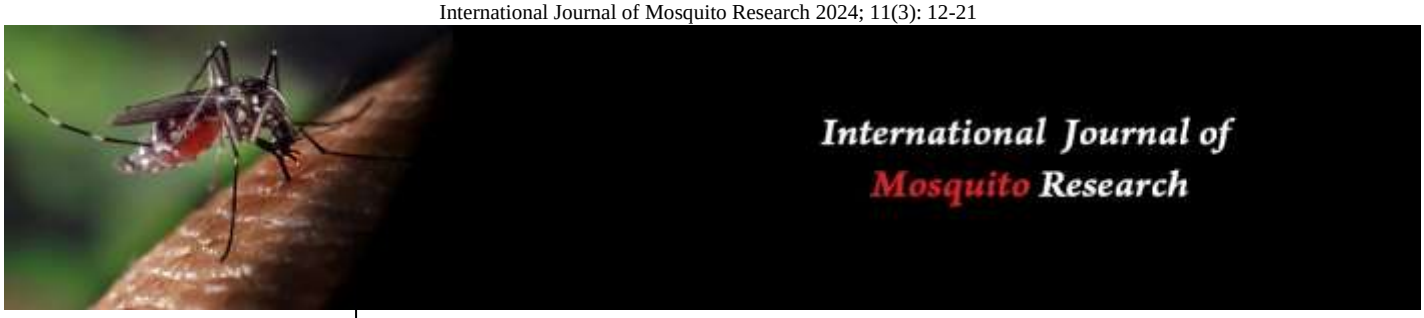

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Evaluation of mosquitocidal efficacy of panchgavya from indigenous and crossbreed cows against dengue vector

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Abstract
The growing incidence of mosquito-borne diseases and insecticides resistance highlights the need for effective mosquito control strategies in the modern era. The present study looks towards reporting the mosquitocidal efficacy of panchgavya (PG) from two distinct breed cows, indigenous and crossbreed, by conducting toxicity tests on larvae and pupae as well as repellency tests against adult female *Aedes aegypti* (L.), the primary vector for dengue. To further investigate potency of the PG for novel active compounds, GC-MS analysis was conducted. Results revealed that PG from indigenous samples displayed a more significant lethal effect on II instar, III instar, and IV instar, as well as pupae than the PG from crossbreed samples in a dose-dependent and time-dependent manner. The reported LC_{50} and LC_{90} values for this sample against II instar, III instar, IV instar and pupae was approximately 1.1- to 3.4-fold lower than those of the PG from crossbreed samples. 100% repellency was reported up to 140 min, 160 min, and 170 min intervals at 50, 100, and 150 ppm dose of PG from indigenous samples, and up to 130 min, 140 min, and 160 min intervals at 50, 100, and 150 ppm dose for PG from crossbreed samples. GC-MS analysis revealed the occurrence of more than 20 active compounds with potent biological actions. These findings revelled that the indigenous PG has the potential to serve as a natural larvicidal, pupicidal and repellent agent at lower doses. Further detailed mechanistic studies should be conducted to elucidate the mechanism underlying such mosquitocidal action.

Keywords: Cow products, natural repellents, vector control, bioactive molecules

1. Introduction
Mosquitoes represent a major threat to public health on the global scale. Every year, millions of people lose their lives due to mosquito-borne viral infections and diseases (Norris and Coats, 2017) [26]. Factors such as unprecedented population growth, uncontrolled urbanization, climate change, and the breakdown of public health infrastructure contribute to the proliferation of mosquito species and the emergence of deadly conditions such as dengue, chikungunya, yellow fever, and Zika infections. Dengue, caused by DENV viruses with more than four serotypes, has become one of the most rapidly spreading mosquito-borne viral diseases in the recent years (Bartlow *et al*., 2019) [7]. DENV serotypes are transmitted to both humans and animals through bites of adult females of *Aedes aegypti* (L.).
Over the past few decades, a number of synthetic insecticides and repellents have been devised and implemented for dengue management, primarily to minimize mosquito populations and avert the transmission of viruses from mosquitoes to humans (Tavares *et al*., 2018) [37]. Despite their effectiveness, these synthetic chemicals have adverse effects on the environment and non-target organisms, particularly humans. Frequent use of synthetic chemicals can also lead to the current state of insecticide resistance (Paaijmansssa and Lobo, 2023) [30]. To overcome these limitations, growing research interest has shifted towards products of plant or animal origin to control mosquitoes and prevent mosquito-borne viral transmission in humans. Natural products offer a selective mode of action and minimal adverse effects on non-target organisms, and are cost-effective and biodegradable in the nature (da Silva and Ricci-Júnior, 2020) [11]. After plants, substances of animal origin, primarily excretory secretions, have proven to be effective against various pathogenic bacteria, fungi and pest (Indriani *et al*., 2023; Amina *et al*., 2024; Carrillo *et al*., 2024) [15, 3, 9].

The well-known products of cow metabolism, including milk and its derivatives (curds, clarified butter, and ghee), as well as waste products such as dung and urine, are known as 'gavya' in Sanskrit. The concept of Panchgavya (PG) encompasses all these five 'gavya,' and hold a unique position in traditional Ayurveda system of medicine for their potent nutritional, therapeutic, and biological properties (Bajaj *et al*., 2022) [6]. Several studies have confirmed that PG exhibits antimicrobial, antibiofilm, antioxidant, anticancer, anticonvulsant, antidiabetic, anti-inflammatory, bioenhancing, nootropic, and immunomodulatory activities (Gajera *et al*., 2024; Totawar *et al*., 2023; Chinniah *et al*., 2024) [13, 39, 10]. Some recent studies also revealed that PG derived nanomaterials displayed antimicrobial, antioxidant and biopesticidal properties (Arumugam *et al*., 2019; Ukkund *et al*., 2021; Sathiyaraj *et al*., 2021) [5, 40, 33]. PG could therefore be used for mosquito control, however, literature on this topic is scarce and limited. Notably, PG comprises a significant amount of milk, with the native cow breed producing A2 milk, whereas crossbreeds produce A1 milk, both of which have distinct differences in their biological functions (Kaskous *et al*., 2020; Xiao *et al*., 2023) [17, 43].

