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Laboratory and simulated semi-field larvicidal efficacy of *Aframomum angustifolium* (*Sonn.) K. Schum* and *Tagetes patula* essential oils against *Anopheles gambiae*

Adelina Thomas [a,e,*], Eliningaya J. Kweka [b,c], Patrick Engeu Ogwang [d,e]

[a] *Department of Medicinal Chemistry & Pharmacognosy, Catholic University of Health and Allied Sciences, Mwanza, Tanzania*
[b] *Pesticides Bioefficacy Section, Tanzania Plant Health and Pesticides Authority, P.O. Box 3024, Arusha, Tanzania*
[c] *Department of Medical Parasitology and Entomology, Catholic University of Health and Allied Sciences, Mwanza, Tanzania*
[d] *Pharm-Biotechnology and Traditional Medicine Centre of Excellence (ACEII), P.O BOX 1410, Mbarara, Uganda*
[e] *Department of Pharmacy, Faculty of Medicine, Mbarara University of Science and Technology, Uganda*

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ABSTRACT

Introduction: Malaria is a vector-borne, important public health problem spread by mosquitoes in tropical regions. The use of synthetic insecticides for mosquito control has been linked to insecticide resistance and environmental consequences. Thus, there is an urgent need to search for new highly selective and biodegradable insecticides lacking long-term toxicity to humans and mammals which are botanical-based compounds from indigenous plant sources available in local communities. Therefore, this study focused on evaluating the larvicidal activity of *Aframomum angustifolium* and *Tagetes patula* essential oils against *Anopheles gambiae* larvae in laboratory and simulated fields.

Methods: The Eos was extracted by the hydro distillation method, and the chemical composition of the Eos was determined using gas chromatography-mass spectrometry (GC-MS). The WHO protocol was followed for the laboratory and the simulated bioassays. Both laboratory-reared and field-collected larvae, as well as non-targeted organisms (*Gambusia affinis),* were used in simulated field conditions. The collected data were analyzed using probit analysis, and their means were compared in one-way analysis of variance (ANOVA) using IBM SPSS Statistic version 26.

Results: The main active ingredients in *T. patula* Eo were terpinolene (20.75 %) and (*Z*)-ocimene (17.10 %) and *A. angustifolium* were cis-pinen-3-ol (58.48 %) β-pinene (31.03 %). The EO of *T. patula* was the most efficacious in the bioassay. The larvicidal bioassay findings demonstrated that the mortality rate was dose- and exposure-dependent. In the laboratory, the EOs of *A. angustifolium* and *T. patula* have larvicidal activity against *An. gambiae* larvae, with LC50 values of 1.71 and 0.71 ppm after 48 h, respectively. Both binary combinations of the EOs showed synergistic interactions at 24 h but antagonistic interactions at 48 h. In the simulated field trial using laboratory-reared larvae, the mortality rate was higher in the simulated setting compared with the laboratory setting for all the tested concentrations for both EOs. Only *A. angustifolium* EO was toxic against *Gambusia affins* at 100 ppm.

Conclusion: The findings of this study have shown that *T. patula* and *A. angustifolium* oils have good larvicidal activities for *An. gambiae*. The two plants are potential sources of larvicidal compounds that could be used to control the malaria vector.

1. Introduction

Mosquitoes are one of the most serious public and veterinary health concerns around the world (Khalighifar et al., 2022). They carry and spread diseases to humans, causing thousands of deaths each year (WHO, 2022). There has been a rapid decline in malaria cases and mosquito populations brought in by indoor residual spray and long-lasting insecticidal net usage (WHO, 2022). Mosquito larval control has a track record of reducing malaria transmission and even eradicating malaria-transmitting mosquitoes (Mutero et al., 2020). Combining

* Corresponding author at: Department of Medicinal Chemistry & Pharmacognosy, Catholic University of Health and Allied Sciences, Mwanza, Tanzania.
E-mail address: adelinathomas45@gmail.com (A. Thomas).

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larval source reduction with adult mosquito control interventions such as long-lasting insecticidal nets has been deemed a highly effective malaria control strategy (Kahamba et al., 2022) The emergence and spread of resistance genes among mosquito species populations to major insecticide classes such as pyrethroids, carbamates, and organophosphates, as well as insecticidal effects on non-targeted organisms and environmental pollution, pose a challenge to malaria vector control using synthetic insecticides (Rezende-Teixeira et al., 2022; Velayudhan et al., 2021). As a result, targeting mosquitoes at the larval stage is the best option because larvae are largely immobile and limited within a geographical area, can't change their behaviour to avoid insecticide effects, and are thus easier to target than adult mosquitoes ((Derua et al., 2018). There is now a focus on shifting to the use of natural or botanical larvicides, which kill mosquito larvae selectively while having little effect on non-target organisms and the environment (Macho, 2021) Plant essential oils are rich sources of active and complex combinations of bioactive chemicals with various mechanisms of action, which may reduce the likelihood of resistance in mosquito populations (da Silva Sá et al., 2023).

Aframomum angustifolium (Sonn.) K. Schum Family (Zingiberaceae) is a perennial herb that grows in moist, shady areas with highly pungent and aromatic seeds in East Africa, the fruits are eaten raw as snacks (Van et al., 2021; Nyakoojo and Tugume, 2020).

Tagetes patula L. (Asteraceae), commonly recognized as French marigold or dwarf marigold, is an annual plant native to North America and widely acclimatized worldwide (Riaz et al., 2020).

Aframomum angustifolium and *Tagetes patula* essential oils' pharmacological potential or medicinal benefits have been well-reported in the literature (Nakayima, 2021; MAKAMWE et al., 2023; Politi et al., 2021; Sadaf et al., 2023). Previous research has extensively explored the diverse activities associated with the essential oils of *Aframomum angustifolium* and *Tagetes patula* (SEED, A.E.O.A.A., 2023; Upoki Anywar and Kirimuhuzya, 2015; Safar et al., 2020a; Villada-Ramos et al., 2021). However, a critical gap exists in the literature regarding the larvicidal potential of these essential oils specifically against the larvae of *Anopheles gambiae*, especially when combined, Additionally, there is a paucity of studies addressing the impact of these essential oil blends on non-targeted organisms, such as mosquito fish *(Gambusia affins*). This dual-pronged approach to examining the larvicidal efficacy and potential ecological impact of these essential oil blends constitutes a novel contribution to the field. By presenting a comprehensive evaluation encompassing both targeted pest control and non-targeted organism safety, this research unveils a fresh perspective on the utilization of essential oils for effective and environmentally conscious mosquito management. Therefore, this study aims to address these gaps by systematically examining the larvicidal activity of *Aframomum angustifolium* and *Tagetes patula* essential oil blends against *Anopheles gambiae* larvae and assessing their potential toxicity to mosquito fish.

