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RMN ^{13}C y ^{1}H Spectrum of toxic pesticides to human health, used in *Annona muricata* crops

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BACKGROUND
Indiscriminate usage of pest control substances on the food crops is a problem that has become a risk for human health, thus, is important to carry through researches that enable the comprehension of pesticides' properties and characteristics and the way they interacts with the environment and all the living organisms.

OBJECTIVE
Obtain the most important signals, displacements and characteristic peaks of each vibration and the structural composition present on Malathion, Glyphosate, Chlorpyrifos, Diazinon, Dichlorvos and Dimethoate, organophosphate pesticides used on *Annona muricata corps*.

RESULTS
The existent data is precise, due to the coefficient of variation is less than the 7%, except hydrogen one of the Dichlorvos in the RMN ^{1}H analysis, which value was 9.313, indicating that this data was acceptable, however, for the rest of studied pesticides there were no significant difference between the three employed databases.
For both Chlorpyrifos and Diazinon RMN ^{1}H, spectrums shows no difference thus confirm that data is close between the three databases and CV <7%.

CONCLUSION
According to the results, for each pesticide an estimated displacement was established, from the possible molecular vibration, for the carbon compounds as for the hydrogens, displacement that corresponds to the data measurement.

KEY WORDS
Pesticide, Annona muricata, RMN ^{13}C, RMN ^{1}H.

INTRODUCTION

Agriculture is one of the main food supply sources for humans. Due to the rate of population growth, agricultural production methods had been implemented, improving food's quality, quantity, and efficiency for the national and international market. Pesticides is one of those methods, according to the United Nation Organization for Food and Agriculture definition, Pesticides are blends of chemical substances set aside for prevent, control or destroy any plague, including human or animal, illnesses, unwanted plants species and animals that interfere for food production, elaboration, storage, shipment or commercialization or that can be given to animals to combat insects, arachnids or other pests in their bodies [1].

Although pesticides have many benefits, its uncontrolled and excessive usage has generated several environmental problems, specially long term problems, the reason is that every day the exposed species get an unknown dose, threatening their health as, so intoxication or poisoning could be unnoticed [2], [3]. In Colombia, an agricultural country by excellence. Pesticide usage on fruit and vegetable crops is essential. However, the misuse of these substances is the most common act, because of the monocrops, where the chemicals are not correctly provided causing increase in the risk level, for the farmers, final consumers and the environment [4].

It´s know that in Colombia, there are about 436 pesticides tons, stored around the country, without the required safety conditions [5]. In addition to this, there are 4.973 of contaminated hectares with an unknown amount of buried toxics. It's been reported that many of this toxic elements are in the middle of industrial and residential areas [6].

Nowadays, in Colombia the toxicological studies, control and supervision of food pesticides, is not completely implemented. Thus the actual researches are focused on monitoring the residues, aiming to determinate all the possible short and long term toxicological consequences and the exposition level of human [7], [8], [9]. This residues are found in concentrations called traces, generally expressed in parts per (µg pesticide/g food). Small quantities of this toxic compounds, blended with the natural chemical food compounds, make their analysis a hard job, taking into account that natural compounds could interfere with the measurement of the pesticides residues [10].

Several quantification ways has been studied, among the most popular exist the liquid chromatography (HPLC) [11], [12], [13] gas (CG), [2] [4], [10], [14], [15] and ultraviolet colorimetry (UV) [16], [17], [18]. Other way is to use the nuclear magnetic resonance spectroscopy ^{13}C y ^{1}H, as a molecular structure identification. The resultant spectrum analysis needs time and it must be done by specialist, this increases the cost for the enterprises.

In this works, one RMN ^{13}C y ^{1}H spectrum recompilation is presented, of six organophosphate pesticides: Malathion, Glyphosate, Chlorpyrifos, Diazinon, Dichlorvos and Dimethoate in *Annona muricata*. Easing the comprehension of the ^{13}C and ^{1}H chromatogram spectroscopic analysis, obtained from the nuclear magnetic resonance spectroscopy food analysis, reducing time and cost of this task.

