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ORIGINAL RESEARCH ARTICLE

Local Variation in Adulticidal Susceptibility of *Anopheles spp.* to *Artemisia scoparia* Essential Oil in Batagarawa LGA, Katsina State, Nigeria

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ABSTRACT
Malaria remains a major public health burden in Nigeria, exacerbated by increasing insecticide resistance among *Anopheles* spp. Plant-derived essential oils are increasingly being investigated as environmentally sustainable alternatives for vector control. This study evaluated the adulticidal susceptibility of *Anopheles* spp. to *Artemisia scoparia* essential oil (ASEO) across five localities in Batagarawa Local Government Area (LGA), Katsina State, Northwestern Nigeria. Larvae and pupae were collected from 31 breeding sites distributed across Batagarawa localities, reared to adulthood under standardized insectary conditions, and tested using WHO tube bioassays. Four replicates of 25 non-blood-fed adult female *Anopheles* spp. were exposed per locality to 0.50% ASEO-impregnated filter papers. Knockdown times (KDT_{50} and KDT_{95}) were estimated using probit regression analysis, while locality differences were analyzed using one-way ANOVA at $p < 0.05$. Breeding-site physicochemical parameters including temperature, pH, dissolved oxygen, conductivity, and turbidity were also evaluated. Significant variation was observed in breeding-site pH ($p = 0.0128$) and temperature ($p = 0.0067$) among localities. Mosquito knockdown responses differed significantly across localities, with KDT_{50} values ranging from 33.08 (95% CI: 29.56 - 37.02) to 59.15 (95% CI: 48.58 - 72.01) minutes ($p = 0.0271$). However, 24-hour mortality remained consistently low across all localities, ranging from 4% to 16% ($p = 0.2167$). The findings indicate that although ASEO induced measurable knockdown effects, its adulticidal efficacy at 0.50% concentration was inadequate under the tested formulation and exposure conditions. These results suggest that *A. scoparia* essential oil may currently serve better as a complementary component in integrated vector management strategies than as a stand-alone adulticide.

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INTRODUCTION
Malaria remains one of the most serious public health challenges globally, with sub-Saharan Africa bearing the brunt of the burden. Nigeria alone accounts for nearly 27% of the world's malaria cases and deaths, with the highest incidence occurring in the northern and north-eastern regions (World Health Organization, WHO, 2023). In 2021, Nigeria recorded approximately 68 million malaria cases and 194 000 deaths, around 80% of which occurred among children under five years (WHO, 2023). Malaria is responsible for about 60% of outpatient visits, 11% of maternal mortality, and 30% of child mortality (approximately under-five) in the country (Dawaki et al., 2016).

Control of malaria largely relies on vector management, particularly through long-lasting insecticidal nets (LLINs) and indoor residual spraying (IRS) (Kleinschmidt et al., 2018). However, the widespread emergence and escalation of insecticide resistance in *Anopheles* spp.—particularly pyrethroid resistance—threatens the effectiveness of these interventions (Ranson & Lissenden, 2016). In Nigeria, resistance to pyrethroids and other common insecticides has been documented across both southern and northern regions, undermining national malaria control efforts (Ranson & Lissenden, 2016).

In light of growing chemical resistance and environmental concerns, there is increasing interest in environmentally friendly alternatives. Biopesticides—derived from plants, microbes, and minerals—offer selective toxicity against pests while minimizing impacts on non-target organisms and environmental contamination (Negahban et al., 2006; Isman, 2019; Bello et al., 2025; Muhammad & Lawal, 2025; Oyedeji et al., 2022, 2024; Ubani & Muhammad, 2025). Essential oils (EOs) derived from plants are particularly promising, demonstrating insecticidal, repellent, larvicidal, and oviposition deterrent properties against various mosquito species (Corzo-Gómez et al., 2024). Compared with many conventional synthetic insecticides, plant-derived essential oils are biodegradable,

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readily available locally, and less likely to persist in the environment, making them attractive candidates for sustainable vector control strategies.

Artemisia scoparia essential oil, in particular, has shown diverse bioactivities. Studies have demonstrated its larvicidal and repellent effects against mosquitoes, with variations in efficacy linked to differences in chemical composition driven by altitude and climatic conditions (Parveen et al., 2024). Additionally, in related studies on stored-product pests, *A. scoparia* EO exhibited fumigant and repellent activity (Negahban et al., 2006). However, most published studies on *Artemisia*-derived essential oils have focused primarily on laboratory-based larvicidal or repellent evaluations, with limited emphasis on the adulticidal responses of field-collected *Anopheles* populations from different endemic localities. Furthermore, available studies rarely examine variation in susceptibility among mosquito populations originating from different endemic localities within the same transmission zone.

Despite this promise, there is limited research on the effectiveness of *A. scoparia* essential oil against *Anopheles* spp., particularly in field-derived mosquito populations under laboratory-controlled bioassay conditions. To the best of our knowledge, no previous study has evaluated the adulticidal susceptibility of field-derived *Anopheles* spp. to *A. scoparia* essential oil in Northwestern Nigeria, particularly within Batagarawa Local Government Area of Katsina State. This represents an important knowledge gap, given the increasing pressure from insecticide resistance and the ecological variability associated with malaria vector populations across endemic communities in the region. Investigating locality-based heterogeneity in susceptibility responses may provide useful baseline information for the development and optimization of environmentally sustainable mosquito control approaches.

In this study, we aimed to assess locality-based variations in the susceptibility of *Anopheles* spp. to *Artemisia scoparia* essential oil in Batagarawa Area, Katsina State, Northwestern Nigeria. The study hypothesized that there is no significant difference among localities in mosquito susceptibility to the essential oil. Findings from this study may contribute to the growing evaluation of plant-derived essential oils as complementary tools within integrated vector management (IVM) programmes, particularly in areas experiencing increasing resistance to conventional synthetic insecticides. Although *A. scoparia* essential oil may not presently serve as a stand-alone replacement for standard insecticides, understanding its adulticidal performance and locality-specific responses could support future formulation improvement, synergistic applications, and sustainable resistance-management strategies.