Therefore, the aim of this study was to assess the mosquitocidal efficacy of PG from both indigenous and crossbred cows by conducting toxicity tests on larvae and pupae as well as repellency tests against adult female *Aedes aegypti* (L.), the primary vector for dengue. To investigate the potency of PG with novel active compounds, GC-MS analysis was conducted. This study offers a sustainable method for controlling mosquito vectors that could lead to the development of new agricultural and public health products.

2. Materials and Methods

2.1 Collection and processing of PG samples: This study examined PG from two cow breeds, Malvi breed and Holstein-Friesian breed. The Malvi breed is an indigenous breed of cow whereas the Holstein-Friesian breed is a crossbreed cow. Both are housed at a local cattle breeding center situated in the Udaipur district of Rajasthan (latitude 24.21965°N, longitude 73.65035°E) and three lactating females from each breed were chosen to collect all the necessary samples required to prepare panchgavya, including urine, dung, milk, and ghee. Panchgavya was prepared by blending urine (300 mL), dung (300 g), milk (300 mL), ghee (100 g), and curd (300g) samples from both breed cow thoroughly with water and some other ingredients (coconut water, jaggery and banana) in a covered wide mouth container as per modified method of Sathiyaraj *et al*. (2022) [32]. The container was placed in a shaded location at room temperature until the final process was completed. The resulting panchgavya was filtered through filter paper and stored at 4 °C for subsequent experiments.

2.2. Larvicidal and pupicidal activity

To conduct larvicidal and pupicidal assays, laboratory rearing of *A. aegypti* mosquitoes were performed in accordance with the standard WHO protocol (WHO, 2005) [41]. The mortality rates of the selected larval stage (II, III, and IV instar) and pupae were determined by exposing them to beakers containing desired concentrations of PG from both indigenous and crossbreed samples (Fig. 1). Each replicate contained 30 larvae and pupae. In the control treatment, larvae and pupae were placed in a beaker containing acetone and water solution (Thomas *et al.*, 2017) [38]. Mortality rate (%) was calculated as.

$$Mortality\ (\%) = \frac{Number\ of\ dead\ larvae\ or\ pupae}{Number\ of\ larvae\ or\ pupae\ introduced} X100$$

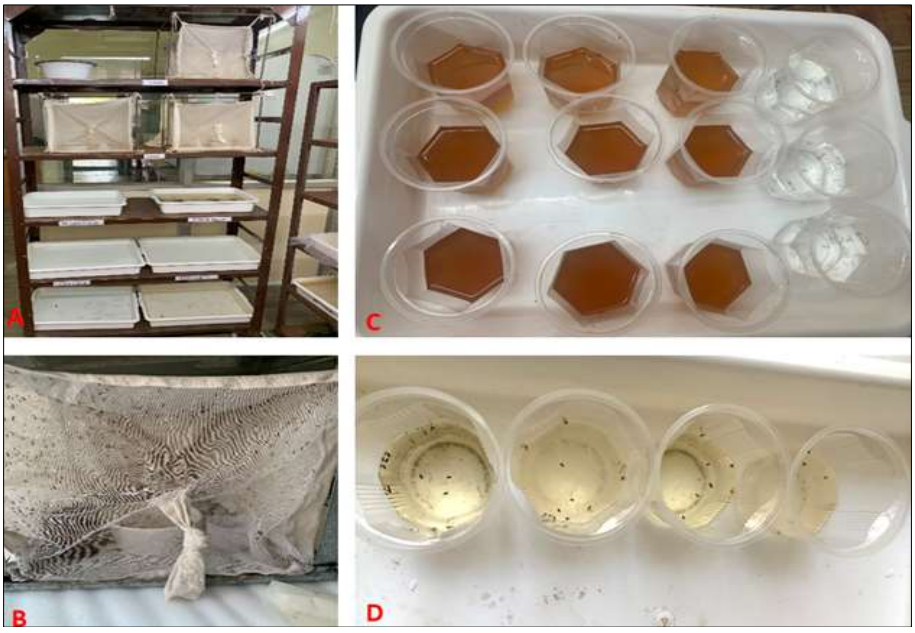


Fig 1: Bioaay experimental setup, A & B= rearing of mosqutioes, C&D= different dose experiment

2.3 Repellent activity

The PG from both indigenous and crossbreed samples were evaluated for repellency against adult mosquitoes using a Y-tube olfactometer constructed according to WHO guidelines (WHO, 2013) [42]. The test arm received 1 ml of PG at the desired concentrations for both indigenous and crossbreed cow. After 24 h of fasting, 30 adult female mosquitoes were placed in the Y-tube olfactometer and observed at 10 min intervals to record the arm of choice. This experiment was conducted in triplicates, with at least three replicates per

treatment. The repellent activity was evaluated for up to 200 min, and the repellency (R %) was evaluated as per following equation.

$$R\ (\%) = \frac{Total\ no.of\ mosquito\ in\ control\ arm - Total\ no.of\ mosquito\ in\ test\ arm}{Total\ number\ of\ mosqutio\ on\ control\ arm} X100$$

2.4 GC-MS analysis

The PG samples from both indigenous and crossbreed cows were analyzed using an Agilent 5977 B GC-MS instrument, which was equipped with an EI/CI interface and loaded with the Agilent Mass Hunter and NIST library. The key parameters applied were sample injection volume (1 µl), injector temperature (250 °C), and carrier gas (helium; flow rate, 1 ml/min). The other parameters applied included column oven temperature, which was initialized at 60°C and gradually increased to 230 °C, ion source temperature (230 °C), scan interval (0.1 s), and a scan mass range of 40-700 m/z (Nautiyal and Dubey, 2021) [24].

2.5. Data Analysis

All experiments were performed in triplicates. Results are stated as mean ± standard deviation (SD). To calculate the LC_{50}, and LC_{90} mean mortality data whereas for ED_{50} and ED_{90} mean repellency data were used at 95% confidence limits of statistical significance (Finney, 1971) [12]. To analyze the results, SPSS statistical software (version 16.0; USA) was used to perform ANOVA (analysis of variance) and DMRT (Duncan's multiple range tests) as well as chi-square tests at p ≤ 0.05.