MATERIALS AND METHODS

A. Materials:

I) Pesticides:

According to the World Health Organization (WHO), the pesticides are classified by the toxicology of the active ingredient, as: IA (extremely dangerous –color red), IB (highly dangerous-color red), II (moderately dangerous-color yellow), III (slightly dangerous-color blue) and U (acute risk are improbable in normal use situations -color green) [19].

In Colombia the same rating is used but the classes are listed from I to IV, depending on the toxicology of the formulated product registered in the ICA [20]. In table 1, the qualification of six pesticides used for the soursop *(Annona muricata*) crops is shown.

Table 1: Toxicology qualification of pesticides used on *Annona muricata*

Pesticide	WHO´s Toxicology	Colombian Toxicology
Malathion	III	II-III
Glyphosate	U	III-IV
Chlorpyrifos	II	III
Diazinon	II	II
Dichlorvos	Ib	I
Dimethoate	II	II

II) Software:

To collect the representative data and the RMN ^{13}C and ^{1}H spectrum for each compound, three databases where used, two of free access and the *nmrdb.org Tool for spectroscopists* software *[21]*, that predicts RMN ^{1}H and ^{13}C, COSY and HSQC spectrums from the proposed molecular structure, at the same time the obtained values could be exported as an image or as determinate values. The second database *Spectral Database for Organic Compounds SDBS [22]*, gives information from the common name or IUPAC, of the molecular formula, CAS register or molecular weight, also information about RMN 1H and 13C specters, masses and IR. Finally, the *MestReNova* software, provides the RMN 1H and 13 C, COSY, HSQC spectrums from the compound molecular, enabling the adjustment of the dissolvent and reading frequency (MHz) parameters

B. Methods:

I) Molecular structure acquisition:

There are studies and software, which permits the prediction of possible molecular peaks and displacements in RMN ^{1}H y ^{13}C spectrums [23]. Existent software draw the required structure and see the RMN spectrums, but by now, just a few of them are capable to show the molecular structure from the RMN ^{1}H and ^{13}C spectrums, and at the same time they are very expensive.

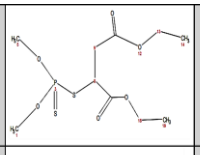
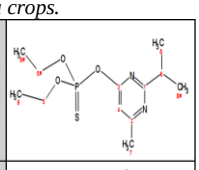
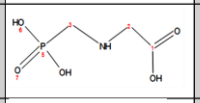
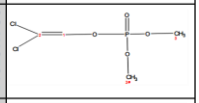
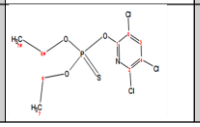
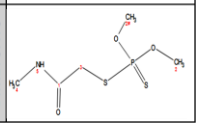
In relation to the above, the studied pesticides molecular structure were drawn as reported in the *SBDS database*, and were numerated in the two other databases, obtaining the structures shown in Table 2**Error! Reference source not found.**.

II) RMN ^{1}H and ^{13}C spectrum obtainment:

Due to the toxicity of the pesticides used for the *Annona muricata´s* pest control, stablish the traces or residues that humans could eat is required. The analysis of those traces are made in laboratories, one of the ways to identify and elucidate the structures and compounds, is by the nuclear magnetic resonance (RMN), this one use the analysis of all the signals, their position, form, and size to indicate the molecular structure of a compound [24], the environment of the hydrogen atoms (or carbon atoms), and gives information on the alkyl groups and the functional groups present in the molecule.

For the RMN ^{1}H and ^{13}C spectrum obtainment of the pesticides mentioned above, the three databases were set with the dissolvent and reading frequency parameters reported by the *SDBS*. This way, for RMN ^{1}H, 399.65 MHz frequency was employed and deuterium chloroform dissolvent ($CDCl_3$), except for glyphosate, which used the deuterium oxide (D_2O). On the other hand, for RMN ^{13}C, the dissolvent was the same of RMN ^{1}H and the frequency was 100.4 MHz.

