 2015). Diverse vector control measures deployed to checkmate threats from these disease vector mosquitoes have continued to be thwarted by their growing resistance to synthetic insecticides due to misuse and overuse (Bass and Jones, 2016; Farooqi *et al*., 2019). The current challenge demands the development of new and environmentally friendly insecticides that can be used as effective larvicides (Borovsky, 2003) hence the need for continued search for novel ones that will be efficacious in checkmating their activities.

So far, plant extracts seem to serve as appropriate alternatives to synthetic insecticides because they are safe, biodegradable, cost effective and readily available worldwide (Susheela, 2016). Though several plants from different families have been reported to be bioactive against different mosquito stages, only very few of them have moved from the laboratory to field use (Murthy and Rani, 2009) which necessitates continued search for appropriate bioactive plant products that can be used in the field. Aquatic macrophytes, some of which pose problems to mosquito control (Kant and Srivastava, 2004), may have allelochemicals detrimental to these organisms (Ghobrial *et al*., 2015). These preliminary results demand in-depth study of their phytochemical constitutions, bioactivities and efficacy as possible mosquito control agents.

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MATERIALS AND METHODS

Test mosquitoes

Mosquito eggs of *A. aegypti* reared in the laboratory to the fifth generation down the line were collected from Arbo-Viral Research Laboratory, Federal Ministry of Health Enugu, Nigeria, while the wild mosquito eggs of the *A. aegypti* were trapped in the Zoological Garden of University of Nigeria, Nsukka. The eggs were hatched and allowed to develop into the 4th instars larvae. The 4th instars larvae appeared within 3 to 4 days after egg-hatching.

Plant collection and extraction

Whole plant (stems, roots and leaves) of *Ipomoea stolonifera* were collected from the 'Ohe' pond in Nsukka Urban (Nsukka Central Local Government Area) of Enugu state, Nigeria. Bio-Resources Development and Conservation Programme, Nsukka centre (BDCP) identified the plants. Absolute methanol of analytical grade (Sigma – Aldrich, Germany) diluted to 70% with distilled water was used for the crude extraction from the plant. A 304 g quantity of *I. stolonifera* dried at room temperature in the laboratory was pulverised in Thomas Wiley Mill, Model. 4 and then macerated in 70% methanol for 24 h. It was first filtered with muslin cloth and then with Whatman no.1 (24.0 cm) filtre papers. The filtrate was then subjected to rotary evaporation to recover the methanol and then all traces of methanol in the resulting jelly extract removed by keeping it at room temperature for complete evaporation to dryness. The percentage yield of the extract was then determined gravimetrically as follows:

$$\%\ Yield = \frac{Mass\ of\ dried\ methanolic\ extract\ (x)}{Mass\ of\ pulverised\ ipomoea\ (y)} \times 100$$

Brine shrimp cytotoxicity assay

The brine shrimp, *Artemia salina* (Leach) was used in bioassay procedure to estimate the cytotoxicity as well as the biological activity of the *I. stolonifera* methanolic extracts using standard methods (Mclaughlin and Chnag, 1991; Meyer *et al*., 2007). The result was analysed using probit analysis (minitab for windows release 12.21) to determine the LC_{50} at 95% confidence interval.

Phytochemical tests

Small dry quantities of the plant extract were subjected to phytochemical tests following standard methods (Iwu and Chiori, 1984).

Egg hatching and larval rearing

Tap water, in a plastic bowl, used for the egg hatching and larval rearing was to stand undisturbed for 24 h to acclimatize. A piece of white linen cloth containing the eggs was soaked in the treated water. The linen was totally submerged in such a way that the surface with the trapped eggs faced up in the bowl. Eggs started to hatch within 30 minutes but were left overnight, to ensure eggs were completely hatched, before removing the white linen. The young larvae appeared, in a plastic bowl, as tiny motile organisms when viewed with hand lens. The emerging wrigglers were fed with fine powder of brewer's yeast (Iyaloo and Facknath, 2017) spread over the water and subsequently with more animal, growers' marsh, chicken feed, product of Vita Feed Nigeria Limited, for quicker generation of detritus which the larvae fed on.

Surface film, scum, which occasionally formed on the surface of the water, was daily skimmed off with a piece of paper. The 4th instars larvae which were reached after the 3rd instars had molted were pooled into a beaker of clean tap water.