2. Materials and methods

2.1. Study design

The research used an experimental research design with only a post-test assessment.

2.2. : Plant sample collection

Fresh plant leaves of *Aframomum angustifolium* were collected from Rukararwe Gardens, a Non-Government organisation for Rural Development in Sheema district (0° 31' 26.9 S, 30° 13.2" E), in the Western Region, Uganda. *Tagetes patula* leaves were collected from Kakoba division (Latitude: 0° 35' 59" S; Longitude: 30° 40' 44" E) in Mbarara District, Western Region, Uganda. All plants were brought to Mbarara University of Science and Technology's analytical laboratory for the extraction of essential oils. Herbarium specimens were collected and identified by Wanyana Olivia, a botanist at Makerere University. Voucher specimen numbers AT-001 for *Aframomun angustifolium* and AT-002 for *Tagetes patula* were provided, and the specimens were deposited at the national herbarium.

The *Anopheles gambiae* larvae and *Gambusia affins* were identified by Eliningaya Kweka, an entomologist from the Tanzania Plant Health and Pesticides Authority (TPHPA), using identification keys established by Coetzee and others (Gilles and Coetzee, 1987). The identification number was assigned as AN-001 for laboratory-reared larvae and AN-002 for field-collected larvae of *An. gambiae* and *G. affinis* were labeled GA-001.

2.3. Study population

The study subjects are the third instar larvae of *An. gambiae* and *Gambusia affins* obtained from the (TPHPA), Insectary unit. Field-collected mosquito larvae were obtained at Mabogini Village, Moshi District, Kilimanjaro Region, Tanzania. and were transported to TPRI for experiments. This collection site is located between latitude 03° 21′ S and longitude 37° 20′ E, at 750 m above sea level and covering 1600 ha. The area is well-known for human activity, particularly rice irrigation, which contributes to mosquito breeding grounds.

2.4. Sample sizes

A total of 9000 laboratory-reared larvae were needed for the laboratory assay(25 larvae *x* 6 (5 concentrations+1 control) X4 replicates for 3 experiments) = 1800 for *A. angustifolium* oil + 1800 larvae for *T.patula* oil + 5400 larvae for blends ((25 larvae X 6 (5 replicates+1 control) X 3 experiments = 1800), thus 1800 × 3 for (1:1, 1:3, and 3:1).).

Then, a total of **4800** larvae were required for the simulated semi-field bioassay (2400 laboratory reared-larvae and 2400 field-collected larvae) (25 larvae X 4 (3 concentrations plus 1 control) X 4 replicates for 3 experiments) = 1200 for each essential oil.

A total of **36** *Gambusia affins* (mosquito fish) for toxicity of non-target organisms, 1 mosquito fish per bowl x 4 bowls (3 +1 control) x 3 treatment doses x 3 experiments).

2.5. Extraction of essential oils

In this study, essential oils from *Aframomum angustifolium* and *Tagetes patula* were extracted at the Mbarara University of Science and Technology Analytical Laboratory in Uganda. The decision to conduct extraction at this facility was influenced by the convenient accessibility of both plant species in Uganda, the presence of suitable extraction equipment, and the expertise of the supervisor in essential oil extraction. Subsequent phases of the research, including larvicidal and toxicity experiments, as well as chemical analysis of the essential oils, took place at the Division of Livestock and Human Disease Vectors Control within the Tanzania Plant Health and Pesticide Authority (TPHPA) in Arusha, Tanzania. The choice of this facility was driven by its specialized focus on Livestock and Human Disease vector control, providing an ideal setting for conducting in-depth experiments related to larvicidal activity, toxicity assessment, and comprehensive chemical composition analysis of the essential oils.

The 100 g of fresh plant material, the leaves of all test plants, were mixed with 500 mL of distilled water and then hydro-distilled using a Clevenger apparatus with continuous extraction for 2 h. The yielded essential oil was separated from water using a separating funnel, dried over anhydrous sodium sulphate, and stored at 4 °C in closed, airtight amber bottles wrapped with aluminium foil. The essential oil yield was calculated (Tahghighi et al., 2019) After extraction, the essential oils were stored in dark glass bottles with airtight caps, using solid caps instead of rubber bulb-capped lids. These dark glasses help reduce the risk of oxidation or rancidity. The essential oils in glass amber bottles were stored in a refrigerator at 5 to 10° C for optimal oil storage at the

Table 1
Dilution of essential oils.

Final concentration (ppm)	Final volume (mL)	Stock solution (ppm)	Initial volume (mL)	Tap water volume (mL)
10	100	10,000	0.2	99.8
25	100	10,000	0.5	99.5
50	100	10,000	1	98
75	100	10,000	1.5	98.5
100	100	10,000	2	98

Mbarara University of Science and Technology analytical laboratory before transportation to Tanzania in an ice box.

2.6. Gas-chromatography–mass spectrometry(GC–MS) analysis of the essential oils

The GC-MS analysis was done at the Tanzania Plant Health and Pesticides Authority's (TPHPA) chemistry analysis laboratory. The GC-MS data was collected using a GCMS-QP 2010 Ultra (Shimadzu instrument) in Electron Ionisation (EI) mode (MS) at 70 eV and a Flame Ionisation Detector (Tilley et al.) for GC. A Restek-5MS column (30 m x 0.25 mm×0.25 μm) was used for the separation of Eos components. One (1) uL of the sample in 1 mL of dichloromethane was injected into the GC-MS by an autosampler. The oven temperature programme was 55 °C to 200 °C and held at 55 °C for half a minute. The temperature was increased to 200 °C for one minute (hold time) at a rate of 10 °C per minute. The injection temperature was 250 °C with split injection mode. The helium carrier gas flow rate was 1.21 mL/min. The ion source temperature and interface temperature in MS were 230 °C and 300 °C respectively. The identification of compounds in the sample was done by the scan method, which involved the use of Mass Spectral Library and Search Software (NIST). The quantification of compounds in the samples was done based on percentage composition derived from the Peak Integration method (area normalisation) (Belluomo et al., 2021).