Table 2: Molecular structures of the pesticides used on *Annona muricata crops.*

MALATION		DIAZINON	
GLIFOSATO		DICLORVOS	
CLORPIRIFOS		DIMETOATO	

Obtained Results:

Aiming to analyze the existence differences between the signal values and displacements (ppm) according to the databases, the standard deviation and coefficient of variation (CV) is calculated for the RMN ^{1}H and ^{13}C spectrums, the obtained results are shown in **Error! Reference source not found.**. They expose that the existent data is precise, due to the coefficient of variation is less than the 7%, except hydrogen one of the Dichlorvos in the RMN ^{1}H analysis, which value was 9.313, indicating that this data was acceptable. However, for the rest of studied pesticides there were no significant difference between the three employed databases.

The above was confirmed by comparing the RMN ^{13}C spectrums of each molecule (see **Error! Reference source not found.**), it can be observe that there is no appreciable displacement between the three databases, ratifying that (ppm) values are close and the coefficients of variation and small and precise.

Table 3: RMN ^{1}H and ^{13}C database comparison.

Análisis RMN	Plaguicida	Numero de H	Media	N	Desviación estándar	CV	Análisis RMN	Numero de Carbono	Media	N	Desviación estándar	CV
1H	Malation	1	3,590	4	0,173	4,830	13C	1	54,293	4	0,136	0,250
		5	3,594	4	0,178	4,945		5	54,310	4	0,131	0,242
		8	4,148	4	0,145	3,494		8	44,043	4	1,424	3,232
		9	3,166	4	0,094	2,956		9	38,327	4	0,717	1,870
		9#	2,983	4	0,181	6,059		10	170,123	4	0,528	0,310
		13	4,371	4	0,286	6,551		13	61,416	4	0,572	0,931
		13*	4,004	4	0,241	6,016		14	14,130	4	0,022	0,153
		14	1,196	4	0,051	4,247		15	171,137	4	1,855	1,084
		18	4,242	4	0,133	3,142		18	61,776	4	0,529	0,856
		18*	4,052	4	0,136	3,359		19	14,130	4	0,094	0,666
		19	1,239	4	0,040	3,234						
	Glifosato	2	3,657	4	0,220	6,006		1	173,413	4	4,516	2,604
		3	3,297	4	0,311	9,431		2	51,747	4	2,230	4,309
								3	44,553	4	0,996	2,236
	Clorpirifos	3	8,005	4	0,129	1,612		1	151,162	8	0,745	0,493
		6	4,163	4	0,189	4,545		2	143,893	4	0,174	0,121
		6*	4,152	4	0,174	4,186		3	141,083	4	0,217	0,154
		6#	4,163	4	0,189	4,545		4	126,510	4	0,113	0,089
		6#*	4,152	4	0,174	4,186		5	120,377	8	0,141	0,118
		7	1,314	4	0,084	6,392		6	65,838	8	0,187	0,283
								7	15,833	8	0,051	0,321
	Diazinon	4	6,446	4	0,222	3,444		1	175,420	4	0,057	0,032
		5	4,168	4	0,142	3,419						
		5*	4,163	4	0,136	3,275		2	169,337	4	0,457	0,270
		5#	4,168	4	0,142	3,419		3	164,747	4	0,646	0,392
		5#*	4,163	4	0,136	3,275		4	106,070	4	0,997	0,940
		6	3,158	4	0,033	1,050		5	65,192	8	0,323	0,496
		7	2,547	4	0,041	1,602		6	37,137	4	0,387	1,041
		8	1,282	4	0,032	2,528		7	24,157	4	0,047	0,195
		8#	1,282	4	0,032	2,528		8	21,077	4	0,332	1,575
		9	1,296	4	0,070	5,372		9	16,347	8	1,086	6,643
		9#	1,296	4	0,070	5,372						
	Diclorvos	1	6,853	4	0,638	9,313		1	133,180	8	0,793	0,595
		3	3,761	4	0,093	2,480		2	113,160	8	1,159	1,025
								3	56,105	8	1,502	2,677
	Dimetoato	2	3,586	4	0,167	4,657		1	167,910	4	0,205	0,122
		3	3,552	4	0,089	2,519		2	54,347	8	0,125	0,231
		4	2,726	4	0,099	3,643		3	36,273	4	0,090	0,248
		5	6,19	3	0,28	4,523		4	26,720	4	0,014	0,053
		2#	3,586	4	0,167	4,657						

