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Insides into molecular structural elucidation on the pesticidal and herbicidal potency of AD biogas slurry

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ABSTRACT

Biogas slurry is the residue liquid after anaerobic fermentation of biomass. The bio-slurry has the potential to promote plant growth and control pests. This study explores the pesticidal potency of anaerobic digestate focusing on structural elucidation through gas chromatography–mass spectrometry analysis. Samples were collected from three anaerobic digesters processing cow dung, mixed substrates, and pig waste. Physicochemical parameters such as pH, electrical conductivity, total dissolved solids, and nitrogen content were measured. The slurries exhibited slightly basic pH values (7.5–7.8) and electrical conductivity (13.8–21.9 mS/m). GC–MS analysis revealed diverse phytochemical compounds in the bio-slurries, such as gramine, 2,2-dimethoxybutane, and 4-methyl-3-penten-2-one, with potential pesticidal properties. Gramine exhibited insecticidal, herbicidal, and algaecidal effects, while 2,2-dimethoxyethane demonstrated fungicidal and herbicidal properties. Gramine was identified and possesses its potential as a natural biopesticide. The identified compounds offer promising alternatives to synthetic pesticides, emphasizing the potential of biogas slurry as a sustainable biopesticide resource.

1. Introduction

Agriculture is a crucial national growth industry affecting people's core interests (Pretty et al., 2010). Since the 20th century, pesticides have been employed to increase crop yields. Although they have significantly increased agricultural productivity, many drawbacks have been identified (Tudi et al., 2021). At the same time, they are being used to seriously harm the natural environment, which includes deterioration of soil properties, pollution of water resources, an increase in pest resistance and damage to people's health (Sarkar et al., 2021; Tudi et al., 2021). This has resulted in the innovative application of organic wastes in agriculture as an alternative to traditional pesticides. Among these are anaerobic digestion biogas slurries.

Biogas slurry (BGS) is the waste product produced by anaerobic biomass fermentation, and it promotes the development and reproduction of animals and plants (Zheng et al., 2012), while also controlling insects owing to its high concentration of nutrients and ammonia nitrogen (Kumar et al., 2023). Biogas slurry is highly effective in controlling aphids and red spiders in fruits and vegetables (Nyakeyo, 2023). Many research studies have reported that bio-slurry possesses pesticide properties (Kebede et al., 2022). For example, Jothi et al. (2003) examined bio-slurry vs. commercial pesticides in a controlled tomato root-knot nematode infection. According to his research, at a dose of 10 % of soil weight, the bio-slurry could suppress the nematode infection more successfully than the commercial pesticide. Even at 5 %, the bio-slurry outperformed the pesticide in several ways (Jothi et al., 2003).

Similarly, Xiao et al. (2007) investigated the effects of anaerobically digested swine dung on soybean cyst nematode (SCN) egg suppression. They focused the analysis on two components of swine bio-slurry: ammonium (NH_4^+) and volatile fatty acids (VFAs), which they found to be active in SCN (Acharya, 2020). They concluded that SCN egg counts decreased as manure doses increased in soil samples collected 35 days after receiving the manure treatments (raw, VFA-enriched, and NH_4^+ enriched manure (Xiao et al., 2007).

Furthermore, BGS was effectively examined for nematicidal, antifungal, termite repellant, and other related actions in a different study. BGS repelled termites and inhibited weed development by roughly 50 %, and its compost has been shown to boost cereal crop productivity by 10–30 % over FYM when kept and applied appropriately (Kumar et al., 2023). With an emphasis on hydrolytic enzymes, biopesticides, and biosurfactants, Cerda et al. (2019) examined the use of solid-state fermentation (SSF) for the valorisation of bio-waste digestate (Cerda

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et al., 2019). Kupper et al. (2006) investigated the possibility of bio-slurry usage viz. the conventional fungicide treatment (copper oxychloride and carbendazim + mancozeb) for managing the citrus black spot causative agent, *Phyllosticta citricarpa*. He sprayed Natal Orange trees with bio-slurry from a digester fed by bovine feces (a bio-slurry mixture was mixed with water and sprayed on the plants) (Kupper et al., 2006). At dosages of 10–20 % in water, bio-slurry had a considerable impact on the management of citrus black spots. Nonetheless, from the study, synthetic fungicide proved to be more successful than bio-slurry treatment, as evidenced by the fact that 95 % of the fruits showed no signs of black spot (Troncoso-Rojas and Tiznado-Hernández, 2014).

According to a similar study by Shekhar (2018), the bio-slurry could be a viable substitute for the fungicide in the management of citrus black spots; nevertheless, further research was required to establish the optimal dosage of the bio-slurry and interval of administration. However, the impact of the bio-slurry on *Fusarium graminearum*, or wheat scab, was found to be effective (Shekhar, 2018).

Research shows that entomopathogenic bacteria are the source of 90 % of commercial biopesticides (Thakur et al., 2020). These biopesticides are more effective at low doses, better targeted at specific pests, and quickly break down without leaving harmful residues than traditional chemical pesticides (Agboola et al., 2022; Gupta and Dikshit, 2010). Thus, there can be significant financial gains from the complete and sensible use of biogas slurry. Several places have started using clean, inexpensive biogas slurry directly to control insects, and it has been discovered to be equally effective as other synthetic pesticides (Mohapatra et al., 2015). Organic fertilisers made from biogas slurries and manure are enriched with ammonium salts, B vitamins, and trace antibiotics, contributing to nutrient supply and pest control. Compared to conventional pesticides, a 26.2 % increase in aphid control on citrus trees when using biogas slurry was achieved (Srivastava, 2023). To increase insecticidal efficacy even further, biogas slurry is frequently combined with additional insecticides. Most of the reviewed literature work has focused on the tests to verify the bio-slurries' potential as biopesticides; however, this study aimed at identifying the molecules and the molecular structures that present the pesticidal and herbicidal potency in AD biogas slurry and its derivatives using gas chromatography (GC) coupled with a mass spectrophotometer (MS).

2. Materials and methods

2.1. Sample collection

The samples were collected from three AD biogas plants in a mesophyll environment. The fresh slurry samples were fetched from the outlet flow channel of the digester and kept tightly in three plastic sample-holding bottles. They were then transported to the Maasai Mara University laboratory, where they were kept at room temperature, awaiting analysis and characterization. The AD biogas plants were of different feedstock: cow dung AD biogas plant, mixed substrates composed of kitchen waste, cow dung droppings, poultry waste AD biogas plant, and pig waste AD biogas plant. The anaerobic biogas digesters were all located along the Narok-Bomet road, Narok County, Kenya (1°05′22.0″S 35°49′19.8″E for cow dung, 1°05′24.4″S 35°49′22.6″E for mixed waste and, 1°05′23.4″S 35°49′21.1″E for pig waste bio-slurry).

2.2. Chemicals and reagents

The chemicals and reagents used were Methyl Orang of pure grade indicator, sulfuric acid (H_2SO_4·99.99 %), sodium hypobromite (NaOBr ≥99.0 %), sodium hydroxide (NaOH ≥97.0 %), and distilled water (H_2O). All the chemicals were purchased from Sigma Aldrich.