3. Results

3.1. Larvicidal and pupicidal activity of PG from indigenous samples

Among the tested doses, the highest percent mortality (80.33%) for II instar larvae was observed at 75 ppm after 24 h, which increased to 95.66% after 48 h. For III instar larvae, the highest percent mortality (70%) was observed at 75 ppm after 24 h, which increased to 93.11% after 48 h. For IV instar larvae, the highest percent motility (72.66%) was observed at 100 ppm after 24 h, which increased to 96.00% after 48 h. Control samples did not exhibit mortality against any of the larval stages tested (Table 1). The LC_{50} values were recorded as 12.475 ppm for II instar larvae, 12.994 ppm for III instar larvae, and 44.990 ppm for IV instar at a 95% confidence interval. Similarly, the LC_{90} values were 78.505 ppm for the II instar larvae, 112.276 ppm for the III instar larvae, and 115.648 ppm for the IV instar at a 95% confidence interval.

For pupae, 48.22% mortality was observed at 50 ppm concentration after 24 h, which increased to 71.44% after 48 h. The highest mortality (68.19%) was recoded at 100 ppm after 24 h, which increased to 90.89% after 48 h. The control samples did not exhibit mortality during the experiment. The LC_{50} and LC_{90} values were 37.345 ppm (with a lower limit of 28.959 ppm and an upper limit of 58.953 ppm) and 119.859 ppm (with a lower limit of 93.541 ppm and an upper limit of 144.932 ppm), respectively (Table 1).

Table 1: Larvicidal and pupicidal activity of PG from indigenous cow

Target stage	Dose (ppm)	Percent mortality (mean ± SD)		LC_{50} (LCL;UCL) ppm	LC_{90} (LCL;UCL) ppm	Probit regression equation	χ^2 (df=4)
		24 h	48 h				
II instar	Control	0.00±0.00	0.00±0.00	12.475 (19.885;34.152)	78.505(71.440;106.594)	y=0.3333+0.0167x	.010
	20	61.12±1.02	82.44±1.15				
	50	68.00±2.00	91.11±1.15				
	75	80.34±2.51	95.66±1.02				
III instar	Control	0.00±0.00	0.00±0.00	12.994(23.00;36.370)	112.276(77.725;126.675)	y=0.2667+0.0133x	.016
	50	58.89±1.52	74.44±2.51				
	75	63.33±2.08	90.66±1.15				
	100	70.00±3.00	93.11±2.88				
IV instar	Control	0.00±0.00	0.00±0.00	44.900(25.845;57.695)	115.648(92.653;139.713)	y=1.0+.025x	.011
	20	24.00±1.52	35.11±1.15				
	50	52.00±2.00	74.44±2.51				
	75	60.00±1.60	92.00±1.52				
	100	72.66±2.15	96.00±2.51				
Pupae	Control	0.00±0.00	0.00±0.00	37.345(28.959;58.953)	119.859(93.541;144.932)	y=0.8+0.02x	.098
	50	48.22±1.52	71.44±2.51				
	75	52.00±2.00	76.67±2.15				
	100	68.19±1.60	90.89±1.52				

Data stated are the mean ± SD from three repeats of every single sample; LC_{50} = concentration at which 50% of the exposed mosquito die; LC_{90} = concentration at which 90% of the exposed mosquito die; LCL = lower confide nce limit, UCL = upper confidence limit; x^2 = chi-square

3.2 Larvicidal and pupicidal activity of PG from crossbreed samples

Among the tested doses, the highest percent mortality (74.44%) for II instar larvae was observed at 75 ppm after 24 h, which increased to 90% after 48 h. For III instar larvae, the highest percent mortality (73.33%) was observed at 100 ppm after 24 h, which increased to 90% after 48 h. For IV instar larvae, the highest percent motility (75.00%) was observed at 125 ppm after 24 h, which increased to 92.22% after 48 h. Control samples did not exhibit mortality against any of the larval stages tested (Table 2). The LC_{50} values were recorded as 40.235 ppm for II instar larvae, 41.883 ppm for III instar larvae, and 78.337 ppm for IV instar larvae at a 95% confidence interval. Similarly, the LC_{90} values were 100.322 ppm for the II instar larvae, 120.889 ppm for the III instar, and 127.198 ppm for the IV instar larvae at a 95% confidence interval.

For pupae, 21.65% mortality was observed at 50 ppm concentration of PG after 24 h, which increased to 34.17% after 48 h. The highest mortality (63.33%) was recoded at 100 ppm after 24 hrs, which increased to 90% after 48 h. The control samples did not exhibit mortality during the experiment. The LC_{50} and LC_{90} values were 70.150 ppm (with a lower limit of 49.848 ppm and an upper limit of 88.950 ppm) and 150.667 ppm (with a lower limit of 122.979 ppm and an upper limit of 209.484 ppm), respectively.

Table 2: Larvicidal and pupicidal activity of PG from crossbreed sample

Target stage	Dose (ppm)	Percent mortality (mean ± SD)		LC_{50} (LCL;UCL) ppm	LC_{90} (LCL;UCL) ppm	Probit regression equation	χ^2 (df=4)
		24 h	48 h				
II instar	Control	0.00±0.00	0.00±0.00	41.833(27.881;53.253)	100.322 (68.510;103.544)	y=1.1+0.028	1.607
	20	22.41±1.58	35.64±1.85				
	50	63.33±3.60	81.11±2.08				
	75	74.44±2.08	90.00±1.00				
III instar	Control	0.00±0.00	0.00±0.00	40.235(40.146; 68.177)	120.889 (94.678;152.731)	y=1.0+0.02	0.21
	50	51.11±2.88	72.22±2.88				
	75	66.67±2.00	81.11±2.08				
	100	73.33±2.11	90.00±1.73				
IV instar	Control	0.00±0.00	0.00±0.00	78.337(67.702;87.445)	127.198 (115.073;182.211)	y=1.8+0.02	2.050
	50	22.22±1.52	31.11±1.15				
	75	50.00±3.00	74.44±2.51				
	100	70.00±3.60	88.89±1.52				
	125	75.00±4.35	92.22±2.51				
Pupae	Control	0.00±0.00	0.00±0.00	70.150(48.848;88.950)	150.667 (122.979;209.484)	y=1.167+0.0167x	0.005
	50	21.65±1.35	34.17±2.11				
	75	44.67±2.88	70.00±2.00				
	100	52.11±1.52	83.22±2.51				
	125	63.33±2.08	90.00±1.52				