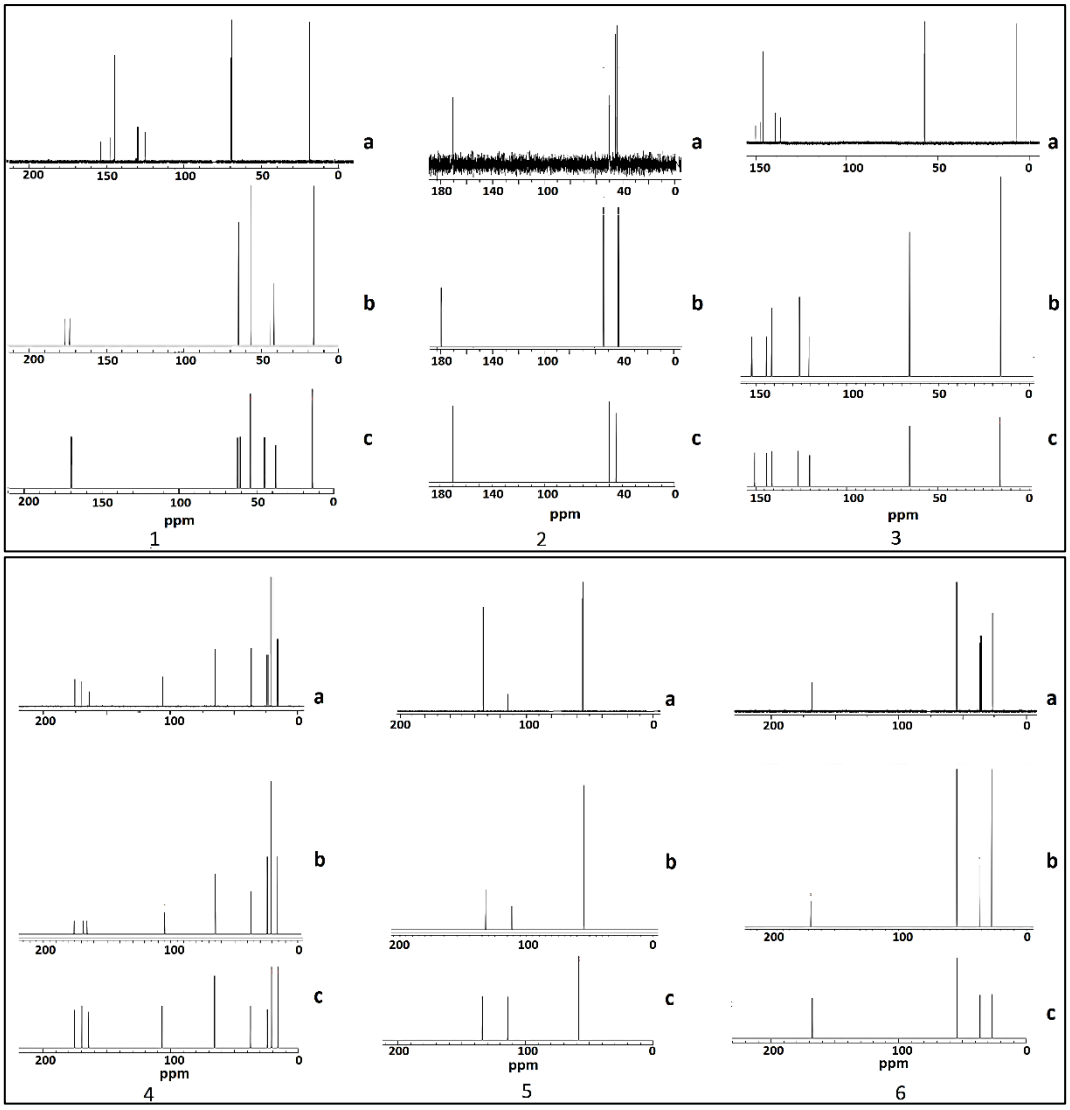
On the other hand, it can be observed that RMN ^{1}H (see Illustration 2), the Malathion spectrum (number 1) presents more peaks with the software *MestReNova* (b), than it does with *SDBS* (a) and the *NMRDB* (c), because the hydrogens 13, 13*, 18 and 18*, from the R-CH_2-X (X= N, O, Halogen) the *MestReNova* present them at different displacements or peaks, 4.770; 3.670; 4.430 and 3.860 ppm, respectably. Instead, this hydrogens appears for the *SDBS* a 4.229 (13, 13*), 4.160 (18, 18*) y el *NMRDB* a y 4.113 (13, 13*) y 4.136 (18, 18*).