MATERIALS AND METHODS

Research Design

This study employed a cross-sectional experimental design to evaluate locality-based variation in the susceptibility of *Anopheles* mosquito populations to *Artemisia scoparia* essential oil (ASEO) within Batagarawa Local Government Area (LGA), Katsina State, northwestern Nigeria. The design integrated field-based larval sampling with laboratory-controlled insecticide-susceptibility bioassays, following World Health Organisation (WHO) guidelines.

Mosquito larvae and pupae were collected from multiple breeding sites across five geographically distinct localities (namely Batagarawa town, Dabaibayawa, Barawa, Babbar Ruga, and Ajiwa), during the peak malaria transmission season (July–August 2025). Field-derived specimens were reared in the Insectary of Umaru Musa Yar'adua University, Katsina, under standardized insectary conditions to minimize environmental and physiological variability prior to testing. Susceptibility to ASEO was assessed using WHO standard tube bioassays, with mortality and knockdown responses as primary outcome variables.

Study Area

This study was conducted in Batagarawa Local Government Area (LGA) of Katsina State, Northwestern Nigeria (Figure 1). Batagarawa LGA lies approximately between latitude 12°55'N and longitude 7°37'E. It shares boundaries with Katsina LGA to the south, Rimi LGA to the north, and Charanchi LGA to the east. The area has an estimated population of over 180,000, predominantly engaged in farming and trade. The climate is characterized by a tropical savannah type, with distinct wet (May–September) and dry (October–April) seasons, and an average annual rainfall of about 850 mm. Malaria remains hyperendemic in the region, with *Anopheles* spp. serving as primary vectors that contribute significantly to the persistent malaria burden (WHO, 2023).

Larval Collection and Measurement of Physicochemical Parameters

Anopheles spp. larvae and pupae were collected in July and August 2025 from five selected localities within Batagarawa LGA: Batagarawa Town, Dabaibayawa, Barawa, Babbar Ruga, and Ajiwa villages. Each locality was surveyed for potential mosquito breeding sites, including stagnant pools, irrigation channels, and temporary rain-filled depressions. A total of thirty-one (31) well-established breeding sites were identified and sampled in the five localities: Batagarawa Town (6), Dabaibayawa (5), Barawa (6), Babbar Ruga (9), and Ajiwa villages (5). Standard dipping techniques were employed, using larval collection sets to collect larvae and pupae into labelled plastic containers.

Anopheles larval sampling was conducted between 09:00 AM and 12:00 PM, a period corresponding to minimal larval vertical movement and reduced disturbance of breeding habitats. At each breeding site, sampling lasted approximately 15–30 minutes, depending on habitat size and larval density. Sampling effort was standardised spatially by covering approximately 1 m² at each breeding site, using repeated dips distributed evenly across the habitat to ensure representative coverage.

Figure 1: **Map of Katsina state showing the study area (Batagarawa L.G.A.)**

This standardized temporal and spatial sampling approach was adopted to allow comparability of larval abundance and species composition across localities. For each breeding site, in situ physicochemical parameters of the water were measured immediately prior to larval collection to minimize disturbance-related bias. Water temperature (°C) and pH were measured using a portable digital pH/temperature meter (HI98107, Hanna Instruments, Romania), while dissolved oxygen (ppm) was measured using a portable dissolved oxygen meter (HI9147, Hanna Instruments, Romania). Measurements were taken at a depth of approximately 5–10 cm below the water surface and at three randomly selected points per breeding site, with mean values used for subsequent analyses.

Approximately 500 mL of water was collected from each breeding site in sterile, labelled polyethene bottles and transported to the laboratory in insulated containers

within 6 hours of collection. In the laboratory, electrical conductivity (µS cm^{-1}) was measured with a digital conductivity meter (HI-2300, Hanna Instruments, Romania), and turbidity (NTU) was measured with a turbidity tube (Lovibond, USA) following manufacturer-recommended protocols.

All meters were calibrated daily using standard reference solutions supplied by the manufacturers. Collected mosquito larvae were then transported to the insectary for rearing under controlled conditions.

Plant Preparation and Essential Oil Extraction

Fresh, healthy leaves of *Artemisia scoparia* were collected from naturally growing populations in Katsina State, Northwestern Nigeria. The plant samples were cleaned to remove dust and debris and were immediately transported to the Department of Biological Sciences, Umaru Musa Yar'adua University (UMYU), Katsina, for taxonomic verification. The species was confirmed at the UMYU Herbarium, where a voucher specimen was prepared and assigned the number UMYUH 492 for reference.

Essential oil (EO) extraction was carried out using hydrothermal distillation, following the protocol described by Bhallah &Vershney (2023). Approximately 500 g of fresh leaves were placed in a hydro-distillation apparatus containing distilled water. The mixture was heated, and steam carrying volatile components was condensed through a cooling system. The resulting EO was separated from the aqueous phase and dried over anhydrous sodium sulfate to remove residual moisture. The extracted oil was stored in airtight amber glass bottles at 4 °C until subsequent preparation for insecticidal bioassays.

A mixture of olive oil (as the carrier oil) and acetone (as the solvent) was used to impregnate filter papers with the essential oil at the desired concentration. All extraction and preparation procedures were conducted under aseptic conditions to preserve the oil's integrity and chemical stability.

Qualitative Phytochemical Screening

Qualitative phytochemical screening of *Artemisia scoparia* essential oil (ASEO) was conducted using standard phytochemical evaluation procedures widely adopted for medicinal plant extracts and essential oils. The analyses for terpenoids, flavonoids, tannins, saponins, alkaloids, sterols, glycosides, phenolic compounds, anthraquinones, proteins, and carbohydrates were performed according to established protocols described by Harborne (1998) and Trease and Evans (2009), with minor laboratory modifications where necessary. The intensity of phytochemical occurrence was qualitatively graded as strongly present (+++), moderately present (++), slightly present (+), or absent (−) based on observable color changes, precipitate formation, or frothing reactions during the screening tests.