Data stated are the mean ± SD from three repeats of every single sample; LC_{50} = concentration at which 50% of the exposed mosquito die; LC_{90} = concentration at which 90% of the exposed mosquito die; LCL = lower confide nce limit, UCL = upper confidence limit; x^2 = chi-square

3.3 Repellent activity of PG from indigenous samples

At a dose of 50 ppm, the treated arm showed 96.66% repellency at the start of the assay (10 min), which increased to 100% at 30 min interval and remained constant for 140 min interval. Subsequently, the percent repellency began to decline and reached 70% after 200 min. At both 100 and 150 ppm concentrations, the treated arm demonstrated 100% repellency at the start of the assay (10 min), which remained constant up to 160 min and 170 min intervals at 100 ppm and 150 ppm concentrations, respectively. The percent repellency then began to decline, reaching 80% and 83.33% at 200 min interval for 100 ppm and 150 ppm, respectively. During the course of the experiments, the control arm demonstrated mosquito entry rates of 5.33%, 4.66%, and 1.66% at maximum 200 min intervals for 50, 100, and 150 ppm concentrations, respectively (Table 3). The mean percent repellency were recorded as 94.33% at 50 ppm, 97.66% at 100 ppm and 98.16% at 150 ppm. The calculated ED_{50} and ED_{90} values were 8.126 ppm and 33.752 ppm, respectively (Fig. 2).

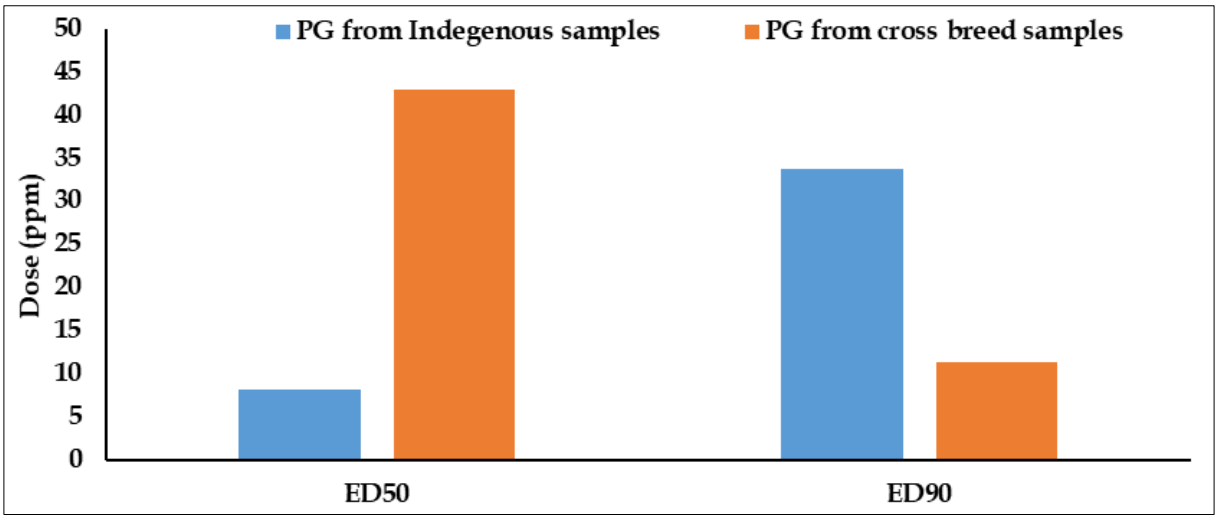


Fig 2: The ED_{50} and E_{90} values for PG from indigenous and crossbreed samples

Table 3: Repellent efficacy of PG from indigenous samples

Exposure Time (Min)	% Repellency (mean ± SD)					
	Dose in ppm					
	Control	50 ppm	Control	100 ppm	Control	150 ppm
10	00.00±0.0	96.66±1.92	00.00±0.0	100.00±0.00	00.00±0.00	100.00±0.00
20	00.00±0.0	96.66±1.92	00.00±0.0	100.00±0.00	00.00±0.0	100.00±0.00
30	00.00±0.0	100.00±0.00	00.00±0.0	100.00±0.00	00.00±0.0	100.00±0.00
40	00.00±0.0	100.00±0.00	00.00±0.0	100.00±0.00	00.00±0.0	100.00±0.00
50	00.00±0.0	100.00±0.00	00.00±0.0	100.00±0.00	00.00±0.0	100.00±0.00
60	00.00±0.0	100.00±0.00	00.00±0.0	100.00±0.00	00.00±0.0	100.00±0.00