2.7. Dilution of the essential oils

The EOs were diluted in acetone, and the final concentrations were obtained from a stock solution of 10,000 ppm (0.5 mL of oil diluted in 49.5 mL) according to Table 1**.** To prepare mixtures, a stock solution (10,000 ppm) for two oil combinations was prepared by mixing 0.5 mL of each essential oil with 49 mL of acetone 0.25 mL with 0.75 mL and 0.75 mL with 0.25 mL for a ratio of 1:1, 1:3 and 3: 1 respectively according to (Wangrawa et al., 2022a).

2.8. Mosquito rearing

The third instar larvae of *Anopheles gambiae* were used as per World Health Organization protocol (WHO, 2005). The Kisumu strain of *An. gambiae* s.s. mosquito colony is maintained in the disease vector control laboratory of the Tropical Pesticide Research Institute (TPRI) at Ngaramtoni, Arusha. Adults were kept in cages at a photoperiod of 12 L:12 D with a temperature of 27 ± 2 °C and a relative humidity of 78 ± 2 %, supplied with a 10 % sucrose solution, and fed by the blood of rabbits. Mosquitoes were allowed to lay eggs on wet filter paper supported by cotton and placed on petri dishes.

2.9. Laboratory larvae bioassay

Laboratory Larvicidal bioassay took place at the Division of Livestock and Human Disease Vectors Control within the Tanzania Plant Health and Pesticide Authority (TPHPA) in Arusha, Tanzania. The choice of this facility was driven by its specialized focus on Livestock and Human Disease vector control, providing an ideal setting for conducting in-depth experiments related to larvicidal activity and toxicity assessment.

Larval bioassay tests to determine the larvicidal activity of single and binary EOs followed the WHO standard guidelines (WHO, 2005). The experiments were set using 5 different concentrations of 10 ppm, 25 ppm, 50 ppm, 75 ppm, and 100 ppm, with a control of 1 % acetone used as solvent to make the test solutions. Each of the concentrations and controls had four replicates. Aliquots of each of the test concentrations at concentrations of 10, 25, 50, 75, and 100 ppm were added to bowls containing 100 mL of normal mosquito-rearing water and 25 third-instar mosquito larvae. Then mosquito larval mortality was recorded in each test concentration at 24- and 48-hour intervals. Both dead and moribund larvae were recorded as dead. The above experiments were repeated three times, each time using new test solutions and new batches of mosquito larvae.

2.10. Simulated semi-field larval bioassay

The larvicidal bioassay in the simulated semi-field and toxicity experiments, as well as chemical analysis of the essential oils, took place at the Division of Livestock and Human Disease Vectors Control within the Tanzania Plant Health and Pesticide Authority (TPHPA) in Arusha, Tanzania.

Test concentrations which were reported larval mortality of more than 80 % in laboratory bioassay were tested in the simulated semi-field tests. Simulated semi-field tests were carried out following standard procedures of the World Health Organization's Pesticide Evaluation Scheme with some modifications as described by (Mdoe et al., 2014). Small bowls with a diameter of 14 cm, a depth of 10 cm, and a capacity of 250 mL were used as microcosms during experiments. The bowls were arranged 1 m apart in four rows. Each bowl was filled with 100 mL of distilled water for laboratory-reared larvae and water from irrigated rice fields for wild larvae. Batches of 25 field-collected third-instar larvae and 25 laboratory-reared larvae were introduced into their respective bowls separately. In each treatment round, 12 of the bowls were served as treated bowls (3 doses with 4 replicates) of the *Aframomum angustifolium* established in laboratory tests. The other four (4) bowls served as untreated controls (1 % acetone) The essential oils were spread in the experimental bowls evenly over the entire water surface by a glass stirring rod. A net-covered emergence trap was placed on top of the bowls to prevent wild insects from laying eggs in the sites and to prevent the escape of any emerging adult mosquitoes. The larvae in the bowls were assessed after 24 and 48 h, and the dead larvae were recorded (Ochola et al., 2022).

2.11. Toxicity of the essential oils against Gambusia affins (mosquito fish)

The toxicity bioassay against the mosquito fish in the simulated semi-field took place at the Division of Livestock and Human Disease Vectors Control within the Tanzania Plant Health and Pesticide Authority (TPHPA) in Arusha, Tanzania. *Gambusia affinis* were reared in tanks with water the temperature of the water was between 20 °C and 30 °C (68°F to 86°F). With a pH level between 7.0 and 8.5 fed with small aquatic insects supplemented with flakes. The test concentrations of 50, 75, and 100 ppm tested on a simulated semi-field were tested on the non-targeted aquatic organism *Gambusia affin* as described in the simulated field bioassay. Only one mosquito fish per bowl was used to avoid cannibalism. Furthermore, surviving fish were observed for 10 days; no additional mortality or changes in the swimming behaviour of *G. affinis* fish were observed during this time.

2.12. Statistical analysis

In larvicidal assays, data collected were recorded and entered in Microsoft Excel and the observed mortality rates were corrected using Abbott's formula (Abbott, 1925) Data from all four replicates was pooled for analysis. The software used for the data analysis was IBM Statistical Programme for Social Scientists (SPSS) version 26.0 for

Table 2
Chemical Composition of *Tagetes patula* essential oil.