2.3. Determination of physicochemical parameters

2.3.1. pH

The pH of the bio-slurry samples was measured in situ using the Universal Multiline Hanna pH 210 Microprocessor (Hanna G-114, Germany). This was done according to a procedure used by (Kinaichu et al., 2021) with slight adjustments.

2.3.2. Electrical conductivity of the bio-slurry

The bio-slurry sample solutions were first filtered for electric conductivity measurement using the Whatman 42 filter paper. The method followed was used by (Kinaichu et al., 2021) in their research, but with some changes. The obtained filtrate was then measured for EC using the INE-EC110B Portable Conductivity EC Meter. The measurements were performed in triplicates for each bio-slurry sample.

2.3.3. Total dissolved solids (TDS)

Total Dissolved Solids (TDS) for the bio-slurry samples was determined by heating an empty evaporating dish for an hour at 180 ± 2 °C and then cooling for 40 min (Ayalew, 2017). Before usage, it was weighed and kept in a desiccator. Briefly, a 50 mL bio-slurry sample was measured and transferred to the evaporating dish. Before evaporation, the samples were filtered via filter paper (Whatman number 42). After the excess water evaporated during the drying process, the dish and its contents were put into an oven preheated to 103 °C. They were weighed after an hour of drying at 150 ± 2 °C in a desiccator and after an hour of chilling. This heating and cooling process was repeated five times until a constant mass was reached. The concentration of total dissolved solids in milligrams per liter was then calculated using the mathematical expression given in Eq. (1).

$$\text{Total dissolved solids}, \frac{mg}{L} = \frac{(\text{A} - \text{B})1000}{\text{Sample volume}, \text{mL},} \quad (1)$$

where A is the weight of dried residue plus dish (mg), and B is the weight of dish (mg).

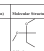
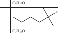
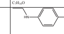
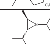
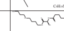
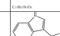
2.4. Total nitrogen determination

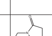
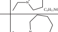
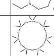
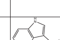
The total nitrogen content in the bio-slurry was measured using the Kjeldahl method with slight modifications (Bremner, 1960). The bio-slurry sample, amounting to 50.0 mL, was meticulously placed in a suitable container. An alkaline digestion step was incorporated to enable precise nitrogen analysis, introducing an alkaline (10.0 mL of solution containing 50 g/L sodium hydroxide (NaOH) and 400 g/L sodium hypobromite NaOBr). This digestion process was essential, aiming to convert all nitrogen-containing species in the bio-slurry into ammonia. Following this, the bio-slurry underwent steam distillation. The resulting distillate was meticulously collected in a receiving flask containing a predefined volume of sulfuric acid (0.020 N H_2SO_4). Subsequently, the titration phase commenced, which involved the titration of the collected distillate (30 mL) using a standardized sulfuric acid solution. The titration reaction encompassed the neutralization of ammonia by the acid, leading to the formation of ammonium ions. The determination of the titration endpoint, symbolizing the completion of the reaction, was conducted using a methyl orange indicator. The color change from yellow to red in the solution indicated the point at which all ammonia reacted. Eq. (2) below was used to calculate the total nitrogen content.

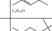
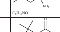
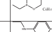
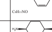
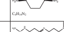
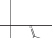
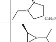
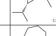
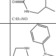
$$TKN, \frac{mg}{L} = \left\{ \frac{[(A - B)N\ x\ F\ x\ 1000\,]}{S} \right\} \quad (2)$$

where A is the standard 0.020 N H_2SO_4 solution used in the titrating sample (mL), B is the standard 0.020 N H_2SO_4 solution used in the titrating blank (mL), N is the normality of sulfuric acid solution, F represents the weight nitrogen (14 mg), and S is the milliliters of sample digested.

Table 1
Physicochemical properties.

Cow-dung Bio-slurry		Physicochemical Parameters				
		pH	E.C(mS/m)	TDS (ppm)	Total N (g/L)	
		7.80±0.02	13.84±0.02	8673±0.002	14.142±0.001	
Cow-dung Bio-slurry AD bio-slurry GC-MS molecules identified						
S/N	RT(min)	Molecular Structure/Formula	Area%	Name	Derivatives	Pesticidal properties
1	3.300	 $C_6H_{14}O_2$	0.68	2,2-Dimethoxybutane	Dimethoxycarbamate Dimethoxybutylamine Dimethoxybutyl acetate Dimethoxybutyl ether	Fungicide Antibacterial Herbicide
2	3.855	 $C_6H_{10}O$	6.50	3-Penten-2-one, 4-methyl-	4-hydroxy-4-methyl-2-pentanone	Insect repellant (fruit flies)
3	4.475	 $C_7H_{16}O$	82.10	2-Hexanol, 2-methyl-	N/A	N/A
4	6.287	 $C_8H_{11}NO$	2.64	Phenol, 4-(ethylamino)-	N/A	Herbicide
5	7.004	 $C_6H_{14}N_2$	0.69	cis-2,5-Dimethylpiperazine	triforine	Herbicide Fungicidal Antibacterial Insecticide Active against plant viruses
6	8.917	 $C_9H_{17}NO$	1.64	4-Piperidinone, 2,2,6,6-tetramethyl-	N-(hydroxyalkyl)-4-chlorophenoxyacetamide 5-butyl-2-ethylamino-6-methylpyrimidin-4-yldimethylsulphamate 2,2,6,6-tetramethylpiperidine-1 -oxyl	Herbicide Fungicide Insect repellant Antibacterial
7	9.333	 $C_7H_{15}N$	1.63	2-Methyl-1-ethylpyrrolidine	N/A	N/A
8.	9.681	 $C_9H_{19}N$	0.60	Aziridine, 1,2-diisopropyl-3-methyl-, trans-	Triazolines	Insecticide
9.	10.798	 $C_{17}H_{37}N_7O_3$	0.95	Deoxyspergualin	N/A	N/A
10.	16.471	 $C_{11}H_{14}N_2$	2.58	Gramine	Gramine	Antibacterial Antifungal Herbicide Algaecide

Mixed waste Bio-slurry		Physicochemical Parameters				
		pH	E. C(mS/m)	TDS (ppm)	Total N (g/L)	
		7.58±0.01	21.88±0.04	6050±0.002	16.247±0.000	
Mixed waste Bio-slurry AD bio-slurry GC-MS molecules identified						
S/N	RT(min)	Molecular Structure/ Formula	Area%	Name	Derivatives	Pesticidal properties
1	3.291	 $C_6H_{14}O_2$	0.56	2,2-Dimethoxybutane	Dimethoxycarbamate Dimethoxybutylamine Dimethoxybutyl acetate Dimethoxybutyl ether	Fungicide Antibacterial Herbicide
2	3.849	 C_7H_{14}	2.86	2-Pentene, 4,4-dimethyl-, (Z)-	N/A	N/A
3	4.467	 $C_7H_{16}O$	91.87	2-Hexanol, 2-methyl-	N/A	N/A
4	5.657		1.00	2-Heptanol, acetate	N/A	N/A
		$C_9H_{18}O_2$				
5	9.332	 $C_6H_{11}NO$	0.84	1-Ethyl-2-pyrrolidinone	2-(nitromethylene)pyrrolidines	Insecticide
6	10.804	 $C_8H_{17}N$	0.35	N-Ethyl-hexahydro-1H-azepine	Molinate	Herbicide
7	12.689	 $C_{14}H_{42}O_7Si_7$	1.05	Cycloheptasiloxane, tetradecamethyl-	Octasiloxane, 1,1,3,3,5,5,7,7,9,9,11,11,13,13,15,15-hexadecamethyl-Heptasiloxane, 1,1,3,3,5,5,7,	Insecticide
					7,9,9,11,11,13,13-tetradecamethyl-Cyclooctasiloxane, hexadecamethyl-Cyclohexasiloxane, Dodecamethyl-(D6)	
8	16.473	 $C_{11}H_{14}N_2$	1.47	Gramine	Gramine	Antibacterial Antifungal Herbicide Algaecide