70	00.00±0.0	100.00±0.00	00.00±0.0	100.00±0.00	00.00±0.0	100.00±0.00
80	00.00±0.0	100.00±0.00	00.00±0.0	100.00±0.00	00.00±0.0	100.00±0.00
90	00.00±0.0	100.00±0.00	00.00±0.0	100.00±0.00	00.00±0.0	100.00±0.00
100	00.00±0.0	100.00±0.00	00.00±0.0	100.00±0.00	00.00±0.0	100.00±0.00
110	00.00±0.0	100.00±0.00	00.00±0.0	100.00±0.00	00.00±0.0	100.00±0.00
120	00.00±0.0	100.00±0.00	00.00±0.0	100.00±0.00	00.00±0.0	100.00±0.00
130	00.00±0.0	100.00±0.00	00.00±0.0	100.00±0.00	00.00±0.0	100.00±0.00
140	00.00±0.0	100.00±0.00	00.00±0.0	100.00±0.00	00.00±0.0	100.00±0.00
150	1.00±0.22	96.66±1.92	00.00±0.0	100.00±0.00	00.00±0.0	100.00±0.00
160	1.33±0.23	90.00±1.94	00.00±0.0	100.00±0.00	00.00±0.0	100.00±0.00
170	2.00±0.32	80.00±1.66	00.00±0.0	96.66±1.77	00.00±0.0	100.00±0.00
180	2.66±0.34	76.66±1.41	1.66±0.22	90.00±1.64	1.00±0.21	93.33±1.33
190	3.00±0.23	80.00±1.27	2.33±0.45	86.66±1.82	1.00±0.21	86.66±1.82
200	5.33±0.27	70.00±1.93	4.66±0.31	80.00±1.77	1.66±0.28	83.33±1.84
Mean % repellency	94.33±0.89c		97.66±0.91b		98.16±0.90a	
Probit equation	y=1.1667+ 0.0167x					
χ^2(df=31)	ns					

3.4 Repellent activity of PG from crossbreed samples

At a dose of 50 ppm, the treated arm showed 93.33% repellency at the start of the assay (10 min), which increased to 100% at 40 min interval and remained constant for 130 min interval. Subsequently, the percent repellency began to decline and reached 70% at 200 min interval. At 100 ppm, the treated arm demonstrated 96.66% repellency at 10 min interval, which increased to 100% at 20 min interval and remained constant up to 140 min interval. The percent repellency then began to decline, reaching 73.33% at 200 min interval. Similarly, at a dose of 150 ppm, the treated arm demonstrated 100% repellency at the start of the assay (10 min), remained constant up to 160 min interval and then started to decline, reaching 80% at 200 min interval. During the course of the experiments, the control arm demonstrated mosquito entry rates of 13.33%, 7.66%, and 3.66% at maximum 200 min intervals for 50, 100, and 150 ppm concentrations, respectively, as the treated arm gradually attracted mosquitoes (Table 4). The mean percent repellency were recorded as 94.33% at 50 ppm, 95.49% at 100 ppm and 97.99% at 150 ppm. The calculated ED_{50} and ED_{90} values were 42.895 ppm and 111.333ppm, respectively (Fig. 2).

Table 4: Repellent efficacy of PG from crossbreed samples

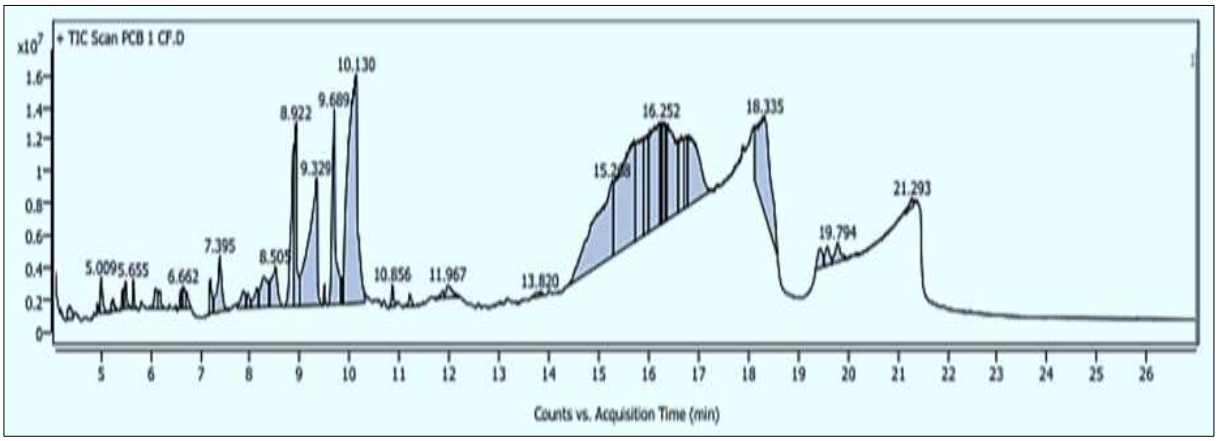
Exposure Time (Min)	**% Repellency (mean ± SD)**					
	Dose in ppm					
	Control	**50 ppm**	**Control**	**100 ppm**	**Control**	**150 ppm**
10	00.00±0.0	93.33±1.33	00.00±0.0	96.66±1.85	00.00±0.0	100.00±0.00
20	00.00±0.0	96.66±1.92	00.00±0.0	100.00±0.00	00.00±0.0	100.00±0.00
30	00.00±0.0	96.66±1.92	00.00±0.0	100.00±0.00	00.00±0.0	100.00±0.00
40	00.00±0.0	100.00±0.00	00.00±0.0	100.00±0.00	00.00±0.0	100.00±0.00
50	00.00±0.0	100.00±0.00	00.00±0.0	100.00±0.00	00.00±0.0	100.00±0.00
60	00.00±0.0	100.00±0.00	00.00±0.0	100.00±0.00	00.00±0.0	100.00±0.00
70	00.00±0.0	100.00±0.00	00.00±0.0	100.00±0.00	00.00±0.0	100.00±0.00
80	00.00±0.0	100.00±0.00	00.00±0.0	100.00±0.00	00.00±0.0	100.00±0.00
90	00.00±0.0	100.00±0.00	00.00±0.0	100.00±0.00	00.00±0.0	100.00±0.00
100	00.00±0.0	100.00±0.00	00.00±0.0	100.00±0.00	00.00±0.0	100.00±0.00
110	00.00±0.0	100.00±0.00	00.00±0.0	100.00±0.00	00.00±0.0	100.00±0.00
120	00.00±0.0	100.00±0.00	00.00±0.0	100.00±0.00	00.00±0.0	100.00±0.00
130	00.00±0.0	100.00±0.00	00.00±0.0	100.00±0.00	00.00±0.0	100.00±0.00
140	00.00±0.0	96.66±1.92	00.00±0.0	100.00±0.00	00.00±0.0	100.00±0.00
150	1.00±0.14	93.33±1.85	00.00±0.0	93.33±1.33	00.00±0.0	100.00±0.00
160	1.33±0.23	86.66±1.39	00.00±0.0	90.00±1.94	00.00±0.0	100.00±0.00
170	2.00±0.28	90.00±1.94	00.00±0.0	93.33±1.33	00.00±0.0	96.66±1.92
180	2.66±0.31	83.33±1.69	1.66±0.28	86.66±1.53	1.00±0.0	90.00±1.94
190	3.00±0.27	80.00±1.22	2.33±0.36	80.00±1.22	1.00±0.16	93.33±1.85
200	13.33±0.49	70.00±1.11	7.66±0.56	73.33±1.77	3.66±0.16	80.00±1.22
Mean % repellency	94.33±0.91		95.49±0.84		97.99±0.71	
Probit equation	y=1.1156+0.0156x					
χ^2 (df=31)	722.547					

