Research Journal of Food Science and Nutrition
Volume 9(3), pages 83-89, June 2024
Article Number: F729C9482
ISSN: 2536-7080
https://doi.org/10.31248/RJFSN2024.177
https://integrityresjournals.org/journal/RJFSN

Full Length Research

Determination of pesticide residues in Cameroonian honey by QuEChERS method and public health significance

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Received 3rd May 2024; Accepted 20th June 2024

ABSTRACT: Pesticides and food contamination has become one of the major health problems throughout the years with almost all agricultural foods exposed over the world. Honey, one of the most consumed products has been described in various parts of the world as exposed to pesticides contamination, but few studies have looked to this issue in Africa. In Cameroon where honey production has known an increase over the past decade, no study has been done to evaluate the presence of pesticides or any toxic compound and its related health significance. Therefore, this study was designed and caried out from January to December 2022, in order to assess the level of honey contamination in the main productive areas of Cameroon respectively (Bimodal Forest, Sudanoguinean and western highlands agroecological areas). 50 samples of 100 g of honey was collected in sterile tubes at the level of hives, after extraction and in the markets and analysed using QuECHERS method. Hazard Index was used to assess health risk associated with the consumption of honey in Cameroon. Thirteen samples were found positive for an overall prevalence of 8.69±0.29% with 23.37±0.32% in the western highlands and 6.67±0.55% in the bimodal forest zone. Satratoxin-h, Methyl-Diclofop, Fumaronitrile and propiolonitrile were identified and no health risk was associated with their presence as the Hazard Index value was estimated to 1.08×10^{-8}. Even if there are no public health hazards associated with pesticide contamination in the country, it should be taken seriously in order to prevent any increase in contamination in the future.

Keywords: Cameroon, honey, toxic compound, Health risk, QuECHERS.

INTRODUCTION

Almost all agricultural foods and livestock have been exposed to pesticide contamination at different levels either resulting from wrong practices, or from various incidents resulting to food safety concern in many countries over the world (WHO, 1990; Carvalho, 2017; Leskovac and Petrovic, 2023). In the same order, abundant and wrong use of pesticides have been described in some studies in Cameroon, with sometimes almost a half of product contaminated with pesticides above the Maximum Residue Limits (MRLs) (Galani *et al*., 2021). Honey, one of the most consumed products has been described as containing pesticide residues linked either to wrong beekeeping practices, to contamination from agriculture or environment (Mitchell *et al*., 2017; Durazzo *et al*., 2021; EFSA *et al*., 2021) or to the adulterations as it is the third most counterfeit agriculture product in the world after milk and olive oil (García, 2018). In Cameroon, one of the leading producing country of honey in the central Africa region (European Commission, 2021) studies have described the physico-chemical and