Mosquito Rearing

Collected *Anopheles* larvae and pupae were transported to the insectary at UMYU, Katsina, for rearing under controlled laboratory conditions. The specimens were maintained in plastic trays containing dechlorinated tap water and kept at a temperature of 25 ± 2 °C with a relative humidity of 80 ± 10%. Larvae were fed daily with a mixture of powdered yeast and finely ground cabin biscuit, following the feeding protocol described by Leite *et al.* (2024).

Upon pupation, mosquitoes were transferred into small cups containing clean water, which were placed inside screened emergence cages. Emerged adults were maintained in the cages and provided with 10% sucrose solution on cotton pads ad libitum, as recommended by Leite *et al.* (2024). Only three-day-old, non-blood-fed adult female *Anopheles spp.* were used for the insecticidal susceptibility bioassays.

To ensure consistency and prevent contamination, all cages, trays, and feeding materials were sterilized before use. Each cohort of mosquitoes was carefully labeled by locality to preserve the geographic origin for subsequent analysis of potential locality-specific differences in susceptibility.

Insecticide Preparation

Essential oil (EO) of *Artemisia scoparia* extracted as described above was formulated for insecticidal susceptibility testing following the World Health Organization (WHO) standard operating procedures (WHO, 2022). A working solution was prepared by diluting the EO with a mixture of olive oil (carrier) and acetone (solvent) to achieve a final concentration of 0.5% (v/v) of the active ingredient (EO).

Whatman No. 1 filter papers (12 × 15 cm) were impregnated with the prepared EO solution in a fume hood to ensure uniform distribution of the active ingredient. Each impregnated paper was allowed to dry completely at room temperature and stored individually in aluminium foil at 4 °C until use. Control papers were prepared in parallel using only the carrier oil-solvent mixture (olive oil and acetone) without the essential oil.

All procedures adhered strictly to WHO guidelines to ensure reproducibility and safety. Impregnated papers were used only once and discarded after the bioassays to avoid cross-contamination and changes in potency. Proper labelling and handling protocols were followed to maintain traceability between batches of impregnated paper and the corresponding mosquito cohorts from different localities.

Bioassay Procedures

Susceptibility bioassays were conducted using the standard WHO tube test method (WHO, 2016). For each of the five study localities (Batagarawa town, Dabaibayawa, Barawa, Babbar Ruga, and Ajiwa), adult female *Anopheles spp.* aged three days, non-blood-fed, and sugar-fed were tested. For each test, four replicates of 25 mosquitoes were

exposed to *Artemisia scoparia* essential oil–impregnated filter papers, with an additional control group of equal size exposed to control papers treated only with the mixture carrier and solvent.

Mosquitoes were introduced into exposure tubes lined with impregnated paper and monitored at 10, 15, 20, 30, 40, 50, and 60 minutes to record knockdown. After 60 minutes, mosquitoes were transferred to clean holding tubes lined with untreated papers and supplied with 10% sucrose solution on cotton pads. Mortality was recorded after 24 hours of recovery.

One complete WHO susceptibility bioassay consisting of four replicates was conducted for each mosquito population collected from the five sampled localities, in an insectary room maintained at 25 ± 2 °C and 80 ± 10% relative humidity, in line with WHO recommendations. To maintain consistency, insecticide-impregnated papers were used only once, and all equipment (cages, tubes, feeders) were sterilized prior to use.

The susceptibility of mosquito populations was classified according to WHO criteria: 98–100% mortality indicating full susceptibility, 90–97% mortality suggesting possible resistance (requiring further investigation), and <90% mortality indicating confirmed resistance.

Ethical Considerations

All procedures conducted in this study complied with institutional, national, and internationally accepted guidelines for research involving mosquito vectors and laboratory-based insecticide bioassays. Ethical clearance for the study was granted by the Departmental Research Ethics Committee of the Department of Biological Sciences, Umaru Musa Yar'adua University, Katsina, Nigeria.

Prior to larval collection, verbal permission and community consent were obtained from local authorities and community representatives within each study locality. Formal ethical approval reference numbers were not applicable because the study did not involve human participants, vertebrate animals, patient samples, or clinical interventions.

Mosquito larvae were collected from natural breeding habitats and subsequently reared and tested under controlled insectary conditions to ensure biosafety and minimize environmental exposure. All insecticidal handling and bioassay procedures were performed in accordance with World Health Organization (WHO) safety recommendations using appropriate personal protective equipment (PPE) and standard laboratory safety protocols. Insecticide-contaminated materials and experimental wastes were disposed of following approved laboratory waste management procedures to prevent environmental contamination and maintain laboratory biosafety standards.

Data Collection and Analysis

Knockdown and mortality data were recorded for each replicate across all study localities. Progressive knockdown responses were documented at 10, 15, 20, 30, 40, 50, and 60 minutes post-exposure, while cumulative mortality was recorded 24 hours after exposure. Each bioassay consisted of four replicates of 25 non-blood-fed adult female *Anopheles* spp. per locality, giving a total of 100 mosquitoes tested per locality. Control groups exposed to filter papers treated only with the carrier-solvent mixture (olive oil and acetone) consistently recorded mortality below 5%; therefore, Abbott's (1952) correction formula was not applied.

The primary endpoints analyzed included:

- Percentage mortality at 24 hours post-exposure.
- Replicate-derived (per bioassay replicates) and aggregate-derived (sum of bioassay replicates) knockdown times for 50% (KDT_{50}) and 95% (KDT_{95}) of the exposed mosquito populations. Replicate-derived knockdown times were used to provide replicate estimates of KDT_{50} & KDT_{95} for statistical comparison of susceptibility outcomes between localities.

