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Quantitative Determination of Organochlorine Pesticide Residues in Sugarcane Obtained from Hawkers in Gusau Metropolis, Zamfara State, Nigeria

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ABSTRACT

In this research work, organochlorine pesticide residues was quantitatively determined in sugarcane juice extracts obtained from hawkers in Gusau metropolis Zamfara state, Nigeria. QuECHERS and GC-MS were used for samples extraction and analysis under standard conditions. Twenty organochlorine pesticide standard were used and only five organochlorine pesticide residues were detected in the extract namely; alpha lindane, endosulfan ether, isodrine, heptachlor epoxide and DDMU with concentration 0.0250 mg/kg, 0.0270 mg/kg, 0.0141 mg/kg, 0.0003 mg/kg, 0.0016 mg/kg respectively. The entire organochlorine pesticide residues detected are within the maximum residual limit (MRL) except alpha lindane and endosulfan ether that are little higher than 0.02 mg/kg of MRL. The presence of organochlorine pesticides residues in the sugarcane juice extract shows that farmers are still using the banned organochlorine pesticide for pest control, therefore, routine monitoring of pesticide residues is necessary for information on the level of environmental pollution, in order to minimize health risk to human.

Keywords: Organochlorine pesticide, Residues, Sugarcane, GC-MS

1.1 INTRODUCTION

Sugar cane (*Saccharum officinarum*) is a species of tall perennial grass that belong to the grass family. The value of Sugar cane in the world cannot be over emphasis, sugar cane as raw material, account for about 60 to 80 % for the world sugar production [1]. Sugar cane can be planted in different variety soils but grown best in organic matter rich soil. It required pH range of 5.0 -8.0 and a temperature of 26 -33^{oC} with rainfall between 1500 and 2500 mm [1].

In Nigeria, sugar cane is commercially grown in the states of Kwara, Niger, Kano, Kaduna, Kastina, Jigawa, Taraba, Sokoto, Adamawa and Zamfara. The help of irrigated agriculture provides more sugar cane even during the dry season. Sugar cane production in Nigeria is about 1.5 million tons in 2020 [1]. Sugarcane is susceptible to the following pests and diseases during its growing process: Shooter Borer, Internodes Borer, Top Shoot Borer, Termites, White Mad, White Woolly Aphid, Whitefly and Meal Beetle. These attacks are of economic importance as they affect the quality and quantity of production [2], [3].

As a result, sugarcane farmers use pesticides such as organochlorine pesticides because it had be identified as cheap pesticide and readily available in smaller quantity for farmer to purchase compared to other pesticides. The deposition of residues of organochlorine pesticides in sugar cane exposed local consumers to the harmful effects of these pesticides such as cancer, respiratory effects, reproductive dysfunction and immune deficiency. Local consumers such as people that household uses are exposure to the effects of these organochlorine pesticides residues when present, because the consumed it raw in order to enjoyed the juice which are mostly rich in natural sugar such as fructose. These are sold by hawkers in mostly in the northern part of Nigeria including Zamfara state, unlike the sugar industries that sourced sugar cane as a raw material [4], [5],.[6].

1.2 MAXIMUM RESIDUE LIMIT FOR ORGANOCHLORINE PESTICIDES (MRL)

Residues are defined by the WHO [7] as any substance or mixture of substances in food for humans or animals after the used of pesticide for pest controlled. Pesticide tends to undergoes different phase of degradation and transformation, metabolites, reaction products. Those residues may found in our food and food products with toxicologically significant impurities. Various countries have set its own Maximum Residue Limit (MRL) and Acceptable Daily Intake (ADI) of pesticide residues in its agricultural products

. Nigeria used residual limits set by the Food and Agriculture Organization of the United Nations (FAO) and the World Health Organization (WHO) (NAFDAC, 2017). The WHO has warned that almost three million pesticide poisonings cases occur annually, which was resulting in 220,000 deaths worldwide. Farmers around the world used both approved and banned pesticides to control pests and diseases, therefore, there is need for routine monitoring of sugar cane for pesticide residues because of the local consumers of direct sugarcane [8] [9].