3.5 GC-MS analysis of PG from indigenous and crossbreed samples

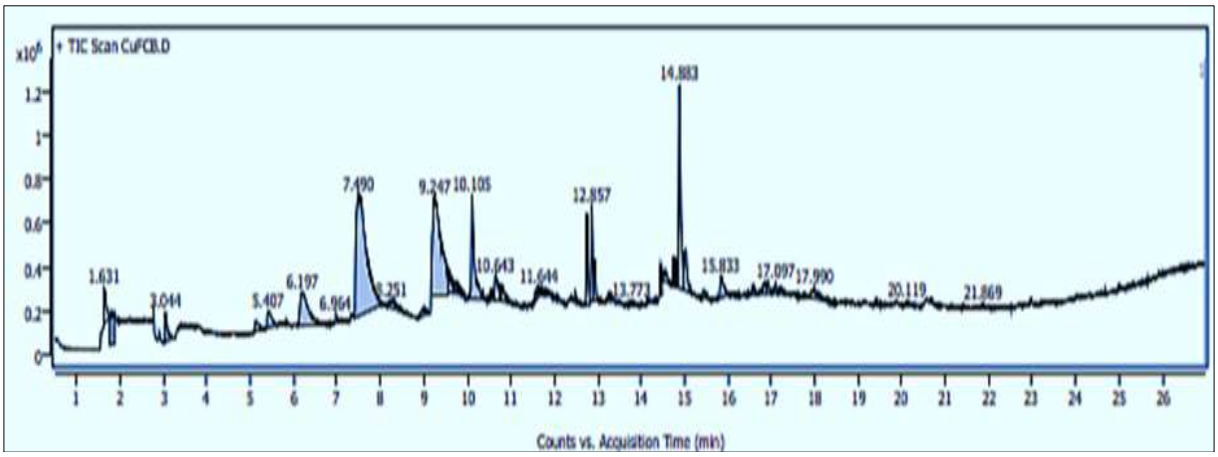
The GC-MS chromatogram of PG from the indigenous sample (Fig. 3a) indicated the presence of ethanol, 1-(2-methyl-2H-tetrazol-5-yl)-2-[(thiophen-2-ylmethyl)amino] with a peak area of 100%, 2-Izopropyl-4,6-dimethyl-1,3,2-oxathiaborinane with a peak area of 74.14%, 1,3,5-Trisilahexane, 5-methyl with a peak area of 68.14%, beta-D-glucosyloxyazoxymethane with a peak area of 56.30%, and benzoic acid silver salt with a peak area of 50.81%. The other major active compounds and their respective retention times (RTs), peak areas, and molecular formulas are presented in Table 5.

Similarly, the GC-MS chromatogram of the crossbreed

sample (Fig. 3b) indicated the presence of ethanol, 1-(2-methyl-2H-tetrazol-5-yl)-2-[(thiophen-2-ylmethyl) amino] with a peak area of 100%, benzoic acid silver salt with a peak area of 62.87%, trans-3-Methyl-2-n-propylthiophane with a peak area of 38.36%, trans-2-Methyl-4-n-butylthiane, S,S-dioxide with a peak area of 13.47% and 3-heptyl isothiocyanate with a peak area of 10.56%. The other major active compounds and their respective retention times (RTs), peak areas, and molecular formulas are presented in Table 6.



a)



b)

Fig 3: GC-MS chromatogram of PG; (a) GC-MS chromatogram of PG from indigenous samples; (b) GC-MS chromatogram of PG from crossbreed sample

Table 5: Active compounds identified by the GC-MS analysis of PG from Indigenous sample

Sr. No.	Retention time	Area (%)	Compound name	Formula
1.	5.009	3.81	trans-2,4-Dimethylthiane, S,S-dioxide	$C_7H_{14}O_2S$
2.	5.655	1.81	3-n-Butylthiolane	$C_8H_{16}S$
3.	6.662	2.65	Trisilane	H_8Si_3
4.	7.395	11.16	3,4-Octadiene, 2,2- dimethyl	$C_{10}H_{18}$
5.	8.282	10.23	Ethanethiol, 2- (diethylboryloxy)	$C_6H_{15}BO_S$
6.	8.505	11.30	Boronic acid, ethyl-, bis(2 mercaptoethyl ester	$C_6H_{15}BO_2S_2$
7.	8.865	19.22	trans-3-Methyl-2-n-propylthiophane	$C_8H_{16}S$
8.	8.922	22.03	1,2,4,5-Tetrazine-3,6- diamine, 1,4-dioxide	$C_2H_4N_6O_2$
9.	9.329	50.81	Benzoic acid, silver(1+) salt	$C_7H_5AgO_2$
10.	9.689	31.15	1,3,2-Dioxaborolane, 2- [(2- methylcyclohexyl)oxy]	$C_9H_{17}BO_3$
11.	10.130	100	Ethanol, 1-(2-methyl-2H-tetrazol-5-yl)-2-[(thiophen-2-ylmethyl)amino]	$C_9H_{13}N_5OS$
12.	10.856	2.08	1-.beta.-d-Ribofuranosyl-3- [5-tetraazolyl]-1,2,4-triazole	$C_8H_{11}N_7O_4$
13.	11.967	3.31	2-Azido-2,4,4,6,6- pentamethylheptane	$C_{12}H_{25}N_3$
14.	15.268	68.14	1,3,5-Trisilahexane, 5- methyl	$C_4H_{16}Si_3$
15.	15.703	74.14	2-Izopropyl-4,6-dimethyl-1,3,2-oxathiaborinane	$C_8H_{17}BOS$
16.	15.886	31.46	.beta.-D-Glucosyloxyazoxymethane	$C_4H_{16}Si_3$
17.	16.252	7.82	1,5-Anhydroglucitol	$C_6H_{12}O_5$
18.	16.373	35.54	2,5-O-Methylene-D-mannitol	$C_7H_{14}O_6$