MestReNova software, presents for Glyphosate (Illustration 2, spectrum 2), a 4.70 ppm signal characteristic of labile protons (-OH, -NH2, -SH) presented in the molecule. Regarding Dimethoate (**Error! Reference source not found.**, spectrum 6), there is a 5.901 ppm signal from the Hydrogen 5 (NH) molecule labile proton, the labile protons are excluded from the other databases.

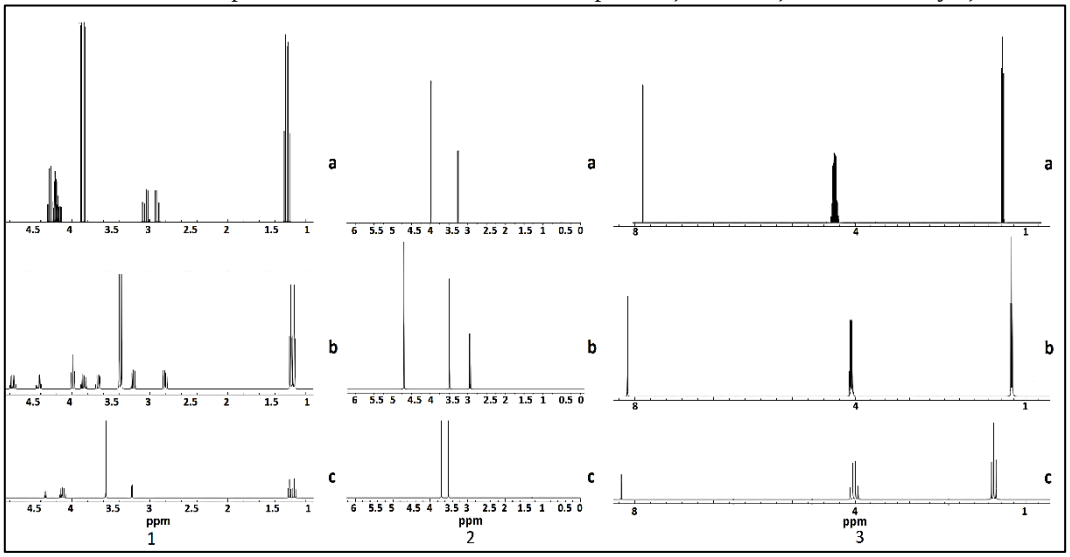
For the al Dichlorvos (Illustration 2, spectrum 5), a difference in the signal of del Hydrogen 1 was observed in each database,

This is verified with the findings above with the coefficient of variation (9.3%). For both Chlorpyrifos and Diazinon (Illustration 2, spectrums 3, 4) RMN ^{1}H, spectrums shows no difference thus confirm that data is close between the three databases and CV <7%.