2.1 MATERIALS METHODS

All reagents and pesticides used were of analytical grade. All glassware were sterilized to ensure is free from interference. Centrifuge and Gas Chromatograph with Mass Spectrometer-Detector (GC-MS) pair are used for extraction and analysis, respectively Centrifuge (30000rpm, Model number: AM-10607) and Gas Chromatograph with Mass Spectrometer-Detector (GC-MS) pair (Model 7890 Agilent) instruments Technologies), balance (electronic, model: ES225SM-DR), oven (model: E028-280v), pH meter (Beckman model 72).

Ten sugar cane samples from different hawkers in different parts of the Gusau metropolis were collected. The samples were brought to the laboratory and kept in a refrigerator at a temperature of 25 °C before extraction.

2.2 SAMPLES PREPARATION

Each cane stalk was washed with distilled water and raised with ethanol to remove all dirt that may causes interference before extraction. All glassware, mortars, pestles and knives used were also sterilized using autoclaved at 121°C for 45 minutes. Each sugar cane sample was crushed using a laboratory mortar and pestle. All of the juice obtained from each cane stalk was blended into a cleaned 500 mL beaker. The composite cane juices were filtered using Whatman 1 filter paper to remove the particles and the filtrate was stored in a cleaned 500 mL flask and covered with aluminum foil, kept in the refrigerator to avoid fermentation before extraction of pesticide residues.

2.3 EXTRACTION OF ORGANOCHLORINE PESTICIDE FROM SUGAR JUICE

The Quick Easy Cheap Effective Rugged Safe (QuEChERS) method was adopted to extract organochlorine pesticide residues from sugarcane juice as described by [10], [11], but slightly modified. 20 cm^3 sugar cane extract was measured into a cleaned 50 ml test tube; 20 cm^3 of acetonitrile was added and shaken vigorously for 30 minutes. To ensure, organic pesticides were dissolved in the solvent. 5 g MgSO4 and 2 g NaCl were added to remove water and increase polarity, respectively. It was divided into three cleaned test tubes and centrifuged at 600 rpm for 10 minutes. The supernatants were transferred to a cleaned test tube, followed by addition of 150 mg of $MgSO_4$ and shaking for 30 seconds and centrifugation at 1500 rpm for 1 minute before clean up.

2.4 CLEAN UP OF EXTRACT OF SUGARCANE EXTRACT

Sugar cane extract was purified using PSA and solid phase extraction with pesticide cartridge. The extract was loaded onto the pesticide cartridge and washed with acetonil. The elute was evaporated to dryness using a rotary evaporator and a nitrogen bleed. The cartridge was loaded with the residue after dilution with 10 mL of acetonitrile and re-evaporated before dilution with acetonile and saved for analysis [12].

2.5 INSTRUMENTATION

The analysis was performed using an Agilent Technologies GC-MS model 7890 equipped with an auto sampler, a capillary column length of HP5 ms with a length of 30 m and an internal diameter of 0.320 mm and 0.25 micron. The temperature was program 60°C held for 5 minutes at 8°C per minute to the final temperature of 300°C held for 0.5 minutes and the MSD transfer line was held at 300°C. one micro liter split injection was performed at 300°C injector temperature with a purge flow of 3 ml/minute, helium was used as the carrier gas. at a flow rate of 2.17 ml/minute, the pressure was 150 kPa. and the interface temperature was 300°C. The ionization mode of the Agilent Technologies Model 5975 mass spectrometer was electron impact with an ion source temperature of 230 °C and ranges from 45-500 M/Z in full scan mode.

The internal standard technique was used to analyze the sugar juice extracts. The organochlorine standards used are Alpha-Lindane, Delta-Lindane, Endulfan I&II, Heptachlor, Aldrin, Isodrin, trans-Chlorine, DDMU, DDT, P,P-DDE, Dieldrin, Endrin, Mitotane, Endrin-Keto, Melhoxychlor and Delta -Pent. The standards are prepared at various concentrations from 0.100 ppm to 2.00 ppm and were used to generate calibration curves for each compound. The efficiency of the method was validated with recoveries of reference material without pesticide residues and with four 0.1 ppm and 1.0 ppm spiked organochlorine pesticide residues