19.	16.682	16.66	1,5-Anhydroglucitol	$C_6H_{12}O_5$
20.	16.744	9.35	1,5-Anhydro-d-mannitol	$C_6H_{12}O_5$
21.	16.796	34.07	Pentanoic acid 1- methylpropyl ester	$C_9H_{18}O_2$
22.	18.335	56.30	beta.-D-Glucosyloxyazoxymethane	$C_8H_{16}N_2O_7$
23.	19.794	4.79	Diethylene glycol, isobutyl ether, trimethylsilyl ether	$C_{11}H_{26}O_3Si$
24.	21.293	1.74	D-Mannitol	$C_6H_{14}O_6$

Table 6: Active compounds identified by the GC-MS analysis of PG from crossbreed sample

Sr. No.	Retention time	Area (%)	Compound name	Formula
1.	4.482	8.25	4-(Pyrrolidin-1-yl)isothiazolidine 1,1-dioxide	$C_7H_{14}N_2O_2S$
2.	4.717	4.16	Cyclobutane, 1,2:3,4-di-O-ethylboranediyl	$C_8H_{14}B_2O_4$
3.	5.506	13.47	trans-2-Methyl-4-n-butylthiane, S,S-dioxide	$C_{10}H_{20}O_2S$
4.	6.038	5.66	trans-3-Methyl-2-n-propylthiophane	$C_8H_{16}S$
5.	6.880	2.20	2-Azido-2,4,4,6,6-pentamethylheptane	$C_{12}H_{25}N_3$
6.	7.612	10.56	3-Heptyl isothiocyanate	$C_8H_{15}NS$
7.	8.133	3.17	2-Isobutoxy-4-methyl-[1,3,2] dioxaborinane	$C_8H_{17}BO_3$
8.	8.831	1.33	Ethanethiol, 2-(diethylboryloxy)-	$C_6H_{15}BOS$
9.	9.168	38.36	trans-3-Methyl-2-n-propylthiophane	$C_8H_{16}S$
10.	9.214	15.60	1,2,4,5-Tetrazine-3,6- diamine, 1,4-dioxide	$C_2H_4N_6O_2$
11.	9.632	62.87	Benzoic acid, silver(1+) salt	$C_7H_5AgO_2$
12.	10.479	100	Ethanol, 1-(2-methyl-2H-tetrazol-5-yl)-2-[(thiophen-2-ylmethyl)amino]	$C_9H_{13}N_5OS$
13.	11.446	3.55	4,5,6,7- Tetrahydroxydecyl isothiocyanate	$C_{11}H_{21}NO_4S$
14.	12.750	1.82	3H-Pyrazole-3-carboxylic acid, 4,5-dihydro-5,5-di-t-butyl-, methyl ester	$C_{13}H_{24}N_2O_2$
15.	13.918	1.07	1-.beta.-d-Ribofuranosyl-3-[5- tetraazolyl]-1,2,4- triazole	$C_8H_{11}N_7O_4$
16.	15.806	5.54	3H-Pyrazole-3- carboxylic acid, 4,5- dihydro-5,5-di-t-butyl-, ethyl ester	$C_{14}H_{26}N_2O_2$
17.	16.767	3.41	Methyl 12,13-tetradecadienoate	$C_{15}H_26O_2$
18.	19.302	1.36	d-Galactono-1,4- lactone, 5,6-O- (ethylboranediyl)-	$C_8H_{13}BO_6$
19.	21.568	1.01	Nona-2,3-dienoic acid, ethyl ester	$C_{11}H_{18}O_2$

4. Discussion

4.1 Larvicidal and pupicidal activity of PG

The most effective approach to managing *Aedes* mosquitoes is to target their earlier developmental stages, particularly their larvae and pupae. The findings of present study demonstrated that PG from indigenous samples displayed a more significant lethal effect on II instar, III instar, and IV instar, as well as pupae than the PG from crossbreed samples in a dose-dependent and time-dependent manner. The reported LC_{50} value for the PG from indigenous samples against II instar, III instar, IV instar and pupae was approximately 1.7- to 3.4-fold lower than those of the PG from crossbreed samples. Similarly, the LC 90 value was approximately 1.1-to 1.3-fold lower than those PG from crossbreed samples. This indicate that the PG from indigenous samples were effective in achieving 50% and 90% larval motility at lower doses than PG from crossbreed samples. This enhanced larvicidal and pupicidal effect may be attributed to the synergistic actions of their bioactive components (Ozege and Omoregie, 2022) [29], which could potentially cause structural and functional damage to larvae and pupae thus, impaired larval survival in present study. The larval phase is a feeding stage, so it is possible that larvae ingest active compounds during treatment, which may cross passively to insect mid-gut and induced extensive damage to its cellular composition (Sharma *et al*., 2022) [35]. On the other side, pupae stage is a non-feeding phase, it is possible that during treatment, active xenobiotic molecules may come into direct contact with the pupae cuticle membrane, leading to disruption of their cellular architecture (Bouabida and Dris, 2022) [8]. The current findings are more convenient than those of a recent study where PG exhibited substantial larval mortality against II Instar, III instar, and IV instar larvae and pupae of *Aedes aegypti*, (Sathiyaraj *et al*., 2022) [32]. In a previous study, complete larval mortality was achieved within 24 h when 15% cow urine samples were used against *Culex quinquefasciatus* (Kumar *et al*., 2009) [19].