Chemical name	RRI calc	RRI lib	% Composition
α-Pinene	931	939	
Sabinene	976	976	0.70
Myrcene	986	991	0.24
α-Phellandrene	1004	1005	0.45
Limonene	1022	1031	7.39
(*Z*)-(β) Ocimene	1033	1040	17.1
(*E*)-(β)-Ocimene	1043	1050	2.28
Dihydrotagetone	1056	1054	1.04
Terpinolene	1088	1088	20.75
Linalool	1098	1098	0.40
P-mentha-1,3,8-triene	1112	1111	0.24
Allo-ocimene	1129	1129	
(*Z*)-ocimenoxide			0.24
(*E*)-ocimenoxide			0.42
(*E*)-tagetone	1147	1146	6.09
(*Z*)-tagetone	1151	1153	5.26
Borneol	1168	1165	0.22
Terpinen-4-ol	1177	1177	0.84
p-cymen-8-ol	1185	1183	0.59
α- terpineol	1189	1176	0.94
(*Z*)-Ocimenone	1231	1231	9.42
(*E*)-Ocimenone	1240	1239	4.81
Piperitone	1257	1252	2.46
Thymol	1288	1289	0.26
Piperitenone	1346		7.33
Piperitenone oxide	1369		0.22
(β)-Caryophyllene	1418	1418	3.53
Germacrene-D	1480	1480	1.35
Bicyclogermacrene	1505	1508	0.89
(*E*)-(β)-farnesene	1459	1458	0.21
spathulenol	1580	1577	0.28
Caryophyllene-oxide	1590	1581	0.46

Windows (SPSS Inc., Chicago, IL). Probit analysis was conducted on mortality data collected at 24 h and 48 h of exposure to different concentrations of oil to determine the lethal concentration for 50 % and 90 % mortality (LC_{50} and LC_{90}) values for the essential oils. An Independent t-test was used to compare the mean values of mortality between laboratory-reared and wild-collected larvae. Fractional effect indices (FEI) were calculated as per (Houghton, 2009); The binary blends' FEIs were interpreted using the classification proposed by (Berenbaum, 1989). If the FE index is less than 1.0, there is synergism; if it is 1.0, there is an additive effect; and if it is greater than 1.0, there is antagonism. The effects of a combination of drugs or insecticides are classified as additive, synergistic, or antagonistic (Liess et al., 2020). The toxicity of essential oils expressed as LC_{50} values for non-target species was compared to Meyer's toxicity index. Meyer's toxicity index classifies extracts with LC_{50} less than 1000 g/mL as toxic, while extracts with LC_{50} greater than 1000 g/mL as non-toxic (Meyer et al., 1982).

3. Results

3.1 Chemical composition identification

The hydro-distillation process was used to extract essential oil from 100 g fresh *Tagetes patula* and *Aframomum angustifolium* leaves, 0.14 mL and 0.8 mL of EOs were obtained respectively. According to GC/MS results, *T. patula* EO was found to contain 32 compounds, as shown in (Table 2), and 14 compounds in *Aframomum angustifolium* essential oil, as shown in (Table 3). In *T. patula* essential oil, the main components were Terpinolene (20.75 %) and (*Z*)-(β-Ocimene (17.10 %). In the oil of *A. angustifolium*, the most abundant component was cis-Pinen-3-ol (58.48 %) followed by beta-pinene (31.03 %).

3.2Larvicidal activity of the essential oils against laboratory-reared An. gambiae in a laboratory

The *Tagetes patula* and *A.angustifolium* essential oils' larvicidal mortalities after 24- and 48-hour exposure were dose-dependent and time-dependent with the highest mortality observed in *T. patula* EO as shown in (Fig. 1.) At 100 ppm, EOs of *T.patula* and *A.angustifolium* caused 100 % mortality to *An. gambiae* larvae at 48 h. Among the oils, *T. patula* was more toxic against *An. gambiae* with LC_{50}s of 12.99 ppm and 0.17 ppm, at 24 h and 48 h respectively whereas *A.angustifolium* was less toxic with an LC_{50} of 25.92 ppm and 1.71 ppm at 24 h and 48 h respectively (Table 4). Fig. 2.

3.3. The larvicidal activity of the essential oils blends against laboratory-reared An.gambiae in a laboratory

Larvicidal efficacy was highest in treatment solutions containing blends containing 1:1 among the blends tested shown in (Fig. 3). The LC_{50} and LC_{90} values were obtained after 24- and 48-hour exposure to blends 1:1, 1:3, and 3:1 against *An. gambiae* s.s. larvae are shown in (Table 6). Blend 1:1 had the lowest concentration required to kill 50 % and 90 % of *An. gambiae* s.s. larvae, followed by blends 3:1 and 1:3. Fractional effect indices (FEI) are presented in (Table 5). There was no mortality in the control group. At 24 h both blends showed synergism and at 48 h both blends showed antagonism. Table 6.

3.4Larvicidal activities of the essential oils against laboratory-reared An. gambiae in a laboratory and semi-field

The mortality trends obtained during Simulated semi-field conditions (SFCs) were consistent with those found in the laboratory and showed dose- and time-dependent patterns as depicted in (Table 7.). Higher mean mortality was reported in *T.patula* EO at both 24 h and 48 h. However, mortality rates in SFCs were significantly higher ($P < 0.05$) than in laboratory conditions at higher doses (75 and

Table 3
Chemical Composition of *Aframomum angustifolium* essential oil.

CHEMICAL NAME	MW	RETENTION TIME	BASE PEAK	% COMPOSITION	RELATIVE INDEX
alpha. -Pinene	136	2.96	93.10	3.19	948.00
beta-Pinene	136	3.46	93.10	31.03	943.00
beta. -Myrcene	136	3.59	93.10	0.36	958.00
alpha. -Phellandrene	136	3.77	93.10	0.37	968.00
o-Cymene	134	4.02	119.20	1.07	1042.00
beta. -Terpinyl acetate	196	4.08	68.05	1.40	1348.00
4-Terpinenyl acetate	196	4.47	93.10	0.76	1327.00
Isopulegol	154	5.66	81.10	0.21	1196.00
Citronellal	154	5.73	69.10	0.22	1126.00
Isoborneol	154	2.96	93.10	3.19	948.00
3-Pinanone, cis	152	3.46	93.10	31.03	943.00
trans-Sabinene hydroxide	154	3.59	93.10	0.36	958.00
Citronellol	156	3.77	93.10	0.37	968.00
cis-Pinen-3-ol	152				