3.1 RESULTS AND DISCUSSIONS

Table 1 GC-MS RESULT OF THE STANDARD

s/n	Organochlorine residues	RT (min)	Area
1	.delta.-Pentachlorocyclohexene	10.028	353817
2	.alpha.-Lindane	11.738	129752
3	.delta.-Lindane	14.29	1142875
4	Endosulfan ether	14.519	276180
5	Heptachlor	15.229	2515872
6	Aldrin	16.047	1901251
7	Isodrin	16.654	86294
8	Heptachlor epoxide	17.128	2447491
9	trans-Chlordane	17.769	3529360
10	DDMU	18.055	357696
11	trans-Nonachlor	18.422	2710779
12	p,p'-DDE	18.622	6311
13	Dieldrin	18.879	1473560
14	p,p'-DDE	19.057	3154273
15	Endrin	19.48	598174
16	Endosulfan	19.892	745763
17	Mitotane	20.482	4536
18	Endosulfan sulfate	21.191	579813
19	Endrin ketone	22.564	777102
20	Methoxychlor	23.405	5700249

KEY; RT = retention time, ND = not detected

Table 2 GC-MS RESULT SUGARCANE SAMPLE EXTRACT

Name of compounds	RT (min)	Area
.delta.-Pentachlorocyclohexene	ND	ND
.alpha.-Lindane	11.761	327
.delta.-Lindane	ND	ND
Endosulfan ether	14.514	739
Heptachlor	ND	ND
Aldrin	ND	ND
Isodrin	16.671	122
Heptachlor epoxide	17.569	72
trans-Chlordane	ND	ND
DDMU	18.004	58
trans-Nonachlor	ND	ND
p,p'-DDE	ND	ND
Dieldrin	18.874	466
p,p'-DDE	ND	ND
Endrin	ND	ND
Endosulfan	19.852	54
Mitotane	ND	ND
Endosulfan sulfate	ND	ND
Endrin ketone	ND	ND
Methoxychlor	ND	ND

KEY; RT = retention time, ND = not detected

Table 3 Concentrations of organochlorine pesticide residues in sugarcane juice extract

Organochlorine residues	RT of standard (min)	RT of sample (min)	Area of standard	Area of sample	Conc.of OCP residues mg/kg
.delta.-Pentachlorocyclohexene	10.028	0	353817	ND	ND
.alpha.-Lindane	11.738	11.761	129752	327	0.0250
.delta.-Lindane	14.29	ND	1142875	ND	ND
Endosulfan ether	14.519	14.514	276180	739	0. 0270
Heptachlor	15.229	ND	2515872	ND	ND
Aldrin	16.047	ND	1901251	ND	ND
Isodrin	16.654	16.671	86294	122	0.0141
Heptachlor epoxide	17.128	17.569	2447491	72	0.0003
trans-Chlordane	17.769	ND	3529360	ND	ND
DDMU	18.055	18.004	357696	58	0.0016
trans-Nonachlor	18.422	ND	2710779	ND	ND
p,p'-DDE	18.622	ND	6311	ND	ND
Dieldrin	18.879	18.874	1473560	466	0.0320
p,p'-DDE	19.057	ND	3154273	ND	ND
Endrin	19.48	ND	598174	ND	ND
Endosulfan	19.892	19.852	745763	54	0.0072
Mitotane	20.482	ND	4536	ND	ND
Endosulfan sulfate	21.191	ND	579813	ND	ND
Endrin ketone	22.564	ND	777102	ND	ND
Methoxychlor	23.405	ND	5700249	ND	ND

KEY; RT = retention time, OCP = organochlorine pesticide, ND = not detected

Table 1 shows the GC-MS result of the organochlorine standard; twenty organochlorine pesticide standards were used for these analysis. The standards were prepared under the same conditions as the samples extract, the result shows the retention time and the area of the chromatogram peaks for all organochlorine pesticide residues standard. The retention time was used to adjust the retention time of the residues in the sample.

Table 2 shows the result of organochlorine pesticide residues present in sugar cane juice. The retention time and area of the chromatogram peaks of individual organochlorine pesticide residues are shown in Table 2. The results show the presence of alpha-lindane, endosulfan ether, isodrine, heptachlorepoxide DDMU, dieldrin and endosulfan. While delta-pentachlorocyclohexane, delta-lindane, heptachlor, aldrin, trans-chlordane, DDE, endrin, mitotane, endosulfan sulfate, methoxychlor were not detected in sugar cane juice extract.