Counting-in of larvae and larvicidal screening tests

Twenty 4th instars larvae were counted into each of all labelled 250ml beakers containing only tap water and the set up left to stand on the bench for 24 h in order to acclimatize. Mortality effects of the methanolic extract of *I. stolonifera* to the larvae were evaluated using graded concentrations method. Stock solution of the methanolic extract was prepared by dissolving 1 g dry-weight of the extracts in 4 ml of dimethyl sulphoxide (DMSO) for every 100 ml stock solution in tap water. Six graded concentrations of 5000mg/L, 2000mg/L, 1000mg/L, 500 mg/L, 100mg/L and 50mg/L were each prepared in triplicates in 100 ml amounts from the stock solution. The control was made up of 40 ml/L of the DMSO solvent.

The larvicidal effects of the different concentrations of the extract were then checked 6 hourly for a period of 24 h. Thus, the larvicidal effects of graded concentrations of the extract were determined within 24 h for the 4th instars larvae of both the laboratory-reared and field-collected mosquitoes.

Statistical analysis

One-way analysis of variance (ANOVA) for comparing the effect of the various concentrations of the extract on the 4th instars larvae was utilised and expressed as mean ± SD. A comparison of lab-reared and field-collected *Aedes* mosquito was carried out using independent samples t-test. The median lethal concentration bioassay value, LC_{50} was obtained using probit analysis (Minitab for windows release 12.21). All analyses were carried out using Stata 20 statistical package.

RESULTS

Phytochemical tests

Phytochemical tests performed on the *I. stolonifera* extracts showed that some compounds were absent (-), present in small concentration (+), present in moderately high concentration (++) or present in very high concentration (+++). The result is as follows: saponins (++), flavonoids (+), glycosides (+++), proteins (+++), tannins (+++), steroids (+), terpenoids (+), reducing sugar (-), carbohydrate (-), resins (-), alkaloids (-), acid (-), fats and oil (-).

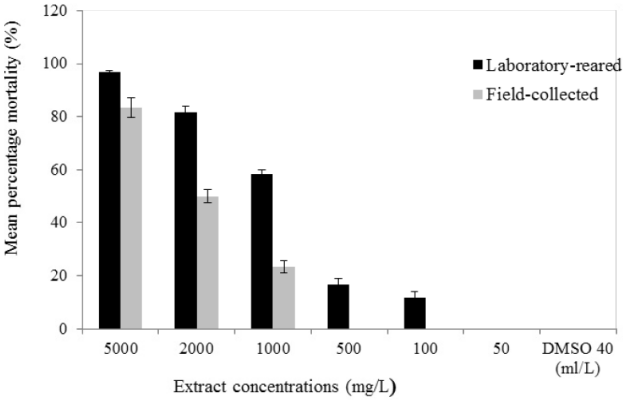

Fig.1. Mean percentage mortality of laboratory-reared and field-collected fourth instars larvae of *A. aegypti* at different concentrations.

Table I. Comparison of mortality effect of substance administered on 4th instars larvae of laboratory-reared and field-collected mosquitoes (Mean ± SD).

Extract concentration	Laboratory-reared	Field-collected	t–Staistic♣
5000mg/L	96.67%±1.67	83.33%±7.26	1.7889
2000mg/L	81.67%±4.41	50.00%±2.87	.0083***
1000mg/L	58.33%±3.33	23.33%±4.41	.3317***
500mg/L	16.67%±4.41	0.00%±0.00	.7796**
100mg/L	11.67%±4.41	0.00%±0.00	2.6458*
50mg/L	0.00%±0.00	0.00%±0.00	--
40ml/L(DMSO)	0.00%±0.00	0.00%±0.00	--

Test statistic testing for the difference in means of number dead within 24 h between laboratory reared and field-collected mosquito larvae at different concentrations; *, **, *** Significant at 10%, 5% and 1% levels respectively.

Percentage yield of the extract used

The percentage yield (w/w) of the crude methanolic extract of *I. stolonifera* was 23.06%.

Brine shrimp mortality bioassay

Result of mortality bioassay of methanolic extract of the plant on brine shrimp was LC_{50} 1625.3 ppm at 95% confidence interval.

Larvicidal evaluation

Mean mortality effects of graded concentrations of the extract on 4th instar larvae of both laboratory-reared and field-collected *Aedes* mosquitoes after 24 h exposure are shown in Figure 1. The result showed that the mean mortality effect of the extract on laboratory-reared mosquitoes was highest (96.67 ± 1.67^{a} %) at 5000mg/L concentration and least (11.67 ± 4.41^{d} %) at 100 mg/L. There were no mortality effects at 50mg/L of the extract. Also, all the larvae in 40ml/L DMSO control were alive and vibrant.