Pig- waste Bio-slurry		Physicochemical Parameters				
		pH	E.C(mS/m)	TDS (ppm)	Total N (g/L)	
		7.70±0.01	21.60±0.05	1534±0.001	12.281±0.001	
Pig waste Bio-slurry AD bio-slurry GC-MS molecules identified						
S/N	RT(min)	Molecular Structure	Area%	Name	Derivatives	Pesticidal properties
1	3.290	 $C_6H_{14}O_2$	0.81	2,2-Dimethoxybutane	Dimethoxycarbamate Dimethoxybutylamine Dimethoxybutyl acetate Dimethoxybutyl ether	Fungicide Antibacterial Herbicide
2	3.850	 $C_6H_{10}O$	10.82	3-Penten-2-one, 4-methyl-	4-hydroxy-4-methyl-2-pentanone	Insect repellant (fruit flies)
3	4.473	 $C_7H_{16}O$	60.36	2-Hexanol, 2-methyl-	N/A	N/A
4	4.914	 $C_6H_{13}NO$	1.91	2-Pentanone, 4-amino-4-methyl-	N/A	N/A
5	5.484	 $C_7H_{14}O_2$	0.59	2-Pentanone, 4-methoxy-4-methyl-	N/A	N/A
6	5.738	 $C_6H_{15}BO_2$	0.33	Boronic acid, ethyl-, diethyl ester	N/A	N/A
7	6.231	 $C_8H_{11}NO$	7.84	Phenol, 4-(ethylamino)-	N/A	N/A
8	6.954	 $C_6H_{14}N_2$	5.28	1,4-Cyclohexanediamine, cis-	N/A	N/A
9	8.012	 $C_8H_{17}N$	0.85	N-[3-[N-Aziridyl]propylidene]-3-dimethylaminopropylamine	N/A	N/A
10	8.908	 $C_9H_{17}NO$	8.65	4-Piperidinone, 2,2,6,6-tetramethyl-	N-(hydroxyalkyl)-4-chlorophenoxyacetamide, 5-butyl-2-ethylamino-6-methylpyrimidin-4-yldimethylsulphamate, 2,2,6,6-tetramethylpiperidine-1 -oxyl	Herbicide Fungicide Insect repellant Antibacterial
11	9.333	 $C_6H_{11}NO$	0.58	1-Ethyl-2-pyrrolidinone	2-(nitromethylene)pyrrolidines	Insecticide Herbicide Fungicide
12	9.676	 $C_9H_{19}N$	0.98	Aziridine, 1,2-diisopropyl-3-methyl-, trans-	Triazolines	Insecticides
13	10.797	 $C_7H_{13}NO$	0.82	2H-Azepin-2-one, hexahydro-6-methyl-	N/A	N/A
14	15.547		0.18	Pyrrolidine-2-carboxylic acid, methyl-phenyl-amide	N/A	N/A
		$C_{12}H_{16}N_2O$				

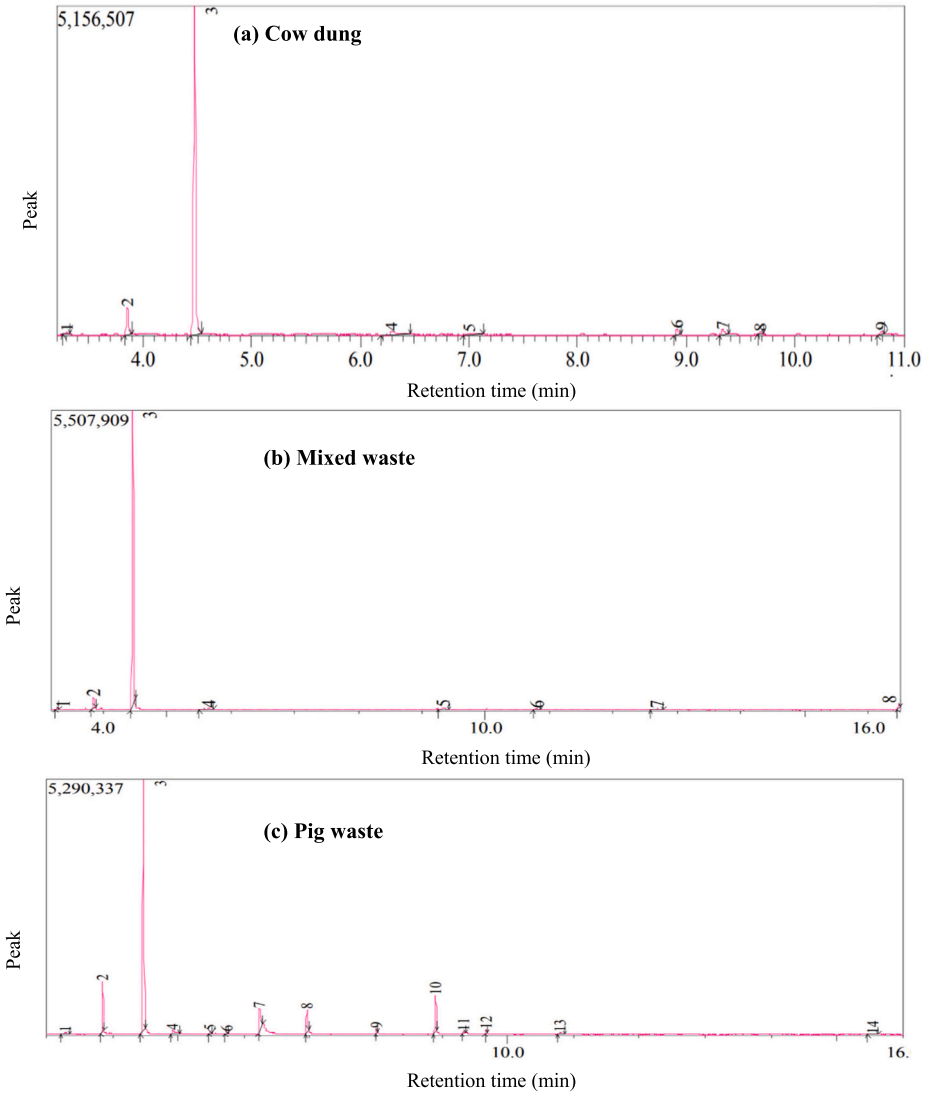


Fig. 1. AD bio-slurry GC spectra of (a) Mixed waste, (b) cow dung waste, and (c) pig waste.