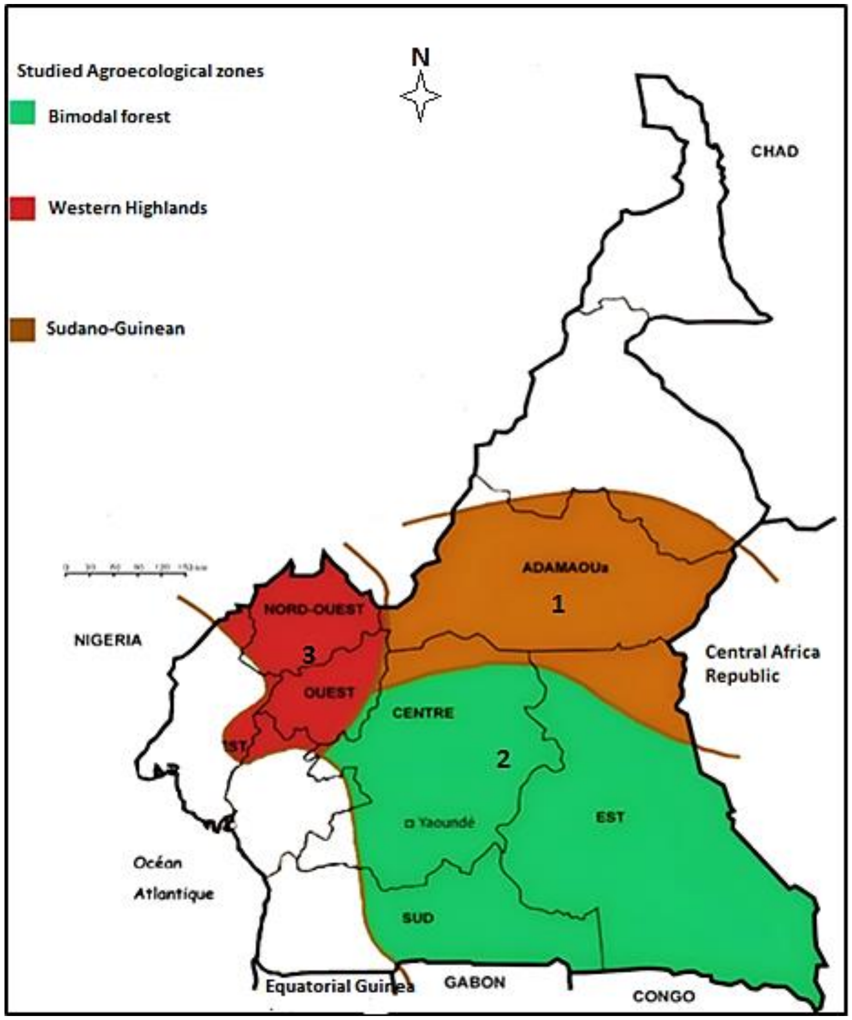

Figure 1. Samples areas (agro ecological areas studied).

microbial properties of honey (Tchoumboué *et al*., 2007; Tatsadjieu *et al.,* 2008; Mbogning *et al.,* 2011) no known studies have been done on the toxicological aspect of local honeys. However, studies in other African countries with similar organisation and agricultural features have shown pesticides contamination of honeys (Darko *et al*., 2017; Fikadu, 2020), leading to a suspicion of a similar situation in the country. Therefore, the investigation of toxic compounds in honeys according to the technological level, from the hive to the final product, was done in order to assess its public health significance in relation with its production and processing.

MATERIALS AND METHOD

Studied areas and period

This study was carried out from January to December 2022 in three agroecological zones of Cameroon respectively the bimodal forest, the Soudano-guinean and the western highlands (Figure 1) which constitute more than 99% of the national honey production (MINEPIA-DEPCS, 2020).

Sampling size and method

The determination of the sampling size was determined according to the methodology of the French Food Safety agency for the research of pesticides residues in honey described in the instruction DGAL/SDSPA/2009 of the 01st of February 2009 “which recommend a minimum size of 10 samples for 300 tons of honey up to 3000 t then 1 sample for 300 tons” (AFSAA, 2009). The number of samples for each area was obtained on the basis of the allocation of the weight of their respective honey production extracted from the databases of the National Statistics Institute and the Ministry of Livestock, Fisheries and Animal Industries. In each region a stratified random