KDT_{50} and KDT_{95} values were estimated using probit regression analysis with corresponding 95% confidence intervals (95% CI). Percentage mortality was calculated using the expression:

$$\text{Percentage mortality} = \frac{\text{Total number of died mosquitoes}}{\text{Total tested}} \times 100$$

Data analyses were conducted using IBM SPSS Statistics Version 20 (IBM Corp., Armonk, NY, USA) and standard Python statistical libraries. Prior to inferential analysis, data normality and homogeneity of variance were assessed using Shapiro–Wilk and Levene's tests, respectively. One-way analysis of variance (ANOVA) followed by Tukey's Honestly Significant Difference (HSD) post-hoc test was applied for normally distributed datasets, whereas the Kruskal–Wallis test followed by Dunn's post-hoc multiple comparison test was used for non-parametric data where assumptions of normality were violated.

Descriptive statistics, including means, standard deviations (SD), ranges, and percentage absolute mean deviation (Abdullahi, 2024), were used to summarise the physicochemical and replicate-derived knockdown estimates. Percentage absolute mean deviation is utilized here as a more robust estimator of statistical dispersion that is scale-invariant (i.e., invariant to both location-shift and scaling of data), making it reliable for comparing dispersion estimates across disparately different groups without relying solely on the size or unit of the mean estimate, as is common with the standard deviation. All statistical analyses were performed at a 95% confidence level, with significance established at $p < 0.05$.

Although WHO susceptibility criteria are primarily established for conventional insecticides, they were used here as a comparative interpretive framework for evaluating relative adulticidal performance. The resistance

status of mosquito populations was interpreted according to World Health Organization (WHO) susceptibility criteria as follows:

- 98–100% mortality: fully susceptible,
- 90–97% mortality: possible resistance requiring confirmation,
- <90% mortality: confirmed resistance.

RESULTS

Physicochemical Characteristics of the Breeding Sites

The physicochemical properties of *Anopheles* mosquito breeding sites across the five study localities in Batagarawa LGA are presented in Table 1. Across all sites, breeding habitats were predominantly temporary to semi-permanent water bodies, including street-side ditches and shallow ponds, largely originating from rainfall. Most sites exhibited earthy to greenish coloration, an earthy or grassy odor, and varied exposure to sunlight (shaded, partially shaded, or full sunlight). Vegetation types were mixed, with floating, submerged, and emergent plants present in all localities.

Among the measured parameters, pH varied significantly among localities ($p = 0.0128$), with Barawa (8.48 ± 0.41) showing slightly more alkaline conditions than the others, while Babbar Ruga (7.24 ± 0.67) and Ajiwa (7.42 ± 0.41) were relatively closer to neutral. Temperature also differed significantly ($p = 0.0067$), with Dabaibayawa (29.45 ± 0.71 °C) and Ajiwa (29.02 ± 0.93 °C) recording slightly higher temperatures than the other localities.

Other parameters, including dissolved oxygen (DO), conductivity, turbidity, and water depth, did not differ significantly across localities ($p > 0.05$). Dissolved oxygen ranged from 5.88 ± 1.70 ppm (Ajiwa) to 8.00 ± 1.36 ppm (Batagarawa). Conductivity values showed wide variability, but no statistically meaningful pattern was observed among sites ($p = 0.9540$). Turbidity and water depth likewise showed broad overlap between localities, reflecting similar habitat types.

Overall, the breeding sites across Batagarawa LGA exhibited similar ecological characteristics, with only pH and temperature showing statistically significant spatial variation. These findings suggest broadly consistent larval habitat conditions across the sampled areas, suitable for *Anopheles* mosquito development.

Phytochemical Screening of Artemisia scoparia Essential Oil

The qualitative phytochemical screening of *Artemisia scoparia* essential oil (ASEO) in Table 2 revealed the strong presence of terpenoids and monoterpenes (+++), indicating that these compounds constitute the dominant phytochemical groups in the oil. Phenolic compounds and flavonoids were moderately present (++), while tannins, saponins, alkaloids, sterols, and glycosides were detected in trace to low amounts (+). Anthraquinones, proteins, and carbohydrates were absent (−) in the essential oil sample analyzed.

Adulticidal Efficacy of *Artemisia scoparia* Essential Oil Against *Anopheles* spp.

The knockdown and mortality responses of *Anopheles* spp. exposed to *Artemisia scoparia* essential oil (ASEO) at 0.50% are summarized in Table 3. The median knockdown Time (KDT_{50}) varied among mosquito populations from the five localities, ranging from 33.08 min (95% CI: 29.56–37.02) in Barawa to 59.15 min (95% CI: 48.58–72.01) in Babbar Ruga. Statistical analysis based on replicate-derived KDT estimates revealed significant differences in KDT_{50} among some localities ($p = 0.0271$).

The KDT_{95} values ranged from 171.29 min (95% CI: 128.25–228.77) in Ajiwa to 521.52 min (95% CI: 254.30–1069.52) in Batagarawa. Although these differences were numerically large, they were not statistically significant ($p = 0.1382$). The unusually high KDT_{95} estimates likely reflect statistical extrapolation associated with incomplete mortality and weak late-phase knockdown responses at the tested concentration.

The 24-hour post-exposure mortality percentages were generally low, ranging from 4.00 ± 2.83% in Batagarawa to 16.00 ± 4.90% in Dabaibayawa. No statistically significant differences in mortality were observed among localities ($p = 0.2167$). Based on the WHO comparative susceptibility criteria applied in this study, the mortality rates observed (<90%) indicate confirmed resistance or tolerance to ASEO at the tested concentration across all mosquito populations examined.

These findings suggest that although ASEO exhibited measurable knockdown activity at 0.50%, it did not achieve lethal effects sufficient for effective adulticidal control of the tested *Anopheles* populations.

Locality-Based Susceptibility Classification

Using WHO insecticide susceptibility criteria (WHO, 2016), mosquito populations were classified based on their 24-hour mortality rates following exposure to *Artemisia scoparia* essential oil (ASEO) at 0.50%. Mortality rates below 90% indicate confirmed resistance, 90–97% suggest possible resistance (requiring confirmation), and ≥98% indicate full susceptibility.