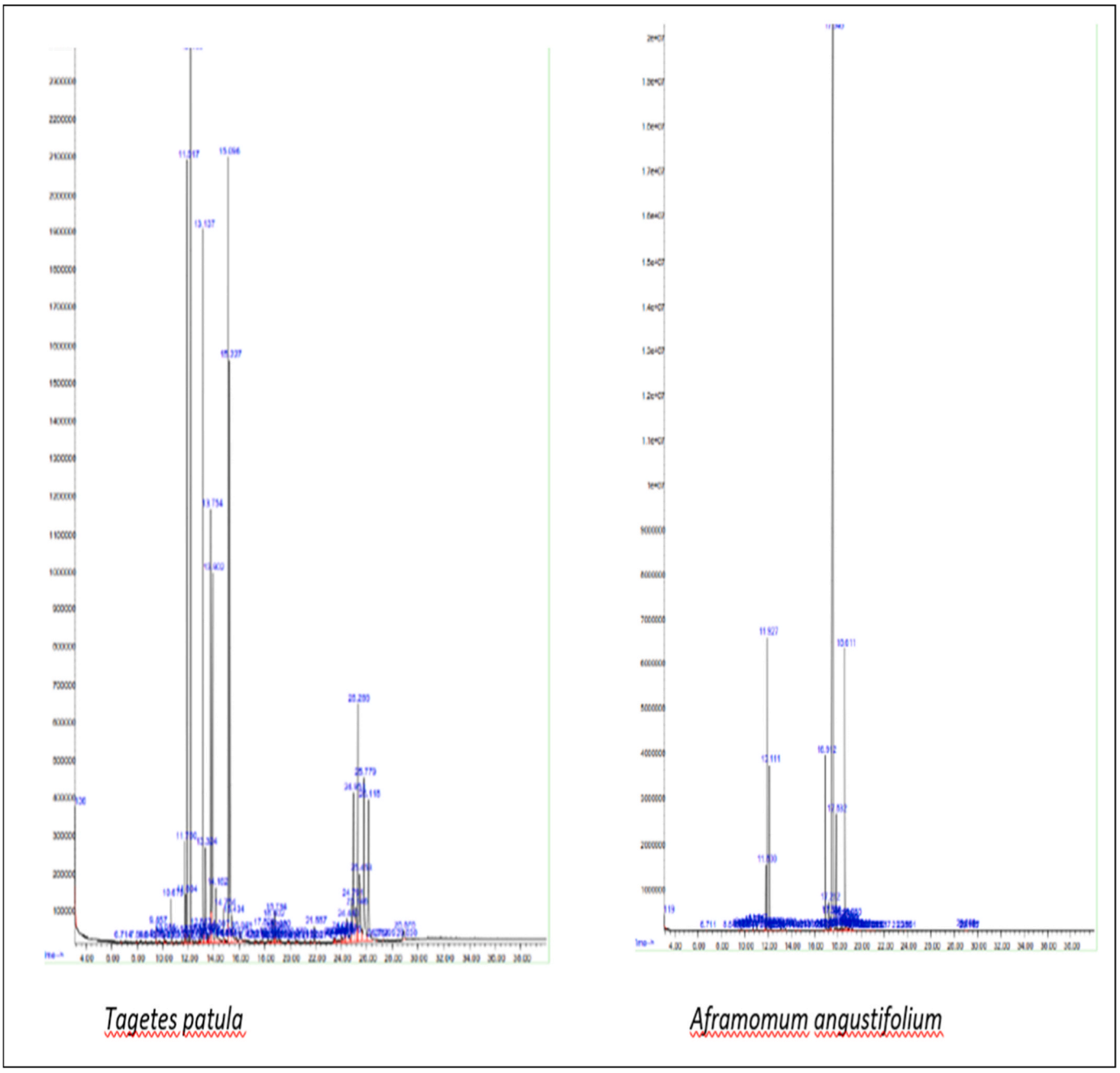


Fig. 1. Total ion chromatogram of compounds in *T. Patula* and *A. angustifolium* essential oils.

Table 4
LC_{50} and LC_{90} of *A. angustifolium* essential oils and *T. patula* against *An. gambiae* larvae.

Essential Oils	Time	Lethal concentration	Parameter Estimate	95 % C. I.		Chi Square	d.f.	P-value
				Lower	**Upper**			
Aframomum angustifolia	24	LC_{50}	25.92[a]	21.57	30.32	101.10	58	<0.001
		LC_{90}	222.60[a]	162.86	345.90			
	48	LC_{50}	1.71[a]	0.31	3.79	142.00	58	<0.001
		LC_{90}	21.89[a]	14.96	30.25			
Tagetes patula	24	LC_{50}	12.99	9.72	16.13	69.85	58	0.137
		LC_{90}	179.50	133.43	272.35			
	48	LC_{50}	0.17	0.01	0.79	68.76	58	0.157
		LC_{90}	14.12	7.07	20.74			

Down the column, values with the same superscripts are significantly different ($P < 0.05$).

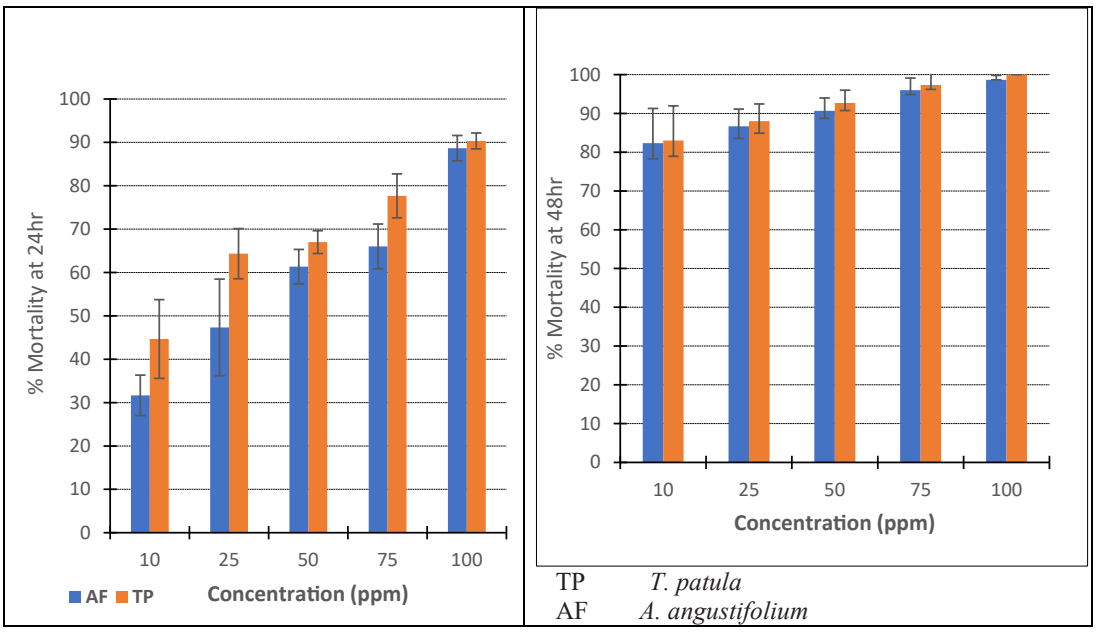


Fig. 2. Bar graphs for larvicidal activity *of T. patula* and *A. angustifolium* against third-instar larvae of *Anopheles gambiae* under laboratory conditions at 24 h and 48 h.