The field-collected mosquitoes also showed highest mortality effect of 83.33±7.26^{a} % at 5000mg/L concentration but least effect of 23.33% ±4.41^{c} at 1000mg/L. None of the larvae at concentrations of 500mg/L, 100mg/L and 50mg/L died. The larvae in the 40ml/L DMSO control of this group were also alive.

The results of dosage responses for the 4th instars larvae of both the laboratory-reared and field-collected mosquitoes at LC_{50} were 1.24±0.09 and 2.81±0.14 respectively at 95% confidence intervals. Test statistic testing for the difference in means of number dead within 24 h between laboratory-reared and field-collected mosquito larvae at different concentrations are shown in Table I.

DISCUSSION

Tannin formed the highest component (+++) of our crude extract while saponin (++) was next to it. Flavonoids (+), steroids (+) and terpenoids (+) were present in minute amounts. Pure samples of all of these extracts have been reported to be bioactive in various degrees and dimensions. Tannin has been reported to be toxic to *Culex. pipiens, Aedes taxa, A. aegypti, A. albopictus and A. rustics* (Rey *et al*., 1999; Tharkur *et al*., 2004) while saponin has been reported as having larvicidal activity (Jawale, 2014) and especially very efficacious against *A. aegypti* and *C. pipiens* (Wiesman and Chapagain, 2003; Chapagain and Wiesman, 2005). Flavonoids, steroids and terpenoids have also been reported to be capable of inducing larval mortality or retarding larval development (Arivoli and Tennyson, 2011; Ghosh *et al*., 2012). The various constituents of our extract combine effectively and most probably synergistically to boost the efficacy of *I. stolonifera* as a bioactive agent against the *Aedes* mosquito sampled. Plant saponins are particularly widely distributed amongst plants and have a

wide range of biological properties (Sparg *et al*., 2004). Since bioactivity of crude plant extracts depend on one or more of its phytochemical constituents, *I. stolonifera* as good reservoir of these constituents might have contributed significantly to the mortality effect of the crude extract on *A. aegypti* larvae recorded. The plant, *I. stolonifera* can therefore be used as bioactive agent in *A. aegypti* control either as purified sample or as crude extract.

We recorded various degrees of mortality on the 4th instars larvae which were concentration and breeding condition dependent. Susceptibility is known to be concentration and breeding conditions dependent (Gutierrez *et al*., 2014). The 4th instar larvae of the laboratory-reared mosquitoes were more susceptible to the extract than those of the field-collected. This could be attributed to several factors including insecticide use for controlling crop pests in agriculture as selective pressures favouring resistance; the presence of anthropogenic pollutants common in urban, agricultural or industrial areas, and the impact of plant chemicals (Nkya *et al*., 2013). The last ones might be more responsible since mosquito larvae are thought to ingest particulate matters like debris or detritus which abound in much of the water bodies where they grow. Interactions between these xenobiotics influence mosquito detoxification pathways (Moon, 1985; Chahine and O'Donnell, 2011) as opposed to the laboratory-reared ones in friendlier and pre-conditioned environment.

The result of log probit analysis showed that LC_{50} values of the extract were 1.24 ± 0.09g/L and 2.81 ± 0.14g/L for 4th instars larvae of both laboratory-reared and field-collected mosquito larvae. This extract has comparably low bioactivity (Promsiri *et al*., 2006) which could be attributed to variation in toxicity potentials of the active components of different plants. Phytochemical constituents and concentrations also vary from plant to plant and screening for larvicidal activity of plant extracts could lead to the discovery of new agents for pest and vector control (Kamaraj *et al*., 2008).

The confinement of the lab-reared *Aedes* mosquito could also have influenced their higher level of susceptibility as opposed to the field-collected ones which enjoyed free-range life and were thus prone to environmental influences. The methods of application of extracts affect their biological activities as well (Boschitz and Grunwald, 1994). Sensitivity is also influenced by the presence and level of some enzymes like cytochrome P-450, esterase, and glutathione-S-transferase. It has been reported that cytochrome P-450 and esterases are involved in the detoxification of tannins (Rey *et al*., 1999), a toxic constituent of many plant species. *Ipomoea stolonifera* is quite available in many aquatic bodies and can be grossly exploited in mosquito control if further research is carried out on how to harness the active ingredients for this purpose.

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Statement of conflict of interest

The authors have declared no conflict of interest.

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