Across all five localities, the mortality rates recorded ranged from **4%** (Batagarawa) to **16%** (Dabaibayawa) (Table 3). These values are well below the WHO susceptibility threshold, confirming that all sampled mosquito populations exhibit resistance to ASEO at the tested concentration.

Table 1. Physicochemical characteristics of *Anopheles* mosquito breeding sites across five localities in Batagarawa LGA, Katsina State, Nigeria.

Parameter	Batagarawa	Dabaibayawa	Barawa	Babbar Ruga	Ajiwa	P-value
Temperature (℃)	27.24 ± 0.57 (22.76)^{b}	29.45 ± 0.71 (11.76)^{a}	27.84 ± 0.37 (8.49)^{ab}	27.61 ± 0.65 (23.81)^{ab}	29.02 ± 0.93 (16.64)^{ab}	0.0067
pH	7.5 ± 0.41 (13.47)^{ab}	7.52 ± 0.38 (15.11)^{ab}	8.48 ± 0.41 (16.63)^{a}	7.24 ± 0.67 (16.39)^{b}	7.42 ± 0.41 (16.56)^{ab}	0.0128
DO (ppm)	8.0 ± 1.36 (14.99)	5.92 ± 0.9 (18.4)	6.94 ± 0.93 (17.83)	6.43 ± 1.13 (16.04)	5.88 ± 1.7 (13.64)	0.1398
Conductivity (µS cm^{-1})	202.8 ± 119.64 (15.16)	201.2 ± 47.56 (6.39)	208.6 ± 67.2 (19.87)	272.88 ± 207.78 (15.53)	235.5 ± 94.51 (13.43)	0.954
Turbidity (NTU)	75.0 ± 23.02 (7.86)	70.0 ± 25.0 (17.14)	82.0 ± 18.87 (14.06)	70.62 ± 29.41 (14.25)	47.25 ± 19.11 (12.4)	0.4293
Water depth (meters)	0.14 ± 0.05 (15.67)	0.13 ± 0.11 (14.83)	0.65 ± 0.85 (15.35)	0.26 ± 0.17 (13.3)	0.27 ± 0.04 (17.89)	0.2002
Water origin	Rainfall	Rainfall	Rainfall	Rainfall	Rainfall	
Water odor	Grassy, Earthy	Grassy, Earthy	Grassy, Earthy	Grassy, Earthy	Grassy, Earthy	
Water color	Greenish, Earthy	Greenish, Earthy	Greenish, Earthy	Greenish, Earthy	Greenish, Earthy	
Water permanence	Semi-permanent, Temporary	Semi-permanent, Temporary	Semi-permanent, Temporary	Semi-permanent, Temporary	Semi-permanent, Temporary	
Exposure to sunlight	Sunlight, Shaded, Partially Shaded	Sunlight, Shaded, Partially Shaded	Sunlight, Shaded, Partially Shaded	Sunlight, Shaded, Partially Shaded	Sunlight, Shaded, Partially Shaded	
Vegetation presence	Submerged, Floating, Emergent	Submerged, Floating, Emergent	Submerged, Floating, Emergent	Submerged, Floating, Emergent	Submerged, Floating, Emergent	
Breeding site nature	Street-side Ditches, Shallow Pond	Street-side Ditches, Shallow Pond	Street-side Ditches, Shallow Pond	Street-side Ditches, Shallow Pond	Street-side Ditches, Shallow Pond	

Values represent mean ± standard deviation (% absolute meanic deviation shown in brackets). Means sharing the same superscript letter(s) in a row are not significantly different ($p > 0.05$). The temperature and turbidity datasets passed the normality test; therefore, ANOVA followed by Tukey' s HSD post-hoc test was applied. Other parameters failed the normality test; therefore, the Kruskal–Wallis test followed by Dunn' s post hoc multiple-comparison test was used. A total of 31 breeding sites distributed across five localities were included in the analyses.

Table 2: Qualitative Phytochemical Constituents of *Artemisia scoparia* Essential Oil (ASEO).

Phytochemical Constituents	Presence Level	Phytochemical Constituents	Presence Level
Terpenoids	+++	Alkaloids	+
Monoterpenes	+++	Sterols	+
Phenolic Compounds	++	Glycosides	+
Flavonoids	++	Anthraquinones	−
Tannins	+	Proteins	−
Saponins	+	Carbohydrates	−

Key: +++ = strongly present/highly abundant; ++ = moderately present; + = slightly present/trace amount; − = absent or not detected consistently.

Table 3. Knockdown time and mortality of *Anopheles* spp. exposed to *Artemisia scoparia* essential oil (ASEO, 0.50%) across five localities in Batagarawa LGA, Katsina State, Nigeria. Replicate-derived knockdown estimates are presented descriptively as mean ± standard deviation.

Locality	*Knock Down Time [95% Confidence Interval]*, Mean Knock Down Time ± STD (SAMD)		
	KDT_{50} (min)	**KDT_{95} (min)**	**Mortality (%)**
Batagarawa	*47.97 [95% CI: 39.82 - 57.79],* 49.28 ± 4.88 (14.87)^{ab}	*521.52 [95% CI: 254.3 - 1069.52],* 808.04 ± 645.6 (15.74)^{a}	4.00 ± 2.83 (22.22)^{a}
Dabaibayawa	*35.84 [95% CI: 31.27 - 41.08],* 36.52 ± 3.57 (7.65)^{b}	*310.03 [95% CI: 181.69 - 529.02],* 554.96 ± 553.17 (13.21)^{a}	16.00 ± 4.90 (17.14)^{a}
Barawa	*33.08 [95% CI: 29.56 - 37.02],* 36.93 ± 12.65 (13.44)^{b}	*206.15 [95% CI: 139.77 - 304.05],* 281.88 ± 185.78 (17.97)^{a}	11.00 ± 8.19 (14.88)^{a}
Babbar Ruga	*59.15 [95% CI: 48.58 - 72.01],* 62.68 ± 13.12 (16.05)^{a}	*472.27 [95% CI: 249.06 - 895.54],* 672.11 ± 428.17 (10.43)^{a}	10.00 ± 7.21 (15.38)^{a}
Ajiwa	*44.49 [95% CI: 40.24 - 49.19],* 46.24 ± 9.84 (13.05)^{ab}	*171.29 [95% CI: 128.25 - 228.77],* 187.76 ± 72.17 (7.74)^{a}	7.00 ± 5.92 (16.09)^{a}
Estimator	ANOVA-T [F(4,15), p= 0.0271]	KW-D [H(4), p = 0.1382]	ANOVA-T [F(4,15), p = 0.2167]