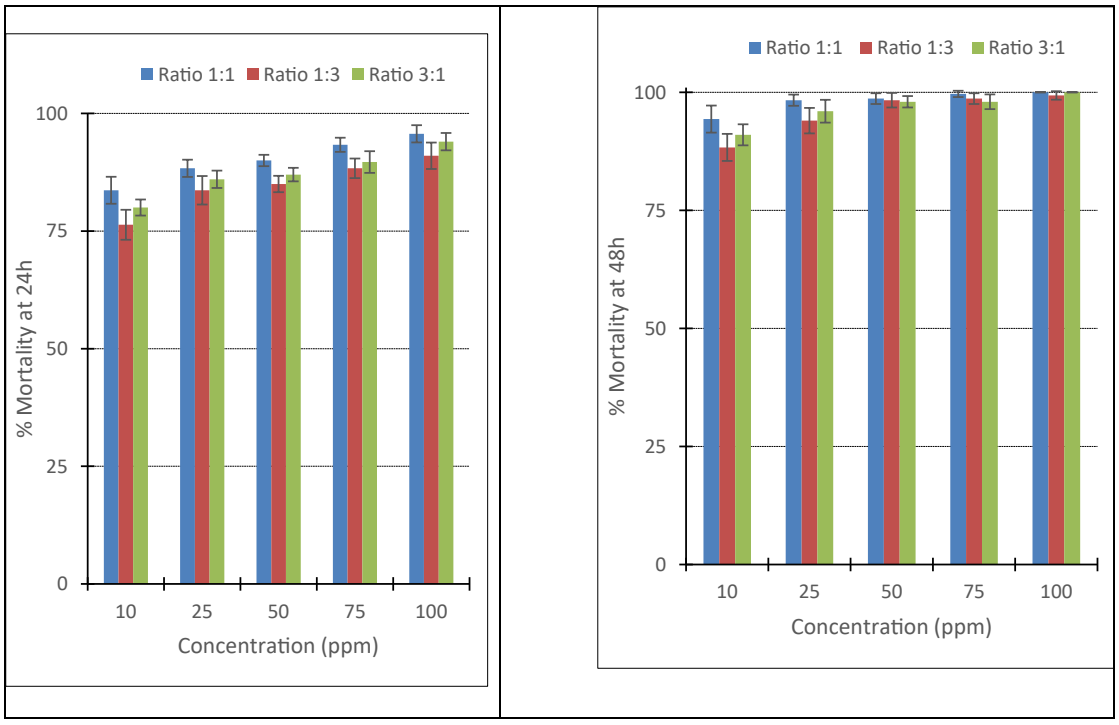


Fig. 3. Bar graphs of larvicidal activity of *T. patula* and *A. angustifolium* (Tp: Af) ratios against third-instar larvae of *Anopheles gambiae* under laboratory conditions at 24 h and 48 h.

Table 5
Binary mixtures of EOs for larvicidal activity against *An. gambiae:* synergistic/additive/indifferent/antagonistic effects.

Time	RATIO	FRACTIONAL EFFECT INDICES	EFFECT
24 h	1:1	0.04	Synergism
	1:3	0.06	Synergism
	3:1	0.04	Synergism
48 h	1:1	3.36	Antagonism
	1:3	7.50	Antagonism
	3:1	3.82	Antagonism

100 ppm) in 24 h exposure and low dose (50 ppm) at 48 h for *T. patula* EO. Significantly higher ($P < 0.05$) mean mortality was observed at (50 and 75 ppm) within 24 h and only 100 ppm within 48 h exposure for *A. angustifolium* EO.

3.5Larvicidal activity of essential oils against laboratory-reared and field-collected larvae of An. gambiae in semi-field

The findings revealed that increasing the concentration of the essential oil solution increased larvae mortality. When compared to

Table 6
LC_{50} and LC_{90} of binary mixtures of EOs for larvicidal activity against *An. gambiae*.

RATIOS	Time	Lethal concentration	Parameter Estimate	95 % C. I.		Chi Square	d.f.	P-value
				Lower	Upper			
RATIO 1:1	24	LC_{50}	0.32	0.19	1.15	19.76	58	1.000
		LC_{90}	33.30	23.65	47.33			
	48	LC_{50}	0.52	0.49	1.16	45.18	58	0.890
		LC_{90}	5.74	2.36	8.89			
RATIO 1:3	24	LC_{50}	0.53	0.04	1.71	24.14	58	1.000
		LC_{90}	100.89	67.13	216.44			
	48	LC_{50}	1.16	0.37	2.26	46.96	58	0.850
		LC_{90}	12.50	8.89	15.95			
RATIO 3:1	24	LC_{50}	0.36	0.20	1.33	18.01	58	1.000
		LC_{90}	62.28	44.12	109.04			
	48	LC_{50}	0.59	0.09	1.52	45.94	58	0.157

Table 7
T. patula and *A. angustifolium* larvicidal activities against third-instar laboratory-reared *Anopheles gambiae* larvae at 24 and 48 h in a laboratory and semi-field environment.