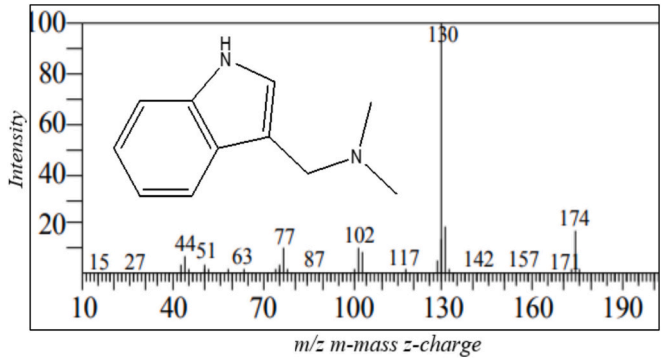


Fig. 2. Mass spectrum of Gramine (*N*,*N*-dimethyl-1*H*-indole-3-methylamine) with retention time (RT) ≈ 16.47.

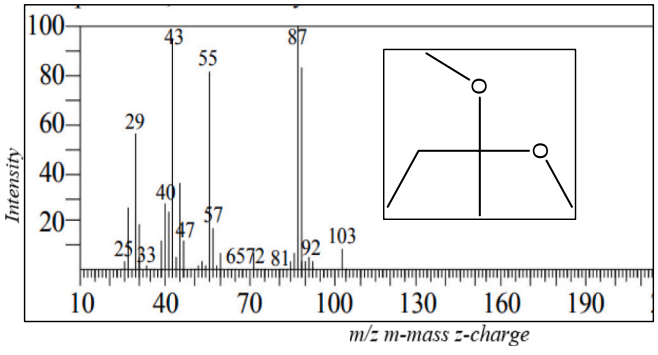


Fig. 3. Mass spectrum of 2,2-Dimethoxybutane with retention time (RT) ≈ 3.290.

2.5. Gas chromatography-mass spectrophotometer analysis

The GC–MS analysis of the study was done using a method by Yu et al. (2008) with slight modifications. In the preliminary stage, bio-slurry samples underwent hydrocarbon extraction using hexane, which was succeeded by solid phase extraction (SPE) with water as the polar solvent. This tailored procedure ensured the effective separation and purification of the bio-slurry components. The SPE process was executed on a specialized, highly retentive alkyl-bonded silica phase, ideal for the unique characteristics of bio-slurry samples encompassing non-polar to moderately polar compounds. The silica phase,

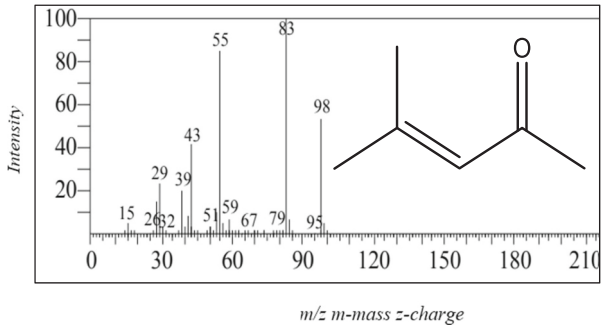


Fig. 4. Mass spectrum of 4-methyl-3-penten-2-one with retention time (RT) ≈ 3.850.

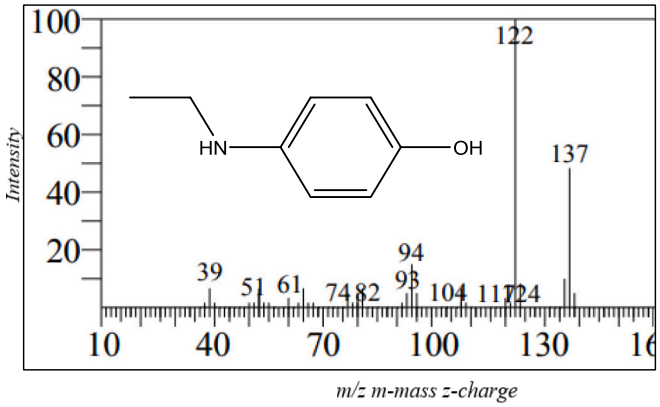


Fig. 5. Mass spectrum of 4-(ethylamino)-Phenol, with retention time (RT) ≈ 6.287.

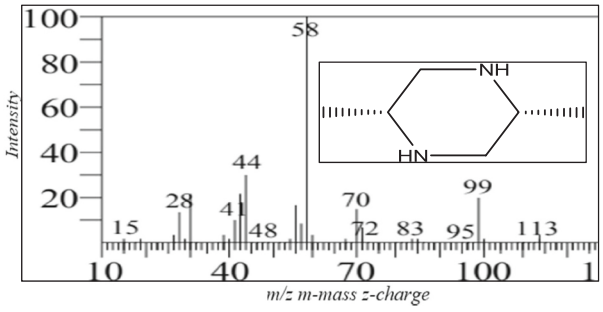


Fig. 6. Mass spectrum of cis-2,5-Dimethylpiperazine with retention time (RT) ≈ 7.004.

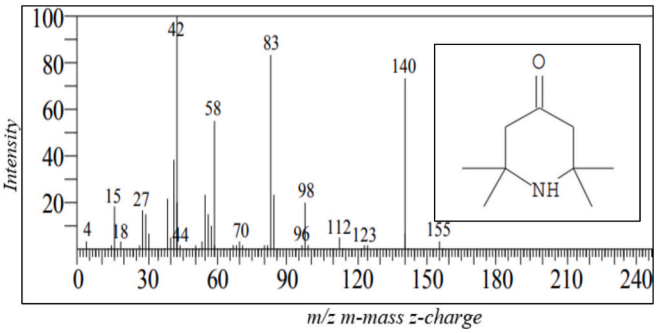


Fig. 7. Mass spectrum of 2,2,6,6-Tetramethyl-4-piperidone with retention time (RT) ≈ 8.917, 8.908.

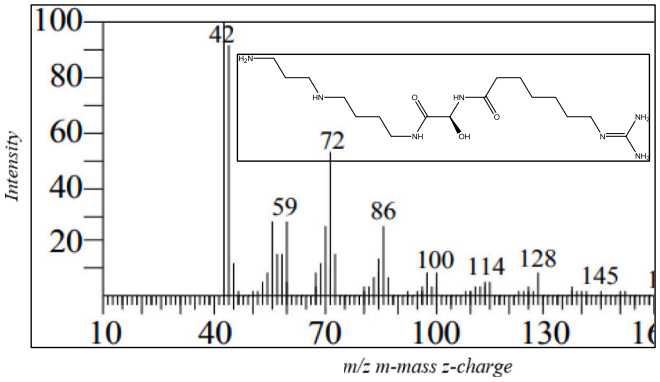


Fig. 8. Mass spectrum of Aziridine, 1,2-diisopropyl-3-methyl-, trans- with retention time (RT) ≈ 9.676.