Keys: KDT_{50} = knockdown time for 50% of mosquitoes; KDT_{95} = knockdown time for 95% of mosquitoes; STD = Standard Deviation; SAMD = Statistical Absolute Meanic Deviation (Abdullahi, 2024); ANOVA-T = ANOVA and Tukey's HSD Comparison; KW-D = Kruskal-Wallis test and Dunn's Post-Hoc tests.

Note: KDT_{50} and KDT_{95} values were estimated from pooled replicate knockdown data using probit regression analysis. Replicate-derived knockdown estimates are additionally presented descriptively as mean ± standard deviation for comparative statistical analysis among localities.

Pearson Chi-square goodness-of-fit for the KDT_{50} and KDT_{95} [Minimum: χ^2 = 4.9635, df = 5, p = 0.4203; Maximum: χ^2 = 1.2781, df = 5, p = 0.9372]. Means sharing the same superscript letter(s) in a row are not significantly different ($p > 0.05$). For each locality, it was 5 localities × 4 replicates = 20 observations.

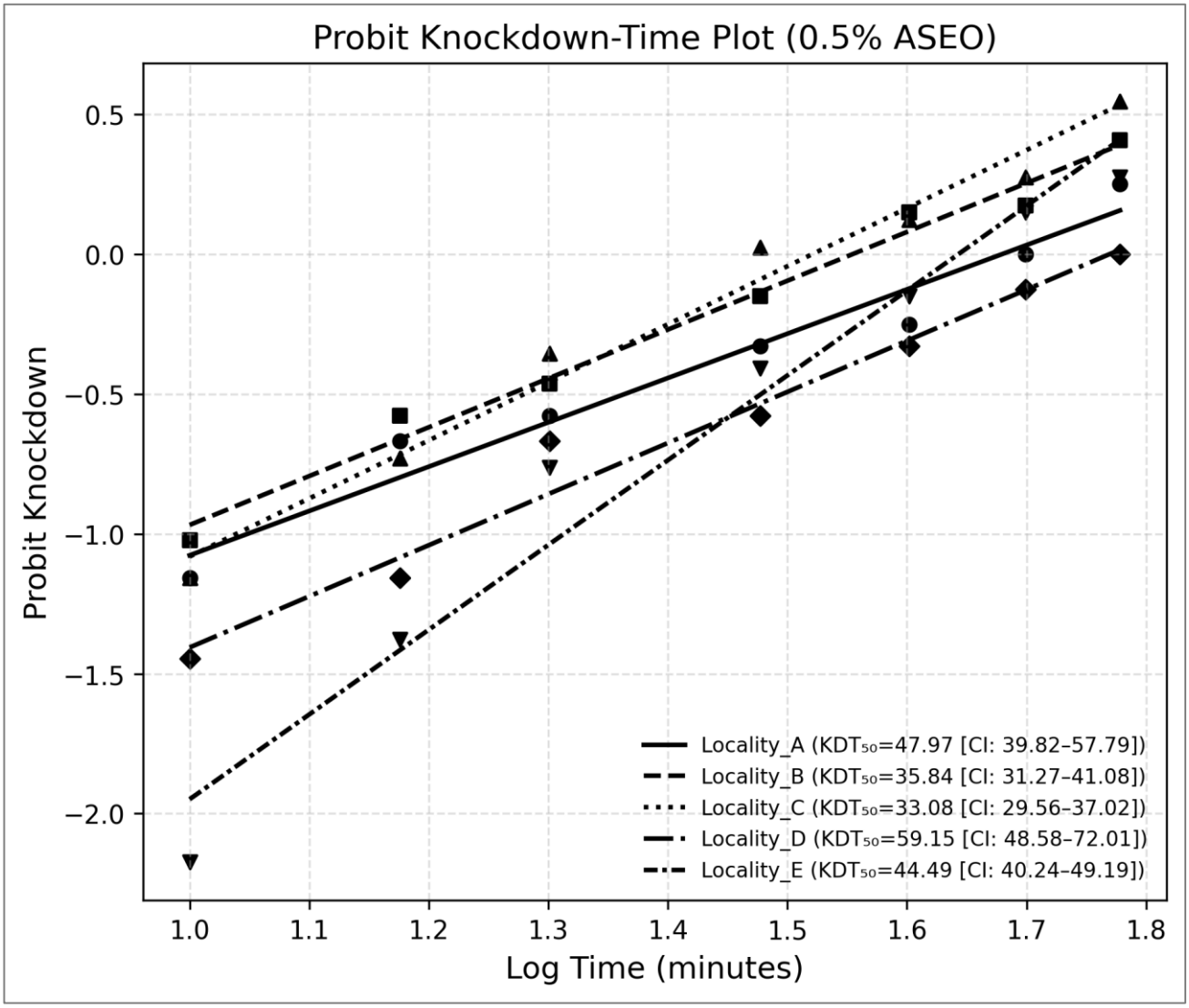

Figure 1: Probit regression of knockdown responses of adult mosquitoes exposed to 0.50% (v/v) of Artemisia scoparia essential oil (ASEO). Knockdown proportions were transformed to probit units and plotted against $\log_{10}$ *T*ime (minutes). Each line represents the fitted regression for a specific locality. Reported KDT_{50} values (with 95% confidence intervals) were derived from probit analysis and are indicated in the legend.