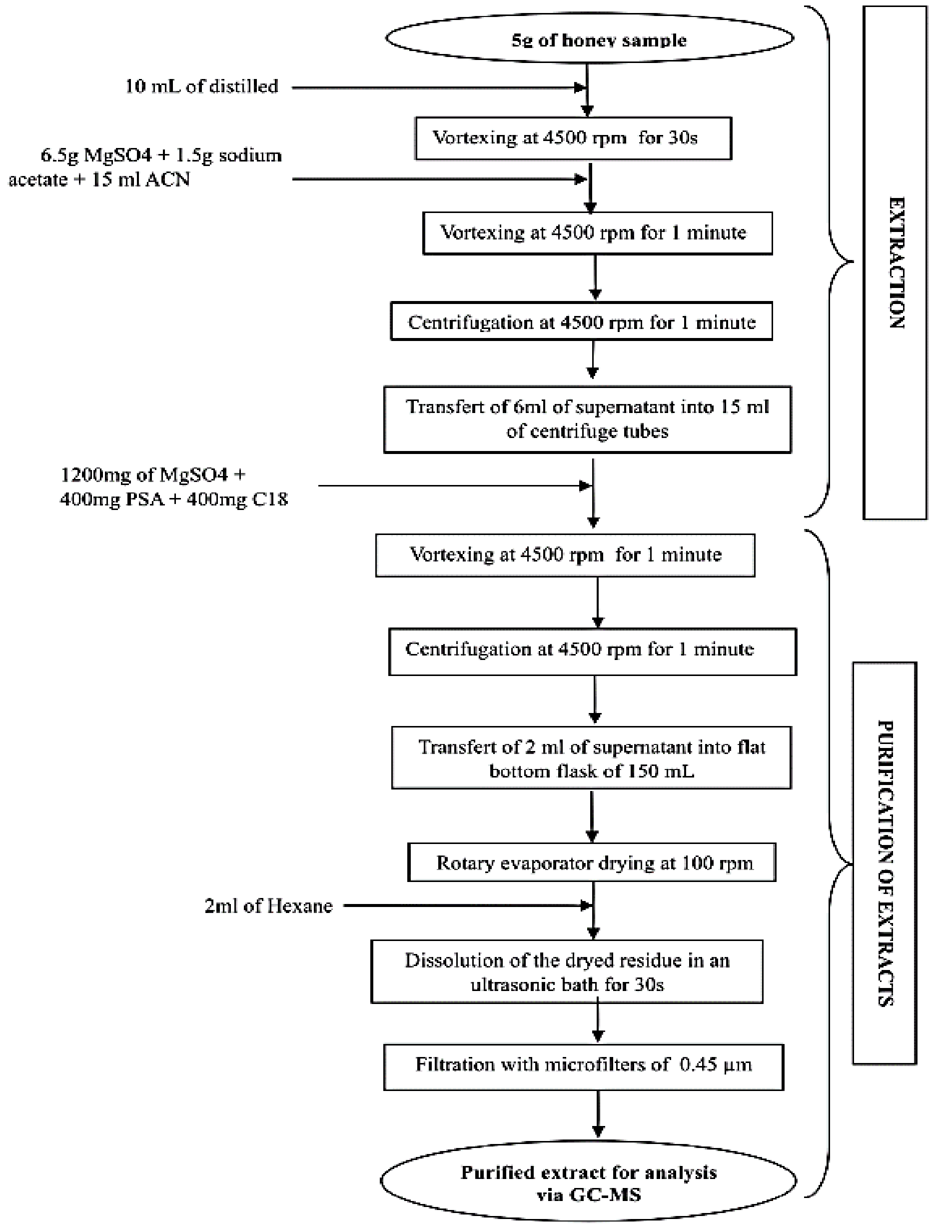

Figure 2. QuEChERS diagram method.

method was used and the samples were collected from the hive, after extraction and from the final product and in the different markets.

Within each area, one sample of approximately 100 g was collected, put in sterile tubes, labelled and brought to the laboratory and stored at 4°C before analysis.

Sample analysis

Toxic components identification

The analysis method used was the Quick, Easy, Cheap, Effective, Rugged, and Safe (QuEChERS 2007.01) one as the extraction technique chosen to maximize the quantity of extractable analytes, associated with gas chromatography coupled with mass spectrometry for quantification (Figure 2) (Martel, 2018; Chin and Sowndhararajan, 2020).

Gas-Chromatography-mass spectrometry: A volume of 1 µl of sample is introduced into the capillary column model: HP-5MS; length: 30 m – internal diameter 0.250 mm – thickness: 0.25 µm by splitless mode, in the injector heated to a temperature of 280°C. The carrier gas, helium, is introduced at a flow rate of 104.2 mL/min. The mass transfer line has a temperature of 250°C, and the data is acquired by SCAN, while the ionization method is by ion impact. The elevation of the temperature of the oven to the gradient is done at a speed of 25°C/min with a first passage from 110°C (initial temperature of the oven) to 140°C, then it is stabilized for 1 minute, then raised up to 250°C at the same speed. The sample is passed through the column for a total of 7.6 minutes. Mass spectra were taken at 70 eV; a scan interval of 0.5 s and fragments from 40 to 950 Dalton.

Identification of components

Interpretation on mass spectrum of GC-MS and identification of various toxic compounds was done using the database of the United States National Center for Biotechnology Information (NCBI).

Health risk assessment: Health risk assessment was done through the estimation of the Mean Daily Intake (MDI) and the calculation of health hazard quotient and hazad index (HI) as described by El-Nahhal (2020).

Estimation of mean daily intake of toxic compounds

It was done through the equation

$$MDI = (PSxQ)/BW$$

Where PS is the average concentration of toxic compounds in honey samples expressed in µg/kg, Q the amount of honey sampled consumed by a person and BW is the consumer's body weight.

