4 Materials and Methods

4.1 Study area, experimental mosquitoes, study design and laboratory conditions

This was an *in vitro* designed study conducted at the Centre for Global Health Research (CGHR) entomology laboratory where immature stages (Eggs, third larval instars (L3s) and pupae) of *An. gambiae* were sourced. The experimental design used was as described (Kothari, 2004; Yugi and Kiplimo, 2017). Briefly, a completely randomized informal 'after-only with control' experimental design was used with the solvent, dose and biopesticide extracts taken as independent while mortalities as dependent variables. Distilled water was taken as negative control. The laboratory temperatures and humidity were 28 ºC~30 ºC and 70%~80% respectively. Photoperiod was 12 hrs light (06.30~18.30 hrs) and 12 hrs darkness (18.30~0630 hrs).

4.2 *Urtica massaica* plant parts source, extraction and stocks solution preparation

U. massaica plant (leaves, stem and roots), voucher specimen number JOY2017/001 were sourced from 35º16' 46'' E, 0º31' 41'' N in Eldoret, Kenya. The extraction and processing of the biospesticide was done as described by Khatoro et al., (2021). Briefly, two grams of crude biopesticide stock's extracts was dissolved in 200 millilitres (mls) of dimethyl sulfoxide (DMSO). 160 mls (with 160 mls (v/v) of extract) of this solution was obtained and topped up with 40 mls of distilled water to make 200 mL (with 160 mls (v/v) of extract). This solution was then aliquoted in two beakers of equal capacity (100 mL) each to give a concentration of 80 ml/100ml (s/w). One of this was picked and 100ml distilled water added to top it up to 200 mls and then aliquoted in equal units of 100 mls to give a concentration of 40 mL/100 mL (s/w). This procedure was repeated until serial dilution of 80 mL/100 mL (s/w), 40 mL/100 mL (s/w), 20 mL/100 mL (s/w), 10 mL/100 mL (s/w), 5 mL/100 mL (s/w), 2.5 mL/100 mL (s/w) were obtained for the leaf, stem and root extracts.

4.3 Toxicity bioassay

Toxicity bioassays was conducted as described by Khatoro et al., (2021) and insecticidal potency of the biopesticide determined following the WHO, (2005) procedures. Briefly, 100 freshly transformed third larval instars (L3) were transferred by means of a dropper to plastic containers measuring 6 cm × 5.7 cm × 3.5 cm and left exposed for 24 hours after which the experiment was stopped. The experiments were replicated four times. This procedure was repeated for eggs and pupae for both methanol and hexane extracts. Eggs were however exposed for 48 hours. Mortality was calculated (i) and corrected (ii) using Abbot's (1925) formula for mortality of 5 % larvae in the control.

$$\% \text{ Mortality} = \frac{\text{Number of dead aquatic stage}}{\text{Total number of aquatic stage introduced}} \times 100 \quad \text{.....(i)}$$

$$\% \text{ Corrected mortality} = \frac{\text{Percent mortality in test-Percent mortality in control}}{\text{100-Percent in control}} \times 100 \quad \text{.....(ii)}$$

4.4 Statistical Analysis

Data was organized in excel spreadsheets and analysed using regression (probit) statistics to determine levels of toxicity (LC_{50} and LC_{90}) for the 50% and 90% respectively for dose and solvent of extraction. Levels of significance were adopted at 95% confident interval (CI) (that is at $p \leq 0.05$). Calculated goodness of fit was compared with critical value ($\chi^2 = 22.4$) at the same CI to inform on the relationship with the hypothesis of no relation. All statistical analysis was performed using statistical package for social scientists (SPSS) version 22.

Authors' contributions

Conceptualization, data analysis, and writing of the original draft done by JOY, supervision, methodology, investigation, data collection, review and editing done by all authors.

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