Table 3 shows the concentration of organochlorine pesticide residues in the sugar cane juice extracts from the samples. In fact, seven organochlorine pesticide residues were determined; Alpha Lindane, Endosulfan Ether, Isodrin, Heptachlorepoxide, DDMU, Deildrin and Endosulfan. The concentration of Deildrin is higher (0.0320 mg/kg), followed by endosulfan ether (0.0270 mg/kg) and alpha-lindane (0.025 mg/kg). . Heptachlorepoxide has a concentration of (0.0003 mg/kg), which is the lowest, followed by (0.0016 mg/kg). All organochlorine pesticide residues identified are within the Maximum Residue Limit (MRL) except for alpha-lindane and deildrin.

The alpha-lindane determined in the extracted sugar cane juice at a concentration of (0.0250 mg/kg) is above the maximum permitted limit of 0.02 mg/kg by [13] in fruit and vegetables. The present of organochlorine pesticide residues in the extract may as result of farmers using the pesticide to control pests during the sugar cane plantation, or the pesticide can be washed out by the runoff water from where it is used by the farmers and absorbed by the sugar cane plant. There is evidence of organochlorine pesticide residues in the soil of sugarcane farms, [14], [115] reported the presence of lindane in the soil of sugarcane farms.

Endosulfan ether and endosulfan were determined at concentrations of (0.027 mg/kg) and (0.0072 mg.kg) respectively, this amount was below the maximum residue level (MRL) of 0.5 mg/kg accepted in food. Endosulfan ether has been reported to remain in soil more than other organochlorine pesticide residues [16].

Isodrin was detected in the sugarcane juice extract (0.0141 mg/kg), which is slightly above the maximum residual limit of 0.01 mg/kg Isodrin may be the result of recent use of the pesticide by the farmer or due to indiscriminate use of combined chlorinated cyclodiene insecticides such as Aldrin, Dieldrin and Endrin as they are all endo-endo isomers of cyclodiene. The organochlorine pesticide Isodrin was developed as an alternative pesticide after the global ban on DDT. It was later banned by the FAO/WHO because of its persistent and slow degradation in soil and its health hazards [17].

Heptachlor epoxide was present in sugarcane juice extract at a level of 0.0003 mg/kg, which is below the maximum residue limit for fruits and vegetables. Heptachlor has been banned by the WHO/FAO because the insecticide causes liver disease in animals and is also suspected of being carcinogenic in humans. WHO/FAO [18]. The presence of heptachchlor indicates that the pesticide is still used for agricultural purposes, despite being banned due to potential health hazards. The indiscriminate use of this banned pesticide and the lack of Good Agricultural Practices (GAP) can lead to high concentrations of heptachlor in the sugarcane plant. Heptachlor has been classified as possibly carcinogenic to humans by the Environmental Protection Agency (EPA) and has a long half-life in soil [19].

The Deildrin detected in the sugar cane juice extract is (0.0320 mg/kg), which is slightly higher than the MRL recommended in food. Deildrin is a synthetic chemical used to kill insects, but due to concerns about environmental damage and potential human health risks, the US Environmental Protection Agency (EPA) has banned the use of this compound for agricultural purposes [20], [21].

2, 2-bis (chlorophenyl)-1-chloroethane (DDMU).Detected in sugar cane juice extract at a concentration (0.0016 mg/kg) lower than the maximum residual limit. The presence of DDMU indicates the farmer's use of dichlorodiphenyltrichloroethane (DDT) for pest control, as DDMU is one of the breakdown products of DDT. Dichlorodiphenyltrichloroethane has been banned for agricultural use worldwide because of its health hazards and unfavorable environmental impact [21],[22].

3.5 CONCLUSION

The result obtained from this research shows the presences of five organochlorine pesticides residues namely; alpha lindane (0.0250 mg/kg), endosulfan ether, (0.0270 mg/kg), isodrine. (0.0141 mg/kg), heptachlor epoxide, (0.0003 mg/kg), and DDMU, (0.0016 mg/kg). all the organochlorine pesticide residues detected were below the maximum residue limit of the US Environmental Protection Agency, except alpha lindane and endosulfan ether that are little above the maximum residual limit. The presence of organochlorine pesticides residues in the sugarcane juice extract may be attributed to illegal use of these pesticides by farmers in the area where this sugarcane are planted. Routine monitoring of pesticide residues is necessary for information on the level of environmental pollution and control, in order to minimize health risk to human, with exposure of pesticide residues

Conflict of interest

The authors hereby declare that there are no conflicts of interest.

Author's contributions

The authors have equal contribution to the work.

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