Although variation in knockdown time (KDT_{50} and KDT_{95}) was observed, it did not translate into sufficient mortality to meet the WHO-defined susceptibility levels. Localities with relatively faster knockdown responses (e.g., Barawa: KDT_{50} = 33.08 min, 95% CI: 29.56–37.02) still exhibited low mortality (11%), indicating that while ASEO may induce transient incapacitation, it does not provide effective adulticidal control at the tested concentration and formulation.

These results highlight **heterogeneous physiological responses** among *Anopheles* populations in Batagarawa LGA but confirm a general trend of **insecticide resistance** to ASEO under the experimental conditions applied.

DISCUSSION

Physicochemical Habitat Characteristics and Local Variability in *Anopheles* Larval Ecology

The analysis of larval breeding habitats across five localities in Batagarawa LGA revealed broadly similar ecological profiles, with pH and temperature standing out as the only significantly variable parameters. Most breeding sites were temporary to semi-permanent, rain-fed water bodies such as roadside ditches and shallow ponds. These habitats typically exhibited earthy to greenish coloration, grassy or earthy odors, and variable sunlight exposure (ranging from shaded to fully exposed). Aquatic vegetation was consistently present, including floating, emergent, and submerged plants, indicating structurally diverse larval environments.

Significant differences were observed in pH ($p = 0.0128$), with Barawa exhibiting slightly alkaline water, whereas Babbar Ruga and Ajiwa were relatively closer to neutral conditions. Water temperature also varied significantly ($p = 0.0067$), with higher averages observed in Dabaibayawa and Ajiwa. In contrast, dissolved oxygen, conductivity, turbidity, and depth showed no significant inter-locality differences ($p > 0.05$). Dissolved oxygen ranged from 5.88 ± 1.70 ppm (Ajiwa) to 8.00 ± 1.36 ppm (Batagarawa), while other parameters displayed overlapping values across localities.

Even modest ecological differences may carry biological significance. Small shifts in pH can alter microbial communities, nutrient cycling, and chemical speciation (e.g., unionized ammonia levels), thereby influencing larval feeding efficiency, survival, and stress physiology (Multini *et al.*, 2021). The slightly alkaline conditions in Barawa could affect microbial availability or impose physiological challenges on larvae, with downstream consequences for adult phenotype and insecticide response.

Temperature is another key determinant of larval development and adult traits. Warmer habitats (Dabaibayawa and Ajiwa) may accelerate development but often yield smaller adults with altered metabolic capacities, including increased detoxification enzyme activity (Rueda *et al.*, 1990; Bayoh & Lindsay, 2003). Elevated rearing temperatures have been shown to modulate pyrethroid resistance by upregulating detoxification genes, potentially reducing insecticidal efficacy (Oliver & Brooke, 2017).

Although dissolved oxygen and vegetation did not differ significantly across sites, both remain ecologically important. *Anopheles* larvae are more dependent on dissolved oxygen than *Culicines*, and low oxygen conditions can alter microbial composition and stress responses (Service, 1993). Vegetation influences food availability, shading, and microclimate, while decaying organic matter fosters microbial growth that may interact with larval detoxification systems (Evans *et al.*, 2019). In agro-ecological contexts, inputs such as fertilizer runoff or pesticide residues may further modify larval environments and select for enhanced detoxification mechanisms that promote cross-resistance in adults (Oliver & Brooke, 2018; Nkya *et al.*, 2013). Given Batagarawa's reliance on farming and trading, such anthropogenic influences on larval habitats cannot be discounted.

Overall, although most physicochemical parameters were stable across sites, the observed variability in pH and temperature may contribute to local differences in larval ecology and adult physiological responses, although the present study was not designed to establish direct causal relationships. These findings underscore the need to integrate environmental monitoring with entomological surveillance to capture locality-specific drivers of insecticide resistance.

Phytochemicals of Artemisia scoparia Essential Oil

The phytochemical screening of *Artemisia scoparia* essential oil (ASEO) revealed a predominance of terpenoids and monoterpenes, with moderate levels of phenolic compounds and flavonoids. This finding agrees with previous phytochemical studies on medicinal plants and essential oils, which identified terpenoids and phenolics as major bioactive constituents responsible for several biological activities (Harborne, 1998). Similar reports from Nigeria by Sofowora indicated that flavonoids, tannins, alkaloids, and saponins are common secondary metabolites in medicinal plants with therapeutic and pesticidal importance (Sofowora, 2008).

The presence of terpenoids and phenolic compounds in high to moderate amounts may explain the potential insecticidal activity of ASEO, as these phytochemicals are known to possess toxic, repellent, and growth-inhibitory effects against insect pests. The observed phytochemical profile is also comparable to the findings of Trease and Evans (2009), who reported that essential oils are typically rich in volatile terpenoid compounds with pharmacological and bioactive properties (Trease & Evans, 2009).

Adulticidal Activity of *Artemisia scoparia* Essential Oil and Locality-Based Resistance Implications

Adult *Anopheles* populations from the five localities in Batagarawa LGA displayed considerable variation in knockdown speed but consistently low 24-hour mortality following exposure to *A. scoparia* essential oil (ASEO) at 0.50%. Probit-derived knockdown times (KDT_{50}) ranged from 33.08 min (95% CI: 29.56–37.02) in Barawa to 59.15 min (95% CI: 48.58–72.01) in Babbar Ruga (Figure 2), indicating that some populations were more rapidly incapacitated than others. In contrast, mortality rates remained low across all sites (4–16%), below the WHO comparative susceptibility threshold applied in this study, confirming resistance or tolerance irrespective of locality.