The value of Q considered was 50 g of honey/person/day for an adult and 9-11 g/day for children as defined by international standards (FAO/WHO, 2009; Wilmart *et al*., 2016; El-Nahhal, 2020). The Hazard Quotient (HQ) was estimated through the equation:

$$HQ = MDI/ARfD$$

With ARfD as the acute reference dose of Toxin compoud expressed in µg/kg/day (El-Nahhal, 2020). The Hazard Index (HI) was calculated as the total risk of multiple chemicals on the assumption of dose additivity (Bommuraj *et al.,* 2019).

$$HI = HQ1 + HQ2 + \cdots HQn$$

With n = compound

If calculated HI≥1 then the risk is high whereas if HI≤1 then the risk is low or ignorable (Bommuraj *et al*., 2019; El-Nahhal, 2020).

Data analysis

The analysis of data was done with SPSS 20 IBM™, and the results were expressed in the form mean ± Standard Deviation at a level of significance of 95%. Chi-square test was done to test if there is a significant difference between the parameters measured.

RESULTS

Sample distribution within the different agroecological areas and technological levels

A total of 150 samples were collected from the three areas, with respectively 90 (63.12%) from the Bimodal Forest Area and 30 from the Soudano-Guinean and Western Highlands Areas. The allocation for the technological level was also obtained as presented in Table 1.

Toxicological profile

Out of the 150 honey samples, 13 were positives in two areas respectively the Bimodal Forest Zone (6.67±0.55%) and the Western Highlands (23.37±0.32%) with an orverall prevalence of 8.67±0.29%. From the collecting level, 5 (10%) samples were positive at the level of the hive, 3 (6%) after extraction, and 5 (10%) at the level of the market as presented in Table 2, with no positive cases for the Sudanoguinean Area. Four compounds were found in the different samples, Satratoxine h being the most found compound in 46.15% followed by Propiolonitrile and Fumaronitrile (20%) while only one sample (6.67%) was containing Diclofop. No significant difference (p>0.05) was found between the prevalences of pesticides between the two agroecological zones even if the prevalence from the western highlands is higher than the one in the bimodal forest area.

Health risk assessment

From the values of the concentration of the different toxic components found, the assessment of the health risks associated with their presence was done but they presented no risk for the health as their concentrations were very low. The overall Hazard Index calculated was $1.08.10^{-8}$ <<<<1 as presented in Table 3.

DISCUSSION

Four toxic components were found in our study, firstly the biological toxin (satratoxin-h) which is synthesised by the molds of the genus Ascomycetes, secondly, pesticide residue: diclofop, propiolonitrile and fumaronitrile.

Table 1. Sample size allocation according to the technological levels in studied areas.

Agro ecological areas	Honey productions (2019) in tons	Weigh (%)	Sample size	Sample size allocated per technological level		
				Level 1 (hives)	Level 2 (extraction)	Level 3 (markets)
Soudano-guinean	987	13.77	30	10	10	10
Bimodal forest	4 522	63.12	90	30	30	30
Western highlands	1 655	23.09	30	10	10	10
Total	7 164	100	150	50	50	50

Table 2. Prevalence of honey contamination by type of pesticide according to the agroecological areas and collection sites.

Zones	technological levels	samples colected	Compounds				Total	Prevalence	Odds ratio	p-value
			Satratoxine-h	Diclofop	Propiolonitrine	Fumaronitrile				
Bimodal forest	hive	30	1	1	2	0	4	6,67±0,55	0.84	0,23
	Extraction	30	0	0	0	0	0			
	market	30	2	0	0	0	2			
	Total 1	90	3	1	2	0	6			
Western highlands	hive	10	1	0	0	0	1	23,37±0,32	1.19	
	Extraction	10	2	0	0	1	3			
	market	10	0	0	1	2	3			
	Total 2	30	3	0	1	3	7			
Total (Total 1 + Total2)		150*	6 (46.15%)	1 (6.67%)	3(20%)	3(20%)	13 (100%)	8,67±0,29		

*The Total of 150 is obtained with the sample (50) of the Sudanoguinean area which were not put in the table as no positive result was obtained.

Table 3. Hazard quotient and index from the concentrations of toxic components according to the technological level and the studies areas.