Essential Oils	Environment	Time	Concentration (ppm)	Mean mortality	95 % C. I.		t-static value	P-value
					Lower	Upper		
Tagetes patula	Laboratory	24 h	50	16.75	16.03	17.47	1.22	0.234
	Simulated			17.42	16.46	18.37		
	Laboratory		75	19.42	18.02	20.81	3.26	0.004
	Simulated			21.92	20.96	22.87		
	Laboratory		100	22.58	22.08	23.09	5.73	<0.001
	Simulated			24.25	23.86	24.65		
	Laboratory	48 h	50	23.17	22.64	23.70	4.05	0.001
	Simulated			24.42	23.99	24.84		
	Laboratory		75	24.33	24.02	24.65	1.22	0.237
	Simulated			24.58	24.26	24.91		
	Laboratory		100	25.00	25.00	25.00	.	.
	Simulated			25.00	25.00	25.00		
Aframomum angustifolium	Laboratory	24 h	50	15.33	14.24	16.43	2.22	0.037
	Simulated			13.83	12.83	14.84		
	Laboratory		75	16.50	15.08	17.92	7.64	<0.001
	Simulated			22.58	21.42	23.75		
	Laboratory		100	22.17	21.36	22.97	2.85	0.009
	Simulated			23.58	22.84	24.32		
	Laboratory	48 h	50	22.67	21.76	23.58	0.55	0.590
	Simulated			22.92	22.49	23.34		
	Laboratory		75	24.00	23.14	24.86	1.34	0.193
	Simulated			24.58	24.16	25.01		
	Laboratory		100	24.67	24.35	24.98	2.35	0.028

field-collected larvae, *T. patula* EO induced the highest larvicidal activity in SFCs at all concentrations, which was statistically significant ($p < 0.05$) at 24 h for laboratory-reared larvae. However, at only a concentration of 50 ppm *A. angustifolium* caused a statistically significant difference in larvicidal activity against laboratory and field-collected larvae at 24 and 48 h. (Table 8).

3.6. Toxicity of the essential oils against Gambusia affins

The essential oils' effects were studied on the non-target aquatic organism *Gambusia affins* (mosquito fish). No mortality was observed for *Tagetes patula* EO. But for *A. angustifolium,* mortality was 25 % at 100 ppm with $LC_{50} = 134.03$ and $LC_{90} = 195.6$, as described in (Table 9). Furthermore, surviving fish were observed for 10 days; no additional mortality or changes in the swimming behavior of *G. affinis* fish were observed during this time.

4. Discussion

Plant essential oils are safer, biodegradable, target-specific alternatives that have been taken into consideration to replace traditional mosquito control methods due to the rise in mosquitoes that are resistant to them and problems with toxicity to both target and non-target organisms.

The difference in oil yield of *T. patula* and *A.angustifolium* obtained in our study are in line with the previous study conducted in Saudi Arabia reported a 0.84 % (v/w) yield of essential oils in *Tagetes patula* flowers (Aati et al., 2022) and a 0.15 % (v/w) yield of essential oils in *Aframomum latifolium* leaves in west-south Nigeria (Olagoke et al., 2023).

The EOs used in this study contain a mixture of major and minor compounds. The chemical composition of *T. patula* and *A. angustifolium EOs* in this study showed heterogeneity from each other but homogeneity from other *Tagetes* and *Aframomum* species, respectively, while there was a slight difference percentage of the chemical composition of compounds. Our results of *A.angustifolium* EO were consistent with previous reports on the essential oils obtained from the leaves of three *Callistemon species* which reported Terpinolene as one of the constituents detected as common constituents in all Callistemon species (Gad et al., 2019) also our *T.patula* EO findings are consistent with the study on larvicidal activity of *Litsea umbellata* and *Litsea iteodaphne* essential oils, which reported β- pinene (18.8 % and 6.6 %, respectively) (Dai et al., 2020). and these compounds are known for their antibacterial and insecticidal properties (Marotti et al., 2004; Safar et al., 2020b). The chemical composition of *T. patula* and *A. angustifolium* varies greatly depending on geographical location, growing conditions, plant parts extracted, plant developmental stage, extraction solvent,

Table 8
The comparison of larvicidal activity of *T. patula and A. angustifolium* EOs against third-instar laboratory-reared and field-collected larvae of *Anopheles gambiae* at 24 h and 48 h in a simulated field environment.

Essential Oils	Larvae	Time	Concentration (ppm)	Mean	95 % C. I.		t-stastic value	P-value
					Lower	Upper		
Tagetes patula	Laboratory larvae	24 h	50	17.42	16.46	18.37	6.03	<0.001
	Field larvae			13.00	11.70	14.30		
	Laboratory larvae		75	21.92	20.96	22.87	4.01	<0.001
	Field larvae			19.50	18.58	20.42		
	Laboratory larvae		100	24.25	23.86	24.65	2.94	0.008
	Field larvae			23.17	22.46	23.88		
	Laboratory larvae	48 h	50	24.42	23.99	24.84	2.97	0.007
	Field larvae			23.33	22.65	24.02		
	Laboratory larvae		75	24.58	24.26	24.91	1.04	0.308
	Field larvae			24.33	23.92	24.75		
	Laboratory larvae		100	25.00	25.00	25.00	1.48	0.152
	Field larvae			24.83	24.59	25.08		
Aframomum angustifolium	Laboratory larvae	24 h	50	13.83	12.83	14.84	5.98	<0.001
	Field larvae			9.92	8.89	10.95		
	Laboratory larvae		75	22.83	21.69	23.98	1.56	0.135
	Field larvae			21.82	20.98	22.66		
	Laboratory larvae		100	23.58	22.84	24.32	0.51	0.614
	Field larvae			23.33	22.55	24.12		
	Laboratory larvae	48 h	50	22.92	22.49	23.34	4.23	<0.001
	Field larvae			21.17	20.36	21.97		
	Laboratory larvae		75	24.58	24.16	25.01	0.56	0.583
	Field larvae			24.42	23.91	24.92		
	Laboratory larvae		100	25.00	25.00	25.00	1.39	0.178
	Field larvae			24.75	24.36	25.15		

Table 9
Toxicity of *T. patula* and *A. angustifolium* EOs against *Gambusia affins* (LC_{50} and LC_{90}).