The differences in knockdown timing may reflect subtle physiological or behavioural heterogeneity associated with larval habitat characteristics. For example, Barawa, with a slightly alkaline pH, produced mosquitoes that knocked down faster than those from more neutral habitats. This observation aligns with prior reports suggesting that water chemistry during larval development can influence adult cuticle permeability and neurophysiological responses to insecticidal agents (Owusu & Müller, 2017). Conversely, Babbar Ruga, under cooler, more neutral conditions, yielded mosquitoes that were slower to succumb to knockdown, suggesting possible differences in detoxification enzyme activity or nervous system sensitivity. However, these physicochemical associations should be interpreted cautiously because the present study was not specifically designed to establish causal ecological mechanisms underlying adult susceptibility variation.

The observed knockdown activity of ASEO may be associated with the phytochemical constituents detected during the qualitative screening of the essential oil. The presence of terpenoids, phenolic compounds, flavonoids, alkaloids, and other bioactive secondary metabolites in the oil suggests potential neurotoxic and physiological effects against mosquitoes. Terpenoids and phenolic compounds, in particular, are widely recognised for their insecticidal and fumigant properties, including interference with neurotransmission, respiratory inhibition, membrane disruption, and behavioural repellency. These compounds may therefore explain the measurable knockdown responses observed across the mosquito populations despite the relatively low overall mortality.

However, the uniformly low mortality across all localities suggests that local physicochemical variations alone do not overcome inherent tolerance mechanisms to ASEO.

Instead, this pattern likely reflects widespread metabolic detoxification, behavioural avoidance, reduced penetration, or volatilisation or degradation of the essential oil, which may allow mosquitoes to recover after transient neurotoxic effects (Norris et al., 2018; 2015). It is also possible that the concentration of active phytochemicals in the 0.50% formulation was insufficient to maintain prolonged toxic action required for irreversible mortality. Similar findings have been reported elsewhere, where plant-derived essential oils induced high knockdown rates within the first hour of exposure but poor sustained mortality after 24 hours (Norris et al., 2018).

Additionally, repeated environmental exposure of mosquito populations to agricultural chemicals and other xenobiotics within endemic communities may contribute to cross-resistance or enhanced detoxification capacity against plant-derived compounds. Such adaptive responses could reduce susceptibility even when bioactive phytochemicals with recognized insecticidal properties are present within the essential oil formulation.

These results emphasize that, while ASEO possesses detectable adulticidal and neurotoxic potential, as evidenced by knockdown responses and its phytochemical composition, it lacks sufficient potency as a stand-alone adulticide under the conditions tested. The observed variation in knockdown suggests local physiological diversity, but the overarching resistance profile points to the need for integrated vector management approaches, possibly combining ASEO with other control measures or using it in alternative roles such as repellents, larvicides, or synergistic botanical formulations.

Broader Implications for Vector Control: Locality Variations and Operational Significance

The variation in knockdown speed across Batagarawa's mosquito populations, when viewed alongside relatively uniform mortality outcomes, provides operational insight for vector control planning. Although the physicochemical characteristics of breeding habitats were broadly similar, the modest differences in pH and temperature may contribute to micro-geographical variation in mosquito susceptibility phenotypes. In practical terms, some localities may produce adult mosquitoes that respond quickly to neuroactive botanical compounds, while others may generate populations that are less behaviorally or physiologically affected**.**

However, the lack of mortality above the WHO-defined susceptibility threshold across all localities underscores that *Artemisia scoparia* essential oil (ASEO), at the tested concentration and formulation, cannot be relied upon for adult mosquito population suppression. Instead, its role may be better suited to complementary or preventive **measures** rather than as a primary killing agent. This is consistent with the essential oil literature, which often highlights their value as repellents**,** larvicides**,** or **synergists** rather than stand-alone adulticides (Negahban *et al.*, 2006; Parveen *et al.*, 2024; Rants'o *et al.*, 2022). For example, areas producing adult mosquitoes with relatively rapid knockdown responses (e.g., Barawa: KDT_{50} = 33.08 min) could benefit from strategies that exploit short-term behavioural incapacitation, such as attractive toxic sugar baits (ATSBs) or contact-irritant push–pull systems.

Ultimately, these findings reinforce the need for localized, evidence-based decision-making in vector control programs. While the ecological similarities among breeding habitats suggest a broadly uniform risk landscape, the observed differences in physiological responses highlight the importance of continuous resistance monitoring and tailored interventions that reflect not only insecticide class but also local mosquito biology shaped by environmental context**.**

Study Limitations

However, this study has some limitations. The chemical composition and yield profile of *Artemisia scoparia* essential oil were not characterised by GC-MS; therefore, the specific bioactive constituents responsible for the observed insecticidal responses could not be identified. In addition, mosquito populations were identified morphologically to the genus level using standard larval identification characteristics, without molecular characterisation to the species-complex level. These limitations may influence interpretation of locality-specific susceptibility variation and should be addressed in future studies. In addition, only a single essential oil concentration and formulation were evaluated, which may limit broader interpretation of concentration-dependent adulticidal efficacy.

CONCLUSION

This study assessed the adulticidal efficacy of *Artemisia scoparia* essential oil (ASEO) against *Anopheles* spp. collected from five localities in Batagarawa LGA, Katsina State, in the context of their larval habitat physicochemical characteristics. We observed subtle but significant differences in pH and temperature across breeding sites, and these were associated with variation in knockdown speed among mosquito populations. However, mortality remained uniformly low across all sites, well below the WHO comparative susceptibility thresholds used in this study. Our null hypothesis (H_0), which stated that no significant differences exist between localities in the resistance/susceptibility of *Anopheles* spp. exposed to ASEO, is only partially supported: there were differences in physiological response speed (knockdown) but not in final mortality classification. Overall, the findings support further optimization of *A. scoparia* essential oil formulations as complementary components within environmentally sustainable integrated vector management programmes.

Further research should evaluate multiple concentrations and formulations of ASEO, explore its role in larval control and repellency, and investigate synergistic formulations, while incorporating molecular and biochemical analyses to clarify how local environmental

and genetic factors interact to shape mosquito susceptibility.

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The authors declare that they have no known competing financial interests or personal relationships that could have influenced the work reported in this paper.

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