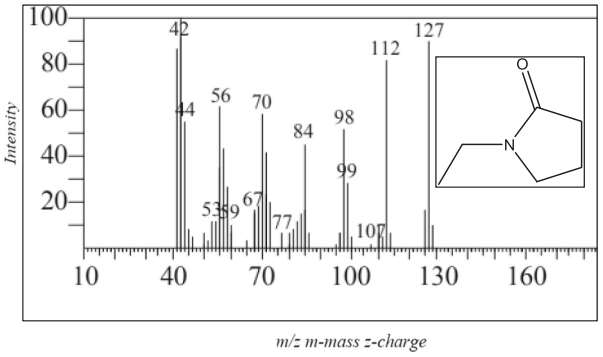


Fig. 9. Mass spectrum of 1-Ethyl-2-pyrrolidinone with retention time (RT) ≈ 9.33.

characterized by a generous surface area ranging from 470 to 530 m^2/g, a particle size of 40 to 60 μm, and a pore size of 60 Å, was chosen to accommodate the specific properties of bio-slurry matrices. These optimized conditions enhanced the efficiency of hydrocarbon extraction and purification from bio-slurry samples.

The analysis was performed using the Shimadzu GCMS-QP2010 SE instrument. Before analysis, control parameters were tailored to address the unique characteristics of bio-slurry matrices. The column oven temperature was initially set at 50 °C, ensuring an optimal starting point for the separation process. Injection of the bio-slurry samples occurred at an elevated temperature of 200 °C in a split mode, where the linear velocity flow control played a crucial role in achieving efficient separation. The pressure was maintained at 9.4 psi, and the total flow reached 13.2 mL/min, with specific attention to the column flow at 0.93 mL/min and a purge flow of 3.0 mL/min. The split ratio was carefully adjusted to 10.0 to enhance sample introduction efficiency. Carrier gas saver features were activated, with a split ratio of 5.0 during a 1.00 min interval, demonstrating a thoughtful approach to resource utilization. The temperature program of the column oven exhibited a strategic gradient, starting at 50 °C for 1 min, followed by a ramp rate of 10 °C/min, eventually reaching a final temperature of 300 °C and maintaining it for 5.00 min. This temperature program aimed to facilitate the optimal separation of biofuel molecules within the bio-slurry samples.

The readiness of critical components, such as the column oven, split 1 (SPL1), and the mass spectrometer (MS), was verified to ensure the system's preparedness for the unique challenges posed by bio-slurry samples. Detector status checks, including the assessment of baseline

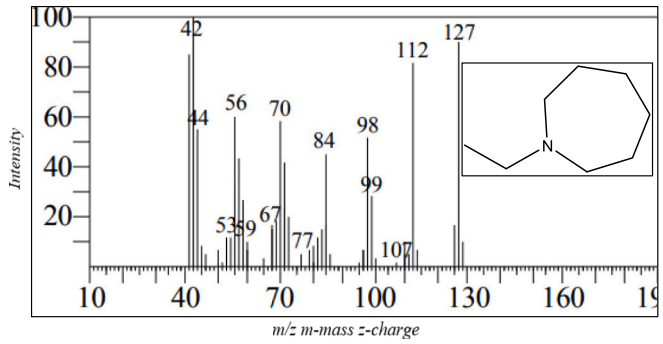


Fig. 10. Mass spectrum of N-Ethyl-hexahydro-1H-azepine with retention time (RT) ≈ 10.80.

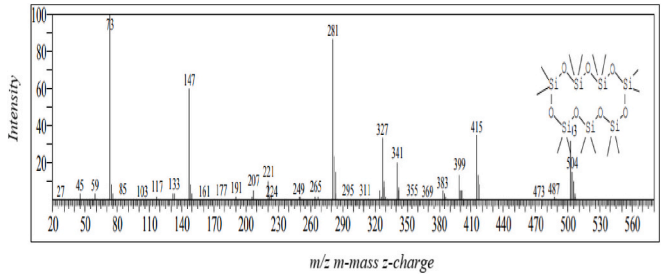


Fig. 11. Mass spectrum of Cycloheptasiloxane, tetradecamethyl- with retention time (RT) ≈ 12.689.

drift, confirmed the reliability of the data generated during the analysis.

3. Results and discussions

3.1. Physicochemical properties

The pH levels of the three AD bio-slurry samples from the findings are slightly basic and close to neutral, with values of 7.8 ± 0.02, 7.58 ± 0.01, and 7.7 ± 0.01 for mixed waste, cow dung slurry, and pig waste bio-slurry, respectively (Table 1). These values are within the standard pH levels reported for similar biogas slurries, which were reported to be around 7.8 (Warnars and Oppenoorth, 2014). Similarly, Rewe et al. (2021) established that the pH of AD bio-slurries was slightly alkaline. Various biological processes likely influence the observed pH levels during the dark fermentation of the feedstock (Warnars and Oppenoorth, 2014). In terms of electrical conductivity (EC), the mixed bio-slurry exhibited the highest EC of 21.88 ± 0.04 mS/m, followed by pig waste slurry with 21.60 ± 0.05 mS/m, and cow dung slurry with the lowest EC of 13.84 ± 0.02 mS/m (Table 1). The differences in EC could be attributed to the presence of different nutrients, dietary salts, and groundwater-containing salts in the samples (Huang et al., 2017; Racz and Fitzgerald, 2001). The variations in the conductivity are primarily due to the presence of ions that can conduct electricity, with pig waste and mixed bio-slurries having higher levels likely due to the presence of different types of ions, such as ammonium ions samples (Huang et al., 2017; Racz and Fitzgerald, 2001).

Total dissolved solids (TDS) levels, as seen in Table 1, varied among the samples, with cow dung bio-slurry having the highest TDS of 8673 ± 0.002 ppm, followed by mixed bio-slurry with 6050 ± 0.002 ppm and pig waste bio-slurry with the lowest TDS of 1534 ± 0.001 ppm. The differences in TDS levels can be attributed to the varying solubility of solids in the samples (Chaka et al., 2020; Roy et al., 2016).

Similarly, as seen in Table 1, the nitrogen concentrations in mixed waste, cow dung, and pig waste bio-slurries are 16.247 ± 0.000 g/L, 14.142 ± 0.001 g/L, and 12.281 ± 0.001 g/L, respectively. This suggests that bio-slurries have potential as a nutrient source for agricultural applications. Higher nitrogen levels, particularly in mixed bio-slurry, indicate richer nutrient content, which is essential for promoting plant growth. Research indicates that certain nitrogen-rich compounds found in bio-slurries possess pesticidal properties. For instance, amino acids, peptides, and proteins derived from organic matter present in bio-slurries have been shown to exhibit insecticidal and nematicidal effects (Puissant et al., 2021). These compounds can disrupt insect feeding, development, and reproduction and inhibit plant-parasitic nematodes' growth (Balmer et al., 2013). Mixed waste bio-slurry, with its higher nitrogen content, may contain a greater abundance of these bioactive compounds, potentially enhancing its bio-pesticidal efficacy. Similarly, although slightly lower in nitrogen concentration than mixed waste, cow dung and pig waste bio-slurries still contain nitrogen-containing compounds with bio-pesticidal properties.