Finally, for the most used pesticides employed on *Annona muricata* crops, a databased recompilation is obtained, estimating the possible displacements (ppm) for both RMN ^{13}C and ^{1}H cases, which are show as a measurement in **Error! Reference source not found.** and that will help the researcher to define the own signals of each molecule.



Ilustration 1: RMN ^{13}C. 1-Malathion, 2- Glyphosate, 3- Chlorpyrifos, 4-Diazinon, 5- Dichlorvos, 6-Dimethoate spectrums. Where databases correspond: a) *SDBS*, b) *MestReNova* y c) *nmrdb*.



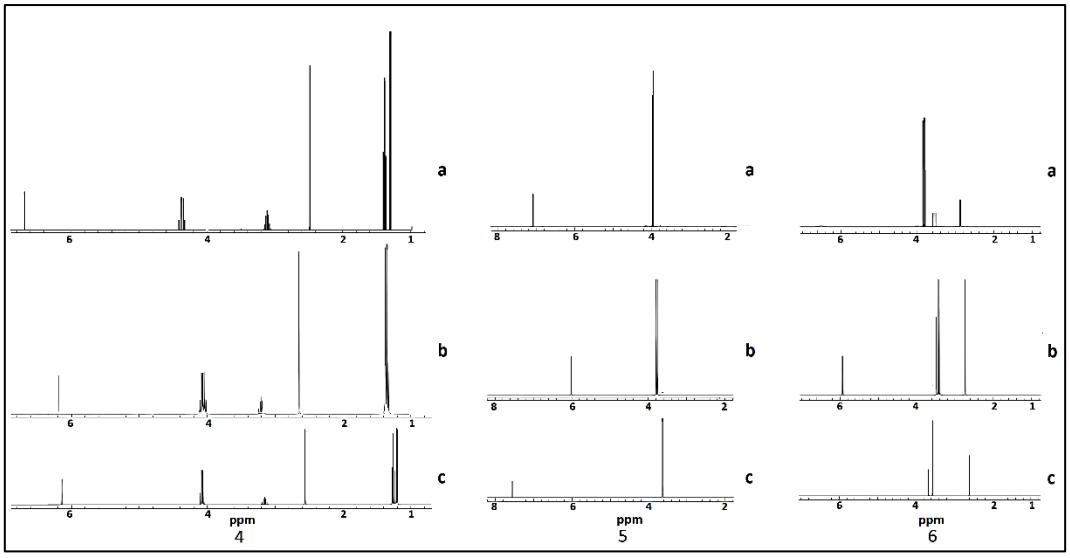


Illustration 2: RMN ^{1}H. 1-Malathion, 2- Glyphosate, 3- Chlorpyrifos, 4-Diazinon, 5- Dichlorvos, 6-Dimethoate spectrums. Where databases correspond: a) *SDBS*, b) *MestReNova* y c) *nmrdb*.

Conclusions and Future Perspectives:

According to the results, for each pesticide an estimated displacement was established, from the possible molecular vibration, for the carbon compounds as for the hydrogens, displacement that corresponds to the data measurement.

With this data recompilation, the researcher has a new RMN spectrum database for verify the pesticide spectrums.

The coefficients of variation found (<7%), when comparing the three employed databases, shows that there isn't a significant difference, hence they are. Even though the Hydrogen 1 CV of Diazinon was > 7%, the data is considered as acceptable.

Using the obtained data, it is proposed a software development that indicates, the presence of one of the pesticides used in the *Annona muricata,* when introducing the spectrums.

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