Essential Oils	Time	Lethal concentration	Parameter Estimate	95 % C. I.		Chi Square	d.f.	P-value
				Lower	Upper			
T. Patula	24	LC_{50}	Because the ratios of response counts to subject counts are the same, the slope is zero, no statistics were computed.				34	
		LC_{90}						
A. angustifolium	24	LC_{50}	134.03			17.72	34	0.99
				–	–			
		LC_{90}	195.67	–	–			

photosensitivity of some of the compounds in the extract, and methods used to isolate the essential oils *(*Shajahan et al., 2022*)*.

Both EOs had larvicidal activities on *An. gambiae* and the mortality was dose and time-dependent and varied with different EOs. Higher mortality was observed in *T.patula* essential oil. This can be explained by the presence of compounds in the oil reported to have insecticidal activity (Politi et al., 2012). The essential oil of *T. patula* has insecticidal action, and the proposed mechanism of action is the inhibition of acetylcholine esterase in insects by the main constituents α-terpinolene, piperitenone, and piperitone (Riaz et al., 2020; e Santos et al., 2022).

Our research demonstrated the synergistic and antagonistic effects of extracted plant EOs. Using an EO combinations ratio of 1:1 showed that *An. gambiae* was highly susceptible. Both combinations of EOs showed a synergistic effect at 24 h and an antagonistic effect at 48 h against the larvae of *An. gambiae.* The synergistic effects of the EOs are thought to be due to the production of additional new molecules after the EOs are mixed, amplifying their effects on the larvae studied (Wangrawa et al., 2022b) and also due to the EOs' simultaneous impacts on multiple targets, which increases the studied EOs' larvicidal effect (Youssefi et al., 2019; Manh et al., 2020). This is in line with the report conducted in India that reported synergistic interactions among the binary mixtures with co-toxicity factors for the binary combinations of the EOs were >20 (Pandiyan et al., 2019). The antagonistic effect observed in our study may be due to the dilution of the most effective EO constituent (s) in the blend over time, or compound decomposition to form new compounds that act on the same target, hence reducing the effect. This is also in line with research conducted in Canada, which found that all other ternary EO blends showed antagonistic interactions except for the ternary blend of cinnamon bark, geranium, and rosemary, which showed a synergistic interaction with an estimated RD50 of 29.50 mg (Peach et al., 2019).

The trends in larval mortality of laboratory-reared larvae found in simulated semi-field trials correlated with those observed in laboratory bioassays being dose and time-dependent. Our concentration-dependent mortality results are consistent with previous findings from a study conducted in Brazil, which found that *C. forbesi* had a high mortality rate in a semi-field environment, with LC_{50} and LC90 values of 0.630 and 2.99 mg/L respectively (dos Santos et al., 2021). Large variations in temperature, humidity, and light are attributed to higher larval mortalities under SFCs (Oguh et al., 2019) in which increased environmental temperatures during the larval stages decreased larval survival (Agyekum et al., 2022). Furthermore, because the larvae used in this study were laboratory-bred rather than wild variants, simply putting them in a different set of habitat settings could make them more vulnerable to the EOs they were exposed to than they were in their usual laboratory environment.

At the same time of exposure, the results obtained with field larvae are lower than those obtained with laboratory larvae. The fact that wild larvae captured in their natural environment have already been exposed to specific active chemicals to which they have evolved behavioral resistance is consistent with the report by (Sougoufara et al., 2020). The current study's findings, on the other hand, are consistent with a study conducted in Nigeria, which found that higher concentrations of *Moringa oleifera* of 0.65 and 2.28 ppm were required to kill 50 % and 95 % of field-collected *An. gambiae* larvae at 24 h post-exposure as compared to

laboratory-reared larvae (Idowu et al., 2021).

The toxicity of *T. patula* and *A. angustifolium* EOs was investigated against (*Gambusia affinis*). In comparison to mosquito larvae, the EOs of both plants were less harmful to non-target organisms. Furthermore, surviving fish were observed for 10 days; no additional mortality or changes in the swimming behaviour of *G. affinis* fish were observed during this time. Within 10 days of observation after the exposure, *Gambusia affinis'* swimming behaviour and survival were unaffected. Given that *A. angustifolium's* LC_{50} for *G. affinis* was calculated to be 134.033 ppm Because the LC_{50} of these EOs is less than 1000 g/mL, they can be considered toxic to fish. Even at higher concentrations, no concentration caused death in *T. patula* (100 ppm). The results of this study agree with the study conducted on ethyl acetate extract of *Sargassum* from Kabung Island West Kalimantan which reported cytotoxic test exhibited that the LC_{50} was more than 1000 ppm, so it was considered into non-toxic category (Sofiana et al., 2023) The observed harmful effects of the *A. angustifolium* essential oil (EO) on non-target species, particularly fishes, at a concentration of 100 ppm may be attributed to the presence of specific chemical constituents within the essential oil that possess inherent toxicity towards aquatic organisms (Ranatunga et al., 2023). Azadirachtin, a key component of neem oil, is recognized for its larvicidal properties. However, at higher concentrations, it may affect non-target organisms such as fish by interfering with their hormonal balance and reproductive processes (Sil and Barman, 2020). The essential oil of *Hedychium larsenii,* containing ar-curcumene, was found to be ten times more toxic to *Poecila reticulata,* with an LC50 of 16, 646.63 μg/mL (de Souza et al., 2020).

5. Conclusion

The findings of this study have shown that *T. patula* and *A. angustifolium* oils have good larvicidal activities for *An. gambiae*. The two plants are potential sources of insecticidal compounds that could be used to control the malaria vector. As a result, the formulation, testing, and commercialization of products derived from these plants is recommended.

Ethics approval and consent to participate

Ethical clearance with reference number MUST-2021-278, for the research, was granted by the Institutional Research and Ethics Committee on Human Research (IREC) of Mbarara University of Science and Technology (MUST), on behalf of the Uganda National Council for Science and Technology (UNCST). Similarly, permission to conduct the experiments and chemical analysis at the Tanzania Plant Health and Pesticides Authority (formerly Tropical Pesticides Research Institute (TPRI)) was also sought.

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CRediT authorship contribution statement

Ogwang Patrick Engeu: Supervision, Writing – review & editing. **Kweka Eliningaya J.:** Supervision, Writing – review & editing. **Thomas Adelina:** Conceptualization, Methodology, Writing – original draft, Writing – review & editing, Investigation.

Declaration of Competing Interest

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

Data Availability

Data will be made available on request.

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