3.2. Gas chromatography-mass spectrum analysis of the bio-slurries

The GC chromatogram and MS spectra from the GC–MS analysis of the cow dung, mixed waste, and pig waste obtained using PSA recorded a total of 8, 10, and 14 peaks, respectively, as shown in Fig. 1(a–c) and Table 1. The findings identified 10 compounds in the cow dung which include; 2,2-Dimethoxybutane (0.68 %), 4-methyl-3-Penten-2-one (6.50 %), 2-methyl-2-Hexanol (82.10 %), 4-(ethylamino)-Phenol (2.64 %), cis-2,5-Dimethylpiperazine (0.69 %), 2,2,6,6-tetramethyl-4-Piperidinone (1.64 %), 2-Methyl-1-ethylpyrrolidine (1.63 %), Aziridine, 1,2-diisopropyl-3-methyl-, trans-(0.60 %), Deoxyspergualin (0.95 %) and Gramine (2.58 %). From the mixed waste bio-slurry the compounds identified are 2,2-Dimethoxybutane (0.56 %), 2-Pentene, 4,4-dimethyl-, (*Z*)-(2.86 %), 2-Hexanol, 2-methyl-(91.87 %), 2-Heptanol, acetate (1.00 %), 1-Ethyl-2-pyrrolidinone (0.84 %), N-Ethyl-hexahydro-1H-azepine (0.35 %), Cycloheptasiloxane, tetradecamethyl-(1.05 %) and Gramine (1.47 %). Additionally, from the pig waste bio-slurry, the 14 molecules identified were 2,2-dimethoxybutane (0.81 %), 3-Penten-2-one, 4-methyl-(10.82 %), 2-Hexanol, 2-methyl-(60.36 %), 2-pentanone, 4-amino-4-methyl-(1.91 %), 2-Pentanone, 4-methoxy-4-methyl-(0.59 %), Boronic acid, ethyl-, diethyl ester (0.33 %), Phenol, 4-(ethylamino)-(7.84 %), 1,4-Cyclohexanediamine, cis-(5.28 %), N-[3-[N-Aziridyl]propylidene]-3-dimethylaminopropylamine (0.85 %), 4-Piperidinone, 2,2,6,6-tetramethyl-(8.65 %), 1-Ethyl-2-pyrrolidinone (0.58 %), Aziridine, 1,2-diisopropyl-3-methyl-, trans-(0.98 %), 2H-Azepin-2-one, hexahydro-6-methyl-(0.82 %), and Pyrrolidine-2-carboxylic acid, methyl-phenyl-amide (0.18 %).

3.3. Pesticidal (insecticidal, herbicidal, fungicidal, algaecidal, rodenticidal, and herbicidal) potency of the bio-slurry bioactive compounds

3.3.1. Gramine

From the findings in Table 1 and Fig. 2, Gramine (*N*, *N*-dimethyl-1*H*-indole-3-methylamine (2.58 %)) was present in cow dung and mixed waste bio-slurry. Gramine is an alkaloid compound found naturally in certain plants such as barley, rye, and some grass families, especially (Poaceae). The molecule has garnered attention for its pesticidal properties, although its effectiveness and safety compared to commercial pesticides warrant careful consideration. Several studies have investigated the pesticidal potential of gramine. For instance, gramine was found to act as an important defensive toxin in plants, exhibiting broad-spectrum insecticidal activity against herbivorous insects, including aphids, cotton bollworms, brown plant hoppers and beetles. Zhang et al. (2023a, 2023b) from their study reported that Gramine exhibited toxicity to *Daphnia magna* with an EC_{50} value of 6.03 μg/mL. Balmer et al. (2013) noted that the *N*, *N*-dimethyl-1*H*-indole-3-methylamine mechanism involves disruption of the pests' feeding behaviour and growth processes.

In addition to its phytotoxicity, *N*, *N*-dimethyl-1*H*-indole-3-methylamine has been reported to be toxic to mammals (Goelz et al., 1980),

insects, bacteria (Schütz, 2022) and fungi (Matsuo et al., 2001). More recently, publications have emphasized its potential as an algaecide (Bährs et al., 2014; Duan et al., 2022). This broad-spectrum toxicity suggests that the effects of *N*, *N*-dimethyl-1*H*-indole-3-methylamine might be mediated by more than one mode of action and/or through action on ubiquitous targets. Liu and Lovett described the effect of *N*, *N*-dimethyl-1*H*-indole-3-methylamine on the root tip ultrastructure of white mustard (de Almeida et al., 2024; Liu and Lovett, 1993). Subsequent studies of *N*, *N*-dimethyl-1*H*-indole-3-methylamine-induced toxicity in algae highlighted an enhancement of oxidative stress by the allelochemical that might be responsible for its algicidal effect (Canton et al., 2019; Laue et al., 2014). Studies indicate that Gramine may affect a range of weed species as an herbicide, given its biochemical properties and mode of action (Soltys et al., 2013). Gramine's herbicidal activity involves mechanisms such as interference with key physiological processes in plants, including photosynthesis, cell division, or nutrient uptake, which are essential for weed growth and development (Soltys et al., 2013).

3.3.2. 2,2-Dimethoxybutane

As seen in Table 1 and in Fig. 3, all of the bio-slurries analyzed contained 2,2-dimethoxybutane. 2,2-Dimethoxybutane is a fatty acid found in Australian plants such as eucalyptus and peppermint (Mani et al., 2021). As a fungicide, 2,2-dimethoxybutane is a supplement with strong antibacterial properties (Sani et al., 2015). 2,2-Dimethoxybutane has been used as a herbicide to prevent triloculare and pubescens from growing. When applied topically to plants, this substance inhibits *Trichophyton mentagrophytes* and *T. rubrum* (Saghafi et al., 2021).

The derivatives of 2,2-Dimethoxybutane include Dimethoxycarbamate, Dimethoxybutylamine, dimethoxybutyl acetate, and dimethoxybutyl ether (Devendar and Yang, 2019; Pyo et al., 2017; Yoshikawa et al., 2011). Dimethoxycarbamate derivatives are frequently employed in agriculture as broad-spectrum fungicides and herbicides (Bai et al., 2020). Dimethoxycarbamate is a widely used herbicide and insecticide in a variety of crops because it is more stable and has longer-lasting effects than its parent chemical (Pyo et al., 2017). Marketed as a specialized herbicide, Dimethoxybutylamine minimizes damage to desired plants while targeting specific weed species (Pyo et al., 2017). Dimethoxybutyl acetate is a derivative that finds application in agricultural formulations where it may be used as an active component in fungicidal and herbicidal treatments as well as a solvent (*3,3-dimethylbutylacetate | C8H16O2*, n.d.). Furthermore, Dimethoxybutyl acetate enhances agricultural formulations' efficacy and shelf life, guaranteeing reliable performance under various environmental circumstances (Gregory and Pasteris, 2009). Similarly, Dimethoxybutyl ether, a derivative of 2,2-Dimethoxybutane, is often used to produce herbicidal and fungicidal formulations (Gregory and Pasteris, 2009).