4.2 Repellent activity of PG

Mosquito repellents have emerged as one of the exceptional way to prevent the spread of vector-bone diseases and mitigate the disquiet caused by mosquito bites. It is widely recognized that natural repellents are considered to be safer, more effective, environmentally friendly, and more easily accessible than their synthetic counterparts (Nogueira Barradas *et al*., 2016; Iliou *et al*., 2019) [25, 14]. In the present study, 100% repellency was reported up to 140 min, 160 min, and 170 min intervals at 50, 100, and 150 ppm dose of PG from indigenous samples, respectively. However, for crossbreed samples, it was up to 130 min, 140 min, and 160 min intervals at 50, 100, and 150 ppm dose, respectively. The reported ED50 and ED90 values for PG from indigenous samples was approximately 5.3-fold and 3.3-fold lower than that of for PG from crossbreed samples. These findings indicate that the PG from indigenous samples was more effective in achieving 50% and 90% repellency at lower doses than the PG from crossbreed samples. It is believed that PG act as repellent agents due to their volatile active substances, which can create toxic vapor barriers that mosquitoes find unpleasant (Legeay *et al*., 2018; da Silva and Ricci-Júnior, 2020) [21, 11], resulting in complete inhibition of mosquitoes landing on the treated arms. The results of the current investigation are align with those of a previous study that examined the repellent efficacy of 21 active ingredients from the Environmental Protection Agency (EPA) 25(b) list using a Y-tube olfactometer, with significantly reduced *Aedes aegypti* mosquito attraction between 60 and 120 min (Mitra *et al*., 2020) [22]. A recent investigation evaluated the repellent effects of unsaturated aldehydes derived from cattle on three disease-carrying mosquito species *Aedes aegypti, Anopheles coluzzii*, and *Culex quinquefasciatus*. The results

demonstrated that the repellent response to these aldehydes was stronger than that of commercially available repellents including DEET, IR3535, PMD, icaridin, and d-allethrin (Isberg and Ignell, 2022) [16]. In a previous study conducted using a Y-tube olfactometer, volatile compounds derived from Holstein-Friesian heifers were evaluated for their ability to elicit an olfactory response in *Haematobia irritans*. The findings revealed that variations in attractiveness can occur in the interactions between *H. irritans* populations and volatile compounds (Oyarzún *et al*., 2009) [28].

4.3 GC-MS analysis of PG from indigenous and crossbreed samples

The GC-MS technique combines the separation efficiency of gas-liquid chromatography with the detection capabilities of mass spectrometry to identify different volatile substances in the test samples. This method offers numerous advantages, including increased molecular ion content, improved sample identification confidence, a broader range of thermally labile and low-volatility samples, faster analysis, and enhanced sensitivity, among other features that make it ideal for various applications (Zeki *et al*., 2020) [44].

The results of GC-MS analysis confirmed the occurrence of several active components in PG from both indigenous and crossbreed samples, at varying concentrations and retention times. The ethanol, 1-(2-methyl-2H-tetrazol-5-yl)-2-[(thiophen-2-ylmethyl) amino ($C_9H_{13}N_5OS$) was the major contributors to PG from both indigenous and crossbreed samples with a peak area of 100%**.** This compound belongs to a class of bioactive transition metal complex ethanolamines, which have received growing interest in the field of modern pharmacology, particularly as potential therapeutic and cytotoxic agents (Amjad *et al*., 2016; Saturnino *et al*., 2017; Kaval and Garsin, 2018) [4, 34, 18].

The most common compounds in the PG from both indigenous and crossbreed samples was benzoic acid silver salt or benzoate ($C_7H_5AgO_2$), accounting for more than 50% of the peak area in both tested samples. Benzoate, an ester or salt of benzoic acid, is a strong aromatic compound with significant antimicrobial and biopesticidal potential (O'Beirne *et al*., 2019; Aboelhadid *et al*., 2023) [1]. In addition to these compounds, several fatty alcohols and esters, including nona-2, 3-dienoic acid ethyl ($C_{11}H_{18}O_2$), methyl 10, 11 tetradecadienoate ($C_{15}H_{26}O_2$), and methyl 9, 10 octadecadienoate ($C_{19}H_{34}O_2$), with peak areas ranging from 5 to 40%, were commonly reported PG from indigenous and crossbreed samples. These compounds play a key role in energy metabolism and possess strong antimicrobial and antioxidant potentials (Paul *et al*., 2022; Mohiuddin *et al*., 2022; Tareq *et al*., 2023) [31, 23, 36]. The findings of the current study are consistent with those of previous studies that have reported several volatile compounds in the urine of dairy cows at various physiological stages using GC-MS analysis (Ramesh Kumar *et al*., 2000; Le Danvic *et al*., 2015; Ahamad *et al*., 2017) [2]. A recent investigation of panchgvaya samples by Sathiyaraj *et al*. (2022) [32] revealed the existence of various bioactive compounds, including 1, 2-propanediol diformate, 2-butanol, 3-chloro-, di-n-propylmalonic acid, and acetaldehyde tetramer, with peak areas ranging from 10 to 15%. Correspondingly, Nutiyal and Dubey (2021) documented the presence of several long-chain fatty alcohols in cow urine, comprising 1-Heneicosanol, n-Heptadecanol-1, and n-Nonadecenol-1, with peak areas of 37.91, 19.05, and 17%, respectively.

5. Conclusion

The PG from the indigenous samples exhibited a more pronounced lethal effect against the II, III, and IV instar larvae and pupae of *Aedes aegypti* (L.), with lower LC_{50} and LC_{90} values than the crossbreed samples tested. This suggests that the indigenous PG has the potential to serve as a natural larvicidal and pupicidal agent at lower doses. The with lower ED_{50} and ED_{90} values than the crossbreed PG, indigenous PG acts as potent repellent agent at lower doses. These activities is due to the presence of various novel and common bioactive compounds. Further detailed mechanistic studies should be conducted to elucidate the mechanism underlying the larvicidal, pupicidal and repellent activity observed in PG. Novel fractions of the PG have to isolated and tested further.

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