Compound (ARfD)	Concentration (µg/kg)		Mean daily intake (µg/kg)		Hazard quotient	
	Bimodal Forest	Western Highlands	Bimodal Forest	Western Highlands	Bimodal Forest	Western Highlands
Satratoxine h (LD_{50} =1 400µg/kg)	20	35	$1,42.10^{-5}$	$1,79.10^{-5}$	$1,01.10^{-8}$	$1,28.10^{-8}$
Diclofop (LD_{50} =563 000µg/kg)	10	0	$7,14.10^{-6}$	0	0	$1,27.10^{-11}$
Propiolonitrile (LD_{50} : 39 000µg/kg)	20	10	$1,42.10^{-5}$	$7,14.10^{-6}$	$3.64.10^{-10}$	$1.89.10^{-10}$
Fumaronitrile (LD_{50} :132 000µg/kg)	0	30	0	$2,14.10^{-5}$	0	$1,62.10^{-10}$
HI					$1,04x10^{-8}$	$3,76.10^{-10}$

The presence of Satratoxin h, a toxin synthesized by molds of the genus Ascomycetes, can be explained by the fact that under certain conditions especially high moisture content, fungi are generally found in honey samples (Tchoumboué *et al*., 2007; Xiong *et al*., 2023).

Furthermore, the study has revealed the presence of this toxin at all the different levels (hive, after extraction and at the market) which implies the fact that throughout the production and processing, hygienic conditions and probably normal physico-chemical characteristics of honey were not met for microbial inhibition of these agents as also described by some studies (Olaitan *et al*., 2007; Al-Waili *et al*., 2012; Nzeh *et al.,* 2022).

The presence of Diclofop, an organochlorine herbicide, is mainly explained by indirect contamination linked to its use in agriculture for the treatment of the crops (Galani *et al*., 2021). It is therefore possible that crops surrounding the apiary were exposed to this pesticide and brought to the hive through bee foraging. However, it is worth noting that this pesticide is not officially approved in Cameroon (MINADER, 2019). Moreover, its presence only at the hive level mays suggest a recent contamination as its also rapidly degradable (NCBI, 2023). The presence of this residue confirms the results of previous studies in Cameroon and elsewhere where pesticides residues have been found in animal products and vegetables sometimes at levels above the acceptable limits (Eissa *et al*., 2014; Cesar *et al*., 2017; Calatayud-Vernich *et al.,* 2018; Galani *et al*., 2021; Albero *et al.,* 2023).

As for propiolonitrile and fumaronitrile, two highly toxic compounds, their presence may be explained either by their use in field activities or more certainly by their formation during gas chromatography. Indeed, these compounds and similar have been described as being able to form during the pyrolysis phases, similar to those which take place during chromatography (Vin, 2019).

The health risk assessment linked to the toxicological contamination in this study showed that different honeys from all the area do no present a danger for consumers. These results are in conformity with many other studies done in Africa, where level of pesticides residues in honey are generally lower than the minimum acceptable levels and do not represent a risk for the public health (Irungu *et al*., 2016; Darko *et al*., 2017).

This is mainly explained by the fact that direct contamination is very low, as beekeepers in Africa in general do not use pesticides to fight against bee predators and pest compared to other parts of the world (Irungu *et al*., 2016; Fikadu, 2020). The main reason for that is the fact that African bee species in general have a lot of diversity, are aggressive and a better resistance against these predators and pest (Pirk *et al*., 2016; Rasoloarijao, 2018).

However, the low presence of pesticides residues in honey does not necessarily a low level of contamination, as they generally are more present in beeswax (Cesar *et al*., 2017; Albero *et al*., 2023).

Conclusion and recommandations

The evaluation of the toxicological characteristic of honey in Cameroon according to the technological levels shown that no health risk is associated to the consumption of honey even if four main toxic compounds were found. Even if the levels were below the acceptable limits, the presence of these compounds is to be taken as a signal as this study has only investigated non polar and volatile compounds through GC-MS. Therefore a complete study at a larger scale shall be done to search for all types of pesticides. Moreover, if these levels do not represent a danger for human consumption, it may not be the same for pollinators in general and bees in particular. Therefore good beekeepings practices should be promoted from the production level to the commercialisation in order to prevent an increase in the contamination process in the future.

ACKNOWLEDGEMENT

We would like to express our thanks to the Ministry of Livestock, Fisheries and Animal Husbandry, to the Ministry of Agriculture and Rural development, so as the different institutions who helped us to gathered the information for the research. The research team is also grateful to all the beekeepers for their collaboration, enthusiasm and willingness to share with us useful information.

CONFLICT OF INTEREST

We declare that we have no any financial or personal interest that inappropriately influences in writing this article.

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