3.3.3. 3-Penten-2-one, 4-methyl-

As seen in Table 1 and Fig. 4, 3-Penten-2-one, 4-methyl- also known as 4-methyl-3-penten-2-one or Mesityl oxide was identified in pig waste and cow dung bio-slurries. 3-Penten-2-one, 4-methyl- is an essential olefinic chemical categorised as an aliphatic ketone and is a useful insect repellent owing to its unique chemical properties and odour (Lewis, 2016). Naturally occurring, 4-methyl-3-penten-2-one functions similarly to acrylic acid and can be found in plants such as *Bistorta manshuriensis* and *Tamarix aphylla* (Lewis, 2016). Its ecological relevance is highlighted by its ability to deter insects, which provides a natural substitute for synthetic repellents with little negative environmental effects (*3-Penten-2-one, 4-methyl-—Substance Details—SRS | US EPA*, n.d.). Furthermore, studies from the literature show that 4-methyl-3-penten-2-one, its derivative 4-hydroxy-4-methyl-2-pentanone, causes a positive repellence on *B. invadens*, and *G. gynandra* flies (Nishida et al., 2000). The findings further revealed the efficiency of Mesityl oxide on *G. gynandra* flies similar to those previously documented for other fruit fly species, where it was shown that many males were drawn to substances released by non-host plants (Niassy et al., 2023; Nishida et al., 2000). This suggests that Mesityl oxide might have significant insecticidal properties that can affect male *B. invadens* and *G. gynandra*. Additionally, in the absence of its host plants, Mesityl oxide may serve as a potential para-pheromone that attracts flies in the field and may even regulate the population of the flies (Kimbokota and Torto, 2013). Similarly, 3-Penten-2-one,4-methyl-(0.45 %) is also used as a pesticide owing to its insecticidal properties (Xu et al., 2011). Therefore, its presence in pig waste and cow dung bio-slurries suggests that, the bio-slurries has a potential to be employed as a substitute to synthetic pesticides.

3.3.4. Phenol, 4-(ethylamino)-

As seen in Table 1 and Fig. 5, Phenol, 4-(ethylamino)-(BHA) was present in cow-dung and pig waste bio-slurries. BHA is an important formulant (non-active ingredient) used as a stabiliser or fragrance in pesticide products (Canada, 2010). Phenol, (1,1-dimethylethyl)-4-methoxy-(BHA) derivative like Atrazine [2-chloro-4-(ethylamino)-6-(isopropylamino)-1,3,5-triazine] is a herbicide used for controlling broadleaf and grassy weeds and is relatively persistent in soils (Neumann et al., 2004). Their high-, middle-, and low-affinity binding explain the triphasic binding features of phenolic herbicides (David Troncoso et al., 2022). Floris et al. (2021) noted that the inhibition constant of photosystem II electron transport in phenols is equivalent to the middle-affinity binding constant. If the hydroxyl group in the phenol is methylated, phenolic herbicides lose more than two orders of magnitude of their inhibitory efficacy (Floris et al., 2021). Thus, to ensure the pesticide efficiency of Phenol, 4-(ethylamino)-, handling should be carefully controlled to preserve the active hydroxyl groups.

3.3.5. Cis-2,5-Dimethylpiperazine

From the GC–MS analysis, as seen in Fig. 6 and Table 1, Cis-2,5-Dimethylpiperazine was present in cow-dung bio-slurry. *Cis*-2,5-Dimethylpiperazine, a piperazine derivative, stands as a pivotal compound within agrochemicals due to its versatile properties and broad spectrum of bioactivities (Zhang et al., 2023a, 2023b). Piperazine derivatives such as triforine exhibit fungicidal activity (Zhang et al., 2023a, 2023b). Similarly, Aryl-formyl piperidinone derivatives have proved to have inhibitory activity on 4-hydroxyphenylpyruvate dioxygenase (HPPD), finding its application in weed control (Fu et al., 2021). These Cis-2,5-Dimethylpiperazine derivatives have emerged as crucial linkers, facilitating the connection of active substructures with promising bioactivities (Zhang et al., 2023a, 2023b). Over two decades, from 2000 to 2022, numerous piperazine-containing compounds have garnered attention for their remarkable efficacy against a wide range of agricultural threats, including fungi, bacteria, insects, plant viruses, and weeds (Zhang et al., 2023a, 2023b). These compounds have demonstrated potent pesticide activities and proven valuable as plant growth regulators, further enhancing their utility in agricultural practices (Zhang et al., 2023a, 2023b).

Furthermore, piperazine derivatives used in fungicidal formulations serve as active components in the fight against fungal diseases that harm crops, including leaf spots, grey mould, and powdery mildew (Zhang et al., 2023a, 2023b). As an insecticide, Cis-2,5-Dimethylpiperazine is used to control aphids, thrips, caterpillars, beetles, etc., in the form of sprays and baits (Zhang et al., 2023a, 2023b). Other derivatives of cis-2,5-Dimethylpiperazine include 4-Cyclohexylaminovirimidine derivative, which is a novel bioactive compound used as a pesticide for agricultural and horticultural use useful as an insecticide, acaricide, fungicide and like (Allen et al., 2010).

3.3.6. 4-Piperidinone, 2,2,6,6-tetramethyl-

Table 1 and Fig. 7 show that 2,2,6,6-Tetramethyl-4-piperidone was present in cow-dung and mixed waste bio-slurries. 2,2,6,6-Tetramethyl-4-piperidone, sometimes referred to as Triacetonamine, is an essential step in creating insecticides (Cao et al., 2010; Kim et al., 2022). 2,2,6,6-

Tetramethyl-4-piperidone derivative Triacetonamine is used in pharmaceutical applications; however, its safety has been questioned due to its oral action and association with acute liver failure (ALF) in rats (Kim et al., 2022). Additionally, studies indicate that Triacetonamine inhibits the proliferation of skin cells by interfering with DNA replication and cell division processes (Łączkowski et al., 2016). Interestingly, Triacetonamine has also demonstrated growth-inhibiting effects on bacteria via interactions with oxidative DNA, suggesting that it may have antibacterial properties (Cao et al., 2010). Furthermore, 2,2,6,6-tetramethylpiperidine derivatives containing selenium and sulfur nitroxyl radicals: 4-isothiocyanato-2, 2,6,6-tetramethylpiperidine-1-oxyl (TEMPO-NCS, 1), 4-isoselenocyanato-2, 2,6,6-tetramethylpiperidine-1-oxyl (TEMPO-NCSe, 3), and N-substituted compounds: 1-thioformyl-2, 2,6,6-tetramethylpiperidine (TMP-CHS, 5), and 1-selenoformyl-2,2,6,6-tetramethylpiperidine (TMP-CHSe, 6) (Asghari-Paskiabi et al., 2019). It was discovered that these compounds exhibit antifungal activity in vitro against four different fungus species. Among all the chemicals examined, TEMPO-NCSe (3) turned out to be the most active. A selenium or sulfur atom may be added in place of an oxygen atom to improve the antifungal activity of the investigated compounds, according to a comparison of the antifungal activity of (Asghari-Paskiabi et al., 2019; Łączkowski et al., 2016).

3.3.7. *Aziridine, 1,2-diisopropyl-3-methyl-, trans-*

As shown in Table 1 and Fig. 8, Aziridine, 1,2-diisopropyl-3-methyl-, trans- was found to be present in cow dung and pig waste bio-slurries. Aziridine, 1,2-diisopropyl-3-methyl-, trans-, is known for its chemosterilant properties and has become a focus of agricultural research due to its potential as an insecticide and chemosterilant (Holloway et al., 1986; Kadaba, 1994). Research on the boll weevil (*Anthonomus grandis*) compared the effectiveness of aflatoxin and aziridine derivatives as chemosterilants and insecticides (Bořkovec, 2019). Aflatoxin, a well-known mycotoxin, was tested to examine its viability as a substitute for Aziridine. Fascinatingly, when other functional groups, such as N—H, N-amino, or N-phthalimido, were added to the chrysanthemate 2-methyl-1-propenyl double bond, the insecticidal activity decreased (Bořkovec, 2019). On the other hand, these changes increased the cockroach ventral nerve cord's in situ potency to produce recurrent discharges (Bořkovec, 2019). The observed effect aligns with previous research on analogues, including cyclopropyl, episulfide, and epoxide, suggesting that structural alterations are crucial in determining insecticidal efficacy and neurotoxicity (Moore et al., 1978).

Additionally, 3,5-diisopropylphenyl methylcarbamate derivative of Aziridine, 1,2-diisopropyl-3-methyl-, trans- is a carbamate used as an insecticide (PubChem, n.d.). Similar to organophosphate insecticides, carbamate pesticides are made from carbamic acid and work by killing insects. They are extensively utilised in gardens, houses, and farming (PubChem, n.d.).

3.3.8. *1-Ethyl-2-pyrrolidinone*

From the findings in Table 1 and Fig. 9, 1-Ethyl-2-pyrrolidinone (NEP) was present in mixed waste and pig waste bio-slurry. NEP is an essential ingredient in pesticide formulations; it functions as a dispersion and solubiliser in the agrochemical industry's creation of pesticides, herbicides, and fungicides (Kim et al., 2019; Martín-García et al., 2023). Its capacity to solubilise and distribute active chemicals improves pesticide applications' uniformity and efficacy, assisting in crop protection and pest control (Kim et al., 2019). NEP-containing pyrrolidine derivatives have strong insecticidal effects, especially on lepidopterous larvae that infest plants (Martín-García et al., 2023). The use of 2-(nitromethylene)pyrrolidines, produced from NEP, has proven beneficial in managing lepidopterous larvae (Maienfisch et al., 2001). This could suggest that mixed waste and pig waste bio-slurry could offer a potential for controlling pests, especially lepidopterous larvae.

3.3.9. *N-Ethyl-hexahydro-1H-azepine*

From the GC–MS results in Table 1 and Fig. 9, N-Ethyl-hexahydro-1H-azepine was present in the mixed waste bio-slurry. N-Ethyl-hexahydro-1H-azepine is a molinate chemical known as S-ethyl hexahydro-1H-azepine-1-carbothioate. It is predominantly used as a selective pre-emergence thiocarbamate herbicide in rice cultivation (Moretto et al., 2001). Molinate is an activated herbicide, exerting its effects by inhibiting enzymes crucial in fatty acid and cholesterol synthesis pathways (Fig. 10). Notably, molinate serves as a cell-specific activator, facilitating its use in vitro assays on bacterial strains (Moretto et al., 2001). Apart from molinate, several derivatives, such as 11-alkoxyimino-5,6-dihydro-dibenzo[*b*,*e*]azepine-6-one, demonstrate remarkable fungicidal activity against Botrytis cinerea (Allen et al., 2010). Compounds like 11-(n-butyloxyimino)-5,6-dihydro-dibenzo[*b*,*e*]azepine-6-one (4A-05), 11-(4-nitrobenzyloxyimino)-5,6-dihydro-dibenzo[*b*,*e*]azepine-6-one (4A-12), and 11-(2-chloro-6-fluorobenzyloxyimino)-5,6-dihydro-dibenzo[*b*,*e*]azepine-6-one (4A-21) exhibit exceptional fungicidal activity, surpassing the effectiveness of commercial fungicides like chlorothalonil and procymidon (Xiao et al., 2013). 11-(2-fluorobenzyloxyimino)-5,6-dihydro-dibenzo[*b*,*e*]azepine-6-one (4A-16) and 11-(3-fluorobenzyloxyimino)-5,6-dihydro-dibenzo[*b*,*e*]azepine-6-one (4A-17) in particular has a significant fungicidal potential compared to procymidon, suggesting their promising role in crop protection strategies (Xiao et al., 2013).

3.3.10. *Cycloheptasiloxane, tetradecamethyl-*

From the findings in Table 1 and Fig. 11, cycloheptasiloxane, tetradecamethyl- was observed in pig waste bio-slurry. Silicon-based cycloheptasiloxane, tetradecamethyl- is usually employed as a pesticide (Jaleel et al., 2021). A study on chemical constituents in e-cigarettes found that cycloheptasiloxane, tetradecamethyl- silicon conjugated compounds act as biocide (Jaleel et al., 2021). Compared to synthetic pesticides, studies show that siloxanes are more advantageous for controlling insect pests. For instance, silica nanoparticles can promote development and trigger defensive responses against bugs. Cycloheptasiloxane, tetradecamethyl- has antimicrobial, antiseptic, hair-conditioning, and skin-conditioning agent-emollient properties (Jaleel et al., 2021). This suggests that the pig waste bio-slurry has a significant potential to be used as an antimicrobial, antiseptic, hair-conditioning agent, and skin-conditioning agent due to the presence of siloxane.

4. Conclusion

The analysis of anaerobic digestion (AD) biogas slurries showed the presence of bio-pesticide molecules and their derivatives, which offers intriguing opportunities for sustainable agricultural and environmental management. The physicochemical examination confirmed the presence of higher nitrogen, which is vital for plant development and insect control. GC–MS analysis revealed various compounds with pesticide capabilities, including Gramine, 2,2-dimethoxybutane, 4-methyl-3-penten-2-one, etc. The order of the abundance of bio-pesticides was Cow dung > mixed waste > pig waste. These findings highlight the potential of AD biogas slurry and its derivatives as viable alternatives to synthetic pesticides, providing tailored pest management options while reducing environmental impact and boosting sustainable agriculture practices. Using the bio-pesticide capability of AD biogas slurry may offset the detrimental effects of conventional pesticides on human health and the environment while improving agricultural yield and food security.

CRediT authorship contribution statement

M.G. Gitonga: Writing – original draft, Validation, Project administration, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **A.M. Osano:** Writing – review & editing, Validation, Supervision, Resources, Methodology, Conceptualization. **M. Sitati:** Writing – review & editing, Supervision, Resources.

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

All the data associated with this research has been provided herein except for the raw data, which can be provided upon request without withholding.

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